

## Research Highlight

# Microbes in the Water Infrastructure: Underpinning Our Society

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How do you feel when you hear the words “wastewater,” “sludge,” “sewer,” and “sewage”? These words may make you feel uncomfortable. However, as Victor Hugo wrote in “Les Misérables,” these words are inevitably related to human society (16):

“... The history of men is reflected in the history of sewers. The *Germoniae* narrated Rome. The sewer of Paris has been an ancient and formidable thing. It has been a sepulchre, it has served as an asylum. Crime, intelligence, social protest, liberty of conscience, thought, theft, all that human laws persecute or have persecuted, is hidden in that hole ...”

Since ancient Roman times, sewer pipe/tunnel networks have been built beneath urban areas to gather sewage/wastewater discharged from human activity (9). Biological treatment technologies such as activated sludge (3–5) and anaerobic digestion (AD) (6, 41) have been developed since the early 1900s to remediate sewage/wastewater constituents. Nowadays, these biological treatment processes are used as the core technology of modern municipal wastewater treatment processes (WWTP) and have become the underlying infrastructure for society. Within these processes, diverse microorganisms (eukaryotes [15, 33] and prokaryotes [35, 56]) form “sludge” and play a critical role in sewage/wastewater remediation. Thus, the characterization of sludge microbial assemblages and identification of their *in situ* functions have been conducted using cultivation, microscopic, and molecular techniques.

Nitrification, the stepwise biological conversion of ammonia to nitrate, is an important step in municipal WWTP. The long-term (*i.e.*, between 5 and 6 years) microbial community monitoring of activated sludge systems by employing the modern massive parallel sequencing of 16S rRNA gene amplicons revealed the temporal dynamics of core community members including nitrifying bacteria (*i.e.*, *Nitrosomonas*, *Nitrospira*, and “*Ca. Nitrotoga*”) (20, 51). Microradiography combined with fluorescent *in situ* hybridization (MAR-FISH) showed that “*Ca. Nitrotoga*”-type nitrite-oxidizing bacteria (NOB) may play a role in nitrite-dependent autotrophic carbon fixation (30). Using the  $\beta$ -subunit of the nitrite oxidoreductase (*nxrB*) gene, amplicon pyrosequencing uncovered the high diversity of *Nitrospira* spp., expanding to lineages I, II, and the novel ‘WWTP Ingolstadt 454 lineage’, in activated sludge systems (13). Fujitani and colleagues (12) enriched *Nitrospira*-type NOB from nitrifying sludge by using a laboratory-scale continuous feeding reactor with porous poly-

ester non-woven fabric materials as biomass carriers. They then successfully isolated the novel bacterial species “*Nitrospira japonica*,” associated with *Nitrospira* lineage II (54). Itoh *et al.* (19) isolated the thermotolerant ammonia oxidizer *Nitrosomonas* sp. JPCCT2, which is capable of growing at temperatures of up to 48°C, from activated sludge in a thermal power station. Until recently, nitrification was considered to be performed by two metabolically distinct organisms, *i.e.*, an ammonia oxidizer and nitrite oxidizer. As if to rewrite the textbook of microbiology, Daims *et al.* (10) isolated “*Ca. Nitrospira inopinata*” as the first complete ammonia oxidizer (comammox) from a microbial biofilm developing on the walls of a pipe under a flow of hot water. In addition to this isolated representative, they identified several comammox-like metagenomic bins derived from a full-scale activated sludge tank, membrane bioreactor, and groundwater wells. Hanada *et al.* (14) also reported that organisms assigned to uncultivated phylum TM7 (“*Ca. Saccharibacteria*”) predominated in laboratory-scale acidophilic nitrifying sequencing-batch reactors (ANSBR), suggesting that TM7 plays a specific role in nitrification in acidic environments. Kindaichi *et al.* (24) very recently reported that “*Ca. Saccharibacteria*” organisms are capable of utilizing glucose under aerobic, anoxic (nitrate-reducing), and anaerobic conditions, as demonstrated using MAR-FISH and enzyme-labeled fluorescence combined with FISH (ELF-FISH) analyses.

An enhanced biological phosphorous removal (EBPR) system is implemented in many municipal WWTP to decrease phosphate concentrations in sewage/wastewater (11, 42). In the EBPR system, polyphosphate-accumulating organisms (PAO) intracellularly synthesize polyphosphate under aerobic conditions and utilize it as an energy resource for polyhydroxyalkanoates (PHA) and/or glycogen biosynthesis from carbon substrates under anaerobic conditions. Glycogen-accumulating organisms (GAO), competitors of PAO, accumulate PHA and glycogen and become predominant in deteriorated EBPR systems. The metabolic capacity and physiological traits of PAO (“*Ca. Accumulibacter*” [2], *Tetrasphaera*-type PAO [26], and *Microlunatus phosphovorus* strain NM-1 [23]) and GAO (“*Ca. Competibacter*”-type GAO [34] and “*Ca. Defluviicoccus tetraformis*” strain TFO71 [43]) have hitherto been characterized by (meta) genomic analyses. A 16S rRNA gene pyrotag-based microbial community analysis of a laboratory-scale EBPR reactor was meticulously conducted to investigate the dynamics of “*Ca. Accumulibacter*”-related PAO (50). Lv *et al.* (32) reported that *Dechloromonas*-related PAO outcompeted “*Ca. Accumulibacter*” in a laboratory-scale sequencing batch EBPR system operated under anaerobic-anoxic (denitrifying) conditions.

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AD technology is widely used to decompose waste activated sludge (WAS; excess sludge generated from an activated sludge process) and remediate industrial wastewater (28, 39, 55). In the AD process, three microbial trophic assemblages basically forge a collaborative relationship to convert organic compounds in WAS/wastewater to CH<sub>4</sub> and CO<sub>2</sub>: ‘fermenters’ degrade high-molecular-weight ingredients (e.g., carbohydrates, proteins, and lipids) to volatile fatty acids (VFAs) and H<sub>2</sub>; syntrophic substrate oxidizers (‘syntrophs’) degrade VFAs to acetate and H<sub>2</sub>; and methanogenic archaea (‘methanogens’) further utilize acetate and H<sub>2</sub> with the production of CH<sub>4</sub> and CO<sub>2</sub> (52). 16S rRNA gene-based microbial community profiling previously revealed that AD sludge embraced a large proportion of uncultivated organisms with unidentified ecophysiology in AD (1, 7, 8, 27, 35, 36, 38, 45, 49, 53, 57). Recent omics-based analyses have provided a deeper insight into how uncultivated AD organisms function and survive. For example, members of the candidate phylum Hyd24-12 (“*Ca. Fermentibacteria*”), which is commonly observed in AD sludge, were metagenomically identified as sugar fermenters (25). The genomics of *Syntrophorhabdus aromaticivorans* strain UI, isolated from methanogenic sludge treating terephthalate wastewater, revealed its metabolic and energy conservation systems for the syntrophic degradation of aromatic compounds (44, 46). The *in situ* ecophysiology and synergistic interactions of uncultivated organisms including the candidate phyla “*Ca. Atribacteria* (OP9)”, “*Ca. Cloacimonetes* (WWE1)”, “*Ca. Hydrogenedentes* (NKB19)”, and “*Ca. Marinimicrobia* (SAR406)” in a methanogenic bioreactor treating terephthalate wastewater were investigated using a combined approach employing metagenomics, metatranscriptomics, and single-cell amplified genomics (45). In addition, Nobu *et al.* (47) successfully reconstructed four metagenomic bins of *Euryarchaeota* clade WSA2 (or ArcI), which is considered to play a role in methanogenesis in the AD process (7), and identified WSA2 as the first methanogen employing the demethylation of methylated thiols for methanogenesis. By considering its metabolic novelty and performing a genome-wide phylogenetic comparison between known *Euryarchaeota* organisms, they proposed the novel taxa “*Ca. Methanofastidiosum methylthiophilus*” of the class “*Ca. Methanofastidiosa*” for the WSA2 lineage. Iino *et al.* (17) also proposed the family *Methanomassiliococcaceae* and order *Methanomassiliicoccales* with the *Methanomassiliococcus*-related methanogen “*Ca. Methanogram caenicola*” enriched from AD sludge.

Besides WWTP, concerted efforts have been made in an attempt to elucidate the microbial community structure of biofilms that develop in drinking water systems because it is important to ensure water quality and prevent health risks. A microbial community survey of water meters/pipes installed in the city of Landskrona, Sweden, showed that biofilms dominated by *Nitrospira*- and *Pedomicrobium*-related organisms developed in a water meter, in which red water (containing relatively high concentrations of iron and manganese) had been reported, while *Sphingomonadaceae*-proliferated biofilms were observed in other water meters without any issues (31). Karwautz and Lueders (22) investigated the effects of high-pressure hydraulic purging on biofilm microbial community structures in drinking water wells.

They found that a significant decrease in the populations of *Pseudomonas* and *Legionellaceae*, which are recognized as potential pathogens of drinking water systems, was observed after the purging event. Ling and Liu (29) reported that a chloramination treatment may affect the dominant bacteria in artificially-developed biofilms in laboratory-scale reactors fed groundwater, and members of *Mycobacterium* that potentially harbor resistance to disinfectants through mycolic acid-containing cell walls may survive or grow under chloramination. Inoue *et al.* (18) found phylogenetically novel, functionally uncharacterized *Legionella*-associated clades from cooling tower/bath water samples using a clone library analysis combined with a DNA-intercalator ethidium monoazide treatment.

As discussed above, omics approaches may contribute to our understanding of the role of microbes in WWTP ecosystems, while cultivation/enrichment/microscopic approaches may facilitate the accuracy and robustness of omics-derived implications (21, 37, 40, 48). In “Les Misérables,” Jean Valjean carries wounded Marius on his shoulder through a long and difficult route in the Paris sewer system in order to save his life, and this leads to an outcome that makes Cosette and, perhaps, Jean Valjean himself happy. Unraveling the complete picture of the ecology and physiology of and interactions between microbes in the water infrastructure is also challenging. The happy ending of this story has not yet been reached.

## References

1. Aida, A.A., K. Kuroda, M. Yamamoto, A. Nakamura, M. Hatamoto, and T. Yamaguchi. 2015. Diversity profile of microbes associated with anaerobic sulfur oxidation in an upflow anaerobic sludge blanket reactor treating municipal sewage. *Microbes Environ.* 30:157–163.
2. Albertsen, M., L.B. Hansen, A.M. Saunders, P.H. Nielsen, and K.L. Nielsen. 2012. A metagenome of a full-scale microbial community carrying out enhanced biological phosphorus removal. *ISME J.* 6:1094–1106.
3. Ardern, E., and W.T. Lockett. 1914. Experiments on the oxidation of sewage without the aid of filters. *J. Soc. Chem. Ind.* 33:523–539.
4. Ardern, E., and W.T. Lockett. 1914. The oxidation of sewage without the aid of filters. Part II. *J. Soc. Chem. Ind.* 33:1122–1124.
5. Ardern, E., and W.T. Lockett. 1915. The oxidation of sewage without the aid of filters. Part III. *J. Soc. Chem. Ind.* 34:937–943.
6. Buswell, A.M. 1947. Important considerations in sludge digestion. 2. Microbiology and theory of anaerobic digestion. *Sewage Works Journal.* 19:28–38.
7. Chouari, R., D. Le Paslier, P. Daegelen, P. Ginestet, J. Weissenbach, and A. Sghir. 2005. Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environ. Microbiol.* 7:1104–1115.
8. Chouari, R., D. Le Paslier, C. Dauga, P. Daegelen, J. Weissenbach, and A. Sghir. 2005. Novel major bacterial candidate division within a municipal anaerobic sludge digester. *Appl. Environ. Microbiol.* 71:2145–2153.
9. Cooper, P.F. 2001. Historical aspects of wastewater treatment. p. 11–38. *In* G. Lettinga, G. Zeeman, and P. Lens (ed.), *Decentralised Sanitation and Reuse: Concepts, Systems and Implementation*. IWA Publishing, London.
10. Daims, H., E.V. Lebedeva, P. Pjevac, *et al.* 2015. Complete nitrification by *Nitrospira* bacteria. *Nature* 528:504–509.
11. Forbes, C.M., N.D. O’Leary, A.D. Dobson, and J.R. Marchesi. 2009. The contribution of ‘omic’-based approaches to the study of enhanced biological phosphorus removal microbiology. *FEMS Microbiol. Ecol.* 69:1–15.
12. Fujitani, H., Y. Aoi, and S. Tsuneda. 2013. Selective enrichment of two different types of *Nitrospira*-like nitrite-oxidizing bacteria from a wastewater treatment plant. *Microbes Environ.* 28:236–243.

13. Gruber-Dorninger, C., M. Pester, K. Kitzinger, D.F. Savio, A. Loy, T. Rattei, M. Wagner, and H. Daims. 2015. Functionally relevant diversity of closely related *Nitrospira* in activated sludge. *ISME J.* 9:643–655.
14. Hanada, A., T. Kurogi, N.M. Giang, T. Yamada, Y. Kamimoto, Y. Kiso, and A. Hiraishi. 2014. Bacteria of the candidate phylum TM7 are prevalent in acidophilic nitrifying sequencing-batch reactors. *Microbes Environ.* 29:353–362.
15. Hirakata, Y., M. Oshiki, K. Kuroda, M. Hatamoto, K. Kubota, T. Yamaguchi, H. Harada, and N. Araki. 2015. Identification and detection of prokaryotic symbionts in the ciliate *metopus* from anaerobic granular sludge. *Microbes Environ.* 30:335–338.
16. Hugo, V. 1862. *Les Misérables* (Translated in 1887 by Hapgood, I.F.), A. Lacroix, Verboeckhoven & Cie., France.
17. Iino, T., H. Tamaki, S. Tamazawa, Y. Ueno, M. Ohkuma, K. Suzuki, Y. Igarashi, and S. Haruta. 2013. *Candidatus* Methanogram caenicola: a novel methanogen from the anaerobic digested sludge, and proposal of *Methanomassiliicoccaceae* fam. nov. and *Methanomassiliicoccales* ord. nov., for a methanogenic lineage of the class *Thermoplasmata*. *Microbes Environ.* 28:244–250.
18. Inoue, H., R. Fujimura, K. Agata, and H. Ohta. 2015. Molecular characterization of viable *Legionella* spp. in cooling tower water samples by combined use of ethidium monoazide and PCR. *Microbes Environ.* 30:108–112.
19. Itoh, Y., K. Sakagami, Y. Uchino, C. Boonmak, T. Oriyama, F. Tojo, M. Matsumoto, and M. Morikawa. 2013. Isolation and characterization of a thermotolerant ammonia-oxidizing bacterium *Nitrosomonas* sp. JPCCT2 from a thermal power station. *Microbes Environ.* 28:432–435.
20. Ju, F., and T. Zhang. 2015. Bacterial assembly and temporal dynamics in activated sludge of a full-scale municipal wastewater treatment plant. *ISME J.* 9:683–695.
21. Kamagata, Y. 2015. Keys to cultivating uncultured microbes: Elaborate enrichment strategies and resuscitation of dormant cells. *Microbes Environ.* 30:289–290.
22. Karwautz, C., and T. Lueders. 2014. Impact of hydraulic well restoration on native bacterial communities in drinking water wells. *Microbes Environ.* 29:363–369.
23. Kawakoshi, A., H. Nakazawa, J. Fukada, *et al.* 2012. Deciphering the genome of polyphosphate accumulating actinobacterium *Microlunatus phosphovor*. *DNA Res.* 19:383–394.
24. Kandaichi, T., S. Yamaoka, R. Uehara, N. Ozaki, A. Ohashi, M. Albertsen, P.H. Nielsen, and J.L. Nielsen. 2016. Phylogenetic diversity and ecophysiology of Candidate phylum Saccharibacteria in activated sludge. *FEMS Microbiol. Ecol.* 92:fiw078.
25. Kirkegaard, R.H., M.S. Dueholm, S.J. McIlroy, M. Nierychlo, S.M. Karst, M. Albertsen, and P.H. Nielsen. 2016. Genomic insights into members of the candidate phylum Hyd24-12 common in mesophilic anaerobic digesters. *ISME J.* doi: 10.1038/ismej.2016.43.
26. Kristiansen, R., H.T. Nguyen, A.M. Saunders, *et al.* 2013. A metabolic model for members of the genus *Tetrasphaera* involved in enhanced biological phosphorus removal. *ISME J.* 7:543–554.
27. Kuroda, K., M. Hatamoto, N. Nakahara, K. Abe, M. Takahashi, N. Araki, and T. Yamaguchi. 2015. Community composition of known and uncultured archaeal lineages in anaerobic or anoxic wastewater treatment sludge. *Microb. Ecol.* 69:586–596.
28. Lacroix, N., D.R. Rousse, and R. Hausler. 2014. Anaerobic digestion and gasification coupling for wastewater sludge treatment and recovery. *Waste Manag Res.* 32:608–613.
29. Ling, F., and W.T. Liu. 2013. Impact of chloramination on the development of laboratory-grown biofilms fed with filter-pretreated groundwater. *Microbes Environ.* 28:50–57.
30. Lucker, S., J. Schwarz, C. Gruber-Dorninger, E. Spieck, M. Wagner, and H. Daims. 2015. *Nitrotoga*-like bacteria are previously unrecognized key nitrite oxidizers in full-scale wastewater treatment plants. *ISME J.* 9:708–720.
31. Lührig, K., B. Canbäck, C.J. Paul, T. Johansson, K.M. Persson, and P. Rådström. 2015. Bacterial community analysis of drinking water biofilms in southern Sweden. *Microbes Environ.* 30:99–107.
32. Lv, X.M., M.F. Shao, C.L. Li, J. Li, X.L. Gao, and F.Y. Sun. 2014. A comparative study of the bacterial community in denitrifying and traditional enhanced biological phosphorus removal processes. *Microbes Environ.* 29:261–268.
33. Matsunaga, K., K. Kubota, and H. Harada. 2014. Molecular diversity of eukaryotes in municipal wastewater treatment processes as revealed by 18S rRNA gene analysis. *Microbes Environ.* 29:401–407.
34. McIlroy, S.J., M. Albertsen, E.K. Andresen, A.M. Saunders, R. Kristiansen, M. Stokholm-Bjerregaard, K.L. Nielsen, and P.H. Nielsen. 2014. ‘*Candidatus* Competibacter’-lineage genomes retrieved from metagenomes reveal functional metabolic diversity. *ISME J.* 8:613–624.
35. Narihiro, T., and Y. Sekiguchi. 2007. Microbial communities in anaerobic digestion processes for waste and wastewater treatment: a microbiological update. *Curr. Opin. Biotechnol.* 18:273–278.
36. Narihiro, T., T. Terada, K. Kikuchi, *et al.* 2009. Comparative analysis of bacterial and archaeal communities in methanogenic sludge granules from upflow anaerobic sludge blanket reactors treating various food-processing, high-strength organic wastewaters. *Microbes Environ.* 24:88–96.
37. Narihiro, T., and Y. Kamagata. 2013. Cultivating yet-to-be cultivated microbes: The challenge continues. *Microbes Environ.* 28:163–165.
38. Narihiro, T., M.K. Nobu, N.K. Kim, Y. Kamagata, and W.T. Liu. 2015. The nexus of syntrophy-associated microbiota in anaerobic digestion revealed by long-term enrichment and community survey. *Environ. Microbiol.* 17:1707–1720.
39. Narihiro, T., M.K. Nobu, R. Mei, and W.T. Liu. 2015. Microbial Community Involved in Anaerobic Purified Terephthalic Acid Treatment Process. p. 31–48. *In* H.H.P. Fang, and T. Zhang (ed.), *Anaerobic Biotechnology: Environmental Protection and Resource Recovery*. Imperial College Press, London.
40. Narihiro, T., and Y. Kamagata. 2016. Anaerobic Cultivation. p. 2.1.2-1–2.1.2-12. *In* M. Yates, C. Nakatsu, R. Miller, and S. Pillai (ed.), *Manual of Environmental Microbiology*, Fourth Edition. ASM Press, Washington, DC.
41. Neave, S.L., and A.M. Buswell. 1928. Treatment and disposal of distillery slop by anaerobic digestion methods. *Industrial and Engineering Chemistry.* 20:837–838.
42. Nielsen, P.H., A.M. Saunders, A.A. Hansen, P. Larsen, and J.L. Nielsen. 2012. Microbial communities involved in enhanced biological phosphorus removal from wastewater—a model system in environmental biotechnology. *Curr. Opin. Biotechnol.* 23:452–459.
43. Nobu, M.K., H. Tamaki, K. Kubota, and W.T. Liu. 2014. Metagenomic characterization of ‘*Candidatus* Defluviicoccus tetraformis strain TFO71’, a tetrad-forming organism, predominant in an anaerobic-aerobic membrane bioreactor with deteriorated biological phosphorus removal. *Environ. Microbiol.* 16:2739–2751.
44. Nobu, M.K., T. Narihiro, H. Tamaki, *et al.* 2014. Draft genome sequence of *Syntrophorhabdus aromaticivorans* strain UI, a mesophilic aromatic compound-degrading syntroph. *Genome Announc.* 2:e01064-13.
45. Nobu, M.K., T. Narihiro, C. Rinke, Y. Kamagata, S.G. Tringe, T. Woyke, and W.T. Liu. 2015. Microbial dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor. *ISME J.* 9:1710–1722.
46. Nobu, M.K., T. Narihiro, H. Tamaki, *et al.* 2015. The genome of *Syntrophorhabdus aromaticivorans* strain UI provides new insights for syntrophic aromatic compound metabolism and electron flow. *Environ. Microbiol.* 17:4861–4872.
47. Nobu, M.K., T. Narihiro, K. Kuroda, R. Mei, and W.T. Liu. 2016. Chasing the elusive *Euryarchaeota* class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. *ISME J.* doi: 10.1038/ismej.2016.33.
48. Puspita, I.D., Y. Kamagata, M. Tanaka, K. Asano, and C.H. Nakatsu. 2012. Are uncultivated bacteria really uncultivable? *Microbes Environ.* 27:356–366.
49. Riviere, D., V. Desvignes, E. Pelletier, S. Chaussonnerie, S. Guermazi, J. Weissenbach, T. Li, P. Camacho, and A. Sghir. 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J.* 3:700–714.
50. Satoh, H., K. Oshima, W. Suda, P. Ranasinghe, N. Li, E.G. Gunawardana, M. Hattori, and T. Mino. 2013. Bacterial population dynamics in a laboratory activated sludge reactor monitored by pyrosequencing of 16S rRNA. *Microbes Environ.* 28:65–70.
51. Saunders, A.M., M. Albertsen, J. Vollertsen, and P.H. Nielsen. 2016. The activated sludge ecosystem contains a core community of abundant organisms. *ISME J.* 10:11–20.
52. Schink, B., and A.J.M. Stams. 2013. Syntrophism among prokaryotes. p. 471–493. *In* E. Rosenberg, E. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (ed.), *The Prokaryotes*. 4th ed. Springer-Verlag Berlin Heidelberg.

53. Sundberg, C., W.A. Al-Soud, M. Larsson, E. Alm, S.S. Yekta, B.H. Svensson, S.J. Sorensen, and A. Karlsson. 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. *FEMS Microbiol. Ecol.* 85:612–626.
54. Ushiki, N., H. Fujitani, Y. Aoi, and S. Tsuneda. 2013. Isolation of *Nitrospira* belonging to sublineage II from a wastewater treatment plant. *Microbes Environ.* 28:346–353.
55. van Lier, J.B., N. Mahmoud, and J. Zeeman. 2008. Anaerobic wastewater treatment. *In* M. Henze, M.C.M. van Loosdrecht, G.A. Ekama, and D. Brdjanovic (ed.), *Biological Wastewater Treatment Principles. Modeling and Design*. IWA Publishing, London.
56. Wagner, M., and A. Loy. 2002. Bacterial community composition and function in sewage treatment systems. *Curr. Opin. Biotechnol.* 13:218–227.
57. Werner, J.J., D. Knights, M.L. Garcia, *et al.* 2011. Bacterial community structures are unique and resilient in full-scale bioenergy systems. *Proc. Natl. Acad. Sci. U.S.A.* 108:4158–4163.