State of the Art Compendium Report on Biocluster Activities

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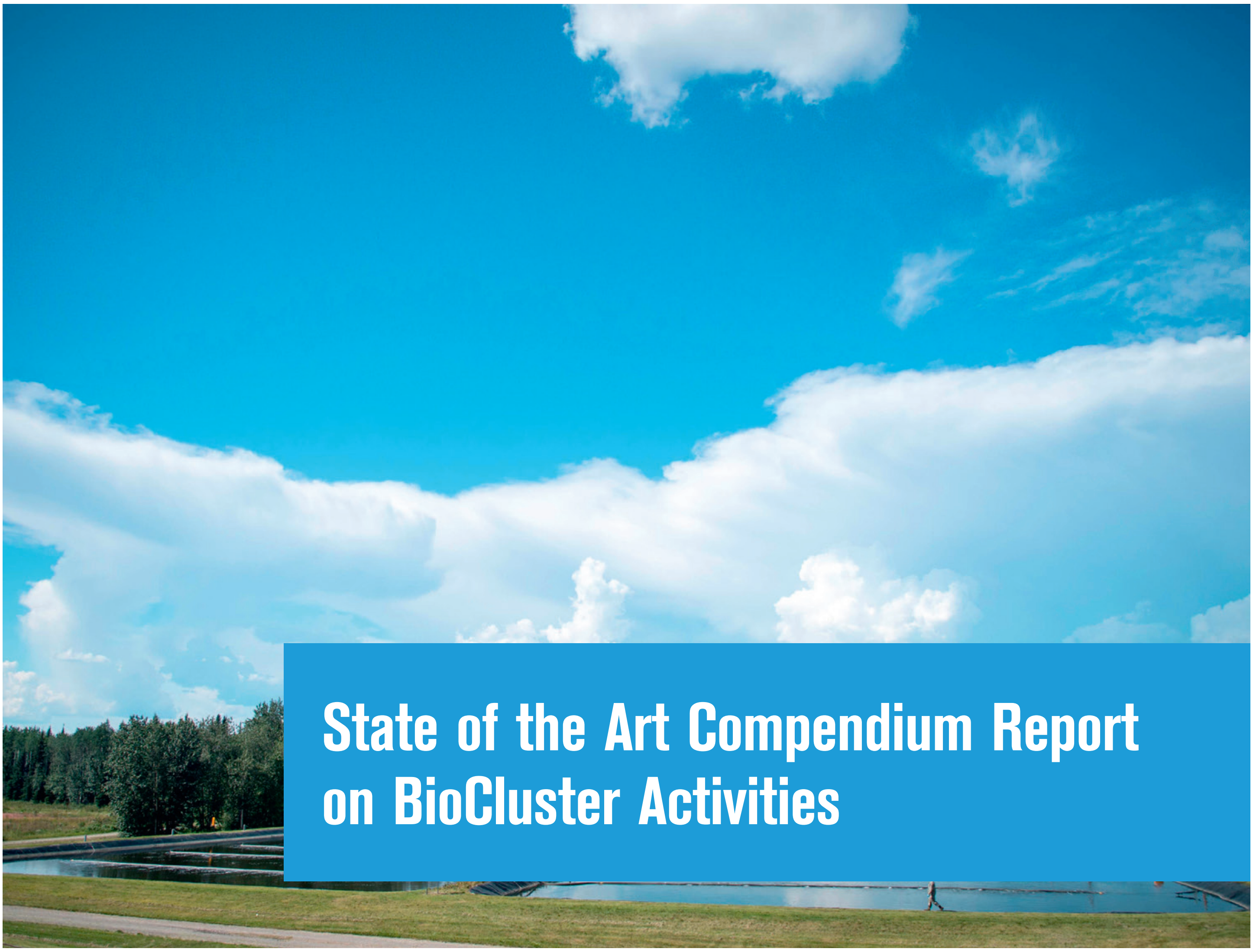
IWA/ISME BioCluster

the international water association



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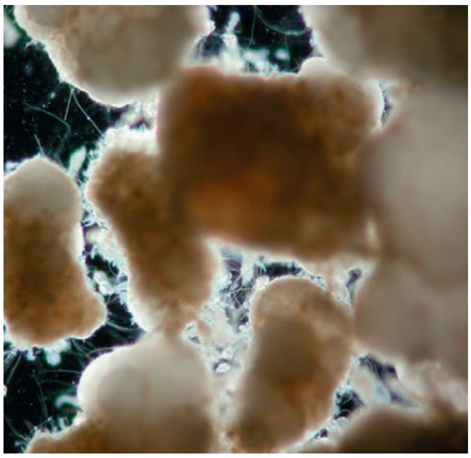

IWA/ISME
BioCluster

STATE OF THE ART COMPENDIUM REPORT ON BIOCLUSTER ACTIVITIES

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International Water Association

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PREFACE

We are facing great challenges in securing global sustainable development. These include sufficient clean water for everybody, sanitation, recovery of energy and nutrients, and good health for humans, animals and the environment. Fortunately, ground-breaking advances are taking place in microbial ecology, including the continuous development of novel technologies, such as DNA sequencing technologies; and with an intensified collaboration between microbial ecologists, water professionals and others, this holds great promise for solve these challenges.

The BioCluster facilitates cooperation among Specialist Groups in the International Water Association (IWA) on the one hand and between IWA and the International Society for Microbial Ecology (ISME) on the other. Many of the challenges IWA faces are related either to microbiological problems, such as human health, or to the application of microbial communities for specific purposes, such as wastewater treatment or bioenergy production. A close collaboration with ISME, with its associated expertise in many fields of microbial ecology, will ensure the transfer and application of the latest knowledge to the water science and management community. In return, the researchers in ISME are exposed to a range of profound and important (and often new) research questions, where their expertise can be applied, new questions asked and where joint research projects can be developed.

The scientific focus comprises studies of identity, physiology, ecology and population dynamics of relevant microbial populations, which also includes viruses, bacteria, archaea and higher organisms. Although many tools are already available, new developments are coming very quickly, and both new and established tools will help us to develop the concepts and theories that are common to all engineered biological water processes, allowing novel insights and practical applications.

This report summarises the aims and activities of the BioCluster and provides many examples where microbial ecology and biotechnology have already solved problems, where new solutions are promising, and where we still are looking for good solutions, primarily in the areas of public health and sanitation, wastewater management, resource recovery and industrial wastewater. The hope is that many students, engineers, scientists and practitioners will be inspired and participate in IWA–ISME activities for the benefit of sustainable development.

Per Halkjær Nielsen, BioCluster Chair
Michael Wagner, former president, ISME

1 INTRODUCTION

1.1 BIOTECHNOLOGY AND THE WATER SECTOR

Microbial ecology, water management and biotechnology are complex fields that have traditionally been distinct and separate in their development. However, in recent years these disciplines have become increasingly intertwined and show even greater promise in their interactions. It is through this collaboration that these seemingly diverse fields can come together to address important issues of sustainability, environmental quality, dwindling resources, health and climate security.

1.2 IWA AND ISME COLLABORATION

The International Water Association (IWA) is the world's largest network of water professionals, situated in 130 different countries. IWA develops innovative solutions- and service-oriented programmes that contribute to the progression of water management worldwide. Among these programmes are world-class events that bring the latest science, technologies and best practices to the water sector at large.

The International Society for Microbial Ecology (ISME) is the principal scientific society for the field of microbial ecology and its related disciplines. ISME fosters the exchange of scientific information by holding international symposia and specialised workshops while also promoting education and research with sponsored publications.

Many practitioners in the water sector have not yet been exposed to the exciting developments in biotechnology and novel molecular technologies. IWA and ISME both acknowledge the value and potential of the ground-breaking advances in microbial ecology and, as such, call for intensified collaboration to foster innovation, encourage research and see this science become common practice. By working together, IWA and ISME are able to connect their vast networks of researchers, scientists, engineers and decision-makers to share knowledge and become exposed to the exciting developments in this field.

1.3 MICROBIAL ECOLOGY: A REVOLUTION

Microbial ecology is a long-standing scientific field that has developed at a steady pace since the 17th century. However, the changes seen currently and in the past 10 years are revolutionary. The foundation of the science is to understand the complex communities of self-sustaining microorganisms and how they interact with the environments that surround them. Up until the 1980s, microbial ecology developed slowly as the existing tools were not capable of answering key questions, such as the identity, abundance or function of the microbes being analysed. These issues were addressed around 1985 when molecular biology tools were introduced that allowed for selective and reliable amplification of defined DNA using polymerase chain reaction. Today, high-throughput DNA sequencing is commonplace, and bioinformatics is key to interpreting the enormous amounts of data produced. It is definitely a revolution as we can now start answering the questions about identity, abundance and function of the microbes of interest.

1.4 FROM THEORIES TO PRACTICE

Translation of current knowledge towards industrial application for process design or optimisation is of great importance. We are only starting to scratch the surface of the potential impact that can be realised with the new knowledge being generated through microbial ecology. It is therefore important with collaboration and interaction between industries and researchers setting up pilot-scale and case studies to be able to go from theories to practice and to use lessons from practice to refine theories.

1.5 AIM OF THIS REPORT

This state of the art report aims to give water professionals a general overview of available technologies in microbial ecology, as well as to outline obstacles and opportunities for water engineering in the context of public health and sanitation, wastewater management, resource recovery, and industrial wastewater. The report further emphasises the need to encourage good practice by introducing several cases, before providing some general suggestions on future trends. It will serve as a roadmap for IWA's BioCluster and for its activities.

2 BIOCLUSTER

2.1 FOUNDATION

The BioCluster was created in 2010 with the mission of inspiring, facilitating and supporting the mutual exchange of knowledge and cooperation among Specialist Groups in IWA on the one hand and between IWA and ISME on the other, all in the field of microbial ecology research and its application for water science and management. In contrast to the IWA Specialist Groups, which work on specific topics or in well-defined areas, an IWA Cluster facilitates systematic ‘conversations’ across IWA Specialist Groups, addressing cross-cutting issues that are of relevance to individual Specialist Groups, but which extend across one or more of them. Clusters also reach out to external partners that are beyond the traditional water sector, such as ISME. The topics and scopes of clusters are reviewed every 5 years to ensure they are still doing relevant work for the water sector. There are currently three clusters: the Alternative Water Resources Cluster, the Resource Recovery Cluster and the BioCluster.

2.2 MEMBERSHIP

The IWA/ISME BioCluster is a community of researchers, scientists, engineers, consultants and developers committed to improving the water cycle by researching and applying novel technologies in microbial ecology. For such a large initiative with members and contributors around the world, a steering committee is required to direct and convene the cluster to achieve its mission. Currently the steering committee comprises Per Halkjær Nielsen as chairman, Michael Wagner as ISME representative, Glen Daigger as IWA representative, and Tom Curtis and Ameet Pinto as representatives for the Specialist Group on Microbial Ecology and Water Engineering.

The BioCluster leverages the knowledge of a diverse group of professionals from around the world to share best practices and the transfer from theory to effective application. This is achieved through a variety of activities such as workshops, conferences, nominated awards, and of course supported knowledge sharing platforms developed by IWA. The catalyst for the growth of the BioCluster is the combination of ground-breaking developments in molecular technologies, particularly those in DNA/RNA sequencing and the need for new solutions to water-related problems that are challenging communities around the world.

2.3 THE FUTURE

The BioCluster understands the need for science-based approaches for identifying and solving practical problems. The foundation of this are the IWA Specialist Groups who are currently focusing on topics such as Microbial Ecology and Water Engineering, Health Related Microbiology, Biofilms, Anaerobic Digestion, Nutrient Removal and Recovery, Waste Stabilization Ponds, and several others.

- Design, Operation and Maintenance of Drinking Water Treatment Plants
- Health Related Water Microbiology
- Design, Operation and Costs of Large Wastewater Treatment Plants
- Sludge Management
- Wastewater Pond Technology
- Anaerobic Digestion
- Biofilms
- Membrane Technology
- Microbial Ecology and Water Engineering
- Nutrient Removal and Recovery
- Water Reuse
- Chemical Industries
- Forest Industry
- Pretreatment of Industrial Wastewaters

Box 2.1 Examples of Specialist Groups interacting in the BioCluster.

3 MICROBIAL ECOLOGY

3.1 FUNDAMENTALS

The study of microbial ecology focuses on understanding the complex self-sustaining communities of microorganisms, bacteria, archaea and protozoa. Microorganisms are present throughout the biosphere and perform key roles in biogeochemical cycles of every ecosystem, which makes understanding them crucial. The science of microbial ecology works to answer fundamental questions such as which microbes are present in a community, what is their relative (and absolute) abundance, what are their possible functions and interactions, and which factors determine their presence and activity. The quest for answers to these questions has been in slow development since the 1950s but that has changed radically in the past 10 years. Thanks to novel techniques in DNA sequencing and a drastic decrease in costs, scientists are now able to quickly and cheaply study microbial communities and access more detailed information than ever before. With the advent of this technology, microbial science can now be integrated and applied to engineering fields to invent new processes and to optimise those previously in use.

Microbial ecology is about understanding more than just a single bacterium: it involves research into relationships, with the environment and within the microbial community. In the control and understanding of microbial populations certain foundational theories are required. These include community resilience and stability, which refer to an ability to withstand environmental change and to having the potential to adapt. Following from this is the concept of community succession or immigration, which is understanding how communities develop and what happens when populations are wiped out or killed. Additional relationships and rules that are being developed by scientists include biogeography, predator–prey interactions, occurrence of immigration–emigration, food webs, functional redundancy, selective pressures and insular disturbance.

There are several key parameters when considering the success and function of a microbial community in controlled environments. Commonly, communities can be selected or favoured by posing the right selection pressure on them. This could, for example, include altering the substrate type and concentration, the presence of electron acceptors such as oxygen and nitrate, or abiotic factors such as salinity, temperature and pH. Some examples of this related to water systems are wastewater treatment nitrifying bacteria, which are best controlled by changing the sludge age (biomass retention time), concentration of substrates and oxygen availability, or polyphosphate-accumulating organisms, which are best controlled by acetate and oxygen availability.

Applying microbial science to the water sector requires a process that may include surveillance, identification, understanding and, finally, control. There are two main components to the surveillance of microbial communities in water systems. The first is sampling and identification. The identification of microbes is essential for understanding the types of community that are present, their function and the proper control responses. The second component is the collection of meta-data from the system of interest. By monitoring the changes in system performance and relating them to the known microbial community, environmental changes to optimise the process can be identified.

The identification of microorganisms has improved greatly in recent years thanks to novel high-throughput methods and the drastic decrease in costs of existing technologies. From identification we may understand the potential function and morphology of a microbe by comparing it with known databases such as the MiDAS field guide (http://midasfieldguide.org/) for the wastewater treatment system (McIlroy *et al*., 2015).

3.2 NOVEL TECHNIQUES

Different methodologies are used to study microorganisms and their communities. Each has its advantages and disadvantages, but some are more useful in the water sector or in combination with one another. High-throughput sequencing technologies are generally the most interesting method in large-scale operations, offering the ability for in-depth detection of almost everything present in the sample and the ability to piece together possible functions contained within it. This method is relatively new and is only now becoming more accessible but it may with time offer the best information in relation to meta-data and system control. Secondly, fingerprinting offers a quick look at microbial communities to evaluate biodiversity or track changes over a period of time. Fingerprinting cannot identify individual organisms but instead develops an overall picture of the sample. This method is generally less favoured than sequencing methods. Lastly, quantification remains an important method in understanding the abundance and population change over time. It does not provide identifying information, but is useful to scientists in understanding selective pressures and control of a community. Four specific technologies currently used in microbial ecology are amplicon sequencing, fluorescence *in situ* hybridisation (FISH), quantitative polymerase chain reaction (qPCR), and flow cytometry, which are described below.

3.2.1 AMPLICON SEQUENCING

High-throughput amplicon sequencing is recommended for routine analyses of microbial communities. Most commonly, the 16S rRNA gene is used as a marker to identify bacteria and archaea. There are multiple steps involved in amplicon sequencing, including DNA extraction, PCR amplification and DNA sequencing, which then provide sequences that can be analysed using public databases and bioinformatics approaches to create a list of all the genera and their relative abundance in the sample (Figure 3.1). The Illumina platform is most widely used and is capable of sequencing hundreds of samples much more cheaply and quickly than previous techniques (Caporaso *et al*., 2012).

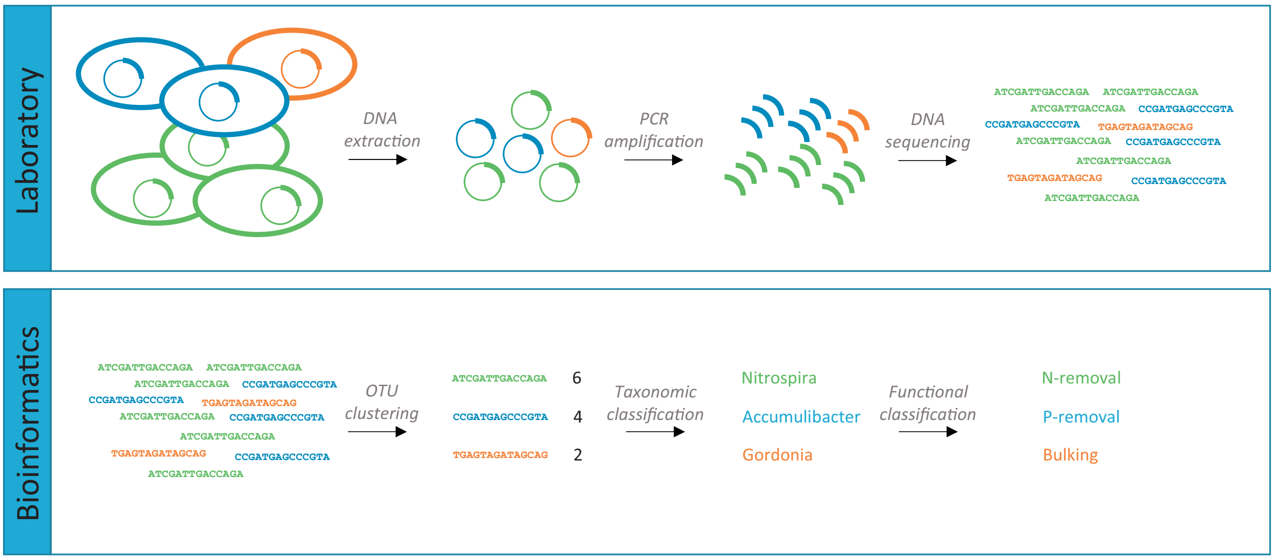

Figure 3.1 Workflow for 16S rRNA amplicon sequencing. From Karst *et al.* (2016).

3.2.2 FLUORESCENCE *IN SITU* HYBRIDISATION

Fluorescence *in situ* hybridisation (FISH) is a method in which microorganisms are labelled with fluorescent oligonucleotide probes for enumeration and organisational visualisation (Figure 3.2). The probes target the 16S or 23S rRNA in the ribosomes and are capable of being designed to target phylogenetic groups from species level and higher. FISH is an effective but labour-intensive method of microbial community analysis, and an excellent supporting technique for validating results from amplicon sequencing. FISH is the only identification and quantification method that provides visual feedback, which can be very important for verifying critical results. Moreover, FISH has been the key tool for functional *in situ* analyses when combined with other methods such as Raman microscopy or microautoradiography (MAR). Nowadays, FISH combined with other methods still enables studies that would not be possible with other approaches (for example the quantification of storage compounds in specific cells). Finally, FISH enables studies of biofilm and floc architecture (spatial distribution of specific organisms) and of the spatial co-localisation of microbes. This can provide important hints on ecophysiology and microbe–microbe interactions.

Although much of this may be less relevant for the daily practical work of engineers, FISH and its extensions can be used to identify most of the known, important functional microbial players in wastewater treatment plants (WWTPs). Many other approaches in wastewater microbiology (such as amplicon sequencing-based identification of important microbes, and ‘omics’) still build on previous insights from FISH-based studies.

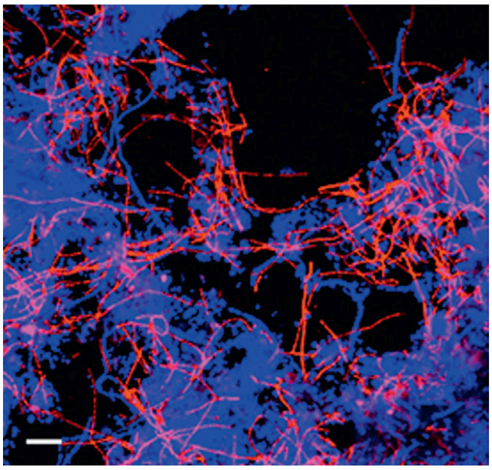

Figure 3.2 FISH image of full-scale sludge biomass from the KČN Kočevje WWTP. Red: novel uncultured chloroflexi associated with 0092 morphotype filamentous bulking in activated sludge. Blue: all bacteria targeted with EUBmix probes. Scale bar, 10 μm. Micrograph from McIlroy *et al.* (2016).

3.2.3 QUANTITATIVE PCR

Real-time or quantitative polymerase chain reaction (qPCR) is the most sensitive technique for quantifying specific genes (small DNA sequences). In optimal conditions it can detect a single target sequence and can be used to obtain absolute quantities from relative abundance data. qPCR is used in water treatment to estimate overall bacterial abundance and the quantity of bacteria belonging to a specific taxon. Furthermore, qPCR can in some cases quantify the abundance of bacteria belonging to specific functional groups such as ammonia or nitrite oxidisers (Baptista *et al.*, 2014). This can also be used to track infectious viruses and antibiotic-resistant genes.

3.2.4 FLOW CYTOMETRY

Flow cytometry is a technique to quantify the total microbial cell counts in samples of interest (Hammes *et al.*, 2008). Over the past 10–15 years, the use of flow cytometric microbial quantification has rapidly expanded in terms of both application type and setting. For instance, staining of samples with various fluorescent dyes followed by flow cytometry can be used to count total microbial cells and to discriminate between cells with intact and damaged cell membranes, although there is still some discussion about the method's reliability (Berney *et al.*, 2007). Methodological developments have resulted in the adoption of standardised flow cytometric protocols (SLMB, 2012), and the small duration of time to obtain results has made this technology extremely attractive for practitioners in water engineering, particularly when the focus is on planktonic bacteria.

3.3 LINKING IDENTITY AND FUNCTION

To learn more about the ecology and function of the important microorganisms present in water systems, comprehensive research is needed. Isolation and studies of pure cultures are extremely useful, but only a few relevant species have so far been isolated. The study of the uncultured majority has especially benefitted from the recent development of numerous molecular tools including powerful bioinformatics methods. The so-called 'omics' techniques have afforded promising new insights into microbial ecology that revolutionise our concepts of microbial diversity and physiology within complex consortia and up to entire ecosystems. Novel sequencing techniques enable the retrieval of whole metagenomes from any sample (DNA from all microorganisms) and from them more or less complete genomes, which is of great importance because they serve as the blueprint for putative functions (Albertsen *et al.*, 2013). Relying on genomic reference databases, post-genomic methods such as transcriptomics, proteomics, and metabolomics may give us information about the potential functions and metabolic pathways being performed in the given environment at any given time (Simó *et al.*, 2014). An obstacle is the lack of a comprehensive reference database with relevant genomes; however, with present rapid technological developments, these challenges may be resolved in a few years. Another obstacle is the lack of knowledge about the function of the protein-encoding genes in the novel genomes; it may take many years to solve this and it will require studies with various single-cell techniques, such as FISH-MAR (Nierychlo *et al.*, 2015) or new more sophisticated methods such as NanoSIMs or Raman microspectroscopy which allow non-invasive *in vivo* biochemical analysis of individual living cells (Wagner, 2009; Singer *et al*., 2017).

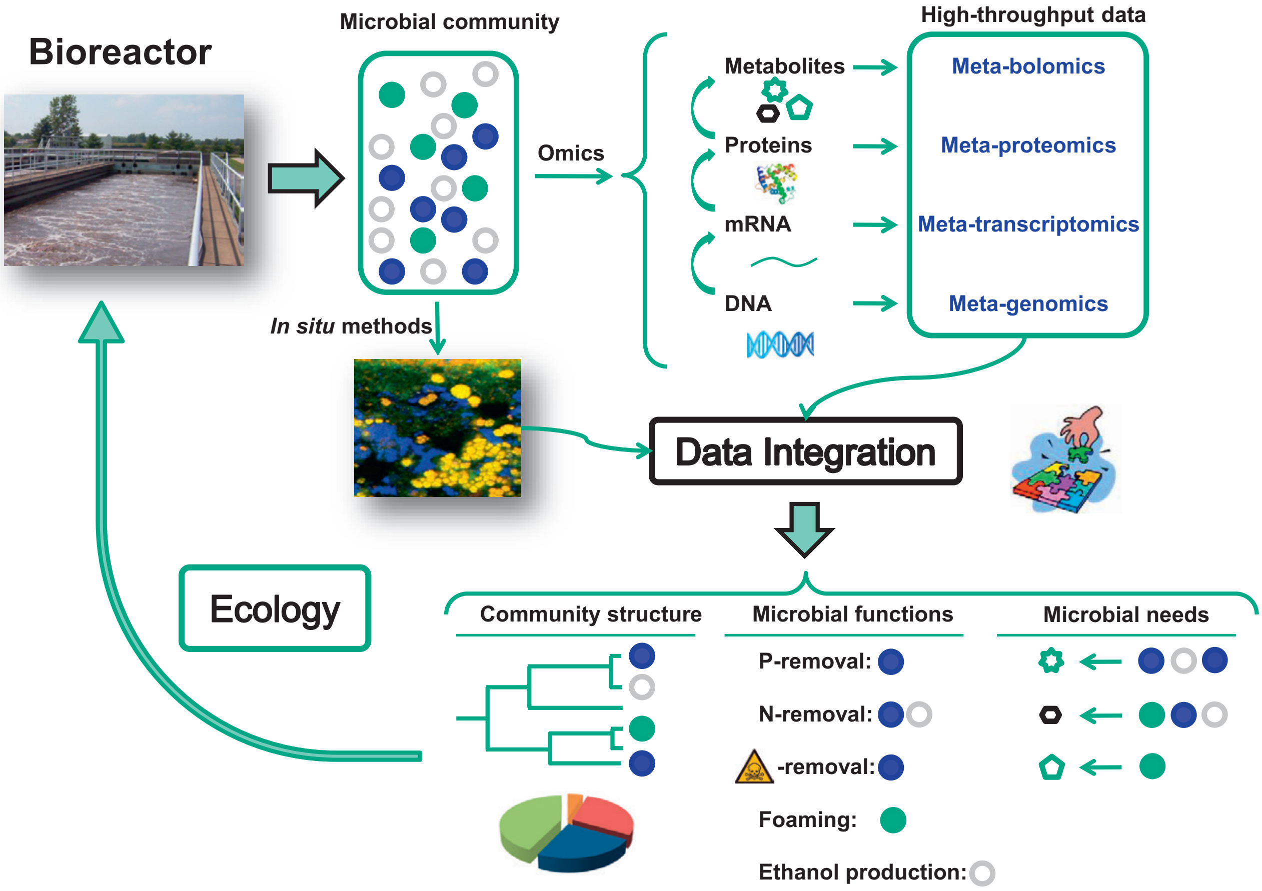

Figure 3.3 Understanding ecosystems using molecular methods.

3.4 SHIFT IN THINKING

The overall approach in using novel molecular methods in the water sector is to shift from a reductionist approach to a more holistic system-based approach, by using a combination of culture-dependent and culture-independent techniques in characterising microbial composition and functions. It is also important to integrate ecological principles into system design to enhance stability, and to re-examine current theoretical explanations of why and how the different key processes occur.

3.5 FUTURE TRENDS

Microbial science still has a long way to go with documenting, identifying and understanding the vast number of microorganisms present in water systems. Databases and libraries, such as MiDAS (McIlroy *et al*., 2015), are continuing to grow at a steady rate but require considerable time investment as well as a high degree of skill. Both of these barriers will become easier to overcome as sequencing technologies become cheaper and more accessible. The development of this field is two pronged, however, and will require data and experience *ex situ* so that scientists can better understand environmental changes and microbial functions. As such, on-site and online surveillance and sampling technology is expected to be available on the market within the next 5 years. This will allow system users, operators and engineers to identify their local microbial communities, to compare them with an existing database and take appropriate control measures. A portable sequencing laboratory from Oxford Nanopore Technologies, the MinION, has already been tested in the field, relying on a portable DNA sequencer, which has shown promise for the near future (Menegon *et al*., 2017). In the wake of improved sequence techniques, the field of meta-omics is continuing to grow as scientists develop high-throughput methods for transcriptomics, proteomics and metabolomics, which will also contribute to microbial community modelling.

4 PUBLIC HEALTH AND SANITATION

4.1 FUNDAMENTALS AND CHALLENGES

Water is fundamental for life on earth. Improved water quality and sanitation worldwide is now of great importance, because in 2017 the United Nations agreed upon 17 Sustainable Development Goals. Of these, Goal number 6 is 'Ensuring availability and sustainable management of water and sanitation for all'. Targets included in this Goal are, among others, 'by 2030, to achieve universal and equitable access to safe and affordable drinking water for all' and 'improving water quality by reducing pollution, eliminating dumping and minimising release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally' (United Nations, 2017).

Political aims such as these call for technologies that can provide cleaner water in systems that are more efficient, with faster surveillance and tracking of contaminants and pathogens. There will also be a need for plans of action when emerging threats such as Ebola virus break out or when extreme events happen such as earthquakes, floods, tsunamis, etc., which all dangerous to public health when water supplies are affected.

The introduction of novel biotechnology into these processes, including a better understanding of the microbial ecology, could provide answers to these common issues and alleviate many of the drinking water challenges that much of the world faces.

4.2 DRINKING WATER DISTRIBUTION SYSTEMS

Good drinking water distribution systems (DWDSs) are key to public health. Most problems in DWDSs are microbial in nature, primarily biofilms, nitrification, bio-corrosion and pathogen persistence. Pathogens that are capable of reproduction in DWDSs can cause various infections (Szewzyk *et al*., 2000). Water inherently contains microorganisms: even ground water and surface water entering the DWDS after treatment is estimated to contain a bacterial concentration up to 10^5 cells per millilitre (Pinto *et al*., 2012). The understanding of microbial ecology in DWDSs is currently limited because of the inaccessibility and previous assumptions that these systems were relatively free of bacterial and fungal cultures. However, reinvigorated by novel molecular-based methods, DWDSs are now more available to study, which will provide an understanding of the diverse microbial life that exists there.

The standards and norms for microbial characterisation are dictated by national and international standards. However, screening methods are 'old-fashioned' and in many cases rely on cultivation. It is well known that culture-dependent methods detect and enumerate faecal coliforms at a reasonable cost but that the outcomes lack information on the greater microbial community, revealing less than 1% of microbial diversity.

Drinking water treatment plants provide clean water to the DWDSs. Their main tasks are the treatment of contaminants (chemical and microbiological) related to health risks, removal of natural organic matter and control of disinfection by-product formation.

More insight into the ecology of DWDSs will facilitate the development of effective control strategies that will ensure safe and high-quality drinking water. Scientists and engineers try to answer the questions of which microbes are present, how abundant they are, how their activities affect other organisms or humans, and how the environment affects the microbial community (Douterelo *et al*., 2014). To answer these questions, culture-dependent and culture-independent methods have been developed, with the former being more commonly used to assess the quality of drinking water. Currently, these culture-independent molecular methods are relatively expensive, time-consuming and require trained personnel, but as the technology becomes more accessible and cheaper, they are expected to be used routinely in the future. Also, flow cytometry seems to have great potential.

In the following paragraphs we describe selected methods and examples of studies providing a look into the complex microbial communities present in DWDSs, together with information into how drinking water systems can be better managed.

4.2.1 PREMISES PLUMBING

While DWDSs transport water from the treatment plant to the built environment, the intricate plumbing systems within the latter, i.e. premises plumbing, have been an emerging focus of research. Plumbing in premises typically has a much smaller surface area to volume ratio, allowing higher contact between bulk water and the biofilm phase. In addition, water can experience extended periods of stagnation in premises plumbing, and the ambient temperature in premises plumbing is likely to be higher than in the DWDS. These factors can have a significant impact on the chemical and biological quality of drinking water, and thus premises plumbing can serve as a hotspot for opportunistic pathogens (Falkinham *et al*., 2015).

4.2.2 BIOFILMS

Biofilms are suspected to be the main source of contamination in treated water streams in the absence of external contamination (LeChevallier *et al*., 1987; Berry *et al*., 2006). Biofilms are a great challenge because the attached cells have distinct advantages over planktonic (free floating) cells such as their ability to metabolise recalcitrant organic compounds and increased resistance to biocides, especially chlorine (Tachikawa *et al*., 2005). The mechanisms of biofilm resistance as still not fully understood, but much research is done into the production of the extracellular matrix and other cell-to-cell interactions (Flemming *et al*., 2016).

4.2.3 PATHOGENS

The detection of pathogens in DWDSs and biofilms is much more sensitive with culture-independent methods than with traditional culture-dependent methods. Amplicon sequencing has already proved to be enlightening in resolving phylogenetic diversity in water samples and has shown new emerging pathogens (Ju and Zhang, 2015; Keely *et al*., 2015; Roeselers *et al*., 2015). The methodology has great potential because it presents the opportunity to get information on the content of all microbes present in the sample simultaneously. However, validation of the approach is still needed before next-generation sequencing (NGS) can become a reliable tool for quick identification of the most significant microorganisms associated with public health risks. In addition, to enable correct interpretation of the huge amount of data output from next-generation sequencing, there is a need for good bioinformatics algorithms and better interfaces for non-experts.

Faecal pollution is a primary health concern in water systems. Development of new indicators of faecal contamination, including viruses that cause asymptomatic infections in humans, is colloquial. Detailed knowledge about the contamination sources is needed for efficient and cost-effective management strategies to minimise faecal contamination in water. Human DNA viruses, adenovirus (HAdV) and polyomavirus, which are associated with persistent infections, are used as the most specific viral human indicator parameters and are currently being quantified by molecular methods. The viruses are excreted by a high proportion of the human population and are present all over the world (Rusiñol *et al*., 2014). In addition to faecal contaminants, there is an increasing emphasis on understanding factors that contribute to the proliferation of opportunistic pathogens (i.e. *Legionella pnuemophila*, *Mycobacterium avium*, etc.) largely because of increasing incidences of illnesses associated with them.

4.2.4 DISINFECTION

A study in 2003 verified that the presence of different disinfectants was not sufficient to eliminate a variety of pathogens, including biofilms of *Escherichia coli* and *L. pneumophila* (Williams and Braun-Howland, 2003). Furthermore, in pilot-scale studies, monochloramine and ultraviolet light did not inhibit *L. pneumophila* from accumulating in biofilms. These studies and many more illustrate the danger of pathogen resilience in biofilms and the need for research to better manage such pathogens. While developing novel disinfection technologies and existing disinfection approaches remains a key focus of drinking water quality, there is also emerging debate on the trade-off between risks and benefits of water disinfection (Rosario-Ortiz *et al*., 2016).

4.2.5 ANTIBIOTIC RESISTANCE

Antimicrobial resistance is an emerging threat to public health. However, bacteria with antimicrobial resistance genes are common in wastewater and faecal wastes. Currently, there are no World Health Organization guidelines assessing the issue. For risk assessments and management strategies to be developed, there is a need for evidence to underpin recommended actions. Therefore, it is crucial to obtain a better understand of the fate and transfer of antimicrobial resistance genes in the water environment by applying novel next-generation sequencing methods and microbial ecology.

4.2.6 MODELLING

The complexity of microbial communities and their associated growth and interactions require the use of mathematical modelling so that they can be monitored and addressed. Multispecies biofilm models are developing in complexity; however, disinfection models almost exclusively address single-populations (Xavier *et al*., 2005). We need to develop multispecies models for disinfection and such advancement will be a key towards operating DWDSs efficiently and reducing contamination.

4.2.7 BIOENHANCED ACTIVATED CARBON

In drinking water plants, granulated activated carbon (GAC) is a common packing material for filters. In conventional treatment processes, bacteria will grow on the surface of GAC, also termed biological activated carbon (BAC) (Servais *et al*., 1992; Brown, 2008). BAC is a type of biofilm capable of biodegrading a significant amount of entrapped nutrients in the carbon pores. Furthermore, it is capable of decreasing backwash times and extending the life of the GAC by biological regeneration. The greatest challenge for BAC, however, is the time required for a sufficient amount of biomass to form. For this reason, research has begun into the immobilisation of dominant microorganisms which are capable of degrading organic matter without lengthy acclimatisation. The immobilisation of these dominant species in GAC is often called bioenhanced activated carbon (BEAC).

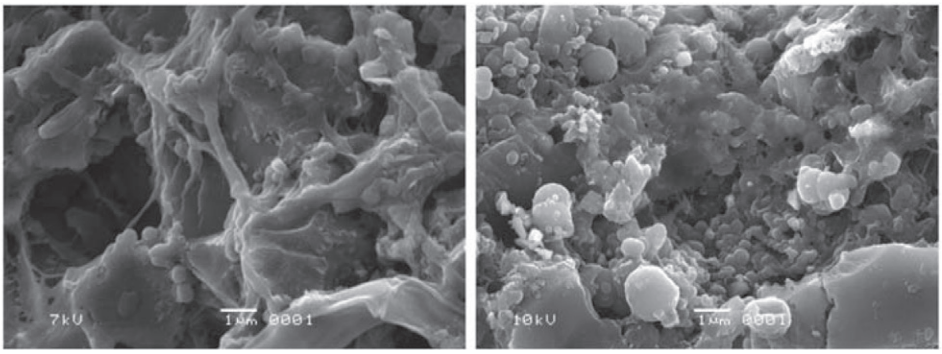

Figure 4.1 Scanning electron micrographs of surface GAC media for drinking water filtration after different backwashing procedures. From Yin *et al.* (2014) with permission from ASCE.

4.2.8 BIODESALINATION

Desalination is an increasingly common method of providing drinking water. In some areas this may be an answer to clean water challenges, for example in relation to drought episodes, but the energy demands and cost prohibit this technology from becoming common practice. As such, an alternative method for water desalination is required. Scientists have been exploring the use of cyanobacteria for their potential to act as an ion exchanger to remove salt. Strains that grow in high density under low nutritional requirements, such as sunlight, carbon dioxide and minerals, are being selected to drive these processes (Amezaga *et al*., 2014). Recent biodesalination designs use a low-salt biological reservoir within seawater that can serve as an ion exchanger. Cyanobacterial transport mechanisms must be manipulated to generate cells that can accumulate sodium intracellularly, instead of sodium export that occurs naturally. Further research is needed to understand the effects of environmental factors, pH, temperature and nutrients on the salt transport of cyanobacteria. Finally, the separation of cyanobacteria from water is difficult owing to the low density and small molecular size; this will require more work into the characterisation of cyanobacteria and their physiology (Amezaga *et al*., 2014).

4.2.9 MYCOFILTRATION

Researchers at Washington State University have demonstrated physically durable and biologically resilient fungal species and low-cost cultivation methods that can be implemented to produce a fungal biofilter, which is capable of filtering *E. coli* from flowing water under laboratory conditions. Mycofiltration technology uses the vegetative growth of bacteria-predating fungi that form an intricate and dynamic three-dimensional web of tube-like cells, called a mycelium. This living microscopic net can strain, adsorb and digest bacteria as a food source, thus reducing effluent bacteria concentrations with a simple, small footprint intervention (Stamets *et al*., 2012). This is an innovative concept and specific fungal strains seem to be sufficiently resilient and biologically active to be considered for stormwater treatment applications against a variety of targets including pathogens. However, more research is needed to clearly define treatment design and operating parameters.

4.3 FUTURE TRENDS

Future perspectives for technical and scientific developments are related to the production of more quantitative information on the pathogens in water, standardisation, multiplex assays and, importantly, developing sensitive protocols for applying next-generation sequencing techniques that facilitate the automation of the processes. Also, building interactive databases for mass sequencing studies available for non-specialised end-users remains a challenge.

5 WASTEWATER MANAGEMENT

5.1 FUNDAMENTALS AND CHALLENGES

A variety of technologies are employed worldwide in WWTPs, with by far one of the most common being the activated sludge process. The activated sludge process was one of the earliest methods of wastewater treatment, discovered in 1913 in the United Kingdom (Ardern and Lockett, 1914; Beychok, 1967) and continues to be used because of its effectiveness and simplicity. Additional technologies that may complement or substitute the activated sludge process include biofilm systems, granular sludge, membrane bioreactors (MBRs), anaerobic digestion reactors and wastewater ponds. These technologies have achieved varying levels of success in their development, with MBRs and anaerobic digestion reactors becoming more common as capital costs decrease owing to novel biological approaches and the reuse of resources. Complementary to all of these processes are the sewer networks that transport the wastewater streams to their final destinations. The networks pose a challenge as microbial and chemical transformations during transportation can be associated with health risks, network deterioration and change in wastewater quality before it reaches the WWTP, with hydrogen sulfide formation being a particular problem.

A requirement for WWTPs and their associated technologies is meeting the effluent standards of their respective regions of operation. These regulations differ drastically from country to country, with many having stringent regulations and others having only very basic practices. The standards heavily influence the design of WWTPs and are a necessary consideration for the development of new processes.

In recent times wastewater management in many countries has been changing focus from simple removal and disposal to reuse, and bioenergy production. This shift is a result of a societal push towards sustainable practices as well as technological advances that allow capture of energy, carbon and nutrients from wastewater streams. Nutrient removal is of particular interest because not only is this potentially hazardous to downstream ecosystems by posing a risk of eutrophication, but also it has the ability to recover both nitrogen and phosphorus.

According to UNESCO only 20% of wastewater receives proper treatment globally, with a treatment capacity of 70% in high-income countries and 8% in low-income countries. As such, there is a pressing need to develop simple cost-effective and efficient systems to better handle wastewater. WWTPs are increasingly being designed in ways that provide higher throughputs, lower ecological footprints, more efficient energy usage and maximisation of the recovery of resources from wastewater streams. Advances in biotechnology support the development of sustainable practices in wastewater management.

5.2 ACTIVATED SLUDGE PROCESS

The activated sludge process relies on microbial communities to remove carbon, nitrogen, phosphorus, micropollutants and, to some extent, pathogens from wastewater. The following section outlines some key processes and current status, and it highlights potential new developments building on microbial ecology, water management and biotechnologies.

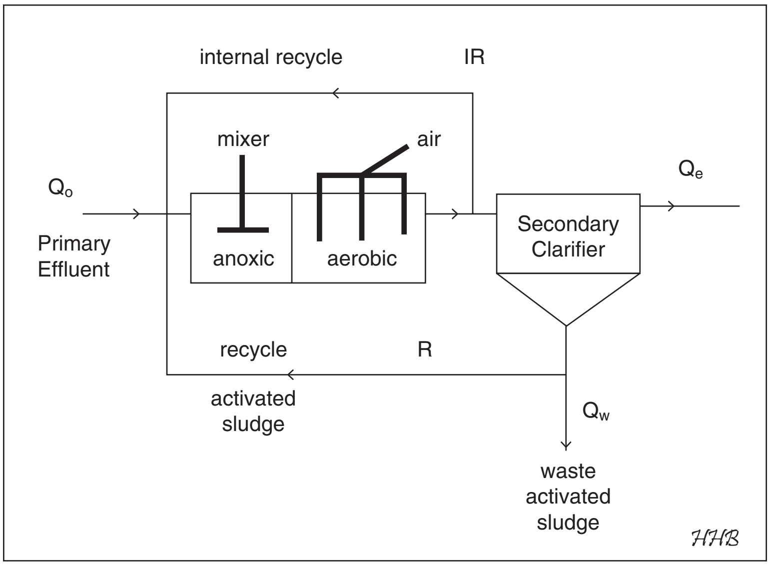

Figure 5.1 Flow diagram of a conventional activated sludge system with anoxic-aerobic processes for N-removal.

5.2.1 NITROGEN REMOVAL

The removal of nitrogen from municipal wastewaters has become a key stage in the treatment process in the past few decades. The removal of nitrogen is traditionally a two-part process of nitrification and denitrification (Fig. 5.1). Through this process, nitrogen in the form of organics, ammonia, and nitrite and nitrate are converted to nitrogen gas and safely released into the atmosphere (Fig. 5.2). Generally, the bacteria involved in the nitrification step are aerobic chemolithoautotrophs, and they are assumed to be well known and well described. However, recent studies using novel molecular methods have shown unexpected diversity and physiologies. One example is the discovery of the genus *Nitrotoga* as a common and often dominant nitrite oxidiser in addition to the canonical *Nitrospira* (Lücker *et al*., 2015). It has also been shown that *Nitrospira* consume organic compounds (Daims *et al*., 2001; Koch *et al*., 2015) as potential carbon and energy sources. Recently, certain species within the genus of *Nitrospira* have been shown to carry out the entire oxidation process from ammonium to nitrate (Daims *et al*., 2015; Pinto *et al*., 2015; van Kessel *et al*., 2015), known as the comammox process (COMplete AMMonium OXidation), which has hitherto been assumed to be a two-step process carried out by two distinct groups of bacteria, the ammonium oxidisers and the nitrite oxidisers (Daims *et al*., 2015). This new knowledge can explain many previously 'strange' observations and may pave the way for better process optimisation. Comparatively, denitrification is an anoxic process that is completed by a wide variety of microorganisms that convert the nitrate from nitrification to nitrogen gas. Some key denitrifiers are described in the MiDAS database, for example *Dechloromonas*, but most are still unknown and undescribed. A major issue related to nitrogen removal is the production of nitrous oxide, N_2O, a strong greenhouse gas. It is still unknown how to control this production and whether it is primarily caused by nitrifiers or denitrifiers in full-scale plants.

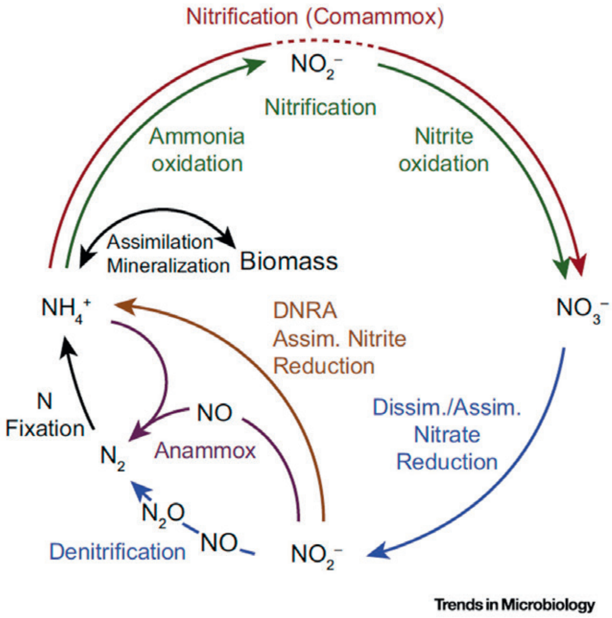

Figure 5.2 Schematic illustration of the key processes of the nitrogen cycle. From Daims *et al.* (2016).

5.2.2 ANAMMOX PROCESS

Nitrogen can also be removed by the anammox (ANaerobic AMMonium Oxidation) process, often called the 'deammonification' process. It is a process where anammox bacteria are capable of taking ammonium as an electron donor and nitrite as an electron acceptor to produce nitrogen gas under anaerobic conditions (Strous *et al*., 1998). Compared with conventional nitrogen removal by the nitrification/denitrification process, anammox bacteria and the associated process have shown very good results as a side-stream process connected to anaerobic digesters; however, the anammox process is still not applicable in the main process, although it may have a potential here in municipal water nitrogen removal. Anammox bacteria do not require any organic carbon; they reduce aeration energy demands by 60% and decrease sludge production by approximately 90% (Mulder, 2003; van Loosdrecht and Salem, 2006; Siegrist *et al*., 2008). The process was first discovered at Delft University of Technology in the 1990s and is today increasingly popular for treating effluent from anaerobic digesters as a side-stream with high ammonium content (Kuenen, 2008). The process can occur in one reactor where bacteria grow on compact granules (Box 5.1) or on a fixed bed (Kartal *et al*., 2010). A key parameter in the anammox process is maintaining appropriate concentrations of oxygen, nitrite, nitrate and ammonium. Intensive experimentation is currently ongoing to include anammox as a main-stream process, not only in warm climates but also under colder conditions. Four anammox microorganisms have been found in the plants (*Candidatus* Brocadia, *Ca.* Kuenenia, *Ca.* Anammoxoglobus and *Ca.* Jettenia (van Niftrik and Jetten, 2012)) but none of them have yet been isolated (Fig. 5.3). Ongoing research is trying to understand their physiology to make the process more stable, to speed up the growth rates of these very slow-growing bacteria, and to understand their interplay with comammox nitrifiers, other nitrifiers and denitrifiers. The major challenge associated with the application of the anammox process is maintaining the balance between these different microbial groups.

Figure 5.3 Anammox reactor with red granules containing *Kuenenia stuttgartiensis*. Image courtesy of Boran Kartal.

5.2.3 ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

One of the most recent advances in nutrient removal is the process configuration called enhanced biological phosphorus removal (EBPR). By removing phosphorus by biological means instead of chemical precipitation, less chemical sludge is produced and the sludge is more suitable for phosphorus recovery (Melia *et al*., 2017). The EBPR configuration adds an anaerobic reactor before the nitrification/denitrification reactors where polyphosphate-accumulating organisms (PAOs) are selectively enriched. PAOs can accumulate three to four times as much phosphorus in their cells as most other bacteria. EBPR then follows the same path in a typical activated sludge process but with considerably more phosphorus removed as the PAOs settle out and are removed by surplus sludge (Blackall *et al*., 2002). Currently two genera of PAOs are known, *Ca*. Accumulibacter and *Tetrasphaera* (He and McMahon, 2011; Nguyen *et al*., 2015; Stokholm-Bjerregaard *et al*., 2017) but there are probably other, not yet detected, species. Present research focuses on a better understanding of their physiology and ecology but there still exist challenges in the control of this process.

One challenge the EBPR process encounters is competition between the PAOs and glycogen-accumulating organisms (GAOs). Both types of organism compete for the same carbon sources, thus reducing the overall capability of PAOs. Research is being conducted into this competition and several factors seem to favour PAOs over GAOs in laboratory-scale systems; however, it is difficult to extrapolate to full-scale plants where many more microorganisms and varying environmental conditions are involved. Because of this, some scientists doubt that GAOs will have a noticeable impact and question whether the phenomenon is limited to laboratory conditions. Two GAO groups are currently recognised, the gammaproteobacterial *Ca.* Competibacter and the alphaproteobacterial *Defluviicoccus*-related tetrad-forming organisms (McIlroy and Seviour, 2009). More research is required in this area.

The Nereda process was discovered in 1995 and was developed and upscaled by Mark van Loosdrecht at Delft University of Technology. Pilot plants were operated in The Netherlands, Portugal and South Africa with promising results. As of June 2015, some 25 additional plants were in operation or being designed globally. One key advantage of the Nereda process is the reduced power consumption (Giesen and Schroers, 2015).

The Nereda process uses granules, 'aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear', instead of the conventional flocs. Granules have better biomass retention, faster settling and can house aerobic and anaerobic reactions for nutrient removal. Nutrient removal occurs first on the outer aerobic layer of the granule where nitrifying bacteria accumulate. The nitrate formed here is then denitrified at the anoxic core of the granule. Phosphorus uptake happens last by PAOs housed inside the granule (Giesen *et al*., 2013).

Box 5.1 Aerobic granular biomass in the Nereda process

5.2.4 SLUDGE SEPARATION

Another very important step in activated sludge systems is the separation of the activated sludge flocs and treated water. This is a two-step process where the sludge is separated in the final clarifier (settling tank) by settling and where most sludge is recirculated to the process tank and some is removed as surplus sludge. The surplus sludge either goes for dewatering before final deposition (typically land field, agriculture or incineration) or it goes to a digester for sludge reduction, energy production and potentially nutrient recovery.

Bulking is one of the greatest challenge to the operators of activated sludge processes. Sludge bulking occurs when the biological sludge floc and water do not separate in the settling tank, resulting in a contaminated effluent water flow. The primary cause of bulking is the growth of filamentous microorganisms such as *Microthrix* (Rossetti *et al*., 2005) (Box 5.2). Filamentous microbes have a relatively large volume and surface area owing to their long thin morphology, which means they are slow to settle and separate from the treated water. Most filamentous microorganisms are now identified by molecular methods and, by knowing their identity and physiology, most can be controlled. However, most of this new knowledge is still not in operation, so the focus over the coming years should be to translate the new knowledge to proper control measures.

The floc formation and dewatering process also deserves special attention, as dewatering is difficult and expensive. Much research has been done in understanding the 'glue' in the flocs, the extracellular polymeric substances excreted by the microorganisms. These determine the floc size distribution and many other properties including the water-binding properties (Flemming and Wingender, 2010). The vision – some years ahead – was to 'design' the right flocs with good floc and dewatering properties through informed manipulation of the microbial communities. However, at present the relative contribution to the floc properties from the different bacteria is still very poorly understood.

Microthrix parvicella is a dominant species in bulking sludges and foams around the word. It is favoured in long sludge retention times, alternating aerated zones and low temperature environments. At the Meridian WWTP, seasonal blooms were reducing the capacity of clarifiers and leading to increased suspended solids which caused filter backwashing. Various methods were explored to control the *Microthrix* growths; these are presented in Table 5.1.

Method	**Advantages**	**Disadvantages**
Eliminate food source	Addresses root cause	Difficult to remove all sources
Decrease sludge retention times	Simple, addresses root cause, washes out filaments	Loss of nitrification
Surface spraying of foam	Quick and easy	Difficult to accomplish in covered tanks, treating a symptom not a cause
Digester modifications	Flexible	Expensive, treats symptom not a cause
Polyaluminium chloride addition	Specific to *Microthrix*, treats root cause	Relatively high chemical costs
Surface wasting/classifying selector	Eliminates *Microthrix* at source	Relatively high capital costs

Table 5.1 *Microthrix* removal methods

The Meridian WWTP selected polyaluminium chloride (PAX) addition as their preferred short-term solution. Through microbial analysis, it was seen that the addition of PAX reduced the ability of *Microthrix* to use up lipids, thus starving them. PAX is preferred as an emergency fix because it has relatively low capital costs, but high chemical costs.

The chosen long-term solution was a classifying selector that removes foam continuously and prevents build-up. The removal of foam eliminates a *Microthrix* breeding ground but at the same time leads to a thin sludge, which is not ideal for the dewatering process. As such, dissolved air flotation tanks are used to thicken the activated sludge before the digestion process (Ayers and Kelly, 2012).

Box 5.2 *Microthrix* at the Meridian WWTP, Idaho, USA

5.3 MBRs

An alternative technology used in place of settling tanks and clarifiers is MBRs. The advantage of MBRs is higher effluent quality and that the process infrastructure requires less space (Henze *et al*., 2008). The greatest challenge facing MBR technology is biofouling. Biofouling is the build-up of unwanted biomass on the membrane that reduces flow, lowers treatment effectiveness and can lead to failure of the membrane. Research into the microbiology of the fouling layer indicates differences in abundance from the bulk sludge using amplicon sequencing; however, the results are still inconclusive (Ziegler *et al*., 2016; Zhang *et al*., 2018). Further investigations of the microbial association with biofouling are needed, together with the development of monitoring and control techniques to address the biofouling issue.

Suggestions for monitoring techniques include analysis of feed-water parameters and system performance in terms of pressure changes, oxygen uptake and biofilm growth by, for example, the use of fluorometry. Ultrasonic time-domain reflectometry uses sound waves to locate biofilm build-up and could also provide useful information about fouling characteristics; however, the technique has yet to be tested in a full-scale application (Sim *et al*., 2012).

Biofouling control strategies are many. One example is the addition of adsorbents or coagulants into sludge suspension, thereby decreasing the level of solutes and colloids, which leads to better filtration performance. However, the potential impacts of coagulants or adsorbents on biomass community or biomass metabolism need to be taken into account (Meng *et al*., 2009). Another example is the addition of chemical agents to the sludge suspension to reduce fouling. Chlorine, chlorine dioxide and ozone are well-known antimicrobial agents, yet they are not preferred because of the sensitivity of the membrane material and possible harmful by-product formation. A third example is the application of quorum quenching. Quorum sensing is very important for communication systems among bacteria during biofilm formation and thus is important for biofouling. Quorum sensing is achieved by releasing and detecting molecules called auto-inducers. The successful application of quorum quenching bacteria, which degrades the auto-inducers, for biofouling control in MBR systems at pilot-scale has already been reported (Waheed *et al*., 2017). Among other strategies for biofilm detachment, still in its early development, are the addition of bacteriophages and nitric oxide. In contrast to adding agents, membrane surfaces can also be modified in multiple ways to minimise or delay biofilm development. This can involve using different polymers, surface coatings or infusing additives to the membrane. Finally, investigation into the use of predatory metazoans has proved successful, increasing the flux over the membrane at laboratory-scale. This was done by changing the biofilm structure as well as significantly reducing the thickness of the layer (Klein *et al*., 2016).

5.4 ANAEROBIC DIGESTION

Anaerobic digestion is the collection of processes by which microorganisms break down biomass in the absence of oxygen, producing useful by-products such as biogas. Anaerobic digestion is used widely in the treatment of organic sludges (for example primary sludge and surplus sludge from WWTPs) and of concentrated organic industrial wastes, in nutrient recovery and bioenergy creation (Fig. 5.4). There are many advantages to anaerobic digestion including the production of renewable biogas, reduction of greenhouse gases, dense/dried sludge and relatively low operating costs. The associated disadvantages are the accumulation of heavy metals and microcontaminants in the sludge, and a narrow temperature control range (Chan *et al*., 2009). Another current challenge of anaerobic digestion reactors is their efficiency and biogas output. Often the gas production is insufficient to offset the capital costs in a reasonable payback period. Furthermore, the quality of the biogas is often low with relatively high sulfide contents. As for activated sludge, foaming problems also occur occasionally.

There are four stages involved in anaerobic digestion: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Currently, few microorganisms responsible for anaerobic digestion processes have been well described and significant research is being undertaken to identify the key players in these reactors. For example, the MiDAS field guide for activated sludge (http://midasfieldguide.org/) has been expanded to contain the most abundant players in anaerobic digesters too. The majority of growing genera are known to be fermentative organisms, including *Coprothermobacter* and *Anaerobaculum* in thermophilic systems, and *Thermovirga*, *Leptolinea* and *Ca*. Fermentibacter in mesophilic systems. The dominant archaea in mesophilic reactors running on primary and surplus sludge from WWTPs was *Methanosaeta*, while *Methanothermobacter* and *Methanosarcina* were the dominant methanogens in thermophilic systems (Kirkegaard *et al*., 2017). More insights into the functions of these organisms are needed and more research needs to be done for a better control of anaerobic digestion processes.

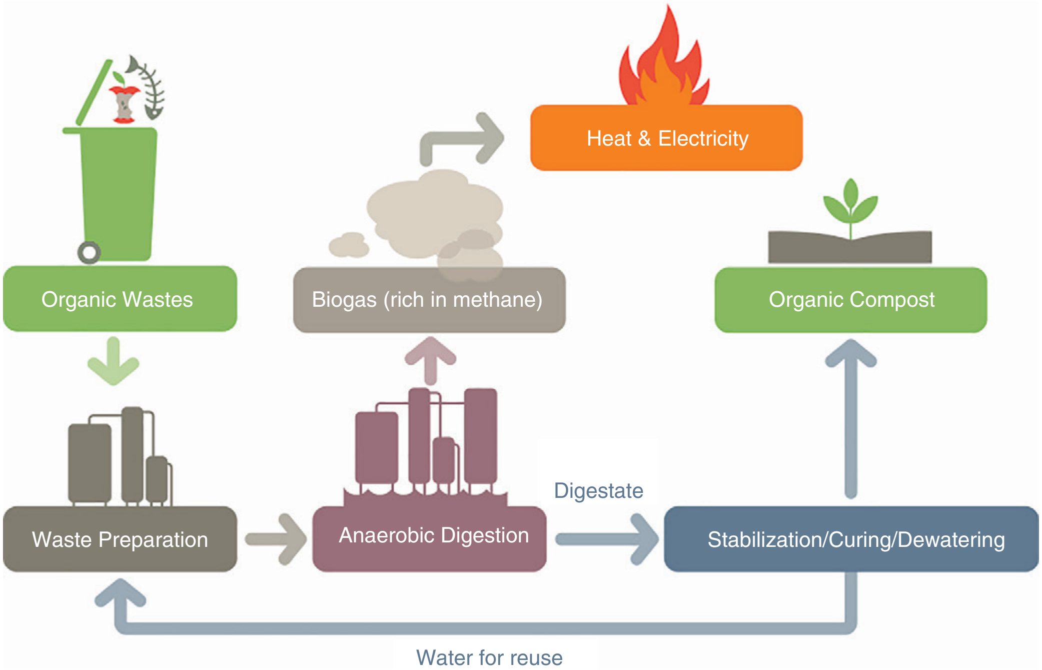

Figure 5.4 The principle of anaerobic digestion.

5.5 FUTURE TRENDS

The microbial reference databases will continue to expand with more novel microorganisms from around the world, including their possible functions (Karst *et al.*, 2018). A better understanding of the physiology and ecology of the microbes based on solid theories in microbial ecology will enable development of more complex control strategies at WWTPs. On-site and online surveillance and sampling technologies together with easy bioinformatics processing are expected to be available on the market within the near future. This will allow system users, operators and engineers to identify their local microbial communities, compare them with an existing database and take appropriate control measures.

6 RESOURCE RECOVERY

6.1 FUNDAMENTALS AND CHALLENGES

Water conservation, energy efficiency and resource recovery from wastewater systems are being more globally recognised. We are entering an era of circular economy, with a focus on sustainability that is supported by various national and international initiatives. However, treatment of waters contaminated with faecal matter, such as sewage, has been subject to a long history of 'disgust'. Until recently, sanitation practice was treating wastewater for disposal and elimination away from the population instead of treating it as a resource. The reputation of wastewater treatment is slowly changing, with visions of rebranding wastewater as 'used water' for better social acceptance. Technically, it is already possible to produce reclaimed water and recover a variety of elements; however, there are still major challenges in terms of economic feasibility, legacy, sufficient amounts of product and 'fit-for-purpose' quality compared with mining nutrients. In addition to the IWA BioCluster, The Resource Recovery Cluster works on all aspects of resource recovery and has produced a state of the art compendium to consult for more information (Holmgren *et al*., 2015).

Resource recovery is a growing field, with rapid developments in methane and biogas production and new initiatives in bioplastics, medium-chain fatty acids and microalgae. With the novel methods available in microbial ecology, biotechnology has become a driver in the field of resource recovery, providing new areas of research and development for an increasingly sustainable industry. This chapter will primarily focus on the role of microbial ecology involved in the recovery of a variety of resources from municipal wastewater streams.

6.1.1 WATER RECOVERY

The recovery of water from effluent streams is in principle similar to the conventional treatment process that would occur in WWTPs. The biological processes involved in this are outlined in Chapter 5, with one additional step, in which the water is separated and returned to serve another resource purpose, depending on its level of treatment.

6.1.2 ENERGY RECOVERY

The most promising developments in energy recovery from wastewater are plants using anaerobic digestion for biogas production as well as biogas incineration for electricity generation. The biological processes involved in anaerobic digestion describing the microorganisms capable of methane production are outlined in Chapter 5. A good example of energy recovery comes from a traditional WWTP in Toronto, Canada, where it was estimated that over nine times as much energy was recovered from the wastewater than was used to treat it (Shizas and Bagley, 2004). Several full-scale WWTPs in Denmark are now net energy producers by reducing operational costs and improving methane yield, and this is now a trend in several countries (Nielsen, 2017).

6.1.3 MICROBIAL FUEL CELLS

Through oxidation processes of the organic matter contained in wastewater streams, microorganisms can generate electricity directly, while at the same time treating the water (Rabaey and Verstraete, 2005; Logan, 2008). Along with the potential for electricity generation, nutrients, contaminants and ammonia can be removed in the processes; however, the process still has challenges. Electrogenic bacteria that power microbial fuel cells include, among others, *Shewanella putrefaciens* IR-1, *Clostridium butyricum* EG3 and *Desulfuromonas acetoxidans* (Logan, 2009) (Fig. 6.1). Laboratory-scale enriched anodic biofilms have generated power densities as high as $6.9\,W\,m^{-2}$.

By combining a microbial electrochemical cell and a forward osmosis cell, the engineers at Virginia Tech created a hybrid biological and electrochemical system. In this system, ammonia-driven forward osmosis was combined with an ammonia-generating microbial electrochemical cell (Lu *et al*., 2014). Now proved at bench-scale, the project is moving to pilot-scale as the researchers currently are collaborating with local water treatment plants to establish a research facility.

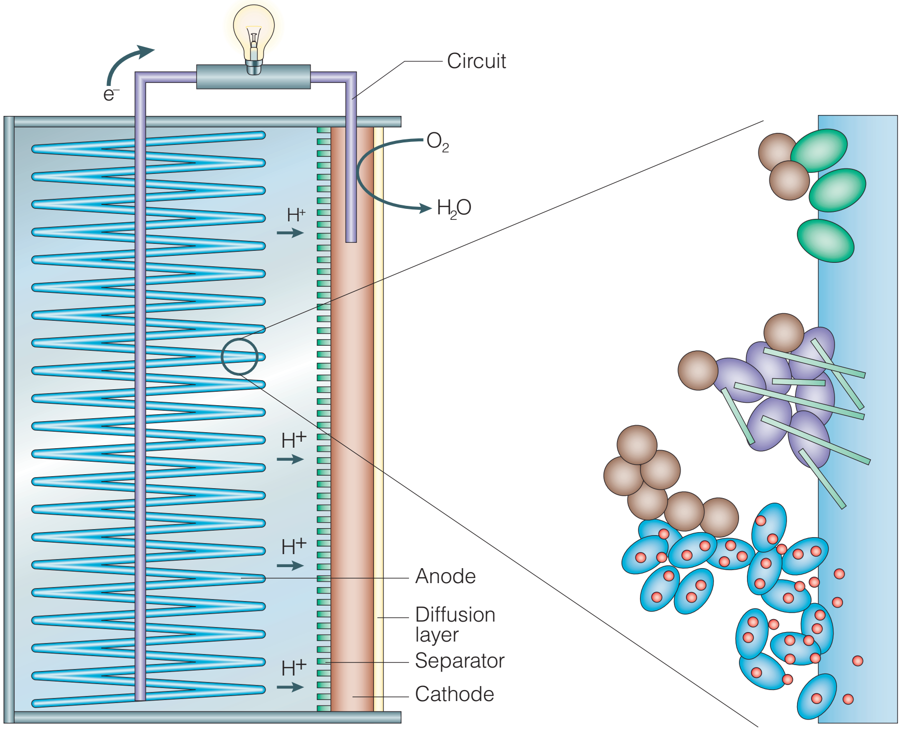

Figure 6.1 Concept of a microbial fuel cell (Logan, 2009). Reprinted by permission from Springer Nature: Nature Reviews Microbiology, Exoelectrogenic bacteria that power microbial fuel cells, B.E. Logan, Copyright, 2009.

6.1.4 NUTRIENT RECOVERY

Nitrogen and phosphorus are the primary nutrients targeted and recovered in water management, particularly because of their application in agriculture and the need to reorganise the biogeochemical flows in a more sustainable manner (Steffen *et al*., 2015). The recovery of nutrients has become a primary focus in the development of wastewater treatment and is one of the drivers in the shift towards wastewater recovery. The primary methods of microbial recovery have already been outlined in Chapter 5; however, more alternative methods are included below.

Constructed wetlands for biological assimilation of excess nutrients are a relatively novel concept. Most constructed wetlands are designed for treatment and do not consider recovery; however, there are examples where nutrient recovery is promoted. Constructed wetlands may vary in water level, types of plant used and flow direction, but the general method is a combination of shallow water, low flow velocity, dense vegetation and narrow channels to create plug flow conditions. Common species of plants and vegetation for surface assimilation are *Eichhornia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce) and several species of Lemnaceae (duckweed). These plants can be harvested after assimilation and sold as a source of nutrients in fish and animal feeds, having removed 83–87% nitrogen and 70–85% phosphorus from wastewater effluents (Ozengin and Elmaci, 2007).

In anaerobic digestion systems a typically unwanted side product is hydrogen sulfide, usually counting for 0.1–2% of the biogas volume (Lastella *et al*., 2002). When hydrogen sulfide is left within the biogas it can contribute to unwanted odours, corrosiveness and sulfur emissions after combustion. Biological treatments have been used heavily in this regard; and focus has been placed on the phototrophic bacterium *Cholorobium limicola*, which is capable of growth under anaerobic conditions using only inorganic compounds and of a very efficient production of elemental sulfur from hydrogen sulfide (Syed *et al*., 2006). A new concept is proposed whereby hydrogen-oxidising bacteria are used to recover nitrogen from water treatment systems by producing microbial proteins (Matassa *et al*., 2015b).

There is a need for growth in available highly nutritious protein, especially as protein requirements are estimated to double by 2050 as the world's population approaches 10 billion people. This increase in population together with vulnerability of conventional crop production to climate change justifies the re-examination of current methods of converting nutritious compounds into biogas in sewage and WWTPs. It appears that for future wastewater treatment, the production of biogas should no longer be directed to energy recovery: rather, biogas and the nutrients present in wastewater must be tuned for reuse by upgrading them into valuable molecules such as proteins. The recent advances in single-cell protein production, which is the production of protein-rich feed and food, using recovered nutrients and organics, open possibilities for new microbial technological applications to short-circuit nutrient and energy cycles into more efficient processes. By upgrading treatment plants to factories in which the incoming materials are first deconstructed to units such as ammonia, carbon dioxide and clean minerals, it is possible to implement a highly intensive and efficient microbial resynthesis process in which the used nitrogen is harvested as microbial protein at efficiencies close to 100% (Matassa *et al*., 2015a; Verstraete and De Vrieze, 2017).

Microbial protein is a promising advancement that addresses impending food challenges (Pikaar *et al*., 2017). In terms of advantages, microbes have a rapid growth rate, a high conversion yield, high protein content and a small environmental footprint. A few challenges remain in the use of microbial proteins as feed, with the greatest being public acceptance. Furthermore, technologies need to be developed for harvesting microbial protein as well as the taste and texture of the final product.

Box 6.1 Microbial protein as new feed

6.1.5 MICROALGAE FOR BIOREMEDIATION AND BIOFUEL PRODUCTION

Microalgae possess a technological option for combined bioremediation of wastewater and algal biomass cultivation. Microalgal biomass can be used as slow-leaching fertiliser or for biofuel production (Mata *et al*., 2010; Wijffels and Barbosa, 2010; Matassa *et al*., 2015a). Large-scale microalgal cultivation for biofuel production is not favourable, owing to the high water and nutrient demand, unless the microalgae are cultivated in wastewater (Markou *et al*., 2014). Microalgae are efficient for removing different types of compound such as nitrogen, phosphorus, potassium, silica, iron, magnesium and other chemicals from municipal and industrial wastewater. In addition, microalgae have a high capacity to accumulate heavy metals (selenium, chromium, lead), metalloids (arsenic) and organic toxic compounds (hydrocarbons) to form microalgal biomass which subsequently can be used for biofuel production. The most commonly used microalgal species for wastewater remediation include *Chlorella* sp. (for example *Chlorella sorokiniana* and *Chlorella vulgaris*) and *Scenedesmus* sp. *Chlamydomonas mexicana* has been suggested as a promising algae for simultaneous nutrient removal and highly efficient biodiesel production (Maity *et al*., 2014). Leading contenders in large-scale microalgal cultivation are open pond systems and closed-air photobioreactors. The benefits coming from open pond systems, such as raceway ponds, are the relatively low cost, low energy requirement and simple maintenance; however, it is difficult to maintain a stable culture composition (Wágner, 2016). Closed photobioreactors have shown high efficiency due to their ability to eliminate environmental pathogens and predators, and providing better light conditions. Further benefits include a low environmental footprint, high productivity, enhanced gas transfer and protection from outdoor-climate-related impacts such as rainfall or evaporation (Maity *et al*., 2014). Disadvantages include more complex and expensive operation, biofouling, oxygen accumulation and overheating (Mata *et al*., 2010).

Coppens *et al*. (2015) assessed the potential to use microalgal biomass as fertiliser, grown on wastewater resources. They reported the improvement of the nutritional level of plants that were fertilised by microalgal flocs. The main advantage of using microalgae as fertilisers is the slow release of nutrients, thus reducing the oversupply of nutrients (Solovchenko *et al*., 2015).

One of the main contributors to production costs in biofuel production is the harvesting of the microalgal biomass. Reducing these costs could be promoted by, for example, floc formation between bacteria and algae (Van Den Hende *et al*., 2016) or by co-flocculation of bacteria and microalgal biomass in combined bacterial–algal systems (Wágner *et al*., 2016), which has beem suggested for use in biogas production.

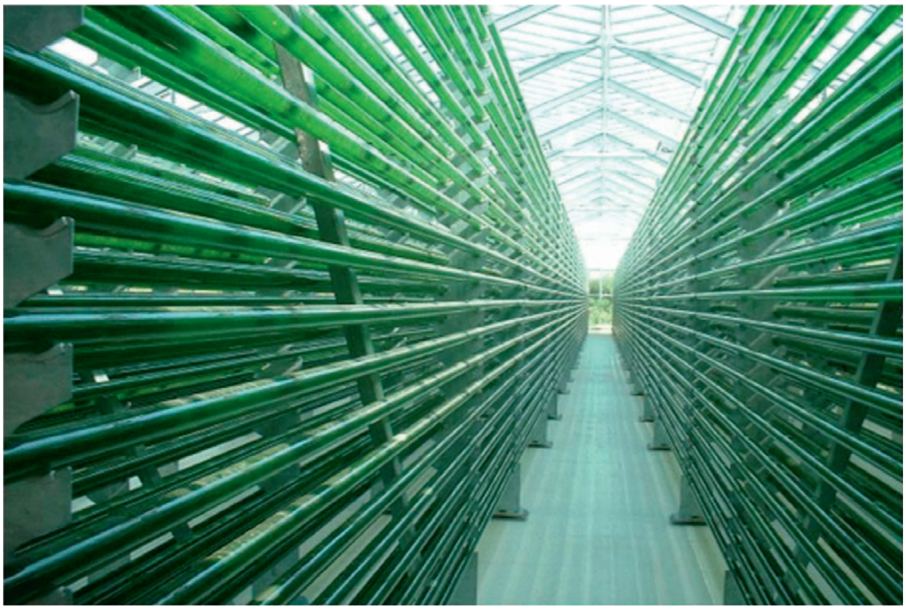

Figure 6.2 Horizontal tubular photobioreactor design (Abdel-Raouf *et al.*, 2012).

6.1.6 BIOPLASTICS

Polymers called polyhydroxyalkanoates (PHAs) can be produced by a range of bacteria by breaking down organic compounds. PHA polymers can substitute traditional petroleum-based plastics with the added benefit of a low environmental impact and the potential for recycling. Bioplastics are commonly used in medical and pharmaceutical industries owing to their biodegradable properties and increasingly in a wider range of industries. PHA production can be achieved in open, mixed microbial cultures, thereby coupled to wastewater treatment. In this context, waste organic matter is utilised as a carbon source in activated sludge biological treatment for biopolymer synthesis (Morgan-Sagastume *et al*., 2014). Very high levels of PHA have been obtained in laboratory set-ups with microbial enrichments. In one case, 77% PHA of cell dry weight was obtained on acidified paper mill wastewater. Biomass in this enrichment included *Plasticicumulans acidivorans* as a main PHA producer (Jiang *et al*., 2012).

More recently, a process was developed for biological treatment of municipal wastewater for carbon and nitrogen removal while producing added-value PHAs. The process comprised steps for pre-denitrification, nitrification and post-denitrification and included integrated fixed-film activated sludge with biofilm carrier media to support nitrification. The process was demonstrated at pilot-scale with 83% removal of chemical oxygen demand and 80% removal of total nitrogen while producing a biomass that was able to accumulate up to 49% PHA of volatile suspended solids. The outcomes show that production of added-value biopolymers may be readily integrated with carbon and nitrogen removal from municipal wastewater (Bengtsson *et al*., 2017).

6.1.7 METAL RECOVERY

Metal-contaminated wastewater posts great health and environmental concerns, but it also provides opportunities for precious metal recovery. Recovery of metals directly from wastewater is considered an economic and environmental win–win scenario, owing to the dual benefits of reduced pollutant loading and creating potential economic value. High-value elements such as platinum have been shown to accumulate in WWTPs, possibly originating from automobile catalysts that drained from roadways into sewers. A recent study in the USA estimated the economic potential of metal recovery from WWTP biosolids, by capturing the 14 most lucrative elements (silver, copper, gold, phosphorus, iron, palladium, manganese, zinc, iridium, aluminium, cadmium, titanium, gallium and chromium) with a combined value of US$280/ton of sludge (Westerhoff *et al*., 2015). A strategy for metal removal could be direct biosorption. Previously, *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus* were found to be capable of removing significant amounts of copper and lead (Leung *et al*., 2000); however, there is still far to go.

Another novel approach to remove metals is a microbial fuel cell that is generates electricity and at the same time produces active electrolyte in this process as a product of wastewater electrolysis. The active electrolyte is highly caustic and can be used for parallel flocculation and precipitation of heavy metals (Gajda *et al*., 2017). This approach seems promising using self-powered electrocoagulation for treatment of wastewater polluted with heavy metals.

6.2 FUTURE TRENDS

Advances in microbial ecology and a better understanding of microbial pathways will have a great impact on biological resource recovery. The large palette of ongoing resource recovery strategies will heavily benefit from new knowledge of novel organisms, biochemical pathways and measures for informed control of microbial ecosystems.

7 INDUSTRIAL WASTEWATER

7.1 FUNDAMENTALS AND CHALLENGES

Industrial wastewater originates from numerous sources. The wastewater composition and flow can vary substantially both daily and seasonally depending on the type of wastewater; thus the treatment can be challenging. Some industries, for example wineries, only produce wastewater seasonally owing to the periodic operation (Ioannou *et al*., 2015). Other industrial sources might contain organic pollutants, for example phenolic compounds that can be inhibitory to biological treatment processes (Guieysse and Norvill, 2014). The main contributors to industrial wastewater production are the food industry, pulp and paper industry, dairy production and farms, breweries and wineries, textile industry, metal processing, pharmaceutical industry and petrochemical industry. Industrial wastewater is differentiated from domestic wastewater on the basis of the high amounts of organic matter and nutrient content. The organic content of industrial sources varies to a great extent, i.e. between 0.2 and 15 g of chemical oxygen demand (COD) per litre (Hamza *et al*., 2016). The contaminants are in some areas discharged illegally to either the sewer or directly to the environment. This results in environmental damage, biodiversity deterioration, contamination of the food chain, etc. Industrial wastewater loads into sewers can also interfere with the processes of conventional wastewater treatment plants, especially in small systems where shock loadings cannot be easily treated (Techobanoglous *et al*., 2004). Global actions have been made as the United Nations in 2017 agreed upon 17 Sustainable Development Goals, with Goal number 12 addressing industrial waste: 'Ensure sustainable consumption and production patterns' (United Nations, 2017). One target by 2030 is substantially to reduce waste generation through prevention, reduction, recycling and reuse. Despite the good intensions, cost-effective treatment is a major issue. Many industrial operators are not interested in treating wastewater unless there is a rapid payback or the core business is affected, often considering the environmental issues last on the agenda. Large industries located in metropolitan or outer urban areas generally discharge to an available sewer, which transfers the waste to a municipal wastewater treatment plant. However, many facilities are located in regional and rural areas, for example mine sites, wineries and abattoirs. In these cases, it is more common that wastewater is treated on-site and discharged to the environment. There is increasing pressure to treat industrial effluents before they enter sewers or the environment.

The degree of treatment required for industrial wastewater depends heavily on the contaminants as well as the receiving body and the potential for recovery and reuse. There is a great potential for reuse of wastewater from, for example, food industries owing to the large volumes and the high quality without faecal contaminant, making it suitable for direct use in production of high-quality product, such as specific enzymes, production of single cell proteins, etc.

The principles of industrial wastewater treatment systems are similar to those of municipal wastewater systems. This chapter will illustrate case examples of technologies, challenges and trends in water management associated with the diverse industrial wastewater.

Several chemical and biological processes have been proposed to treat industrial wastewater, for example advanced oxidation processes (AOPs), coagulation–flocculation, electrocoagulation, MBRs and moving bed biofilm reactors (MBBRs), anaerobic digestion, upflow anaerobic sludge blankets (UASBs), constructed wetlands and microalgae cultivation (Lofrano *et al*., 2013; Guieysse and Norvill, 2014; Ioannou *et al*., 2015; Hamza *et al*., 2016; Van Den Hende *et al*., 2016; Shi *et al*., 2017). Biological processes are considered more economical than chemical ones, and they promote energy recovery and the recovery of other valuable resources. Depending on the source of wastewater, the biodegradability of contaminants can vary considerably: for example, dairy industry effluents are high in readily biodegradable organics whereas petrochemical and pharmaceutical effluents can contain high amounts of non-readily biodegradable fractions (Hamza *et al*., 2016). Aerobic and anaerobic biological processes can be both considered; however, the oxygen requirement for treating high organic content wastewater, the volatilisation of organic matter and the additional sludge handling costs due to the high amount of sludge produced make anaerobic processes favourable (Buntner *et al*., 2013). Conventional activated sludge processes might be limited in the removal of, for example, pharmaceuticals and hormones.

Combined anaerobic–aerobic processes reduce energy requirements and treatment costs. The organic pollutants are removed in the anaerobic stage while the aerobic process polishes the effluent from the first step. Moreover, the combined process produces less sludge (Hamza *et al*., 2016).

Biological processes can be combined with chemical processes, for example using an AOP on industrial wastewater as the pretreatment step following a biological treatment for organic matter and nutrient removal, reducing the cost and increasing treatment efficiencies of chemical treatment processes (Ioannou *et al*., 2015).

Techniques are applied to reduce the amount of wastewater that is produced. Addition of enzymes in food production has been proved to reduce water consumption and emissions to the environment in many cases. Examples include phospholipases in de-gumming soybean oil, pectinases in fruit juice production, and amylases in the bakery and brewery industries (Jegannathan and Nielsen, 2013). The addition of enzymes to industrial processes eliminates the use of harsh chemicals, resulting in cleaner wastewaters.

7.2 UASBs

Among other technologies, UASB reactors are commonly used in treating wastewater. The technology is based on the formation of microbial granules. Microbial granules have better resistance against shock loadings than suspended cultures owing to their good settling ability which allows retention of the biomass in the system. The system has a low energy requirement and low sludge production (Hamza *et al*., 2016). It has high biomass concentrations and high microbial diversity. It can withstand variation in pH and temperature (Shi *et al*., 2017). The start-up of the system is challenging and requires a long time (Hamza *et al*., 2016). UASB effluent removes more than 60% of COD in most wastewater types; however, the effluent does not meet the discharge level and a polishing step is needed for nutrient removal. Anaerobic treatment processes have been used in pharmaceutical wastewater treatment and studies of the microbial communities involved have taken place (Shi *et al*., 2017).

7.3 MBRs

MBR technology offers another solution for treating industrial wastewater. It is most often a combination between a biological process and membrane filtration. The membrane filtration makes the separation of sludge faster, reducing the hydraulic retention time (HRT), it produces a high-quality effluent, it can handle high volumetric loading rates and reduces the sludge production (Hamza *et al*., 2016). The major drawback is membrane fouling, which increases the maintenance costs (Lofrano *et al*., 2013). Anaerobic membrane bioreactors (AnMBRs) combine a high-rate anaerobic digestion step with a membrane reactor. There is great potential for using this technology owing to its high-quality effluent characteristics, high tolerance of load variations and the production of solid-free effluent (Jensen *et al*., 2015).

The possibility to treat winery wastewater with a side-stream AnMBR was assessed by Basset *et al*. (2016). Winery wastewater has high biodegradable organic content, low nutrients and acidic pH. Owing to the low nutrient content, the anaerobic treatment of winery wastewater has great potential. The process has to be flexible as the winery wastewater characteristics greatly vary seasonally based on the production. COD removal of 96% was found while producing biogas that could cover the costs of electricity requirements during the high loading season. The presence of methanogenic archaea was studied in detail and *Methanosaeta* sp. with a filamentous morphology was found attached to the membrane surface and not growing in the membrane cake layer. Also, slaughterhouse wastewater can be used for recovery of nutrients by the AnMBR (Jensen *et al*., 2015). Ninety-five per cent of COD was removed from the wastewater, and up to 90% of nitrogen and 74% of phosphorus remained in the permeate; thus a subsequent recovery of nutrients with other technologies is possible.

Buntner *et al*. (2013) presented a combined UASB and MBR system to treat dairy wastewater. The system was successful in producing high amounts of biogas with 73% methane content while the total COD removal was above 99%. Different genera of methanogens (*Methanosaeta* and *Methanosarcina*) were studied to understand their dependence on the organic load.

7.4 MBBR

The MBBR is a technology that is based on biofilms grown on small plastic carriers which are mixed in the reactor. MBBRs offer the combination of biofilm reactors and activated sludge, resulting in a robust technology. MBBRs are efficient at removing pharmaceuticals and other complex compounds. Bering *et al*. (2018) described a successful use of the MBBR process to treat laundry wastewater. Removal efficiencies of up to 98% of biological oxygen demand (BOD) and 94% of COD were obtained. The removal efficiency for surfactants, contaminants present in laundry wastewater in high amounts, was up to 99%.

Hospital wastewater contains high amounts of pharmaceuticals and it is typically treated with municipal wastewater. However, to remove pharmaceuticals more efficiently and to decrease the amount in WWTPs, there is pressure to treat the wastewater on-site. Similar or better removal efficiencies of common compounds have been found compared with other technologies, i.e. activated sludge or MBRs. Casas *et al*. (2015) used pilot-scale MBBRs to treat hospital wastewater; removal efficiencies were dependent on the compounds and substantially variable. Torresi *et al*. (2018) operated a staged and non-staged denitrifying MBBR for pharmaceutical removal from wastewater. They found several bacteria that were previously reported to be associated with pharmaceutical removal. A core community was found to be shared in both systems, consisting of Burkholderiales, Xanthomonadales, Flavobacteriales and Sphingobacteriales. The last part of the staged reactor selected for Candidate division WS6 and Deinococcales.

7.5 MICROALGAE AND MACROALGAE CULTIVATION

Microalgae cultivation has been proposed to treat industrial wastewater resources. The produced microalgal biomass can be used, for example for biodiesel or biogas, fertiliser and pigment production, thus promoting resource recovery. Microalgae cultivation is especially useful in treating wastewater high in nitrogen and phosphorus or as a polishing step after organic carbon has been removed.

Safafar *et al*. (2015) showed the possibility of cultivating microalgae on industrial wastewater from an industrial site in Kalundborg, Denmark, where the wastewater was rich in nitrogen (190 mg/L total nitrogen) and nutrients were recovered in the form of pigments. The microalgae grown on the wastewater contained high amounts of carotenoids, for example lutein and β-carotene, and antioxidants. The highest amounts of total carotenoids were detected in *Desmodesmus* sp. Light intensity had a significant effect on the amount of carotenoids in *Chlorella sorokiniana*.

An effluent of a UASB reactor treating food industry wastewater was subsequently treated by an outdoor raceway pond containing microalgal–bacterial biomass (MaB-flocs) (Van Den Hende *et al*., 2016). The most abundant microalgal species in the MaB-floc was *Desmodesmus* sp. when the flocs were grown on UASB effluent. The wastewater contained high amounts of COD and nutrients (nitrogen and phosphorus). The UASB reactor removed more than 50% of the COD. The effluent was polished with the MaB-flocs, whereby the microalgae reduced the amount of nitrogen and phosphorus; however, the effluent quality did not meet regulations, thus further optimisation is needed. The potential in the technology lies in the coexistence of microalgae and bacteria, whereby the oxygen produced by the algae is used by the bacteria, and the microalgae use the carbon dioxide produced by the bacteria. Moreover, owing to the floc characteristics, the biomass can be easily separated by gravity settling, thus further promoting use of the biomass and nutrients recovery.

Heavy metal pollution is a serious environmental concern today. The use of biological materials such as bacteria, yeast, algae and fungi has shown promising and cost-effective results in the removal of heavy metals from high-volume and low-concentration wastewaters via biosorption. A study from Australia in 2014 showed that biosorption with macroalgae may be a promising technology for the bioremediation of industrial effluents. The biosorbents were produced from the freshwater macroalga *Oedogonium* sp. (Chlorophyta), which is native to the industrial site from which the effluent was sourced and which has been intensively cultivated to provide a feedstock for biosorbents. The biomass of *Oedogonium* proved to be an effective substrate for the production of biosorbents to remediate both metals and metalloids from a complex industrial effluent (Kidgell *et al*., 2014).

7.6 CONSTRUCTED WETLANDS

Constructed wetlands can be another treatment option for industrial wastewater that is used in, for example, leather tannery wastewater (Lofrano *et al*., 2013). The three main components of constructed wetlands are substrate, plants and microbes; however, the possible removal mechanisms involved are still not fully elucidated. The plants should be carefully chosen according to the wastewater and the toxic components. The application of constructed wetlands provides an alternative method for removing pharmaceutical contaminants from wastewater or as a polishing mechanism. There is a general consensus among many researchers that constructed wetlands hold great potential for use as an alternative secondary wastewater treatment system or as a wastewater polishing treatment system for the removal of pharmaceuticals, but relevant reported studies are scarce and are not conclusive in their findings (Li *et al*., 2014).

7.7 OTHER TECHNIQUES

Another interesting strategy for petroleum hydrocarbon degradation is the symbiotic collaboration between plants and their associated microorganisms. This symbiotic collaboration has proved advantageous for remediating soil contaminated with petroleum hydrocarbons in terms of overall cost and success rates for *in situ* implementation in a diversity of environments. However, in a practical and applied sense, biological unknowns still remain that present challenges for applying these technologies in the industry (Gkorezis *et al*., 2016). Recently, two types of yeast have been enriched and isolated from industrial refinery wastewater. These strains were observed for their ability to utilise several classes of petroleum hydrocarbon substrates, such as *n*-alkanes and aromatic hydrocarbons as a sole carbon source. The strains were found to be members of the genera *Candida* and *Trichosporon*. Both could be very useful for the bioremediation process and for decreasing petroleum pollution in wastewater contaminated with petroleum hydrocarbons (Gargouri *et al*., 2015).

7.8 FUTURE TRENDS

Several technologies exist for the remediation of industrial wastewater. Optimisation of anaerobic pretreatment steps in a wide range of industries for energy generation and cost-efficiency is an area of great interest. The combination of different treatment processes should be considered where the effluent does not meet discharge levels. Research should focus on the optimisation of removal of pharmaceuticals by, for example, MBBR technology, as their removal potential still depends on the type of pharmaceutical. The reuse of clean effluents should be promoted for optimal water usage. Microalgae cultivation has a high potential for industrial wastewater remediation and more research is needed. Research and development in the specific biological degradation processes is of key importance for these types of optimisation. There is great potential to apply novel molecular methods to investigate the microbial composition of treatment systems to understand better their identity, function and to develop control measures.

8 CONCLUSION

Ground-breaking advances in microbial ecology, including the continuous development of cutting-edge methods and, at the same time, the recognition of the need for new solutions to water-related problems, strongly call for an intensified collaboration between microbial ecologists and water professionals to foster innovation in water and wastewater treatment processes.

Translation of current knowledge towards industrial application for process design or optimisation has just started and the potential impact that can be realised with the new knowledge through microbial ecology is huge. However, we still need more fundamental knowledge on microbial communities and the scientific focus needs to be on the identity, physiology, ecology and population dynamics of relevant microbial populations (including viruses, bacteria, archaea and higher organisms). Many of the tools, concepts, theories and challenges of the work that are common to all engineered biological water treatment processes can be combined and employed, allowing novel insights and practical applications. Furthermore, novel technologies will soon allow high-throughput, cheap and reliable identification of microbes in any system, so surveillance and control of drinking water, receiving waters and various technical systems are now within our reach.

This report summarises cases where the theories and findings from microbial ecology are directly used in the areas of public health and sanitation, wastewater management, resource recovery and industrial wastewater. The cases also illustrate that there is fantastic untapped potential through a stronger collaboration among IWA and ISME members, and we hope many readers are inspired to do so!

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