

# 饮用水系统硝化微生物分布规律、环境影响因素及调控应用的研究进展

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**摘要:** 硝化微生物广泛存在于饮用水系统中。水处理过程中, 硝化微生物对含氮污染物的去除有突出贡献; 而输配水过程中, 硝化微生物会加剧消毒剂氯胺的降解, 造成一系列饮用水微生物安全问题。本文介绍了常用硝化微生物检测方法, 综述了硝化微生物在滤池、市政主管网、二次供水系统中的分布特征和规律, 分析了环境因子及工程条件对硝化微生物的影响机制, 探讨了硝化微生物强化应用及管控的实际工程措施, 展望了未来饮用水系统中硝化微生物的研究重点与应用前景。

**关键词:** 饮用水系统; 硝化微生物; 硝化作用

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# Distribution characteristics, environmental influencing factors and engineering application of nitrifiers in drinking water systems: a review

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**Abstract:** Nitrifiers are omnipresent in drinking water systems. While being capable of degrading nitrogenous contaminants in drinking water treatment process, nitrifiers accelerate the consumption of disinfectant in drinking water distribution systems, thus posing a serious threat to public health. This paper introduces the methodological techniques currently used in the detection of nitrifiers and summarizes the distribution characteristics of nitrifiers in filters, drinking water distribution systems, and secondary water supply systems. Further, it elucidates the influencing mechanism of environmental and engineering factors on nitrifiers, discusses the augmentation and suppression of nitrification in drinking water system and prospects the future research and application of nitrifiers.

**Keywords:** drinking water systems; nitrifiers; nitrification

生物硝化作用是自然界氮循环过程的关键步骤, 一般认为该过程由两大类微生物分步完成<sup>[1-2]</sup>, 即由氨氧化微生物(ammonia-oxidizing microorganisms, AOM)完成氨氮向亚硝酸盐的转化后, 再由亚硝酸盐氧化细菌(nitrite-oxidizing bacteria, NOB)将亚硝酸盐氧化成硝酸盐。AOM包括氨氧化细菌(ammonia-oxidizing bacteria, AOB)和氨氧化古菌(ammonia-oxidizing archaea, AOA)<sup>[3]</sup>。然而, 近期发现了一种能够独立完成上述两步硝化作用的全程硝化细菌(complete ammonia-oxidizing bacteria, comammox), 它们在自然环境和工程水系统中广泛存在<sup>[4]</sup>。基于 16S rRNA 基因序列比对结果, 目前已知的 comammox 均属硝化螺菌属(*Nitrospira*)系统发育谱系II<sup>[5-6]</sup>。Comammox *Nitrospira* 的发现验证了 Costa 等<sup>[7]</sup>

利用最佳代谢路径长度理论对全程硝化微生物存在的预测, 增进了人们对于自然界微生物硝化作用的认知。

硝化微生物对饮用水系统的生物处理过程及管网生物稳定性具有重要作用。一方面, 在水处理过程中, 硝化微生物有助于原水中氨氮等含氮污染物的去除, 提高出水水质; 另一方面, 在以氯胺为消毒剂的市政主管网(distribution mains)和二次供水系统(secondary water supply systems, SWSSs)中, 硝化微生物可引发硝化作用, 加剧氯胺的耗损<sup>[8-10]</sup>。以一氯胺( $\text{NH}_2\text{Cl}$ )为例, 硝化微生物可利用其自分解反应( $\text{NH}_2\text{Cl} \rightarrow \text{N}_2 + 3\text{H}^+ + 3\text{Cl}^- + \text{NH}_3$ )<sup>[8]</sup>产生的氨氮引起反应平衡移动, 从而加剧氯胺降解<sup>[9]</sup>。其次, 硝化作用生成的亚硝氮可与氯胺反应( $\text{NH}_2\text{Cl} + \text{NO}_2^- + \text{H}_2\text{O} \rightarrow$

$\text{NO}_3^- + \text{NH}_3 + \text{HCl}$ )一步加剧氯胺损耗<sup>[10]</sup>。此外,某些硝化微生物如 *Nitrosomonas europaea* 还可通过共代谢(cometabolism)的方式降解氯胺<sup>[11-12]</sup>。由硝化微生物引发的消毒剂降解可造成致病菌滋生等一系列水质恶化问题(图 1),对饮用水的供水安全性带来严峻挑战。因此,研究饮用水系统中硝化微生物的分布规律及其影响因素对实现对硝化作用的调控有重要意义。

## 1 硝化微生物的检测方法

### 1.1 培养法

培养法是研究微生物最传统、最常用的手段之一。含不同氮源的培养基可实现对特定硝化微生物的分离培养。例如,以 $(\text{NH}_4)_2\text{SO}_4$ 或 $\text{NaNO}_2$ 为唯一氮源的培养基能够分别实现对 AOB 或 NOB 的分离培养<sup>[13]</sup>。最大自然数(most-probable-number, MPN)法常被用于饮用水样品中硝化微生物的培养计数<sup>[14-15]</sup>,文献曾报道其检测范围为

$10^2-10^6$  MPN/cm<sup>2</sup>、 $10-10^5$  MPN/g (生物膜)<sup>[14-15]</sup>和  $10^2-10^5$  MPN/L (水样)<sup>[13]</sup>。

培养法是从环境中富集、纯化硝化微生物并对其进行生理生化特性研究的重要手段。Könneke 等<sup>[3]</sup>将水族馆鱼缸水中的微生物抽滤富集后,于含 1 mmol/L  $\text{NH}_4\text{Cl}$  的培养基中暗培养 6 个月,获得含 90% 泉古菌门(*Crenarchaeota*)和 10% 细菌的富集物;再通过以重碳酸盐为唯一碳源、氨氮为唯一氮源的培养基筛选,得到了具有氨氧化能力的古菌,将其命名为 *Nitrosopumilus maritimus* SCM1。Daims 等<sup>[16]</sup>将石油探井热水管壁上的生物膜培养于含 0.5 mmol/L  $\text{NH}_4\text{Cl}$  的培养基中,通过连续的梯度稀释、纯化获得了能够将氨氮氧化为硝酸盐的微生物,并进一步利用分子生物学手段证实了该微生物能够独立完成硝化反应全程,将其命名为 *Nitrospira inopinata*。鉴于环境中的硝化微生物常与异养菌同时存在,一些特殊的微生物分离技术,如

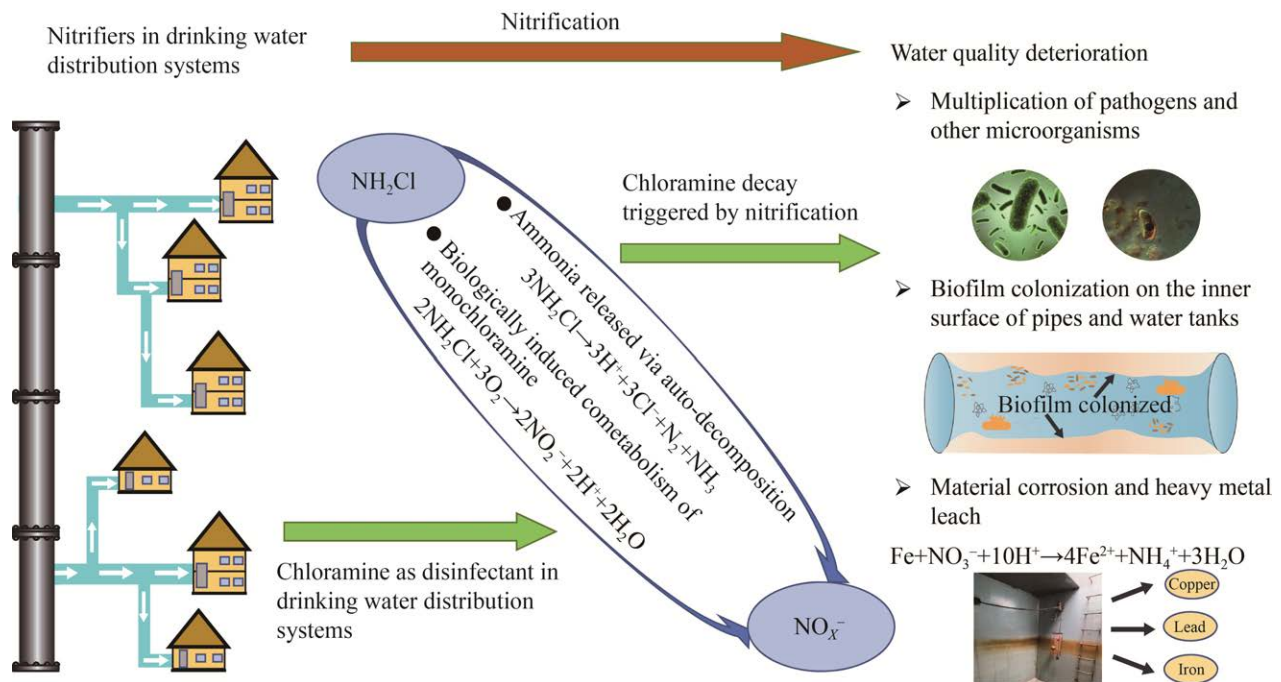


图 1 硝化作用导致供水管网水质恶化过程示意图

Figure 1 Water quality deterioration triggered by nitrification in drinking water distribution systems.

拉曼光镊(Roman tweezers)<sup>[17]</sup>、拉曼稳定同位素探针(Roman stable isotope probing)法<sup>[18]</sup>也常被用于环境中硝化微生物的分离纯化。

## 1.2 分子生物学的方法

与培养法相比,分子生物学方法具有检测速度快、通量高、灵敏度高优点,广泛用于不同环境介质中硝化微生物的检测分析。

实时荧光定量 PCR (quantitative real-time PCR, RT-qPCR)常用于硝化微生物的定量,结果以目标基因的拷贝数表示<sup>[19-23]</sup>。氨单加氧酶(ammonia monooxygenase, AMO)、羟胺脱氢酶(hydroxylamine dehydrogenase, HAO)等硝化过程中关键酶的编码基因可作为检测氨氧化微生物的基因标记物<sup>[16,24-25]</sup>。在 comammox 被发现前,

编码亚硝酸盐氧化还原酶(nitrite oxidoreductase)的 *nxB* 基因常用于环境中 NOB 的检测<sup>[26]</sup>。后来为了区分 comammox 和严格亚硝酸盐氧化菌(strict NOB, sNOB)<sup>[27-28]</sup>, Jiang 等<sup>[29]</sup>设计了靶氰化酶(cyanase)编码基因 *cynS* 的引物用于 sNOB 的检测。常用扩增硝化微生物特征基因的 PCR 引物见表 1。

以 *amoA* 基因为例,引物对 Arch-*amoA*F/Arch-*amoA*R<sup>[30]</sup>和 *amoA*-1F/*amoA*-2R<sup>[31]</sup>可分别用于氨氧化古菌和氨氧化细菌 *amoA* 基因的扩增,从而实现对 AOA 和 AOB 的定量。Comammox 的 *amoA* 基因与典型氨氧化微生物相似度低,可用于区分 comammox 与典型氨氧化微生物,因此也发展出了许多扩增 comammox

表 1 硝化微生物特征基因的 PCR 引物

Table 1 PCR primer sets targeted marker genes of nitrifiers

Targeted organisms	Targeted genes	Sequences (5'→3')	References
Ammonia oxidizing archaea	<i>amoA</i>	Arch- <i>amoA</i> F: STAATGGTCTGGCTTAGACG Arch- <i>amoA</i> R: GCGCCATCCATCTGTATGT	[30]
Ammonia oxidizing bacteria	<i>amoA</i>	<i>amoA</i> -1F: GGGG TTTCTACTGGTGGT <i>amoA</i> -2R: CCCCTCKGSAAAGCCTTCTTC	[31]
Strict NOB	<i>cynS</i>	Ntspa- <i>cynS</i> F: TSATCGGHGTSTAYGGMGA Ntspa- <i>cynS</i> R: CCGTTCARSGTRATCTTGCA	[29]
Comammox <i>Nitrospira</i> clade A	<i>amoA</i>	CA377F: GTGGTGGTGGTCBAAAYTA C576R: GAAGCCCATRTARTCNGCC	[29]
	<i>amoA</i>	comaA-244F: TAYAAYTGGGTSAAAYTA comaA-659R: ARATCATSGTGCTRTG	[27]
Comammox <i>Nitrospira</i> clade B	<i>amoA</i>	CB377F: GTACTGGTGGGCBAAYTT C576R GAAGCCCATRTARTCNGCC	[29]
	<i>amoA</i>	comaB-244F: TAYTTCTGGACRTTYTA comaB-659R: ARATCCARACDGTGTG	[27]
Comammox <i>Nitrospira</i>	<i>amoA</i>	Ntsp- <i>amoA</i> 162F: GGATTTCTGGNTSGATTGGA Ntsp- <i>amoA</i> 359R: WAGTTNGACCACCASTACCA	[32]
<i>Nitrospira</i> spp.	<i>nxB</i>	<i>nxB</i> 169F: TACATGTGGTGGGAACA <i>nxB</i> 638R: CGGTTCTGGTTCRATCA	[26]
	16S rRNA	Eub338F: ACTCCTACGGGAGGCAGC Ntspa0685: CGGGAATTCCGCGCTC	[33]
<i>Nitrobacter</i> spp.	16S rRNA	Nitro-1198F: CCTAGCAAATCTCAAAAAACCG Nitro-1423R: CTTCACCCAGTCGCTGACC Nitro-1374Taq: ACCCGCAAGGAGGCAGCCGACC	[34]

的 *amoA* 基因的 PCR 引物<sup>[27,29,32,35-37]</sup>。也有学者利用反转录 PCR (reverse transcription-PCR, RT-PCR) 进一步研究硝化功能基因在环境中的表达情况<sup>[38]</sup>。近年来, 随着多组学研究方法的日趋成熟, 将宏基因组 (metagenome)<sup>[32,39-43]</sup>、宏转录组 (metatranscriptomic)<sup>[44]</sup> 等方法应用于硝化微生物的研究也逐渐增多, 进一步提供了不同环境介质中硝化微生物存在丰度、硝化功能基因原位表达等方面的信息。此外, 也存在一些特殊的分子生物学方法, 如基于液态悬浮生物芯片技术 (luminex xMAP) 的 mRNA 直接多重检测法<sup>[45]</sup> 可用于同时检测环境中 *amoA* 基因的表达及氨氧化细菌的活动情况。

## 2 硝化微生物在饮用水系统中的分布特征

硝化微生物广泛存在于给水厂各构筑物中<sup>[46-47]</sup>, 因生物滤池 (biological filters) 对氨氮转化有较大的贡献, 有大量研究围绕生物滤池中硝化微生物展开<sup>[20,22,40,48-49]</sup>。此外, 在以氯胺为消毒剂的市政管网和二次供水系统中, 硝化作用是导致水质恶化的重要原因<sup>[50-51]</sup>, 因此, 其中硝化微生物的丰度与分布情况也广受关注。各硝化微生物标记基因在饮用水系统中的分布与检出丰度见表 2。

### 2.1 生物滤池

各类硝化微生物在水厂滤池中均有检出, 但其丰度在不同水厂存在明显差异。以滤料中 AOA 与 AOB 的丰度为例, 当 AOA 作为滤池中的主导氨氧化微生物时, AOA *amoA* 基因的拷贝数可达  $10^7$  GC/g-carbon, 而且滤池中无 AOB *amoA* 基因检出<sup>[52]</sup>。不过, 滤池中 AOB *amoA* 基因数远高于 AOA *amoA* 基因的情况也常有报道<sup>[53,56]</sup>, 某些砂滤池中, AOB *amoA* 基因拷贝

数可达 AOA *amoA* 基因拷贝数的 446 倍<sup>[56]</sup>。*Nitrospira* 一直被认为是滤池中完成亚硝酸盐氧化的优势属, 其丰度高于 *Nitrobacter* 等其他 NOB 的情况常有发生<sup>[46,53,57]</sup>。在滤料表面生物膜中, *Nitrospira* 占总细菌群落的相对丰度可达到 40% 以上<sup>[58]</sup>。除了典型硝化微生物外, 越来越多近年来的研究开始关注 comammox 在水厂中的存在。Pinto 等<sup>[39]</sup> 通过宏基因组技术从炭砂滤池的活性炭层样品中获得了一个具有全程硝化能力的 *Nitrospira* 拼接基因组 (metagenome-assembled genomes, MAGs), 证明了饮用水系统中 comammox 的存在。但 comammox 不同簇 (即 clade A 和 clade B) 在不同水厂中的分布也有明显区别。例如, 丹麦某给水厂砂滤池中 comammox clade B 为主导硝化微生物, 其丰度约占总 comammox 的 75%<sup>[32]</sup>。然而, 也有文献报道砂滤池中 comammox clade A *amoA* 基因拷贝数 ( $10^5-10^6$  GC/g-sand) 比 clade B *amoA* 基因拷贝数 ( $10^3-10^4$  GC/g-sand) 高出 1-2 个数量级<sup>[49]</sup>。这些研究证实了各类硝化微生物在滤池中的广泛存在, 反映了滤池在饮用水处理过程中对含氮污染物去除的贡献, 但不同硝化微生物间的丰度可能受水源类型、滤池构型、氨氮浓度等多种因素影响。

### 2.2 市政主管网与二次供水系统

市政主管网是出厂水配送的关键设施, 以氯胺为消毒剂的管网中, 硝化微生物的滋生可加速氯胺降解, 从而导致管网中条件致病菌的繁殖<sup>[54,59]</sup>, 威胁饮用水生物安全性。各类硝化微生物在市政主管网检出情况如表 2 所示。亚硝化单胞菌属 (*Nitrosomonas*) 是氯胺消毒管网中常见的优势氨氧化菌属<sup>[15,60]</sup>, 其在管内壁生物膜中的丰度可达 68.8%<sup>[15]</sup>。*Nitrospira* 在市政主管网也常被检出, 文献中汇报的相对丰度为 0.3%-22.4% (水相)<sup>[54,61]</sup> 和小于 27.4% (生物膜)<sup>[15,23]</sup>。

表 2 饮用水系统中硝化微生物标记基因检出丰度

Table 2 Densities of nitrifier gene markers in drinking water systems

Targeted organisms	Sampling sites	Sample types	Targeted gene markers and copy numbers	References
Ammonia oxidizing archaea	Biological activated carbon filters	Carbon	AOA <i>amoA</i> $10^6-10^7$ GC/g	[52]
			AOA <i>amoA</i> $10^{5.2}-10^{8.9}$ GC/g	[22]
	Rapid sand filters	Water	AOA <i>amoA</i> $10^4$ GC/L	[29]
		Sand	AOA <i>amoA</i> $<10^5$ GC/g	[53]
		Sand	AOA <i>amoA</i> $2.5 \times 10^3-1.4 \times 10^7$ GC/g	[49]
	Distribution mains	Water	AOA <i>amoA</i> $10^4$ GC/L	[29]
		Water	AOA <i>amoA</i> $<10^3$ GC/mL	[54]
Household taps	Water	AOA <i>amoA</i> $10^4-10^5$ GC/L	[29]	
Ammonia oxidizing bacteria	Biological activated carbon filters	Carbon	AOB <i>amoA</i> $10^{1.3}-10^4$ GC/g	[22]
		Water	AOB <i>amoA</i> $10^3$ GC/L	[29]
	Rapid sand filters	Sand	AOB <i>amoA</i> $10^6-10^8$ GC/g	[53]
		Sand	AOB <i>amoA</i> $1.5 \times 10^5-8.9 \times 10^5$ GC/g	[49]
		Water	AOB <i>amoA</i> $10^3$ GC/L	[29]
	Distribution mains	Water	AOB <i>amoA</i> $<10^4$ GC/mL	[54]
	Simulated water distribution systems	Biofilm	AOB <i>amoA</i> $<7.8 \times 10^2$ GC/cm <sup>2</sup>	[55]
Household taps	Water	AOB <i>amoA</i> $10^4-10^5$ GC/L	[29]	
Comammox <i>Nitrospira</i> clade A	Biological activated carbon filters	Water	Comammox clade A <i>amoA</i> $10^4-10^5$ GC/L	[29]
	Rapid sand filters	Water	Comammox clade A <i>amoA</i> $10^4$ GC/L	[29]
		Sand	Comammox clade A <i>amoA</i> $1.2 \times 10^3-4.2 \times 10^6$ GC/g	[49]
Household taps	Water	Comammox clade A <i>amoA</i> $10^3-10^4$ GC/L	[29]	
<i>Nitrobacter</i> spp.	Rapid sand filters	Sand	<i>Nitrobacter</i> 16S rRNA $10^4-10^6$ GC/g	[53]
<i>Nitrospira</i> spp.	Distribution mains	Water	<i>Nitrospira</i> 16S rRNA $<10^5$ GC/mL	[54]
sNOB	Biological activated carbon filters	Water	sNOB <i>cynS</i> $10^3$ GC/L	[29]
	Household taps	Water	sNOB <i>cynS</i> $10^3-10^4$ GC/L	[29]

二次供水系统是市政管网向用户端的延伸, 包括建筑管道与水箱系统。硝化微生物在二次供水系统中广泛存在。在家用水龙头出水中, AOA、AOB 和 comammox 的 *amoA* 基因均有检出(表 2)。在中国河南省和中国香港特别行政区的水龙头出水中, comammox *amoA* 序列的丰度可达到 AOA 和 AOB 的 *amoA* 序列的 1.7 倍和 4.4 倍<sup>[40]</sup>。此外, 在水箱水样甚至淋浴软管内壁生物膜样品中也有硝化菌属如 *Nitrospira* 被检出<sup>[62-63]</sup>。水箱是二供系统的关键设施, 其内底沉积物也是硝化微生物滋生的温床。Liu 等<sup>[64]</sup>利用微电极技术在水箱沉积物中检测到了硝化作用,

而且由于沉积物为硝化微生物的繁殖提供了保护性环境, 使用氯胺或氯消毒均无法完全穿透沉积物灭活全部硝化微生物, 但水箱沉积物中硝化微生物的丰度及群落结构暂未完全解析。

### 3 影响饮用水系统中硝化微生物的环境因子及工程条件

#### 3.1 温度

温度通过影响酶的活性从而影响微生物的生命活动<sup>[65]</sup>。低温条件下, 硝化微生物对氨氮的转化能力降低。例如, 在活性炭滤池运行过程中, 5 °C 时滤池的单位容积氨去除率降低至

25 °C时的 6.1%<sup>[66]</sup>。当环境温度升高时, 部分硝化微生物的生长可能受到抑制。以建筑管道为例, 当热水管道的温度上升时(51–58 °C), 管道内壁生物膜中 *Nitrospira* 的丰度呈现下降趋势<sup>[67]</sup>。然而, 目前对 *Nitrospira* 的最适生长温度尚无准确定论, 文献报道 *Nitrospira lenta*、*Nitrospira* sp. KM1 和 *Nitrospira inopinata* 的最适温度分别为 28<sup>[68]</sup>、30<sup>[69]</sup>和 37 °C<sup>[70]</sup>。

### 3.2 碳源

硝化微生物属自养型微生物, 但外界碳源可通过影响异养菌的数量与活性间接影响硝化微生物。由于碳源充足条件下异养菌的活性增加, 硝化微生物在与异养菌争夺氮源的过程中处于劣势, 限制了硝化微生物的生命活动, 所以在高碳氮比水体中的硝化作用相对减弱<sup>[71]</sup>。碳源也可以直接影响硝化微生物的生命活动。在氨氧化古菌 *Nitrosopumilus maritimus* SCM1 的培养过程中, 即使添加低浓度的有机化合物也会抑制 SCM1 的生长<sup>[3]</sup>。有学者认为, 某些有机化合物如多酚类(polyphenols)、单萜类(monoterpenes)对硝化作用的抑制机理可能与某些铜离子螯合剂类似<sup>[72]</sup>。

### 3.3 氨氮

由于不同硝化微生物对氨氮的亲合力存在明显差异, 所以环境中的氨氮浓度影响着硝化微生物的分布和丰度。一般来说, AOA ( $K_{\text{NH}_4^+}=0.36\text{--}4.40 \mu\text{mol/L}$ )<sup>[6]</sup>对氨氮的亲合力高于 AOB ( $K_{\text{NH}_4^+}=1.9\text{--}200.0 \mu\text{mol/L}$ )<sup>[6]</sup>, 更易在低氨氮条件下( $\text{NH}_4^+\text{-N}<0.02 \text{ mg-N/L}\text{--}0.26 \text{ mg-N/L}$ )<sup>[22,52]</sup>生存。有研究表明, 氨氧化古菌的氨氮亲合力与其细胞的比表面积相关, 细胞比表面积较大的氨氧化古菌往往具有更高的氨氮亲合力<sup>[73]</sup>。除个别海洋性氨氧化古菌(*Nitrosopumilus maritimus* SCM1,  $K_{\text{NH}_4^+}=0.003 \mu\text{mol/L}$ )<sup>[70,74]</sup>具有极高的氨氮亲合力外, comammox (*Nitrospira inopinata*,

$K_{\text{NH}_4^+}=0.049 \mu\text{mol/L}$ ; *Nitrospira krefftii*,  $K_{\text{NH}_4^+}=0.040 \mu\text{mol/L}$ )<sup>[70,75]</sup>相比其他典型氨氧化微生物能更能够适应低氨氮环境, 因此, 在饮用水系统中( $\text{NH}_4^+\text{-N}<0.53 \text{ mg-N/L}$ )常观察到 comammox 作为主导硝化微生物的情况<sup>[76]</sup>。然而, comammox 不仅存在于寡营养环境中, 在一些高氨氮的污水处理装置( $\text{NH}_4^+\text{-N}: 58\text{--}264 \text{ mg-N/L}$ )<sup>[38,77]</sup>中也能检测到较高丰度的 comammox *amoA* 基因。但也有研究发现, 当氨氮浓度高于  $0.025 \mu\text{mol/L}$  时, comammox 的氨氮氧化能力将受到抑制<sup>[75]</sup>。由于目前 comammox 的纯化菌株少, 对其生理生化特性的研究受到限制<sup>[70,75]</sup>, 因此, 环境中 comammox 全部特征的解析尚需进一步研究。

### 3.4 消毒剂

消毒剂是影响管网中硝化微生物分布和丰度的重要因素。在以氯胺为消毒剂的管网中, 常出现硝化微生物的滋生, 影响供水安全性。与无消毒剂管网( $1.1\times 10^3 \text{ amoA GC/cm}^2$ )相比, 氯胺消毒管网生物膜中的 AOB *amoA* 基因数量 ( $4.7\times 10^4 \text{ amoA GC/cm}^2$ )显著增高<sup>[60]</sup>。由于硝化微生物对消毒剂的耐受性差异, 不同消毒剂浓度可筛选出不同的硝化微生物群落。例如, 在市政主管网中, 由于氯胺沿程降解, 管道内部形成氯胺浓度梯度, 因而促成了水厂近端和远端管道内硝化微生物群落的显著差异<sup>[15]</sup>; 而在无消毒剂的管网中, 水厂近、远端输水管道内硝化微生物的丰度却无显著变化<sup>[19]</sup>。消毒剂类型(氯胺与氯消毒)对不同种属硝化微生物的灭活能力存在差异。一项基于 CDC 生物膜反应器的研究发现 AOA 对氯与氯胺消毒剂的抗性均优于 AOB<sup>[20]</sup>, 但具体机理尚不清楚, 这可能与 AOB 和 AOA 的细胞壁与细胞膜组成成分差异相关。

### 3.5 无机盐与金属离子

除了碳、氮等基本的营养物质外, 无机盐

也对硝化微生物至关重要。磷酸盐是限制硝化作用的无机盐之一。当滤池中的生物可利用磷(microbially available phosphorus, MAP)浓度低于 10  $\mu\text{g/L}$  时, 滤池中的硝化作用基本停止, 此时 AOB 的生长速率几乎为零; 这可能是由于 AOB 的磷酸盐亲和力( $K_p < 3 \mu\text{g/L}$ )低于其他异养菌, 使得 AOB 在低磷酸盐浓度下与异养菌的竞争处于劣势<sup>[78]</sup>。

铁、铜等金属元素也可以影响硝化微生物代谢活性。环境中的游离无机铁(free inorganic Fe, Fe')限制着氨氧化古菌的生长。AOA (*Nitrosopumilus maritimus* SCM1)对 Fe'的半饱和常数( $K_{Fe} = 361.5 \text{ pmol/L}$ )比异养菌和浮游微生物高 1–2 个数量级, 而且其自身无法产生铁载体(siderophore)从外界攫取 Fe'; 较低的 Fe'亲和力使 AOA 在与异养菌竞争 Fe'过程中处于劣势, 因此, AOA 更易在异养菌稀少的区域(如水体的透光区低层)生存<sup>[79]</sup>。铜离子是氨氧化步骤的关键金属离子。氨单氧化酶中存在多个铜离子结合位点<sup>[80]</sup>, 有研究表明, 铜离子螯合剂如双氰胺(dicyandiamide, DCD)、3,4-二甲基吡唑磷酸盐(3,4-dimethylpyrazole phosphate, DMPP)能够螯合结合位点上的铜离子<sup>[81–82]</sup>, 抑制氨单氧化酶的活性从而削减硝化作用。在水处理过程中, 常常通过外加铜离子来强化滤池的硝化能力<sup>[41]</sup>。

### 3.6 工程因素

饮用水系统的其他工程环境参数, 如水源、滤池构型和管道材料等因素都可对硝化微生物的群落特征造成影响<sup>[22,49]</sup>。水源中原始的硝化微生物丰度和群落特征影响着滤池中硝化微生物的组成。Hu 等<sup>[49]</sup>比较了河水、湖水两种水源对砂滤池中硝化微生物组成的影响, 发现在以河水为水源的砂滤池中, AOA *amoA*、comammox *amoA* 基因丰度显著高于以湖水为

水源的砂滤池, 而且两者氨氧化微生物的组成存在显著差异。滤池的构型是影响滤池中硝化微生物活性的关键因素。Rui 等<sup>[22]</sup>发现滤池流态可对池中硝化微生物的丰度和群落组成造成影响; 与下向流滤池相比, 上向流滤池具有更高的硝化微生物丰度和硝化潜力, 这与不同流态在池内营造的微生物生存条件的差异有关。此外, 不同材料供水管道内壁生物膜中硝化微生物的生长趋势存在差异。Kitajima 等<sup>[83]</sup>比较了黄铜、不锈钢、聚氯乙烯和聚甲醛这 4 种材料表面生物硝化微生物的生长情况, 发现 AOB 在不锈钢表面生物膜中丰度最高( $10^{3.94} \text{ GC/cm}^2$ ), 黄铜表面( $10^{2.14} \text{ GC/cm}^2$ )最少, 这可能与管道材料释放的某些金属离子有关。

## 4 硝化微生物的工程应用和管理

### 4.1 硝化过程的强化

新建成的滤池从启动到具备完整硝化能力所需的时间从几周到几个月不等<sup>[84]</sup>, 缩短滤池的启动时间能够有效减少能耗、提高经济效益<sup>[85]</sup>。对新滤池进行生物强化(bioaugmentation)是缩短滤池启动时间的有效手段。例如, Albers 等<sup>[85]</sup>利用接种硝化微生物的方式对新建滤池进行生物强化, 使得新建滤池具备完整硝化能力的时间提前了 11 d。此外, 在实验室规模的滤池装置运行过程中, 也可通过接种硝化微生物的方式减少装置启动时间, 缩短实验周期<sup>[86]</sup>。

在滤池实际的运行中, 常出现氨氮去除率低、亚硝酸盐积累等问题。向滤池中补充铜<sup>[41]</sup>、磷<sup>[78]</sup>等元素能够有效提高滤池的硝化能力。Wagner 等<sup>[41]</sup>研究了铜离子对砂滤池去除氨氮能力的影响, 发现一定剂量的铜离子( $< 1 \mu\text{g/L}$ )能够增加滤池中 AOB (10 倍)和 comammox (1.3–1.8 倍)的丰度, 而且能够显著提高滤池对氨氮的去除率(63%提高至 97%)。



## 4.2 硝化过程的监测

硝化微生物的丰度是反映供水管网硝化作用的重要指标。目前,大多数研究仍然以典型氨氧化菌 AOA 或 AOB 作为反映供水管网硝化作用的指示生物<sup>[54,87]</sup>。然而,与典型氨氧化微生物相比,新型全程硝化微生物 comammox 在供水管网中分布广、丰度高<sup>[29,40]</sup>,而且对供水系统的低氨氮环境具有更强适应性<sup>[70]</sup>。因此,将新型硝化微生物 comammox 纳为指示生物可为硝化作用监控提供更为完善的信息。

二次供水系统是硝化微生物的繁殖热区,是供水系统中氯胺降解最为剧烈的位点<sup>[62]</sup>。然而,目前对二供系统中硝化微生物关注度不高。因此,有必要加强对二供系统硝化微生物监测和硝化反应的监控,并采取相应措施确保用户端具有一定浓度的氯胺残留。

## 4.3 硝化过程的控制

在氯胺消毒管网中常采用短期内转换为氯消毒(free chlorine disinfection period, CIP)的方式控制硝化作用,这一方法被称为 chlorine burn。Wang 等<sup>[54]</sup>对实际管网研究表明,将氯胺消毒转换为氯消毒后,每毫升水样中 AOA、AOB 和 *Nitrospira* 的标记基因分别减少了 6.3、251.2 和 125.9 copies,说明将氯胺消毒转换为氯消毒能够抑制管网中硝化微生物的滋生;然而再次恢复至氯胺消毒后,管网中硝化微生物数量出现回升趋势,甚至会超过初始水平。

## 5 结语与展望

饮用水系统中,硝化微生物影响原水中含氮污染物的去除及管网中氯胺消毒剂的稳定。本文综述了硝化微生物的常用检测方法,分析了其在饮用水系统中的存在规律和影响因素,并探讨了硝化微生物的工程应用案例与管控方案。针对饮用水系统中的硝化微生物,目前仍

存在一些值得深入研究的关注点。

### (1) 供水系统硝化微生物的动态迁移规律

目前,大多数研究仅关注特定硝化微生物种群在饮用水系统中各位点的分布特征,尚不清楚硝化微生物在供水全流程中的动态迁移规律及种间生态位分离。未来研究应着眼于剖析不同类群硝化微生物在供水系统中的动态变化规律,阐明环境因素和工程条件对硝化微生物群落组成的影响机制,为供水系统硝化微生物的调控提供理论依据。此外,鉴于二次供水系统属性有利于硝化微生物的滋生和硝化反应发生,有必要对二次供水系统硝化微生物生长规律、影响因素和控制措施展开进一步探究。

### (2) 硝化微生物原位硝化作用贡献度

目前,国内外研究大多以 MPN 计数、qPCR 测定或扩增子测序等方法测定 AOA、AOB 等硝化微生物的丰度,以此为基础判定其在饮用水系统中的硝化作用贡献度。近年来,随着全程硝化微生物 comammox 的发现,饮用水系统中不同类别硝化微生物对硝化作用的具体贡献度及相互作用关系成为新的科学问题。不同硝化微生物对底物亲和力、比利用率不同,对环境压力的具体响应机制存在差异,仅以硝化微生物的丰度评估其对硝化作用的贡献度可能存在较大误差<sup>[76,88]</sup>。未来研究可结合反转录 PCR、转录组、荧光原位杂交等技术手段进一步探究各类硝化微生物对硝化作用的原位贡献率。

### (3) 氯胺消毒管网硝化过程的控制

氯胺消毒管网中的硝化作用是加剧氯胺降解的重要原因。现阶段研究大多采用 chlorine burn 抑制管网中硝化微生物繁殖。然而,高频次的 chlorine burn 可能会造成饮用水中重金属离子浓度升高<sup>[89]</sup>、消毒副产物生成<sup>[90]</sup>等问题,对饮用水安全性造成潜在威胁。同时, chlorine burn 还可能筛选具有消毒剂抗性的硝化微生

物, 进一步降低该举措的效果。因此, 未来研究应阐明不同硝化微生物对 chlorine burn 的响应差异性, 关注高频次 chlorine burn 条件下微生物群落变化规律, 结合实际工况进一步优化 chlorine burn 实施频次和持续时间, 从而降低其负面影响。此外, 应在出厂水投加消毒剂的过程中优化氯与氨的投加比例, 尽可能减少管网中原始氨氮的引入, 同时强化管网中氨氮、亚硝酸盐浓度及硝化微生物丰度等指标的动态监测, 从而为供水管网提供实时化、精准化和高效化的硝化作用管控手段。

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