Review in recent researches and applications of technology of environmental microbiology metagenomics in water treatment engineering

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Abstract. Metagenomics is an emerging research area that combines environmental biology and genomics. It is featured by unique biomolecule detection technique and high-throughput gene screening methods, not only has achieved a series of breakthroughs in the environmental microbial community dynamics and resource exploitation but also is gradually applied in multiple fields of environmental science and engineering. This paper provides a brief introduction to metagenomics technology, summarizes its latest applications and progresses in the field of water treatment engineering, and also provides more theoretical basis and methodological ideas for the improvement of biological treatment technology in this field. Finally, the application and development of metagenomics in water treatment engineering are prospected.

1. Introduction to metagenomics

Environmental microorganisms, including bacteria, archaea, fungi, etc., serve as an important part of the ecosystem and play a vital role in eco-systematic energy and material cycles on the planet. Environmental microbiology focuses on microbial communities in terms of their ecological distribution, species composition, diversity structure, population genetic characteristics and interaction with the environment. In recent years, this discipline has attracted significant attention from the field of environmental science. However, providing the features of microorganisms including tiny sizes, vigorous metabolism as well as highly adaptable and variant ability, our understanding about the action mechanism, population effect and genetic mechanism of environmental microorganisms at present remains limited. In the laboratory environment, only a small part of the naturally available
microorganisms can be cultured. Studies have shown that the culturable bacterial species in the soil only account for 1% to 10% [1]. Since the traditional microbial isolation and culture techniques fail to fully reflect the structural composition and diversity characteristics of microbial communities in the environment, a technology that obtains the genomes of these microorganisms through directly extracting nucleic acid samples from the environment comes into being and exhibits its competence in collecting the composition, structure and function information of microbial communities in the environment.

Metagenomics is both a system of research methods and a research field [2]. First proposed by Handelsman et al in 1998, the concept of metagenome referred to a collection of all genomes of all microorganisms in soils [3], and it is also known as environmental genome [4]. Nowadays, the term "metagenomics" covers all studies that analyze the total DNA obtained directly from the environment [5]. Theoretically, the DNA-based metagenomics includes all microorganisms in environmental samples and thus can reflect the composition of microbial communities more comprehensively and authentically. Furthermore, this technology has greatly expanded the sources for screening new genes or bioactive substances. Metagenomics reflects the characteristics of the entire communities in different organisms or environments and contains all genetic information of environmental microorganisms. Compared with 16S rRNA, metagenomics can not only offer the classification information of various microorganisms in the community but also embodies the functional gene information of all microorganisms, thus contributing to an in-depth analysis of the potential functions of microbial communities [6].

![Diagram of microbial community composition and function revealed by environmental microbial metagenomics and environmental geochemical parameters.](image)

Figure 1. Microbial community composition and function revealed by environmental microbial metagenomics and environmental geochemical parameters.

At present, the high-throughput detection methods in metagenomics mainly include gene chip technology and high-throughput sequencing technology. Gene chip technology, which conducts sequencing by hybridization based on a microchip consisting of massive molecular probes with known sequences, is characterized by high microbial detection depth, high throughput and quantifiability. Nevertheless, this technology can only obtain the gene information of known species in the sample while fails to detect new genes or new species [7]. By contrast, high-throughput sequencing technology finds wider applications. Known as the second-generation sequencing technology represented by Roche-454 sequencing technique and Illumina sequencing technique, it is featured by high accuracy, reliable information identification, as well as the ability to screen new genes and
discover new species. However, further improvement is required to enhance its competence of discovering low-abundance microorganisms in the community [7-9]. After storage and sequence analysis, the data obtained by sequencing is then compared and refined to analyze microbial community composition, structures and ecological impact. The combination of environmental microbial metagenomics with environmental geochemical parameters demonstrate great potential in exploring microbial community composition and diversity identification, microbial community functions, and microorganism-environment interactions (figure 1) [10]. With the decline in sequencing costs and the increase in sequencing demands, the high-throughput data volume of metagenomics is increased dramatically. At the same time, the fact that sequence assembly as well as sequence and gene prediction often require huge computing resources puts forward a higher requirement for the analysis of metagenomics [11].

At present, microbial metagenomics has become a research hotspot and frontier subject in the fields of microbiology and environmental science and engineering [10,12]. Water treatment engineering, as a subfield of environmental engineering, was discussed in our study by summarizing the latest research achievements of metagenomics technology in environmental engineering subfields like water treatment engineering, demonstrating the application prospect of metagenomics in environmental engineering, and envisioning its development trend and research direction in the future.

2. Studies on water treatment engineering using metagenomics

In recent years, the output of various types of wastewater, such as domestic sewage and industrial wastewater, has witnessed a continuous increase; meanwhile, heavy polluting enterprises propose a strong demand for discharge reduction. In this context, water treatment engineering gradually becomes a particularly important subfield of environmental engineering. At the present stage, the water treatment generally adopts anaerobic or aerobic processes based on biological treatment. Several processes involved in wastewater treatment are often thought of as black boxes, and with the development of microbial metagenomics, the genomic analysis of complex communities has become much more accessible [13]. The research on the microbial community composition and functions in water treatment system can not only provide an in-depth understanding towards the mechanism of treatment processes but also predict the changes in the processing capacity of reactors themselves, thereby offering a theoretical support for the development of wastewater treatment technology [14].

2.1. Refractory pollutants

Metagenomics technology can isolate the gene clusters with ability to degrade pollutants or specific activities from the environment, thereby providing a guidance for the degradation of refractory pollutants, mainly including petroleum hydrocarbons, heavy metals, and various toxic and harmful compounds [15]. Metagenomic DNA was extracted by Jia et al [16] from the activated sludge of a tannery wastewater treatment plant, where Illumina sequencing technique was used to acquire the key genes for corresponding degradation. After that, the functional microorganisms capable of processing the cadmium and copper ions in sewage were analyzed, which provided an effective approach to improve the biological treatment efficiency of the copper and cadmium ions in sewage. Wang et al [17] analyzed the microbial community functional structures and characteristic genes in the activated sludge samples from 4 WWTPs in Beijing using gene chip technology; they found that different microorganisms shared similar genes in the refractory substances in sewage. Furthermore, the microbial functions were closely associated with the sewage temperature, dissolved oxygen concentration, ammonia concentration and COD loading rate; their work also discerned the relationship between microbial communities and environmental variables in WWTPs. Based on Illumina high-throughput sequencing, Ye et al [18] revealed that the overall patterns of metabolic pathways in different bioreactors were similar while the gene sequences between these pathways varied. Significant differences in the abundance and diversity of microbial genes degrading pollutants were observed between different wastewater treatment reactors and different time, which provided a powerful evidence for monitoring and evaluating the ability of activated sludge to degrade pollutants.
and purify wastewater. According to the investigation on 3 microbial communities in the aeration tank sludge from different pharmaceutical wastewater treatment systems, Ouyang et al [19] found that the uncultured Candidatus Saccharibacteria bacterium, Anaerolineaceae bacterium and Blastocatella contributed to removing ammonium nitrogen from wastewater. For the sake of facilitating researchers to quickly identify and classify complex uncultured microorganisms, Konstantinidis et al [20] proposed a new genome-based classification method in 2017 to fully describe the uncultured microorganisms. This method could be used to explore the degradation mechanism of pollutant Polycyclic Aromatic Hydrocarbons (PAHs) by studying the species, community structures and action mechanisms of uncultured microorganisms in the environment. Through enriching anaerobic microbial consortium from a petrochemical refinery wastewater, Sanches et al [21] explored their biodegradability to phenanthrene and acenaphthene. In their study, the microbial communities were characterized by sequencing technique and fluorescence in situ hybridization. The results demonstrated that PAH-removing consortium was mainly consisted of Diaphorobacter and Paracoccus genera. In the study by Hesham et al [22], yeasts were employed to remove PAHs from wastewater, where the activated sludge was inoculated to three different systems, followed by analyzing the structure and abundance of yeasts in these systems via PCR-DGGE and FISH. The results indicated that five yeast strains inoculated to two bioaugmentation systems served as the dominant populations to remove HMW PAHs, and these two bioaugmentation systems inoculated with exotic yeasts exhibited high performance in degrading HMW PAHs. Ye et al [23] analyzed the microbial community composition of different treatment areas in municipal WWTPs using 454 pyrosequencing and noticed that the bacterial diversity in activated sludge samples was higher than that in the samples from influent, effluent and digestion sludge samples; the microbial communities in these samples mainly involved Alphaproteobacteria, Thermotogae, Deltaproteobacteria and Gammaproteobacteria.

2.2. Nitrogen removal

In addition to degrading the organic matters in the sewage, the water treatment engineering is also required to remove nitrogen and phosphorus from the sewage and reduce the possibility of eutrophication. Based on 37 metagenomes, Zhao et al [24] found that there were multiple potential cross-feedings during anammox reactor start-up, which reflected the importance of symbiotic bacteria in the anammox reaction of WWTPs. Fontana et al [25] first conducted a comprehensive study on the thermophilic anaerobic degradation of cheese wastewater and evaluated the process efficiency according to different reactor configurations, where metagenomics was used for the in-depth characterization of microbial community structures. For these 22 population genomes newly identified by metagenomic analysis, a notable difference in microbial composition was reported between these two configurations, where the two-stage configuration was featured by higher methanogen diversity. Through combining nitrosation and anammox reaction to construct a coupling process for denitrification, Zeng et al [26] detected the corresponding dominant bacteria using metagenomics and found that in the anammox phase, the original genus of anammox bacteria (Candidatus Kuenenia stuttgartiensis) was transformed into another genus (Candidatus Brocadia fulgida). Under the conditions of limited-oxygen and high influent organic concentration, Xu et al [27] explored diversity for sulfate-reducing and nitrate-reducing microbial communities using gene chip technology and concluded that under the limited-oxygen condition, no significant differences were identified in the functional genes of sulfate-reducing microorganisms, whereas the abundance of nitrate-reducing genes was decreased. In addition, sulfate-reducing bacteria might oxidize sulfides more efficiently through altering their own populations or genes, which provided a new idea for wastewater treatment engineering. Denitrification refers to the process that microorganisms gradually reduce nitrate or nitrite (NO\textsuperscript{−}) to nitrogen through specific intermediates nitric oxide (NO) and nitrous oxide (N\textsubscript{2}O). Denitrification-enhanced biological phosphorus removal (EBPR) reactors can enrich denitrifying phosphate accumulating organisms (DPAOs). Despite the fact that studies have reported massive N\textsubscript{2}O enrichment, few knowledge has been acquired regarding the underlying mechanism of N\textsubscript{2}O
production, the prevalence of complete denitrification pathways and truncated denitrification pathways, as well as the influence of NO$_3^-$ feed on DAPO-enriched composite system. Gao et al [28] examined the nitrogen conversion potential via the metagenomic approach in a NO$_3^-$ feed denitrification EBPR bioreactor, which was enriched with Candidatus Accumulibacter and prone to N$_2$O accumulation. Their analysis acquired 41 nearly complete metagenomes (MAGs). The results suggested that the unusually high levels of N$_2$O production observed in this Accumulibacter-enriched consortium were partially linked to the selection of non-PAO flanking microorganisms with truncated denitrification pathways. Zhao et al [29] adopted the gene chip technology to study the microbial communities in a combined nitritation-anammox reactor and revealed that nitrogen compounds, C/N ratio and the operation parameters functioned as key variables for microbial denitrification. During the investigation in methanogenesis, denitrification and anammox in the reactor, Liu et al [30] noticed that the microbial communities in the reactor presented high diversity by utilizing high-throughput sequencing, where the contents of bacteria, archaea and eukaryotic microbes were 87.9%, 6.3% and 5.3%, respectively. Alyne et al [31] found some microbial communities that are symbiotic with Planctomycetes in the anammox reactor, such as Proteobacteria, Chloroflexi, Chlorobi, Acidobacteria and Bacteroidetes. It is very challenging to determine the role and interaction of these microorganisms in anammox reactor in future research.

2.3. Phosphorus removal

He et al [32] found that Candidatus Accumulibacter was a dominant polyphosphate-accumulating organism in the wastewater biological phosphorus removal system. They conducted further study on the metabolism, physiological characteristics, ecological distribution and dephosphorization mechanism of this population in the community. Through employing the polyphosphate kinase 1 gene (ppk1, the functional gene encoding polyphosphate kinase) as a genetic marker, Zhang et al [33] examined the abundance and community structure of Accumulibacter at the clade level based on the 12 activated sludge samples from 9 WWTPs. Both QPCR and phylogenetic analysis confirmed that the effect of biological phosphorus removal was closely associated with the community structure of Accumulibacter; the insufficient phosphorus removal effect might be related to the higher percentage of IID to the total Accumulibacter. Denitrifying sulfur conversion-associated enhanced biological phosphorus removal (DS-EBPR) system proves to be not only a novel wastewater treatment process but also an ideal microbial ecological model. For the DS-EBPR system, however, varying insights has not reached a consensus regarding the effects of functional microorganisms on carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) biotransformation and their interactions. Zhang et al [34] adopted the metagenomic approach and established a microbial community ecological model in a lab-scale DS-EBPR system, which could stably operate for more than 400 days.

2.4. Antibiotics

The treatment of sewage and drinking water containing antibiotics has also become a research hotspot, and microbial populations may exert very special effects and roles in this field. Although antibiotics have a lethal effect on most microorganisms, some special microorganisms appear to be antibiotic-resistant and can transmit this ability to other microorganisms.

Good results can be obtained in treating wastewater containing antibiotics if microbial metabolic pathways are rationally utilized [35]. By combining with quantitative PCR and gene cloning technique, Zhang et al [36] conducted an analysis of microbial populations in biochemical wastewater containing oxytetracycline using high-throughput gene chip technology. The results showed that the degradation genes in microorganisms were highly correlated with the antibiotic concentration in wastewater. In addition to antibiotic resistance genes (ARGs), functional genes capable of degrading carbon had also been identified in these microorganisms. The structures of microbial communities were featured by rich functional diversity, which provided a factual basis for the diversity of microbial communities in wastewater treatment systems containing antibiotics. Yang et al [37] applied Illumina sequencing and bioinformatic analysis to reveal that the ARGs could migrate in WWTPs, although
approximately 99.82% of the ARGs would be removed during the process of wastewater treatment. Christgen et al [35] also explored ARGs in anaerobic, aerobic and anaerobic-aerobic reactors using Illumina sequencing and discovered that the anaerobic-aerobic reactor achieved the best treatment efficiency at the influent ARG concentration of 198 mg·L⁻¹ and when the effluent ARG concentrations were reduced to 74, 34 and 29 mg·L⁻¹ respectively. By combining high-throughput sequencing-based 16S rRNA gene and metagenomic analysis, Zhao et al [38] carried out a lab-scale batch processing of six antibiotics for their dynamic changes under different concentrations of microbial communities and ARGs. As a result of significantly lowering microbial diversity, the presence of antibiotics leads to a dramatic change in microbiota structure and places a selective pressure on the enrichment of potential antibiotic-resistant bacteria (ARB); the high selective pressure by antibiotics increases the abundance of ARGs while reducing their quantity. Upon specific antibiotic treatment, the increase in the abundance of corresponding and non-corresponding ARGs demonstrates the side effects of antibiotic selective pressure. Microbial communities may play an important role in the composition of ARGs. Free nitrous acid (FNA) has a broad antibacterial effect, but its sensitivity varies greatly between different microorganisms. For the nitrifying bacteria found in activated sludge during the wastewater treatment process, nitrite-oxidizing bacteria (NOB) were more susceptible to FNA than ammonia-oxidizing bacteria (AOB). However, the molecular mechanism controlling AOB’s atypical tolerance to FNA remains unclear. By integrating metagenomics and label-free quantitative sequential windowed acquisition of all theoretical fragment ion mass spectra (SWATH-MS), Laloo et al [39] studied the effect of antimicrobial FNA on activated sludge containing AOB and NOB. While exposed to FNA, AOB Nitrosomonas genus would maintain the homeostasis through upregulating certain known oxidative stress enzymes like pteridine reductase and dihydrolipoyl dehydrogenase. Upon exposure to FNA, the increase in denitrifying enzymes suggested that nitrite had a detoxification function against nitric oxide. DNA and protein repair enzymes, phage prevention proteins, iron transport proteins and other proteins involved in the stress response mechanisms were all upregulated upon the exposure to FNA. In addition, enzymes related to energy generation were also upregulated when being exposed to FNA. These findings allow us to gain knowledge of the adaptive mechanism of AOB Nitrosomonas genus to bactericide FNA.

2.5. Drinking water
High-throughput sequencing technology has also been applied to the studies of microbial communities for the chlorine disinfection of drinking water. Upon the influence of chlorine disinfection on microbial community structure, both resistant microorganisms and resistant genes are enriched with proteobacteria as the top dominant bacteriophyta [40]. The proliferation of ARGs in drinking water and their horizontal transfer to pathogens may result in antibiotic failure. Unfortunately, monitoring of antimicrobial resistance in drinking water has not been set as a routine procedure at present. The bacterial hosts of ARGs in drinking water, especially the small-sized microorganisms, may not be removed via membrane filtration disinfection, so posing a threat to human health. Ma et al [41] performed a metagenomics-based investigation on the antibiotic resistome of small-sized microorganisms (0.2-0.45 μm) in 20 household drinking water samples from 12 cities in China and Singapore. A total of 265 ARG subtypes belonging to 17 ARG types were detected; their abundances ranged from 0.04 to 1.0 copies/cell. The dominant genes included multidrug, bacitracin and aminoglycoside resistance genes, where 43 ARG subtypes turned to be specific to small-size microorganisms. According to the presence of ARGs, pathogens and ARG-carrying pathogens, the collected drinking water samples were divided into three groups. All these new findings of antibiotic resistome of small-sized microorganisms in drinking water demonstrate the necessity to implement a more comprehensive ARGs monitoring for the drinking water supply. Water recycling provides a valuable resource for the consumption of non-drinking water. However, few insights into the potential of wastewater reuse in spreading ARGs have been achieved. Garner et al [42] conducted seasonal sampling during 2014 to 2015 from the reclaimed and drinking water distribution systems of 4 American utilities before treatment, after treatment and at five points of use. The resistance was
analyzed using shotgun macrogenomic sequencing technology. According to their findings, bacterial community composition, horizontal gene transfer, and selection pressure were identified as potential factors regulating the presence of ARGs. The correlation between bacterial community composition and ARGs proved to be weak; 193 ARGs were found to be related with plasmid-related genes; ARGs in the reclaimed water were detected of a greater abundance compared with those in the drinking water.

2.6. Reactor

The investigation on the degradation mechanism of environmental microorganisms for pollutants in sewage contributes to predicting operating conditions of the reactor. Vuono et al [43] compared the expression levels of 16S rRNA genes (rDNA) and rRNA gene in an activated sludge treatment plant (sequencing batch membrane bioreactor) at solids retention times (SRTs) of 20 and 5 days respectively. By examining the changes in the rates of micropollutant biotransformation and nutrient removal at these two SRTs, they concluded that compared to abundant taxa (≥1%), rare taxa (<1%) had disproportionately high ratios of rRNA and rDNA, which indicated higher protein synthesis. These results demonstrated that rare taxa might exert an effect in bioreactor performance. According to these findings, differential expression of rRNA at high SRTs probably offered an explanation for the promoting effect of high SRTs on higher rates of micropollutant biotransformation. The metagenomic analysis on micropollutant-related degradation genes and the direct measurements of a group of micropollutants and nutrients further confirmed that the enhanced functions would be lost after 5-day SRT operation. Membrane bioreactors (MBRs) enjoy a broad market in the field of wastewater treatment and have been widely applied to municipal wastewater treatment at present [44]. The performance of MBRs mainly relies on the microbial community structure in the activated sludge. However, further exploration is required to clarify the functional structures of microorganisms in MBRs. Sun et al [45] analyzed microbial genes in four MBRs using gene chip technology and discovered that the microbial functional genes in different reactors had antibiotics resistance, metal resistance and organic degradation. Furthermore, they described the relationship between microbial functional structures and reactor variables, including hydraulic retention time, influent COD concentration, NH4+-N concentration, temperature and humic substances concentration. Along with these achievements, their study also proposed an approach to investigate the microbial communities in WWTPs via gene chip technology.

2.7. Landfill leachate

Due to the complicated and changeable composition, the treatment of landfill leachate has always been an important research direction in the field of water treatment engineering. In situ microbial communities have a critical role in pollutant degradation and reflect the geochemical effects of pollutants. At the same time, the composition of the microbial communities implies to certain extent the huge potential of the microorganisms from the landfill leachate in bioremediation [46]. Lu et al [47] conducted an analysis on the diversity of functional genes and structures of microbial communities in landfill leachate by gene chip technology. The functional diversity of microbial communities tended to vary with the changes in landfill leachate geochemistry, such as pH, sulfate concentration, ammonia concentration as well as dissolved organic matter concentration. Their efforts provided a basis for solving leachate problems via biological treatment. Zhao et al [48] examined the spectral characteristics of ARGs in landfill leachate from 12 Chinese cities using network analysis and high-throughput sequencing-based metagenomic analysis. A total of 526 ARG subtypes that belonged to 21 ARG types were identified in their study. It was found that SU1, Su 2, AADA and Baca could be used as indicators for predicting total abundances of ARGs. Furthermore, a set of core ARGs were widely distributed in landfill leachate and the landfill environment, and an obvious co-occurrence pattern was found among ARGs and between ARGs and microbial populations.

2.8. Bioelectrochemical system (BES)
Bioelectrochemical system (BES) is a system unit, where the electrochemical reaction at either the electrochemical system’s anode or cathode is completed under the catalysis of microorganisms. In this system, the biomass energy, mainly including the electricity-producing microbial fuel cells (MFCs) [49] and the hydrogen energy-producing microbial electrolysis cells (MECs) [50], can be recycled via microbial extracellular electron transfer capacity. BES systems are considered as a new technology with great development potential in the treatment of environmental engineering wastewater owing to the advantage of recycling large amount of energy contained in waste biomass while processing the wastewater. There remains a huge research space in terms of microbial community structures and their working mechanisms in BES systems. Constructing efficient BES systems and optimizing the regulation of wastewater treatment efficiency requires analyses based on the electron-transferring microbiota and systematic microbiota functions. To this end, metagenomics has become an important analytical tool for BES system development and optimization. Liu et al [51] analyzed the functional structures of microbial communities in MEC reactors under different conditions using gene chip technology. After 4 months of culture in the single MEC reactor substrate, the functional and phylogenetic diversities of microorganisms were significantly improved, where the reactors with larger amount of hydrogen production enjoyed higher microbial diversity. Meanwhile, alternations in the initial operation and culture conditions of different MEC reactors would lead to significant changes in microbial community structures, which were correlated with Coulombic efficiencies (CEs) and COD degradation in the reactors. Wang et al [52] conducted a study on the state of planktonic communities in the MEC reactor, where wastewater was taken as the substrate to generate biogas. The analysis via gene chip technology and related biostatistical methods revealed the functional flora characteristics of high-yield hydrogen reactors, where the 4 specific communities included Shewanella, Pseudomonas, Geobacter and Bra-dyrrhizobium. It was found that in response to pH changes, the microorganisms in the high-yield hydrogen MEC system exhibited differences in system recovery and that the electricigens determining hydrogen yields had higher pH sensitivity than other functional flora. By inoculating to wastewater, Varrone et al [53] established MEC reactors that could simultaneously process wastewater and yield hydrogen. The gene chip technology was adopted to analyze the microorganisms and the results showed that the microbial strains with high hydrogen yields and high wastewater treatment efficiency could be domesticated and obtained by taking wastewater as inoculum. Besides, the hydrogen production efficiency could be controlled by electron transferring process in MEC reactors. In addition, they also pointed out that dissimilatory metal-reducing bacteria and antagonistic methanogens played a key role in efficient hydrogen production in MEC reactors. Yun et al [54] using Domestic wastewater established a kind of multifunctional biological treatment method, through the polarity inversion reduce the co-occurrence of pollutants. Using cathodic biofilm as substrate, the model pollutants can be effectively reduced in open or closed loop. When mixed together, the reduction reaction can be catalyzed simultaneously, and the reaction efficiency is not affected. The relevant phyla Bacteroidetes, Firmicutes and Synergistetes existed in biocathodes. Members of proteobacteria such as Pseudomonas, Thauera, and Comamonas, responsible for the reduction of different substrates, were significantly enriched in biocathodes. In combination with other technologies, it is possible to remove other pollutants from wastewater. Cai et al [55] found that the pH polarization region in the range of 2mm around the cathode was between 6.9 and 10.1 using a pH microsensor. This greatly affected methane production and the structure microbial community. Miseq sequencing data in the highly conserved region of MCRA gene showed that, at the applied potential, the alpha diversity of methanogens in the concentration of the electrode biofilm changed significantly, which confirmed that the dominant microorganisms in the cathode were Hydrogenotrophic methanogens. These results suggest that the pH variation in the microenvironment around the electrode is a an ecological niche enriched with Methanobacterium.

3. Prospects
Microorganisms regulate the biogeochemical cycle on a global scale and affect the function of ecosystem. For the sake of understanding ecosystem functions, it is essential to reveal the types and
functions of microorganisms in the natural environment as well as the interactions among microorganisms and between microorganisms and environmental factors at both community and single cell levels. Occupying an important position in microbial research, metagenomics have harvested certain achievements in multiple fields including water treatment engineering by combining different gene library screening methods with the two gene sequencing technologies, which further improves its application and promotion in the field of water treatment engineering.

However, it should also be noted that metagenomics, by providing the basis for biological diversity and revealing the interrelationships between microorganisms and environmental factors, cannot fully explain the theoretical mechanism of water treatment, that is, it cannot replace further biochemical research work.

With the rapid development of biotechnology, the research contents and means of metagenomics are also deepening. However, how to process high-throughput data quickly and efficiently becomes the main bottleneck during its development. This problem turns to be more striking for water treatment engineering, as most environmental engineering researchers lack the knowledge related to bioinformatics mining and statistical analysis. Under such predicament, it is difficult for researchers to perform a quick analysis of high-throughput metagenomic data and process them into intuitive information, thus greatly restricting the further promotion of this technology. Therefore, it is necessary for environmental engineering researchers to master the relevant knowledge of bioinformatics mining and statistical analysis as much as possible. At current stage, we should not only promote metagenomics technology but also construct a public analytic platform for environmental science and engineering researchers as quickly as possible to help them interpret the core data contained in the genomes in a simple and time-saving way, thus contributing to a better understanding for mankind on the world of environmental microorganisms as well as further development of environmental science and engineering practice.

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