

异养硝化-好氧反硝化菌新型固定化方式的研究进展

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摘要: 异养硝化-好氧反硝化(heterotrophic nitrifying-aerobic denitrifying, HN-AD)菌可在有氧条件下同时完成硝化与反硝化过程, 极大地简化了污水处理流程。该菌株生长速度快、耐极端环境, 被广泛应用于各类污水处理中。本文综述了HN-AD菌的脱氮途径, 着重介绍了在废水领域中应用脱氮的新型固定化载体(复合载体材料、磁性纳米载体材料和生物炭载体材料)的潜能; 重点梳理了新型固定化技术(仿生矿化固定化技术、电纺纤维固定化技术和3D打印载体固定化技术)的基本原理、应用实例、研究现状及未来发展潜力; 讨论了新型固定化菌株技术在提高脱氮处理效果和增强稳定性等方面的强化作用; 展望了HN-AD菌固定化技术在制备过程及应用中面临的挑战和未来发展趋势。

关键词: 固定化; 异养硝化好氧反硝化菌; 生物脱氮; 固定化技术

Research advances in novel immobilization methods for heterotrophic nitrifying-aerobic denitrifying bacteria

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Abstract: Heterotrophic nitrifying-aerobic denitrifying (HN-AD) bacteria can simultaneously complete nitrification and denitrification processes under aerobic conditions, significantly

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simplifying wastewater treatment procedures. These strains exhibit rapid growth, tolerance to extreme environments, and are widely applied in various wastewater treatments. This review summarizes the nitrogen removal pathways of HN-AD bacteria and highlights the potential of novel immobilization carriers (composite materials, magnetic nanocarriers, and biochar) for nitrogen removal across diverse wastewater sectors. It focuses on elucidating the fundamental principles, application cases, current research status, and future prospects of emerging immobilization technologies, including biomimetic mineralization immobilization, electrospun fiber immobilization, and 3D printing carrier immobilization. The enhanced effects of novel immobilization strategies on improving nitrogen removal efficiency and system stability are discussed. Finally, challenges and future prospects for HN-AD bacterial immobilization technology during fabrication and application are outlined.

Keywords: immobilization; heterotrophic nitrifying-aerobic denitrifying bacteria; biological nitrogen removal; immobilization technology

工业、农业和人类活动导致过量氮污染物排放至水生生态系统, 对水质和人类健康造成严重负面影响^[1]。在污水处理中, 高效脱氮起着至关重要的作用。我们需要采用合理高效的方法从废水中去除氮, 而生物修复脱氮是最有效、最节能且最常用的方法之一^[2]。然而, 这一传统工艺需分别构建有氧和无氧环境以满足硝化和反硝化细菌的不同需求, 处理流程繁琐复杂^[3]。在此过程中工艺参数的控制和原料添加是分开的, 这也带来了高昂的建设成本和运行成本^[4]。

异养硝化-好氧反硝化(heterotrophic nitrifying-aerobic denitrifying, HN-AD)菌的发现为氮污染治理开辟了新途径。HN-AD 菌可在好氧条件下同时完成硝化和反硝化过程, 极大地简化了污水处理流程, 显著提高了处理效率^[5-6]。研究表明此类菌株生长速度快、生物量高^[7]。HN-AD 菌环境适应性强, 例如在低温强碱^[8]、重金属^[9]和高盐度^[10]等环境下均能高效脱氮, 这也为筛选和培育具有更优良性能的菌株提供了丰富的基因资源。目前筛选出的 HN-AD 菌种类繁多, 由于 HN-AD 细菌存在个体差异, 其 HN-AD 机制存在相互矛盾的结果。典型的氮代谢途径包括硝化、反硝化、异化硝酸盐/亚硝酸盐还原和

氮同化^[11-12]。HN-AD 菌中检测到存在硝化和反硝化作用的基因, 包括氨单加氧酶(ammonia monooxygenase, AMO)、羟胺氧化还原酶(hydroxylamine oxidoreductase, HAO)、周质硝酸盐还原酶(periplasmic nitrate reductase, NAP)和亚硝酸盐还原酶(nitrite reductase, NIR)等, 具有完整的脱氮途径: $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ^[13]。还有研究表明 HN-AD 菌关键基因 HAO 和 AMO 缺失^[14], 因此氨氮去除可直接通过羟胺还原为氮气^[15]。此外, 在 HN-AD 菌中无机氮主要通过同化和异化转化为有机氮和气态氮^[12]。由于 HN-AD 菌种类繁多且脱氮机制不同^[16], 因此可通过合适的载体材料以及载体修饰来增强 HN-AD 菌的脱氮能力和稳定性。

菌株能否在实际废水的脱氮应用中发挥作用, 其环境适应性、生长繁殖能力及氮素降解能力是关键性决定因素。游离菌株极易流失或受到复杂环境因子的显著影响, 这直接导致菌株活性降低、生物量下降, 进而影响脱氮效率^[17]。特别是在处理贫营养废水^[18]以及面对高盐^[19]、抗生素^[20]、重金属^[9]等不利因素胁迫时高效菌株稳定的脱氮能力会出现波动。因此, 为使微生物脱氮系统整体达到稳定状态, 通过

固定化减少功能细菌的损失至关重要。微生物固定化是一种生物技术，它采用物理或化学方法将选定的游离微生物限制在有限的空间区域内以保持高密度和良好的生物活性，增强其对不利环境因素的抵抗能力^[21]。此外，能为菌株提供营养的方式，可选择包埋固体碳源或通过吸附有机物来实现^[22]。常见的固定化方法包括吸附法、包埋法、交联法和共价结合法等^[23]。前期固定化方法，如单一聚乙烯醇包裹、生物炭吸附等，虽然能保留大量 HN-AD 菌株，但普遍存在载体强度低、传质阻力大、亲水性降低和难以回收利用等缺陷^[24-26]。载体的选择会显著影响性能，因为材料和配制会影响其特性^[27]。HN-AD 菌的新型固定化系统需要调控载体内部的微环境(如溶解氧等)以满足其同时进行需氧硝化和厌氧反硝化的矛盾需求。因此，深入探究菌株的固定化技术及其研究进展对于推动其在污水处理等领域的大规模应用具有重要意义。本文将对 HN-AD 菌固定化的最新研究进展进行全面综述，例如仿生矿化固定化技术、电纺纤维固定化技术及 3D 打印载体固定化技术等新型固定化方式，以期为 HN-AD 菌的固定化研究和实践提供参考。

1 HN-AD 菌的脱氮途径分析

1.1 HN-AD 菌的氮异化途径

1.1.1 HN-AD 菌异养硝化好氧反硝化脱氮途径

该途径是一个多酶参与、有序进行的复杂生物化学反应过程。早在 1993 年 Wehrfritz 等^[28]提出了 HN-AD 的耦合模型，后续被广泛应用。氮代谢途径如图 1 所示，异养硝化途径为 $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$ 。HN-AD 菌内的氨单加氧酶(AMO)发挥关键作用，催化氨氮转化为羟胺(NH_2OH)，生成的羟胺在羟胺氧化还原酶(HAO)的催化下继续转化为亚硝酸盐(NO_2^-)，然后在亚硝酸氧化酶(nitrite oxidoreductase, NXR)的作用下会进一步被氧化为硝酸盐(NO_3^-)，进入 HN-AD 菌的硝酸盐-亚硝酸盐还原脱氮关键环节^[29]。

异养硝化途径之后的好氧反硝化途径： $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ^[30]，通过一系列酶如硝酸盐还原酶(nitrate reductase, NAR)、NAP、NIR、一氧化氮还原酶(nitric oxide reductase, NOR)和一氧化二氮还原酶(nitrous oxide reductase, NOS)等将物质转化为氮气。其中在好氧条件下，NAP

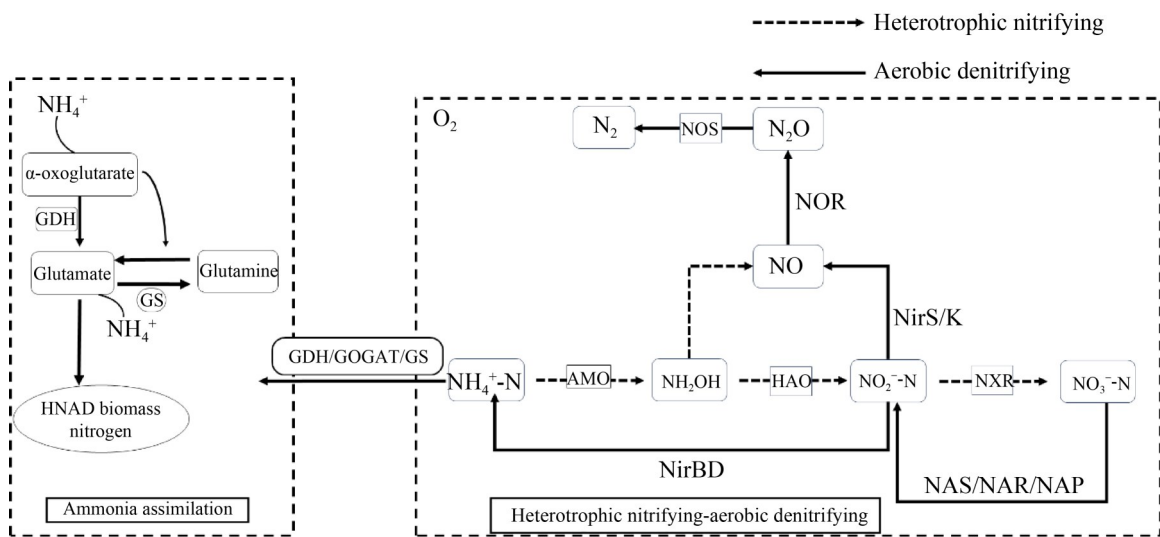


图1 氮代谢途径

Figure 1 Nitrogen metabolism pathway.

基因占主导地位, 对氧气具有较高耐受性, NAP 有助于微生物在有氧条件下将电子顺利传递给硝酸盐, 将 NO_3^- 还原为 NO_2^- ^[31-32], NirK 在低氧条件下仍可催化 $\text{NO}_2^- \rightarrow \text{NO}$, 随后通过 NOR、NOS 相关酶将物质转化为 N_2 。

1.1.2 HN-AD 羟胺脱氮的还原途径

许多研究表明, 在一些微生物中还存在羟胺的还原途径, 即通过氨的氧化产生羟胺, 而后直接越过亚硝酸盐阶段逐渐反硝化为氮气。最新研究发现一种新的脱氮途径, 氨氮直接被还原为氮气, 其中未检测到 NO_3^- -N 和 NO_2^- -N 的产生, 该途径被定义为直接氨氧化^[33]。Zhao 等^[15]的研究表明, 粪产碱菌(*Alcaligenes faecalis*)的硝酸还原酶(nitrate reductase, NR)无法去除硝酸盐和亚硝酸盐, 但能够将羟胺还原为含氮气体。近期还有研究发现, 粪产碱菌 TF1 缺乏硝酸盐还原所必需的周质硝酸盐还原酶(NapA)基因, 导致硝酸盐和亚硝酸盐无法降解, 但是遵循羟胺还原途径去除氮素^[34]。相较于传统脱氮微生物, HN-AD 菌的这一羟胺还原脱氮途径在有氧条件下即可高效进行, 无需严格的厌氧环境, 极大地拓宽了其在不同生态环境中的生存和作用空间。这一特性使其在污水处理等领域展现出巨大的应用潜力, 为解决各类含氮污染问题提供了新的思路和方法。

1.1.3 其他脱氮途径

HN-AD 菌还存在硝化和反硝化的联合作用对氮进行不完全去除的过程。在这一过程中, 微生物通过异养硝化作用将氨氮氧化为亚硝酸盐, 然后通过好氧反硝化作用将亚硝酸盐进一步还原, 但这种过程未必完全转化为氮气, 常停留在亚硝酸盐、一氧化氮等中间产物状态, 形成不完全脱氮。另外环境因素也会导致代谢过程不完整, 如高溶解氧(dissolved oxygen, DO)、低碳源会影响亚硝酸盐还原酶的基因表达和酶活性, 导致亚硝酸盐的积累^[35]。

1.2 HN-AD 菌氮同化

氮同化可以回收氮源用于细胞生长并且极大地减少氮源的损失^[36]。目前一些报道指出, 部分 HN-AD 菌的硝化和反硝化的脱氮相关基因不完整, 缺少硝化相关的基因(AMO 和 HAO 基因)和反硝化的主要基因(NIR 和 NOS 基因)^[37]。在氮同化起始阶段, 环境中的含氮化合物如铵盐、亚硝酸盐和硝酸盐等被 HN-AD 菌摄入细胞内。最近的报道发现, 一些 HN-AD 菌株可以通过一种新的硝酸盐还原途径初步将 NO_3^- -N 转化为 NH_4^+ -N, 具体过程为: 细胞通过特定的转运蛋白, 利用质子动力势将其逆浓度梯度转运至细胞内; 进入细胞后, 硝酸盐在 NR 的催化下被还原为亚硝酸盐, NR 是一种依赖于辅酶(如 NADH 或 NADPH)的酶, 此还原过程需消耗能量, 是氮同化途径的关键限速步骤之一; 随后, 亚硝酸盐在亚硝酸还原酶(NIR)作用下进一步还原为铵离子, 然后同化 NH_4^+ -N 到生物氮^[38]。

生成的铵离子是氮同化的核心中间产物, 可通过多种途径参与细胞物质合成。其中, 谷氨酸合成途径是主要的同化方式之一。如假单胞菌(*Pseudomonas reactans*) WL20-3 的氮同化就是在谷氨酰胺合成酶(glutamine synthetase, GS)和谷氨酸合成酶(glutamate synthase, GOGAT)的协同作用下铵离子与谷氨酸反应生成谷氨酰胺, 谷氨酰胺再与 α -酮戊二酸反应重新生成谷氨酸, 同时将氮固定于谷氨酸分子中^[8]。

2 新型固定化技术载体的选择

载体材料的选择是决定固定化效果的核心因素之一。传统的固定化载体选择大多集中在单一的天然或简单合成载体(如海藻酸钠、聚氨酯海绵和生物炭), 通过物理吸附或包埋实现菌株和材料的固定化^[39-41]。其优势在于成本低、操作简单, 但存在诸多缺陷, 例如微生物易暴露、重复使用性低且易发生二次污染^[42], 传质

阻力受限^[43]。Zhou 等^[44-45]利用扬水曝气技术对周村水库土著异养硝化-好氧反硝化菌进行原位强化,虽然实现了水体氮素的高效去除,但可能存在一些缺陷,如由于菌株暴露导致功能菌活性受环境(温度、pH)制约,脱氮效率会受到影响。目前,载体材料多采用改性的复合材料,例如 NaHCO_3 被用作改性剂来优化聚乙烯醇(polyvinyl alcohol, PVA)/海藻酸钠(sodium alginate, SA)的传质效率和溶胀性能^[46]。新型少量的纳米金属材料的使用可有效促进微生物的生长和代谢,提高微生物活性^[47-48]。此外,还有研究探究不同热解温度生物炭与菌株的耦合使用,以及生物炭去除氮的增强特性和机制。

2.1 复合载体材料

多种天然聚合物(如琼脂、海藻酸钠和壳聚糖)和合成聚合物[如聚氨酯、聚丙烯酰胺、聚乙烯醇和聚乙二醇(polyethylene glycol, PEG)]已成功应用于微生物固定化^[49]。虽然天然有机载体对微生物的毒性较低,但它们易分解会显著降低生物修复效率^[50]。合成聚合物具有良好的机械性能,但与天然聚合物相比扩散能力较差^[51]。目前,应用较多的聚合物是 PVA、SA 和壳聚糖,常见的是聚乙烯醇/海藻酸钠(PVA/SA)的结合。PVA/SA 凝胶珠具有成本低、耐用性好、机械强度高优点^[52]。交联 PVA 水凝胶具有良好的生物兼容性和高机械强度,对生物体无毒,且可廉价地进行工业规模生产,在废水处理中引起了极大关注。研究中还会加入一些改性剂来优化 PVA/SA 的传质效率和溶胀性能,如 NaHCO_3 、聚乙二醇和 CaCl_2 。加入 CaCl_2 改性的 PVA/SA 交联门多萨假单胞菌(*Pseudomonas mendocina*) A4 在 10% 的盐度环境下能去除 87% 的氨氮,几乎是游离细菌去除量的 2 倍^[53]。然而,仅利用 PVA 和 SA 复合体系在长期应用中存在显著局限性,会导致包埋的微生物在长时间使用后脱保护,进而降低生物反应系统效能。因此有报道在 PVA/SA 中添加黏土矿物,如层状双氢氧化物(layered double hydroxide, LDH),有

助于合成载体材料的增强发展,使其具有更广泛的适用性,通过添加 MgAl-LDH 对 PVA/SA 进行修饰与固定化微生物形成新型生物复合物可使化学需氧量(chemical oxygen demand, COD)和总氮(total nitrogen, TN)的去除率达到 90% 以上^[54]。此外,还有生物-无机复合的固定化材料应用研究。微生物诱导钙沉淀(microbial-induced calcium precipitation, MICP)是一种常见的生物矿化形式,是利用细菌代谢过程进行废水修复的新兴技术。研究发现通过 MICP 技术结合 HN-AD 过程, *Pseudomonas* sp. 能够实现同时去除铵态氮和磷酸盐^[55]。地表水低 C/N 比制约生物脱氮效率,外源碳源的投加面临经济成本与生态安全的双重限制,因此有必要寻找有效的额外电子供体。最近一项研究报道,Fe 和腐殖质(humus, HS)螯合制备不溶性络合物(Fe-HS)可避免 HS 溶于水和损失的缺点,Fe-HS 络合物可捕获金属离子和含氧阴离子^[56]。通过载体 Fe-HS@PVA/SA 固定菌株动胶菌属(*Zoogloea* sp.) ZP7 能够实现 85% 以上的总氮去除^[57]。

新型复合载体材料通过将不同材料进行复合设计形成具有协同效应的多功能材料,既能防止固定的菌株损失以避免生物活性减弱,又能增加载体的重复使用性,更加环保经济。此外,对于不同水体(如天然水体、贫营养水体、工业废水等)也应采用不同的固定化方法并选择合适的填料,以确保固定化微生物能够在不同环境中可靠运行。

2.2 磁性纳米载体材料

纳米材料凭借其纳米级尺寸和独特的原子排列方式展现出区别于宏观材料的特殊性能。其表面原子和近表面原子比例随尺寸减小而增加,这些表面原子因配位不饱和而形成高活性位点,这种表面主导的特性使纳米材料在催化、传感、能源等领域展现出不可替代的优势^[58]。研究表明 Fe_3O_4 磁性纳米材料对氮转化关键酶系具有显著调控作用,该材料不仅可增强硝化反应中氨单加氧酶(AMO)和亚硝酸盐氧化酶(NXR)

的催化效能, 还能同步提升反硝化途径中周质硝酸盐还原酶(NapA)与亚硝酸盐还原酶(NirS)的酶促反应速率^[59], 其作用机制可能涉及纳米颗粒表面电子传递促进效应及酶蛋白构象稳定化调控。也有报道称, 添加磁性纳米颗粒有助于改善材料的多孔结构、吸附性能和生物兼容性^[60]。有研究使用 PVA、羧甲基纤维素钠(sodium carboxymethyl cellulose, CMC)、硅藻土和 Fe₃O₄ 通过封装 HN-AD 菌制备磁性固定化载体, 在同时含有硝氮和氨氮的系统中磁性载体能去除 98.6% 的 NH₄⁺-N 和 86.7% 的 TN, 而非磁性载体能去除 84.7% 的 NH₄⁺-N 和 72.8% 的 TN^[61]。这说明添加 Fe₃O₄ 纳米颗粒显著增强了微生物对载体的吸附, 使载体具有较高的氨氧化活性, 生物降解反应引发更快。

2.3 生物炭载体材料

近年来, 生物炭凭借其独特的多孔结构、丰富的化学组分以及活性表面官能团等特性, 已成为提升氮污染物净化效率的新型功能材料。这种碳基材料通过物理吸附和化学反应的协同作用在污水深度脱氮处理中展现出显著的应用潜力^[62-63]。生物炭内部的孔隙为微生物的附着和生长提供了理想环境, 进而提高了生物量。此外, 生物炭表面的氧化还原官能团和电导率可作为电子穿梭体, 促进电子转移, 从而提高脱氮性能^[64]。目前已有许多关于生物炭负载微生物高效去除污染物的报道, 但大多数报道是基于对生物炭作为吸附剂和微生物附着物的物理特性的调查^[65]。本课题组 Di 等^[66]利用不同材料制备的生物炭(玉米秸秆、芦苇和竹子)耦合微生物, 在低 C/N 条件下实现高效脱氮, 并研究了其脱氮系统性能的差异以及微生物反应, 这对于揭示生物炭增强微生物脱氮的机制至关重要。未来还需进一步深入探索生物炭利用自身化学特性去除氮的增强特性和机制。有实验报道, 热解温度为 300 °C 的椰壳生物炭更有利于 HN-AD 菌的代谢活性, 在好氧系统中其对 TN 和总磷(total phosphorus, TP)的去除率分别提高

了 68% 和 88%^[67]。高热解温度的生物炭强化效果不明显, 这表明高温会导致产生更多挥发性物质, 脂肪族碳水平显著降低^[68]。研究表明生物炭的这些物理化学性质受制备温度和原材料等因素的影响^[69]。因此, 有必要研究生物炭的制备方法, 包括原料的选择、热解温度, 以及生物炭通过 HN-AD 菌去除氮的增强效率和机制。

3 新型固定化技术的种类及特点

近几年报道的仿生矿化固定化技术、电纺纤维技术及 3D 打印载体固定化技术正推动该领域向高效化、智能化方向发展, 与传统包埋固定化方法形成鲜明对比。传统固定化技术(如 PVA/SA 包裹、海藻酸钠包裹和生物炭吸附等)虽然应用广泛, 但仍存在一定局限性, 例如缺乏功能设计、生物亲和力差和传质性能差等^[43], 且固定化过程缺乏精细调控^[25]。相比之下, 新型固定化技术在传质限制与稳定性方面以及精准优化方面实现了显著突破。

3.1 仿生矿化固定化技术

仿生矿化固定化技术通过模拟生物体内矿化机制, 将微生物等活性物质嵌入无机-有机复合载体中实现高稳定性与功能可控性的统一。与传统的固定化技术相比, 仿生矿化提供了更温和的条件、更高的负载率, 是一种经济高效且对环境无害的微生物固定化技术^[70]。常见的仿生矿化是将天然高分子(如海藻酸钠、壳聚糖等)与无机矿物(如高岭土、CaCO₃ 等)复合, 构建多孔矿化层。有研究使用海藻酸钠-高岭土作为载体包裹 *Pseudomonas* sp. DM02, 在处理实际水产养殖废水时, 当 C/N 为 10 时 TN 可从 (21.4±1.7) mg/L 降至 (6.9±1.3) mg/L, 且无其他氮素积累^[5]。高岭土属于传统低成本高效吸附剂, 其化学成分和内部孔隙大小不同, 具有从废水中去除污染物的巨大潜力^[71]。高岭土的添加可增强海藻酸钠凝胶的机械强度, 降低传质

阻力。截至目前,在报道的新型层状蜂窝仿生结构中其具有孔隙率高、材料用量少、稳定性好、比表面积大等特点,在细胞支架、抗菌材料、分子阵列和生物传感方面具有广泛应用^[72]。因此,已有实例将层状蜂窝仿生结构与异养硝化好氧反硝化菌结合以强化脱氮。Wu等^[73]采用新型交叉流蜂格仿生微生物载体,在同时硝化反硝化系统中氮去除率可达95.4%。然而,当前也面临一些问题,如矿化过程动力学调控精度不足、易出现结构缺陷、复杂体系下的多组分协同机制不明确等。仿生矿化固定化技术正从“结构仿生”向“功能仿生”跃迁,其与合成生物学、纳米技术的深度融合可进一步提高微生物的活性,实现有效的氮去除。

3.2 电纺纤维固定化技术

静电纺丝技术利用高压电场将聚合物溶液[如聚乳酸(poly(lactic acid), PLA)、聚乙烯醇]纺成具有高比表面积、多孔结构的纤维膜,为微生物提供理想的附着与生长环境。静电纺丝作为一种生产从几十纳米到几微米的连续纤维的技术^[74],可为制备微生物固定化载体提供新思路。此外,静电纺丝区别于其他制备生物材料的技术,具有独特优势,可通过调节纺丝液配方及工艺参数精确调控纤维支架的微观结构、孔隙特征与机械性能,进而构建适配细菌生长需求

的微环境,促进其黏附、增殖及分化行为。Lencova等^[75]以电纺聚酰胺纤维为生物载体,研究了其对3种细菌的固定效果(图2),这些微生物在静电纺丝法制备的聚酰胺纤维表面成功定殖并构建了生物膜结构,该现象证实了此类纤维材料在生物膜支撑体系构建中具备应用潜能。目前,大多报道集中在材料改性方面,材料改性不仅在改善这些纳米纤维的机械性能、亲水性和其他基本特性方面发挥着关键作用,而且在优化其微观结构方面为细菌的附着和生长提供了更有利条件。Li等^[76]通过SiO₂和聚己内酯(polycaprolactone, PCL)的混合静电纺丝制备纳米纤维,并采用低温等离子体和硅烷偶联剂对纳米纤维进行改性,提高了纳米纤维的物理化学性能,制作的静电纺丝负载人苍白杆菌(*Brucella anthropi*) N2-2在混合氮源中32 h内总氮去除效率可达90.6%。静电纺丝技术虽在优势功能菌的选择和保护、提高菌群的生物效率等方面展现出巨大潜力,但在制作和应用过程中仍面临一些核心问题,例如生产率低、纤维均匀性难以控制,还面临着结构精准调控难题。未来,还需向功能性-可靠性-经济性多目标协同优化方向发展。

3.3 3D打印载体固定化技术

3D打印(增材制造)技术通过逐层堆叠材料

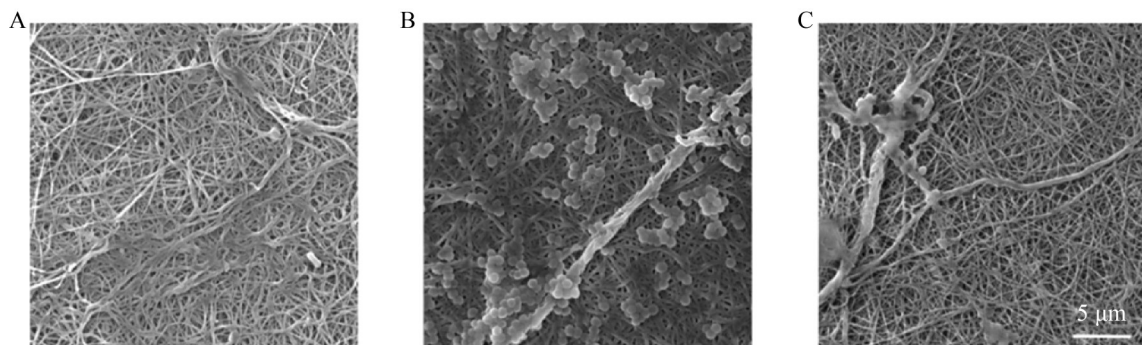


图2 8%聚酰胺纤维上形成生物膜的SEM图像(10 000×)。A: 大肠杆菌CCM 4517; B: 金黄色葡萄球菌CCM 3953; C: 表皮葡萄球菌CCM 4418^[75]。

Figure 2 SEM image of the biofilm formed on 8% polyamide fibers (10 000×). A: *Escherichia coli* CCM 4517; B: *Staphylococcus aureus* CCM 3953; C: *Staphylococcus epidermidis* CCM 4418^[75].

构建三维结构, 为微生物固定化载体设计带来革命性突破。其借助高度受控的过程实现了对材料几何构型与微观孔隙的精确调控, 为有生命材料的可控制造提供了新策略^[77]。传统载体制造方法在材料功能性与几何设计方面存在局限, 促使人们转向具有复杂多材料结构等优势 的 3D 打印技术。此外, 在减废方面, 3D 打印技术基于逐层增材(而非减材)的制造原理, 能显著降低固体废弃物的产生^[78]。3D 打印生物载体因其独特的形状和表面纹理促进了营养物质的扩散, 提升了微生物对底物的吸收, 加速了生物膜的形成^[26]。有研究报道, 将回收的废料涂上活性炭制备成新的载体, 活性生物量显著提高了 20.2%, TN 的去除率显著提高了 16.8%^[79]。由于载体结构会影响溶解氧的扩散, 并且在相同的溶解氧值下结构复杂的载体比结构简单的载体更有可能实现稳定的溶解氧供应^[80]。因此, 基于通过载体结构在生物膜上创建缺氧区的设想设计了具有狭窄通道的圆形载体用于单级移动床生物膜反应器, 提高了同步硝化反硝化的效率, TN 去除率高于 67.8%^[81]。3D 打印技术通过结构精准设计与材料创新为 HN-AD 菌固定化提供了突破传质限制、提升生物活性的新范式, 标志着微生物固定化技术向定制化、智能化迈进的必然趋势。未来, 需通过跨学科协作(如运用先进计算模拟工具等)在载体材料设计和工艺参数调控中展现出日益显著的价值。

4 新型固定化技术对 HN-AD 菌的强化作用

固定化技术通过将 HN-AD 菌限制于特定空间内显著提升了其环境适应性与功能稳定性, 成为废水生物脱氮领域的前沿研究方向。传统包埋法因载体传质阻力大、菌体活性易受损等缺陷限制了其工程化应用, 而新型固定化技术通过材料创新与结构优化实现了突破。表 1 汇总了固定化 HN-AD 菌在污水处理中的效果。

4.1 提高菌株活性和稳定性

固定化技术通过优化微生物的微环境、增强抗逆性及促进代谢协同效应显著提升了菌株的活性和稳定性。以 PVA 作为固定化载体包裹菌株 *Pseudomonas sp.* Y1, 通过扫描电镜(scanning electron microscope, SEM)进行形态观察发现, PVA 水凝胶的内部结构呈现出明显的多孔形状, 这为细菌生长提供了更合适的栖息地, 并增强了系统对各种冲击负荷的生物响应抗性^[88]。这表明刚性载体(如硅胶、PVA 凝胶等)可以为菌株提供栖息地、抵抗水力冲击, 减少菌体流失。然而, 研究还发现 PVA 中加入一些黏土矿物(例如膨润土)更有利于菌株附着, 因为膨润土颗粒均匀地分布在 PVA 基质中导致孔径显著减小、孔壁粗糙度增加^[89]。新型固定化技术静电纺丝也有应用, 最近有研究将静电纺丝材料先通过物理改性, 再浸入 2% 的 3-氨基丙基三甲氧基硅烷水溶液中浸泡进行化学改性, 然后加热到 800 °C 进行稳定性测试, 结果发现未改性的纳米纤维膜损失了 99.24% 的质量, 而改性材料则有 17.59% 的质量留存^[76]。这说明经改性形成的二氧化硅-氧-碳(Si-O-C)键在化学改性过程中增强了原有构型与功能特性, 其稳定的共价键网络使材料在高温环境中表现出显著增强的热耐受性^[90]。固定化载体材料除了能够保持菌株的稳定性, 还能添加一些导电材料(如石墨烯、添加 Fe²⁺改性的材料等)作为“电子桥梁”加速胞外电子传递, 增强能量代谢效率。乔楠等^[91]通过使用 FeSO₄ 改性的硅藻土负载 *Pseudomonas sp.* H1, 结果发现 Fe²⁺ 的加入使硅藻土表面带有更多的正电荷, 不仅提升了菌株 H1 的吸附性, 还提高了菌株的活性; 同时, 负载改性硅藻土的菌株对 pH 和温度的耐受性也更强。综上所述, 生物量的增长主要表现为载体表面生物膜的增厚与内部孔隙的填充。有研究表明, HN-AD 菌 FH1 积聚在低热解温度玉米生物炭(300 °C)的表面和孔隙中, 一些菌株单独黏附, 一些聚集形成生物膜, 载体中的蛋白质含

表1 固定化技术负载HN-AD菌的强化案例

Table 1 Enhanced cases of fixed-base technology for enriching HN-AD bacteria

| Strain type | Wastewater type | Reactor | Carrier material | Treatment effects | References |
|---|---|----------------------------|--|---|------------|
| <i>Pseudomonas</i> sp. DM02 | Aquaculture wastewater | Semi-continuous culture | Sodium alginate (SA), Kaolin | When treating actual aquaculture wastewater with a C/N ratio of 10, the total nitrogen (TN) can be reduced from (21.4±1.7) mg/L to (6.9±1.3) mg/L, with no accumulation of other nitrogen compounds | [5] |
| <i>Brucella anthropi</i> | Domestic wastewater | None | Electrospun fibers | Immobilized bacteria cultured under NH ₄ ⁺ -N as the sole nitrogen source, in which 100 mg NH ₄ ⁺ -N was completely degraded within 16 h | [76] |
| <i>Paracoccus</i> sp. QD-21 | Domestic wastewater | None | Polyvinyl alcohol (PVA), SA, Biochar, SiO ₂ | In practical wastewater treatment, the ammonia nitrogen removal rate has increased by 63.31% | [82] |
| <i>Enterobacter</i> , <i>Pseudomonas</i> , and <i>Bacillus</i> | Landfill leachate | None | PVA, Fe ₃ O ₄ nanoparticles Diatomite | The removal rates of NH ₄ ⁺ -N and TN were 93.7% and 78.3%, respectively | [61] |
| <i>Acinetobacter pittii</i> SY9 | Low C/N wastewater | Sequencing batch reactor | PVA, SA, corn cob | At a low C/N ratio of 2.8, there was still efficient heterotrophic nitrification and aerobic denitrification performance. The TN removal rate was over 90% | [83] |
| <i>Acinetobacter pittii</i> , <i>Stenotrophomonas maltophilia</i> , and <i>Klebsiella oxytoca</i> | Domestic wastewater | Sequencing batch reactor | Low-temperature coconut shell biochar | The removal efficiencies of TN and TP in the aerobic system were increased by 68% and 88%, respectively | [67] |
| <i>Acinetobacter</i> sp. TAC-1 | Swine wastewater | Moving bed biofilm reactor | PVA | For the actual swine wastewater treatment, the removal efficiencies of COD, NH ₄ ⁺ -N, and TN within 24 hours were 84.85%, 95.01%, and 86.40%, respectively | [84] |
| <i>Delftia</i> sp. Y19 | Low C/N wastewater | None | Magnetite | The system achieved removal rates of 100% for nitrate and chemical oxygen demand, and 36% for ammonium | [85] |
| <i>Zobellella</i> sp. B307 | Marine aquaculture wastewater | Moving bed biofilm reactor | Cylindrical hollow filler | The abundance of functional genes related to nitrogen metabolism in the strains was increased, and the removal rates of COD, NH ₄ ⁺ -N, and NO ₃ ⁻ -N reached 95.6%, 94.4%, and 85.7%, respectively | [86] |
| <i>Flavobacterium</i> , <i>Azoarcus</i> | Livestock and poultry breeding wastewater | Membrane bioreactor | The rotating biological contactor forms a biofilm | The removal rates of NH ₄ ⁺ -N and TN were respectively 29.23% and 31.03% higher than those of the pure activated sludge biofilm | [87] |
| <i>Flavobacterium</i> , <i>Hydrogenophaga</i> | Rural domestic wastewater | Moving bed biofilm reactor | Thermoplastic | At a DO concentration of 1.7–2.3 mg/L, the COD and TN removal rates of the carrier were respectively higher than 88.4% and 67.8% | [81] |

量为 (19.50 ± 2.36) mg/g^[67]。尽管载体负载能力存在物理极限,它主要取决于载体的材料和结构(如孔径、孔隙率、比表面积)、机械强度及微生物的生长特性^[27]。例如,不动杆菌(*Acinetobacter* sp.) TSH1 在膜-生物反应器(membrane bio-reactor, MBR)反应器系统中发现 *Acinetobacter* sp. 的相对丰度并不高(4.02%),说明微生物之间的竞争会影响强化菌株的相对丰度^[92]。另一方面,一些研究通过增加接种剂量和频率或应用新型固定化技术提高了生物强化微生物的相对丰度和生物反应器的长期稳定运行^[93]。有些固定化利用凝胶(PVA、SA 等)对载体及菌株进行包埋,包埋在凝胶内的菌株极易产生胞外聚合物,使其与凝胶珠内部的网络结构结合更牢固、更致密,使菌株不易流失^[94]。总之,固定化技术不仅延长了菌株寿命,还激活了其代谢潜能,为环境修复、生物制造等领域提供了高效、可持续的解决方案。

4.2 经济可行性和环境评估

在最新的研究中发现,HN-AD 菌具有较强的环境耐受性,展现出对极端环境的高度适应性,一株新型鞣鞣酮丛毛单胞菌(*Comamonas testosteroni*) HR5 在 5 °C 和 pH 10.0 的双重极端条件下混合氮系统的氮去除率均大于 91%^[95]。更具抗重金属能力的耐重金属贪铜菌(*Cupriavidus metallidurans*) TX6 在 Cu^{2+} 浓度低于 15 mg/L 时能够去除超过 75% 的 NH_4^+ -N 和 TN。此外,还有一些具有耐高氨氮浓度、高盐等特性的 HN-AD 菌株,除了能够直接在极端环境下表现出抗性,还能通过固定化技术延长时间及提供电子等,使其免受极端环境的影响,提高系统的稳定性,达到高效脱氮的目的。Fe-C 微电解耦合 HN-AD 菌在低 C/N (3) 时,8 h 内 TN 去除率为 88.6%,不含 Fe-C 物质的仅有 55.2% TN 去除率;无 Fe-C 物质时只发生硝氮的去除,氨氮和 TN 的去除率仅为 14.4%,说明硝氮的去除基于 Fe-C 物质的电解^[96]。Fe-C 物质的耦合减少了有机碳源的增加,增强了菌株的使用寿命,并且

全流程无二次污染风险。然而,传统的生物处理技术,包括活性污泥工艺、好氧/厌氧生物反应器等,存在一些缺点,如对经营管理要求高、对极端环境耐受性低和承受负荷低,生物过程中产生的污泥会造成新的污染^[97]。其中,活性污泥法被认为是不环保的废水处理系统,因为其曝气过程中大量能源消耗了化石燃料,同时排放了温室气体^[98]。此外,HN-AD 的固定化避免了传统工艺中缺氧/好氧分区切换的能耗需求,运行能耗大大降低;固定化载体可以重复使用,也大大减少了材料成本。例如,传统 HN-AD 菌固定在纳米载体材料(如氧化铁纳米颗粒)上,固定化材料在修复完后可使用磁吸进行分离和回收,但大型污水处理厂难以使用磁场发生器^[99]。因此可采用新型的固定化技术,如添加藻酸盐和纳米物质到膨胀黏土制作可漂浮的载体材料,运行完后可直接进行过滤或用清扫网进行分离,转移到新污染地^[100],以实现固定化载体的回收利用。

4.3 维持营养物质的稳态输送

HN-AD 固定化技术不仅提高了脱氮效率,还实现了废水处理过程中碳、氮等营养物质的稳态输送与动态平衡。这种稳态调控机制在复杂废水处理系统中尤为重要,能够有效避免传统工艺中因底物波动导致的处理效能不稳定问题。静电纺丝纳米纤维独特的结构为 *Brucella anthropi* N2-2 构建了稳定的微生态位,载体内部材料可减缓水流对菌体的损伤,同时实现氨氮、硝酸盐的去除,相较于游离细菌具有更高的降解率^[76]。这种缓释-吸附”双重机制使载体表面持续有营养物质输送,避免了游离菌系统中因底物冲击引发的代谢抑制。此外,HN-AD 菌通过代谢重构实现了有机碳源的高效利用。在固定化体系中如添加生物炭固体碳源,可以不断释放营养物质,减少了对有机碳源的添加量。有研究表明,通过嵌入 HN-AD 菌和玉米芯的生物微胶囊用于强化污水脱氮过程具有多孔结构的生物微胶囊(孔径 2 579.74 – 3 725.44 nm; 孔隙率

53.6%–79.9%)可以减缓玉米芯碳源释放达120 d,即使在低 C/N (2.8)条件下也能确保生物微胶囊具有较高的 HN-AD 性能^[83]。此外,加入铁无机电子供体, Fe-C 微电解耦合 HN-AD 菌是解决电子供体缺乏的低 C/N 废水处理问题的有效策略^[96]。固定化 HN-AD 技术通过载体结构设计、氧梯度构建及代谢网络优化实现了氮素与碳源的稳态输送与高效转化,为低营养废水处理提供了新思路。

5 总结与展望

近年来,大量高效 HN-AD 菌被引入污水处理设施。因其能够进行同时硝化和反硝化脱氮,且部分菌株能够在极端环境下高效脱氮,在含氮废水处理中具有极大的应用前景。本文主要综述了 HN-AD 菌的氮异化及氮同化的脱氮途径、HN-AD 菌常见的固定化载体、新型固定化技术在 HN-AD 领域的深入应用。其在极端环境中高效脱氮、多污染物协同去除及资源回收利用等方面展现出显著优势,但仍面临着技术瓶颈与规模化应用挑战。

未来发展中应注重以下 4 方面研究。(1) 仿生矿化固定化技术应加快从“结构仿生”向“功能仿生”的跃迁,可与纳米技术深度结合,进一步提高微生物的活性。(2) 电纺纤维技术是当前研究的热点,可用于低碳氮废水同步去除硝氮和氨氮,但菌株与载体界面作用机制尚不明确。(3) 3D 打印载体的引入非常适合 HN-AD 菌复杂的脱氮流程,该技术融合了材料科学、微生物学和先进制造的交叉优势,有望解决传统生物脱氮工艺中的诸多痛点。未来可在单一载体上设计不同的功能区。例如,载体外层设计为高氧环境区利于硝化;内层或特定区域设计为微氧/缺氧微环境区利于反硝化,更贴近 HN-AD 菌的理想代谢微环境。(4) 还需进一步评价实际废水特性(如盐、碱、重金属和抗生素等)对载体材料物理化学稳定性及微生物活性的影响。更多地关注材料载体与菌株的互作机制,研究菌

株的群落结构演替,为实际废水应用提供理论基础。

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作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

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