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INTRODUCTION

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Wastewater treatment forms a crucial link in the services that the sanitation sector delivers to society. For centuries, sanitation largely consisted of transporting fresh, clean water to the cities, and using this water to transport the waste out of the city and discharge it into the natural environment. However, with the increase in human populations in cities as a result of the industrial revolution in the 19th century, this could no longer be maintained. The occurrence of epidemic diseases facilitated the development of wastewater treatment facilities and their implementation since the early 20th century. This development has been largely an empirical activity with theoretical approaches following experimental observations (Figure 1.1).

Figure 1.1 Noyes Laboratory on the campus of the University of Illinois in Urbana was arguably the most important in promoting research in wastewater in the early 20th century (photo: University of Illinois, 1902).

The discovery and development of activated sludge technology (described in detail in Jenkins and Wanner, 2014) was crucial as it triggered the rapid development and application of various analytical and experimental methods. Experimental work in the Lawrence Experimental Station in Massachusetts, USA, which at that time (1912) was a unique facility aimed at the experimental verification of different possible wastewater treatment procedures, inspired Gilbert Fowler to request Edward Ardern and William Lockett to repeat the experiments with wastewater aeration in the UK that he had seen in the USA. In 1913 and 1914 Lockett and Ardern carried out lab-scale experiments at the Manchester - Davyhulme wastewater treatment plant (Figure 1.2). Glass bottles were used to represent lab-scale aeration basins 'fed' by sewage from different districts of Manchester. Contrary to the experiments that Fowler saw in Massachusetts, in the Manchester aeration tests the sediment that remained after decantation was left in the bottle and a new dose of sewage was added to the sediment for the next batch. Lockett and Ardern soon found that the amount of the sediment increased with the increasing number of batches. At the same time the aeration time necessary for 'full oxidation' of sewage (full oxidation was a term used to describe the removal of degradable organics and for complete nitrification) was reduced. By using this technique of repeated batch aeration with the sediment remaining in the bottle, Lockett and Ardern were able to shorten the required aeration time for 'full oxidation' from a few weeks to less than one day, which made the process technically

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feasible. The sediment formed during the aeration of sewage was called activated sludge due to its appearance and activity. Lockett and Ardern published their results in a famous series of three papers (Ardern and Lockett 1914a, 1914b, 1915). This was the 'birth' of activated sludge, which is today the workhorse of wastewater treatment and the most widely applied sewage treatment technology in the world.

Figure 1.2 The Davyhulme Sewage Works Laboratory, where the activated sludge process was developed in the early 20th century (photo: United Utilities).

Wastewater engineering is a profession that is extremely experiment-based, and therefore it has always had the need to develop and standardise methods. This seemingly simple activity is strongly hampered by two factors, namely: (*i*) wastewater engineering is a typical interdisciplinary activity where chemical engineers, civil engineers, microbiologists and chemists interact to develop and understand the processes; the challenge here is to integrate methods and approaches from these disciplines, and, (*ii*) in addition, wastewater and its treatment processes are by their nature difficult to define with exactitude. It is for instance virtually impossible to measure all the individual compounds in the wastewater itself. Identifying all the relevant microorganisms in the processes has long been impossible and is still a complicated challenge. Defining all the potentially occurring chemical conversions is, due to the myriad of chemicals present, again an almost impossible task.

Due to the undefined nature of the experimental system, research has tended to progress slowly and it heavily depends on standardised methods that may not be exact but, when used in a standardised way, are very helpful and useful to compare experimental results. Examples are the commonly used chemical or biological oxygen demand tests. The iconic 'Standard Methods for the Examination of Water and Wastewater' (APHA *et al.,* 2012, Figure 1.3) has for generations of sanitary engineers been the resource for analysing their experimental systems and full-scale operations. These methods focus heavily on the chemical characterization and measurement of specific microorganisms.

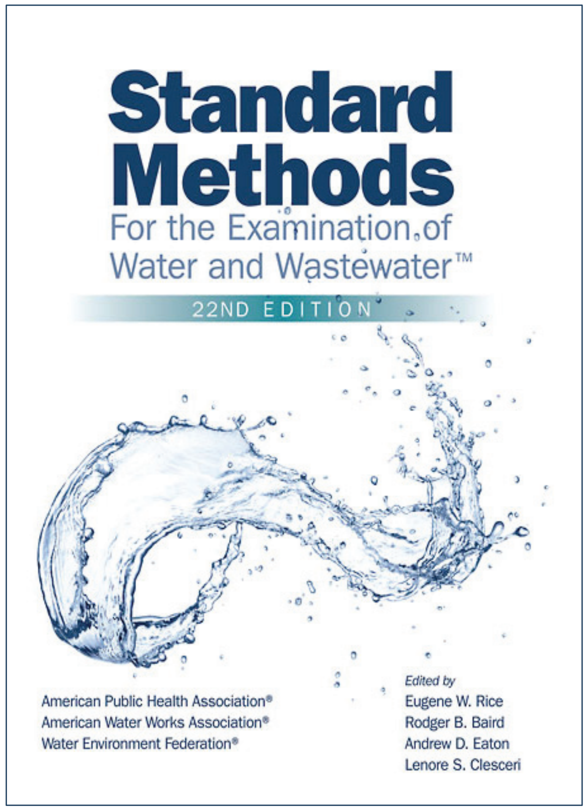

Figure 1.3 The Standard Methods for the Examination of Water and Wastewater. The first edition appeared in 1905 (image: APHA *et al.,* 2012).

Societal demands on the efficiency of wastewater treatment plants have advanced, moving from public health protection to water resources and environmental protection and nowadays to integrated resource and energy recovery. Therefore the need to accurately characterize the microbial processes in the wastewater treatment processes has increased over recent decades. Certainly, it is a challenge to develop standardized methods for experimental work that can be easily repeated in different laboratories. In many cases, the exact handling is important, but it is not easy to be written down in a practical protocol.

Therefore, to avoid these problems, it was decided to develop not only a book describing all the experimental methods but also a video catalogue with the methods described in this book actually being demonstrated in the laboratory. This book and its associated video-based material are designed to support the research and development field with a manual for characterizing the biological processes in wastewater treatment. The editors have decided in this first edition of the 'Experimental methods in wastewater treatment' book to focus on the activated sludge process since this is worldwide by far the most applied technology. Nevertheless, most of the methods presented in this book can also be applicable to biofilm-based technologies or anaerobic digestion processes.

The decision to focus on experimental methods related to the activated sludge process has resulted in seven chapters describing the key experimental methods. The content and focus of these chapters are summarised in Table 1.1. Activated sludge consists of a myriad of microorganisms, converting a range of important compounds (organic matter, oxygen, nitrogen and phosphate compounds). The first three chapters focus on characterizing the conversion capacities of the microbial communities for the major microbial processes. A distinction has been made between full liquid-phase-based methods and methods where the conversion are characterized by measuring the respiration of the organisms, usually gas-phase measurements. Since there is an increasing focus on and interest in assessing the environmental impact of wastewater treatment plants, a separate chapter has been added for measuring greenhouse gas emissions from wastewater treatment plants. These chapters are followed by a chapter describing data handling techniques. Measurements often, certainly from full-scale or pilot plants, have relatively large uncertainties. With adequate data handling techniques the measurements can be used to derive associated (difficult to directly measure) process data or to minimise their uncertainty.

Activated sludge processes mainly depend on settling of the flocculent sludge to separate the biomass from the cleaned wastewater. This is often the Achilles heel of the treatment process and a key factor in the process design. One chapter is therefore devoted to characterization of the sludge settling properties.

As said earlier, microorganisms are the workhorses in the activated sludge process. Therefore the microscope is unavoidably the main technique to observe them directly, not only for individual organisms but also for the floc morphology related to settling characteristics. For a long time the microscope has been the main method of choice when observing which bacteria are present in activated sludge. However, although very helpful, it cannot show the full complexity of the microbial community. The last decade's advance in molecular DNA-based techniques has revolutionized the way one can observe microorganisms. These generic novel methods are described in the final chapter of this book.

Within the chapters the authors have tried to describe especially those methods that are experimentally complex and not standard analytical procedures. Therefore, standard analytical methods for e.g. organic matter, ammonium, phosphate etc. are not described in detail. On the other hand, it was also decided to include some analytical techniques recently developed and/or improved that are becoming frequently used but are scattered across scientific literature (e.g. glycogen and poly-hydroxy-alkanoates determination). In addition, methods that could be of academic interest but currently have limited practical application have not been included in detail in the text.

In terms of symbols and notation, an attempt has been made to standardize them as much as possible. While this was achieved at the chapter level, full standardization was not possible across all the chapters due to their diverse nature and heterogeneity of items as well as lack of global agreement on the use of symbols and notations, although the most common guidelines were quite closely followed (e.g. Corominas *et al.,* 2010).

The book is conceptualized so as to satisfy users with high demands who are able to handle complex analytical and experimental equipment. However, the content is equally suited to the requirements of less advanced laboratories and less experienced experimenters; in particular, the complementary, freely available video materials address the execution of experiments in more challenging environments, such as those usually prevailing in most less developed countries.

"To measure is to know."
Lord Kelvin

Table 1.1 A simplified overview of the experimental methods presented in the book per process of interest.

Process	Chapter							
	Introduction	Activated sludge activity tests	Respirometry	Off-gas emission tests	Data handling and parameter estimation	Settling tests	Microscopy	Molecular methods
Organic matter removal	Overview and rationale to experimental methods	Kinetics	Biochemical oxygen demand (BOD) Short-term biochemical oxygen demand Wastewater characterization and fractionation Biomass characterization Toxicity and inhibition	Sampling methods for nitrogen GHG emissions Methods for off-gas measurements Aqueous N_2O and CH_4 concentration determination methods Gas measurement methods in open tanks	Data handling and validation Parameter estimation Uncertainty analysis Local sensitivity analysis and identifiability analysis	Settling velocity distributions in primary settling tanks Sludge settleability in secondary settling tanks Flocculation properties Settling behaviour of granular sludge	Light microscopy Confocal microscopy Morphological investigations Staining techniques Fluorescence in situ Hybridization (FISH) Combined staining techniques	DNA extraction Real-time quantitative PCR Amplicon sequencing
Nitrification		AOO and NOO activity Kinetics Stoichiometry	Wastewater characterization and fractionation Biomass characterization AOO and NOO activity Toxicity and inhibition Kinetics Stoichiometry					
Denitrification		Denitrification over NO_2 and NO_3 Denitrification on RBCOD and SBCOD Stoichiometry Kinetics	Denitrification over NO_2 and NO_3 Toxicity and inhibition Stoichiometry Kinetics					
Anammox		AMX activity Kinetics Stoichiometry						
EBPR		PAO, GAO, and DPAO activity Kinetics Stoichiometry	Aerobic kinetics and stoichiometry Toxicity and inhibition					
Anaerobic treatment		SRB activity Kinetics Stoichiometry	Specific methanogenic activity Biomethane potential Toxicity and inhibition Kinetics Stoichiometry					
Settling								

AMX Anammox organisms
AOO Ammonium oxidizing organisms
CH_4 Methane
DNA Deoxyribonucleic acid
DPAO Denitrifying poly-phosphate accumulating organisms
EBPR Enhanced biological phosphorus removal
FISH Fluorescence in situ hybridization
GAO Glycogen accumulating organisms
GHG Greenhouse gas emissions
N_2O Nitrous oxide
NO_2 Nitrite
NO_3 Nitrate
NOO Nitrite oxidizing organisms
PAO Poly-phosphate accumulating organisms
PCR Polymerase chain reaction
RBCOD Readily biodegradable COD also known as readily biodegradable organics
SBCOD Slowly biodegradable COD also known as slowly biodegradable organics
SRB Sulphate reducing bacteria or SRO Sulphate reducing organism

Figure 1.4 The mission of UNESCO-IHE is to contribute to the education and training of professionals, to expand the knowledge base through research and to build the capacity of sector organizations, knowledge centres and other institutions active in the fields of water, the environment and infrastructure in developing countries and countries in transition. The photos depict the illustrative example of the Institute's latest project in Cuba where the laboratory of the Instituto de Investigaciones para la Industria Alimenticia (IIIA) in Havana has been equipped with new state-of-the-art technology and where the local staff has been trained on how to operate the equipment and prepare and carry out experimental work (photo: Brdjanovic, 2015).

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The section on activated sludge historical development presented in this chapter is adapted from Jenkins and Wanner (2014).