微生物学通报
Microbiology China
tongbao@im.ac.cn
http://journals.im.ac.cn/wswxtbcn

Aug. 20, 2019, 46(8): 1988−1997
DOI: 10.13344/j.microbiol.china.190317

专论与综述

厌氧氨氧化颗粒污泥研究进展

许冬冬 康达 郭磊艳 郑平*

浙江大学环境工程系 浙江 杭州 310058

摘 要：厌氧氨氧化(Anaerobic ammonium oxidation，Anammox)工艺是一种新的生物脱氮技术。一经问世即得到人们青睐，现已成为废水脱氮的升级技术。厌氧氨氧化菌(Anaerobic ammonium oxidation bacteria，AnAOB)是 Anammox 工艺的功能之源。以颗粒污泥形态存在的 AnAOB 是 Anammox 颗粒污泥床脱氮系统的重要支柱。由于 AnAOB 生长缓慢且对环境条件变化敏感，Anammox 脱氮系统不仅启动缓慢，而且运行极易失稳甚至崩溃。值得庆幸的是，AnAOB 可自主选择、组合和固定功能菌群落而形成 Anammox 颗粒污泥，并通过其优良的重力沉降性能和高效的基质转化性能保障 Anammox 脱氮系统的持续工作。本文综述了 AnAOB 的种类和特性及 Anammox 颗粒污泥的组成、结构和功能，以期为 Anammox 工艺的优化和拓展提供参考。

关键词：厌氧氨氧化菌，厌氧氨氧化颗粒污泥，组成，结构，功能

Research progress on Anammox granular sludge

XU Dong-Dong KANG Da GUO Lei-Yan ZHENG Ping*

Department of Environmental Engineering, Zhejiang University, Hangzhou, Zhengjiang 310058, China

Abstract: Anammox process is a new biotechnology for nitrogen removal from wastewaters. It was favored in the field of environmental engineering once it came out and now it has become the upgrading technology. Anammox bacteria (AnAOB) are the function source of Anammox process and Anammox granular sludge (AnGS) formed by AnAOB is the vital pillar of Anammox granular sludge bed system. However, due to the slow growth of AnAOB and their sensitivity to the change of environmental conditions, Anammox nitrogen removal system not only starts up slowly, but runs easily to be unstable and even collapses. Fortunately, AnAOB can select, combine and fix the functional bacterial community freely to form AnGS, thus ensuring the continuous work of the Anammox nitrogen removal system for its excellent gravity settling property and high efficient substrate conversion property. In this paper, the taxonomy and characteristic of AnAOB, compopsition, structure, and function of AnGS are reviewed so as to give guidance for the optimization and expansion of Anammox process.

Keywords: Anammox bacteria, Anammox granular sludge, Composition, Structure, Function

Foundation item: National Natural Science Foundation of China (51778563)
***Corresponding author:** Tel: 86-571-88982819; E-mail: pzheng@zju.edu.cn
Received: 13-04-2019; **Accepted:** 20-05-2019; **Published online:** 29-05-2019
基金项目：国家自然科学基金(51778563)
*通信作者：Tel：0571-88982819；E-mail：pzheng@zju.edu.cn
收稿日期：2019-04-13；接受日期：2019-05-20；网络首发日期：2019-05-29

厌氧氨氧化(Anaerobic ammonium oxidation，Anammox)是厌氧氨氧化菌(Anaerobic ammonium oxidation bacteria，AnAOB)将氨和亚硝酸转化成氮气的生物反应[1-2]。与传统硝化反硝化脱氮工艺相比，Anammox 工艺具有无需外源电子供体(如有机物)、容积负荷高、运行费用低等优点，已广泛应用于含高氨氮工业废水处理[3-4]，并作为主流脱氮工艺向城市污水拓展[5-6]。2002 年，全球首座 Anammox 工程在荷兰鹿特丹 Dokhaven 污水处理厂建成[7]。迄今全球已有 200 余座 Anammox 工程投产[8]，应用前景广阔。

然而，由于 AnAOB 生长缓慢且易受环境条件的影响，Anammox 工艺的实际应用仍然面临着多方面的巨大挑战[9-11]。AnAOB 是 Anammox 工艺的根本, Anammox 工艺的成功有赖于 AnAOB 的鼎力支持。研究证明，AnAOB 具有自主选择和自主固定功能菌群并形成颗粒污泥的能力。颗粒污泥不仅赋予了 Anammox 反应器高效菌群, 也赋予了 Anammox 反应器足够的功能菌量，它为 Anammox 反应器的高速运行和有效脱氮提供了有力保障[12-14]。因此，颗粒污泥研究成为推动 Anammox 工艺深入发展的重要因素。

对于 Anammox 颗粒污泥的研究，国内外已有不少文献报道，但未见专题文献综述[15-16]。本文拟结合文献报道和自身研究，对 AnAOB 的种类和特性及 Anammox 颗粒污泥的组成、结构和功能作一综述，以期为 Anammox 工艺的性能优化和应用拓展提供参考。

1 厌氧氨氧化细菌的种类和特性

1.1 厌氧氨氧化细菌的种类

已鉴定的 AnAOB 属于浮霉菌纲(*Planctomycetia*)厌氧氨氧化菌目(*Brocadiaceae*), 共 6 属 22 种。AnAOB 的种类、倍增时间、生境及亲和力常数见表 1。

1.2 厌氧氨氧化细菌的形态和结构

AnAOB 细胞呈不规则球状、卵状，直径约为 0.8 μm −1.1 μm，革兰氏染色阴性。由于细胞含有大量细胞色素，AnAOB 呈红色[39-40]。AnAOB 细胞内有独特的区室结构，由细胞质膜、胞浆内膜、厌氧氨氧化体膜将细胞物质分隔成 3 个部分，从外到内分别为外室细胞质(Paryphoplasm)、核糖细胞质(Riboplasm)、厌氧氨氧化体(Anammoxosome)[41]。其中厌氧氨氧化体是 AnAOB 特有的细胞器，占细胞体积的 50%−80%，是物质代谢和能量转换的场所。厌氧氨氧化体膜含有致密的梯形烷，可防止代谢中间产物 NO 和 N_2H_4 泄漏[42]。

1.3 厌氧氨氧化细菌的生理、生化和生态特性

AnAOB 均具有化能自养功能，在厌氧条件下氧化氨氮/亚硝氮获得能量，并以 CO_2 作为碳源[2]。*Candidatus* B. fulgida、*Candidatus* A. propionicus、*Candidatus* K. stuttgartiensis 等还可利用乙酸和丙酸等有机物作为电子供体，将硝酸盐异化还原为氨[43-44]。

如图 1 所示, 厌氧氨氧化途径分为 3 步: (1) Cyt cd_1 型亚硝酸还原酶(Nir)将亚硝酸还原为 NO; (2) 联氨合成酶(Hzs)将氨和 NO 转化成联氨; (3) 联氨脱氢酶(Hdh)将联氨转化为氮气。联氨氧化释放 4 个电子，经细胞色素 c、辅酶 Q、细胞色素 bc_1 传递给 Nir、Hzs，用于亚硝酸还原和联氨合成，伴随电子传递，在厌氧氨氧化体膜内外建立质子梯度，驱动 ATP 合成[45]。

AnAOB 分布广泛，海洋水体及沉积物、淡水水体及沉积物、污水处理厂构筑物以及陆地等生境中均被发现(表 1)。厌氧氨氧化发生的前提条件是氨和亚硝酸共存于缺氧环境中。在自然水体及其沉积物中，氨会在好氧/缺氧界面转化为亚硝酸，从而为 AnAOB 的生存提供条件；而在人工废水处理系统中，氨常因氧气供应不足而容易氧化成亚硝酸，从而为 AnAOB 的生长提供适宜场所[16,46]。

2 厌氧氨氧化颗粒污泥的组成与结构

颗粒污泥最早发现于上流式厌氧污泥床反应器(Upflow anaerobic sludge blanket，UASB)[47]，后来也发现于好氧反应器[48-49]。根据 2006 年荷兰代尔夫特好氧颗粒污泥研讨会的定义，颗粒污泥是尺寸大于 0.2 mm 的生物颗粒[50]。AnAOB 易团聚形成颗粒污泥[51]，并借助自身的重力沉降堆积形成污泥床[52]，赋予 Anammox 反应器高效脱氮功能[53-54]。

表 1 厌氧氨氧化菌的种类和倍增时间

Table 1 The taxonomy and doubling time of AnAOB

属 Genus	种 Species	倍增时间 Doubling time (d)	来源 Source	氨氮亲和力常数 K_S for NH_4^+ (μmol/L)	亚硝氮亲和力常数 K_S for NO_2^- (μmol/L)	参考文献 References
Brocadia	*Candidatus* Brocadia anammoxidans	9−11	废水 Wastewater	<5	<5	[17-18]
	Candidatus Brocadia fulgida	18	废水 Wastewater	640±130	350±90	[19]
	Candidatus Brocadia sinica	7	废水 Wastewater	28±4	34±21	[20]
	Candidatus Brocadia brasiliensis	−	废水 Wastewater	−	−	[21]
	Candidatus Brocadia caroliniensis	−	废水 Wastewater	530±50	370±40	[22]
	Candidatus Brocadia sapporoensis	3.5	废水 Wastewater	−	−	[23]
Kuenenia	*Candidatus* Kuenenia stuttgartiensis	8.3−11	废水 Wastewater	−	0.2−3	[24]
Jettenia	*Candidatus* Jettenia asiatica	−	淡水 Freshwater	−	−	[25]
	Candidatus Jettenia caeni	14.2	废水 Wastewater	17.1±4.3	35.6±0.92	[26]
	Candidatus Jettenia moscovienalis	28	废水 Wastewater	−	−	[27]
Scalindua	*Candidatus* Scalindua brodiae	−	废水 Wastewater	−	−	[28]
	Candidatus Scalindua sorokinii	−	海水 Seawater	−	−	[29]
	Candidatus Scalindua wagneri	−	废水 Wastewater	−	−	[28]
	Candidatus Scalindua sinooifed	−	油藏 Oil reservoirs	−	−	[30]
	Candidatus Scalindua marina	−	海洋沉积物 Marine sediments	−	−	[31]
	Candidatus Scalindua arabica	−	海水 Seawater	−	−	[32]
	Candidatus Scalindua profunda	−	海水 Seawater	−	−	[33]
	Candidatus Scalindua zhenghei	−	海水 Seawater	−	−	[34]
	Candidatus Scalindua richardsii	−	亚缺氧海区 Sub hypoxic sea area	−	−	[35]
Anammoxoglobus	*Candidatus* Anammoxoglobus propionicus	−	废水 Wastewater	−	−	[36]
	Candidatus Anammoxoglobus sulfate	−	废水 Wastewater	−	−	[37]
Anammoximicrobium	*Candidatus* Anammoximicrobium moscowii	32	淡水 Freshwater	<29	<27	[38]

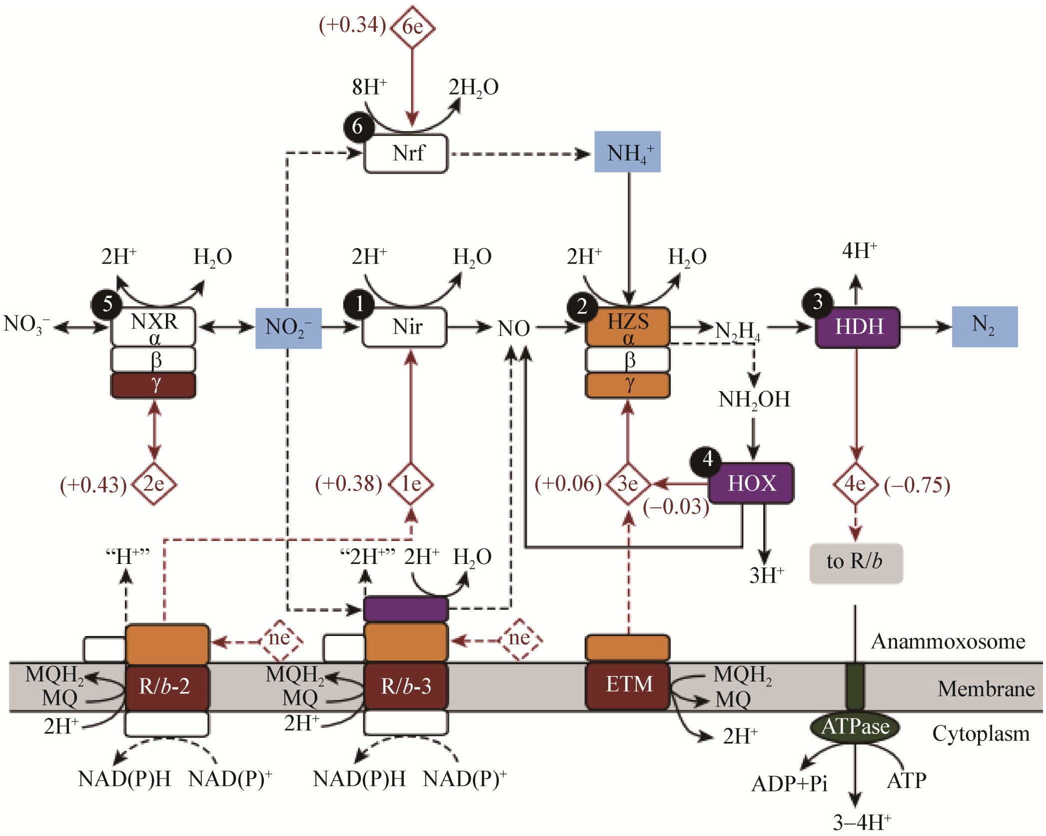

图 1　厌氧氨氧化代谢模型

Figure 1　Metabolic model of anaerobic ammonuim oxidation

2.1　厌氧氨氧化颗粒污泥的组成

根据物理状态，Anammox 颗粒污泥的组成可分为气、液、固三相。气相部分包括颗粒污泥空腔和孔道内的气体，AnAOB 将氨和亚硝酸转化成氮气，可积累于空腔内，并通过孔道由内向外传输[55]。液相包括颗粒污泥空腔和孔道内的液体。空腔和孔道不是被气体占据，就是被液体占据。Xu 等研究发现，在颗粒污泥的反应过程中，空腔和孔道中气液比会发生周期性的变化，其原因是颗粒污泥的产气和释气循环[56]。根据化学成分，固相部分可分为无机固体和有机固体。有机固体又可细分为菌体和胞外多聚物(Extracellular polymeric substance，EPS)。Anammox 颗粒污泥气、液、固三相的比例是功能菌活性和数量的显示。

Anammox 颗粒污泥可自主选择、组合和固定功能菌群，赋予颗粒污泥高效的生化反应性能和优良的重力沉降性能。Anammox 颗粒污泥中的功能菌群可分为 AnAOB 和其他伴生菌。其中 AnAOB 为优势菌，相对丰度较高(一般超过 50%)。此外，绿弯菌门(*Chloroflexi*)、绿菌门(*Chlorobi*)、变形菌门(*Proteobacteria*)、酸杆菌门(*Acidobacteria*)和拟杆菌门(*Bacteroidetes*)等菌群也有一定的丰度(30%–70%)[43,57]。Lawson 等采用宏基因组、宏转录组等分子生物学手段，检测了 Anammox 颗粒污泥中 AnAOB 和异养菌的基因分布和表达水平，并推测了它们之间的相互作用；研究结果显示，绿菌门可高效分解胞外多肽并将硝酸盐还原成亚硝酸，从而为 AnAOB 提供反应物，同时为 AnAOB 清除代谢产物[58]。Zhao 等研究发现，装甲菌门(*Armatimonadetes*)和变形菌门可为 AnAOB 提供生长因子叶酸和钼辅因子[59]。AnAOB 与伴生菌的共存是 Anammox 颗粒污泥组成的重要成分。

Anammox 颗粒污泥中的功能菌合成并分泌的EPS 不仅利于自我保护，用作碳源和能源，还可用作粘结剂促使细胞团聚[60]。EPS 的主要成分是蛋白质和多糖，还有少量脂质、核酸以及腐殖酸类物质[53]。一般认为 EPS 是污泥颗粒化的重要致因，它积累于细胞外，提高了细胞的粘附性，促进了细胞与细胞之间、细胞与颗粒之间粘连聚集[61]。迄今令人费解的是，自养型 Anammox 颗粒污泥的 EPS 含量高于异养型厌氧颗粒污泥(表 2)，可推测 EPS 在 Anammox 颗粒污泥的形成、维持和工作中发挥着重要作用。然而，具体到 EPS 的两大宏量组分(蛋白质和多糖)在污泥颗粒中的作用，目前认识不一。Liu 等[67]研究发现，蛋白质含量与细菌细胞表面的疏水性呈正相关；Hou 等[61]则发现，蛋白质中含有大量疏水性氨基酸且其结构松散，能够充分暴露内部的疏水基团，从而促进 Anammox 颗粒污泥的形成；另外一些研究表明，多糖在污泥颗粒化过程中发挥着更为重要的作用，多糖中含有羧基、羟基等带负电荷的官能团，具有细胞之间的架桥作用，可促进颗粒污泥形成[65]。有关 EPS 中蛋白质和多糖的组成及其动态变化，依然存在许多盲点，值得深入研究。

2.2 厌氧氨氧化颗粒污泥的结构

关于 Anammox 颗粒污泥的结构，目前普遍认为可分为 4 个层次：细胞单体、菌胶团(细胞簇)、亚单位(细胞簇复合体)和颗粒污泥(图 2)[56,59,68]。在扫描和透射电镜下观察 AnAOB 细胞(1.2 所述，图 2A)可见，细胞间通过 EPS 粘连，形成菌胶团(几 μm 到几十 μm，图 2B)；菌胶团进一步在 EPS 和丝状菌的桥接下形成亚单位(几十 μm 到几百 μm，图 2C)；多个亚单位最终整合成颗粒污泥(几百 μm 到几 mm，图 2D)。

据文献报道，按接种物类型可将污泥团聚机理分为两种类型(图 3)。第一种类型，采用颗粒污泥(好氧、厌氧或厌氧氨氧化颗粒污泥)作为接种物，在厌氧氨氧化系统中，由于生境条件的变化，接种颗粒污泥逐渐解体，解体的细小颗粒污泥被用作内核，在 EPS 的作用下促进 AnAOB 粘附，伴随着基质的利用，AnAOB 生长繁殖，形成菌胶团，多个菌胶团在 EPS 和丝状菌的粘接下形成亚单元，多个亚单元团聚成颗粒污泥。第二种类型，采用非颗粒性混培物作为接种物，生境中以沉淀物形式存在的多价阳离子(如钙、铁、镁离子)被用作 AnAOB 的粘附剂和颗粒化的内核，后续颗粒化步骤类同于上述第一种类型[15]。

表 2 不同颗粒污泥中的胞外多聚物含量

Table 2 Extracellular polymeric substances (EPS) content in different microbial granules

颗粒污泥 Granular sludge	胞外多聚物 Extracellular polymers (mg per g VSS)			参考文献 References
	蛋白质 Proteins	多糖 Polysaccharides	蛋白质/多糖 PN/PS	
厌氧氨氧化颗粒 Anammox granules	164.4±9.3	71.8±2.3	2.29	[53]
下沉厌氧氨氧化颗粒 Settling anammox granules	234.25	90.78	2.57	[62]
上浮厌氧氨氧化颗粒 Floating anammox granules	323.37	76.84	4.15	[62]
厌氧颗粒 Anaerobic granules	42.7±38.7	17.3±0.8	2.80	[63]
好氧颗粒 Aerobic granules	40	16	2.50	[64]
酚类降解颗粒 Phenol-degrading granules	240±13	61.0±9.4	3.93	[65]
产氢颗粒 Hydrogen-producing granules	70.9±4.5	115.6±5.2	0.60	[66]

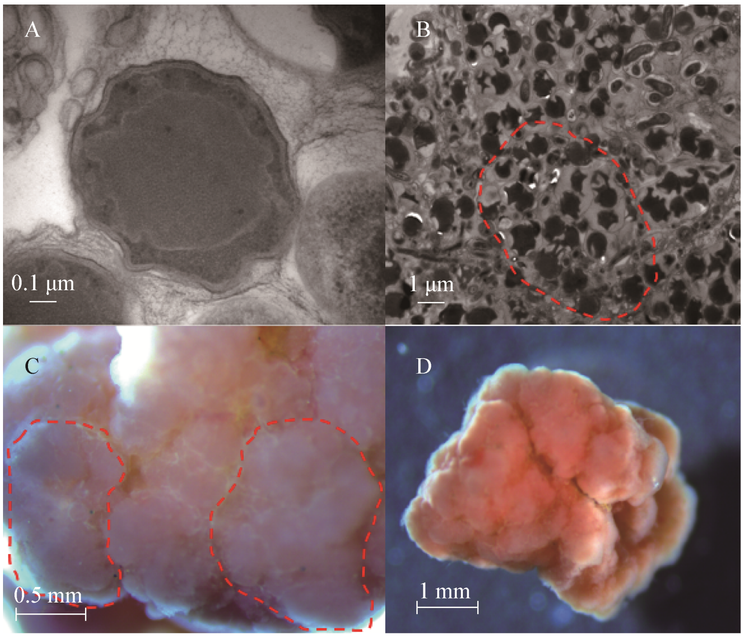

图 2　厌氧氨氧化颗粒污泥的四级结构

Figure 2　Four-level structure of Anammox granular sludge

注：A：厌氧氨氧化菌；B：菌胶团；C：亚单位；D：颗粒污泥.

Note: A: AnAOB; B: Cell clusters; C: Subunits; D: Granule.

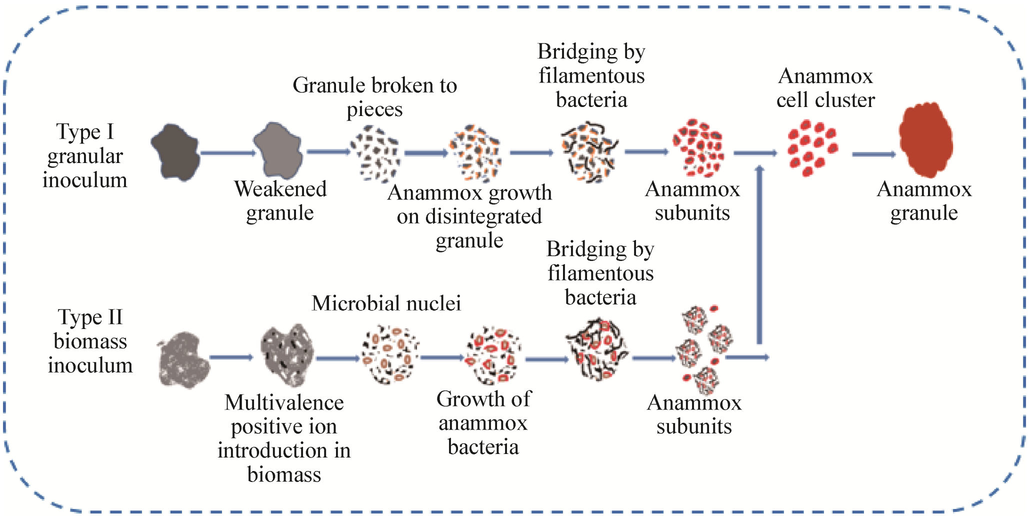

图 3　厌氧氨氧化污泥颗粒化机理

Figure 3　Granulation mechanism of Anammox sludge

由于 Anammox 颗粒污泥以外表面与环境接触，颗粒污泥外层会发育形成类似“皮肤”边界层。由于 AnAOB 富含血红素，通常 Anammox 颗粒污泥显现红色[69]。由于受基质传递的限制，Anammox 颗粒污泥生长到一定尺度(mm 级)时趋向稳定。若基质供不应求，颗粒污泥内部细胞死亡、水解，一方面可作为营养物质，支持异氧菌生活，另一方面产生空穴，持留气体产物。颗粒污泥内部积累气体产物，既可导致颗粒密度下降而漂浮，又可造成内压过大而破裂。前者随出水流失，后者成为新的颗

粒污泥组合单元，进入 Anammox 颗粒污泥的下一轮生命周期[70]。

3 厌氧氨氧化颗粒污泥的功能

Anammox 脱氮系统的效能与功能菌群的活性和数量密切相关。因此，理想的颗粒污泥应有较高的代谢活性和较好的沉降性能。

3.1 厌氧氨氧化颗粒污泥的沉降性能

AnAOB 生长缓慢，倍增时间长(表 1)，形成沉降性能优良的颗粒污泥有利于功能菌在反应器中的有效持留和循环使用。因此，Anammox 颗粒污泥的沉降性能关乎反应器的成败。Anammox 颗粒污泥的沉降性能可用自由沉降速度表征。在液相中，颗粒污泥的自由沉降速度是重力、浮力、黏滞阻力等综合作用的结果。在上流式颗粒污泥床反应器中，当颗粒污泥的沉降速度大于上升水流速度时，颗粒污泥沉降持留于反应器底部；当颗粒污泥的沉降速度小于上升水流速度时，颗粒污泥被上升水流洗出反应器；当颗粒污泥的沉降速度等于上升水流速度时，颗粒污泥悬浮于反应器中[40]。根据斯托克斯公式，颗粒污泥的自由沉降速度与粒径和密度相关。Lu 等[71]研究证实，颗粒污泥的粒径和密度是决定沉降速度的关键因素，颗粒污泥的沉降速度随粒级的增大而提高，但随着粒径的增大，颗粒污泥内部聚集氮气，导致颗粒污泥密度下降，产生颗粒污泥漂浮。Lu 等[71]认为，粒径在 1.75 mm−2.20 mm 范围内时，Anammox 颗粒污泥具有最优沉降性能。

3.2 厌氧氨氧化颗粒污泥的反应性能

Anammox 反应器的脱氮能力源于颗粒污泥内的功能菌群。颗粒污泥的反应性能关乎反应器的成效。Anammox 颗粒污泥的反应性能可用比厌氧氨氧化活性(Specific Anammox activity，SAA)来表征[72]。迄今为止，文献报道的最高 Anammox 颗粒污泥比活性为 5.6±0.9 kg N/(kg VSS·d)[16]，具有良好的脱氮潜力。SAA 是基质传递速率和菌种代谢速率的综合体现。显然，要获得高活性的 Anammox 颗粒污泥，不仅要有理想的菌种，还要有理想的传质通道。

功能菌群对基质的代谢速率主要取决于菌种类型、菌体数量以及基质浓度[73-74]。据 Strous 等研究，AnAOB 只有在细胞密度高于 10^{10}−10^{11} 个/mL 时，才能够显现 Anammox 活性[75]，颗粒污泥中可固定大量 AnAOB，这是其显现高活性的根本。McArthur 等根据进化对策，将生物区分为 *r* 对策者和 *K* 对策者[76]。对于 AnAOB，一般认为 *Kuenenia* 是 *K* 对策者，基质亲和力较大，但基质转化速率较慢；而 *Brocadia* 是 *r* 对策者，基质转化速率较快，但基质亲和力较小[77]。利用 *r* 对策者和 *K* 对策者的生理特性，可通过调控基质(氨和亚硝酸)来优化颗粒污泥中功能菌群落的组成和结构。

Anammox 颗粒污泥的基质传递速率受颗粒污泥结构(如粒径大小、内部孔隙大小及其分布)、功能菌群在颗粒污泥中的空间分布以及流体湍流程度等的影响[40,66,78]。颗粒污泥的传质过程可分为外部传质和内部传质。基质(氨和亚硝酸)先由液相扩散并附着至颗粒表面(外部传质)，再通过孔道由颗粒表面向内部扩散(内部传质)[79]。对于外部传质，反应器产气以及水流上升速度提高都可增大流体湍流程度，从而加快液固界面传质[73-74]。对于内部传质，由于颗粒污泥内部孔隙结构的复杂性，迄今传质机理没有完全探明。陆慧锋通过比较不同粒径的 Anammox 颗粒污泥的比活性发现，当粒径超过 1 mm 时，颗粒污泥比活性受到抑制，据此推测随着颗粒污泥粒径的增大，其比表面积降幅增加，基质传递速率的受限程度加剧[68]。Zhu 等研究得出，在 0.5−0.9 mm 的粒级范围内，Anammox 颗粒污泥的活性最高[80]。胡倩怡发现，颗粒污泥的孔隙率随粒径的增大而减小，传质阻力则因此而增大[55]。由于存在基质浓度梯度，在颗粒内部传质推动力逐渐降低，位于颗粒污泥核心的功能菌群极易受基质传递(供给)速率的严重限制[70]。综上可知，要保证优良的反应性能，宜将 Anammox 颗粒污泥粒径控制在一定粒级范围内。

4 总结与展望

Anammox 工艺因其容积效能高、运行费用低、

污泥产量低等优点而受到人们青睐。其中，基于颗粒污泥的 Anammox 工艺又因高效、经济、简便而广泛应用。对于 Anammox 污泥颗粒床工艺，颗粒污泥的质量和数量是其高效、稳定运行的根本保障。本文综述了 AnAOB 的种类和特性及 Anammox 颗粒污泥的组成、结构和功能，以期深入理解 Anammox 脱氮过程，助力 Anammox 工艺的优化和应用。对于 Anammox 颗粒污泥的研究，目前仍有不少盲区，值得今后深入研究：

(1) 有关 Anammox 颗粒污泥的功能菌群，已有不少研究报道，但依然缺乏足够的信息。例如，在 Anammox 系统中，究竟涉及多少种功能菌？它们各有什么作用？它们有怎样的动态变化规律。

(2) 有关 Anammox 颗粒污泥床工艺的应用，已在高氨废水脱氮中取得了成功，但有待拓展应用于低氨废水脱氮。在低氨废水脱氮中，面临功能菌饥饿胁迫、颗粒污泥解体、颗粒污泥入不敷出等挑战。

(3) 有关 Anammox 颗粒污泥活性的优化，已经关注基质转化速率方面的研究，但没有关注基质传递速率方面的研究。若能引进微型 CT 技术，探明颗粒污泥中的孔隙大小、分布及动态变化，将助力颗粒污泥传质性能的改善。

REFERENCES

[1] Kuenen JG. Anammox bacteria: from discovery to application[J]. Nature Reviews Microbiology, 2008, 6(4): 320-326

[2] Kartal B, Maalcke WJ, de Almeida NM, et al. Molecular mechanism of anaerobic ammonium oxidation[J]. Nature, 2011, 479(7371): 127-130

[3] Kartal B, Kuenen JG, van Loosdrecht MCM. Sewage treatment with Anammox[J]. Science, 2010, 328(5979): 702-703

[4] van Loosdrecht MCM, Brdjanovic D. Anticipating the next century of wastewater treatment[J]. Science, 2014, 344(6191): 1452-1453

[5] Malovanyy A, Trela J, Plaza E. Mainstream wastewater treatment in integrated fixed film activated sludge (IFAS) reactor by partial nitritation/anammox process[J]. Bioresource Technology, 2015, 198: 478-487

[6] Trojanowicz K, Plaza E, Trela J. Pilot scale studies on nitritation-anammox process for mainstream wastewater at low temperature[J]. Water Science & Technology, 2016, 73(4): 761-768

[7] van der Star WRL, Abma WR, Blommers D, et al. Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam[J]. Water Research, 2007, 41(18): 4149-4163

[8] Cao YS, van Loosdrecht MCM, Daigger GT. Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies[J]. Applied Microbiology and Biotechnology, 2017, 101(4): 1365-1383

[9] Strous M, Heijnen JJ, Kuenen JG, et al. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms[J]. Applied Microbiology and Biotechnology, 1998, 50(5): 589-596

[10] Chen QQ, Sun FQ, Guo Q, et al. Process stability in an anammox UASB reactor with individual and combined thiocyanate and hydraulic shocks[J]. Separation and Purification Technology, 2017, 173: 165-173

[11] Jin RC, Yang GF, Yu JJ, et al. The inhibition of the Anammox process: a review[J]. Chemical Engineering Journal, 2012, 197: 67-79

[12] Ali M, Chai LY, Tang CJ, et al. The increasing interest of anammox research in China: bacteria, process development, and application[J]. BioMed Research International, 2013, 2013: 134914

[13] Xing BS, Guo Q, Yang GF, et al. The properties of anaerobic ammonium oxidation (anammox) granules: Roles of ambient temperature, salinity and calcium concentration[J]. Separation and Purification Technology, 2015, 147: 311-318

[14] Zhang ZZ, Xu JJ, Hu HY, et al. Insight into the short-and long-term effects of inorganic phosphate on anammox granule property[J]. Bioresource Technology, 2016, 208: 161-169

[15] Manonmani U, Joseph K. Granulation of anammox microorganisms for autotrophic nitrogen removal from wastewater[J]. Environmental Chemistry Letters, 2018, 16(3): 881-901

[16] Tang CJ, Duan CS, Yu C, et al. Removal of nitrogen from wastewaters by anaerobic ammonium oxidation (ANAMMOX) using granules in upflow reactors[J]. Environmental Chemistry Letters, 2017, 15(2): 311-328

[17] Strous M, Fuerst JA, Kramer EHM, et al. Missing lithotroph identified as new planctomycete[J]. Nature, 1999, 400(6743): 446-449

[18] Strous M, van Gerven E, Kuenen JG, et al. Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge[J]. Applied and Environmental Microbiology, 1997, 63(6): 2446-2448

[19] Kartal B, van Niftrik L, Sliekers O, et al. Application, eco-physiology and biodiversity of anaerobic ammonium-oxidizing bacteria[J]. Reviews in Environmental Science and Bio/Technology, 2004, 3(3): 255-264

[20] Hu BL, Zheng P, Tang CJ, et al. Identification and quantification of anammox bacteria in eight nitrogen removal reactors[J]. Water Research, 2010, 44(17): 5014-5020

[21] Araujo JC, Campos AC, Correa MM, et al. Anammox bacteria enrichment and characterization from municipal activated sludge[J]. Water Science & Technology, 2011, 64(7): 1428-1434

[22] Magrí A, Vanotti MB, Szogi A. Anammox treatment of swine wastewater using immobilized technology[A]//Technologies/ Systems for Different Manure and Organic Waste Treatment Options[C]. Lisbon, Portugal: USDA, 2010: 2-5

[23] Narita Y, Zhang L, Kimura ZI, et al. Enrichment and physiological

characterization of an anaerobic ammonium-oxidizing bacterium '*Candidatus* Brocadia sapporoensis'[J]. Systematic and Applied Microbiology, 2017, 40(7): 448-457
[24] Schmid M, Twachtmann U, Klein M, et al. Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation[J]. Systematic and Applied Microbiology, 2000, 23(1): 93-106
[25] Quan ZX, Rhee SK, Zuo JE, et al. Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor[J]. Environmental Microbiology, 2008, 10(11): 3130-3139
[26] Ali M, Oshiki M, Awata T, et al. Physiological characterization of anaerobic ammonium oxidizing bacterium '*Candidatus* Jettenia caeni'[J]. Environmental Microbiology, 2015, 17(6): 2172-2189
[27] Nikolaev YA, Kozlov MN, Kevbrina MV, et al. *Candidatus* "Jettenia moscovienalis" sp. nov., a new species of bacteria carrying out anaerobic ammonium oxidation[J]. Microbiology, 2015, 84(2): 256-262
[28] Schmid M, Walsh K, Webb R, et al. *Candidatus* "Scalindua brodae", sp. nov., *Candidatus* "Scalindua wagneri", sp. nov., two new species of anaerobic ammonium oxidizing bacteria[J]. Systematic and Applied Microbiology, 2003, 26(4): 529-538
[29] Kuypers MMM, Sliekers AO, Lavik G, et al. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea[J]. Nature, 2003, 422(6932): 608-611
[30] Li H, Chen S, Mu BZ, et al. Molecular detection of anaerobic ammonium-oxidizing (anammox) bacteria in high-temperature petroleum reservoirs[J]. Microbial Ecology, 2010, 60(4): 771-783
[31] Brandsma J, van de Vossenberg J, Risgaard-Petersen N, et al. A multi-proxy study of anaerobic ammonium oxidation in marine sediments of the Gullmar Fjord, Sweden[J]. Environmental Microbiology Reports, 2011, 3(3): 360-366
[32] Woebken D, Lam P, Kuypers MMM, et al. A microdiversity study of anammox bacteria reveals a novel *Candidatus* Scalindua phylotype in marine oxygen minimum zones[J]. Environmental Microbiology, 2008, 10(11): 3106-3119
[33] van de Vossenberg J, Woebken D, Maalcke WJ, et al. The metagenome of the marine anammox bacterium '*Candidatus* Scalindua profunda' illustrates the versatility of this globally important nitrogen cycle bacterium[J]. Environmental Microbiology, 2013, 15(5): 1275-1289
[34] Levy-Booth DJ, Prescott CE, Grayston SJ. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems[J]. Soil Biology and Biochemistry, 2014, 75: 11-25
[35] Fuchsman CA, Staley JT, Oakley BB, et al. Free-living and aggregate-associated *planctomycetes* in the Black Sea[J]. FEMS Microbiology Ecology, 2012, 80(2): 402-416
[36] Kartal B, Rattray J, van Niftrik LA, et al. *Candidatus* "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria[J]. Systematic and Applied Microbiology, 2007, 30(1): 39-49
[37] Liu ST, Yang FL, Gong Z, et al. Application of anaerobic ammonium-oxidizing consortium to achieve completely autotrophic ammonium and sulfate removal[J]. Bioresource Technology, 2008, 99(15): 6817-6825
[38] Khramenkov SV, Kozlov MN, Kevbrina MV, et al. A novel bacterium carrying out anaerobic ammonium oxidation in a reactor for biological treatment of the filtrate of wastewater fermented sludge[J]. Microbiology, 2013, 82(5): 628-636
[39] van Niftrik L, Geerts WJC, van Donselaar EG, et al. Linking ultrastructure and function in four genera of anaerobic ammonium-oxidizing bacteria: cell plan, glycogen storage, and localization of cytochrome *c* proteins[J]. Journal of Bacteriology, 2008, 190(2): 708-717
[40] Kang D, Zheng P, Hu QY. Structure, morphology and function of Anammox granular sludge[J]. CIESC Journal, 2016, 67(10): 4040-4046 (in Chinese)
康达, 郑平, 胡倩怡. 厌氧氨氧化结构体、形态与功能[J]. 化工学报, 2016, 67(10): 4040-4046
[41] Oshiki M, Shimokawa M, Fujii N, et al. Physiological characteristics of the anaerobic ammonium-oxidizing bacterium '*Candidatus* Brocadia sinica'[J]. Microbiology, 2011, 157(6): 1706-1713
[42] Jia FX, Peng YZ, Wang SY, et al. Ultrastructure and function of anaerobic ammonium oxidation bacteria cells[J]. Chinese Journal of Applied and Environmental Biology, 2014, 20(5): 944-954 (in Chinese)
贾方旭, 彭永臻, 王衫允, 等. 厌氧氨氧化菌细胞的超微结构及功能[J]. 应用与环境生物学报, 2014, 20(5): 944-954
[43] Feng Y, Zhao YP, Guo YZ, et al. Microbial transcript and metabolome analysis uncover discrepant metabolic pathways in autotrophic and mixotrophic anammox consortia[J]. Water Research, 2018, 128: 402-411
[44] Hu QY, Zheng P, Kang D. Taxonomy, characteristics, and biotechniques used for the analysis of anaerobic ammonium oxidation bacteria[J]. Chinese Journal of Applied and Environmental Biology, 2017, 23(2): 384-391 (in Chinese)
胡倩怡, 郑平, 康达. 厌氧氨氧化菌的种类、特性与检测[J]. 应用与环境生物学报, 2017, 23(2): 384-391
[45] Kartal B, Keltjens JT. Anammox biochemistry: a tale of heme *c* proteins[J]. Trends in Biochemical Sciences, 2016, 41(12): 998-1011
[46] Wang ZX, Chai LY, Yang ZH, et al. Identifying sources and assessing potential risk of heavy metals in soils from direct exposure to children in a mine-impacted city, Changsha, China[J]. Journal of Environmental Quality, 2010, 39(5): 1616-1623
[47] Hulshoff Pol LW, de Zeeuw WJ, Velzeboer CTM, et al. Granulation in UASB-Reactors[J]. Water Science & Technology, 1983, 15(8/9): 291-304
[48] Adav SS, Lee DJ, Show KY, et al. Aerobic granular sludge: recent advances[J]. Biotechnology Advances, 2008, 26(5): 411-423
[49] Franca RDG, Pinheiro HM, van Loosdrecht MCM, et al. Stability of aerobic granules during long-term bioreactor operation[J]. Biotechnology Advances, 2018, 36(1): 228-246
[50] de Kreuk MK, Kishida N, van Loosdrecht MVM. Aerobic granular sludge–state of the art[J]. Water Science & Technology, 2007, 55(8/9): 75-81
[51] Ni BJ, Hu BL, Fang F, et al. Microbial and physicochemical characteristics of compact anaerobic ammonium-oxidizing granules in an upflow anaerobic sludge blanket reactor[J]. Applied and Environmental Microbiology, 2010, 76(8):

2652-2656

[52] Schmidt JE, Ahring BK. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors[J]. Biotechnology and Bioengineering, 1996, 49(3): 229-246

[53] Tang CJ, Zheng P, Wang CH, et al. Performance of high-loaded ANAMMOX UASB reactors containing granular sludge[J]. Water Research, 2011, 45(1): 135-144

[54] Zhang ZZ, Cheng YF, Xu LZJ, et al. Evaluating the effects of metal oxide nanoparticles (TiO_2, Al_2O_3, SiO_2, and CeO_2) on anammox process: performance, microflora and sludge properties[J]. Bioresource Technology, 2018, 266: 11-18

[55] Hu QY. Finger-print of anammox sludge activity[D]. Hangzhou: Master's Thesis of Zhejiang University, 2018 (in Chinese)
胡倩怡. 厌氧氨氧化污泥活性指纹的研究[D]. 杭州: 浙江大学硕士学位论文, 2018

[56] Xu DD, Kang D, Yu T, et al. A secret of high-rate mass transfer in anammox granular sludge: "Lung-like breathing"[J]. Water Research, 2019, 154: 189-198

[57] Lawson CE, Wu S, Bhattacharjee AS, et al. Metabolic network analysis reveals microbial community interactions in anammox granules[J]. Nature Communications, 2017, 8: 15416

[58] Bhattacharjee AS, Wu S, Lawson CE, et al. Whole-community metagenomics in two different anammox configurations: process performance and community structure[J]. Environmental Science & Technology, 2017, 51(8): 4317-4327

[59] Zhao YP, Liu SF, Jiang B, et al. Genome-centered metagenomics analysis reveals the symbiotic organisms possessing ability to cross-feed with anammox bacteria in anammox consortia[J]. Environmental Science & Technology, 2018, 52(19): 11285-11296

[60] Yang M, Hu XW, Ning P, et al. Research progress in extracellular polymeric substances applied to biological wastewater treatment[J]. Industrial Water Treatment, 2011, 31(7): 7-12 (in Chinese)
杨敏, 胡学伟, 宁平, 等. 废水生物处理中胞外聚合物(EPS)的研究进展[J]. 工业水处理, 2011, 31(7): 7-12

[61] Hou XL, Liu ST, Zhang ZT. Role of extracellular polymeric substance in determining the high aggregation ability of anammox sludge[J]. Water Research, 2015, 75: 51-62

[62] Chen JW, Ji QX, Zheng P, et al. Floatation and control of granular sludge in a high-rate anammox reactor[J]. Water Research, 2010, 44(11): 3321-3328

[63] Wu J, Zhou HM, Li HZ, et al. Impacts of hydrodynamic shear force on nucleation of flocculent sludge in anaerobic reactor[J]. Water Research, 2009, 43(12): 3029-3036

[64] Zheng YM, Yu HQ. Determination of the pore size distribution and porosity of aerobic granules using size-exclusion chromatography[J]. Water Research, 2007, 41(1): 39-46

[65] Adav SS, Lee DJ, Tay JH. Extracellular polymeric substances and structural stability of aerobic granule[J]. Water Research, 2008, 42(6/7): 1644-1650

[66] Mu Y, Yu HQ. Biological hydrogen production in a UASB reactor with granules. I: physicochemical characteristics of hydrogen-producing granules[J]. Biotechnology and Bioengineering, 2006, 94(5): 980-987

[67] Liu YQ, Liu Y, Tay JH. The effects of extracellular polymeric substances on the formation and stability of biogranules[J]. Applied Microbiology and Biotechnology, 2004, 65(2): 143-148

[68] Lu HF. Performance and mechanisms of salt-tolerant ANAMMOX process[D]. Hangzhou: Doctoral Dissertation of Zhejiang University, 2015 (in Chinese)
陆慧锋. 耐盐厌氧氨氧化工艺性能及其机理研究[D]. 杭州: 浙江大学博士学位论文, 2015

[69] Chen H, Ma C, Yang GF, et al. Floatation of flocculent and granular sludge in a high-loaded anammox reactor[J]. Bioresource Technology, 2014, 169: 409-415

[70] Lin XM, Wang YY. Microstructure of anammox granules and mechanisms endowing their intensity revealed by microscopic inspection and rheometry[J]. Water Research, 2017, 120: 22-31

[71] Lu HF, Ji QX, Ding S, et al. The morphological and settling properties of ANAMMOX granular sludge in high-rate reactors[J]. Bioresource Technology, 2013, 143: 592-597

[72] Dapena-Mora A, Fernández I, Campos JL, et al. Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production[J]. Enzyme and Microbial Technology, 2007, 40(4): 859-865

[73] Shi ZJ, Guo Q, Xu YQ, et al. Mass transfer characteristics, rheological behavior and fractal dimension of anammox granules: the roles of upflow velocity and temperature[J]. Bioresource Technology, 2017, 244: 117-124

[74] Reino C, Carrera J. Low-strength wastewater treatment in an anammox UASB reactor: effect of the liquid upflow velocity[J]. Chemical Engineering Journal, 2017, 313: 217-225

[75] Strous M, Kuenen JG, Jetten MSM. Key physiology of anaerobic ammonium oxidation[J]. Applied and Environmental Microbiology, 1999, 65(7): 3248-3250

[76] MacArthur RH, Wilson EO. The Theory of Island Biogeography[M]. Princeton: Princeton University Press, 1967

[77] Kang D, Xu DD, Yu T, et al. Texture of anammox sludge bed: composition feature, visual characterization and formation mechanism[J]. Water Research, 2019, 154: 180-188

[78] Liu L, Sheng GP, Liu ZF, et al. Characterization of multiporous structure and oxygen transfer inside aerobic granules with the percolation model[J]. Environmental Science & Technology, 2010, 44(22): 8535-8540

[79] Vangsgaard AK, Mauricio-Iglesias M, Gernaey KV, et al. Sensitivity analysis of autotrophic N removal by a granule based bioreactor: influence of mass transfer versus microbial kinetics[J]. Bioresource Technology, 2012, 123: 230-241

[80] Zhu GB, Wang SY, Ma B, et al. Anammox granular sludge in low-ammonium sewage treatment: not bigger size driving better performance[J]. Water Research, 2018, 142: 147-158