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专论与综述

活性污泥中菌群多样性及其功能调控研究进展

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摘　要：活性污泥是污水处理厂生物处理工艺的功能主体，活性污泥中菌群的种类、数量及活性是提高污水处理能力与效果的重要基础。本文综述了活性污泥处理工艺中的主要功能细菌(絮凝菌、脱氮菌、除磷菌等)生物群落的多样性与生态特征，并对目前主流的菌群鉴定方式进行总结，最后从运行条件、定向驯化及生物强化 3 个方面对菌群调控进行论述，以期为活性污泥法污水处理工艺提供一些理论指导。

关键词：活性污泥，菌群多样性，生物鉴定，定向驯化，生物强化

Research progress of bacterial diversity and functional regulation in activated sludge

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Abstract: Activated sludge is the main function body of biological treatment process in wastewater treatment plant. The species, quantity and activity of bacteria in activated sludge are the important basis for improving wastewater treatment capacity and effect. Based on the summary and analysis of the relevant literatures, the diversity and ecological characteristics of the main functional bacteria (flocculants, denitrifying bacteria, phosphorus removal bacteria, etc.) in activated sludge treatment process were summarized, and the current mainstream identification methods of bacteria were also summarized. Finally, the regulation and control of micro flora in activated sludge are discussed from three aspects: operation conditions, directional domestication and biological reinforcement, providing a certain theoretical basis for improvement of the functional stability of sewage treatment plants.

Keywords: Activated sludge, Microbial diversity, Biological identification, Directional domestication, Bioaugmentation

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活性污泥法是应用广泛、运行稳定的污水处理工艺，已有 100 多年的历史。迄今为止，世界各地至少有 5 万座基于活性污泥法的污水厂在运行，每天处理的污水量超过 5 亿 m^3[1]。活性污泥由多种好氧微生物、兼性厌氧微生物(少量厌氧微生物)与废水中的有机、无机固体物混凝交织在一起所形成，外观呈黄褐色絮绒状，具有较高的生物多样性和活性，是活性污泥工艺的主体。菌胶团是活性污泥的核心物质，在其上生活着放线菌、真菌、原生动物和微型后生动物等多种微生物[2]。这些微生物与非生物体构成众多微生态系统，成为污水处理的功能单元，其中细菌起主导作用，数量可占到微生物总量的 90%以上[3]。活性污泥菌群结构多样性以及优势菌群之间的相互作用决定了污水处理厂生物处理功能的稳定性，群落的改变常常影响污水厂的处理效率与出水水质[4]，甚至出现污泥膨胀[5]、污泥泡沫[6]等故障，导致污水厂无法正常运行。因此，了解活性污泥中细菌群落结构组成及多样性，选择性地对菌群功能进行调节，使其中的微生物更好地发挥净化作用，对于提升污水处理系统整体效果、降低处理成本具有重要意义。

1 活性污泥菌群多样性的研究

1.1 活性污泥菌群组成

一个城市污水处理厂中的微生物数量多达 10^{18} 个[7]，这些不同种类微生物协同作用实现水质净化，解析这些微生物的组成与多样性，是认识污水生物处理过程与微生物生态学规律的基础。早期对活性污泥菌群的检测多采用光学显微镜及传统的分离培养法，该方法发现的主要优势菌群有动胶杆菌属(*Zoogloea*)、丛毛单胞菌属(*Comamonas*)、不动杆菌属(*Acinetobcter*)、螺菌属(*Spirillum*)、产碱杆菌属(*Alcaligenes*)、短杆菌属(*Brevibacterium*)、黄杆菌属(*Flavobacterium*)和假单胞菌属(*Pseudomonas*)等[8-10]。但随后大量研究表明：常规培养条件下只有不到 15%的细菌种类能够正常生长，难以对活性污泥微生物种群进行完整分析[11-13]。近年来，由于分子生物学技术的应用，越来越多的微生物被发现，可较全面地了解活性污泥菌群结构。

1.2 活性污泥功能菌群

1.2.1 絮凝微生物

活性污泥中一部分细菌能够分泌胞外聚合物(Extracellular polymeric substance，EPS)，EPS 将大量分散的细菌、阳离子(如 Ca^{2+}、Mg^{2+})和其他细颗粒桥联，形成活性污泥絮体骨架[14]，这类细菌被称为絮凝菌，主要包括动胶菌属(*Zoogloea*)[15]、芽孢杆菌属(*Bacillus*)[16]、黄杆菌属(*Flavobacterium*)[17]、诺卡氏菌属(*Nocardia*)和假单胞菌属(*Pseudomonas*)等[18]，种类多达数十种，其特性见表 1。

丝状菌也是一种重要的絮凝菌[19]，是构成菌胶团不可或缺的一部分，目前发现的丝状细菌约 58 种，其中变形杆菌 21 种、放线菌 14 种、拟杆菌 8 种、厚壁菌 5 种、浮霉菌 5 种、绿弯菌 3 种、热微菌 2 种[18]。丝状菌对营养物质的摄取能力较强，当丝状菌过度繁殖时，大量的菌丝会阻碍污泥絮体之间的压缩，导致絮体松散、破碎，造成严重的污泥膨胀[20]。相关研究表明，低 DO、低 F/M、低 pH、高硫化物等均是丝状菌污泥膨胀的诱因，但丝状菌自身对液氯、过氧化氢等杀毒剂的抵抗力较弱，在生产上可利用这些生理特性控制此类污泥膨胀[21-23]。

1.2.2 脱氮微生物

自然界的氮循环包括同化作用、氨化作用、硝化作用、反硝化作用、固氮作用以及厌氧氨氧化等。活性污泥中微生物实现氮素转化主要依赖体内存在的代谢酶，编码这些酶的基因可作为相应的功能标记基因(图 1)[24]，并通过宏基因组等分子生物学技术进行鉴定，从而确定相应的微生物菌种。在污水处理中，硝化和反硝化作用被认为是最主要的脱氮机制。

(1) 硝化作用

氨经过微生物作用氧化成亚硝酸，再进一步氧化成硝酸，该过程被称为硝化作用(Nitrification)，是由两类细菌分两阶段进行[25]。第一阶段，氨被氧化成亚硝酸，由亚硝酸菌(氨氧化菌 Ammonia oxidizing

表 1　活性污泥中主要絮凝菌种

Table 1　The main flocculating bacteria in activated sludge

菌属 Genus	所属门类 Phylum	生理特征 Physiological characteristics
动胶菌属	变形杆菌门	细胞杆状，无孢子或孢囊；成熟细胞上有指状或树枝状突起物；革兰氏阴性；化能异养
Zooglobin	*Proteobacteria*	Rod-shaped, no spores or sporangia; Fingerlike or dendritic projections on mature cells; Gram-negative; Chemotrophic heterotrophy
埃希氏杆菌属	变形杆菌门	直杆菌，单个或成对；以周生鞭毛运动或不运动
Escherichia	*Proteobacteria*	Straight bacilli, Single or paired; Moving with peritrichaete or not moving
假单胞菌属	变形杆菌门	直或弯；单鞭毛或多鞭毛；无鞘或突起物；革兰氏阴性；化能异养
Pseudomonas	*Proteobacteria*	Straight or curved; Single or multiple flagella; No sheath or protuberance; Gram-negative; Chemotrophic heterotrophy
芽孢杆菌属	厚壁菌门	菌体杆状；鞭毛侧生；革兰氏反应在生长早期为阳性；化能异养
Bacillus	*Firmicutes*	Rod-shaped; Lateral flagellum; Gram-positive in early growth stage; Chemotrophic heterotrophy
黄杆菌属	拟杆菌门	直杆状，端圆，不形成内生孢子；革兰氏阴性；无滑动或泳动；化能异养
Flavobacterium	*Bacteroidetes*	Straight bacilli, end circle, No endospore formation; Gram-negative; No sliding or swimming; Chemotrophic heterotrophy
诺卡氏菌属	放线菌门	早期分裂成类球菌状和杆菌状，产生茂盛的菌丝体；革兰氏阳性；不运动
Nocardia	*Actinobacteria*	Coccoidor bacterioid forms at early stage and produce flourishing mycelia; Gram-positive; Not moving

bacteria，AOB)完成；第二阶段，亚硝酸被氧化为硝酸，由硝酸菌(亚硝酸氧化菌 Nitrite oxidizing bacteria，NOB)完成。这两类硝化细菌均是化能自养型，生长极其缓慢，且易受到 pH 值、温度等外界条件的影响。活性污泥中 AOB 主要包括亚硝化单胞菌(*Nitrosomonas*)和亚硝化螺菌(*Nitrosospira*)[26]，其中亚硝化单胞菌包括 *N. europaea/eutropha*、*N. communis*、*N. oligotropha*、*N. marina* 和 *N. cryotolerans* 在内的 5 个种。亚硝化螺菌包括亚硝酸螺旋菌属、亚硝酸颤菌属和亚硝酸叶菌属[27]。随着菌群鉴定技术的发展，一些氨氧化细菌相继被发现(表 2)，极大地充实了硝化细菌的种群库。

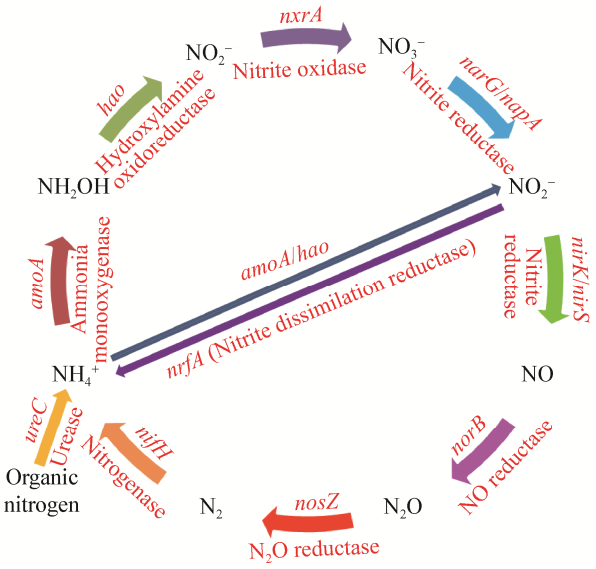

图 1　微生物氮循环及其功能基因

Figure 1　Nitrogen cycling and microbial functional genes

NOB 均为革兰氏阴性，一般与 AOB 分布在一起，尤其喜欢有机质含量低而富含无机氮的环境。通常认为 NOB 的优势菌群为硝化杆菌(*Nitrobacter*)，有 4 类菌属已定名为 *Nitrobacter winogradskyi*、*Nitrobacter hamburgensis*、*Nitrobacter vulgaris* 和 *Nitrobacter alkalicus*。一些研究表明，硝化螺菌(*Nitrospirae*)也有可能是优势 NOB，这些细菌能够利用有机碳源和无机碳源，至今无法通过传统培养方法进行分离。除此之外，NOB 还包括两种海洋种属 *Nitrococcus mobilis* 和 *Nitrospina gracilis*，分别归类为变形菌纲(*Proteobacteria*)中的 γ 和 δ 亚类。NOB 各个属代表性菌株的形态特征见表 3。

(2) 反硝化作用

反硝化作用的实现主要依赖反硝化细菌，反硝化细菌广泛存在于自然界中且数量巨大，它们占土壤、水体和水底淤泥中微生物总量的 10%−15%，分属于假单胞菌属(*Pseudomonaceae*)、产碱杆菌属

表 2 近年来研究发现的氨氧化细菌

Table 2 Ammonia-oxidizing bacteria discovered in recent years

细菌名称 Bacteria name	年份 Year	环境 Environment	基因序列 Genome sequence	参考文献 References
Candidatus Nitrosoglobus terrae	2017	酸性土壤 Acid soil	Y	[28]
Nitrosococcus wardiae D1FHS	2016	海洋沉积物 Marine sediments	N	[29]
Nitrosomona sp. NP1	2017	活性污泥 Activated sludge	N	[30]
Nitrosomonas mobilis Ms1	2016	污水厂污泥 Sewage sludge	Y	[31]
Candidatus Nitrosocaldus cavascurensis	2018	温泉 Hot spring	Y	[32]
Candidatus Nitrosocaldus islandicus	2018	温泉 Hot spring	Y	[33]
Nitrosomarinus catalina SPOT01	2017	海洋 Ocean	Y	[34]
Nitrosopumilus cobalaminigenes HCA1	2017	海洋渔场 Ocean fisheries	N	[35]
Candidatus Nitrosocosmicus exaquare G61	2017	污水厂 Sewage treatment plant	Y	[36]
Candidatus Nitrosopumilus sp. NF5	2016	海洋 Ocean	Y	[37]
Candidatus Nitrosocosmicus franklandus	2016	中性土壤 Neutral soil	N	[38]
Nitrospira inopinata	2017	热水管道 Hot water pipeline	Y	[39]

注：Y：该菌种的基因序列已测，可从基因数据库中查到；N：菌种基因序列未测或数据库中无此菌种.

Note: Y: The gene sequence of the strain has been determined and can be found in the gene database; N: Undetected strain gene sequence or no strain in the database.

表 3 各种硝酸菌形态特征

Table 3 Morphological characteristics of various nitrite-oxidizing bacteria

细菌名称 Bacterial name	特征 Features							
	形态 Shape	细胞尺寸 Cell size (μm)	内膜 Intima	鞭毛 Flagella	温度 Temperature (°C)	pH	游动性 Moving ability	繁殖方式 Reproduction
Nitrobacter	梨状或多形态杆状 Pyriform or multiform rod	(0.5−0.9)×(1.0−2.0)	扁平泡囊 Flat vesicle distribution	极生或侧生 Polar or lateral	5−37	6.5−8.5	有 Yes	出芽或二分裂 Budding or bipartition
Nitrococcus	球状 Globular	1.5−1.8	管状分布 Tubular distribution	极生 Polar flagella	15−30	7.5−8.0	有 Yes	二分裂 Bipartition
Nitrospina	细杆状 Rhabditiform	(0.3−0.5)×(1.7−6.6)	无内膜 No intima	未观察到 No observation	20−30	7.0−8.0	无 No	二分裂 Bipartition
Nitrospira	疏松螺旋状 Liciform	(0.2−0.4)×(0.9−2.2)	无内膜 No intima	未观察到 No observation	20−30	7.6−8.0	无 No	二分裂 Bipartition

(*Caicaiigenes*)、芽孢杆菌属(*Bacillus*)等 50 多个属[40]。反硝化细菌大致可分为厌氧反硝化细菌和好氧反硝化细菌两大类。传统上，反硝化被认为是一个严格厌氧的过程，O_2 的存在会阻碍 NO_3^-和 NO_2^-作为电子受体[41]，同时抑制反硝化还原酶的活性[42]。20 世纪 80 年代 Robertson 等[43]首次发现好氧反硝化细菌并证明好氧反硝化酶系的存在以来，随后好氧反硝化细菌得到广泛关注。表 4 列举了近年来关于好氧反硝化细菌的一些研究成果。

1.2.3 除磷微生物

除磷是污水处理过程中很重要的一部分，磷对水体富营养化的贡献远大于氮素。目前在污水处理厂中应用较多的是强化生物除磷(Enhanced biological phosphorus removal，EBPR)工艺。EBPR 主要通过聚磷菌(Phosphate accumulating organisms，PAOs)在厌氧条件下释磷，在好氧条件下过量吸磷，然后以

表 4 好氧反硝化菌来源及其功能

Table 4 Sources and functions of aerobic denitrifying bacteria

来源 Source	菌种 Bacteria	除氮 Nitrogen removal	除磷 Phosphorus removal
活性污泥 Activated sludge	粪产碱菌 *Alcaligenes faecalis*[44]	+	–
	假单胞菌 *Pseudomonas* C-17[45]	+	–
	Shinella zoogloeoides[46]	+	–
	不动杆菌属 *Acinetobacter* sp. J6[47]	+	+
		–	–
污水处理系统 Sewage treatment system	假单胞菌属 *Pseudomonas* sp.[48]	+	–
	陶厄氏菌属 *Thauera*[49]	+	+
	副球菌 *Paracoccus denitrificans*[50]	+	–
	肺炎克雷伯氏菌 *Klebsiella pneumonia*[51]	+	–
		–	–
养猪废水处理系统 Pig wastewater treatment system	假单胞菌 *Pseudomonas stutzeri*[51]	+	–
	红球菌属 *Rhodococcus* sp.[52]	+	–
	Citrobacter diversus[53]	+	–
土壤 Soil	芽孢杆菌 *Bacillus*[54]	+	–
	Mesorhizobium sp.[55]	+	–
垃圾渗滤液 Landfill leachate	*Zobellella taiwanensis* DN-7[56]	+	–
富营养水体 Eutrophic water	阴沟肠杆菌 *Enterobacter cloacae* HW-15[57]	+	+
盐碱湖 Saline soda lake	*Halomonas campisalis*[58]	+	–
太湖底泥 Taihu Lake sediment	假单胞菌 *Pseudomonas stutzeri* YG-24[59]	+	+
二沉池污泥 Secondary clarifier sludge	不动杆菌属 *Acinetobacteria*[60]	+	+

注：+：具备脱氮或除磷功能；−：不具备脱氮或除磷功能.

Note: +: Bacterium has the function of nitrogen (or phosphorus) removal; −: Bacterium doesn't have.

剩余污泥形式将富磷的聚磷菌排出,进而达到将磷从污水中去除的目的[61]。聚磷菌不是生物分类单位，而是厌氧释磷、好氧超量吸磷的异养型细菌的统称。

由于部分聚磷菌菌种不能以单菌种存在，早期的分离培养方式无法对所有聚磷菌种属进行鉴定，近年来利用非培养方法发现了许多不能培养的 PAOs，揭示了 PAOs 在活性污泥中的重要作用，已发现的 PAOs 种类主要有：不动杆菌属(*Acinetobacte*)、气单胞菌属(*Aeromona*)、假单胞菌属(*Pseudomonas*)、小月菌属(*Microlunatus*)、俊片菌属(*Lamproprdia*)、红环菌属(*Rhodocyclus*)、产碱杆菌属(*Alcaligenes*)、酵母菌型 PAOs。此外，丙酸杆菌、克雷伯氏菌、枯草芽孢杆菌等也具有除磷功能。

大量研究表明，在缺氧条件下一些微生物可以利用硝酸盐或亚硝酸盐作为电子受体完成过量吸磷，这一类具有缺氧除磷功能的微生物被称为反硝化聚磷菌(Denitrifying phosphate-removal bacteria，DPB)[62]，主要存在于细菌中，在放线菌中也有少量报道。已鉴定的反硝化聚磷菌有假单胞菌属、不动杆菌属、芽孢杆菌属、红环菌属、副球菌属等。研究发现一些反硝化细菌可在好氧条件下聚磷，即好氧反硝化聚磷菌，但其脱氮机制目前尚无统一定论。我们从黑臭底泥中分离出一株施氏假单胞菌(*Pseudomonas stutzeri*),经驯化后具有良好的好氧反硝化除磷能力，并能够在低 C/N 比的污水中稳定运行，为低 C/N 比生活污水的同步脱氮除磷提供了一种新途径。

1.2.4 其它功能性微生物

活性污泥中还分布着一些具备特有功能属性的菌群，这些菌群有的能降解污水中难降解的污染物质，有的由于特定的生理生态特征在污水处理系统运行时起到指示作用。如嗜酸寡养单胞菌(*Stenotrophomonas acidaminiphila*)可降解拟除虫菊酯[63]，红球菌(*Rhodococcus*)常用来修复被石油污染的土地[64]，芽孢杆菌可利用聚丙烯酰胺作为碳源和氮源并对其进行生物降解[65]，β-变形杆菌(*Betaproteobacteria*)的大量出现往往意味着活性污泥系统存在污泥膨胀风险[66]。这些功能菌的存在为活性污泥的广泛应用提供了基础，是活性污泥工艺百年来经久不衰的源泉。

2 活性污泥菌群鉴定方法

早期对污泥中菌群种类识别主要采用分离培养法[8]，通过分离纯化使微生物群体分开从而获得单一菌群，其对菌种的识别主要通过形态观察，可鉴定的微生物种类有限。20 世纪 80 年代后 PCR 技术、克隆文库、荧光原位杂交(FISH)等免培养的分子生物学技术(Cultivation-independent technique)逐渐成为菌群鉴定的主要方法。表 5 列举了一些常见的菌群鉴定方法，其中使用较广泛的主要有分离培养法、16S rRNA 基因序列分析、分子探针及高通量测序技术。

3 活性污泥菌群调控研究

3.1 运行条件

活性污泥是一种复杂的生命体，运行条件的改变会导致微生物群落结构随之改变，而活性污泥生物群落的重建需要一定时间，污水处理厂常常更加关注出水的化学指标(如氮磷、COD 等)，将活性污泥视为化学系统而不是生物系统，在运行参数的调整中造成原有污泥生物群落的破坏。污水处理反应条件对微生物群落的影响研究表明：溶氧、污泥负荷、温度等因素对活性污泥中菌群数量、种类及活性均存在较大影响[67-69]，如何降低进水波动对活性污泥运行的影响，并为活性污泥中的微生物提供适宜的生长繁殖条件，使活性污泥中的生物群落处于一个相对稳定的状态，是活性污泥法污水处理中需要重点研究解决的问题。

3.2 定向驯化

污泥定向驯化采用成熟的活性污泥作为对象，驯化为具有处理特定污染物能力的污泥。驯化的方法是在混合液中逐步提高含污染物质废水的比例，直至达到对特定废水所要求的满负荷及很高的处理效率为止。驯化过程中，能分解废水的微生物得到发展，不能适应的微生物被淘汰。菌群多样性是活性污泥驯化的基础[70]，随着驯化条件不断强化，菌群多样性呈现递减的趋势，驯化条件越极端，菌群的多样性越小，说明活性污泥的驯化是外部条件对细菌种群定向选择的结果[71]。自活性污泥法问世以来，研究者们定向驯化出各种功能菌种，这些菌种能够降解特定的污染物，为工业废水的处理做出了巨大贡献[72-74]。

3.3 生物强化

活性污泥中细菌数量巨大，一般来说，污水处理效果的好坏取决于菌群活性是否发挥了最大效能，微生物菌群活性的提高除优化反应条件外还可采用生物强化的方式。生物强化主要包括两个方面：(1) 添加特殊的微生物菌剂，这些菌剂是从污染环境中筛选驯化的较普通微生物更有活性的菌种，因此菌剂的加入通常可以提高活性污泥的整体活性；(2) 通过添加微生物制剂(如酶制剂、次级代谢产物)改善活性污泥菌群结构，从而提高整体污泥的活性，即所谓“微生态调控”。Wang 等[75]从处理造纸废水的活性污泥中分离出降解苯酚的真菌(*Magnusiomyces capitatus*)，经过驯化培养并将其接种到含酚废水处理中，结果证明接种后活性污泥菌落丰度和多样性增加，形成一个稳定的微生态系统，显著提高了含酚废水的处理效率。Roy 等[76]通过添加生物表面活性剂及产甲烷复合菌剂，强化活性污泥对富烃炼油废水的处理，结果表明采用生物强化活性污泥的方法可使炼油废水中的石油烃分解速度提高 46%−55%。

表 5 活性污泥菌种主要的鉴定方法

Table 5 The main identification methods of activated sludge bacteria

鉴定方法 Identification methods	方法介绍 Method introduction	优点 Advantages	缺点 Disadvantages
分离培养法 Isolation of pure culture	采用接种及菌落计数的方法,通过观察微生物的生理特性及形态构造等进行分类鉴定 Using inoculation and colony counting, through observing the physiological characteristics and morphological structure of microorganisms to classify and identify microorganisms	要求简单,适合分离特定功能的微生物 Simple, suitable for isolating microorganisms with certain functions	局限性大，不能单独分离共生细菌 Limited to isolate symbiotic bacteria
16S rRNA 基因序列 16S rRNA gene sequence analysis	通过比较分析 16S rRNA 基因序列，从而在微生物系统发育上进行分类 Classification of microbial phylogeny by comparative analysis of 16SrRNAgene sequences	能动态观察群落变化 Dynamic observation of community changes	需要与已有的序列比对 Need to compare with existing sequences
高通量测序 High throughput sequencing	将 DNA(cDNA)随机片段化、加接头，制备测序文库，对文库中数以万计的克隆进行延伸反应,检测对应的信号，最终获取序列信息 Preparing DNA sequencing library and extending the library to detect corresponding signals and obtain sequence information	快捷，处理量大，一次可对几百万条 DNA 分子进行分析 Quick and large processing capacity	信息的分析和解读能力不足 Insufficient analysis and interpretation of information
克隆文库 Clone library	以微生物基因组 DNA 序列信息为依据，通过分析样品中 DNA 分子种类和数量来反映微生物区系的组成 Reflect the microflora group by analyzing the species and quantity of DNA molecules in the samples	16S rRNA 基因扩增较易，能全面地反映样品中的微生物组成 Easy to amplify and can fully reflect the microbial composition	只能建立已知基因的文库，通量很低 Only a library of known genes can be established with low throughput
核酸分子杂交 Molecular hybridization of nucleic acid	使用已知序列的单链核酸片段作为探针，去查找各种不同来源的基因组 DNA 分子中的同源基因或同源序列 Using single-stranded nucleic acid fragments as probes to locate homologous genes or sequences in DNA molecules	特异性强，杂交速度快 High specificity and fast hybridization	探针分子所带标记物少则灵敏度较低 Sensitivity of probes is lower if there are fewer labels on probes
荧光原位杂交 Fluorescence *in situ* hybridization	通过荧光标记的寡核苷酸探针特异地和互补核酸序列在完整的细胞内结合,用显微镜和流式细胞术等荧光检测技术进行观察和分析 Fluorescent probes are specifically binded to complementary nucleic acid sequences, using fluorescence detection to observe and analyze	安全、简便、灵敏、快速，可同时检测几种微生物 Safe, simple, sensitive and fast	应用较短探针时，效率下降 Efficiency decreases if a shorter probe were used
生物芯片 Biochip	通过固化生物大分子与待测样品中靶分子杂交,检测杂交信号强度从而判断靶分子的数量 Detection of hybridization signal intensity by hybridization of solidified biological macromolecule with target molecule	高度并行性、微型性、自动化和快速检测 Miniaturization, automation, rapidity	定量准确性及重现性不太好 Quantitative accuracy and repeatability are not good
宏基因组学 Metagenomics	对环境样品中全部微生物的总 DNA(宏基因组，metagenomic)进行克隆，并通过构建宏基因组文库和对比筛选等手段获得该环境中微生物的遗传多样性和分子生态学信息 Cloning the metagenomic gene and obtaining the genetic diversity of microorganisms by constructing the macrogenomic library	可发现难培养或不可培养微生物以及相关的功能基因 Difficult or uncultured microorganisms and related functional genes can be found	文库所包含的微生物基因不全面 Microbial genes contained in the library are incomplete
DGGE/TGGE	依据双链 DNA 片断熔解行为的不同,分离 PCR 产物中长度相同但序列不同的 DNA 标记片断(rRNA 或 rDNA) Separation of DNA marker fragments with different sequences in PCR products according to different melting behavior of double-stranded DNA fragments	可直观反映微生物群落的结构和多样性 Visually reflects the structure and diversity of microbial communities	对有多个不同碱基序列差异的 DNA 片段的分离效果较差 Difficult to isolate DNA fragments with different base sequences

(待续)

(续表 5)

脂肪酸图谱 Fatty Acid Methyl Esters Analysis	通过分析脂肪酸谱图的特异性来监测菌群变化 Changes in flora are monitored by analyzing the specificity of fatty acid profiles	简便、灵敏、可重复性强 Simple, sensitive and repeatable	分类水平较低 Lower classification level
醌类图谱 Quinone profile	以特征化合物醌为标记来解析微生物群体组成的方法 Analyzing microbial population composition using characteristic quinone as label	简便、灵敏、可重复性强 Simple, sensitive and repeatable	不能具体属或种 Unable to specify genus or species
现代培养 Modern culture and separation technology	模拟自然环境，构建类似于污泥运行环境的生长条件，得到无法人工培养或环境中丰度较低的微生物 Constructing sludge environment conditions to obtain microorganisms that cannot be cultured artificially	可培养传统方法未能培养的微生物 Get microorganisms that could not be cultured by traditional methods	无法定向操作，结果存在不确定性 Unable to operate directionally

4 结论与展望

活性污泥中微生物种类主要包括絮凝菌、脱氮菌、除磷菌及一些其他的功能菌，菌群种类和结构的多样性决定着系统的稳定性与处理能力。活性污泥菌群鉴定方法从传统的分离培养发展到现在的分子生物学分析，应用较广泛的技术有 16S rRNA 基因序列分析、分子探针技术及高通量测序技术。国外目前已开始应用的免疫培养技术和现代培养技术，将传统的培养分离法与非培养技术相结合，克服二者在菌群鉴定方面各自的缺陷，是未来污泥菌群鉴定的一个重要方向。

活性污泥菌群的调控对于优化菌群结构、提高菌群活性和减少剩余污泥产量有着重要意义，目前常采用控制运行条件、定向驯化及生物强化的方式进行菌群调控。现阶段的菌群调控水平依旧较低，未来菌群调控应向分子方向发展，通过调节代谢酶及功能基因达到菌群性能精准调控的目的。另一方面，构建活性污泥法生物场数学模型，并结合人工智能学习不断提高调控水平，也是活性污泥调控的一个重要研究方向。

污水厂现有的监测多针对出水水质，而在出水水质改变前，作为污水处理功能主体的活性污泥可能已受到严重损害，菌群结构与功能发生改变，因此现有的污水处理过程监测存在很大滞后性。未来可以运用灵敏、快捷的分子生物学检测方法，结合生物信息工程技术，直接在线监测活性污泥菌群结构与数量，及时进行调控，从而有效保障污水处理效果。

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