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INFLUENCE OF THE PRESENCE OF LONG CHAIN FATTY ACIDS (LCFAs) IN THE SEWAGE ON THE GROWTH OF *M. PARVICELLA* IN ACTIVATED SLUDGE WASTEWATER TREATMENT PLANTS

D. MAMAIS[1*]
G, NIKITOPOULOS[1]
E. ANDRONIKOU[1]
E. GAVALAKIS[1]
A. ANDREADAKIS[1]
C. NOUTSOPOULOS[1]
C. GIOTAKIS[2]
G. TSIMARAKIS[2]

[1] *Sanitary Engineering Laboratory, Department of Water Resources Hydraulics and Maritime Works, Faculty of Civil Enging., National Technical University of Athens, 5 Iroon Polytechniou Str., Zografou Athens 15773, Greece*
[2] *Municipal Enterprise for Water and Sewage of Ioannina Ioannina, Greece*

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*to whom all correspondence should be addressed
Tel.: +30 210 7722897; Fax: +30 210 7722899
e-mail: mamais@central.ntua.gr

ABSTRACT

Foaming and bulking problems in activated sludge treatment plants are associated to the presence of a variety of filamentous bacteria. However, it has been observed that *M. parvicella* is the most frequent filamentous microorganism causing sludge bulking and foaming, especially in treatment plants involving nutrients removal.

High sludge retention time, low DO, low temperature, presence of anoxic, anaerobic, and intermittently aerated zones, are the most commonly cited conditions associated with its growth. Substrate composition is another significant factor, as it has been found that slowly degradable organic material may favour the growth of *M. parvicella*. If has also been suggested that *M. parvicella* may preferably store long chain fatty acids (LCFAs) under anaerobic conditions and subsequently use it for growth.

The paper presents the results of an investigation conducted at the wastewater treatment plant of Ioannina, aiming to establish a cause-effect relationship between the presence of LCFAs and the abundance of *M. parvicella*. This investigation is a part of a wider study sponsored by the Greek Secretariat for Research, under the PENED programme.

The duration of the investigation covers 8 months, from January 2004 to August 2004. During this period samples were taken from the sewage collection network and wastewater treatment plant of Ioannina and analysed for, among other parameters, LCFAs and total fatty acids while samples of the mixed liquor and the foam in the biological reactors were microscopically analysed in order to determine the presence and amount of various types of filamentous bacteria.

Two main conclusions were drawn. The first is related to the effect of temperature on the growth of *M. parvicella*, indicating that the growth of this specific filamentous bacterium is favoured by low temperatures (generally below 20 ^{o}C), while higher temperatures cause the practical elimination of *M. parvicella*, irrespectively of other factors. This conclusion verifies previous studies in pilot units and full scale plants. The second conclusion is that during winter periods there seems to be a positive correlation, between the presence of fatty acids and more specifically LCFAs and the amount of *M. parvicella*.

KEYWORDS: Activated sludge; filamentous bulking; long chain fatty acids; foaming

INTRODUCTION

Foaming and bulking problems in activated sludge treatment plants are associated to the presence of a variety of filamentous bacteria. However, it has been observed that *M. parvicella* is the most frequent filamentous microorganism causing sludge bulking and foaming, especially in treatment plants involving nutrients removal [1, 2].

High sludge retention time, low DO, low temperature, presence of anoxic, anaerobic, and intermittently aerated zones, are the most commonly cited conditions associated with its growth [2]. Substrate composition is another significant factor, as it has been found that slowly degradable organic material may favour the growth of *M. parvicella*. If has also been suggested that *M. parvicella* may preferably store long chain fatty acids (LCFAs) under anaerobic conditions and subsequently use it for growth [3,4].

Sewer systems may often operate as bioreactors causing considerable wastewater quality changes, influencing the composition of influent wastewater and particularly the relative magnitudes of readily biodegradable, easily hydrolysable and slowly hydrolysable fractions [5]. A change in the composition of influent wastewater that will result in the increase in the concentration of hydrolysed lipids especially in the form LCFAs may be responsible for the excessive growth of filamentous bacteria such as *M. parvicella*.

The paper presents a new method for the analysis of LCFAs in wastewater, which constitutes a "tool" for the investigation of the relationship between filamentous bacteria and the existence of LCFAs in urban wastewater. The LCFAs determination method that was developed can be utilized for the calculation of both free LCFAs (sample without saponification) and total LCFAs contained in the samples (after sample saponification). The method was applied to the wastewater treatment plant of Ioannina, aiming to establish a cause-effect relationship between the presence of LCFAs and the abundance of *M. parvicella*. This investigation is a part of a wider study sponsored by the Greek Secretariat for Research, under the PENED programme.

MATERIALS AND METHODS

The duration of the investigation covers 8 months, from January 2004 to August 2004. During this period samples were taken from the sewage collector and treatment plant of Ioannina and analysed for, among other parameters, LCFAs and total fatty acids while samples of the mixed liquor and the foam in the biological reactors were microscopically analysed in order to determine the presence and amount of various types of filamentous bacteria.

Ioannina sewage treatment plant

The sewage treatment plant (STP) of Ioannina serves a population equivalent of approximately 135,000. Sewage is transported to the plant via a main collector with a diameter of 1.2 m and 4 km in length. In addition to the sewage, the plant receives septage which enters the plant after preliminary treatment. Subsequently the mixture receives primary treatment and following this biological treatment by the activated sludge process. The treated effluent after chlorination is mixed with surface waters and is mainly used for irrigation. The produced sludge is anaerobically digested, dewatered and disposed off by landfilling.

Long chain fatty acids measurements

Measurements were performed with a Perkin Elmer AutoSystem XL gas chromatographer fitted with a split/splitless injector for capillary columns and a Flame Ionization Detector. The column was a BP21-SGE fused silica capillary column (50 m x 0,22 mm i.d., 0,25 μm film thickness) with Polyethylene glycol as a stationary phase combined with a pre-column of 5 m length and 0,22 mm i.d. Carrier gas was helium (purity 99,999 %) and the whole chromatographic system was controlled by Turbochrom software of PE Nelson.
Samples were collected in glass bottles previously cleaned with HCl and stored at 4 ^{0}C until analysis. Analyses were performed with the least possible delay. All standards of LCFAs were analytical grade >99 % and purchased from Labor Dr. Ehrenstorfer. In more detail, four different fatty acids were studied, three of them with even number of carbon atoms (palmitic, stearic and oleic acid) and one with odd number of carbon atoms (margaric acid). For the saponification of samples sodium hydroxide from Merck kGaA was used and hydrochloric

acid from Riedel-de Haën (min 37 %) was added to bring the sufficient pH to the samples. Finally, chloroform from Riedel-de Haën (assay min 99,8 %) was used as extraction solvent.

Extraction method: 100 mL of wastewater were transferred to a volumetric flask which was placed in a sonicated bath for 6 h. Then, HCl (1:1) was added to bring the pH to 1. The sample was transferred to a separatory funnel and 20 mL chloroform were added. The mixture was shaken for 2 minutes and the organic phase was collected after decantation. The extraction was repeated two times. The extracts were mixed, filtered with glass fibre filter 47 mm and concentrated to dryness using nitrogen gas at 40 ^{0}C. The residue was dissolved in a suitable volume of chloroform and homogenized.
Saponification: Hydrolysis of glycerides by sodium hydroxide (saponification) before the use of the extraction method was required for the calculation of total LCFAs that exist in sewages samples. 100 mL of sample were mixed with 20 mL deionised water, 20 mL ethanol 96 % and 5 g NaOH in a distilling flask. Using vertical refluxing device, the mix was boiled for 30 minutes in a hot water bath. The flask was then removed from water bath, cooled at room temperature and HCl (1:1) was added to bring the pH to 7.
Calibration: Each of standards of LCFAs was dissolved in chloroform in order to construct calibration curves for each fatty acid (1-10 ppm and 10-100 ppm).
GC analysis: A 1 µL aliquot of the extract was injected in the gas chromatograph, using split mode 50:1. The GC parameters were: Pressure of carrier gas: 60 psi, Injector temperature: 280 ^{0}C, Oven temperature program: 180 ^{0}C (1 min), 8 ^{0}C/min, up to 220 ^{0}C, Detector temperature: 280 ^{0}C.

The concentration of long chain fatty acids in the samples was calculated using the constructed calibration curves for each fatty acid.

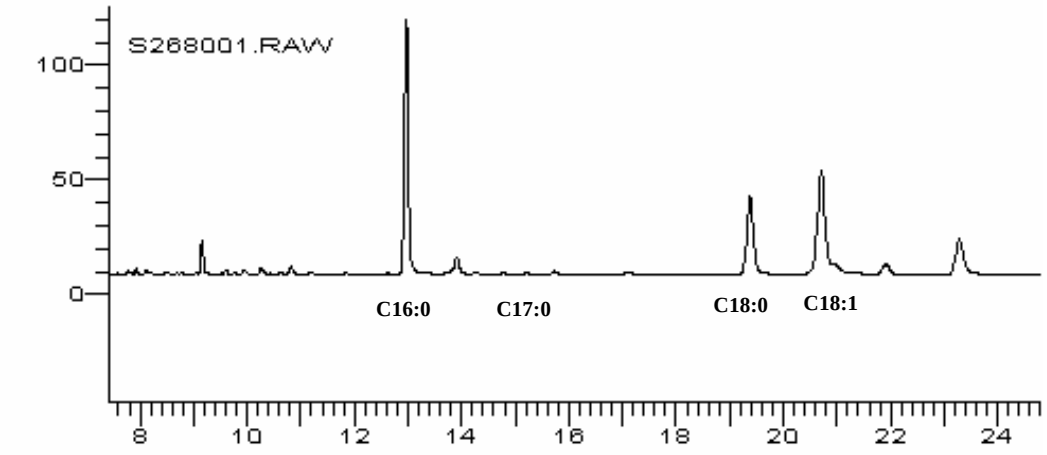

Figure 1. Typical chromatogram of wastewater LCFAs analysis from the influent in the wastewater treatment plant of Ioannina

Identification and enumeration
Microscopic examination of the sludges was performed according to [6] and [7]. *M.parvicella* levels in the activated sludge samples were estimated in terms of intersections of gram positive non-branched filaments > 3 µm in length and < 1.5 µm wide, per gram of mixed liquor suspended solids [3].

RESULTS

Total LCFAs concentration in the untreated wastewater from the city of Ioannina ranged from 8 to 50 mg l^{-1} The main LCFAs detected were palmitic acid, followed by stearic and oleic acids (Figure 2). All three long chain fatty acids have been reported to promote the growth of *M parvicella* in activated sludge systems [3, 4, 8, 9,10, 11].

According to the results of analyses conducted following saponification of samples, free LCFAs concentration ranged from 41% to 94% of the total LCFAs with an average of 76%. Both free and total LCFAs were reduced during transportation through the 4 Km long main sewerage collector, by 45% and 48% respectively. Average total LCFAs at the start of the main collector and at the influent to the treatment plant was 26 mg l^{-1} and 13,7 mg l^{-1}., respectively. This decrease through the sewer system can be attributed to both settlement and consumption of LCFAs by the biofilm and the suspended biomass in the sewer network [12]. According to the results it appears that the uptake rate of LCFAs is somewhat higher than the rate of lipids and fats hydrolysis. However it was not possible to establish a correlation between the ratio of free to total LCFAs and the residence time in the sewer.

Average free and total LCFAs concentrations measured in raw wastewater of Ioannina, in the preliminary treated and the primary effluent wastewater are shown in Table 1. According to these measurements it appears that there is a significant increase in both free and total LCFAs concentrations in preliminary treated wastewater compared to the raw wastewater. This increase is attributed to the septage and the recycle stream from the sludge treatment facilities that are discharged ahead of the grit removal facilities. Therefore both septage and sludge recycle streams may contribute significantly to the amount of LCFAs available to filamentous bacteria in the activated sludge system.

Table 1. Average free and total LCFAs concentrations in the Sewage Treatment Plant of Ioannina

Sample site	**Free LCFAs, (mg l^{-1})**	**Total LCFAs, (mg l^{-1})**
Raw wastewater	10,4	13,7
Preliminary treated wastewater	23,6	36,5
Primary treated wastewater	13.7	16

As shown in Table 1, a significant removal of both free and total LCFAs is achieved during primary sedimentation due to the combined effects of settlement, hydrolysis and uptake of LCFAs. Average reduction of free and total LCFAs is 42% and 54%, respectively.

For the assessment of the settling characteristics of the activated sludge routine measurements of the Sludge Volume Index (SVI) were conducted. Furthermore microscopic analysis of the mixed liquor and foam samples was carried out throughout the monitoring period to determine the dominant filamentous microorganisms. According to the results of the microscopic analysis excessive growth of low F/M filamentous organisms was evidenced especially during winter months (Jan – Apr) with Filament Index (FI) values ranging from 3 to 4 with an average value of 3,7. Throughout the winter period the relatively high FI values were followed by bulking phenomena. SVI values varied between 145 - 380 ml/gSS with an average value of 277 ml/gSS. During winter months the dominant filamentous organisms in activated sludge samples in decreasing order of magnitude were *M. parvicella*, *Type 0092*, *Type 0041* and *Type 0675* (Figure 3) with average Specific FI (SFI) values of 3,8, 3,3, 1 and 0,9 respectively. The same filamentous microorganisms were also found in the foam samples (Figure 4) in the same order, with *M.parvicella* exhibiting an even higher SFI value (average SFI = 4,4) due to the hydrophobic nature of its cell membrane.

The composition of filamentous bacteria in sludge biocenosis observed during summer months (May – Aug) was significantly different (Figures 3 and 4). The dominant filamentous microorganisms in decreasing order were *Type 0092*, *Type 0675* and *Type 0041*. During summer the activated sludge exhibited better settling characteristics with SVI values in the range fro 115 to 147 ml/gSS. The higher temperatures observed during summer months resulted in a significant decrease in *M.parvicella*. As shown in Figure 2 *M.parvicella* counts progressively decreased during summer months from values of 27 x 10^6 intersections/gVSS, at temperatures lower than 18^{o}C, to less than 1 x 10^6 at temperatures above 20^{o}C. It is interesting to note that during summer, as *M.parvicella* presence progressively decreased to almost complete disappearance another low F/M bacterium, *Type 0092* became dominant in the sludge biocenosis.

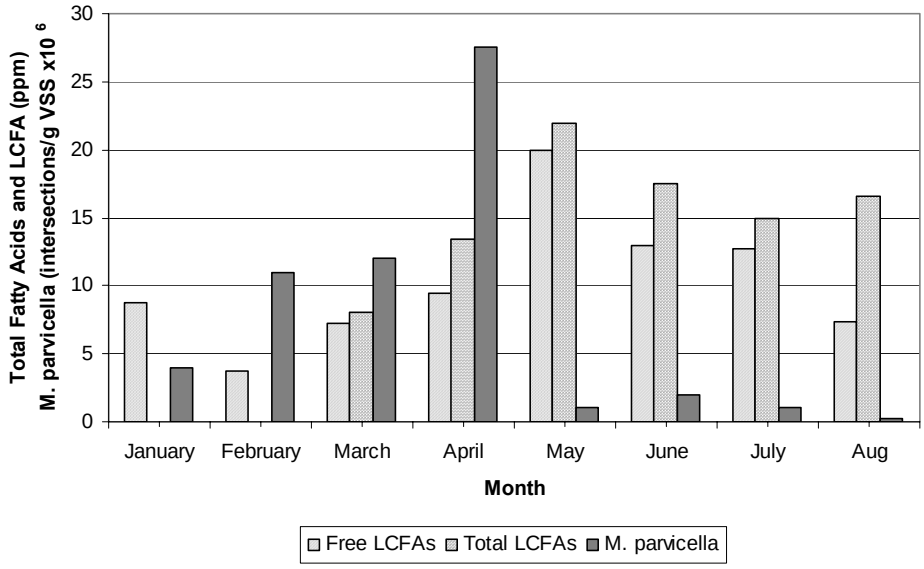

Figure 2. Correlation between *M. parvicella* abundance and free and total LCFAs concentrations

As shown in Figure 2 during winter period, appears to be a positive correlation between the presence of LCFAs and *M. parvicella*. However, the positive effect of LCFAs on the growth of *M. parvicella* is not as significant as the effect of temperature, since as shown in Figure 2, despite the fact that higher LCFAs were measured during the summer period, the elevated temperatures had a much more profound negative effect on the growth of *M. parvicella*. The verification and explanation of the seasonal variation of *M. parvicella* requires further investigation.

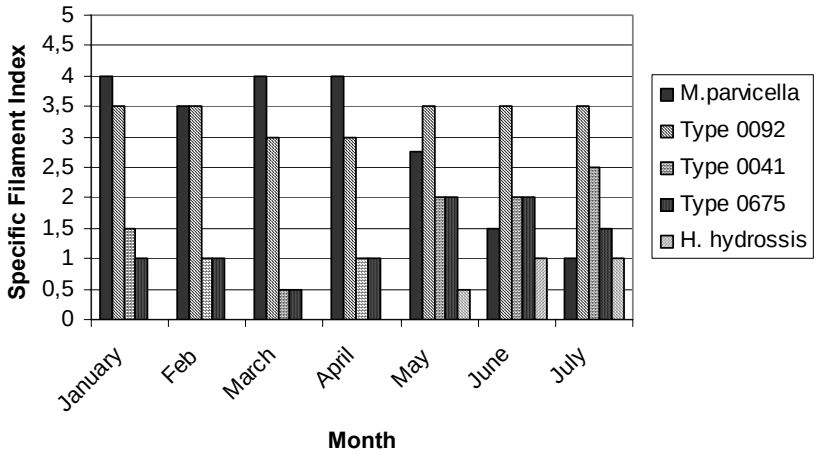

Figure 3. Dominant filamentous bacteria in activated sludge samples

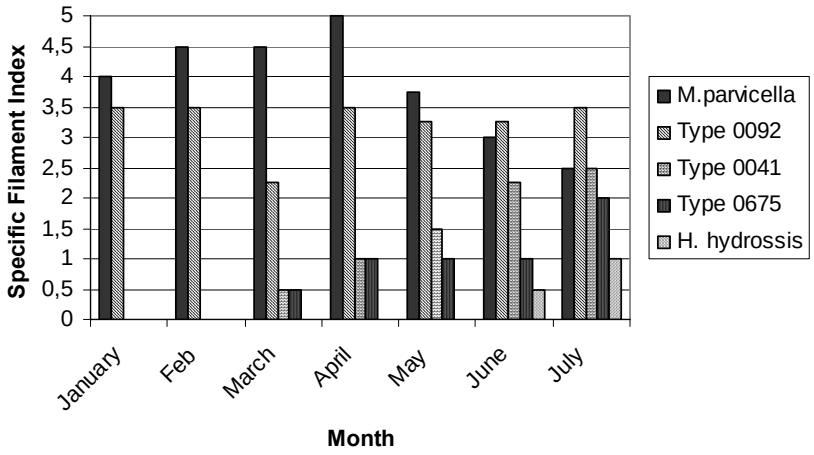

Figure 4. Dominant filamentous bacteria in the scum layer

CONCLUSIONS

The main conclusions can be summarised as follows:

- The main LCFAs detected in wastewater samples were palmitic acid, followed by stearic and oleic acids. All three acids have been reported to promote the growth of *M parvicella* in activated sludge systems. Free LCFAs represent on average 76% of the total LCFAs, the percentage ranging from 41% to 94%.
- Both free and total LCFAs were reduced during transportation through the 4 Km long, sewer system by 45% and 48% respectively. However it was not possible to establish a correlation between the removal of LCFAs during transportation and the residence time in the sewer.
- Septage and sludge recycle streams appear to contain high amounts of LCFAs and contribute significantly to the amount of LCFAs available to filamentous bacteria in the activated sludge system.
- Primary sedimentation results in reduction of both free and total LCFAs by 42% and 54% respectively, due to the combined effects of settlement, hydrolysis and uptake.

With respect to the growth of filamentous bacteria and more specifically the growth of *M. parvicella* and the effect of LCFAs on the growth rate, two main conclusions were drawn.

- The first is related to the effect of temperature on the growth of *M. parvicella*, indicating that the growth of this specific filamentous bacterium is favoured by low temperatures (generally below 20 ^{o}C), while higher temperatures cause the practical elimination of *M. parvicella*, irrespectively of other factors. This conclusion verifies previous studies in pilot units [2 and 4].
- The second important conclusion is that during winter periods there seems to be a positive correlation, between the presence of fatty acids and more specifically LCFAs and the amount of *M. parvicella*. However, the effect of LCFAs on the growth of *M. parvicella* is not as significant as the effect of temperature, since despite the fact that higher LCFAs were measured during the summer period the growth of *M. parvicella* is inhibited at the elevated summer temperatures.

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