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An Overview of the Effects of Heavy Metals Content in Wastewater on Anammox Bacteria

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Abstract

The application of Anammox process in treating nitrogen rich wastewater had been more preferable since the discovery of Anammox process and Anammox bacteria at 1999 due to the advantages of energy saving and cost reduction compared to the conventional nitrification/denitrification process. However, often nitrogen-laden wastewater such as metal refinary wastewater, swine, industrial wastewater, and landfill leachate contain various concentrations of heavy metal ions such as Cadmium, Copper, Lead, Mercury, Nickel, Zinc, Silver and Ferrous iron. Trace amount of Zn(II), Co(II), Mn(II), Cu(II) and Ni(II) are recommended to be added in substrate during cultivation of Anammox bacteria as essential micronutrients. These metals are crucial co-factors for certain enzymes and metalloproteinase. Nevertheless, regardless of stimulation effect of some metals on the growth of bacteria at low concentration, a high concentration of metals ions might cause a negative effect on long term. The inhibition effects of various heavy metals were compared in this study. It was found that nine heavy metal, Pb^{2+} has the lowest inhibition effect on Anammox process, Cu^{2+} might be having the most inhibition effect on Anammox with the IC_{50} inhibition concentration. The IC_{50} inhibition concentration of Fe (II) was found at 0.20 mM. It was found Pb^{2+} has the lowermost inhibition effect, AT > 75 mg/L of Pb^{2+}, while, Cu^{2+} might be having the supreme inhibition effect with the IC_{50} inhibition concentration at 1.9 mg/L. The IC_{50} of Fe (II) was found at 55.6 mg/L. More effort should be dedicated to understand the profound knowledge of heavy metal on Anammox bacteria.

Keywords

Anammox, Wastewater, Heavy metal, Inhibition, IC_{50}, Stimulation

Abbreviations

Anammox: Anaerobic Ammonium Oxidizing; AOB: Ammonia Oxidizing Bacteria; COD: Chemical Oxygen Demand; EDX: Energy Dispersive X-ray; Hao: Hydroxylamine Oxidoreductase; HDH: Hydrazine Dehydrogenase; hh: Hydrazine Hydrolase; HZO: Hydrazine Oxidizing Enzyme; HZS: Hydrazine Synthase; IC_{50}: Half Inhibition Concentration; N: Element Nitrogen; NH_4^+: Ammonium; NO: Nitric Oxide; N_2O: Nitrous Oxide; NOB: Nitrite-Oxidizing Bacteria; SAA: Specific Anammox Activity; TN: Total Nitrogen

Introduction

The concept where ammonium can be oxidized under anoxic condition initially came from calculations based on theoretical thermodynamic [1] and the Redfield ratio in marine ecosystems [2]. The concept was proven 20 decades later by Mulder, et al. [3] who discovers the anaerobic ammonium oxidizing (anammox) process (Eq. (1)) and Strous, et al. [4] who found the responsible microorganisms, anammox bacteria. Anammox bacteria are Planctomycete type bacterium with anaerobic (no need oxygen) and autotrophic (no need organic carbon) metabolism, which combines ammonium (as electron donor) and nitrite (as an electron acceptor) to generate dinitrogen gas in the absence of oxygen.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.256NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

The process of Anammox has become essential at the global level as it contributes to oceanic nitrogen loss level

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Table 1: Various concentration of nitrogen in different type of wastewater.

Type of Wastewater	Ammonium Nitrogen Concentration (mg/L)	References
Industry:		
Metal Plating	50-600	Stankovic, et al. [40]
Rubber Manufacturing	150-200	Subbiah, et al. [41]
Domestic	20-40	Davis, et al.; Santos, et al. [42,43]
Landfill Leachate	50-2200	Kjeldsen, et al.; Renou, et al. [44,45]
Swine	400-800	Nicholson, et al.; Vanotti, et al. [46,47]

and has multiple advantages compared to the conventional nitrification/denitrification process in the removal of nutrient from wastewater [5]. In addition, Anammox process is critical in the ocean's nitrogen cycle, wherein the continental shelf sediment, Anammox contributes up to 67% of the nitrogen gas and only 33% of nitrogen gas formed due to denitrification process [6]. Anammox process is considered as most cost effective biological nitrogen removal processes, and the advantages of Anammox process over conventional nitrification/denitrification process are: i) Save 90% operation cost (reduces 64% aeration, 80-90% sludge production and 100% exogenous electron donor (e.g organic carbon)) [7-11] reduced CO_2 or N_2O emissions [5]. Anammox process is a promising method for treating nitrogen rich and low chemical oxygen demand (COD) content wastewater such as landfill leachate and metal refinery wastewater. Table 1 shows various concentration of nitrogen in different type of wastewater. However, these type of wastewaters contain a high concentration of heavy metals [12-14]. Heavy metals such as iron, zinc, copper, nickel, and cobalt can either cause stimulation, inhibition or even toxic effect in biochemical reactions dependent on the concentrations and species [13]. Therefore, it is crucial to obtain more knowledge about the effect of heavy metal on the microbial activity and the performance of the reactor. This aims to provide a compressive review concerning the stimulation and inhabitation effects of heavy metals on anammox process and anammox bacteria activities.

Removal of Nitrogen Compounds from Wastewater

The element nitrogen (N) in its various redox forms are essential macronutrients for all living organisms on earth. Our land's atmosphere is made up of 78% of dinitrogen gas (N_2). However, living organisms cannot access the nutrients of nitrogen from the atmosphere directly. A specialized group of bacteria can fix dinitrogen gas from the air to ammonium (NH_4^+), then, the fixed nitrogen, in the form of NH_4^+, will either enter food chain directly by assimilation process for macromolecule biosynthesis or it will be used as a substrate for bacteria which acquired energy for growth throughout the oxidation. Figure 1 shows the nitrogen cycle in nature that is responsible for all the reaction of the bacteria that convert nitrogen to its various redox forms, from +5 (NO_3^-) to -3 (NH_4^+/NH_3). Nitrification (autotrophic conversion of ammonium to nitrite then further to nitrate), is a stepwise oxidation process where ammonia oxidizing bacteria (AOB) oxidize NH_4^+ to NO_3^- and nitrite oxidizing bacteria (NOB) oxidize NO_2^- to NO_3^-. Denitrification (heterotrophic conversion of nitrate to dinitrogen gas) is a stepwise reduction process where denitrifying bacteria reduced NO_3^- to N_2 with NO_2^-, nitric oxide (NO) and nitrous oxide (N_2O) as intermediate. Furthermore, in the process of nitrogen fixation, some non-symbiotic and some symbiotic with leguminous plants will fixate the atmospheric N_2 to NH_4^+. In Anammox process, autotrophic bacteria utilize NH_4^+ as an electron donor and NO_2^- as an electron acceptor and convert into nitrogen gas and some nitrate under anaerobic conditions in the absence organic carbons.

Conventional nitrification/Denitrification process

Due to the elevation of industry or/and agriculture activities, and discharge of excess nitrogen from wastewater directly into water bodies such as river and lake without advanced treatment, nature suffered from eutrophication and hypoxia. Nowadays worldwide environmental authorities enforcing stringent nitrogen discharge criteria. The conventional nitrogen removal system implemented nitrification and denitrification process to helped in treating nitrogen-rich wastewater before discharging to nature. In autotrophic nitrification process, aeration is necessary as both AOB and NOB need either oxygen or sulfate and ferric as other oxidized compounds, as an electron acceptor to oxidize NH_4^+ to NO_3^- and oxidize NO_2^- to NO_3^-. While in heterotrophic denitrification process (from NO_3^- to N_2), addition external electron donor (e.g. organic carbon) such as methanol (in majority case) is required for the denitrifying bacteria. Intermediate such as NO_2^-, NO and N_2O will be produced in denitrification process. When applying nitrification/denitrification in the process of nitrogen removal, a significant amount of oxygen is needed, large amount of sludge produced, and the considerable volume of N_2O generated a greenhouse gas with a potential roughly 300 times higher than CO_2.

Anaerobic ammonium-oxidizing (Anammox) process

Anammox bacteria is a coccoid-shaped *Planctomycete* type bacterium with anaerobic (absence of oxygen) and autotrophic (not require organic carbon) metabo-

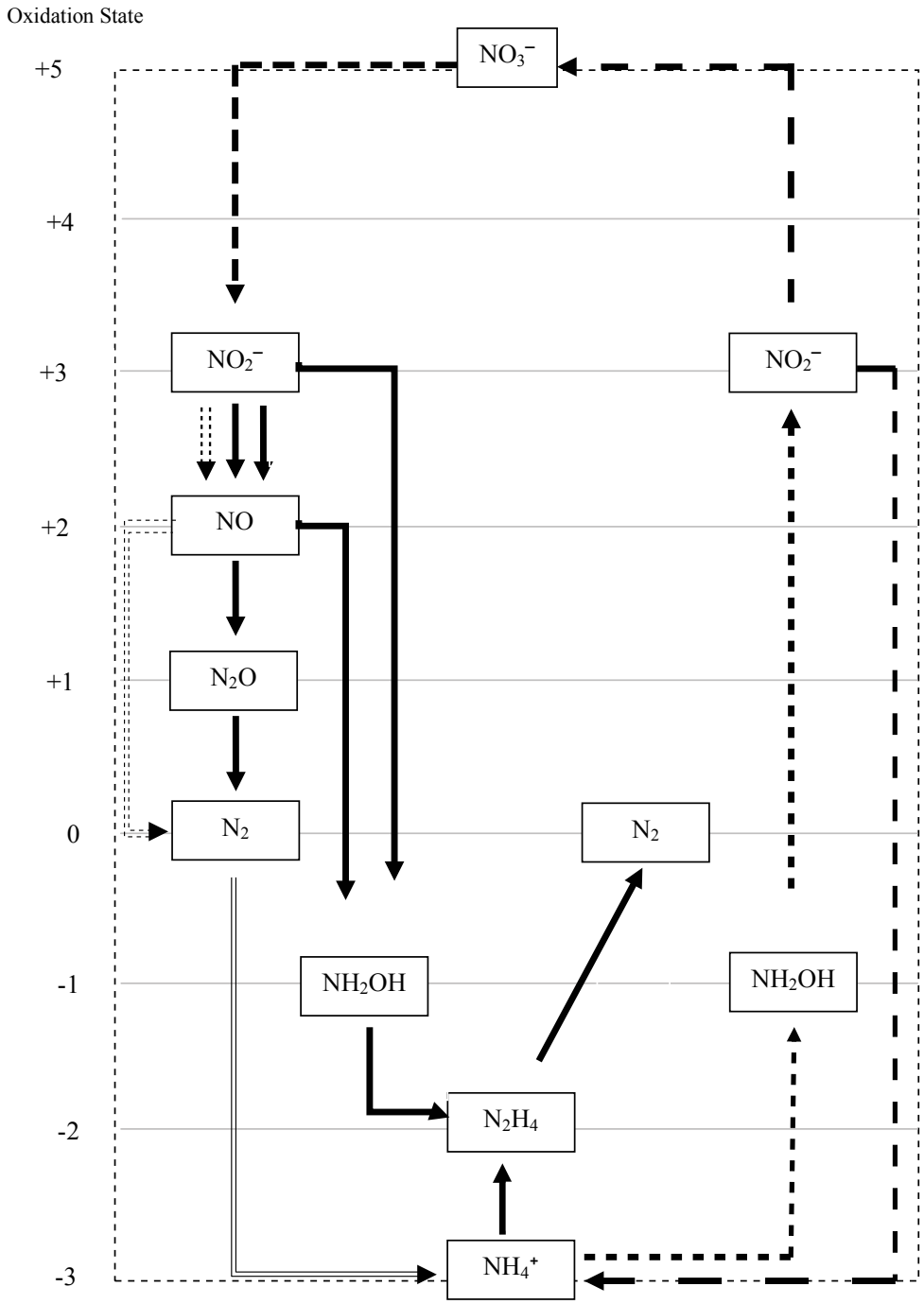

Figure 1: The Nitrogen Cycle. The proposed biochemical pathway of the anammox process is as follows: first, NO_2^- is reduced to nitric oxide (NO) then, the produced NO is reduced to hydroxylamine (NH_2OH) and coupled with NH_3 to form hydrazine (N_2H_4) by hydrazine synthase (HZS) and finally, the N_2H_4 is oxidized to N_2 gas. However, in 2016, Oshiki proposed another pathway of Anammox bacteria with N-tracer experiments which demonstrated that "*Candidatus Brocadia sinica"* cells could reduce NO_2^- to NH_2OH, instead of NO, with as yet unidentified nitrite reductase(s).

lism, which utilize ammonium (NH_4^+) (as electron donor) and nitrite (NO_2^-) (as electron acceptor) in a ratio of 1:1.3 during the growth phase to generate dinitrogen gas (N_2) and some nitrate (NO_3^-). Anammox used bicarbonate as carbon sources to produce biomass ($CH_2O_{0.5}N_{0.15}$) (see Eq. (1)), which also acts as a buffering agent in Anammox process. Nitrite not only functions as an electron acceptor for ammonium oxidation but also as an electron donor for the reduction of carbon dioxide. The central catabolic Anammox metabolism can be described as a three-step reaction. It involves nitric oxide (NO) and hydrazine (N_2H_4), a well-known rocket fuel, as intermediates [15]. First, nitrite (NO_2^-) will be reduced to nitric oxide (NO), catalyzed by nitrite reductase (Nir) (which also named nitrite reducing enzyme (NR)) (Eq (2)). Then the nitric oxide (NO) and ammonium (NH_4^+) will be

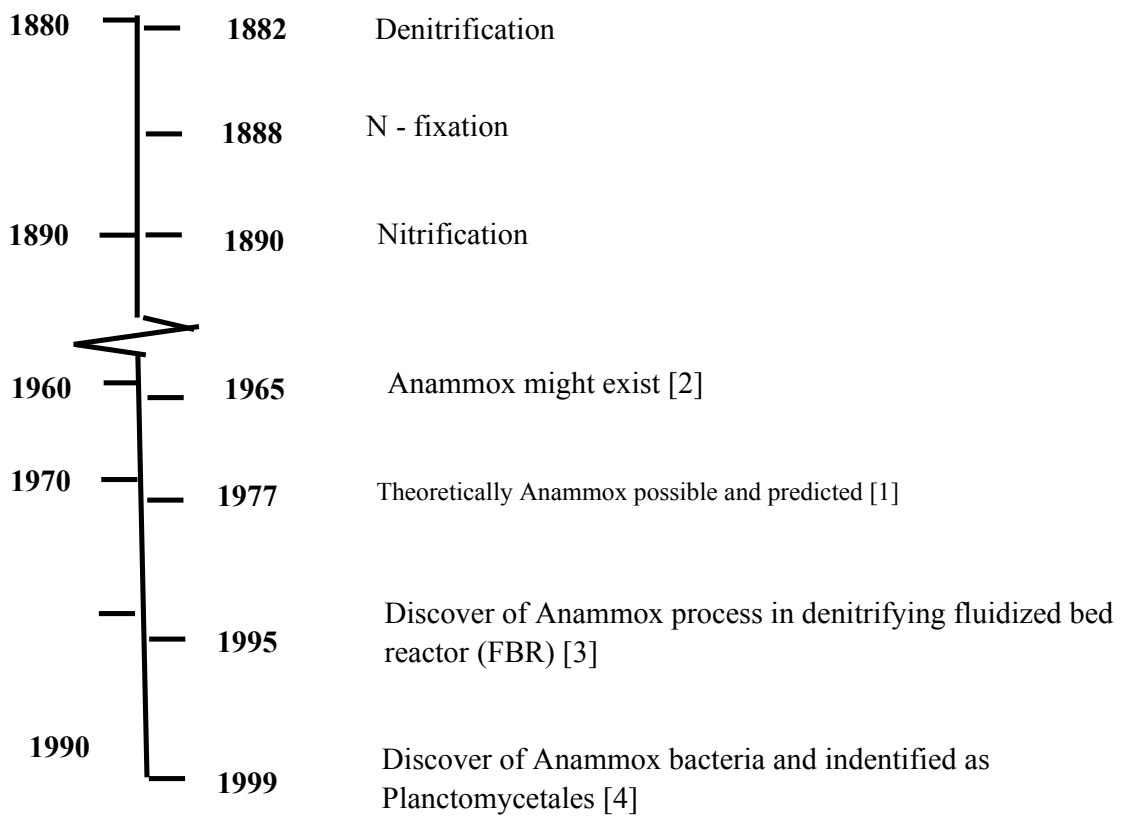

Figure 2: Timeline of discovery of Anammox and Electro-dense Anammoxosome particles containing iron.

condensed to hydrazine (N_2H_4) through hydrazine synthase (HZS) enzyme (Eq (3)), followed by oxidation of hydrazine (N_2H_4) to nitrogen gas (N_2) catalyzed by hydrazine oxidizing enzyme (HZO) (Eq (4)).

$$NO_2^- + 2H^+ + e \rightarrow NO + H_2O \ (E_0' = +0.38\ V) \quad (2)$$

$$NO + NH_4^+ + 2H^+ + 3e \rightarrow N_2H_4 + H_2O \ (E_0' = +0.06\ V) \quad (3)$$

$$N_2H_4 \rightarrow N_2 + 4H^+ + 4e \ (E_0' = -0.75\ V) \quad (4)$$

$$NO_2^- \rightarrow NO^- + 2H^+ + 2e \ (E_0' = +0.42\ V) \quad (5)$$

Until now five genomes have been identified: '*Candidatus Brocadia', 'Candidatus Kuenenia', 'Candidatus Scalindua', 'Candidatus Anammoxoglobus' and 'Candidatus Jettenia'*. Together, they form the monophyletic order Brocadiales that branches deeply in the phylum Planctomycetes [11]. Timeline of discovery of Anammox and Electro-dense Anammoxosome particles containing iron are illustrated in Figure 2.

Conventional nitrification/denitrification vs. Anammox process

Before the discovery of Anammox process and Anammox bacteria, conventional nitrification/denitrification process was one of the milestones in nitrogen removal technology that helped to prevent environmental disasters such as eutrophication and hypoxia. However, there are several drawbacks in conventional nitrification/denitrification process in treating nitrogen rich wastewater include: i) Requirement of aeration for conversion of NH_4^+ to NO_3^- in autotrophic nitrification process, ii) Heterotrophic denitrification process requires external carbon source (e.g., electron donor) such as methanol (in majority cases), that ultimately derived from fossil fuel and iii) Both process release N_2O, which is the primary cause of ozone depletion [16] and iv) Produce large amount of sludge. In addition, besides being a novel-method, Anammox process are environmentally friendly and cost effective compared to conventional nitrification/denitrification. Anammox directly convert ammonium, NH_4^+ (electron donor) and nitrite, NO_2^- (electron acceptor) to nitrate, NO_3^- and dinitrogen gas, N_2 without both oxygen and organic carbon.

Genera and Species of Anammox

Ten species of Anammox bacteria had been identified (Table 2) based on culture dependent methods and 16S rRNA analysis, which belong to five genera which together form the order of *Brocadiales*, branching deeply in the bacteria phyl*um Planctomycetes.* Among five genera of Anammox bacteria, *Brocadia, Kuenenia, Anammoxoglobus,* and *Jettenia* are belong to fresh water species while *Scalindua* belong to marine species [17].

Table 2: Ten species of Anammox bacteria in five genera.

Genera	Species	References
Candidatus Brocadia	*Candidatus Brocadia anammoxidans*	Strous, et al. [4]
	Candidatus Brocadia fulgida	Kartal, et al. [25]
	Candidatus Brocadia sinica	Oshiki, et al. [48]
Candidatus Kuenenia	*Candidatus Kuenenia stuttgartiensis*	Strous, et al. [24]
Candidatus Scalindua	*Candidatus Scalindua brodae*	Schmid, et al. [49]
	Candidatus Scalindua sorokinii	Woebken, et al. [50]
	Candidatus Scalindua wagneri	Van der Vossenberg, et al. [51]
	Candidatus Scalindua profunda	
Candidatus Anammoxoglobus	*Candidatus Anammoxoglobus propionics*	Kartal, et al. [52]
Candidatus Jettenia	*Candidatus Jettenia asiatica*	Quan, et al. [53]
		Hu, et al. [54]

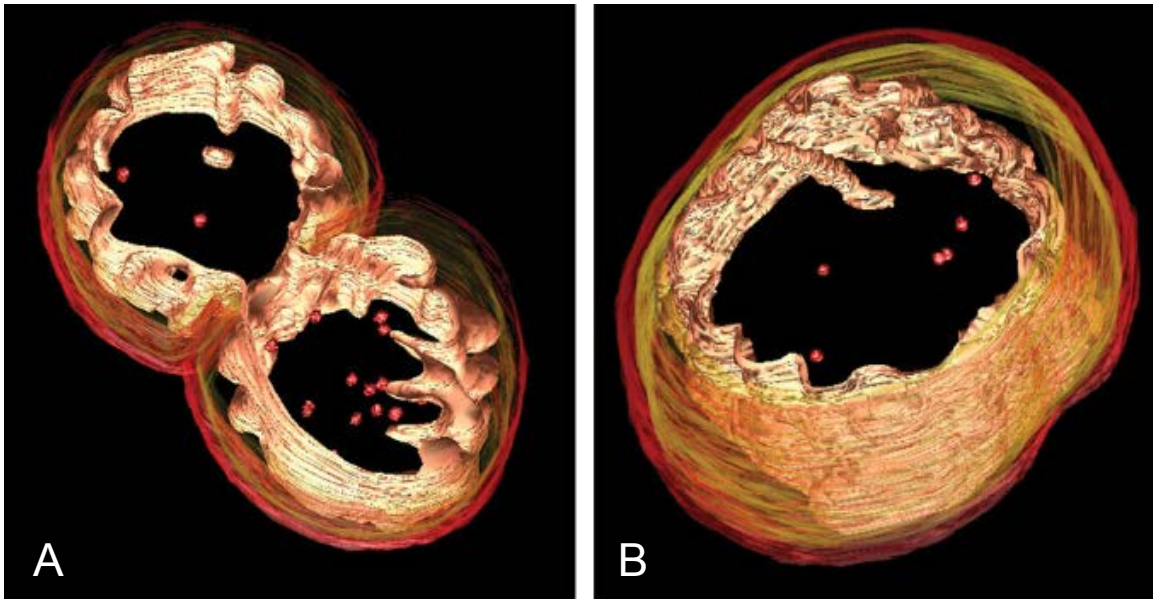

Figure 3: Snapshots of ''*Candidatus K. stuttgartiensis*'' electron tomography models showing the curved Anammoxosome membrane and iron particles inside the Anammoxosome (A) Dividing Anammox cell (Supplementary material Movie S1.1-1.3); (B) Single cell with deep protrusions of the Anammoxosome membrane into the Anammoxosome (Supplementary material Movie S2.1-2.3). Models show (from out to inside) cell wall (in transparent red), intracytoplasmic membrane (in transparent yellow), Anammoxosome membrane (in pink), and Anammoxosome particles (in red).

Source: Van Niftrik, et al. [21].

Morphology of anammox and Anammoxosome

In Anammox cell, there are three compartments divided by bilayer membrane; they are from inside to the outside: The Anammoxosome, riboplasm and paryphoplasm [18]. While the function of paryphoplasm remains unknown [19]. Inside riboplasm, there isribosomes and nucleoid and the role of riboplasm is similar to the cytoplasm of other bacteria, in which the translation and transcription will take place. The mechanism that sorted and transports the protein synthesized in riboplasm to other compartment remain unknown [20]. Anammoxosome is a membrane-bounded intracytoplasmic compartment in Anammox bacteria, with the majority of its membrane in a curved configuration as shown in Figure 3. Anammoxosome occupied most of the volume of Anammox cell [21], and the catabolism metabolism of Anammox bacteria are assumed to take place inside this compartment [20]. The researcher had discovered the existence of the iron-containing electron-dense Anammoxosome particles in the Anammoxosome compartment [21]. The storage of iron inside Anammoxosome compartment was for the excess supply of iron for further Heme c synthesis [22].

Effect of Heavy Metals on Anammox Bacteria

Stimulation effect of heavy metals on Anammox

It was found that the existance of limited amount of several heavy metals during the cultivation of Anammox cell will enhance anammox activity [23]. The common trace element solutions addded to substrate of Anam-

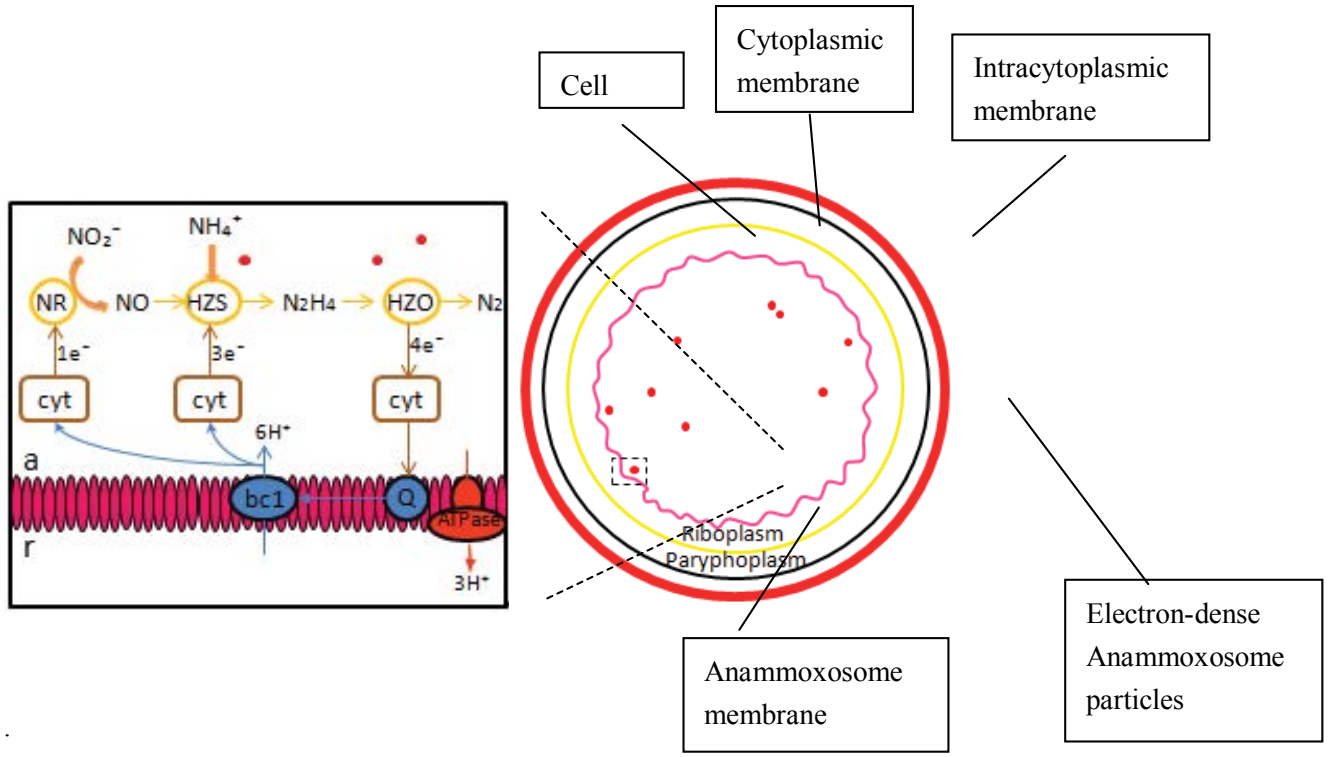

Figure 4: Proposed morphology of Anammox cell and model for catabolic Anammox reaction.

mox bioreactor (1.25 ml/L) contained EDTA (15 mg/L), $FeSO_4 \cdot 7H_2O$ (5 mg/L), $NiCl_2 \cdot 6H_2O$ (0.05 mg Ni/L), $CuSO_4 \cdot 5H_2O$ (0.06 mg Cu/L), $CoCl_2 \cdot 6H_2O$ (0.06 mg Co/L), $ZnSO_4 \cdot 7H_2O$ (0.10 mg Zn/L), $NaMoO_4 \cdot 2H_2O$ (0.10 mg Mo/L), $MnCl_2 \cdot 4H_2O$ (0.28 mg Mn/L), $NaSeO_4 \cdot 10H_2O$ (0.05 mg Se/L), and H_3BO_3 (0.014 mg/L) [24]. Heavy metal ions can enhance Anammox activity through stimulating the metabolism of Anammox cell by the fact that those metal ions can either be the component of many enzymes or the co-enzyme. Both molybdenum and copper are vital constituents of enzyme associated with the catabolism of Anammox cell, such as nitrite oxidoreductase [25] and nitrite reductase [23]. Besides that, enzyme of Anammox bacteria such as nickel-dependent hydrogenase [23] zinc-containing dehydrogenase [24] and ATP-dependent zinc metalloprotease Fts Hare also reliant on heavy metal. When starvation for those required heavy metal occurs, specific metal transport can be induced via different tranporter families suah as those driven by ATP.

In addition, Fe (II) is one of the essential nutrients for growth of Anammox. Since the discovery of Anammox process by Van de Graff, et al. [26], the Fe (II) concentration was set as 0.03 mM or 0.04 mM in most of the feeding medium of enriched Anammox sludge system. Energy dispersive x-ray (EDX) analysis revealed that several electron-dense Anammoxosome particles contained iron. In which the possible function of these Anammoxosome particles are for energy generation and as an iron storage facility for the heme-c enzyme involved in electron transport chain [21]. Therefore, Fe (II) can be the potential factor to affect the growth and activity of Anammox bacteria. Electron-dense An anammoxosome particles containing iron was found within the Anammoxosome compartment of Anammox [21]. Fe (II) is an essential nutrient for Anammox as it helps synthesize of heme c-containing enzyme of Anammox by forming the active region of heme c-containing enzyme. Hydrazine synthase (HZS), also named hydrazine hydrolase (hh) and hydrazine oxidizing enzyme (HZO) also called hydroxylamine oxidoreductase (hao), or hydrazine dehydrogenase (HDH) are two of the heme c-containing enzymes. In HZS enzyme catalyze formation of hydrazine (N_2H_4), the intermediates of Anammox from ammonia (NH_4^+) and nitric oxide (NO), while hydrazine oxidizing enzyme (HZO) helps in the formation of dinitrogen gas (N_2) by oxidizing hydrazine, at the same time providing electrons needed for hydrazine synthase and nitrite reduction Figure 4. shows the morphology of Anammox cell and model for catabolic Anammox reaction.

Since 2013, the researcher had evaluated the effect of different concentration of Fe (II) on Anammox. Liu, et al. [22] had study the relationship between the effect of various Fe (II) concentration and the growth rate of Anammox by batch test, and they found that growth rate (i.e. 0.172 d^{-1}) was maximum at 0.09 mM of Fe (II).

Similarly, Zhen, et al. [27] had investigated Anammox start-up period and they found that the shortest start-up (i.e. 50 d) was attained at 0.09 mM of Fe (II). Sen, et al. [28] investigated the total nitrogen (TN) removal percentage by batch test at different concentrations of Fe (II), and they found that the maximum total nitrogen removal percentage (63%) was observed at 0.09 mM of Fe (II) concentration. From the above studies, it can be presumed that 0.09 mM Fe (II) concentration has the most positive effect on Anammox.

The inhibition factors of heavy metal on Anammox bacteria

After the Anammox process and Anammox bacteria had been discovered, researcher from around the world have been tried to study Anammox due to the advantages of low cost and its capability of removing high ammonium nitrogen wastewater when comparing to conventional nitrification/denitrification process [29]. The Anammox process have been successfully applied from the lab-scale reactor to full-scale treatment plant to treat ammonium or nitrogen rich wastewater since the last two decades [30]. On the other hand, the application and operation of Anammox process in treating real wastewater can be restricted by few factor such as the slow growth rate of Anammox cell and the present of some inhibition factor in wastewater such as heavy metals, substrate, etc.

The inhibitory effects of heavy metals may show great variations in synthetic wastewater, contaminated wastewater and natural environment based on the type and concentration [31].

Toxicity occurs when microorganism's uptake excess amount of heavy metals or metal partitioning through extracellular sorption, transmembrane transport, and intracellular accumulation [10,32,33]. Depending on the viability of the biomass and concentrations of the heavy metals, the transmembrane transport can active, passive or both. The toxicity of heavy metals towards Anammox cell was through their bioaccumulation in cells. When there is present of heavy metals, diffusion of metals will occur across the outer wall of Anammox bacteria through porins and subsequently enter transport across the cytoplasmic membrane in various ways. When the metal ions are inside the Anammox cell, they can interact with both nucleic acids, enzyme active sites and lead to a rapid decline in membrane integrity, which is usually demonstrated as leakage of mobile cellular solutes and cell death.

Zhen Bi, et al. [27] had investigated the inhibition effects of Cd, Ag, Hg and Pd on Anammox activity. Result of the study illustrated that deterioration of crude enzyme activity occurred due to the accumulation of heavy metals inside Anammox cell, which eventually lead to the decrease of nitrogen removal rate of the Anammox system. Furthermore, the heme c concentration also susceptible to short-term exposure to heavy metals. Therefore, investigations are necessary for the comprehend of the inhibitors so that the inhibition effect can be minimize at the same time improve Anammox process.

Specific Anammox Activity (SAA) Test

According to Daverey, et al. [34] the effect of heavy metals on Anammox bacteria is more prominent when the biomass concentration is low (< 2000 mg-MLVSS L^{-1}). Most of the specific anammox activity (SAA) tests perform according to Dapena-Mora, et al. [35] methods. The production of N_2 gas is normally tracked bymeasuring the overpressure in the headspace with a time-frequency depending on the biomass activity in each batch test.

Evaluation of SAA

The total amount of N_2 gas produced is normallycalculated from the overpressure measured in the headspace of each serum bottle at the end of the assay by using the ideal gas law equation. The N_2 gas production rate, dN_2/dt can be calculated from the maximum slope of the curve describing the pressure increase in the vial along the time (α) (Eq. (6)) [35]

$$\frac{dN_2}{dt} = \frac{\alpha \times V_g}{R \times T},\ molN_2hr^{-1}$$

α = slope of pressure increase in the bottle along the time (atm)

V_G = volume of gas phase (0.01 L)

R = ideal gas constant 0.0820575 (atm L mol^{-1} K^{-1})

T = temperature (K)

Then, SAA determined by dividing the N_2 gas production rate, dN_2/dt by the concentration of biomass in the serum bottle, X (g VSS L^{-1}) (Eq. (7)) [35]

$$SAA = \frac{\frac{dN_2}{dt} \times 28}{X \times V_L} \times 24,\ gN_2gVSS^{-1}d^{-1}$$

28 = molecular weight of N_2 (g N/mol)

24 = unit conversion factors from hour to days

X = biomass concentration in the bottle (g VSS L^{-1})

V_L = volume of liquid phase in the bottle

Percentage of activity increase and IC_{50}

Comparison had been done between inhibition effect, IC_{50} inhibition concentration of various heavy metals on Anammox bacteria based on specific anammox activity

Table 3: Half inhibition concentration of heavy metals on Anammox bacteria.

Heavy Metal ions	Half Inhibition Concentration, IC_{50} (mg/L)	References
Cadmium, Cd^{2+}	11.2	Zhen Bi, et al. [27]
Copper, Cu^{2+}	1.9-30	Yang, et al.; Yang, et al. [9,39]
Lead, Pb^{2+}	NT	Li, et al. [55]
Mercury, Hg^{2+}	60.35	Zhen Bi, et al. [27]
Molybdate, MoO_4^{2+}	NT	Li, et al. [55]
Nickel, Ni^{2+}	48.6	Li, et al. [55]
Silver, Ag^{+}	11.52	Zhen Bi, et al. [27]
Zinc, Zn^{2+}	3.9-25	Achlesh, et al.; Li, et al. [37,55]
Fe (II), Fe^{2+}	55.6 (0.20 mM)	Mak, et al. [36]

NT = was not toxic at the highest concentration tested (75 mg Pb/L and 23.8 mg Mo/L).

(SAA) as shown in Table 3. The inhibition effects of nine various heavy metals had been compared included ferrous ion (Fe^{2+}), Cadmium (Cd^{2+}), Copper (Cu^{2+}), Lead (Pb^{2+}), Mercury (Hg^{2+}), Molybdate (MoO_4^{2+}), Nickel (Ni^{2+}), Silver (Ag^{+}) and Zinc (Zn^{2+}) respectively. Among the nine heavy metal, Lead ion, Pb^{2+} has the lowest inhibition effect on Anammox process, in which even 75 mg/L of Lead ion do not cause any inhibition effects on Anammox bacteria, while copper ion, Cu^{2+} might be having the most inhibition effect on Anammox with the IC_{50} inhibition concentration ranged start from 1.9 mg/L. The IC_{50} inhibition concentration of Fe (II) was found to be approximately 0.20 mM, which, after conversion is equal to 55.6 mg/L [36]. Thus, from the comparison, the inhibition effects of Fe (II) on Anammox process is not as intense as most of the heavy metals tested and the inhibition effects of Fe (II) was found lower than Cadmium (Cd^{2+}), Copper (Cu^{2+}), Nickel (Ni^{2+}), Silver (Ag^{+}) and Zinc (Zn^{2+}).

Recovery

Achlesh, et al. [37] had studied the long term effect of zinc on SNAD system of Anammox bacteria, the system was able to recovered the nitrogen (total nitrogen and ammonium nitrogen) removal efficiency to around 90% after inhibited by 20 mg/L of zinc which had reduce the nitrogen (total nitrogen and ammonium nitrogen) removal efficiency to around 70%, due to the fact that the microbial communities in the reactor were well acclimated. In the study of Kimura and Isaka [38] a continuous Anammox bioreactor (lab-scale) with gel-carrier had been operated to investigate the effects of Ni, Cu, Co, Zn and Mo on Anammox activity. It was demonstrated that high concentrations of those heavy metal is inhibitory to Anammox cell, however the effects were reversible. Yet, the inhibition effect of Mo on Anammox cell was irreversible. Therefore, it is suggested that extra attention should be paid to Mo concentrations in the wastewater subject to Anammox bacteria. In addition, Anammox activity and performance subjected to copper inhibition for long time (almost 200 days) were restorable and the recovery process lasted for short time (nearly 50 days) due to the accumulation of Anammox cell [39].

Conclusion

Anammox process has become essential in treating ammonium rich wastewater due to its multiple advantages compared to the conventional nitrification/denitrification process. Anammox process is a promising method for treating nitrogen rich and low COD content wastewater. The presence of heavy metals in wastewater can either affect stimulation, inhibition or even toxic in biochemical reactions. It was found that heavy metal ions can improve Anammox activity through stimulating the metabolism of Anammox cell as those metal ions can either be the component of many enzymes or the co-enzyme. Nevertheless, toxicity occurs when microorganism uptake extra quantity of heavy metal through extracellular sorption, transmembrane transport, and intracellular accumulation. In this review, IC_{50} inhibition concentration of various heavy metals on Anammox bacteria based on SAA. Based on literature review, it was found that Lead ion, Pb^{2+} has the lowest inhibition effect, in which even 75 mg/L of Pb^{2+} do not cause any inhibition effects on Anammox bacteria, while copper ion, Cu^{2+} might be having the most inhibition effect on Anammox with the IC_{50} inhibition concentration of 1.9 mg/L. The IC_{50} of Fe (II) was found at 55.6 mg/L. It can be concluded that that the high concentrations of those heavy metal has inhibitory effect on Anammox cell, on the other hand the effects are generally reversible.

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