



好氧氨氧化过程中的关键酶及 N₂O 排放研究进展

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摘要: 氧化亚氮(nitrous oxide, N₂O)排放量的持续增加对全球生态平衡造成了严重的威胁。微生物 N₂O 排放占主要来源。其中, 好氧氨氧化过程是氨在有氧的条件下氧化为亚硝酸盐, 其直接或间接地影响着全球产生 N₂O 与释放量。氨氧化古菌(ammonia-oxidizing archaea, AOA)、氨氧化细菌(ammonia-oxidizing bacteria, AOB)、全程氨氧化菌(complete ammonia oxidation, Comammox)和异养氨氧化菌(heterotrophic ammonium oxidizing bacteria, HAOB)是氨氧化过程中主要的参与者, 明确这四类微生物 N₂O 产生的机制对缓解全球 N₂O 排放是必要的。本文综述了 AOA、AOB、Comammox 和 HAOB 在好氧氨氧化过程中驱动的 N₂O 产生途径, 并结合酶学分析了一些关键酶在 N₂O 产生途径中的作用。本文旨在为调控生物 N₂O 排放提供理论基础。

关键词: 好氧氨氧化; 氨氧化微生物; 酶学; 氧化亚氮; 一氧化氮; 氮循环

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Key enzymes and N₂O emission in aerobic ammonia oxidation process

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Abstract: The increasing emission of nitrous oxide (N₂O) poses a serious threat to global ecological balance, to which microbes contribute a lot. Aerobic ammonia oxidation is the oxidation of ammonia to nitrite under aerobic conditions, which directly or indirectly affects the global production and release of N₂O. Ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), complete ammonia oxidizers (Comammox), and heterotrophic ammonia-oxidizing bacteria (HAOB) are major contributors to ammonia oxidation. It is important for the mitigation of N₂O emission to clarify how N₂O is produced by the four types of microorganisms. This paper reviewed the N₂O production pathways in AOA, AOB, Comammox and HAOB driven by the aerobic ammonia oxidation process. The roles of some key enzymes in the N₂O production pathways were elucidated through enzymatic analysis. Ammonia monooxygenase (AMO) is the first key enzyme in the autotrophic aerobic ammonia oxidation process, and the direct product of hydroxylamine oxidation is NO instead of NO₂⁻, which is then converted to NO₂⁻ by an unknown enzyme. The aerobic ammonia oxidation process and related enzymes of HAOB are not completely clear, and the issue of N₂O emission needs to be further studied. This paper aims to lay a theoretical basis for regulating biological N₂O emission.

Keywords: aerobic ammonia oxidation; ammonia-oxidizing microorganisms; enzymology; nitrous oxide; NO; nitrogen cycle

一氧化二氮(nitrous oxide, N₂O)是大气中的一种长寿命痕量温室气体,目前在大气中的寿命为(116±9)年^[1]。N₂O成为了继CO₂和CH₄之后第三大最重要的温室气体,对全球变暖的贡献高达6%^[2]。同时,N₂O的增温潜势分别约为CO₂和CH₄的300倍和10倍^[2-3]。在过去的40年中,N₂O排放量增加了30%以上^[4],并且人为因素增加的排放量占全球总量的30%–45%^[5]。因此,更好地研究微生物N₂O产生过程对实现人工调控减少N₂O排放、减缓温室效应、维护地

球生态平衡均具有重要意义。

微生物N₂O排放的主要过程包括硝化作用、硝化反硝化作用和反硝化作用^[1,6]。如图1所示,硝化作用是微生物将铵(NH₄⁺)氧化为硝酸盐(NO₃⁻),N₂O作为副产物排放出来。硝化反硝化是将亚硝酸盐(NO₂⁻)还原为一氧化氮(NO),最后转化为N₂O或N₂。反硝化是将NO₃⁻转化为N₂O,最后转化为惰性N₂。而硝化过程中的好氧氨氧化形成的NO₂⁻或NO₃⁻为异养反硝化过程提供底物,因此好氧氨氧化直接或间接

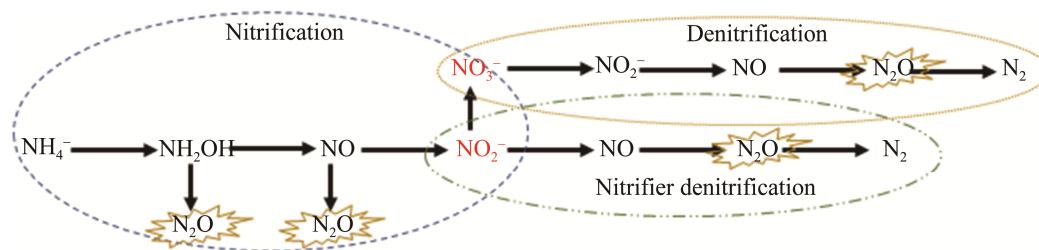


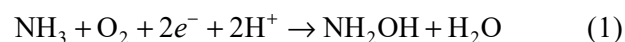
图 1 微生物 N₂O 排放过程

Figure 1 Microbial N₂O emission process.

地影响着全球产生 N₂O 与释放量^[7]。Hink 等^[8]证实好氧氨氧化是土壤 N₂O 排放的主要原因。好氧氨氧化菌分为自养和异养两大类，自养氨氧化菌包括氨氧化古菌 (ammonia-oxidizing archaea, AOA)、氨氧化细菌 (ammonia-oxidizing bacteria, AOB) 和新发现的全程氨氧化菌 (complete ammonia oxidation, Comammox)。因早期认为自然界异养氨氧化弱，因此好氧氨氧化及形成 N₂O 的途径和机理主要依据自养氨氧化菌的研究结果。

1 自养氨氧化菌的好氧氨氧化过程及 N₂O 排放

好氧氨氧化是硝化作用的一个限速步骤。在这个过程中 AOA、AOB 和 Comammox 都使用保守的氨单加氧酶 (ammonia monooxygenase, AMO) 将氨 (NH₃ 而不是 NH₄⁺) 氧化成羟胺 (NH₂OH) [公式(1)]; 进一步地将 NH₂OH 氧化成 NO₂⁻，但该途径所涉及的酶学和中间反应过程仍未完全解决，有待进一步研究。但总的来说 AMO 是三大类氨氧化微生物 (AOA、AOB 和 Comammox) 中氨氧化途径唯一共有的酶，NH₂OH 和 NO 是必经的中间产物^[9-11]。



1.1 AMO 是自养氨氧化反应第一步关键必须酶

好氧氨氧化的第一步是 NH₃ 在 AMO 的作用下氧化为 NH₂OH。通过 16S rRNA 和 *amoA* 基因发现，AMO 是 AOA^[12]、AOB^[13] 和 Comammox^[14-16] 这三类氨氧化微生物唯一所共有的关键酶。AMO 是一种铜依赖性多聚体跨膜酶，与颗粒甲烷单加氧酶 (particulate methane monooxygenase, pMMO) 属于同一铜依赖性膜单加氧酶超家族^[11]。自养氨氧化菌的 AMO 在蛋白提取液中稳定性差，尽管其细胞裂解物的体外活性可以在某些条件维持 (如避光、添加 Mg²⁺ 和牛血清蛋白等)^[17-18]，但 AMO 全酶尚未以纯化的活性形式分离出来，这极大地阻碍了对该蛋白质的结构和机制知识的扩展^[19-20]。关于 AMO 结构和功能的见解主要是从全细胞或细胞提取液的实验中推导而来^[21] 或基于与其同源性较好的 pMMO 推导而来的^[22]。例如，细菌好氧氨氧化成羟胺的活性被铜螯合剂烯丙基硫脲 (ATU) 抑制；少量 Cu 的加入可以明显刺激氨氧化，并能稳定细胞提取液中 AMO 的活性，这表明 AMO 是一种铜依赖性酶^[23]。然而硝化抑制剂对 AOA 和 AOB 的活性影响存在差异，这表明 AOA 和 AOB 在 AMO 结构和功能上存在差异^[24]。

AMO 为多聚体膜结合蛋白，目前已分离到的确定为 AMO 亚基的有 3 个 (*amoA*、*amoB* 和

amoC), 并且在所有的 AOB 都以 *amoC*、*amoA*、*amoB* 的顺序排列在同一操纵子中^[10,22,25]。此外, 在 *amoCAB* 操纵子的下游也有关于 *AmoD* 和 *AmoE* 的报道^[26]。相关研究认为, *amoA* 和 *amoC* 亚基是完整的膜蛋白, 而 *amoB* 亚基包含铜结合催化位点^[27]。由于 *amoD* 和 *amoE* 的确切功能未知, 因此 *amoCABED* 基因簇通常被称为 *amoCAB* 操纵子。*amoA* 基因是最保守的亚基, 常被用于其他亚群中系统发育研究^[21,28-29]。当然也存在其他操纵子的可能性。如, El Sheikh 等^[30]研究发现, 在氨氧化细菌 *Nitrosococcus oceani* ATCC 19707 的 *amoCAB* 操纵子的上游和下游分别存在另外的 2 个保守基因 *amoR* 和 *amoD*, 形成了特异性 *amoRCABD* 操纵子。Bollmann 等^[26]报道了在 *Nitrosomonas* sp. Is79 的 *amoCAB* 操纵子的下游存在 2 个单拷贝的 *amoC* 基因。Kozlowski 等^[31]通过对比 5 株 AOB 发现都含有 1-2 个编码 AMO 的 *amoCABED* 簇。

在 AOA 的 AMO 中除了常见的 *amoA*、*amoB* 和 *amoC* 亚基外, 通常还包括 *amoX*^[28,32],

并且这 4 个亚基的顺序在不同的 AOA 中的排列顺序多变。一些菌的亚基因按 *amoAXCB* 排列 (图 2)。一些 AOA 包含多个分离的 *amoA*、*amoB* 和 *amoC* 亚基副本。同时 AOA 中 *amoC* 的氮端和 *amoA* 的碳端比 AOB 短^[22]。也正是因为 AOA 的 AMO 亚基排列顺序与 AOB 的 AMO 亚基排列顺序的不同, 有人认为 AOA 氨氧化的产物可能不是 NH_2OH 而是亚硝酸(nitroxyl, HNO), 但缺乏直接的证据^[28,33]。

迄今为止发现的所有可培养的 Comammox 都是自养型微生物, 属于 *Nitrospira*^[14,34], 从完全氨氧化为硝酸盐中获得能量^[29]。与 AOA 和 AOB 的 *amoA* 基因相比, Comammox 的 *amoA* 基因具有不同的基因序列^[35-36], 表现出更高的多样性^[37]。但与颗粒甲烷单加氧酶基因 *pmoA* 更为相同^[16]。Comammox 中的 AMO 由单个 *amoCAB* 基因簇和其他基因组位点的 2 个额外 *amoC* 基因编码^[14]。由于目前分离纯化得到的 Comammox 菌株较少, 多通过组装基因组学获得相关基因, 并且多关注于 *amoA*, 因此有关 Comammox 的 AMO 的其他性质有待进一步研究。

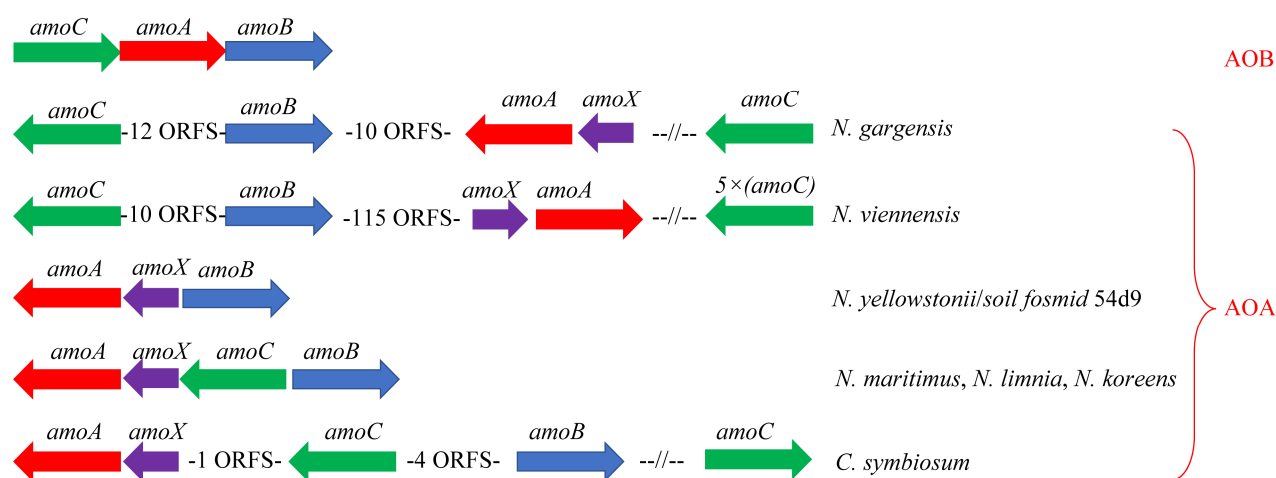


图 2 *amo* 操纵子在 AOB 和 AOA 中的顺序^[28]

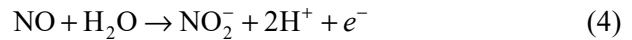
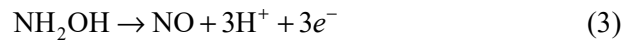
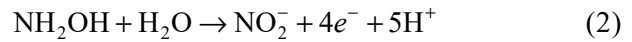
Figure 2 Sequence of *amo* operons in AOB and AOA^[28].

1.2 羟胺氧化为 NO_2^- 在不同的好氧氨氧化菌中存在差异, 但 NH_2OH 和 NO 是必经中间产物

1.2.1 AOB 中羟胺经 HAO 转化为 NO

好氧氨氧化的普遍观点主要基于对 AOB 亚硝化单胞菌模型的研究, 通常认为氨氧化的第二步是 NH_2OH 在羟胺氧化还原酶(hydroxylamine oxidoreductase, HAO)的作用下转化为 NO_2^- , 并产生 4 个电子, 其中 2 个返回到 AMO [公式(2)]^[38-39]。随着 Caranto 和 Lancaster^[40]研究结果的发现打破了这一定论(图 3)。他们认为羟胺受到 HAO 和细胞色素 P_{460} (厌氧)的共同作用。当 NH_2OH 受到 HAO 和细胞色素 P_{460} 的催化时分别产生 3 个和 2 个电子, 并且形成的直接产物是 NO 而不是 NO_2^- ^[40]。羟胺在 HAO 的催化下产生 NO 和 3 个电子, 其中的 2 个电子回到 AMO, 1 个电子进入细胞呼吸[公式(3)]。接着 NO 在一氧化氮氧化还原酶(nitric oxide oxidoreductase, NOO)的作用下氧化为 NO_2^- [公式(4)]。最近通过对比不同 AOB 研究发现, 亚硝基蓝蛋白(nitrosocyanin, NcyA)在氨氧化过程中同 AMO 和 HAO 一样高度表达, 并能够与 NO 结合, 被认为是缺失的第 3 种酶^[13,41-42]。发现 NcyA 参与将电子从醌池循环到 AMO 或作为羟胺到氧气的电子中继器^[13]。但是在 *Nitrosomonas sp. Is 79* 的全基因组中没有发现 *ncyA* 基因^[26]。此外, 也有人认为是由反向操作的含铜亚硝酸盐还原酶(copper nitrite reductase, Cu-NirK)行使由 NO 到 NO_2^- 的功能^[42]。但在某些 AOB 的全基因组中也找不到相关基因^[41-42]。因此有必要进一步研究在

AOB 中行使功能的第 3 种酶。



在 AOB 的羟胺氧化过程中确定功能的蛋白只有 HAO。由于 HAO 可溶性质, 其是氨氧化过程中研究最清楚的功能组分。HAO 蛋白已结晶, 其结构也已解析。HAO 是一种多血红素酶, 由同质三聚亚基组成, 每个亚基的分子量为 67.1 kDa^[43]。编码 *hao* 的基因表达为单顺反子转录本。从 *N. europaea* 中鉴定出 3 个 *hao* 基因拷贝: *hao1*、*hao2* 和 *hao3*。并且 *hao1* 和 *hao2* 的核酸序列几乎相同, 而 *hao3* 与其他两个存在差异^[44]。HAO 酶的每个亚基包含 8 个 c 型血红素: 7 个是电子转移辅助因子, 第 8 个是由细胞色素 P_{460} 组成的羟胺氧化的活性位点^[44]。来自 HAO 的电子流通过细胞色素 c_{554} 被传至细胞色素 c_{m552} ^[45]。值得注意的, 最近的一项新研究发现, *DnfA* 编码的羟胺氧化酶在有氧的条件下能将羟胺直接转化为 N_2 ^[46]。

1.2.2 AOA 中羟胺经含铜的未知酶转化为 NO

在 AOA 好氧氨氧化过程中也观测到了 NO 的产生^[47-48], 表明 NO 是该过程中的中间产物。通过添加 NO 的清除剂(如 PTIO)显著抑制 AOA 氨氧化形成 NO_2^- ^[41]。但由于 AOA 基因组中缺乏任何可识别的与 AOB 中 HAO 相近的同源物, 这意味着 AOA 中有其他酶来负责羟胺氧化^[5,22]。并且进一步由 NO 氧化为 NO_2^- 所涉及的酶也有待进一步确定^[39,41]。目前, 提出了 2 个可能的 AOA 羟胺氧化模型。一种模型认为羟胺和 NO 共同作用催化产生 2 分子 NO_2^- (图 4A)^[47]。另一



图 3 AOB 好氧氨氧化过程的中间产物和相关酶^[41]

Figure 3 AOB intermediate products and related enzymes of aerobic ammoxidation process^[41].

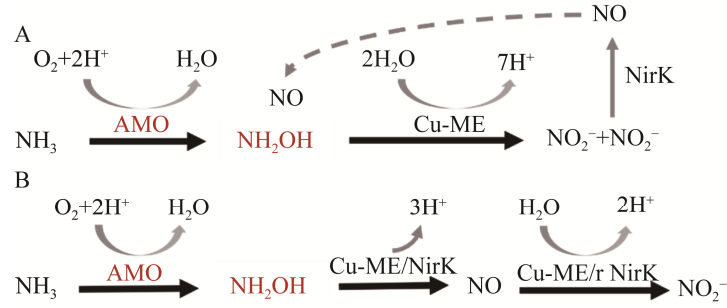


图4 AOA好氧氨氧化涉及的中间产物和相关酶^[41]

Figure 4 Intermediates and related enzymes involved in AOA aerobic ammoxidation^[41].

种模型是羟胺连续通过2种酶先氧化为NO再氧化为NO₂⁻(图4B)^[49]。羟胺通过未知酶氧化产生5个电子。其中2个电子被运送到膜相关醌还原酶(membrane-associated quinone reductase, QUED), 然后通过一个蓝色的含铜蛋白载体的作用转移到AOA的AMO进行氨氧化^[50-51]。剩余的电子被转移到电子传递链上用于呼吸。目前认为NH₂OH氧化为NO₂⁻过程中涉及的酶必须是铜基的^[41]。有两位可能的候选者, 即亚硝酸盐铜还原酶(copper nitrite reductase, Cu-NirK)和铜羟胺氧化还原酶(copper hydroxylamine oxidoreductase, Cu-HAO)^[28]。由于Cu-HAO与AOB的HAO是同源物, 但编码Cu-HAO的基因尚未被识别^[28,33], 因此不确定AOA中HAO是否存在。而Kobayashi等^[52]通过异源表达NirK发现, 重组NirK蛋白能够催化NO₂⁻还原为NO和催化羟胺氧化为NO。因此, Cu-NirK认为可能是与细菌HAO的对应物。但现有推导的古菌NirK与细菌NirK的相似度较低, 确切的生理作用尚不确定^[28], 因此这也是今后需要解决的问题。

1.2.3 Comammox中羟胺经未知酶转化为NO

Comammox菌在好氧氨氧化过程中产NO也得到了验证^[5]。目前认为Comammox的羟胺氧化过程与AOB类似。Dimitri Kits等^[5]通过对

宏基因组组装基因组分析发现, 15个Comammox菌均含有HAO酶, 并且推断羟胺在HAO酶的作用下产生NO。并且在一种未知的NOO酶作用下将NO氧化为NO₂⁻。但是并未在Comammox菌的基因组中找到类似AOB中的*ncyA*基因^[5]。因此, 有其他的基因行使NOO酶的功能。此外, Dimitri Kits等^[5]在所有Comammox菌的基因组中发现了*nirK*基因, 但是认为在细胞内pH值和氧化还原电位下, 反应动力学非常不利, 表明NirK不是NOO的理想候选物。总之, Comammox菌有通过HAO或NirK产生NO的遗传潜力, 但缺乏形成N₂O的关键酶。

1.3 自养好氧氨氧化过程中N₂O的排放

大量研究证实AOA和AOB是N₂O的主要产生源。而Comammox介导的过程被认为是“绿色过程”^[36,53], 其对N₂O排放量的贡献没有明确的报告, 即使有产生N₂O, 量也特别少^[54]。因此, 主要讨论AOA和AOB在好氧氨氧化过程中涉及的N₂O排放。

好氧氨氧化过程中参与N₂O排放的物质有NH₂OH、NO和NO₂⁻。(1) NH₂OH以及NH₂OH和NO共同在细胞色素P₄₆₀的作用下直接转化为N₂O是在厌氧情况下发生的^[55-56], 不在本次的讨论范围。(2) NO经一氧化氮还原酶(nitric oxide reductase, NOR)产生N₂O似乎是AOB所独有的

过程^[57]。目前在所有的 AOA^[47]和 Comammox 菌^[16]中都没有发现 *nor* 基因的同源物。(3) NO_2^- 经过硝化反硝化过程逐步产生 NO 和 N_2O 。由于 AOA 缺乏 NOR 酶, 通过同位素研究发现 N_2O 中的 N 来自 NH_4^+ 和 NO_2^- ^[33]。因此认为 AOA 中的 N_2O 是由非生物反应产生的^[31,47]。此外, Comammox 菌是较新发现的, 有关其 N_2O 排放的研究较少。Dimitri Kits 等^[5]发现 Comammox 菌对 NO 清除剂很敏感, 不能反硝化为 N_2O , 并且不含有 *nor* 基因。因此 Comammox 菌在好氧氨氧化过程中的 N_2O 排放是非生物转化的^[36]。总的来说, 好氧氨氧化过程中生物 N_2O 排放关键酶是 NOR, 其催化 NO 到 N_2O 的转化。

2 异养氨氧化菌的好氧氨氧化过程及 N_2O

异养氨氧化早在一百多年前就有报道。近年来, 异养氨氧化菌 (heterotrophic ammonium oxidizing bacteria, HAOB) 因其在酸性土壤氨氧化及其产生 N_2O 量中起重要作用而得到极大关注^[58]。已从旱地、水田、废水处理厂和养殖场等多种场所分离纯化种类繁多的异养硝化菌, 对氮转化途径及特性、影响因素和功能应用等方面进行了详细研究^[59-63]。

2.1 异养氨氧化菌的好氧氨氧化过程存在差异

相较于自养氨氧化菌, HAOB 的底物范围广泛^[56]。并且异养氨氧化往往与好氧反硝化耦联发生^[64], 这使得从中间产物推断异养硝化机理异常困难。目前对 HAOB 好氧氨氧化途径一般是基于不同氮源的利用和代谢产物、功能酶或基因的检测而提出的^[63]。但异养氨氧化发生的代谢途径没有共识。即使是同一种属的 HAOB 好氧氨氧化过程都存在差异。例如, 有研究认为假单胞菌 (*Pseudomonas* sp.) 的好氧氨

氧化途径为 $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ^[65-66], 也有研究认为 *Pseudomonas* sp. 的好氧氨氧化途径为 $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow$ 气态氮^[67]。但是, 也有学者认为异养硝化跨过了 NH_4^+ 分解, 即有机氮 $\rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ^[68]。值得注意的是, 通过基因推测好氧氨氧化过程似乎也存在一些问题。Silva 等^[69]对具有氨氧化能力的菌株 *Pseudomonas stutzeri* 进行测序发现, 基因中未检测到与自养硝化菌氨氧化过程中已知的基因和酶, 但发现反硝化过程中所需的基因和酶存在。

2.2 异养氨氧化菌好氧氨氧化过程的酶学不完全清楚

HAOB 好氧氨氧化过程的基因和酶学是不完善的, 那么对 HAOB 好氧氨氧化第一步是否由 AMO 催化产生 NH_2OH 存在质疑。一方面由于 NH_2OH 含量低较难检测到, 如在 *Streptomyces mediolani* EM-B2^[23]、*P. aeruginosa*^[64]、*Pseudomonas putida* Y-9^[70] 和 *Acinetobacter* sp. ND7^[71] 氨氧化过程中均没有检测到 NH_2OH 的存在。那么推断可能的氨氧化过程为 $\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ^[72]。另一方面异养氨氧化过程是基于自养氨氧化过程推测的, 普遍接受了氨在 AMO 的作用下产生 NH_2OH 。但目前仅在少数 HAOB 中发现了 AMO。如 Lang 等^[73]使用 *amoA* 基因在 3 株 HAOB 中扩增出 AMO 条带。虽然 Wang 等^[74]也扩增出 AMO 条带, 但 *amoA* 引物来自自身高通量数据。相较于自养氨氧化菌, HAOB 的 AMO 在细胞提取液中稳定, 由 2 个亚基^[75], 或单一蛋白^[18]组成。但值得注意的是, 也有一些异养硝化菌不含 *amoA* 基因或基因组缺乏传统硝化途径关键基因^[76]。例如, 本实验室前期在粗酶液中检测到 AMO 酶活, 但使用 AOB 的 *amoA* 引物没有扩增出 *Pseudomonas putida* Y-9 的

AMO 条带^[70]。Cui 等^[77]也是相同的结果。此外, 现有的硝化抑制剂(主要针对自养氨氧化菌设计)对 HAOB 作用效果不明显^[70,78]。如本实验室发现, 0.5 mmol/L ATU 对 AOB 有显著的抑制作用, 而对 *Arthrobacter arilaitensis* 无抑制作用; 但随着浓度的增加, 抑制作用增强^[79]。而 *Pseudomonas putida* Y-9 对 ATU 极不敏感^[70]。以上结果说明在某些异养氨氧化菌中可能存在不同于自养氨氧化菌的氨氧化过程。

总的来说, HAOB 氨氧化过程所涉及的基因和酶都不是很清楚, 可能针对不同种属的 HAOB 需要提出特定的氨氧化过程。

2.3 异养氨氧化过程中 N₂O 的排放

目前关于不同生态系统及体系中 N₂O 的产生量及机理有很多报道, 发现不少系统中异养硝化作用排放 N₂O 强烈。如 Liu 等^[80]研究发现异养硝化作用对水稻土释放 N₂O 的贡献比自养硝化作用更大。Zhang 等^[81]使用 ¹⁵N 同位素标记证明异养硝化作用会诱导我国亚热带酸性森林土壤和温带森林土壤产 N₂O, 并且是亚热带酸性森林土壤产 N₂O 的主要原因。Pan 等^[60]通过高通量测序发现异养硝化菌在处理高盐废水脱氮中发挥了关键作用。Fan 等^[82]研究表明, 3,4-二甲基吡唑磷酸酯(3,4-dimethylpyrazole phosphate, DMPP, 一种硝化抑制剂)对红壤产 N₂O 仅能抑制 41.7%, 而对灰漠土抑制率高达 90.0%。究其原因与酸性红壤含异养硝化菌多有关。异养硝化菌的氨氧化酶与自养菌不同, 其活性对传统意义的硝化抑制剂可能不敏感^[82-83]。

目前认为 HAOB 氨氧化排放 N₂O 的途径有 2 条。(1) 与自养氨氧化类似, 往往针对异养硝化和好氧反硝化相耦合的 N₂O 排放: $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O}$ 。如本实验室发现菌株 *Pseudomonas putida* Y-9^[70]在氨氧化过程中会有 NO₂⁻的积累, 并且生产的气态氮均为

N₂O。(2) 是 HAOB 仅进行异养氨氧化的 N₂O 排放途径: $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO} \rightarrow \text{N}_2\text{O}$ 。如 *Acinetobacter calcoaceticus* HNR^[84]能够去除 NH₄⁺和 NH₂OH, 但不能利用 NO₂⁻和 NO₃⁻, 故推测第(2)途径为可能的 N₂O 排放途径。类似的还有 Zhao 等^[59]报道的 *Alcaligenes faecalis* NR, 不从 NO₂⁻和 NO₃⁻产生 N₂O, 而是 NH₂OH 直接产生 N₂O。然而, 异养硝化菌的氮代谢过程的关键基因及酶都是根据自养硝化菌的研究结果来推测的, 异养氨氧化菌的 AMO 亚基组成、羟胺还原酶等的结构及生理作用都不清楚, 无法从异养氨氧化产 N₂O 机理方面进行分析, 导致研究结果不能更加全面地反应氮代谢途径及其影响因素对 N₂O 产生的贡献, 对人们寻找减少 N₂O 排放的有效方法带来极大的阻碍。

3 展望

好氧氨氧化是氮循环过程中的一个重要过程, 该过程直接或间接的影响着全球 N₂O 的排放量。本文系统的综述了好氧氨氧化过程中参与的微生物, 包括该过程中涉及的中间体、关键酶及 N₂O 的排放问题。并且发现了现有研究中存在的不足, 需要各位研究者进一步的探究:

(1) 羟胺氧化的直接产物 NO 已经得到证实, 但有关从 NO 到 NO₂⁻的过程中所涉及的酶及基因还有待进一步的确定。

(2) 新发现的 Comammox 菌的大部分基因没有功能注释, 缺乏对相关蛋白的纯化和表征, 需要进行生理实验验证; 并且 16S rRNA 测序无法区分 Comammox 和亚硝酸盐还原菌^[39,85], 需要针对 Comammox 设计专门的引物。

(3) 异养氨氧化菌可能具有氨氧化酶, 但现有 AMO 引物不能很好的对其进行表征^[63]。因此需要针对异养氨氧化菌开发特异引物; 此外, 异养氨氧化菌种类繁多, 过程复杂, 往往与其他过

程同时发生(如好氧反硝化、碳循环等), 并且相关酶学和基因不清楚, 而其在全球 N_2O 排放的贡献不可忽略。而现有的硝化抑制剂对其效果不显著。因此需要研发针对异养氨氧化菌的特异性抑制剂。

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