the relative abundance number does not offer. Furthermore, if sequencing data suggests that a particular plant has only 2–3 predominant filamentous bacteria, these may easily be recognized by microscopy. However, one great advantage of using sequencing is that not only are the filaments identified and quantified, but all other important players in N and P removal are also included in the analyses for further info and use.

We do not know much about the stability of the microbial communities in the WWTPs, including the filaments, and what controls them. Therefore, there is a need to carry out surveys and time-series analyses with at least weekly resolution and correlate this information with 'metadata', that is, all the data available that describe the wastewater composition, plant operation, and performance. We have recently started a 'Biobank' project, in which many participating Danish WWTPs are asked to freeze activated sludge samples weekly and store them for potential later DNA analyses. If problems occur or interesting changes in microbial population or plant operation take place, the samples can be analyzed, and correlations may be established for future knowledge generation and development of suitable control measures.

6.8 FRANCE

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6.8.1 General situation

According to the national database provided by the Ministry of Ecology, Sustainable Development and Energy (BDERU, 2012), in 2012 metropolitan France accounted for 19,500 wastewater treatment plants (WWTPs), designed to treat the wastewater of 98 million of population equivalent (P.E.). The actual load reached a value corresponding to 76 million of p.e. in 2012. Activated sludge processes represent 36% of the French WWTPs and treat 73% of the total load generated as urban wastewater. In addition, the French National Agency for Water and Aquatic Environments (Onema) pointed out that 91% of the WWTPs with a capacity higher than 2000 P.E. used the activated sludge process (Golla *et al.* 2010). This process is well controlled and appropriate to reach the level of treatment specified by the European Urban Waste Water Treatment Directive, especially concerning nutrient removal in sensitive areas.

Metropolitan France accounted for around 6500 activated sludge plants operated under low or very low food to microorganisms (F/M) ratio in 2012 (BDERU, 2012). They provide high treatment performances in terms of nitrogen removal thanks to the high sludge retention times (SRT > 15 days at 12°C).

As shown in Fig. 6.48, 11% of the activated sludge systems with low and very low F/M ratio treated 76% of the French urban wastewater in 2012.

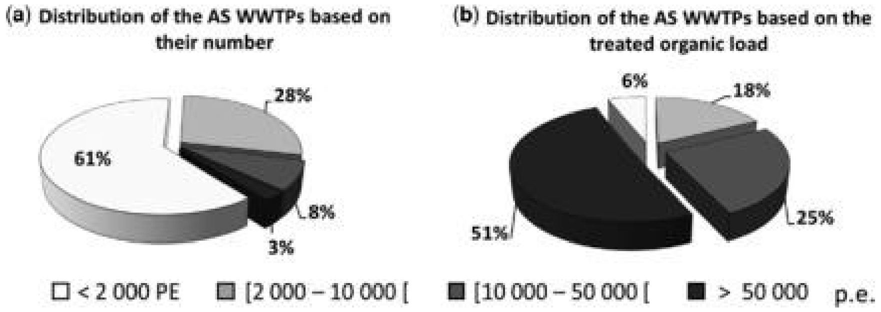

Figure 6.48 Distribution of the activated sludge WWTPs operated under low and very low F/M ratio based on their number (**a**) and on the treated organic pollution (**b**).

6.8.1.1 Bulking and foaming situation before 2005

Activated sludge processes operated under low F/M ratios are known to encounter bulking and foaming issues due to the growth of specific filamentous bacteria, such as M. parvicella or Type 0092 (Jenkins *et al.* 2004). Several surveys related to settling and foaming problems have been performed in France during the last thirty years. In terms of bulking, the first survey, carried out in 1984 on 3860 activated sludge plants (regardless of the F/M ratio), showed that 25% of these plants were subjected to bulking and, in 91% of the cases, the WWTPs were operated at a low or very low F/M ratio. Around 15 years later, a second survey performed on 1000 activated sludge plants showed that an equivalent proportion of WWTPs (25%) had sludge volume index values highlighting settling problems (above 200 mL g^{-1}) (Graveleau *et al.* 2005). As far as foaming was considered, 20% of the 6000 activated sludge plants (considering all F/M ratio) were subjected to foaming (Pujol *et al.* 1991) and these events occurred in majority in WWTPs operated at low or very low F/M ratio (97%) (Pujol *et al.* 1991; Pujol & Boutin, 1989). The dominant filamentous bacteria identified in the different surveys performed in France before 2005 are reported in Table 6.13.

M. parvicella, Type 0041/0675 and Type 0092 were the dominant filamentous organisms in activated sludge bulking and/or foaming in France. This result is coherent with recent publications on filamentous bacteria diversity among Danish activated sludge plants (Mielczarek *et al.* 2012). Indeed, low F/M ratio (<0.2 kg BOD_5 kg^{-1} MLVSS d^{-1}) and slowly biodegradable substrate promote the growth of these filamentous bacteria (Eikelboom, 2000a, b; Jenkins *et al.* 2004; Richard, 1989).

The frequency of bulking or foaming problems did not evolve significantly between 1984 and 2000. A new survey was carried out in 2012, in order to update the previous results as well as to inventory the control strategies employed. Particular attention was paid to the process configuration implemented in the surveyed WWTPs, especially in relation to phosphorus removal.

Table 6.13 Filamentous bacteria identified during bulking and foaming events according to previous surveys with their rank of occurrence and their frequency (%).

	Bulking		Foaming
	Pujol and Boutin (1989)	**Graveleau *et al.* (2005)**	**Pujol *et al.* (1991)**
Type 0041/0675	1	4 (6%)	2 (26%)
Type 0092	2	1 (47%)	4 (5%)
M. parvicella	3	2 (26%)	1 (55%)
Thiothrix sp. and Type 021N	4–5	3 (11%)	–
Sphaerotilus natans	6	0% as major 2% as secondary	–
Mycolata (*Nocardia* sp.)	–	0% as major 5% as secondary	3 (14%)

6.8.2 Current settling and foaming problems and control measures (2012 survey)

The national survey carried out in 2012–2013 aimed at estimating the occurrence of bulking and/or foaming problems due to filamentous bacteria in activated sludge plants operated at low or very low F/M ratios. A questionnaire was sent to 1000 plants taking into account the heterogeneity of French WWTPs, that is, the proportion of treated load (Fig. 6.48), and geographical locations (Durban, 2015). To this aim, five samples were defined according to the WWTP's size; 370 responses were obtained, as shown in Table 6.14. Sample 5 corresponds to the 19 WWTPs treating more than 250,000 P.E., this group was studied separately.

Table 6.14 Response rate according to the WWTP's size.

Maximum Organic Load Treated (P.E.)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Total**	**Sample 5**
	<2000	**[2000; 10,000]**	**[10,000; 50,000]**	**[50,000; 250,000]**	**<250,000**	**≥250,000**
N. WWTPs	3955	1829	552	150	6486	19
N. questionnaires sent	609	282	86	23	1000	19
Response rate (%)	29	41	67	48	36	63

6.8.2.1 Characteristics of the surveyed WWTPs

The majority of the WWTPs include a nitrification/denitrification step (87% for WWTPs below 250,000 P.E. and 100% for the others) (Durban, 2015). Concerning phosphorus, 73% of WWTPs with a capacity lower than 2000 P.E. did not implement any specific treatment whereas 64% of the WWTPs between 2000 and 250,000 P.E. use metallic salt addition to remove phosphorus (Fig. 6.49a). Excluding Sample 5, WWTPs mainly use iron salts (Fig. 6.49b). The proportion of WWTPs treating phosphorus only through enhanced biological removal (EBPR) is very low (around 3%).

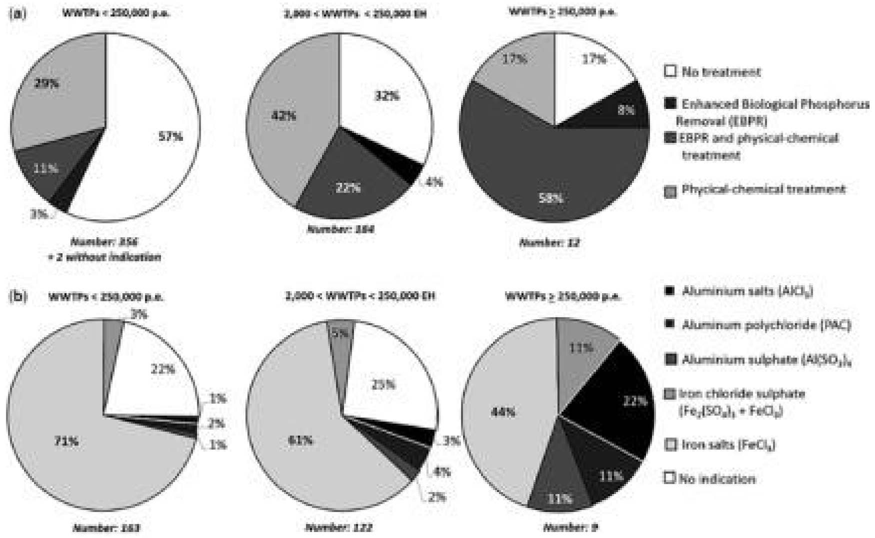

Figure 6.49 Distribution of the WWTPs according to (**a**) phosphorus removal and (**b**) type of chemical product added.

Biological treatment coupled with metallic salt addition is the main process for the WWTPs treating more than 250,000 P.E. (58%) (Fig. 6.49a). The combination of these two processes guarantees a good phosphorus treatment while reducing cost due to chemical addition. These WWTPs generally used metallic salt composed of aluminium or iron in the same proportion for physical–chemical phosphorus treatment (Fig. 6.49b).

An important point to notice is that French wastewater treatment plants are operated at relatively low effective load (in comparison to their design load): 71% and 53% of the design hydraulic and organic capacities respectively, which is coherent with recent data provided by Pujol and Arnaud (2015). A few values of F/M ratios and SRTs were provided in the collected data: the average F/M ratio was 0.07 kg BOD_5 kg^{-1} MLVSS d^{-1}. The average SRT was 29 days for WWTPs with a capacity below 250,000 P.E. and 19 days for Sample 5.

6.8.2.2 Occurrence of settling and/or foaming problems

The settling and/or foaming problems were divided in three categories:

Non-biological origin: a settling and/or foaming problem was observed but was not induced by an excessive growth of filamentous bacteria;
Biological origin: a settling and/or foaming problem was observed as well as an excessive growth of filamentous bacteria;
Unidentified origin: a settling and/or foaming problem was observed but the origin could not be identified.

Results reported in Fig. 6.50 show that settling and/or foaming problems concerned 37% of the WWTPs below 250,000 P.E., and that settling problems were dominating in 30% of the cases. This result is coherent with the surveys of Graveleau *et al.* (2005) and Pujol and Boutin (1989). Settling problems of unidentified origin are relatively important, especially for small WWTPs (<2000 P.E.). Microscopic observations are rarely carried out at this size of WWTPs, making it difficult to see if the occurrence of settling and/or foaming problems was due to excessive growth of the filamentous bacteria.

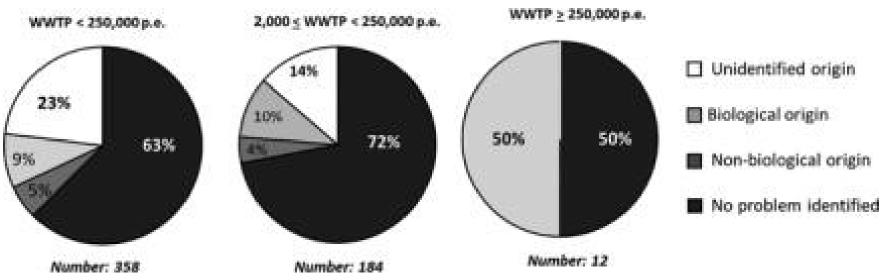

Figure 6.50 Proportion of WWTPs encountering a settling and/or foaming problem in 2012.

Fifty per cent of the WWTPs treating more than 250,000 P.E. encountered settling and/or foaming problems. In three cases out of six, an excessive growth of filamentous bacteria (with two due to M. parvicella) was observed and led to bulking and foaming. One case was due to recurrent bulking induced by M. parvicella and mycolata without foaming. In the two remaining cases, foaming was observed but no filament identified.

According to the survey results, bulking and/or foaming show a seasonal variation and are observed from November to April (winter time). In these large plants, sludge dewatering problems induced by bulking require the addition of larger amounts of polymers. To limit the costs associated with sludge treatment, a curative treatment using chlorination is set up when the diluted sludge volume index (DSVI) reaches a value of around 110–130 mL g^{-1}.

6.8.2.3 Factors influencing settling and/or foaming problems

Similarly to the results reported by Graveleau *et al.* (2005), the main factors inducing settling and/or foaming problems listed by the respondents to the questionnaire are the following (the average frequencies are indicated in brackets):

- Dilution of the influent by clean water infiltration (24%)
- Aeration shortage (22%)
- An important proportion of industrial influent in the wastewater (15%)
- A high concentration of suspended solids in the bioreactors due to sludge extraction/treatment problems (13%)
- Hydraulic overloads (10%)

Contrarily to the previous surveys, the plant's obsolescence does not seem to be a factor inducing settling problems anymore, as upgrades and/or new constructions have been performed to comply with the legislation.

6.8.2.4 Correlation between settling/foaming problems and the process configuration of the surveyed WWTPs

Statistical tests (ANOVA) have been performed in order to highlight the link between the occurrence of settling problems and the process configurations. No significant correlation was found with nitrogen removal or with the presence of industrial effluents even if it had been reported as an influencing factor (see section 6.8.2.3). The only factor impacting significantly the occurrence of settling/foaming was the process employed to remove phosphorus (Fig. 6.51).

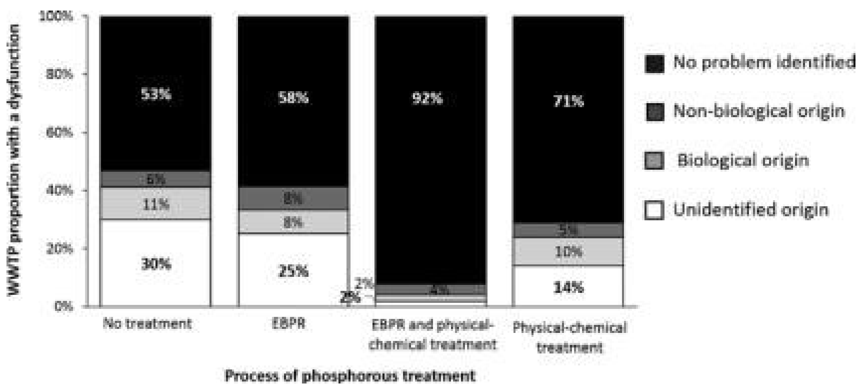

Figure 6.51 Occurrence of bulking/foaming according to the process used to remove phosphorus (WWTPs below 250,000 P.E.).

The influence of enhanced biological P removal was not statistically significant. As is well-known, the addition of metallic salts significantly enhanced settling and therefore reduces settling problems, especially when coupled with EBPR.

6.8.2.5 Control measures

According to the collected data during the last survey, corrective measures to face settling and/or foaming problems mainly consist in modifying operating parameters (64% of the responses) rather than setting up a curative treatment (17%) or both (19%).

Among modified operating parameters, reducing the mixed liquor suspended solids concentration is the most frequent measure (increasing the flow of extracted sludge or the sludge recirculation rate). Secondly, the aeration is upgraded in the cases where oxygen shortage has been identified. In terms of curative treatment, chlorination and metallic salt addition are dominant (27 and 33% of the answers, respectively).

The employed control measures are in agreement with the recommendations reported in the conclusions of the previous French survey (Graveleau *et al.* 2005; Tandoi *et al.* 2006): control the aeration/mixing conditions, sludge return septicity and sludge concentration.

6.8.3 A case study: metallic salt addition in an industrial size pilot-plant subject to M. parvicella bulking and foaming

Durban *et al.* (2016) report the impact of aluminium and iron chloride addition on the performance and the activated sludge microbiology of a BNR industrial-scale pilot plant. The pilot plant was located in the largest wastewater treatment plant of the Parisian area (Seine Aval) and consisted of an activated sludge system designed to treat the wastewater of 1500 P.E. It was continuously fed with pre-treated influent from the WWTP Seine Aval, with an average flow rate of 313 $m^3\ d^{-1}$.

In order to control recurrent M. parvicella bulking and foaming, metallic salts (Me) were added and two commercial solutions were used, containing (i) only $AlCl_3$ [30.5% (w/v) – series 1] and (ii) $AlCl_3$ and a small fraction of $FeCl_3$ [24% (w/v) of $AlCl_3$ and 8% (w/v) of $FeCl_3$, – series 2 called Al-Fe-Cl_3]. The applied dosages and the main results of these tests are reported in Table 6.15. It was shown by the authors that metallic salt addition induced no negative impact on the plant removal efficiencies, except a decrease of P-removal when additions were stopped (potentially due to a wash-out of PAOs during the dosage). The improvement of the settling conditions was certainly due to flocculation mechanisms.

A concentration between 0.2 and 0.5 mmol Me g^{-1} MLSS promotes a stabilization of settling conditions and M. parvicella growth was not affected. This dosage could be used as a preventive treatment. At a salt concentration higher than 0.7 mmol Me g^{-1} MLSS, the DSVI value decreases quickly and filamentous

Table 6.15 Metallic salt addition – applied dosage and main results.

Chemical Solution	Phase	Dosage (mmol Me kg^{-1} MLSS d^{-1})	Duration (*d*)	Observations
$AlCl_3$ (*30.5%*)	Initial state	0	18	DSVI ≈ 157 mL g^{-1} – filamentous index (FI) ≈ 5 with M. parvicella as dominant filamentous bacteria.
	Trial 1	41	70	Stabilization of the DSVI value below 150 mL g^{-1} and foam disappearance without significant impact on filamentous bacteria or floc morphology.
	Intermediate state	0	24	Increase in DSVI and FI – Progressive reappearance of foam.
	Trial 2	137	14	Significant decrease in DSVI (<110 mL g^{-1}) and FI (<3) with no longer free filamentous bacteria in the outside of the flocs, which became larger and more compact. The filamentous bacteria are embedded into the biologival aggregates. Foam disappeared in less than 1 day.
	Final state	0	54	Settling characteristics progressively deteriorated.
$AlCl_3$ (*24%*) **$FeCl_3$** (*8%*)	Initial state	0	44	DSVI ≈ 173 mL g^{-1} – FI ≈ 4 with M. parvicella as dominant filamentous bacteria.
	Phase I	16	21	Stabilization of DSVI and foam disappearance without significant impact on filamentous bacteria or floc morphology.
	Phase II	94	35	Significant decrease in DSVI (<100 mL g^{-1}) with no free filamentous bacteria in the outside of the flocs, which became larger and more compact. The filamentous bacteria were embedded into the biological aggregates.
	Phase III	16	24	Progressive re-opening of flocs with a DSVI increase during Phase III
	Final state	0	54	

bacteria were embedded in the aggregates. Microbiological analyses pointed out the decrease in M. parvicella quantity and suggested a bacterial growth limitation in addition to coagulation effects.

According to these results, aluminum salts should be added progressively and over a few weeks in order to efficiently control M. parvicella bulking without impacting the remaining biomass.

In addition, an interesting correlation between the sludge volume index and the copy number of M. parvicella 16S rRNA gene was highlighted in the work of Durban *et al.* (2016), as shown in Fig. 6.52.

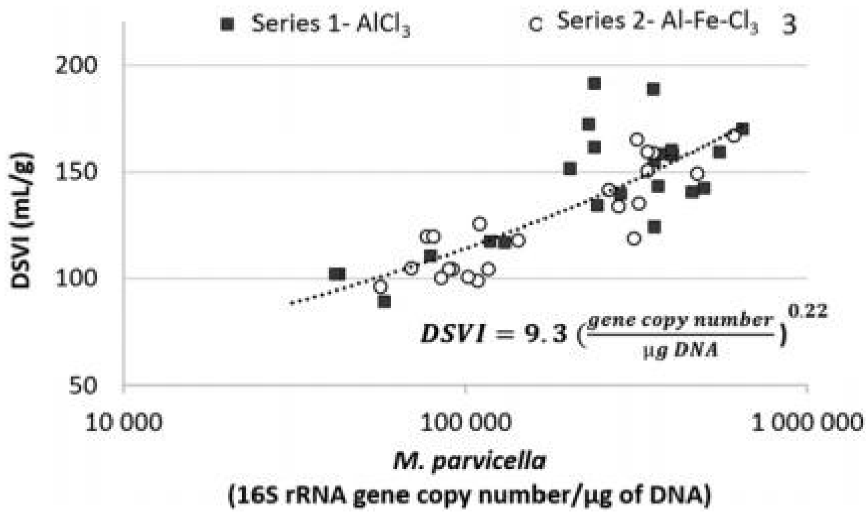

Figure 6.52 DSVI as a function of the number of gene copies of M. parvicella (Durban *et al.* 2016).

Given this correlation, measuring M. parvicella abundance by qPCR would be interesting in order to develop an early warning method to detect bulking and foaming and to follow the impact of control strategies.

This approach deserves to be deepened and extended. Other works are ongoing, developing for example the usage of phages that specifically target filamentous bacteria (Choi *et al.* 2011). Other teams are investigating the possible mechanisms involved in filament development, in order to define the intrinsic parameters for the control of the filamentous growth and to induce a hypothetical reversibility of this phenomenon (Seder-Colomina *et al.* 2015).

6.8.4 Conclusions

Several surveys carried out over the last 30 years showed that 25 to 30% of the French activated sludge wastewater treatment plants still encounter foaming and/or bulking problems. The dominant bulking filamentous bacteria were shown to be M. parvicella, Type 0041/0675 and Type 0092, in agreement with the operating conditions of the facilities (low to very low F/M ratios). The last survey performed in 2012–2013 pointed out that metallic salt addition in order to remove phosphorus significantly reduces the occurrence of bulking problems. Enhanced biological phosphorus removal coupled with metallic salt addition was also shown to be even more beneficial to settling. Control measures are still empirical, although new methods based on 16S rRNA data are being developed to early detect bulking and/or foaming issues and to follow the performance of implemented control strategies.

6.8.5 Acknowledgments

The authors are grateful to the persons involved in the completion of the last survey and especially to David Delage and Adrien Petit for their contribution.

6.9 GREECE

A. Andreadakis, D. Mamais and C. Noutsopoulos

6.9.1 General situation

The adoption of Urban Wastewater Treatment Directive (91/271/EEC) has led to the construction and the operation of a great number of WWTPs in Greece during the last twenty years. The great majority of the Greek WWTPs (77%) operate as extended aeration activated sludge systems with sludge stabilization in the biological stage, while others (23%) enable primary treatment along with biological treatment at high organic loadings and sludge stabilization through anaerobic digestion. Secondary treatment for C and N removal is applied in most of the WWTPs (either as pre-denitrification or as simultaneous nitrification/denitrification systems, e.g. Carrousel, oxidation ditches, etc.) and serve more than 75% of total P.E. (Table 6.16), while phosphorus removal (mainly by EBPR and/or by chemicals addition) is being implemented in WWTPs serving 16.3% of total equivalent P.E. Advanced treatment (sand filtration, micro-filtration, etc.) is applied in 15% of the WWTPs (serving 7.6% of the total P.E.). Chlorination is the main disinfection method (80% of the WWTPs) while UV (10%) and ozonation (3%) are alternative methods also applied.

6.9.2 Reason for dysfunctions and filamentous bacteria identified

The major settling problems encountered in Greek WWTPs are filamentous bulking and foaming. A national survey aiming to record bulking problems and dominant filamentous bacteria in 20 of the largest WWTPs of Greece was conducted by the Sanitary Engineering Laboratory of NTUA. Based on the