FEMS Microbiology Ecology 12 (1993) 277–284

Published by Elsevier

FEMSEC 00475

Control of *Microthrix parvicella* growth in activated sludge

Paolo Madoni [a] and Donatella Davoli [b]

[a] *Istituto di Ecologia, Università di Parma, Viale delle Scienze, Parma, Italy and* [b] *AGAC, Reggio Emilia, Italy*

(Received 23 December 1992; revision received 4 May 1993, accepted 17 May 1993)

Abstract. Effects of aerobic and anaerobic conditions on the growth of *Microthrix parvicella* in the activated sludge were studied to prevent bulking caused by this filamentous bacterium. The study was conducted on a pilot plant with selector and the data were compared with those observed in a full scale plant subjected to severe bulking due to a massive growth of *M. parvicella* Both plants were fed with the same settled waste water. A substantial suppression of the growth of *M parvicella* was observed in only the experiments where returned activated sludge was mixed with waste water under aerobic conditions. Both the number of filaments and the sludge volume index (SVI) were lower in the pilot plant than in the full scale plant. Under anaerobic conditions, the selector was not able to improve the settleability and avoid the growth of *M parvicella*.

Key words: Filamentous bacteria; *Microthrix parvicella*; Bulking; Activated sludge process; Aerobic and anaerobic conditions

Introduction

The successful operation of the activated sludge process depends on the ability of the specialized microbial community present to form flocs which then rapidly settle out to provide a clear final effluent [1]. Often this process can be disrupted by bulking, which occurs when filamentous microorganisms extend from flocs into the bulk solution and interfere with sludge settlement in the clarifier [2]. The filamentous bacteria involved in bulking have been well documented [3–7], but they are usually differentiated solely on morphology, as many have not yet been isolated and are grown in pure culture, and hence are largely uncharacterized.

The suppression of the growth of filamentous bacteria in systems with a substrate concentration gradient can be explained in the terms of Monod kinetics, since most of the filamentous microorganisms belong to the so-called K-strategists. These microorganisms exhibit very low values of the half-saturation constant for substrate, dissolved oxygen and nutrients. The values of the μ_{max}/K ratio are therefore higher for the filamentous than for the floc-forming bacteria. Under low concentrations of substrate, DO and nutrients, the growth of filaments will be favoured, whereas under higher substrate concentrations they will be outcompeted by the floc-formers.

Correspondence to: Paolo Madoni, Istituto di Ecologia, Università di Parma, Viale delle Scienze, 43100 Parma, Italy.

In recent years some researchers have demonstrated that filamentous bulking can be effectively suppressed in a selector-type reactor [8–16]. The selector is an inlet area in which higher concentrations of substrate are maintained to support the growth of floc-forming bacteria and to suppress the growth of filamentous microorganisms. Nevertheless, some filamentous bacteria such as *Microthrix parvicella* exhibit kinetic parameters similar to those of floc-formers and are able to accumulate substrates, which decreases the selective pressure under aerobic and anaerobic conditions [17,18].

M. parvicella nom. prov. was first isolated by van Veen [3] on a mineral salt medium containing small amounts of glucose, vitamin B_{12} and thiamin. Growth was very slow with microcolonies developing in 6–8 weeks, although Eikelboom [4] achieved slightly better growth, using a sludge based medium. This bacterium forms thin, strongly curled filaments of up to 300–500 μm. The internal structure of the Gram-positive trichomes is only visible by the use of an electron microscope. The metabolism of this organism is unusual as it cannot use common carbon sources, such as glucose, and fructose. According to Slijkhuis [17] this microorganism uses oleic acid as sole carbon and energy source, but Wanner (personal communication) found *M. parvicella* in many cases when no fatty acids were present in the waste waters. Growth of *M parvicella* in activated sludge occurs when aeration is intermittent and the food/microorganism ratio (F/M) is low. More exhaustive studies on both the physiology and ecology of *M. parvicella* are due to Slijkhuis et al. [19–21].

The work reported in this paper was directed towards a further understanding of the effect of the aerobic and anaerobic environment on the growth of *M. parvicella* in the activated sludge.

Materials and Methods

Description of the plants

The research was conducted on a nitrogen removing, activated sludge plant affected by severe bulking due to the massive growth of *M. parvicella*, and on a pilot-plant. Both the experimental pilot-plant and full-scale plant comprised a plug-flow reactor, a denitrification tank and a secondary sedimentation basin. The plants were both fed with the same influent waste after primary sedimentation. In the presettled waste water the COD values and the COD/BOD ratio ranged from 490 to 583 mg l^{-1} and from 2.0 to 3.7 respectively. To test the influence of aerobic and anaerobic zones on the growth of *M. parvicella*, a selector was installed upstream of the pilot-plant reactor. The selector used in the test system consisted of five 0.20 m diameter cylindrical tanks in series (Fig. 1). The total volume of the selector was 35 litres. Influent and return sludge were directed to the first selector tank. When the selector was operated under aerobic conditions, four ceramic diffuser stones near the bottom of each tank provided both aeration and mixing. The air flow rate to the diffuser in each tank was adjusted to maintain the DO concentration with a range of 4–6 mg l^{-1}. The substrate removal rate in the selectors was 60%. A sludge age of 25 days was maintained during the experimental period. The experimental test conditions and operational parameters of both pilot-plant and full-scale plant are show in Table 1.

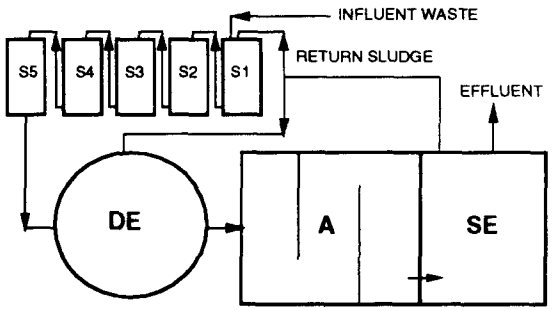

Fig 1 Flow-scheme of the experimental pilot-plant system S_{1-5}: selector tanks, DE denitrification basin, A. aeration basin, SE settling tank

Laboratory analysis

Two samples per week were taken from the mixed liquor in the aeration basin of both plants. Standard methods [22] were used for measuring

Table 1

Operational parameters of both pilot plant and full-scale plant

Parameter	Pilot plant	Full-scale plant
Inflow waste ($m^3\ h^{-1}$)	0 11	200
Return sludge to the selector ($l\ h^{-1}$)	30	–
Volume·		
– selector (l)	35	–
– denitrification basin (m^3)	1.4	2000
– aeration basin (m^3)	1.8	5000
Residence time (h)		
– selector	0 2	–
– denitrification basin	13	10
– Aeration basin	17	13
– sedimentation tank	10	12
Sludge loading ($kgBOD\ kgMLSS^{-1}\ d^{-1}$):		
– aeration basin	0.03– 0 05	0 02– 0.03
– selector	18 –19	–
Aeration basin:		
– Temperature (°C)	9 –24	9 –23
– Dissolved oxygen ($mg\ l^{-1}$)	5 – 6	4 – 5
– MLVSS ($g\ l^{-1}$)	3 – 4	3.5 – 4 5

suspended solids (SS), MLSS, SVI, COD and DO. When the sludge volume after 30 min settling (SV_{30}) exceeded 200 ml in a 1-litre cylinder, the diluted sludge volume index (DSVI) was used with one or more serial dilutions until an SV_{30} of less than 200 ml was achieved. The DO was measured by a calibrated DO probe and oxygen meter. The oxidation-reduction potential was measured by means of platinum and calomel reference electrodes. When the selector operated under anaerobic conditions, the oxidation-reduction potential dropped to below −150 mV.

Microscopic observations of samples obtained from the reactors were made with phase-contrast at 200× and 1000× magnifications. The filamentous microorganisms were identified according to Eikelboom and van Buijsen [6]; their abundance was estimated using the simplified filament counting techniques [23]. The Gram and Neisser staining techniques were also used for the identification of filamentous bacteria.

Results

Effect of the anaerobic selector

The change in filamentous abundance and SVI values during the 75 days of the first experiment with the selector run anaerobially are shown in Fig. 2. In both plants *M. parvicella* was the dominant filamentous microorganism (Table 2), but numbers of other species such as Type 0041 and *Nostocoida limicola* were low. *M. parvicella* growth was slightly lower in the pilot-plant than in the full-scale plant. During the experiment the filamentous microorganisms in both plants increased slightly. In the pilot plant the maximum density of filamentous bacteria was reached on the 60th day with 120 filaments μl^{-1}, whereas in the full-scale plant a peak of 186 filaments μl^{-1} was reached on day 67. As the filament density increased the SVI ranged from 150 to 220, which corresponded to poor sludge settling. Neverthe-

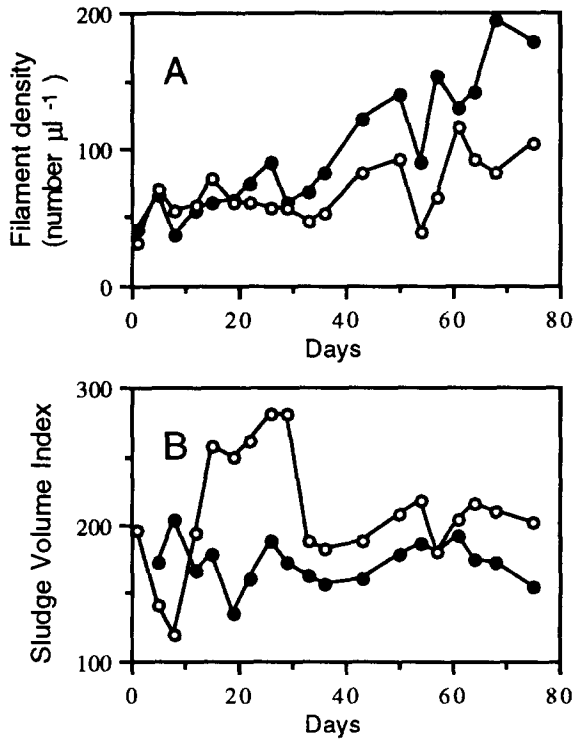

Fig. 2. Variation of filament density and SVI in the first experiment with an anaerobic selector, (○) pilot-plant, (●) full-scale plant.

less, SVI values were always higher in the pilot plant than in the full-scale plant, and this means that the anaerobic selector was not able to suppress *M. parvicella* growth and, consequently, could not substantially improve the settling properties of the activated sludge.

The test with the anaerobic selector was repeated for a further period of 40 days. The trends showed in Fig. 3 substantially confirmed the results obtained in the first experiment. So under anaerobic conditions the selector was unable to improve the settling properties of the sludge, and the growth of *M. parvicella* was limited, but not suppressed. In both plants high SVI values were observed for the whole period. In the full-scale plant the SVI values ranged from 190 to 270, in the pilot plant from 165 to 465. All these values corresponded to bulking conditions.

Effect of the aerobic selector

The effect of an aerobic selector was tested in a 50 day experiment. DO in the selector tanks during the experiment ranged from 4 to 6 mg l^{-1}. The numbers of filamentous bacteria and SVI values are shown in Figs. 4a and b. After the starting phase (after day 15), the settling properties of the sludge were better in the pilot plant than in the full-scale plant. In fact, the SVI values in the pilot plant ranged from 90 to 100 ml g^{-1}, whereas in the full-scale plant, the SVI values were between 120 and 150 ml g^{-1}, although the mixed liquor was treated with $FeCl_3$ to improve settleability. The positive effect of the aerobic selector was also shown by the lower number of filamentous microorganisms observed in the pilot plant compared to the full-scale plant. In the full-scale plant the mean number of filaments (150 filaments μl^{-1}) was three times greater than in the pilot plant (50 filaments μl^{-1}). The activated sludge of the full-scale plant was dominated by *M. parvicella* for the whole period of study; by contrast, in the pilot plant, this microorganism was completely suppressed (Table 2). Among the few filamentous microorganisms present, Type 0041 was the most abundant, although no dominant forms were observed.

The experiment with an aerobic selector was repeated for a further period of 70 days. The trends of both filament density and SVI values

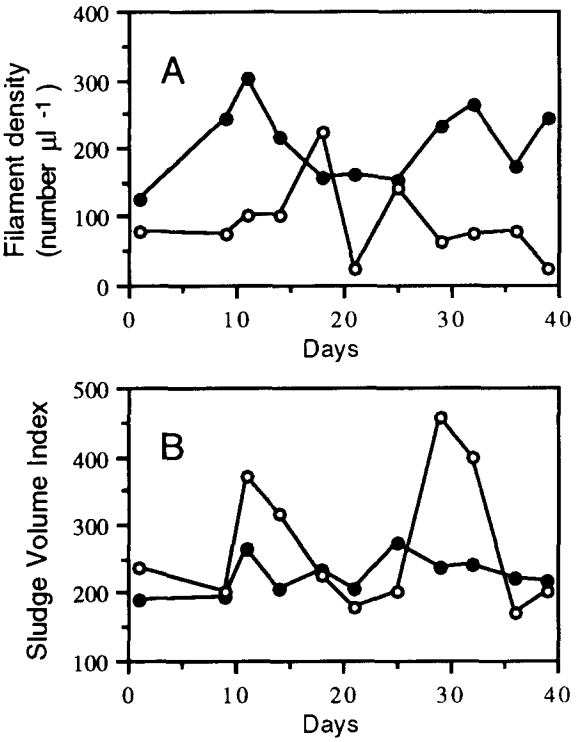

Fig 3 Variation of filament density and SVI in the second experiment with an anaerobic selector, (○) pilot-plant, (●) full-scale plant

Table 2

Filamentous microorganisms observed in the sludge of the pilot-plant and full-scale plant during experiments with anaerobic and aerobic selectors (d. dominance; p: presence)

Experiment	Pilot plant	Full-scale plant
Anaerobic selector		
1st test	*M parvicella (d)*	*M parvicella (d)*
	Type 0041 (p)	Type 0041 (p)
	N limicola (p)	*N limicola (p)*
2nd test	*M parvicella (d)*	*M. parvicella (d)*
	Type 0041 (p)	Type 0041 (p)
	Thiothrix (p)	*Thiothrix (p)*
Aerobic selector		
1st test	Type 0041 (p)	*M parvicella (d)*
	Type 0675 (p)	Type 0041 (p)
2nd test	Type 0041 (p)	*M parvicella (d)*
	Type 0092 (p)	Type 0041 (p)

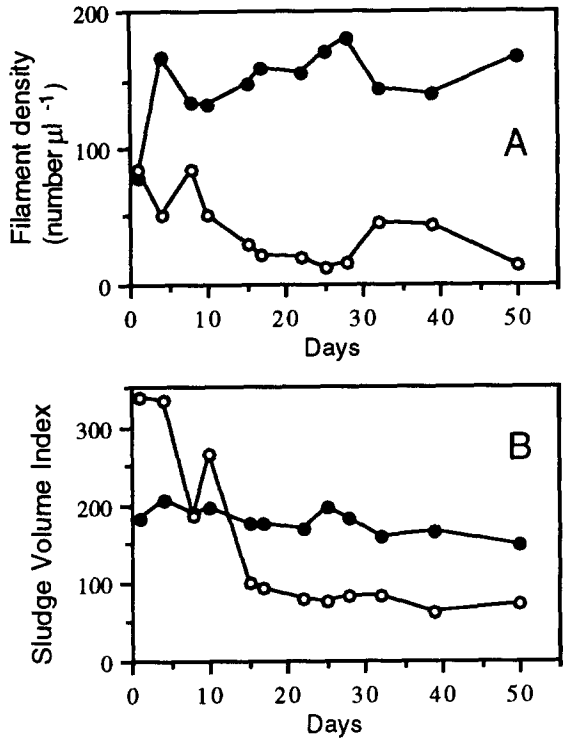

Fig. 4. Variation of filament density and SVI in the first experiment with an aerobic selector, (○) pilot-plant, (●) full-scale plant.

(Fig. 5) were similar to those observed during the first experiment. The abundance of filamentous microorganisms in the pilot plant was decidedly lower than in the full-scale plant. The maximum density of filaments in the pilot plant was below 50 filaments μl^{-1}, with a mean number of 25 filaments μl^{-1}. On the other hand, the mean abundance of filamentous bacteria in the full-scale plant was 100 filaments μl^{-1}, with maximum values reaching 150 filaments μl^{-1}. *M. parvicella* was completly dominant in the full-scale plant, whereas in the pilot plant this species was totally suppressed. Filamentous microorganisms observed in the pilot plant belonged to the Type 0041 and Type 0092.

Discussion

Filamentous microorganisms in activated sludge are generally slow-growers, having low values for the saturation constant K_s and of the maximum substrate removal rate $r_{x,m}$. Floc-forming microorganisms in activated sludge, on the other hand, are fast-growers, having high values of K_s and $r_{x,m}$ [24]. At high substrate concentrations, the fast-growers remove the substrate at much higher rates than the slow-growers do. The research reported in this paper shows that the full-scale plant was always affected by a massive growth of *M. parvicella*. This was because it was operated at very low substrate concentrations (sludge load of about 0.02–0.03 kgBOD kgMLSS^{-1} d^{-1}) to achieve complete nitrification. The experiments showed many advantages of an aerated selector. Using an aerated upstream zone with a sludge load of 18–19 kgBOD kgMLSS^{-1} d^{-1}, the growth of *M. parvicella* was avoided and the settling properties were improved. Conversely, the tests clearly indicated that the anaerobic selector was not able to supress the growth

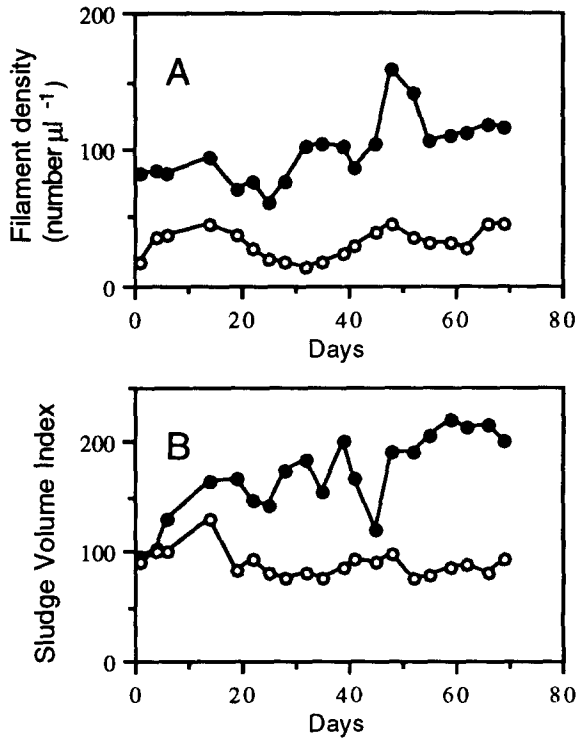

Fig 5. Variation of filament density and SVI in the second experiment with an aerobic selector, (○) pilot-plant, (●) full-scale plant.

of filamentous microorganisms such as *M. parvicella* and consequently did not improve sludge settleability.

Nevertheless, little information on the control of *M. parvicella* growth is available from the literature. According to some authors [25,26], *M. parvicella* can not only utilize substrate in oxic and anoxic conditions, but is also able to accumulate a significant portion of degradable substrate in anaerobic conditions and thus competes effectively against floc-forming bacteria. Moreover, *M parvicella* was considered one of the most common filamentous microorganisms in nutrient removal plants, as it can grow better with alternating anoxic-oxic conditions than in completely oxic systems [27]. Gabb et al. [28], tested the selector effect on filamentous bulking in a long sludge age, activated sludge system. They concluded that under completely aerobic conditions low F/M filaments did not proliferate. So the selector effect appeared to be irrilevant in the suppression of the low F/M filaments because amelioration of bulking by these filaments occurred both when the selector effect was present or absent.

Nevertheless, the results obtained in the present research are in agreement with those of Daigger et al. [10], in which bulking, caused by *M. parvicella*, was also prevented by using an aerated selector.

The success of the aerobic selector and the failure of the anaerobic zone on the suppressing the excessive growth of *M. parvicella* can possibly be explained by metabolic principles. The main mechanism of substrate sequestration in the anaerobic zone is a conversion of readily biodegradable compounds into the internal storage products performed by poly-phosphate accumulating (poly-P) bacteria. Nevertheless, *M. parvicella* does not behave as a typical filament, because it is capable of utilizing substrate under anaerobic conditions. When the anaerobic selector was placed at the head of the denitrification-nitrification system, *M. parvicella*, in competition with poly-P bacteria, used the energy from the depolymerization of accumulated polyphosphate to store substrate in the form of PHB which was subsequently utilized as an intracellular source of energy in oxic conditions. The ability of *M. parvicella* to syntesize both polyphosphates and internal storage products with a rate comparable to that of floc-formers, can explain the frequent occurrence of these filaments in nutrient removal systems [27] Nevertheless, more supporting experimental evidence is needed.

When the aerobic selector was placed at the head of the denitrification-nitrification system, most of the influent soluble substrate was removed in the aerobic selector. In this contact tank, where the returned sludge was mixed with the influent waste, the cells of activated sludge microorganisms were exposed to an environment with a substrate concentration higher than in the main aeration basin. These conditions (high F/M and dissolved oxygen) supported the growth of floc-forming microorganisms that appeared better able, than *M. parvicella*, to accumulate substrate. The substrate taken up in the aerobic selector was metabolized further in the denitrification basin, where microorganisms utilized oxidized nitrogen as a final electron acceptor in their electron transport system. This did not limit the denitrification efficiency in the DE basin, since the abatement of N-compounds was similar in both plants. Nevertheless, since the proper balance between substrate consumed under anoxic and under oxic conditions is always important for succesful denitrification, this phenomenon deserves to be studied in more detail in our next experiments. Finally, floc-forming microorganisms grew under low substrate concentrations in the aeration basin with high rates of nitrification. Under such circumstances, there was no substrate left for the growth of *M parvicella*. This resulted in a high selective pressure in comparison with the metabolic selection under anaerobic conditions, so that *M. parvicella* growth was completely suppressed.

Acknowledgements

We are indebted to J. Wanner (Prague Institute of Chemical Technology) for valuable comments on the paper, and to technicians of the AGAC laboratory for their skilful assistance. Two anonymous reviewers offered constructive sugges-

tions on an earlier version of the manuscript. This research was supported in part by AGAC and MURST.

References

1 Curds, C.R. and Hawkes, H.A (1983) Ecological Aspects of Used-Water Treatment Vol. 2, Biological Activities and Treatment Processes, pp. 82–91, Academic Press, London

2 Chiesa, S.C. and Irvine, R.L. (1985) Growth and control of filamentous microbes in activated sludge: an integrated hypothesis. Wat. Res 19, 471–479

3 van Veen, W.L (1973) Bacteriology of activated sludge, in particular the filamentous bacteria Antonie Leeuwenhoek 39, 189–205

4 Eikelboom, D H. (1975) Filamentous organisms in activated sludge. Wat Res. 9, 365–388

5 Eikelboom, D H (1977) Identification of filamentous organisms in bulking activated sludge Progr Water Technol. 8, 153–161

6 Eikelboom, D.H and van Buijsen, H.J J. (1983) Microscopic sludge investigation manual, 2nd. Delft, TNO Research Institute of Environmental Hygiene

7 Williams, T.M. and Unz, R F. (1985) Isolation and characterization of filamentous bacteria present in bulking activated sludge Appl. Microbiol. Biotechnol 22, 273–282

8 Chudoba, J., Grau, P. and Ottova, V. (1973) Control of activated sludge filamentous bulking II Selection of microorganisms by means of a selector Wat. Res. 7, 1389–1406

9 Chudoba, J , Cech, J.S , Farkac, J. and Grau, P. (1985) Control of activated sludge filamentous bulking Experimental verification of kinetic selection theory Wat Res. 19, 191–196.

10 Daigger, G T., Robbins, M.H and Marshall, B.R. (1985) The design of a selector to control low F/M filamentous bulking. Journal WPCF 57, 220–226

11 Hoffman, H. (1987) Influence of oxic and anoxic mixing zone in compartment system on substrate removal and sludge characteristics in activated sludge plants. Wat. Sci Technol. 19, 897–910

12 Wanner, J , Chudoba, J., Kucman, K. and Proske, L (1987) Control of activated sludge filamentous bulking. VII. Effect of anoxic conditions Wat. Res 21, 447–451

13 Linne, S.R , Chiesa, S.C , Rieth, M.G. and Polta, R.C (1989) The impact of selector operation on activated sludge settleability and nitrification pilot-scale results. J WPCF 61, 66–72

14 Salameh, M.F. and Malina, J.F (1989) The effect of sludge age and selector configuration on the control of filamentous bulking in the activated sludge process J. WPCF 61, 1510–1522

15 Patoczka, J. and Eckenfelder, W W. (1990) Performance and design of a selector for bulking control. J WPCF 62, 151–159.

16 Davoli, D. and Madoni, P (1991) Effect of aerobic and anaerobic zone on the growth of filamentous microorganisms in an activated sludge plant, in Biological Approach to Sewage Treatment Process Current Status and Perspectives (Madoni, P., Ed), pp 163–167, Centro Bazzucchi, Perugia.

17 Slijkhuis, H (1983) The physiology of the filamentous bacterium *Microthrix parvicella* Thesis, Agricultural University, Wageningen, The Netherlands

18 Wanner, J and Grau, P (1989) Identification of filamentous microorganisms from activated sludge· a compromise between wishes, needs and possibilities Wat Res 23, 883–891

19 Slijkhuis, H and Deinema, M H. (1982) The physiology of *Microthrix parvicella*, a filamentous bacterium isolated from activated sludge, in Bulking of Activated Sludge: Preventative and Remedial Methods (Chambers, B. and Tomlinson, E , Eds.), pp 75–83, Ellis Horwood, Chichester.

20 Slijkhuis, H. and Deinema, M. (1988) Effect of environmental conditions on the occurrence of *Microthrix parvicella* in activated sludge Wat. Res. 22, 825–828.

21 Slijkhuis, H., Van Groenestijn, J.W and Kylstra, D J (1984) *Microthrix parvicella*, a filamentous bacterium from activated sludge growth on Tween 80 as carbon and energy source J. Gen. Microbiol 130, 2035–2042

22 A P H.A. (1985) Standard methods for examination of water and waste water, 16th Edn., APHA, AWWA, WPCF, Washington

23 Jenkins, D., Richard, M G. and Daigger, G T. (1986) Manual on the causes and control of activated sludge bulking and foaming. WRC, Pretoria and USEPA, Cincinnati

24 Chudoba, J (1985) Control of activated sludge filamentous bulking. VI Formulation of basic principles Wat Res. 19, 1017–1022.

25 Gabb, D M D., Still, D A , Ekama, G A , Jenkins, D., Wetzel. M C and Marais, G v R (1988) Development and full scale evaluation of preventive and remedial methods for control of activated sludge bulking Final Research Report W62, Univ. of Cape Town.

26 Wanner, J. and Grau, P (1988) Filamentous bulking in nutrient removal activated sludge system Wat Sci. Technol 20, 1–8.

27 Blackbeard, J.R., Gabb, D.M.D., Ekama, G A and Marais, G.v R (1988) Identification of filamentous microorganisms in nutrient removal activated sludge plants in South Africa Water SA 14, 1–18

28 Gabb, D.M.D , Still, D.A , Ekama, G A , Jenkins, D. and Marais, G.v R (1991) The selector effect on filamentous bulking in long sludge age activated sludge systems Wat Sci. Technol 23, 867–877.