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Review

Strategies for Controlling Filamentous Bulking in Activated Sludge Wastewater Treatment Plants: The Old and the New

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Abstract: Filamentous bulking and foaming are the most common settling problems experienced in activated sludge (AS) wastewater treatment plants (WWTPs). The quality of the final effluent is poor during episodes of bulking and foaming, which is an environmental, human health and economic burden. Remedial measures are often ineffective, and traditional non-specific methods such as chlorination may also negatively impact important functional bacterial species such as nitrifiers. Modifications to older methods as well as new strategies are required for controlling filamentous bulking. Laboratory testing needs to be followed by testing at scale in WWTPs. This review describes the filamentous bacteria responsible for filamentous bulking, with a focus on their global distribution and known factors which are selective for the growth of specific filaments. Traditional and new non-specific and biological control strategies are reviewed and discussed. Research gaps are identified with the aim of promoting continued efforts to establish effective control strategies for filamentous sludge bulking.

Keywords: filamentous; bulking; activated sludge; chlorination; rotifer; bacteriophage; selector

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1. Introduction

Filamentous bulking and foaming are caused by the excessive growth of filamentous bacteria in wastewater treatment plants (WWTPs) and are the most common and yet complex sludge separation problems experienced globally in activated sludge (AS) WWTPs [1–3]. Ideally, there should be an optimal balance between the growth of filamentous bacteria and floc-forming bacteria [4,5]. In moderate amounts, filamentous bacteria are beneficial to AS settle-ability in clarifiers as they serve as a structural base for robust floc formation [6–8] (Figure 1A). However, filamentous overgrowth can result in the presence of either open flocs and/or inter-floc bridging, depending on the type of filamentous microorganisms that are present (Figure 1B). The former occurs when copious filaments grow inside flocs which are poorly consolidated, thus capturing water inside the flocs. The latter occurs when filaments protrude from the flocs into the bulk liquid, forming bridges between the flocs and preventing the compaction of individual flocs [6,7,9]. Excessive growth of filamentous bacteria is promoted by the presence of a variety of physicochemical factors and/or changes in process conditions that indirectly contribute to the chemical status of the AS [10,11]. Based on studies using laboratory-scale sequencing batch reactors (SBR) with synthetic wastewater under low dissolved oxygen (DO) conditions, researchers have found that the quantity and quality of the extrapolymeric substances (EPS) present during excessive filamentous bacterial growth have a significant effect on settling [12,13]. Similar results on EPS quality during bulking have been noted with granular AS under nitrogen (N) deficient conditions [14]. Notably, during bulking episodes: (i) overall decreases in EPS, (ii) relative increases in the polysaccharide to protein ratios in the EPS, (iii) relative increases in the ratio of proteins responsible for the synthesis of hydrophilic to hydrophobic proteins, and (iv) increased surface electrostatic charges have been found [12,13]. These factors ostensibly retard bacterial agglomeration, causing loose floc structures and bulking.

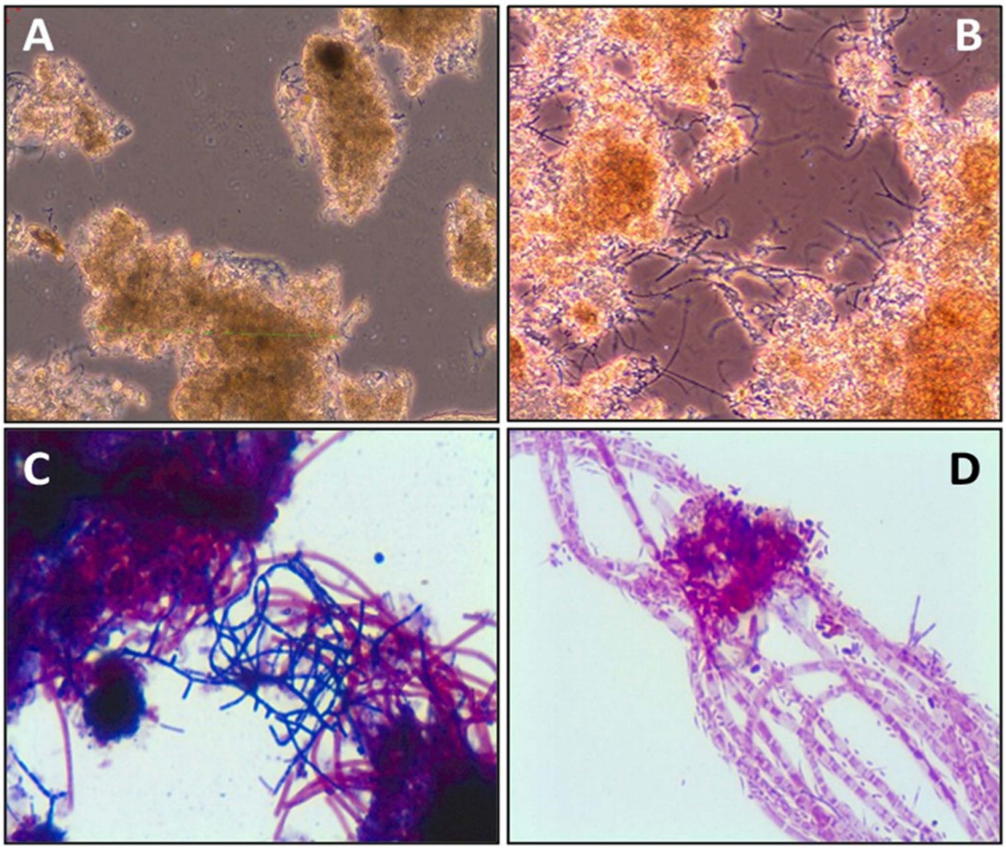

Figure 1. Wet mount of large, compact, firm, well-settling flocs (**A**), loose flocs with inter-floc filament bridging (**B**), Gram stain of Gram-negative Eikelboom Type 0092 and Gram-positive *Gordonia amarae*-like organisms, and (**C**) Gram stain of Gram-negative Type 021N (**D**).

Foaming sludge is characterised by a considerable volume of foams and solids accumulating on the surface of aeration basins and settling tanks [15,16]. Biotic foaming caused by certain types of bacteria must be clearly distinguished from abiotic foaming caused by high contents of surface-active compounds such as grease and oil in wastewater and sludge [17]. Biological foaming is a well-recognised AS operational problem that results from the presence of microorganisms that have cell walls with hydrophobic properties, typically due to the presence of mycolic acids. It is these hydrophobic properties that enable the cells to selectively float on the surface of the mixed liquor [17]. Although most biological foaming has been attributed to filamentous bacteria, non-filamentous bacteria with hydrophobic cell walls may also be involved in foaming [18].

Prevention of filamentous bulking and foaming is challenging, despite extensive research devoted to this topic [16]. Different types of physical, chemical, and biological methods have been used to counter these settling problems. However, none of the current methods are completely effective [5,19,20]. For example, biocides used to suppress the growth of filamentous bacteria are non-specific and can also have detrimental effects on other functional members of the sludge community such as floc-formers and nitrifiers [4]. This review describes filamentous sludge bulking, the global distribution of filamentous bacteria in AS WWTPs, important key factors affecting filamentous bacterial growth in AS, and the conventional and more recently described strategies for preventing and/or controlling filamentous bulking in AS WWTPs.

2. Filamentous Bacteria: Identification and Global Distribution

Bulking and foaming only became widely problematic in AS WWTPs after the introduction of biological nutrient removal (BNR) AS WWTPs in the 1970s [21]. Around 75% of 33 BNR WWTPs studied in South Africa in the 1980s experienced bulking and foaming [21]. Later studies conducted in Denmark, Greece, the Netherlands and Germany confirmed that the new BNR configurations that included alternating anoxic/aerobic or aerobic/anoxic zones played a significant role in promoting the growth of filamentous over floc-forming bacteria [22].

Due to difficulties with culturing filamentous bacteria, many were traditionally categorised using microscopic morphological and staining features and classified alphanumerically as Eikelboom 'types' [23] (Figure 1C,D). Although the use of light microscopy as an identification tool is cost-effective and rapid, it has been found that more than one bacterial genus and/or species may have the same morphological and/or physiological features and vice versa [24,25]. Common filamentous bacteria in AS samples have therefore been more definitively identified using fluorescent in situ hybridisation (FISH). However, the taxonomic identities of several filaments have not been elucidated and probes have not been designed for many filamentous morphotypes. Routine use of clone libraries from denaturing gradient gel electrophoresis (DGGE) as well as high throughput amplicon sequencing for identifying filamentous bacteria causing bulking in AS samples also have some limitations [26]. In some cases, dominant taxa in genomic DNA extracted from bulking sludge are assumed to be the causative agents of filamentous bulking although they have not been visualised microscopically as being filamentous [12]. This has been seen, for example, with the genera *Saprospiraceae*, *Tetrasphaera* and *Trichococcus* [27,28].

Despite the current limitations of the available technologies, results obtained using more novel strategies are continually adding to the pool of knowledge. For example, the use of bioinformatic information from amplicon sequencing can be used to design FISH probes [24], qPCR may be used for filament quantification [29,30], and whole genome sequencing can provide insight into the metabolic capabilities of filaments [29]. In time, sufficient information will be available to complete taxonomic and functional databases such as 'MIDAS' [31]. In the interim, it may be argued that the use of Eikelboom morphotypes is still relevant, especially in routine laboratories. In order to interrogate and compare results from historical and more current studies, Eikelboom morphotypes have been used to describe many of the filamentous bacteria in this manuscript

Several national surveys have focused on determining the prevalence of filamentous bacteria in AS WWTPs (Table 1). Some filamentous bacteria are ubiquitously dominant, while others, such as Eikelboom Type 0092 (designated as members of the *Chloroflexi* phylum) have been documented as dominant in AS WWTPs in some countries across the world, but not others (Table 1). Studies suggest that certain filamentous bacteria may be associated with WWTPs with different design configurations, operational practices, and influent characteristics [32,33]. The regional variations in filamentous bacterial distributions have therefore been ascribed to differences in combinations of influent biochemistries, environmental conditions, and operational factors [34–36].

The most frequently observed filamentous bacteria in bulking sludge include: *Microthrix parvicella/Candidatus* Microthrix, *Sphaerotilus natans*, *Nostocoida limicola* (designated as a member of the genus *Tetrasphera*), and the morphotypes Eikelboom Type 021N (designated as member/s of the genus *Thiothrix*), Type 0041 (designated as member/s of the phylum *Chloroflexi*), Type 0092, Type 1851 (designated as member/s of the genus *Kouleothrix*) and the Gram-positive branching *Gordonia amarae*-like organisms (GALO), which includes *G. amarae* (formerly *Nocardia amarae*), and the so-called 'nocardioform' organisms which are typically Actinobacteria [17,24,25,37–39]. The primary filamentous bacteria responsible for biological foaming are GALOs, *M. parvicella*/*Ca.* Microthrix and, to a lesser extent, some Eikelboom morphological types [28,40–42].

Table 1. Dominant filamentous bacteria in activated sludge wastewater treatment plants in different countries.

Country	Ranking (in Order of Dominance)			Reference
	1	2	3	
		Bulking		
Australia	*M. parvicella*	Type 0041/0675	Type 0092	[43]
Denmark	*Ca.* Microthrix	*Trichococcus*	*Chloroflexi*	[44]
	M. parvicella	Type 0041	*N. limicola*	[22]
Czech Republic	*M. parvicella*	*N. limicola*	Type 0092	[45]
France	*M. parvicella*	Type 0675	GALO	[46]
Germany	Type 0092	*M. parvicella*	Type 0041	[47]
Italy	*M. parvicella*	GALO	Type 0675	[17]
Netherlands	*M. parvicella*	Type 0041	Type 021N	[22]
	Type 0092	*M. parvicella*	Type 0041	[48]
South Africa	Type 0092	Type 1851	GALO	[49]
	GALO	Type 0041	Type 0675	[50]
	Type 0092	Type 0675	Type 0041	[21]
Switzerland	S. *natans*	Type 021N	Type 0961	[51]
		Foaming		
Australia	*M. parvicella*	GALOs	Type 0092	[52]
Czech Republic	*M. parvicella*	*N. limicola*	GALO	[53]
France	*M. parvicella*	Type 0675	GALO	[46]
Italy	*M. parvicella*	GALO	Type 0675	[17]
Netherlands	*M. parvicella*	GALO	*N. limicola*	[54]
South Africa	Type 0092	*M. parvicella*	GALO	[21]
United Kingdom	*M. parvicella*	*N. limicola*	GALO	[55]

"Type" refers to Eikelboom morphological types. GALO (*Gordonia amarae*-like organisms), including *G. amarae* (previously *N. amarae*), filamentous bacteria previously named *Nocardia* spp., and other bacteria with the same morphological cellular and staining properties.

3. Factors Favouring the Growth of Filamentous Bacteria

Theoretically, filamentous bacterial types dominate under a variety of conditions in BNR WWTPs (Figure 2). Some, such as food-to-microorganism (F/M) ratios, appear to be almost universally selective, while others are selective for fewer filament types [56]. Multiple factors are usually responsible for filament selection, typically confounding correlative analyses between filament types and physicochemical parameters. For this reason, most literature descriptions are based on laboratory studies with defined synthetic wastewaters and have often proven difficult to validate in 'real world' scenarios.

3.1. Wastewater and Mixed Liquor Composition

3.1.1. Food-to-Microorganism Ratio/Substrate Availability

Low substrate availability (and related low F/M ratios) are features of BNR AS WWTPS that are commonly associated with filamentous bulking [57–59]. Not only the quantity but also the quality of the organic substrates (readily biodegradable/slowly biodegradable/particulate/soluble) play a fundamental role in microbial selection [36,58,60]. There may also be some correlation between bulking and the degree of endogenous decay taking place in the reactors because it has a direct impact on the quality of the organic substrate [58]. Theories have been expounded to try and understand the link between organic substrate availability and the promotion of floc forming and/or filamentous bacterial growth. These include the filamentous backbone theory (FBT), the kinetic selection theory (KST), the hydrolysis of slowly biodegradable organics theory (HSBO) and the substrate diffusion limitation (SDL) theory [61–63]. For both the KST and the SDL theories, the proposed main driving force for filamentous overgrowth is a low substrate concentration [60,61]. For the former, this is based on the premise that filamentous bacteria have higher substrate affinity constants (Ks) and maximum growth rates (μmax) than floc formers, while the latter is based on the premise that morphologically filamentous bacteria can take up more substrate because they have larger external surface areas [60]. The HSBO theory is based on the experimentally validated fact that flocs can internalise and then later hydrolyse slowly biodegradable substrates [62]. A group of researchers [60] have recently proposed an expansion of the Activated Sludge Model (ASM) no 1 (ASM1) to describe filamentous bulking, as well as to model the effects of incorporating aerobic selectors in the AS process configuration (Section 4.2.1). The ASM1 was expanded by classifying: (i) the chemoorganotrophic (heterotrophic) bacteria in AS into free filaments and floc-formers, and (ii) the soluble substrate into that present in the influent and that generated within the flocs via hydrolysis as per the HSBO model. In combination with the KST, SDL, and FBT theories, the expanded model allows the concentration of free filaments and the associated likelihood of bulking to be predicted. A possible flaw with the model is the fact that *M. parvicella* has also been shown to utilise long-chain fatty acids (slowly biodegradable substrates) at similar rates to floc formers under aerobic, anoxic, and anaerobic conditions [64]. This has been advanced as a reason why selector tanks (Section 4.2.1) may fail to prevent bulking by this organism [64].

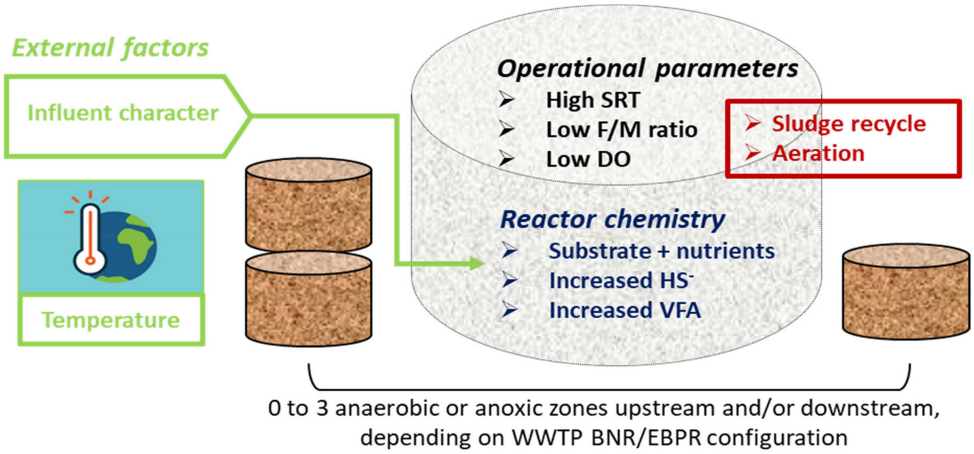

Figure 2. Schematic showing (i) the relationships between the main factors that play roles in filamentous bulking (external factors shown in **green**, aerobic reactor chemical parameters shown in **blue**, and operational parameters shown in **black**), and (ii) means of controlling some of these factors (in **red**). SRT—sludge retention time; F/M—food-to-microorganism ratio; DO—dissolved oxygen; HS—hydrogen sulphide; VFA—volatile fatty acids.

3.1.2. Inorganic Nutrients: Nitrogen and Phosphorus

To promote robust, well-settling flocs in clarifiers, it has been recommended that the ratio of biological oxygen demand (BOD) to N to phosphorus (P) in AS should be around 100:5:1 [65]. Correlations have been found between biotic non-filamentous viscous/zoogloeal bulking and N and P deficiencies [66], especially in AS WWTPs treating industrial wastewater [7,67,68]. Mechanisms responsible for purported filamentous bulking associated with N and/or P deficiencies are less clear. In laboratory studies, filamentous bulking caused by *Thiothrix* species due to an increase in the polysaccharide to protein ratio in the EPS content in N-deficient granular AS has been reported [14]. However, P deficiency did not cause bulking. In complete contrast, a correlation between P deficiency but not N deficiency and filamentous bulking was found in SBRs [36]. In the latter, the researchers established a negative correlation between the sludge volume index (SVI) and polyhydroxyalkanoate (PHA) concentrations. These results suggest indirect rather than direct links between N and/or P deficiencies and sludge settle-ability.

3.1.3. Sulphides and Volatile Organic Acids

Under oxygen (O_2) limited conditions caused by long sewer retention times, in anaerobic reactors in enhanced biological phosphate removal (EPBR) plants, or insufficient aeration in AS reactors, oxidised sulphur (S) compounds such as sulphates (SO_4^{2-}) in wastewater are reduced to sulphides (S^{2-}) which are then present as HS^- in the mixed liquor [69]. Anaerobic environments also lead to the accumulation of volatile organic acids (VOAs), mostly the short-chain volatile fatty acids (VFA), acetate and propionate from fermentative microbial metabolic processes [36]. As discussed in Section 3.1.1, the quality and quantity of organic substrates are key factors associated with filamentous versus floc-forming bacterial selection and proliferation. *Thiothrix* species (which includes Type 021N), are often metabolically versatile, allowing them to adapt to a range of environments. Different species have been shown to obtain energy heterotrophically from organic substrates using O_2 or nitrate (NO_3^-) as terminal electron acceptors and/or chemolithotrophically by taking up and oxidising reduced S compounds, which may also be stored as poly-S [70]. Similarly, some *Thiothrix* species may compete with non-filamentous phosphate accumulating organisms (PAOs) for VFAs and phosphates which they store as PHAs and utilise when O_2 is available [71,72]. A significant correlation between the SVI, Type 021N abundance and propionic acid concentration has been shown [73]. Indeed, the correlations between *Thiothrix* species abundance and increased HS^- and/or VFAs (as found in 'septic' wastewater) have been well documented [71]. Bulking by *Thiothrix nivea* and *S. natans* has been shown to occur in laboratory reactors when fed with VFAs under low substrate availability [36], confirming similar results found by other researchers [62,74]. A positive correlation between SO_4^{2-} reducing bacterial (SRB) activity and *Thiothrix* abundance has also been established. For example, it has been found that abundant growth of *Thiothrix eikelboomii* followed the growth of SRB in reactors fed with acetate and peptone [75], supporting the results of earlier studies that showed that filamentous bulking could be caused by increased SO_4^{2-} reduction [76], and reversed by adding ferric chloride ($FeCl_2$) in order to reduce the HS^- concentration by favouring biological Fe-reducing over SO_4^{2-} reducing reactions by "filamentous sulphur bacteria" [77].

3.1.4. Toxic Metals

Toxic metals can accumulate and cause microbial growth inhibition and/or biological toxicity in AS WWTPs [78]. Different inhibitory concentrations of Cu, Ni, and Zn have been reported for filamentous bacteria, including *Thiothrix* species, Eikelboom Type 1701 and Type 021N [79]. However, only as few studies on the inhibition threshold and mechanism of heavy metal effects on filamentous bacteria have been conducted, and further research is required.

3.2. Other Operational Parameters

3.2.1. Dissolved Oxygen

The typical target DO concentration in AS is approximately 2 mg/L. Overgrowth of certain filaments has been correlated with O_2 limitation at lower DO concentrations [80–83]. Oxygen limitation may be exacerbated once filamentous overgrowth occurs because O_2 mass transfer rates are impeded due to an associated increase in the apparent viscosity of the mixed liquor in filamentous bulking sludge [84,85].

Various filaments, including some *Thiothrix* species and Type 0092, can synthesize and store PHAs, allowing them to proliferate in O_2-deficient environments and compete with heterotrophic bacteria for organic substrates [38,71,72,86]. *Microthrix parvicella*/*Ca.* Microthrix is metabolically active under a wide range of DO concentrations [40,87], but the typically long and regular filaments become deformed when the DO concentration is high [40,88]. Although *M. parvicella* has been reported to grow preferentially in low DO reactors [88], this may be related to some degree to other factors such as substrate loading [87,89]. It has also been postulated that their facultative ability to grow anoxically/anaerobically, together with their high specific surface area affords them more of a competitive advantage when the O_2 concentrations are inadequate for the growth of aerobic floc-formers [78]. Other filamentous bacteria that have also been associated with low DO environments include Type 0041 [86,90] and Type 1851 (*Kouleothrix* sp.) [86].

3.2.2. Sludge Retention Time and Sludge Load

The sludge retention time (SRT) is directly linked with the F/M ratio and sludge age. These are all key operational parameters linked to the performance of AS WWTPs [91,92]. Fortunately, the SRT is easily manipulated by adjusting the sludge wasting rate [91,92]. Laboratory studies suggest that the length of filaments within and/or extending from flocs has a significant impact on the size of flocs and that this is related to the SRT [93]. Apart from the ancillary effect of the SRT on substrate availability (F/M), microorganisms that have shorter generation times than the SRT are more likely to dominate than those with longer generation times [94]. Certain filamentous bacteria, notably Type 1851 (*Kouleothrix* sp.) [39], *Microthrix parvicella/Ca.* Microthrix [43,89,95], and Type 0092 [7,38] have been found to dominate in WWTPs with long SRTs and the introduction of shorter SRT (<5 days) has been shown to suppress the overgrowth of *M. parvicella*, Type 0041, and Type 0675, but not Type 0092 in a laboratory environment at 14–18 °C [64].

3.3. Climatic and Seasonal Factors

Temperature

Climatic features that may impact microbial selection and proliferation in AS WWTPs are rainfall and temperature. However, rainfall is only significant for WWTPS that receive stormwater together with domestic and/or industrial influents, or where the integrity of sewer systems is compromised and groundwater levels fluctuate to allow intermittent groundwater ingress [96,97]. Given microbial temperature preferences, it is expected that more pronounced seasonal shifts may be seen in countries with larger temperature gradients and colder winters. However, the range of outdoor ambient temperatures measured in the different seasons is not necessarily reflected in the influent (wastewater) temperatures. In a recent study, a large range of ambient temperatures were measured over two winters (-33.9 to -20.6 °C, n = 30) and summers (13.3 to 27.8 °C, n = 30), but the influent temperatures in a municipal SBR in the area fell within a substantially more moderate and narrower range, with the lowest being measured in spring (11.56 $\pm$ 0.58 °C, n = 30), and the highest in summer (17.53 $\pm$ 0.51 °C, n = 30) [98]. Although the authors reported seasonal shifts in the overall filamentous bacterial composition, the study was only conducted for one calendar year so confounding variables could not be statistically excluded. However, recurrent seasonal microbial succession was noted in a WWTP in Denmark [99], and in full-scale WWTPs in China, with the SVI being negatively correlated with temperature [35,100]. However, winter bulking is not globally universal, as evidenced by studying a full-scale WWTP in Kuwait, where filamentous bulking was only noted in summer [101].

Low-temperature bulking is usually ascribed to *M. parvicella*, but low temperature alone is not sufficient to promote poor settling. For example, *M. parvicella* proliferated profusely at 13° but not at 20 °C in a bottle experiment [102]. However, bulking only occurred when oleic acid was used as a substrate under alternating anaerobic-aerobic-conditions, while no bulking occurred when other mixed carbon substrates were used under aerobic conditions. In a bench-scale AS reactor fed with real domestic wastewater and operated with anaerobic, anoxic and aerobic tanks at 15 °C and SRT of 11–15 days, it was found that at low sludge loading (0.04 $\pm$ 0.004 kg COD/(kg MLSS.day^{-1}), the SVI increased to 164 mL/g due to proliferation of *M. parvicella* and deterioration of floc structure [89]. This was reversed at medium and high sludge loading rates of 0.07 $\pm$ 0.015 COD/kg MLSS.day^{-1} and 0.12 $\pm$ 0.016 COD/kg MLSS.day^{-1}, respectively. The hypothesis that the combination of low temperature and low sludge load was responsible for bulking by *M. parvicella* was validated at full-scale in a A2O (anaerobic–anoxic–oxic) configured AS WWTP in China that had experienced filamentous bulking due to *M. parvicella* (SVI = 265 $\pm$ 55 mL/g) in winter when the sludge loading rate, as well as the temperatures, were lower. When the sludge discharge rates were manipulated to maintain a higher and more stable sludge loading rate (0.14 ± 0.04 kg COD/kg MLSS.day^{-1}), bulking was alleviated over a 2-year experimental period, despite low temperatures [89].

Other filamentous bacteria have also occasionally been associated with low temperatures. In laboratory-enhanced biological phosphorus removal (EBPR) reactors fed with synthetic wastewater and operated at 15° and 25 °C, significant ($p < 0.05$) increases in abundance of *Thiothrix* species and Type 0041 occurred at 15 °C and were attributed to 'septic' conditions in the anaerobic reactor as described in Section 3.1.3 [100]. In Hong Kong, which has a warmer climate (average ambient 13–30 °C) than Northern Europe and parts of China, *M. parvicella* and *N. limicola* abundances were negatively correlated with temperature [103]. In contrast to other studies, no correlation with low F/M was found. This anomaly may be climate-related or influent-related, as seawater is used to flush toilets in Hong Kong [103].

4. Control Strategies for Filamentous Bulking Sludge

Control of filamentous bulking and foaming remains a challenge facing the field of AS wastewater treatment globally [38,104–106]. General methods used to suppress the overgrowth of filamentous bacteria include chemical methods such as the addition of chlorine (Cl), hydrogen peroxide (H_2O_2), aluminous or ferrous salts [107,108], the addition of selectors [109] operational adjustments such as manipulating the SRT [64,110], and water sprays or steam application [111]. However, most non-specific methods are aimed at reducing the levels of filamentous bacteria without addressing the aetiology of over-proliferation [112], often only providing a temporary solution [108]. In addition, they can have a negative impact on the functional microbial ecology within the AS [113,114]. Specific methods are aimed at removing the cause of filamentous proliferation and are targeted to specific microorganisms or groups of microorganisms. Both specific and non-specific methods have advantages and disadvantages (Table 2).

Table 2. Advantages/disadvantages of some methods used to control filamentous bulking.

Method	Advantages	Disadvantages
Chlorination	- Simple - Cost effective - Well tested at scale	- Addition of chemicals required - Some filamentous species are resistant to chlorination - Toxic substances may be formed (THM) - Can reduce nitrification, COD removal - May cause zoogloeal bulking
Hydrogen peroxide	- Simple - No toxic by-products	- More expensive than chlorination - Dosage and contact times are critical (skilled operation required)
Ozonation	- Can be retro-fitted to existing WWTPs - No need for addition of chemicals - Promising results at lab-scale	- High energy requirements - Some filamentous species are resistant to ozone - More long-term studies needed at scale
Metal addition	- Simple - Simultaneous removal of P	- Addition of chemicals required - Not a long-term solution - Excess sludge production
Selectors	- Proven technology for the control of filamentous bulking - Can be retro-fitted to existing WWTPs - No need for addition of chemicals - Performance can be optimized by changing operational parameters *in-situ*	- Skilled operators required to respond to upsets - Prevention of bulking by some filament types may be replaced by bulking with others - Questionable performance for reducing *M. parvicella*

Table 2. *Cont.*

Method	Advantages	Disadvantages
Rotifers	- No need for addition of chemicals - No need to adjust operational parameters - Promising results at lab-scale - Species can be selected according to WWTPs physicochemical parameters - Universal filament consumers	- Technology has yet to be tested at scale - Skilled personnel required to maintain live stocks of rotifers - May be sensitive to bulking chemicals and other toxins
Bacterio-phages	- No need for addition of chemicals - No need to adjust operational parameters - Promising results at lab-scale - Host specific, allowing targeting of species causing bulking	- Technology has yet to be tested at scale - Skilled personnel required to maintain live stocks of an array of species - May be sensitive to bulking chemicals and other toxins - Effect on functional microbial species still needs to be elucidated

4.1. Non-Specific Methods

4.1.1. Chlorination-Based Control

Chlorination as a control strategy for the overgrowth of filamentous bacteria has been well documented [7,115–118]. Chlorination works by inhibiting the proliferation of the exposed outer filaments branching out of the flocs while the floc formers are protected within the floc and remain viable [7,119,120]. The common forms of Cl used for disinfection at wastewater treatment plants are Cl_2 gas, liquid (sodium hypochlorite (NaOCl) solution) and pellets/granules (calcium hypochlorite ($CaClO_2$)) [121]. Effective disinfection is dependent on the exposure time and Cl dosage, which is usually between 10 and 20 mg/L Cl_2 [122]. Using the respirometry technique and INT-dehydrogenase activity test, it was demonstrated that the effect of Cl on filamentous bacteria depends on the location of the filaments in the flocs as well as the presence of EPS [113]. In 2020, researchers reported a significant improvement in sludge settle-ability through the addition of 10 g/L NaCl to experimental SBRs fed with different mixtures of organic acids as substrates [123]. They found that NaCl addition suppressed the growth of the dominant filament, *Meganema,* and enriched the PHA-producing *Thauera* and *Paracoccus* genera, resulting in PHA productivity in the range of 0.244–0.298 g/L.day^{-1} [123].

However, due to the non-selective nature of chlorination, both nitrification and biodegradation of organic matter in AS WWTPs may be hindered, resulting in poor effluent quality [5]. Despite many successful reports of bulking alleviation when chlorination has been used in accordance with recommendations [116,124], failures have also been reported. For example, researchers investigated the susceptibility of *S. natans* and the floc-forming *Acinetobacter anitratus* to NaOCl at concentrations from 5.4 to 8.5 mg/L Cl_2 and contact times ranging from 2–20 min [116]. In this study, the researchers found that the floc-forming species were more susceptible to Cl than the filamentous species [116]. Another study that investigated the effect of treatment with NaOCl for 8–12 hrs on 0.6 L filamentous bulking sludge found the causative bacterium, Eikelboom type 021N, to be resistant to chlorination up to a dosage of 80 mg Cl/g total suspended solids (TSS) [4].

Molecular techniques have also been explored to determine the effects of chlorination during wastewater treatment. One such study used assessed the effect of propidium monoazide treatment by high throughput MiSeq sequencing. In this study, chlorination induced immediate changes in the composition of the bacterial communities accompanied by further impacts on WWTP performance over 24 h [118]. These results were echoed in a more recent study [114] where it was found that chlorination had a significant effect on filamentous bacteria ("*Nocardioides* and *Gordonia*"), but promoted the proliferation of *Zoogloea*, the bacterium responsible for viscous bulking (Hennessy, 2020).

Notwithstanding the advantages that chlorination is a relatively cheap and simple means to mitigate bulking, it is not a preventative measure, and it does not solve the underlying cause of bulking [116]. Another negative effect of using this biocide as a control measure is that it produces undesirable by-products such as trihalomethanes which can be dangerous to human health [7,116].

4.1.2. Hydrogen Peroxide-Based Control

Hydrogen peroxide has been used to mitigate sludge bulking since the mid-1980s/early 1990s [7,125] in a similar way to chlorination [7,126]. The use of H_2O_2 results in the fragmentation of the exposed filamentous bacterial strands, thereby improving the settling of flocs [127]. Unlike chlorination, no toxic residual by-products are formed because any excess H_2O_2 dissociates into water (H_2O) and O_2 [120]. Although H_2O_2 dosages and application times for effective filament reduction vary between WWTPs, they are often higher than for chlorination [128], making H_2O_2 application a more expensive option [129]. In one study, a dosage of 20 mg/L H_2O_2 added to the mixed liquor at 5 min flow time from the exit of the reactor resulted in foaming in the clarifier. When this was increased to 15 min at a dosage of 12 mg/L, the SVI was reduced within 2 days [127]. In a laboratory study conducted by the US Environmental protection agency (EPA), the efficacy of H_2O_2 on filamentous bulking caused by *Sphaerotilus* was demonstrated [130]. At H_2O_2 concentrations of 20–200 mg/L, bulking was controlled for several days. At 200 mg/L H_2O_2, the free-growing filamentous bacteria were eliminated, while the spherical aerobic flocs were unaffected. However, when the H_2O_2 concentration was increased to 400 mg/L, partial de-flocculation of the spherical flocs occurred. In a more recent study, a 0.1% (v/v) H_2O_2 solution added to AS influent with a contact time of 2 weeks resulted in a sharp decrease in SVI, confirming the feasibility of this approach in controlling filamentous bulking sludge in some instances [126].

4.1.3. Ozone

The addition of ozone (O_3) to return activated sludge (RAS) can be used to control the growth of most filamentous bacteria [99]. In a similar way to chlorination and peroxidation, ozonation improves the floc structure by inhibiting the growth of the exposed filamentous bacteria thereby leading to improved sludge settle-ability [99,131,132]. A dosage of 5 g O_3/kg TSS has been shown to significantly improve sludge settling [131,132]. At this concentration, a 1.7-fold reduction in filamentous bacterial abundance without any adverse consequences on the rates of biological nitrification and phosphorous (P) removal, or non-filamentous microbial community composition has been demonstrated [133]. A decrease in diluted SVI from 7–35% by application of 3–4.8 gO_3/kg TSS has also been shown [133]. However, it has recently been found that the effects of ozonation on different filaments are not universal [133,134]. Researchers found that *M. parvicella* is susceptible to ozonation while Type 0041 filaments are more resistant. This suggested that higher dosages of O_3 would be required to control sludge bulking relative to the latter morphotype [133].

4.1.4. Metal Application (Aluminium and Iron)

Salts of aluminium (Al^{3+}) and ferric ions (Fe^{3+}) ions are widely used in WWTPs to chemically remove P by precipitation [135]. It has been found that the addition of Fe^{3+} salts such as $FeCl_2$ can simultaneously suppress filamentous growth and improve floc density [76,77,136,137] while also retarding $SO_4{}^{2-}$ reduction [76]. In a study comparing changes in sludge settle-ability with the addition of 30 mg/L ferrous sulphate ($FeSO_4$) or 11 mg/L $AlCl_3$, it was found that only the latter was able to reduce the stirred specific volume index (SSVI) of AS to <100 mL/g [138]. However, the effect was transient as there was a sudden increase in the SSVI to >160 mL/g 4 weeks later, intimating that the addition of metal ions may not be a long-term solution to filamentous bulking [138]. In addition, Al^{3+} and Fe^{3+} coagulants can alter the pH in AS and lead to excess sludge generation via precipitation of P [137].

4.1.5. Synthetic Polymer-Based Control

Synthetic high molecular weight anionic polymers can either be added individually or in combination with cationic polymers at the exit of the aeration tank or to the clarifier as a control measure for filamentous bulking [65,139]. While one study found that a combination of alumina-silicate, a cationic polymer (50–67% MLSS) and a biocide (quaternary ammonium salt) destroyed problematic filaments and improved sludge settle-ability [140], another demonstrated some resistance to the biocide by *N. limicola* II [139]. It has also been shown that while the addition of synthetic polymer can solve poor sludge settle-ability, it may cause a microbial shift and negatively affect the growth of floc-formers [141]. In addition, when the polymer is no longer added, more severe bulking than previously can occur, leading some researchers to question the suitability of polymer addition for bulking control [1,141].

4.1.6. Magnetic Field Application

With this approach, a magnetic field is used to improve AS settle-ability by strengthening polymeric interactions and electrostatic forces that promote the aggregation of bacterial cells [142,143]. Exposure of AS to a magnetic field intensity of 15 mT for 48 h has been shown to increase COD removal efficiency, increase cellular aggregation to 90% and retain 54% surface hydrophobicity in AS within 10 h, while in another study, a magnetic field intensity of 88 mT has been shown to promote COD removal, nitrification and denitrification in an AS SBR while simultaneously alleviating filamentous sludge bulking [139]. Although these studies have demonstrated that the application of magnetic fields can inhibit the growth of 'nuisance' bacteria responsible for bulking and foaming [139], further research is required to determine how exposure to magnetic fields affects the growth, morphology, EPS and cell hydrophobicity of different filamentous species, and how to apply such technology at scale [139].

4.1.7. Quorum Sensing

Bacteria produce, detect and release chemical signalling molecules known as autoinducers (AIs) [144]. Information received via AIs is used to determine changes in cell numbers, and the bacteria respond by altering gene expression to optimise cellular growth and reproduction [145]. It was recently found that the expression of synthesis (hdtS), receptor (lasR and cciR), and associated metabolic genes were associated with the proliferation of filamentous bacteria during AS bulking [145]. Furthermore, a quorum-sensing-based strategy mediated by N-acyl homoserine lactone (AHL) was used to control filamentous bulking in AS and it was found that N-hexanoyl-L-homoserine lactone levels increased from 31.39 ± 3.52 ng/g VSS to 125.29 ± 6.70 ng/g VSS as the population of filamentous bacteria increased. Ultrasonic time-domain reflectometry has been used to monitor the regulation of exogenous AHLs on sludge settling. In this study, the AHLs C12-HSL, 3OC6-HSL and 3OC14-HSL improved the settling of AS by 2.03, 1.90 and 1.62 times, respectively [146]. In addition, it was found that the use of exogenous 3OC6-HSL decreased the abundance of filamentous bacteria by 2.7%. These fundamental studies show that AHL-mediated quorum sensing is a promising new strategy for controlling filamentous bulking.

4.2. Specific Methods

Specific methods can be defined as the preventative measures used to obtain suitable environmental conditions to control the proliferation of nuisance bacteria such as bulking filaments [139].

4.2.1. Selector-Based Control

Biological selectors are upstream aerobic, anaerobic, or anoxic reactors comprising <10% of the volume of the aerobic tanks in AS WWTPs [16,34,60,147]. The primary function of selectors is to prevent filamentous bulking, but they can also reduce the chemical toxicity of influent to the AS process as shown with phenol, 2-chlorophenol, 2,4-dichlorophenol and 1,2,4-trichlorobenzene [148].

Selector tanks are fed by RAS and influent wastewater which creates concentration gradients similar to those found in plug flow systems. They have been successfully applied for alleviating bulking in municipal (e.g., [34,60,149]) and industrial WWTPs, including those treating beet sugar mill [150], petrochemical [151], food processing [152], and slaughterhouse [153] effluents. Studies suggest that the use of more than one selector in a series is advantageous [60,154].

The principle of aerobic selectors has been well elucidated and is based on the premise that the high F/M to low F/M ratio gradient in the selector creates a 'feast' to 'famine' gradient. Initially, the high substrate availability promotes preferential growth of floc-formers and concurrent storage of substrates that they utilise for growth when the F/M ratio decreases, giving them a competitive advantage [34,120,155]. The quality of the organic matter also plays a role in the function of aerobic selectors, and the presence of high amounts of particulate organic matter (POM) can be detrimental to performance [156].

The ASM model No. 1 has recently been modified to predict filamentous bulking sludge and to model the effects of incorporating aerobic selectors in AS systems based on organic substrate availability [60] (Section 3.1). Although the model requires more validation, in a study at a full-scale WWTP in Jordan, the filament score was reduced from 4 (filaments very common) to 1.5 (very few filaments) as predicted by the model with the addition of 3 aerobic selector tanks [60]. Such models are not valid for non-aerated selectors because kinetics only play a significant role in bulking in aerated, but not anoxic or anaerobic selectors [157]. Anoxic selectors have been shown to reduce bulking by increasing the degree of denitrification in the selector [158] and anaerobic selectors by hydrolysis of particulate organic matter (POM) to increase substrate bioavailability [159].

Selectors are not always effective in controlling bulking, and the operational parameters often need to be adjusted to achieve satisfactory performance [34,157]. Prevention of bulking by different filamentous bacteria using selectors has been reported, including Types 021N and 0961 [149], and *Thiothrix* species [153]. However, it appears that the proliferation of some filament types may be difficult to control, most notably *M. parvicella*, ostensibly because of its ability to utilise long-chain fatty acids (LCFA) as substrates and the fact that these are not completely removed in selectors [87,160]. Notwithstanding filament prevalence, prevention of bulking may not be possible in WWTPs operated with long mean cell residence time (MCRT > 10 days) or with very high influent BOD [157].

In a comprehensive study conducted in the United States and the United Kingdom, only 47 of 87 AS WWTPs reported improved sludge settleability after the addition of selectors [157]. To understand the dynamics for selector failure, regression analyses were performed on data collected from 48 WWTPs divided into 3 categories based on the MCRT and aeration. Short and long MCRT WWTPs were classified as those with MCRTs of <4 and >10 days, respectively, while those with MCRTs of 4–10 days were classified according to the degree of nitrification (full = long MCRT), and/or the type of dominant filaments (Short MCRT: *S. natans, Thiothrix* species, Types 1863, 021N, 1701, Long MCRT: *M. parvicella, N. limicola*, Types 0092, 0041, 0675, 0914, 1851). Based on the results, it was recommended that for WWTPs operated with short MCRT, non-aerated selectors and high mixed liquor suspended solids (MLSS $\geq$ 1 500 mg/L), bulking could be prevented by adjusting the total reactor MCRT to >4.5 days, the selector MCRT to 2–3 days and maintaining initial contact zone (ICZ) F/M ratios at <1.0 kg BOD/kg MLSS.day^{-1}.

Previously, low F/M ratios (0.7–1.2 BOD/kg MLSS.day^{-1}) have also been applied to control bulking in 5 full-scale WWTPs operated with anoxic selectors in the United States [161]. In contrast, high ICZ F/M ratios (15 kg BOD/kg MLSS.day^{-1}) have been recommended for short MCRT WWTPs operated with aerated selectors [157]. In addition, the %RAS (recommended 25–35%) has been found to be a significant factor for WWTPs operated with aerated selectors, but not significant for those operated with non-aerated selectors [157]. Such studies provide valuable insight into how to improve the functioning of selectors in the 'real world'. However, additional studies in different geographical locations are required to assess whether suggested changes in operational parameters translate into long-term bulking alleviation in full-scale WWTPs.

4.2.2. Condition-Based Control

Condition-based control is based on the theory that the physicochemical conditions in the mixed liquor of AS WWTPs can be manipulated by changing selected operational parameters to create environments conducive to the growth of floc formers at the expense of filaments [17,43,66]. As nutrient deficiency can cause filamentous bulking, some strategies have been directed at changing F/M ratios and/or the biodegradability of organics and/or other nutrient ratios to mitigate bulking [66]. Some examples based on laboratory studies have been reported: low F/M bulking was mitigated by adding substrates with higher particulate to soluble nutrient ratios (such as waste flour) to AS reactors [162], and when the synthetic substrate was changed from particulate (starch) to soluble (glucose), the total extended filament length (TEFL) of Eikelboom type 1851 increased [35]. Another suggestion for control of a specific filament type is to employ methods to reduce the concentration of LCFA in the influent to reduce the proliferation of *M. parvicella* [64,87,160]. However, such strategies have their limitations for long-term control because if, for example, the F/M is increased to counter the growth of 'low F/M' filaments, it may cause excessive proliferation of 'high F/M' filaments such as Eikelboom Type 021N/*Thiothrix* species and *N. limicola* instead [17,43].

4.3. Biological Control

Biological control of filamentous bulking is based on the use of biotic agents native to AS to control the growth of filaments, and currently includes research into the use of rotifers and bacteriophages. Although these measures show promise, uptake in full-scale WWTPs has yet to be realised, probably because of the technical skills required to grow and maintain these organisms, especially the host-specific bacteriophages.

4.3.1. Rotifer-Based Control

Some rotifers are well adapted to AS environments, including factors such as low pH and the presence of toxic metals; they are also voracious feeders, being able to ingest several times their body weight per day [163,164]. These factors make them good potential biological control agents to limit filamentous bacterial growth. The capability of rotifers to control bulking while simultaneously reducing sludge volume was first demonstrated using the rotifer *Lecane inermis* [165]. It was found that this Monogonont rotifer transferred from pure culture was able to proliferate rapidly in AS and ingest filamentous bacteria [165]. Subsequently, a number of studies have shown that *L. inermis* has the ability to significantly reduce the abundance of *M. parvicella* [165], *N. limicola* [166], Eikelboom Type 021N [167] and Type 0092 [168], *Thiothrix* species [169], and *Haliscomenobacter hydrossis* [170], suggesting that it is a universal filament consumer.

Temperature and food availability have been shown to be the main factors that affect the grazing rate of rotifers [171–173]. Therefore, rotifers need to be selected according to requirements, for example, different species are required in colder climates as most rotifers, including the well-studied *L. inermis*, usually grow better and are more active at higher temperatures in AS [166]. The influence of temperature (8 °C, 15 °C and 20 °C) on different rotifers from AS systems was therefore investigated [165]. The results showed that *Lecane tenuiseta* exhibited high growth rates and better adaptability to 8 °C than *L. inermis* and members of the genus *Cephalodella*, suggesting that it may be a good candidate for bulking control in cold climates/seasons. Subsequently, in a lab experiment [174], it was confirmed that *L. tenuiseta* reduced the abundance of *M. parvicella* with no negative effect on the chemical parameters of the effluent at temperatures of 13 °C and 20 °C. If control of bulking using rotifers is to be implemented, simultaneous use of chemical bulking control measures such as chlorination or chemical flocculation would need to be carefully considered as they may be toxic to rotifers. The effects of two common flocculants ($AlCl_3$ and aluminium sulphate ($Al_2(SO_4)_3$) on *L. inermis* at 8 °C, 15 °C and 20 °C have already been investigated [175]. Both negatively affected the rotifer population at low concentrations, and the temperature was the key factor that modified the toxicity of the flocculants to the rotifers. The reduced sensitivity of rotifers to the flocculants at temperatures below 15 °C supported the idea that the combined application of rotifers and chemicals is reasonable and effective for filamentous bulking control at low temperatures [175].

4.3.2. Bacteriophage-Mediated Control

Bacteriophages are bacterial viruses that are highly specific in their host-cell recognition. They are found in all habitats where their host bacteria proliferate [108,176,177]. Virulent bacteriophages adsorb onto host cells by recognising surface receptors that are common to closely related species, implying that some species may be lysed by the same bacteriophage/s [20]. Bacteriophages are capable of influencing microbial community structures by specifically infecting and rapidly lysing host bacteria [178,179] and have therefore been proposed as candidates for biological control of filamentous bulking [180].

A number of bacteriophages isolated from AS systems have been shown to be effective for filament control under laboratory conditions [181]. A bacteriophage specific to *H. hydrossis* was isolated from the mixed liquor of a WWTP [182]. At a bacteriophage-to-host ratio of 1:1000, the host death rate was 54% and the virus was able to reduce the SVI in AS from 150 mL/g to 105 mL/g. In another study, the application of a WWTP bacteriophage (SnaR1) isolate specific for *S. natans* reduced the abundance of this filament up to 83% after 72 h of infection [183]. Similarly, four bacteriophage isolates specific to *Gordonia* species were able to achieve a significant 10-fold reduction in the *Gordonia* host when compared with non-treated controls [20].

5. Conclusions and Recommendations

This review summarises what is currently known about filamentous bulking sludge, factors that influence the growth of filamentous bacteria, as well as the current and proposed control strategies. Although filamentous bulking has been well-studied, it still occurs widely in AS WWTPs across the world. To develop specific, efficient, and affordable strategies to solve bulking sludge problems, a variety of factors need to be considered. Due to the number of confounding variables in full-scale WWTPs, research into new control strategies has only really been conducted at a lab scale.

Many filaments have traditionally been described using morphological features, and the quest to link taxonomic identities and physiological functioning to many of these is ongoing. As culture on artificial media is challenging, molecular approaches are being refined to acquire fundamental knowledge of filamentous bacteria that will assist with formulating bulking control measures. New control strategies need to continue and promising strategies such as the use of rotifers, quorum sensing strategies and the use of magnetic fields need to be tested long-term and at scale.

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