The development in biological wastewater treatment over the last 50 years

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ABSTRACT

The paper summarizes the development in the understanding and practical application of the activated sludge process over the last 50 years. Since its invention, the activated sludge process has been a big challenge to design engineers. Traditionally, the technology was covered by sanitary engineers. However, with the development in the understanding of activated sludge process principles, further progress was not possible without knowledge of reaction kinetics and reactor theory. The shift from BOD removal only to combined removal of organic pollution, nitrogen and phosphorus required a chemical engineering approach with outputs of activated sludge microbiology and microbial ecology. Molecular biology enabled more accurate identification of important activated sludge microorganisms. The development in activated sludge process also required more efficient activated sludge separation and thickening. The paper describes the development from secondary clarifiers to membrane separation. Increasing water stress around the globe has also changed the main wastewater paradigm from wastewater treatment and safe discharge to safe reuse.

Key words | activated sludge, nitrogen, organic pollution, phosphorus, separation, water reuse

HIGHLIGHTS

- Wastewater treatment since the 1970s when the IWA Specialist Group on the Design, Operation and Costs of Large Wastewater Treatment Plants was established.
- Reactor engineering and kinetics.
- Biological nutrient removal and application of microbial ecology and molecular biology.
- Development in activated sludge mathematical modelling.
- Development in activated sludge separation and water reuse.

ACTIVATED SLUDGE PROCESS

Transition from sanitary to chemical engineering approach

The beginning of the 1970s, when the SG LWWTP (the specialist group on the Design, Operation and Costs of Large Wastewater Treatment Plants) came into being, was the period when the activated sludge process definitely became the dominant biological process in municipal wastewater treatment plants. The activated sludge process replaced biological filtration, which was at that time used less and less for sanitation of towns in both major modifications; that is, as soil filtration or trickling filters.

A typical activated sludge plant at the turn of the 1960s and 1970s was designed solely for BOD₅ removal or a
combination of BOD₅ removal with nitrification. The first modification of pre-denitrification followed by nitrification was already known at that time (Ludzack & Ettinger 1962). The change from carbon removal to combined carbon and nitrogen removal also required a deeper understanding of the processes occurring in activated sludge and of the rules governing the behavior of activated sludge systems. Although the inventors of the activated sludge process were chemists and formulated basic principles of its design and operation (Ardern & Lockett 1914a, 1914b, 1915), the further development of the process was mainly affected by a civil/sanitary engineering approach. However, the introduction of the nitrification process required the application of reaction kinetics and reactors theory. Fortunately, the basic knowledge on the design and behavior of chemical reactors was already available at that time, especially thanks to the pioneering work of Levenspiel (1962, 1972). The reaction kinetics was also applied to biological treatment to explain the difference in cultivation requirements of organotrophic and chemolithotrophic nitrification bacteria and to define the basic technological parameter controlling the conditions in their mixed culture (Jenkins & Garrison 1968). The theory of the hydraulic regime of chemical reactors was also applied in the explanation of the competition between major morphological groups of activated sludge organotrophic microorganisms; that is, floc-formers and filaments, by the kinetic selection theory (Chudoba et al. 1973a, 1973b).

The end of the 1960s and beginning of the 1970s was the period when the further development of the activated sludge process started moving from sanitary (civil) to chemical engineering and this combination gave rise to a new discipline, called environmental engineering today. The practical application of kinetic selection theory in the 1970s (aeration basins with plug-flow hydraulic regime or aeration tanks with contact zones (selectors)) helped not only to control the excessive growth of certain types of filamentous microorganisms but also contributed to higher metabolic diversity of activated sludge. This was observed and practically used especially by Barnard in South Africa. His findings were employed in the design of the four-stage Bardenpho process, which was later on completed with an anaerobic zone for enhanced biological phosphorus removal (EBPR). The phenomenon of EBPR was already known at that time as phosphate ‘luxury uptake’ (Levin & Shapiro 1965; Milbury et al. 1971) but was used only in phosphorus removal in side-stream processes (e.g. Phostrip). The works of Barnard (Barnard 1973, 1974, 1975) triggered the development of many modifications of the activated sludge process for biological removal of nitrogen and phosphorus. A detailed survey of these practical applications can be found, for example, in Cooper & Downing (1998) or in chapters 3, 5 and 6 of the IWA jubilee publication Activated Sludge – 100 Years and Counting (Barnard & Comeau 2014; Khunjar et al. 2014; Stensel & Makinia 2014). The activated sludge process with nutrient removal in the mainstream was operated not only with physically separated nitrification and denitrification zones. The phenomenon of so-called simultaneous nitrification and denitrification was observed (Matsché 1972; Kayser & Ermel 1984) even in unbaffled aeration tanks.

Application of microbial ecology in the further development of activated sludge process

Growing practical experience from the operation of biological nutrient removal (BNR) activated sludge plants in the 1970s and 1980s indicated certain limits of the chemical engineering approach in explaining observed operational problems like instability of nitrogen and phosphorus removal efficiency and persistent difficulties in activated sludge separation in gravity clarifiers. The extent of problems the engineers had to solve at that time was clearly illustrated at two important IWA conferences organized in September 1987:

- 5th LWWTP conference in Budapest
- Biological Phosphate Removal from Wastewaters, Rome

Some of the papers presented at these conferences described new selection mechanisms (metabolic selection) and factors affecting the composition of activated sludge consortium, which were later studied by a new IWA specialist group established in 1988 under the name ‘Activated Sludge Population Dynamics’, which was renamed in 2009 to ‘Microbial Ecology and Water Engineering’. The main topics covered in the 1980s and 1990s by this new group were explained in two principal publications of the group (Wanner 1995, 1997). The worldwide expansion of the BNR activated sludge process was accompanied by new separation problems (Wanner 1998):

- activated sludge bulking caused by filamentous microorganisms, which were not so widespread in conventional BOD₅ removal plants and which were obviously not so easy to control by kinetic selection
- activated sludge foaming.

Any progress in the understanding of bulking and foaming problems could not be expected without rapid and
reliable identification of causative filamentous microorganisms. Eikelboom (1975) based the first practically usable method of filamentous microorganism identification on their characteristic morphological features and developed a systematic procedure of microscopic examination of activated sludge samples (Eikelboom & van Buijsen 1981). According to this procedure, the organisms are identified to the so-called types. Although the taxonomic position of most types was very unclear and uncertain, the identification by types meant a big breakthrough in the studies of bulking and foaming problems. Biological foams were observed already at the end of the 1960s; however, foams as a massive activated sludge problem are connected with the global application of the BNR activated sludge process (Wanner 1994). The common application of Eikelboom’s identification technique worldwide helped in understanding that certain filamentous types are connected with certain conditions and can cause both or either of the problems (bulking and foaming), which helped very much in development of more successful control strategies (regeneration zones, control of filamentous growth in anaerobic and/or anoxic zones, selective foam removal, etc.). Because of its simplicity and practical value, the identification by types is still used especially in solving urgent separation problems (for instance, Jenkins et al. 2004).

The progress in microbiology and microbial ecology of activated sludge, as summarized for example by Seviour & Blackall (1999), Jenkins et al. (2004) and Seviour & Nielsen (2010), scientifically substantiated the formerly postulated principles of activated sludge bulking and foaming control design. However, the design itself would not be enough to achieve and guarantee good separation properties of activated sludge. It was also very important to bring and explain the knowledge of activated sludge microbiology and microbial ecology to practitioners operating activated sludge plants. In this respect, courses organized for operators played a crucial role in teaching them the proper operational practice. One of the most traditional courses was the course on ‘Operation and control of activated sludge processes using microbiological analysis’ organized by the Italian National Committee of the IWA and Dr Valter Tandoi in the period of 1991 to 2014 (for the course programme see http://www.villaumbra.gov.it/media/operation-and-control-of-activated-sludge-processe). In this way, the operators learnt how to properly operate contact zones, anoxic and anaerobic zones for both biological nutrient removal and for bulking and foaming control, and how to efficiently use remedial methods like chlorination, coagulant and flocculant addition, foam removal methods, etc., in case of serious operational problems.

**Activated sludge mathematical models**

Gradual understanding of activated sludge kinetics and microbial ecology also enabled the development of activated sludge mathematical models, as described in detail by Ekama & Takács (2014). A great impulse in the development was the formation of the IWA (at that time LAWPRC) task group in 1982, which formulated the basic principles of a model that is now known as ASM1 – Activated Sludge Model No. 1 (Henze et al. 1987). The mathematical description of the model is based on a simple matrix describing the processes in which the individual reacting components are consumed or produced. The model operates with various fractions of substrate (readily degradable, slowly degradable, inert) and biomass (e.g. organotrophic and chemolithotrophic fractions). The main processes in the simple ASM1 are aerobic and anoxic growth of organotrophs and their decay, aerobic growth and decay of chemolithotrophs (nitrifiers), hydrolysis of entrapped organics and organic nitrogen, and ammonification of soluble organic nitrogen. The growth of biomass is described by using Monod saturation kinetics (as to the substrate concentration); the differentiation of the growth under different cultivation conditions is also done by means of saturation-type switching functions. The biomass decay is simulated by first-order kinetics (as to the biomass concentration). The form of matrix notation enables a simple mass balance check over the simulated reactor and to quickly see the process rate equations, overall conversion equations for each component and also whether a component is consumed or produced by particular processes. The philosophy of the matrix allows the simple adding of additional components or processes. The ASM1 was soon expanded to ASM2 and ASM2d for mathematical simulation of biological phosphorus removal (ASM2d with phosphate uptake also under anoxic conditions, Bortone et al. 1996). ASM1 and ASM2 models postulated the growth of organotrophs with the balanced growth only, which is not always the case, especially in plug-flow activated sludge systems or systems with a regeneration zone in the stream of the return sludge. Therefore, the ASM3 model also covers the storage and regeneration phenomena. The introduction of storage also allowed more correct simulation of the process of EBPR and thus there are several other modifications of ASM available (Hauduc et al. 2010).

The mathematical models of the activated sludge process are today not only the tools of researchers or designers but can be already directly applied in the on-line control of the activated sludge process (Bartáček &
Wanner 2019). The efficient use of mathematical models in the activated sludge process control requires real-time measurement of basic process parameters.

**Activated sludge in the age of molecular biology**

The identification procedure based on microorganism morphology can be applied only for a limited fraction of the activated sludge population, such as filamentous bacteria or PAOs (polyphosphate-accumulating organisms). But even so, the method says nothing about the taxonomic position of a given organism. For bacteria like nitrifiers without pronounced morphology, it cannot be used at all. Conventional cultivation methods for the identification of activated sludge microorganisms are often misleading because they do not provide the true picture about the most important organisms in the mixed culture, but on the ‘best culturable’ organisms.

The development of molecular methods targeting nucleic acids in the late 1980s and early 1990s finally provided some more specific identification tool. Fluorescence *in situ* hybridization (FISH) uses DNA oligonucleotides and fluorochromes to probe the abundant rRNA molecules in active cells, thereby allowing visualization of whole microbial cells. Amann et al. (1990) optimized the method, making it possible to identify, visualize and quantify bacteria without any isolation procedure. It was possible to differentiate among different phylogenetic groups of microorganisms in any mixed culture by microscopy. The FISH technique was soon used also for the identification of activated sludge microorganisms (Nielsen & McMahon 2014) and is becoming more and more used also in laboratories of companies operating activated sludge plants. Further on, the FISH technique was combined with microautoradiography (MAR) and radiolabeled substrates provided information about the physiology of many probe-defined organisms (Wagner et al. 2006). The recent development in this field is aiming to provide a generalized guide through all important groups of microorganisms in activated sludge in respect to their metabolic functions, morphology, phylogeny and role in activated sludge consortia (Nierychlo et al. 2019).

The application of molecular biology methods improved significantly our knowledge not only of nitrification bacteria and filamentous microorganisms responsible for bulking, but helped us to understand better problems connected with enhanced biological phosphorus removal (EBPR). The instability of EBPR was empirically explained with competition between PAOs (polyphosphate accumulating organisms) and the so-called G-bacteria (Cech et al. 1995) which were later called GAOs (glycogen accumulating organisms). Both PAOs and GAOs compete for readily biodegradable substrates under anaerobic conditions in EBPR activated sludge systems. In the past, these groups of organisms could be distinguished only by using Neisser stain (Jenkins et al. 2004). However, the application of molecular biology methods revealed that both PAOs and GAOs are formed by various different microorganisms with seemingly similar morphology and with different substrate preferences (Blackall et al. 2002; Maszenan et al. 2005; Oehmen et al. 2006).

Similar to EBPR activated sludge systems, the application of molecular biology methods improved our understanding of biological foam formation and practical control methods of foams in activated sludge systems. By using conventional microscopic examination, the foam-forming filaments were identified principally based on their pronounced morphology. This group of foam-forming filaments was identified as the so-called GALOs; that is, *Gordona amarae*-like organisms. However, the application of comparative rRNA sequence analysis clearly demonstrated that morphologically similar organisms identified as GALOs were phylogenetically diverse also with different growth preferences (de los Reyes et al. 1998; Seviour & Nielsen 2010; Nielsen & McMahon 2014). The exact identification of foam-forming filaments makes the foam control measures more targeted, especially in the case of the use of enzymes in anti-foam agents.

**ACTIVATED SLUDGE SEPARATION**

**Secondary clarification**

Activated sludge separation has changed during the last 50 years from separation in rather simple secondary clarifiers via more sophisticated clarifiers with activated sludge flocculation and better thickening up to membrane filtration. A more detailed description of this development can be found in the literature on history of activated sludge process (e.g. Parker et al. 2014; Stensel & Makinia 2014).

A typical secondary clarifier (both circular and rectangular) at the end of the 1960s did not differ very much from a primary clarifier. Both circular and rectangular tanks were built rather shallow (sidewall depth about 2.5 m) with very simple inlet structures whose only role was to dissipate excess kinetic energy and evenly distribute incoming activated sludge mixed liquor. Settled sludge was transported to a sludge hopper by straight scrapers mounted on a
travelling bridge, both in circular and rectangular tanks. Also, the outlet structure was rather simple – the effluent launders were built either with peripheral design or in-board design in about 2/3 of the tank radius and effluent weirs were not protected by scum baffles. The floating biomass was simply escaping to the effluent, and thus the clarifiers were not equipped with scum skimmers. The 1970s started changes in secondary clarifier design resulting from a better understanding of settling and thickening mechanisms. Keinath et al. (1977) introduced the settling flux approach and state point concept to the secondary clarifier design. Parker et al. (1971) studied the importance of activated sludge quality for the efficiency of activated sludge sedimentation and thickening. These studies resulted later on in the concept of flocculators installed in secondary clarifiers (Parker 1983). The gradual understanding of flocculation, sedimentation and thickening processes resulted in typical design features (Albertson 1992; Wanner & Torregrossa 2017):

1. The effectiveness of separation of activated sludge in secondary clarifiers depends on the quality of activated sludge flocs. The principles of controlling good floc structure and sedimentation properties are summarized by Wanner & Jobbágy (2014).
2. Secondary clarifiers consist of four principal construction parts: (i) inlet with energy dissipation and activated sludge flocculation, (ii) outlet structure with mostly peripheral launders; (iii) efficient transportation and removal of settled and thickened activated sludge, (iv) removal of floating biomass – foam skimming.
3. Secondary clarifiers are much deeper than in the past with sidewall depth around 4.0–4.5 m, so that the clarifier (i) can better absorb the flow peaks, (ii) accumulates more activated sludge during the peak flow conditions, (iii) is less sensitive to changes in activated sludge separation properties.
4. The inlet structure is equipped with a flocculation zone where the kinetic energy of the incoming mixed liquor is used for agitation and subsequent flocculation.
5. The removal of settled sludge is more efficient, which reduces the risk of sludge floating and/or secondary phosphate release. In circular clarifiers, spiral blade scrapers are used most commonly, in rectangular clarifiers (endless) chain scrapers are becoming dominant in new or upgraded installations. The blades of chain scrapers act as settled sludge scrapers and floating biomass skimmers at the same time.

6. The weirs of effluent launders are protected by scum baffles from floating biomass leakage to final effluent.
7. The effluent launders are protected from floc release caused by density currents by means of the so-called Stamford baffles (deflectors).
8. Once the effluent launders are equipped with scum baffles, the secondary clarifiers must also be equipped with skimmers and a scum box for collecting floating biomass.
9. The secondary effluent contains residual concentrations of nitrogen and phosphorus, which can support the growth of algae. The effluent launders can be covered or equipped with cleaning brushes to prevent excessive algae growth.

The difference in the progress in secondary clarifier design can be illustrated by clarifiers of the older water line of the Central WWTP of Prague. The original clarifiers were designed and built in the early 1960s. In 1997 the plant was upgraded, and four new clarifiers were added (Figures 1 and 2).

The secondary clarifiers form one system with aeration basin(s) and thus the operation and control of the clarifiers have to be coordinated with the operation of aeration basins (Wanner & Torregrossa 2017).

**Membrane separation**

The secondary clarifiers reached at the turn of the 21st century probably their optimum design and performance based on the development in sedimentation and thickening theory. Even so, secondary clarification is still the limiting factor in
the design of the whole activated sludge system because of several inherent deficiencies of secondary clarifiers:

(i) Gravity sedimentation is an extensive process and secondary clarifiers occupy large built-up areas in wastewater treatment plants.

(ii) The performance of secondary clarifiers is strongly affected by activated sludge separation properties, which are not stable.

(iii) Even with very good activated sludge separation properties, the effluent from secondary clarifiers always contains residual concentrations of suspended solids (unsettleable biomass particles, individual bacterial cells).

(iv) The thickening capacity of clarifiers is limited and thus the concentration of return activated sludge usually does not exceed 10 kg/m³.

All the above factors initiated the research and development in alternative activated sludge separation methods. The most promising method proved to be membrane filtration with pore sizes in the range of microfiltration. The first full-scale municipal MBR installations appeared in Japan and Canada in the early 1990s (Judd 2010). Membrane modules (immersed membranes) are either placed directly into aeration basins or in a separate tank with biomass recycle to the aeration tank. Membranes are made either of plastic or ceramic materials. The most common membrane design is in the form of flat plates (desks) or hollow fibers. The combination of activated sludge process and activated sludge separation by means of membranes is called a membrane bioreactor (MBR). The use of MBRs is now becoming more common, especially in cases when the main advantages and benefits of MBRs can be fully used (Crawford et al. 2014; Park et al. 2015). The main benefits are:

- more flexible and efficient control of activated sludge age
- less dependence on activated sludge separation properties
- smaller built-up area in comparison with gravity sedimentation
- very high quality of effluent (permeate) with no suspended solids and practically disinfected; the permeate of such quality is suitable for water reuse
- concentrated waste activated sludge.

The activated sludge separation by membrane filtration is used especially in the case of water reuse when the permeate can be sold as a valuable product. This justifies higher investment and operation costs of membrane filtration in comparison with secondary clarifiers.

**Granulated activated sludge**

Another way to intensify the process of activated sludge separation is the application of granular sludge. Granular sludge was originally developed for anaerobic reactors but in the 21st century, the activated sludge in the form of granules was developed also for aerobic/anoxic carbon removal, nitrification and phosphorus removal (Giesen et al. 2013; Barnard & Comeau 2014). The granular sludge is mostly used in sequencing batch reactor (SBR) modification of the activated sludge process. The main advantage of the SBR mode with granular sludge is in the very short time needed for efficient separation of activated sludge in the settling phase of the main reactor.

**LARGE WASTEWATER TREATMENT PLANTS AS WATER RECLAMATION FACILITIES**

The perception of wastewater treatment and the main roles of wastewater treatment plants (WWTPs) has significantly changed during the 50 years of the IWA LWWTP SG. The main role of the WWTP was traditionally seen as the safe disposal of treated wastewater. The effluent of municipal WWTPs was just discharged into receiving waters. However, during the last two decades, the water stress in many parts of the world increased to such an extent that people looked for some alternative water resources. Thus, the effluent of municipal WWTPs has been understood in countries suffering from serious water shortages as such an alternative source. The WWTP effluents are suitable for this purpose because:
the amount of wastewater produced in large towns is rather stable, even in periods of long droughts

thanks to the progress in wastewater treatment technology, the quality of municipal WWTP effluents is steadily increasing.

The classical ‘secondary’ biological wastewater treatment can be improved by additional operations and processes of further treatment which can be covered under the terms of:

(i) tertiary treatment: processes like coagulation, removal of residual suspended solids, tertiary precipitation of phosphorus, disinfection

(ii) quaternary treatment: removal of residual specific organic compounds (e.g. pharmaceuticals, hormones, etc.)

The change in the wastewater treatment paradigm is also accompanied by the change in terminology, where the term ‘wastewater’ is replaced by ‘used water’ and ‘wastewater treatment plant’ with ‘water reclamation facility’.

There are many successful examples of municipal wastewater reuse in agriculture, industry or in the water management of large towns (e.g. Lazarova et al. 2015; Eslamian 2015). In the member states of the European Union, the use of recycled water will be facilitated by a new regulation which was accepted in 2020 and is called Regulation of the European Parliament and of the Council on minimum requirements for water reuse. Another great impulse for growing interest in large wastewater treatment plants as reliable water resources was the IWA specialized conference held in June 2019 in Berlin with the name ‘Water Reuse 2019: Overcoming Water Stress by Water Reclamation and Reuse’, where many successful case histories of water reuse in all sectors of water management including direct and indirect potable reuse were presented.

THE ROLE OF THE SG LWWTP IN WASTEWATER TREATMENT DEVELOPMENT

The specialist group on the Design, Operation and Costs of Large Wastewater Treatment Plants played an irreplaceable role in the described development of biological wastewater treatment. The main contribution of the group can be seen in the information exchange. We have to realize the situation in information exchange 50 years ago. No internet, no digitalized information databases, the only communication means available were rather slow post letters and expensive and not always reliable telephones. The main information resources were books and printed professional journals. The production time of books was in years, the period between submitting a journal paper and its final publication lasted for many months, often more than one year. Under such situation, there was really a ‘hunger for information’. At that time, the fastest way of communication was participation in seminars and conferences and personal information exchange among participants.

The seminars, later on called conferences, on LWWTP created such a suitable platform for information exchange. The participants were made of a very well-balanced mixture of academicians, researchers on one side and practitioners from wastewater treatment plants, design and engineering companies, and suppliers on the other side. In the first years of the LWWTP conference series the lectures on design and operational experience came mostly from large wastewater treatment plants in ‘traditional countries’ from (i) Europe, for example:

- Germany (WWTP operated by companies like Ruhrverband, Emschergenossenschaft, Erfteverband, Niersverband)
- Sweden (Göteborg, Stockholm)
- UK (London), France (Paris), Denmark, Austria, Czech Republic, Hungary and other countries;

and also, from (ii) overseas countries, mainly from:

- South Africa (Cape Town, Pretoria, Johannesburg)
- USA (Philadelphia, Blue Plains, Chicago, Los Angeles, San Francisco)
- Israel, Japan and others.

During the long history of the LWWTP conferences, the specific topic of the group has become relevant also in other parts of the world when large wastewater treatment plants started to be built in countries of Central and Latin America (especially Mexico and Brazil), in China but also in eastern Europe. Therefore, the attendees of LWWTP conferences represent today the whole world and this unique environment justifies the reason for these conferences even in the age of developed information technologies.

The SG on LWWTP has always collaborated with other specialist groups within the IWA because the design and operation of large wastewater treatment plants also requires knowledge from other fields. Typically, the LWWTP conferences are dealing also with nutrient removal and recovery, carbon deficiency in nutrient removal systems, activated sludge population dynamics (microbial ecology), mathematical modelling, instrumentation – control – automation, membrane technology and most recently also with water reuse.
CONCLUSIONS

The IWA specialist group on the Design, Operation and Costs of Large Wastewater Treatment Plants celebrated, in 2020, fifty years of its existence. During this period, the activated sludge process has become a dominant wastewater treatment technology used in large wastewater treatment plants worldwide. The activated sludge process has developed from its empirical beginnings into sophisticated modern biotechnology employing scientific findings of many science and engineering disciplines, such as reaction kinetics, reactor theory, microbiology, microbial ecology and mathematical modelling. This development required rather intense information exchange, which was also the main role of the IWA SG LWWTP. Thus, the current level of wastewater treatment plants in large towns and cities was achieved also thanks to the information exchange platform provided by the specialist group and its regular conferences.

ACKNOWLEDGEMENT

With this paper the author wants to remember Dr. David Jenkins, Professor Emeritus at the University of California at Berkeley (1935–2021). Dr. Jenkins, known also as “FlocDoc”, was one of the greatest personalities in the history of activated sludge process. Dr. Jenkins has to be remembered for his enormous contribution to improving our understanding of nutrient removal and of activated sludge bulking and foaming. David was also recognized for his activities in educating academics and practitioners about how to solve operational problems of activated sludge process. He led workshops around the world on filamentous microsopic identification and control, including the annual course in Perugia, Italy, mentioned in this paper.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES


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