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The Morphological Characteristics of Anammox Granular Sludge: a Potential Indicator of Reactivity and Settleability

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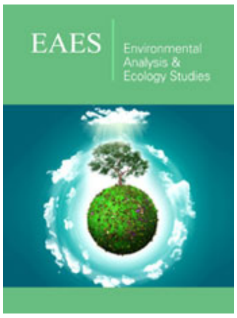

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Abstract

Anaerobic ammonium oxidation (Anammox) process is a promising biotechnology which has been successfully applied in the nitrogen removal from wastewaters. Anammox granular sludge (AnGS) plays a key role in the high-rate anammox processes due to its high nitrogen removal rate and excellent settling ability. AnGS is a complex microbial aggregate and could show the unique vision like the color, size and shape. The structure of microbial aggregate, which was composed of microbial community, extracellular polymeric substance and gas/water voids, is the link between function and morphology. By correlating the morphological characteristics with the function of AnGS or bioreactor, a visible indicator could be developed to guide the optimization and control of anammox process. Also, the influence of heavy metals (Fe^{2+}, Cu^{2+}, Zn^{2+}) on the activity and morphology of AnGS was reviewed.

Keywords: Anammox; Granular sludge; Morphology; Reactivity; Settleability; Heavy metals

Introduction

The discovery of anaerobic ammonium oxidation (anammox) 20 years ago has greatly promoted the development of biological nitrogen removal from wastewaters and the biogeochemical nitrogen cycle [1]. Because anammox process can transform ammonium and nitrite to dinitrogen gas [2], it has been widely applied in the wastewater treatment. So far, more than 200 full-scale facilities have been put into operation all over the world [3]. The anammox process is acknowledged to be a cost-saving process and is hopeful to replace the conventional nitrification-denitrification process. Granular sludge is special self-immobilized microbial aggregates [4] which strongly support the function of anammox reactor due to the high nitrogen removal rate and excellent settling ability [5].

So far five candidate genera of AnAOB (anaerobic ammonia oxidation bacteria) have been reported based on phylogenetic analysis including Candidatus Brocadia, Candidatus Anammoxoglobus, Candidatus Jettenia, Candidatus Kuenenia and Candidatus Scalindua [6].

Metagenomics have become a powerful tool to investigate the microbial community and metabolic pathway of anammox granule [7]. The anammox granular sludge is made up of subunit, microbial cell cluster and single cell, whose arrangement form its structure. In turn, the structure endows the granular sludge the specific morphology, reactivity and settleability. However, anammox process is vulnerable and heavy metals existed in the wastewaters could be strong inhibitors which in turn shape the morphology of AnGS.

Discussion

The morphology of AnGS

Macroscopic characteristics: The AnGS has the specific physical characteristics which are easy to be recognized by naked eyes, such as the color, size and shape. At their high activity, AnAOB show their typical red color due to the abundance of heme c-containing proteins in their cell [8]. However, the color of AnGS could change from carmine, via brown, to black at various nitrogen loading rates. With reference to the color in typical state, the color of AnGS could serve as an indicator to roughly evaluate the specific activity and reactor capacity [9-11]. The average diameter of granular sludge is reported to be 0.5-4mm while the largest diameter could be up to 16mm [12,13]. The morphology of AnGS is like broccoli shape with the average sphericity and roundness factor of 0.6949±0.0771 and 0.6408±0.0735, respectively [14]. Li et al. [15] found that the granular sludge was streamlined at high nitrogen loading rate simulated by the boundary layer theory.

Microscopic characteristics: AnGS was composed of microbial community, extracellular polymeric substance (EPS) and gas/water voids. It includes four layers of structure (Granule, Subunit, Microbial cell cluster and single cell) under the electron microscope [16]. EPS is an important component of AnGS, which makes cells stick to each other and promotes the microbial aggregation [17]. EPS also helps to form the interstitial voids (0.4-18.9μm) of AnGS, which serve as water channels or gas tunnels and affect the mass transport and the density of AnGS [16].

The function of AnGS

Reactivity: The reactivity of AnGS refers to the specific anammox activity which is most concerned by environmental engineers. The effect of granular size on the reactivity is studied extensively but no consistent conclusions have drawn so far. The granular size determines the ratio of granule surface to volume, which is an important parameter for mass transport and thereby affects the nitrogen removal rate. An et al. found that the AnGS with diameter of 1.0-1.5mm got the highest activity [18].

Zhu et al. also observed that the abundance, activity and specific anammox rate were enhanced with granular diameter of 0.5-0.9mm. Ni et al. [19] suggested that the optimum granular diameter for the maximum N-removal should be 1.0-1.3mm based on the mathematical model simulation. The difference of optimal diameter may result from the diversity of AnAOB and the different flow pattern of bioreactor.

Settleability: AnGS has an advantage of higher settling velocity with the range of 10-100m/h than the floc sludge with only 1m/h. The Stokes' law is often used to describe the settling velocity of a granule considering the balance of the buoyancy, gravity and drag force. According to this equation, the settling velocity is determined by the water viscosity, the size and shape of particle, and the density difference between water and particle. Lu et al. [14] pointed out that AnGS with diameter of 1.74-4.00mm had the maximum activity for the anammox process based on the improved settling model considering the granular shape [14]. Small granules are easy to be washed out at the high upflow velocity, while large granules are prone to producing the gas pocket that leads to the sludge floatation. In order to understand the complex settling behavior, some influencing factors, such as back mixing, granules collision, wake effect of floating bubbles, should be put into consideration together.

The inhibition of heavy metals

Anammox process has been successfully applied in treating high-strength ammonium-containing wastewaters, like industrial, swine wastewater and landfill leachate where heavy metal ions could reach a high level [20-22]. Heavy metal ions are essential for bacteria growth, but if overloaded, they can accumulate and cause severe biological toxicity influencing the specific morphology, reactivity and settleability of AnGS.

Fe^{2+} inhibition

AnAOB has been proved to possess large amounts of heme c proteins and ferrous iron (Fe^{2+}) is an essential part for heme biosynthesis. Liu et al. [23] demonstrated that appropriate Fe^{2+} dosing (i.e., 0.09mM) could significantly enhance the specific anammox growth rate by 45.8% [23]. Qiao et al. [24] also proved that with 0.09mM Fe^{2+}, the start-up time of anammox process could be shortened and nitrogen removal rate could increase 32.2%, but Fe^{2+} over 0.18mM would deteriorate the reactivity [24].

Cu^{2+} inhibition

Cu is an essential constituent of nitrite reductase of AnAOB [25]. Several previous researches reported the half maximal inhibitory concentration (IC50) of Cu^{2+} on anammox activity varying from 1.9-12.9mg/L [26, 27]. Yang et al. [27] investigated the long-term effects of Cu^{2+} on the anammox process and found that 5mg/L could strongly inhibited the anammox activity dropped by 94%. Accordingly, the anammox sludge under the Cu^{2+} stress had lower settling velocity and larger diameter. The electron microscope observation revealed that Cu^{2+} could induce the EPS secretion and cause cell membrane damage [28]. Similar results have been also proved with the long-term joint effects of Cu and Zn. The high PN/PS ratios of EPS caused the worse settling property and AnAOB showed serve shrinkage and large amounts of precipitation existed around the cell aggregates [28].

Zn^{2+} inhibition

Zn^{2+} acts as a vital cofactor of metalloproteinases and enzymes and is common in many ammonium-rich wastewaters. Zhang et al. [29] stated that AnAOB could adapt to 1-10mg/L Zn^{2+}, but were inhibited over 20mg/L. The secretion of EPS was enhanced at first due to the self-protection strategy, then the amount of EPS decreased because of the bacteria death when Zn^{2+} entered the cell [29]. Zhang et al. [29] found that the IC50 of Zn^{2+} was 25mg/L, the sludge diameter declined and the settling property was enhanced due to the lower PN/PS which was inconsistent with the Cu^{2+} inhibition. However, the cell membrane damage and cell lysis were also observed which could be the essential cause of the activity loss [30].

Conclusion

AnGS is the structural and functional element of anammox process. It shows some visual characteristics like the color, diameter and shape. AnGS is a complex structure composed of microbial community, extracellular polymeric substance and gas/water voids, which determines the morphology as well as the reactivity and settleability. It is helpful to link the morphological characteristics with the specific activity of AnGS and the conversion capacity of anammox reactor for the optimization and control of anammox process. Appropriate dose of heavy metals could enhance the anammox activity, but excessive amount could cause the cell lysis. Different heavy metals have different suppression threshold and the corresponding variation of morphology of AnGS should be further investigated.

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