Survey of filamentous bacteria in activated sludge plants in KwaZulu-Natal

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Abstract

The objective of this investigation was to conduct a survey of filamentous bacteria present in activated sludge plants in Durban and surrounding areas (KwaZulu-Natal, South Africa). A diverse population of filamentous bacteria was identified. Dominant filamentous bacteria identified from mixed liquor samples in descending order of frequency included: (1) *Nocardia* spp., (2) Type 0041, (3) Type 0675, (4) Type 1851, (5) Type 021N, (6) *Nosticola limicola II*, (7) *Sphaerotilus natans*, (8)*Thiothrix I* and *II* and (9) *Beggiatoa*. *Nocardia* spp. were the only dominant filamentous bacteria present in foam samples, while Type 0914, *Microthrix parvicella* and *Sphaerotilus natans* occurred incidentally. All filamentous bacteria identified were present throughout the year. *Nocardia* spp. and *Microthrix parvicella* were found to be dominant during winter months. It can be concluded that filamentous populations are significantly affected by seasonal and influent variations.

Introduction

Activated sludge comprises a diverse population of micro-organisms which include eubacteria, filamentous bacteria, rotifers, protozoa and algae (Jenkins et al., 1984). In order for the activated sludge process to operate successfully, it is essential that the resident microflora form flocs which settle out readily, thereby producing a clear effluent with a low suspended solids concentration (Curds and Hawkes, 1983). Filamentous bacteria form the floc macrostructure that facilitates adhesion to floc forming bacteria. Most activated sludge plants around the world suffer from bulking and/or foaming, operational disorders caused by the proliferation of certain filamentous bacteria. Foaming is a well-recognised problem originally thought to be caused by *Nocardia amarae* (Lechavalier and Lechavalier, 1975; Pipes, 1978). However, more recent surveys in South Africa (Blackbeard et al., 1986) and Europe (Lemmer and Kroppenstadt, 1984; Goddard and Forster, 1987) have identified a wider range of filamentous bacteria in foam samples. These include other Nocardioforms, *Microthrix parvicella* and several Eikelboom morphological types (Eikelboom, 1975). In a study conducted by Blackbeard et al. (1988) on filamentous bulking in 33 South African nutrient removal plants, approximately eight plants experienced such problems. The five most frequently dominant filamentous organisms isolated from mixed liquor samples during the study included: Type 0092, dominant in 82% of plants, Type 0675 in 45%, Type 0041 in 39%, *M. parvicella* and Type 0914, both in 33%.

These five filamentous organisms were also the most frequently dominant in foam samples in 18 of the plants (Blackbeard et al., 1988). Type 0092 had the highest frequency of dominance in foam at 78%, followed by *M. parvicella* in 50%, Type 0041 in 33%, and Types 0675 and 0914 in 22% each. Only *M. parvicella* and *Nocardia* spp. and Type 0092 were found to accumulate in foam selectively (Blackbeard et al., 1988).

Domestic and industrial activated sludge plants in South Africa have been extensively studied yet little quantitative information describing filamentous populations has been communicated. KwaZulu-Natal wastewater treatment installations have received scant attention regarding their filamentous populations, resulting in the need for this study to be conducted. The objectives of this study were therefore to:

- identify filamentous bacteria from mixed liquor and foam;
- determine the effect of seasonal variations on filamentous bacteria; and
- determine the frequency of dominance and occurrence of the various filamentous bacteria identified.

Materials and method

Sample collection and handling

Grab samples of mixed liquor (1 000 mℓ) and foam (250 mℓ) were collected in sterile bottles from aeration tanks at the following nutrient removal activated sludge systems: Amanzimtoti, Northern Works, Kwa-Mashu, Umbilo, Darvill and Hammarsdale. The plants were sampled on a fortnightly basis from May through to October. Samples were stored at 4°C and microscopically analysed within 24 h of collection. Triplicate samples of 100 mℓ liquid sludge were dried overnight at 105°C on pre-weighed filter paper to determine the mixed liquor suspended solids concentration.

Identification techniques for filamentous bacteria

Activated sludge wet mounts and smears were prepared for examination (triplicate). Wet mounts were studied under direct illumination at 1000x magnification to determine morphological characteristics of various filaments. Smears were stained using Gram and Neisser staining techniques. Stained smears were examined under oil immersion and direct illumination at 1000x magnification. The individual abundance level of each filament was determined using the scoring technique outlined by Jenkins et al. (1984). Each filament was scored on a scale between 0 and 6 (integer scores had the following meanings: 0 = none; 1 = few; 2 = some; 3 = common; 4 = very common; 5 = abundant; and 6 = excessive). The system rated filament abundance on an average "per floc" basis. Filamentous bacteria with individual abundance

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TABLE 1
MORPHOLOGICAL STAINING CHARACTERISTICS OF FILAMENTOUS BACTERIA OBSERVED IN ACTIVATED SLUDGE

FT	GS	NS	PHB	TL	TLL	CS	I	SH	AG	CL/S
Type 1701	-	-	+	E	20-80	+	+	+	+	round rods 0.8 x 1.2
Type 0041	+/-	+/-	-	IN/E	100-600	+	-	+	++	squares 1.4 x 1.5
Type 0675	+/-	+/-	-	IN	60-160	+	-	+	++	squares 1 x 1
Type 021N	-	+/-	+	E	60->500	+	+	-	-	barrel 1-2 x 1.5
Type 1851	-	_	-	E	100->500	+/-	-	-	-	rectangle 0.8 x 1.5
Type 0914	+/-	+/-	+	E/F	50-200	+	-	-	+	square 1 x 1
Beggiatoa	+/-	+/-	+	E	100->500	+/-	-	-	-	rectangle 2 x 6
Thiothrix I	+/-	+/-	+	E	100- >500	+	-	+	-	rectangle 2 x 3-5
Thiothrix II	-	-	+	E	50-200	+	-	+	-	rectangle 1 x 1.6
M. parvicella	+	+	+	IN	100 - 400	-	-	-	-	variable
N. limicola II	+/-	-	+	IN/E	100 - 200	+	+	-	-	oval 1.2 x 1
H. hydrossis	-	-	-	E/F	20 - 100	-	-	+	+	variable
Nocardia spp.	+	+	+	IN	10-20	+/-	-	-	-	variable
S. natans	-	-	+	E	>500	+	+	+	-	rods 1 x 1-2

[+]Source: Jenkins D, Richard MG and Daigger GT (1984) Manual on the Causes and Control of Activated Sludge Bulking and Foaming. Water Research Commision, PO Box 824, Pretoria, 0001, RSA.

FT= filament type; **GS**= gram stain; **NS**= neisser stain; **PHB**= presence (+) or absence (-) of polyhydroxy butyrate; **TL**= trichome location; **TLL**= trichome length (µm); **CS**= cell septa present (+) or absent (-); **I**= indentation present (+) or absent (-); **SH**= sheath present (+) or absent (-); **AG**= attached growth present (+) or absent (-); **CL &S** = cell length and shape; **E**= extended growth; **IN**= interbridging; **++**= excessive attached growth.

levels of 4 or more were classified dominant while those with individual abundance levels of 3 or less were classified secondary. The same procedures were carried out during foam sample analysis.

Phenotypic variances of filamentous bacteria were elucidated according to various characteristics outlined in Table 1 (Jenkins et al., 1984). These bacteria have all previously been observed in domestic and industrial activated sludge plants (Cyrus and Sladka, 1970; Farquhar and Boyle, 1971; Eikelboom, 1975; Strom and Jenkins, 1984).

Sludge volume indices were determined according to techniques outlined by Jenkins et al. (1984). Dissolved oxygen concentrations were also measured from aeration tanks with the aid of an Oxi 597 S DO meter.

Results and discussion

Filamentous organisms identified in mixed liquor samples

Comparisons indicate that a diverse population of filamentous organisms was present in the activated sludge process. In the present study, a total of 14 filamentous bacteria were identified. Results of percentage frequency of occurrence and dominance of various filamentous organisms identified in mixed liquor samples are shown in Fig. 1. The graph shows that the six most frequently dominant filamentous bacteria identified in descending order are:

- *Nocardia* spp. and
- Type 0041 in 83% of the plants sampled,
- Types 0675 (Fig. 3),
- 1851 and
- 021N in 67% of plants, and
- *N. limicola II* in 50% of plants.

The following series has been arranged in descending order of frequency:

- *N. limicola 11* in 83% of plants,
- *Nocardia* spp.,
- Types 0041,
- 0675 and
- 1851 in 67% and
- Type 02IN in 50% of the plants sampled.

The absence of Type 0092 did cause concern as it is a very common filament in nutrient removal plants. Equally surprising was the high frequency of *N. limicola II.* Although these two filamentous organisms have similar morphologies and staining reactions, the latter filament had distinct PHB granules with oval shaped cells as apposed to rectangular forms observed in Type 0092.

Blackbeard et al. (1988) observed Type 0092 and *M. parvicella* (Fig. 4) as dominant filamentous types in nutrient removal plants in South Africa. Type 0092 was never observed in any of the mixed liquor or foam samples of the present study. *M. parvicella*

had one of the highest percentage frequencies of occurrence, yet was never observed as a dominant filament. Blackbeard's results show that *M. parvicella* was observed as a dominant filament in 33% of sampled plants. The present results were not anticipated regarding the percentage frequency of dominance of *M. parvicella* as it is one of a restricted number of filamentous bacteria that can survive the anaerobic and anoxic conditions present in the activated sludge process before entering the aerobic zone (Mulder and Resink 1987; Resink and Donker 1982). The high frequency of occurrence of *M. parvicella*, Type 0041 and Type 0675, which were also the most frequently occurring filamentous organisms observed by Blackbeard et al. (l988) and Bux and Kasan (1994), can be attributed to their ability to withstand and adapt to wide parameter ranges (pH, temperature, dissolved oxygen variations etc.) present in the activated sludge system.

Table 2 shows various filamentous bacteria identified at specific plants. It is evident that filaments such as *Sphaerotilus natans*, *Thiothrix I* and *II*, *Beggiatoa* spp. and *Haliscomenobacter hydrossis* appeared at specific plants only. This can be attributed to the nature of the influent composition of the wastewater and to the configuration of the plant i.e. whether it is a nutrient or a non-nutrient removal plant. *S. natans* is not common in South African nutrient removal plants due to its inability to survive under anoxic conditions. It was, however, observed on one occasion at Amanzimtoti. The filament's ability to easily utilise complex carbohydrates present in brewery waste has been documented (Richard et al., 1984). High volumes of brewery waste may have been pumped into the plant during the time of sampling, providing ideal substrate for the filament. Wastewater from a neighbouring brewery is controlled so that only 30% of the total wastewater composition at Amanzimtoti contains brewery waste in order to avoid proliferation of certain filamentous bacteria such as *S. natans*.

Table 2 shows that *Thiothrix II* was observed at Umbilo and Hammarsdale Wastewater Works, yet the filament was not observed by Bux and Kasan (1994) and Blackbeard et al. (1988). A possible explanation for these contradicting results may be the fact that sampling was limited only to the KwaZulu-Natal region which has undergone a rapid rate of

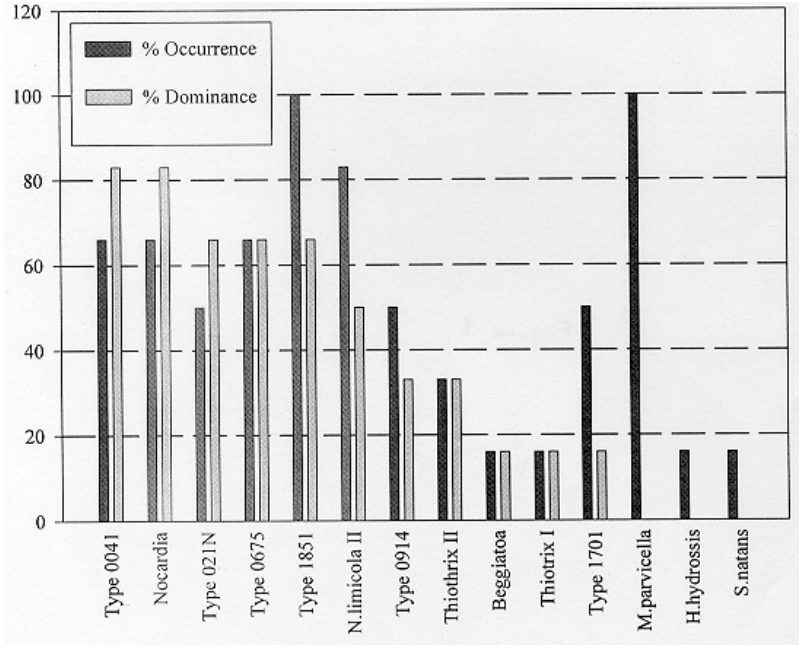

Figure 1
Percentage frequency of occurrence and dominance of filamentous bacteria identified in mixed liquor

TABLE 2
FILAMENTOUS BACTERIA IDENTIFIED IN MIXED LIQUOR SAMPLES (MEAN OF NUMBER OF SAMPLES TAKEN FROM EACH PLANT)

Name of bacterium	DAR	NW	TOTI	UMB	HAM	KWA
Type 1701	*	*	*			
Type 1851	*	*	*	*	*	*
Type 0041	*	*	*	*	*	*
Type 0914		*	*	*	*	*
Type 0675	*	*	*	*	*	*
Type 021N	*	*	*	*	*	
Nocardia spp.	*		*	*	*	*
Thiothrix I			*			
Thiothrix II				*	*	
Beggiatoa spp.		*				
N. limicola II	*	*	*	*	*	*
M. parvicella	*	*	*	*	*	*
H. hydrossis					*	
S. natans			*			

* = Filament type identified at specified plant.
KEY: DAR=Darvill; NW=Northern Works; TOTI=Amanzimtoti; UMB=Umbilo; HAM=Hammarsdale; KWA=Kwa-Mashu.

TABLE 3
FILAMENTOUS BACTERIA IDENTIFIED IN FOAM SAMPLES

Plant name	*M. parvicella*	Season	NALO	Season	Other bacteria		Season
DAR	+/-	Aug, Sep	+++	Jul, Aug, Sep	Nil	Nil	Nil
TOTI	+/-	T	+++	Apr, May, Jun	Type 0914, *S. natans*	+	T
KWA	+/-	Jul, Aug, Sep	+++	Jul, Aug, Sep	Nil	+/-	4/1/97
HAM	Nil	Nil	+++	T	Type 0914	+	Apr, May, Jun
UMB	Nil	Nil	+++	Jul, Aug, Sep	Type 0914	++	Jul, Aug, Sep

NALO = *Nocardia amarae* like organisms; T = present throughout the year; +++ = abundant growth; ++ = very common; + = common; +/- = incidental growth

TABLE 4
PROCESS PARAMETERS MEASURED AT VARIOUS ACTIVATED SLUDGE PLANTS

Plant	Average temperatures measured during sampling (^{0}C)	Sludge volume index (mg/ℓ MLSS)	Dissolved oxygen concentration measured from aeration tanks (mg/ℓ)
NW	20 - 24	111	0.3317
DAR	20 - 25	94	0.6550
UMB	20 - 24	114	0.6550
TOTI	20 - 24	115	0.5733
KWA	20 - 25	115	0.3411
HAM	20 - 25	76	0.1017

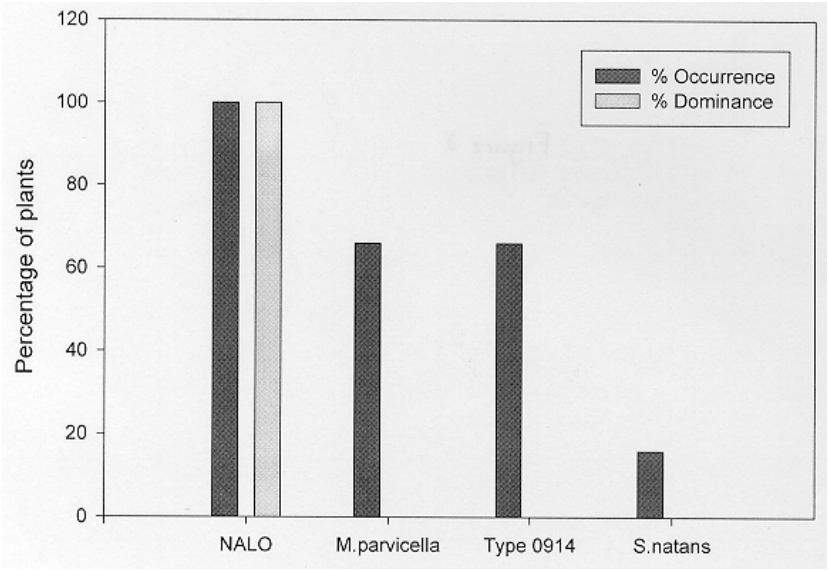

Figure 2
Percentage frequency of occurrence and dominance of filamentous bacteria identified in foam samples

industrialisation with the result that the composition and nature of various wastewaters may have altered. Table 2 also shows that *Thiothrix I* was observed on one occasion (out of a total of 12 samples analysed) at Amanzimtoti. This does not confer with Bux and Kasan's (1994) findings, where the filament was observed at KwaMashu. In both studies the filament had a low percentage frequency of occurrence. This can be attributed to the fact that most treatment plants in KwaZulu-Natal contain wastewaters low in sulphides and the majority do not experience nutrient deficiencies, conditions known to favour this particular filament's growth (Jenkins et al., 1984).

All the filamentous bacteria identified were present throughout the year. Table 3 shows that *Nocardia* spp. and *M. parvicella* were found to be dominant during the winter season (July, August and September).

Filamentous organisms identified in foam sample

Studies have shown that bulking and foaming are related to the increased presence of certain filamentous types such as Type 0041, Type 0675, *M. parvicella and Nocardia* spp. (Jenkins et al., 1984; Eikelboom, 1975). The present study substantiated the above findings. Although none of the plants sampled experienced bulking problems as depicted by the sludge volume index (SVI) readings which occurred within reasonable ranges below 150 mg·ℓ^{-1} (Table 4), there was foaming in 83% (6) of the plants. Northern Works was the only plant found not to experience foaming problems.

The fact that *M. parvicella* and *Nocardia* spp. were never observed as dominant filamentous organisms at Northern Works may explain the absence of foam at the plant. Past studies have shown *M. parvicella* to

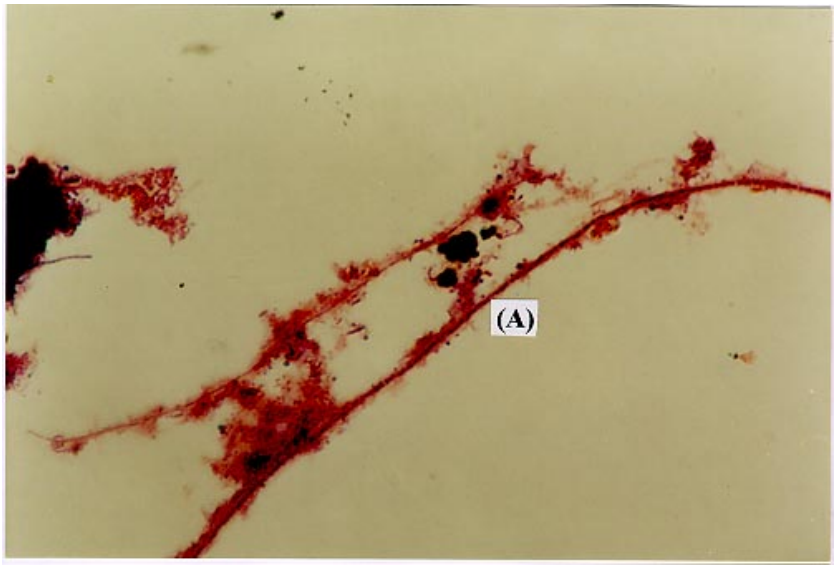

Figure 3
Type 0675, Gram negative with heavily attached growth

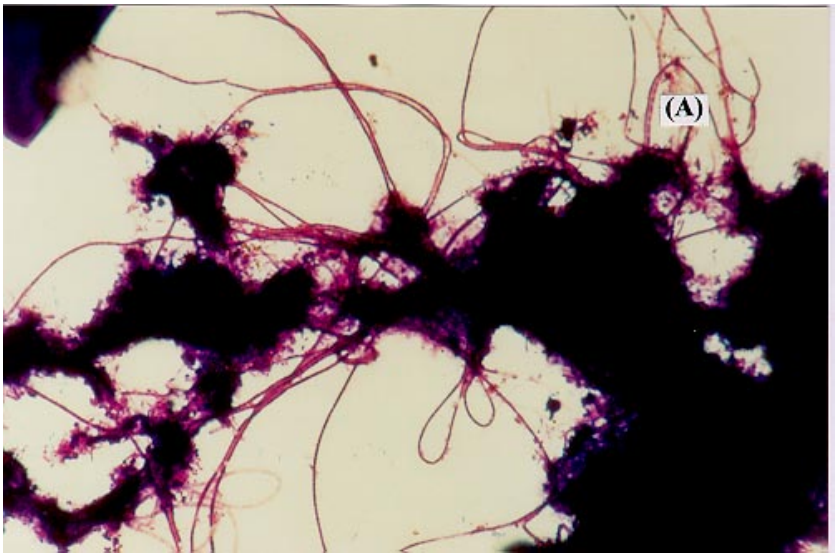

Figure 4
M. parvicella*, a strongly Gram positive organism. It can be distinguished by the presence of spaces between the cells, indicating the presence of a sheath*

grow in systems fed with wastewaters rich in fatty acids and low dissolved oxygen (DO) concentrations (Slijkhuis and Deinema, 1988). It is possible that the influent composition at Hammarsdale Wastewater Works had a high fatty acid content from a neighbouring poultry processing plant. The plant also experienced the lowest DO readings from all the plants sampled from. This explains the plant's excessive foam production (Hammarsdale experienced the worst foaming problem of all the plants sampled). Although the filament was never dominant at Hammarsdale, it did show the highest percentage frequency of occurrence compared to other plants. Mamais et al. (1996) showed that low temperatures and long chain fatty acids favoured *M. parvicella's* growth. Wastewaters from Hammarsdale and Umbilo contained high concentrations of fatty acids from neighbouring food industries and low DO (Table 4) and yet this filamentous organism was not observed to be dominant at the plants. A possible explanation could be that temperature variations have a greater impact on this filament's growth, more so than DO and substrate concentration. According to Hao et al. (1983), *M. parvicella* favours warmer temperature ranges between 26 and 35°C. Table 4 shows that these temperature ranges were never observed in any of the plants sampled. This explains why the filament never appeared dominant in any of the plants surveyed.

Figure 2 shows the filamentous organisms identified in foam samples with respective percentage frequencies of dominance and percentage frequencies of occurrence. Discrepancies existed between the present study and Blackbeard et al. (1988). *Nocardia amarae* like organisms (NALO) were the only dominant species present in foam samples i.e. had a score below 4). Fig. 2 also shows that *M. parvicella* and Type 0914 had the highest frequency of occurrence whilst *S. natans* was less common. The following filamentous bacteria were identified in foam samples:

- NALO in 100% of plants,
- Type 0914 and
- *M. parvicella* in 66% of plants and
- *S. natans* in 16% of plants.

Many nocardioforms have similar or identical morphologies and cannot be microscopically distinguished (Seviour et al., 1990). As a result, *Nocardia* species are referred to as *Nocardia amarae* like organisms. Blackbeard et al. (1988), related the presence of

NALO and *M. parvicella* to foaming problems.

The present study demonstrates that excessive growth of *Nocardia* spp. could be related to foaming problems experienced at Darvill, Hammarsdale, Umbilo and KwaMashu Wastewater Treatment Plant. *M. parvicella* and Type 0914 occurred incidentally at Darvill, Hammarsdale and Umbilo. Findings differed with Blackbeard et al. (1988) where *Nocardia* spp. and Type 0914 were dominant in 22% of plants sampled. However, in this investigation these two organisms occurred at high percentage frequencies of 66% each. *S. natans* was observed on one occasion only in foam obtained from Amanzimtoti. The filamentous bacterium occurred simultaneously in the mixed liquor sample suggesting that the filament is not a true foam causing organism but rather a contaminant from mixed liquor.

Conclusions

The study shows that a diverse population of filamentous bacteria was identified and that the filamentous bacterial population composition is not fixed. This was reinforced by the fact that different filamentous bacteria were observed in addition to those identified by Blackbeard et al. (1988) and Bux and Kasan (1994). Changes in the filamentous microbial population can be attributed to seasonal and wastewater composition variations i.e. waste composition, pH, temperature etc. This has also proved to influence the dominance of filamentous bacteria in wastewater treatment systems. Conventional methods for identifying filamentous bacteria (i.e. microscopic techniques) have shown limitations regarding the degree of accuracy of results obtained. This may largely be attributed to human error particularly with regards to staining techniques used, inexperience with regards to filament morphologies and the fact that only limited areas are analysed under the microscope not giving a true representation of the population composition. The development of new identification techniques, such as RNA oligonucleotide probes, will allow the identification of filamentous bacteria to occur with a greater degree of accuracy and reliability.

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