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10

Bulking sludge

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10.1 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 amount of biomass which is produced in wastewater treatment plant is relatively low. An influent carbon content of 500 mg COD generates 200-300 mg suspended solids. Without biomass retention this would also be the actual sludge concentration in the treatment process. Therefore biomass retention is used in order to increase the biomass concentration in the biochemical stage and obtain higher volumetric conversion rates. Since bacteria form flocs which can be separated from the treated wastewater by gravity forces, this energy-friendly and economical option is the standard technology applied for solid-liquid separation. 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. This separation is therefore based on the formation of compact flocs. The relatively low force of gravity means that the required settling area (*i.e.* settling tanks) becomes a large part of the total treatment plant; it easily consists of 30-50% of the total treatment area (Figure 10.1).

Figure 10.1 A modern biological nutrient removal plant in The Netherlands (BCFS® process) showing the importance of sludge separation on the total process layout. The settling tank design was based on a sludge volume index (SVI) of 120 ml/g (photo: Van Loosdrecht *et al.*, 1998).

The relation between sludge settling and settler design is treated in detail in Chapter 12. The sludge volume index (SVI) is used as an empirical measure which links the sludge characteristics and settler design (Ekama and Marais, 1986). This value is obtained by settling a sludge sample in a 1 litre measurement cylinder for 30 minutes. The volume of the sludge layer can be read and divided by the original suspended solids content of the sludge sample. In this way one obtains the volume which is taken by a gram of sludge after settling. The SVI has a major effect on the required settler size (Figure 10.2), and an increase in SVI from 100 to 150 ml/g will result in almost doubling the design area needed for settlers. Details on this measurement are provided in Van Loosdrecht *et al*., 2016.

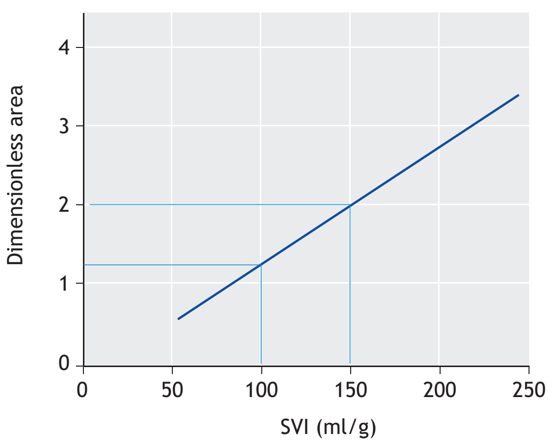

Figure 10.2 Relation between sludge volume index and surface area needed for a settler according to the STOWA (STOWA, 1994) design guidelines for settlers.

Bulking sludge, a term used to describe the excessive growth of filamentous bacteria, is a common and longstanding problem for activated sludge processes (*e.g.* Donaldson, 1932). When the sludge flocs are open and porous the settling is hindered and the settled sludge will contain low amounts of solids. In practice bulking is associated with a high SVI. The critical value for the SVI above which bulking sludge occurs is heavily dependent on the local practise in design and construction for settlers. In effect bulking sludge is defined in general when suspended solids cannot be maintained in the settler, although different regions have different traditions in settler design. For instance, nowadays in the Netherlands an SVI above 120 ml/g is considered bulking sludge since this index value is currently used in the design guidelines for settlers. Bulking sludge is typically an operational or empirical problem, so there is not an exact scientific index value to distinguish bulking sludge from non-bulking sludge. Open and porous sludge flocs settle more slowly so require larger settlers in order to be maintained in the process and/or prevent solids from occurring in the effluent. The growth of filamentous bacteria is especially detrimental and leads to many problems in practice. The volume fraction of filamentous bacteria in the activated sludge community which causes settling problems can be very small; volume fractions of 1-20% are sufficient to cause bulking sludge (Palm *et al.,* 1980; Kappeler and Gujer, 1994b). Although filamentous bacteria are often not the dominant metabolic bacterial group in the treatment plant, they still cause bulking sludge (Figure 10.3).

Despite a vast amount of research on bulking sludge it continues to be a problem in operating wastewater treatment plants. This is likely caused by several conditions which cause filamentous organisms to proliferate. Many filamentous bacteria are not available in pure cultures, preventing a detailed microbiological study of these organisms. The status 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 (Eikelboom, 1975; 2000). By studying and understanding the ecophysiology of the filamentous bacterium (either in pure culture or by applying *in-situ* techniques, such as microautoradiography: MAR), it is hoped that a solution to avoid the occurrence of the specific filament can be found.

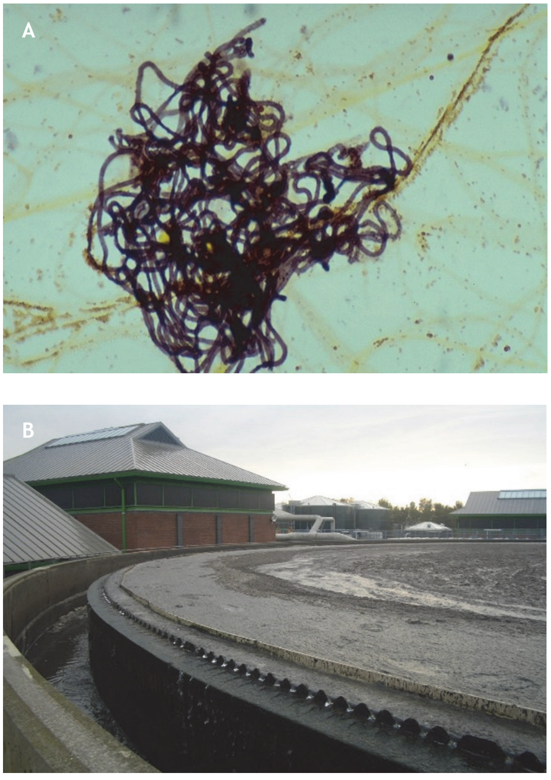

Figure 10.3 Nightmares for the wastewater treatment plant operator: (A) filamentous bacteria, (B) bulking sludge leaving the plant with the effluent (photos: D.H. Eikelboom and D. Brdjanovic).

A different approach is by recognising that the general characteristic is the cell morphology. Realising how the microbial cell morphology affects the ecology of the bacteria can lead to a general solution independent of the species involved (Chudoba *et al.,* 1973a; Rensink, 1974). In this approach the occurrence of a specific kind of filamentous bacterium is a second order problem. The problem is therefore that process engineering as well as microbiology knowledge is needed in order to solve the problem and that the solution cannot be obtained from either of these two fields in isolation.

10.2 HISTORICAL ASPECTS

It is not the intention in this chapter to fully describe the history and developments of activated sludge systems. For this the reader is invited to read the reviews provided, for instance, by Allemann and Prakasam (1983) or Albertson (1987). We will just highlight some of the most important historical facts which have contributed to the understanding of the bulking sludge problem.

The activated sludge process was developed in the early 1900s in England (Ardern and Lockett, 1914). Initially fill-and-draw systems were brought into operation but they were quickly converted into continuous flow systems. Despite the increased occurrence of settling problems, continuous flow systems became popular and spread worldwide. Donaldson (1932) 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 the development of bulking sludge. As a corrective measure he suggested that the aeration basin should be compartmentalised (*i.e.* a plug flow reactor) to promote the development of satisfactorily settling sludge. Nevertheless, continuously-fed completely mixed activated sludge systems remained the preferred design. Clearly civil engineering advantages in the construction phase prevailed over process engineering advantages during operation. However, the discussion on the effect of the feeding pattern on sludge settleability was reopened in the 1970s. Studies showed the advantage of using compartmentalised tanks with a plug flow pattern over continuously-fed completely mixed systems (Chudoba *et al.,* 1973b; Rensink, 1974; and many others), confirming the earlier recommendations of Donaldson (1932).

Pasveer (1959) went back to the original fill-and-draw technology of Ardern and Lockett, from which he developed the Pasveer or 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 relatively popular in Europe for a few years, but once more, almost all these systems were soon converted to continuous-flow oxidation ditches by the addition of a secondary settler and solids recycling. Nevertheless, during the 1960s Pasveer showed that intermittently-fed full-scale oxidation ditches produce sludge with better settleability than continuously-fed completely mixed systems (Pasveer, 1969).

In the 1970s Chudoba and his co-workers (1973b) and Rensink (1974) developed the selector reactor, which became the most widespread engineering tool to control bulking sludge. However, although the use of selectors has been successful and has reduced bulking problems in many activated sludge systems, there are still regular reports of their failure.

10.3 RELATIONSHIP BETWEEN MORPHOLOGY AND ECOPHYSIOLOGY

One of the most intriguing and complex questions on bulking sludge is whether microbial morphology, physiology and substrate kinetics are related and how these 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? Is it possible to design reactor conditions which prevent all the filaments from proliferating and still achieve the effluent quality required biologically? Even though some plants have never been observed to have bulking sludge, for decades scientists, engineers, and microbiologists have failed to find a definitive answer to these questions. However, some relationships can be inferred and they will be briefly discussed here.

10.3.1 Microbiological approach

The lack of success in finding a general solution to bulking sludge control led many researchers to look at the microbial population and search for the predominant filamentous bacteria responsible for bulking. Identification keys were developed (Eikelboom, 1977, 2000) to identify filamentous bacteria based on microscopic characterisation.

Despite several limitations these identification methods produced a systematic tool which allowed 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, food/microorganism ratio - F/M, etc.) in order to define (specific) strategies for its control (Jenkins *et al.,* 1993) (Table 1.1). The distribution of filamentous microorganisms varies considerably between different geographical areas (Martins *et al*., 2004a) 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 bulking sludge episodes, supposedly due to the abundance of *Microthrix parvicella*, were more frequent in winter and spring than in summer and autumn (*e.g.* Kruit *et al.,* 2002). It was also confirmed that the morphotypes type 021N, type 0961, *Sphaerotilus natans* and *Thiothrix sp.*, are controlled by anaerobic and anoxic stages, as typical in bio-P and denitrifying systems (Ekama *et al*., 1996b). However, these conditions seem to be inefficient for the dominant filamentous microorganisms found in biological nutrient removal systems. Curiously the morphotype filamentous bacteria found in biological nutrient removal systems are usually Gram-positive which implies that their likely hydrophobic cell surface could easily adsorb compounds with a low solubility. It is however unclear whether low-loaded systems also can be enriched with Gram-positive floc-forming bacteria.

Table 10.1 Proposed groups of model morphotype filamentous microorganisms (Wanner and Grau, 1989; Jenkins *et al.*, 1993).

Microorganisms	Features	Control
Group I: Low DO aerobic zone growers		
Sphaerotilus natans, type 1701, *Haliscomenobacter hydrossis*	Use readily biodegradable substrates; grow well at low DO concentrations; grow over wide range of SRTs.	Aerobic, anoxic or anaerobic plug flow selectors; increase SRT; increase DO concentration in the aeration basin (> 1.5 mg O_2/l).
Group II: Mixotrophic aerobic zone growers		
Thiothrix sp., type 021N	Use readily biodegradable substrates, especially low molecular weight organic acids; present at moderate to high SRT; capable of sulphide oxidising to stored sulphur granules; rapid nutrient 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, *Nostocoida 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		
Microthrix parvicella, type 0092, type 0041/0675	Abundant in anaerobic-anoxic-aerobic systems; present at high SRTs; 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 the entire system; several stages (anaerobic/anoxic/aerobic) should be well defined; maintain a relatively high oxygen concentration in the aerobic phase (1.5 mgO_2/l) and a low ammonium concentration (<1mgN /l) (Kruit *et al*., 2002) and a low nitrate and nitrite in the anoxic reactor before the aerobic reactor (Casey *et al*., 1999; Musvoto *et al*., 1994).

During the 1990s molecular methods based on DNA and RNA analyses were introduced into biological wastewater treatment (Chapter 2). These methods enable the correct identification of a filamentous bacteria population. Therefore, it is advisable to apply specific gene probes, whenever they exist, in bulking sludge surveys. Their use, together with filamentous bacteria characterisation and definition of the correct control and operational conditions (*e.g.* selector reactor), are considered major challenges to control bulking sludge.

10.3.2 Morphological-ecological approach

Filamentous bacteria grow preferentially in one or two directions. This morphological feature appears to give competitive advantages to filamentous organisms under substrate limiting concentrations (*e.g.* diffusion-limiting environments). It is thought that these organisms have a higher outward growth velocity and win the competition because they gain easy access to bulk liquid substrate (Martins *et al.*, 2003a). This is in line with some other studies which also connect the excessive growth of filamentous microorganisms with substrate diffusion resistance inside biological flocs (Pipes, 1967; Kappeler and Gujer, 1994a).

Given these views, their morphology as such gives these 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 has even led to suggestions that actually filamentous organisms form the backbone of activated sludge flocs (Jenkins *et al.*, 1993a). This type of filamentous skeleton structure would promote the attachment of other cells by their extracellular polymeric substances (EPS).

10.4 FILAMENTOUS BACTERIA IDENTIFICATION AND CHARACTERISATION

The basis for understanding and characterising bulking sludge is generally thought to depend on the correct identification of the filamentous bacteria involved. This is briefly discussed below.

10.4.1 Microscopic characterisation *versus* molecular methods

Many types of bacteria have still not been identified and are not recognised taxonomically. Therefore, these bacteria are not documented in standard microbiological identification manuals such as Bergey's Manual of Systematic Bacteriology. Eikelboom (1975, 1977) 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 (Eikelboom, 2000) that, together with a slightly different manual by Jenkins *et al.* (1993a, 2006), are used as worldwide references on filamentous bacteria identification.

However, although very useful, this type of identification has its limitations. For instance, many filamentous bacteria (*e.g.* the morphotypes *Sphaerotilus natans*, 1701, 0092 and 0961) can change morphology in response to changes in environmental conditions and although some of them can look the same morphologically, they probably vary considerably in their physiology and taxonomy. For instance, the filamentous bacterial morphotype *Nostocoida limicola* encompasses several phylogenetically different bacteria (Seviour *et al.*, 2002) belonging to the following groups: low mol% G+C Gram-positive bacteria, high mol% G+C Gram-positive bacteria, *Planctomycetes*, green non-sulphur bacteria and the alpha subclass of *Proteobacteria* (Martins *et al.*, 2004b). This also applies to the filamentous morphotype Eikelboom type 1863.

Microscopic identification of filamentous bacteria based on morphology requires a well-trained and experienced person; otherwise a wrong judgement can easily be made. Furthermore, about 40 new morphotypes of filamentous bacteria were identified in a survey study in industrial activated sludge systems (Eikelboom and Geurkink, 2002), making the identification of filamentous bacteria even more complex. This misleading and difficult identification by traditional microscopic techniques has directed research towards molecular methods. Molecular methods based on analysing the DNA or RNA of the bacteria have developed rapidly. For activated sludge several methods are presently commonly used. In order to characterise the complexity of a microbial community the 16S rRNA of the bacteria can be used. Details of these methods are out of the scope of this chapter and are briefly treated in Chapter 2. Details on the methodology for microscopic evaluation is described in Van Loosdrecht *et al.* (2016).

10.4.2 Physiology of filamentous bacteria

As stated above, most filamentous organisms are still very poorly characterised and described, mainly due to the problems of cultivation and maintenance of cultures. However, recent developments in combining micro-autoradiography with fluorescent *in-situ* hybridisation (FISH) are promising for elucidating the exact physiology of filamentous bacteria. Nonetheless there exists no obvious intrinsic relation between the morphology of filamentous bacteria and a specific physiology. Therefore, there general ecological behaviour of filamentous bacteria is more linked with the morphology then with a specific metabolism.

A general problem we face is that old physiological data are described for morphotype filamentous bacteria, which are likely to be phylogenetically unrelated bacteria with large physiological differences, and, consequently, old physiological data (*e.g.* the morphotype '*Nostocoida limicola*') might or might not be correct. Therefore, 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 the electron acceptor. To our knowledge, only the morphotypes type 0961, type 1863, type 1851 and *Nostocoida limicola* have the capacity to perform a fermentative metabolism and therefore may have competitive advantages in systems with anaerobic stages. However, these morphotypes are believed to be only minor components of the total microbial population and they are in general not responsible for bulking sludge episodes.

Some filamentous bacteria, such as *Microthrix parvicella*, *Sphaerotilus natans*, *Thiothrix spp.*, type 021N and type 1851, are able to use nitrate as the electron acceptor, reducing it only to nitrite but the substrate uptake rate and denitrification rate for the filamentous bacteria analysed so far (type 021N and *Thiothrix spp.*) are much lower (by more than 80 times) than for floc-forming bacteria (Shao and Jenkins, 1989). Type 0092, a filamentous bacterium dominant in many nutrient removal activated sludge systems, seems to be incapable of using nitrate as an electron acceptor. Furthermore, in the case of *Microthrix parvicella* it is reported that growth is not sustained under anoxic conditions. Anoxic contact zones have been using this physiological information to control bulking sludge particularly due to bulking caused by type 021N and *Sphaerotilus natans* (Ekama *et al.,* 1996a). Of the predominant filamentous bacteria found in biological nutrient removal activated sludge systems only the morphotype type 0092 and *Microthrix parvicella* were grown in a pure culture, and significant difficulties are encountered in the isolation of the latter. *Microthrix parvicella* seems to be the most dominant problematic organism in biological nutrient removal processes (Nielsen *et al*., 2002). The main difference is that their preferred substrate are long chain fatty acids rather then volatile fatty acids. The organism needs reduced sulphur compounds for protein synthesis and is described as microaerophilic (Slijkhuis and Deinema, 1988; Rossetti *et al*., 2005). When anoxic or anaerobic–anoxic conditions are introduced to stimulate biological nutrient removal, *M. parvicella* can therefore proliferate. It was observed that selectors indeed could not eliminate *M. parvicella* bulking under anoxic-aerobic conditions. When in a system with a selector the main reactor was made fully aerobic and *M. parvicella* bulking was properly controlled, whereas an anoxic-aerobic main reactor led to the proliferation of *M. parvicella* (Ekama *et al*., 1996a). This laboratory experience is supported by a full-scale evaluation by Kruit *et al*. (2002). They concluded that the main criterium to prevent *M. parvicella* bulking was to have distinctly separate aerated (DO > 1.5 mg/l) and anoxic stages (no detectable oxygen).

A metagenomics approach (McIllroy *et al*., 2013) gave further insight into the metabolism of *Microthrix parvicella.* The dominance of long-chain fatty acid accumulation as lipids under anaerobic or microaerophilic conditions was highlighted, including the need for glycerol in the medium. Polyphosphate is likely the main energy supply for this anaerobic/microaerophilic metabolism. The organism produces exocellular lipases, indicating that fats are indeed the main carbon source.

10.5 CURRENT GENERAL THEORIES TO EXPLAIN BULKING SLUDGE

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

10.5.1 Diffusion-based selection

Several researchers have pointed out that the morphology of filamentous bacteria aids the substrate uptake under low nutrients or oxygen concentrations. Until 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 (Pipes, 1967). Especially at low substrate concentration this high A/V ratio is advantageous to organisms since it apparently facilitates mass transfer to cells with a high A/V ratio. At lower substrate concentrations this would lead to a relatively higher growth rate.

Later theories proposed that filaments could easily penetrate outside the flocs. When the flocs are growing at a low substrate concentration, the filamentous bacteria detect effectively a higher substrate concentration than the floc formers inside the floc (Sezgin *et al.,* 1978; Kappeler and Gujer, 1994a). Micro-gradients of substrate concentration in flocs have been theoretically predicted (*e.g.* Beccari *et al.,* 1992) and experimentally observed in sludge flocs. Later Martins *et al.* (2004c) extended this theory by comparing floc growth with biofilm growth. Van Loosdrecht *et al.* (1995) and Picioreanu *et al.* (1998) 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.* (1994) showed that the colony morphology of a pure culture also depends on substrate micro-gradients, with low substrate concentrations leading to filamentous colony morphology. Therefore, it can be that the low substrate concentration leads to a floc being more open and filamentous (Martins *et al.,* 2003b). Filamentous bacteria fit excellently in such a structure.

10.5.2 Kinetic selection theory

Similarly to Donaldson (1932), Chudoba *et al.* (1973a) 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.* (1973a) showed that 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 sludge with satisfactory settling properties. The authors concluded that the primary cause of the selection of floc-forming microorganisms in a mixed culture is the macro-gradient of substrate concentration at the inlet part of the system.

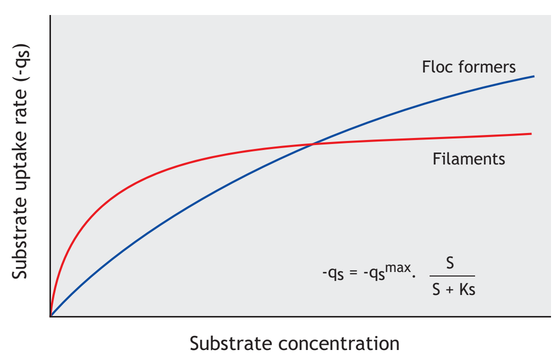

Figure 10.4 Relation between substrate uptake rate (q_S) and substrate concentration (S) for floc-forming and filamentous bacteria according to the kinetic selection theory.

Based on these results, Chudoba *et al.* (1973b) formulated the kinetic selection theory to explain the occurrence or suppression of filamentous bacteria in activated sludge systems. Their explanation was based on a selection criterion for the limiting soluble substrate by filamentous and floc-forming bacteria. Chudoba *et al*. (1973b) hypothesised that filamentous microorganisms (K strategists) are slow-growing organisms which can be characterised as having maximum growth rates (μ_{max}) and an affinity constant (K_S) lower than floc-forming bacteria (r strategists) (Figure 10.4). In systems where the substrate concentration is low (typically $C_S<K_S$), as 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, as in plug-flow reactors and sequencing batch reactor (SBR) systems, 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 filamentous bacteria (*e.g.* Sphaerotilus natans, Haliscomenobacter hydrossis, type 1701, type 021N, Microthrix parvicella) and floc-forming bacteria (Arthrobacter globiformis, Zoogloea ramigera) supported this theory (*e.g.* Van den Eynde *et al.,* 1983). It is however questionable whether these floc-forming bacteria are representative of activated sludge systems. Use of molecular probes has shown that regularly non-dominant bacteria have been enriched from activated sludge. A technique based on quantitative MAR and FISH was applied *in situ* to measure the kinetics of filamentous bacteria (*Candidatus* Meganema perideroedes and *Thiothrix sp.*) (Nielsen *et al.,* 2003). 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 filamentous bacteria have in general a lower maximum growth rate than other bacteria present in 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 kinetic selection theory is also not proven yet for the general case. 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, as in the diffusion-based A/V hypothesis of Pipes (1967), then it is fully in agreement with the kinetic selection theory. In flocs the K_S value based on bulk liquid measurements is anyway an apparent coefficient influenced by floc morphology. The more diffusion resistance (the larger and denser the flocs), the higher the measured apparent K_S value (Beccari *et al.,* 1992; Chu *et al.,* 2003). For filaments extending from the floc this would mean a lower apparent K_S value compared to bacteria inside the flocs. Based on this reasoning it might well be that the diffusion-related theories (Pipes, 1967; Sezgin *et al.,* 1978; Kappeler and Gujer, 1994a; Martins *et al.,* 2004c) and the kinetic selection theory (Chudoba *et al.,* 1973b) are two sides of the same coin, and therefore have the same descriptive power.

One experiment which indicates that both theories are potentially correct was performed by Martins *et al.* (2008). When bacteria grow on starch the soluble substrate concentration is always low. The hydrolysis product (maltose) is directly taken up by the actively growing cells. In this case there is substrate uptake at low concentrations, but without forming a substrate gradient because the starch is hydrolysed inside the floc and not in the bulk liquid.

In this case satisfactorily settling sludge was obtained (according to the diffusion-based theory) but the flocs were dominantly formed by *nostocoida* cells (according to the kinetic selection theory). This observation would indicate that the competition between filamentous and non-filamentous bacteria is correctly described by the kinetic selection theory, but the floc morphology is dependent on the same diffusion gradient formation (Figure 10.5) as for biofilm systems (Van Loosdrecht *et al.,* 1995).

Figure 10.5 (A) Left: sludge containing starch stained blue with iodide, right: supernatant, no staining because of the absence of starch, (B) microscopic graph of sludge floc from a starch grown activated sludge culture dominated by *Nostocoida* cells. The blue line represents the substrate concentration for a normal soluble substrate, and the red line the substrate concentration for a hydrolysis product (photos: A.M. Martins).

10.5.3 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 (*e.g.* Van den Eynde *et al.,* 1983). However, recent studies have showed that bulking sludge could have a similar or even higher storage capacity than satisfactorily settling sludge (Beccari *et al.,* 1998; Martins *et al.,* 2003b). Studies of pure and mixed cultures also show that some filamentous bacteria, such as *Microthrix parvicella*, can have a high storage capacity under all the possible environmental conditions (*i.e.* aerobic, anoxic and anaerobic) (Nielsen *et al.,* 2002). The filamentous organism *Thiothrix caldifontis* was shown to have the regular metabolism of phosphate-accumulating bacteria, as well as sulphide storage potential (Rubio-Rincon *et al.,* 2017). This stored material can be metabolised for energy generation or protein production during the aerobic famine periods, which would represent a major advantage in selecting these microorganisms instead of 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 but although they may not be prime selection parameters, storage and regeneration (depletion) are intrinsic processes which play a key role in selector-like systems (Van Loosdrecht *et al.,* 1997). Therefore, they should be considered in the description of the metabolic processes which take place in bulking and non-bulking systems.

10.6 REMEDIAL ACTIONS

There are two principal strategies that can be followed to control bulking sludge, *i.e.* non-specific and specific methods. The non-specific methods comprise techniques such as chlorination, ozonation and the 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 floc-forming bacteria. Note that this explanation is in line with the diffusion-based hypothesis for competition by filamentous bacteria. Chlorination is widely used in the USA and the procedures for its implementation are well documented (*e.g.* Jenkins *et al.,* 1993b). However, its application in Europe is limited due to environmental concerns about 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, non-specific methods do not remove the causes of 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. On the other hand, the specific methods are preventive methods which are intended to encourage 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 these specific methods allow the permanent and sustainable control of bulking in activated sludge systems, they should be developed and adopted in preference to non-specific methods.

Until now preventive action for bulking sludge has not been based on knowledge of the physiology and/or kinetics of a specific type of filamentous bacteria. This is despite the major emphasis on studies to identify 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 readily degradable COD is consumed, and thereafter a completely mixed tank can be used. If oxygen is consumed at low concentrations it leads to bulking sludge in a similar manner as for readily biodegradable COD.

The combined effect of oxygen concentration and readily biodegradable substrate concentration on sludge properties is depicted in Figure 10.6.

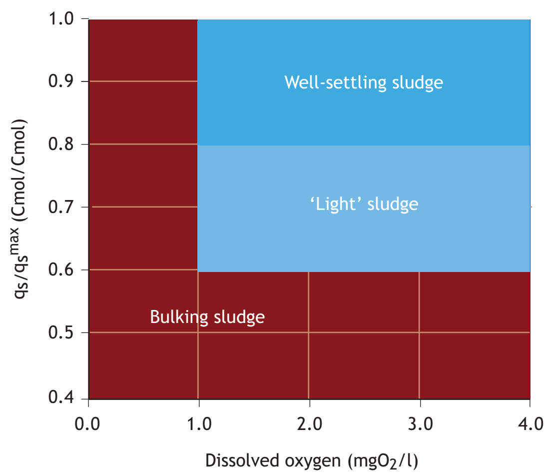

Figure 10.6 Effect of concentration of oxygen and readily available substrate (the latter expressed as the actual rate for substrate uptake relative to its maximum rate) on the type of sludge formed in an activated sludge process (Martins *et al.,* 2003b).

The effective substrate concentration should be seen in relation to the affinity constant for the substrate, and therefore the ratio between the actual and the maximum substrate rate is used here. The dissolved oxygen content seems only to be relevant for the period where readily biodegradable substrate is available. The prerequisite of a plug-flow initial part of the activated sludge process has resulted in the development of selectors to prevent bulking. Both the theories for sludge bulking (A/V or diffusion-based selection as well as kinetic selection theory) support this approach.

10.6.1 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 (Chudoba *et al.,* 1973b; Rensink, 1974). It can also be a small separate initial zone of a biological reactor which receives the influent and sludge return flows and has a high readily biodegradable COD uptake rate, with virtually complete readily biodegradable COD removal (Jenkins *et al.,* 1993a). 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 a SBR fed in a static way is the ideal selector system.

It has been shown that indeed in such systems the opposite of bulking sludge, *i.e.* aerobic granular sludge, can be formed (Beun *et al.,* 1999). 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 any available external substrate (a low growth rate or famine environment) should then exist (aerobic stage) to re-establish the storage capacity of the cells (Van Loosdrecht *et al.,* 1997; Beun *et al.,* 1999). However, although selectors have been widely installed and are still the most applied engineering tool world-wide in full-scale activated sludge systems for the prevention of the bulking sludge phenomena, nevertheless there are regularly still reports citing selector failure in the control of bulking sludge (*e.g.* in Ekama *et al.,* 1996b). 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 which have somehow affected the population dynamics, giving competitive advantage to filamentous bacteria. For the control of *M. parvicella*-type bulking in biological nutrient removal processes, selectors seem to fail (Eikelboom, 1994; Ekama *et al*., 1996b; Kruit *et al*., 2002) or are not sufficient (see also Section 10.4.2 on physiology). The different selectors and their potential pitfalls will be briefly described here in the following sections. A general overview of selector design guidelines can be found in Table 10.2.

10.6.1.1 Aerobic selectors

Until 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 the USA, the systems were mainly a high loading 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 type 021N and type 1701. In Europe and South Africa, low loading rate plants such as oxidation ditch systems and extended aeration systems were constructed. By the 1990s more stringent regulations regarding nutrient emissions, particularly ammonia emissions, were required in Europe and the 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 *Microthrix parvicella* and types 021N, 0041/0675 0092, and 0581. These observations led to the definition of what is referred to as the low F/M filamentous bacteria group by Jenkins *et al.* (1993a,b). Aerobic selectors, a 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.*, *Sphaerotilus natans*, but not always successfully in the case of *Microthrix parvicella*.

The contact time, a typical design parameter for selectors, has a significant and non-linear effect on sludge settleability (Martins *et al.,* 2003a). When the contact time is insufficient, soluble substrate is not fully consumed in the contact zone, and is carried over into the main aeration basin. In this case the growth of filamentous microorganisms will occur due to substrate uptake at a low substrate concentration in the aeration basin. 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 major effect of a contact tank that is either too large or small on the sludge volume index (SVI) makes a good design difficult (Figure 10.7).

Figure 10.7 Aerobic selector (photo: M. van Loosdrecht).

In systems with highly dynamic feeding patterns, such as temperature and flow, and load variations such as wastewater treatment systems, a good design is not easy and may be a plausible reason for the regular reports on the failing of aerobic selector tanks. Therefore, in practice it is expected that only plug flow systems, as in long channels (length-to-width ratio larger than 10:1), compartmentalised contact tanks, or a SBR fed in a pulse-feed, can guarantee a significant macro-gradient of substrate concentration and will function properly under highly dynamic conditions. Furthermore, proper staging can improve the performance of activated sludge systems which are kinetically limited (Scuras *et al.,* 2001). 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 have been proposed (Figure 10.5). Although the recommended contact time in an aerobic selector tank is very short, the amount of oxygen required is approximately 15 to 30% of the soluble COD removed (Jenkins *et al*., 1993a; Ekama *et al*., 1996a; Martins *et al*., 2003b). This underlines the importance of a 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 (Martins *et al*., 2003b). Furthermore, the aeration control is very important and the sensors should be placed in the first compartment where the oxygen consumption is highest (Table 10.2) and not, as is often the case, at the end of the selector.

10.6.1.2 Non-aerated selectors

As in the aerobic selectors, all the readily biodegradable COD should be removed in anoxic and anaerobic (selector) reactors, preventing any readily biodegradable COD entrance into the aerobic stage, which if it occurs might give advantages to filamentous bacteria (Kruit *et al.,* 2002). Furthermore, oxygen and nitrate should both be absent from the anaerobic reactor, and oxygen from the anoxic reactor. Recycle flows might unintentionally add to the introduction of oxygen in such selectors. In addition to disruption of EBPR and/or denitrifying activity, the presence of microaerophilic conditions in the anaerobic and/or anoxic stages, which for instance can be attributed to the diffusion of oxygen through the liquid surface (Plósz *et al.,* 2003) or to the aeration of the returned sludge/liquid stream in screw pumps or at overflow weirs, can lead to worsening sludge settling characteristics.

Table 10.2 Selector design guidelines recommended for aerobic, anoxic and anaerobic selectors in municipal wastewater treatment systems.

Parameter	Value	Reference
Aerobic selector		
Number of compartments	≥ 3	Jenkins *et al.* (1993a)
Contact time	10-15 min., depending on load, temperature and wastewater composition (*i.e.* fraction of readily biodegradable COD).	Still *et al*. (1996)
Sludge loading rate	12 (1st compartment), 6 (2nd comp.) and 3 (3rd comp.) kgCOD/kgMLSS.d	Jenkins *et al.* (1993a)
Floc loading	50-150 gCOD/kgTSS (1st comp.)	Kruit *et al.* (1994)
DO concentration	≥ 2 mgO_2/l, but it depends on the sludge loading rate, floc loading rate and/or substrate uptake rate. Sensor should be placed in the 1st comp.	Sezgin *et al.* (1978), Albertson (1987), Martins *et al.* (2003b)
Anoxic selector		
Number of compartments	≥ 3	Jenkins *et al.* (1993a)
Sludge loading rate	6 (1st comp.), 3 (2nd comp.) and 1.5 (3rd comp.) kgCOD/kgMLSS.d	Jenkins *et al.* (1993a)
Contact time	45-60 min.	Kruit *et al.* (2002)
$(RBCOD/NO_3\text{-}N)_{consumed}$	Usually around than 7-9 mg readily biodegradable COD per $mgNO_3$-N due to substrate storage.	Jenkins *et al.* (1993a), Ekama *et al.* (1996a), Van Loosdrecht *et al.* (1997)
Anaerobic selector		
Number of compartments	≥ 3, long channels (length-to-width ratio larger than 10:1)	Albertson (1987), Kruit *et al.* (2002)
Contact time	1-2 h	Kruit *et al.* (2002)
$(COD_{VFA+fermentable}/PO_4\text{-}P)_i$	9-20 gCOD/gP	Wentzel *et al.* (1990), Smolders *et al.* (1996)

10.6.1.3 Anoxic selectors

The design criterion of anoxic selectors (Table 10.2) is primarily based on the ratio of readily biodegradable COD *versus* nitrate entering the reactor (Ekama *et al*., 1996b). Since in selectors an important fraction of readily biodegradable COD is expected to be converted into storage products, the ratio is higher than the typical range for direct denitrification (approximately 7-9 mg readily biodegradable COD per mg NO_3-N). The type of mixing has been found to be of less or no influence compared to aerated selectors. Anoxic selector designs are therefore in principle more robust and can therefore cope with variations in flow, as long as nitrate remains in surplus (Martins *et al.,* 2004b). However, in full-scale systems it is difficult to balance the nitrate load to readily biodegradable COD 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 readily biodegradable COD can either be stored by ordinary heterotrophic organisms (Beun *et al.,* 2000), or used by the phosphorus accumulating organisms (PAOs) or by glycogen accumulating non-polyphosphate organisms (GAOs).

However, leakage of readily biodegradable COD into 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 in the competition between these microorganisms. In the meantime, in order to design a reliable full-scale anoxic selector it is advisable to first perform pilot-plant studies and only then to scale up the system. Nevertheless, despite all the above, the struggle for even more reduced effluent nitrate concentrations will anyway lead to the recycling of sludge with low nitrate content, limiting the use of anoxic selectors.

10.6.1.4 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 the readily biodegradable COD uptake rate to the phosphorus release rate, which is needed for phosphorus removal, ensuring that virtually no readily biodegradable COD enters the main aeration basin (Table 10.2). These conditions are created in activated sludge systems to promote the growth of PAOs. However, another group of bacteria, known as glycogen accumulating organisms (GAOs), can grow quite well in similar conditions. Both types of bacteria are capable of taking up simple soluble substrates in the anaerobic stage and store it as polyhydroxyalkanoates (PHA). The energy reserve which allows the uptake and storage mechanisms is however different in both types of bacteria. Polyphosphate is used in the case of PAOs and glycogen in the case of GAOs. This metabolic diversity gives considerable flexibility to the anaerobic selector in removing the organic load, independently of the occurrence of phosphorus removal. However, in spite of the great diversity of PAOs and GAOs, no filamentous bacteria have been unequivocally identified so far as having this metabolism. As a result of the availability and consumption of readily biodegradable COD in the anaerobic stage, PAOs and GAOs accumulate in the sludge and therefore force the aerobic microorganisms, which lack substrate in the aerobic phase, to decrease in number. Thus, the more that substrate is removed from the anaerobic stage, which also means less substrate available in the oxic stage, the better the settling characteristics of the activated sludge should be. Furthermore, sludge rich in polyP bacteria usually settles better because they form dense clusters, and also intracellular polyphosphate, in combination with chemical phosphorus precipitation, increases the sludge density even more. The mixing conditions in anaerobic selectors, as in anoxic selectors, do not seem to be critical. Moreover, the carryover of COD into the aerated stage is much less detrimental than in aerobic conditions; this means that the anaerobic selector design is not critical (Martins *et al.,* 2004a). Recent reports have confirmed the success of anaerobic selectors in controlling sludge bulking, even when *Microthrix parvicella* is the most dominant filamentous bacteria (Kruit *et al.,* 2002). An anaerobic selector, however, cannot always be used. For instance, its application is not recommended for waste streams rich in sulphur compounds. This is because anaerobic conditions can increase the production of reduced sulphur compounds, which can be used in the aerobic stage by filamentous sulphur-oxidising bacteria (Eikelboom, 2000).

Recent studies in the Netherlands have showed that satisfactorily settling sludge (SVI<120 ml/g with common values below 100 ml/g) can be achieved in full-scale biological nutrient removal systems by implementing carefully controlled strictly anaerobic and anoxic plug-flow selectors (Kruit *et al.,* 2002). A potentially important factor which has 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 mgN/l) and a high DO concentration (> 1.5 mgO_2/l) (Kruit *et al.,* 2002; Tsai *et al.,* 2003). An example of a treatment system based on these considerations is the BCFS® concept (Van Loosdrecht *et al.*, 1998) which is currently being successfully applied to twelve full-scale plants in the Netherlands.

10.7 MATHEMATIC MODELLING

To study complex ecosystems such as activated sludge cultures, in which many factors are acting together, mathematical modelling can be a very useful tool. A lot of 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 are examples of useful models to study population dynamics in activated sludge systems. As the knowledge of bacterial physiology increases these models are continuously being upgraded (Figure 10.8).

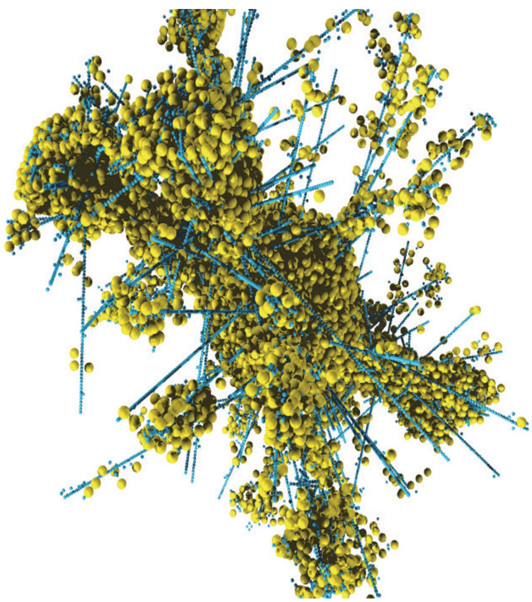

Figure 10.8 Modelled floc structure with filamentous and floc-forming bacteria (image: Martins *et al.*, 2004c).

An example is the incorporation of storage processes in ASM 3. This is a first attempt to allow for modelling of storage polymer metabolism and to better describe the conversions occurring in selector-like systems. In addition, recently developed metabolic models provide a better link between the kinetics and the biochemistry of storage (Beun *et al.,* 2000) and will certainly contribute to the description and modelling of the metabolic processes which take place in selectors. However, despite the great detail in these models, the growth of filamentous bacteria and, thus bulking sludge, still cannot be predicted.

Models which can predict the settling characteristics of 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 existence of a dual species or a group competition (*e.g.* floc formers, filaments, low-dissolved oxygen filaments, low F/M filaments) for a single substrate or for a group of substrates (readily biodegradable COD or slowly biodegradable COD) (Kappeler and Gujer, 1994a; Takács and Fleit, 1995). These models can be basically categorised into two groups: the first one considering the bacterial physiology and kinetic-biokinetics, and the second considering both the physiology and kinetics, as well as the morphology of the bacteria. Diffusional transport of substrates into the activated sludge flocs is an important mechanism in the competition between floc-forming bacteria and filamentous bacteria. Kappeler and Gujer (1994a) proposed that readily biodegradable COD could favour the growth of filamentous microorganisms due to substrate diffusional resistance in the biological flocs. They suggested the integration of this behaviour in traditional activated sludge models (Chapter 14). Observed readily biodegradable COD 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. This approach gives realistic qualitative results. However, it is still not possible to predict the SVI of the sludge or the sludge settling properties.

Later studies have taken both the micromorphology of the floc and the oriented growth characteristics of the filamentous bacteria (preferential unidirectional growth) into account (Takács and Fleit, 1995). 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 filamentous bacteria predominate in soluble substrate and DO-limited environments. The authors did not specifically differentiate between the effect of kinetic parameters and the effect of cell morphology.

More recently Martins *et al.* (2004c) adopted a previous model for predicting biofilm morphology (Picioreanu, 1998) for activated sludge flocs. This approach showed that the diffusion gradient is more important for floc morphology than the differences in affinity constants between different organisms, supporting the diffusion gradient-based theory for selection of filamentous bacteria.

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 micro-gradient in sludge flocs, under a wide range of kinetic parameters. More research should be carried out 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 study may lead to the better understanding of the competition between filamentous and non-filamentous bacteria in the gradient-governed microenvironments that are very typical of activated sludge systems.

10.8 GRANULAR SLUDGE

With the understanding that bulking sludge occurs when readily biodegradable COD is removed under conditions where significant substrate gradients occur over the sludge floc, it was realized that granules should form when these conditions are minimised (Beun *et al*., 1999). In effect, granular sludge and bulking sludge are at opposite ends of the sludge morphology scale (Figure 10.9).

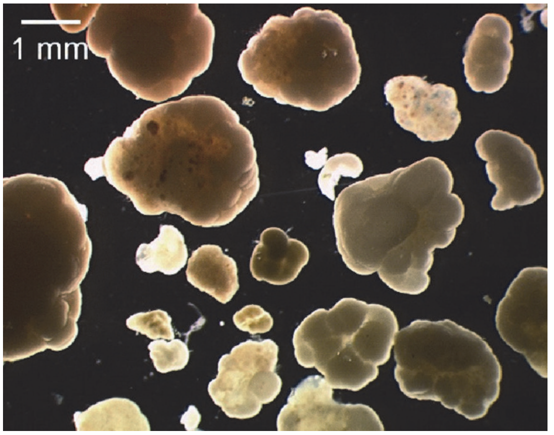

Figure 10.9 Aerobic granular sludge (photo: M.R. de Kreuk).

For biofilms it was already hypothesized that biofilm morphology depends on the ratio between substrate transport rate and biomass growth (Van Loosdrecht *et al.,* 1995, 2002). This not only means that minimising substrate gradients over the sludge floc will improve the SVI, but also selecting slow-growing bacteria will improve the SVI too. Therefore it was *e.g.* always relatively easy to obtain anaerobic granular sludge or nitrifying granular sludge (Figure 10.10), and difficult to achieve this under full aerobic conditions.

The application of anaerobic selectors results in a group of bacteria (phosphate and glycogen accumulating bacteria) with a lower maximum growth rate than ordinary heterotrophic bacteria. They have therefore an additional advantage over aerobic selectors. Selecting for this type of conditions has also been shown to lead to more stable aerobic granular sludge formation (De Kreuk *et al.,* 2004).

A further description of aerobic granular sludge processes is given in Chapter 11.

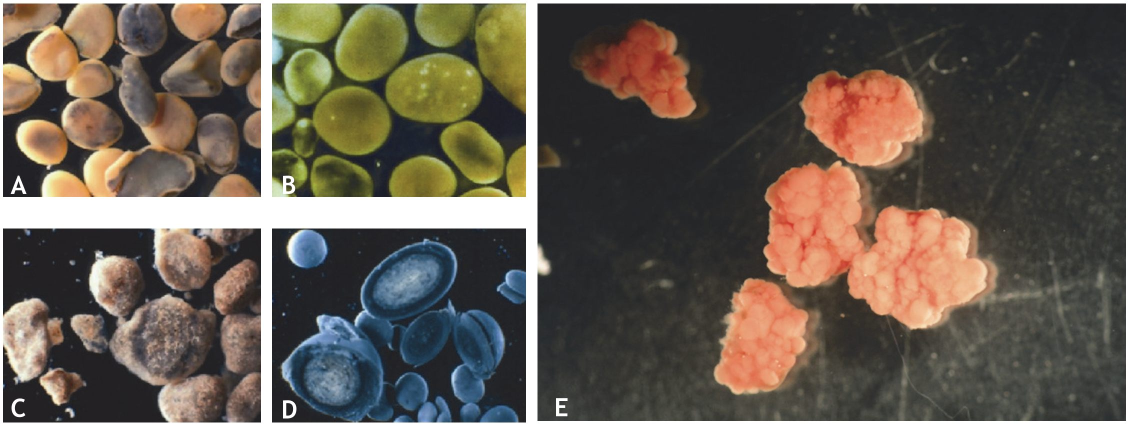

Figure 10.10 Varieties of granular sludge: (A) nitrifying, (B) heterotrophic, (C) denitrifying, (D) methanogenic (photos: Biothane B.V), and (E) anammox (photo: Paques B.V.).

10.9 CONCLUSIONS

Bulking sludge is one of the main problems of activated sludge properties, but in practice there is a sufficient level of understanding at least at the level needed to control the problem. For instance, an activated sludge BNR system designed to minimise bulking sludge problems should have the following general characteristics: (*i*) a pre-treatment step to remove complex substrates (*e.g.* lipids), (*ii*) plug-flow selector reactors to allow a significant macro-gradient of substrate concentration along the system, (*iii*) 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, (*iv*) the avoidance of intermittent aeration and microaerophilic conditions, and (*v*) sufficient aeration to maintain high DO concentration (> 1.5 mgO_2/l) and low ammonium concentration (< 1mg N/l) in the final aerobic stage.

The basic ideas outlined in Chapter 11 have even led to processes based on the opposite of bulking sludge: granular sludge. Even in well-designed systems, operational weaknesses can easily lead to cases of bulking sludge. Therefore, as long as the basic processes governing sludge morphology are not fully taken into account, the statement made by Albertson (1987), *'In spite of all we learn and understand, some sludge will still bulk'*, will still be valid (Figure 10.11).

Figure 10.11 Extreme manifestation of sludge bulking (photos: Eikelboom, 2006).

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NOMENCLATURE

Symbol	Description	Unit
C_S	Substrate concentration in bulk liquid	mgCOD/l
K_S	Half saturation concentration for substrate utilization	mgCOD/l
q_S	Substrate uptake rate	mgCOD/l.h

Abbreviation	Description
AS	Anaerobic digestion model
ASM	Activated sludge model
A/V	Surface to volume ratio
BOD	Biological oxygen demand
COD	Chemical oxygen demand
EPS	Extracellular polymeric substance
FISH	Fluorescence *in-situ* hybridization
GAO	Glycogen accumulating organism
IWA	International Water Association
MAR	Microautoradiography
MLSS	Mixed liquor suspended solids
PAO	Phosphorus accumulating organism
PHA	Polyhydroxyalkanoate
RBCOD	Readily biodegradable COD
SRT	Sludge retention time
SVI	Sludge volume index
UCT	University Cape Town
VFA	Volatile fatty acid