Effect of solids retention time on *Microthrix parvicella* growth

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Abstract

The objective of this study was to evaluate the effect of solids retention time (SRT) on *M. parvicella* growth and to calculate growth kinetic parameters of this filamentous species. Bench-scale continuous-flow experiments showed that *M. parvicella* growth can be significantly suppressed at an SRT of lower than 5.7 d for temperatures of between 14 and 18°C. According to the continuous-flow experiments the maximum sludge age for the avoidance of filamentous foaming problems caused by *M. parvicella* is 6 d for temperatures lower than 18°C. At this sludge age *M. parvicella* loses its hydrophobicity and therefore its foaming potential. However, even lower SRTs are required in order to achieve a significant suppression of its growth. At SRT values of less than 5.7 d *M. parvicella* initially forms a shorter filament (< 150 μm) with clear spaces inside filaments and variable Gram stain reaction and eventually is eliminated from activated sludge biocoenosis. According to kinetic studies presented in this paper, *M. parvicella* is a slow growing bacterium with a low maximum specific growth rate of 0.67 1/d and 0.53 1/d under aerobic and anoxic conditions respectively. Maintenance energy requirements of *M. parvicella* were found to be significantly lower than the maintenance energy of floc forming micro-organisms as well as other filamentous species, thus providing the micro-organism with a significant advantage under starvation conditions prevailing at the majority of the extended aeration activated sludge systems.

Keywords: bulking, filamentous micro-organisms, foaming, growth kinetics *Microthrix parvicella*, solids retention time (SRT)

Nomenclature

BNR	biological nutrient removal
BNRA	experimental system
BNRB	experimental system
BNRC	control system
COD	chemical oxygen demand (mg COD/ℓ)
DO	dissolved oxygen concentration (mg O_2/ℓ)
DSVI	dissolved sludge volume index (mℓ/g TSS)
f_{av}	active fraction of the MLVSS
f_{cv}	COD/VSS ratio of activated sludge (mg COD/mg VSS)
FI	filament index
K_d	decay coefficient (1/d)
m	maintenance coefficient (mg COD/mg COD·d)
MLSS	mixed liquor suspended solids concentration (mg SS/ℓ)
MSTW	Metamorphosis Sewage Treatment Works
NH_4-N	ammonia nitrogen concentration (mg N/ℓ)
NO_3-N	nitrate nitrogen concentration (mg N/ℓ)
NUR	nitrate – nitrogen uptake rate (mg NO_3-N/ ℓ·d)
NUR_i	initial nitrate – nitrogen uptake rate (mg NO_3-N/ ℓ·d)
OUR	oxygen uptake rate (mg O_2 /ℓ·d)
OUR_i	initial oxygen uptake rate (mg O_2 /ℓ·d)
P	total phosphorus concentration (mg P/ℓ)
SFI	specific filament index
S_o	initial substrate concentration (mg COD/ℓ)
SRT	solids retention time (d)
TSS	concentration of total suspended solids (mg TSS/ℓ)
[VSS]	concentration of volatile suspended solids (mg VSS/ℓ)
VSS	volatile suspended solids (mg/ℓ)
X_o	initial biomass concentration (mg VSS/ℓ)
X_t	biomass concentration at time t (mg VSS/ℓ)
Y_H	yield coefficient of heterotrophic biomass (mg VSS/mg COD)
μ_{max}	maximum specific growth rate (1/d)

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Introduction

Microthrix parvicella is the most common filamentous species responsible for bulking and foaming problems in biological nutrient removal systems. So far only a few studies on *M. parvicella* pure cultures have been reported (Slijkhuis 1983; Slijkhuis et al., 1984; Seviour et al., 1994; Blackall et al., 1996; Tandoi et al., 1998, Rossetti et al., 2002), due to difficulties in maintaining the micro-organism in culture.

Kinetic experiments on pure and mixed cultures indicate that *M. parvicella* is a slow-growing bacterium with a μ_{max} rate of between 0.3 and 0.66 1/d (Slijkhuis, 1983; Slijkhuis et al., 1984; Tandoi et al., 1998, Rossetti et al., 2002), which is able to proliferate in BNR systems, especially when operated in the extended aeration mode.

Richard (1989) postulates that the operation of an activated sludge system at sludge ages of greater than 10 d promotes *M. parvicella* growth and thus stimulates filamentous bulking and/or foaming problems. Based on full- and laboratory-scale experiments Knoop and Kunst (1998) suggest that *M. parvicella* growth and occurrence of settling and foaming problems will appear at wastewater treatment plants operating at a sludge loading rate of less than 0.1 kgBOD_5/kgSS·d and at low temperatures (<15°C). However, no systematic study on the effect of sludge age on *M. parvicella* is available, nor have the lower SRT limits of *M. parvicella* been determined. In view of the above, the objectives of this study were to:

- Evaluate the effect of SRT on *M. parvicella* growth
- Calculate growth kinetic parameters of this filamentous species.

Materials and methods

Continuous-flow experiments

Bench-scale continuous-flow experiments were conducted in order to evaluate the effect of SRT on *M. parvicella* growth. Activated sludge cultures were established in three bench-scale BNR activated sludge systems, inoculated with sludge containing high amounts of *M. parvicella* (Specific Filament Index = 4.0), from the Metamorphosis Sewage Treatment Works (MSTW) in Athens. Continuous-flow experiments were conducted using primary effluent from MSTW supplemented with oleic acid in the form of Tween 80 to promote *M. parvicella* proliferation. The ability of long-chain fatty acids to stimulate the growth of *M. parvicella* is well documented for both activated sludge studies (Mamais et al., 1998; Andreasen and Nielsen., 1998) and pure cultures (Slijkhuis et al., 1984; Slijkhuis and Deinema, 1988). Activated sludge samples were periodically taken from the continuous-flow systems to conduct aerobic, and anoxic substrate uptake batch tests (OUR and NUR) as developed by Kristensen et al. (1992). For anoxic experiments N_2 gas was blown above the liquid to avoid surface aeration. Air supply was controlled to maintain a dissolved oxygen (DO) concentration of between 3.0 and 4.0 mg/ℓ. Temperature was also controlled at values lower than 18°C to avoid any negative temperature effect on *M. parvicella* growth. All configurations included one 3 ℓ anoxic and one 5 ℓ aerobic reactor followed by a 5 ℓ settling tank.

According to the experimental protocol one system (BNRC) was maintained as control and operated at a constant solids retention time (SRT) of 15.5 d (average organic loading = 0.19 kgCOD/kgVSS·d) , while its performance was compared to two experimental units (BNRA and BNRB). The two experimental units were operated for 43 d at constant SRT equal to 10 d (average organic loading = 0.22 kgCOD/kgVSS·d). After day 43 the experimental system BNRA was operated for 67 d at a sludge age of 8.5 d (average organic loading = 0.25 kgCOD/kgVSS·d), for the next 35 d at a sludge age of 7 d (average organic loading = 0.33 kgCOD/kgVSS·d), for 27 d at a sludge age of 4.5 d (average organic loading = 0.40 kgCOD/kgVSS·d) and finally for 16 d at an SRT equal to 3 d (average organic loading = 0.45 kgCOD/kgVSS·d). Accordingly after day 43 the experimental system BNRB was operated for 67 d at a sludge age of 7.0 d (average organic loading = 0.33 kgCOD/kgVSS·d) and for the next 78 d at sludge ages between 5 to 6 d (average organic loading = 0.38 kgCOD/kgVSS·d). Steady state conditions, evidenced by relatively constant MLSS concentrations and OUR values, were established in a period of 2 to 3 SRT after changing the SRT at each experimental system.

The performance of the bench-scale units was assessed by routine measurements of COD, TSS, VSS, NH_4-N, NO_3-N, P and DSVI as well as microscopic examination of the sludges throughout the experimental period. All analyses were done in accordance with *Standard Methods* (1992); DSVI values were measured in unstirred 1 ℓ cylinders; soluble fractions were obtained by 0.45 μm membrane filtration. Microscopic examination of the sludges was performed according to Eikelboom and Van Buijsen (1981) and Jenkins et al. (1993). The abundance of the filamentous population was determined by the Filament Index (FI) on a scale of 0 to 5. To quantify the presence of *M. parvicella* the microscopic counting method developed by Pitt and Jenkins (1990) for *Gordona amarae* and modified by Mamais et al. (1998) was applied. The foam coverage percentage was measured on a daily basis as the percentage of aerobic reactor's surface covered by foam. To control SRT the sludge mass balance method was used.

Growth kinetic studies

In order to determine the kinetic parameters of activated sludge samples that contained different amounts of *M. parvicella*, batch experiments were conducted with an initial substrate to biomass ratio (S_o/X_o) of 1.5. Foam and mixed liquor samples from an activated sludge system (BNR unit) which encountered serious bulking and foaming problems (Chalkida Sewage Treatment Works) were used to determine the kinetic parameters of *M. parvicella*. In order to produce activated sludge samples highly enriched with *M. parvicella*, mixed liquor samples were placed in a 1 ℓ cylinder and after addition of a small quantity of oleic acid, were aerated at moderate rates to promote filamentous foam formation. The floating foam layer was continuously removed from the surface of the cylinder and was added to the foam samples. Air was provided for several minutes until a change in the colour of the foam was observed (brown to white). Following this procedure two activated sludge stock solutions were collected, one that contained high amounts of *M. parvicella* (foam sample) and the other with a very low *M. parvicella* content (mixed liquor sample). Both samples were microscopically analysed in order to determine the *M. parvicella* content of the foam (*M. parvicella* counts > 1×10^7 intersections/gVSS) and the mixed liquor sample (*M. parvicella* counts < 2×10^6 intersections/gVSS), respectively.

To estimate maximum specific growth rate (μ_{max}), batch tests with an initial S_o/X_o ratio of 1.5 were conducted following the experimental protocol suggested by Ekama et al. (1986). Therefore μ_{max} was calculated based on the initial OUR_i by the following equation (Ekama et al., 1986; Pollard and Greenfield, 1997; Pollard et al., 1998):

$$\mu_{\max} = \frac{24}{\left(1 - f_{cv} \times Y_H\right)} \times OUR_i \times \frac{Y_H}{f_{av} \times X}$$

Accordingly μ_{max} calculations under anoxic conditions based on the initial nitrate NUR_i were performed according to the following equation (Sozen et al., 1998):

$$\mu_{\max} = \frac{24}{\left(1 - f_{cv} \times Y_H\right)} \times NUR_i \times \frac{Y_H}{f_{av} \times Xv} \times 2.86$$

The active fraction (f_{av}), the COD/VSS ratio of activated sludge (f_{cv}) and the heterotrophic yield (Y_H) were determined for each test as described by Ekama et al. (1986). Following depletion of substrate the experiments were carried out for a period of 30 d in order to evaluate the decay coefficient (K_d) of each culture (foam and mixed liquor samples). The decay coefficient was determined according to the observed decrease in VSS concentration over time by assuming that the following equation described the VSS reduction over time:

$$X_t = X_o \times e^{-K_d \times t}$$

where:

X_t is the VSS concentration at time t (mgVSS/ℓ)

X_o is the VSS concentration at time t=0 when all substrate has been depleted (mgVSS/ℓ).

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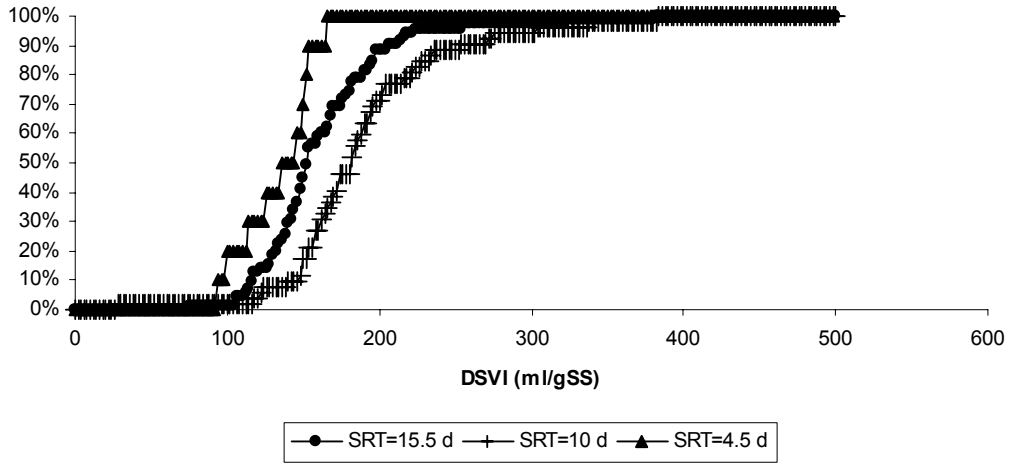

Figure 1
Cumulative distribution of DSVI for alternative SRT values

Results and discussion

The effect of SRT on M. parvicella growth

The effect of SRT on *M. parvicella* growth was studied by monitoring the operation of three BNR bench-scale units for a period of 188 d. All experimental units received MSTW primary effluent supplemented with 100 mg/ℓ Tween 80 (polyoxyethylene sorbitan mono-oleate) a water soluble carbon source containing 0.22 g of oleic acid /g of Tween 80, to promote the growth of *M. parvicella*. Average COD, NH_4-N and P concentrations in the influent were 513 mg/ℓ, 53 mg/ℓ and 20 mg/ℓ respectively, whereas the COD: N: P ratio was in the order of 25.7:3.2:1. All BNR systems exhibited satisfactory total COD and nitrate removal rates in the range of 72 to 77% and 54 to 89% respectively. Ammonia nitrogen removal obtained at SRT values greater than 8.5 d was high with an average value of 89%. Following the decrease of SRT ammonia nitrogen removal gradually decreased, reaching an average value of 64% at an SRT of 4.5 d.

Sludge settleability was assessed in the form of cumulative distributions of the DSVI values (Fig. 1). Additional information related to DSVI values of the experimental units is given in Table 1. According to the results the increase in the organic loading as realised by the decrease of the SRT from 15.5 d to 4.5 d resulted in a relatively moderate improvement of the settling characteristics of systems biomass, measured in terms of average DSVI values. On the other hand a significant improvement of settling properties with regard to 90 percentile DSVI values at lower SRT values was evidenced (Table 1). Furthermore, the percentage of excessive bulking incidents (DSVI>200 mℓ/gTSS) was 10% to 29% for an SRT of greater than 8.5 d, whereas no bulking incidents were recorded for SRTs of lower than 7 d. However, it should be mentioned that throughout the experimental period and even at low SRT values, the average DSVI never fell below 100 to 120 mℓ/gTSS due to the continuous presence of *Type 0092* in all systems.

Average filament indices (FI) for SRT values of 15.5 d, 10 d, 8.5 d, 7 d, 5.7 d and 4.5 d were 3.1, 2.8, 3.6, 2.6, 1.8 and 2.0 respectively. Microscopic analyses revealed that the dominant filamentous species for the control system (SRT=15.5 d) throughout the experimental period was *Microthrix parvicella* followed by *Type 0041*, *Type 0092*, *Type 0675* and *Nostocoida limicola*. Average SFI values of *M. parvicella* varied between 3.0 for SRT greater than 7 d to less than 1 for an SRT of lower than 5.7 d. Accordingly average SFI values for *Type 0041* and *Type 0675* ranged between 0.6 and 1.2 and 0.3 and 1.0 respectively with the higher values associated with SRTs greater than 7 d and the lower values associated with SRT lower than 5.7 d (organic loading equal to 0.38 kgCOD/kgVSS·d). The aforementioned organic loading rate of 0.38 ± 0.06 kgCOD/kgVSS·d is in very good agreement with threshold values for *Type 0041* proliferation (0.23 kgBOD5/kgVSS·d) reported by Scruggs and Randall (1998) and Richard (1989).

TABLE 1
Effect of SRT on DSVI values

SRT (d)	15.5	10	8.5	7	5.7	4.5
$DSVI_{50}$* (mℓ/gSS)	152	181	123	153	136	133
$DSVI_{10}$** (mℓ/gSS)	205	253	199	157	154	177
DSVI >200 mℓ/gSS (%)	12%	29%	10%	0%	0%	0%

* *DSVI correspond to 50% of all recorded values*
** *DSVI correspond to 10% of all recorded values*

The effect of the organic loading on *Type 0092*, surprisingly enough, was insignificant for the range of organic loadings studied (0.19 to 0.45 kgCOD/kgVSS·d). Although this filamentous micro-organism was not dominant in the sludge biocoenosis of the three bench-scale systems (mainly due to the unfavourable conditions for its growth temperatures of less than 18°C), its presence was stabilised at moderate values (SFI=1to 2) even at SRT values of 4.5 d.

Figures 2 to 5 summarise the effect of SRT on *M. parvicella* growth in the temperature range 14 to 18°C. More specifically, Figs. 2 to 4 present the variation of *M. parvicella* counts (in terms of intersections/gVSS) for BNRC, BNRA and BNRB experimental units for a period of 188 d. According to the results, *M. parvicella* exhibited substantial growth in the control system for the entire experimental period with average counts of 8.2×10^6 intersections/gVSS (Fig. 2). On the other hand *M. parvicella* content in the two experimental units was highly dependent on the SRT established during each time period. Figure 5 presents the average *M. parvicella* content and their 95% significance interval for the SRT values examined. As is illustrated in Fig. 5, *M. parvicella* growth is significantly suppressed at SRT values of lower than 5.7 d.

Microscopic examination of mixed liquor samples at periods when the systems operated at SRT values of lower than 5.7 d indicate that *M. parvicella* was present at very low concentrations forming short filaments with lengths of no more than 150 µm, with clear areas in the filament visible, and exhibited a variable Gram stain reaction. This short filament growth form

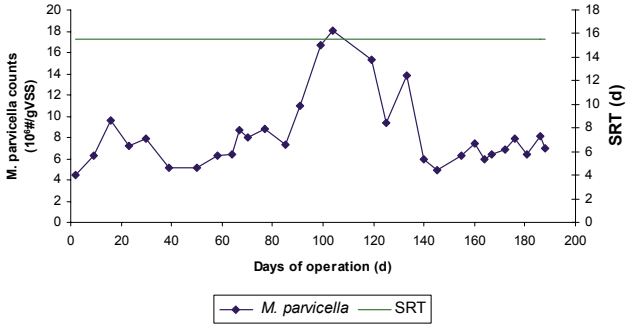

Figure 2
M. parvicella *counts for control system BNRC*

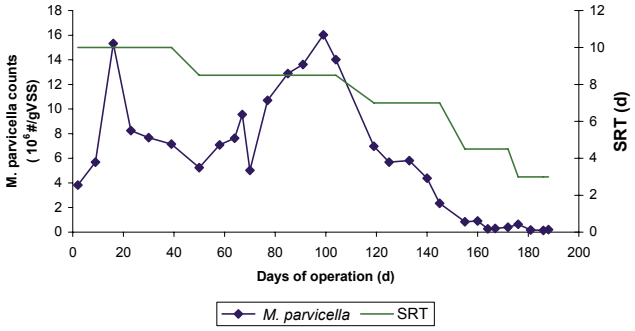

Figure 3
M. parvicella *counts for experimental unit BNRA*

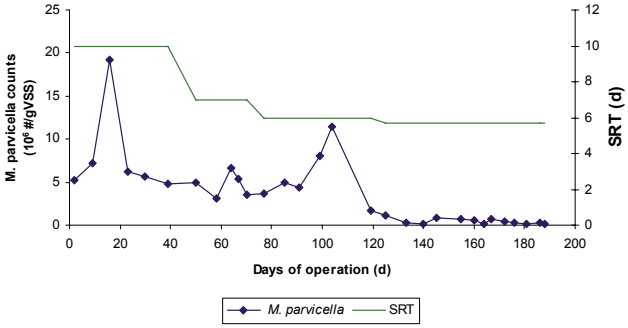

Figure 4
M. parvicella *counts for experimental unit BNRB*

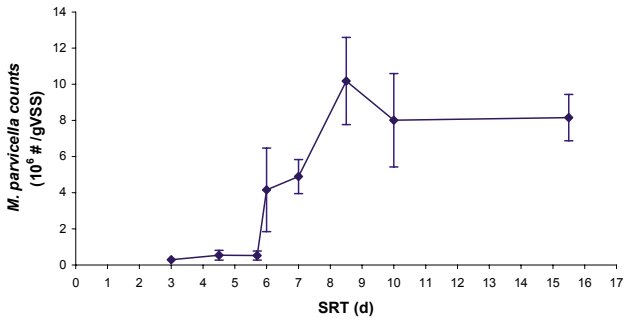

Figure 5
M. parvicella *counts as a function of SRT*

and the variable Gram stain reaction were not recorded for the control system (SRT=15.5 d) and the two experimental systems when operated at SRTs of greater than 6 d. These findings are in good agreement with data given by Foot et al. (1992) and Knoop and Kunst (1998). According to the results obtained from batch experiments Foot et al. (1992) reported that under high organic loading conditions (2 kgCOD/kgSS·d) *M. parvicella* proliferated initially in the form of individual cells of 3 µm length and 2 µm width, that gradually developed a filament form with a length of less than 100 µm, presenting a variable Gram stain reaction. The authors postulated that the variable Gram stain reaction is directly related to the surface chemistry of the filament cells and is attributable to the stress conditions created due to the high organic loading. However, this interpretation does not seem very stable as the Gram stain reaction of a micro-organism is highly related to the physical rather than the chemical properties of its cell walls (Brock et al., 1994). Thus the variation in Gram stain reaction of *M. parvicella* should be attributed to the decrease of the cell wall thickness as a result of the adverse conditions for its growth imposed on the experimental systems (SRT<5.7 d). Similar observations have been reported for other Gram-positive filamentous micro-organisms causing foaming problems in activated sludge plants (Pitt and Jenkins, 1990; Cha et al., 1990). It has been observed that *Gordona amarae* will only weakly retain a Gram-positive reaction when subjected to environmental stress in an activated sludge system (Cha et al., 1990).

In addition to microscopic analyses the foam coverage percentage in the aeration zone of the three bench-scale BNR activated sludge units was also recorded on a regular basis (daily). Figure 6 presents the average foam coverage percentages and their 95% significant intervals for the SRT values examined. All bench-scale units encountered filamentous foaming problems. More specifically the control system faced intense foaming incidents throughout the experimental period of 188 d

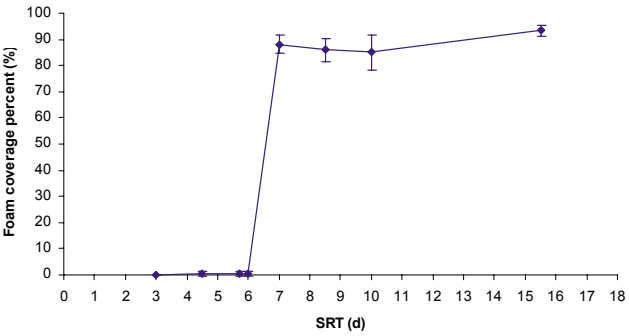

Figure 6
Foam coverage percent as a function of SRT

(average foam coverage 93%). Accordingly the two experimental units exhibited equally high foam coverage with average values of between 85 to 88% when operated at SRTs of between 7 and 10 d. However, the decrease in the SRT from 7 d to 6 d resulted in a rapid reduction of the foam coverage from 85% to 50% over a period of 10 d, while foam was completely eliminated from the BNRA after an additional period of 9 d. This rapid elimination of the filamentous foam was recorded also for the second experimental unit (BNRB) when the SRT was changed from 7 d to 5.7 d. Thus it appears that although *M. parvicella* loses its hydrophobic properties (enabling it to produce biological foam) at an SRT of 6 d, its washout is a slower process initiated at even lower SRT values (< 6 d).

Growth kinetic studies

Batch experiments were performed to measure the maximum specific growth rate (μ_{max}), the decay coefficient (K_d) and the yield coefficient (Y_H) of activated sludge samples by monitor-

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TABLE 2
Average and 95% significance level of *M. parvicella* and floc-forming micro-organisms kinetics

Parameter	Units	Floc formers	*M. parvicella*
f_{av}		0.425	0.55
f_{cv}	mgCOD/mgVSS	1.29 ± 0.16	1.60 ± 0.27
Y_H (VSS)	mgVSS/mgCOD	0.53 ± 0.08	0.38 ± 0.05
K_d (based on endogenous respiration)	1/d	0.026	0.0164
K_d (based on μ_{max})	1/d	0.075 ± 0.011	0.031 ± 0.006
m	mgCOD/mgCOD·d	0.04	0.027
μ_{max}	1/d	1.58 ± 0.23	0.67 ± 0.12

ing the changes in OUR, VSS and substrate utilisation rate. The experiments were performed under low initial substrate (acetate) to biomass ratios (S_o/X_o<2) because as postulated by Grady et al., (1996), Chudoba et al., (1992) and Stasinakis et al., (2003), a high value of the S_o/X_o ratio will significantly alter the composition of initial sludge biocoenosis.

As already described after treatment with aeration (at moderate rates), two stocks of activated sludge samples were collected; one with a low content of *M. parvicella* and another with a high *M. parvicella* content (> 1×10^7 intersections/gVSS). Figure 7 illustrates the effect of the *M. parvicella* content on biomass μ_{max}. All the kinetic parameters, determined at low initial substrate to biomass ratios (S_o/X_o=1.5), are summarised in Table 2.

According to the results, as the *M. parvicella* content of the biomass increases up to a count of 1×10^7 intersections/gVSS, the maximum specific growth rate of biomass gradually decreases, whereas relatively constant μ_{max} values are evidenced for higher *M. parvicella* counts. The μ_{max} values determined using sludges enriched with *M. parvicella* cultures (*M. parvicella* counts > 1×10^7 intersections/gVSS) appear to be consistently lower with an average value of 0.67 1/d. It was postulated that the kinetic parameters determined using sludge enriched with *M. parvicella* closely reflect the characteristics of this filamentous species that highly dominated the biocoenosis of the activated sludge. Hence, measurements of kinetic parameters using sludge with a low *M. parvicella* content are attributed to floc forming micro-organisms, while measurements on sludge samples with a high *M. parvicella* content (>1×10^7 intersections/gVSS) are attributed to *M. parvicella* (Table 2). As presented in Table 2, average μ_{max} of *M. parvicella* is 0.67 1/d, a typical value of the slow growing micro-organisms found in BNR systems operating at extended aeration mode (high sludge age). Moreover it should be pointed out that this value is almost identical with the μ_{max} (0.66 1?/d) measured by Rossetti et al. (2002) in mixed culture samples, while slightly higher of the range (0.3 to 0.5 1/d) reported for pure culture studies (Slijkhuis 1983; Slijkhuis et al., 1984; Tandoi et al., 1998, Rossetti et al., 2002).

The decay coefficient (K_d) values determined according to endogenous respiration experiments were significantly lower than those calculated from μ_{max} values by assuming a 5% K_d to μ_{max} ratio (Table 2). Decay coefficient values of *M. parvicella* are much lower than those attributed to floc formers and lower than values given for other filamentous micro-organisms (Richard et al., 1985). It should be mentioned that as measurements of kinetic parameters were performed in mixed cultures the kinetic values attributed to floc formers were lower than those reported in the literature (Wanner, 1994).

Based on the μ_{max} and K_d values presented in Table 2, the specific maintenance coefficient (defined as K_d over Y_H ratio) of both *M. parvicella* and floc formers was calculated. The resulting maintenance coefficient (m) of *M. parvicella* was significantly lower (30%) than the maintenance coefficient which was calculated for floc-forming bacteria, thus reflecting lower maintenance energy requirements (defined as the minimum energy input necessary for a cell to perform its basic biochemical processes). The calculated maintenance coefficient for *M. parvicella* is comparable to the 0.035 mg/mg·d (in COD units) value which is reported by Wanner (1994) for the same micro-organism and much lower than reported maintenance coefficients for other filamentous species and floc formers (Wanner, 1994).

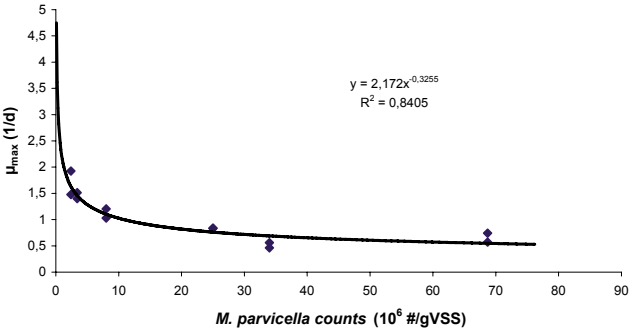

Figure 7
Effect of M. parvicella content on biomass μ_{max}

By using similar experimental procedures, batch tests were also performed under anoxic conditions and the average maximum specific growth rate of *M. parvicella* as well as its 95% significance level was calculated equal to 0.53 ± 0.12 1/d. By dividing μ_{max} values for anoxic and aerobic conditions an average n_g value of 0.80 was anticipated, which is equal to the default value suggested by Henze (1992) for use in the IWA Activated Sludge Model No. 1 (Henze et al., 1987).

As discussed earlier all the aforementioned kinetic studies were conducted at low S_o/X_o ratios. To evaluate the effect of the initial S_o/X_o on the determination of μ_{max}, a limited number of batch assays using foam and mixed liquor samples were performed at high initial S_o/X_o ratios (S_o/X_o=15) following the experimental procedure proposed by Kappeler and Gujer (1992). The values of μ_{max} determined were significantly higher (over the range of 6.5 to 7.5 1/d) than μ_{max} values determined at low initial S_o/X_o. This difference is in good agreement with findings reported by Grady et al. (1996), Chudoba et al. (1992), Novak et al. (1994) and Stasinakis et al. (2003). According to the authors of these studies a high value of S_o/X_o ratio will significantly alter the composition of initial sludge biocoenosis. Assuming there is sufficient substrate available, the species growing faster will be favoured during the batch assays conducted at high S_o/X_o ratios and the proportion of these species will increase in sludge

biocoenosis with time. Therefore the μ_{max} values determined at high S_o/X_o ratios will more closely reflect the characteristics of the culture developed at the end of the batch assay and not those of the original culture. On the other hand, μ_{max} values determined at low S_o/X_o ratios more closely resemble the initial characteristics of activated sludge biocoenosis. *M. parvicella* being a slow-growing bacterium is not expected to maintain its high content in batch assays conducted at high S_o/X_o ratios. For these reasons the results of the batch assays conducted at low S_o/X_o ratios are thought to give representative values for the kinetic parameters of sludges enriched with *M. parvicella* cultures as they correlate better with the characteristics of the initial sludge biocoenosis.

Conclusions

The objective of this study was to evaluate the effect of SRT on *M. parvicella* growth and to calculate growth kinetic parameters of this filamentous species. Bench-scale continuous-flow experiments showed that *M. parvicella* abundance can be significantly reduced at an SRT of lower than 5.7 d. According to the experiments the maximum sludge age for the avoidance of filamentous foaming problems caused by *M. parvicella* is 6 d for temperatures of lower than 18°C. On the basis of the above it can be postulated that even though *M. parvicella* loses its hydrophobicity at sludge ages as low as 6 d (elimination of foaming problems), a significant suppression of its growth is achieved at slightly lower SRT values (5.7 d). Under these operating conditions *M. parvicella* initially forms a shorter filament (< 150 μm), with clear spaces inside filaments and variable Gram stain reaction, which is eventually eliminated from activated sludge biocoenosis.

In addition, at SRT values of lower than 5.7 d, growth of *Type 0041* and *Type 0675* is suppressed whereas this is not the case for *Type 0092*.

A rather simple experimental technique was employed to calculate growth kinetics for *M. parvicella*. According to the results *M. parvicella* has a low maximum specific growth rate of 0.67 1/d, whereas under anoxic conditions μ_{max} becomes even lower (0.53 1/d). Maintenance energy requirements of *M. parvicella* were found to be significantly lower than the maintenance energy of floc forming micro-organisms as well as other filamentous species, thus providing the micro-organism with a significant advantage under starvation conditions prevailing at the majority of the extended aeration activated sludge systems.

From the results it can be concluded that a possible effective strategy for *M. parvicella* control is SRT reduction to fewer than 5.7 d for temperatures of between 14 and 18°C. In practice this may be a promising approach for systems aiming at carbon removal, as required by the European Union for discharge into non-sensitive recipients. The situation is more complicated whenever nutrient removal (especially nitrogen) is required, as is the case for sensitive recipients. The problem arises due to the need for operation under sufficiently high sludge ages for complete nitrification. However, taking into consideration the favourable temperature conditions under Mediterranean conditions, with mixed liquor temperatures during most of the critical winter period about 16 to 17°C, it may be possible to achieve the nitrification goal at sludge ages of 5.5 to 5.7 d, although admittedly through very carefully balanced conditions and provided that the sewage does not contain substances inhibitory for the nitrification process.

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