Microbial Community Composition and Functions in Activated Sludge Treatment System

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Abstract

Activated sludge is the most popular biological method for treatment of wastewater. This process has successfully eliminated detrimental environmental impacts, such as toxicity, persistent organic materials, depletion of oxygen, and formation of algal blooms. However, it is often considered as economically and environmentally unsustainable wastewater treatment technology. The advent of latest technologies and improvements in metagenomics and metaproteomics study has provided a detailed insight into the microbiome of activated sludge treatment system. The present chapter mainly deals with the microbial community present in activated sludges and its composition. The seasonal modulation of the microbial communities in activated sludge is also discussed in detail along with the abundance of different microbial groups and their role and physiological activities in activated sewage sludge are reviewed. Antibiotic resistance genes present in activated sludge have also been discussed in detail.

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8.1 Introduction

A huge amount of wastewater is produced continuously by urban, agricultural, and industrial sectors. This wastewater is characterized by elevated levels of nitrogen, carbon, and other organic elements which leads to the eutrophication of aquatic bodies. The inputs of the wastewater vary greatly leading to a constant change in the composition of wastewater (Kumar and Thakur 2020; Kumar et al. 2021a, c, 2022a, b). Chemically, the wastewater composed of organic and inorganic components is very complex in nature and in any wastewater system only 16% of the water is reused and only 35.8%, 35.8%, and 35.7% of organics, ammonical nitrogen (NH_4^+-N), and total phosphate (TP) can be recovered. Thus, detoxification of both domestic and industrial wastewater is considered as a crucial step for protection of environment. The activated sludge technique is currently the widely accepted process for biological treatment of wastewater which is effective for removal of organic pollutants and petroleum product, benzopyrene, and toluene. The activated sludge process is a favored process for the treatment of wastewater as it is considered to be very cost effective and the microbes in the sludge helps in pollutants removal and detoxification. Activated sludge is characterized by the presence of a wide range of bacteria, archaea, viruses, and protists which have very closely interconnected trophic interactions. Since its proposal by Arden and Lockett in 1913, this process has undergone several changes and has been extensively remodeled. The process is broadly divided into two phases including the aeration phase and sludge settlement phase. Settlement is not allowed during the first phase and the wastewater is passed from primary settlement tank into the aerobic tank which is characterized by wide range of microbial population. The aerobic tank is mainly aerated by surface agitation or addition of oxygen via diffuser which is essential for the growth of aerobic microorganisms in the reactor. This oxygen is vital for the maintenance of the microbial flocs and maximizes the contact time between the surface of floc and wastewater. Moreover, oxygen facilitates mass transfer and efficiently dissipate the metabolic products trapped in the flocs. The main function of this activated biomass is the production of a wide range of enzymes which helps in the degradation of the organic pollutant and also perform ammonification, nitrite and nitrate oxidation, and denitrification process which help in a considerable reduction in the nitrogen content. In the second stage, flocculated biomass settles to form sludge which clears the effluent from solids and is discharged as the final effluent. In an activated sludge process, for every kilogram of biological oxygen demand (BOD) removed around 0.5 kg and 0.8 kg dry weight (DW) of sludge is produced. Most of the activated sludge is then returned to maintain a sufficient microbial population to oxidize the upcoming wastewater. The

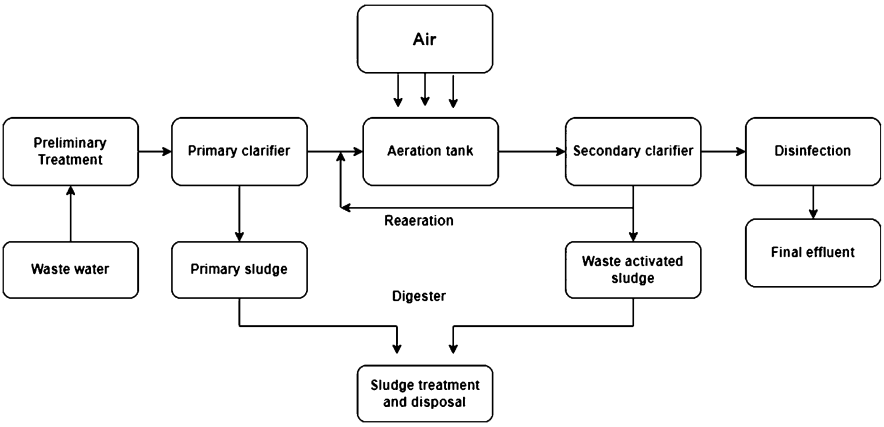

Fig. 8.1 Flow diagram for the activated sludge process

maintenance of microbial flocs is very crucial in any activated sludge process. They not only help in the adsorption of organic matter but also help in the rapid and effective separation of effluent in sedimentation tank itself. The detailed flow diagram of the activated sludge process is presented in Fig. 8.1 which explains the main two stages. The main component of activated sludge consists of flocculant suspension bacteria, other microfauna, and microflora along with adsorbed particulate matter. It is noted that any changes in the operation parameters may alter the nature of microbial floc which may generate turbid effluents due to scanty settlement leading to a subsequent loss in biomass. Activated sludge works efficiently in food limited conditions and each microbe uses its own cellular content and reduces the biomass produced. The two principles for removal mechanism in an activated sludge process are assimilation and mineralization. Assimilation process is carried out by utilizing the waste materials to create biomass associated with the rapid removal of BOD. Mineralization occurs by conversion of waste material to inert end products that are left in solution in the effluent and requires longer aeration times.

The present chapter mainly deals with the functions and composition of the microbial community present in activated sludge. The seasonal variation of the communities of microbes in activated sludge has been discussed in detail along with the abundance of different microbial groups, their role, and physiological activities in activated sewage sludge were reviewed. Antibiotic resistance genes present in activated sludge have also been discussed in detail.

8.2 Characteristics of Activated Sludge

Carbohydrates, lipids, and proteins are the chief organic components present in municipal wastewater, which provide nutrients to the bacterial community and help in floc formation. The inputs of the wastewater vary greatly leading to a

constant change in the composition of wastewater. Chemically the wastewater is composed of organic and inorganic components which is very complex in nature and it is difficult to completely define it. In several research works, it was found that carbohydrate was associated with particles of size greater than 63 μm (Sophonsiri and Morgenroth 2004). Huang et al. (2010) also reported that size fraction lesser than 0.1 μm contribute to nearly 62% of the total organic carbon (TOC) which is mainly complexed with proteins or carbohydrates. Nitrogen can be present in both inorganic forms that is in form of ammonium or nitrate or else present in organic forms. Generally, nitrate is presence in activated sludge are in a soluble form which is the most concern for groundwater pollution. On the other hand, inorganic nitrogen in the form of ammonium is volatile and is lost. Organic nitrogen found in activated sludge can be considered as inert and needs to be degraded by microorganisms, or mineralized to inorganic ammonia (NH_4^+ and NO_3^-). Some other sludge constituents, including calcium (Ca), magnesium (Mg), phosphorus (P), and iron (Fe), are known to form insoluble compounds with sludge solids, and are present at high concentrations. Other sewage sludge constituents, such as potassium and sodium, being water-soluble, are normally discharged with the treated wastewater. Suspended solids present in activated sludge mostly comprise 70% organic solids and 30% inorganic solids which includes food particles, fecal matter, garbage associated with sand, grit, and clay, which can only be removed from the wastewater using physical or mechanical processes, such as sedimentation or filtration. Other compounds, such as surfactants, humic acids, tannic acids, volatile fatty acids (VFAs), amino acids, RNA, and DNA, has been recorded in activated sludge.

8.3 Microbial Diversity in Activated Sludge

Activated sludge is constituted of a plethora of anaerobic and aerobic bacteria, fungi, archaea, and protists which are able to degrade organic pollutants and also reduce toxic metals to its related nontoxic forms. Activated sludge is considered as a complex medium having interconnected trophic relationships between microorganisms. Activated sludge harbors great biodiversity having a functionally important population. In complex ecosystems, bacteria accounts for nearly 95% of the total microbes, which play a crucial role in wastewater treatment. The microbial community of activated sludge was previously studied by culture-dependent methods (Zhang et al. 2018a, b; Yang et al. 2020); however, it does not give a thorough idea due to the incapability to grow most of the microbes in any specific culture conditions. With the advent of different molecular biology methods, the domain of microbial diversity has been revolutionized. Different techniques such as PCR-based techniques provide detailed information on the expression and diversity of ribosomal as well as protein coding genes in the activated sludge environment.

The advent of the "-omics era" has been considered as a breakthrough in the study of microbial diversity, both phylogenetically and functionally. High-throughput sequencing (HTS) using 454-pyrosequencing and Illumina has generated millions of sequence reads in a cost-effective way for superior understanding of the microbial

diversity and their genomic-potential in environmental samples (Kumar et al. 2020, 2021b). Also, methods like DNA-fingerprinting, clone-library, quantitative polymerase chain reaction (qPCR), and fluorescence in situ hybridization (FISH) studies based on functional genes or 16S rRNA gene segments have helped in developing idea on the microbial community of the activated-sludge (Johnston et al. 2019).

Normally sludge is characterized by the presence of floc made up of highly complex microbial communities comprising of archaea, bacteria, and viruses. The bacterial population plays a crucial role in the degradation of nutrients and organic pollutants containing both phosphorus and nitrogen. Moreover, they have the ability to tolerate adverse environmental impact, toxicity, and oxygen depletion. Metabolically they are diverse and perform a crucial role in biological nitrification and oxidizes ammonia to nitrate and nitrite then to nitrogen via denitrification and was found to be dominated by both ammonia-oxidizing bacteria (AOB) (Park et al. 2006; Gao et al. 2014; Pang et al. 2016) and nitrite-oxidizing bacteria (NOB) (Lucker et al. 2010). Research has been conducted on ammonia-oxidizing microorganisms, nitrite-oxidizing bacteria, denitrifiers (Zielinska et al. 2016; Pang et al. 2016), and phosphorus-accumulating organisms (PAOs) (Mielczarek et al. 2013). They have several biomarker genes such as ammonia monooxygenase (*amo*) (Ye et al. 2011) and nitrite reductase subunits (*nirK* and *nirS*) (Geets et al. 2007).

The activated microbial-community comprises Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes along with the presence of Actinobacteria, Chloroflexi, Planctomycetes, Acidobacteria, and Verrucomicrobia (Gao et al. 2016). Yu and Zhang (2012) in their study suggested that bacteria were dominant accounting for nearly 92% and 69% of DNA- and cDNA sequences, respectively, whereas eukaryotes account for approximately 43 and 30.97% of the total sequences in DNA and cDNA, respectively. They also reported that the bacterial community was dominated mostly by Proteobacteria, followed by Actinobacteria, Bacteroidetes, and Firmicutes, representing nearly 22%, 15%, 6%, and 3% of small subunit ribosomal DNA (SSU rDNA) reads, respectively. Both *Verrucomicrobia* and *Nitrospirae* exhibited high occurrence in protein-coding DNA reads. Among Archaea, Euryarchaeota also represented a very high amount of SSU rDNA (19.38%). Actinobacteria, Firmicutes, Planctomycetes, and Euryarchaeota showed a % SSU rRNA–% SSU rDNA ratio of less than one. Bacterial SSU rDNA and rRNA sequence reports show a high abundance of Proteobacteria which was followed by phyla, such as Bacteroidetes, Verrucomicrobia, and Actinobacteria. The main genera occurring in activated sludge are *Nitrosomonas*, *Nitrosospira*, *Methylocystis*, and *Methylosinus* having high ammonia monooxygenase activity. Similarly, *Nitrosomonas*, *Nitrosospira*, *Methylocystis*, and *Anaeromyxobacter* account for the activity of nitrification enzyme. Genera like *Acidovorax*, *Cupriavidus*, *Leptothrix*, *Alicycliphilus*, *Paracoccus*, and *Escherichia* were also reported which have high hydroxylamine reductase activity. On the other hand, *Riemerella*, *Dyadobacter*, *Dechloromonas*, *Candidatus accumulibacter*, and *Acidovorax* reported high nitrous oxide reductase activity. The wastewater treatment plants contain *Curvibacter*, *Azoarcus*, *Thauera*, *Zoogloea*, and *Accumulibacter*, which are mainly denitrifiers, *Tetrasphaera* and *Accumulibacter*, which are reported

to be phosphorus-accumulating organisms. Filamentous bacteria such as *Microthrix parvicella* and *Gordonia* was also abundant.

Zielinska et al. (2016) identified the presence of 38 orders from microbial consortia of wastewater treatment plants (WWTPs) which include Anaerolineales, Burkholderiales, Rhodocyclales, Planctomycetales, Rhizobiales, and so on and six core genera, such as *Prosthecobacter*, *Ferruginibacter*, and *Zooglea*. The presence of denitrifying populations, such as *Azoarcus*, *Thauera*, *Curvibacter*, and *Dechloromonas*, was also evident (Thomsen et al. 2004, 2007). *Candidatus accumulibacter* belonging to the family Rhodocyclaceae were designated as phosphorus-accumulating organisms were also identified along with *Pseudomonas* having denitrifying properties. *Halomonas* was also present in large numbers comprising 5% of the microbes (Nguyen et al. 2012). Kristiansen et al. (2013) reported Tetrasphaera of family Intrasporangiaceace which contained functional genes for denitrification. Similarly, Nielsen et al. (2009) reported the abundant presence of *Dechloromonas* spp. which was also a denitrifier and a putative PAO. Moreover, Acinetobacter (*Moraxellaceae)* was also an abundant bacterial genus which is strictly aerobic and chemoorganotrophic in nature with oxidative metabolism (Vandewalle et al. 2012).

Later in a study, Zielinska et al. (2016) reported the presence of Alphaproteobacteria, and Betaproteobacteria. Among Alphaproteobacteria, Rhizobiales, and among Betaproteobacteria, Burkholderiales were present. Their findings also show lesser presence of gamma (6.5%) and deltaproteobacterial sequences (9.9%) compared to the previous studies conducted by previous researchers. Apart from them, bacterial reads belonging to the order Rhodobacteriales, Rickettsiales, and Rhodocyclales were also reported in this study. The microbes were reported to contain genes coding for periplasmic nitrate-reductase (*napA)* and a gene coding for membrane-bound nitrate reductase (*narG*) (Heylen et al. 2006). However, Actinobacteria accounts for nearly 11% narG.

In a much later study, Zhang et al. (2019) reported the presence of bacterial operational taxonomic unit (OTU) assigned to 14 different phyla including Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes, Chlorobi, Planctomycetes, Verrucomicrobia, Saccharibacteria, and Proteobacteria. Proteobacteria consisted of a total of 47% of the OTUs, followed by Bacteroidetes (30%), Firmicutes (7%), Acidobacteria (2.2%), and Chlorobi (1.2%). Among Proteobacteria, classes Gammaproteobacteria (25%) and Betaproteobacteria (24%) were the most prominent. In addition, Flavobacteriia (18%) and Cytophagia (13%) were also significantly abundant. Gammaproteobacteria, being more sensitive to antibiotics, was present in much less quantity (Novo et al. 2013).

Core-microbial OTUs existing in activated sludges were studied and identified by the Global Water Microbiome Consortium (GWMC) (http://gwmc.ou.edu/) which reports the presence of 28 core taxa; however, nearly half of them are annotated only at genus or family level. Song et al. (2020) reported OTU_16 of *Betaproteobacteria* could not be annotated to any taxa. While working they isolated 830 isolates of which Strain SJ-1 was characterized and reported as a novel species, *Casimicrobium huifangae*, of the novel family *Casimicrobiaceae*.

Johnston and Behrens (2020) reported the core microbial community largely comprises Saprospiraceae, *Trichococcus*, *Microthrix*, *Tetrasphaera*, and Fibrobacteraceae. However, only constant activity was visible in Bacteroides, *Hypnocyclicus*, and *Tolumonas*. *Kouleothrix*, *Chloroflexi*, and *Gordonia* showed extensive growth in activated sludge, which is associated with sludge bulking and degrading various xenobiotic compounds. Apart from them *Leptotrichia*, *Arcobacter*, and *Acinetobacter* were also reported which are enteropathogenic bacteria related to human infections.

The details of the microbial community available in activated sludges obtained from different studies are presented in Table 8.1.

8.4 Enzyme Activity and Associated Physiological Function of Microbiome in Activated Sludge

A wide range of enzymatic activity was seen by the microbial community in the wastewater. In earlier research done by Nybroe et al. (1992), it was reported that esterase and dehydrogenase activities were correlated with the presence of heterotrophic bacteria. In activated sludge, they did an extensive study in which four different enzymes including α-glucosidase, alanine-aminopeptidase, esterase, and dehydrogenase were obtained from different types of wastewater. The enzyme profile showed the existence of a diverse group of bacteria with a wide range of activities. Konneke et al. (2005) and Park et al. (2006) reported the presence of diverse bacterial communities which perform a vital role in different types of nitrogen metabolisms. Most of the microbes perform a crucial role in ammonification, nitrite and nitrate oxidation, and denitrification, which help in a considerable reduction in the nitrogen content of the wastewater.

With the advent of modern technologies and metaproteomic study, it has helped in providing a more detailed insight of the microbial community and helped in detection of different types of enzyme variants, which indicated the degree of genetic diversity in sludges. Metaproteomic study of the extracellular polymeric substances present in activated sludge also revealed the presence of several cytoplasmic proteins, which may play various roles in the treatment of activated sludge biomass.

The process of nitrification is carried out by two diverse domains of microbes: ammonia-oxidizing microorganisms (Konneke et al. 2005; Park et al. 2006), which oxidize ammonia into nitrate, and nitrite-oxidizing bacteria, which oxidize nitrite into nitrate (Lucker et al. 2010). Ammonia-oxidizing microorganisms lead to the complete oxidation of ammonia (comammox), which oxidizes ammonia via nitrite to nitrate (Jiang et al. 2020). Under anaerobic conditions, denitrifying bacteria reduce nitrite to gaseous-forms like nitrous-oxide and dinitrogen gas which in turn may reduce the wastewater nitrogen concentration. These group of bacteria is represented by bacteria *Curvibacter* within Comamonadaceae, apart from which genera like *Azoarcus*, *Thauera*, *Dechloromonas*, and *Accumulibacter* (Zielinska et al. 2016).

Both DNA and cDNA show the presence of a wide range of ammonia assimilation, nitrite/nitrate ammonification, denitrification, and nitrogen fixation–related

Table 8.1 Microbial abundance in activated sludge

Phylum	Class	Genera	Method of study	References
Halobacterota Micrarchaeota Nanoarchaeota Proteobacteria Bacteroidota Patescibacteria Myxococcota Actinobacteriota Planctomycetota Chloroflexota Acidobacteriota Firmicutes	–	–	–	Ye et al. (2020)
Proteobacteria Nitrospirae Chloroflexi Chlamydiae Chlorobi Chloroflexi Elusimicrobia Ignavibacteriae Latescibacteria Parcubacteria Spirochaetae Armatimonadetes Bacteroidetes Chlamydiae Chlorobi Cyanobacteria Fibrobacteres Verrucomicrobia	–	*Nitrosomonas* sp., *Hyphomicrobium* sp., *Nitrospira* sp., *Bdellovibrio* sp., *Thauera* sp., *Halochromatium* sp., *Terrimonas* sp., *Ferruginibacter* sp., *Dechloromonas* sp., *Hyphomicrobium* sp., *Phaeodactylibacter* sp., *Parafilimonas* sp., *Nitrosomonas* sp., *Nitrospira* sp.	Illumina HiSeq 2500	Yang et al. (2020)

Proteobacteria Actinobacteria Acidobacteria Bacteroidetes		*Sphingopyxis* sp., *Bradyrhizobium* sp., *Candidatus* sp., *Saccharimonas* sp., *Mesorhizobium* sp., *Bosea* sp., *Niastella* sp., *Acidovorax* sp., *Alicycloiphilus* sp., *Sphingomonas* sp., *Thauera* sp., *Azoarcus* sp., *Candidatus Contendobacter* sp., *Candidatus Competibacter* sp., *Pyrinomonas* sp., *Piscicoccus* sp., *Kineosphaera* sp., *Microlunatus* sp., *Dehalobacter* sp., *Nitrospira* sp., *Tetrasphaera* sp., *Nakamurella* sp., *Propionicella* sp., *Friedmanniella* sp.	Illumina sequencing	Ai et al. (2019)
Proteobacteria Bacteroidetes Firmicutes Chlorobi Chloroflexi	Alphaproteobacteria Betaproteobacteria Albirhodobacter	*Dyadobacter* sp., *Variovorax* sp., *Nitrosomonas* sp., *Nitrosospina* sp., *Brocadia* sp., *Nitrobacter* sp., *Nitrotoga* sp.	Illumina MiSeq sequencing system	Johnston et al. (2019)
Acidobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Firmicutes, Planctomycetes, Chlorobi, Saccharibacteria, Verrucomicrobia, and Proteobacteria	Gammaproteobacteria (25%) Betaproteobacteria (24%) Flavobacteriia (18%) Cytophagia (13%) Clostridia (18%) Sphingobacteriia (12%) Epsilonproteobacteria Mollicutes Bacteroidia Chloroflexia Cytophagia	*Flavobacterium* sp. (13%), *Pseudomonas* sp. (8%), *Alcaligenes* sp., *Acinetobacter* sp., *Legionella* sp., *Acidovorax* sp., *Dokdonella* sp., *Bacillus* sp., *Lactobacillus* sp., *Terrimonas* sp., *Pseudofulvimonas* sp., *Nitrospira* sp., *Nitrosococcus* sp., *Nitrosomonas* sp., *Brevundimonas* sp., *Pedobacter* sp., *Chryseobacterium* sp.,	Illumina Miseq sequencing platform	Zhang et al. (2019)

(continued)

Table 8.1 (continued)

Phylum	Class	Genera	Method of study	References
	Nitrospira Negativicutes Ignavibacteria Spartobacteria Spingobacteriia	*Comamonas* sp., *Archrobacter* sp., *Stenotrophomonas* sp., *Dyadobacter* sp., *Caulobacter* sp., *Cohnella* sp., *Massilia* sp., *Exiguobacterium* sp.		
Proteobacteria, Acidobacteria, Chloroflexi, and Bacteroidia Actinobacteria Synergistetes and Thermi		*Caldilinea* sp., *Dechloromonas* sp., *Thiobacillus* sp., *VadinCA02* sp., *Thauera* sp., *Nitrospira* sp.	Illumina shotgun DNA library	Zhang et al. (2018a, b)
Proteobacteria Nitrospirae Bacteroidetes Actinobacteria Firmicutes Euryarchaeota	Betaproteobacteria (46.19) Gammaproteobacteria (11.14) Alphaproteobacteria (8.19) Deltaproteobacteria (1.51) Epsilonproteobacteria (0.07) Nitrospira (15.4) Flavobacteriia Sphingobacteriia Cytophagia Bacteroidia Ignavibacteria Actinobacteria Gemmatimonadetes Acidobacteriia Solibacteres Clostridia Bacilli Negativicutes	*Rhodobacter* sp., *Caulibacter* sp., *Pseudomonas* sp., *Geobacter* sp., *Rhodopseudomonas* sp., *Thauera* sp., *Dechloromonas* sp., *Bordetella* sp., *Nitrosomonas* sp., *Nitrospira* sp., *Mycobacterium* sp., *Placntomyces* sp., *Bradyrhizobium* sp., *Burkholderia* sp., *Xanthomonas* sp.	Illumina HiSeq. 2000	Guo et al. (2017)

Actinobacteria Proteobacteria Nitrospirae Bacteroidetes Firmicutes Verrumicrobia Spirocheate Cyanobacteria Deinococcus-Thermus Chloroflexi		*Nitrospira* sp., *Nitrosomonas* sp., *Pseudomonas putida* sp., *Nitrosovibrio* sp.	GeoChip 4.2	Xia et al. (2016)
Actinobacteria Proteobacteria Verrumicrobia Firmicutes Bacteroidetes Fusobacteria Nitrospirae Planctomycetes Deinococcus-Thermus Chloroflexi Tenericutes Euarchaeota	Deltaproteobacteria Epsilonproteobacteria Betaproteobacteria Gammaproteobacteria Alphaproteobacteria Dienococci Clostridia Thermomicrobia Methanobacteria Methanomicrobia	*Acinetobacter* sp., *Akkermansia* sp., *Acidaminococcus* sp., *Cloacibacterium* sp., *Megasphaera* sp., *Prevotella* sp., *Streptococcus* sp., *Trichococcus* sp., *Gemmatimonas* sp.	GS Junior system (Roche)	Shchegolkova et al. (2016)
Actinobacteria Proteobacteria Nitrospirae Bacteroidetes Firmicutes Verrumicrobia	Betaproteobacteria Gammaproteobacteria Alphaproteobacteria Deltaproteobacteria Flavibacteria Nitrospira	*Mycobacterium* sp., *Clostridium* sp., *Hyphomicrobium* sp., *Tissierella* sp., *Sphingomonas* sp., *Desulfobacterium* sp., *Kribbella* sp., *Deinococcus* sp.,	Metagenomics-rapid annotation using Subsystem Technology server (MG-RAST)	Yadav et al. (2014)

(continued)

Table 8.1 (continued)

Phylum	Class	Genera	Method of study	References
Spirocheate Cyanobacteria Deinococcus-Thermus Chloroflexi Tenericutes Verrumicrobia	Bacteroidia Solibacteres Chloroflexi Clostridia Cytophagia Deinococci Sphingobacteria	*Saccharopolyspora* sp., *Myroides* sp.		

genes. DNA sequences related to a wide range of enzymes such as hydroxylamine reductase, ammonia monooxygenase, nitrate reductase, hydroxylamine oxidase, nitrilase, formamidase, carbamate kinase, nitrous oxide reductase, nitrite reductase, nitric oxide reductase, and nitrogenase were obtained. Ammonification genes such as *amoCAB* which encodes enzyme ammonia monooxygenase increase with the rise in temperature from 20 °C to 35 °C, which was associated with a concomitant reduction in enzymes related with denitrification. At much lower temperature (20 to 5 °C) the genes connected to nitrogen metabolism were increased. Moreover, at lower temperature genes related to carbamate kinase, glutamate dehydrogenase, and glutamine synthetase were increased. Enzyme nitrite reductase (*nrfA*), associated with reduction of nitrite to ammonia, along with hydroxylamine reductase (*hcp*), associated with reduction of hydroxylamine to ammonia, was increased.

Yu and Zhang (2012) also reported the abundance of hydroxylamine reductase (*har*), ammonia monooxygenase (*amo*), nitrate reductase (*nar*), hydroxylamine oxidase (*hao*), nitrite reductase (nir), nitrous oxide reductase (*nos*), nitric oxide reductase (*nor*), and nitrogenase (*nif*) genes. In a 2.4 Gbp DNA *nir* gene was found in abundance, followed by *nor* and *nos* coding gene sequences. The prevalence of nitrification enzyme coding gene sequences along with *amo* and *hao* was found to be the lowest. Nitrifying virus was expressed in a higher amount than that of denitrification enzymes. In the case of hydroxylamine oxidase, the cDNA–DNA ratio was around 0.09. Nitrification enzyme genes, such as *amo*, showed much higher expression activities in activated sludges, which was mainly due to the higher concentration of ammonia in sewage.

Xia et al. (2016) reported a total of 528 genes which showed phosphorus utilization activity including polyphosphate kinase (*ppk*; 37.3%), exopolyphosphatase (*ppx* 57.6%), and *phytase* (5.1%). Exopolyphosphatase (*ppx*) was found to be highly capable of catalyzing the anaerobic hydrolysis of terminal residues of long-chain polyphosphate to inorganic phosphate (Pi). Apart from this, the genes related to a wide number of functions like carbon, phosphorus, and sulfur cycling, and also of organic pollutant remediation were reported. The genes related to processes such as denitrification, ammonification, nitrogen fixation, assimilatory, and dissimilatory nitrogen reduction were also found.

According to studies made by Song et al. (2020), they reported a novel species, *Casimicrobium huifangae*, which belonged to the core microbial community of activated sludge. The isolate was found to reduce nitrate into nitrite but neither into ammonia or into N_2, NO, and N_2O. Genes encoding nitrogen regulation sensor (*ntrB*), nitrate transport (*nasD* and *nrtA*), nitrite reductase (*nirBDS*), nitrate reductase (*narGHV*), and other proteins (*narJKL*) were annotated which was associated with nitrogen metabolism. This strain also has a wide range of phosphate transporters and conversion genes, such as *pstABCS* and *phnEC* for removal of phosphorus. Apart from them, one *ppx*, two *ppk*, and one poly(3-hydroxyalkanoate) polymerase gene (*phaC*) are also present which may help in phosphorus accumulation. Moreover, this isolate was also able to tolerate a wide range of heavy metals and have genes for p-type ATPase for efflux of metals and multidrugs (*mrcA, acrAB,* and *oprM*).

The GWMC recorded the universal occurrence of *Nitrospira* in a global survey of wastewater treatment plants. *Nitrotoga* and *Nitrobacter* were the most abundant nitrite oxidizers. Similarly, *Nitrosomonas* was also present which is the most prevalent ammonia-oxidizer. *Nitrosomonas*, *Nitrotoga*, and *Nitrobacter* were the nitrification bacteria.

8.5 Antibiotic Resistance Genes of Activated Sludge

Antibiotic resistance has been considered as a global problem and in developing nations like India, poor waste management and inadequate sanitary practices leads to the further spread of antibiotic resistance genes (ARGs) in environment. They are mostly persistence nature, have slow decaying rate, and are reckoned as chemicals of upcoming concerns or as potent pollutants. Wastewater treatment plants contain microbes from both human and environmental sources and can be a rich source of ARGs, which are developed by natural selection or by adaptation in bacteria due to constant exposure to antibiotics. Moreover, wastewater treatment plants receive water from households, and pharmaceutical industries which contains antibiotic residues and antibiotic-resistant bacteria at higher concentrations. All these exert a selective pressure on antibiotic-resistant bacteria and expression of ARGs (Nnadozie et al. 2017; Karkman et al. 2017), thus acting as a hotspot for the spread of antibiotic resistance in different groups of bacteria. Activated sludge, being rich in nutrient concentration, is ideal for bacterial growth and facilitates horizontal (lateral) gene transfer. Mainly resistance against antibiotic classes, such as β-lactams, fluoroquinolones, tetracyclines, and macrolides is most prevalent (Almakki et al. 2019).

Mobile genetic elements, such as a plasmids, transposons, and integrons, contribute largely to the dissemination of ARGs. However, till now very few studies have been conducted on the host cells which harbor such ARGs. As much as thirty ARGs encoding resistance to quinolones, sulfonamides, tetracycline, or macrolides were identified in activated sludge of two wastewater treatment plants of China by Mao et al. (2015). Mao et al. (2015) reported a significant enrichment of 10 ARG including *sulI, sulII, qnrB, tetG, tetB, tetS, tetH, tetX, tetT,* and *ermC*.

In a recent study by Liu et al. (2019), they have identified around 22 bacterial phyla which can act as a putative host for these genes. Genera, such as *Mycobacterium* and Burkholderiaceae family harbors around 14–50 ARGs. Metatranscriptome analysis showed nearly 65.8% of the identified ARGs were being expressed showing that they are transcriptionally active in the bacterial population of which most were plasmid associated rather than being within bacterial chromosomes. Several researchers like Bengtsson-Palme et al. (2016), Karkman et al. (2016), and Yang et al. (2014) showed the presence of antibiotic resistance genes associated with beta-lactam, sulfonamide, vancomycin, and tetracycline. Metagenomics analysis was found to be the most efficient method for the analysis of antibiotic resistance genes by researchers like Pal et al. (2016) and Van Goethem et al. (2018). Liu et al. (2019) in an extensive study on ARGs in activated sludge reported 24 different

classes of antibiotics in activated sludge and genes associated with antibiotics like acriflavines, aminoglycosides, betalactams, bacitracin, multidrug resistance (MDR), daunorubicin, macrolide–lincosamide–streptogramin (MLS), polymyxin, and sulfonamide. Inactivated sludge multidrug resistance genes were most abundant followed by betalactams, macrolide–lincosamide–streptogramin, and bacitracin. A similar research carried out by Zhao et al. (2018). Yang et al. (2013) reported aminoglycosides and tetracycline resistance to be most prominent in activated sludge. Twenty different antibiotic resistance genes, such as bacitracin (*bacA*, *bceA*), acriflavine (*acrB*, *acrF*), bleomycin (*ble*), beta-lactam (*pbp2*), fosmidomycin (*rosA*), kasugamycin (*ksgA*), daunorubicin (*drrA*), MDR (*mdtC*, *mdtB*, *mexK mexW*), polymyxin (*arnA*, *arnC*), sulfonamide (*sul1*, *sul2*), MLS (*macA*, *macB*), and trimethoprim (*dfrA3*), accounted for nearly 70% of the total types of ARGs, of which Gene *macB* (macrolide resistance gene) was very predominant in nature. Several genera of antibiotic resistance bacteria have also been reported in activated sludge, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* spp., Enterobacteria, *Pseudomonas*, and *Acinetobacter*, among others (Bouki et al. 2013; Figueira et al. 2011). Typically, members of Enterobacteriaceae reported resistance to 13 different antibiotics (Amador et al. 2015). Apart from them, opportunistic pathogenic bacteria such as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, Enterobacteriaceae, *Staphylococcus aureus* with ARGs were also reported (Alexander et al. 2015).

Korzeniewska and Harnisz (2018) reported resistance to cefotaxime which is a relatively new antibiotic and its resistance was easily transmitted in Gram-negative *E. coli*.

Song et al. (2020) reported a novel species, *Casimicrobium huifangae*, carrying resistance to a wide range of antibiotics which included bacitracin (uppP), tetracycline (typA and lepA), streptogramin (vat), macrolides (macB), polymyxin (yfbG), kasugamycin (rsmA), aminoglycosides (aacA), and β-lactams.

Qi et al. (2021) reported different functional microbiomes, one associated with complete catabolism of sulfamethoxazole, and the second one was associated with complete catabolism of phenyl part of sulfamethoxazole (SMX). They also reported *Paenarthrobacter* and Nocardiodes as primary degraders of sulfonamide functional group (–C–S–N– bond) and (3-amino-5-methylisoxazole) (3A5MI). Yan et al. (2022) reported SMX and ARGs from both autotrophic and heterotrophic microorganisms. It was found that heterotrophic bacteria contributed crucially to SMX degradation; however, ammonia-oxidizing bacteria displayed a superior metabolic rate and contributed much to SMX removal by cometabolism.

8.6 Future Challenges and Opportunities

The activated sludge microbiome consists of a plethora of bacteria, archaea, viruses, and protists which play a crucial role in the degradation of toxic organic pollutants. Most of these microbial communities are interconnected at trophic levels and also related in their degradation and metabolic pathways. Earlier, it was always difficult

to assess them using culture dependent methods. With the advent of omics technology and the availability of the metagenomics and metatranscriptomic datasets, it has become possible to assess the whole community composition of activate sludge in detail. Moreover, the identification and assessment of ARGs present and actively transcribed have increased our understanding the fate of highly expressed ARGs and multidrug-resistant hosts from wastewater treatment plants. Also, both metagenomics and metatranscriptomic datasets have provided us with ample information on the influence of environmental factors in the activated sludge process. It has provided us with a detailed idea on the shift of alpha and beta community diversity due to variations in temperature, which is considered crucial for the effectiveness of the activated sludge process. We could also assess the key functional groups present in activated sludge, which largely include ammonia-oxidizing bacteria, denitrifiers, and nitrogen-fixing bacteria and their potential role in activated sludge. Further research on the microbial community of activated sludge will broaden our knowledge and help in better application and further modification of the process.

8.7 Conclusion

The activated sludge process is a process of biological treatment of wastewater which is popular all over the world. This entire process can be divided into the aeration phase and sludge settlement phase. The wastewater from the primary settlement tank is passed into the aerobic tank having a wide range of microorganism populations. The aeration phase helps in the maintenance of microbial flocs and maximizes the oxidation of the contaminant which is followed by sludge formation and separation. The advent of omics technology has helped us to gain a wide knowledge of the microbial community present in activated sludge. This bacterial community is a repository of many antibiotic resistance genes. Moreover, this microbial community has several physiological functions, performs several types of biogeochemical cycles, and sequestration of nutrient from the sludge. A detailed understanding of the microbial community assembly will help us to develop deeper understanding on the microbial-ecological theories.

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