Practical Paper

The cationic polyelectrolite use in the bulking control: a case of study

R. A. Nastro, M. Guida and G. B. Fincato

ABSTRACT

The solution for a filamentous bulking episode (associated with foaming) at a municipal wastewater plant in La Spezia (North-West Italy) is reported. Two main bacterial Types were involved: one belonging to the group of the so-called *Nocardia Amarae Like Organisms* (NALO); the other was identified as *Type 0092*. The identification was made according to Jenkins (1986) by the observation of Mixed Liquor fresh samples at direct microscopy before and after Gram and Neisser Stains. To evaluate treatment plant performance, the Sludge Biotic Index (SBI) was also calculated. As a result of different environmental conditions existing between the two treatment lines currently in use, the population dynamics of both protozoan ciliates and filamentous bacteria in the oxidation tanks were also different. The sludge rehabilitation procedure included the use of a cationic polyelectrolite instead of NaClO thus allowing the bulking problem to be solved within 20 hours, without significant negative alterations on treatment performance which improved in the following days. Hence, the proposed method would represent an alternative to the disinfection of Mixed Liquor in bulking control.

Key words | bulking, cationic polyelectrolite, foaming, NALO, SBI, Type 0092

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NOMENCLATURE

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DSVI	Diluted Sludge Volumetric Index
F/M	Food to microrganisms ratio
FISH	Fluorescence In Situ Hybridization
GALO	Gordona Amarae Like Organisms
GS	Gram Stain
HRT	Hydraulic Retention Time
ML	Mixed Liquor
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solids
NALO	Nocardia Amarae Like Organisms
NS	Neisser Stain
PLTO	Pine Like Tree Organisms
SBI	Sludge Biotic Index
SRT	Sludge Retention Time
SVI	Sludge Volumetric Index
TSS	Total Suspended Solids

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INTRODUCTION

The filamentous bulking is greatly widespread, and it represents the main performance problem in activated sludge plants. Recent studies have shown that the use of H_2O_2 and NaClO are not very effective in the proliferation control of filamentous bacteria (Madoni *et al.* 2000; Vismara 1998). Some bacteria such as *M. parvicella*, in fact, are resistant to chlorination, some grow in the sludge

flocs (for example, *Type 0092*) and the sludge is not exposed to a high enough concentration of NaClO to be lethal. H_2O_2 seems less effective on the filamentous growth control (Madoni *et al.* 2000) while it has a positive effect by increasing dissolved oxygen. 7 days of NaClO and 2 days of H_2O_2 treatment were reported to control a bulking episode in activated sludge (Vismara 1998). A great concern of using disinfectants for the bulking control problem is that disinfectants could inactivate both filamentous and floc-forming bacteria and damage the protozoan community in the activated sludge (Madoni *et al.* 2000;Vismara 1998).

This study proposes the use of a cationic polyelectrolite for the control of a filamentous bulking episode (associated with foaming) at a municipal wastewater plant in La Spezia (North-West Italy) in which two main bacteria were involved, (*Nocardia Amarae Like Organisms* [NALO] and *Type 0092*), in the bulking phenomenon.

MATERIAL AND METHODS

Treatment plant

The sewage plant of La Spezia currently receives daily 10,800 m^3 (with a maximum capacity of 24,000 m^3) of wastewater. It consists of three parallel treatment lines, but currently, only two are in use with the capacity of 6,000 m^3/d (line 1) and a 12,000 m^3/d line (line 2). Each line is composed of screen, sand grinder and oil removal, then anoxic (pre-denitrification) and aerobic (pre-oxidation) selectors and finally of an oxidation and a secondary settling tank. During monitoring, primary sedimentation, effluent chlorination units were not active. The system is operated at a F/M ratio of 0,13–0,15 as kg BOD_5/Kg MLSS and with Hydraulic Retention Time (HRT) of 14 hours. The treatment plant receives wastewater from urban sewerage (large part) and industrial activities (small part). During this study, influent BOD load was of 3240 kg/d and the Sludge Retention Time (SRT) was approximately 14 days while SVI was 180 ml/g in both lines.

Analysis

The sewage treatment plant was subjected to monitoring between October and December 2001 period by weekly analysis. During November a bulking problem occurred. The monitoring included mainly filamentous bacteria identification both in Mixed Liquor (ML) and in foam samples. Wet mounts (in duplicate) were studied under direct illumination in contrast phase at 1000 × magnification to determine morphological characteristics of various filaments. Slides were stained using Gram and Neisser staining techniques. Stained slides (in duplicate) were examined under oil immersion and direct illumination at 1000 × magnification. The individual abundance level of each filament was determined using the scoring technique outlined by Jenkins *et al.* (1986). Each filament was scored on a scale between 0 and 6 (integer scores had the following meanings: 0 = none; 1 = few; 2 = some; 3 = common; 4 = very common; 5 = abundant; and 6 = excessive). The system is referred to as filament abundance on an average "per floc" basis. Filamentous bacteria with individual abundance levels of 4 or more were classified dominant while those with individual abundance levels of 3 or less were classified as secondary. The same procedures were carried out during foam sample analysis (Lacko *et al.* 1999).

Protozoa in the ML were identified according to previously established methods (Madoni 1994). A 50 μl of sample analysed under direct microscopy. Subsequently the amount of each taxonomical unit in two replicate fresh smears containing 25 μl was determined. Small flagellates were encountered at Fuchs-Rosenthal encountering-chamber.

Sludge Biotic Index (SBI) was calculated to evaluate plant performance (Madoni 1994; Nicolau *et al.* 2005). This index is based on the abundance and diversity of the protistan community in the activated sludge of the aeration tank and on the different sensitivity revealed, by some of the micro fauna groups, to physical-chemical and operational factors prevailing in the system as shown in Table 1. To calculate the SBI, it is necessary to find previously the number of species (or supra-specific level whenever species identification cannot be achieved), the abundance of each species and of total micro fauna and to estimate the number of small flagellates. All the analysis were carried out daily, maintaining the ML samples (approximately 500 ml) well oxygenated in 1 litre containers for no more than 4 hours (Madoni 1994).

Table 1 | The statement among SBI, quality classes and diagnosis (Madoni 1994)

SBI	Class	Diagnosis
8–10	I	Stable and well colonized sludge, optimum treatment performance; optimal biological activity
6–7	I	Stable and well colonized sludge, sub-optimal biological activity; fairly good treatment performance
4–5	III	Insufficient biological treatment; moderate treatment performance
0–3	IV	Scarce biological treatment; inadequate treatment performance

The chemical parameters were determined according *Standard Methods* (1998).

During this investigation, the following parameters were considered:

- SVI (Sludge Volumetric Index),
- COD, BOD_5, NH_4^+, NO_2^-, NO_3^- (measured in the influent and effluent),
- Sludge Retention Time (SRT).
- Dissolved oxygen (DO) in the oxidation basins.

Dissolved oxygen concentration was detected by a multi parametric probe (YSI°®)

RESULTS AND DISCUSSION

Chemical analysis

Table 2 shows the influent chemical parameters measured with an automatic sampling system within 24 hours on the 4th of November. The ratio of COD:N:P varied (COD/N from 5,4 to 67,0 with an average of 11,1; COD/Tot-P from 1007 to 11,7 with an average of 117,6; N/Tot-P from 87,2 to 2,1 with an average of 10,6) due to excess nitrogenous and phosphorous components. In fact, a ratio of 200:5:1 was considered balanced in previous studies (Madoni *et al.* 2000; Vismara 1998).

Moreover, the daily variations of COD were noticed. $COD/BOD_5 = 2.0$, in other terms, $BOD_5/COD = 0.45$ ratio indicated a low biodegradable substances content. The pH was about 7,3. The performance of the wastewater

Table 2 | Influent chemical characteristics

Parameters	Unit	Maximum	Minimum	Average
COD	mgO_2/l	925	75	400
BOD_5	mgO_2/l	310	50	190
NH_4^+	mg/l	56	13,4	30,8
NO_3^-	mg/l	22,5	0,4	5,2
Tot-P	mg/l	6,4	0,9	3,4
TSS	mg/l	660	65	225

plant was approximately 90% for COD, 92% for BOD, 98% for NH_4^+ parameters. As a result of filamentous proliferation, Total Suspended Solids (TSS) concentration in the effluent progressively increased, therefore, effluent quality progressively deteriorated. While filamentous growth increased, Diluted Sludge Volumetric Index (DSVI) was also calculated. This parameter is independent from TSS in ML and, hence, it is more useful to estimate the total length of filaments for each floc (Vismara 1998).

Biological analysis: the microfauna

A wastewater treatment plant can be considered as an artificial ecosystem (subjected to extreme environmental conditions) that makes purification possible. A natural population dynamic in ML can be summarized as the following: particulate and dissolved organic matter are used by bacteria for their metabolism, ciliates feed on bacteria while some ciliates and small metazoans feed on bacteriophage ciliates. Ciliates (*Ciliophora*) are highly present in ML (they form approximately 9% of MLSS) (Madoni 1994). The subclasses such as *Gymnostomatia, Hypostomatia, Suctoria, Hymenostomatia, Peritrichia, Heterotrichida* and *Hypotrichida* are also present. Three functional ciliates group (all feeding on bacteria) are recognizable in ML:

- free-swimmers: ciliates swimming in ML liquid fraction and so in suspension in the sedimentation tanks (e.g. *Paramecium spp, Colpidium colpoda*);
- crawlers: they lives on flocs surface (e.g. *Chilodonella uncinata, Aspidisca cicada, Trochilia minuta*);

- attached: they are fixed to the flocs by a peduncle; during the secondary sedimentation, they fall down with the flocs (e.g. *Epistylis plicatis, Vorticella convallaria, Zoothaminium spp., Opercularia spp.*)

As well as testate and naked Amoebas, little flagellates and small metazoa (Rotiphers, Nemathods, Terdigrads) are normally present in activated sludge.

Protozoan community composition enables the collection of relevant information about the treatment performance (Madoni 1994; Madoni *et al.* 1993). Madoni (1994) demonstrated a significant correlation between the prevalence of each group and the treatment plant performance as given in Table 3.

After studying protozoan colonization dynamics and correlation between the single functional group and the operational parameters, SBI was defined according to Madoni (1994). It takes into account both protozoan community density and biodiversity. Species particularly resistant to environmental stresses (presence of toxic substances, low oxygen concentrations) as *Opercularia microdiscum.* and *Vorticella microstoma* are considered in the SBI calculation separately from the attached growth group (Table 4).

In this study, SBI has been calculated since the 31st of October, in order to collect further data about the treatment plant dynamics. In fact chemical-physical parameters, even if essential, cannot describe what is really happening in the plant, especially in the oxidation tanks. The protozoan community analysis showed a very good treatment efficiency. The activated sludge, in fact, presented first class quality (Table 1). A small increment in the number of flagellates was detected on the 7th November in both lines (probably caused by an anomalous inflow). Thus, the SBI dropped to second class quality untill the end of the present study.

The analysis of the ciliates population revealed differences between the lines (Tables 5 and 6, Figures 1 and 2), caused by different environmental conditions in the two lines currently used. The prevalence of Testate amoebas (*Arcella spp. Euglypha spp*) in line 1 indicated a high nitrification rate and low F/M ratio (KgCOD/KgMLSS) (Madoni 1994; Madoni *et al.*1993). SRT was decreased by the 14th November resulting, in line 1, in testate amoebas decrease and attached increase (Figure 1, Table 5). Perithrics ciliates were present as the main group in line 2 (Figure 2, Table 6).

Biological analysis: the filamentous bacteria

Since October, a brown dense foam has been present in the oxidation and sedimentation basins with consequently,

Table 3 | Some particular conditions related to micro fauna present in the activated sludge (Madoni 1994)

Dominant Group	Efficiency	Probable causes
Small flagellates	Scarce	Low DO in ML; very high F/M; septic outflow
Small swimmers (<50 μm)	Intermediate	Sludge contact time is insufficient, Low DO in ML
Great swimmers (>50 μm)	Intermediate	Very high F/M
Crawlers	Good	
Attached + crawlers	Good	
Attached	Decreasing	fleeting episode (F/M not constant, high sludge return) High F/M not easily biodegradable
Small naked amoebas and flagellate	Scarce	
Testate amoebas	Good	Low F/M, good nitrification rate, diluted wastewater

Table 4 | Scheme for SBI calculation

Microfauna groups and density on the horizontal entry table		Microfauna species number (except flagellates) in activated sludge and small flagellates (F) in the Fuchs-Rosenthal encounting chamber diagonal							
		>10		8–10		5–7		<5	
Dominant or prevailing group	**Density (ind/l)**	**F < 10**	**10 < F < 100**	**F < 10**	**10 < F < 100**	**F < 10**	**10 < F < 100**	**F < 10**	**10 < F < 100**
Crawlers + Attached* e/o Testate Amoebas	$\geq 10^6$	10	8	9	7	8	6	7	5
	$\leq 10^6$	9	7	8	6	7	5	6	4
Attached* > 80%	$\geq 10^6$	9	7	8	6	7	5	6	4
	$\leq 10^6$	8	6	7	5	6	4	5	3
Opercularia spp	$\geq 10^6$	7	5	6	4	5	3	4	2
	$\leq 10^6$	6	4	5	3	4	2	3	1
Vorticella microstoma	$\geq 10^6$	6	4	5	3	4	2	3	1
	$\leq 10^6$	5	3	4	2	3	1	2	0
Swimmers	$\geq 10^6$	5	3	4	2	3	1	2	0
	$\leq 10^6$	4	2	3	1	2	0	1	0
Small flagellates (>100⊗)	$\geq 10^6$	4		3		2		1	
	$\leq 10^6$	3		2		1		0	

*_Opercularia spp._ and _Vorticella microstoma_ not abundant.
⊗Flagellates number in Fuchs-Rosenthal encounting chamber (diagonal).

partial escape of solids in the effluent. The seasonal drop in temperature, decreased F/M, and reduced or increased sludge age were the reasons for the foaming problem. Consequently, high amounts of *Actinomycetes* different from *Gordonia amarae* like organisms (*GALO*) and Pine Like-Tree Organisms (*PTLO*), resulting from the ramifications angulation (Ferrarese *et al.* 2003), were present in ML and foam samples.

The first ML analysis (on 16th October,) was focused only on the filamentous bacteria and evidenced an excessive amount of *Nostocoida limicola II* in both lines (Figures 7 and 8). As a result of changes in the environmental conditions, *Type 0092* and *NALO* became the co-dominant species in both lines. Main bacterial Types identified and their descriptions are reported in Table 7. Many authors, using the flourescent *in situ* Hybridisation (FISH) technique, have proved the phylogenetic diversity (Thomsen *et al.* 2002) or, at least, the number of different species of filamentous bacterial types as classified by Jenkins (Blackall *et al.* 2000; Howarth *et al.* 1998; Seviour *et al.* 2002; Thomsen *et al.* 2002). It seems they belong in great part to the *Cloroflexi* Phylum (Björnsson *et al.* 2002). Studies of filamentous physiology and isolation *in vitro* (Kohno *et al.* 2002; Ramothokang *et al.* 2003) are available.

According to Figures 3 and 4, Line 2 was characterized by a greater biodiversity than Line 1 at which abundance of *Type 1851* and in a less amount of *Type 1701* was found. Occasionally, *N. limicola I*, *H.hydrossis*, *Thiotrix II* and *Type 021N* have been identified in both lines. During the first sampling, *N. limicola II* was co-dominant with *NALO* in foam, but after two weeks it was replaced by *Type 0092*. *N. limicola II* is often involved in bulking and foaming

Table 5 | Line 1 micro fauna composition and species abundance (n.r.: non relevated)

Identified species (ind/l)	31 Oct.	7 Nov.	14 Nov.	22 Nov.	29 Nov.
Arcella spp	960×10^3	800×10^3	1400×10^3	360×10^3	480×10^3
Euglypha spp.	280×10^3	120×10^3	120×10^3	240×10^3	200×10^3
Emophyris spp.	40×10^3	n.r.	n.r.	40×10^3	80×10^3
Epistylis plicatis	n.r.	80×10^3	n.r.	n.r.	80×10^3
Epistylis spp.	40×10^3	n.r.	1×10^3	n.r.	1×10^3
E. urceolata	n.r.	1×10^3	1×10^3	n.r.	120×10^3
E. thiemanni	40×10^3	n.r.	n.r.	80×10^3	n.r.
V. convallaria	n.r.	40×10^3	n.r.	n.r.	1×10^3
V.fragilis	n.r.	n.r.	n.r.	80×10^3	240×10^3
Vorticella octava	320	n.r.	40×10^3	200×10^3	360×10^3
V. microstoma	n.r.	1×10^3	1×10^3	n.r.	n.r.
Vorticella spp.	n.r.	160×10^3	n.r.	200×10^3	80×10^3
Aspidisca linceus	40	n.r.	40×10^3	160×10^3	n.r.
Aspidisca cicada	560×10^3	520×10^3	600×10^3	40×10^3	80×10^3
T. pusillum	120×10^3	40×10^3	n.r.	n.r.	n.r.
C. uncinata	n.r.	360×10^3	40×10^3	40×10^3	400×10^3
O. microdiscum	n.r.	n.r.	n.r.	n.r.	n.r.
O.coartata	n.r.	120×10^3	n.r.	n.r.	n.r.
Opercularia spp.	1×10^3	n.r.	320×10^3	n.r.	40×10^3
Zoothamnium spp.	n.r.	n.r.	120×10^3	80×10^3	n.r.
Dexiotrica spp.	n.r.	n.r.	n.r.	1×10^3	1×10^3
Euglena spp.	80×10^3	40×10^3	200×10^3	n.r.	40×10^3
Nemathods	n.r.	n.r.	n.r.	n.r.	80×10^3
Rotiphers	200×10^3	360×10^3	360×10^3	320×10^3	200×10^3

episodes (Lemmer 2000; Madoni *et al.* 2000; Meric *et al.* 2003), although the produced foam seems to be less stable than the one caused by *NALO* for lower cellular wall hydrophobicity (Toprak 2004). *Type 1851* was constantly present in foam but in a lesser amount than *NALO* and *Type 0092*. The role of *Type 1851* in foam formation is still to be discussed; it is not clear if it is the cause of its formation or simply it contributes to support it (Lemmer 2000). In

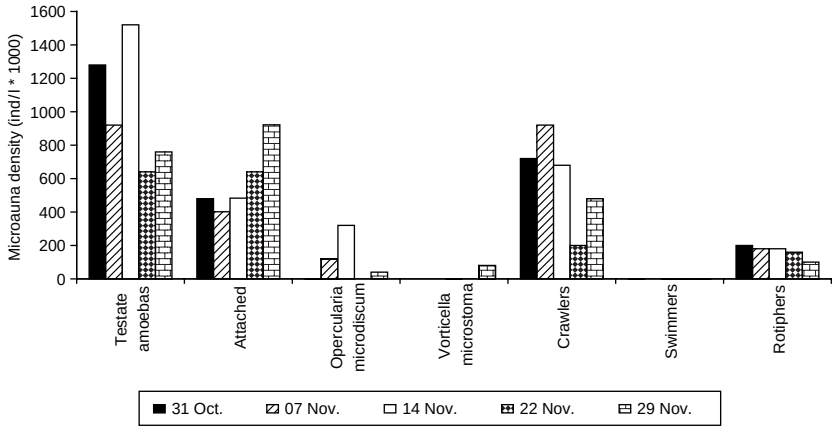

Figure 1 | ML micro fauna composition by time in Line 1.

Figures 3 and 4, dominancy means the percentage of samples in which every type had an abundance index equal or higher than 4 (Lacko *et al.* 1999). The differences between filamentous bacteria found in the oxidation tanks, do not concern the number of species present as much as their relative abundances. For instance, in Line 1, even if Type 1701, Type 0092 and NALO were present in 100% of samples, *Type 0092* has been prevalent in 83% of ML samples. In Line 2 *NALO* and *Type 0092* were found in all samples (100% abundance) but they have been dominant at 50% while *Type 1851* was dominant at 33% of ML samples.

The differences found in both ciliates and bacterial populations between the lines, could be caused by the following factors:

- Different aeration systems. In the oxidation tank of Line 1, fine bubbles of DO are infused by plastics plate shaped diffusers, while in Line 2, aeration is supplied by submerged turbines supercharged with air compressors. As a result, different DO concentrations were measured in the lines (an average of 3 mgO_2/l for Line 1 and 2,3 mgO_2/l for Line 2).
- Unequal influent flow rates between two lines. Sludge return rates were not balanced: Line 1 was characterized by a higher amount of solids and therefore activated sludge age was higher while F/M was lower than Line 2 (Andreottola *et al.* 2000).

Abatement of filamentous bacteria

- NaOCl application

As *Type 0092* abundance index reached the fifth class, the option to chlorinate the return sludge was considered. An experiment was made in order to calculate the minimal

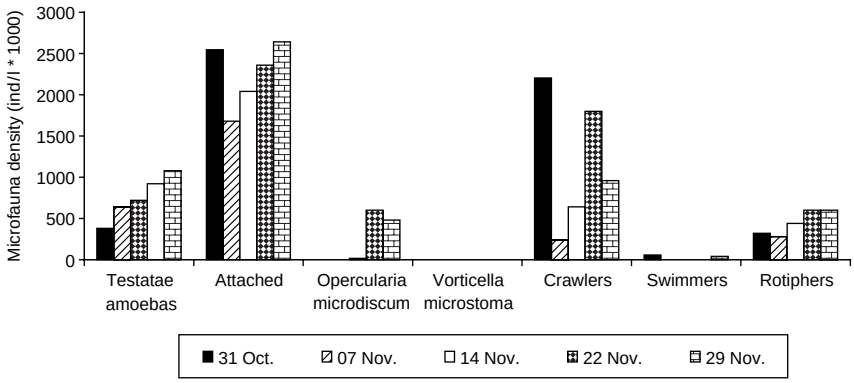

Figure 2 | ML micro fauna composition by time in Line 2.

Table 6 | Line 2 micro fauna community composition and identified species abundance (n.r.: non relevated)

Identified species (ind/l)	31 Oct.	7 Nov.	14 Nov.	22 Nov.	29 Nov.
Arcella spp	260×10^3	520×10^3	680×10^3	640×10^3	760×10^3
Euglypha spp.	120×10^3	120×10^3	140×10^3	80×10^3	160×10^3
Emophyris spp.	n.r.	n.r.	n.r.	n.r.	160×10^3
Epistylis plicatis	540×10^3	360×10^3	400×10^3	n.r	1080×10^3
Epistylis spp.	140×10^3	200×10^3	260×10^3	40×10^3	440×10^3
E. urceolata	1260×10^3	800×10^3	1020×10^3	680×10^3	n.r.
E. thiemanni	80×10^3	n.r.	200×10^3	400×10^3	1×10^3
V. convallaria	140×10^3	80×10^3	60×10^3	n.r.	80×10^3
V.fragilis	n.r.	n.r.	n.r.	40×10^3	40×10^3
Vorticella octava	220×10^3	120×10^3	100×10^3	320×10^3	520×10^3
V. microstroma	40×10^3	n.r.	1×10^3	n.r.	n.r.
Vorticella spp.	n.r.	120×10^3	n.r	1×10^3	n.r.
Aspidisca linceus	n.r.	40×10^3	80×10^3	80×10^3	n.r.
Aspidisca cicada	600×10^3	n.r.	420×10^3	480×10^3	320×10^3
T. pusillum	140×10^3	n.r.	40×10^3	80×10^3	120×10^3
C. uncinata	320×10^3	200×10^3	100×10^3	640×10^3	520×10^3
O. microdiscum	n.r.	n.r.	16×10^3	600×10^3	480×10^3
Zoothamnium spp.	1×10^3	n.r.	1×10^3	1×10^3	n.r.
Paramecium spp.	n.r.	n.r.	n.r.	n.r.	40×10^3
Rotiphers	600×10^3	280×10^3	440×10^3	600×10^3	600×10^3

NaClO concentration necessary for filamentous growth control, estimating at the same time the possible toxic effects on protozoa. 3, 6 and 9 g ClO^-/KgMLSS of NaClO concentrations have been tested with a 10.5% NaClO starting solution and MLSS equal to 3.3 g/l ML (Vismara 1998). NaClO was dosed in ML of Line 1 oxidation tank, where *Type 0092* was very abundant. Four containers with a 30 cm border were prepared, 1 litre of ML was added in each container and oxygenated by two air-vents with porous septum set as far as possible from each other, and stirred by pipette every 15 minutes to maintain MLSS (Mixed Liquor Suspended Solids) in suspension. HRT in oxidation compartment was calculated as 2.5 hours. After 2.5 hours of contact the results given in Table 8 were obtained.

Ciliates present in ML during these experiments were: *Chilodonella uncinata*, *Aspidisca cicada* (belonging to crawlers group and so mobile), *Vorticella acquadulcis* and

Table 7 | Characteristics of main filamentous bacterica identified in ML and in foam samples

FT	GS	NS	PHB	TL	TLL	CS	I	SH	AG	CL/S
N. limicola II	+/−	−	+	IN/E	100/200	+	+	−	−	O Oval 1.2 × 1
NALO	+	+	+	IN	10/20	+/−	−	−	−	Variable
Type 0092	−	+	−	IN	10/60	−	−	−	−	Rettan. 0.8 × 1.5
Type 1701	−	−	+	E	20/80	+	−	+	+	Round rods 0.8 × 1.2
Type1851	+	−	−	E	100– > 500	+/−	−	−	+	Rettan. 0.8 × 1.5
Type 0041	+/−	+	−	IN/E	100–600	+	−	+	++	Squares 1.4 × 1.5
Type 0675	+/−	+/−	−	IN	60–160	+	−	+	+	Squares 1 × 1
Type 1863	−	−	−	E	20/50	+	−	−	−	Oval 0.8 × 1.5
Type 021N	−	+/−	+	E	60– > 500	+	+	−	−	Barrel 1.2 × 1.5
Thiotrix II	−	−	+	E	50/200	+	−	+	−	Rectan. 1 × 1.6

GS = Gram Stain; NS = Neisser Stain; PHB: Polihydroxy butyrate, presence (+) o absence (−); TL = Tricome location; TLL = Tricome length (μm); CS = cell septa present (+) or absent (−); CL/S = Cell Length and Shape; IN = interbridging; AG: Attached Growth present (+), absent (−), excessive (++); SH: Sheath present (+) absent (−); I = indentations present (+) absent (−). (Lacko *et al.* 1999 modified)

Epistylis plicatis (belonging to the attached group, forming resistant forms and telotrocs). *Opercularia coarctata* was not considered in this experiment because it resists several environmental stresses (toxic agents, O_2 deficiency). According to Salvadò *et al.* (2001), NaClO concentrations between 3gClO^-/KgMLSS and 5gClO^-/KgMLSS were the best tolerated by protozoa, especially by Testate amoebas (forming the main protozoan group), *Vorticella aquadulcis* and *Aspidisca cicada*. NaClO toxicity was related to protozoan dimensions and saprobity (Madoni *et al.*1998; Salvadò *et al.* 2001).

A concentration of 6gClO^-/kg MLSS of NaClO caused an important impact also on the floc-formers. Since it was not possible to carry out a further test to verify filamentous

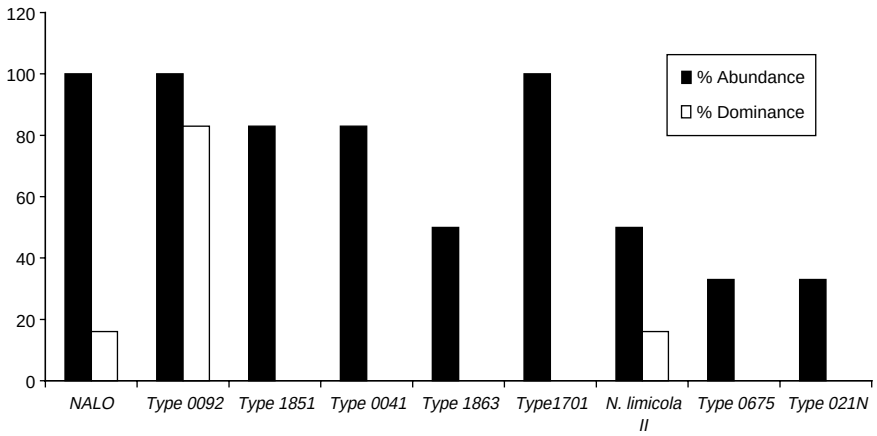

Figure 3 | Percentage frequency of occurrence and dominance of filamentous identified in Line 1 ML.

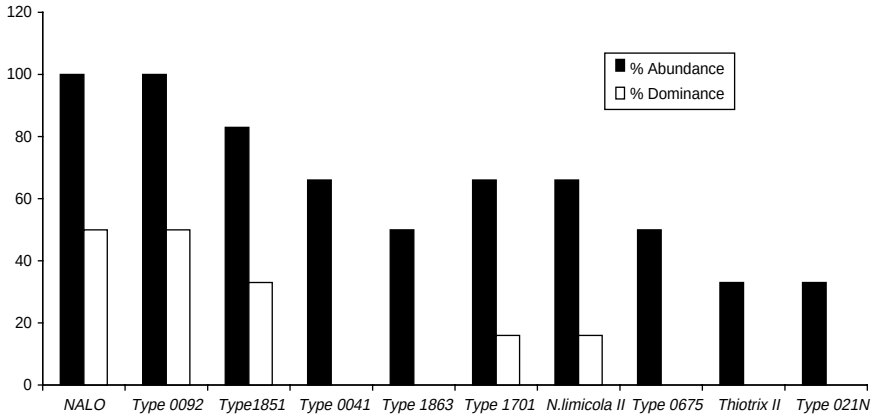

Figure 4 | Dominance and abundance of filamentous in line 2. The values on the y axis are expressed as percentage.

bacteria vitality, this experiment would be indicative of toxic effects of NaClO on activated sludge micro fauna.

- Use of cationic polyelectrolite

Whenever sludge floating in secondary settling compartments was observed, it was decided to add cationic polyelectrolite in the sludge return line as previously applied for foaming control. This method essentially works by neutralizing negative charges present on the surface of organic suspended particles in water and to "consolidate" excess sludge formed in the wastewater treatment plants (Kam & Gregory 2001; Meric *et al.* 2002).

As bulking arose, the following countermeasures were taken:

- cationic polyelectrolite was added at the amount of 1 mg/l to the sludge returned from oxidation compartments. Its concentration was gradually decreased to 0,3 mg/l in the following days (about 3 weeks);
- as both *NALO* and *Type 0092* growth were favoured by high SRT and F/M values between 0.1 and 0.2 kg BOD/kg MLVSS-d, the commonly adopted methods to control their growth are decreasing SRT by increasing F/M values (Fincato 1991) and chlorination of sludge return flows (Madoni *et al.* 2000; Ramothokang *et al.* 2003; Vismara 1998). In this study, the influent load was increased only in the anoxic selectors, passing from three usually in use to two, thus increasing floc-forming bacteria growth for their anoxic metabolism (denitrification). Floc formers are generally thought to have low substrate affinity and high substrate utilization rate, hence they favour higher substrate concentrations. The most common filamentous bacteria use only oxygen as a terminal electron acceptor,

Table 8 | Population dynamics after NaOCl application

Reactor	Population dynamics
Control no NaClO added):	flocs were rather compact, dark coloured, with alive ciliates, excessive filamentous bacteria. In particular, *NALO*. (prevailing) *Type 0092* (prevailing), *Type 021N*, *Type 0675*, *Type 0041* were present
(3gClO^-/KgMLSS)	sludge flocs were still compact with alive ciliates
(6gClO^-/KgMLSS)	a moderate flocs disaggregation was noted with stress of ciliates (increasing of telotrocs, insufficient mobility of crawlers) and decrease of Testate amoebas
(9gClO^-/KgMLSS)	superficial foam formed due to filamentous bacteria but also to sludge flocs came up as shown by microscope analysis of fresh slides. In fact the flocs in ML were few, while groups of filamentous bacteria were noticed

while many of the floc formers can take up readily biodegradable organic matter under either anoxic or anaerobic conditions. Therefore, anaerobic and anoxic conditions can provide a powerful selective pressure against filamentous bacteria. This metabolic selection (Liao *et al.* 2004) is used more frequently to contrast filamentous abnormal growth (Guida *et al.* 2002).

- Aerobic selectors were decreased because DO was too high (about 4,5 mgO_2/l).
- Sludge return flows were regulated to balance their dissolved and suspended inorganic and organic substances contribution to the influent of the two treatment lines.

Microscopic analysis showed that although *NALO* was present permanently in the ML, *Type 0092* became prevailing, occupying completely the space among flocs. It can be supposed, therefore, that both *NALO* and *Type 0092* played an important role in foam production (where *NALO* was highly concentrated) and for the bulking episode. However, it cannot be excluded, that *Type 0092* and *Type 1851* themselves have had a role in foam formation and support.

As F/M was increased in anoxic tanks, DO decreased in aerobic selectors. Effluent NO_3^-, NO_2^- and NH_4^+ concentrations were regularly measured to detect possible nitrification process alterations caused by the polyelectrolite application and by the managerial regulation adopted. The values of those parameters were measured within limits.

During the 20 hours, bulking was drastically regressed and floating solids in the secondary sedimentation tanks became absent. Consequently, an important TSS decrease in the effluent was observed. There was a drastic reduction of *NALO* and *Type 1851* abundance while *Type 0092* became prevailing in both the ML tanks (Figures 7 and 8). On the other hand, an increment of *Thiotrix II* abundance was also noticed in line 2 on the 22nd of November.

As mentioned above, during bulking insurgence floc-formers in ML were very scarce, but due to F/M increase on anoxic selectors, they quickly increased. 48 hours after the addition of cationic polyelectrolite to the sludge return line direct microscopic examinations of ML revealed an increase of floc number and dimension. In particular, an increase of *Zooglea digitata* and *Zooglea amorpha* (several filamentous bacteria main competitors) colonies (Figures 5 and 6).

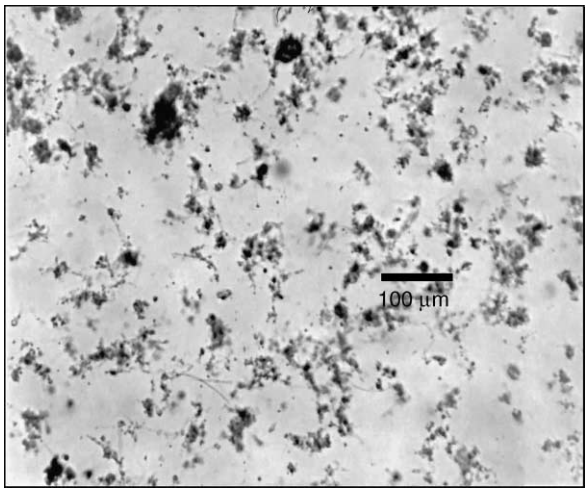

Figure 5 | (100 ×): ML appearance after 24 hours of treatment with cationic polyelectrolite and managerial modifications implementation. (Line 1).

DSVI values decreased as Type 0092 abundance index dropped under the 5th class, and it kept above 150 ml/g during the next two weeks (Figures 7 and 8). The abundance of *NALO* decreased (with the increase of Type 0092 not over the 5th class of abundance in line 2) but the effect on *Type 0092* proliferation was lower and essentially caused by floc-formers competition (Figure 8). *N. limicola II* was also observed in ML samples of both treatment lines. However, neither a treatment performance decrease nor significant toxic effects on protozoa and on floc-forming bacteria were found. It was noticed that Testate amoebas and *Type 0092* found particularly favourable conditions for

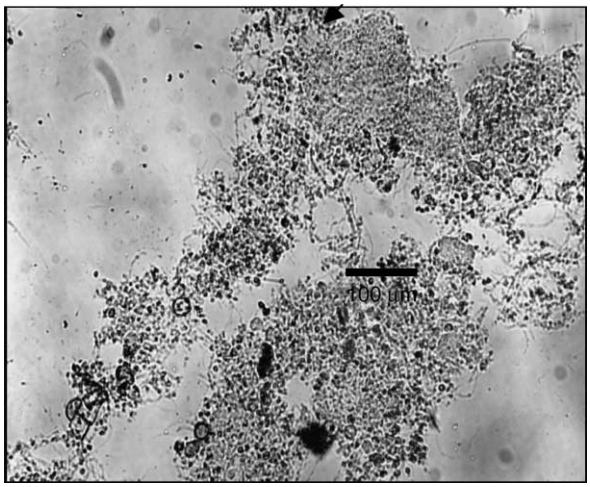

Figure 6 | (100 ×): ML appearance 48 hours after starting polyelectrolite addition and managerial modifications implementation. (Line 1).

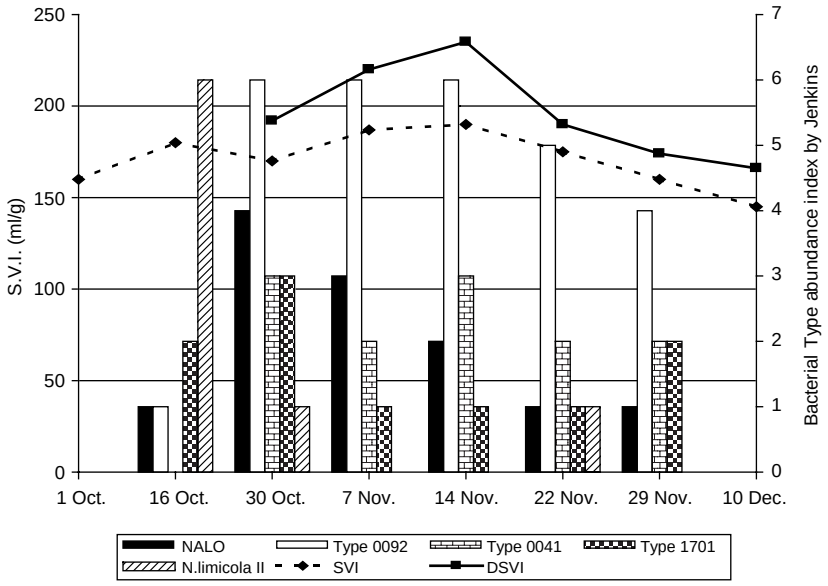

Figure 7 | SVI and DSVI values variation and main bacterial types abundances by time in Line 1.

re-growth in Line 1. As it frequently happens, it was not possible to identify only one cause of the plant dysfunction (Madoni *et al.* 2000; Vismara 1998). For instance, a sudden increase of little flagellates protozoa was observed on the 7th November, probably due to a septic outflow and between the 15th and the 16th of November due to electric blackouts for 10 hours. However, electric blackouts did not reasonably damage *NALO* and not even *Type 0092*. On the contrary, their growth, especially *Type 0092* could have been stimulated (Lakay *et al.* 1999). According to some authors, *Type 0092* could use reduced nitrogen as an electron source in the presence of high oxygen concentrations in as much as it increases when nitrification is complete (Madoni 1994; Musvoto *et al.* 1999) and when reduced nitrogen sources are in excess in the influent and in anoxic tanks (Musvoto *et al.* 1999).

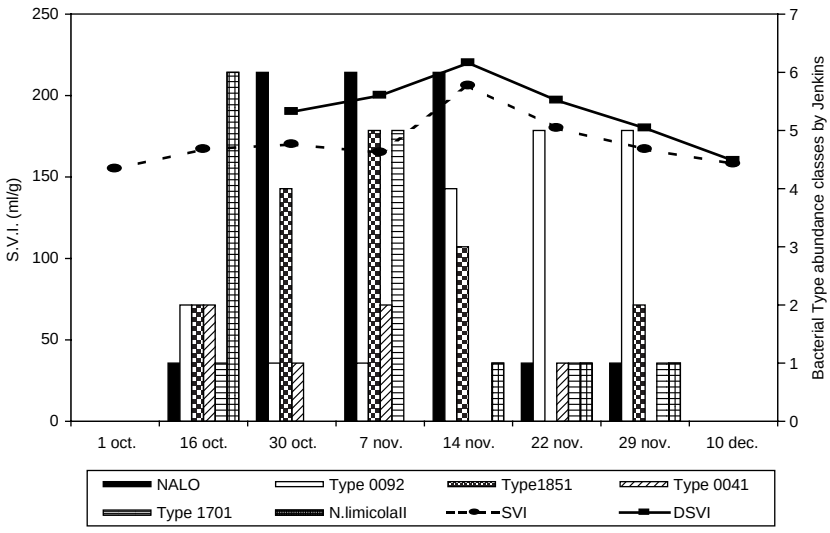

Figure 8 | SVI and DSVI values variation and main bacterial types abundances by time in Line 2.

CONCLUSIONS

This study summarizes the experience of defining the microbial population dynamics and control of foaming and bulking phenomena in a sewage treatment plant which is composed of two aeration lines. *Type 0092* proliferation was strongly abundant in Line 1 where SRT was higher than 10 days. Besides, low F/M and high nitrification efficiency, electricity block-out and a septic outflow have contributed to bulking problem.

The use of cationic polyelectrolyte, proposed in this study, was very effective in foaming and bulking control, although that implies an increase in operational costs; furthermore, it did not negatively affect the treatment efficiency. The NaOCl concentration (an alternative) tolerated by the main protozoan species identified in activated sludge was investigated: it was included between 3 and 5gClO^-/KgMLSS (as 6gClO^-/Kg MLSS of NaOCl concentration seemed to have significative toxic effects, also on floc-forming bacteria). Its effects on filamentous microrganisms were not tested. Moreover, the adjustment of inflow rates between the two treatment lines and sludge return flows improved the performance of the treatment plant and reduced the frequency of foaming and bulking occurrence.

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