Enviromental Ordination of Filamentous Bacteria in Activated Sludge

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Introduction

New strategies and/or biological treatment systems are in continuous development, in order to decrease the operational costs and to compliance the energy efficiency and quality objectives of wastewater treatment plants (WWTPs). One of the frequently occurring operational problems in WWTPs is foaming and bulking (fig. 1a, b), which is mainly associated with excessive growth of filamentous bacteria (fig. 1c). Studies about the dynamics of filamentous bacteria population in activated sludge, often related to bulking and foaming episodes, have mainly focused on the elucidation of the taxonomic position, in situ ecophysiology, presence and distribution, as well as strategies for controlling the populations. However, the published works about the biological process from the point of view of the environmental interpretation is still scarce. Therefore, the aim of this study was the environmental ordination of the relationships between biological variables (filamentous bacteria) and physicochemical and operational variables in WWTPs.

Figure 1. Activated sludge separation problems. (a) Foaming. (b) Bulking. (c) Type 021N. Phase contrast, 1000x.

Material & Methods

Sampling: Samples from activated sludge (n=140), influent (n=420) and treated effluent (n=140) were collected every fifteen days during a year from six bioreactors belonging to four different WWTPs located in Spain (QB, CX, DN and CT).

Filamentous bacteria identification: Fluorescent in situ hybridization (FISH) and conventional microscopy were used to identity filamentous microorganisms present in all samples. A range of oligonucleotide probes targeting different filamentous species was applied (table 1). The procedure was performed according to the guidelines by Nielsen *et al*. (2009). Filamentous bacteria were quantified according to a subjective scoring of filament abundance (range from 0 to 5, no filaments - very many filaments) (Eikelboom, 2000).

Multivariate analysis: Non-metric multidimensional scaling (nMDS) and hierarchical cluster analysis (cluster) were used to evaluate the spatial-temporal variability of bacterial communities by examining the relative distances among samples in the ordination (abundance square-root transformed data; Bray-Curtis similarity; group-average linking). To assess the contribution of the environmental variables to the variability observed in the filamentous bacteria community structure, we carried out distance-based linear models (DISTLM), using parsimonious methods (e.g. BIC, AIC_C). Environmental variables were log-transformed and normalized to eliminate their physical units, prior to multivariate data analyses (euclidean similarity). Distance-based redundancy analysis (dbRDA) was used to visualize the DISTLM. All multivariate analyses were performed with PRIMER v7 (Clarke & Gorley, 2015) with PERMANOVA+ (Anderson *et al.*, 2008).

Table 1. Overview of the specificity of oligonucleotide probes and abbreviatios used in this study.

Morphotypes	Probe name	Coverage	Abbreviation	Reference
Type 021N	G1B	*T. disciformis* (grupo I)	Tdisci	Kanagawa *et al*. (2000)
Type 021N	G2M	*T. eikelboomii* (grupo II)	Teikelb	Kanagawa *et al*. (2000)
Type 021N	G3M	*T. flexilis* (grupo III)	Tflexi	Kanagawa *et al*. (2000)
Thiothrix	TNI	*T. nivea*, *T. unzii*	Tniv	Wagner *et al*. (1994)
Thiothrix	TFR	*T. fructosivorans*, 'T. ramosa'	Tfruct	Kim *et al*. (2002)
Nostocoida limicola I	NlimI91	*Trichococcus* sp.	Trichoc	Liu & Seviour (2001)
Nostocoida limicola II	Noli-644	Ca. 'Alysiosphaera europaea'	Aeurop	Levantesi *et al*. (2004)
Nostocoida limicola II	MC2-649	*Ca.* 'Monilibacter batavus'	Mbatav	Levantesi *et al*. (2004)
Nostocoida limicola II	AHW183	*Nostocoida limicola*	NlimII-chl	Schade *et al*. (2002)
Nostocoida limicola II	Nlim192	*Tetrasphaera japonica*	Tjapon	Liu & Seviour (2001)
Nostocoida limicola III	NlimIII 301+729+830	*Isosphaera* sp.	Isosph	Liu & Seviour (2001)
Type 0803	Caldi-0678	*Caldilinea*	T0803-cal	Kragelund *et al*. (2011)
Type 0803	T0803-0654	Type 0803	T0803-D	Kragelund *et al*. (2011)
Type 0803	T0803ind-0642	Type 0803	T0803-I	Kragelund *et al*. (2011)
Type 0914	CFX67a	Type 0914	T0914-a	Speirs *et al*. (2009)
Type 0914	CFX67b	Type 0914	T0914-b	Speirs *et al*. (2009)
Type 0092	CFX197	Variant A	T0092-A	Speirs *et al*. (2010)
Type 0092	CFX223	Variant B	T0092-B	Speirs *et al*. (2010)
Microthrix parvicella	MPA645	*M. parvicella*	MPA645	Erhart *et al*. (1997)
Microthrix parvicella	MPA60	*M. parvicella*	MPA60	Erhart *et al*. (1997)
Microthrix parvicella	MPAall-1410	*Microthrix*	MPA1410	Levantesi *et al*. (2006)
Microthrix parvicella	MPA-T1-1260	*M. calida*	Mcalid	Levantesi *et al*. (2006)
GALO	Gor0596	*Gordonia* sp.	Gord	de los Reyes *et al*. (1997)
H. hydrossis	HHY	*Haliscomenobacter hydrossis*	HHY	Wagner *et al*. (1994a, b)

Results & Discussion

Seventeen filamentous morphotypes were identified and twenty seven probe FISH hybridized positively in the active sludge samples investigated. As shown in the nMDS plot, the results revealed some differences in filamentous bacteria population between bioreactors (fig. 3), while no seasonal variations were observed (fig. 4). A total of seventeen predictive models (DISTLM) were constructed from the six bioreactors. The dbRDA plot of the bioreactor CX revealed a strong association of several filamentous bacteria with the solids retention time (SRT) (figure 5). Bacteria correlated with increasing SRT (>10 days) were T0803-cal, T0041, T0581, T0092-A/B and *A. europea* (fig. 7a), whereas that *M. parvicella* (fig. 7c), *N. limicola* II (*Chloroflexi*) (fig. 7d), *M. batavus* (fig. 7b), T0041 and CX-02/03 (T1852) were correlated with decreasing SRT.

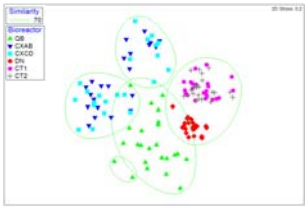

Figure 3. nMDS based on filamentous bacteria abundance data, including clusters at 70% of similarity (circles), according to the bioreactor factor.

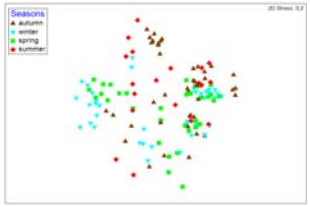

Figure 4. nMDS based on filamentous bacteria abundance data, according to the seasonal factor.

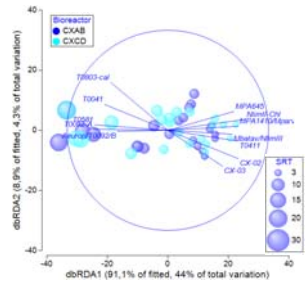

Figure 5. Distance-based redundancy analysis (dbRDA) bubble plot illustrating the DISTLM based on the relationship between SRT (solids retention time) and the filamentous bacteria community structure (CX).

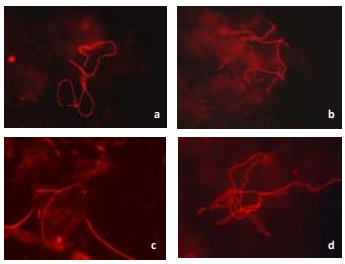

Figure 7. Filamentous bacteria. Epifluorescence, 1000x. (a) *Alysiosphera europea* (Noli-644 probe). (b) *Monilibacter batavus* (MC2-649 probe). (c) *Microthrix* (Mpa-all-1410 probe). (d) *Nostocoida limicola* II-*Chloroflexi* (AHW183 probe).

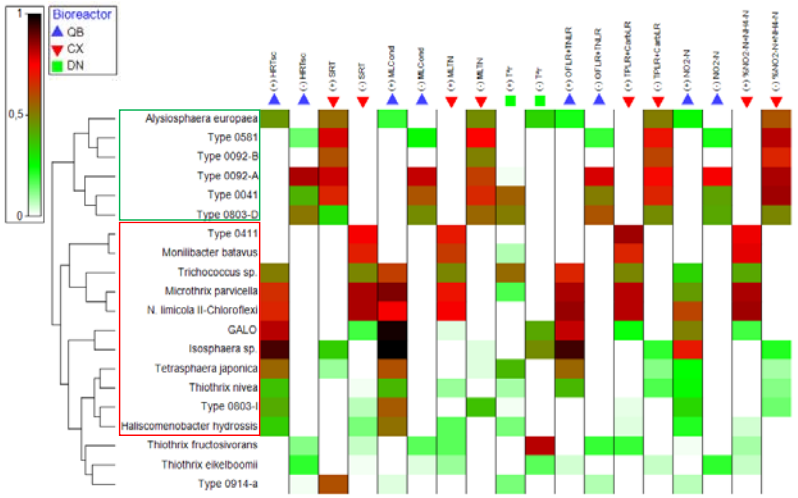

Figure 6. Cluster analysis of the filamentous bacteria according to the nine best DISTLM. The shade plot illustrates the Pearson correlation coefficients (0-1) of the bacteria with the environmental variables. The sign (+) indicates an increase of the variable and the sign (-) indicates a decrease. HRTsc, hidraulic retention time in secundary clarifier; SRT, solids retention time; MLCond, mixed liquour conductivity; MLTN, mixed liquour total nitrogen; T°r, temperature in the reactor; OFLR, oils and fats loading rate; TNLR, total nitrogen loading rate; TPLR, total phosphorus loading rate; CarbLR, carbohydrates loading rate; NO2-N, nitrite nitrogen (effluent); %NO2-N, nitrite nitrogen percentaje (effluent); NH4-N, ammonia nitrogen (effluent).

The figure 6 shows the cluster analysis of the filamentous bacteria according to the nine best DISTLM. The shade plot illustrates the Pearson correlation coefficients of the bacteria with the environmental variables most correlated with the dbRDA1 axes of each of the nine models. The results revealed two groups of filamentous bacteria associated with different ecological characteristics. Group I (green) was associated mainly with high nitrogen removal efficiency, high SRT and low mixed liquor total nitrogen (MLTN), oils and fats loading rate (OFLR), total nitrogen loading rate (TNLR), total phosphorus loading rate (TPLR) and carbohydrates loading rate (CarbLR). In contrast, group II (red) was associated with high hydraulic retention time in secondary clarifier (HRTsc), mixed liquor conductivity (MLCond), MLTN, OFLR, TNLR, TPLR, CarbLR, and low nitrogen removal efficiency and SRT.

Conclusions

The construction of models allows associating the filamentous bacteria to environmental ranges, obtaining valuable information to the knowledge of these dynamic populations. This has allowed to carried out an ecological interpretation of the processes that take place in the biological reactors, and to advance in the knowledge of those filamentous bacteria that cause problems in the treatment plants.

References

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