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异养硝化细菌硝化途径及氮转化功能研究进展

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摘要 异养硝化细菌 (heterotrophic nitrifying bacteria) 是重要的硝化微生物类群之一, 在生长过程中可以通过硝化作用快速去除污水中多余的氮, 因而成为生物脱氮领域的研究热点. 近10年来异养硝化细菌的研究获得了较大进展, 本文系统总结异养硝化细菌类群的硝化酶系及其编码基因特点, 梳理异养硝化代谢途径; 同时总结其他氮转化功能酶及其编码基因在整个类群中的分布, 探讨异养硝化细菌的脱氮机理. 迄今为止, 已发现的异养硝化功能酶比自养硝化酶的多样性更高, 具有更多硝化途径; 且异养硝化细菌普遍具有氮转化功能多样性, 硝化、反硝化、氨同化作用对脱氮作用贡献很大. 目前还有部分异养硝化细菌的氮转化功能相关的基因信息仍然缺乏, 生物脱氮过程的分子机制尚不明确. 从分子水平对异养硝化细菌的氮转化功能活性进行验证, 进一步阐明异养硝化细菌进行脱氮作用的生理机制是今后的重要研究方向. (图2 表1 参96)

关键词 异养硝化细菌; 硝化酶系; 异养硝化途径; 氮转化基因; 生物脱氮机制

Research status and prospect on the nitrification pathway and nitrogen-transforming function of heterotrophic nitrifying bacteria

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Abstract Heterotrophic nitrifying bacteria are an important group of nitrifying microbes and have been a hotspot in nitrogen bio-removal because of their high efficiency in removing sewage nitrogen. Research on heterotrophic nitrifying bacteria has made considerable progress during the last decade. This review summarizes the key enzymes that catalyze nitrification and other nitrogen-transforming processes in heterotrophic nitrifying bacteria, organized heterotrophic nitrification pathways, and underlying molecular and biochemical mechanisms of nitrogen bio-removal. The diversity of nitrifying functional genes is higher in heterotrophic nitrifying bacteria than in autotrophic nitrifiers. Heterotrophic nitrifying enzyme cluster includes ammonia monooxygenase (AMO), hydroxylamine dehydrogenase (HAO), pyruvic oxime dioxygenase (POD), and nitric oxide dioxygenase (NOD). Heterotrophic nitrifying bacteria possess different nitrifying genes and produce nitrate via different nitrification pathways. Moreover, heterotrophic nitrifying bacteria have multiple nitrogen metabolic functions and thus play a key role in sewage nitrogen turnover. Ammonia assimilation and nitrification-denitrification are important ammonia removal pathways. Nonetheless, the genetic information on the nitrogen transformation and nitrogen removal pathways of some heterotrophic nitrifying bacteria remains unclear. In future, more molecular investigations of nitrogen metabolism are required to reveal the nitrogen metabolic pathways of heterotrophic nitrifying bacterial strains and explore bacterial nitrogen removal mechanisms.

Keywords heterotrophic nitrifying bacteria; nitrifying enzymes; heterotrophic nitrification pathway; nitrogen-transforming gene; nitrogen bio-removal mechanism

硝化微生物是介导氮元素生物地球化学循环的重要功能类群, 异养硝化细菌偏好富营养环境, 主要通过联合硝化-反硝化用于消耗还原当量 (NADH)^[1], 从而在氧气受限、能源物质过剩条件下保持较快的生长速度. 在森林土等物质能源丰

富环境中, 异养硝化作用对氮的转化总量远高于自养硝化过程^[2-3]. 随着工业和农业等生产活动加剧, 产生了大量氮素超标的废水, 引起了一系列环境安全问题, 去除水体中过量的氮素一直都是亟须解决的重大环境问题. 而部分异养硝化细菌

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可以快速降低环境中氮的浓度,受到生物脱氮领域的高度关注^[4-7]。到目前为止,已报道的异养硝化细菌物种多样性极高,在生物脱氮系统中检测到的异养硝化细菌即超过45种,各菌株的氮转化途径不同,脱氮效率差异较大^[8]。近年来,分子生物技术不断成熟,基因组学(全基因组、功能基因组、16S rRNA等)和蛋白质组学(蛋白质分离纯化)等技术被广泛应用于异养硝化细菌的研究中,使更多异养硝化细菌的氮代谢功能得以揭示,加深了人们对异养硝化细菌类群脱氮生理机制的认识。本文对近10年来异养硝化细菌类群氮转化功能的相关研究进行总结,包括硝化、反硝化、氨同化、硝酸盐异化还原为铵、硝酸盐同化还原5个氮转化过程的关键酶及其编码基因,对可能进行的异养硝化代谢途径进行梳理,同时剖析各氮代谢过程对脱氮作用的贡献率,最后对今后异养硝化细菌类群的研究方向进行展望,以期以优质脱氮菌种的筛选和改造提供理论依据。

1 异养硝化作用代谢途径及其代谢产物

1.1 异养细菌中的硝化酶系

异养硝化细菌进行的硝化过程涉及多种硝化酶,其中所包含的氨单加氧酶(ammonia monooxygenase, AMO, *amoCAB*)和羟胺氧化还原酶(hydroxylamine dehydrogenase, HAO, *hao*)也是自养硝化类群的硝化功能酶。已有的文献报道了异养硝化细菌与自养硝化细菌类群中AMO和HAO的结构及生理活性之间存在明显差异^[9]。近年来的研究发现了更多具有*amo*和*hao*基因的异养硝化细菌(表1), *Stenotrophomonas maltophilia* DQ01等异养硝化细菌基因组的*amo*基因与已知的*Nitrosomonas*、*Nitrospira*等自养硝化细菌差异较大,无法采用自养硝化细菌的引物进行扩增^[10-12]。然而,也有研究发现*Sphingomonas* sp. YY2, *Acinetobacter* sp. YY1^[13]和*Janthinobacterium* sp. M-11等多个菌株^[14-15]的*amo*基因可以被自养硝化细菌的*amo*引物成功扩增,表明其*amo*基因与自养硝化细菌相似度较高。有学者对*Sinorhizobium meliloti* 1021全基因组序列进行分析比对,发现一段序列可能是*amo*基因(putative *amo*, *P-amo*),根据该*P-amo*设计的特异性引物成功扩增到*Sinorhizobium* sp. NP1的*P-amo*全基因序列,与原始菌株相比, *P-amo*基因被敲除的菌株对NH₄⁺的去除率降低了35%^[16],证明*P-amo*的确是氨氧化作用相关的功能基因,同时也表明*Sinorhizobium* sp. NP1通过氨氧化作用可以转化35%的NH₄⁺。此后,在*Rhizobium etli*基因中也检测到*P-amo*,与*Sinorhizobium* sp. NP1的*P-amo*全基因序列相似度为66%-77%^[5]。由于*P-amo*还未被正式确定为*amo*功能基因,KEGG、GO等微生物基因库中还未进行注释,在已有的数据库中进行比对还不能鉴定出菌株中是否含有*P-amo*基因。

随着更多异养硝化细菌*amo*基因序列的揭示,为深入研究AMO的功能结构提供了新的依据。有研究根据基因库中已有的异养硝化细菌的*amo*设计引物扩增到了*Alcaligenes faecalis* NR的*amo*全基因序列,并根据该序列预测到AMO蛋白序列,并构建了该酶的三级结构模型,发现此AMO是包含12个跨膜区的跨膜蛋白^[12]。也有研究参照*Paracoccus*、*Pseudomonas*、*Arthrobacter*等8个属中的异养硝化细菌基因设计了异养*amo*基因引物heteroamo 378f/634r,但是只能扩增到*Paracoccus*、*Alcaligenes*、*Aquamicrobium*菌株中的

目的基因^[17],表明该引物只适用于特定异养硝化细菌类群。Wang根据*Pseudomonas* sp. JQ-H3^[18]和*Acinetobacter* sp. JQ1004^[11]的分别设计了两对*amoA*基因引物,这两对引物对其他异养硝化细菌类群*amoA*基因的扩增效果还有待进一步验证。上述研究结果明确了多种异养硝化细菌都可以编码氨单加氧酶,且异养硝化细菌类群中的*amo*基因家族系统发育多样性较高。目前, *Klebsiella* sp. TN-10等多个具有氨氧化活性菌株的AMO蛋白结构仍未揭示,也未检测到相应的*amo*基因或相似序列^[6-7],我们推测异养硝化细菌类群中可能还存在与已知*amo*基因序列差异较大的氨单加氧酶基因还未被识别。

异养硝化细菌催化羟胺氧化的主要功能酶是羟胺氧化还原酶(hydroxylamine dehydrogenase, HAO)。一直以来在硝化作用的研究中均认为HAO催化NH₂OH氧化生成的产物是NO₂⁻,后来的研究表明HAO氧化羟胺的直接产物不是NO₂⁻而是NO^[19]。2018年, Carantou等人发现含铜的亚硝酸还原酶(Cu-NIR, *nirK*)可以催化NO氧化为NO₂⁻^[20](图1)。由于NO被氧化为NO₂⁻的过程极易发生,因此目前的研究仍然将NO₂⁻视为氨氧化作用的产物之一。已有的研究检测到*Acinetobacter junii*^[21]、*Klebsiella pneumoniae* CF-S等菌株^[15, 22-23]含有*hao*基因(表1),可以用根据自养硝化细菌(*Nitrosomonas europaea*)所设计的*hao*基因引物进行扩增,表明这部分异养硝化细菌的羟胺氧化还原酶与自养硝化细菌相似度较高。在异养硝化细菌类群中,羟胺还可以通过丙酮肟(C₃H₇NO)双加氧酶(pyruvic oxime dioxygenase, POD)途径氧化生成NO₂⁻(图1)。已有的研究对POD的氨基酸序列进行分析,并结合基因序列初步预测了POD的编码基因序列,同时将所得序列在基因库进行比对,发现*Pseudomonas aeruginosa* PAO1、*Achromobacter xylosoxidans*等菌株也含有相似的基因^[24], *A. faecalis*中也已经检测到具有*pod*和*podh*基因^[25],此外*Cupriavidus pauculus* UM1等菌株进行的硝化作用是以丙酮肟为底物生成硝酸盐^[26],可能也会编码丙酮肟双加氧酶(表1)。一些缺乏HAO的异养硝化细菌则可以通过POD将NH₂OH转化为NO₂⁻。至今还未在自养硝化微生物中检测到POD,因此POD可能是异养硝化细菌所特有的酶。也有研究表示多个异养硝化细菌被报道具有氧化羟胺的功能^[27],却并未检测到*hao*基因,而POD的存在可能会造成HAO活性检测结果的假阳性,在这些菌株中主导羟胺氧化过程的是HAO还是POD仍不明确。

一氧化氮双加氧酶(nitric oxide dioxygenase, NOD)的本质是黄血血红蛋白(flavohemoglobin, flavoHb),是一种古老的酶,编码基因简称为*hmp*(有的称为*fhp*),在好氧条件下可以催化NO直接氧化为NO₃⁻^[28],因此NOD也属于硝化酶系。Arai等人在针对*P. aeruginosa*一氧化氮解毒机理的研究中发现了该细菌基因组中存在*hmp*基因^[29],此后检测到异养硝化细菌*Bacillus subtilis*^[30]和*Vibrio fischeri*^[31]也包含*hmp*基因(表1)。到目前为止,自养硝化功能类群的研究还未报道过*hmp*基因,因此NOD可能是除POD以外的另一种异养硝化类群所特有的硝化酶。*hmp*基因还未引起异养硝化细菌研究领域的重视,该基因在异养硝化细菌中的分布情况还有待进一步研究。根据以上研究可知,异养硝化细菌类群硝化酶系所包含的功能酶比自养类群更丰富,异养硝化代谢途径也比自养硝化更复杂;异养硝化细菌中的氨单加氧酶的基因族成员也比自养类群更多,这也预示着异养硝化细菌类群中可能还蕴藏着更多未被发现的异养硝化功能基因。

表1 分离自不同环境的异养硝化细菌的氮转化功能基因

Table 1 Nitrogen-cycling genes of heterotrophic nitrifying bacteria isolates from diverse environmental samples

门 Phylum	纲 Class	种 Species	采样点 Sample site	硝化基因或 硝化活性 Nitrification gene or activity	其他氮转化基因 Other nitrogen- cycling gene	参考文献 Reference
α-变形菌纲 α-proteobacteria		<i>Ochrobactrum anthropi</i>	活性污泥 Activated sludge	<i>P-amo</i>	<i>nosZ</i>	[5]
		<i>Paracoccus denitrificans</i>	污水处理厂 WWTP	<i>P-amo</i>	<i>nar, nir, norZ, nos</i>	[32-34]
		<i>Sinorhizobium medicae</i>	酸性土壤 Acidic soils	<i>amo</i>	/	[16]
		<i>Sinorhizobium meliloti</i>	根际土壤 Soil surrounding roots	<i>amo, hmp</i>	<i>glnAT</i>	[16, 35]
		<i>Sphingomonas paucimobilis</i>	己内酰胺废水处理系统 Caprolactam wastewater treatment system	<i>amo, hao</i>	<i>napA, nirK</i>	[13]
	β-变形菌纲 β-proteobacteria	<i>Alcaligenes faecalis</i>	活性污泥 Activated sludge 焦化废水 Coking wastewater	<i>amoA, pod, HAO</i>	<i>nirK, norB, nosZ</i>	[25, 36-37]
		<i>Achromobacter xylosoxidans</i>	生活污水 Domestic sewage water	/	<i>gln, napA, nirK, norB, nosZ</i>	[38]
		<i>Achromobacter</i> sp. HNDS-1	水稻土 Paddy soil	<i>p-amo</i>	<i>gltABD, narGHJI, nasA, nirBD</i>	[39]
		<i>Cupriavidus pauculus</i>	/	POD	/	[26]
		<i>Diaphorobacter nitroreducens</i>	焦化厂废水池 Coking-plant wastewater ponds	HAO	NAP, NIR	[40]
假单胞菌门 Pseudomonadota		<i>Diaphorobacter polyhydroxybutyrativorans</i>	反硝化反应器 Denitrification reactor in laboratory	HAO	NIR, NAR	[27]
		<i>Janthinobacterium</i> sp. M-11	松花江 Songhua Rive	<i>amoA</i>	<i>napA, narG</i>	[14, 41]
		<i>Acinetobacter calcoaceticus</i>	生物膜反应器 MBR 鲈鱼养殖场 Commercial sea bass farm	HAO	/	[42-43]
		<i>Acinetobacter haemolyticus</i>	污水处理厂 Sewage treatment plant	<i>amoA, hao</i>	<i>napA, nirS</i>	[44-45]
		<i>Acinetobacter harbinensis</i> HITLi7	松花江 Songhua River	<i>amoA, hao</i>	<i>napA</i>	[46]
	γ-变形菌纲 γ-proteobacteria	<i>Acinetobacter junii</i>	畜禽粪污沼液 Livestock and poultry manure sewage	<i>hao</i>	<i>napA, narG, nirK, nirS, nosZ</i>	[21, 47]
		<i>Acinetobacter</i> sp. JQ1004	污水处理厂 WWTP	<i>amoA</i>	/	[11]
		<i>Acinetobacter</i> sp. JR1	酸性制药废水 Acidic pharmaceutical wastewater	HAO	NR, NIR	[6]
		<i>Acinetobacter</i> sp. DN1	河流沉积物 River sediments	<i>hmp</i>	<i>gdhA, nasAB, nirBD</i>	[48]
		<i>Enterobacter</i> sp. HNDS-6	水稻土 Paddy soil	<i>hao</i>	<i>gltABD, narGHJI, nasA, nirBD</i>	[39]
厚壁菌门 Bacillota		<i>Klebsiella pneumoniae</i>	生活污水 Domestic sewage water	<i>hao</i>	<i>gln, napA, nas, NIR</i>	[23]
		<i>Klebsiella</i> sp. TN-10	序批式反应器 SBR	/	NIR, NAR	[7]
		<i>Providencia rettgeri</i>	实验室序批式反应器 Laboratory-scale SBR	/	NIR	[49]
	芽孢杆菌纲 Bacilli	<i>Pseudomonas mohnii</i>	新鲜生物活性炭样品 Fresh biological activated carbon	<i>amoA</i>	<i>napA, narG</i>	[14, 41]
		<i>Pseudomonas aeruginosa</i>	石油泄漏区域 Oil spill site	<i>pod, AMO, HAO, NXR</i>	<i>narG, nasA, nirS, norC, norZ</i>	[24, 34, 50-52]
		<i>Pseudomonas bauzanensis</i>	深海沉积物 Deep-sea sediment	<i>P-amo</i>	<i>nasA, nirS, norB, nosZ</i>	[53]
		<i>Pseudomonas mendocina</i>	活性污泥 Activated sludge	/	/	[54-55]
		<i>Pseudomonas putida</i>	焦化废水 Coking wastewater 水稻土 Paddy soil	<i>amoA, HAO</i>	<i>napA, nirS, nasA, nasB</i>	[56-58]
	放线菌纲 Actinomycetes	<i>Pseudomonas stutzeri</i>	污水处理厂 WWTP	/	<i>napA, nirS, narG, norB, nosZ</i>	[59-62]
		<i>Pseudomonas</i> sp.YY3	己内酰胺废水处理系统 Caprolactam wastewater treatment system	<i>amoA, hao</i>	<i>napA, nirK</i>	[13]
		<i>Pseudomonas</i> sp. JQ170	污水 Sewage	/	<i>napAB, nas, nirBD</i>	[63]
		<i>Stenotrophomonas maltophilia</i>	垃圾填埋渗滤液污泥 Landfill leachate sludge	<i>amoA</i>	<i>napA, narG, nirK, nirS</i>	[10, 64]
		<i>Vibrio diabolicus</i> SF16	海洋沉积物 Marine sediment	/	<i>napA</i>	[65]
放线菌门 Actinomycetota		<i>Vibrio fischeri</i>	污水处理厂 WWTP	<i>hmp</i>	/	[31]
		<i>Zobellella taiwanensis</i>	垃圾填埋区渗滤液收集池 Leachate collecting tanks from landfill	/	/	[66]
	芽孢杆菌纲 Bacilli	<i>Bacillus haynesii</i>	生物滤池的生物膜 Biofilm from biofilter	<i>nxB, AMO, HAO</i>	<i>nirK</i>	[67]
		<i>Bacillus litoralis</i>	海水养虾场 Shrimp mariculture water	<i>hao</i>	<i>napA, nirS</i>	[68]
		<i>Bacillus megaterium</i>	生物滤池的生物膜 Biofilm from biofilter	AMO, HAO	/	[67]
		<i>Bacillus</i> sp. LY	生活污水处理厂 Domestic WWTP	<i>amoA</i>	/	[69]
	放线菌纲 Actinomycetes	<i>Bacillus subtilis</i>	市政污水处理厂 Municipal WWTP	<i>hmp</i>	<i>glnA, nap, narGH, nasABCDE</i>	[30, 70]
		<i>Bacillus tequilensis</i>	生物滤池的生物膜 Biofilm from biofilter	<i>nxB, AMO, HAO</i>	<i>nirK</i>	[67]
		<i>Bacillus wiedmannii</i>	生物滤池的生物膜 Biofilm from biofilter	AMO, HAO	/	[67]
		<i>Arthrobacter</i> sp. HHEP5	海水养殖池 Mariculture ponds	<i>amoA, hao</i>	<i>narG, nirS, nosZ</i>	[15]
		<i>Rhodococcus pyridinivorans</i>	养猪厂废水池 Swine wastewater ponds	/	/	[22]

/: 没有相关信息。MBR: 生物膜反应器; SBR: 序批式反应器; WWTP: 污水处理厂 大写字母: 检测到相应的酶活性; 斜体字母: 具有对应的功能基因。
/: Not mentioned. MBR: Membrane bioreactor; SBR: Sequencing batch reactor; WWTP: Wastewater treatment plant. Uppercase letters indicate the corresponding enzyme activity; italic letters Indicate the corresponding functional genes.

1.2 异养硝化途径

1970年Odu等人从土壤中分离到一株异养硝化细菌,在以 NH_4^+ 为底物的培养条件下会产生 NO_3^- [71]。而最近的研究表明,以 NH_4^+ 为唯一氮源对*Rhodococcus pyridinivorans* CPZ24、*K. pneumoniae* CF-S9等多个菌株进行培养,同样可以检测到产物 NO_3^- ,并认为这些菌株具备将 NH_4^+ 氧化为 NO_3^- 的功能 [1, 15, 23, 32, 54, 68]。由于硝化活性不同,所以不同异养硝化细菌的培养体系中 NO_3^- -N浓度差异很大 [49, 68, 72]。多数菌株培养液中 NO_3^- -N的浓度始终较低 [7, 23, 65], *Acinetobacter calcoaceticus*在培养过程中无论是改变 NH_4^+ -N的浓度还是将底物更换为 NH_2OH ,检测到 NO_3^- -N的浓度变化不大(1.5-2.0 mg/L) [42-43],由于这些细菌往往含有多个硝酸盐还原酶编码基因(*nap*, *nar*, *nas*) [15, 41],无论在好氧还是厌氧条件下均可还原硝酸盐,这可能是造成 NO_3^- -N累积量较低的原因之一,同时也表明在培养期间 NO_3^- 的合成总量远高于检测值,仅通过 NO_3^- 的累积量来计算微生物的硝化过程会导致硝化活性被低估。*Rhodococcus* sp. B83等少数菌株的培养过程中 NH_4^+ -N转化为 NO_3^- -N的净转化率较高(8.0%-36.7%), NO_3^- -N净合成量较大 [22, 27, 72],以上的研究结果表明多种异养硝化细菌都能将 NH_4^+ -N转化为 NO_3^- -N。而自养硝化类群中,直到2015年才发现存在全程氨氧化细菌(complete ammonia-oxidizing bacteria, comammox)同时具备*amo*、*hao*和*nrx*基因,可以独自将 NH_4^+ 氧化为 NO_3^- [73],目前发现的comammox都属于*Nitrospira*,系统发育较为单一 [74]。根据功能基因和细菌活性的研究结果可知,*P. aeruginosa*同时具有AMO、HAO、NXR活性 [50],*B. haynesii*和*B. tequilensis*同时具有AMO、HAO活性和*nrx*基因 [67],这些菌株的硝化途径可能与自养全程氨氧化过程相似 [73] (图2 ①);而*A. faecalis*等含有AMO和POD编码基因的菌株可能是经丙酮脒再生成 NO_3^- [10, 24] (图2 ②);*B. subtilis*含有*hmp*、*nap*和*narG*却没有*nirK* [70],可能是通过一氧化氮双加氧酶产生 NO_3^- (图2 ③)。但是迄今为止,还没有在异养细菌中同时检测到*amo*、*hao/pod*和*nrx/hmp*基因,上述3条异养硝化代谢途径还需要基因水平的证据进一步验证。

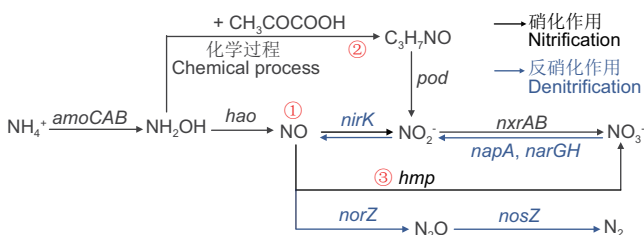


图1 异养硝化作用及其产物的代谢途径。

Fig. 1 The metabolic pathways of heterotrophic nitrification and their metabolites. ① $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$; ② $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{C}_3\text{H}_7\text{NO} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$; ③ $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO} \rightarrow \text{NO}_3^-$.

2 反硝化功能基因及活性

2.1 硝酸盐和亚硝酸盐还原作用

1988年Robertson等人发现了*Paracoccus denitrificans*具有好氧反硝化功能,并认为只有极少数微生物可以进行硝化-反硝化作用 [75],而后续的研究结果发现绝大多数异养硝化细菌都具有反硝化功能。部分异养硝化细菌具有将 NO_3^- 转化为 NO_2^- 的功能,该过程主要由硝酸盐异化还原酶(dissimilatory nitrate reductases)主导,根据酶蛋白所在位置可细分为膜结合硝酸盐异化还原酶(NAR, *narGH*)和

周质硝酸盐异化还原酶(NAP, *napA*),两种酶的结构组成完全不同 [9]。NAR对氧分子敏感,主要参与厌氧反硝化;而NAP对氧分子不敏感,与好氧反硝化紧密相关。*Agrobacterium tumefaciens* LAD9和*Sphingomonas paucimobilis*等超过16个异养硝化细菌菌株含有*nap*基因 [6, 13, 22, 36, 47, 63, 68] (表1),这些微生物主导好氧条件下的硝酸盐异化还原反应;*Achromobacter* sp. strain HNDS-1和*Enterobacter* sp. HNDS-6等超过10种细菌被检测到*nar*基因 [15, 32, 39, 50, 65] (表1),主导微好氧和缺氧环境中的硝酸盐异化还原过程。此外,*Pseudomonas mohnii* M-8、*Janthinobacterium* sp. M-11 [14, 41]、*S. maltophilia* DQ01 [10]和*Pseudomonas stutzeri* [59-60, 76]等超过5个菌株同时具有编码NAP和NAR的基因,可以根据环境中氧气浓度变化调动不同代谢途径催化 NO_3^- 还原为 NO_2^- 。 NO_2^- 又进一步被亚硝酸盐还原酶(nitrite reductases, NIR, *nirS/nirK*)还原为一氧化氮(NO),在污水处理厂的活性污泥中*nirK/S*的丰度很高,*A. calcoaceticus*和*K. pneumoniae*等超过19种异养硝化细菌已被确认含有*nir*基因 [6-7, 10, 32] (表1),氨氧化作用和硝酸盐还原产生的 NO_2^- 迅速被NIR还原为 NO ,培养液中 NO_2^- 的浓度往往很低,有的接近于0.0 mg/L [23, 27, 42, 48, 65]。异养硝化细菌硝化产 NO_3^- 的活性很强,而广泛存在的反硝化功能可以快速降低环境中硝酸盐和亚硝酸盐浓度,作为一种保护机制使自身免受 NO_3^- 和 NO_2^- 的毒害,这种保护机制也促使水体中氮转化过程更活跃。

2.2 反硝化作用产生 N_2O 和 N_2

N_2O 和 N_2 是水体中反硝化作用的终产物,已知的异养硝化细菌通过硝化和反硝化作用产生的含氮气体(N_2O , N_2)占总氮的27.4%-83.7% [5, 42-43] (图2),是重要的生物脱氮途径。其中 N_2O 是温室气体,其温室效应是 CO_2 的265倍,同时 N_2O 也会损耗臭氧层。 N_2O 是由亚硝酸盐还原的产物 NO 经一氧化氮还原酶(nitric oxide reductases, NOR)还原产生,目前已知的NOR有5种,其中氧化氮细胞色素c依赖性(cNOR, *cnorB*)和喹啉依赖性(qNOR, *norZ*)一氧化氮还原酶在自然水体和污水处理系统中广泛分布。最近的研究发现,异养硝化细菌类群中的*Cupriavidus*和*Sphingomonas*两个属的菌株含有*norZ*,*Mycobacterium*和*P. stutzeri* KCB有多个*cnorB*基因拷贝,而*Diaphorobacter*同时具有qNOR和cNOR的编码基因 [62],*A. faecalis* WY-01的一氧化氮还原酶基因也被成功扩增 [36] (表1)。在氮充足的水生环境中一氧化氮还原活性很高,最近,Liang等人报道了富营养水体中硝化联合反硝化作用会排放大量 N_2O [77],由于环境中产生 N_2O 的生物酶的数量和种类极为丰富,识别 N_2O 的具体来源是十分具有挑战性的工作,要明确混菌环境中异养硝化细菌类群对 N_2O 排放的贡献率还有较大难度。已有的报道多采用独立培养方法对菌株的一氧化氮还原活性进行研究,当以硝酸盐为底物对*Enterobacter asburiae* GS2进行培养,排放的 N_2O 占总氮的30.6% [78],*Acinetobacter haemolyticus* ZYL将超过4.6%的 NH_4^+ 转化为 N_2O 等含氮气体 [45];也有研究采用同位素标记技术,发现*Providencia rettgeri* YL在好氧生物反应器中将大量 NH_4^+ 转化为 N_2O [79],这些异养硝化细菌是污水处理过程中 N_2O 的重要来源。

部分异养硝化细菌可以编码氧化亚氮还原酶(nitrous oxide reductase, NOS, *nosZ*),催化 N_2O 还原生成无毒性的 N_2 ,NOS是目前已知的唯一可以直接将 N_2O 还原为 N_2 的具有生物学意义的酶 [80]。*Ochrobactrum anthropic* [5]、*Pseudomonas stutzeri* [61]和*A. faecalis* WY-01 [36]等都可

以编码NOS^[47](表1)。已有的研究采用稳定性同位素核酸探针技术(DNA stable-isotope probing, DNA-SIP)发现*Achromobacter* sp. HNDS-1和*Enterobacter* sp. HNDS-6参与生物脱氮的基因包括*norB*和*nosZ*,具有将氮转化为N₂O再转化为N₂的潜力^[39](表1)。在独立培养体系中*A. haemolyticus* ZYL^[45]可以将自身所产生的绝大部分N₂O转化为N₂,*A. calcoaceticus* HNR在培养后所产生的N₂占总氮的比例可以达到40.2%^[42]。虽然这些异养硝化细菌表现出较高的氧化亚氮还原活性,但是仍然会释放出少量N₂O,不能完全氧化自身产生的N₂O。与环境中N₂O的排放通量相比,目前已知的微生物所编码的NOS的氧化能力都十分有限^[81],不能处理环境中大量产生的N₂O,这也是污水处理系统中N₂O排放高的原因之一。

2010年, Ettwig等人首次发现一氧化氮歧化酶(nitric oxide dismutase, NO-D, *norZ*),催化NO氧化还原为N₂和O₂^[82],该反应不生成N₂O,是较为理想的生物脱氮过程。目前针对异养硝化细菌类群NO-D的研究较少,只确定了少数菌株具有编码NO-D的功能,*P. aeruginosa*和*Paracoccus denitrificans*已被证实含有*norZ*基因^[34],*P. aeruginosa*在氧气充足的环境经过硝化-反硝化作用转化生成的含氮气体主要为N₂^[83](表1)。多个研究发现一些异养硝化细菌在适宜的培养环境中也只产生N₂,如*Zobellia taiwanensis* DN-7^[66],*Vibrio diabolicus* SF16^[65]在单独培养时不产生N₂O,48.8%-65.6%的NH₄⁺-N最终转化为N₂;以及*Acinetobacter* sp. YY-5^[84]和*Diaphorobacter polyhydroxybutyrativans*^[27]等菌株也只产生N₂,可能具有编码一氧化氮歧化酶的功能,这些菌株催化生成N₂的代谢途径尚不明确,还有待进一步研究。自然水生系统的沉积物和工程水生系统中的活性污泥都可以检测到较高丰度的*norZ*^[34],这些区域是异养硝化细菌的热点分布区域,根据已有的研究结果我们推测*norZ*基因可能广泛存在于异养硝化细菌类群中。总体而言,*Z. taiwanensis* DN-7, *V. diabolicus* SF16., *Acinetobacter* sp. YY-5, *D. polyhydroxybutyrativans*, *P. aeruginosa*和*P. denitrificans*在适宜的环境中可以将污水中的无机氮(NH₄⁺, NH₂OH, NO₂⁻, NO₃⁻)转化为N₂,不产生或只产生微量N₂O,对环境的负面影响较小,是潜在的优质脱氮菌种。2022年, Müller等人报道了*Pseudomonas stutzeri*组装NOS酶的分子机制^[58],这一研究加深了对N₂O还原生成N₂这一关键过程的认识,为今后通过生物工程改造氧化亚氮还原酶体系以减少大气中的N₂O含量提供了重要依据。

3 其他氮转化功能

3.1 异养硝化细菌的氨同化活性

异养硝化细菌普遍具有氨同化功能,纯菌株*A. haemolyticus*^[45]、*A. junii*^[21, 85]以及*V. diabolicus* SF16^[65]在培养初期10-20 h内可以观察到细胞生长的对数时期,该时间段氨/铵的去除速率达到最高,随后生物量累积速度变慢,氨/铵的去除速率也随之降低,氨同化活性与脱氮/铵过程呈正相关关系。不同菌株氨同化对脱氮作用有着不同的贡献率,如*E. asburiae* GS2和*P. rettgeri*同化的氨分别占67.8%^[78]和54.5%^[49],*A. calcoaceticus* HNR在C/N为20条件下可以把41.7%的NH₄⁺转化为胞内有机氮^[49],而*R. pyridinivorans* CPZ24的氨同化量更低,占总NH₄⁺的24.0%^[22]。部分异养硝化细菌的氨同化功能基因已经得到确认,*K. pneumoniae*

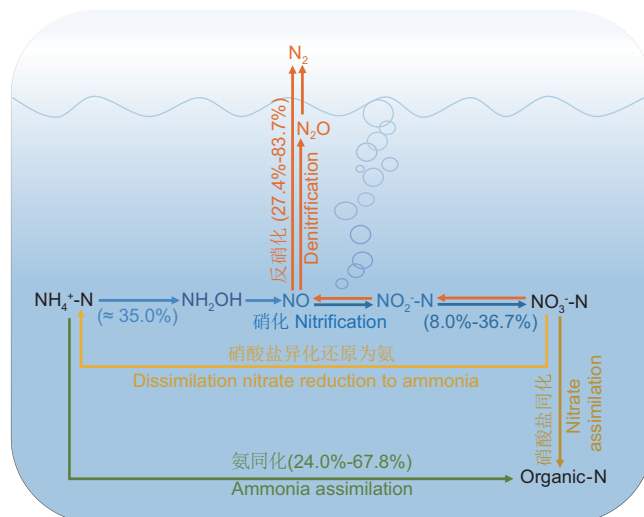


图2 异养硝化细菌生物脱氮代谢途径及脱氮贡献率。

Fig. 2 The ammonia removal pathways of heterotrophic nitrifying bacteria and contribution of these pathways.

和*A. xylosoxidans* CF-S36基因组中包含*glnA*基因^[23, 38](表1),*glnA*是谷氨酰胺合酶(glutamine synthetase, GS)的编码基因。本课题组在分析水稻土微生物宏基因组学信息,发现*Providencia*属中细菌的氨同化基因也是*glnA*,而*R. pyridinivorans*含有*GDH2*基因(未发表),此外,*Acinetobacter* sp. DN1包含*gdhA*基因^[48](表1)。*GDH2*和*gdhA*参与编码谷氨酸脱氢酶(glutamate dehydrogenase, GDH),GS和GDH催化氨氧化生成谷氨酰胺和谷氨酸,使NH₄⁺进入有机氮库。与GDH相比,GS对氨具有更高的亲和力,其米氏常数(K_m)在微摩尔(μmol)范围内,而GDH的K_m在毫摩尔(mmol)范围内^[86],这种对氨亲和力的差别导致GDH在氨浓度高的环境中对氨同化作用有显著贡献,而GS在NH₄⁺浓度较低的环境中发挥重要作用^[87]。由于这种差异,*A. haemolyticus*、*A. junii*、*Acinetobacter* sp. JR1以及*R. pyridinivorans* CPZ24仅在前期NH₄⁺浓度较高的短时间内进行氨同化作用,当氨浓度降低后氨同化活性减弱;而*P. rettgeri*等可以编码谷氨酰胺合酶的菌株在NH₄⁺浓度偏低的环境中仍能继续将氨同化为有机氮,固定的NH₄⁺超过外源NH₄⁺总量的一半,可以消耗更多的氨/铵。上述研究所发现的异养硝化细菌在单独培养过程中对氨/铵的去除率范围为24.0%-67.8%^[22, 49, 78](图2),但是仍有多数异养硝化细菌的氨同化活性及其编码基因的信息尚缺乏,有待进一步探究。

除了纯培养体系之外,混菌培养系统中的脱氮效果同样受到氨同化作用影响显著。最近,对垃圾渗滤液处理系统中的氮转化功能基因的研究结果显示,微生物的氨同化功能基因丰度是所有氮转化功能基因中最高的;且当处理器中氨态氮浓度降低时有机氮的含量随之增加,在所有氮转化功能中氨同化对去除全氮和氨/铵起着主导作用^[4],该研究进一步验证了氨同化功能是脱氮的重要生理过程之一。与硝化-反硝化作用相比,氨被固定为有机氮则可以避免产生温室气体副产物,是更为环保的脱氮过程。硝化作用和氨同化作用需要竞争共同的底物氨,促进氨/铵进入同化途径可以减少硝化-反硝化作用从而抑制N₂O排放。当向污水处理装置中添加碳源时,处理系统中的氨基酸合成量会进一步提高^[88],氨同化量可以达到环境中总氮的77.9%^[10],而缺乏磷酸盐的环境会强烈抑制氨同化过程^[89]。因此通过添加碳源,减少磷酸盐浓度等方式调节培

养环境,增加活性污泥中微生物氨同化过程对氨的利用率,也是减少污水处理过程中 N_2O 排放的有效方法。

3.2 硝酸盐同化/异化还原

硝酸盐是异养硝化作用的主要产物, NO_3^- 除了经过反硝化作用被转化为 N_2O 和 N_2 之外,还可以被异养硝化细菌同化吸收(图2)。*Pseudomonas putida*含有*nasA*和*nasB*基因^[58],*nasA*编码硝酸盐同化还原酶(assimilatory nitrate reductase, NAS; *nasA/nirA*),催化 NO_3^- 同化还原为 NO_2^- ; *nasB*参与编码亚硝酸盐同化还原酶(assimilatory nitrite reductase, cNIR; *nasB/nirB*),催化亚硝酸盐同化还原为 NH_4^+ ,然后 NH_4^+ 最终被固定为细胞的组分。除此之外,*K. pneumoniae*^[23]、*S. meliloti*^[53]和*Acinetobacter* sp. DN1^[48]等多个异养硝化细菌基因组中包含NAS和cNIR的编码基因,均可通过NAS复合体同化吸收环境中的 NO_3^- (表1)。由于*nasAB*结构基因的表达会受到 NH_4^+ 的抑制,硝酸盐同化还原为氨/铵的反应在低氨/铵环境中更为活跃。硝酸盐异化还原为铵(dissimilatory nitrate reduction to ammonium, DNRA)是转化 NO_3^- 的另一代谢途径。微生物采用DNRA在 NO_3^- 和 NO_2^- 浓度较高的环境中进行异养发酵产能^[90],或者消耗环境中过量的 NO_3^- 和 NO_2^- 避免细胞遭受毒害;在低氨/铵环境中,微生物通过硝酸盐同化还原作用将 NO_3^- 转化为氨/铵,再作为自身的氮源,两个途径的作用并不相同。但是有研究发现*Mycobacterium tuberculosis*通过膜结合硝酸盐异化还原酶系也可以同化吸收 NO_3^- ^[91],表明微生物的硝酸盐同化还原和异化还原两种代谢途径没有绝对区别,任一途径都可以吸收 NO_3^- , NO_3^- 被还原吸收并不产生 N_2O ,也是脱氮中较为环保的氮转化过程之一。DNRA的功能基因*narGH*和*nrfABCDH*在污水处理器和河流沉积物中丰度都非常高^[4, 92-93],*S. meliloti*和*P. putida*都具有DNRA功能^[53, 57](表1)。*Acinetobacter* sp. JR1、*Pseudomonas mendocina* X49、*A. xylosoxidans*等在以 NO_3^- 为唯一氮源的培养基中快速增殖($\text{OD}_{600} > 1.0$),并且能在产物中检测到 NH_4^+ ^[6, 38, 54-55],这些菌株可能也具有DNRA功能,有待进一步验证。

DNRA会截留环境中的氮,使其以氨/铵的形态重新进入内循环,可以提高氮素利用率,有利于农业生产,但对于氨/铵浓度超标的污水处理似乎没有积极的效果。Wang等人采用 ^{15}N 同位素技术对 NH_4^+ -N浓度为18.4-46.0 mg/L的8个污水处理装置进行分析,结果显示尽管具有DNRA代谢功能的微生物数量很多,但是DNRA的速率(以N计, $0.4\text{-}2.1 \text{ nmol g}^{-1} \text{ h}^{-1}$)远低于反硝化速率($9.5\text{-}15.7 \text{ nmol g}^{-1} \text{ h}^{-1}$),对水体中的氮转化过程没有显著贡献^[94],这也表明在氨/铵充足的环境中,包括异养硝化细菌在内的整个微生物类群主导的DNRA代谢作用对脱氮的贡献可以忽略不计。而在氨浓度较低($0.0\text{-}5.7 \text{ mg/L}$),硝酸盐浓度较高($18.6\text{-}36.8 \text{ mg/L}$)的环境中可以观察到DNRA活性较高($1.3\text{-}33.0 \text{ nmol m}^{-2} \text{ h}^{-1}$)^[95]。此外,在特定的培养条件下部分异养硝化细菌也具有较高的DNRA活性,*P. putida* Y-9在以葡萄糖为碳源,C/N比为9,pH为7,摇床转速为150 r/min的培养条件下,81.8%的 NO_3^- 被还原为氨/铵或被同化吸收,10.6%通过反硝化作用流失^[96]。当污水处理后期进入氨/铵浓度较低,硝化作用产物累积的阶段,*P. putida*和*S. meliloti*等细菌可以用于还原吸收残余的硝酸盐,进一步降低水体中含氮化合物的浓度;也可以控制反应器中的培养条件,诱导菌株的硝酸盐同化或异化还原为铵代谢活性,减少反硝化作用,从而减少 N_2O 的生成量。

4 结论与展望

近年来分子生物技术的应用揭示了异养硝化细菌普遍具有多种氮转化功能,包括硝化、反硝化、氨同化、硝酸盐同化还原和硝酸盐异化还原为铵等,在多个氮周转过程中都十分活跃,是生物脱氮技术中优质工程菌的重要微生物资源库。总结异养硝化细菌的氮转化功能活性及相应的调控基因有利于明确各菌株的脱氮特性,为筛选出优质脱氮菌种提供理论依据。近10年的研究表明异养硝化细菌中的硝化酶系比自养硝化酶系更丰富,氨单加氧酶编码基因(*amo*)的多样性也更高;且多数异养硝化细菌都可以催化 NH_4^+ 氧化生成 NO_3^- ,根据已有的研究结果,我们预测异养硝化细菌可能通过3种代谢途径将 NH_4^+ 转化为 NO_3^- (图2),是营养物质充足的环境中 NO_3^- 的主要产生者。这些研究阐明了部分异养硝化细菌的脱氮分子机制:(1)*S. meliloti*等菌株具有硝酸盐异化还原为铵和硝酸盐同化吸收功能,在氨氮浓度较高的污水中,这两种功能对脱氮/铵作用几乎没有贡献;而在铵态氮浓度低,硝化产物累积量较大时对生物脱氮具有积极作用;(2)异养硝化细菌主要通过硝化联合反硝化降低环境中的氨/铵浓度,氨/铵去除率可以达到27.4%-83.7%,但是会产生有害气体 N_2O ;反硝化过程中的一氧化氮歧化酶途径可以将氮转化为 N_2 ,是比较环保的脱氮过程;(3)异养硝化细菌还可以通过GS或GDH等途径进行氨同化作用,目前已知的菌株通过氨同化作用去除的氨/铵占24.0%-67.8%,该过程也不会产生副产物。在所有氮转化功能中,氨同化和硝化-反硝化是异养硝化细菌类群的主要脱氮途径,*P. rettgeri*和*E. asburiae* GS2的氨同化活性很高,仅通过氨同化作用就可以除水体中大部分游离的氨/铵;在适宜的环境中*Z. taiwanensis* DN-7、*V. diabolicus* SF16、*Acinetobacter* sp. YY-5、*D. polyhydroxybutyrativorans*、*P. aeruginosa*和*P. denitrificans*等菌株一氧化氮活性或氧化亚氮歧化酶活性较强,将大量无机氮转化为 N_2 ,对环境的负面影响较小,以这些细菌为脱氮菌剂有望减少污水处理过程中 N_2O 的排放量。

异养硝化细菌是包含多个物种的庞大功能类群,还有很多菌株的氮转化活性特点尚未揭示,为了更准确、全面地认识异养硝化细菌对自然生境和人工系统中氮周转的作用,今后的研究可以从以下3个方面进行:(1)从分子水平上对菌株的异养硝化代谢途径进行验证。目前催化氨、羟胺、亚硝酸盐、一氧化氮、丙酮肟等物质氧化的调控基因都已被确认,氨单加氧酶编码基因(*amo*)的相关信息最为丰富,今后还需要采用高通量二代或三代测序技术对*hao*、*pod*、*hmp*等异养硝化功能基因在整个细菌类群中的分布情况进行确认;同时还需要通过室内模拟培养或者原位监测探究上述功能酶的表达条件,明确异养硝化功能对细菌的意义。(2)各氮转化功能与异养硝化细菌脱氮性能之间的联系。目前很多菌株的氮转化功能酶活性和功能酶的编码基因仍不明确,如*P. mendocina*、*R. pyridinivorans*和*Z. taiwanensis* DN-7等多个菌株在硝化作用和硝酸盐异化/同化还原等氮代谢作用的认识仍停留在产物水平上,具体的代谢机理还存在诸多疑问,需要从分子水平找到确切的证据,进而明确菌株通过何种氮转化代谢途径进行脱氮作用。(3)各氮转化功能对生物脱氮的相对贡献率。氮代谢产物 NO 、 NO_2^- 的化学性质非常活泼,在环境中不能稳定存在, NO_2^- 、 NO_3^- 在硝化作用中是产物,同时也是反硝化作

用和硝酸盐同化/异化还原的底物, 这些中间产物在水体中的浓度波动很大, 导致难以获得各氮代谢活性的准确数据。今后可采用培养实验, 采用稳定性同位素核酸探针技术 (DNA-SIP)、代谢组学以及转录组学等技术探究不同菌株的各个氮

代谢功能在菌株脱氮过程中的相对贡献率。这些研究可以加深对异养硝化细菌类群功能活性特点的认识, 为评估各菌株的脱氮性能提供更准确的信息, 有助于后续对潜在的脱氮菌株进行筛选、改造和利用。

参考文献 [References]

- Wehrfritz JM, Reilly A, Spiro S, Richardson DJ. Purification of hydroxylamine oxidase from *Thiosphaera pantotropha*, identification of electron acceptors that couple heterotrophic nitrification to aerobic denitrification [J]. *Febs Lett*, 1993, **335** (2): 246-250
- Schimel JP. Identification of heterotrophic nitrification in a sierran forest soil [J]. *Appl Environ Microb*, 1984, **48** (4): 802-806
- Zhang JB, Müller C, Zhu TB, Cheng Y, Cai ZC. Heterotrophic nitrification is the predominant NO_3^- production mechanism in coniferous but not broad-leaf acid forest soil in subtropical China [J]. *Biol Fert Soils*, 2011, **55** (2): 288-336
- Xu L, Chen YT, Wang Z, Zhang Y, He YL, Zhang A, Chen H, Xue G. Discovering dominant ammonia assimilation: implication for high-strength nitrogen removal in full scale biological treatment of landfill leachate [J]. *Chemosphere*, 2022, **312** (312): 137245
- Lei X, Jia YT, Chen YC, Hu YY. Simultaneous nitrification and denitrification without nitrite accumulation by a novel isolated *Ochrobactrum anthropic* LJ81 [J]. *Bioresour Technol*, 2019, **272**: 442-450
- Yang JR, Wang Y, Chen H, Lyu YK. Ammonium removal characteristics of an acid-resistant bacterium *Acinetobacter* sp. JR1 from pharmaceutical wastewater capable of heterotrophic nitrification-aerobic denitrification [J]. *Bioresour Technol*, 2019, **274**: 56-64
- Li D, Liang XH, Jin Y, Wu CD, Zhou RQ. Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying-denitrifying bacterium, *Klebsiella* sp. TN-10 [J]. *Appl Biochem Biotech*, 2019, **188** (2): 540-554
- Duan SP, Zhang YY, Zheng SK. Heterotrophic nitrifying bacteria in wastewater biological nitrogen removal systems a review [J]. *Crit Rev Sci Technol*, 2022, **52**: 2302-2338
- 张明霞, 李安章, 陈猛, 徐帅帅, 朱红惠. 异养硝化-好氧反硝化菌脱氮相关酶系及其编码基因的研究进展[J]. 生物技术进展, 2020, **10** (1): 40-45 [Zhang MX, Li AZ, Chen M, Xu SH, Zhu HH. Progress on nitrogen removal related enzymes and their coding genes in heterotrophic nitrifying and aerobic denitrifying bacteria [J]. *Curr Biotechnol*, 2020, **10** (1): 40-45]
- Jia YT, Zhou MM, Chen YC, Luo J, Hu YY. Carbon selection for nitrogen degradation pathway by *Stenotrophomonas maltophilia*: Based on the balances of nitrogen, carbon and electron [J]. *Bioresour Technol*, 2019, **294**: 122114
- Wang XJ, Wang WQ, Li Y, Zhang J, Zhang Y, Li J. Biofilm activity, ammonia removal and cell growth of the heterotrophic nitrifier, *Acinetobacter* sp., facilitated by exogenous N-acyl-homoserine lactones [J]. *RSC Adv*, 2018, **8** (54): 30783-30793
- 黄源生, 赵彬, 程丹阳, 安强. 异养硝化菌 *Alcaligenes faecalis* NR 氨单加氧酶 PCR 扩增及生物信息学分析[J]. 微生物前沿, 2017, **6** (2): 44-51 [Huang YS, Zhao B, Cheng DY, An Q. PCR amplification and bioinformatics analysis of AMO from a heterotrophic nitrifier, *Alcaligenes faecalis* NR [J]. *Adv Microbiol*, 2017, **6** (2): 44-51]
- Lang XD, Li QW, Xu YC, Ji MM, Yan GX, Guo SH. Aerobic denitrifiers with petroleum metabolizing ability isolated from caprolactam sewage treatment pool [J]. *Bioresour Technol*, 2019, **290**: 121719
- Yang M, Lu DW, Yang JX, Zhao YM, Sun Y, Liu HL, Ma J. Carbon and nitrogen metabolic pathways and interaction of cold-resistant heterotrophic nitrifying bacteria under aerobic and anaerobic conditions [J]. *Chemosphere*, 2019, **234**: 162-170
- Zhang MY, Pan LQ, Liu LP, Su C, Dou L, Su ZP, He ZY. Phosphorus and nitrogen removal by a novel phosphate-accumulating organism, *Arthrobacter* sp. HHEP5 capable of heterotrophic nitrification-aerobic denitrification: safety assessment, removal characterization, mechanism exploration and wastewater treatment [J]. *Bioresour Technol*, 2020, **312**: 123633
- 刘钰莹, 邱枫, 宋琴, 窦鑫, 许雷. 中华根瘤菌 NP1 氨单加氧酶基因的克隆与功能鉴定[J]. 中国生物化学与分子生物学报, 2010, **26** (8): 768-775 [Liu YY, Qiu F, Song Q, Dou X, Xu L. Cloning and identification of ammonia monooxygenase gene of *Sinorhizobium* sp. NP1 [J]. *Chin J Biochem Mol Biol*, 2010, **26** (8): 768-775]
- Liu XY, Shu ZF, Sun DZ, Dang Y, Holmes DE. Heterotrophic nitrifiers dominate reactors treating incineration leachate with high free ammonia concentrations [J]. *ACS Sustain Chem Eng*, 2018, **6** (11): 15040-15049
- Wang XJ, Wang WQ, Zhang Y, Sun ZT, Zhang J, Chen GH, Li J. Simultaneous nitrification and denitrification by a novel isolated *Pseudomonas* sp. JQ-H3 using polycaprolactone as carbon source [J]. *Bioresour Technol*, 2019, **288**: 121506
- Wijma HJ, Canters GW, Vries SD, Verbeet MP. Bidirectional catalysis by copper-containing nitrite reductase [J]. *Biochemistry*, 2004, **43** (32): 10467-10474
- Carantoa JD, Lancaster KM. Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase [J]. *PNAS*, 2018, **115** (35): E8325
- 高艳辉, 谭娜, 赵天涛, 张云茹, 张千, 彭绪亚, 艾铄. 同步异养硝化好氧反硝化细菌 *Acinetobacter* sp. TAC-1 的氮代谢途径研究[J]. 重庆理工大学学报自然科学版, 2022, **36** (1): 204-214 [Gao YH, Tan N, Zhao TT, Zhang YR, Zhang Q, Peng XY, Ai S. New insight into the nitrogen metabolism pathway of simultaneous heterotrophic nitrification-aerobic denitrification bacteria *Acinetobacter* sp. TAC-1 [J]. *J Chongqing Univ Technol (Nat Sci)*, 2022, **36** (1): 204-214]
- Chen PZ, Li J, Li QX, Wang YC, Li SP, Ren TZ, Wang LG. Simultaneous heterotrophic nitrification and aerobic denitrification by bacterium *Rhodococcus* sp. CPZ24 [J]. *Bioresour Technol*, 2012, **116**: 266-270
- Padhi SK, Tripathy S, Sen R, Mahapatra AS, Mohanty S, Maiti NK. Characterisation of heterotrophic nitrifying and aerobic denitrifying *Klebsiella pneumoniae* CF-S9 strain for bioremediation of wastewater [J]. *Int Biodeter Biodegr*, 2013, **78**: 67-73
- Tsujino S, Uematsu C, Dohra H, Fujiwara T. Pyruvic oxime

- dioxygenase from heterotrophic nitrifier *Alcaligenes faecalis* is a nonheme Fe(II)-dependent enzyme homologous to class II aldolase [J]. *Sci Rep-UK*, 2017, **7**: 39991
- 25 Tsujino S, Dohra H, Fujiwara T. Gene expression analysis of *Alcaligenes faecalis* during induction of heterotrophic nitrification [J]. *Sci Rep-UK*, 2021, **11**: 23105
 - 26 Ramirez M, Obrzydowski J, Ayers M, Virparia S, Wang MJ, Stefan K, Linchangco R, Castignetti D. Pyruvic oxime nitrification and copper and nickel resistance by a *Cupriavidus pauculus*, an active heterotrophic nitrifier-denitrifier [J]. *Sci World J*, 2014, **2014**: 901702
 - 27 Zhang SS, Sun XB, Fan YT, Qiu TL, Gao M, Wang XM. Heterotrophic nitrification and aerobic denitrification by *Diaphorobacter polyhydroxybutyrativorans* SL-205 using poly (3-hydroxybutyrate-co-3-hydroxyvalerate) as the sole carbon source [J]. *Bioresource Technol*, 2017, **241**: 500-507
 - 28 Ouellet H, Ouellet Y, Richard C, Labarre M, Wittenberg B, Wittenberg J, Guertin M. Truncated hemoglobin HbN protects *Mycobacterium bovis* from nitric oxide [J]. *PNAS*, 2002, **99** (9): 5902-5907
 - 29 Arai H, Hayashi M, Kuroi A, Ishii M, Igarashi Y. Transcriptional regulation of the flavohemoglobin gene for aerobic nitric oxide detoxification by the second nitric oxide-responsive regulator of *Pseudomonas aeruginosa* [J]. *J Bacteriol*, 2005, **187** (12): 3960-3968
 - 30 Rogstam A, Larsson JT, Kjelgaard P, Wachenfeldt CV. Mechanisms of adaptation to nitrosative stress in *Bacillus subtilis* [J]. *J Bacteriol*, 2007, **189** (8): 3063-3071
 - 31 Wang Y, Dunn AK, Wilneff J, McFall-Ngai MJ, Spiro S, Ruby EG. *Vibrio fischeri* flavohaemoglobin protects against nitric oxide during initiation of the squid-*Vibrio* symbiosis [J]. *Mol Microbiol*, 2010, **78** (4): 903-915
 - 32 Medhi K, Singhal A, Chauhan DK, Thakur IS. Investigating the nitrification and denitrification kinetics under aerobic and anaerobic conditions by *Paracoccus denitrificans* ISTOD1 [J]. *Bioresource Technol*, 2017, **242**: 334-343
 - 33 Gaimster H, Chalklen L, Munnoch J, Alston M, Richardson D, Gates A, Rowley G. Genome-wide discovery of putative sRNAs in *Paracoccus denitrificans* expressed under nitrous oxide emitting conditions [J]. *Front Microbiol*, 2016, **7**: 01806
 - 34 Zhu BL, Bradford L, Huang SC, Szalay A, Leix C, Weissbach M, Tancsics A, Drewes JE, Lueders T. Unexpected diversity and high abundance of putative nitric oxide dismutase (NOD) genes in contaminated aquifers and wastewater treatment systems [J]. *Appl Environ Microb*, 2017, **83** (4): 2750-2716
 - 35 Del Giudice J, Cam Y, Damiani I, Fung-Chat F, Meilhoc E, Bruand C, Brouquisse R, Puppo A, Boscari A. Nitric oxide is required for an optimal establishment of the *Medicago truncatula*-*Sinorhizobium meliloti* symbiosis [J]. *New Phytol*, 2011, **191** (2): 405-417
 - 36 Chen H, Wang Y, Lü YK. Simultaneous removal characteristics of ammonium and phenol by *Alcaligenes faecalis* strain WY-01 with the addition of acetate [J]. *Bioproc Biosyst Eng*, 2021, **44** (1): 27-38
 - 37 Wang Y, Chen H, Liu YX, Ren RP, Lv YK. Effect of temperature, salinity, heavy metals, ammonium concentration, pH and dissolved oxygen on ammonium removal by an aerobic nitrifier [J]. *RSC Adv*, 2015, **5** (97): 79988-79996
 - 38 Padhi SK, Maiti NK. Molecular insight into the dynamic central metabolic pathways of *Achromobacter xylosoxidans* CF-S36 during heterotrophic nitrogen removal processes [J]. *J Biosci Bioeng*, 2017, **123** (1): 46-55
 - 39 Liu, XT, Zhang QC, Yang XY, Wu D, Li Y, Di HJ. Isolation and characteristics of two heterotrophic nitrifying and aerobic denitrifying bacteria, *Achromobacter* sp. strain HNDS-1 and *Enterobacter* sp. strain HNDS-6 [J]. *Environ Res*, 2023, **220** (18): 115240
 - 40 Ge Q, Yue XP, Wang GY. Simultaneous heterotrophic nitrification and aerobic denitrification at high initial phenol concentration by isolated bacterium *Diaphorobacter* sp. PD-7 [J]. *Chin J Chem Eng*, 2015, **23** (5): 835-841
 - 41 Yang M, Lu DW, Qin BD, Liu QL, Zhao YM, Liu HL, Ma J. Highly efficient nitrogen removal of a coldness-resistant and low nutrient needed bacterium, *Janthinobacterium* sp. M-11 [J]. *Bioresource Technol*, 2018, **256**: 366-373
 - 42 Zhao B, He YL, Hughes J, Zhang XF. Heterotrophic nitrogen removal by a newly isolated *Acinetobacter calcoaceticus* HNR [J]. *Bioresource Technol*, 2010, **101** (14): 5194-5200
 - 43 Sarioglu OF, Suluyayla R, Tekinay T. Heterotrophic ammonium removal by a novel hatchery isolate *Acinetobacter calcoaceticus* STB1 [J]. *Int Biodeter Biodegr*, 2012, **71**: 67-71
 - 44 金敏, 王景峰, 孔庆鑫, 赵祖国, 王新为, 谌志强, 陈照立, 邱志刚, 李君文. 好氧异养硝化菌 *Acinetobacter* sp. YY-5 的分离鉴定及脱氮机理[J]. 应用与环境生物学报, 2009, **15** (5): 692-697 [Jin M, Wang JF, Kong QX, Wang XW, Zhan ZQ, Chen ZL, Qiu ZG, Li JW. Isolation and denitrification mechanism of an aerobic heterotrophic bacterium *Acinetobacter* sp. YY-5 [J]. *Chin J Appl Environ Biol*, 2009, **15** (5): 692-697]
 - 45 Wang Y, Zou YL, Chen H, Lv YK. Nitrate removal performances of a new aerobic denitrifier, *Acinetobacter haemolyticus* ZYL, isolated from domestic wastewater [J]. *Bioproc Biosyst Eng*, 2021, **44** (2): 391-401
 - 46 马银鹏, 李伟光, 孟利强, 姜威, 张淑梅. 异养硝化菌 *Acinetobacter harbinensis* HITLi 7^T 冷休克蛋白基因生物信息学分析与不同温度下的表达[J]. 基因组学与应用生物学, 2020, **39** (9): 4003-4009 [Ma YP, Li WG, Meng LQ, Jiang W, Zhang SM. Bioinformatics analysis of cold shock protein gene from heterotrophic nitrifying bacteria *Acinetobacter harbinensis* HITLi 7^T and its relative expression under different temperatures [J]. *Genom Appl Biol*, 2020, **39** (9): 4003-4009]
 - 47 文刚, 王彤, 黄廷林, 万琪琪, 曹瑞华, 寇莉青, 杨尚业. 贫营养好氧反硝化菌株的脱氮特性及氮/碳平衡分析[J]. 环境科学, 2020, **41** (5): 2339-2348 [Wen G, Wang T, Huang TL, Wan QQ, Cao RH, Kou LQ, Yang SY. Nitrogen removal performance and nitrogen/carbon balance of oligotrophic aerobic denitrifiers [J]. *Environ Sci*, 2020, **41** (5): 2339-2348]
 - 48 Tang Q, Zeng MJ, Zou WG, Jiang WY, Kahaer A, Liu SX, Hong C, Ye YY, Jiang W, Kang JX, Ren YZ, Liu DQ. A new strategy to simultaneous removal and recovery of nitrogen from wastewater without N₂O emission by heterotrophic nitrogen-assimilating bacterium [J]. *Sci Total Environ*, 2023, **872** (2023): 162211
 - 49 Zhao B, He YL, Huang J, Taylor S, Hughes J. Heterotrophic nitrogen removal by *Providencia rettgeri* strain YL [J]. *J Ind Microbiol Biot*, 2010, **37** (6): 609-616
 - 50 Zhu ZQ, Yang Y, Fang AR, Lou Y, Xie GJ, Ren NQ, Xing DF. Quorum sensing systems regulate heterotrophic nitrification-aerobic denitrification by changing the activity of nitrogen-cycling enzymes [J]. *Environ Sci Ecotechnol*, 2020, **2**: 100026

- 51 Wei R, Hui C, Zhang YP, Jiang H, Zhao YH, Du LN. Nitrogen removal characteristics and predicted conversion pathways of a heterotrophic nitrification-aerobic denitrification bacterium, *Pseudomonas aeruginosa* P-1 [J]. *Environ Sci Pollut*, 2020, **28** (6): 7503-7514
- 52 Noriega C, Hassett DJ, Rowe JJ. The *mobA* gene is required for assimilatory and respiratory nitrate reduction but not xanthine dehydrogenase activity in *Pseudomonas aeruginosa* [J]. *Curr Microbiol*, 2005, **51** (6): 419-424
- 53 Zhang MX, Li AZ, Yao Q, Wu QP, Zhu HH. Nitrogen removal characteristics of a versatile heterotrophic nitrifying-aerobic denitrifying bacterium, *Pseudomonas bauzanensis* DN13-1, isolated from deep-sea sediment [J]. *Bioresource Technol*, 2019, **305**: 122626
- 54 Xie F, Thiri M, Wang H. Simultaneous heterotrophic nitrification and aerobic denitrification by a novel isolated *Pseudomonas mendocina* X49 [J]. *Bioresource Technol*, 2021, **319**: 124198
- 55 He XL, Sun Q, Xu TY, Dai M, Wei DS. Removal of nitrogen by heterotrophic nitrification-aerobic denitrification of a novel halotolerant bacterium *Pseudomonas mendocina* TJPU04 [J]. *Bioproc Biosyst Eng*, 2019, **42** (5): 853-866
- 56 Zhang N, Chen H, Lyu YK, Wang Y. Nitrogen removal by a metal-resistant bacterium, *Pseudomonas putida* ZN1, capable of heterotrophic nitrification-aerobic denitrification [J]. *J Chem Technol Biot*, 2018, **94** (4): 1165-1175
- 57 Huang X, Xu Y, He T, Jia H, Feng M, Xiang S, Wang S, Ni J, Xie D, Li Z. Ammonium transformed into nitrous oxide via nitric oxide by *Pseudomonas putida* Y-9 under aerobic conditions without hydroxylamine as intermediate [J]. *Bioresource Technol*, 2019, **277**: 87-93
- 58 Eberl L, Ammendola A, Rothballer M. Inactivation of *gltB* abolishes expression of the assimilatory nitrate reductase gene (*nasB*) in *Pseudomonas putida* KT2442 [J]. *J Bacteriol*, 2000, **182** (12): 3368-3376
- 59 Zhou MH, Ye HR, Zhao XW. Isolation and characterization of a novel heterotrophic nitrifying and aerobic denitrifying bacterium *Pseudomonas stutzeri* KTB for bioremediation of wastewater [J]. *Bioproc Biosyst Eng*, 2014, **19** (2): 231-238
- 60 Ji B, Wang HY, Yang K. Tolerance of an aerobic denitrifier (*Pseudomonas stutzeri*) to high O₂ concentrations [J]. *Biotechnol Lett*, 2014, **36** (4): 719-722
- 61 Müller C, Zhang L, Zipfel S, Topitsch A, Lutz M, Eckert J, Prasser B, Chami M, Lu W, Du J, Einsle O. Molecular interplay of an assembly machinery for nitrous oxide reductase [J]. *Nature*, 2022, **608** (7923): 626-631
- 62 Woo SG, Sewell HL, Criddle CS. Phylogenetic diversity of NO reductases, new tools for nor monitoring and insights into N₂O production in natural and engineered environments [J]. *FESE*, 2022, **16** (10): 23-37
- 63 Xu SQ, Zhang FY, Jiang YH, Zhang KY, Hong Q, Qiu JG, He Y. Characterization of a new heterotrophic nitrification bacterium *Pseudomonas* sp. strain JQ170 and functional identification of nap gene in nitrite production [J]. *Sci Total Environ*, 2022, **806** (2022): 150556
- 64 Zeng XY, Huang JHJ, Hua BB, Champagne P. Nitrogen removal bacterial strains, MSNA-1 and MSD4, with wide ranges of salinity and pH resistances [J]. *Bioresource Technol*, 2020, **310** (2020): 123309
- 65 Duan JM, Fang HD, Su B, Chen JF, Lin JM. Characterization of a halophilic heterotrophic nitrification-aerobic denitrification bacterium and its application on treatment of saline wastewater [J]. *Bioresource Technol*, 2015, **179**: 421-428
- 66 Lei Y, Wang YQ, Liu HJ, Xi CW, Song LY. A novel heterotrophic nitrifying and aerobic denitrifying bacterium, *Zobellella taiwanensis* DN-7, can remove high-strength ammonium [J]. *Appl Microbiol Biot*, 2016, **100** (9): 4219-4229
- 67 Mendoza LFD, Quimi Mujica JG, Risco Cunayque JM, Aroni Lucana GW, Intriago Angulo JJ, De la Cruz VIS, Cedido Escobar VA, Mialhe ME. Assessment of heterotrophic nitrification capacity in *Bacillus* spp. and its potential application in the removal of nitrogen from aquaculture water [J]. *J Pure Appl Microbio*, 2019, **13** (4): 1893-1908
- 68 Huang F, Pan LQ, Lv N, Tang XM. Characterization of novel *Bacillus* strain N31 from mariculture water capable of halophilic heterotrophic nitrification-aerobic denitrification [J]. *J Biosci Bioeng*, 2017, **124** (5): 564-571
- 69 Zhao B, He YL, Zhang XF. Nitrogen removal capability through simultaneous heterotrophic nitrification and aerobic denitrification by *Bacillus* sp. LY [J]. *Environ Technol*, 2010, **31** (4): 409-416
- 70 Kunst F, Ogasawara N, Moszer I. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis* [J]. *Nature*, 1998, **390** (6657): 249-256
- 71 Odu C, Adeoye KB. Heterotrophic nitrification in soils—a preliminary investigation [J]. *Soil Biol Biochem*, 1970, **2** (1): 41-45
- 72 Chen J, Zhao B, An Q, Wang X, Zhang YX. Kinetic characteristics and modelling of growth and substrate removal by *Alcaligenes faecalis* strain NR [J]. *Bioproc Biosyst Eng*, 2016, **39** (4): 593-601
- 73 Van Kessel MAHJ, Speth DR, Albertsen M, Nielsen PH, Den Camp HJMO, Kartal B, Jetten MSM, Lückers S. Complete nitrification by a single microorganism [J]. *Nature*, 2015, **528** (7583)
- 74 石秀丽, 郭萌萌, 张莹, 秦华, 万琪慧, 谢德体, 蒋先军. 单步硝化作用与全程氨氧化微生物研究进展[J]. 草业学报, 2018, **27** (7): 196-203 [Shi XL, Guo MM, Zhang Y, Qin H, Wan QH, Xie DT, Jiang XJ. Progress in understanding of one-step nitrification (complete ammonia oxidizing) microorganisms: a review [J]. *Acta Pratacult Sin*, 2018, **27** (7): 196-203]
- 75 Robertson LA, van Niel EWJ, Torremans RAM, Gijs J. Simultaneous nitrification and denitrification in aerobic chemostat cultures of *Thiosphaera pantotropa* [J]. *Appl Environ Microb*, 1988, **54** (11): 2812-2818
- 76 Ji B, Yang K, Wang HY, Zhou J, Zhang HN. Aerobic denitrification by *Pseudomonas stutzeri* C3 incapable of heterotrophic nitrification [J]. *Bioproc Biosyst Eng*, 2015, **38** (2): 407-409
- 77 Liang X, Wang BL, Gao DZ, Han P, Zheng YL, Yin GY, Dong HP, Tang YL, Hou LJ. Nitrification regulates the spatiotemporal variability of N₂O emissions in a eutrophic lake [J]. *Environ Sci Technol*, 2022, **56** (23): 17430-17442
- 78 Xu TY, Song SM, Ren JH, Yang JY, Bai L, Piao ZY. Fungus *Pichia kudriavzevii* XTY1 and heterotrophic nitrifying bacterium *Enterobacter asburiae* GS2 cannot efficiently transform organic nitrogen via hydroxylamine and nitrite [J]. *Front Microbiol*, 2022, **13**: 1038599
- 79 Ye J, Zhao B, An Q, Huang YS. Nitrogen removal by *Providencia rettgeri* strain YL with heterotrophic nitrification and aerobic denitrification [J]. *Environ Technol*, 2016, **37** (17): 2206-2213
- 80 Zumft WG, Kroneck PMH. Respiratory transformation of nitrous oxide (N₂O) to dinitrogen by bacteria and archaea [J]. *Adv Microb*

- Physiol*, 2006, **52**: 107-227
- 81 Pauleta SR, Carepo MSP, Moura I. Source and reduction of nitrous oxide [J]. *Coordin Chem Rev*, 2019, **387**: 436-449
 - 82 Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kuypers MMM, Schreiber F, Dutilh BE, Zedelius J, De Beer D, Gloerich J, Wessels HJCT, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HM, Janssen-Megens EM, Francoijs KJ, Stunnenberg H, Weissenbach J, Jetten MSM, Strous M. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria [J]. *Nature*, 2010, **464** (2010): 543-548
 - 83 Sonderholm M, Kragh KN, Koren K, Jakobsen TH, Darch SE, Alhede M, Jensen PO, Whiteley M, Kuhl M, Bjarnsholt T. *Pseudomonas aeruginosa* aggregate formation in an alginate bead model system exhibits *in vivo*-like characteristics [J]. *Appl Environ Microbiol*, 2017, **83** (9): e00113- e00117
 - 84 王景峰, 金敏, 谌志强, 邱志刚, 张斌, 孔庆鑫, 李君文. 1株好氧脱氮菌的筛选与脱氮特性研究[J]. 环境科学, 2011, **32** (8): 5 [Wang JF, Jin M, Shen ZQ, Qiu ZG, Zhang B, Kong QX, Li JW. Screening of the aerobic simultaneous denitrobacteria and its denitrification characteristics [J]. *Chin J Environ Sci*, 2011, **32** (8): 2409-2413]
 - 85 刘方剑, 杨海龙, 周化斌. 异养硝化细菌 *Acinetobacter junii* WZ17 的脱氮特性及动力学研究[J]. 环境科学学报, 2021, **41** (3): 951-959 [Liu FJ, Yang HL, Zhou HB. Nitrogen removal and kinetics research by heterotrophic nitrification bacterium *Acinetobacter junii* WZ17 [J]. *Acta Sci Circumst*, 2021, **41** (3): 951-959]
 - 86 Reitzer L, Magasanik B. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine and D-alanine in *Escherichia coli* and *Salmonella typhimurium* [J]. *Cell Mol Biol*, 1987, **1987**: 302-320
 - 87 Meng LQ, Li WG, Zhang SM, Wu CD, Wang K. Effects of sucrose amendment on ammonia assimilation during sewage sludge composting [J]. *Bioresour Technol*, 2016, **210**: 160-166
 - 88 Wang S, Chen MF, Zheng KK, Wan CL, Li J. Promising carbon utilization for nitrogen recovery in low strength wastewater treatment: ammonia nitrogen assimilation, protein production and microbial community structure [J]. *Sci Total Environ*, 2020, **710**: 136306
 - 89 Chou WS, Chang TC, You SJ, Sie YC. Nutrient removal performance in an intermittent aerated sequencing batch membrane bioreactor [J]. *J Chin Inst Eng*, 2010, **33** (4): 581-590
 - 90 Chèneby D, Hartmann A, Hénault C, Topp E, Germon JC. Diversity of denitrifying microflora and ability to reduce N₂O in two soils [J]. *Biol Fert Soils* 1998, **26**: 19-26
 - 91 Malm S, Tiffert Y, Micklinghoff J, Schultze S, Joost I, Weber I, Horst S, Ackermann B, Schmidt M, Wohlleben W, Ehlers S, Geffers R, Reuther J, Bange FC. The roles of the nitrate reductase *NarGHJI*, the nitrite reductase *NirBD* and the response regulator *GlnR* in nitrate assimilation of *Mycobacterium tuberculosis* [J]. *Microbiology*, 2009, **155** (4): 1332-1339
 - 92 Zhang K, Zhang YL, Deng MC, Wang PC, Yue X, Wang PD, Li WJ. Monthly dynamics of microbial communities and variation of nitrogen-cycling genes in an industrial-scale expanded granular sludge bed reactor [J]. *Front Microbiol*, 2023, **14**: 1125709
 - 93 彭强, 关傲梅, 齐维晓, 曲久辉. 基于同位素示踪和分子生物学技术对潮白河沉积物中氮还原功能特征的研究[J]. 环境科学学报, 2021, (11): 4615-4624 [Peng Q, Guan AM, Qi WX, Qu JH. The characteristics of nitrogen reduction process in sediments of Chaobai River based on isotope tracer approach and molecular biology technology [J]. *Acta Sci Circumst*, 2021, (11): 4615-4624]
 - 94 Wang SY, Liu CL, Wang XX, Yuan DD Zhu GB. Dissimilatory nitrate reduction to ammonium (DNRA) in traditional municipal wastewater treatment plants in China: widespread but low contribution [J]. *Water Res*, 2020, **179**: 115877
 - 95 Scott JT, McCarthy MJ, Gardner WS, Doyle RD. Denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation along a nitrate concentration gradient in a created freshwater wetland [J]. *Biogeochemistry*, 2008, **87** (1): 99-111
 - 96 Huang XJ, Tie WZ, Xie DT, Jiang DH, Li ZL. Certain environmental conditions maximize ammonium accumulation and minimize nitrogen loss during nitrate reduction process by *Pseudomonas putida* Y-9 [J]. *Front Microbiol*, 2021, **12**: 764241