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Long chain fatty acids removal in selector tanks: Evidence for insufficient *Microthrix parvicella* control

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ABSTRACT

Microthrix parvicella is the dominant filamentous microorganism prevailed in biological nutrient removal (BNR) activated sludge systems experiencing temporarily or even permanently filamentous bulking and foaming problems. Reports regarding the ability of *M. parvicella* to uptake and utilize long chain fatty acids (LCFA) are rather controversial. In the context of this study a series of carbon uptake batch experiments with oleate as the external substrate under aerobic, anoxic and anaerobic conditions were performed in order to investigate the biosorption capacity of activated sludge samples experiencing serious filamentous bulking and foaming problems due to *M. parvicella* proliferation. According to the results LCFA removal efficiency is reversely proportional to the applied organic loading. Removal efficiencies in the order of 70–90% were observed under aerobic conditions for floc loadings lower than 100 mgCOD/gSS, whereas lower removal efficiencies were experienced (30–40%) for significantly higher floc loadings. Similar correlation of removal efficiencies with floc loading was evidenced under anaerobic and anoxic conditions (50% LCFA removal for floc loadings lower than 100 mgCOD/gSS). Based on these findings it can be stated that under the conditions prevailing in anaerobic, anoxic and aerobic selector tanks, only a portion of LCFA is removed, and therefore the remaining LCFA under the completely mixed conditions prevailing at the aeration zone of a bioreactor would establish low enough floc loadings, thus stimulating *M. parvicella* growth.

Keywords: Biosorption; Bulking; Filaments; Floc loading; Foaming; Long chain fatty acids; *Microthrix parvicella*; Selector tanks

1. Introduction

Filamentous bulking and foaming of activated sludge are the most important and widespread sludge separation problems. Evidently, more than 50% of the activated sludge systems around the world realize either periodically or systematically such problems. Based on the need to achieve low nitrogen and phosphorus effluent concentrations most of the activated sludge plants are operated as extended aeration systems. Filamentous bulking and foaming in such systems are highly correlated to the proliferation of low F:M filamentous microorganisms (i.e. *Microthrix parvicella*, *Type 0041*, *Type 0675* and *Type 0092*).

Several national surveys conducted in The Netherlands [1], Denmark [1], Italy [2], Czech Republic [3], Greece [4], Germany [5], France [6] and UK [7] show that *M. parvicella* is the most dominant filamentous microorganism prevailed in biological nutrient removal (BNR) activated sludge systems experiencing temporarily or even permanently filamentous bulking and foaming problems. Table 1 summarizes the dominant filamentous species identified in the aforementioned extensive national surveys.

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Table 1
Dominant filamentous microorganisms in activated sludge systems from different European countries

Rank	The Netherlands [1]	Denmark [1]	Italy [2]	Czech Rep. [3]	Greece [4]	France [6]	UK [7]
1	*M. parvicella*	*M. parvicella*	*M. parvicella*	*M. parvicella*	*M. parvicella*	*M. parvicella*	*M. parvicella*
2	*Type 021N*	*Type 0041*	*Type 0041*	*Type 0092*	*Type 0092*	*Type 0041*	*Type 021N*
3	*H. hydrossis*	*N. limicola*	*N. limicola*	*GALOs*	*GALOs*	*N. limicola*	*N. limicola*
4	*Type 0092*	*Type 0092*	*H. hydrossis*	*Type 0041*	*Type 0041*	*Type 0092*	*S. natans*
5	*Type 1701*	*Type 0803*	*Type 021N*	*Type 0675*	*N. limicola*	*Type 0803*	*GALOs*

Reports regarding the ability of selector tanks to provide for effective *M. parvicella* proliferation control are rather controversial. Evidently, there are several studies reporting on the positive effect of the incorporation of a selector tank (in most cases an aerobic selector) to control low F:M filamentous bulking [8–13], whereas there are also experimental studies reporting on the opposite [14–17].

Accordingly, reports regarding the ability of *M. parvicella* to utilize readily and slowly biodegradable organic substances are also controversial. Early pure culture studies on the physiological characteristics of *M. parvicella* indicate that the microorganism utilizes long chain fatty acids (LCFA) as carbon and energy source [18,19]. This finding was also confirmed under in situ conditions [20,21] and in continuous flow lab scale experiments [16]. On the other hand, late pure culture experiments indicate that *M. parvicella* is able to utilize a wide range of organic carbon sources including organic acids, complex substrates and fatty acids but not LCFA as sole carbon and energy sources [22–24]. As postulated by Rossetti et al. [24] the high storage capacity of a variety of different carbon sources exhibited by *M. parvicella* under all environmental conditions (aerobic, anoxic and anaerobic) suggests that the use of selector tanks cannot provide for effective control of this bacterium.

In view of the above, the objective of this study was to assess LCFA removal efficiency of an activated sludge with a high *M. parvicella* content under conditions prevailing in aerobic, anaerobic and anoxic selector tanks.

2. Experimental materials and methods

A total of 90 carbon uptake batch tests were conducted, 45 under aerobic conditions, 10 under anoxic conditions and 35 under anaerobic conditions. In all batch assays LCFA in the form of oleate (Tween 80) were added as the external substrate and the concentration of soluble COD was measured 5 min and 30 min after substrate addition.

Biosorption was calculated by the following equation:

$$q = \frac{\mathrm{COD}_t = \mathrm{o} - \mathrm{COD}_t}{\mathrm{SS}}$$

where $\mathrm{COD}_t = 0$ and COD_t are the soluble COD concentration at time 0 and t, respectively, and SS is the concentration of the suspended solids of the activated sludge sample.

For each batch experiment the floc loading (FL) was calculated as the ratio of soluble COD at the beginning of the experiments ($t = 0$) over the concentration of suspended solids.

To establish aerobic conditions, air was provided at a constant rate, whereas anoxic experiments were acquired through the addition of nitrate nitrogen solution.

In all the experiments activated sludge samples collected from a wastewater treatment plant which encountered serious bulking and foaming problems (Fig. 1) were used under constant temperatures (20–22°C). Based on microscopic observations of activated sludge samples, the dominant filamentous microorganisms, in decreasing order of magnitude, were *M. parvicella* (specific filament index between 3 and 5), *Type 0041* (specific filament index between 1 and 2) and *Type 0092* (specific filament index between 1 and 2). Furthermore, the majority of activated sludge flocs with sizes between 150 and 500 μm exhibited an open and loose structure with noticeable bridging.

To quantify the presence of *M. parvicella* the microscopic counting method developed by Pitt and Jenkins [25] for Gordona amarae and modified by Mamais et al. [16] was applied. Measurements of total and soluble COD,

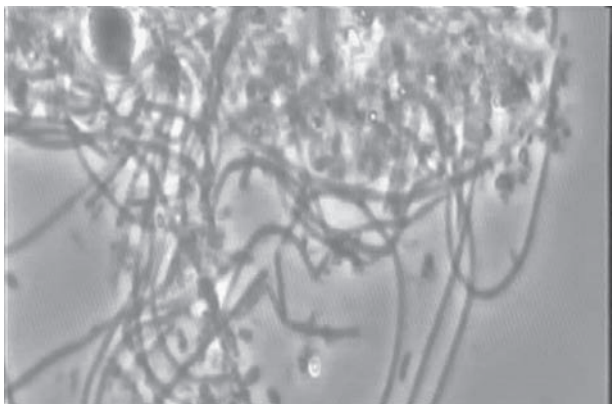

Fig. 1. Microscopic picture of a biomass with a very high *M. parvicella* content (1000 × phase contrast).

suspended solids and nitrate nitrogen concentrations were performed according to Standard Methods [26].

3. Results and discussion

A series of carbon uptake batch experiments with oleate as the external substrate under aerobic, anoxic and anaerobic conditions were performed in order to investigate LCFA removal efficiency of an activated sludge experiencing serious filamentous bulking and foaming problems due to *M. parvicella* proliferation.

Biosorption was expressed as the soluble COD removed at time t over the concentration of the suspended solids of the activated sludge sample. As postulated by Eikelboom [27] biosorption accounts for all the physicochemical and biological processes participating in the overall phenomenon of instantaneous removal of organic matter from the liquid phase when wastewater come in contact with mixed liquor.

For the interpretation of the experimental results the floc loading (FL) described as the ratio of soluble COD at the beginning of the experiments over the concentration of suspended solids was employed.

Experimental results under aerobic, anaerobic and anoxic conditions are presented in Figs. 2–4.

According to the results, the biosorption capacity of the biomass, under all environmental conditions (anaerobic, anoxic and aerobic), is highly dependent on the applied floc loading. More specifically under aerobic conditions and for floc loadings lower than 150 mgCOD/gSS biosorption is almost linearly proportional to the applied organic loading (first order). Due to the increased floc loading substrate penetrates deeper in the floc thus providing more active sites available for additional substrate removal on the external area of the flocs. On the other hand, for very high floc loadings (>150 mgCOD/gSS) biosorption was almost constant (zero order), obviously due to saturation phenomena (saturation of adsorption sites and membrane transport proteins). Under anaerobic and anoxic conditions biosorption appears to be linearly correlated with organic floc loading for floc loading values lower than 100 mgCOD/gSS, whereas for greater floc loading values saturation phenomena are presented.

Thus, a Monod-like curve may be applied to correlate biosorption capacity of LCFA with the floc loading that biomass biocenosis is experiencing. This Monod-like dependence of biosorption with respect to floc loading was observed for all the environmental conditions examined (aerobic, anaerobic and anoxic).

Based on the experimental results, biosorption capacity of activated sludge increased with contact time. However, the difference in biosorption between 5 and 30 min contact time, although increased in proportion to FL, was moderate in the order of 20–25% for all environmental conditions examined. On the other hand, there are researchers reporting that according to their experimental results biosorption was almost doubled for an increase of contact time from 1 to 30 min [28,29] and from 1 to 10 min [27].

However, it should be mentioned that the experimental results of these authors were based on batch tests in which wastewater was used as the external substrate

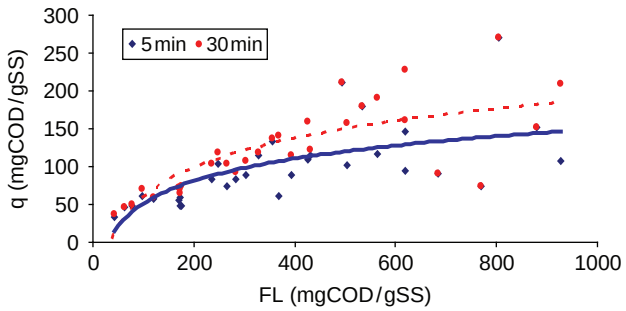

Fig. 2. Biosorption of activated sludge samples as a function of floc loading and contact time under aerobic conditions.

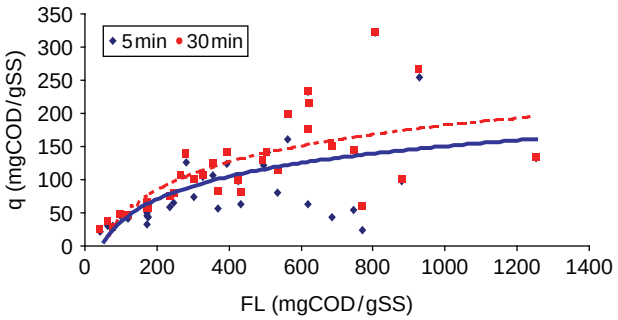

Fig. 3. Biosorption of activated sludge samples as a function of floc loading and contact time under anaerobic conditions.

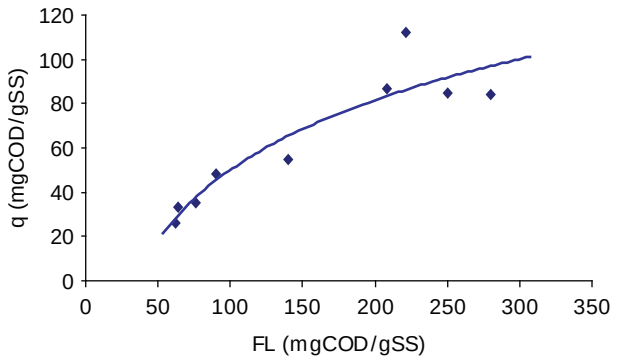

Fig. 4. Biosorption of activated sludge samples as a function of floc loading for a contact time of 5 min under anoxic conditions.

and not LCFA. Hence in these experiments activated sludge samples were supplied, among others, with readily catabolized sources of energy which stimulate biodegradation thus providing the required energy for the transfer of substances inside the flocs and the initiation of storage processes.

Figs. 5–7 present the variation of LCFA removal with respect to floc loading and contact time under aerobic, anaerobic and anoxic conditions, respectively.

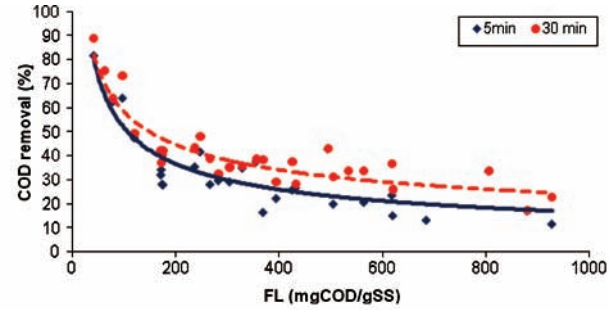

Fig. 5. LCFA removal with respect to floc loading and contact time under aerobic conditions.

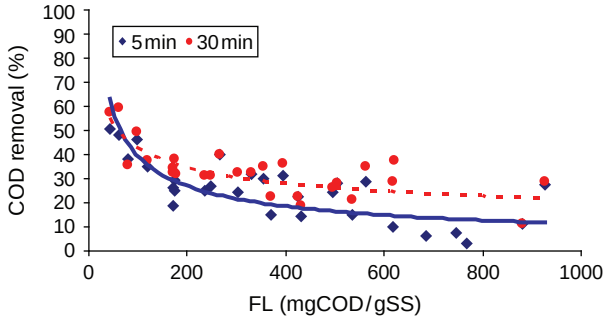

Fig. 6. LCFA removal with respect to floc loading and contact time under anaerobic conditions.

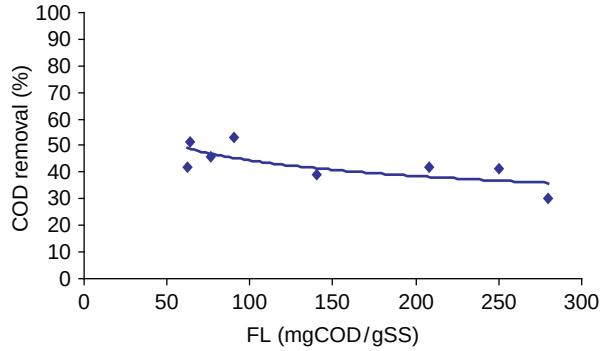

Fig. 7. LCFA removal with respect to floc loading for a contact time of 5 min under anoxic conditions.

As illustrated in Fig. 5, LCFA removal efficiency is reversely proportional to the applied organic loading. Removal efficiencies in the order of 70–90% were observed under aerobic conditions for FL lower than 100 mgCOD/gSS, whereas lower removal efficiencies were experienced (30–40%) for significantly higher FL (>150 mgCOD/gSS).

Similar correlation of removal efficiencies with FL was evidenced under anaerobic and anoxic conditions (Figs. 6 and 7). More specifically for FL lower than 100 mgCOD/gSS removal efficiencies were in the order of 45–55% and 50% for anaerobic (Fig. 6) and anoxic conditions (Fig. 7), respectively, whereas at higher FL removal efficiencies fell down to 20–25% and 40% for anaerobic and anoxic conditions respectively.

Floc loadings greater than 100 mgCOD/gSS can be achieved in fully compartmentalized reactors simulating plug flow pattern, in selector tanks and in SBR systems where influent is discontinuously supplied to the bioreactors [27,30].

Based on the above it can be stated that under the conditions prevailing in anaerobic, anoxic and aerobic selector tanks, only a portion of LCFA is removed (40–50%), and therefore the remaining LCFA under the completely mixed conditions prevailing at the aeration zone of a bioreactor would establish low enough FL (i.e. low food to microorganisms ratios), thus stimulating *M. parvicella* growth.

Furthermore, as biosorption related to biological activity under aerobic conditions was 30% greater than biosorption calculated for anaerobic and anoxic experiments, it can be concluded that hydrolysis, uptake, storage and utilization of LCFA under aerobic conditions proceed with higher rates than those under anoxic and anaerobic conditions. The necessary energy for the storage of dissolved COD (LCFA) under aerobic conditions can be obtained from β-oxidation reactions which are taking place intracellularly in order to catabolize LCFA to acetyl-CoA prior to its incorporation in PHA inclusions. Accordingly under anaerobic conditions the required energy for the storage of soluble COD can be obtained from the hydrolysis of polyphosphate and other organic carbon inclusions (PHA, PHB, glycogen, lipids). As the majority of the organotrophic bacteria like *M. parvicella* could not grow in anaerobic conditions [20,21,31], it can be assumed that anaerobic biosorption of LCFA by *M. parvicella* is energetically supported by microorganism's unique ability to use intracellular reserve material like lipids. The ability of *M. parvicella* for LCFA uptake may be attributed also to the morphological properties (no sheath present), the hydrophobic nature of species surface, the ability of species to grow outside flocs (decreased diffusional resistance) and the lipase activity exhibited on its filament surface.

Based on additional carbon uptake tests with LCFA as external substrate under anoxic conditions, it was observed that although a significant portion of soluble COD was removed from the liquid phase after 5 min of contact, a partial redissolution took place after 4 h as evidenced by a moderate increase of soluble COD. Following hydrolysis hypothesis suggested by Ekama and Marais [32] redissolution of COD should be attributed to the diffusion of LCFA hydrolysis products in the bulk solution. Redissolution of COD was followed by a further removal of soluble COD and the initiation of denitrification process as evidenced by the decrease of nitrate nitrogen concentration. All the above provide sufficient indication that anoxic hydrolysis of LCFA proceeds with much lower rates compared to aerobic hydrolysis. Thus, in the case of an anoxic selector a significant portion of the sorbed LCFA will be transferred to the following completely mixed reactor thus stimulating *M. parvicella* growth.

The inability of anoxic and anaerobic selector tanks to provide for significant LCFA removal and thus for effective *M. parvicella* control was fully verified with lab scale experiments [16,33,34]. Both completely mixed continuous flow systems with anaerobic and anoxic selectors developed biomass with high *M. parvicella* content. The failure of both selector systems to achieve satisfactory *M. parvicella* control can be interpreted in terms of the relatively low portion of LCFA removed in the selector tanks which counted for almost 40–50% of the total external LCFA added to the two systems.

Hence it seems reasonable that in the presence of selector tanks the compartmentalization of the aerobic zone (at least the first part of it) is required as well, to effectively control *M. parvicella* growth in BNR systems.

4. Conclusions

The objective of this study was to evaluate the LCFA removal efficiencies of activated sludge samples with a high *M. parvicella* content under anaerobic, anoxic and aerobic conditions prevailing in selector tanks. According to the results of a series of carbon uptake batch experiments, the biosorption capacity of the biomass, under aerobic, anoxic and anaerobic conditions, is highly dependent on the applied floc loading. LCFA removal efficiencies under conditions prevailed in selector tanks are in the order of 40% for aerobic and anoxic conditions and 25% for anaerobic conditions.

By considering that high floc loadings (i.e., high food to microorganisms ratios) favor the differential growth of floc forming microorganisms, it can be concluded that the complete compartmentalization of the anoxic and anaerobic reactors should be anticipated to favor uptake of LCFA by floc forming microorganisms. However even in this case, as only a portion of LCFA is removed under anoxic and/or anaerobic conditions, the remaining LCFA under the completely mixed conditions prevailing at the aeration zone of a bioreactor would establish low enough FL, thus stimulating *M. parvicella* growth. Therefore, the compartmentalization of the aerobic zone (at least the first part of it) is required as well, to effectively control *M. parvicella* growth in BNR systems.

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