Isolation and cultivation of filamentous bacteria implicated in activated sludge bulking

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Abstract

Filamentous bacteria have long been associated with activated sludge bulking and foaming and are known to be the main cause of this problem. Chemical control methods such as chlorination and the use of hydrogen peroxide have been, and still are, used to cure bulking and foaming but are only effective as interim measures. More detailed understanding of the physiology and biochemistry of filamentous bacteria is still required for effective long-term control of bulking and foaming. Isolation and cultivation of filamentous bacteria in pure culture have shown promise as methods to gaining better understanding of bulking and foaming. The aim of this project was, therefore, to assess different techniques for effective isolation and cultivation of filamentous bacteria in pure culture. Activated sludge samples from Durban and surrounding areas were screened microscopically to identify constituent filamentous bacterial populations. Samples with varieties of different filamentous bacteria were subjected to various floc break-up procedures (nonidet surfactant treatment, sonication and cellulase hydrolysis) in conjunction with physical separation (centrifugation) to separate filamentous bacteria from floc-forming bacteria. Both treated and untreated samples were serially diluted and plated onto a variety of different solid media, whereafter discrete bacterial colonies were isolated and screened microscopically for filamentous morphology. Cellulase hydrolysis proved unsuccessful for filament isolation while direct inoculation, nonidet pretreatment and sonication resulted in the isolation of five different filaments, one via sonication and two each via the other methods. The filaments were provisionally identified as *Sphaerotilus natans, Microthrix parvicella*, Type 1863, Type 0092 and *Haliscomenobacter hydrossis*.

Keywords:

Introduction

The activated sludge process has found vast application as an effective means of wastewater treatment. Due to public concern about environmental protection, and its (the public's) increasing exposure to pollution and subsequent consequences, an extensive re-evaluation of existing wastewater management concepts has come about (Orhon and Artan, 1994). The objective of the activated sludge process is to remove soluble and insoluble organics from the wastewater and to convert this material into a flocculent microbial suspension that settles well in a conventional gravity clarifier. As a general rule, the nature of the wastewater will dictate the preferred process modifications, primarily for the purpose of maintaining mixed liquor settling quality (Eckenfelder and Musterman, 1995). Basically, activated sludge comprises a microbiological enrichment culture consisting of a mixed, and largely uncontrolled, consortium of micro- and macro-organisms that remove wastewater inorganics and organics and transform them into environmentally acceptable forms (Richard, 1989).

It is important to maintain the growth of floc-forming bacteria on wastewater organics, which will settle under gravity in the final clarifier so as to obtain or sustain a clarified supernatant (final effluent) and a thickened return sludge. However, not all bacteria in the activated sludge process are floc-formers. Many different types of filamentous bacteria have been identified in activated sludge and play important roles in wastewater treatment. Filamentous bacteria directly affect sludge settling as they make provision for the rigid support network or backbone upon which floc-forming bacteria can adhere and grow into suitable activated sludge flocs (Richard, 1989). Filamentous bacteria may be considered detrimental to wastewater treatment when they occur in excessive quantities (sludge bulking), but are just as important in the development of activated sludge flocs with proper settling and clarification properties (Gerardi et al., 1990). Filamentous microorganisms can also be good indicators of conditions prevailing in an activated sludge system on a microbiological level. The indications given by the filamentous bacteria could be of low dissolved oxygen (DO) (e.g. *Sphaerotilus natans*), low food-to-micro-organism (F/M) ratio (e.g. *Microthrix parvicella*, Type 0092), presence of septic waste (e.g. *Thiothrix* spp.), nutrient deficiency (e.g. *Haliscomenobacter hydrossis*) and low pH in the system (e.g. fungi) (Jenkins et al., 1986).

Since the introduction of continuous-flow reactors, sludge bulking has been one of the major problems affecting biological waste treatment (Slykes, 1989 as cited by Bitton, 1999). A bulking sludge may be defined as one which settles and compacts slowly due to proliferation of filamentous organisms and resultant interfloc bridging, which in turn produces sludge with a poor settling rate (Richard, 1989). Two toxicants, chlorine and hydrogen peroxide, have been used to selectively kill filamentous organisms and therefore alleviate symptoms of activated sludge bulking (Jenkins et al., 1986). Chlorination is however, a last resort in the control of bulking. It should only be contemplated when effluent from the bulking plant is likely to cause environmental damage to the receiving water. If used correctly it provides a rapid alleviation of bulking, but, if used incorrectly may completely inhibit all treatment (Horan, 1990). The formation of foams or scums, on the surface of activated sludge aeration basins and secondary clarifiers, has also been ascribed to the possible presence of undegraded surfactants in the aeration basin and the presence of large numbers

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TABLE 1
Pre-screening of treatment plants for constituent filamentous populations for sample selection

Northern works	Southern works	Amanzimtoti works	Darvill works	Laboratory-scale MLE
Type 0041 Type 0675 *H. hydrossis* *N. limicola* II	Type 0041 Type 0675 *H. hydrossis*	Type 0041 Type 0675 *H. hydrossis* *N. limicola* II Type 021N *Thiothrix* II	Type 0041 Type 0675 Type 1701 *N. limicola* II Type 1851 *S. natans* *Nocardia* spp.	*Sphaerotilus* spp. *H. hydrossis* *S. natans* *N. limocola* Type 1851

of filamentous bacteria, such as *Nocardia* spp. and *M. parvicella* (Jenkins et al., 1986; Hwang and Tanaka, 1998). The causes of *Nocardia* growth and foaming in activated sludge are not well understood with much contradictory information on the subject. Many of the suggested causes are indeed hypotheses that still need to stand the test of experimental verification (Jenkins et al., 1986). In covered aeration basins *Nocardia* foam has been known to accumulate such that the water content of the foam trapped in the basin exceeds the available head for gravity flow of sewage through the aeration basin. Process control can be compromised if a significant fraction of the solids inventory is present in the foam trapped in the aeration basin (Jenkins et al., 1986). In many domestic sewage treatment plants in northern Europe and Australia, *M. parvicella* has been predominantly observed as a foaming organism (Seviour et al., 1990, Pujol et al., 1991 and Eikelboom, 1993, as cited by Hwang and Tanaka, 1998). A report by Seviour et al., (1990) (as cited by Hwang and Tanaka, 1998) indicated that 62% of the foam samples in Australia contained *M. parvicella*, and in 24% of those it was predominant (Hwang and Tanaka, 1998). Hwang and Tanaka (1998) showed that the application of chlorination had no effect on *M. parvicella* reduction or foaming suppression and that a more effective and economical method for the control thereof still needs to be established.

With the current state of knowledge, control of filamentous bulking remains a challenge facing all engineers, chemists and microbiologists working in the field of wastewater treatment (Beccari and Ramadori, 1996). Isolation of filamentous organisms is becoming a promising solution for the investigation of these organisms and factors that promote or inhibit their growth. Because the majority of filamentous bacteria are overgrown by more rapid growers, samples are diluted and pretreated before plating on solid media (Kämpfer, 1997). Narrow and short filaments or filaments scarce in the activated sludge may be concentrated by centrifugation. Another method for selective isolation of filamentous bacteria is micromanipulation with special microtools under a microscope (Kämpfer, 1997). Although the latter method is one of the latest used methods for isolation of filamentous bacteria, it requires highly specialised equipment, is very expensive and therefore inaccessible to many laboratories. It also tends to be very labour intensive and difficult (Seungbum, 2001). It is important that the flocs be broken up before isolation so as to facilitate easier separation of filamentous bacteria from floc-forming bacteria. This is so as to avoid or minimise competition by faster growing floc-formers on different solid media. The basis to gaining a proper understanding of the role of filaments in bulking and foaming lies in obtaining these organisms in pure culture and studying them on the level of their physiology, biochemistry and commensal relationships.

It was therefore the aim of this project to assess different techniques for effective isolation and cultivation of filamentous bacteria in pure culture, with particular emphasis on the assessment of floc break-up procedures and filament separation from floc-forming bacteria.

Experimental

Sampling

Four different activated sludge treatment plants in the Durban and surrounding areas (Northern Works, Southern Works, Amanzimtoti Works, Darvill Works - Pietermaritzburg) were screened for constituent filamentous bacteria. Sterile Schott bottles were used for 250 ml grab samples (× 3) of mixed liquor, obtained from the aerobic zones of the various plants. The mixed liquor samples were stored half-full in the Schott bottles so as to maintain aerobic conditions for filamentous survival during sample transit (Eikelboom, 2001). The procedures employed for identification of filaments were those used for typical identification of filaments (Jenkins et al., 1986). From Northern Works four different types of filaments were observed, Southern Works three different types, Amanzimtoti Works six different types and seven different types from Darvill Works (Table 1). Darvill works was selected for further samples (× 3 grab samples) because of its larger variety in filamentous bacterial population as opposed to the other plants screened. The Darvill system is similarly configured to the Johannesburg system, for biological nitrogen and phosphorus removal, with an anaerobic zone followed by informal anoxic and then aerobic zones. A pre-anoxic zone is also incorporated to protect the anaerobic zone from nitrate/nitrite restriction on phosphorus release (Lilley et al., 1997; De Haas, 1998). Typically, this configuration has the potential to select for twice the amount of denitrifying organisms than conventional anoxic zones, thus resulting in very good nitrogen removal capacity (Osborn et al., 1989). The nature of the influent substrate composition to Darvill is made up of a mixture of industrial and domestic waste streams, typically of low organic strength. The system treats 60-160 Ml/d (De Haas, 1998). In addition to Darvill sludge, grab samples of bulking activated sludge were also obtained from a laboratory-scale system treating soybean and sunflower oil effluent. The laboratory-scale system was configured as a modified Ludzack-Ettinger process for carbonaceous organics and nitrogen removal and operated at a 24 h hydraulic retention time with 15 d sludge age. During

intermittent soybean effluent additions, to the laboratory-scale system, extensive filamentous bulking was obtained with sludge volume index (SVI) measurements as high as 770 ml/g (Reddy et al., 2003).

Sample pretreatment (floc break-up)

In addition to direct inoculation of mixed liquor samples to solid media, sample pretreatment was performed so as to reduce surface tension between filaments and floc-formers, therefore, facilitating the separation of floc-formers from filaments. The aim of this step was to ultimately reduce the number of floc-formers in the mixed liquor samples. The mixed liquor samples were pretreated with nonidet surfactant, sonication and cellulase prior to physical separation (by centrifugation) and isolation of filaments.

Direct inoculation

Untreated mixed liquor samples were serially diluted (10^{-1}-10^{-5}), homogenised with a vortex mixer for approximately 10 s, and then directly inoculated onto solid media. Media that were used for isolation have all been reported to be successful in supporting the growth of a wide range of filamentous bacteria (Table 2). The techniques employed for the inoculation of agar plates were the streak-plate and spread-plate techniques (Mulder and Deinema, 1981; Ziegler et al., 1990, Kämpfer, 1997). Plates were incubated at 20^{0}C for 10 d followed by isolation of well-defined colonies onto fresh agar plates. Pure cultures of isolates were obtained with continuous sub-culturing and incubation at 20^{0}C.

TABLE 2
Media used for inoculation and isolation of filamentous bacteria

#	Media	Filaments reported to grow
1	R2A agar (Atlas, 1993)	*M. parvicella*, *S. natans*, Types 1701, 0803, 1863, 0092, 0411 and *Leptothrix* spp. (Kämpfer, 1997)
2	CGYA (Casitone glycerol yeast agar) (Bridson, 1995)	*S. natans* (Kämpfer, 1997)
3	I Medium (Van Veen, 1973 as cited by Mulder and Deinema, 1981; Atlas, 1993)	*Thiothrix* spp., *Beggiatoa* spp. and Type 021N (Kämpfer, 1997) and *H. hydrossis* (Mulder and Deinema, 1981)
4	TYGA (Tryptone yeast glucose agar) (Atlas, 1993)	*Nocardia* spp. (Kämpfer, 1997)

Nonidet pretreatment

Nonidet (Igepal CA- 630, Sigma - Germany) is a non-ionic surfactant that alleviates cell clumping without any obvious damage to the cells when used at correct concentrations i.e. 0.1% (Stahl and Amann, 1991). Different concentrations (0.1, 1, 10, and 100%) of nonidet were mixed with the mixed liquor samples (9 parts sample: 1 part nonidet) to assess which concentration achieved effective floc break-up. Following pretreatment, samples were evaluated microscopically (× 1000, oil immersion) so as to determine the effects of the various nonidet concentrations on the integrity of the filaments and floc structure. The undiluted (100%) nonidet proved to be the better concentration for floc break-up. The samples were then homogenised for 1 min using a vortex mixer so as to assure complete homogenisation and optimal floc break-up. Samples were then centrifuged at 1 500 r·min^{-1} for 10 min to separate floc-formers from filaments via gradient separation. Serial dilutions (10^{-1}-10^{-5}) of the pretreated samples were made and inoculated onto fresh agar plates. Inoculation, media, cultivation and isolation procedures were the same as those for the direct inoculation samples.

Sonication

Sonication of activated sludge samples is a convenient method for dispersing sludge flocs (Pike, 1975 and Jorand et al., 1995 as cited by Munch and Pollard, 1997; Kämpfer, 1997). Mixed liquor samples were sonicated at 20W for 10, 15, 30, 45, and 60 s. Following sonication samples were evaluated microscopically (× 1000, oil immersion) so as to determine the effects of the various sonication times on the integrity of the filaments and floc structure. Sonication at 20W for 10 s was selected due to least disruption of filament integrity. Sonicated samples were not centrifuged because filaments broke down into small pieces (almost the same size as the floc-formers) thus making gradient separation of filaments and floc-formers impossible. Serial dilutions (10^{-1}-10^{-5}) of the pretreated samples were made and inoculated onto fresh agar plates. Inoculation, media, cultivation and isolation procedures were the same as those for the direct inoculation samples.

Cellulase hydrolysis

Cellulase is an enzyme complex capable of decomposing cellulosic polysaccharides into smaller fragments (http://www.nutrition-focus.com, accessed on 06/04/2001). The enzyme was dissolved in distilled water at a concentration of 1 mg/l. Mixed liquor samples were then centrifuged and 200 mg of the pellet sludge measured into a clean dry test tube. To this, 4.0 ml of a 0.05M acetic acid solution and 1.0 ml of the enzyme dilution were then added and mixed, followed by incubation at 37^{0}C for 2 h (http://www.worthington-biochem.com/manual/C/CEL/, accessed 06/04/2001). Following pretreatment, samples were evaluated microscopically (× 1000, oil immersion) so as to determine the effects of the cellulose hydrolysis on the integrity of the filaments and floc structure. The samples were then washed three times with sterile distilled water by centrifugation at 1 500 r·min^{-1} for 10 min so as to minimise the amount of floc-formers present. Serial dilutions (10^{-1}-10^{-5}) of the pretreated samples were made and inoculated onto fresh agar plates. Inoculation, media, cultivation and isolation procedures were the same as those for the direct inoculation samples.

Isolation, characterisation and identification

After 10 d incubation well-defined bacterial colonies were isolated onto fresh agar plates. Pure cultures of isolates were obtained with continuous sub-culturing and incubation at 20^{0}C. All isolates were screened microscopically for filamentous morphology using gram staining as well as a simple crystal violet staining procedure. Characterisation of cellular morphological characteristics of filamentous bacterial isolates was conducted according to Jenkins et al. (1986) in conjunction with assessment of colonial morphology as seen on agar plates (Cappuccino and Sherman, 1992). Broth

Results

TABLE 3
Isolated filamentous bacteria and their presumptive identification

Pretreatment procedure	No. of filaments isolated	Codes of filaments isolated	Media of isolation	Presumptive filamentous identifications
Direct inoculation	2	R021 and T013	R2A and TYGA	R021- *S. natans* T013- Type 1863
Nonidet	2	R023 and T022	R2A and TYGA	R023- Type 0092 T022- *H. hydrossis*
Sonication	1	OSI 004a	I medium	*M. parvicella*
Cellulase hydrolysis	0	-	-	-

TABLE 4
Morphological characteristics of isolated filaments

Features	R021	R023	T013	T022	OSI 004a
Gram reaction	Negative	Negative	Negative	Negative	Positive
Trichome Ø (µm)	1-2	<1	0.8	<1	0.5-0.8
Trichome length (µm)	>500	20	20	20-50	20-ca.300
Trichome shape	Straight	Straight	Curved	Straight	Slight curve coiled
Cell septa clearly observed	Yes	No	Yes	No	Yes
Sheath	Yes	No	No	Yes	Yes
Cell shape/size (µm)	Round-ended rods (1-2 × 3-5)	Rectangles (0.8 × >1)	Oval rods	-	Rectangles (0.5 × ca. 1.5)

TABLE 5
Colonial morphology of isolated filaments

Features	R021	R023	T013	T022	OSI 004a
Growth Medium	R2A	R2A	TYGA	TYGA	I medium
Colonial Ø (mm)	<1	<1	<1	ca. 3	<0.5
Shape/Form	Rhizoid	Circular	Circular	Circular	Circular
Elevation	Flat	Flat	Flat	Flat	Flat
Margin	Filament	Entire	Entire	Undulate	Entire
Surface/Texture	Rough/ Matt	Glossy/ Smooth	Rough/ Matt	Glossy/ Smooth	Glossy/ Smooth
Colour	Cream	Cream	Yellow	Cream	Colourless
Odour	Fresh sewage	Fresh sewage	Fresh sewage	None noted	None noted
Effect on medium	None noted	None noted	None noted	None noted	None noted

cultures of the presumptive filamentous bacteria were then prepared so as to assess the difference in growth on both solid and liquid media.

Discussion

The filamentous bacteria isolated during the course of this study were provisionally identified as *S. natans*, *M. parvicella*, Type 1863, Type 0092 and *H. hydrossis* (Table 3). Although direct inoculation of samples resulted in cultivation of two isolates, isolation of filamentous bacteria proved to be a difficult task. This is probably due to filamentous bacteria being slow-growers as opposed to the fast-growing heterotrophic floc-formers. Pretreatment of the mixed liquor samples did help in reducing cell clumping from the filamentous bacteria thus leading to reduction of contamination by floc-formers. Although a lot of floc-formers were still in the sample after pretreatment with nonidet, it was found to be effective as two filamentous isolates were obtained with a significant reduction of floc-formers noted (microscopic evaluation of centrifuged supernatant). The undiluted nonidet (100% concentration) was found to be most effective and, therefore, selected for optimal cell alleviation. Sonication as a pretreatment method, while successful in the isolation of one filamentous isolate, was found to break the filaments such that a great majority

would be the same size as the floc-formers, thus making it impossible to separate filaments and floc-formers via gradient separation. Mixed liquor samples were initially sonicated at different times, so as to find the more effective time for this procedure, and 10 s at 20W was found to be least damaging to the physical structure of filamentous bacteria. Although sonication has been reported to be the least disruptive to bacterial cell integrity (Pike, 1975 and Jorand et al., 1995 as cited by Munch and Pollard, 1997), it was noted to be severely disruptive to filamentous bacterial integrity. Even at 10s filaments were severely shortened and broken. Only very short and quick bursts of sonication are therefore recommended. Success achieved with sonication in this study was most likely due to the fact that the sludge sample was bulking and contained a high concentration of filamentous bacteria. There would, however, still be the need to establish means of separating filaments from floc-formers in sonicated samples. A substantial amount of fungal growth was observed with cellulase-hydrolysed samples, probably due to the low pH (about pH 4) at which hydrolysation is carried out. The low pH could also have had a negative effect on the filamentous bacteria during hydrolysation. Apart from the pretreatment procedures already mentioned, another way of obtaining significantly concentrated amounts of filamentous bacteria would be by enriching the mixed liquor samples prior to pretreatment or isolation, or alternatively use bulking sludge. Centrifugation of the pretreated samples, which also served as a sample washing step, also enhanced reduction of floc-formers whilst concentrating the filamentous bacteria, which led to a denser filamentous population. Enhancement of the reduction of floc-formers by centrifugation was microscopically observed while evaluating the supernatant. A lot of free swimming floc-formers were found to be in the bulk liquid following each of the washing steps. Direct inoculation and centrifugation have also been found to be successful by other researchers such as Ziegler et al. (1990) in the isolation of filamentous bacteria from activated sludge. The spread-plate technique proved to be more efficient for initial inoculation, as opposed to the streak-plate technique, owing to the larger surface area available for microbial growth in conjunction with the larger sample size that can be applied (0.1 ml – spread-plate; 10 μl drop – streak-plate). However, the streak-plate technique was more efficient for sub-culturing of isolated colonies. Broth cultures were found to be no different from those grown on plates after 10d of incubation.

The presumptive filaments were characterised in greater detail so as to obtain a more efficient identification of the isolated filaments. The isolated filaments are thus far presumed to be *S. natans*, *M. parvicella*, Type 1863, Type 0092 and *H. hydrossis* due to the cellular characteristics noted (Tables 3 and 4). R2A managed to grow both *S. natans* and Type 0092 while Type 1863 and *H. hydrossis* were isolated on TYGA and *M. parvicella* on I medium. Type 0092 has been grown on R2A before (Kämpfer, 1997). The other isolates have been reported to grow on different media (Kämpfer, 1997) as opposed to the above mentioned in this study. These isolated filaments still require conclusive identification using fluorescent *in situ* hybridisation, to ascertain their identification. Four of the five presumptive filaments isolated, i.e. *M. parvicella*, Type 0092, Type 1863 and *H. hydrossis*, were not detected during microscopic screening of the mixed liquor samples prior to pretreatment. These filamentous bacteria may not have been present in large numbers in the mixed liquor samples to begin with or alternatively, perhaps constituent filaments exhibited different growth characteristics on artificial media. Solid media that were used might be having a tremendous effect on the growth of filamentous bacteria and, were probably not selective enough for or favourable for the successful growth of the detected bacteria. The colonial morphology of the filaments (Table 5) could also serve as an important factor in basic filament identification, especially when the biochemistry of the filaments is well established. Though Type 0041 and Type 0675 were always found to be very abundant prior to pretreatment, they have been reported to be very difficult to isolate and cultivate on artificial media (Seungbum, 2001). Another important consideration that should not be ruled out is the design of a medium that is chemically representative of sewage and the activated sludge process where the sample was taken. This would involve taking the mixed liquor and autoclaving it, inoculating it with the appropriate sample or adding agar to the liquid medium, if required in solid form. Taking samples from bulking or foaming sludge would also be of benefit, as it would be expected that filaments would be more prevalent in such conditions, than in non-bulking and non-foaming activated sludge, and be able to outcompete the faster growing floc-formers.

The isolated filaments, *S. natans*, *M. parvicella*, Type 1863, Type 0092 and *H. hydrossis*, have often been implicated in bulking and foaming and are, therefore, believed to be a part of bulking and foaming problems (Jenkins et al., 1986). Low DO can cause the growth of several filamentous organism types in activated sludge. *S. natans* is associated with low DO and it is also possible that *H. hydrossis* may contribute to bulking at low DO concentrations in low F/M systems (Jenkins et al., 1986). *S. natans* is nearly always present in activated sludge flocs, its amount depending on the composition of the influent of the purification plant. Poorly settling activated sludge often contains many filaments of this bacterium (Mulder and Deinema, 1992). *Thiothrix* spp., Type 021N and *H. hydrossis*, occur at dominant levels under several causative conditions of bulking and foaming, for instance nutrient deficiency and septic wastewater. These filaments are, in general, not good indicator organisms for the diagnosis of the causes of bulking (Jenkins et al., 1986). Careful observation of the morphological characteristics of these filaments could give clues to the conditions that cause their dominant growth. *M. parvicella* and Type 0092 has also been associated with low F/M bulking (Jenkins et al., 1986) while *M. parvicella* is also known to contribute to activated sludge foaming and scum formation (Seviour et al., 1990, Pujol et al., 1991 and Eikelboom, 1993, as cited by Hwang and Tanaka, 1998). The importance of obtaining and studying filamentous bacteria in pure culture cannot be overemphasised at this stage. Greater understanding of these organisms, and their physiological characteristics, is needed for more efficient and long-term methods for controlling and curing activated sludge bulking and foaming. With it being difficult to obtain filaments in pure culture, more work still remains to be done in developing more efficient and inexpensive isolation procedures.

Conclusion

The filamentous bacteria isolated in this study are of significant importance to further research as they are implicated in activated sludge bulking and foaming problems. Direct inoculation and pretreatment using nonidet surfactant and sonication were successful techniques for isolation of these filaments. However, floc break-up procedures employed require further research and optimisation for successful filament isolation. Success with sonication was limited due to its destructive nature on the integrity of the filamentous bacterial structure. The pH at which cellulase hydrolysis takes place could be too low to maintain the survival of filamentous bacteria during incubation, therefore, inhibiting filamentous growth. Isolation of filamentous bacteria from bulking samples is also possibly advantageous in that less floc-former

competition exists when plated onto solid media. The findings obtained thus far should serve as a significant basis for future studies on filamentous bacteria and factors promoting or inhibiting bulking and foaming.

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