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Nocardia foaming control in activated sludge process treating domestic wastewater

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Abstract

In this study, filamentous bacteria (*Nocardia amarae*) were identified as the major causal microorganism in foaming sludge. The results of growth kinetics study indicated that *N. amarae* had a relatively strong affinity for non-readily biodegradable fatty acids. *N. amarae* was able to consume various fatty acids at a constant growth yield from 0.413 to 0.487 g/gCOD. Under common *F/M* ratio (less than 0.5 gBOD/gMLSS/d) used in activated sludge processes, specific growth rate of *N. amarae* was found to be more significant than that of non-filamentous bacteria. Based on this feature, a novel technique feast–fast operation (FFO) was developed for the foaming control. The sludge volume index (SVI) rapidly decreased from 300 to 80 mL/g and further stabilized at about 70 mL/g and the system was free from stable foam, while the BOD removal efficiency was maintained above 95%. This control technology effectively suppressed the overgrowth of filaments and improved the settleability of activated sludge without adverse effects on the treatment performance and the process stability.

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Keywords: Foaming control; Wastewater treatment; Filamentous bacteria; Long-chain fatty acids

1. Introduction

In recent decades, activated sludge process is accredited to be the most widespread technology for treating various types of wastewater such as domestic sewage and industrial effluent (Martins et al., 2004). However, since its development in 1914, operation problems and deterioration of treatment efficiency due to bulking sludge have been cited as common problems in activated sludge processes around the world (Graveleau et al., 2005; Krhutkova et al., 2002).

Activated sludge process is a biological process which depends on the profound balance ecosystem among the floc-formers, such as *Pseudomonas* spp., *Zoogloea* spp., *Alcaligenes* spp., and *Achrombacter* spp., and the filaments, such as *Nocardia* spp., *Rhodococcus* spp., Type 1863 and *Microthrix* spp. (Martins et al., 2004; Rossetti et al., 2005; Wagner et al., 2002). They are the key organic

degraders and offer the skeletal matrix for the formation of compact flocs. However, the overgrowth of filamentous microorganisms is generally identified as the main origin of foaming, which deteriorates sludge settleability, and results in decreased sludge settling rate and incompact sludge blanket (Jenkins et al., 2004; Jolis et al., 2006; Tsang et al., 2007).

Physical factors such as process design parameters (e.g. aeration basin configuration and feeding regime) and operating conditions (e.g. high mixed liquor suspended solids (MLSS), low sludge return rate and low dissolved oxygen) are contributed to the growth of filamentous bacteria (Martins et al., 2003, 2004; Metcalf and Eddy, 2003). Chemical factors such as nutrient balance and substrate composition also play an important role in governing the filamentous overgrowth (Liu and Liu, 2006; Tsai et al., 2003). The nonionic and self-producing surfactants also significantly boosted the foam generation and stability (Iwahori et al., 2001; Pagilla et al., 2002; Theander and Pugh, 2003).

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Nomenclature

Biochemical oxygen demand (BOD) (mg/L)
 Chemical oxygen demand (COD) (mg/L)
 Dry cell mass (g/L)
 F/M ratio (gBOD/gMLSS/d)
 Growth yield ($Y_{x/s}$) (g/gCOD)

Mixed liquor suspended solid (MLSS) (mg/L)
 Saturation constant (K_s) (gCOD/L)
 Sludge volume index (SVI) (mL/g)
 Specific growth rate (μ_m) (1/h)

Bulking and foaming problems have been alleviated by addition of toxic chemicals such as chlorine or hydrogen peroxide to the aeration tank or the return sludge line (Chang et al., 2004; Ramothokang et al., 2003). Metal ions such as calcium, magnesium, iron (Philips et al., 2003; Thompson and Forster, 2003; Agridiotis et al., 2007), synthetic polymer (Juang, 2005), and multi-component additive (Seka et al., 2001) are observed to control bulking effectively. However, these chemical treatment methods are costly and, most importantly, they only offer short-term solution as bulking and foaming will resume when chemical additions are stopped. Kinetic selection is an alternative to control filamentous overgrowth specifically under low F/M condition, it was first published by Chudoba et al. (1973). The theory stated that substrate concentration gradient favors the growth of floc-formers, instead of filamentous bacteria, among the activated sludge microbes.

Although many studies have been carried out, the root causes of filamentous overgrowth are still not fully understood and contradicting views remain (Martins et al., 2004), and not much detailed comparison of the growth characteristics is done between floc-formers and filaments. The effects of the amount and the types of substrates on growth kinetics of floc-formers and filaments are also not clearly identified. Therefore, a comprehensive understanding of filamentous growth is essential for more effective control. In addition, universal filamentous control strategy is still unavailable; a cost-effective method should be developed to solve this problem, which commonly occurs in the activated sludge process treating domestic wastewater with high concentration of fatty acids, without affecting the treatment performance.

In this study, the predominant microorganisms in the foaming sludge collected from Shatin and Tai Po Sewage Treatment Works in Hong Kong were identified. The effects of common fatty acids on the filamentous bacteria and the foaming mechanism were investigated. Studies and comparisons of growth kinetics of filamentous and non-filamentous bacteria in activated sludge were also carried out. By the use of growth kinetic of *N. amarae* as a theoretical basis, a new activated sludge operation strategy was interpreted for foaming control.

2. Methods

This study was divided into three parts. In Part A, the cell culture was prepared from the activated sludge pro-

cesses in Shatin and Tai Po Sewage Treatment Works for the determination of the predominated filamentous micro-organism. The growth of filaments under various chain lengths and concentrations of the carbon source was also investigated. In Part B, the pure cultures of *N. amarae* and *Pseudomonas aeruginosa* were sourced and examined in order to monitor their growth rates under different fatty acids as sole carbon source. In Part C, according to the results obtained from Parts A and B, a novel activated sludge system was designed and studied by altering the process operation parameters to investigate the effectiveness for foaming control.

2.1. Part A

2.1.1. Cell cultures

The microorganisms in sludge samples collected from local wastewater treatment plants were isolated by plated culture with Czapek's agar supplemented with 0.4% yeast extract, and incubated at 28 °C for 3 weeks. The bacteria were identified according to the criteria adopted by Blackbeard et al. (1988) and Jenkins et al. (2004). The isolated bacteria were cultured in 500 mL shake flasks with 100 mL sterilized LB on an orbital shaker at 28 °C and 200 rpm for 5 days. The pure cultures were then separately inoculated in shake-flasks with (i) minimum salt medium (MSM) added with various general sewage fatty acids as sole carbon source, and (ii) autoclaved raw sewage added with different fatty acids.

2.1.2. Foaming stability test

Fatty acid solutions (0.5 g/L) were solubilized by 1 N NaOH, 20 mL of acid solution was added into 980 mL of activated sludge with MLSS of 3400 mg/L. Five-hundred millilitre of the mixture was placed in a 1 L graduated cylinder and aerated with an air flow rate of 2 L/min through a sintered-sand diffuser for 60 s. The foam height was recorded at 15 s intervals during aeration and stationary periods (Tsang et al., 2006). This procedure was repeated for the concentrations of fatty acids of 1.0 g/L and 1.5 g/L.

N. amarae in shake-flask cultures were yielded by centrifugation, then washed twice and re-suspended in distilled water to give a 20 g/L suspension. 5, 10, and 15 mL of the suspension were added to 500 mL of activated sludge with MLSS of 3400 mg/L, respectively. The same test procedures as described above were carried out.

2.2. Part B

2.2.1. Cell cultures

N. amarae (ATCC 27810) and *P. aeruginosa* (CRCC 10261) were adopted to represent filamentous and non-filamentous bacteria in activated sludge, respectively. The cultures were maintained on yeast-extract-glucose agar slants at 2–4 °C. The inocula were derived from agar slants and maintained at 30 °C for 72 and 24 h, respectively. The prepared MSM was adjusted to pH 7 with NaOH and autoclaved. Fatty acids were prepared separately with different length of carbon chain and adjusted to pH 7.0 with NaOH for solubilization, and then filtered through a 0.45 µm membrane filter and sterilized. Ninety millilitre of MSM, 10 mL of fatty acid solution and 5 mL of inoculum were mixed in a baffled conical flask, and cultured at 30 °C and 200 rpm for a certain period to achieve stationary phase.

2.3. Part C

2.3.1. Seed and influent

Activated sludge with excessive filamentous growth used as inoculum was collected from the return sludge of Shatin Sewage Treatment Works. The collected sludge was then screened with a 2 mm-sieve to remove coarse particles before transferring it to the simulator system for start-up.

The synthetic wastewater was prepared as the influent with 0.8 g/L of glucose, 76 mg/L of NH₄Cl, and 22 mg/L of KH₂PO₄, and maintained at a BOD: N: P ratio of 100: 5:1. The mean BOD and COD concentrations were 405 ± 13 mg/L and 673 ± 24 mg/L (*n* = 160), respectively.

2.3.2. Activated sludge simulator

A two-phase activated sludge simulator consisted of two 3 L-aeration columns and a sedimentation tank

(Fig. 1). Both the columns received the synthetic wastewater influent and the returned sludge from settling tank at the predetermined flow rates. This system was operated under conventional conditions for 86 days to achieve a stable performance before the commencement of FFO programme. Each aeration column was operated at different HRT, MLSS and return sludge rates, resulting in relatively high and low F/M ratios. The simulator system was operated in an air-conditioned laboratory at the temperature of 25 ± 1 °C. The operating conditions of conventional operation and FFO mode are summarized in Table 1.

2.4. Analytical methods

All parameters of mixed liquor and effluent quality were determined according to standard methods (APHA, 1998). Bacterial growth was monitored by optical density with Milton Roy Spectrophotometer (Spectronic 601). Substrate utilization was monitored by measuring the residual COD in culture medium (APHA, 1998).

Table 1
Operating conditions under conventional and FFO mode

Parameters	Conventional operation	Feast fast operation	
		Feasting unit	Fasting unit
Effective volume (L)	6	3	3
Hydraulic retention time (h)	10	8.3	12.5
Sludge retention time (d)	15	15	15
pH	6–8	6–8	6–8
Dissolved oxygen (mg/L)	2–4	2–4	2–4
MLSS concentration (mg/L)	2200	1700	5000
Influent flow rate (L/h)	0.6	0.36	0.24
Return sludge rate (L/h)	0.25	0.06	0.21
F/M ratio (gBOD/gMLSS/d)	0.4	0.75	0.16
Sludge wastage rate (L/d)	0.4	0.2	0.2

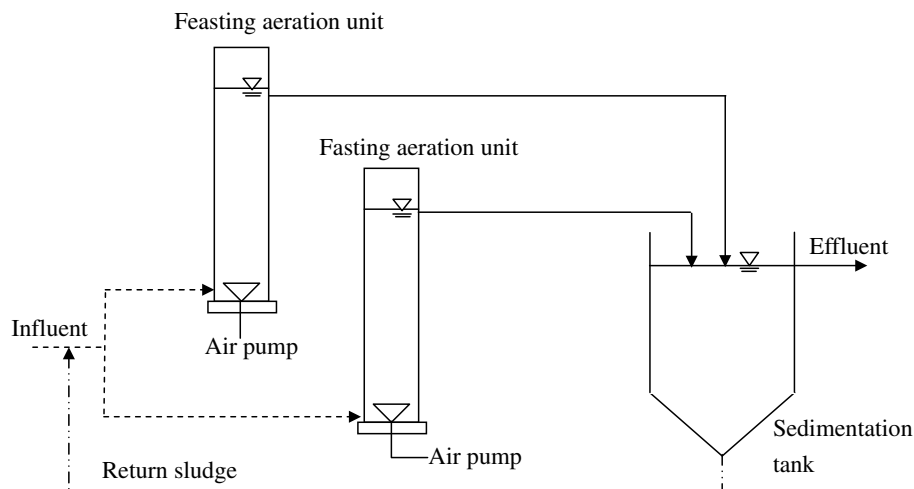


Fig. 1. Schematic diagram of the activated sludge simulator.

3. Results and discussion

3.1. Part A

3.1.1. Identification of foam-causing organisms

The appearances of different activated sludge foam can be diagnosed by the use of microscopic examinations (Richard et al., 2003). The microscopic examination of the sludge sample of bulk floc from Shatin and Tai Po Sewage Treatment Works indicated an overgrowth of filamentous microorganisms with right-angled branching, and the filaments were $0.5\text{--}1.0 \times 80\text{--}160$ microns. The colonies on plate cultures illustrated aerial hyphae that gave a superficially matte and chalky appearance. The generation time of the isolated bacteria in shake-flask cultures at 28°C was 10.5 h. The isolated bacteria were found to be gram-positive and Neisser-negative, and could not survive after an 8 h-incubation at 50°C in heat resistance test. These morphological and physiological characteristics identified the bacteria as *N. amarae* (Blackall et al., 1991; Jenkins et al., 2004; Strom and Jenkins, 1984). This filament also proves its abundance in activated sludge treatment system (Pagilla et al., 2002).

3.1.2. Effects of fatty acids on the growth of *N. amarae*

The effects of fatty acids on the growth of *N. amarae* were investigated using autoclaved raw sewage with different fatty acids. The biomass concentrations obtained from the media using sewage with fatty acids C_8 , C_9 and C_{14} were 4, 3 and 1.5 times higher than that obtained from sole sewage medium, respectively. The results showed that stimulated growth of *N. amarae* was more promoted for fatty acids with shorter length of carbon chain. This also suggests that high fatty acids concentration (e.g. 1 g/L) in sewage might cause an overgrowth of *N. amarae*, and hence foaming in activated sludge systems.

3.1.3. Effects of fatty acids and *Nocardia* overgrowth on foaming and foam stability

Fatty acids in the influent of activated sludge process can promote foaming. The foam could remain stable for a period of time after aeration was cut off at higher concentration of fatty acids (e.g. 1 and 1.5 g/L) (Table 2). The degree of foaming increased with the increase of fatty acid concentrations. This could be attributed to the surface-active properties of fatty acids, which induce biosurfactants and increase the stability and the resistance against breaking-up of liquid films of air bubbles.

Table 3 shows the effects of quantity the of filaments on the foam stability. The degree of foaming and its stability increased with the increase in the amount of filamentous bacteria in activated sludge. The foam height was also stable when aeration was cut off.

Table 2

Foam height under different concentrations of fatty acids

Time (s)	Foam Height (mL)			
	Fatty Acid Concentration (g/L)			
	0	0.5	1	1.5
<i>Aeration</i>				
0	0	0	0	0
15	15	20	30	35
30	20	30	35	50
45	20	30	40	60
60	20	30	40	60
<i>No Aeration</i>				
75	0	10	30	50
90	0	0	15	30
105	0	0	10	25
120	0	0	5	20

Table 3

Foam height under different quantities of filaments

Time (s)	Foam Height (mL)			
	<i>N. amarae</i> Suspension Volume (mL)			
	0	0.5	1	1.5
<i>Aeration</i>				
0	0	0	0	0
15	10	20	25	30
30	15	30	40	40
45	20	40	55	60
60	20	40	55	60
<i>No Aeration</i>				
75	0	20	40	45
90	0	0	15	20
105	0	0	2	10
120	0	0	5	5

3.2. Part B

3.2.1. Growth of filamentous and non-filamentous bacteria on different carbon sources

For the acetic acid (C_2) culture, the logarithmic growth phase was indicated to last for about 50 h, the biomass concentration gradually reached a maximum of 0.22 g/L. Other fatty acids, including C_{12} , C_{14} , C_{16} and C_{18} , except C_{24} , also encouraged the growth of *N. amarae*, but in a less pronounced level for the long-chain fatty acids. According to the results, only a very little growth of *N. amarae* occurred under the culture of C_{24} with the maximum biomass concentration of about 0.033 g/L after the 110 h-incubation period.

Similar to the results of *N. amarae*, *P. aeruginosa* grew rapidly with the acetic acid culture and reached the logarithmic growth phase that lasted for about 5 h and achieved the maximum biomass concentration of 0.21 g/L at the 7th h. However, *P. aeruginosa* hardly utilized other fatty acids for the bacterial growth and maintained a constant dry cell mass (less than 0.05 g/L) for the 9 h-opera-

tion, particularly for C₂₄ in which only a slight growth was detected.

3.2.2. Kinetic selection studies of filamentous and non-filamentous bacteria

The maximum specific growth rate (μ_m) and the saturation constant (K_s) of the Monod kinetics model were obtained from the batch culture growth data for filaments and floc-formers followed by processing with Lineweaver–Burk equations and linear-regression techniques (Table 4). The results of the growth yields ($Y_{x/s}$) of bacteria are illustrated in Table 4. For the filamentous *N. amarae*, the maximum specific growth rates decreased and the saturation constants increased with the increase of the length of carbon chain. The results also indicated that the specific growth rates of *N. amarae* were smaller than those of *P. aeruginosa* with the magnitude ranging from 3 to 37 times, especially with the availability of readily biodegradable acetic acid. This result was found to be in agreement with the study by Contreras et al. (2002), indicating that filamentous bacteria (*Sphaerotilus natans*) have smaller values of μ_m and K_s than floc-formers (*Acinetobacter anitratus*). It is evidenced to the reliability of this experiment and also the unique growth characteristics for both filaments and floc-formers.

The specific growth rates of filaments and floc-formers were determined under various *F/M* ratios. The cut-off value of *F/M* ratio was 0.56 gBOD/gMLSS/d, below which *N. amarae* grew faster than *P. aeruginosa*. While at *F/M* ratio above the cut-off value, the growth rate was higher for *P. aeruginosa*. These results indicated that filamentous *N. amarae* grew faster, but had a strong affinity towards the substrate and turned out to be the predominant strain at lower concentration of fatty acids. On the contrary, *P. aeruginosa* grew rapidly but required higher concentration of fatty acid for growth and dominated at higher substrate levels. This finding is in agreement with the previous study that the specific growth rate of filamentous bacteria was higher than non-filamentous bacteria under the cut-off *F/M* ratio (Tsang et al., 2006).

The dominance of filamentous bacteria is based on the availability of readily biodegradable substrates or refrac-

tory substrates (Chudoba et al., 1973). However, this phenomenon was not observed in the growth of *N. amarae* with fatty acid as carbon source. For the readily biodegradable substrate (acetic acid), the maximum specific growth rate and the half velocity constant of *N. amarae* were much lower than those of *P. aeruginosa*. The growth yields of *N. amarae* were fairly constant among various fatty acids used in this study and ranged from 0.413 to 0.487 g/gCOD. On the contrary, the growth yield of *P. aeruginosa* was the highest for acetic acid, but it dropped drastically from 0.665 to less than 0.105 g/gCOD for longer chain length of fatty acids.

The results demonstrated that the growth of *P. aeruginosa* is not in proportion to the utilization of existing substrate with the increase in the chain length of fatty acids. *N. amarae* was more favorable for the growth in sewage that contained a higher proportion of long-chain fatty acids. Long-chain fatty acids are commonly produced from the microbial hydrolysis of oils and fats and account for more than 30% by weight of total organic content in municipal sewage. They would encourage the overgrowth of *N. amarae* and lead to its profusion in sewage treatment plant. However, non-filamentous floc-formers including the genera of *Pseudomonas*, and presumably *Zoogloea*, *Achromobacter*, *Alcaligenes*, and *Flavobacterium*, cannot or hardly utilize the long carbon chain fatty acids for growth and proliferation.

3.3. Part C

3.3.1. Development of feast–fast operation programme

The growth behavior indicated that filamentous overgrowth was inevitable in normal activated sludge processes, which are continuously exposed to the usual *F/M* ratio of 0.2–0.6 gBOD/gMLSS/d. The kinetics selection results revealed that higher *F/M* ratio (>0.56 gBOD/gMLSS/d) rendered filamentous bacteria to be at a competitive disadvantage relative to the non-filamentous microbes. Hence, high *F/M* ratio is regarded as a prerequisite for effective selection of the desired floc-former from the sludge population. Moreover, other studies found that the sludge bacteria, after exposing to high *F/M* ratio conditions, required sufficient time for oxidation of adsorbed/accumulated sub-

Table 4
Growth characteristics of *N. amarae* and *P. aeruginosa*

Carbon source	<i>N. amarae</i>			<i>P. aeruginosa</i>		
	Specific growth rate, μ_m (h ⁻¹)	Saturation constant, K_s (gCOD/L)	Growth yield, $Y_{x/s}$ (g/gCOD)	Specific growth rate, μ_m (h ⁻¹)	Saturation constant, K_s (gCOD/L)	Growth yield, $Y_{x/s}$ (g/gCOD)
Acetic acid	0.095	0.320	0.487	3.557	2.004	0.665
Lauric acid	0.073	1.097	0.426	1.003	7.435	0.105
Myristic acid	0.071	1.156	0.428	0.997	8.724	0.097
Palmitic acid	0.070	1.308	0.433	0.724	10.457	0.070
Stearic acid	0.069	1.400	0.413	0.347	11.155	0.041
Lignoceric acid	0.048	1.520	0.453	0.123	12.625	0.009

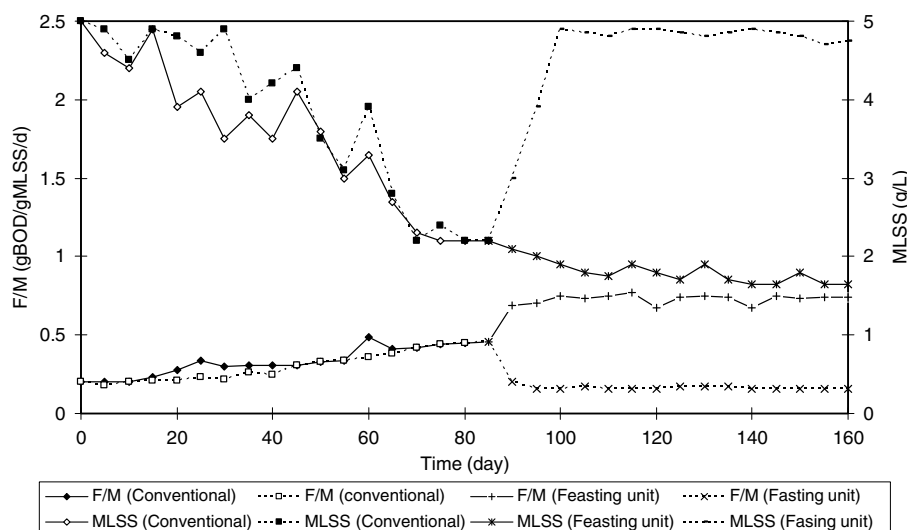


Fig. 2. F/M ratio and MLSS concentration during operation.

strates (Čech and Chudoba, 1983; Chudoba et al., 1982). It was also investigated that activated sludge with good settleability was best achieved when the microorganisms were periodically exposed to high substrate concentration and long-lasting near-starvation conditions (Wilderer and Schroeder, 1986). This information provided the theoretical basis for FFO development in this study.

Based on the observation in the previous sections, operation design for the control of filamentous growth was investigated in this section. In the dual-reactor system, the feasting column was operated at a higher F/M ratio of 0.75 gBOD/gMLSS/d, while the fasting column was run at a lower F/M ratio of 0.16 gBOD/gMLSS/d. The FFO mode allowed an overall process F/M ratio to maintain within the normal range while avoiding prolonged

exposure of the activated sludge to an F/M ratio below the cut-off value of 0.56 gBOD/gMLSS/d.

3.3.2. Process performance

The system, which was operated with the conditions as shown in Table 1, was started with conventional operation for microbial acclimation. The variations of MLSS concentrations and F/M ratios during the conventional operation and the FFO programme are shown in Fig. 2. During the 86 d start-up period, F/M ratio increased from 0.18 gBOD/gMLSS/d to a steady value of 0.45 gBOD/gMLSS/d. At the same time, MLSS concentration decreased from the initial level of 5000 mg/L to a stable level of 2200 mg/L. The two aeration columns showed a similar trend of F/M ratio and MLSS concentration under

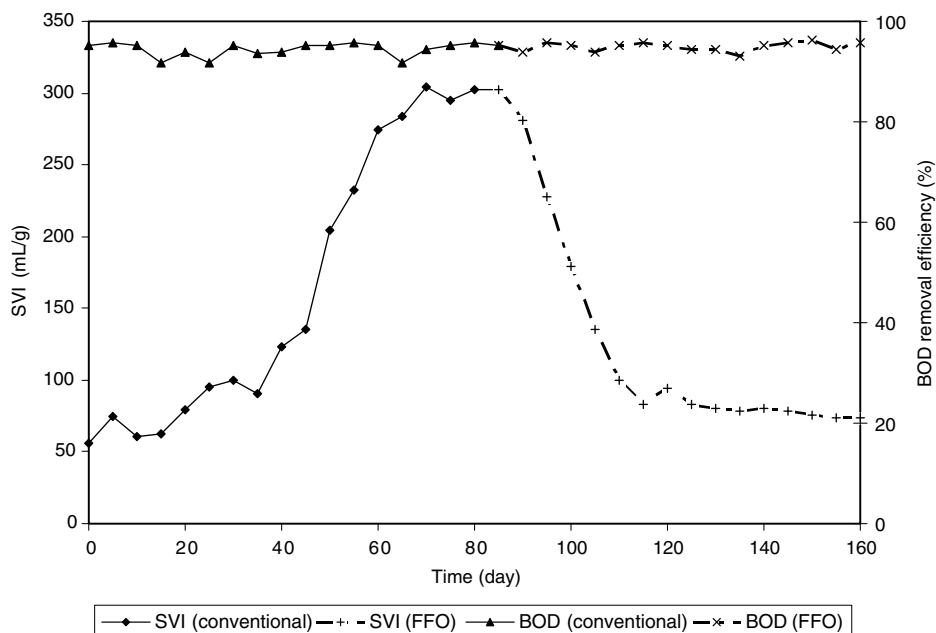


Fig. 3. SVI and BOD removal efficiency during operation.

conventional operation. From day 87, the two columns were operated under two different routes by the FFO mode with various F/M ratios.

Sludge settleability can be presented by the mean of sludge volume index (SVI) (Jenkins et al., 2004; Tsang et al., 2007). Fig. 3 shows the variations of SVI and BOD removal efficiency throughout the whole process. During the conventional operation period, SVI increased from the initial value of about 60–70 mL/g to a level up to a maximum of 300 mL/g after exposing the activated sludge to F/M ratio of 0.4 gBOD/gMLSS/d. The overgrowth of filamentous *N. amarae* occurred during the conventional operation when the SVI increased to more than 230 mL/g.

Within 20 d-operation after the initiation of the FFO programme, SVI dropped back to a healthy level of 80 mL/g rapidly and further stabilized at about 70 mL/g during the next 30 d-operation. This result is consistent with the observation in the previous section that non-filamentous bacteria grew better than filamentous bacteria under a higher F/M ratio. SVI profile also proved the effectiveness of the FFO strategy, which can quickly suppress the filamentous overgrowth and maintain good sludge settleability.

The design of FFO strategy enables the settled activated sludge from the sedimentation tank to be randomly recycled between the feasting and the fasting reactors, avoiding the prolonged exposure of the sludge to a consistently low F/M ratio. Hence, filaments cannot acclimate to a suitable condition for growth that induces foaming problem in activated sludge process. Simultaneously, this strategy creates a favourable environment for the non-filamentous bacteria to gain dominance in the ecosystem.

High BOD removal efficiency was achieved during the treatment process when the system was switched from the conventional operation to the FFO programme (Fig. 3). The BOD concentration in the effluent was maintained within 20 mg/L, resulting in a stable BOD removal efficiency (>95%). The FFO strategy successfully suppressed the foaming problem and improved the sludge settleability without adversely affecting the treatment efficiency on BOD removal.

4. Conclusion

The predominant filamentous microorganisms that encouraged bulking and foaming sludge in the domestic wastewater treatment plants were identified as *N. amarae*. These organisms were able to utilize the fatty acids with different length of carbon chain for their growth. They had lower maximum specific growth rates and smaller saturation constants, and can survive on a low concentration of fatty acids. A novel control technique (FFO) based on the growth kinetics and the inter-species interaction was developed to suppress the overgrowth of *N. amarae* and to improve microbial population balance without deteriorating the treatment efficiency and the process stability of

activated sludge process that receives wastewater with a high concentration of long-chain fatty acids, oil and fats.

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