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Review

Application of metagenomics to biological wastewater treatment

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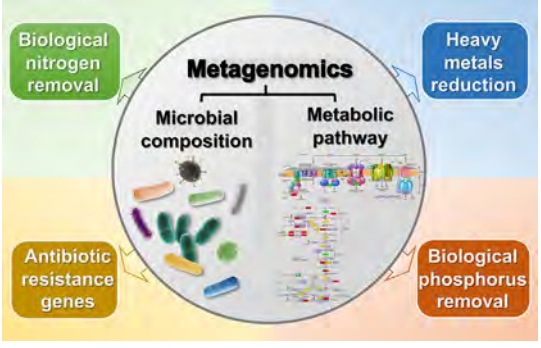
HIGHLIGHTS

- Metagenomics is used to evaluate mi crobial communities in environmental research.
- Metagenomics can elucidate biological removal of phosphorus and nitrogen.
- Metagenomics elucidates the distribu tion, transfer, and removal of ARGs.
- Metagenomics elucidates microbial re mediation and response of heavy metals.
- Multi omics will be important research tools for biological wastewater treat ment.

GRAPHICAL ABSTRACT



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ABSTRACT

Biological wastewater treatment is a process in which the microbial metabolism of complex communities trans forms pollutants into low or non toxic products. Due to the absence of an in depth understanding of the diver sity and complexity of microbial communities, it is very likely to ignore the potential mechanisms of microbial community in wastewater treatment. Metagenomics is a technology based on molecular biology, in which mas sive gene sequences are obtained from environmental samples and analyzed by bioinformatics to determine the composition and function of a microbial community. Metagenomics can identify the state of microbes in their na tive environments more effectively than traditional molecular methods. This review summarizes the application of metagenomics to assess microbial communities in biological wastewater treatment, such as the biological re moval of phosphorus and nitrogen by bacteria, the study of antibiotic resistance genes (ARGs), and the reduction of heavy metals by microbial communities, with an emphasis on the contribution of microbial diversity and met abolic diversity. Technical bottlenecks in the application of metagenomics to biological wastewater treatment are elucidated, and future research directions for metagenomics are proposed, among which the application of multi omics will be an important research method for future biological wastewater treatment.

Contents

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1. Introduction

Biological wastewater treatment using complex microbial communities to transform pollutants into low or non toxic products is the most important technique for purifying wastewater, and the microbial community is the key factor affecting treatment performance (Wu and Yin, 2020; Wu et al., 2019). Various methods (Table 1) have been applied to explore the microbial ecology, that is, diversity, genetics, metabolic processes, and interactions of microbes in biological wastewater treatment systems. Cultivation dependent techniques that have traditionally been used to investigate microbial ecology cannot comprehensively identify the ecology of complex microbial communities (Wommack and Ravel, 2013). Among approximately one billion reported microbial species in wastewater worldwide, only 1% can be artificially cultured, making it difficult to completely understand the microbial composition and metabolic pathways, and it is easy to overlook potential microbial mechanisms for wastewater treatment (Shade, 2017; Wu et al., 2019). Therefore, obtaining information on the microbial community in biological wastewater treatment more directly, comprehensively, and quickly become an urgent issue.

Metagenomics was first defined by Handelsman et al. in 1998 (Handelsman et al., 1998) and is the determination of total genetic material in a microbial community to analyze the microbial and the genetic diversity, and metabolic processes of microbes in a specific environment (Bengtsson Palme, 2018; Boulund et al., 2018; Mardanov et al., 2018; Méndez García et al., 2018; Ramazzotti and Bacci, 2018). Unlike traditional cultivation dependent techniques for microbiological research, metagenomics directly extracts several nucleotide fragments from environmental samples and uses DNA sequencing to obtain the target information directly, which can more effectively identify the state of microbes in a specific environment. Moreover, polymerase chain reaction (PCR) bias will not occur by metagenomics, which is much more advantageous than by 16S amplicon sequencing, as shown in Table 2.

Two strategies, functional and sequence based metagenomics, are currently used to acquire metagenomes from environmental samples (Mardanov et al., 2018). Functional metagenomics mainly uses an expression library formed by heterologous expression of several cloned DNA fragments in insert holding vectors to screen enzymatic functions and activities related to microbes and metabolic pathways (Allen et al., 2009; Felczykowska et al., 2015; Liebl et al., 2014; Mardanov et al., 2018). Therefore, functional metagenomics is widely applied to identify functional genes, such as ARGs, genes for heavy metal resistance, and genes related to nitrogen/phosphorus metabolism and pollutant degradation (Allen et al., 2009; Guo et al., 2017). In addition, functional metagenomics can characterize genes encoding enzymes with a particular activity, allowing for the discovery of novel enzymes whose functions may not be predicted using DNA sequences (Ferrer et al., 2007).

Sequence based metagenomics uses next generation sequencing technology to sequence DNA fragments on a large scale and annotates genes using bioinformatics analysis (Bharagava et al., 2019). Sequence based metagenomics can provide genetic information for an entire community and can thus be applied to construct metabolic processes and predict the function of potential genes (Chong et al., 2020). For example, shotgun metagenomics has been used to investigate microbial community interactions in anammox systems (Lawson et al., 2017; Speth et al., 2016).

After over 20 years of development, metagenomics has begun being applied in environmental research. From 2011 to 2020, the number of articles indexed in the Web of Science on "metagenomics" and "wastewater treatment" increased from 20 to 180 per year. These studies have mainly focused on nitrogen and phosphorus removal, pollutant degradation, and ARGs. Therefore, this study summarizes the application of metagenomics to biological wastewater treatment for the biological removal of phosphorus and nitrogen, the study of ARGs, and the reduction of heavy metals (Fig. 1), with an emphasis on the contribution of biodiversity and biochemical pathways of functional genes to demonstrate the broad applicability of metagenomics in the environmental field.

2. Application of metagenomics to biological wastewater treatment

2.1. Metagenomics expands the understanding of biological phosphorus removal process

The discharge of high concentration phosphorus wastewater into natural waters causes eutrophication and destroys ecological functions and structures (Conley et al., 2009). Biological removal of anthropogenic phosphorus has been widely used in wastewater treatment to alleviate eutrophication because of its low cost and environmental friendliness. Phosphate accumulating organisms (PAOs, also known as polyphosphate accumulating organisms) are important functional bacteria used in biological phosphate removal, and research on PAOs can help to optimize this process. Biological phosphorus removal involves processing phosphate stored by PAOs in wastewater through bacterial metabolism to form polyphosphate which is discharged as a high phosphorus sludge (Mino et al., 1998). Despite years of development, limitations in biological phosphorus removal remain, such as the unclear interaction mechanism between PAOs and glycogen accumulating organisms (GAOs), which is an important factor affecting reactor performance. In addition, an insufficient understanding of the biodiversity, metabolic function, interactions among functional microbes, and niche differentiation affect the overall phosphorus removal capability and the stability of the system.

The biodiversity of PAOs should be considered for phosphorus removal during wastewater treatment (Mino et al., 1998). However, PAOs are considerably less diverse in laboratory cultures than in practical environments, and only genera *Accumulibacter*, *Thioploca*, and *Tetrasphaera* have been found to be able to store phosphorus (Fernando et al., 2019; Holmkvist et al., 2010; Qiu et al., 2020), which significantly limits our understanding of the diversity and phylogeny of PAOs. The metagenomic binning technology has advanced our understanding (Albertsen et al., 2016; Skennerton et al., 2015; Zhang et al., 2016), showing that *Candidatus Accumulibacter* is the main clade of PAOs. For example, Skennerton et al. enriched *Ca. Accumulibacter* from different clades by modifying the laboratory culture and assembling the genome using metagenomic binning technology to obtain seven clades for the first time, thereby increasing the biodiversity of *Ca. Accumulibacter* (Skennerton et al., 2015). Later, Kolakovic et al. adjusted the community structure of *Accumulibacter* through operating parameters and measured that the phosphorus removal effect when different clades were dominant was 33%, 99%, and >99%, respectively (Kolakovic et al., 2021). This indicates that the understanding of the *Accumulibacter* diversity, the dominant species in the biological phosphorus removal system, will likely play a significant role in promoting

Table 1
Comparison of microecological research methods.

Method		Advantage	Disadvantage
Cell observation	Microscopy	Simple and direct observation of microbial morphology	Difficult to quantify and qualitative, not suitable for mixed bacterial communities
	Flow cytometry	Accurate quantification, analysis of cell size, granularity, DNA/RNA/protein content, *etc.*	Not suitable for mixed bacterial communities, difficult to identify bacterial species
Metabolic cultivation	Biolog metabolic fingerprinting	High sensitivity, strong resolving power, simple determination, and ability to obtain microbial function information	Imperfect standard database , inaccurate
	Stable isotope labeling	The composition and function of microbes can be linked, and the operation is simple, efficient, and accurate	High cost, few isotope internal standard compounds
Biochemical method	Quinones profiling	Conveniently and quickly obtain community diversity and quantitative biomass	The classification level is low, and the species cannot be identified
	PLFA method	Reliably and quickly obtain the diversity and structural changes in the microbial community	The classification level is low, no species can be identified, only living microbes can be detected
Omics method	16S amplicon sequencing	Low cost, simple and fast obtain species composition	PCR biased, single function
	Metagenomics	Obtain the species composition and function of microbes and discover unknown genes	Difficult to assemble, high computational cost, and difficult to find unexpressed genes
	Metatranscriptomics	Distinguish the temporal and spatial characteristics of gene expression	Difficulty in building a database, lack of reference genome
	Metaproteomics	Study the actual function of genes	Difficulty in total protein extraction, narrow detection range, lack of reference genes

fine adjustments in reactor performance. Although GAOs and PAOs have been considered to have no homology in the phylogenetic tree, Albertsen et al. (2016) found that *Ca. Propionivibrio*, which had previ ously been thought of as a PAOs that coexisted favorably with *Ca. Accumulibacter* in laboratory scale sequencing batch reactors, is a new type of GAOs (Fig. 2). In addition, it is used as the PAOmix FISH probe to evaluate *Accumulibacter* abundance, which leads to the overestima tion of *Accumulibacter* abundance *in situ*. This finding provided a more accurate quantification of PAOs species abundance and evaluation of the phosphorus removal potential of the reactor. GAOs are some of the important microbial members in the enhanced biological phospho rus removal system, and their role in the community also affects the bi ological phosphorus removal system performance; therefore, it is important to explore GAOs diversity. In addition, it has always been a challenging task to elaborate and strengthen the competition mecha nism between PAOs and GAOs in biological phosphorus removal sys tems, and the application of metagenomics will facilitate this research.

Biological phosphorus removal systems often exist in various com plex environments, which lead to different ecological niches of the PAOs community and metabolic differences and specific functions. The application of metagenomics in conjunction with other omics tech niques can advance our understanding of PAOs metabolic diversity (Barr et al., 2016; Camejo et al., 2016; Gao et al., 2019; Law et al., 2016; Zheng et al., 2020). For example, Barr et al. used metagenomics and metaproteomics to comparatively analyze biofilm protein through flocculated and granular enhanced biological phosphorus removal, and the proteins involved in the transition were annotated, which expounded the mechanism behind the change in the enhanced biolog ical phosphorus removal metabolic pathway from flocculation to gran ules (Barr et al., 2016). This study helps us to improve the phosphorus removal stability of the reactor by regulating the formation of biofilms. In addition, different electron acceptors in the reactor affect the phosphorus removal capability of PAOs in the biofilm. For example, Camejo et al. used metagenomics and qPCR to analyze the metabolism of different evolutionary clades of *Ca. Accumulibacter* under cyclic anaer obic and microaerobic conditions and found that different operating pa rameters produced different dominant clades in the reactor. When clades IC (clades based on the ppk1 gene) are dominant, oxygen, nitrite, and nitrate can serve as electron acceptors to uptake phosphorus, but the utilization efficiency is low (Camejo et al., 2016). Although we reviewed the metagenomics to explore PAOs in different engineering designs, and used different electron acceptors for different ecological niches in the contribution metabolism, there are still some problems worth further discussion. For example, PAOs in complex ecological niche differentiation in practical projects and control PAOs improve per formance and stability in research reactors.

2.2. Metagenomics advances our knowledge of nitrogen cycling microor ganisms for nitrogen removal

The rapid development of industries and modern agriculture has re sulted in the emission of nitrogen containing compounds that far ex ceed environmental capacity, resulting numerous environmental problems, such as acid rain, soil acidification, and water eutrophication. Therefore, the efficient removal of nitrogen from wastewater is a critical issue for modern treatment systems. Biological nitrogen removal is low cost and is therefore widely applied to reduce nitrogen emission from wastewater treatment systems. As nitrogen cycling microorganisms are a critical component in biological nitrogen removal, increasing our knowledge of microbial nitrogen cycling networks can help to accu rately control microbes and increase operational efficiency.

Using metagenomics to identify the genetic information of microbes in their native environments more effectively has been extremely useful in determining the diversity of nitrogen cycling microorganisms. For

Table 2
Comparison of amplicon sequencing and metagenomics sequencing.

	Low-throughput amplified sequencing	Metagenomics sequencing
Target segment	16S rRNA gene fragment	Non-targeted, random access to gene fragments in the environment
Function	The species composition and diversity of microbes	The composition, diversity, genome structure, and potential functions of microbial communities
Cost	Lower	Expensive
Data analysis	Simpler analysis of 16S rRNA gene fragment analysis	The analysis is difficult, requires bioinformatics knowledge and an excellent server
Advantages	Simple, fast, and universal	Obtain isolate difficult species metagenome-assembled genome, discover novel functional genes/ enzymes
Disadvantages	PCR is biased, single function	Difficult to assemble, high computational cost, and difficult to find unexpressed genes, complex information analysis

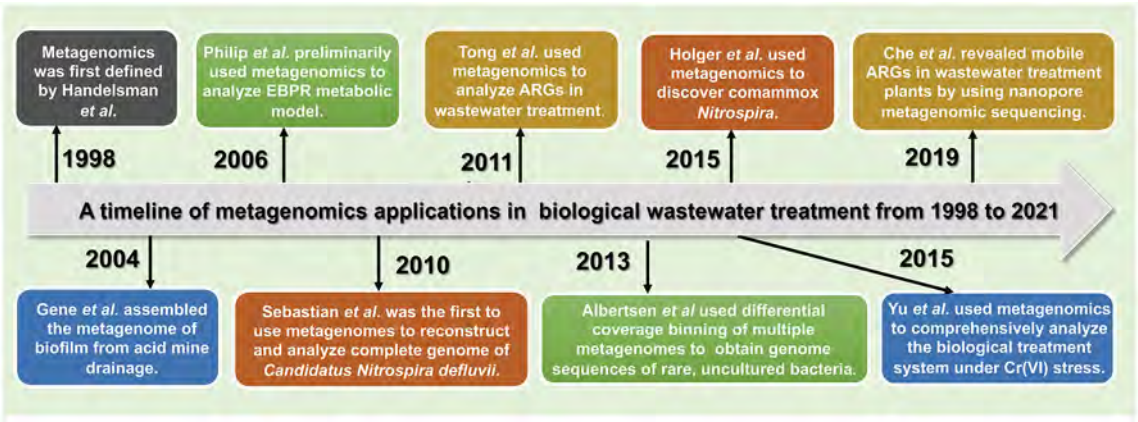

Fig. 1. Timeline of Landmark Developments in the application of metagenomics to wastewater biological treatment, including the biological removal of phosphorus and nitrogen, the study of resistance genes, and the reduction of heavy metals (Albertsen et al., 2013; Che et al., 2019; Daims et al., 2015; Handelsman et al., 1998; Luecker et al., 2010; Martin et al., 2006; Miao et al., 2015; Skennerton et al., 2015; Tyson et al., 2004; Zhang et al., 2011).

example, Speth et al. used metagenomics to analyze the partial nitration anammox (PNA) process, obtained 23 metagenome assemble genomes of denitrification microbes, and classified these genomes in terms of functional genes, thereby expanding our knowledge of denitrification microbe diversity (Speth et al., 2016). Later, Chen et al. (2020) used metagenomics to determine the effect of particle size on granule based PNA performance and found that large particles produced high biodiversity and functional diversity in a reactor, which may be the reason for the good denitrification perfor mance of large granular based PNA (Fig. 3). The discovery of key micro organisms in the nitrogen cycle network often leads to changes in traditional understanding. Daims et al. used metagenomic binning technology to assemble the genome of comammox *Nitrospira* which only presents in wastewater treatment systems, and *Nitrospira* was found to be capable of achieving complete nitrification by expressing genes related to ammonia oxidation and nitrite oxidation simulta neously during nitrification (Daims et al., 2015). This study led to the discovery of a completely new metabolic process of comammox which refreshed our understanding of the traditional nitrification denitrification process.

Metagenomics can both enrich our knowledge of nitrogen cycling microorganism diversity and provide a novel way of structuring micro bial ecology. For example, Jia et al. predicted the potential structure and function of the microbial community in different anammox aggregates and used metagenomics to construct the metabolic pathway for anammox bacterial aggregation at the gene level, which provided new insight into stable anammox reactor operation (Jia et al., 2021). Compe tition between microorganisms in the biofilm can also affect nitrogen removal performance. For example, Zhao et al. used metagenomics and metatranscriptomics to elucidate the mechanism of substrates competition between *Ca. Jettenia* and *Ca. Brocadia* in an immobilized an aerobic baffled reactor. *Ca. Brocadia* was found to have more complete functional genes and higher gene expression for nitrogen metabolism and chemotaxis than *Ca. Jettenia*, which may be attributed to the niche advantage of *Ca. Brocadia* and substrate competition to promote tran scription activity, thereby increasing the removal rate of ammonia ni trogen (Zhao et al., 2019). It has been reported that interspecific competition can affect the ability of extracellular electron transfer of mi croorganisms (Xiao et al., 2021), and extracellular electron transfer can also enhance the removal of ammonia nitrogen to a certain extent

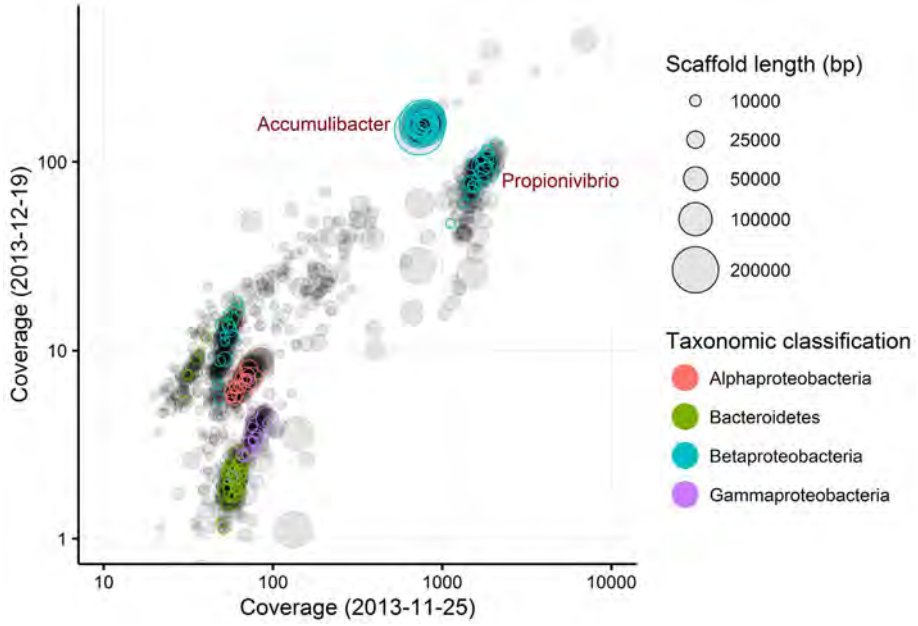

Fig. 2. Metagenomes of *Propionivibrio* and *Accumulibacter* were extracted by the differential coverage approach (Albertsen et al., 2016).

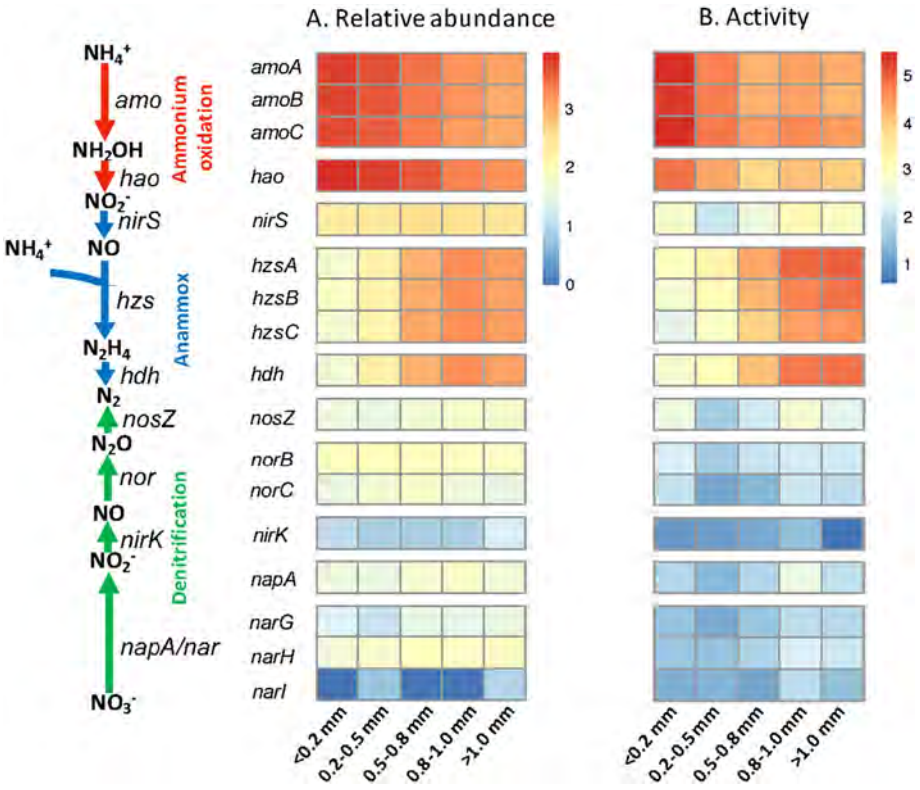

Fig. 3. Expression of nitrogen metabolism-related genes in size-fractionated anammox granules was analyzed based on metagenomics (Chen et al., 2020).

(Vilajeliu Pons et al., 2018), but whether the ability of extracellular electron transfer of anammox bacteria has not been confirmed. The study conducted by Shaw et al. showed that the extracellular electron transfer mechanism of anammox bacteria, which were found to transfer electrons to an external insoluble electron acceptor by oxidizing NH_4^+, and an alternative pathway for NH_4^+ oxidation with electrodes as electron acceptors, which provided theoretical support for the development of an anammox process based on microbial electrochemistry (Shaw et al., 2020). As reviewed above, the application of metagenomics has made important contributions to our understanding of the biodiversity and functional diversity of nitrogen cycling microorganisms that have not been cultivated, but it is far from sufficient. There remain many unknown nitrogen cycling microorganisms and nitrogen metabolic pathways, and their exploration may help us develop more efficient and sustainable biological nitrogen removal systems.

2.3. Using metagenomics to study antibiotic resistance genes in wastewater treatment systems

Drug resistance and ARGs of microbes are highly likely to occur in biological wastewater treatment, which is mainly derived from pharmaceuticals, stock farming, and other ventures (Kim and Carlson, 2007). Wastewater treatment plants (WWTPs) serve as receiving sites for resistance genes (Munir et al., 2011) to integrate and transfer, producing multiple resistance genes in a bacterium and accelerating their spread in the environment (Berendonk et al., 2015). The development of gene related resistance is an active subject of research. Metagenomics has been used to elucidate the distribution, horizontal transfer, and degradation related mechanisms of ARGs in WWTPs.

Using metagenomics to detect and analyze functional gene fragments has advanced research on the formation and distribution of ARGs in wastewater treatment (Liu et al., 2019; Luo et al., 2017; Raza et al., 2021; Rodriguez et al., 2021) (Fig. 4). Raza et al. showed that the complex ecological environment of WWTPs facilitated ARGs enrichment by increasing the diversity and abundance of these genes (Raza et al., 2021). This conclusion was further confirmed by Rodríguez and his co authors. They used metagenomics to detect ARGs in four different treatment reactors in a WWTPs; significantly different ARGs were found in each reactor, and the activated sludge in the treatment reactor was found to be selective for specific ARGs (Rodriguez et al., 2021). The similar phenomenon was also found in the anaerobic sludge reactors. The study conducted by *Lou* and his co authors showed that the distribution of ARGs in three anaerobic digestion reactors. Environmental factors, such as the substrate type, temperature, hydraulic retention time, and presence of volatile fatty acids, as well as the microbial community composition, were found to play decisive roles in determining the ARGs composition in the digestion reactor, which provided important information for identifying the influencing factors for ARGs formation (Luo et al., 2017).

The horizontal transfer of gene fragments is not only an important means of microbial evolution but is also a main approach for spreading ARGs. Meanwhile, horizontal gene transfer of ARGs causes microorganisms to produce multiple drug resistance, which leads to environmental health problems, and has become an increasingly popular research topic (Bellanger et al., 2014; Jin et al., 2020). Metagenomics enables the assembly of mobile genetic elements and allows researchers to explore the heterogeneity of these elements within and across genomes (New and Brito, 2020). Therefore, metagenomics has been used to elucidate the horizontal transfer of ARGs in wastewater. The study conducted by Che and his co authors showed that most of the ARGs in WWTPs were carried by plasmids and the highest relative abundance of ARGs was found for plasmids and conjugate transposons, which may be the main means of promoting the horizontal transfer of resistance genes in wastewater (Che et al., 2019). The similar phenomenon was also found in medical wastewater. Marathe et al. used metagenomics to identify 112 different types of mobile ARGs in medical wastewater and showed that a complex wastewater environment could induce several different resistance genes carried by microbes(Marathe et al., 2019), which may accelerate the spread of super resistant microorganisms.

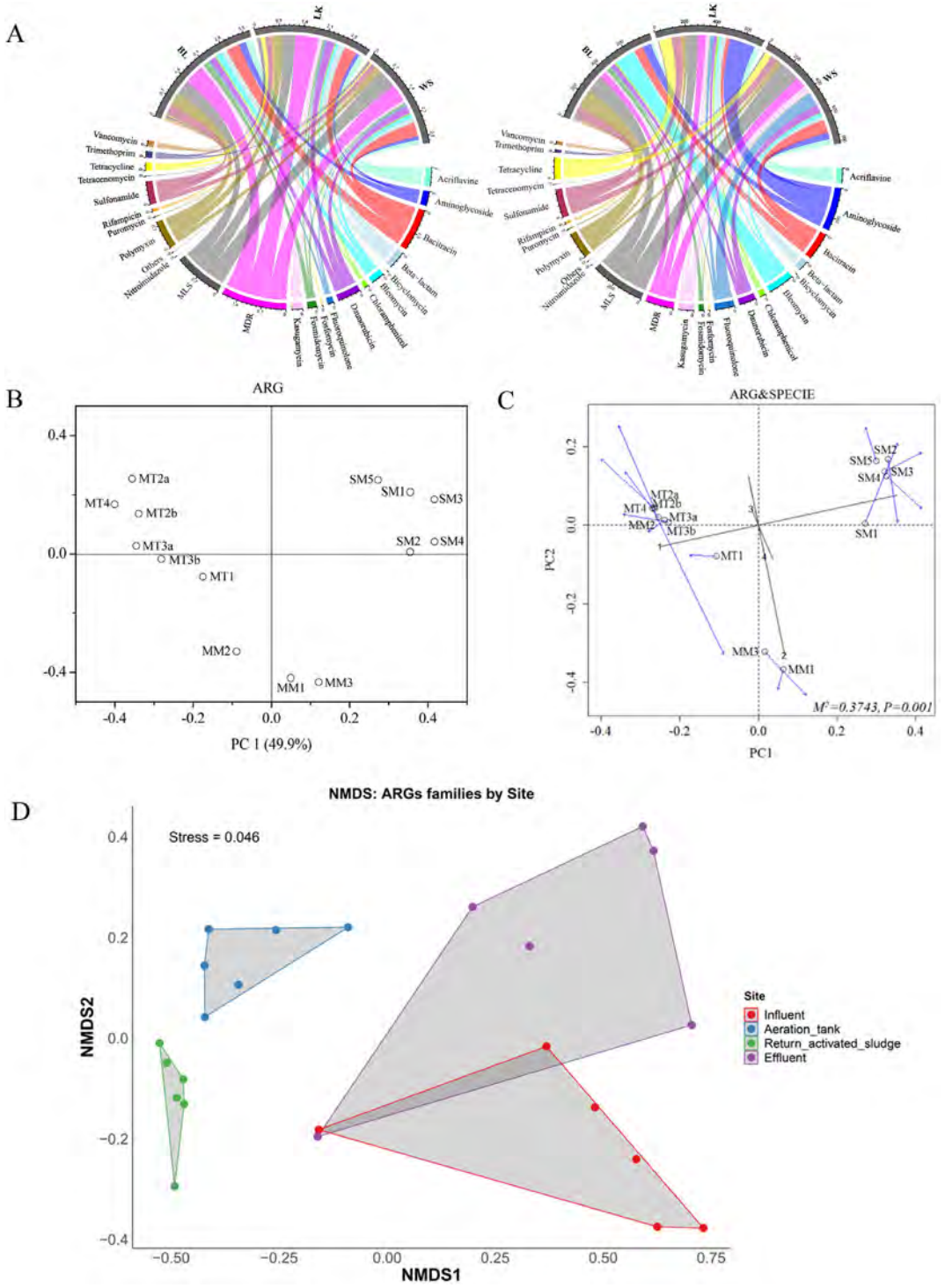

Fig. 4. Analysis of the distribution and formation of ARGs in WWTPs based on metagenomics. (A) Distribution of antibiotic classes in the activated sludge. Influence of (B) Substrates and (C) Microbial communities on the formation of ARGs. (D) Spatial variations in ARGs in the WWTPs (Liu et al., 2019; Luo et al., 2017; Rodriguez et al., 2021).

The removal of ARGs in wastewater and the reduction in the spread of ARGs in the environment are currently active research areas (Krzeminski et al., 2019; Zhu et al., 2021). Ultraviolet disinfection has been reported to partially remove ARGs from wastewater. (Aminov, 2011; McKinney and Pruden, 2012; Munir et al., 2011). The similar con clusions were obtained by Hu's work. They combined metagenomics with molecular biology technology to investigate the diversity and abundance of ARGs in ultraviolet disinfected wastewater and found that ultraviolet irradiation reduced the number of microbes with ARGs but increased the abundance of microbes carrying multiple resistance genes (Hu et al., 2016). Anaerobic digestion of waste activated sludge is also one of the effective methods to remove ARGs. Jang et al. used metagenomics to explore resistance gene degradation in an anaerobic digestion reactor under different temperature sequences: good removal of both ARGs and heavy metal resistance genes was found using thermophilic thermophilic sequences, whereas a thermophilic mesophilic sequence was found to be more suitable for removing resis tance genes and pathogenic bacteria carried by class 1 integrons (Jang et al., 2018). Traditional research methods have been used to study ARGs with limited results. Metagenomics has considerably expanded

our understanding of ARGs, enabling an in depth study of the impact of horizontal transfer of resistance genes, transmission route of resistance genes, formation of multidrug resistant bacteria, and impact of antibi otics on microbial communities.

2.4. Metagenomics has transformed the research strategy of microbial treatment of heavy metals

The rapid development of modern industry has made heavy metal pollution one of the most severe environmental problems. Metals in in dustrial wastewater are highly biotoxic and bioaccumulative and can severely damage the ecological environment upon entry into the envi ronment and food chain. Therefore, a series of physical and chemical methods have been developed to remove toxic heavy metals (Barakat, 2011; Fu and Wang, 2011; Kurniawan et al., 2006), such as chemical precipitation, ion exchange, adsorption, and membrane filtration. Al though these methods achieve good removal in specific pollution situa tions, high operation and maintenance costs preclude large scale applications. Microbial remediation of heavy metals has been exten sively studied because of its low cost and good results. The main princi ple of microbial remediation is the reduction of the toxic heavy metal valences to non toxic or low toxic values by microbial metabolism for resource recycling. Research has been performed on heavy metal reduc tion by microbes for decades, but knowledge of the mechanisms of mi crobial remediation of heavy metals and the response of microbial communities to heavy metal stress remains limited. Metagenomics has been used to significantly advance the development of microbial heavy metal remediation.

Metagenomics has played a significant role in elucidating the re sponse mechanisms of microbial communities under heavy metal stress. Some results revealed that the microbial community, including the structure, function, and metabolic processes, changes to some ex tent under heavy metal stress (Sharma et al., 2021). For example, Zhao et al. used metagenomics to analyze a sequencing batch bioreactor for Cu(II) containing wastewater treatment and found that 5 mg/L Cu (II) inhibited the degradation of organic matter by activated sludge (Zhao et al., 2021). The results showed that Cu(II) did not destroy the metabolic process of microbes, but inhibited organic degradation by inhibiting the expression of genes encoding enzymes related to refrac tory organic compound degradation. Similarly, Aktan et al. applied metagenomics to assess the toxicity level of the anammox process used to treat wastewater with high nitrogen concentrations containing Ni and Zn, which showed that the microbial community inhibition is re lated to the valence states of heavy metals and dominant bacteria (Aktan et al., 2018). Under Cr(VI) stress, the microbial community may have different response mechanisms under different process oper ations. Sun et al. used metagenomics and networks to analyze different concentrations of Cr(VI) on the performance of the A_2O process and showed that high concentrations of Cr(VI) not only inhibited the expression of nitrogen metabolism related genes, but also reduced the diversity of nitrogen metabolism related microbes, thus reducing the removal performance of NH_3 N and total nitrogen (TN) (Sun et al., 2019). However, Reddy et al. obtained granular sludge capable of both denitrification and reduction of nitrate and chromate under long term hexavalent chromium stress, and metagenomic analysis showed that *Halomonas sp.*, which had a good ability to remove chromate and ni trate, was substantially enriched in the activated sludge (Reddy and Nancharaiah, 2018). We have preliminarily reviewed the response mechanism of microbial communities under heavy metal stress; how ever, industrial wastewater often contains several or even dozens of heavy metals, so further study of the response mechanism of microbial communities under such complex conditions is needed.

Metagenomics has been used for the microbial remediation of heavy metals in wastewater treatment. Shi et al. used metagenomics to inves tigate the biological reduction process of Sb(V) in membrane bioreac tors, where the *sbrA* gene in the dimethyl sulfoxide reductase gene family and genes encoding cytochrome *C*, response regulators, and fer ritin were all found to participate in Sb(V) reduction (Shi et al., 2019). Similarly, Lai et al. (2018) used metagenomics to study the vanadate re duction process in a membrane biofilm reactor and showed that the se cretion of extracellular polymeric substances and biological metabolic processes are related to V(V) reduction, and the generation of methane

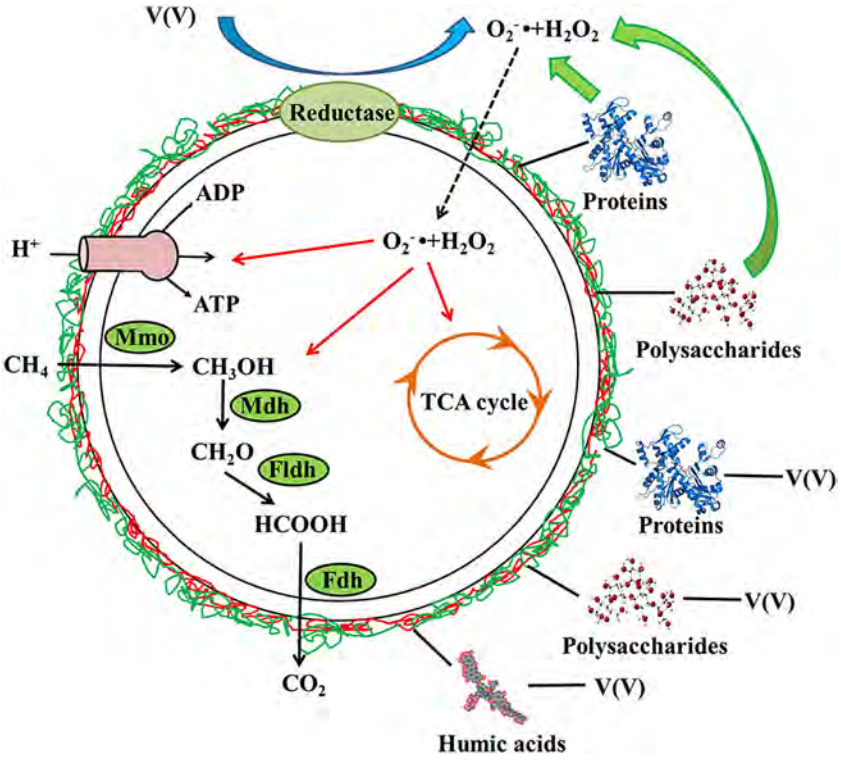

Fig. 5. Reduction mechanisms of V(V) in biofilm reactors were elucidated using metagenomics (Lai et al., 2018).

oxidation ATP in the TCA cycle and EPS may alleviate the oxidative stress response induced by V(V) and protect microorganisms from V (*V*)toxicity (Fig. 5). Wang et al. further explored the reduction mecha nism of microorganism V(V) in the reactor, and metagenomic analysis found that the functional genes related to methane oxidation and acetic acid production were substantially enriched, suggesting that methane oxidizing microorganisms may provide electron donors for other micro organisms to reduce V(V) by producing intermediates such as acetic acid through methane oxidation (Wang et al., 2019). Metabolism among microorganisms and the catalysis of related enzymes are very important for the reduction of heavy metals by microorganisms. Metagenomics has provided us with information to study the mecha nisms involved, but there remain problems worthy of further study.

3. Outlook

Metagenomics has become an indispensable tool in microbial stud ies on the biological removal of phosphorus, nitrogen, and resistance genes and the reduction of heavy metals in water treatment engineer ing. Metagenomics has been used to expand the boundaries of knowl edge obtained using traditional methods, and the results have facilitated optimization of biological treatment performance. However, in depth study is still required for some aspects of the wastewater bio logical treatment process. (1) Microbes need to be controlled in a suit able niche to remove nitrogen and phosphorus more efficiently. (2) The mechanism by which a microbial community transforms pollut ants and evolves corresponding functions in complex wastewater needs to be elucidated. (3) The interactions of microbial communities in com plex wastewater and whether these interactions facilitate the positive evolution of microbial communities need to be determined. (4) The evolution of a microbial population under heavy metal stress needs to be investigated. (5) The mechanism by which microbes or pathogens carry multiple resistance genes that are spreading in nature remains to be elucidated and whether this spread is as harmful as anticipated needs to be determined. (6) The impact of emerging contaminants on the biodiversity and metabolic diversity of microbial communities needs to be determined. In addition, the use of metagenomics to provide flexible solutions to these problems requires further consideration.

Metagenomics can be used to obtain a significant amount of infor mation on the structure and function of microbial communities but can not fully describe the role played by microbes during some metabolic processes in specific environments. Metagenomics can be combined with multiple omics technologies, such as metagenomics, metatranscriptomics, and metaproteomics, to determine the differences between communities and individual metabolic capabilities at the ge nome, transcriptome, and proteomics levels, resulting in a more com plete interpretation of the interaction between microbial populations.

Declaration of competing interest

There are no conflicts to declare.

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