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Identification and abundance of filamentous bacteria in a membrane bioreactor treating domestic wastewater

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INTRODUCTION

Recent studies have suggested that the concentration of filamentous bacteria in the operation of a membrane bioreactor (MBR) should be controlled due to its negative effect on membrane fouling [1]. Other authors [2] found that the elevated presence of filamentous bacteria did not affect the quality of effluent or the permeability of the membranes. Identification of filamentous based on morphological identification can be ambiguous, so molecular methods such as fluorescence in situ hybridization (FISH) with oligonucleotide probes targeting different filamentous species are necessary for reliable identification. The aim of the present study was to determine the abundance of filamentous bacteria on MBR sludge samples using FISH.

MATERIAL AND METHODS

The sampling period was conducted between February to November 2012 in an MBR (Figure 1) treating domestic water equipped with microfiltration membranes. The biological reactor volume was 251 m^3. Fortnightly samples of mixed liquor were taken for the identification of filamentous bacteria with the FISH technique.

Figure 1. Membrane bioreactor wastewater treatment plant (MBR WTP)

In the FISH analysis 15 rRNA-targeted nucleic acid probes were used (Table 1) . The abundance of filamentous bacteria in the 14 activated sludge samples was measured according to the subjective scoring method of Eikelboom (2000) where observations are rated on scale from 0 (none) to 5 (extensive growth). The hybridizations were performed at 46 ° C for 2 hours except for *Microthrix* that has extended the period of hybridization until 3 h. All the probes labelled at the 5' end with Tamra, were purchased from TibMobiol, Germany.

Table 1. FISH probes used for the identification of filamentous bacteria

Specificity	Probe	Secuencia	% FA*	Reference
Caldilinea	Caldi-0678	TTCCACCACTACACCGGG	30	Kragelund *et al.* (2011)
	Comp1-Caldi-0678	TTTCACCACTACACCGGG	-	Kragelund *et al.* (2011)
	Comp2-Caldi-0678	TTCCACCGCTACACCGGG	-	Kragelund *et al.* (2011)
Type 0803	T0803-0654	ACACC CTCTCACYRCCT	30	Kragelund *et al.* (2011)
	T0803ind-0642	CTGCCTCAAGCTACTCAG	30	Kragelund *et al.* (2011)
	h1 T0803ind-0607	AGTTAAGCCAGGAGATTT	-	Kragelund *et al.* (2011)
	h2 T0803ind-0625	TTTCCAACGACCCCTCCC	-	Kragelund *et al.* (2011)
	h3 T0803ind-0662	GAATTCTACACCCCTCTC	-	Kragelund *et al.* (2011)
	h4 T0803-0680	ATTCCACCACTACACCGG	-	Kragelund *et al.* (2011)
Type 0092	CFX197	TCCCGGAGCGCCTGAACT	40	Speirs *et al.* (2009)
	CFX197comp	TCCCGAAGCGCCTGAACT	-	Speirs *et al.* (2009)
	CFX223	GGTGCTGGCTCCTCCCAG	35	Speirs *et al.* (2009)
	CFX223 H202	AGCGCCTGAGCTTCAGTCATC	-	Speirs *et al.* (2009)
	CFX223 H241	CGTTACCTTACCAACTAGCTGATGG	-	Speirs *et al.* (2009)
	CFX67a	TTCCGAAGATCAGGTTCG	35	Speirs *et al.* (2010)
	CFX67-H46	TTCGACTTGCATGTGTTARGC	-	Speirs *et al.* (2010)
	CFX67 comp	TTCCGAAGATCGGGTTCG	-	Speirs *et al.* (2010)
	CFX67b	TTCCGAAGATTAGGTTCG	35	Speirs *et al.* (2010)
	CFX67-H95	CCGTRCGCCACTAACCYT	-	Speirs *et al.* (2010)
Phylum *Chloroflexi*	CFX1223	CCATTGTAGCGTGTGTGTMG	35	Björnsson *et al.* (2002)
	CFX1223-H1206	CCCWGGAYATAAAGGCC	-	Speirs *et al.* (2010)
	CFX1223-H1243	TTTAGCAACYYATTGTACCGR	-	Speirs *et al.* (2010)
	GNSB914	AAACCACACGCTCCGCT	35	Gich *et al.* (2001)
	GNSB914-H927	GCTTGTGCGGGCCC	-	Speirs *et al.* (2010)
	GNSB941-H958	TTCTTCGYGTTGCATCGAATT	-	Speirs *et al.* (2010)
Type 1851	CHL1851	AATTCCACGAACCTCTGCCA	35	Beer et al.(2002)
Microthrix parvicella	Mpa645	CCGGACTCTAGTCAGAGC	20	Erhart et al. (1997)
C. "Monillibacter batavus"	MC2-649	CCGGACTCTAGTCAGAGC	35	Snaidr et al. (2002)
Type 0041/0675	TM7905	CCGTCAATTCCTTTATGTTTTA	20	Hugenholtz et al. (2001)
Thiothrix spp.	G123T	CCGTCAATTCCTTTATGTTTTA	40	Kanagawa et al. (2000)
Haliscomenobacter hydrossis	HHY	GCCTACCTCAACCTGATT	20	Wagner et al. (1994)

*FA: Formamide percentage; Comp: Competitor probe; H: Helper probe

RESULTS

Many filaments were present in 100% of the samples, which implies that bulking still frequently occurs in MBR plants treating municipal wastewater. In 14 (100%) of the samples, several *Chloroflexi* (GNSB941+CFX1223 probe) filamentous species were observed (figure 2). Members of *Chloroflexi* are responsible for degradation of soluble microbial products (SMP) including carbohydrates and cellular materials, which consequently reduces membrane fouling potential [3]. The transmembrane pressure was not significantly increased from February (0.04 bar) to May (0.11 bar). Co-dominating (FI>5) populations of *Caldilinea* (probe caldi0678), Type 0092 (probe CFX197) (figure 3), *Candidatus* "Monilibacter batavus" (figure 4) and Type 0803 (probe T0803-0654 (figure 5), were observed. Type 0914 (probe CFX67a) filaments were common (FI>3). Type 0092 variante B (probe CFX223) and *Thiothrix* spp. (probe G123T) were observed in 10 of the samples (FI<3). Occurrence of Type 0041/0675, *Haliscomenobacter hydrossis* (4 samples with FI>2), and Type 1851 (4 samples with FI>5) were estimated by conventional microscopy. No fluorescence signal was detected with the probes TM7905 (Type 0041/0675), HHY (H. hydrossis), CHL1851 (Type 1851). Canonical correspondence analysis (CCA) was used to reveal the relationships between filamentous bacteria occurrence, operational parameters (figure 6) and influent characteristics (figure 7). In the investigated range of reactor temperatures (Tr) (18-28ºC), a rise in temperature resulted in a higher *Thiothrix* abundance. Conversely, Type 0092 and Type 0914 were negatively related with Tr. The CCA biplot showed a positive relationship between the abundance of *C.* "M. batavus"and *Thiothrix* spp., and organic loading rate (OLR) and excess sludge production (ESP). The influent increase in the availability of C and N might have contribute positively to the change in relative abundance of C."M. batavus" and Type 0092, while *Thiothrix* and Type 0914 were negatively related. Type 0914 was positively related with hydraulic retention time (HRT), contrary C. "M. batavus"and *Thiothrix* were negatively related with HRT.

Table 2. Operational and physico-chemical parameters of MBR WTP

Parameters	Unit	Acronym	Mean	Range
Excess sludge production	Kg d^{-1}	ESP	1004	180-2326
Hydraulic retention time in reactor	h	HRT	56	47-65
Organic loading rate	Kg BOD/kg MLVSS day	OLR	0.014	0.010-0.020
Mixed liquor suspended solids	mg l^{-1}	MLSS	9853	8232.-11648
Mixed liquor volatile suspended solids	mg l^{-1}	MLVSS	7626	6127-8796
Temperature in reactor	°C	Tr	22.0	18.3-28.4
Percentage of Mixed liquor volatile suspended solids	%	%MLVSS	77.3	74.0-80.5
pH in influent		pH_inf	8.18	8.04-8.36
Biological oxygen demand (5 days) in influent	mg l^{-1}	BOD_5_inf	234	180-290
Chemical oxygen demand in influent	mg l^{-1}	COD_inf	521	371-636
Conductivity in influent	µS/cm	Cond_inf	2780	2680-2830
Total nitrogen in influent	mg l^{-1}	TN_inf	69.6	60.1-79.7
Total nitrogen loading rate	Kg TN/kg MLVSS day	TNLR	0.003	0.00-0.01

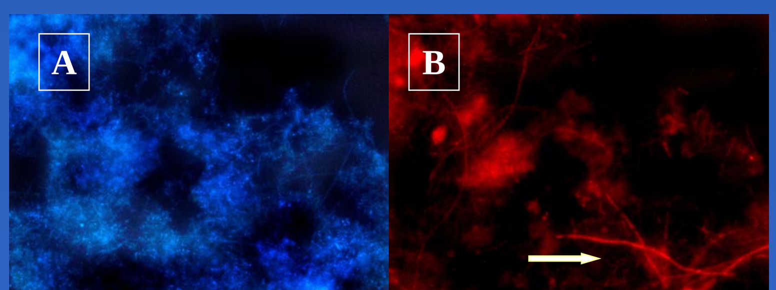

Figure 2: *Chloroflexi*, A: DAPI staining, B: CFX1223/GNSB941 mix probes, 1000X

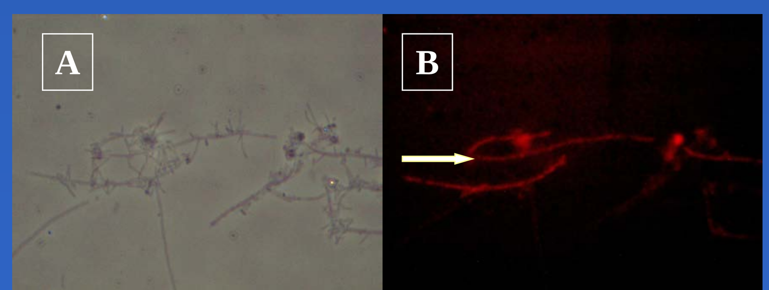

Figure 3: Type 0092 variant A, A: phase contrast, B: CFX197 probe, 1000X

Figure 4: *Candidatus* 'Monilibacter batavus', A: MC2-649 probe, B: phase contrast, 1000X

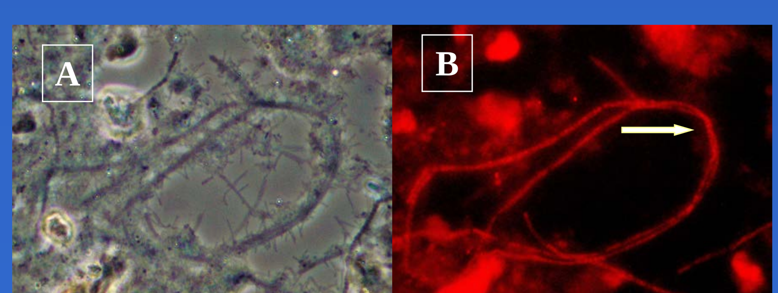

Figure 5: Type 0803 variant A, A: phase contrast, B:T0803-0654 probe, 1000X

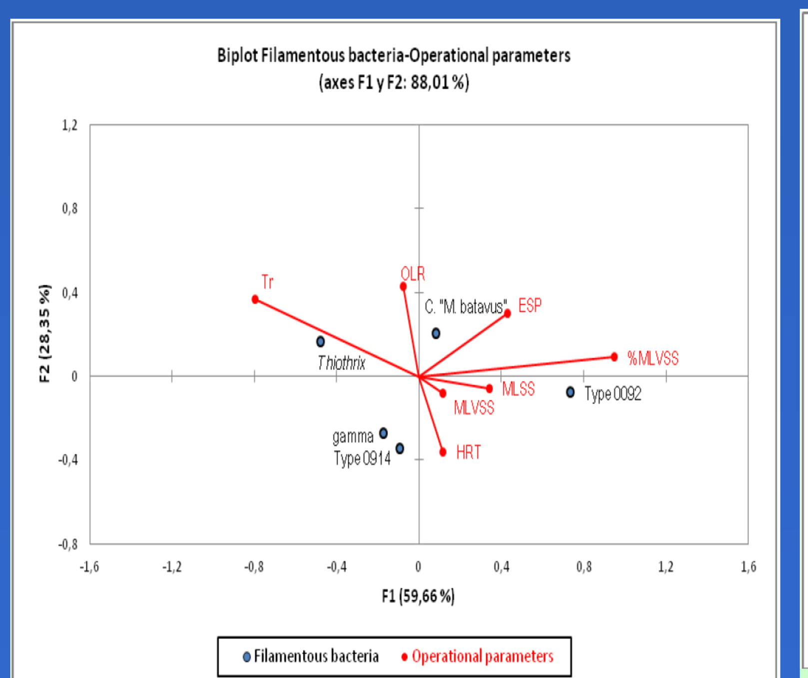

Figure 6: CCA between filamentous bacteria and operational parameters

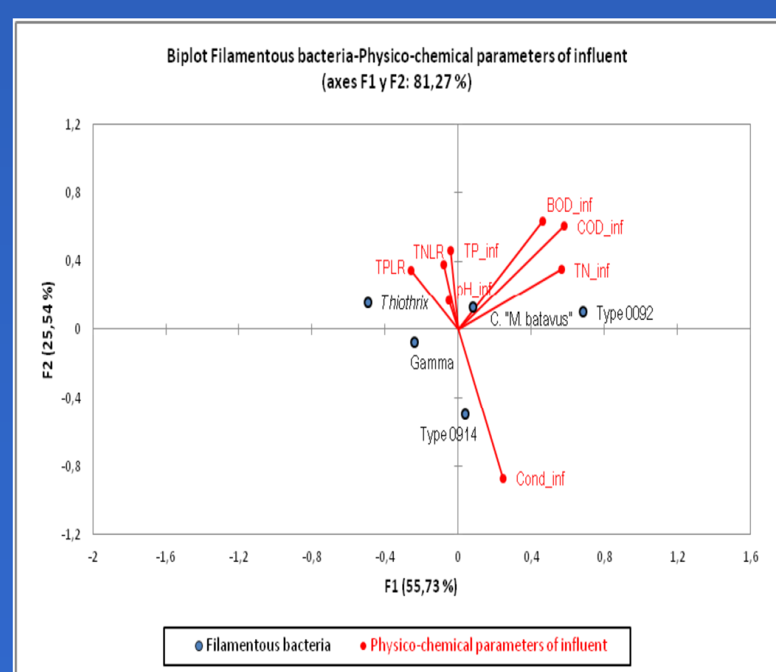

Figure 7: CCA between filamentous bacteria and physico chemical parameters

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REFERENCES

[1] Meng F., Zhang H., Yang F., Li Y., Xiao J., Zhang X. (2006) Effect of filamentous bacteria on membrane fouling in membrane bioreactor. J. Membr. Sci. 272:161-168

[2] Miura Y., Watanabe Y., Okabe S. (2007) Significance of Chloroflexi in performance of submerged membrane bioreactors (MBR) treating municipal wastewater. Environ. Sci. Technol. 41:7787-7794.

[3] Parada-Albarracín J., Marin E., Pérez J, Moreno B., Gómez M. (2012) Evolution of filamentous bacteria during urban wastewater treatment by MBR. J. Environ. Sci. Hlth. (A) 47:863-872