Critical review

Filamentous bulking sludge—a critical review

António M.P. Martins[a,b], Krishna Pagilla[c], Joseph J. Heijnen[a], Mark C.M. van Loosdrecht[a,*]

[a] *Department of Biochemical Engineering, Delft University of Technology, Julianalaan 67, Delft 2628 BC, The Netherlands*
[b] *Environmental Technologies Centre, ISQ, Av. Prof. Cavaco Silva, No. 33, Talaíde, Tagus Park, Oeiras Apartado 012, CTT Porto Salvo, 2780-994 Porto Salvo, Portugal*
[c] *Illinois Institute of Technology, Chemical and Environmental Engineering, Suite 127, Perlstein Hall, 10 West 33rd Street, Chicago, IL 60616, USA*

Received 7 July 2003; received in revised form 31 October 2003; accepted 6 November 2003

Abstract

This paper reviews the long-standing bulking sludge problem in activated sludge systems. Despite the extensive amount of research that has been done on bulking sludge, it still occurs world-wide and a comprehensive solution does not seem to be available. Bulking sludge can be approached as a microbiological problem (occurrence of a specific filamentous bacterium) or as an engineering problem (growth of bacteria with a filamentous morphology). In the first case species-specific solutions should be found, whereas in the latter case, a generic approach might be available. Since bulking sludge is caused by a group of bacteria with a specific morphology, but not a specific physiology we believe that a generic approach would be feasible. Several theories for bulking sludge are discussed. Based on these theories the application and associated problems with the use of biological selectors are critically evaluated. Finally, a set of open research questions is identified.

Keywords: Filamentous bulking sludge; General theories; Morphology; Physiology; Substrate kinetics and storage; Selector design guidelines

Contents

1. Introduction 794
2. Historical aspects 795
3. Relationship between morphology and ecophysiology 795
   3.1. Microbiological approach 795
   3.2. Morphological–ecological approach 798
4. Filamentous bacteria identification and characterisation 798
   4.1. Microscopic characterisation versus molecular methods 798
   4.2. Physiology of filamentous bacteria 799

*Corresponding author. Tel.: +31-15-278-1618; fax: +31-15-278-2355.
E-mail addresses: a.martins@tnw.tudelft.nl, martins.antonio@netc.pt (A.M.P. Martins), pagilla@iit.edu (K. Pagilla), m.c.m.vanloosdrecht@tnw.tudelft.nl, mark.vanloosdrecht@tnw.tudelft.nl (M.C.M. van Loosdrecht).

0043-1354/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2003.11.005

5. Current general theories to explain bulking sludge . . . 802
5.1. Diffusion-based selection . . . 802
5.2. Kinetic selection theory . . . 802
5.3. Storage selection theory . . . 803
5.4. Nitric oxide (NO) hypothesis . . . 803
6. Remedial actions . . . 803
6.1. Selector . . . 804
6.2. Aerobic selectors . . . 804
6.3. Non-aerated selectors in BNR systems . . . 805
6.3.1. Anoxic selectors . . . 805
6.3.2. Anaerobic selectors . . . 805
6.3.3. Contradictory observations about the effectiveness of selectors in BNR systems 807
7. Mathematic modelling . . . 807
8. Research questions . . . 809
8.1. Sludge architecture . . . 809
8.2. Bacteria identification and physiology . . . 809
8.3. Role of particulate substrates . . . 809
8.4. Storage polymers . . . 809
8.5. Selectors . . . 810
8.6. Control and monitoring . . . 810
8.7. Mathematical modelling . . . 810
9. Conclusions . . . 811
Acknowledgements . . . 811
References . . . 811

1. Introduction

The activated sludge process is the most commonly used technology for biological wastewater treatment. It consists of two stages, a biochemical stage (aeration tank) and a physical stage (secondary clarifier). In the aeration tank, organic carbon, ammonium and phosphate are removed from the wastewater by the activated sludge. The diversity of the biological community is very large, containing many species of viruses, bacteria, protozoa, fungi, metazoa and algae. In this complex ecosystem bacteria, which are usually about 95% of the total microbial population [1], play a key role. Hence, bacteria kept in the activated sludge process under the correct environmental conditions efficiently remove the organic material and nutrients from wastewater. A good separation (settling) and compaction (thickening) of activated sludge in the secondary clarifier is a necessary condition to guarantee a good effluent quality from the activated sludge process.

Bulking sludge, a term used to describe the excessive growth of filamentous bacteria, is a common problem in activated sludge process (e.g., [2,3]). The term bulking sludge often also is used for non-filamentous poor settling, but in this study it refers only to filamentous sludge. The volume fraction of extended filamentous bacteria in the activated sludge culture that causes settling problems could be minor. According to Palm et al. [4] and Kappeler and Gujer [5] volume fractions of 1–20% are sufficient to cause bulking sludge. Kaewpipat and Grady [6] even suggest that the number of filamentous bacteria in bulking sludge can be too low to detect by denaturing gradient gel electrophoresis (DGGE). This would indicate that the filamentous bacteria regularly do not represent the dominant metabolic bacterial group in the treatment plant, but still cause bulking sludge.

Despite much research bulking sludge seems to be a continuous problem in operating wastewater treatment plants. This is likely caused by several facts. Many filamentous bacteria are not available in pure cultures, preventing a detailed study of these organisms. The condition of the plant operation under which bulking sludge occurs is usually only marginally documented.

One reason for not finding a good general solution to bulking sludge might be the absence of a consensus on the exact level at which the problem should be approached. The dominant approach found in the literature is by trying to identify the specific filamentous bacterium in a bulking sludge [7–11]. By studying and

understanding the ecophysiology of the filamentous bacterium (either in pure culture or by applying in situ techniques, such as microautoradiography), it is hoped that a solution to avoid the occurrence of the specific filament can be found. Another approach is the recognition that the general characteristic is the cell morphology. Realising how the morphology affects the ecology of the bacteria could lead to a general solution independent of the species involved [12,13]. In this approach, the occurrence of a specific filamentous bacterium is a second-order problem.

In this review the present state of bulking sludge research is evaluated. The proposed theories are critically evaluated. The design (and pitfalls herein) of different selectors is discussed based on a generalised view of bulking sludge. Finally, specific research themes to find solutions to bulking sludge problem are identified.

2. Historical aspects

It is not our intention to fully describe the history and developments of activated sludge systems. For this the readers are invited to read the excellent reviews provided, for instance, by Allemann and Prakasam [14], Albertson [15], Wanner [16], Orhon and Artan [17] and Casey et al. [18]. We will just stress some of the most important historical facts that contributed to the understanding of the bulking sludge problem.

The activated sludge process was developed in the early 1900s in England [19]. Initially, fill-and-draw systems were brought into operation but they were quickly converted into continuous-flow systems. Despite more frequent occurrence of settling problems, continuous-flow systems became popular and spread worldwide. Donaldson [2] suspected that back-mixing in plug-flow aeration basins, which changes the hydraulic behaviour and the substrate regime to a completely mixed mode, was an important factor promoting to the development of bulking sludge. As a corrective measure he suggested that the aeration basin should be compartmentalised (i.e. plug-flow reactor) to promote the development of well settling sludge. Nevertheless, continuously fed completely mixed activated sludge systems remained the preferred design. The discussion on the effect of feeding pattern on the sludge settleability was reopened in the 1970s. Several studies showed the advantage of using compartmentalised tanks with plug-flow pattern (i.e. high food-to-microorganism ratio—F/M environments) over continuously fed completely mixed systems [13,20–26], confirming the early recommendations of Donaldson [2].

Pasveer [27] studied the use of fill-and-draw technology, from which he developed the oxidation ditch system. This reopened the discussion on the advantages of utilising these systems in the treatment of municipal wastewater. The fill-and-draw oxidation ditch became quite popular in Europe for a few years, but once more, almost all the systems were soon converted to continuous-flow oxidation ditches by the addition of a secondary settler and solids recycle. Pasveer during the 1960s showed that intermittently fed full-scale oxidation ditches produce sludge with better settleability than continuously fed completely mixed systems [28]. In the late 1960s and during the 1970s Irvine and his co-workers renamed the periodic operated processes as sequencing batch reactor (SBR) [29] and were largely responsible for the world-wide dissemination of this technology [30–33].

In the 1970s Chudoba et al. [12] developed the selector reactor, which became the most widespread engineering tool to control bulking sludge. Although the use of selectors has been successful and has reduced bulking problems in many activated sludge systems, there were regular reports of their failure [25,34–43].

3. Relationship between morphology and ecophysiology

One of the most intriguing and complex questions on bulking sludge is whether morphology, physiology and substrate kinetics are related and how do they contribute to the dominance of filamentous bacteria in activated sludge. Is there a general mechanism that could explain the growth of filamentous bacteria or does each filamentous microorganism need to be identified and physiologically, morphologically, kinetically and taxonomically described in order to develop strategies for bulking sludge control? For decades scientists, engineers and microbiologists failed to find a definitive answer to these questions. Some relationships can be inferred and they will be briefly discussed here.

3.1. Microbiological approach

The lack of success in finding a general solution to bulking sludge control led many researchers to look to the microbial population and search for the predominant filamentous bacteria responsible for bulking. Identification keys were developed [7–11] to identify morphotype filamentous bacteria. Although with several limitations these identification methods produced a systematic tool that allowed a relative confidence in the identification of filaments. The next step was finding relationships between the most predominant filaments and their physiology and the operational conditions (e.g., dissolved oxygen concentration— DO, F/M, etc.) in order to define (specific) strategies for its control [1,11,44] (Table 1).

Table 1
Proposed groups of model morphotype filamentous microorganisms [1,45]

Microorganisms	Features	Control
Group I: Low DO aerobic zone growers		
S. natans[a], type 1701, *H hydrossis*	Use readily biodegradable substrates; grow well at low DO concentrations; grow over wide range of SRTs.	Aerobic, anoxic or anaerobic plug-flow selectors; and increase SRT; increase DO concentration in the aeration basin ($>1.5\,mg\ O_2\,L^{-1}$).
Group II: Mixotrophic aerobic zone growers		
Thiothrix sp.[b] Type 021N[b]	Use readily biodegradable substrates, especially low molecular weight organic acids; present at moderate to high SRT; capable of sulphide oxidising to stored sulphur granules; and rapid nutrients uptake rates under nutrient deficiency.	Aerobic, anoxic or anaerobic plug flow selectors; nutrient addition; eliminate sulphide and/or high organic acid concentrations (eliminate septic conditions).
Group III: Other aerobic zone growers		
Type 1851, *N. limicola* spp.	Use readily biodegradable substrates; present at moderate to high SRTs.	Aerobic, anoxic or anaerobic plug-flow selectors; reduce SRT.
Group IV: Aerobic, anoxic, anaerobic zone growers		
M parvicella, types 0092, type 0041/0675	Abundant in anaerobic–anoxic–aerobic systems; present at high SRTs; and possible growth on hydrolysis of particulate substrates.	Still uncertainty but the most recommended solutions are: install a skimmer to remove particulate substrate; maintain a plug-flow regime in all the system; the several stages (anaerobic/anoxic/aerobic) should be well defined; maintain a relatively high oxygen concentration in the aerobic phase ($1.5\,mg\ O_2\,L^{-1}$) and a low ammonium concentration ($<1\,mg\ N\,L^{-1}$)[c].

[a] Also abundant (identified by specific gene probe SNA23a) in fully well-aerated systems under low soluble organic substrate (acetate) concentration [46].
[b] Also abundant (identified by specific gene probes TNI and 21N) in badly aerated systems (SO_2 $<0.5\,mg\ O_2\,L^{-1}$) where a high load of soluble organic substrate (acetate) concentration was instantaneously applied [47].
[c] STOWA [48] and Kruit et al. [49].

Table 2
World-wide surveys of filamentous microorganisms in activated sludge systems

Region/country	Main filamentous microorganisms	Reference
Africa		
South Africa	*M. parvicella* and Types 1851, 0041/0675 and 0914	Blackbeard et al. [50,51]
Asia		
Japan	Type 021N, NALO, *S. natans*, Type 0041/0675, and *Thiothrix* sp.	Mino [52]
Thailand	Types 021N, 1701, 0092, 0041/0675, and NALO	Mino [53]
Europe		
Czech republic	*M. parvicella* and Type 0092	Krhutková et al. [3]
Denmark	*M. parvicella*, and Types 0041/0675, 021N, 0092, 0914, and 1851	Kristensen et al. [54]
Denmark, Germany, Greece and The Netherlands	*M. parvicella*, Type 0041/0675, *N. limicola*, and Types 0092, 0803, and 0914.	Eikelboom et al. [55]
France	*M. parvicella*, Types 0041/0675, 0092, and *N. limicola*	Pujol and Canler [56]
Germany	*M. parvicella* and Types 1701, 0041/0675, and 0092	Kunst and Reins [57]
Italy	*M. parvicella*, NALO, Types 0092, and 0041/0675	Rossetti et al. [58] and Madoni et al. [59]
The Netherlands	*M. parvicella*, Type 021N, *H. hydrossis*, and Types 0092, 1701, and 0041/0675	Eikelboom [8,60] and Kruit et al. [61]
United Kingdom	*M. parvicella*, Type 021N, *N. limicola*, and NALO	Foot [62] and Lavender et al. [63]
North America		
USA	Types 1701, 021N, 0092, 0041/0675, NALO, and *M. parvicella*	Strom and Jenkins [44] Switzenbaum et al. [64]
Oceania		
Australia	*M. parvicella* and Types 0041/0675, 0092, and *H. hydrossis*	Seviour et al. [65]
South America		
Argentina	Type 1701, *S. natans*, NALO, *M. parvicella*, and Type 0041/0675	Di Marzio [66]

Several surveys have been performed to establish the occurrence of filamentous microorganisms in wastewater treatment plants from different countries (Table 2)

In all the surveys filamentous bacteria were identified by morpho-based methods, which could have led to misleading or incorrect results. In addition, the quantification was based on the subjective qualitative scoring method [1,9,11]. Therefore, these data should be interpreted with caution.

Although the distribution of filamentous microorganisms varies considerably between different geographical areas and seasonally, it can be concluded that *Microthrix parvicella* and Types 0092 and 0041/0675 are apparently the major morphotype filaments, mainly responsible for the bulking events observed in biological nutrient removal (BNR) activated sludge systems. These surveys also showed that the bulking sludge episodes, supposedly due to the abundance of *M. parvicella*, were more frequent in winter and spring than in summer and autumn (e.g., [49,54,55]). It was also confirmed that the morphotypes Type 021N, Type 0961, *Sphaerotilus natans* and *Thiothrix* sp. are controlled by anaerobic and anoxic stages [67–69], as typical in bio-P and denitrifying systems. These conditions seem, however, to be inefficient for the dominant filamentous microorganisms found in BNR systems. Curiously, the morphotype filamentous bacteria found in BNR systems are usually Gram positive, which implies that their likely hydrophobic cell surface could easily adsorb compounds with a low solubility [55]. It is, however, unclear whether low-loaded systems also enrich for Gram-positive floc-forming bacteria.

During the 1990s molecular methods based on DNA and RNA analyses were introduced to biological wastewater treatment (e.g., [70,71]). These methods allow a correct identification of the filamentous bacteria population. Therefore, it is advisable to apply specific gene probes, whenever they exist, in future surveys. Their use together with filamentous bacteria characterisation and definition of the right control and operational conditions (e.g., selector reactor) are considered major challenges to control bulking sludge in the coming decade.

3.2. Morphological–ecological approach

Filamentous bacteria grow preferentially in one or two directions. This morphological feature apparently gives competitive advantages to filamentous organisms under substrate limiting concentrations (e.g., diffusional-resistant environments). It is foreseen that these organisms have a higher outward growth velocity and win the competition because they gain easy access to bulk liquid substrate [46]. This is in line with some studies that also connect the excessive growth of filamentous microorganisms with substrate diffusional resistance inside biological flocs [72–74]. In these views, the morphology as such gives the organisms an ecological advantage. It would also imply that under non-bulking process conditions filamentous bacteria can still be present inside the floc. If substrate limitation occurs they will then quickly grow out of the floc. The almost ubiquitous presence of filaments in activated sludge even led to suggestions that actually filamentous organisms form the backbone of activated sludge flocs [74–76]. This type of filamentous skeleton structures would promote the attachment of other cells by their extracellular polymeric substances (EPS) [77].

In general, floc morphology is still not well studied. With the advance of microscopic techniques such as transmission electron microscopy (e.g., [78]) and confocal laser scanning microscopy (CLSM), and molecular tools like fluorescent in situ hybridisation (FISH) technology is available to study floc morphology in detail [79]. These studies would greatly help in defining floc architecture and the role of filamentous bacteria therein.

4. Filamentous bacteria identification and characterisation

The basis for understanding and characterising bulking sludge is generally thought to depend on a proper identification of the filamentous bacteria involved (see Section 3). This is briefly discussed below.

4.1. Microscopic characterisation versus molecular methods

Many types of bacteria are still not identified and taxonomically not recognised. Therefore, these bacteria are not documented in the standard microbiological identification manuals like Bergey's manual of systematic bacteriology [80]. Eikelboom [7,8] developed the first identification key to identify filamentous bacteria in activated sludge systems. This identification is mainly based on morphological characteristics and on the response of the filamentous bacteria to a few microscopic staining tests. The procedures, techniques and identification keys were compiled in a microscopic sludge investigation manual [9,10] that, together with a slightly different manual by Jenkins et al. [1,11], have been used as world-wide references on filamentous bacteria identification. Although very useful this type of identification has its limitations. For instance, many filamentous bacteria (e.g., the morphotypes *S. natans*, 1701, 0092 and 0961) can change morphology in response to changes in environmental conditions [81–83] and although some of them can look morphologically the same, they probably vary considerably in their

physiology and taxonomy (e.g., [84,85]). For instance, the filamentous bacterial morphotype '*Nostocoida limicola*' has several phylogenetically different bacteria, belonging to the following groups: low mol% G+C Gram-positive bacteria [86–88], high mol% G+C Gram-positive bacteria [87–89]), *Planctomycetes* [87,88,90], green non-sulphur bacteria [91] and alpha-subclass of *Proteobacteria* [92]. Similar conditions occur for the filamentous morphotype Eikelboom type 1863 [83]. A new genus, *Alisphaera*, has been recently proposed for the '*N. limicola*' belonging to alpha-subclass of *Proteobacteria* [92]. Because this genus contains large and robust filamentous bacteria which were found to be dominant in many industrial activated sludge systems [93], further studies about their characteristics are relevant to define (specific) strategies to control bulking.

Microscopic identification of filamentous bacteria based on morphology requires a well-trained person, otherwise a wrong judgement can easily be made. Furthermore, about 40 new morphotypes of filamentous bacteria were recently identified in a survey study in industrial activated sludge systems [93], making the identification of filamentous bacteria more complex. Misleading and difficult identification by traditional microscopic techniques directs research towards molecular methods. Molecular methods based on analysing DNA or RNA of the bacteria have developed rapidly. For activated sludge two methods are presently commonly used. In order to characterise the complexity of a microbial community, the 16S rRNA of the bacteria can be used. In a DGGE [94] the 16S rRNA, unique for each organism, can be separated. Each rRNA molecule can then be sequenced, after which the taxonomy of the organisms can be determined, even if no pure culture is available. With DGGE changes in the microbial population can easily be followed, without the anomalies associated to traditional plating techniques. Based on a known 16S rRNA sequence, probes that react with a specific sequence can be designed. These can be used to stain specific bacteria. In this way it is possible to uniquely identify bacteria with the FISH method (e.g., [71,95]). Although being relatively quick, DGGE has some limitations and can often not be used. A full rRNA cycle is then preferred. It combines two different rRNA-based techniques: direct rRNA retrieval followed by in situ hybridisation with oligonucleotide probes based on the retrieved sequences. The full rRNA cycle is considered to be the best approach to characterize the community structure of activated sludge [70,204]. A huge research effort, however, has to be undertaken in the development of more specific gene probes since from a total universe of about 80 different morphotypes of filamentous microorganisms, only less than 20 species can be currently identified with specific gene probes by FISH (Table 3).

Automated molecular methods like microarray/DNA chips and flow cytometry in combination with automated image analysis in epifluorescence microscopy or CLSM are currently being developed and will hopefully become available for routine use in the coming years (e.g., [84,105]).

4.2. Physiology of filamentous bacteria

Unfortunately, most of the filamentous organisms are still very poorly characterised, mainly due to the problems of cultivation and maintenance of cultures. Recent developments in combining microautoradiography with FISH [106–108] are a promise for elucidating the exact physiology of filamentous bacteria. In general, there is no obvious relation between filamentous morphology and physiology of the bacteria.

A general problem we faced is that the old physiological data are described for morphotype filamentous bacteria, which are likely to be phylogenetically unrelated bacteria (e.g., '*N. limicola*' in [86–89,91,92] with deep physiological differences, and, consequently, old physiological data (e.g., the morphotype '*N. limicola*' in [109]) might or might not be correct. Therefore, the old physiological data should be interpreted with caution and future bacterial physiological studies should unequivocally show the taxonomy of the studied organisms.

The few physiological studies with pure cultures of chemoheterotrophic filamentous bacteria showed that most of them appear to have a strictly aerobic respiratory metabolism, with oxygen as electron acceptor. To our knowledge only the morphotypes Type 0961, Type 1863, Type 1851 and *N. limicola* are claimed to have the capacity to perform a fermentative metabolism [109–111], and therefore may have competitive advantages in systems with anaerobic stages. Anyway, these morphotypes are believed to be minor components of the total microbial population and they are, in general, not responsible for bulking sludge episodes.

Some of the filamentous bacteria are able to use nitrate as electron acceptor, reducing it as far as nitrite, like *M. parvicella* [112,113], *S. natans* [114], *Thiothrix* spp. [115,116], Type 021N [115,116] and Type 1851 [111], but the substrate uptake rate and denitrification rate for the filamentous bacteria analysed so far (Type 021N and *Thiothrix* spp.) are much lower (more than 80 times) than for floc-forming bacteria (*Zoogloea ramigera*) [115]. Type 0092, a filamentous bacterium dominant in many nutrient removal activated sludge systems, seems to be incapable of using nitrate as electron acceptor [110]. Furthermore, in case of *M. parvicella,* it is reported that growth is not sustained under anoxic conditions [112]. Anoxic contact zones have been using this physiological information to control bulking sludge

Table 3
List of some important FISH probes currently available for filamentous bacteria identification

Taxonomic group/microorganism	Oligonucleotide probe name	Reference
Alpha-subclass of *Proteobacteria*		
Alisphaera europea (EU24)	Noli-644	Snaidr et al. [92]
Alisphaera MC2	MC2-649	Snaidr et al. [92]
Alisphaera PPx3	PPx3-1428	Snaidr et al. [92]
Beta-subclass of *Proteobacteria*		
Leptothrix discophora and other members of the β1 group of *Proteobacteria*	LDI	Wagner et al. [71]
S. natans and other members of the β1 group of *Proteobacteria*	SNA23a	Wagner et al. [71]
Gamma-subclass of *Proteobacteria*		
Acinetobacter spp., some Eikelboom type 1863	ACA	Wagner et al. [96]
Eikelboom type 021N	21N	Wagner et al. [71]
T. nivea, T. unzii	TNI	Wagner et al. [71]
Eikelboom type 021N group I	G1B	Kanagawa et al. [97]
Eikelboom type 021N group II (*T. eikelboomii*)		Kanagawa et al. [97]
Eikelboom type 021N group III (*T. defluvii*)		Kanagawa et al. [97]
Eikelboom type 021N *T.*		Kanagawa et al. [97]
Leucothrix mucor	LMU	Wagner et al. [71]
T. fructosivorans, T. ramosa	TFR	Kim et al. [98]
Competitor against TNI and TFR	TEI	Kim et al. [98]
Cytophaga–flavobacterium–Bacteroides		
Haliscomenobacter spp.	HHY	Wagner et al. [71]
Green non-sulphur bacteria		
'*Chloroflexi*': Eikelboom type 1851 (BEN 52)	CHL 1851	Beer et al. [99]
'*N. limicola*'-like bacteria	AHW183	Schade et al. [91]
High mol% G + C Gram-positive bacteria: *Actinomycetes*		
Corynebacterineae	Myc657	Davenport et al. [100]
Genus *Gordona*	Gor-0596	De los Reyes et al. [101]
Gordona amarae	G.am-0192	De los Reyes et al. [101]
Gordona amarae	G.am-0205	De los Reyes et al. [102]
Gordona amarae group 1 strains	G.am1-0439	De los Reyes et al. [102]
Gordona amarae group 2 strains	G.am2-0439	De los Reyes et al. [102]
N. limicola II	NlimII175, NlimII192	Liu and Seviour [87]
M. parvicella	MPA60, MPA223, MPA645, MPA650, CompMPA650.1 and CompMPA650.2	Erhart et al. [103]
Low mol% G + C Gram-positive bacteria		
N. limicola I	NlimI91	Liu and Seviour [87]
Planctomycetes		
N. limicola III	NlimIII301, NlimIII792, NlimIII830	Liu and Seviour [87]
TM7, candidate division of the domain *Bacteria*		
Eikelboom type 0041/0675	TM7305, TM7905	Hugenholtz et al. [104]

particularly due to Type 021N and *S. natans* [67,115,117,118]).

Of the most predominant filamentous bacteria found in BNR activated sludge systems only the morphotype Type 0092 and *M. parvicella* were grown in pure culture [81,110,112,113,119–121] and significant difficulties are encountered in the isolation of the latter. Table 4 summarizes some important characteristics of

Table 4
Physiology, stoichiometry and kinetics of the mostly dominant filamentous bacteria in BNR-activated sludge systems

Filamentous bacteria	Physiology	Stoichiometry and kinetics	Reference
'Microthrix parvicella'	The strains RN1 and 4B can grow in R2A medium (with glucose, casaminoacids, yeast extract, sodium pyruvate, starch, and proteose peptone) but not under anoxic (presence of nitrate and/or nitrite) or anaerobic conditions. Although these strains can store the substrate (PHA granules) under aerobic, anoxic (nitrate and/or nitrite) and anaerobic conditions, no growth occurs in anoxic and anaerobic conditions. Both strains are able to reduce nitrate to nitrite but not to N_2 and have a high resistance to long periods of anoxic and anaerobic conditions.	$\mu_{max} = 0.46\,day^{-1}$ (4B), 0.37 (RN1), 0.66–0.67 day (*Microthrix parvicella* from activated sludge); $k_s = 3.9\,mg\ COD\,L^{-1}$ (4B and RN1).	Rossetti et al. [112]
	The organism grows in R2AM medium, a modified medium of R2A. Able to take up and to store the long-chain fatty acids (oleic acid and palmitic acid) and a lipid (trioleic acid) under oxic, anoxic, and anaerobic conditions. Not able to take up simple substrates such as acetate, propionate, butyrate, glucose, ethanol, glycine, and leucine. Not able to store labelled orthophosphate under alternating anaerobic and anoxic/oxic conditions applied for enhanced biological phosphorus removal.	Doubling time (t_g) = 128 days.	Connery et al. [120] Andreasen and Nielsen [107,122] and Nielsen et al. [108]
	Strain RN1 is able to use a wide range of different substrates including organic acids, complex substrates, and fatty acids as the sole carbon source but glucose and either pure oleic acid or its esters are not carbon sources for its growth. It contains intracellular lipid granules and accumulates polyphosphate. Able to reduce nitrate to nitrite but unable to denitrify to N_2.	$\mu_{max} = 0.3-0.5\,day$	Tandoi et al. [113].
	Strains DAN1-3 and Ben43 are able to grow on non-Tween medium (NTM—with peptone and succinate) and R2A, respectively. It contains polyphosphate inclusions.		Blackall et al. [119,123]
	Able to use long-chain fatty acids (e.g., Oleic acid) and their esters as carbon and energy source. No growth occurs on organic acids and sugars. Excess carbon is stored as a lipid granule under oxic conditions. It is a micro-aerophilic bacterium with a high affinity for oxygen and might be dependent on reduced N and S compounds for growth. Its lipid composition is very high, at times approaching 35% of the dry weight of the organism.	$\mu_{max} = 1.44\,day^{-1}$ (chemostat), $0.38\,day^{-1}$ (batch); $Y_{SX}^{max} = 1.41\,g\,g$ cell d.w. g^{-1} oleic acid; $m_s = 1.46\,mg$ oleic acid g^{-1} cell d.w. h^{-1}; $m_o = 4.48\,mg$ $O_2\,g^{-1}$ cell d.w. h^{-1}	Slijkhuis [121]
Eikelboom type 0041/0675	Able to take up several sugars (glucose, galactose and manose) and amino acids (more leucine than glycine) under aerobic and anoxic conditions but not acetate.		Thomsen et al. [124]
Eikelboom type 0092	Grow poorly on sugars and has enzyme activities capable of degrading some proteins. It is a strict aerobic bacterium.	$\mu_{max} = 7.92\,day^{-1}$, 0.37 (RN1), $k_S = 350\,mg\ COD\,L^{-1}$, $k_d = 0.04\,h^{-1}$	Horan et al. [110] and Buali and Horan [81]

the dominant filamentous bacteria found in BNR systems.

5. Current general theories to explain bulking sludge

Several hypotheses about bulking sludge were formulated in the hope of finding a general explanation for this problem. Unfortunately, none of them led to a definitive solution. Moreover, most of the theories still lack experimental verification. Nevertheless, they form the current basic theoretical framework to approach and understand bulking sludge and, therefore, they will be discussed further.

5.1. Diffusion-based selection

Several researchers have pointed out that the morphology of filamentous bacteria aid in substrate uptake under low nutrients or oxygen concentrations. Till the early 1970s, the competition between filamentous and non-filamentous bacteria was based on the fact that the surface-to-volume (A/V) ratio is higher for filamentous bacteria [73]. Especially, at low substrate concentration this high A/V ratio gives advantages to the organisms since the mass transfer to the cells with a high A/V ratio is more facilitated. At lower substrate concentrations this would lead to a relatively higher growth rate.

In later theories it was stated that the filaments could easily penetrate outside the flocs. When the flocs are growing at a low substrate concentration the filamentous bacteria would observe effectively a higher substrate concentration than the floc formers inside the floc [72,74,125]. Micro-gradients of substrate concentration in flocs have been theoretically predicted (e.g., [126]) and experimentally observed in sludge flocs [127]. Later, Martins et al. [46,47] extended this theory by comparing floc growth with biofilm growth. Van Loosdrecht et al. [128] and Picioreanu et al. [129] indicated that in diffusion-dominated conditions (i.e. low substrate concentrations) open, filamentous, biofilm structures arise. At high substrate concentrations compact and smooth biofilms arise. Ben-Jacob et al. [130] showed that the colony morphology of a pure culture also depend on substrate micro-gradients, with low substrate concentrations leading to filamentous colony morphology. Therefore, it could be that the low substrate concentration would lead to a floc to become more open and filamentous [46]. Filamentous bacteria would excellently fit in such a structure.

5.2. Kinetic selection theory

Similar to Donaldson [2], Chudoba et al. [21] related the settling characteristics with the mixing characteristics of the activated sludge aeration tank. Using mixed cultures with defined substrate under laboratory-controlled conditions, Chudoba et al. [21] showed that the aeration systems with a low degree of axial mixing and higher macro-gradients of substrate concentration along the system suppress the growth of filamentous bacteria and lead to the development of well settling sludge. The authors concluded that the primary cause of the selection of floc-forming microorganisms in the mixed culture is the macro-gradient of substrate concentration at the inlet part of the system.

Based on these results, Chudoba et al. [12] formulated the kinetic selection theory to explain the occurrence or suppression of filamentous bacteria in activated sludge systems. The explanation was based on a selection criterion for the limiting soluble substrate by filamentous and floc-forming bacteria. Chudoba et al. [12] hypothesised that the filamentous microorganisms (K-strategists) are slow-growing organisms that can be characterised as having maximum growth rates (μ_{max}) and affinity constant (K_s) lower than the floc-forming bacteria (*r*-strategists). In systems where the substrate concentration is low (typically $C_s < K_s$), like in continuously fed completely mixed systems, filamentous bacteria have a higher specific growth rate than floc-forming bacteria, and thereby win the competition for substrate. In systems where the substrate concentration is high, like in plug-flow reactors and SBR systems, the filamentous bacteria should be suppressed since their growth rate is expected to be lower than that for floc-forming bacteria. Pure culture studies with some of the filamentous bacteria (e.g., *S. natans* in [125,131]; *Haliscomenobacter hydrossis* in [132] Type 1701 in [82] Type 021N in [133] *M. parvicella* in Slijkhuis et al., 1983, [112,113] and floc-forming bacteria (*Arthrobacter globiformis* in [24] *Z. ramigera* in [133] supported this theory. It is, however, questionable whether these floc-forming bacteria are representative for activated sludge systems. Use of molecular probes has shown that regularly non-dominant bacteria have been enriched from activated sludge [95]. A technique based on quantitative MAR and FISH was recently developed and applied to measure in situ the kinetics of filamentous bacteria ('*Candidatus Meganema perideroedes*' and *Thiothrix* sp.) [134]. This approach is promising and efforts should be made to extend it to other filamentous and non-filamentous bacteria.

Until now no one has unequivocally shown that the filamentous bacteria have in general a lower maximal growth rate than other bacteria present in the sludge. Moreover, there is no theoretical explanation why a filamentous morphology would lead to a lower growth rate. The generally lower K_s value for filamentous bacteria as proposed in the kinetic selection theory is also not proven yet. If the K_s is seen as a property of the substrate uptake enzymes, there again seems to be no direct relation between K_s and filamentous morphology.

If, however, the K_s is seen as an apparent mass transfer parameter describing mass transfer to the cell the A/V hypothesis of Pipes [73], then it is fully in agreement with the kinetic selection theory. In flocs, the K_s value is anyway an apparent coefficient influenced by the floc morphology. The more the diffusional resistance (the larger and denser the flocs) the higher the measured apparent K_s value. For filaments extending from the floc this would mean a lower K_s value. Based on the reasoning above it might well be that the diffusion-related theories [46,47,72–74,125] and the kinetic selection theory [12] are two side of the same coin.

5.3. Storage selection theory

Traditionally, non-filamentous microorganisms are supposed to exhibit the ability to store substrate under high substrate concentrations. This ability presumably gives an extra advantage to non-filamentous bacteria in highly dynamic activated sludge systems such as plug-flow reactors, SBR and selector systems [25,131,135–139]). However, recent studies showed that bulking sludge could have similar or even higher storage capacity than well settling sludge [46,47,140]. Pure and mixed culture studies also show that some filamentous bacteria, like *M. parvicella*, can have a high storage capacity under all the environmental conditions (aerobic, anoxic and anaerobic) [107,108,112,121]. The stored material can be metabolised for energy generation or protein production during the famine periods, which would represent a strong selective advantage for these microorganisms in competition with other filamentous and non-filamentous bacteria. A lower storage capacity by filamentous bacteria can clearly not be considered as an absolute rule in the selection mechanism for filamentous bacteria. Although they may not be the prime selection parameters, storage and regeneration (depletion) are intrinsic processes that play a key role in selector-like systems. Therefore, they should be considered in the description of the metabolic processes that take place in bulking and non-bulking systems.

5.4. Nitric oxide (NO) hypothesis

Based on extensive experiments at laboratory scale and at full scale, Casey et al. [141–143] proposed a new hypothesis for the proliferation of (low F/M) filamentous bacteria in BNR systems. The hypothesis considers two groups of bacteria, i.e. filamentous and floc-forming bacteria, which are assumed to compete for organic substrate under different denitrification mechanisms. They hypothesised that nitrite and NO, both intermediates of denitrification, accumulate in the floc-forming bacteria and not in the filamentous bacteria. It was postulated that filamentous bacteria only perform denitrification till nitrite and, therefore, do not accumulate the intermediate inhibiting compound NO. In these conditions filamentous bacteria have competitive advantages over floc-forming bacteria since they can utilise the slowly biodegradable COD (SBCOD) under aerobic conditions. Since nitrite is the precursor of NO and because intracellular NO is very difficult to measure, bulk liquid nitrite concentration was used by the authors as a possible indicator of the presence of NO. The inhibition of floc-forming bacteria under aerobic conditions is sustained by the presence of nitrite and low rate of readily biodegradable COD (RBCOD) addition in the aerobic zone, which is continuously produced by the hydrolysis of SBCOD [42,43]. Until now, however, no experimental evidence exist that nitrite and NO levels during denitrification are coupled. Recent results indicate an alternative hypothesis, which appears to be additional to the NO hypothesis, based on the requirement of ammonia for growth by *M. parvicella* [144].

The NO hypothesis has its merits but still needs to be verified. Type 0092, a low F/M filamentous bacteria dominant in many BNR activated sludge systems, seems not to be capable of using nitrate as electron acceptor [110] and microautoradiography studies suggest that nitrite under in situ conditions might be used by *M. parvicella* as an electron acceptor providing energy for the uptake of oleic acid [107], which makes the validity of such hypothesis at least questionable. More detailed biochemical and microbiological studies are needed to definitively discard or prove this hypothesis.

6. Remedial actions

Basically, there are two strategies that can be followed to control bulking sludge, i.e. specific or non-specific methods. The non-specific methods comprise techniques such as chlorination, ozonation and application of hydrogen peroxide. The application principle of these methods is quite simple: since filamentous bacteria causing bulking sludge are placed mostly outside the floc, they are more susceptible to oxidants than the floc-forming bacteria. Chlorination is widely used in USA and the procedures for its implementation are well documented (e.g., [1]). Its application in Europe is limited due to environmental concerns in several countries with the potential formation of undesirable by-products such as halogenated organic compounds. Another negative aspect is that slow-growing bacteria such as nitrifiers when affected by oxidants take a long time to recover, which could potentially lead to effluent quality deterioration. Furthermore, the non-specific methods do not remove the causes for the excessive growth of filamentous microorganisms and their effect is only transient. The same applies to short-term control methods, such as redistribution of biomass from the clarifiers to the aeration tanks and/or increase in the

sludge wasting rate. Specific methods are preventive methods that have the goal to favour the growth of floc-forming bacterial structures at the expense of filamentous bacterial structures. The challenge is to find the right environmental conditions in an activated sludge treatment plant to reach this goal. Because the success of its application would allow a permanent control of bulking in activated sludge systems, in a sustainable way, these methods should be developed and preferentially be adopted.

Until now preventive actions for bulking sludge are not based on the knowledge of the physiology and/or kinetics of a specific type of filamentous bacteria, this despite the great emphases in process monitoring on recognising the filamentous bacteria present. Generalised preventive actions seem to agree that readily biodegradable substrates need to be consumed at high substrate concentrations. This means that in the entrance part of the activated sludge process a plug-flow type of hydraulics is needed until the RBCOD is consumed, thereafter a completely mixed tank can be used. If oxygen is consumed at low concentrations it leads in a similar manner as for RBCOD to bulking sludge. Adequate aeration in the plug-flow stage, therefore, is essential to prevent bulking. The prerequisite of a plug-flow, initial part of the activated sludge process, has resulted in the development of selectors to prevent bulking. Both theories for sludge bulking (A/V or diffusion-based selection as well as kinetic selection theory) support the above approach.

6.1. Selector

A selector is defined as the initial part of a biological reactor, characterised by a low dispersion number and by an adequate macro-gradient of substrate concentration [12]. It can also be a small separate initial zone of a biological reactor that receives the influent and sludge return flows and has a high RBCOD uptake rate, with virtually complete RBCOD removal [1]. In selector-like systems, the microorganisms are subjected to periods with (feast) and without (famine or regeneration) external substrate. In essence a pulse fed SBR or an SBR fed in a static way is the ideal selector system. It has been shown that in such systems even aerobic granular sludge can be formed [145]. In the selector, the microorganisms are subjected to high growth rate environments and are able to accumulate substrate as internal storage products in their cells (storage). A sufficiently long period without external substrate available (low growth rate or famine environment) should then exist (aerobic stage) to reestablish the storage capacity of the cells [131,133,135,136,146–148]. Selectors were quickly installed in full-scale activated sludge systems and are still world-wide the most applied engineering tool for the prevention of bulking sludge phenomena. Nevertheless, there are still regular reports citing selectors failure in the control of bulking sludge [25,34–37] cited by Ekama et al. [38]. It is unclear if such failures were due to a bad design of the selector tank, to transient conditions in the biological treatment system or to other factors that somehow affected the population dynamics, giving competitive advantage for filamentous bacteria. The different selectors and their potential pitfalls will be briefly described here in the following. It is clearly not the authors' aim or intent to review all reported experiments.

6.2. Aerobic selectors

Till the end of the 1980s only organic carbon removal was required in most countries, and fully aerobic systems usually with a completely mixed feeding pattern were preferred. In USA, the systems were mainly high rate with a sludge retention time (SRT) lower than 5 days. Under these conditions, the occurrence of bulking sludge was mainly attributed to the excessive growth of filamentous bacteria such as Types 021N and 1701 [44]. In Europe and South Africa, low-rate plants like oxidation ditch systems and extended aeration systems have been constructed. In the 1990s more stringent regulations with respect to nutrient emissions, particularly ammonia emissions, were required in Europe and USA. In order to fulfil these requirements wastewater treatment plants had to be upgraded and improvements for biological nitrification capability were made. The aeration systems were improved and to keep the nitrifying bacteria in the system, the SRT was usually increased to over 10 days. Furthermore, intermittent aeration systems became more common since they allowed a certain degree of denitrification. In these conditions, bulking sludge was mainly due to the proliferation of the morphotypes *M. parvicella* and Types 021N, 0041/0675 0092 and 0581 [60,61,64,149–151]. These observations led to the definition of the so-called low F/M filamentous bacteria group by Jenkins et al. [1,11].

Aerobic selectors, small mixing zone (aerobic or anoxic) or contact zone (without aeration), were implemented to control bulking sludge attributed in many cases to the excessive growth of Type 021N, *Thiothrix* spp., *S. natans*, but not always successfully in the case of *M. parvicella* [1,13,22,26,36,39,46,47,56,61,67,68,115,117,133,138,149–160].

Many continuous-flow, controlled dynamic systems impose sub-optimal selective pressures on the microbial community and are therefore unable to respond well even during the peak loading periods for which they were originally designed [33]. The contact time, a typical design parameter for selectors, has a very strong and non-linear effect on the sludge settleability [46]. The knowledge of this trend is relevant to define strategies to prevent sludge bulking. When the contact time is

insufficient, soluble substrate is not consumed in the contact zone, and may penetrate into the main aeration basin. In this case the growth of filamentous microorganisms will occur. On the other hand, when the contact time is even slightly too long, the concentration of substrate will be low, approaching the typical level of completely mixed tanks, which also favours the growth of filamentous microorganisms. The strong effect of a too large or small contact tank on the sludge volume index (SVI) makes a proper design difficult. In systems with highly dynamic feeding patterns, like temperature, flow and load variations such as wastewater treatment systems, a good design is not easy and may be a plausible reason for regular failing of selector tanks. Therefore, in practice it is expected that only plug-flow systems, like long channels (length-to-width ratio larger than 10:1) [161], compartmentalised contact tanks or an SBR fed in a static way [33,135], can guarantee a strong macro-gradient of substrate concentration and will function properly under highly dynamic conditions. Furthermore, proper staging can improve the performance of activated sludge systems that are kinetically limited [162].

The necessity to maintain a minimum DO concentration as a function of the soluble organic loading rate or soluble substrate uptake rate in the aeration basin and in the aerobic selector has been recognised and verified in several studies and working diagrams were proposed [4,47,74,163–165]. Although the recommended contact time in an aerobic selector tank is very small, the amount of oxygen required is about 15–30% of the soluble COD removed [1,47]. This underlines the importance of sufficient oxygen supply in the aerobic selector. If a compartmentalised (plug-flow) aerobic selector tank has a too low aeration rate, the negative impacts on the sludge settleability could be worse than with an "overdesigned" (too large) completely mixed selector tank [47]. Furthermore, the aeration control is very important and the sensors should be placed in the first compartment where the oxygen consumption is highest (Table 5).

6.3. Non-aerated selectors in BNR systems

With the introduction of BNR systems factors such as long SRTs (low F/M), unaerated (anoxic and/or anaerobic) sludge mass fraction, hydrolysis of SBCOD, kinetics and storage in the unaerated reactors, frequency of alternation between anoxic and aerobic conditions and low DO concentration in the simultaneous nitrification/denitrification reactor (SND) or in the aerobic tank may have contributed to serious bulking sludge problems that are reported in many BNR-activated sludge systems [39,41,42,51,54,55,57,117].

Like in the aerobic selectors, all the RBCOD should be removed in the anoxic and anaerobic (selector) reactors, preventing any RBCOD entrance into the aerobic stage, which if occurs might give advantages to filamentous bacteria [1,49,115]. Furthermore, oxygen and nitrate should be absent from the anaerobic reactor and the former from the anoxic reactor. In addition to disruption of bio-P and/or denitrifying activity, the presence of microaerophilic conditions in the anaerobic and/or anoxic stages, which for instance can be attributed to diffusion of oxygen through the liquid surface [169], or to the aeration of the returned sludge/liquid stream in screw pumps, can lead to worsening sludge settling characteristics [170] cited by Chiesa [171].

6.3.1. Anoxic selectors

The design criterion of anoxic selectors (Table 5) is primarily based on the ratio RBCOD/NO_3-N entering the reactor. Since in plug-flow reactors an important fraction of RBCOD is expected to be converted to storage products the ratio $\left(\mathrm{RBCOD}/\mathrm{NO_3\text{-}N}\right)_{\mathrm{consumed}}$ is higher than the typical range for completely mixed systems (7–9 mg RBCOD/mg NO_3-N) [1,16,146,148,161,166].

In full-scale systems, it is difficult to balance the nitrate load to RBCOD load since there are daily variations and some degree of denitrification takes place in the secondary clarifier. Periods with lower nitrate concentration or temporarily anaerobic conditions in the anoxic selector are expected. These conditions are not necessarily harmful for the sludge settling characteristics because in a plug anoxic selector an important fraction of RBCOD can be stored by ordinary heterotrophic organisms [118,146,172] or used by the phosphorus-accumulating organisms (PAOs) [173] cited by Marten and Daigger [118,174] or by glycogen-accumulating non-polyphosphate organisms (GAOs) [175–179]. However, the leakage of RBCOD to the aeration basin, and subsequently bulking sludge, can occur if the anoxic selector has a reduced storage capacity (e.g., in completely mixed systems). More research is needed to uncover the key factors on the competition between these microorganisms. In the meantime to design a reliable full-scale anoxic selector it is advisable to first perform pilot-plant studies and only then scale-up the system.

6.3.2. Anaerobic selectors

Under strictly anaerobic conditions (e.g., in UCT-type processes) the soluble substrate, mainly volatile fatty acids and other simple substrates, are taken up and mostly stored. The design of anaerobic selectors follows the ratio of RBCOD uptake rate to phosphorus release rate, which is needed for phosphorus removal, making sure that virtually no RBCOD enters the main aeration basin (Table 5). These conditions were created in activated sludge systems to promote the growth of PAOs. However, another group of bacteria, known as GAOs, can grow quite well in similar conditions (e.g., [176,179]. Both types of bacteria are capable of taking

Table 5
Selector design guidelines recommended for aerobic, anoxic, and anaerobic selectors in domestic wastewater treatment systems

Parameter	Value	Reference
Aerobic selector		
Number of compartments	$\geqslant 3$	Jenkins et al. [1]
Contact time	10–15 min, But it depends on load, temperature, and wastewater composition (i.e. Fraction of RBCOD)	Eikelboom [154], Daigger et al. [149] Van Niekerk et al. [133], and Martins et al. [46]
Sludge loading rate	12 (1st comp.), 6 (2nd comp.). and 3 (3rd comp.) Kg COD kg^{-1} MLSS d^{-1}	Jenkins et al. [1]
Floc loading	50–150 g COD kg TSS^{-1} (1st comp)	Heide and Pasveer [155], Eikelboom [154], and Kruit et al. [61]
DO concentration	$\geqslant$ 2 mg O_2 L^{-1}, but it depends on the sludge loading rate, floc loading rate, and/or substrate uptake rate. Sensor should be placed in the 1st comp	Casey et al. [163], Sezgin et al. [74], Palm et al. [4], Albertson [15], and Martins et al. [47]
Anoxic selector		
Number of compartments	$\geqslant 3$	Jenkins et al. [1]
Sludge loading rate	6 (1st comp.), 3 (2nd comp.), and 1.5 (3rd comp.) kg COD kg^{-1} MLSS d^{-1}	Daigger and Nicholson [150], Albertson [152], and Jenkins et al. [1]
Contact time	45–60 min	Kruit et al. [49]
$(RBCOD/NO_3\text{-}N)_{consumed}$	Usually higher than –79 mg RBCOD mg NO_3-N^{-1} due to storage	Randall et al. [166], Jenkins et al. [1], Wanner [16], Van Loosdrecht et al. [148], WEF [161], and Beun et al. [146]
Anaerobic selector		
Number of compartments	$\geqslant 3$, long channels (length-to-width ratio larger than 10:1)	Albertson [15] and Kruit et al. [49]
Contact time	1–2 h	WEF [161] and Kruit et al. [49]
$(COD_{VFA+fermentable}/PO_4\text{-}P)_{inf}$	9–20 g COD g P^{-1}	Wentzel et al. [167] and Smolders et al. [168]

up simple soluble substrates in the anaerobic stage and store it as poly hydroxy-alkanoates (PHA). The energy reserve that allows the uptake and storage mechanisms is, however, different in both types of bacteria. Polyphosphate is used in case of PAOs and glycogen in case of GAOs. This metabolic diversity gives a great flexibility to the anaerobic selector in removing the organic load, independently of the occurrence of phosphorus removal. Furthermore, in spite of the great diversity of PAOs and GAOs no filamentous bacteria have been unequivocally identified so far as having this metabolism.

As result of the availability and consumption of RBCOD in the anaerobic stage, PAOs and GAOs accumulate in the sludge and obligate aerobic microorganisms supposedly decrease in number, as they lack substrate in the aerobic phase. Thus, the more substrate is removed from the anaerobic stage, which also means less substrate available in the oxic stage, the better should be the settling characteristics of the activated sludge. Furthermore, sludge rich in poly P bacteria settle usually better because they form dense clusters and intracellular polyphosphate [180], in combination with chemical phosphorus precipitation, and increases the sludge density even more. Recent reports have been confirmed the success of (plug-flow) anaerobic selectors in controlling sludge bulking [49,181], even when *M parvicella* is the most dominant filamentous bacteria [49]. An anaerobic selector, however, cannot be always used. For instance, its application is not recommended for waste streams rich in sulphur compounds. Anaerobic conditions can favour even more the production of reduced sulphur compounds, which can be used in the aerobic stage by filamentous sulphur oxidising bacteria [182].

6.3.3. *Contradictory observations about the effectiveness of selectors in BNR systems*

Even though we might think that the incorporation of (plug-flow) anoxic and/or anaerobic stages into activated sludge systems could impose a strong selective pressure in bacteria, giving competitive advantages to non-filamentous bacteria, it turned out to be not always the case, at least not for all the filamentous bacteria. In fact, a shift in the predominant filamentous bacteria seems to have occurred with the introduction of BNR systems (e.g., [3,50,51,55]). Therefore, questions arise about the effectiveness of selectors, mostly anoxic and anaerobic, in bulking control of BNR systems.

Albertson [15] argued that bulking sludge occurs in many BNR systems because the anaerobic, anoxic and aerobic zones in these systems are in the form of single completely mixed reactors. He suggested that bulking could be controlled if high macro-gradients of substrate concentration were imposed in the systems by a proper compartmentalisation. Recent studies in The Netherlands showed that well settling sludge (SVI $<120\,ml\,g^{-1}$ with common values below $100\,ml\,g^{-1}$) could be achieved in full-scale BNR systems by implementing a well- controlled strictly anaerobic and anoxic plug-flow selectors [49]. Further possible factor which led to better sludge settleability was the introduction of an aerobic reactor after the anoxic/aerobic stage to create simultaneously a low ammonium concentration ($<1\,mg\,N\,L^{-1}$) and a high DO concentration ($>1.5\,mg\,O_2\,L^{-1}$) [49,144,183]. These observations are in agreement with the general assumption that compartmentalised anoxic or anaerobic selectors, designed to create not only the metabolic selection [67] but also the selection by adopting a plug-flow configuration, leads to well settling sludge. An example of a treatment system based on the considerations is the BCFS® concept [184] of which 12 full-scale plants are currently successful in operation in The Netherlands.

However, there are also studies that show that the introduction of anoxic or anaerobic compartmentalised selectors did not control bulking. Ekama et al. [38] stated that a compartmentalised anaerobic zone (four equally sized completely mixed reactors) with a mass fraction of 0.16, which seems to resemble a plug-flow anaerobic selector [34] cited by Ekama et al. [38] or a first plug-flow anoxic zone with a mass fraction of 0.14, resembling an anoxic selector [35] cited by Ekama et al. [38] were not successful in bulking sludge control. Aerobic selectors (DO concentration in the range 2.0–$4.0\,mg\,O_2\,L^{-1}$) were also tested. Both with and without aerobic selectors present, low F/M filamentous bacteria (mainly the morphotypes *M. parvicella* and Types 0092, 0041/0675, 0914 and 1851) always proliferated in systems with alternating short periods of anoxic–aerobic conditions such as that occurring in oxidation ditch systems [39–43]. The authors concluded that neither kinetic selection nor metabolic selection control bulking by low F/M microorganisms and that the aeration pattern, namely short alternating anoxic/oxic periods, typical of oxidation ditch-type systems, appeared to be the most important factor promoting the growth of the low F/M filamentous bacteria. From these results together with a literature review the authors proposed the NO hypothesis (formerly described) to explain the competition between low F/M filamentous bacteria and floc-forming bacteria [141–143]. Further research work is needed to verify this hypothesis but some concerns, previously mentioned, do exist about its validity.

7. Mathematic modelling

To study complex ecosystems, like activated sludge cultures, in which many factors are acting together, mathematical modelling can be a very useful tool. Much progress has been achieved in this field in spite of the extreme complexity of activated sludge population

dynamics. The Activated Sludge Models (ASM 1, 2, 2D and 3) published by the IWA task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment [185–188] are examples of useful models to study population dynamics in activated sludge systems. As the knowledge of bacterial physiology increases, the models are continuously upgraded. An example is the incorporation of storage processes in ASM 3 [185]. This is a first attempt to allow for modelling of storage polymer metabolism and to better describe the conversions occurring in selector-like systems. Also recently developed metabolic models provide a better link between the kinetics and the biochemistry of storage [146,189], and will certainly contribute to the description and modelling of the metabolic processes that take place in selectors. Despite the great detail in these models the growth of filamentous bacteria and, thus, bulking sludge, still cannot be predicted.

Models that can predict the settling characteristics of the activated sludge are in an early phase of development. Some models already exist to predict the development of filamentous and non-filamentous bacteria considering either a dual species or a group competition (e.g., floc formers, filaments, low DO filaments, low F/M filaments) for single substrate or for group of substrates (RBCOD or SBCOD) [5,72,125,159,190–200]. These models can be basically grouped into two groups: one considering the bacterial physiology and kinetics–biokinetic models; and another one considering both the physiology and kinetics, and the morphology of bacteria.

Diffusional transport of substrates into the activated sludge flocs is an important mechanism in the competition between floc-forming bacteria and filamentous bacteria. Lau et al. [125] developed the first bulking sludge mathematical model incorporating simultaneous diffusion of soluble organic substrate and DO through flocs with predetermined shape. Parameters like bulk liquid soluble organic substrate and DO concentration and floc shapes and sizes were used to predict the volume-averaged growth rate of filamentous bacteria (*S. natans*) and non-filamentous bacteria (*Citrobacter* sp.). The kinetic parameters, which were experimentally measured, had values according to the kinetic selection theory. The results of this model cannot be extrapolated because either the kinetic parameters do not apply to other filamentous or non-filamentous bacteria [85], or the representativeness of the model microorganisms in activated sludge systems can be questioned. In spite of these limitations the model illustrates some aspects that may match the reality. For instance, the model predicts that a cylindrical floc has less resistance to substrate diffusion than equal-volume spherical flocs, which is in line with recent experimental observations about the roughness of bacterial floc structures usually found in bulking sludge systems [46]. Furthermore, the study warned that the one-dimensional (unidirectional) growth of filamentous bacteria might lead to a floc geometry that is better for substrate diffusion. Also Kappeler and Gujer [72] proposed that RBCOD could favour the growth of filamentous microorganisms due to substrate diffusional resistance in the biological flocs. Unlike the former model, similar kinetic parameters, i.e. maximum growth rate and intrinsic substrate half-saturation coefficient, were adopted. Apparent RBCOD half-saturation coefficients for filamentous microorganisms were considered to be lower than those for non-filamentous bacteria to represent the differences in substrate diffusion resistance.

Later studies took into account both the micromorphology of the floc and the oriented growth characteristics of the filamentous bacteria (preferential unidirectional growth) [200]. This study was the first attempt to combine the morphological characteristics with the physiology of filamentous and non-filamentous bacteria. Three groups of microorganisms (floc formers, low dissolved oxygen filaments and low F/M filaments) were considered, with kinetic parameters following the trend indicated by the kinetic selection theory, and different scenarios of soluble substrate and DO were simulated. The simulation of the activated floc structure under diffusion governed conditions showed, as expected, that the filamentous bacteria predominate in soluble substrate and DO limited environments. The authors did not differentiate between the effect of kinetic parameters and the effect of cell morphology as such.

Recent studies showed that the coexistence of floc formers and filaments could not be predicted in activated sludge systems with simple models according to kinetic selection theory [190]. The kinetics, solids retention time and the substrate feed concentration determine which type of bacteria will remain in the system. The two types of bacteria could only coexist at a single solids retention time which is not feasible in practice [190]. The introduction of the backbone theory in the model allowed coexistence of both organisms [191]. Others factors (i.e. storage and decay rates) were later added to model the competition [197]. It is evident that experimental verification is needed with respect to all these factors. Furthermore, diffusion, a well-known physical process, of substrates into the activated sludge flocs should be considered and evaluated through the models.

In summary, modelling can be used to better evaluate the role of unidirectional growth of filamentous bacteria together with the expected higher capacity of filamentous bacteria to grow according to the substrate microgradient in sludge flocs, under a wide range of kinetic parameters. More research efforts should be placed on the role of bacterial morphology and diffusion on this competition because the kinetic parameters, namely the

intrinsic substrate half-saturation coefficient, storage capacity and decay rates, are largely unknown. This kind of studies may lead to a better understanding in the competition between filamentous and non-filamentous bacteria in gradient-governed microenvironments so typical of activated sludge systems.

8. Research questions

Despite the great amount of research that was done on bulking sludge, it still occurs world-wide and a definitive solution does not seem to be available. Partly this is due to the fact that the problem has usually been approached from either an engineering (general solution) or a microbiological (species specific) point of view. To have more insight on the factors promoting the growth of filamentous bacteria both disciplines have to be integrated. An increased knowledge about processes like bacterial morphology and physiology, diffusion, substrate kinetics and substrate storage, hydrolysis and role of particulate substrate, bacteria identification and quantification by molecular methods, flocculation, detachment and attachment, competition for multiple limiting substrates, and other microbial interactions such as commensalism, mutualism, parasitism or predation, as well as models refinement in order to improve their predictions, is needed to better understand the complex bulking sludge phenomena.

8.1. Sludge architecture

Do filamentous bacteria form a structural element of floc architecture? More insight is needed to describe the internal architecture of the flocs (filamentous bacteria/non-filamentous bacteria/EPS). The application of different microscopic and molecular techniques, namely transmission electron microscopy in conjunction with specific methods and CLSM together with specific gene probes and microelectrodes, are useful tools to accomplish this task.

8.2. Bacteria identification and physiology

Can the metabolism of representative filamentous bacteria be sustained under anaerobic and/or anoxic-aerobic cyclic conditions with fully organic substrate removal in the anaerobic and/or anoxic stages? At least some filamentous bacteria can store the organic substrate in anaerobic or anoxic conditions, which potentially give them advantages in biological nutrient removal systems (e.g., *M parvicella* in [107,108,112,122]). Additional research is also needed to verify if growth is sustained under these conditions.

Great research efforts should be made to enable automatic detection by molecular methods (e.g., FISH), and quantification (e.g., microarray/DNA chips and flow cytometry) for the rapid screening of filamentous bacteria in wastewater treatment systems and on bacterial physiology. Possible changes in the bacterial morphology with the growth conditions should also be studied by molecular methods. Some microorganisms are very difficult or even impossible to grow in pure culture. In situ techniques such as FISH-MAR (e.g., [106–108,124,134]) have to be applied in this case. Microsensor measurements combined with FISH, and eventually MAR, can also give some important information about the functional state, like metabolic activities, and bacterial growth over time. Recently, the combined use of FISH and microsensors allowed the analyses of bacterial communities and metabolic activities simultaneously, thereby revealing anaerobic and anoxic microniches in flocs within aerobic environments [127,201]. Growth of filamentous bacteria in substrate gradient environments (e.g., sludge flocs) could also be better studied by combining these techniques. The development of a large data bank with information about the wastewater treatment system and population structure and function [105] is also recommended.

8.3. Role of particulate substrates

Do particulate substrates (and which ones) lead to bulking sludge? Although particulate substrate, mainly SBCOD, is an important fraction of the total COD present in the wastewater [61,72,143,202] little research has been done about its effect on the development of filamentous bacteria. From a mechanistic point of view SBCOD is believed to be advantageous for floc-forming bacteria [72], since it is unlikely that hydrolysis products could diffuse to outside the floc as hypothesised by others [203]. Unfortunately, most of the adsorption, hydrolysis, uptake and storage mechanisms of complex substrates, such as lipids, are not well known. Further research is clearly needed to unravel these mechanisms and to clarify their role in the growth of filamentous bacteria.

8.4. Storage polymers

Is there a difference in storage metabolism between floc-forming bacteria and filamentous bacteria? Storage polymers are an important aspect of activated sludge processes, especially in selector-like systems. Literature data regarding quantification of storage polymers in such highly dynamic conditions and the relation with sludge bulking are still scarce and the exact role of substrate storage in sludge bulking control is still not fully clarified. Some results from lab-scale systems are already available but especially in full-scale systems more research is needed. Although techniques to

measure important storage polymers such as PHA, glycogen and poly phosphate are available, more analytical methods are needed to measure other internal storage polymers (e.g., lipids). On the other hand, the analytical methods, and thus the studies, should show clearly how a distinction is made between the internal stored substrate and the adsorbed and intracellular non-stored material (e.g., internal stored lipid and membrane phospholipids; glycogen and other adsorbed and intracellular sugars).

8.5. Selectors

Can safe design guidelines be well defined for selectors? What are the safe design guidelines? Since selectors are the only known preventive engineering tools capable of minimising bulking sludge more research should be directed to better define its design and operating criteria. For instance, some doubts still exist about the necessity to design anoxic and anaerobic selectors with a plug-flow regime. At present the design criteria are mostly based on empirical observations. Further research studies should aim to define design guidelines based on understanding the mechanism of selector function, like bacterial physiology and storage processes, in order to define reliable and efficient operating strategies and not trial-and-error approaches. More research is also needed to clarify the role of short anaerobic/anoxic/aerobic cycles, typical of oxidation ditch systems, on the growth of filamentous bacteria.

Comparative effectiveness selector studies could be performed in cases where either an aerobic and, specially, anoxic or anaerobic selector could be applied. To make a good assessment of the selector, performance mass balances, rates and ratios between several parameters (e.g., COD, NO_3^-, P, OUR, NUR, $P_{released}/RBCOD_{consumed}$, F:M, storage polymers) should be performed in the selector, as well as in the control system, in parallel with the sludge settleability evaluation and filamentous bacteria identification and quantification.

8.6. Control and monitoring

How can selectors be safety and robust controlled? Most of the selectors currently in place in full-scale systems are merely seen as an additional stage, and hence the operational working conditions are often forgotten. Selector's role for the proper operation of the biological system can be huge; therefore, control parameters for operation of selectors are needed. The use of a reliable, robust and proactive process control, based, for instance, on redox measurements [184], is needed to prevent microaerophilic conditions or the introduction of nitrate and oxygen in the anaerobic and oxygen in the anoxic stages, respectively. These are example of essential process control measures, which are needed to develop good control strategies for bulking sludge.

Is an early detection system for filamentous bacteria possible and useful? Predictive methods of bulking sludge have also to be developed and implemented in control strategies of wastewater treatment plants. For instance, the early detection and quantification of filamentous bacteria by molecular tools together with automated analytical techniques like microarray/DNA chips and flow cytometry, could allow routine and precise filamentous bacteria identification and quantification. Population shifts could then serve as indicators for upcoming bulking sludge events. Control strategies could then be developed and implemented in a more proactive way, instead of the commonly used feedback control. However, it should be kept in mind that a full-scale system has usually a limited flexibility and the operational staff is many times reluctant to changes in the process. Besides safe and robust, control measures should be as simple as possible.

8.7. Mathematical modelling

Can a universal model (e.g., general activated sludge model—ASM type) for bulking sludge be developed? Mathematical modelling is a must in the study of population dynamics of activated sludge systems. In such complex systems where many factors are acting together, mathematical modelling can help in the understanding of the biological processes. For instance, selectors are hardly ever modelled and its design guidelines are still mainly based on empirical observations. More insights into selector systems could be achieved if modelling was applied, leading to a faster control and optimisation of the selector and, therefore, of the wastewater treatment system.

The key for the study of different bulking sludge scenarios is considered to be the interaction between bacterial morphology and bacterial physiology in gradient-governed microenvironments, usually present in activated sludge systems. A floc model is needed where the micro-gradients of nutrients are the key factor in the growth of filamentous bacterial structures. The simulation of preferential uni- or bidirectional growth of filamentous bacteria together with the development of dynamic floc structures could help in the understanding of the different sludge settleability characteristics. More knowledge about other processes like multiple limiting substrates or other microbial interactions could also allow better model predictions. Further challenge will be to integrate all this knowledge in a general activated sludge model type.

9. Conclusions

The main conclusions of the review are:

- There are hypothesis to explain the development of filamentous bacteria but no final mechanistic proof exists about their validity.
- Bulking filamentous sludge appears related to the occurrence of steep micro-gradients of nutrient concentration in flocs. Providing these gradients using sufficient high concentration of both electron donor and electron acceptor, and remaining nutrients, (i.e. plug-flow selector systems) seems to avoid the morphological ecological advantage of filamentous bacteria.
- Modelling key processes such as floc morphology, growth conditions, diffusion and substrate kinetics of both electron donor and electron acceptor and substrate storage is a framework needed to predict floc properties and selector behaviour.
- A state-of-the-art activated sludge BNR system, designed to minimise bulking sludge problems, is proposed with the following general characteristics: a pre-treatment step to remove complex substrates (e.g., lipids), plug-flow selector reactors to allow a strong macro-gradient of substrate concentration along the system; well-defined anaerobic, anoxic and aerobic plug-flow stages and exclusion of oxygen from the anoxic stage, and nitrate and oxygen from the anaerobic stage; avoid systems with intermittent aeration and microaerophilic conditions; good aeration to maintain high DO concentration ($>1.5\,mg\ O_2\,L^{-1}$) and low ammonium concentration ($<1\,mg\ N\,L^{-1}$) in the final aerobic stage.
- A set of open research questions related with sludge architecture, bacteria identification and physiology, role of particulate substrates, storage polymers, selectors, control and monitoring and mathematical modelling, is identified.

Acknowledgements

At the time this paper was prepared, António Martins and Krishna Pagilla were respectively a Ph.D. student and a visiting researcher at the Department of Biochemical Engineering, Delft University of Technology. António Martins received financial support from the Portuguese State in the context of PRAXIS XXI by the Doctoral Scholarship BD/19538/99.

References

[1] Jenkins D, Richard MG, Daigger GT. Manual on the causes and control of activated sludge bulking and foaming, 2nd ed.. Michigan: Lewis Publishers; 1993.

[2] Donaldson W. Use of activated sludge increasing. Civil Eng 1932;2(3):167–9.

[3] Krhutková O, Ruzickova I, Wanner J. Microbial evaluation of activated sludge and filamentous population at eight Czech nutrient removal activated sludge plants during year 2000. Water Sci Technol 2002;46(1/2):471–8.

[4] Palm JC, Jenkins D, Parker DS. Relationship between organic loading, dissolved oxygen concentration and sludge settleability in the completely mixed activated sludge process. J Water Pollut Control Fed 1980;52: 2484–506.

[5] Kappeler J, Gujer W. Verification and applications of a mathematical model for "aerobic bulking". Water Res 1994;28(2):311–22.

[6] Kaewpipat K, Grady Jr CPL. Population dynamics in laboratory-scale activated sludge reactors. Water Sci Technol 2002;46(1–2):19–27.

[7] Eikelboom DH. Filamentous organisms observed in activated sludge. Water Res 1975;9:365–88.

[8] Eikelboom DH. Identification of filamentous organisms in bulking activated sludge. Prog Water Technol 1977; 8:153–62.

[9] Eikelboom DH, Van Buijsen HJJ. Microscopic sludge investigation manual. TNO report A 94A, 2nd Edition, 1981.

[10] Eikelboom DH. Process control of activated sludge plants by microscopic investigation. London, UK: IWA Publishing; 2000.

[11] Jenkins D, Richard MG, Daigger GT. Manual on the causes and control of activated sludge bulking and foaming. Water Research Commission, P.O. Box 824, Pretoria 0001, 1984.

[12] Chudoba J, Grau P, Ottová V. Control of activated sludge filamentous bulking — II. Selection of microorganisms by means of a selector. Water Res 1973;7(10):1398–406.

[13] Rensink JH. New approach to preventing bulking sludge. J Water Pollut Control Fed 1974;46(8):1888–94.

[14] Alleman JE, Prakasam TBS. Reflections on seven decades of activated sludge history. J Water Pollut Control Fed 1983;55(5):436–43.

[15] Albertson OE. The control of bulking sludges: from the early innovators to current practice. J Water Pollut Control Fed 1987;59(4):172–82.

[16] Wanner J. Activated sludge bulking and foaming control. Lancaster, PA: Technomic Publishing Co. Inc.; 1994.

[17] Orhon D, Artan N. Modelling of activated sludge systems. Lancaster, PA: Technomic Publishing Co. Inc.; 1994.

[18] Casey TG, Ekama GA, Wentzel MC, Marais GvR. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 1: a historical overview of causes and control. Water SA 1995;21(3):231–8.

[19] Ardern E, Lockett WT. Experiment on the oxidation of sewage without the aids of filters. J Soc Chem Ind 1914;33:523–39.

[20] Chambers B. Effect of longitudinal mixing and anoxic zones on settleability of activated sludge. In: Chambers B, Tomilson EJ, editors. Bulking of activated sludge preventative and remedial methods. Chichester, UK: Ellis Horwood Limited; 1982. p. 166–86.

[21] Chudoba J, Ottová V, Madera V. Control of activated sludge filamentous bulking — I. Effect of the hydraulic regime or degree of mixing in an aeration tank. Water Res 1973;7(9):1163–82.
[22] Houtmeyers J, Van den Eynde E, Poffé R, Verachtert H. Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes I. Influence of process parameters. Eur J Appl Microbiol Biotechnol 1980;9:63–77.
[23] Tomlinson EJ. Bulking—a survey of activated sludge plants. Technical report TR35, Elder Way, Stevenhage, Herts, England: Water Research Centre, Stevenhage Laboratory; 1976.
[24] Van den Eynde E, Vriens L, Wynants M, Verachtert H. Transient behaviour and time aspects of intermittently and continuously fed bacterial cultures with regard to filamentous bulking of activated sludge. Appl Microbiol Biotechnol 1984;19:44–52.
[25] Van den Eynde E, Vriens L, De Cuyper M, Verachtert H. Plug flow simulating and completely mixed reactors with a premixing tank, in the control of filamentous bulking. Appl Microb Biotechnol 1984;19:288–95.
[26] Verachtert H, Van den Eynde E, Poffé R, Houtmeyers J. Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. II. Influence of substrate present in the influent. Eur J Appl Microbiol Biotechnol 1980;9:137–49.
[27] Pasveer A. A contribution to the development in activated sludge treatment. J Proc Inst Sewage Purif 1959;4:436.
[28] Pasveer A. A case of filamentous activated sludge. J Water Pollut Control Fed 1969;41(7):1340–52.
[29] Irvine RL, Davis WB. Use of sequencing batch reactors for waste treatment. In CPC International Corpus Christi, Texas, 26th Annual Purdue Industrial Waste Conference, Purdue University. West Lafayette: Ann Arbor Science Publ.; 1971. p. 450–62.
[30] Irvine RL, Busch AW. Sequencing batch biological reactors — an overview. J Water Pollut Control Fed 1979;51(2):235–43.
[31] Irvine RL, Ketchum Jr LH. Sequencing batch reactors for biological wastewater treatment. CRC Crit Rev Environ Control 1989;18(4):255–94.
[32] Irvine RL, Wilderer PA, Flemming H-C. Controlled unsteady state processes and technologies—an overview. Water Sci Technol 1997;35(1):1–10.
[33] Wilderer PA, Irvine RL, Goronszy MC. Sequencing batch reactor technology. Scientific and Technical Report No. 10. Great Britain: IWA Publishing; 2001.
[34] Bagg WK, Burke RA, Wentzel MC, Dold PL, Loewenthal RE, Ekama GA, Marais GvR. Executive Summary Report to the Water Research Commission on the Research Contract Biological phosphorus removal in the activated sludge process, Department of Civil Engineering, University of Cape Town.
[35] Clayton AJ, Wentzel MC, Ekama GA, Marais GvR. Denitrification kinetics in biological nitrogen and phosphorus removal systems treating municipal wastewaters. Water Sci Technol 1991;23(4-6):567–76.
[36] Lee S-E, Koopman BL, Jenkins D, Lewis RF. The effect of aeration basin configuration on activated sludge bulking at low organic loading. Water Sci Technol 1982;14(6/7):407–27.
[37] Linne SR, Chiesa SC. Operational variables affecting performance of the selector-complete mix activated sludge process. J Water Pollut Control Fed 1987;59(7):716–21.
[38] Ekama GA, Wentzel MC, Casey TG, Marais GvR. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 6: review, evaluation and consolidation of results. Water SA 1996;22(2):147–52.
[39] Gabb DMD, Still DA, Ekama GA, Jenkins D, Marais GvR. The selector effect on filamentous bulking in long sludge age activated sludge systems. Water Sci Technol 1991;23:867–77.
[40] Gabb DMD, Ekama GA, Jenkins D, Wentzel MC, Casey TG, Marais GvR. Filamentous organism bulking in nutrient removal activated sludge. Paper 4: system configurations and operating conditions to develop low F/M filament bulking sludges at laboratory-scale. Water SA 1996;22(2):127–37.
[41] Gabb DMD, Ekama GA, Jenkins D, Wentzel MC, Casey TG, Marais GvR. Filamentous organism bulking in nutrient removal activated sludge. Paper 5: experimental examination of aerobic selectors in anoxic–aerobic systems. Water SA 1996;22(2):139–46.
[42] Lakay MT, Hulsman A, Ketley D, Warburton C, de Villiers M, Casey TG, Wentzel MC, Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 7: exploratory experimental investigations. Water SA 1999;25(4):383–96.
[43] Musvoto EV, Lakay MT, Casey TG, Wentzel MC, Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 8: the effect of nitrate and nitrite. Water SA 1999;25(4):397–407.
[44] Strom PF, Jenkins D. Identification and significance of filamentous microorganisms in activated sludge. J Water Pollut Control Fed 1984;56(5):449–59.
[45] Wanner J, Grau P. Identification of filamentous microorganisms from activated sludge: a compromise between wishes, needs and possibilities. Water Res 1989;23(7):883–91.
[46] Martins AMP, Van Loosdrecht MCM, Heijnen JJ. Effect of feeding pattern and storage on the sludge settleability under aerobic conditions. Water Res 2003;37(11):2555–70.
[47] Martins AMP, Van Loosdrecht MCM, Heijnen JJ. Effect of dissolved oxygen concentration on the sludge settleability. Appl Microbiol Biotechnol 2003;62(5–6):586–93.
[48] STOWA. Control of the sludge settleability in municipal wastewater treatment plants with biological nutrients removal. Report 2001-02, 2001 Stichting Toegepast Onderzoek Waterbeheer, Postbus 8090, 3503 RB Utrecht, The Netherlands [in Dutch].
[49] Kruit J, Hulsbeek J, Visser A. Bulking sludge solved?!. Water Sci Technol 2002;46(1/2):457–64.
[50] Blackbeard JR, Ekama GA, Marais GvR. A survey of bulking and foaming activated sludge plants in South Africa. J Water Pollut Control Fed 1986;85(1):90–100.
[51] Blackbeard JR, Gabb DMD, Ekama GA, Marais GvR. Identification of filamentous organisms in nutrient

removal activated sludge plants in South Africa. Water SA 1988;14(1):1–18.
[52] Mino T. Bulking, foaming problems in Japan. Internal document, Department of Environmental and Urban Engineering, The University of Tokyo, 1999.
[53] Mino T. Survey on filamentous microorganisms in activated sludge processes in Bangkok, Thailand. Water Sci Technol 1995;31(9):193–202.
[54] Kristensen GH, Jorgensen PE, Nielsen PH. Settling characteristics of activated sludge in Danish treatment plants with biological nutrient removal. Water Sci Technol 1994;29(7):157–65.
[55] Eikelboom DH, Andreadakis A, Andreasen K. Survey of filamentous populations in nutrient removal plants in four european countries. Water Sci Technol 1998;37(4/5):281–9.
[56] Pujol R, Canler JP. Contact zone: French practice with low F/M bulking control. Water Sci Technol 1994; 29(7):221–8.
[57] Kunst S, Reins M. Practical investigations on bulking and foaming in activated sludge plants with biological phosphorus removal. Water Sci Technol 1994;29(7): 289–94.
[58] Rossetti S, Carucci A, Rolle E. Survey on the occurrence of filamentous organisms in municipal wastewater treatment plants related to their operating conditions. Water Sci Technol 1994;29(7):305–8.
[59] Madoni P, Davoli D, Gibin G. Survey of filamentous microorganisms from bulking and foaming activated-sludge plants in Italy. Water Res 2000;34(6):1767–72.
[60] Eikelboom DH. The *Microthrix parvicella* puzzle. Water Sci Technol 1994;29(7):271–9.
[61] Kruit J, Boley F, Jacobs LJAM, Wouda TWM. Prediction of the O_2 conditions in the selector. Water Sci Technol 1994;29(7):229–37.
[62] Foot RJ. The effects of process control parameters on the composition and stability of activated sludge. J Inst Water Environ Management 1992;6:215–28.
[63] Lavender P, Cowley E, Horan NJ. Experiences with activated sludge bulking in UK. In: Růžičková I, Wanner J, editors. Conference Proceedings Poster Papers of the Ninth IWA Specialised Conference on Design, Operation and Economics of Large Wastewater Treatment Plants. Praha, Czech Republic; 2003. p. 171–4.
[64] Switzenbaum MS, Plante TR, Woodworth BK. Filamentous bulking in Massachusetts: extent of the problem and case studies. Water Sci Technol 1992;25(4-5):265–71.
[65] Seviour EM, Williams C, DeGrey B, Soddell JA, Seviour RJ, Lindrea KC. Studies on filamentous bacteria from Australian activated sludge plants. Water Res 1994; 28(11):2335–42.
[66] Di Marzio WD. First results from a screening of filamentous bacteria in Buenos Aire's activated sludge plants. Water Sci Technol 2002;46(1/2):119–22.
[67] Wanner J, Chudoba J, Kucman K, Proske L. Control of activated sludge filamentous bulking—VII. Effect of anoxic conditions. Water Res 1987;21(12):1447–51.
[68] Wanner J, Kucman K, Ottová V, Grau P. Effect of anaerobic conditions on activated sludge filamentous bulking in laboratory systems. Water Res 1987;21(12): 1541–6.
[69] Wanner J, Grau P. Filamentous bulking in nutrient removal activated sludge systems. Water Sci Technol 1988;20(4-5):1–8.
[70] Amann R, Ludwig W, Schleifer K-H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 1995;59(1): 143–69.
[71] Wagner M, Amann R, Kampfer P, Assmus B, Hartmann A, Hutzler P, Springer N, Schleifer K-H. Identification and in situ detection of gram-negative filamentous bacteria in activated sludge. Syst Appl Microbiol 1994; 17:405–17.
[72] Kappeler J, Gujer W. Development of a mathematical model for "aerobic bulking". Water Res 1994;28(2): 303–10.
[73] Pipes WO. Bulking of activated sludge. Adv Appl Microbiol 1967;9:185–234.
[74] Sezgin M, Jenkins D, Parker DS. A unified theory of filamentous activated sludge bulking. J Water Pollut Control Fed 1978;50(2):362–81.
[75] Jenkins D. Towards a comprehensive model of activated sludge bulking and foaming. Water Sci Technol 1992; 25(6):215–30.
[76] Parker DS, Kaufman J, Jenkins D. Physical conditioning of activated sludge floc. J Water Pollut Control Fed 1971;43(9):1817–33.
[77] Urbain V, Block JC, Manem J. Bioflocculation in activated sludge: an analytic approach. Water Res 1993;27(5):829–38.
[78] Liss SN, Droppo IG, Flannigan DT, Leppard GG. Floc architecture in wastewater and natural riverine systems. Environ Sci Technol 1996;30(2):680–6.
[79] Schmid M, Thill A, Purkhold U, Walcher M, Bottero J, Ginestet P, Nielsen P, Wuertz S, Wagner M. Characterization of activated sludge flocs by confocal laser scanning microscopy and image analysis. Water Res 2003;37(9):2043–52.
[80] Murray RGE, Williams ST, Sharpe ME. Bergey's manual of systematic bacteriology. Baltimore: Williams and Wilkins; 1989.
[81] Buali AM, Horan NJ. Variable morphology in certain filamentous bacteria and implications of this for theories of activated sludge bulking. Environ Technol Lett 1989;10:941–50.
[82] Richard MG, Hao O, Jenkins D. Growth kinetics of Sphaerotilus species and their significance in activated sludge bulking. J Water Pollut Control Fed 1985;57: 68–81.
[83] Seviour EM, Blackall LL, Christensson C, Hugenholtz P, Cunningham MA, Bradford D, Stratton HM, Seviour RJ. The filamentous morphotype Eikelboom type 1863 is not a single genetic entity. J Appl Microb 1997;82:411–21.
[84] IWA. Microorganisms in activated sludge and biofilm processes III. In: Tandoi V, and the International Programme Committee, editors. Selected Proceedings of the Third IWA International Specialized Conference on Microorganisms in Activated sludge and Biofilm Processes. Rome, Italy. Water Science Technol Vol. 46 (1/2). London, UK: IWA Publishing; 2002.
[85] Seviour RJ, Blackall LL. The microbiology of activated sludge. Dordrecht: Kluwer Academic Publishers; 1999.

[86] Liu JR, Burrel P, Seviour EM, Soddell JA, Blackall LL, Seviour RJ. The filamentous bacteria morphotype '*Nostocoida limicola*' I contains at least two previously described genera in the low G + C Gram positive bacteria. Syst Appl Microbiol 2000;23:528–34.

[87] Liu JR, Seviour RJ. Design and application of oligonucleotide probes for fluorescent in situ identification of the filamentous bacterial morphotype *Nostocoida limicola* in activated sludge. Environ Microbiol 2001;3(9):551.

[88] Seviour RJ, Liu JR, Seviour EM, McKenzie CA, Blackall LL, Saint CP. The "*Nostocoida limcola*" story: the phylogeny of this morphotype responsible for bulking in activated sludge. Water Sci Technol 2002;46(1/2): 105–10.

[89] Blackall L, Seviour E, Bradford D, Rossetti S, Tandoi V, Seviour R. '*Candidatus Nostocoida limicola*', a filamentous bacterium from activated sludge. Int J Syst Evol Microbiol 2000;50(2):703–9.

[90] Liu RL, McKenzie CA, Seviour EM, Webb RI, Blackall LL, Saint CP, Seviour RJ. Phylogeny of the filamentous bacterium "*Nostocoida limicola*" III from activated sludge. Int J Syst Evolut Microbiol 2001;51:195–202.

[91] Schade M, Beimfohr C, Lemmer H. Phylogenetic and physiological characterization of a "*Nostocoida limicola*"-like organisms isolated from activated sludge. Water Sci Technol 2002;46(1/2):91–7.

[92] Snaidr J, Beimfohr C, Levantesi C, Rossetti C, Waarde JVD, Geurkink B, Eikelboom D, Lemaitre M, Tandoi V. Phylogenetic analysis and in situ identification of "*Nostocoida limicola*"-like filamentous bacteria in activated sludge from industrial wastewater treatment plants. Water Sci Technol 2002;46(1/2):99–104.

[93] Eikelboom DH, Geurkink B. Filamentous microorganisms observed in industrial activated sludge plants. Water Sci Technol 2002;46(1/2):535–42.

[94] Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 1993;59:695–700.

[95] Wagner M, Amann R, Lemmer H, Schleifer K-H. Probing activated sludge with oligonucleotides specific for *Proteobacteris*: inadequacy of culture-dependent methods for describing microbial community structure. Appl Environ Microbiol 1993;59(5):1520–5.

[96] Wagner M, Erhart R, Manz W, Amann R, Lemmer H, Wedi D, Schleifer K-H. Development of an rRNA-targeted oligonucleotide probe specific for the genus *Acinetobacter* and its application for in situ monitoring in activated sludge. Appl Environ Microbiol 1994;60(3): 792–800.

[97] Kanagawa T, Kamagata Y, Aruga S, Kohno T, Horn M, Wagner M. Phylogenetic analysis of and oligonucleotide probe development for Eikelboom Type 021N filamentous bacteria isolated from bulking activated sludge. Appl Environ Microbiol 2000;66(11):5043–52.

[98] Kim SB, Goodfellow M, Kelly J, Saddler GS, Ward AC. Application of oligonucleotide probes for the detection of *Thiothrix* spp. in activated sludge plants treating paper and board mill wastes. Water Sci Technol 2002;46(1–2): 559–64.

[99] Beer M, Seviour EM, Kong Y, Cunningham M, Blackall LL, Seviour RJ. Phylogeny of the filamentous bacterium Eikelboom Type 1851, and design and application of a 16S rRNA targeted oligonucleotide probe for its fluorescence in situ identification in activated sludge. FEMS Microbiol Lett 2002;207:179–83.

[100] Davenport RJ, Curtis TP, Goodfellow M, Stainsby FM, Bingley M. Quantitative use of fluorescent in situ hybridization to examine relationships between mycolic acid-containing actinomycetes and foaming in activated sludge plants. Appl Environ Microbiol 2000;66(3): 1158–66.

[101] De los Reyes FL, Ritter W, Raskin L. Group-specific small-subunit rRNA hybridization probes to characterize filamentous foaming in activated sludge systems. Appl Environ Microbiol 1997;63:1107–17.

[102] De los Reyes MF, De los Reyes III FL, Hernandez M, Raskin L. Quantification of Gordona amarae strains in foaming activated sludge and anaerobic digester systems with oligonucleotide hybridization probes. Appl Environ Microbiol 1998;64(7):2503–12.

[103] Erhart R, Bradford D, Seviour R, Amann R, Blackall LL. Development and use of fluorescent in situ hybridization probes for the detection and identification of "*Microthrix parvicella*" in activated sludge. Syst Appl Microbiol 1997;20:310–8.

[104] Hugenholtz P, Tyson GW, Webb RI, Wagner AM, Blackall LL. Investigation and candidate division TM7, a recently recognized major lineage of the domain Bacteria with no known pure-culture representatives. Appl Environ Microbiol 2001;67(1):411–9.

[105] Wilderer PA, Bungartz H-J, Lemmer H, Wagner M, Keller J, Wuertz S. Modern scientific methods and their potential in wastewater science and technology. Water Res 2002;36:370–93.

[106] Andreasen K, Nielsen PH. Application of microautoradiography to the study of substrate uptake by filamentous microorganisms in activated sludge. Appl Environ Microbiol 1997;63:3662–8.

[107] Andreasen K, Nielsen P. Growth of *Microthrix parvicella* in nutrient removal activated sludge plants: studies of in situ physiology. Water Res 2000;34(5):1559–69.

[108] Nielsen PH, Roslev P, Dueholm TE, Nielsen JL. *Microthrix parvicella*, a specialized lipid consumer in anaerobic–aerobic activated sludge plants. Water Sci Technol 2002;46(1/2):73–80.

[109] Nowak G, Brown GD. Characteristics of *Nostocoida limicola* and its activity in activated sludge suspension. Res J Water Pollut Control Fed 1990;62:137–42.

[110] Horan NJ, Bu'Ali AM, Eccles CR. Isolation, identification and characterisation of filamentous and floc-forming bacteria from activated sludge flocs. Environ Technol Lett 1988;9:449–57.

[111] Kohno T, Sei K, Mori K. Characterization of Type 1851 organism isolated from activated sludge samples. Water Sci Technol 2002;46(1–2):111–4.

[112] Rossetti S, Tomei MC, Levantesi C, Ramadori R, Tandoi V. "*Microthrix parvicella*": a new approach for kinetic and physiological characterization. Water Sci Technol 2002;46(1/2):65–72.

[113] Tandoi V, Rossetti S, Blackall LL, Majone M. Some physiological properties of an Italian isolate of "*Microthrix parvicella*". Water Sci Technol 1998;37(4/5):1–8.
[114] Pellegrin V, Juretschko S, Wagner M, Cottenceau G. Morphological and biochemical properties of a *Sphaerotilus* sp. Isolated from paper mill slimes. Appl Environ Microbiol 1999;65(1):156–62.
[115] Shao Y-J, Jenkins D. The use of anoxic selectors for the control of low F/M activated sludge bulking. Water Sci Technol 1989;21:609–19.
[116] Williams TM, Unz RF. Isolation and characterization of filamentous bacteria present in bulking activated sludge. Appl Microbiol Biotechnol 1985;22:273–82.
[117] Ekama GA, Wentzel MC, Casey TG, Marais GvR. Filamentous organism bulking in nutrient removal activated sludge. Paper 3: stimulation of the selector effect under anoxic conditions. Water SA 1996;22(2): 119–26.
[118] Tampus M. The effect of anoxic selectors on sludge bulking. Master of Science Thesis, The Netherlands: Technical University of Delft; 2002.
[119] Blackall LL, Seviour EEM, Cunningham MA, Seviour RJ, Hugenholtz P. "*Microthrix parvicella*" is a novel, deep branching member of the Actinomycetes subphylum. Syst Appl Microbiol 1994;17:513–8.
[120] Connery T, Thompson AS, Patrick S, Larkin MJ. Studies of *Microthrix parvicella* in situ and in laboratory culture: production and use of specific antibodies. Water Sci Technol 2002;46(1-2):115–8.
[121] Slijkhuis H. The physiology of the filamentous bacterium *Microthrix parvicella*. Ph.D. thesis, The Netherlands: Agriculture College of Wageningen; 1983.
[122] Andreasen K, Nielsen PH. In situ characterisation of substrate uptake by *Microthrix parvicella* using microautoradiography. Water Sci Technol 1998;37(4/5):19–26.
[123] Blackall LL, Stratton H, Bradford D, Therese DD, Sjörup C, Seviour EM, Seviour RJ. "*Candidatus Microthrix parvicella*", a filamentous bacterium from activated sludge sewage treatment plants. Internat. J Syst Bacteriol 1996;46(1):344–6.
[124] Thomsen TR, Kjellerup BV, Nielsen JL, Hugenholtz P, Nielsen PH. *In situ* studies of the phylogeny and physiology of filamentous bacteria with attached growth. Environ Microbiol 2002;4(7):383–91.
[125] Lau OA, Strom PF, Jenkins D. Growth kinetics of *Sphaerotilus natans* and a floc former in pure and continuous culture. J Water Pollut Control Fed 1984; 56:41–51.
[126] Beccari M, Di Pinto AC, Ramadori R, Tomei MC. Effect of dissolved oxygen and diffusion resistances on nitrification kinetics. Water Res 1992;26(8):1099–104.
[127] Schramm A, Santegoeds CM, Nielsen HK, Wagner M, Pribyl M, Wanner J, Amann R, de Beer D. On the occurrence of anoxic microniches, denitrification, and sulfate reduction in aerated activated sludge. Appl Environ Microbiol 1999;65(9):4189–96.
[128] Van Loosdrecht MCM, Eikelboom D, Gjaltema A, Mulder A, Tijhuis L, Heijnen JJ. Biofilm structures. Water Sci Tech 1995;32(8):35–43.
[129] Picioreanu C, Van Loosdrecht MCM, Heijnen JJ. Mathematical modelling of biofilm structure with a hybrid differential-discrete cellular automaton approach. Biotechnol Bioeng 1998;58:101–16.
[130] Ben-Jacob E, Schochet O, Tenenbaum A, Cohen I, Czirók A, Vicsek T. Generic modelling of cooperative growth patterns in bacterial colonies. Nature 1994; 368(3):46–9.
[131] Van den Eynde E, Geerts J, Maes B, Verachtert H. Influence of the feeding pattern on the glucose metabolism of *Arthrobacter* sp. and *Sphaerotilus natans*, growing in chemostat culture, simulating activated sludge bulking. Eur J Appl Microbiol Biotechnol 1983;17:35–43.
[132] Van Veen WL, Krul JM, Bulder CJEA. Some growth parameters of *Haliscomenobacter hydrossis* (SYN. *Streptothrix hyalina*): a bacterium occurring in bulking activated sludge. Water Res 1982;16:531–4.
[133] Van Niekerk AM, Jenkins D, Richard MG. The competitive growth of *Zoogloea ramigera* and Type 021N in activated sludge and pure culture — a model for low F:M bulking. J Water Pollut Control Fed 1987;59(5):262–73.
[134] Nielsen JL, Christensen D, Kloppenborg M, Nielsen PH. Quantification of cell-specific substrate uptake by probe-defined bacteria under in situ conditions by microautoradiography and fluorescence in situ hybridisation. Environ Microbiol 2003;5(3):202–11.
[135] Chiesa SC, Irvine RL, Manning JF. Feast/famine growth environments and activated sludge population selection. Biotechnol Bioeng, 1985;27:562–8.
[136] Chiesa SC, Irvine RL. Growth and control of filamentous microbes in activated sludge: an integrated hypothesis. Water Res 1985;19(4):471–9.
[137] Majone M, Massanisso P, Carucci A, Lindrea K, Tandoi V. Influence of storage on kinetic selection to control aerobic filamentous bulking. Water Sci Technol 1996;34(5-6):223–32.
[138] Prendl L, Kroiβ H. Bulking sludge prevention by an aerobic selector. Water Sci Technol 1998;38(8/9):19–27.
[139] Krishna C, Van Loosdrecht MCM. Effect of temperature on sludge volume index. Water Res 1999;33(10):2374–82.
[140] Beccari M, Majone M, Massanisso P, Ramadori R. A bulking sludge with high storage response selected under intermittent feeding. Water Res 1998;32(11):3403–13.
[141] Casey TG, Wentzel MC, Loewenthal RE, Ekama GA, Marais GvR. A hypothesis for the cause of low F/M filament bulking in nutrient removal activated sludge systems. Water Res 1992;26(6):867–9.
[142] Casey TG, Wentzel MC, Ekama GA, Loewenthal RE, Marais GvR. An hypothesis for the causes and control of anoxic–aerobic (AA) filament bulking in nutrient removal activated sludge systems. Water Sci Technol 1994;29(7):203–12.
[143] Casey TG, Wentzel MC, Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 11: a biochemical/microbiological model for proliferation of anoxic-aerobic (AA) filamentous organisms. Water SA 1999;25(4):443–51.
[144] Tsai M-W, Wentzel MC, Ekama GA. The effect of residual ammonia concentration under aerobic conditions on the growth of *Microthrix parvicella* in biological nutrient removal plants. Water Res 2003;37(5):3009–15.

[145] Beun JJ, Hendriks A, Van Loosdrecht MCM, Morgenroth E, Wilderer PA, Heijnen JJ. Aerobic granulation in a sequencing batch reactor. Water Res 1999;33(10): 2283–90.

[146] Beun JJ, Paletta F, Van Loosdrecht MCM, Heijnen JJ. Stoichiometry and kinetics of poly B hydroxybutyrate metabolism under denitrifying conditions in activated sludge cultures. Biotechnol Bioeng 2000;67: 379–89.

[147] Van den Eynde E, Vriens L, Verachtert H. Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. Part III: applications with industrial waste waters. Eur J Appl Microbiol Biotechnol 1982;15:246–51.

[148] Van Loosdrecht MCM, Pot MA, Heijnen JJ. Importance of bacterial storage polymers in bioprocesses. Water Sci Technol 1997;35(1):41–7.

[149] Daigger GT, Robbins MH, Marshall BR. The design of a selector to control low F/M filamentous bulking. J Water Pollut Control Fed 1985;56(3):220–6.

[150] Daigger GT, Nicholson GA. Performance of four-scale nitrifying wastewater treatment plants incorporating selectors. Res J Water Pollut Control Fed 1990;62(5): 676–83.

[151] Marten WL, Daigger GT. Full-scale evaluation of factors affecting the performance of anoxic selectors. Water Environ Res 1997;69(7):1272–81.

[152] Albertson OE. Bulking sludge control - progress, practice and problems. Water Sci Technol 1991;23:835–46.

[153] Chudoba J, Bláha J, Madera V. Control of activated sludge filamentous bulking – III. Effect of sludge loading. Water Res 1974;8(4):231–7.

[154] Eikelboom DH. Biosorption and prevention of bulking sludge by means of a high floc loading. In: Chambers B, Tomilson EJ, editors. Bulking of activated sludge: preventative and remedial methods. Chichester, UK: Ellis Horwood Limited; 1982. p. 90–104.

[155] Heide BA, Pasveer A. Oxidation ditch: prevention and control of filamentous sludge. H_2O 1974;7(18):373–7.

[156] Pujol R, Boutin P. Control of activated sludge bulking: from the lab to the plant. Water Sci Technol 1989;21: 717–26.

[157] Rensink JH, Donker JGW. The effect of contact tank operation on bulking sludge and biosorption processes. Water Sci Technol 1991;23:857–66.

[158] Still DA, Ekama GA, Wentzel MC, Casey TG, Marais GvR. Filamentous organism bulking in nutrient removal activated sludge. Paper 2: stimulation of the selector effect under aerobic conditions. Water SA 1996;22(2): 97–118.

[159] Van Niekerk AM, Jenkins D, Richard MG. A mathematical model of the carbon-limited growth of filamentous and floc-forming organisms in low F/M sludge. J Water Pollut Control Fed 1988;60(1):100–6.

[160] Wheeler ML, Jenkins D, Richard MG. The use of a "selector" for bulking control at the Hamilton, Ohio, USA, water pollution control facility. Water Sci Technol 1984;16:33–53.

[161] WEF. Design of municipal wastewater treatment plants, vol. II. Liquid treatment processes. Manual of Practice No. 8 and ASCE Manuals and Reports on Engineering Practice 76. Alexandria: Water Environment Federation; 1998. p. 1–114 [chapter 15].

[162] Scuras SE, Jobbágy A, Grady Jr CPL. Optimization of activated sludge reactor configuration: kinetic considerations. Water Res 2001;35(18):4277–84.

[163] Casey JP, McDowell CS, Spector ML, Zupko AJ. Nonbulking activated sludge process. US patent 3,864,246, 1975. Air Products and Chemicals, Inc., Allentown, USA.

[164] Gaval G, Pernelle J-J. Impact of the repetition of oxygen deficiencies on the filamentous bacteria proliferation in activated sludge. Water Res 2003;37(9):1991–2000.

[165] Pernelle J-J, Gaval G, Cotteux E, Duchene P. Influence of transient substrate overloads on the proliferation of filamentous bacterial populations in an activated sludge pilot plant. Water Res 2001;35(1):129–34.

[166] Randall CW, Barnard JL, Stensel HD. Design and retrofit of wastewater treatment plants for biological nutrient removal. Lancaster, PA: Technomic Publishing Co. Inc.; 1992.

[167] Wentzel MC, Dold PL, Ekama GA, Marais GvR. Biological excess phosphorus removal—steady state process design. Water SA 1990;16(1):29–48.

[168] Smolders GJF, Van Loosdrecht MCM, Heijnen JJ. Steady-state analysis to evaluate the phosphate removal capacity and acetate requirement of biological phosphorus removing mainstream and sidestream process configurations. Water Res 1996;30(11):2748–60.

[169] Plósz BGy, Jobbágy A, Grady CPL. Factors influencing deterioration of denitrification by oxygen entering an anoxic reactor through the surface. Water Res 2003; 37:853–63.

[170] Alleman JE. Nitrogen removal from wastewater using sequencing batch reactors. Ph.D. dissertation. Notre Dame, ID: University of Notre Dame; 1978, unpublished.

[171] Chiesa SC.Growth,control of filamentous organisms in activated sludge systems. Ph.D. Dissertation. Notre Dame, ID: University of Notre Dame; 1982.

[172] Dionisi D, Majone M, Ramadori R, Beccari M. The storage of acetate under anoxic conditions. Water Res 2001;35(11):2661–8.

[173] Wightman D, Daigger GT, Frankenfield RC, Spani C, Read JM, Simonson SE. Upgrading with selector technology. Oper Forum 1994;11:20.

[174] Marten WL, Daigger GT. Comment and author's reply on "Full-scale evaluation of factors affecting the performance of anoxic selectors". Water Environ Res 1998; 70(6):1225–31.

[175] Cech JS, Hartman P. Competition between polyphosphate and polysaccharide accumulating bacteria in enhanced biological phosphate removal systems. Water Res 1993;27(7):1219–25.

[176] Filipe CDM, Daigger GT, Grady Jr CPL. A metabolic model for acetate uptake under anaerobic conditions by glycogen accumulating organisms: stoichiometry, kinetics and the effect of pH. Biotechnol Bioeng 2001;76(1):17–31.

[177] Maszenan AM, Seviour RJ, Patel BKC, Rees GN, McDougall B. The hunt for the G-bacteria in activated sludge biomass. Water Sci Technol 1998;37(4/5):65–9.

[178] Mino T, Van Loosdrecht MCM, Heijnen JJ. Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Res 1998;32(11):3193–207.

[179] Zeng RJ, Yuan Z, Keller J. Model-based analysis of anaerobic acetate uptake by a mixed culture of polyphosphate-accumulating and glycogen-accumulating organisms. Biotechnol Bioeng 2003;83(3):293–302.
[180] Schuler AJ, Jenkins D, Ronen P. Microbial storage products, biomass density, and settling properties of enhanced biological phosphorus removal activated sludge. Water Sci Technol 2001;43(1):173–80.
[181] Parker D, Appleton R, Bratby J, Melcer H. North American performance experience with anoxic and anaerobic selectors for activated sludge bulking control. In: Růžičková I, Wanner J, editors. Conference Proceedings Selected Papers of the Ninth IWA Specialised Conference on Design, Operation and Economics of Large Wastewater Treatment Plants, Praha, Czech Republic; 2003. p. 307–15.
[182] Eikelboom DH. Sulfur-storing bacteria and bulking of activated sludge. In: Lens PNL, Pol LH, editors. Environmental technologies to treat sulfur pollution. London, UK: IWA Publishing; 2000. p. 449–66.
[183] Pitman AR. Settling of nutrient removal activated sludges. Water Sci Technol 1984;17:493–504.
[184] Van Loosdrecht MCM, Brandse FA, De Vries AC. Upgrading of waste water treatment processes for integrated nutrient removal — the BCFS process. Water Sci Technol 1998;37(9):209–17.
[185] Gujer W, Henze M, Mino T, Van Loosdrecht MCM. Activated sludge model no. 3. Water Sci Technol 1999;39(1):183–93.
[186] Henze M, Grady Jr CPL, Gujer W, Marais GVR, Matsuo T. Activated Sludge Model No. 1. IAWPRC scientific and technical report no. 1. London: IAWPRC; 1987.
[187] Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC. Activated sludge model no 2 IAWQ Scientific and Technical Report No 3. London: IAWQ; 1995.
[188] Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC, Marais GVR, Van Loosdrecht MCM. Activated sludge model No 2D, ASM2D. Water Sci Technol 1999; 39(1):165–82.
[189] Dircks K, Beun JJ, Van Loosdrecht MCM, Heijnen JJ, Henze M. Glycogen metabolism in aerobic mixed cultures. Biotechnol Bioeng 2001;73(2):85–94.
[190] Cenens C, Smets IY, Van Impe JF. Modelling the competition between floc-forming and filamentous bacteria in activated sludge waste water treatment systems—I. Evaluation of mathematical models based on kinetic selection theory. Water Res 2000;34(9):2525–34.
[191] Cenens C, Smets IY, Van Impe JF. Modelling the competition between floc-forming and filamentous bacteria in activated sludge waste water treatment systems—II. A prototype mathematical model based on kinetic selection and filamentous backbone theory. Water Res 2000;34(9):2535–41.
[192] Gujer W, Kappeler J. Modelling populations dynamics in activated sludge systems. Water Sci Technol 1992;25(6): 93–103.
[193] Hermanowicz SW. Dynamic changes in populations of the activated sludge community: effects of dissolved oxygen variations. Water Sci Technol 1987;19:889–95.
[194] Kappeler J, Gujer W. Bulking activated sludge systems: a qualitative simulation model for *Sphaerotilus natans*, Type 021N and Type 0961. Water Sci Technol 1992; 26(3–4):473–82.
[195] Kappeler J, Gujer W. Scumming due to Actinomycetes: towards a better understanding by modelling. Water Sci Technol 1994;31(2):225–34.
[196] Kappeler J, Brodmann R. Low F/M bulking and scumming: towards a better understanding by modelling. Water Res 1995;28(4):763–79.
[197] Lou IC, De los ReyesIII FL. Modeling the competition between filaments and floc formers: integrating decay rate, storage, kinetic selection, and filamentous backbone theory. In: *Proceedings of the Conference WEFTEC 2002*. Chicago, IL, USA: Water Environment Federation; 2002.
[198] Magbanua SM, Bowers AR. Effect of recycle and axial mixing on microbial selection in activated sludge. J Environ Eng 1998;124(10):970–8.
[199] Sheintuch M, Tartakovsky B. Activated-sludge system design for species selection: analysis of a detailed multispecies model. Chem Eng Sci 1997;52(17):3033–46.
[200] Takács I, Fleit E. Modelling of the micromorphology of the activated sludge floc: low DO, low F/M bulking. Water Sci Technol 1995;31(2):235–43.
[201] De Beer D, Schramm A, Santegoeds CM, Nielsen HK. Anaerobic process in activated sludge. Water Sci Technol 1998;37(4/5):605–8.
[202] STOWA. Selector design: the role of the influent characterization. Report 94-16, 1994 Stichting Toegepast Onderzoek Waterbeheer, Postbus 8090, 3503 RB Utrecht, The Netherlands [in Dutch].
[203] Ekama GA, Marais GvR. The implications on the IAWPRC hydrolysis hypothesis on low F/M bulking. Water Sci Technol 1986;18:11–9.
[204] Snaidr J, Amann R, Huber I, Ludwig W, Schleifer K-H. Phylogenetic analysis and in situ identification of bacteria in activated sludge. Appl Environ Microbiol 1997;63(7): 2884–96.