

Occurrence of *Microthrix parvicella* in sequencing batch reactors

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ABSTRACT

Sequencing batch reactors (SBRs) are known for high process stability and usually have a good sludge volume index (SVI). Nevertheless, in many SBRs in Germany for municipal wastewater treatment, scum and foam problems can occur, and SVI can be larger than 200 mL/g. The microscopic investigations of the activated sludge from plants with nitrogen and phosphorus removal have shown that *Microthrix parvicella* is dominant in the activated sludge in most of them. Studies showed that the optimum growth of *M. parvicella* is performed at a high sludge age (>20 d) and low sludge load in the range of 0.05–0.2 kg of biochemical oxygen demand per kg of total suspended solids per day (kg BOD₅/(TSS·d)). The investigations in 13 SBRs with simultaneous aerobic sludge stabilization (most of them are operated with a system called differential internal cycle strategy sequential batch reactor (DIC-SBR)) show that *M. parvicella* is able to grow in sludge loads less than 0.05 kg BOD₅/(kg TSS·d) as well. To optimize the operation of those SBRs, long cycle times (8–12 h) and dosing of iron salts to eliminate long-chain fatty acids are both recommended. This leads to better SVI and keeps *M. parvicella* at a low frequency.

Key words | DIC-SBR, *Microthrix parvicella*, SBR, simultaneous aerobic sludge stabilization

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INTRODUCTION

The biological wastewater treatment is based on two main processes, the biological conversion of the pollutants caused by the activity of the bacteria in the biological reactor, and the separation of purified wastewater from the sludge. This separation is usually performed in the clarifier or can be combined with the biological process in the same unit such as in sequencing batch reactors (SBRs), which have been mainly used in the recent years as treatment plants for areas with more than 5,000 population equivalents (PE) (Lemmer & Lind 2000; Jenkins *et al.* 2004).

Most wastewater treatment plants (WWTP) require low sludge loads and the establishment of aerobic, anoxic and anaerobic zones to enable biological nitrogen and phosphorus removal. This has led to new biological communities in activated sludge. The most common filamentous bacteria which can live under these conditions have high flexibility and can exist in all the zones, for example *Microthrix parvicella*, nocardioforms, type 0092, type 1851 and *Nostocoida limicola* (Ekama *et al.* 1996; Lemmer & Lind 2000).

Large amounts of filamentous microorganisms may build bridges between or around the flocs, which has a negative effect on the settling characteristics of the activated

sludge (high sludge volume index (SVI) >150 mL/g). This phenomenon is called filamentous bulking. As a result, many WWTP suffer under bulking-sludge or scum problems, which result in serious operational problems and might cause contamination of the rivers because of overflow of sludge from the WWTP (Casey *et al.* 1995; ATV-AG 2.6.1 1998; Lebek 2003; Jenkins *et al.* 2004).

The most common filamentous microorganism, which is responsible for bulking sludge, scum and foam problems in 93% of low load plants with nutrient removal in the north of Europe, is *M. parvicella* (Knoop 1997). The appearance of *M. parvicella* is associated with the following conditions (Slijkhuys 1983; Casey *et al.* 1995; Eikelboom *et al.* 1998; Kunst *et al.* 2000; Kunst 2002; Nielsen *et al.* 2002; Paris 2004; Rossetti *et al.* 2004):

- low sludge loading (0.05–0.2 kg of biochemical oxygen demand per kg of total suspended solids per day (kg BOD₅/(TSS·d))
- low temperatures (5–15 °C)
- large quantities of oil and grease
- the change between aerobic, anoxic and anaerobic phases

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- low oxygen concentration (less than 1 mg/L O₂)
- recirculation of process water from anaerobic sludge treatment
- plants with primary clarifier.

There are many different studies about the methods to control the growth of *M. parvicella* in WWTP, but not all were successful. All studied methods can be summarized by the following points (Jenkins *et al.* 1993, 2004; Ekama *et al.* 1996; Knoop 1997; Kunst *et al.* 2000; Lemmer & Lind 2000; Kunst 2002; Lebek & Rosenwinkel 2002; Lebek 2003; Paris 2004):

- increasing the sludge load of 0.2 kg BOD₅/(kg TSS · d) could have inhibiting effect on the growth of *M. parvicella*
- dosage of iron chloride (low impact on *M. parvicella*)
- dosage of acidic aluminum components (the most successful way)
- application of aerobic, anoxic and anaerobic selector (has no influence on the growth of *M. parvicella*).

Although many scientists such as Knoop (1997), Lemmer & Lind (2000), Kunst *et al.* (2000), Kunst (2002), Lebek (2003) and Jenkins *et al.* (2004) mentioned that low sludge loads 0.05–0.2 kg BOD₅/(kg TSS · d) favor the growth of *M. parvicella*, studies about the influence of the very low sludge load <0.05 kg BOD₅/(kg TSS · d) on the growth of *M. parvicella* are still limited. These low loads correspond to plants with simultaneous aerobic sludge stabilization (Wanner *et al.* 1998; Lebek 2003).

In order to define the effect of low sludge loads (<0.05 kg BOD₅/(kg TSS · d)) on the growth of *M. parvicella*, 13 SBRs in Germany with simultaneous aerobic sludge stabilization were studied.

According to the batch process in the SBR, the substrates can be stored by the floc-forming and most of the filamentous bacteria in the form of glycogen and acids. There are two groups of these bacteria: high F/M bacteria (high food/microorganism ratio) and low F/M bacteria (Lemmer & Lind 2000). The high F/M bacteria such as *Sphaerotilus natans* and type 021 N can store the substrates as reserve materials only in limited amount. This causes a reduction in frequency of these high F/M bacteria in low sludge load plants. They are replaced by low F/M bacteria such as *M. parvicella*.

Most of the investigated SBRs are working with the differential internal cycle strategy sequential batch reactor method (DIC-SBR) (Figure 1). This method was developed by the company LimnoTec in Lübbecke, Germany, in

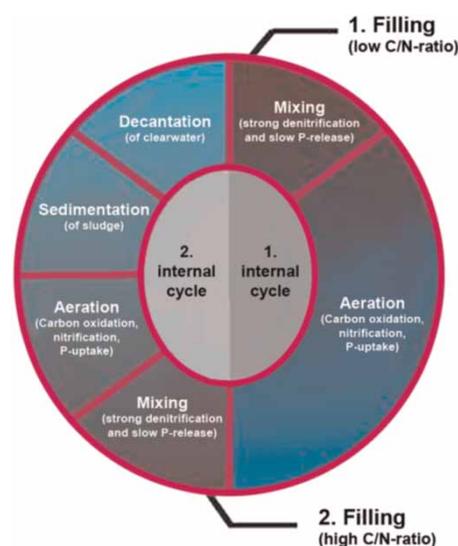


Figure 1 | DIC-SBR cycle (SH + E GROUP 2012).

order to optimize the wastewater treatment. Only 20 SBRs in Germany are operating with this method. The difference between this method and a conventional SBR is characterized by two fillings during one cycle with water having different C/N ratios. The different C/N ratios are adjusted in a multi-functional buffer reservoir before the SBR. The water in the buffer is separated from the solid particles by sedimentation. This supernatant water has a low C/N ratio (which can be called thin water) and a low amount of solids. During the first filling the thin water is applied to the reactor to an amount of 60–90% of the total filling amount during the cycle. During this filling anoxic and anaerobic zones exist, which encourage phosphorus removal and denitrification, if nitrate exists. Before the second filling the water in the buffer is mixed. The rest in the buffer (about 20% of the total amount) is characterized by a high C/N ratio. During the second filling the rest of the nitrate is denitrified (Rönner-Holm & Holm 2009; SH + E GROUP 2012).

It was proved that this method has a lot of advantages such as improvement in the elimination of nitrate and phosphorus, high flexibility and the opportunity of longer charging times.

MATERIALS AND METHODS

Thirteen SBRs in Germany (1,000–25,000 PE) were investigated during cold and dry weather conditions. From each plant just two samples from two locations were taken. One sample was taken from the plant's inflow to measure

BOD₅. The second sample was taken during the second aeration phase to measure SVI and to be investigated under a microscope. Unfortunately, the duration of the project was too short for taking another sample. But the results were compared with the results that were measured in the plant, in order to validate them.

The main task was the evaluation of the operation diaries of these plants and the identification of *M. parvicella* to give a statement about the best operation of SBR and the influence of the very low sludge loads on this bacterium.

Microscopic investigations

For microscopic investigations a Zeiss microscope type Axiolab was used. By using one drop of crystal violet solution on air-dried smear preparation the total filament abundance (filament category) was determined according to the seven total filament categories (0, 1, 2, 3, 4, 5, 6)

Table 1 | Determination of the frequency of one filament type (Jenkins *et al.* 2004)

Frequency (-)	Description
0	No filaments were observed
1	Few filaments from the same type were observed
2	Some filaments from the same type were observed
3	Common filaments from the same type were observed
4	Very common filaments from the same type were observed
5	Plenty of filaments from the same type were observed
6	Excessive filaments from the same type were observed

(Jenkins *et al.* 2004). The microscopic investigation at 100× magnification using darkfield microscopy shows violet flocs and orange filaments (Knoop & Kunst 1998).

Some filamentous organisms can clearly be identified according to their morphology, mobility, branching, thread form, attached growth, length and width, appearance of the cells, etc. (Schmid-Schmieder 2006; Remde 2010). Some organisms are difficult to be identified by their morphology; in these cases Gram and Neisser staining have to be used.

By using Gram und Neisser staining the frequency of *M. parvicella* was determined according to Table 1. *M. parvicella* has a strong Gram-positive staining reaction, Neisser-positive granules, coiled growth, no branching and no movement. Its filaments are between 50 and 500 μm in length and 0.8 μm in width. They can be found inside the floc, surrounding the floc or dispersed (see Figure 2) (Eikelboom 1975; Knoop 1997; Lemmer & Lind 2000; Paris 2004; Jenkins *et al.* 2004).

Calculation of sludge load

To investigate the influence the low sludge load (<0.05 kg BOD₅/(kg TSS · d)) has on *M. parvicella*, the sludge load was calculated in every investigated SBR over some years using the following formula (DWA-M 210 2009)

$$B_{TS,BOD} = (B_{d,BOD,in} / (n \cdot V_R \cdot TSS_R)) \cdot (t_Z / t_R) \quad (1)$$

(kg BOD₅ / (kg TSS · d))

$B_{d,BOD,in}$: daily BOD₅ load in the input of SBR (kg/d), n : number of SBR basins (-), V_R : maximum volume of SBR (m³), TSS_R : dry suspended solids content in relation to the maximum volume of the SBR (g/L), t_Z : cycle duration (h), t_R : duration of reaction phase (h).

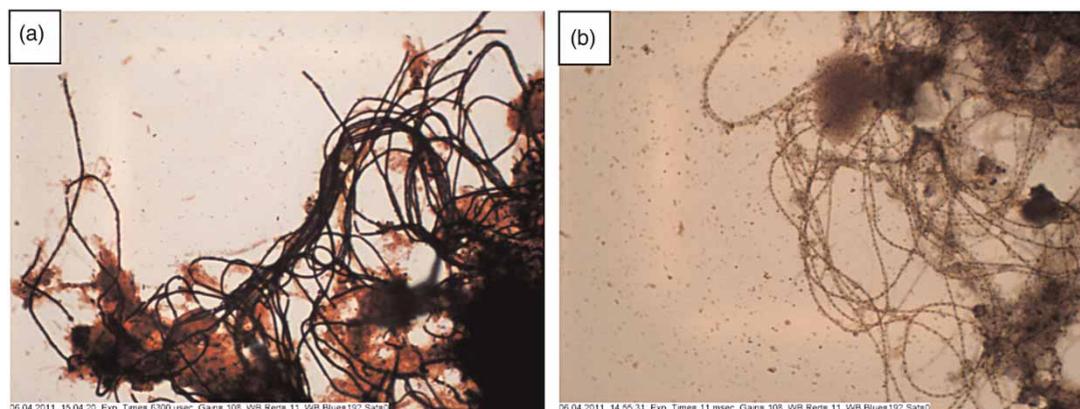


Figure 2 | *M. parvicella* in the plant Z: (a) Gram-positive; (b) Neisser-positive granules; ×1000 enlargement.

According to the development of the sludge load over years and the frequency of *M. parvicella*, it is possible to determine the impact of the low sludge load ($<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$) on the growth of *M. parvicella*.

RESULTS AND DISCUSSION

SBRs are known to have $\text{SVI} < 150 \text{ mL/g}$ with very compact flocs (Schreff & Hilliges 2013). The development of the SVI due to temperature in some SBRs such as in plant A (see Figure 3) shows that SVI can reach higher values in winter ($>150 \text{ mL/g}$) and floating sludge may occur. The investigation under the microscope of activated sludge in plant A shows that *M. parvicella* is dominant.

In order to optimize the operation of these plants, 13 SBRs with aerobic sludge stabilization were investigated and analyzed and the frequency of *M. parvicella* was determined. All examined plants are treating municipal wastewater. Because of the operation with simultaneous aerobic sludge stabilization, these SBRs do not have a primary clarifier. The investigations of Lebek (2003) indicated that 10% of the long-chain fatty acids in urban wastewater are not dissolved. The pre-treatment in the primary clarifier is used to hydrolyze the fats, which improves the growth of *M. parvicella*. Due to the absence of primary clarifiers, the frequency of this bacterium in the systems with aerobic sludge stabilization could be lower. However, the investigations in some SBRs showed that this was not always the case (Helmreich *et al.* 2000).

Unfortunately, SBRs in Germany do not have any microscopic investigations in the plants. For this reason, as mentioned in 'Materials and methods', samples were taken

during the second aeration phase in each investigated plant to measure SVI and to determine the existence of *M. parvicella* under the microscope. The self-measured SVI values, which are shown in Table 2, were compared with the plants' measurements (operation diaries) and the results were very similar. In order to determine the impact of the sludge load on the growth of *M. parvicella*, the sludge load was calculated according to Equation (1). For this calculation the data for a minimum of 4 years were taken from the operation diaries. The results are shown in Table 2.

Table 2 shows that *M. parvicella* exists in all investigated SBRs, but in different frequencies and it can grow in plants with sludge loads more than $0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ and less than $0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ as well.

Comparison of SBRs according to operational process

Table 2 shows that *M. parvicella* can grow in most SBRs with different operations. All these SBRs, except F and U, are operated as DIC-SBR.

In SBR F wastewater is fed continuously during the cycle time. In plant U there is no buffer and wastewater is fed parallel to both reactors. The cycle time in this plant is always 6 h and does not depend on the influent amount. The reaction phase is 3 hours; the sedimentation and decanting phase is 3 hours too. During the sedimentation phase in the first tank the water is charged to the second one.

Comparison of SBRs according to the sludge load

The frequency of *M. parvicella* in SBRs which permanently have low sludge load $<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ is 1–4,

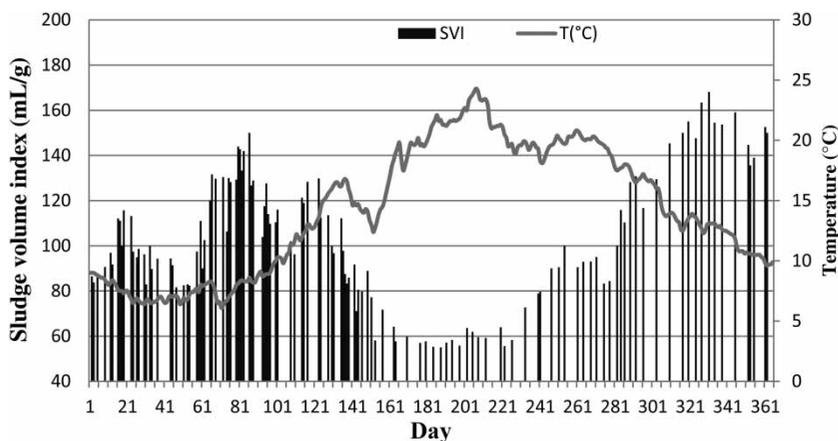


Figure 3 | Development of SVI according to the temperature in the SBR A in 2006 (operation diaries).

Table 2 | Sludge load, SVI, used precipitant, filament category and frequency of *M. parvicella* in the investigated SBRs

Plant	Sludge load (kg BOD ₅ /(kg TSS · d))	SVI (mL/g)	Precipitant	Filament category	Frequency of <i>M. parvicella</i>	PE
A	<0.05	96	FeCl ₃	2–3	2–3	5,000
H	<0.05	72	FeClSO ₄	2–3	1–2	10,000
C	<0.05	98	–	3	2–3	3,000
D	<0.05	79	–	2	2	4,500
P	<0.05	74	NaAl(OH) ₄	1–2	2–3	5,000–10,000
R	<0.05	90	NaAl(OH) ₄	3	3–4	16,000
B	0.03–0.072	74	FeCl ₃	2–3	3	9,200
O	0.03–0.1	86	FeCl ₂	2	1–2	16,000
S	0.05–0.12	122	NaAl(OH) ₄	3–4	4	22,500
Z	0.006–0.09	142	–	4–5	5	5,200
N	– ^a	86	aluminum salts + FeCl ₃	2	1	7,850
F ^b	– ^a	129	(Al ₂ (SO ₄) ₃)	3	3	4,000
U ^b	<0.05	259	FeCl ₃	5	4–5	8,200

^aNo information available.

^bNot working according to DIC-SBR (see 'Comparison of SBRs according to operational process').

except for plant U. It is less than in plants with higher loads of >0.05 kg BOD₅/(kg TSS · d) (frequency = 3–6). Although the plant U works with sludge load less than 0.05 kg BOD₅/(kg TSS · d), it still has a high frequency of *M. parvicella* and nocardioforms as well. Plant U shows very high SVI: >200 mL/g.

Comparison of SBRs according to the precipitant

However, the growth of *M. parvicella* can also be influenced by the used precipitant (see Table 2). The aluminum salts have different effects on *M. parvicella*. In SBR N the dosing of FeCl₃ over the complete year reduces the amount of long-chain fatty acids, and aluminum salts are also dosed in winter when *M. parvicella* begins to occur. This variation shows the best effect on *M. parvicella* and the other filaments. This fact corresponds to the investigations of Lebek (2003).

At the same time the sodium aluminate has obviously no influence on this bacterium (plants P, R and S). This trend

matches with the results of Misera (2002) and Lebek (2003). On the other hand, in low load plants iron salts show a good influence on *M. parvicella* but these salts cannot completely remove this bacterium from the plant (plants A, B, H and O) (Bidder 1999; Lebek 2003).

The dosing amount of the precipitant is variable. It depends on the phosphorus load in the input of the SBR. In order to give a better statement about the recommended precipitant which should be used to optimize the SBR, the dosing amount of the previous named precipitants was determined depending on the 85% value of TSS and the active substance content (see Tables 3 and 4).

Table 3 indicates that the dosage of 0.28 g Fe/(kg TSS · d) during both aeration times has the best effect against the growth of *M. parvicella* (plant H). A higher dosage amount (1.0 g Fe/(kg TSS · d)) during the second aeration can have good results too (plant O). On the other hand, Table 4 shows that high dosage amount of sodium aluminate (during the second aeration) can affect the growth of this bacterium.

Table 3 | Comparison of DIC-SBRs which use ferrous precipitants

Plant	Precipitant	Fe content (g Fe/kg)	Dosing amount (g Fe/(kg TSS · d))	Frequency of <i>M. parvicella</i>	Notice
A	FeCl ₃	135 ^a	0.13	2–3	Dosing in buffer
B	FeCl ₃	135 ^a	0.26	3	Dosing after the second aeration
H	FeClSO ₄	123	0.28	1–2	Dosing during the first and second aeration
O	FeCl ₂	85	1.0	1–2	Dosing after the second aeration

^aFe content was calculated according to ATV-DVWK A 202 E (2004).

Table 4 | Comparison of DIC-SBRs which use sodium aluminate precipitants

Plant	Al content (g Al/kg)	Dosing amount (g Al/(kg TSS · d))	Frequency of <i>M. parvicella</i>	Notice
P	96	0–1.9	2–3	Dosing after the second aeration
S	98	0.15–0.47	4	Dosing after the second aeration

The microscopic investigations show that flocs are still compact when using iron salts; in contrast, the flocs in SBR with sodium aluminate are more disrupted. Figure 4 shows that the filament category of the activated sludge in the plant O is 2. The iron salt does not affect the floc morphology and it has a good influence against the other filamentous bacteria. That is not the case in plant S, which uses sodium aluminate.

According to the results in Table 2, it can be concluded that the operation of DIC-SBR is better than the other SBRs. The outflow quality of plant F may suffer because of the continuous filling. The mode of operation of the plant U encourages the growth of *M. parvicella* and nocardioforms and the dosage of FeCl_3 is not effective in this plant.

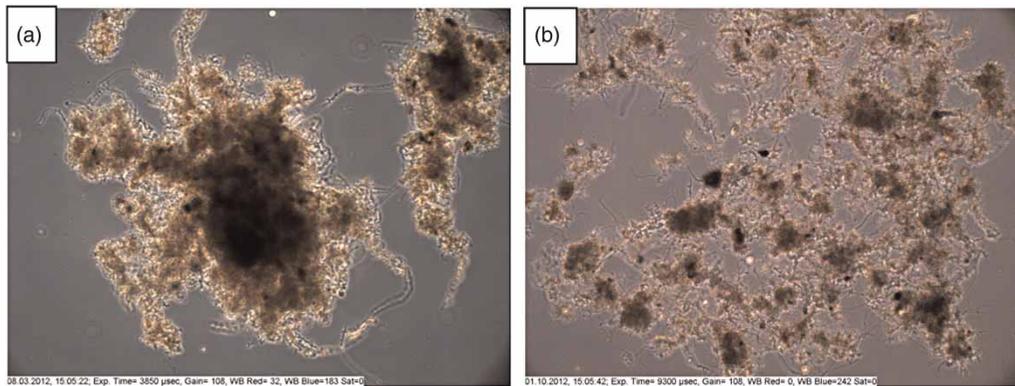
In addition to that, low sludge load of $<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ could have negative effect on the growth of *M. parvicella*. To prove this theory, one SBR with high load and high frequency of *M. parvicella* was chosen and the operation of this plant was converted (plant S).

Characteristics of SBR S

Plant S operates as a DIC-SBR. The elimination rates of BOD_5 , $\text{NH}_4\text{-N}$ and P_{tot} in plant S are usually $>80\%$ (see Table 5). The change in SVI in 2010 due to the temperature is shown in Figure 5. It shows that SVI increased in winter, while it decreased in summer. This development is a sign that *M. parvicella* is dominant in activated sludge (Knoop 1997).

Figure 6 shows the summation curve of sludge load in SBR S over 8 years (2005–2012). Although, SBR S is operated with simultaneous aerobic sludge stabilization, the sludge load in this plant is more than $0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ ($>75\%$). The 85% percentile of sludge load is $0.1221 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$.

The aforementioned results were calculated according to the operation diaries of the plant S. The microscopic investigations of the sludge in SBR S show that the filament category is 3 and the frequency of *M. parvicella* is 4.

**Figure 4** | Activated sludge, original magnification $\times 100$: (a) SBR O; (b) SBR S.**Table 5** | Elimination rates of BOD_5 , $\text{NH}_4\text{-N}$ and P_{tot} in plant S from 2005 until 2012

Years	Elimination rate %								
	BOD_5			$\text{NH}_4\text{-N}$			P_{tot}		
	Min	Max	Average	Min	Max	Average	Min	Max	Average
2005–2012	81.2	99.2	95.1	86.7	99.8	97.7	42.4 ^a	97.2	80.8

^aThe low elimination rate of phosphorus was caused by the maintenance procedure.

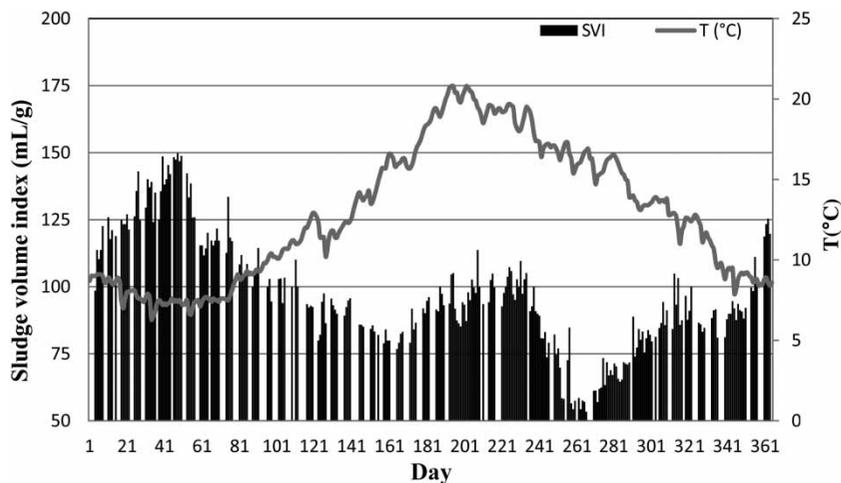


Figure 5 | SVI development according to temperature in SBR S in 2010.

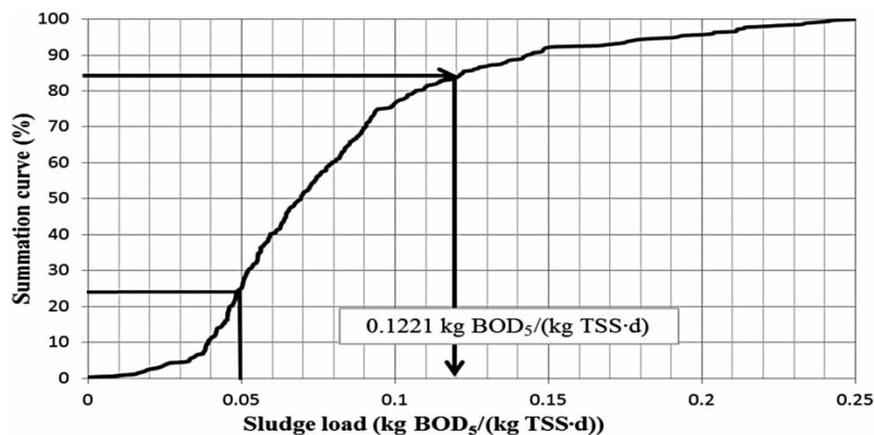


Figure 6 | Summation curve of sludge load in plant S, 2005–2012.

Reducing the sludge load in SBR S

To study the influence of low sludge load on the growth of *M. parvicella* in plant S, TSS was increased from 2.5 g/L to a value more than 4 g/L. This caused the reduction of the sludge load to a value less than 0.05 kg BOD₅/(kg TSS · d). This investigation was carried out in the period 01.10.2012–06.12.2012. Within the conversion period, two samples were taken once a week (see ‘Materials and methods’). TSS, SVI and sludge load were self-determined.

Influence of sludge load <0.05 kg BOD₅/(kg TSS · d) on SVI

During the conversion process (reducing the sludge load), it was noticed that SVI increased (see Table 6 and Figure 7).

Actually, the temperature was decreasing in this period too (from 14 to 10 °C) (see Table 6). This could provide a more suitable environment for the growth of *M. parvicella*.

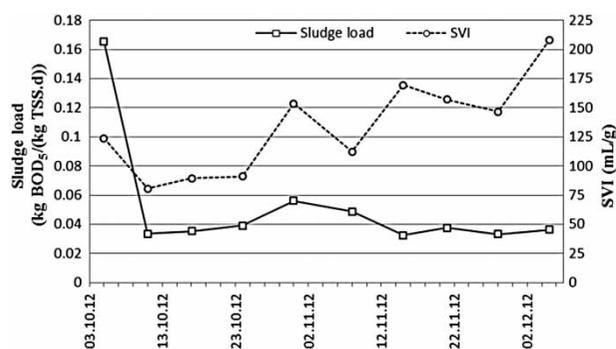
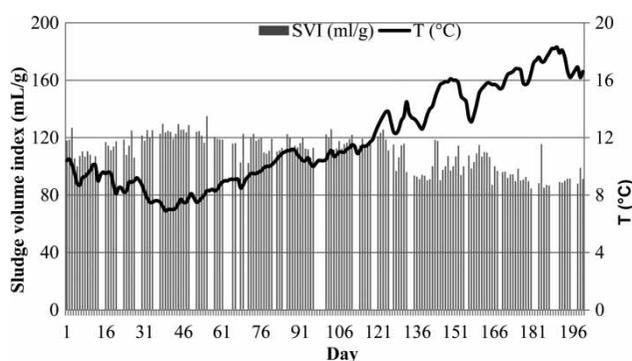
But at the beginning of year 2012 the temperature was very low (<10 °C) and in this period SVI was better than the values during the conversion (see Figure 8 and Table 6). This indicates that both the temperature and low sludge load affected the sludge negatively and led to high SVI.

The elimination rates of BOD₅, NH₄-N and total phosphorus (P_{total}) were not affected by the conversion (see Figure 9). The main problem during this conversion was the appearance of floating sludge.

A few weeks after the raising of biomass concentration started, it was noticed that floating sludge appeared (see Figure 10). The risk of sludge overflow in the case of

Table 6 | SVI while reducing the sludge load in plant S

Date	Temperature (°C)	t_z (h)	SVI (mL/g)
05-10-2012	14.1	4	124
11-10-2012	14.1	8	81
17-10-2012	14.2	8	90
24-10-2012	15.7	6	91
31-10-2012	13.3	8	154
08-11-2012	12.5	8	112
15-11-2012	12.8	8	169
21-11-2012	12.2	8	157
28-11-2012	12.1	8	147
05-12-2012	9.9	8	208

**Figure 7** | SVI development by reducing the sludge load.**Figure 8** | Development of SVI and temperature in plant S in the first 6 months of the year 2012 according to the operation diary of plant S.

strong rain was high, which could lead to deterioration of the outflow quality. For this reason and because of the risk that a further reduction of the sludge load might cause a further increase of SVI, the conversion process was stopped after 10 weeks.

Influence of sludge load $<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ on *M. parvicella* and on filament category

The aim of the operational conversion was to determine the influence of the very low load sludge ($<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$) on the growth of *M. parvicella*. Figures 11 and 12 show that the frequency of *M. parvicella* increased by decreasing the sludge load to a value $<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$, and the filament category as well increased from 3 to 4 which led to higher SVI. The effluent quality of plant S was deteriorated and also floating sludge problems appeared.

Reducing the sludge load in SBR S showed that *M. parvicella* can grow in low loads of $<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$. Sodium aluminate could not affect this bacterium. Table 7 shows a comparison between SBR S (22,500 PE) and the other DIC-SBR plants which have sludge load $<0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ and phosphorus is eliminated biologically or by using sodium aluminate. Small plants (C, D and P) (1,000–10,000 PE) have fewer problems than the bigger ones (R and S). This may be due to the existence of higher concentration of long-chain fatty acids in bigger plants because of the longer flow times to the more centrally located WWTP. In this case it is recommended to increase the sludge load to over $0.05 \text{ kg BOD}_5/(\text{kg TSS} \cdot \text{d})$ or to use suitable precipitants such as iron salts or acidic aluminate precipitants (PAC or AlCl_3). However, these aluminum precipitants must be used continuously (Paris 2004), which results in very high costs. For this reason, using iron salts is recommended in these plants during the regular operation. This causes improvement of SVI and reducing the other filamentous bacteria. In the case of using sodium aluminate and the appearance of filamentous bulking the dosing amount should be raised.

Subdominant filamentous bacteria in SBR

The microscopic investigations show that in addition to the growth of *M. parvicella* in SBR, many other filamentous bacteria, which can cause scum and bulking problems, are present and appear subdominant. The most common ones are type 0041/0675, type 0581, type 0914, *Haliscomenobacter hydrossis*, and in some plants also the sulfate bacteria (type 021N and *Thiothrix* sp.). The filling with low chemical oxygen demand content encourage the growth of filamentous bacteria (Chudoba *et al.* 1973). Figure 13 shows some of these bacteria in the investigated SBRs. The favorable conditions which encourage the growth of these filaments are different,

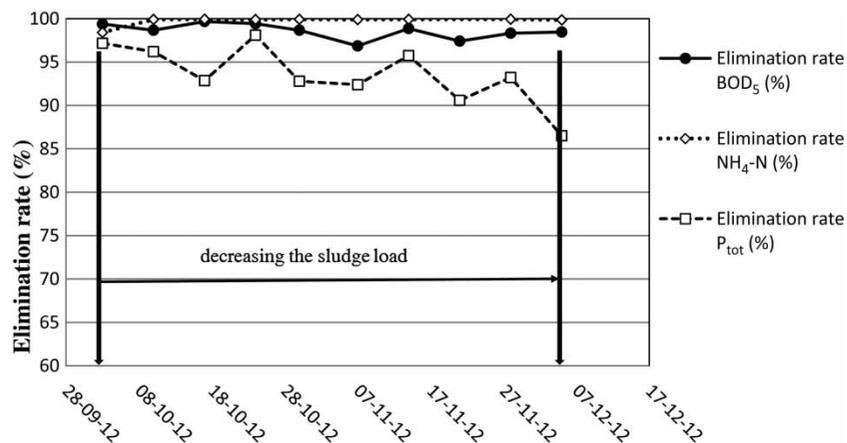


Figure 9 | Elimination rates of BOD₅, NH₄-N and P_{tot} in SBR S during the conversion.



Figure 10 | Floating sludge in plant S during the conversion (left) and in the laboratory after 25 min of sedimentation (right).

but they still have the following connected factors (Kunst *et al.* 2000; Lemmer & Lind 2000):

- low oxygen concentration
- low sludge load <0.2 kg BOD₅/(kg TSS · d)
- unsettled C:N:P ratio.

DIC-SBR provided oxygen until the concentration in the water is 2 mg/L O₂. Then the aeration stops until the concentration becomes <1 mg/L O₂. This offers a suitable environment for many of these bacteria to grow in these plants. Kunst *et al.* (2000), Lemmer & Lind (2000), Jenkins *et al.* (2004) and Remde (2010) mentioned that the optimization of the aeration in the plants can prevent the growth of these bacteria.

Influence of the long cycle time

In the majority of the investigated DIC-SBRs the cycle time is 8 h. In SBR O it is 12 h. Table 8 shows a comparison between the occurrence of *M. parvicella* in plant O and the other plants which use precipitants with iron content. Plant O shows low frequency of *M. parvicella* and a lower filament category.

Gabb *et al.* (1996) showed that a long aeration phase reduces the frequency of *M. parvicella*. The total sludge

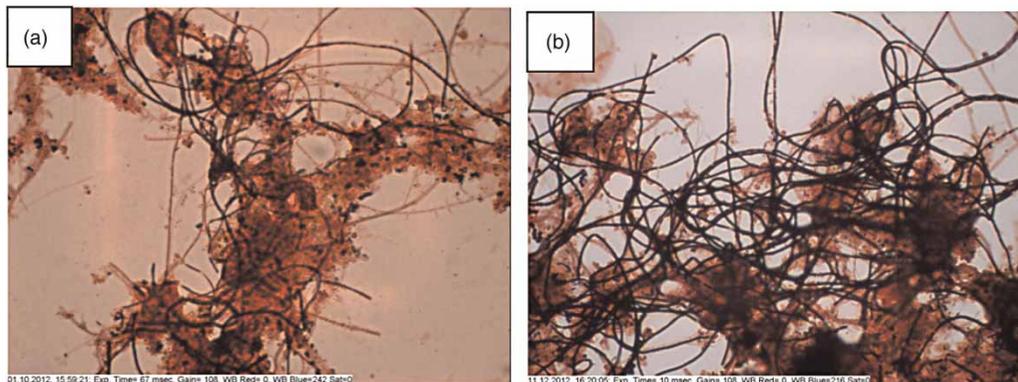


Figure 11 | Frequency of *M. parvicella*: (a) at the beginning of the conversion (frequency = 3); (b) at the end of the conversion (frequency = 5).

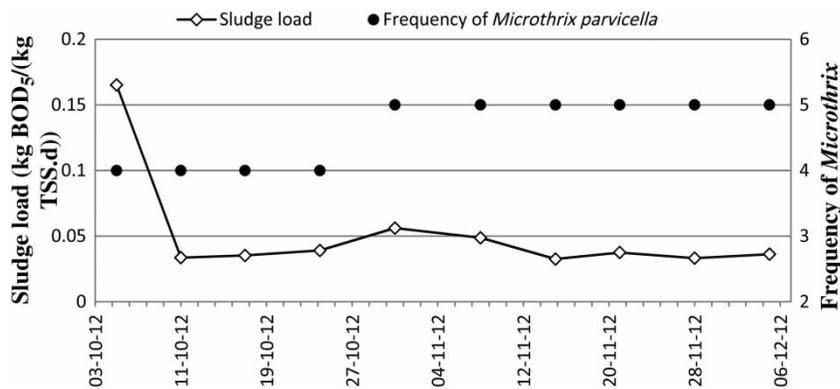


Figure 12 | Development of the frequency of *M. parvicella* during the conversion in plant S.

Table 7 | Comparison between DIC-SBR plants with low loads and phosphorus elimination is biological or by using sodium aluminate

Plant	Sludge load (kg BOD ₅ /(kg TSS · d))	Precipitant	Frequency of <i>M. parvicella</i>	PE
C	<0.05	–	2–3	3,000
D	<0.05	–	2	4,500
P	<0.05	NaAl(OH) ₄	2–3	5,000–10,000
R	<0.05	NaAl(OH) ₄	3–4	16,000
S	<0.05 ^a	NaAl(OH) ₄	4–5	22,500

^aDuring the conversion.

age in the investigated SBRs is 25 d. In DIC-SBR with 8 h cycle time the reactor should be aerated in the first filling for about 2.5 h and in the second filling for 1 h. This means it is aerated for about 3.5 hours per cycle according to the ammonium concentration. In this case the aerated sludge age is about 11 d (44% of the total age). In DIC-SBR with 12 h cycle time the aeration time is more (aerated sludge age is about 50% of the total age).

In the majority of the investigated SBRs in Germany the reactor is aerated until the dissolved oxygen

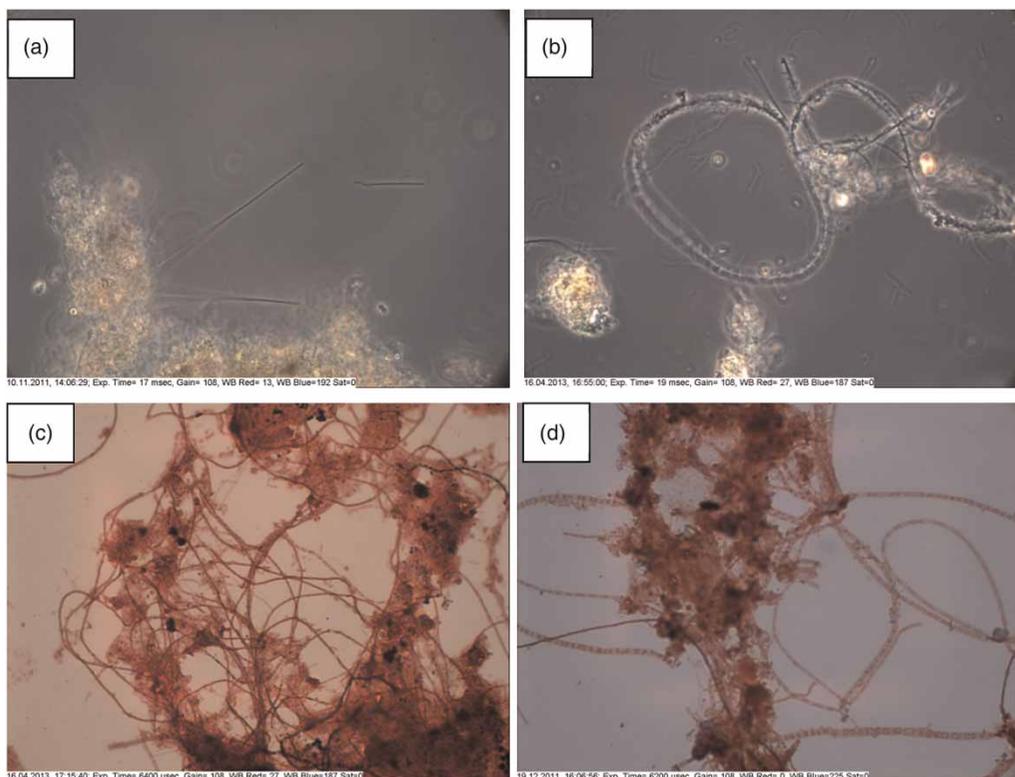


Figure 13 | Filamentous bacteria in different SBRs: (a) *Haliscomenobacter hydroxysis*; (b) type 0041/0675 (original magnification, $\times 100$); (c) type 0581; (d) type 0914 (Gram staining, $\times 1000$).

Table 8 | Comparison between the plants according to cycle time

Plant	Cycle time (h)	SVI (mL/g)	Precipitant	Filament category	Frequency of <i>M. parvicella</i>
A	8	96	FeCl ₃	2–3	2–3
B	8	74	FeCl ₃	2–3	3
H	8	72	FeClSO ₄	2–3	1–2
O	12	86	FeCl ₂	2	2

concentration (DO) is 2 mg/L O₂ and then it is stopped. In order to optimize the operation of these plants longer cycle times and longer aeration phases can be used (such as in plant O). It is recommended that the reactor can be aerated until DO reaches 1 mg/L O₂ and for a longer time (aerated sludge age >44%) until the nitrification is completed. This means more energy costs for the aerator, but at the same time the frequency of bulking filaments can be reduced (D. Schreff, Office for Water, Wastewater and Energy, Germany, personal communication).

CONCLUSION

From the aforementioned discussion, it can be concluded that *M. parvicella* can grow in plants with nitrogen and phosphorus removal in all zones (aerobic, anaerobic and anoxic) and low sludge loads. Many studies showed that the optimal sludge load for *M. parvicella* is 0.05–0.1 kg BOD₅/(kg TSS · d). To study the influence of very low loads of <0.05 kg BOD₅/(kg TSS · d) on *M. parvicella* 13 SBRs with simultaneous aerobic sludge stabilization in Germany were investigated. *M. parvicella* was found with different frequencies in most of the plants in addition to other filaments. These filamentous bacteria can cause scum and bulking sludge under some conditions.

The conversion of plant S showed that *M. parvicella* can grow in low loads of <0.05 kg BOD₅/(kg TSS · d). Its frequency is higher in plants with 10,000–25,000 PE because of the existence of higher concentration of long-chain fatty acids. Due to the high costs of PAC or AlCl₃ the use of iron salts in these plants is recommended. The dosing of the precipitant should be carried out during the aeration, the dosing amount can vary between 0.28 and 1 g Fe/(kg TSS · d). This can lead to better SVI, low filament category and low frequency of *M. parvicella*.

Additionally, even though DIC-SBR shows in comparison to the other types of SBR better nitrogen elimination, biological elimination of phosphorus and good effluent

quality, the filling with different C/N ratios can improve the development of other filamentous bacteria such as type 0041/0675, type 021 N and type 0581. This can be considered as a drawback for DIC-SBR. To prevent the growth of these filaments, longer cycles of 8–12 h and longer aeration times are recommended.

REFERENCES

- ATV-AG 2.6.1 1998 Blähschlamm, Schwimmschlamm und Schaum in Belebungsanlagen-Ursachen und Bekämpfung. (Bulking, floating sludge and scum in aeration system – reasons and reduction). Korrespondenz Abwasser, issue 10. Work report of the ATV working group 'Blähschlamm, Schwimmschlamm, biologische Zusatzstoffe' in the ATV technical committee 2.6: aerobic and biological wastewater treatment, Hennef, Germany.
- ATV-DVWK-A 202 E 2004 *Chemical-Physical Methods for the Removal of Phosphorus from Wastewater*. German Association for Water, Wastewater and Waste, Hennef, Germany.
- Bidder, H. G. 1999 *Blähschlamm bekämpfung mit Eisensalzen-Gestern und Heute (Control of bulking sludge with iron salts, yesterday and today)*. Technological Aspects for Water, Wastewater and Sludge Treatment (98), Institute for Water Quality and Waste Management, University of Karlsruhe, Germany.
- Casey, T. G., Ekama, G. A., Wentzel, M. C. & Marais, G. vR. 1995 Filamentous organism bulking in nutrient removal activated sludge systems. Paper 1: a historical overview of causes and control. *Water SA* 21 (3), 321–338.
- Chudoba, J., Grau, P. & Ottova, V. 1973 *Control of activated sludge filamentous bulking I*. *Water Research* 7 (8), 1163–1182.
- DWA-M 210 2009 *Belebungsanlagen mit Aufstaubetrieb (SBR) (Activated Sludge Plants with Impoundage Operation (SBR))*. German Association for Water, Wastewater and Waste, Hennef, Germany.
- Eikelboom, D. H. 1975 *Filamentous organisms observed in activated sludge*. *Water Research* 9, 365–388.
- Eikelboom, D. H., Andreadakis, A. & Andreasen, K. 1998 *Survey of filamentous populations in nutrient removal plants for European countries*. *Water Science and Technology* 37 (4–5), 281–289.
- Ekama, G. A., Wentzel, M. C., Casey, T. G. & Marais, G. vR. 1996 Filamentous organism bulking in nutrient removal activated sludge systems. Paper 6: review, evaluation and consolidation of results. *Water SA* 22 (2), 147–152.
- Gabb, D. M. D., Ekama, G. A., Jenkins, D., Wentzel, M. C., Casey, T. G. & Marais, G. vR. 1996 Filamentous organism bulking in nutrient removal activated sludge systems. Paper 5: experimental examination of aerobic selectors in anoxic-aerobic systems. *Water SA* 22 (2), 139–146.
- Helmreich, B., Schreff, D. & Wilderer, P. A. 2000 Full scale experiences with small sequencing batch reactor plants in Bavaria. *Water Science and Technology* 41 (1), 89–96.

- Jenkins, D., Richard, M. G. & Daigger, G. T. 1993 *Manual on the Causes and Control of Activated Sludge Bulking and Foaming*. Ridgelines Press, Lafayette, CA, USA.
- Jenkins, D., Richard, M. G. & Daigger, G. T. 2004 *Manual on the Causes and Control of Activated Sludge Bulking and Foaming and other Solids Separation Problems*. 3rd edn, Lewis Publishers, Washington, DC.
- Knoop, S. 1997 *Untersuchungen zum Vorkommen von Microthrix parvicella in Kläranlagen mit Nährstoffelimination. (Researches about the Development of Microthrix parvicella in Wastewater Treatment System with Nutrient Removal)*. Issue 101, Institute for Sanitary Engineering and Waste Management, University of Hannover, Hannover, Germany.
- Knoop, S. & Kunst, S. 1998 [Influence of temperature and sludge loading on activated sludge settling, especially on *Microthrix parvicella*](#). *Water Science and Technology* **37** (4–5), 27–35.
- Kunst, S. 2002 Vorkommen von *Microthrix parvicella* in kommunalen Kläranlagen (Development of *Microthrix parvicella* in municipal wastewater treatment plants). *Microthrix parvicella Symposium. Operational Experiences and New Researches to Control Bulking Sludge Caused by Microthrix parvicella*. University of Hannover, Hannover, Germany.
- Kunst, S., Helmer, C. & Knoop, S. 2000 *Betriebsprobleme auf Kläranlagen durch Blähschlamm, Schwimmschlamm, Schaum (Operational Problems in Wastewater Treatment Systems Caused by Scum, Bulking and Foaming Sludge)*. Springer, Berlin.
- Lebek, M. 2003 *Bekämpfungsmaßnahmen von Blähschlamm verursacht durch Microthrix parvicella (Measures to Control Bulking Sludge Caused by Microthrix parvicella)*. Book 125. Institute for Sanitary Engineering and Waste Management, University of Hannover, Hannover, Germany.
- Lebek, M. & Rosenwinkel, K.-H. 2002 Ergebnisse von halb- und großtechnischen Versuchen zur *Microthrix parvicella* Bekämpfung auf der Kläranlage Köln-Langel (Results of half and large scale experiments to reduce *Microthrix parvicella* in WWTP Köln-Langel). *Microthrix parvicella Symposium. Operational Experiences and New Researches to Control the Bulking Sludge Caused by Microthrix parvicella*. University of Hannover, Hannover, Germany.
- Lemmer, H. & Lind, G. 2000 *Blähschlamm, Schaum, Schwimmschlamm, Mikrobiologie und Gegenmaßnahme. (Scum, Bulking and Floating Sludge, Microbiology and Countermeasure)*. F. Hirthammer, Munich, Germany.
- Misera, R. 2002 *Problemlösung in der Praxis durch die Dosierung von HEIFLOC NB 90 oder Aluminiumchlorid (Problem solution in the practice by dosage of HEIFLOC NB 90 or aluminate-chloride)*. Scum and Bulking Sludge (108), Institute for Water Quality and Waste Management, University of Karlsruhe, Germany.
- Nielsen, P. H., Roselv, P., Dueholm, T. E. & Nielsen, J. L. 2002 *Microthrix parvicella*, a specialized lipid consumer in anaerobic-aerobic activated sludge plants. *Water Science and Technology* **46** (1–2), 73–80.
- Paris, S. 2004 *Bekämpfung von Schwimmschlamm verursacht durch Microthrix parvicella (Measures against floating sludge caused by Microthrix parvicella)*. PhD thesis, University of Munich, Institute for water quality and waste management, Munich, Germany.
- Remde, A. 2010 DWA microscope course in Nordrhein-Westphalia Bottrop, Germany, 10–12 November 2010.
- Rönnner-Holm, S. G. E. & Holm, N. C. 2009 [Special automation and regulation strategies for enhancing sequencing batch reactor \(SBR\) performances](#). *Water Science and Technology* **60** (5), 1161–1172.
- Rossetti, S., Tomi, M. C., Nielsen, P. H. & Tandoi, V. 2004 '*M. parvicella*', a filamentous bacterium causing bulking and foaming in activated sludge systems, a review of current knowledge. *FEMS Microbiology Reviews* **29** (1), 49–64.
- Schmid-Schneider, V. 2006 Die Ursachen für Blähschlamm (Causes of bulking sludge). *Wasserwirtschaft, Wassertechnik* **11–12**, 17–21.
- Schreff, D. & Hilliges, R. 2013 *SBR-Verfahren-Hinweise zur Bemessung und zum Betrieb (SBR-notes on design and operation)*. 10. Course 'Operation of SBR'. German Association for Water, Wastewater and Waste, Bayern, Germany.
- SH + E GROUP 2012 Differential internal cycle strategy (DIC-SBR). <http://www.she-group.com>.
- Slijkhuis, H. 1983 *Microthrix parvicella*, a filamentous bacterium isolated from activated sludge; cultivation in a chemically defined medium. *Applied and Environmental Microbiology* **46** (4), 832–839.
- Wanner, J., Ruzicková, I., Jetmarová, P., Krhutková, O. & Paraniaková, J. 1998 [A national survey of activated sludge separation problems in the Czech Republic: filaments, floc characteristics and activated sludge metabolic properties](#). *Water Science and Technology* **37** (4–5), 271–279.

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