Applied Water Science (2024) 14:215
https://doi.org/10.1007/s13201-024-02268-2

REVIEW ARTICLE

Potential of complex microbial community in aerobic granular sludge as a bio-startup approach for next-generation wastewater treatment

Adel Al-Gheethi[1,2] · Efaq Ali Noman[3,6] · Mohammed Alsahari[4] · Qais Al-Maqtari[4] · Dai-Viet N. Vo[5,7]

Received: 7 April 2024 / Accepted: 5 August 2024

Abstract
Activated sludge has a high diversity of infectious agents. However, many of these microorganisms contribute greatly to the biological treatment process due to the enzyme production and capability for degrading a wide range of organic compounds in wastewater. The present review discusses the potential of aerobic granular sludge as a bio-startup (AGS-BS-up) for new wastewater treatment plants. The complex microbial community in activated sludge was investigated using 16S metagenomic analysis. The potential of aerobic granular sludge prepared from activated sludge to be used as a bio-starting medium for food wastewater treatment plants (FWWTP) was discussed. AGS-BS-up appears to have high applicability to enhance the biological treatment of food wastewater (FWW). The hypothesis behind using aerobic granular sludge as a bio-startup for FWWTP lies in the high microbial biodiversity in the aerobic granular sludge, which contributes effectively to the biological treatment of food wastewater. Based on the previous studies, the AGS-BS-up is efficient in improving the quality of FWW to meet international standards required for safe disposal into the environment.

Keywords Activated sludge · Bio-startup · 16S Metagenomic · Microbial community · Wastewater

Abbreviations

AGS-BS-up	Aerobic granular sludge bio-startup
BOD_5	Biochemical oxygen demand
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DSP	Dewatered sludge particles
EPS	Extracellular polymeric substances
FTIR	Fourier-transform infrared spectroscopy
FWW	Food wastewater
FWWTP	Food wastewater treatment plant
gDNA	Bacterial genomic DNA
GMOs	Genetically modified organisms
MLSS	Suspended solid mixed liquor
MLVSS	Volatile suspended solid mixed liquor
SBR	Sequencing batch reactor
SOUR	Specific oxygen uptake rate
SPGS	Spherical pelletising granular sludge
STPs	Sewage treatment plants
TEA	Techno-economic analysis
TSS	Total suspended solids
WWTP	Wastewater treatment plants

✉ Adel Al-Gheethi
adel.abduh@newcastle.edu.au; adelalghithi@gmail.com

✉ Dai-Viet N. Vo
vndviet@ntt.edu.vn; vo.nguyen.dai.viet@gmail.com

1 Global Centre for Environmental Remediation (GCER), University of Newcastle, Newcastle, Australia

2 CRC for Contamination Assessment and Remediation of the Environment (CRC CARE), Newcastle, Australia

3 Department of Applied Microbiology, Faculty of Applied Sciences, Taiz University, Taiz, Yemen

4 Micropollutant Research Centre (MPRC), Institute of Integrated Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

5 Institute of Environmental Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City 755414, Vietnam

6 School of Chemical & Biomolecular Engineering, University of Sydney, Sydney, NSW, Australia

7 Graduate School of Energy and Environment, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

Introduction

Activated sludge has a high microbial diversity (bacteria, fungi, protozoa, and viruses). Some of these microbes are pathogenic and cause diseases for humans, animals, and plants, while others are non-pathogenic and contribute effectively to the sewage treatment process. The high microbial diversity effectively contributes to the organic pollutants degradation during biological treatment. The current direction is to use the aerobic granular sludge as a bio-startup for a new wastewater treatment plant. This step represents the most important stage of operation. In this process, some sludge from the treatment plant is added to the new system as seeds. The studies in the literature have reported that the bio-startup added to the new wastewater treatment plants (WWTP) improved the chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and suspended solid mixed liquor (MLSS) removal from 40 to 90% and total suspended solids (TSS) reached 96% (Khalili et al. 2013). The advantages of bio-starting included less foam forming, using less seeds, saving costs in sludge transport and better process control (Khalili et al. 2013). Aerobic granular sludge bio-startup (AGS-BS-up) has a dense physical structure, high biomass content, excellent settling property, and a strong ability to withstand unfavourable conditions (Wang et al. 2020).

Huge amounts of activated sludge are generated annually (Kiselev et al. 2019; Hanum et al. 2019). A total of 12.56 mtDM/year of activated sludge was generated in the USA (Seiple et al. 2017), while was 6.25 mtDM/year in China (Yang et al. 2015). The commercial sewage treatment startup (using bacteria and enzymes) has been performed by several companies in the world such as the UK, Canada, Germany, the USA, France, the Netherlands, and India, and the total estimated investment exceeded USD 100 million. The bacterial strains were selected based on the high production of lipase, cellulase, amylase, and protease enzymes (Hydra International Ltd 2023).

On the other hand, thousands of food manufactures are available in the world, and each manufacturer needs to have a wastewater treatment plant (WWTP) for the food processing wastewater before final disposal in the environment. These WWTPs are operated using ASBS-up for each treatment cycle, and this is because the sludge generated from the food wastewater treatment plant (FWWTP) has very low microbial diversity and is not sufficient to be recycled in the next biological treatment process.

The imported startups from other environments are associated with many issues in biodiversity. The startups with different microorganisms might negatively affect nature conservation, especially if the microbial diversity used in the startups has been subjected to genetic engineering to enhance the degradation properties of the bacterial cells. Genetically modified organisms (GMOs) have the potential to reproduce and survive with a long-term effect on natural ecosystems and biological communities. GMOs develop virulence factors that increase their threat to infection, such as antibiotic resistance and pathogenicity and the ability to avoid adaptive immunity caused by the vaccine and change in transmission properties (Caplanova and Sirakovova 2023). One more limitation of using sludge startup from different environments is the efficiency of the microbial diversity to achieve a high reduction in wastewater parameters such as COD, BOD, and nutrients (nitrogen and phosphorus) due to the differences in the environmental conditions which may need a long period to adopt with new environmental conditions. It was revealed that the efficiency of sewage startup might take three months to waste sludge (Khalili et al. 2013).

The selected microbial in AGS-BS-up must have the ability to survive under extreme conditions (pH, osmotic factor, nutrient, moisture, and redox) and compete with indigenous microbial populations and predators (Liu et al. 2023). The application of AGS-BS-up has received great attention in the last few years. In previous studies, biofringe loaded with activated sludge microorganisms was investigated in the sequencing batch reactor (SBR) to improve the removal of ammonium and organic material from leachate from sanitary landfill leachate and poultry wastewater (Aziz et al. 2018; Baker et al. 2020). More studies are required to deeply understand the mechanism of AGS-BS-up with the role of microbial diversity in enhancing the degradation of organic materials with high-efficiency preparation methods for AGS-BS-up to be easily applicable in wastewater treatment. The local dewatered granular particles with indigenous microorganisms as bio-starting (AGS-BS-up) has high applicability.

The hypothesis behind using aerobic granular sludge as a bio-startup for food wastewater treatment process lies in the high microbial biodiversity in the aerobic granular sludge, which contributes effectively to the biological treatment of food wastewater (FWW). The process depends on the efficiency of the microbial consortium to produce hydrolysis enzymes such as cellulase, cutinases, lipases, proteases, esterases, laccase, peroxidases, oxygenases, dehydrogenases, amidases, hydrolases, and carboxylesterases. These enzymes are the keys to the biodegradation of organic compounds in the FWW as a function of synergistic effects between the microbial consortia.

Application of aerobic granular sludge as a bio-startup obtained from a similar environmental condition to the FWW accelerates the treatment process with high efficiency in reducing COD and BOD as well as nutrients of the effluents to meet the standard limits required by national and international regulations. The preparation of AGS-BS-up effectively achieves high efficiency in the degradation of different organic compounds in the FWW. Queries still need to be answered, including the microbial diversity in different activated sludge generated from sewage treatment plants (STPs) in different countries, the best operating parameters for achieving high degradation efficiency of the organic compounds in the FWW, the safety of AGS-BS-up as a final product without negative impact on the environment, the applicability to be used for FWW compared to sludge generated from FWW treatment plants, and the economic cost.

The present review aims to highlight the applicability of AGS-BS-up in the analysis of the complex microbial community of activated sludge which has not been extensively covered in the literature review. The role of machine learning applications in the optimisation process of ASBS-up was discussed. The life cycle assessment and techno-economic studies conducted in the previous studies on AGS-BS-up were reviewed. The current review provides insight into aerobic granular sludge as a bio-startup for food wastewater treatment to empower resource recovery and circular economy and future perspectives for the application of AGS-BS-up on a large scale. In comparison with previous studies, the present work offers a new view for recycling of sludge as a products for new wastewater treatment plant under them of circular economy.

A complex microbial community in the activated sludge

The activated sludge is generated from a biological or secondary wastewater treatment process. As a suspended growth biological treatment process, active sludge uses suspension density microcultures to degrade organic matter under aerobic conditions and forms a biological bubble for solid separation in the stabilisation unit (Kim et al. 2023). The degree of sewage treatment processes varies from the main process of a very polluted waste to the advanced or higher processes of removal of trace concentrations and continues after the main process (Nazif et al. 2023). The final choice of treatment technology is determined by the source of wastewater and the treatment objective (Fig. 1). The scheme of a sewage treatment plant, which produces activated sludge, is depicted in Fig. 2. The generated treated effluent has 10 mg L^{-1} of total ammonia nitrogen, 20 mg L^{-1}

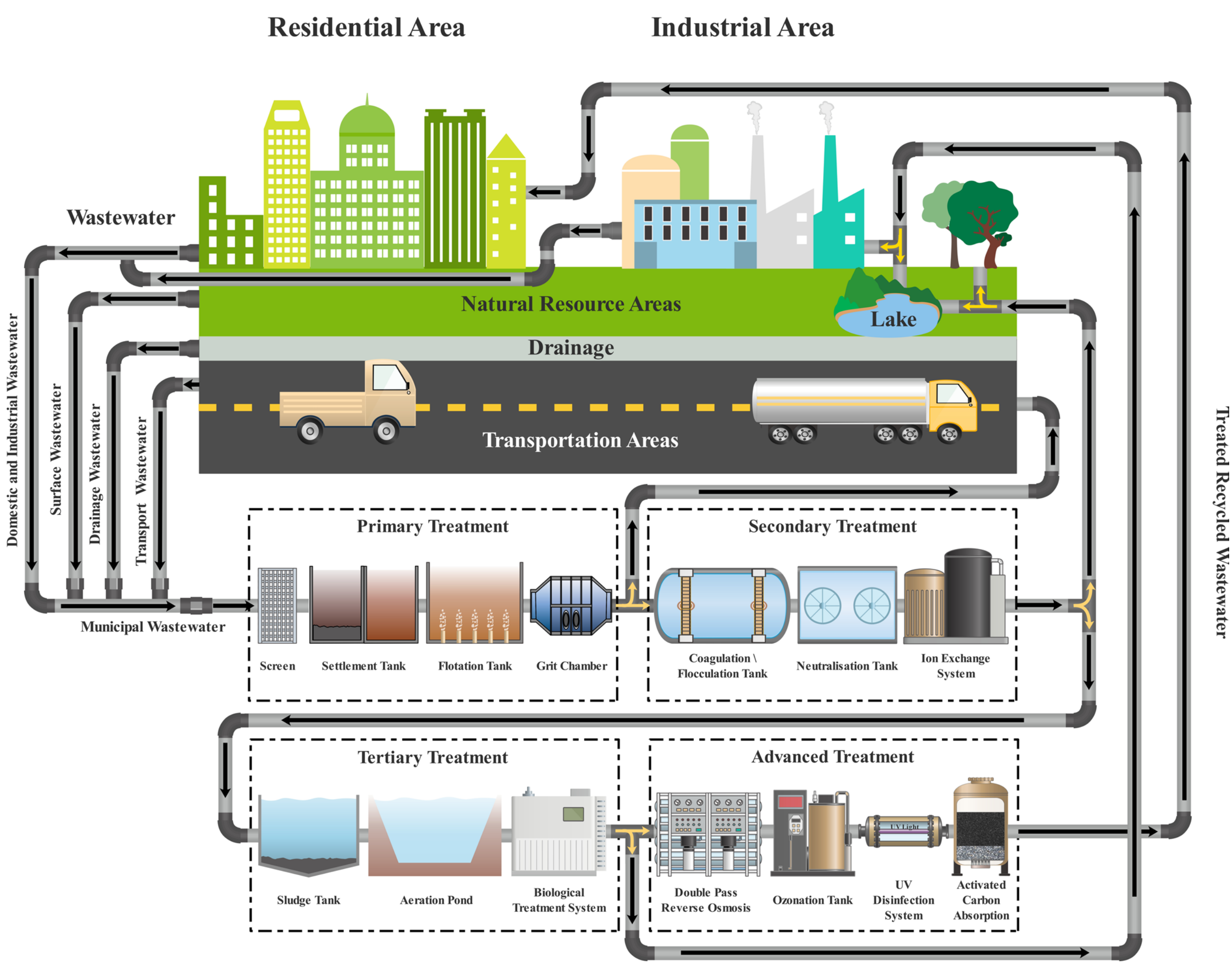

Fig. 1 Treatment technologies of sewage based on the purpose as described by (Al-Gheethi 2014)

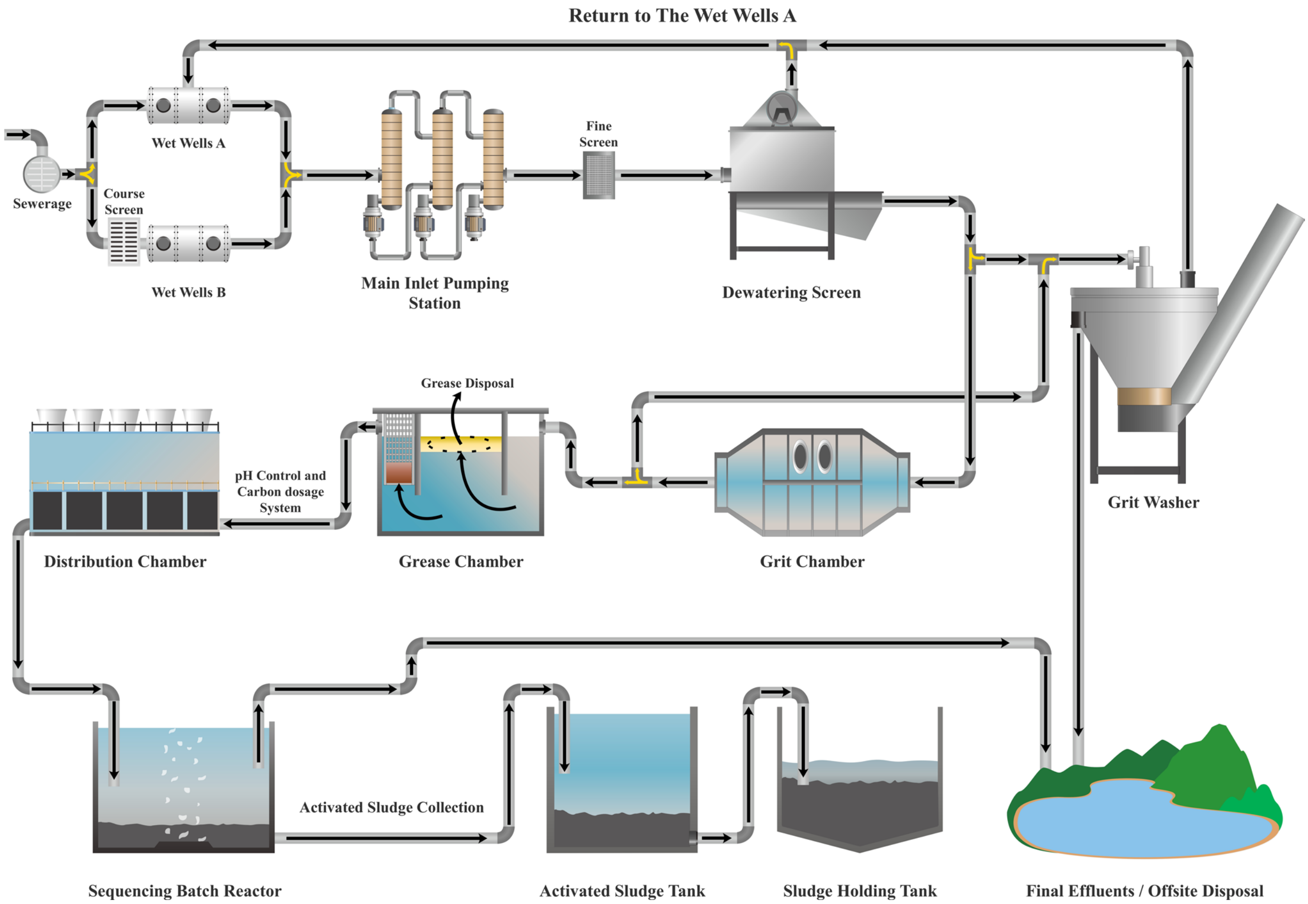

Fig. 2 Activated sludge generation process during sewage treatment process, an example of Jelutong sewage treatment plant, Penang Malaysia (as described by Al-Gheethi 2014)

of BOD, 50 mg L^{-1} of TTS, and 5 mg L^{-1} of nitrate nitrogen (Al-Gheethi 2014). Due to restrictions on land availability, most STPs adopt the SBR activated sludge technology with an anaerobic sludge digester and dewatering system (Fig. 3).

Activated sludge has high microbial diversity, which could enhance the biological treatment of wastewater. Table 1 shows previous studies which have investigated the microbial diversity in the activated sludge. In our preliminary study, an activated sludge was subjected to 16S metagenomic analysis. In this study, an activated sludge was obtained from a sewage treatment plant and submitted to CCB-USM to extract DNA sequences and determine the complex microbial community. For the metagenomics study, bacterial genomic DNA (gDNA) was extracted from activated sludge, quantified, purified, and prepared its libraries. The bioinformatics analysis was computed using Prodigal and open reading frames annotated against highly curated protein databases (i.e. KEGG, UniRef90, and UniProt). The draft genome sequences were used to identify each organism. According to data analysis of 16 metagenomic which was conducted in the present work, the results revealed that 95% of microorganisms in the activated sludge belonged to bacteria with more than 450 bacterial strains, while 0.5% belonged to Archaea. The bacterial diversity was classified into 21 bacterial phyla (Table 2). The most common bacterial strains belonged to *Bacteroidetes* (32.2%), *Proteobacteria* (32%), *Firmicutes* (20.2%), *Chloroflexi* (6.1%), *Actinobacteria* (3.4%), and *Acidobacteria* (1.3%). Many of these microbes are not culturable. *Proteobacteria* were classified into *Alpha-Proteobacteria, Beta-Proteobacteria, Gama-Proteobacteria,* and *Delta-Proteobacteria* (Fig. 4A), where 17% of *Alpha-Proteobacteria* (Fig. 4B), 6% of *Beta-Proteobacteria* (Fig. 4C), 5% of *Gama-Proteobacteria* (Fig. 4D), and 9% of *Delta-Proteobacteria* (Fig. 4E) are non-culturable bacteria. On the contrary, 20% of *Bacteroidetes* (Fig. 4F), 32% of *Firmicutes* (Fig. 4G), 43% of *Clostridiales* (Fig. 4H), 7% of *Actinobacteria* (Fig. 4I), 21% of *Acidobacteria* (Fig. 4J), 24% of *Chloroflexi* (Fig. 4K), and 40% of Anaerolinea (Fig. 4L) are not culturable. These findings indicate that microbial diversity depends on environmental conditions, the size of the local community, public health,

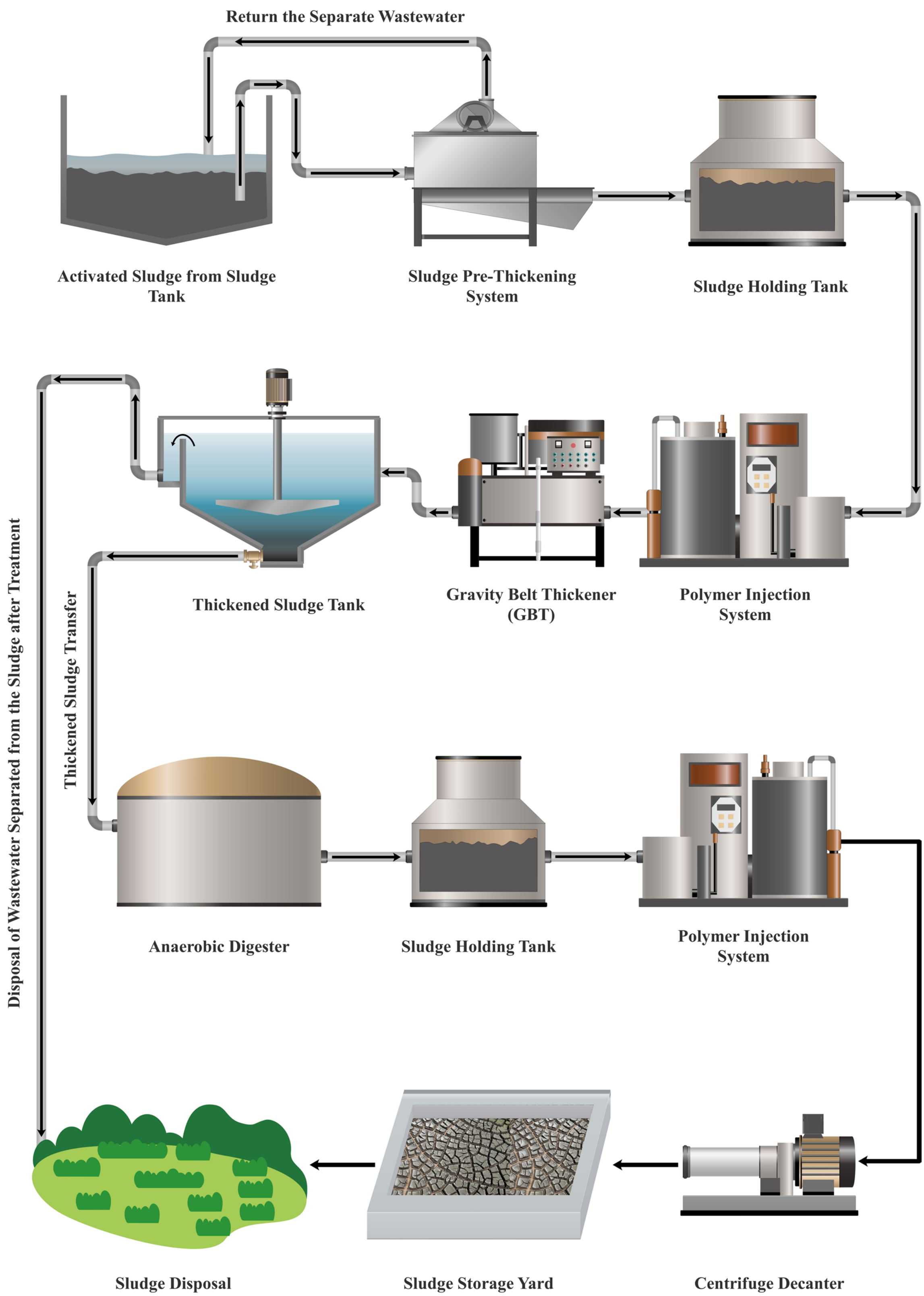

Fig. 3 Activated sludge technology with anaerobic sludge digester and dewatering system, an example of Jelutong sewage treatment plant, Penang Malaysia (as described by Al-Gheethi 2014)

Table 1 Type of activated sludge around the globe and the microbial diversity using metagenomic analysis of sewage

Type of activated sludge	Country origin	Microbial diversity using metagenomic analysis	Method used for Metagenomic analysis	References
Granular sludge from anaerobic digester	WTP, China	*Smithella* sp., *Syntrophomonas* sp., *Syntrophobacter* sp., *Methanosaeta* sp., *Methanolinea* sp., *Methanospirillum* sp., *Methanolinea* sp., *Methanomicrobiales* sp., *Methanospirillum* sp.	16S rRNA	Feng et al. (2023a, 2023b)
Solid sludge from anaerobic digester fed with pig manure and food residue	WTP, China	*Youngiibacter* sp., *Hydrogenophaga* sp., *Sedimentibacter* sp., *Clostridium_sensu_stricto Clostridium* sp., *Methanosaeta* sp., *Methanolinea* sp., *Methanofastidiosum* sp., *Methanospirillum* sp.	16S rRNA	Feng et al. (2022)
Sludge from aeration tank	WTP, China	*Proteobacteria* sp., *Firmicutes* sp., *Bacteroidetes* sp., *Chloroflexi* sp., *Actinobacteria* sp., *Saccharibacteria* sp.	16S rRNA	Yan et al. (2022)
Sludge seed from anaerobic digester	WTP, Malaysia	Synergistetes, Bacteroidetes, Planctomycetes, Firmicutes, Chloroflexi, Proteobacteria, Tenericutes, Thermotogae, Cyanobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Euryarcheaota, Fibrobacteres, Spirochaetes, Lentisphaerae, Nitrospirae, Crenarchaeota, Deferribacteres, Caldiserica	oligonucleotide primers illumine V3/V4F and illuminaV3/V4R	Audu et al. (2021)
Activated sludge From anaerobic tank	WTP, Japan	Accumulibacter sp.	16S rRNA	Nittami et al. (2020)

and the presence of factories and hospitals in the same area. This variation occurs geographically and over time at the same place. Analysis of the complex microbial community in activated sludge provides a greater understanding of their ecology and role in wastewater treatment.

Compared with similar studies in other countries, Kim and Cui (2023) investigated the complex microbial community in six sludge samples, including anaerobic digestion sludge, denitrification sludge, livestock sludge, and activated sludge in the Republic of Korea. The study revealed that *Proteobacteria* were present in all sludge samples, representing 15.21% of the total bacteria in the activated sludge generated from municipal wastewater with anoxic and aerobic oxidation process, among them and *Shingobacteria* (17%), *γ-Proteobacteria* (12%) and *β-Proteobacteria* (22%), in aerobic oxidation. In sludge from anoxic oxidation *Fimbriimonadia* (13%), *Phycisphaerae* (19%), *β-Proteobacteria* (25%), and *Anaerolineae* (18%) were detected. Meanwhile, *Bacteroidetes, Acidobacteria, Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Clostridia*, *Anaerolineae*, *Bacteroidia*, and *Chloracidobacterium* were detected in anaerobic digestion sludge, denitrification sludge, and livestock sludge.

In another study by Raza et al. (2023) in Korea, two sludge samples were collected from STPs dealing with domestic wastewater, with a small portion of the wastewater from poultry farms in STP 1 and pretreated industrial wastewater from steel and electronic manufacturing industries in STP2. *Proteobacteria, Bacteroidetes*, *Actinobacteria,* and *Firmicutes* were the most common. *Acinetobacter, Prevotella, Cloacibacterium,* and *Arcobacter* were most common in sludge sample 1, while *Flavobacterium, Acidovorax,* and *Arcobacter* were more abundant in sludge sample 2. In a study conducted in Finland, sludge samples from domestic municipal wastewater from households, hospitals, a dairy, and fish processing plant, the most common bacterial phyla were *Fusobacteria* (1–10%), *Bacteroidetes* (35–44%), *Proteobacteria* (15–20%), and *Firmicutes* (30–37%). In

Table 2 Bacterial and Archaea phyla in one activated sludge sample from a sewage treatment plant in Malaysia based on metagenomic analysis

Taxonomy	%
Bacteroidetes	32.20
Proteobacteria	32.00
Firmicutes	20.20
Chloroflexi	6.10
Actinobacteria	3.40
Acidobacteria	1.30
Cyanobacteria	0.80
Epsilonbacteraeota	0.80
Patescibacteria	0.70
Euryarchaeota	0.50
Spirochaetes	0.50
Synergistetes	0.30
Other	0.20
Caldiserica	0.20
Latescibacteria	0.20
Calditrichaeota	0.10
Fusobacteria	0.10
LCP-89	0.10
Modulibacteria	0.10
Planctomycetes	0.10
TA06	0.10
Total (%)	100

February, with snowy winters, the most common was *Proteobacteria* (84%) and *Bacteroidetes* (9%) (Leiviskä and Risteelä 2022). Begmatov et al. (2022) investigated the complex microbial community in a sludge sample from a household and industrial wastewater treatment plant in Moscow. The study revealed that *Archaea* represented less than 2%. *Proteobacteria* were detected at 27.8%, among the alpha (4.3%) and gamma (23.5%); *Bacteroidetes* were detected at 15.7%, *Nitrospirota* (2.7%), *Verrucomicrobiota* (4.5%), *Patescibacteria* (5.5%), *Myxococcota* (5.9%), *Planctomycetota* (1.3%), *Chloroflexi* (6.6%), *Actinobacteriota* (12.5%), *Firmicutes* (5.6%), and *Bdellovibrionota* (3.9%). In a study performed in Poland by Kalinowska et al. (2022), two activated sludges from two domestic STPs with biological treatment processes were subjected to complex microbial community analysis with the 16S metagenomic method. The study revealed that *Archaea* was detected with less than 0.2% among different bacterial phyla. *Actinobacteria* ranged from 4.5 to 41.0%, *Bacteroidetes* from 0.5 to 12.4%, *Proteobacteria* ranged from 5.5 to 56.9%, *Cyanobacteria* ranged from < 0.01 to 60.9%, and *Firmicutes* were between 0.03 and 16.6%, while *Verrucomicrobia* ranged from 0.1 to 9.7% and *Planctomycetes* were between 0.04 and 8.5%. In a study carried out in China, *Proteobacteria*, *Bacteroidota*, *Chloroflexi,* and *Actinobacteriota* were the most dominant in activated sludge from a sequencing reactor (Feng et al. 2022). In another study in China, the most common bacterial phyla in activated sludge were *Proteobacteria*, *Bacteroidetes, Acidobacteria, Chloroflexi*, *Saccharibacteria*, *Planctomycetes*, and *Nitrospirae* (Yang et al. 2020). In a study conducted by Wang et al. (2021) in Hong Kong, the most common bacterial phyla were *Myxococcota, Verrucomicrobiota, Planctomycetota, Bacteroidota, Proteobacteria*, *Chloroflexota, Patescibacteria, Actinobacteriota,* and *Bdellovibrionota*.

In our preliminary study, the phyla of these bacteria were detected from sludge generated from an oxidation process. These findings confirmed that different sludge samples from different locations and treatment processes have different complex microbial communities, while sludge from Malaysia has high microbial diversity (Table 3). On the basis of the carbon and energy sources used by bacteria, complex microbial communities are photolithotrophic autotrophs, photoorganotrophic heterotrophs, chemolithographic autotrophs, and chemo-organotrophic heterotrophs. These bacterial communities could degrade a wide range of organic compounds in wastewater, including nutrients and persistent compounds with various metabolic activities, even better than using only microalgae.

Food processing wastewater characteristics and treatment

The main challenges associated with ensuring clean and safe water for communities include the absence a regular monitoring technologies as well as the climate change and presence several of emerging pollutants such as microplastics (MPs), pharmaceuticals and personal care products (PPCPs), and per- and polyfluoroalkyl substances (PFAS), which still have no regulations technophiles for their removal. The treatment of FWW is gaining more attention as a result of the environmental consequences associated with the FWW disposal without treatment. FWW treatment difficulties lie in the high constitution of organic carbon, suspended solids, and nutrients, which provide unique medium for microbial growth and transmission (Al-Gheethi et al. 2023). Untreated industrial waste is discharged into the environment; operating costs increase the attention among the owners of these companies to find effective ways to treat wastewater (Sathinathan et al. 2023).

In order to determine the main components, microbial diversity and current treatment technologies of food processing wastewater, 226 articles were downloaded from Scopus databases. These articles were subjected to bibliometric analysis based on keyword occurrence. The most common keywords were subjected to segregation in clusters such as main components, microbial diversity, and treatment technologies, as presented in Fig. 5. It was noted that FWW contains organic and inorganic compounds. Among these compounds are acetates, organic and inorganic acids, alcohol,

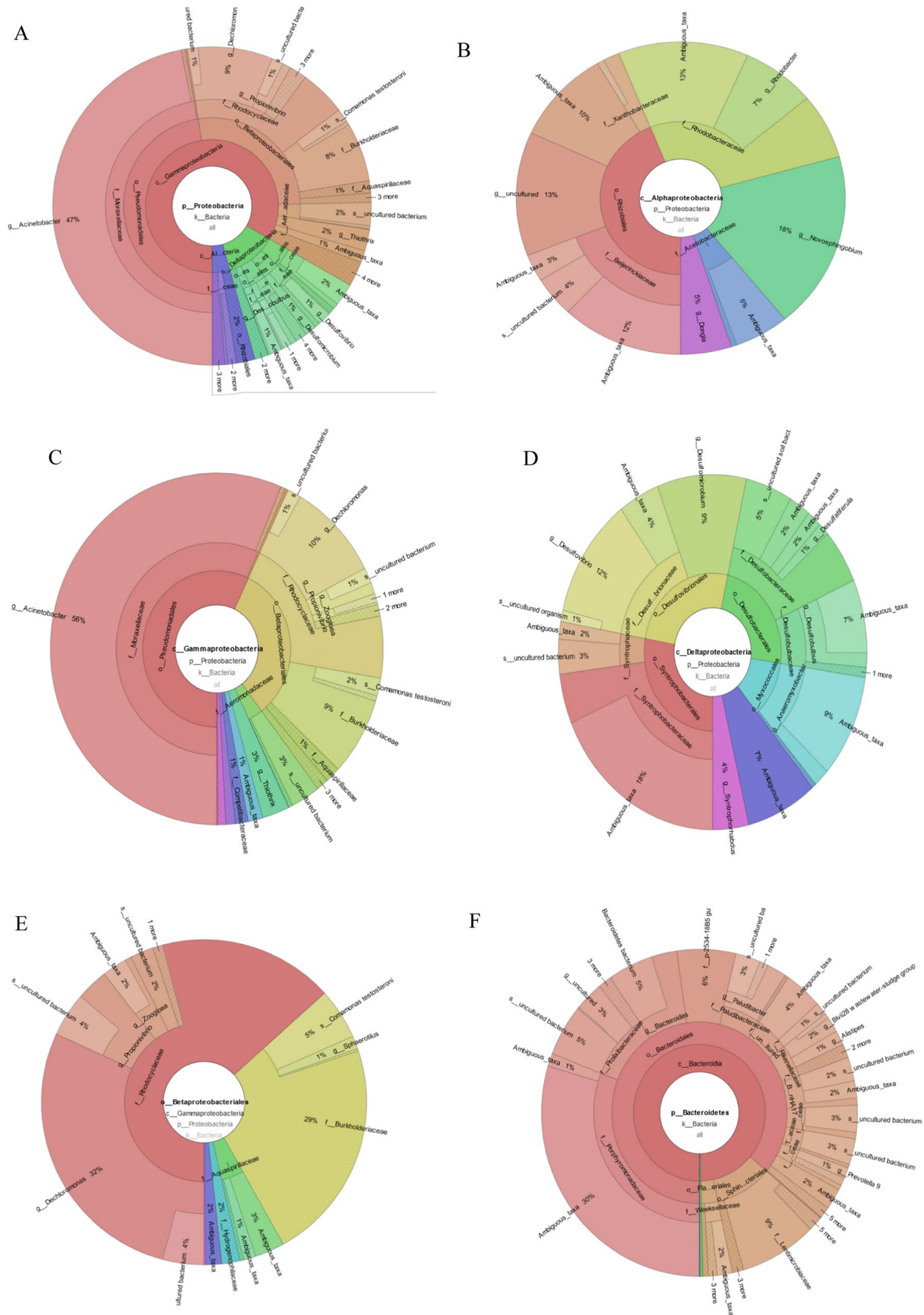

Fig. 4 Complex microbial community in the activated sludge; Proteobacteria (**A**); beta-Proteobacteria (**B**); Gama-Proteobacteria (**C**); Delta-Proteobacteria (**D**); Alpha-Proteobacteria (**E**); Bacteroidetes (**F**); Firmicutes (**G**); Clostridiales (**H**); Actinobacteria (**I**); Acidobacteria (**J**); Chloroflexi (**K**); and Anaerolinea (**L**)

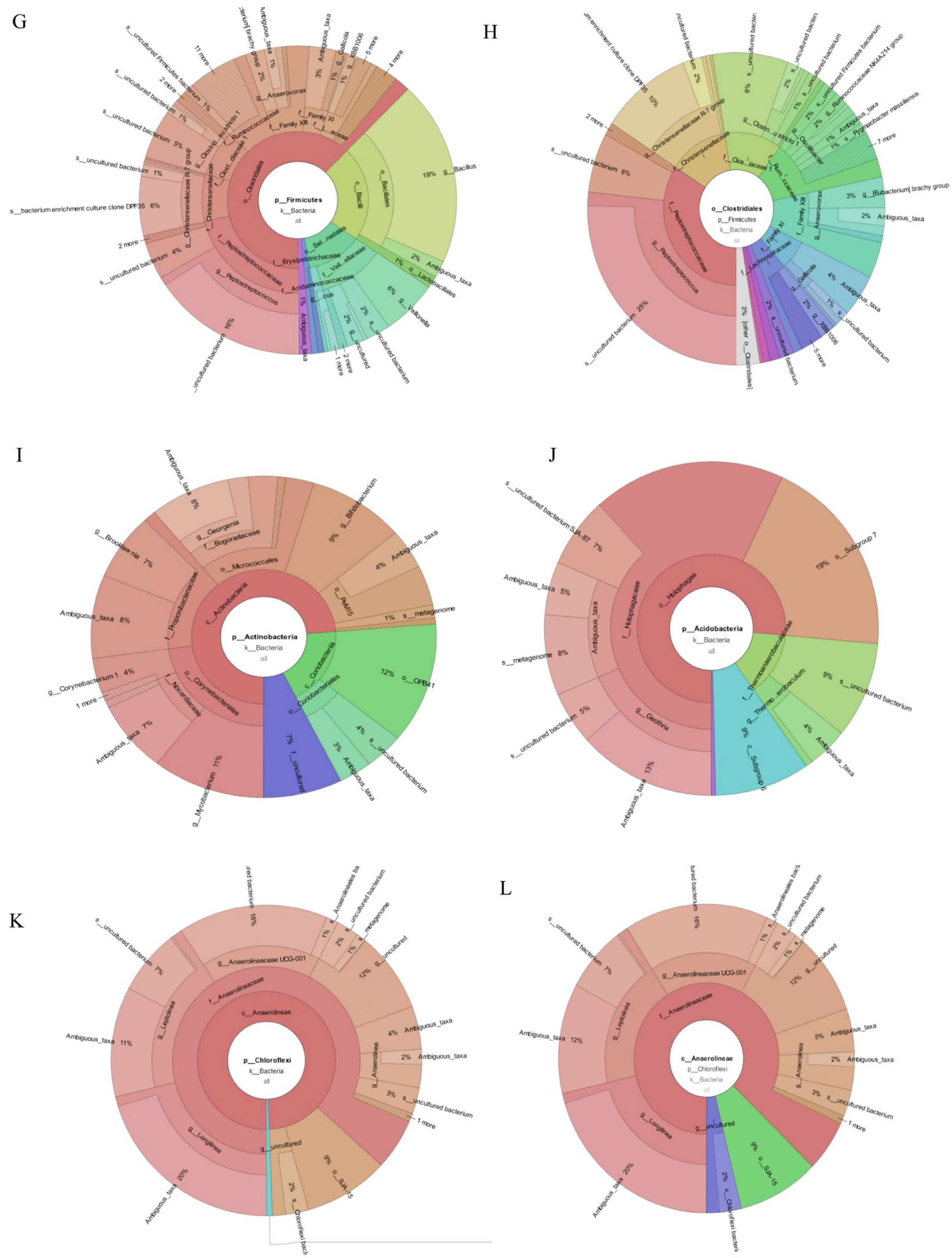

Fig. 4 (continued)

Table 3 Comparison of bacterial and Archaea phyla in activated sludge in Malaysia and other countries based on metagenomic analysis

	Malaysia Our preliminary study	Korea Kim and Cui (2023) and Raza et al. (2023)	Finland Leiviskä and Risteelä (2022)	Moscow Begmatov et al. (2022)	Poland Kalinowska et al. (2022)	China Feng et al., (2022) and Yang et al. (2020)	Hong Kong Wang et al. (2021)
Bacteroidetes	√	x	√	√	√	√	√
Proteobacteria	√	√	√	√	√	√	√
Firmicutes	√	x	√	√	√	x	x
Chloroflexi	√	x	x	√	x	√	√
Actinobacteria	√	x	x	√	√	√	√
Acidobacteria	√	x	x	x	x	√	x
Fimbriimonadia	x	√	x	x	x	x	x
Phycisphaerae	x	√	x	x	x	x	x
Fusobacteria	√	x	√	x	x	x	x
Archaea	√	x	x	√	√	x	x
Nitrospirota	x	x	x	√	x	x	x
Verrucomicrobiota	x	x	x	√	x	x	x
Patescibacteria	√	x	x	√	x	x	√
Myxococcota	x	x	x	√	x	x	√
Planctomycetota	√	x	x	√	√	√	√
Bdellovibrionota	x	x	x	√	x	x	√
Cyanobacteria	√	x	x	x	√	x	x
Verrucomicrobia	x	x	x	x	√	x	√
Epsilonbacteraeota	√	x	x	x	x	x	x
Spirochaetes	√	x	x	x	x	x	x
Synergistetes	√	x	x	x	x	x	x
Caldiserica	√	x	x	x	x	x	x
Latescibacteria	√	x	x	x	x	x	x
Diversity percentage (%)	70	17	17	52	35	26	39

polysaccharides, oil, and grease. In addition to nutrients, ammonium compounds, antibiotic agent, heavy metals, benzenesulfonamide derivatives and bicarbonates. Moreover micro- and bioplastics, polymers and biopolymers, carbohydrates, molybdenum compounds, chlorinated polyethylene, cinnamaldehyde, n(3-oxododecanoyl) homoserine lactone, *n*-acylhomoserine lactone, lipids, and proteins were among the most common compounds. These compounds require more advanced treatment technologies to be removed from FWW before safe disposal into the environment.

Treatment technologies that depend on physical, chemical, and biological concepts have been used for FWW treatment. The advanced oxidation process, aerobic biological treatment, aerobic granular sludge, anaerobic fluidised bed reactor, lagoons, membrane bioreactors, and thermophilic treatment as well as bioflocculation, ultrafiltration membranes, and ceramic membranes exhibited high efficiency in the treatment processes. The microbes-based treatment included fungal wastewater treatment, microalga-based wastewater treatment, microbial electrolysis cells, photobioreactors, upflow anaerobic sludge blanket reactors, and adsorption processes recorded high ability to degrade the organic contents in FWW.

The biological treatment methods are carried out as functions of microorganisms such as bacteria, fungi, and microalgae. In view of the bacterial diversity in FWW based on the previous studies and database analysed using bibliometric analysis keywords, it was found that the most common bacterial phyla and species are *Alpha-Proteobacteria, Beta-Proteobacteria, Delta-Proteobacteria, Planctomycetes, Archaea, Acinetobacter nosocomialis, Aurantiochytrium sp.Bacillus cereus, Burkholderia cenocepacia, Clostridium collagenovorans,* and Coliform bacterium. The findings show that the bacterial diversity in the FWW is very low compared to the activated sludges from domestic STP; recycling sludge generated from the FWW treatment process is insufficient to achieve a high degradation of different organic compounds in the raw FWW.

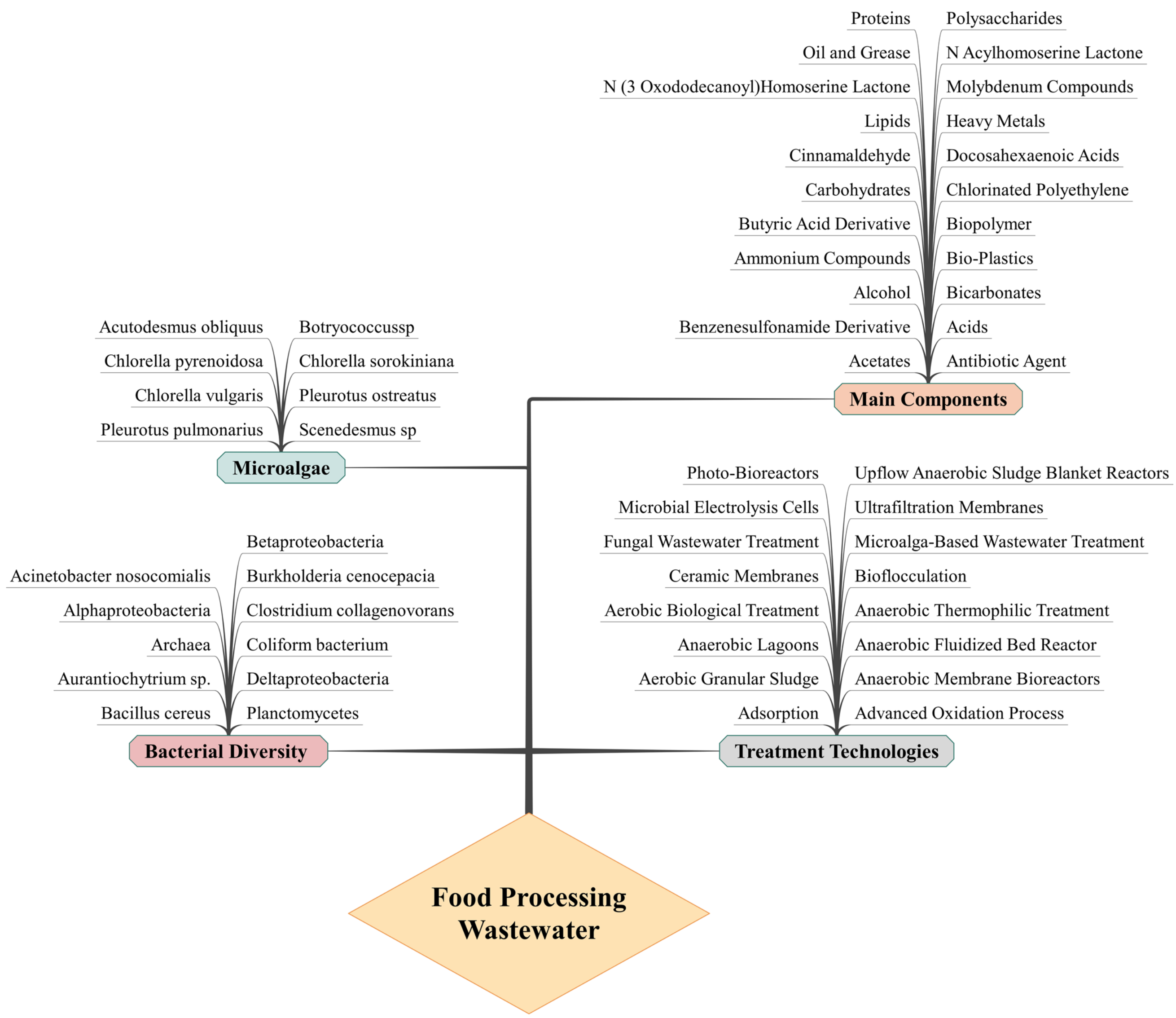

Fig. 5 Main components, microbial diversity, and treatment technologies used for FWW treatment process

The direction is to inoculate activated sludge from domestic STPs in the biological treatment stage of FWW. In comparison, the treatment technologies using microalgae (phycoremediation) might effectively reduce nutrients (phosphorus and nitrogen). Among microalgae strains which have been investigated in the photobioreactors of FWW, the most common are *Acutodesmus obliquus, Botryococcus* sp., *Chlorella pyrenoidosa, Chlorella sorokiniana, Chlorella vulgaris, Pleurotus ostreatus, Pleurotus pulmonarius,* and *Scenedesmus* sp.

The main goal of WWTP is to reduce organic pollutants, nutrients, and heavy metals in wastewater (Shahid et al. 2020). Conventional wastewater treatment has advantages and disadvantages depending on the chemical composition of the wastewater. The choice of wastewater treatment processes depends on costs, reliability, operational difficulties, feasibility, efficiency, and environmental impacts (Crini and Lichtfouse 2019). Conventional wastewater treatment limitations lie in efficiency, sensitive operational conditions, high energy requirements, and expensive disposal procedures (Chai et al. 2021). Traditional WWTPs are facing more problems because the circular economy concept requires a high level of pollution removal before the wastewater is reused for different applications such as irrigation. Alternative solutions to the treatment of industrial waste must be investigated. Many of the reports support the application of microalgae in the photobioreactor system for wastewater treatment and biomass production. Peter et al. (2023) investigated *Chlorella vulgaris* in food processing waste from milk and biscuit manufacturing facilities as an organic carbon source for the growth of microalgae. The microalga growth was optimised for the FWW compared to the growth in the BG-11 medium. *Chlorella vulgaris* produced 44% biomass concentration and 57% more carbohydrates (57%), 20% of proteins, and 11% lipids in FWW (Peter et al. 2023).

In fact, microalgae have achieved promising results in the treatment of industrial wastewater. The biomass produced is used for a variety of sustainable applications. The cultivation of microalgae with different types of industrial waste is still uncertain. Few studies have been conducted on microalgae cultivation using industrial wastewater and controlled operating conditions (Sathinathan et al. 2023). In addition, the harvesting of microalgae biomass from wastewater is still insufficient to avoid eutrophication generated from the final disposal of wastewater into the natural water system.

Dewatered sludge as aerobic granular particles bio-startup for new wastewater treatment plants

Active sludge is a promising technology with high-efficiency performance in biological wastewater treatment compared to conventional biological wastewater treatment technology (Sun et al. 2023). The disadvantages of traditional biological wastewater treatment included being influenced by the external environment, having low pollution-carrying capacity, and having large footprints. In contrast, granular sludge particles as bio-startup exhibited high efficiency of pollutant reductions with good adaptability to the environment and because of microbial diversity, which can act effectively in aerobic, anaerobic, and facultative conditions and enable to remove of various nutrients simultaneously in one reactor which reduces the operating cost (Han et al. 2022). Based on the Scopus patent database with the specific keyword “aerobic granular particles”, a total of 120 patents were recorded between 1997 and 2022, and 60.83% of these patents were recorded between 2019 and 2022, reflecting the new direction in the preparation and the use of AGS in the wastewater treatment process. Among these patents, 55.83% were recorded in the USA, 23.33% were recorded with the World Intellectual Property Organization, 15% were recorded in the European Patent Office, and only 5.83% were recorded in Japan.

Currently, 43.48% of the total studies conducted in the last years have been performed in China, 5.9% in the Netherlands and 5.54% in the USA. AGS is used for treating different types of wastewater, including acrylic acid wastewater, pulp and paper industry wastewater, pulping wastewater, real and low-strength wastewater, textile wastewater, refining wastewater, rubber wastewater, saline wastewater, salty wastewater, seawater-based wastewater, slaughterhouse wastewater, soft drink wastewater, soy sauce wastewater, winery wastewater, meat-processing wastewater, tomato paste processing wastewater, tapioca processing wastewater, and swine wastewater. These wastewaters have low microbial diversity with high contents of inorganic and organic compounds.

The application of AGS-BS-up improves the treatment process of these wastes with high efficiency in degrading organic pollutants. According to Al-Gheethi et al. (2018), the treatment process should be a simple procedure, with no chemical additives or toxic by-products, and economical and could remove a wide range of challenging contaminants only during one stage. Sun et al. (2023) investigated the efficiency of an integrated spherical pelletising granular sludge (SPGS) for a continuous upflow reactor. The study revealed that the removal rates for COD were 90%, nitrogen 70%, phosphorus 85%, and ammonia nitrogen 95%. The efficiency was related to extracellular polymeric substances (EPS) with concentrations of 90.65–209.78 mg/gVSS by microorganisms. These findings indicated that microbial population dynamics play an important role in achieving high treatment. Selecting the microbial community in the AGS-BS-up represents the core factor for the success of the treatment process.

Preparation and characteristics of aerobic granular particle bio-startup

The preparation process represents the main factor in AGS-BS-up production in previous studies. The preparation of AGS-BS-up depends on the drying of the sludge using the syringe-driven strip sludge apparatus, the sludge is produced in strips and cut into 2 mm sections, and the particles were modelled by the boards (Sun et al. 2023). Wang et al. (2020) reduced the water content of activated sludge to 70% at 40 °C before it was prepared as dewatered sludge particles (DSP) and added into reactors. Both studies reported acceptable organic compound degradation. The sludge subjected to the drying process lost much of the microbial community, which plays an important role in enchanting the organic matter reduction.

The selection of microbial communities to be added to the sludge used as a bio-startup based on the culture-based method has limitations. The drying of the sludge used in the preparation of AGS-BS-up or adding more microbes based on the culture-based method to the AGS-BS-up might negatively affect the performance of the AGS-BS-up and might lead to unbalance and increase the competition between microbes with a negative impact on the general performance. Indeed, a new patented AGS-BS-up is required to ensure efficacy and make the AGS-BS-up more applicable for new wastewater treatment plants. Zou et al. (2021) described an efficient method for preparing AGS-BS-up. In

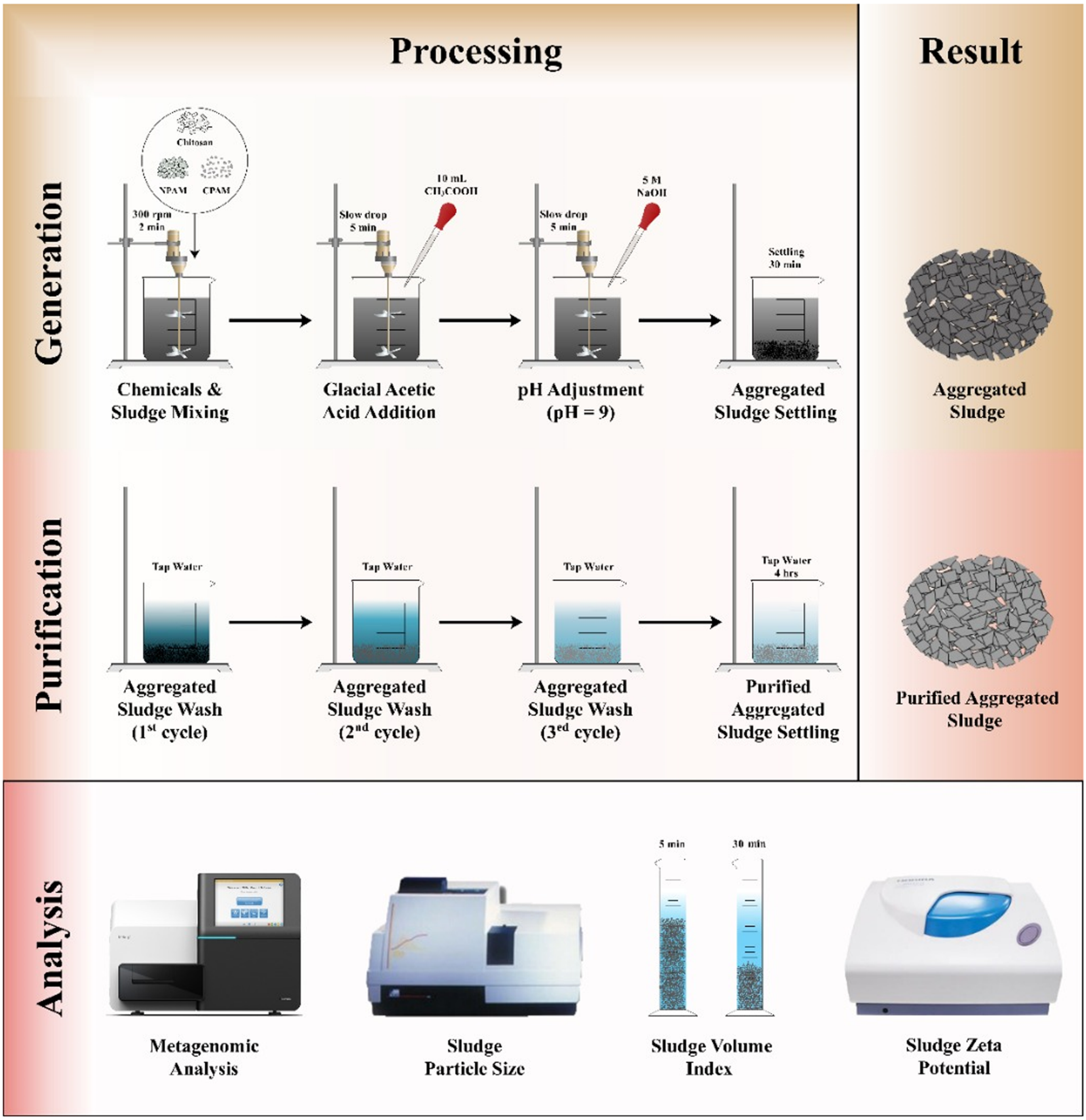

Fig. 6 Preparation methods of AGS-BS-up and the main parameters used for testing the validity of AGS-BS-up as described by Zou et al. (2021) and He et al. (2023)

this method, the activated sludge was collected in a liquid form (before the drying process) and mixed with non-ionic polyacrylamide and chitosan to increase the stability of AGS-BS-up and to be more applicable in the wastewater treatment plant. More advanced preparation processes might enhance the efficiency of AGS-BS-up in the biological treatment process of wastewater and increase the degradation of organic compounds. Hou et al. (2021) reviewed different

Table 4 Type of software used for techno-economic analysis (TEA) for several study goals and the key approaches during application

Software	Goal	Key approaches used to perform TEA	Advantages	Disadvantages	References
SuperPro Designer®	This software is used for analysing the feasibility of large-scale bioprocesses	1. Modelling, evaluation, and optimisation of the production process. 2. Simulation of industrial process especially the continuous processes. 3. Develop the commercial products based on the conceptual design. 4. Estimate the raw material and energy, chemical components, equipment required for the production process	1. A simple use without experience	1. Lack of database examples	Canizales et al. (2020)
ASPEN Plus®	Specified for analysing microalgae-based liquid fuel production	1. Focus on the hydrothermal liquefaction. 2. Depend on the data from literature and estimate the minimum selling price (MSP). 3. Provide a sensitivity analysis to optimise the process factors	1. Has a limitation in the optimisation of product quality, and energy. 2. Steady-state sequential modular simulation	1. Need high experience for simulation. 2. Need deep details and time-consuming. 3. Constraints in the economic and downstream evaluations of large-scale processes. 4. More software programs are required to perform the analysis	Mahmud et al. (2021)
Excel®	Used for the comparison between different treatment strategies	1. Efficient in the pilot-scale test result and have been used for alkaline cleaning wastewater	1. Availability and easy access	1. Spreadsheets estimation restrict the analysis. 2. Need templates which represent the main disadvantage.	

preparation methods used for AGS, such as the zoogloea type, which is formed by adsorbing metal ions, organic matter, and free microbes in water to form zoogloea due to the use of adhesive substances and polysaccharides secreted by microorganisms. Filamentous type formed as a function of filamentous bacteria that produce the AGS as a porous network structure having a large specific surface area. Another type is the integrated type, formed as a function of protein and polysaccharide, which acts as the core and skeleton. He et al. (2023) prepared AGS with a ferric tetroxide and robust nitrogen-doped graphene (Fe_2O_3/GO) for enhancing synchronous nitrification–denitrification in wastewater by 37% and improving the sedimentation performance, with 74% increases in the polysaccharide (PS) and production of substances which in role contributed to the increase of nutrients removal from industrial wastewater by more than 80%.

The main characteristics of the sludge samples that are tested to study the efficiency of AGS include the volume index at 5 and 30 min, particle size, MLSS, volatile suspended solid mixed liquor (MLVSS), specific oxygen uptake rate (SOUR) and mechanical strength, settling velocity, morphological properties with scanning electronic microscope, and atomic flame microscope, chemical composition with Raman spectroscopy and FTIR, and zeta potential of the sludge (Su and Yu 2005; Zhang et al. 2016). Figure 6 shows the preparation methods of AGS-BS-up and the main parameters used for testing the validity of AGS-BS-up, as described by Zou et al. (2021), Su and Yu (2005), and Zhang et al. (2016). In order to improve the efficiency and recycling process of AGS, some studies have loaded it onto different types of bio-carrier. Bio-carriers improve the adherence of microbial load and accelerate the biotransformation of organic contaminants during the treatment process (Al-Amshawee et al. 2020). According to Lin et al. (2021), the utilisation of bio-carrier contributes in the improvement of diversity, and uniformity of aerobic granular microbes in the sequencing batch reactors. The potential of commercialisation for AGS-BS-up is depending on the operational cost, capital cost, and revenue which is conducted using techno-economic analysis (TEA). TEA is conducted using software modelling (Burk 2018). Table 4 displays the type of software used for TEA for study goals and the key approaches during application.

Future perspectives

The AGS-BS-up has a high potential to be used for different wastewater treatments with low microbial diversity. Different preparation methods are associated with different AGS-BS-up properties. The applicability of AGS-BS to wastewater will depend on the chemical, physical, and biological characteristics of the wastewater. One important note is the environmental conditions of the wastewater and source of AGS-BS-up. To achieve high applicability, the aerobic granular sludge used in the AGS-BS-up preparation must be obtained from similar environmental conditions to avoid the long treatment process, since the microorganisms take time to acclimate to new environmental conditions. This is one of the main limitations of AGS-BS-up application since aerobic granular sludge from a hot climate will not effectively treat wastewater in a cool environment. Different indigenous microbes have different efficiency, while the microbial diversity in the activated sludge depends on the source of the raw sewage and the treatment process. The applicability of AGS-BS-up is associated with the cost; the preparation of the AGS-BS-up as a reusable product might facilitate the utilisation of AGS-BS-up in different wastewater treatment plans.

Conclusion

The microbial diversity in the activated sludge was analysed in the present work; it was noted that 95% of microorganisms was belonging to bacteria (450 bacterial strains) and 0.5% was belonging to Archaea. These findings make AGS-BS-up high efficient in the degradation several of organic constitutes and nutrients due to the ability of the microbial consortium to produce several enzymes simultaneously. AGS-BS-up prepared as strips or as dewatered sludge particles (DSP) is the most common. However, the sludge subjected to the drying process lost the microbial community, which plays an important role in enchanting the organic matter reduction. Therefore, this topic still need more studies in order to prepare AGS-BS-up with high efficacy such as the mixing liquid sludge with non-ionic polyacrylamide and chitosan to increase the stability of AGS-BS-up or as zoogloea and filamentous type with high surface area. The most efficient form of AGS-BS-up was prepared with a ferric tetroxide and robust nitrogen-doped graphene (Fe2O3/GO) which has enhanced nitrification–denitrification by 37% and improved the sedimentation performance and nutrients removal by more than 80%. Based on the machine learning analysis, AGS-BS-up has high applicability on large scales. The TEA represents the main step that should be conducted to confirm the absence of negative environmental impacts and large-scale application.

Author contributions Adel Al-Gheethi contributed to conceptualisation and manuscript draft; Efaq Noman was involved in methodology, created tables, investigation, and review and editing; Mohammed Alsahari contributed to figures design and review and editing; and Qais Al-Maqtari and Dai-Viet N. Vo were involved in writing, review, and editing.

Funding The authors did not receive support from any organisation for the submitted work.

Declarations

Conflict of interest The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Ethical approval We confirmed that the current work is an original for the authors and has not been published or under review in any journals.

Consent to participate All authors have read and contributed to this manuscript and agreed for the submission.

Consent to publish All authors have read and contributed to this manuscript and agreed for the publication.

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