Chapter 1
Introduction

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Considering the large number of advances in the area of biological processes for wastewater treatment, to approach them all in a single book would be a rather daunting task. Therefore, we decided it would be best to focus on processes and techniques whose application appears to be spreading and which could alter, in the near future, the scenario of wastewater treatment.

The subject of biological wastewater treatment never attained the status of consolidated knowledge. In fact, new approaches frequently appear which often render established concepts obsolete and allow the development of new technologies for the treatment of different types of wastewater. This is exemplified by the advancement of knowledge related to anaerobic treatment, starting in the late 1970s, as well as more recent findings which verified the versatility of nitrifying bacteria and new forms of nitrogen transformation and also a deepening of our understanding of microbiology and biochemical processes for the removal of phosphorus and sulfur.

These findings and the advancement of knowledge have led to important technological developments. In the case of anaerobic treatment, there has been a remarkable increase in the implementation of reactors which promote microbial retention (UASB, IC, EGSB), and in the case of nitrogen removal, different processes have been proposed including Sharon-Anammox, which has found an interesting niche for its application. Insights regarding the removal of phosphorous and sulfur have provided a new perspective in relation to wastewater treatment, which aims not only at the removal but also the recovery of these elements for reuse, since they are important chemical consumables.

As previously mentioned, in this book we approach some selected themes which we consider to be extremely relevant to the current context of biological treatment processes, these being membrane biofilm reactors (MBR), moving bed biofilm

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reactors (MBBR), new biological nitrogen removal processes, aerobic granulation technology, and molecular biology techniques applied to the study of microbial diversity.

We consider that by approaching these themes we will provide our readers with an insight into the current trends in relation to treatment processes. The themes discussed should guide the conception of new treatment plants, which must fulfill the growing demands for treated effluent quality and confront the current challenges to not only remove macropollutants but also the so-called emerging pollutants present in wastewaters in concentrations of micrograms or nanograms per liter.

In this introduction, we would like to highlight the evolution of effluent treatment processes, which has occurred as a result of targets to be reached. Over many years, the treatment processes were simply aimed at removing particulate material in suspension (suspended solids—SS) or biodegradable organic matter (biochemical oxygen demand—BOD). Later, however, the oxygen demand associated with the oxidation of ammonium nitrogen gained attention as an important factor, and, consequently, nitrification was considered in the conception of treatment plants.

Over time, the need to remove not only ammonium nitrogen but also inorganic oxidized forms of this element (nitrite and nitrate), along with phosphorous, was verified, since these nutrients have adverse effects on the receiving water bodies, one of which is the intensification of the eutrophication of aquatic systems. This led to the development of different processes which combine anoxic and oxic environments in different tanks (Bardenpho process and others) or in different periods in the same tank (sequencing batch reactor).

More recently, treatment processes have had to be adjusted to meet the demands for the removal of emerging pollutants and the production of water for reuse. At the same time, as mentioned above, there has been an interest in the recovery of certain pollutants in the form of industrial consumables, as in the case of phosphorus and sulfur, or even in the production of other consumables such as organic acids and hydrogen.

In the current scenario, particularly in developed countries, there are strict demands regarding effluent treatment processes, and in this context it is evident that the combination of biological and physicochemical processes is imposed as a requirement in the conception of modern wastewater treatment plants.

Figure 1.1 shows the trends in the evolution of wastewater treatment processes. The physicochemical processes are also included in this figure. It should be considered that certain processes can still be improved; however, in the case of those which are well established in the market, such as activated sludge, the improvements must be carried out gradually over time. As new processes appear, such as membrane reactors (MBRs) or Anammox, a wave of innovation follows; however, the consistency and applicability of a new approach will only be determined over time. The horizontal bars representing each process in Fig. 1.1 are closed at their right extremity. This means that the process reached a high degree of maturity in a certain period of time, although improvement could and should still occur.

In order to face the challenges associated with removing emerging pollutants or obtaining water for reuse in applications which require high quality, the combining

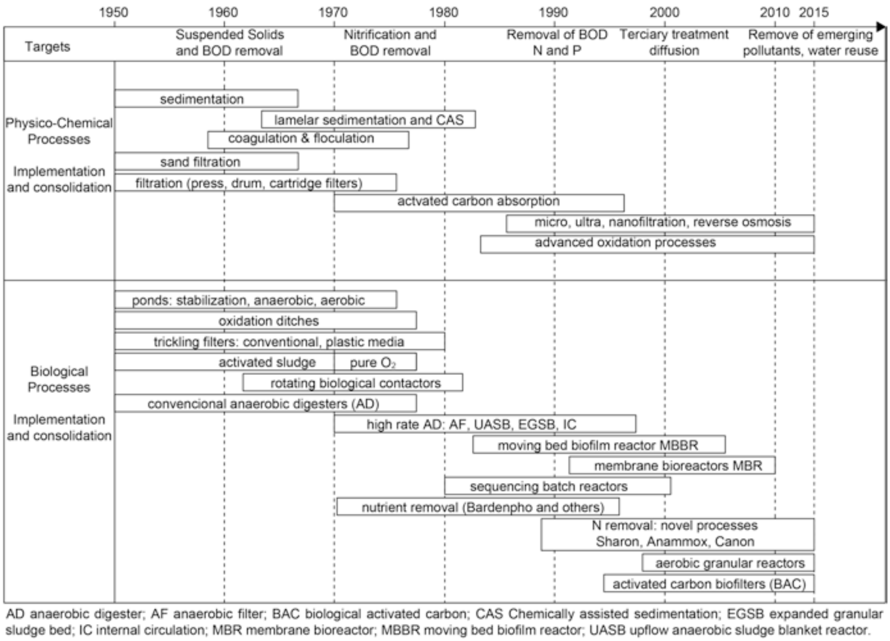

Fig. 1.1 Trends in the evolution of wastewater treatment processes

of processes is imperative. In this regard, the combination of biological processes, membrane filtration, advanced oxidation processes (AOPs), and adsorption onto activated carbon, in different sequential configurations, has been adopted in several countries by companies and local governments.

The appropriate conception of a process is a fundamental, but not the only, step to ensure that the treatment aims are achieved. The monitoring and control of the process are essential for operational success. A treatment process needs to be continuously monitored; however, unfortunately this aspect does not, in general, receive due attention. Although several types of sensors and measuring instruments are available in the market, the degree of instrumentation and automation of many wastewater treatment plants is still deficient.

Although there are sensors and other measuring equipment available for the online or offline monitoring of several variables of interest (pH, temperature, dissolved oxygen, redox potential, flow rate, methane production, ammonia, nitrite, nitrate, phosphorus, and others), challenges remain regarding the characteristics of the main agent of biological processes: the microbial community.

A useful, albeit extremely limited, parameter used to determine the quantity or concentration of microbial agents present in a given process is the volatile suspended solid (VSS) content. The number of design and operational parameters of biological treatment systems which are associated with this highly inconsistent indicator is impressive. Specific removal rates for nitrogen, phosphorus, and organic matter are commonly expressed in specific terms, and the variable used to quantify the biomass of interest is generally volatile solids.

It can be argued that for a given biological sludge sample, it is very difficult to quantitatively determine the presence of each microbial group. For instance, in a sludge sample taken from a reactor which promotes the removal of BOD and nutrients, it is difficult to evaluate the percentages of some microbial groups of interest, such as heterotrophic, ammonia-oxidizing, nitrite-oxidizing, and phosphate-accumulating bacteria. The same applies to a sample taken from an anaerobic reactor regarding methanogenic, homoacetogenic, acetogenic, and sulfate-reducing organisms.

An additional factor which renders the challenge even more complex is that besides the quantification it is important to obtain information regarding the physiological state of the microorganisms of these groups. In relation to obtaining more detailed information on the microbial communities typically present in wastewater treatment processes, the complexity of the problem is enormous and the challenges immense.

Nevertheless, science and technology have contributed to broadening our knowledge regarding these complex microbial communities. This has been achieved due to the development of different molecular techniques and advances on microscopy. In this book, a chapter specifically on molecular techniques has been included. As an introductory and illustrative aid, some of these techniques are detailed in Fig. 1.2. The evolution of most of these techniques was based on fundamental knowledge regarding DNA and RNA molecules obtained during the second half of the twentieth century. In particular, the development of the polymerase chain reaction (PCR) and later the use of thermostable DNA polymerase enabled researchers to gain an insight into microbial diversity. The area known as metagenomics relates to the knowledge which permits the functional analysis of the nucleotide sequences of the collective microbial genomes present in a certain environmental sample. Thus, it allows studies to be carried out on the microbial ecology and an exploration of the diversity of complex communities such as those associated with biological treatment processes.

Although the PCR technique allows the amplification of the number of DNA fragments of a given sample, other operations are still required to further elucidate the diversity. It is necessary to separate the fragments, identify them, and compare the sequences obtained with databanks available on the Internet. The technique of denaturing gradient gel electrophoresis (DGGE) developed by Fischer and Lerman (1983) is the most widely used in the separation stage and in the obtainment of molecular fingerprints. Muyzer et al. (1993) were pioneers in the use of this technique applied to environmental samples.

As shown in Fig. 1.2, several techniques have been developed and refined to study the microbial ecology and diversity, such as respiratory quinone profile (RQP), fluorescent in situ hybridization (FISH), and microautoradiography (MAR). It should be noted that the FISH technique is currently widely employed in academic studies on the microbial groups present in biological treatment systems. This method has become popular due to the development and commercialization of specific

1970–1980	Establishment of the basic principle of PCR (Polymerase Chain Reaction) technique. Kleppe *et alii* (1971)
1980–1990	Utilization of DGGE (Denaturing Gel Gradient Electrophoresis) to separate DNA fragments. Fisher and Lerman (1983)
	Amplification, detection and/or cloning nucleic acid sequences - US patent. Mullis *et alii* (1987)
	Detection of polymorphims of human DNA by gel electrophoresis as single-strand conformation polymorphisms (SSCP). Orita *et alii* (1989)
1990–2000	Development of ARDRA (Amplified Ribosomal DNA Restriction Analysis). Veneechoutte *et alii* (1992)
	Development of FISH (Flouorescent *in-situ* Hybridization). Lengauer *et alii* (1992)
	DGGE utilization for profiling complex microbial populations. Muyzer *et alii* (1993)
	Genome analysis with DNA microarrays. Schena *et alii* (1995)
	Utilization of RQP (Respiratory Quinone Profile) for microbiota characterization. Hu *et alii* (1997)
	Pyrosequencing. Ronaghi *et alii* (1996, 1998)
	Development of TRFLP (Terminal Restriction Fragment Length Polymorphism) technique. Liu *et alii* (1997)
	Analysis of microbial diversity by using RISA (Ribosomal Intergenic Spacer Analysis) method. Borneman and Triplett (1997)
	Combination of FISH and MAR (Micro Autoradiography) for microbial ecology analysis. Lee *et alii* (1999)
2000–2015	Development of high-throughput next-generation sequencers. Shendure *et alii* (2005)
	Development and commercialization of high-throughput sequencers by different companies (2005-2015)
	Nanopore DNA sequencing. Stoddart *et alii* (2009)
	Utilization of Gene FISH technique for linking gene presence and cell identity in environmental microorganisms. Kawakami *et alii* (2010)
	DNA sequencing using electrical tunnelling currents. Di Ventra (2013)

Fig. 1.2 Evolution of different molecular techniques and microscopy applied in the study of microbial diversity

probes for different groups of interest. Its combination with the MAR technique provides a powerful tool for the study of microbial communities.

The use of the abovementioned techniques is still restricted to research of an academic nature or industrial R&D. However, their use as a monitoring and control tool for industrial-scale processes will certainly become a reality in the near future.

New processes, the improvement of established processes, and new tools for the monitoring and control of the variables and parameters of interest are appearing in the area of effluent treatment. These will be of great value in terms of obtaining treated effluent with the best possible quality.

This book is committed to this vision of the future.

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