PERSPECTIVE OPEN

Microbial niche nexus sustaining biological wastewater treatment

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Biological wastewater treatment has developed for more than 100 years, and new concepts about future wastewater treatment have been put forward worldwide. Environmental biotechnology is still the key contributor for wastewater management. However, these biotechnologies are facing challenges due to stringent discharging standards and the removal of emerging pollutants. Here, a new concept of microbial niche nexus sustaining biological wastewater treatment was proposed, which can achieve the efficient removal of known and unknown pollutants through tuning microbial niches to accommodate diverse microbial communities. Microbial niche nexus could be applied to solve emerging challenges besides infrastructure construction. In addition, the co-enrichment of r/K-strategists and the establishment of microenvironments with substrate gradients could be adopted for the design and operation of biological wastewater treatment processes. Finally, future development and perspectives were presented through aspects of microbial enrichment, microbial function and metabolism identification, system design and operation control, and new technology development and application.

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INTRODUCTION

Wastewater treatment plants (WWTPs) are important infrastructure to ensure the efficient management of wastewater for supporting sustainable development of our society. After 100 years of development of the activated sludge process, new concepts about future wastewater management have been put forward worldwide[1], including resources recycling and energy recovery from wastewater, recognizing 'wastewater' as the 'used water', and so on[2]. The ultimate purpose of most concepts is to simultaneously achieve water purification and energy/nutrients recovery.

On the other hand, wastewater treatment is still facing challenges due to stringent discharging standards and the emergence of new pollutants. For example, various types of micro-pollutants including pesticides, pharmaceuticals and personal care products, endocrine-disrupting chemicals (EDCs), and nanoparticles have been continuously emerging in WWTP effluents or residue activated sludge[3–5]. Usually, these emerging compounds have characteristics of low concentrations and are difficult to be removed by conventional wastewater treatment technologies. However, from the evolutionary viewpoint, certain types of microorganisms may be adapted to the emergence of new chemical compounds, promoting the removal of these emerging compounds. Therefore, how to optimize microbial niches to remove more types of pollutants would help address present and future challenges during wastewater treatment.

Recently, the concept of nexus has been applied in the field of sustainable development[6,7]. In the nexus system, water, food, energy, and ecosystem could be interconnected and interlinked. By adopting the nexus concept to wastewater management, the potential target is to achieve the sustainable development of wastewater treatment, with the consideration of other components in the system, such as the integrated and healthy ecosystem receiving the treated wastewater. For the ecosystem protection, not only the water quality but also the productivity of water bodies should be considered. Therefore, similar to the water nexus related systems, the concept of microbial niche nexus could be proposed for biological wastewater treatment. The microbial niche nexus means tuning microbial niches to accommodate diverse microbial communities for the efficient removal of known and unknown pollutants.

Biological processes with the accommodation of microbial niche nexus are still and will be the key foundation for wastewater management[8]. By proper regulation, suitable functional microorganisms can be selected and enriched in WWTPs to fulfill the removal of various types of pollutants (both conventional and emerging compounds), the recovery of energy and the recycling of resources, and also the achievement of the sustainable development of the nexus system.

MICROBIAL NICHE NEXUS BESIDES INFRASTRUCTURE CONSTRUCTION TO SOLVE EMERGING CHALLENGES FOR BIOLOGICAL WASTEWATER TREATMENT

Diverse microbial communities contribute to the removal of various pollutants

Revisiting the history of biological wastewater treatment, with the removal of carbon, nitrogen, and phosphorus from wastewater, the key is to provide different microbial niches to enrich diverse functional microorganisms, such as heterotrophs, nitrifiers, denitrifiers, and polyphosphate accumulating organisms (PAOs). Figure 1 shows the redox potential distribution of typical reactions carried out by different types of functional microorganisms. Reactions for the biological nitrogen cycle usually occur at potentials ranging from 0.34 to 0.97 V, while reactions for anaerobic sulfate reduction and methanogenesis occur at potentials ranging from −0.22 to −0.14 V and −0.43 to −0.25 V, respectively. By suitable management, these functional microorganisms can carry out biological metabolisms sequentially in time or space, which can be applied to achieve successful wastewater purification[9–12].

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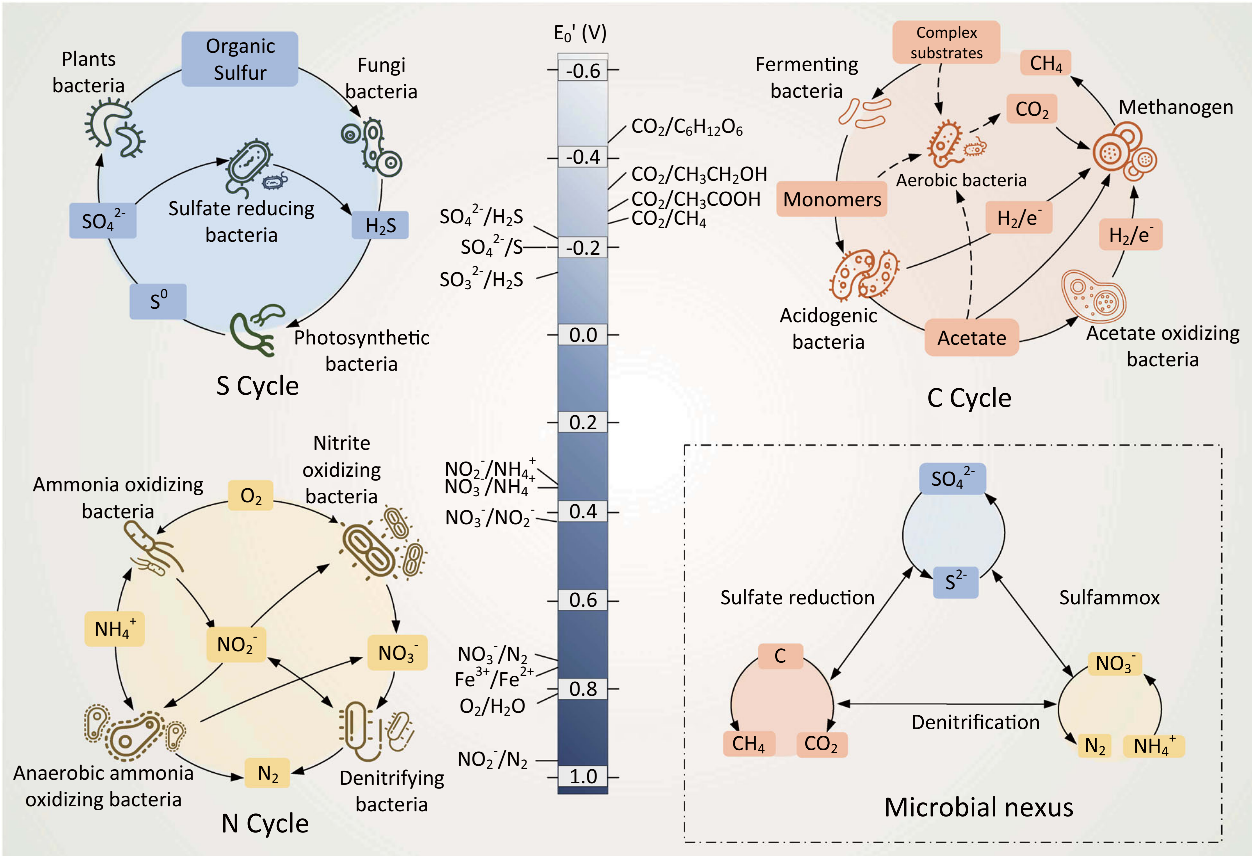

Fig. 1 Redox potentials distribution of typical reactions, and element cycles of sulfur, carbon, and nitrogen. The dotted lines in the carbon cycle represent the aerobic reaction. The lower right corner shows an example of how these cycles are correlated with each other. Sulfate reduction/denitrification enables the simultaneous carbon removal and sulfate/nitrogen removal; the sulfammox process enables the simultaneous sulfate removal and nitrogen removal.

For the removal of organic carbon, anaerobic treatment shows a good example of how functional microorganisms cooperate with each other to achieve the conversion from organic carbon to renewable bioenergy methane (CH_4) (Fig. 1). During anaerobic treatment, fermenting bacteria first degrade complex organic substrates such as protein and sugar into monomers which are subsequently utilized by acidogenic bacteria to produce acetate and hydrogen. Finally, methanogens consume acetate and hydrogen/carbon dioxide (CO_2) to generate the end product CH_4. Success to maintain the microbial population and the growth of these microorganisms is the primary cause of anaerobic system stability. In addition, in aerobic biological wastewater treatment processes, organic carbon is mainly degraded by heterotrophs to produce CO_2 and synthesize biomass.

For typical municipal wastewater treatment, cultivating microbial communities through a serial of anaerobic, anoxic, and aerobic (A^2O) reactors enable the enrichment of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifiers, and PAOs, resulting in the efficient removal of organic carbon, nitrogen, and phosphorus from wastewater[13]. In the anaerobic reactor, volatile fatty acids (VFAs) could be stored by PAOs with energy supplied from intracellular stored polyphosphate. In the anoxic reactor, denitrification will occur with organic carbon in wastewater as the electron donor, and recirculated oxidized nitrogen as the electron acceptor. In the aerobic reactor, phosphorus is uptaken by PAOs, ammonia is nitrified by nitrifiers, and also activities of heterotrophs will occur for organic carbon removal. Through activities of all these diverse microorganisms, wastewater can be efficiently treated.

When treating sulfate-containing wastewater, sulfate-reducing bacteria (SRB) which regulate the sulfidogenic bioprocess will become crucial microbes for sulfate removal (Fig. 1). During sulfate reduction, sulfate is first reduced to sulfite and then to sulfide by SRB. The reduction of sulfite to sulfide can be accomplished via the direct pathway in which sulfite is directly reduced to sulfide by receiving six electrons or another pathway that trithionate and thiosulfate are acted as intermediates[14]. Carbon sources as electron donors can be involved in the SRB metabolism. For example, some SRB metabolize organic compounds as electron donors through Acetyl CoA or a modified TCA pathway[15]. Many intermediate products originating from anaerobic fermentation/hydrolysis such as amino acids, sugars, long-chain fatty acids, and VFAs, can also be metabolized by SRB[14]. In this case, organic carbon can be simultaneously removed efficiently with sulfate.

Furthermore, the synergistic removal of contaminants may be completed by cooperative interactions, and the biological element cycle could be interlinked to each other (Fig. 1). It is well-known that denitrification can remove carbon and nitrogen simultaneously, sulfur-based denitrification can remove sulfur and nitrogen, and denitrifying PAOs can remove organic carbon, nitrogen, and phosphorus together. Rios-Del Toro et al.[16] found that anaerobic denitrification and ammonium oxidation could be coupled with the reduction of sulfate in marine sediments (sulfammox). Free sulfide, elemental sulfur, and sphalerite were produced during the ammonium oxidation with the reduction of sulfate[16]. To achieve the niche development of sulfammox, it is obvious that certain concentrations of sulfate and ammonium should be present in wastewater. However, it remains difficult to connect specific microbes to these functions. Metagenomic

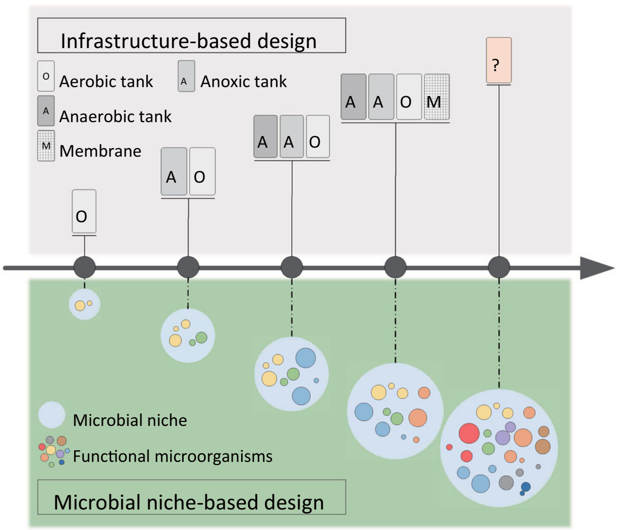

Fig. 2 Infrastructure and microbial niche concepts should be considered during WWTP design and upgradation. Development of wastewater treatment plants based on infrastructure-based design or microbial niche-based design.

analysis needs to be implemented to discover uncultivated functional microbes for further application. On the other hand, for the coupling of sulfate removal and CH_4 production, conductive materials were reported to be able to alleviate the inhibition of sulfate on methanogenesis, which can enhance the diverse biogeochemical reactions. Liu et al.[17] found that the addition of conductive materials could re-enrich syntrophic partners inactivated by sulfate. This new syntrophic community could efficiently produce CH_4 in sulfate-containing environment. In this case, the proper addition of conductive materials in anaerobic systems is the key for achieving the coupling of CH_4 production and sulfate removal. All these show that the interconnections between biogeochemical cycles such as carbon, nitrogen and sulfur would be potentially applied for developing novel environmental biotechnologies through the optimization of microbial niches (Fig. 1). Similar concepts could be developed for other biological element cycles.

Deciphering functions of known and unknown microorganisms

Biological treatment processes would be successfully functioned once that targeted microbial communities are enriched through microbial niches optimization. Therefore, the understanding of key microbial players is the fundamental step. With the application of novel molecular and bioinformatics techniques, more and more uncultured microbes and microbial functions have been and will be identified. For instance, the concept that nitrification is carried out by AOB and NOB sequentially has been accepted for more than a century and the known AOB and NOB are phylogenetically not closely related. However, some *Nitrospira* (NOB) species were found to possess all genes encoding enzymes necessary for ammonia oxidation via nitrite to nitrate, completely revising the picture of the nitrogen cycle[18,19]. The expression of genes during growth through ammonia oxidation to nitrate suggested that *Nitrospira* might be the key bacteria responsible for nitrification, and metabolic labor division in nitrification is not strictly required.

The niches of novel functional microbes may be different from 'conventional' microbes, thus investigating metabolic kinetics, diversity, and microbial interactions of these new microorganisms are crucial for developing novel wastewater treatment technologies based on the optimization of microbial niches.

Clarification of novel microbial metabolic mechanisms

Wastewater treatment processes can be improved through clarifying biological metabolic mechanisms. For example, interspecies hydrogen and formate transfer have been considered as the common pathways for syntrophic methanogenesis. Recently, it has been reported that some syntrophic bacteria and methanogens could exchange electrons directly by conductive pili or outer membrane cytochromes for syntrophic CH_4 production[20,21]. Since electron carriers are not required during direct interspecies electron transfer (DIET), it was considered as a faster and potentially more energy-conserving pathway for CH_4 production[22]. Therefore, DIET may be a crucial approach to improve the energy conversion from wastewater.

By discovery of this new microbial mechanism, several strategies have been proposed that can be potentially applied to achieve the stimulation of DIET so as to improve methanogenesis. The first one is the microbiology-based regulation. A high abundance of DIET-capable microorganisms often implies the good performance of DIET. Enriching DIET players by optimizing their niches can result in the dominance of DIET pathway in methanogenic systems. For example, the well-known bacteria with the DIET ability, *Geobacter*, which syntrophically consumes ethanol as the organic substrate for growth, could be enriched in an up-flow anaerobic sludge blanket reactor treating brewery wastewater[21]. In many cases, high CH_4 production efficiency could be attributed to the high abundance of *Geobacter*. Conducting the pretreatment of ethanol-type fermentation may be a useful approach for cultivating *Geobacter* species[23]. Second, promoting the excretion of extracellular compounds and adjusting the syntrophic interaction could be also applied for better DIET performance[24]. Finally, applying conductive materials as electron conduits in methanogenic systems can provide a good external conductive environment for syntrophic partners with the DIET ability. In this case, electrons released from syntrophic bacteria can be directly transferred to methanogens via conductive materials without contacting closely, enhancing the efficiency of CH_4 production[25]. In the wastewater treatment system, the addition of conductive materials could enhance the conductivity of anaerobic sludge, and stimulate the activity of respiratory chain and the extracellular electron transfer rate of syntrophic partners, thereby promoting the methanogenic efficiency[26].

Strategies for emerging compounds removal through microbial niche tuning

Providing suitable niches for specific microbes can also enhance the removal of emerging compounds and alleviate their toxicity. Conventional AOB can remove EDCs due to their enzyme of ammonia monooxygenase, which can degrade certain types of micro-pollutants, and heterotrophs could be also responsible for the degradation of synthetic estrogen[10]. In addition, the recently discovered complete ammonia-oxidizing bacteria which could oxidize ammonia to nitrate via nitrite were also found to be able to degrade micro-pollutants[27]. Therefore, by tuning all these functional microorganisms, not only conventional pollutants will be removed efficiently, but also emerging compounds will be well controlled.

On the other hand, suitable microbial niches could be applied to alleviate the biological toxicity induced by emerging compounds. For example, the alternate operation of aerobic and extended anaerobic treatment resulted in the enhanced removal of endocrine activities and better control of biological toxicity[4]. Different redox situations of wastewater under aerobic and anaerobic conditions might be one of the reasons for promoting endocrine degradation[4]. In addition, the change of organic loading rate could lead to a niche variety of microbes as well, thus affecting the removal efficiencies[4]. Recently, it was confirmed that cysteine produced during the sulfate reduction could alleviate the nano-metal particle toxicity[5]. This shows that the microbial interactions during biological processes could be functioning diverse for achieving different purposes.

Microbial niche-based design of the wastewater treatment system

For wastewater treatment, if only organic carbon is removed, one aerobic reactor is adequate. While for organic carbon and nitrogen removal, anoxic combined with aerobic reactors would be applied. Furthermore, for organic carbon, nitrogen, and phosphorus removal, anaerobic, anoxic, and aerobic reactors would be adopted. With more types of pollutants removal, the numbers of biological reactors would be extended for wastewater treatment system design.

Besides biological reactors, the microbial niche nexus concept should be incorporated during wastewater treatment system design. For the upgradation of conventional WWTPs, novel microbial communities could be explored and utilized for solving new challenges, including emerging compounds removal. All these could be achieved through microbial niche optimization to enrich diverse microorganisms in the present WWTPs besides to build new infrastructure (Fig. 2). To achieve this purpose, it is essential to further explore the unrevealed biological processes or functions. For example, rare species in biological treatment processes should be paid attention to, which may act as the seed and would be dominant with varied environmental conditions[28,29]. In some cases, species with a low abundance may also contribute a lot to the key function of a microbial system. For instance, Pester et al.[30] reported that *Desulfosporosinus* with only 0.006% of the total relative abundance was an important sulfate reducer in a peatland system.

ENHANCING MICROBIAL COOPERATION WITHIN THE MICROBIAL NICHE NEXUS-BASED SYSTEM

Co-enrichment of r/K-strategists

From the evolutionary perspective, two types of microbes could be selected in each ecosystem, including r- and K-strategists. Figure 3a shows the characteristics of these two types of microbes. The r-strategist microorganisms can grow rapidly with excess nutrients, while the K-strategist microorganisms have a slow growth rate but a high substrate affinity. Both types of microorganisms can survive and have their specific niches in each ecosystem[9].

In wastewater treatment systems, r-strategist microbes can be enriched with high substrate concentrations and K-strategist microbes will be dominating in substrate-limiting conditions (Fig. 3b). Therefore, when optimizing microbial niches, except focusing on biochemical redox conditions, the substrate-based strategy (feast and famine) should be also taken into consideration. For example, r-strategist microorganisms may be enriched in the front part of a plug flow reactor and responsible for the efficient removal of pollutants under high nutrient concentration conditions, while K-strategist microorganisms may be dominant in the latter part of the reactor where contaminants are mostly degraded and oligotrophic conditions dominate (Fig. 3b). In addition, with the ability to grow under oligotrophic conditions, K-strategist microorganisms may have a high affinity for micro-pollutants if they possess relevant genes and enzymes (Fig. 3b). Based on this theory, systems with high substrate gradients to provide specialized niches for both r- and K-strategists may simultaneously achieve high removal rate and removal efficiency of more types of pollutants in wastewater.

r/K-strategists could be also adopted for microbial selection and system control. For example, autotrophic nitrogen removal systems are promising for future wastewater treatment. The key for these processes is to achieve nitrification at the nitrite stage rather than the nitrate stage by inhibiting activities of NOB.

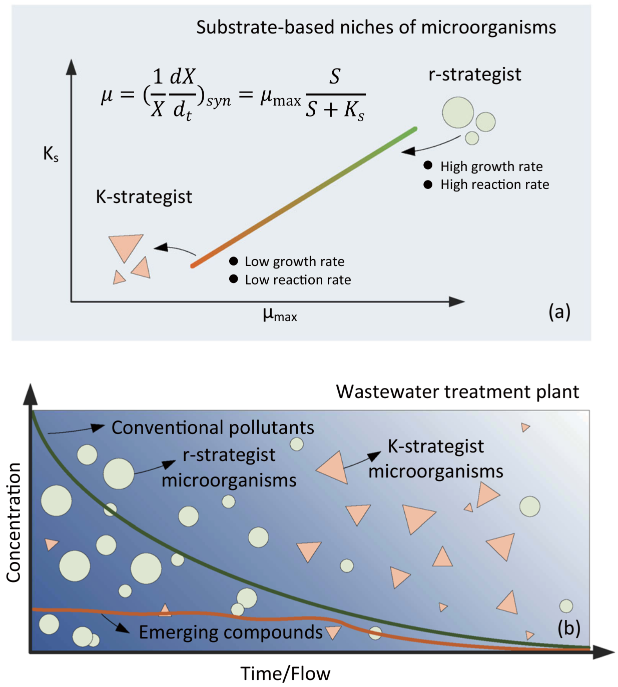

Fig. 3 Substrate-based microbial niches and their application in wastewater treatment processes. Characteristics of r-strategist and K-strategist microbes, and their possible niches and functions in wastewater treatment plant based on time (sequencing batch reactor) or flow (plug flow reactor) series.

Conventionally, it has been considered that AOB has a high affinity for oxygen than NOB, and low dissolved oxygen (DO) concentrations can inhibit NOB[31]. From the r/K theory, for each type of bacteria, two strategists should be included, i.e., one should be enriched under high substrate conditions, and the other one could be enriched under low substrate concentrations. This means that low DO concentrations cannot inhibit NOB efficiently for NOB of the K-strategist. From another aspect, the microbial interactions of cooperation or competition could be applied for inhibiting NOB. For example, Laureni et al.[32] found that if the produced nitrite could be used by anaerobic ammonia-oxidizing bacteria or denitrifiers, the activity of NOB could be inhibited, thereby enhancing nitrogen removal. On the other hand, inhibition of NOB through combined approaches, such as temperature control, and intermittent aeration[33], could be applied, which would be more efficient for nitrite-based nitrogen removal. For example, autotrophic nitrogen removal through the integrated nitritation and anammox process could be efficiently achieved by combining with intermittent aeration[34].

Micro-environment establishment for promoting diverse microbial functions

Microorganisms usually aggregate to form suspended flocs or biofilm in wastewater treatment systems. Microorganisms with a faster growth rate may prefer to live in suspended flocs, while other slow growers may survive in biofilm (Fig. 4). For instance, the complete ammonia oxidizer was considered as a slow grower, which was suggested to be enriched in biofilm systems[9]. Later, this was confirmed through 4 years of acclimation or 12 months of enrichment in biofilm systems[18,19]. Thus, the coupling of suspended flocs and biofilm may be a good way to provide diverse microbial niches and enhance microbial interactions among different microbes.

In addition, for biofilm systems, it is also necessary to control the environmental variables carefully for achieving stable process

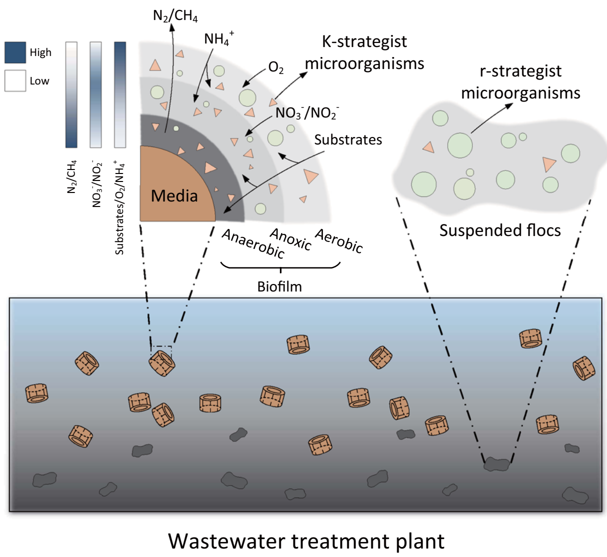

Fig. 4 Microbial niches in suspended flocs and biofilm. The blue and white columns represent the change of compound concentrations in aerobic, anoxic, and anaerobic layers in biofilm.

operation. The gradient of DO is one of the crucial factors needs to be controlled cautiously because too much oxygen supply may affect the anaerobic niches inside the biofilm and limit some microbial functions. As shown in Fig. 4, according to oxygen concentration, a biofilm can usually be divided into three layers including aerobic, anoxic, and anaerobic layers. Different types of microbes grow and metabolize in different layers, resulting in an integrated degradation of various types of pollutants. The important of DO can be also confirmed from a recent study, showing that highly conductive fibers embedded in the cell envelope of cable bacteria conducted electron transfer over centimeter distances under anaerobic conditions, while the conductance declined when exposure to air conditions[35].

Materials on which biofilms attach may additionally affect microbial niches. Functional groups on the surface of materials can change the redox activities, benefiting some bacteria suitable for the specialized redox environment as well as affecting their metabolisms[36]. For example, bacteria with the DIET ability may need to initially produce conductive pili and other biological electrical connections, which make them grow slower. In this case, conductive materials can establish an excellent electron-transfer environment, helping enrich DIET players and form a conductive biofilm. Furthermore, the anaerobic condition inside the biofilm is also beneficial to the performance of conductive fibers. Therefore, the proper selection of biofilm carriers can support the design and operation of innovative processes by affecting microbial niches.

FUTURE DEVELOPMENT AND PERSPECTIVES

The types of pollutants in wastewater determine the enriched diverse functional microorganisms. To advance the idea of microbial niche nexus in specific ways, the prerequisite is the understanding of microbial ecology, including the function, regulation, and interaction of known and unknown microorganisms. Exploring and re-constructing diverse microbial niches particularly based on time and space sequences in wastewater treatment systems is the crucial way to solve the increasing challenges and meet future demands. Further researches on microbial niche-based environmental biotechnology should be carried out by focusing on the following aspects:

(1) Microbial enrichment. Long-term operation systems with diverse microbial niches may help discover more unknown functional key players as well as develop the optimization strategies. Besides, some natural environments such as intertidal zones, which can provide alternative anaerobic and aerobic conditions, are also valuable microbial sources for discovering new microbial metabolisms and functions.

(2) Microbial function and metabolism identification. It is necessary to combine DNA, RNA, and proteomics-based techniques to explore microbial ecological functions and regulation strategies. Apart from the carbon, nitrogen, phosphorus, and sulfur cycles, the elucidation of other metabolic pathways and the regulation of micronutrients such as amino acids and vitamins should be also considered.

(3) System design and operation control. The design of new systems should follow the rule of microbial niche nexus, providing more space or time substrate gradients to ensure microbial diversity and function diversity. For system operation, proper control and optimization of environmental conditions are the prerequisites for achieving efficient system performance with different functional microbes.

(4) New technology development and application. The discovery of new technologies and new principles (e.g., new functional materials and new biological metabolism principles) will flourish the development of biological wastewater treatment technologies. Interdisciplinary cooperation is the key driver to achieve this purpose.

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AUTHOR CONTRIBUTIONS

G.W. conceived the paper. G.W and Q.Y. wrote the draft of the paper and commented on subsequent drafts and revisions.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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