Microorganisms are key players in the development of circular economy in wastewater handling. The understanding of their identity, physiology and ecology is essential for informed optimization and control of these systems.

Circular economy in wastewater handling

In the last few decades, the treatment of wastewater from cities and industries in Denmark has primarily been to remove unwanted pollutants and production of clean water using wastewater treatment plants (WWTP). However, within the last couple of years, several large water utilities have initiated an exciting and sustainable development: they want to develop their treatment plants so they can become a part of the circular economy, where energy production and the recovery of resources become integrated into the removal of pollutants and production of clean water (Figure 1). Several WWTPs are now energy-neutral or even net energy producers, and they do not only produce enough electricity to run the plant, they also send electricity to the common grid.

In addition, recovery of phosphorus (P) takes place, so far at three Danish WWTPs and more are coming. P is typically recovered as struvite from the digester effluent, and the dewatered digester sludge with high P content and other nutrients is used in agriculture. In the future, high-value products such as chemicals and bioplastics may also be produced.

This development is not only taking place in Denmark, but we are in many ways in the forefront in research and implementation. Microorganisms are key working horses in this new development as they carry out most of the processes. At the Center for Microbial Communities at Aalborg University we are currently establishing a better understanding of these microbial communities in order to support the development of a circular economy in wastewater handling. This article briefly highlights some of the approaches, results, and challenges.

Microbial communities are key players

Microorganisms are key players in the development of a circular economy in wastewater handling. Today, almost all wastewater is purified of carbon (C), nitrogen (N), phosphorus (P), micropollutants, and pathogens by the activated sludge process. Activated sludge consists of aggregates ("flocs") of microorganisms that carry out the processes and are separated from the purified water in settling tanks before discharge.

A biogas reactor (a digester) forms an integrated part of most larger WWTPs producing biogas from pre-settled incoming wastewater along with surplus sludge from the activated sludge process. Methane is formed through a complicated food web of microorganisms. In future process design, other types of organic waste, such as food waste and manure, may also be included for energy production (Figure 1).

The future challenges include optimization and troubleshooting of existing process.
systems, e.g., minimizing the foot print, adding chemical and energy inputs, and improvement of operational stability or finding new approaches and solutions. Fundamental to this is an improved understanding of the identity of the microbes involved, their physiology, ecology, and ways to manipulate them for desired functions.

Most microorganisms are “microbial dark matter”

The recent revolution in DNA sequencing technologies has made it possible to study the microbial communities in WWTPs and digesters in great details by culture-independent techniques. Interestingly, although there exist thousands of different microbial species in each WWTP, most are present in very low abundance and likely unimportant, and there seems to exist a core community with only 100-200 abundant microbes (each > approx. 0.1% relative abundance) (1). They are commonly present across most Danish WWTPs and most likely also worldwide. However, when we take a closer look at these microbes, most of them are uncultured and not closely related to known microorganisms. Nothing is known about their physiology or their function in the plants. Such microbes are collectively called “Microbial dark matter”, and it is a great challenge to learn more about these process-critical microbes.

Illumination of the microbial dark matter

Microbial dark matter can be studied by the approach illustrated in Figure 2. The identities of the microbes in a large number of wastewater treatment systems are surveyed. This includes incoming wastewater, activated sludge, mesophilic and thermophilic digesters. We learn about the most abundant core species, often just characterized by a short piece of DNA. We visualize those of interest by fluorescent markers and microscopy (fluorescence in situ hybridization, FISH) to look for morphology and localization in the aggregates (Figure 3).

Subsequently, we try to obtain the entire genome of the individual organisms by “metagenomics”. By extracting all DNA from the samples, sequencing this, and applying sophisticated bioinformatics tools, it is often possible to extract the genomes from key microbes (2,3). On the basis of these genomes, we can reconstruct aspects of their physiology, and establish a metabolic model that will provide a hypothesis for their function in the systems. This can be verified by different in situ studies, e.g., gene expression under specific conditions. Finally, full-scale experiments can be performed to study the process-critical factors that control their presence and activity. This understanding may lead to potential control and optimization of the systems.

What have we learned?

Many microbes involved in nitrogen removal (nitrification, denitrification, and the anammox process) are now fairly well understood, although surprises, such as the discovery of the comammox organisms that oxidize ammonium completely to nitrate, still occur (3,6). Several microbes involved in enhanced biological phosphorus removal, which is essential for a good recovery of P, have now been described. However, it is still uncertain which species are most important in full-scale plants (6).

Likewise, a serious problem in the daily operation in Denmark, and rest of the world, is poor settling of flocs or foam formation due to overgrowth of filamentous microorganisms (6), see Figure 3. Many species have now been identified and characterized so they can be controlled, but some still lack basic characterization and no control strategies exist. However, it is clear that the uncharacterized majority of microbes in WWTPs may play important and so far unknown roles, which need to be revealed.

Digesters at WWTPs are even less well understood (4). Many digesters suffer from low gas yield, low stability, foaming events, and other operational problems that are likely tightly connected to the microbial communities,
so a better understanding is needed to carry out informed control or manipulations of the digester communities.

We have developed the online database and public resource MiDAS (5) in order to collect all relevant knowledge about the microbes in wastewater, activated sludge, and digesters and make this available to everyone working in the field. It also includes a proposal for using a unified taxonomy that will improve the possibility of comparing results from different studies. This is a problem today, where the application of various public databases in different studies may result in different “identities” of the same microbe (5).

**Perspectives**

The fantastic development of the methods for studying the uncultured dark matter provides exciting new possibilities of studying and understanding microbial communities to form a circular economy. It will soon be possible to obtain complete genomes of all important microbes in the systems, including eukaryotes, and learn about their physiology and ecology. This will give us the next level of understanding, helping to establish selective principles for control of specific populations and management of the community. Furthermore, “online” surveillance and possibly control of the microbial communities will be possible. Novel DNA sequencing technologies, e.g., Nanopore MinION can be used on site that can identify the complete community within minutes or hours. It is still uncertain how such data can be applied for informed control of the engineered systems, but with an increased research effort across the world, a build-up of experience and much better understanding of the microbes’ functions will be build up, and hopefully the results will be made accessible to the community for practical use, e.g., through MiDAS or other open resources.

**References**