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CHARACTERIZATION AND CONTROLLING OF FOAM AND SCUM IN ACTIVATED SLUDGE SYSTEMS

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Summary

Introduction

Activated sludge foaming refers to the formation of stable foam, which is a widespread problem in biological wastewater treatment plants (WWTPs) all over the world. This foam is highly enriched with floating biomass and covers activated sludge tanks, secondary clarifiers and sometimes also occurs in anaerobic digesters. It causes severe operating problems, increases maintenance efforts and may lead to poor effluent quality. It is widely accepted that the formation and stabilization of these foams require gas bubbles, surface active compounds and hydrophobic particles. Gas bubbles in the system originate from aeration and mixing or from gas production in denitrification reactors and anaerobic digesters. Surfactants enter the plant by the wastewater as detergents from households or industry but are also produced by bacteria in the activated sludge. The hydrophobic particles are assumed to be bacteria with a hydrophobic cell surface and hydrophobic molecules adsorbed to the flocs.

Decades of research could not fully clarify the key processes behind this phenomenon. The complexity of the topic and the systems, combined with inaccurate measuring methods, imprecise definitions of terms and confusion of phenomena led to plenty of partially contradicting descriptions and hypotheses.

Goal of thesis

The goal of this thesis is to systematically identify and investigate factors that play a crucial role in activated sludge foaming. This includes studying the growth of suspected foam producing bacteria as well as elucidating factors that control the foaming potential of activated sludge. The gained insights shall support the validation or falsification of common hypotheses in order to suggest future research directions and to point out promising strategies to prevent or control foaming in WWTPs.

Methodology

The research presented in this thesis is based on the identification of three major sub-processes of activated sludge foaming: (1) The development of sludge that is able to produce foam, (2) the actual foam formation and stabilization, and (3) the foam accumulation and distribution in the plant.

Therefore, two parallel pilot-scale plants were operated. They were fed with the same wastewater as a full-scale WWTP in which foaming occurs, but operated under strict control of temperature, dissolved oxygen concentration and sludge retention time. Additionally, several full and pilot-scale WWTPs were monitored in order to find correlations among the identified sub-processes.

To enable these investigations, suitable methods had to be developed: A novel method was developed to quantify the abundance of specific bacteria in activated sludge based on fluorescence in-situ hybridization (FISH). To determine the foaming potential of activated sludge under reproducible laboratory conditions, a protocol was developed combining two different test procedures and including the separation of the solid and liquid fraction of the sludge.

Results

A preliminary study revealed distinct seasonal variations of the bacteria “*Microthrix parvicella*” (*Microthrix*) and different types of nocardioform actinomycetes, organisms that are generally assumed to play a crucial role in the formation and stabilization of stable foams. *Microthrix* was dominating in winter and spring, nocardioform actinomycetes in summer and autumn. Surprisingly, there was no unambiguous correlation of the foam coverage of the reactors with the abundance of any of these organisms.

A mathematical model was developed, describing the observed seasonal variation of *Microthrix* abundance. It is based on the hypothesis that low temperature inhibits lipid uptake of non-specialized heterotrophic bacteria and therefore favors *Microthrix* which is a specialized lipid consumer. This model is not proposed to be used for prediction; it is rather a valuable research tool to identify the relevant mechanisms allowing *Microthrix* to successfully compete in activated sludge systems.

In the pilot-scale plants, nocardioform actinomycetes were not able to remain in the system at a sludge retention time of 10 days and a temperature of 10 or 20°C. Also *Microthrix* could hardly grow under these conditions. It was surprising, however, that *Microthrix* suddenly significantly increased during a period of 20°C. This was possibly enabled by incomplete denitrification. Also unexpected was that *Microthrix* was not found to be responsible for poor floc structure or foaming in the pilot plants. Both phenomena were rather correlated with the operating conditions.

The foaming potential was demonstrated to be controlled by the solids concentration and properties. It was not correlated with the foam coverage of the reactors, the floc structure and surprisingly not with the abundance of the suspected foam producing bacteria *Microthrix*. Furthermore, it is particularly interesting that foam samples and sludge from membrane bioreactors (MBRs) exhibited a considerably higher foaming potential than mixed liquor from conventional plants.

Considering the above mentioned three major sub-processes of foaming, it is remarkable that no correlations were found among the abundance of the identified bacteria, the foaming potential and the foam coverage of the activated sludge tanks.

Conclusions

Particularly important findings of this thesis are the fact that widespread assumptions about causes of activated sludge foaming, particularly about the role of *Microthrix*, were not valid in the investigated systems, as well as the key role of the surface properties of the sludge solids for foaming.

To prevent or control foaming in WWTPs, the results of this thesis strongly support the following recommendations:

- Continuously removing the foam selectively eliminated the flocs that are able to float.
- Chemical additives to improve sedimentation, particularly poly-electrolytes, are suitable as emergency means to reduce the foaming potential of the sludge.
- It seems that nutrient removal WWTPs provide ideal conditions for foaming. Therefore, one should also focus on the implementation of measures to increase the foam tolerance of existing and future plants.

For future research the following conclusions can be drawn:

- A detailed classification system of different types of foam should be developed.
- The dynamics of the microbial population and the foaming intensity have to be considered.
- Foaming should be understood as series of sub-processes which are to be distinguished.
- A promising direction towards the understanding of foaming will be to study the reasons for the increased foaming potential in foam and MBR samples. A starting point could be the hypothesis of accumulated colloidal substances from microbial activity and biomass decay.
- Last but not least, communication and cooperation among experts from different fields should be improved. Promising disciplines are such that intend to prevent foam, e.g. biotechnology where foaming is a common nuisance, or technologies where stable foams are produced, such as mineral flotation and food production.

Zusammenfassung

Einleitung

Die Bildung von stabilem Schaum stellt eine verbreitete Betriebsstörung in Abwasserreinigungsanlagen (ARAs) auf der ganzen Welt dar. Dabei bildet sich ein viskoser, stark mit Biomasse angereicherter Schaum, welcher Belebungsbecken und Nachklärbecken bedeckt und manchmal auf in Faulbehältern auftritt. Dieser Schaum verursacht erhebliche Betriebsbeeinträchtigungen, führt zu mehr Unterhaltsarbeiten und kann die Gewässer verunreinigen. Es wird allgemein angenommen, dass für die Bildung solcher Schäume Gasblasen, oberflächenaktive Stoffe und hydrophobe Partikel notwendig sind. Gasblasen stammen aus der Belüftung und Schlammumwälzung. Oberflächenaktive Stoffe gelangen in die ARA als Detergenzien aus Haushalten und Industrie, werden aber auch zu einem grossen Teil von den Mikroorganismen im Belebtschlamm gebildet. Hydrophobe Partikel sind vermutlich Mikroorganismen mit hydrophoben Zellwänden sowie an die flocken adsorbierte Stoffe.

Trotz jahrzehntelanger Forschung konnten die entscheidenden Mechanismen der Schaumbildung in ARAs bis heute nicht eindeutig eruiert werden. Die Komplexität der Prozesse und Systeme sowie ungenaue Messmethoden und unpräzise Definitionen von Begriffen und Phänomenen führten zu einer Vielzahl von teilweise widersprüchlichen Beobachtungen und Hypothesen.

Ziel der Dissertation

Das Ziel dieser Dissertation ist eine systematische Identifizierung und Untersuchung von Faktoren, welche in der Schaumbildung eine wichtige Rolle spielen. Dies beinhaltet das Wachstum von Bakterien, von welchen man vermutet, dass sie die Schaumbildung fördern, ebenso wie die Bestimmung von Faktoren, welche das Schaumbildungsvermögen steuern. Daraus gewonnene Erkenntnisse sollen aufzeigen, welche verbreiteten Annahmen für die untersuchten Systeme gültig sind, Folgerungen für zukünftige Forschungsanstrengungen erlauben und Ansatzpunkte zur Schaumbekämpfung bieten.

Methodik

Die Forschung im Rahmen dieser Dissertation basiert auf der Unterteilung der Schaumbildung in drei Teilprozesse: (1) Die Bildung von Belebtschlamm, welcher fähig ist Schaum zu bilden, (2) die eigentliche Schaumbildung und -stabilisierung, sowie (3) die Anreicherung und Verteilung des Schaums in der Anlage.

Dazu wurden zwei Kläranlagen im Pilotmassstab betrieben. Diese wurden mit dem gleichen Abwasser beschickt wie eine grosstechnische ARA, in welcher Schaum auftritt, aber unter strikter Kontrolle von Temperatur, Sauerstoffkonzentration und Schlammalter betrieben. Zusätzlich wurden mehrere grosstechnische ARAs und Pilotanlagen untersucht um Korrelationen zwischen den identifizierten Teilprozessen zu finden.

Um diese Untersuchungen durchzuführen, mussten geeignete Methoden entwickelt werden: Einerseits wurde eine Methode entwickelt, um Bakterien im Belebtschlamm zu quantifizieren, basierend auf Fluoreszenz in-situ Hybridisierung (FISH). Andererseits wurde ein Verfahren zur Bestimmung des Schaumbildungsvermögens von Belebtschlammproben unter reproduzierbaren Laborbedingungen entwickelt. Dieses basiert auf zwei unterschiedlichen Belüftungs-Prinzipien sowie auf der Auftrennung der Schlammproben in Fest- und Flüssigphase.

Resultate

Eine Voruntersuchung zeigte ausgeprägte saisonale Variationen der Bakterien „*Microthrix parvicella*“ (*Microthrix*) und verschiedener Typen von nocardioformen Actinomyceten, Mikroorganismen, welche allgemein für die Schaumbildung verantwortlich gemacht werden. *Microthrix* herrschte im Winter und Frühling vor, nocardioforme Actinomyceten im Sommer und Herbst. Überraschenderweise korrelierte die Schaumbedeckung der Becken nicht mit der Häufigkeit dieser Organismen.

Ein mathematisches Modell wurde formuliert, welches die saisonalen Variationen von *Microthrix* abbildet. Es basiert auf der Annahme, dass bei tiefen Temperaturen die nicht-spezialisierten heterotrophen Bakterien in der Aufnahme von hydrophoben Stoffen behindert sind, und dadurch der Spezialist *Microthrix* indirekt gefördert wird. Dieses Modell wurde als Forschungstool entwickelt um die Mechanismen zu identifizieren, welche *Microthrix* erlauben sich im Belebtschlamm zu behaupten; es ist daher nicht für Prognosen einzusetzen.

In den Pilotanlagen konnten sich die nocardioforme Actinomyceten nicht halten bei einem Schlammalter von 10 Tagen und einer Temperatur von 10 oder 20°C. Auch *Microthrix* war nur knapp in der Lage unter diesen Bedingungen zu wachsen. Umso erstaunlicher ist die Tatsache, dass sich *Microthrix* in einer Phase mit 20°C plötzlich stark vermehrte. Möglicherweise wurde dies ermöglicht durch eine unvollständige Denitrifikation. Ebenso unerwartet war, dass *Microthrix* nicht für eine Verschlechterung der Flockenstruktur oder für die Schaumbildung in den Pilotanlagen verantwortlich gemacht werden konnte; vielmehr konnten unterschiedliche Betriebsbedingungen als Ursachen identifiziert werden.

Das Schaumbildungsvermögen in den untersuchten Pilotanlagen und grosstechnischen ARAs war deutlich bestimmt durch die Konzentration und die Oberflächeneigenschaften der Feststoff-Fraktion im Belebtschlamm. Es war nicht korreliert mit der Schaumbedeckung auf den Belebungsbecken, der Flockenstruktur und erstaunlicherweise auch nicht mit *Microthrix*, welches häufig für die Schaumbildung verantwortlich gemacht wird. Besonders interessant ist die Beobachtung, dass

Schaumproben und Schlamm aus Membran-Bioreaktoren (MBRs) ein deutlich höheres Schaumbildungspotenzial aufweisen als Schlammproben aus konventionellen Anlagen.

Betrachtet man die drei oben genannten Teilprozesse der Schaumbildung, so ist es bemerkenswert, dass kein Zusammenhang zwischen dem Auftreten der beobachteten Mikroorganismen, dem Schaumbildungsvermögen und der Schaumbedeckung der Becken gefunden werden konnte.

Schlussfolgerungen

Als wichtige Erkenntnisse dieser Dissertation können folgende Aussagen getroffen werden:

- Weit verbreitete Annahmen über die Mechanismen der Schaumbildung, insbesondere was die Rolle von *Microthrix* betrifft, waren in den untersuchten Systemen nicht gültig.
- Unbekannte Oberflächeneigenschaften der Belebtschlammflocken spielen eine dominante Rolle in der Schaumbildung.

Für die Schaumbekämpfung bekräftigen die Resultate dieser Arbeit die folgenden Empfehlungen:

- Kontinuierliches Abziehen von Schaum entfernt selektiv die flotierberen Flocken.
- Chemische Zusatzstoffe zur Verbesserung der Sedimentation, insbesondere Polyelektrolyte, können das Schaumbildungsvermögen reduzieren und somit für den kurzfristigen Notfalleinsatz geeignet.
- Da man in näherer Zukunft mit Schaum in ARAs leben muss, sollten vermehrt Massnahmen vorgesehen werden, um bestehende und zukünftige Anlagen weniger anfällig für die Auswirkungen von Schaum zu machen.

Für die zukünftige Forschung können aus dieser Dissertation folgende Schlüsse gezogen werden:

- Eine detaillierte Klassifikation verschiedener Schaumtypen sollte erarbeitet werden.
- Die ausgeprägte Dynamik der Schaumbildung sowie der Zusammensetzung der mikrobiellen Population muss unbedingt berücksichtigt werden.
- Die Schaumbildung in ARAs ist eine Serie von Teilprozessen, welche in allen Untersuchungen strikt unterschieden werden sollten.
- Um das Verständnis der massgeblichen Prozesse der Schaumbildung zu verbessern, dürfte es vielversprechend sein, die Ursachen des erhöhten Schaumbildungsvermögens von Schaumproben und MBR-Schlamm zu identifizieren. Die Hypothese, dass in diesen Systemen angereicherte kolloidale mikrobielle Produkte eine Rolle spielen, stellt einen ersten Ansatz dar.
- Nicht zuletzt sollte der Austausch und die Zusammenarbeit mit anderen Forschungsfeldern intensiviert werden. Besonders interessant sind dabei Disziplinen, die Schaum zu bekämpfen versuchen, z. B. Biotechnologie, oder Technologien, welche stabilen Schaum erzeugen, z.B. Lebensmittetechnologie oder Flotation in der Bergbauindustrie.

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Thanks are due to several experts from outside Eawag, particularly to Linda Blackall, Hilde Lemmer, Lisa Müller and Michi Wagner, for their valuable inputs and stimulating discussions.

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A big part of the investigations was performed at two full-scale wastewater treatment plants. I wish to thank the WWTP-Kloten-Opfikon, its manager Christoph Liebi and all staff members for allowing me to operate my pilot plant at their ground, to use their lab and workshop and for the many and diverse support. I am also grateful to the WWTP Thunersee, particularly Adrian Baumann and Gabi Eugster, for their analyses. I would like to thank all staff members from both plants for the willingness to support my project and for the many interesting discussions.

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Last but not least, I would like to thank my wife Sibylle for tolerantly accepting the many long evenings I spent in the office or the lab.

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Chapter 1

Introduction

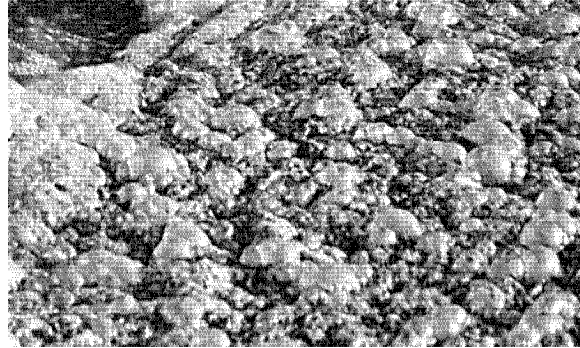
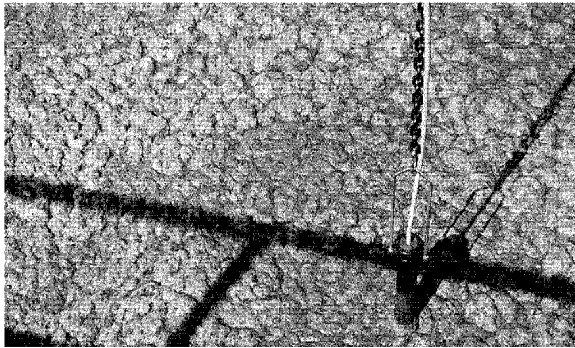
1.1 Foam and scum in activated sludge systems

Activated sludge foaming stands for the formation of stable foam in wastewater treatment plants (WWTPs) covering activated sludge tanks and secondary clarifiers, and sometimes occurring in anaerobic digesters. It causes serious operating problems, increases maintenance and may lead to poor effluent quality. The phenomenon was first reported 1969 (Anonymous, 1969) and has become wide-spread all over the world (Pitt and Jenkins, 1990; Seviour *et al.*, 1990; Pujol *et al.*, 1991; Eikelboom *et al.*, 1998; Wanner *et al.*, 1998), particularly in biological nutrient removal plants. Also membrane bio-reactors (MBRs), a novel technology for wastewater treatment, are often affected.

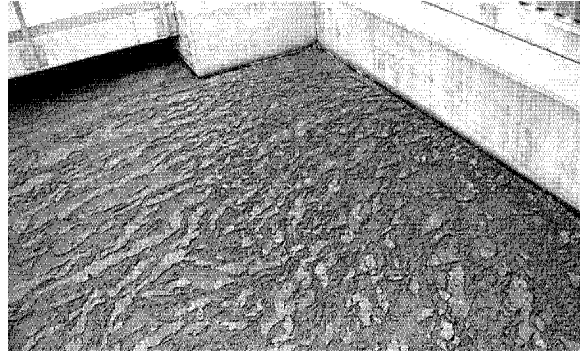
Before describing the goal and approach of this thesis, it is important to provide an overview over the nature of foam in WWTPs and about hypotheses of its formation as well as to discuss the specific challenges of this research field and causes of confusion that exist in literature.

Due to the large number of articles, books and reports from decades of research on activated sludge foaming, this introductory chapter cannot provide a complete literature review. Good reference books on sludge separation problems (bulking and foaming) are Wanner (1994) and Jenkins *et al.* (2004) that are written from the engineers' point of view, as well as Seviour and Blackall (1999), focusing more on the microbiology. Recently, Tandoi *et al.* (2006) published a reference book on sludge separation problems including experience from many countries. Lemmer *et al.* (2000) wrote a recommendable review paper about foam producing bacteria, discussing their growth as well as hypothesized foam forming mechanisms. Martins *et al.* (2004) presents and discusses the knowledge about filamentous bacteria in general, which includes also suspected foam forming bacteria, while Rossetti *et al.* (2005) summarizes what is known about the growth of the suspected foam forming bacterium *Microthrix*.

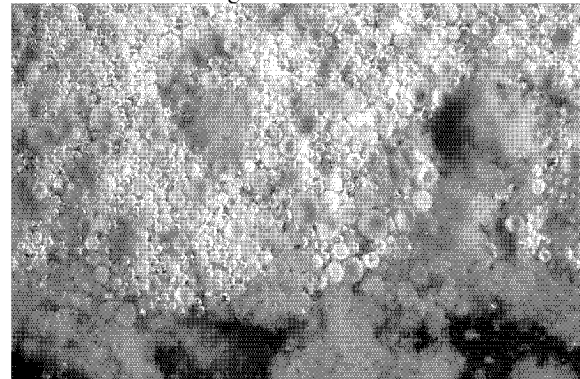
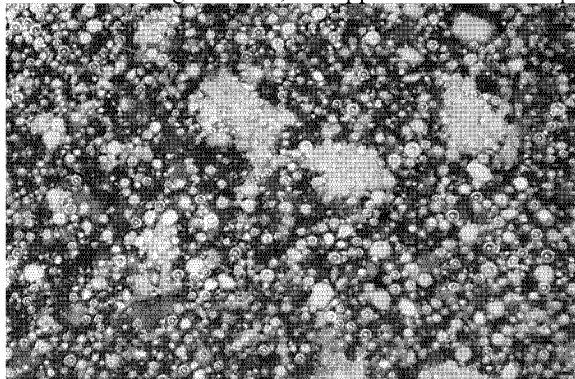
a) foam on aeration basin (bubble size about 5 cm)



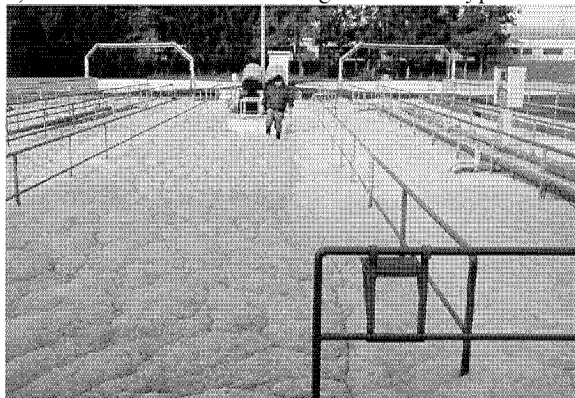
b) stable layer of dense foam on anoxic and anaerobic reactors



c) foam on secondary clarifier consisting of floating sludge covered bubbles ($d=0.5-2.0\text{mm}$), the color is light brown, but appears white on the picture due to the flashlight



d) loose white foam consisting of bacteria type 1863



e) Microthrix forms a net around a bubble (Gram stain)

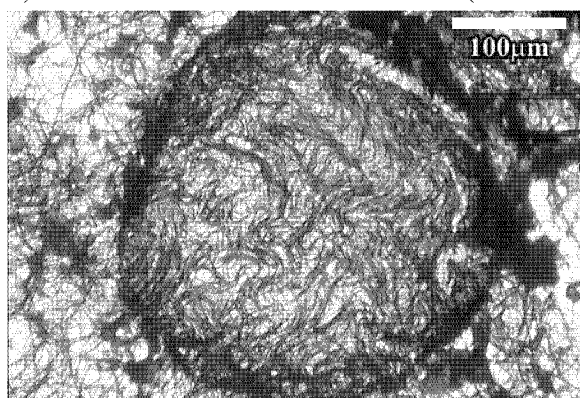


Figure 1-1: Appearance of biological foams in activated sludge systems (photos by T. Hug, photo d by C. Liebi, WWTP Kloten-Opfikon)

1.2 Types of foam

The biological foams investigated in this thesis are viscous and highly enriched with floated biomass. They exhibit very different appearance as shown in Figure 1-1: In the aeration basin, the foam usually consists of relatively large bubbles covered with sludge (bubbles size 1-10 cm, Figure 1-1a), while in anoxic or anaerobic reactors or on secondary clarifiers stable layers with small bubbles and high solids concentration occur (Figure 1-1b). Foam may be formed by agglomeration of individual sludge covered bubbles (Figure 1-1c). These foams are often associated with high concentrations of the Gram-positive filamentous bacteria “*Microthrix parvicella*” (referred to as *Microthrix* in the introduction and conclusions sections of this thesis, chapter 1 and 7) or nocardioform actinomycetes. Sometimes a net of these filamentous bacteria is formed around the bubbles (Figure 1-1e). However, it is not clarified if these particular organisms cause the foam. There exist also other types of biological foam such as the loose but viscous, surfactant-like foam shown in Figure 1-1d that contains mainly the Gram-negative filamentous bacterium type 1863 (Eikelboom and van Buijsen, 1981; Seviour *et al.*, 1997) and occurs in high loaded plants. Furthermore, white loose foam often arises during plant startup, and in the past intensive non-biological foaming was caused by non-biodegradable detergents in the wastewater.

Additionally to the confusion of different types of foam, the phenomenon is often confused with other sludge separation problems, particularly with filamentous bulking, i.e. inhibited sedimentation of activated sludge due to excessive growth of filamentous bacteria. Although both phenomena may occur at the same time and sometimes even the same filamentous bacteria are assumed to be responsible, they are different phenomena and should be strictly distinguished.

Although there are different appearances of foams, there is no unique classification of these types and no unambiguous definitions of terms. The term “*foam*” describes a two-phase system of gas bubbles in a liquid, “*scum*” stands for a layer of froth, impurities or other matter on the surface of a liquid. Although “*scum*” might be the more correct term, in the literature the phenomenon is mostly called “activated sludge foaming”. Hence, in this chapter I use the term “foam” for all foam-like structures in activated sludge systems, including not only white loose surfactant foam, biological surfactant-like foam (Figure 1-1d) and foam with attached biomass on top of aeration basins (Figure 1-1a), but also the stable layer on anaerobic and anoxic reactors (Figure 1-1b) and secondary clarifiers. The term “foaming” I use for the general phenomenon. Because the branched filamentous types of nocardioform actinomycetes (by engineers often called “*Nocardia*” as the most common species was identified as *Nocardia amarae*, today re-classified as *Gordona amarae*) are often highly enriched in the foam, the phenomenon used to be called “*Nocardia* foaming” or “nocardioform foaming” (Jenkins *et al.*, 2004). Rising sludge on the secondary clarifier is often called “floating sludge” or “blanket rising” and might be caused by different reasons. It is important to mention that the terms are not standardized and different people (including researchers from different disciplines and plant operators) might understand different things by the same term.

1.3 Problems caused by foaming

In most affected WWTPs, the occurrence of foam is not only an aesthetical nuisance but causes serious problems:

Environmental pollution:

- If floating sludge cannot be held back, it considerably increases the effluent solids concentration and pollutes the receiving waters.

Operational problems:

- A considerable fraction of the active biomass can be trapped in thick foam layers and therefore be excluded from the intended biological processes. This may limit the plant performance and makes control of solids and sludge retention time (SRT) more difficult.
- Sticky foaming sludge often leads to malfunctioning of electrodes to measure dissolved oxygen and therefore may seriously affect treatment performance and process stability.
- In winter foam can freeze and possibly damage mechanical equipment.
- Intensive foaming reduces the effectiveness of surface aerators.
- Severe foaming in anaerobic digesters, that often occurs simultaneously to foaming in the activated sludge tanks, may significantly reduce the usable reactor volume and therefore lead to serious sludge disposal problems.

Safety risks:

- Foam may overflow onto walkways, creating slippery areas.
- Aerosols may spread suspected pathogenic bacteria that are accumulated in the foam (Stratton et al., 1996).
- Severe foaming in anaerobic digesters may block gas pipes.

Nuisance:

- Foaming requires increased cleaning efforts (and therefore increased operating costs).
- In summer, stable foam may putrefy and cause odor problems.

1.4 Theory and hypotheses about foam formation and stabilization

Foam is generally a dispersion of gas bubbles in a liquid (in “solid foams”, e.g. Styrofoam or bread, the liquid has changed into a solid phase after making the dispersion). Foam of air bubbles in water collapses due to gas diffusion that leads to a growth of large bubbles at the expense of smaller ones (Ostwald ripening) and by rupturing of the thin water layer (lamellae) around the bubbles due to drainage by gravity water flow. Surfactants in the liquid are necessary to produce a stable foam; they reduce the Ostwald effect, lead to a self-stabilizing mechanisms by inducing a liquid flow towards the lamellae region that is becoming thinner (Marangoni effect), and allow the lamellae to become thinner before rupturing. Extremely stable foams are stabilized by an insoluble adsorption layer around the bubbles (e.g. composed of denatured proteins or hydrophobic particles); this prevents Ostwald ripening, may hinder drainage (due to large particles), and mechanically stabilizes the bubbles surface (Walstra, 1989).

Stable foams play an important role in different industries, either as nuisance or as intended product. Many types of foam in biotechnology or food technology are stabilized by proteins and surfactants (Vardar-Sukan, 1998; Bos and van Vliet, 2001). Closer to foams in activated sludge are those containing a high solids concentration, although proteins might play an important role, too. In food technology, foams are often stabilized by a combination of proteins, surfactants and fat globules (e.g. whipped cream and cake batter Brooker, 1993); ice cream additionally contains ice crystals as second solid phase (Goff, 1997). Another type of solids rich foam is produced in “froth flotation” in mining industries (Rao, 2004) to separate valuable ore from unwanted rock by adding particular surfactants to a suspension of grinded mineral in water. Surfactants working as “collector” specifi-

cally adsorb to the ore surfaces making them hydrophobic and allowing them to adsorb to the bubbles in the flotation process. Other surfactants (“frother”) are added to produce a foam in which the floated mineral accumulate in order to be removed.

Foam on activated sludge plants consists of high solid concentration. Therefore, based on the above mentioned theories and technologies, it is widely accepted that the formation of stable foam in activated sludge plants requires gas bubbles, surfactants and hydrophobic particles (e.g. Jenkins *et al.*, 2004).

Gas bubbles are present in WWTPs as air bubbles in the aerated reactors, as nitrogen gas in the denitrification reactors and as carbon dioxide and methane in anaerobic digesters.

Surfactants enter WWTPs with the wastewater as detergents from households or industry, but are also produced by certain bacteria (Desai and Banat, 1997; Lemmer *et al.*, 2000; Pagilla *et al.*, 2002). It is suggested that increased surfactant loads are able to intensively produce foam that is then stabilized by hydrophobic flocs and cells. This would explain the anecdotal experiences of intensive foaming starting within a few hours which cannot be explained by a change of the composition of the microbial population (Wanner, 2000; Jenkins *et al.*, 2004; Joss, 2004).

Only *hydrophobic particles* can be floated by air-bubbles (Gochin and Solari, 1983). In foaming activated sludge systems it has been observed that certain hydrophobic filamentous bacteria, such as nocardioform actinomycetes or *Microthrix* are highly enriched in the foam compared to the mixed liquor. They seem to be selectively floated due to their hydrophobic cell surface. If protruding from flocs, hydrophobic microorganisms make the floc surface more hydrophobic and probably increase their floating ability. Analogously to froth flotation (see above) these organisms might be the actual “collector” compounds in the system. However, freely dispersed hydrophobic filaments seem to be more efficient foam producers (Jenkins *et al.*, 2004).

The ability to produce surfactants and to increase cell surface hydrophobicity is a strategy of bacteria to make hydrophobic substrates available and to attach to the air-water interface where the substrate concentration is considerably higher (Blanchard and Parker, 1977; Dahlbäck *et al.*, 1981; Kjelleberg and Hermansson, 1984).

The role of the extracellular polymeric substances (EPS) and other large organic molecules originating from microbial activity and cell decay is not clarified. These polymers cover the bacteria in the flocs and might therefore define the surface properties of the flocs that are critical for foaming.

Critical for severe foaming in a WWTP is the possibility of *foam trapping*. Trapping and accumulation in certain parts of the plant lead not only to an increased amount of foam on the reactors but also provides a partly isolated compartment with increased solids retention time. This enables slowly growing suspected foam forming bacteria to remain in the plant, collects freely floating suspended hydrophobic bacteria which otherwise would be washed out of the system and accumulates colloidal products of microbial activity and cell decay. From the foam layer, a continuous inoculation of strongly foaming flocs into the mixed liquor occurs, increasing the floating ability of the mixed liquor.

Although the above mentioned hypotheses and experiences suggest that there exists a sound knowledge on foam formation and stabilization, many important factors influencing activated sludge foaming are not known or sufficiently studied. Even in the established technology of froth flotation, mixtures of surfactants often lead to unpredicted effects (Rao, 2004), and controlling of foaming in biotechnology remains to a great extent an empirical art (Vardar-Sukan, 1998) although these systems are better understood than activated sludge in full-scale WWTPs.

1.5 Current strategies to control foaming in WWTPs

Despite decades of research, no universally reliable strategy to prevent or control foaming in WWTPs is known. Many approaches have been reported to be successful in some plants but not to be effectual in others. Since the mechanisms behind foaming are not fully clarified, it is not clear where to start foam prevention.

Current strategies to prevent or control foaming try (1) to reduce the foaming potential of the sludge by inhibiting or damaging foam producing bacteria or changing their surface properties, (2) to control the actual foam formation and stabilization, (3) to limit foam trapping and accumulation, or (4) to reduce the detrimental consequences on the plant. Some approaches intend to solve the problem on a long-term basis, others are suitable as emergency measure.

Common control approaches are the following:

- The continuous removal of foam from the surface, after controlled accumulation in one point of the plant is probably the most successful long-term approach. This does not only keep the problem small by preventing accumulation, but also selectively removes bacteria that are able to float.
- A reduction of hydrophobic substances in the wastewater (oil, grease, long chain fatty acids) which are preferred substrates for nocardioform actinomycetes and *Microthrix* and increase the hydrophobicity of certain bacteria can be achieved e.g. by consequent implementation of oil and grease separation in food processing industries and by banning enzyme addition to grease separators which increases the load of partially digested fat to the WWTP. Long chain fatty acids may also be produced in deposits of sedimented and putrefying sludge in unmixed zones of the tanks.
- Reducing the sludge retention time (SRT) can wash out slowly growing suspected foam producing bacteria. This is successful to suppress nocardioform actinomycetes or *Microthrix*, but nitrifiers will be washed out, too. As the SRT in a stable foam layer is considerably higher than in the mixed liquor, it has to be removed first to prevent inoculation.
- Reducing the SRT also decreases the solids concentration in the mixed liquor which reduces the foaming potential.
- Selectors (contact zones) have sometimes been reported to suppress growth of nocardioform actinomycetes or *Microthrix* although numerous reports of unsuccessful trials exist, too.
- Treating the return sludge with strong oxidants (e.g. chloride, peroxide) or spraying them on the foam surface selectively damages filamentous bacteria protruding out of the flocs. However, disadvantageous effects may be turbid effluent, damage of nitrifiers, and production of chlorinated hydrocarbons.
- Poly-electrolyte alters the floc structure to improve sedimentation but also reduces the foaming potential of the sludge. Aluminum salts and particularly poly-aluminum often additionally inhibit the growth of suspected foam producing bacteria (Nielsen *et al.*, 2005; Paris *et al.*, 2005). Because each sludge has different properties, the product composition and dosage has usually to be adjusted to the particular sludge.
- Antifoaming agents have not found to be successful as most of them were developed for foams without or with low solids content.
- Water sprays are able to partially collapse certain foams.

- Baffles at the effluent of the secondary clarifiers prevent floating sludge from escaping the plant into the receiving waters.
- In anaerobic digesters, foam formation may be reduced by installing mechanical mixers instead of the widespread gas mixing. Lowering the liquid level reduces the risk of overflow and blocking pipes. The foaming potential of the waste sludge can be reduced by thermal treatment prior to digestion (e.g. Westlund *et al.*, 1998; Barjenbruch and Kopplow, 2003).

1.6 Previous research

Since the first report of the phenomenon (Anonymous, 1969), an enormous number of scientific articles and reports about the topic have been published, additional to the anecdotal knowledge and the many unpublished experiences by engineers and plant operators.

Generally, research efforts in connection with activated sludge foaming can be divided into the following groups:

- Surveys reporting and characterizing the foam phenomena and trying to link the problem with specific processes and plant designs; often combined with surveys on filamentous bulking (Pitt and Jenkins, 1990; Seviour *et al.*, 1994; Eikelboom *et al.*, 1998; Wanner *et al.*, 1998).
- Identification systems for filamentous bacteria. A widely used classification was developed by Eikelboom and van Buijsen (1981). Recently, microbiologists developed valuable tools to identify and study bacteria in-situ (summarized by Wilderer *et al.*, 2002).
- Microbiological studies isolating and identifying bacteria from foam and investigating their growth characteristics, in pure or mixed culture (summarized in Seviour and Blackall, 1999).
- Studies measuring and investigating the actual foaming potential of activated sludge using lab-scale “foam tests” (summarized in chapter 6 of this thesis, Hug *et al.*, 2006).
- Engineers’ and plant operators’ efforts to control and prevent excessive foaming problems in their plants. These include not only scientific studies but also reports of empirical trials or descriptions of newly developed technical devices. They cover measures such as the use of different chemical additives, the implementation of selectors to suppress the growth of particular organisms, or how to remove the foam from the surface.

Interestingly, this large number of publications contains a confusing variety of partially contradicting findings and hypotheses. Furthermore, many findings were forgotten and investigations were repeated because of the long period of research but also due to the fact that most research efforts were not continued due to lack of success and therefore limiting financial resources.

1.7 Challenges and causes of confusion

The following section identifies the challenges of research on activated sludge foaming that leads to the confusion in the literature.

- Biological foams occurring in different compartments of WWTPs exhibit different appearance and accordingly may be formed by different mechanisms. However, neither the different phenomena are clearly classified nor the terms describing different types of foam are defined. This leads to a confusion of phenomena and terms. Today, different people (including researchers from different disciplines and plant operators) often understand different things by the same term.

- From the beginning of research on activated sludge foaming, it was observed that filamentous bacteria, often nocardioform actinomycetes or *Microthrix* were accumulated in the floating fraction and it was concluded that they must cause the foaming. This hypothesis might be true, but it has to be noted that only filamentous bacteria could be accurately identified by microscopic methods in those days; nocardioforms are among the easiest to identify. Because the mentioned filamentous bacteria often form an actual net around the bubbles (Figure 1-1e), it is widely assumed that this mechanism stabilizes the bubbles in the foam. However, the effectiveness of froth flotation (Rao, 2004), and the stabilizing effect of oil droplets (Koczo *et al.*, 1991) or milk fat globule in whipped cream (Walstra, 1989; Brooker, 1993) demonstrate that a filamentous nature of the hydrophobic particles is not necessarily required for stabilization. Moreover, extracellular polymeric substances (EPS) or generally macromolecules from microbial activity and cell decay may play an important role in foaming rather than the pure existence of the mentioned bacteria (Forster, 1996).
- Because activated sludge foaming is often associated with filamentous bacteria, the phenomenon is often confused with filamentous bulking, i.e. inhibited sedimentation of activated sludge due to excessive growth of filamentous bacteria. Although both phenomena may occur at the same time and sometimes even the same filamentous bacteria are assumed to be responsible, they are completely different phenomena and should be strictly distinguished.
- The observed foam covering activated sludge tanks and clarifiers in WWTPs is the result of a series of different processes. The following three major sub-processes were identified in this thesis (paragraph 1.9 and Figure 1-2): (1) Generation of activated sludge of a certain foaming potential, (2) the actual foam formation and stabilization and finally (3) the accumulation and distribution of foam in the plant. Not rigorously distinguishing these sub-processes may be a major source of confusion in the literature.
- Research of bulking and foaming seems to be considerably more or at least differently challenging than the traditional investigation of biological degradation processes: (1) *Investigation of biological degradation* processes to remove undesired compounds from the wastewater (COD degradation, nitrification, nutrient removal) is based on the measurement of those particular substances and intermediate products. Often, several different organisms are able to perform these biochemical reactions. Accordingly, mathematical models were developed that are based on virtual concentrations of groups of organisms, which represent total transformation activity rather than actual biomass (e.g. ASM models, Henze *et al.*, 2000). (2) In *research on filamentous bulking* particular organisms have to be investigated, because the physical occurrence of these filamentous organisms inhibits sludge sedimentation. Moreover, in most cases it is unknown which substrates these organisms consume under the competition in the activated sludge. Hence, research on filamentous bacteria needs to identify and quantify the actual biomass of the specific interesting organisms. (3) Even more challenging is *research on foaming*. Not only the physical occurrence of foam producing bacteria is critical, but also their surface properties and surfactant production rate. These properties are furthermore subject to dynamic changes depending on the environmental conditions (Lemmer *et al.*, 2002; Pagilla *et al.*, 2002). In contrast to filamentous bulking where it is easily recognizable which organisms inhibit the sedimentation, it is still not fully clarified which organisms are responsible for foaming. It is likely that bacteria contributing to foaming by the production of bio-surfactants are different from those stabilizing the foam due to their hydrophobic cell surface. Moreover, it is not clarified if the cell surface properties are crucial or if other adsorbed molecules define the relevant surface characteristics of the flocs.

- Inaccurate methods are another major factor inhibiting research, increasing the confusion additionally to the above mentioned challenges. The uncertainty and limitations were only rarely discussed regarding methods to characterize and quantify foam in the plant, to measure the foaming potential of a particular sludge (Haarhoff and Bezuidenhout, 1999; Jenkins *et al.*, 2004), to identify and quantify particular organisms (Daims, 2001; Wilderer *et al.*, 2002) and to characterize the extremely complex surface of activated sludge flocs including the analysis of EPS (Wilén *et al.*, 2003). Due to the inherent uncertainty and variability of these methods, one has to be careful not to take every statistically significant result as prove for a causal connection.
- Another important reason for the observed confusion in literature is the usually undocumented dynamics of relevant parameters. These are diurnal and seasonal variation of wastewater flow and composition, environmental and operational conditions, but also changes in the bacterial population, the sludge surface properties and changes of unknown foaming properties with time constants between hours and months. Furthermore, each WWTP is unique; even if the general design is the same, there will be different hydraulic conditions leading to different residence time distributions, dead zones with deposits of sedimented and putrefying sludge and the possibility of foam trapping. To simplify the investigated system many studies were performed on pure cultures or activated sludge fed with a constant flow of synthetic wastewater. While this revealed valuable knowledge about bacterial growth or foam formation, it is unclear whether those mechanisms and organisms are the relevant ones for foaming in full-scale activated sludge systems. This is because the wastewater composition and environmental conditions are not fully known but complex and dynamic, numerous microorganisms are competing and the floc structure is complex and dynamic. Compared to full-scale WWTPs, conditions in reactors in biotechnology are much better defined. Nevertheless, one successful strategy to control foaming is not necessarily suitable for a similar process elsewhere and even batch-to-batch variations occur (Vardar-Sukan, 1998).

1.8 Goal of this thesis

Based on the identified challenges, limitations and confusion (as discussed above, paragraph 1.7), the goal of this thesis was to systematically identify and investigate factors that play an important role in foaming in activated sludge systems. The following questions were addressed:

- Where are gaps in previous research? What are the reasons that the mechanisms behind foaming are still not fully clarified?
- How can suspected foam producing bacteria be reliably and rapidly quantified?
- How can the foaming potential of particular sludge be quantitatively measured?
- What controls the growth of the suspected foam producing bacteria in a WWTP? What is the role of the wastewater composition and the operating conditions?
- How is the foaming potential of the activated sludge correlated with the abundance of the suspected foam producing bacteria *Microthrix* and nocardioform actinomycetes? Is the foam coverage on the plant defined by the foaming potential of the sludge?
- What is the role of the solid and liquid fraction on the actual foam formation and stabilization?
- What are promising strategies to control and prevent foaming?

1.9 Research approach

The approach to tackle these questions consisted of the following parts:

- As discussed in paragraph 1.7, the observed foam on the surface of activated sludge tanks is the result of a series of sub-processes (Figure 1-2) which were identified to structure the investigations presented in this thesis: (1) The first step is the generation of mixed liquor of a certain foaming potential. This includes the production of surface-active compounds and the generation of hydrophobic floc surfaces, possibly by bacteria with hydrophobic cell surfaces. This step is controlled by the wastewater composition, the plant design as well as operational and environmental conditions. (2) The second step is the actual foam formation and stabilization, which is influenced by the mixed liquor properties and the gas production and distribution. In stable foam layers, the population composition and environmental conditions are different from the mixed liquor. The properties of the foam liquid and solids do not only influence the foam stability but also affect the foaming potential of the mixed liquor if re-mixed. (3) The last and possibly most critical sub-process is the distribution of the foam in the plant. The hydraulics, influenced by water flow and aeration pattern, and the possibility of foam trapping eventually define the amount of foam visible on the surface of the plant.
- To accurately study these sub-processes, appropriate methods were developed including assessments of the uncertainties of their results.
- The studies were based on long-term monitoring of suspected foam producing bacteria, foaming potential and foam coverage of reactors. Investigations were performed at different pilot and full-scale WWTPs (Table 1-1) with different design, all fed with real wastewater; some of them were operated in parallel to others allowing a direct comparison.

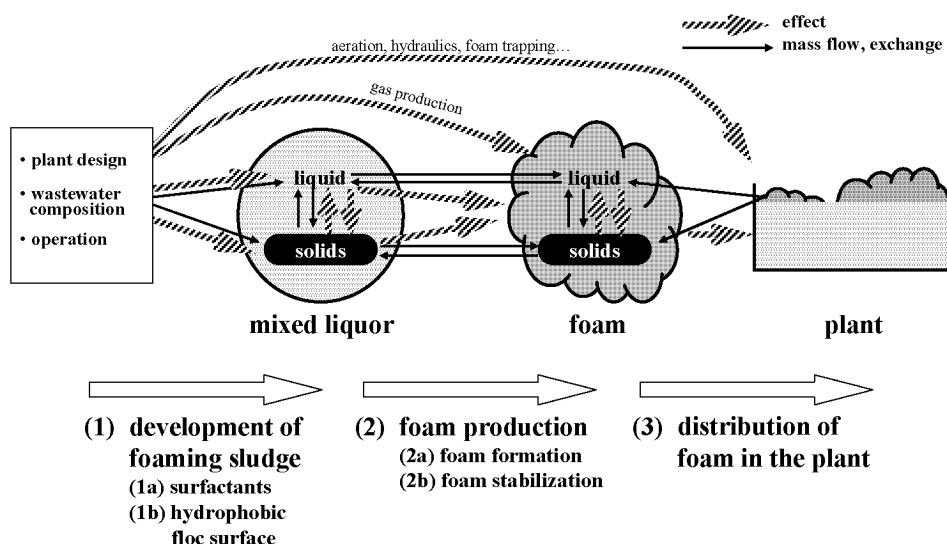


Figure 1-2: The observed foam in WWTPs is the result of many and diverse interactions which can be structured into three major sub-processes: (1) growth and development of foaming sludge, (2) foam formation and stabilization, (3) distribution of foam in the plant.

Table 1-1: In this project, the following pilot and full-scale activated sludge plants in Switzerland were analyzed. Some of them were fed with the same wastewater (indicated by the same name).

name ¹⁾	scale	design	PE ²⁾	SRT _{tot} ³⁾
CAS Kloten-Opfikon	full	pre-denitrification	54,000	12 - 18 d
CAS Kloten-Opfikon	pilot	pre-denitrification, two parallel plants	2 each	10 / 20 d
MBR Kloten-Opfikon	pilot	denitrification, partial EBPR ⁴⁾	100	30 - 70 d
CAS Thunersee	full	EBPR ⁴⁾ (AAO design)	130,000	20 - 50 d
CAS Eawag Dübendorf	pilot	pre-denitrification	200	20 d
MBR Eawag Dübendorf	pilot	pre-denitrification, two parallel plants	2 each	20 / 40 d

¹⁾ CAS: conventional activated sludge plant, MBR: membrane bioreactor.

²⁾ population equivalents

³⁾ total solids retention time

⁴⁾ enhanced biological phosphorous removal

1.10 Outline of the thesis

In the **introduction (chapter 1)** of this thesis, the existing knowledge about the causes and effects of activated sludge foaming is summarized and particular challenges and limitations are identified and discussed in order to derive the goals of this thesis.

Chapter 2 presents and discusses the newly developed rapid **quantification method** that allows to measure long time-series of suspected foam producing bacteria.

A **preliminary study (chapter 3)** analyzed seasonal variation of these microorganisms in the WWTP Kloten-Opfikon (Table 1-1) as well as the dynamics of the foam coverage of the activated sludge tanks.

A **pilot-scale plant** was operated in parallel to the above mentioned WWTP as a controlled model system (**chapter 4**) to study the role of the temperature, season, dissolved oxygen concentration and sludge retention time on the population, floc structure and foaming potential.

In **chapter 5** a **mathematical model** is proposed to further elucidate the mechanisms leading to the observed seasonal variation of the filamentous bacteria “*Microthrix parvicella*”.

The sub-processes of the foam formation and stabilization (Figure 1-2) are studied and discussed in **chapter 6**; for this purpose, lab-scale tests to measure the **foaming potential** of sludge samples were developed and applied to the plants in Table 1-1.

The **conclusions** section (**chapter 7**) presents a synthesis putting together the findings, draws conclusions on foaming control and suggests directions and approaches for further research on activated sludge foaming.

This thesis is structured as “paper dissertation”, i.e. it consists of a number of scientific articles. Therefore, some repetitions are unavoidable, particularly in the introduction sections of the individual papers, and instead of cross-references to other chapters, the papers are cited.

Chapter 2

Rapid Quantification of Bacteria in Activated Sludge using Fluorescence In Situ Hybridization and Epifluorescence Microscopy

Thomas Hug, Willi Gujer, Hansruedi Siegrist

Water Research, 39(16), 3837-3848. (2005)

Abstract

A rapid quantification method for bacteria in activated sludge has been developed, based on fluorescence in situ hybridization (FISH) and epifluorescence microscopy. Samples are hybridized on slides and analyzed by direct microscopic observation. Abundance categories were designed based on digital images of the target organisms. These rating systems were developed for the filamentous bacteria "*Microthrix parvicella*" and for different morphotypes of nocardioform actinomycetes, but can easily be adapted to other types of microorganisms. Due to the quantification by direct microscopic observation, this method is suitable for samples that are difficult to be processed by semi-automated image analysis techniques, such as samples containing fluorescent debris, cells of different fluorescence intensities and target organisms that need partial enzymatic digestion prior to FISH. In contrast to commonly used rating systems consisting of photographs, the newly developed categories allow to quantitatively compare results of different categories and different organisms. The uncertainties of the results were calculated by a non-parametric bootstrap procedure; a thorough uncertainty analysis was performed including sample variability and operator subjectivity.

2.1 Introduction

Activated sludge is a complex and highly variable mixture of different microorganisms. Many recent research questions require identification and quantification of specific bacteria in the activated sludge. To investigate e.g. the population dynamics of organisms involved in scumming (foaming) or bulking, it is necessary to measure time series of their abundance with a high temporal resolution (de los Reyes III and Raskin, 2002; Hug *et al.*, 2005c). To model population dynamics, quantitative data and selective identification is needed (Wilderer *et al.*, 2002).

To identify and quantify bacteria in activated sludge, various methods have been developed, all of them having specific limitations:

Filamentous bacteria have traditionally been identified by their morphology (Eikelboom and van Buijsen, 1981; Jenkins *et al.*, 1993; Eikelboom, 2000). Unfortunately, this widely used identification system does not distinguish different organisms with the same morphology and cannot deal with bacteria exhibiting variable morphology. For quantification, the same publications contain arbitrary rating systems consisting of microphotographs. This procedure is rapid but limited to filamentous bacteria occurring outside the flocs and having a similar morphology like the ones on the images. Furthermore, these rating systems do not allow the quantitative comparison between different categories and morphotypes. More quantitative are methods to measure filament length (Jenkins *et al.*, 1993; de los Reyes III and Raskin, 2002), but they are limited to one morphotype.

Overcoming the limitations of light-microscopy, fluorescent in situ hybridization (FISH) allows the identification of specific bacteria independent of their morphology and, therefore, has become the new state of the art in research of bacteria in activated sludge (Wagner *et al.*, 2002; Wilderer *et al.*, 2002). Moreover, cells inside flocs are easier to recognize using FISH compared to Gram-staining.

The above mentioned quantification methods for filaments can also be applied for fluorescently labeled bacteria, but their limitations are not eliminated. Manual counting of fluorescent cells is often applied but fails for many organisms in activated sludge because cells in filaments or dense agglomerates cannot be distinguished.

Very promising is the recently developed quantification method using confocal laser scanning microscopy (CLSM) and digital image analysis (Kuehn *et al.*, 1998; Bouchez *et al.*, 2000; Daims, 2001) that works completely independent of the morphology of the target cells. Unfortunately, it is not accurate for samples with very inhomogeneous fluorescence intensities (Daims, 2001) and for bacteria that require cell wall permeabilization prior to hybridization which destroys a part of other bacteria. Another big disadvantage for many research groups and plant operators is the requirement of an expensive CLSM to obtain the requested image quality.

The need to measure long time series of suspected scum forming bacteria in activated sludge led to the following demands on a new quantification protocol using FISH:

- rapid
- possible with an epifluorescence microscope - no need of an expensive CLSM
- suitable for difficult matrix containing debris and cells of different fluorescence intensity
- suitable for FISH protocols requiring cell wall permeabilization
- allowing quantitative comparison between different concentrations of one organism as well as between different target organisms
- small uncertainty due to subjectivity of operators
- accurate enough to identify typical seasonal changes in the population composition

This paper presents a newly developed quantification protocol based on drawn abundance categories that fulfills the above stated goals. The paper describes the design and application of the rating systems, presents the results of an uncertainty analysis that included different operators and finally leads to a general discussion about uncertainties of quantification methods for bacteria in activated sludge. Guidelines for the application can be found in the appendix.

2.2 Methods

2.2.1 Samples

For the development of the rating system and for the uncertainty analysis, grab samples from mixed liquor and scum of different full scale wastewater treatment plants (WWTP) in Switzerland were taken.

2.2.2 Fluorescence in situ hybridization (FISH)

The following CY3 labeled oligonucleotide probes were used: MPA223 and MPA645 (Erhart et al., 1997) for "*Microthrix parvicella*", MYC657 (Davenport et al., 2000) for nocardioform actinomycetes. FITC labeled EUB338mix (Amann et al., 1990; Daims et al., 1999), specific for most bacteria, was used as positive control. As negative control, hybridization without probe and NonEUB338 (Wallner et al., 1993), complementary to EUB338, was performed. Since the cell walls of "*M. parvicella*" and nocardioform actinomycetes are difficult to be penetrated by the oligonucleotide probes, the fixed samples (1 min PFA; de los Reyes *et al.*, 1997) were treated with Lysozyme (Beimfohr et al., 1993) for 30 min at room temperature, for "*M. parvicella*" additionally with Mutanolysin (Erhart et al., 1997) for 15 min at room temperature. Hybridization was performed according to Manz *et al.* (1992).

2.2.3 Rating systems

Rating systems were designed for different potentially scum forming bacteria: long filaments of "*M. parvicella*" and three different morphotypes of nocardioform actinomycetes (Figure 2-1). These systems consist of simplified drawings that represent one field of view (1000 fold magnification, diameter of 200 μ m), containing flocs as gray shapes and fluorescent target bacteria as black lines or dots (Figure 2-2). The background of each drawing contains flocs that cover exactly 50 percent of the area. (As an exception, in category 9 of the long filaments, the floc area is reduced to 25 percent because "*M. parvicella*" occurs in such high concentrations mainly outside the flocs.)

Each rating system for a specific target organism consists of 10 categories (appendix, Figure 2-4 to Figure 2-7). The abundance of the target cells exactly doubles from one category to the next; category 0 contains no target cell. To ensure that the abundance categories are quantitatively comparable, the "pixel concentrations" on the digital drawings (ratio of the number of pixels of target cells to the sum of target cells and flocs) is equal for all morphotypes (from 0.2% for category 1 to 51.2% for category 9). For the same reason, the drawings were designed to scale, using digital images of the target organisms (Figure 2-2).

2.2.4 Microscopy and quantification of bacteria

Microscopy was performed on an Olympus BX50 epifluorescence microscope, equipped with filtersets HQ-CY3 and HQ-FITC (Analysetechnik AG, Tübingen, Germany) and an Olympus objective (UplanFl 100x, 1.30) under oil immersion.

To prevent systematic errors, the samples were randomly distributed onto different spots ("wells") of the microscope slides so that the operator did not know what sample was being quantified.

The operator randomly selected several fields of view from each well. For each field, the abundance of fluorescent organisms (CY3) was visually assigned to a category of the according rating system (half numbers were allowed to be chosen). The criterion was the ratio of target cells compared to the floc area (concentration of target organisms), not the absolute number of cells per field.

Particles or cells with a bright signal that were obviously not target cells and bacteria that were fluorescent in the negative controls (no probe or NonEUB338) were not taken into account. To reduce errors due to variable sludge content of the analyzed fields, the operator made sure that the sludge-coverage was at least 30 percent. Furthermore, if the analyzed floc was thicker than the depth of focus (a few micrometers), an average abundance was estimated by considering layers at different depths.

To investigate the *subjective uncertainty* the operator assigned not only one value ("best estimate") for each field, but determined additionally a minimum and maximum value, representing his or her subjective uncertainty range of 95 percent probability.

The *operator to operator variability* was assessed by letting five different operators analyze exactly the same fields of view. To prevent systematic effects, the different persons did not know the values of the others. The sequence of the persons was changed for every field in order to prevent systematic effects due to fading of the fluorescence signals caused by bleaching of the sample.

To study the *spatial variability* within a sludge sample and to assess the *repeatability* of the method, one operator additionally analyzed all four morphotypes in 100 fields of a new hybridization of the same samples several months later, using the same equipment.

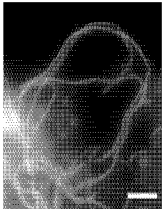
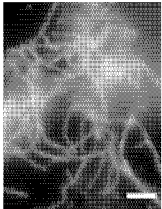
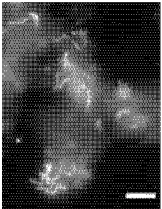
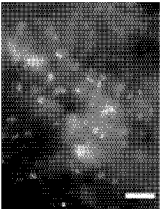
	long filaments	branched filaments	short filaments	single cells (rods)
				
width of filament [μm]	0.50-0.67	0.50-0.67	0.33-0.50	0.67 (length:1.0-1.2)
width of filament [pixels]	3-4	3-4	2-3	4 (length: 6-7)

Figure 2-1: Dimensions of the target organisms in the rating systems. Length of bar in pictures: 5 μm .

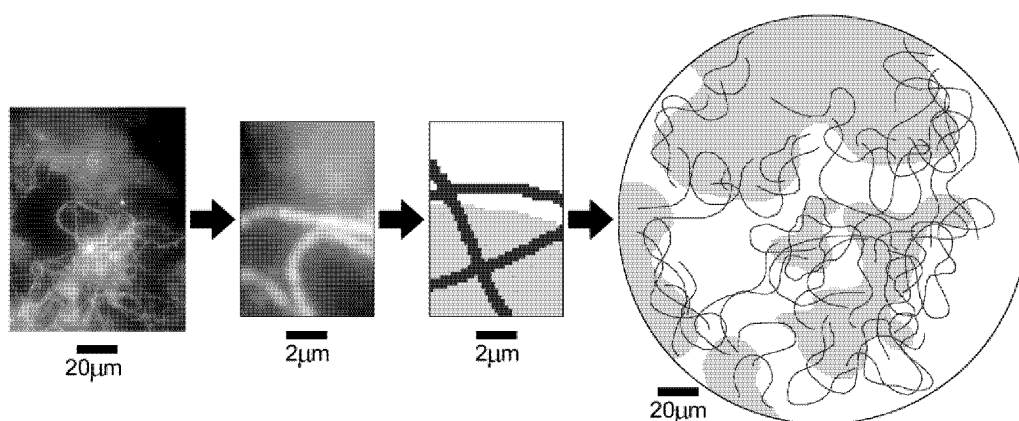


Figure 2-2: The rating systems consist of simplified drawings that represent one field of view with flocs and target organisms, which were designed to scale according to a microphotograph.

2.2.5 Analysis of quantification results

For the presented uncertainty analysis, the following calculation was done. Once the uncertainties are known, the simplified procedure as described in the appendix can be applied.

The calculations were performed on R 1.9.1 (an open source statistical software, freely available at <http://www.r-project.org>). A program code used for the following calculations is available on request.

The target value of the quantification is the *mean abundance* of an organism in a sludge sample and its confidence interval. Taking into account that the distribution of the assigned values is unknown, we applied a non-parametric bootstrap (Efron and Tibshirani, 1993). The principle of the bootstrap is to create many new data sets by sampling (with replacement) from the original data set. This leads to an empirical distribution of possible mean values and, therefore, allows to estimate a confidence interval.

The original data set, consisting of all assigned values (n_{tot} "best estimates" by one operator) for one sludge sample and morphotype was written as vector $\mathbf{x}$ of the length n_{tot} :

$$\mathbf{x} = \begin{pmatrix} x_1 \\ \vdots \\ x_{n_{\text{tot}}} \end{pmatrix}$$

From the elements x_k of this vector, $m \cdot n$ "bootstrap samples" X_{ij} were drawn (with replacement), resulting in $m = 10,000$ sets of length n (usually $n = n_{\text{tot}}$):

$$\mathbf{X} = \begin{pmatrix} X_{11} & \dots & X_{1n} \\ \vdots & & \vdots \\ X_{m1} & \dots & X_{mn} \end{pmatrix}, \quad X_{ij} \in \mathbf{x}, \quad \text{Prob}(X_{ij} = x_k) = \frac{1}{n_{\text{tot}}}$$

Calculating the mean of each set ($X_{i1} \dots X_{in}$) led to a distribution of m estimated mean values $\bar{X}_i$ (see below). However, due to the logarithmic scale of the rating systems, a transformation was necessary to obtain the mean of the concentration. Consequently, the "bootstrap samples" $\mathbf{X}$ were transformed into "concentrations" $\mathbf{C}$. Calculating the mean of each series ($C_{i1} \dots C_{in}$) resulted in $m = 10,000$ estimated mean "concentration" values which represent an empirical distribution of the mean abundance. The results were finally transformed back to the category scale, resulting in a vector $\bar{\mathbf{x}}$ that contains m possible mean values of the abundance:

$$\begin{aligned} \mathbf{C} &= \begin{pmatrix} C_{11} & \dots & C_{1n} \\ \vdots & & \vdots \\ C_{m1} & \dots & C_{mn} \end{pmatrix} = 2^{\mathbf{X}} = \begin{pmatrix} 2^{X_{11}} & \dots & 2^{X_{1n}} \\ \vdots & & \vdots \\ 2^{X_{m1}} & \dots & 2^{X_{mn}} \end{pmatrix} \\ \bar{\mathbf{c}} &= \begin{pmatrix} \bar{C}_1 \\ \vdots \\ \bar{C}_m \end{pmatrix} = \begin{pmatrix} \frac{1}{n} \sum_{j=1}^n C_{1j} \\ \vdots \\ \frac{1}{n} \sum_{j=1}^n C_{mj} \end{pmatrix} \\ \bar{\mathbf{x}} &= \log_2(\bar{\mathbf{c}}) = \begin{pmatrix} \bar{X}_1 \\ \vdots \\ \bar{X}_m \end{pmatrix} \end{aligned}$$

We characterized the resulting distribution by its median as measure of the expectation value of the mean abundance and by the 2.5% and 97.5% percentiles representing an estimated confidence interval of $p = 0.95$ (Figure 2-3).

If the presented categories are used, "pixel concentration" can be calculated as

$$\bar{c}_{\text{pix}} = 0.1\% \cdot 2^{\bar{x}}$$

where $\bar{c}_{\text{pix}}$ is the "pixel concentration" as percent, corresponding to the category value $\bar{x}$.

In order to include the *subjective uncertainty* range of one operator, the recorded ranges (minimum, "best estimate", maximum) were visually analyzed and found to be symmetrically distributed. Therefore, we considered the subjective uncertainty as normally distributed random error with a standard deviation according to the width of the recorded subjective uncertainty range. Since the recorded range represented the estimated 95% confidence range of the operators, we used half of that interval as standard deviation s_{subj} . Consequently, this uncertainty range was included into the calculation of the mean abundance by adding to each "bootstrap sample" X_{ij} a random sample ε_{ij} from a normal distribution with mean = 0 and s_{subj} as standard deviation (values outside the category range $\{0 \dots 9\}$ were set to 0 or 9):

$$\mathbf{X}' = \begin{pmatrix} X'_{11} & \dots & X'_{1n} \\ \vdots & & \vdots \\ X'_{m1} & \dots & X'_{mn} \end{pmatrix} \quad X'_{ij} = X_{ij} + \varepsilon_{ij}, \quad \varepsilon_{ij} \sim N(0, s_{\text{subj}})$$

These new values were object of the above described transformation into a linear "concentration" scale and calculation of the means:

$$\bar{x}' = \log_2 \begin{pmatrix} \frac{1}{n} \sum_{j=1}^n 2^{X'_{1j}} \\ \vdots \\ \frac{1}{n} \sum_{j=1}^n 2^{X'_{mj}} \end{pmatrix}$$

To assess the *operator to operator variability*, the described calculation was performed for the values assigned by five different operators to the same fields. The five resulting probability distributions were combined to one multimodal distribution from which the confidence interval was calculated (Figure 2-3c).

Due to the *spatial variability* of the sludge sample, the confidence interval of the resulting mean abundance narrows with an increasing number of analyzed fields. To assess this effect, the calculations were repeated with different length n ($2 \leq n \leq n_{\text{tot}}$) of the bootstrap data sets, sampling always from the total number of n_{tot} available values (Figure 2-3).

2.3 Results

The developed method is described in detail above in the methods section of this paper, including design of the rating systems, microscopic analysis, calculation of results and uncertainty analysis using a bootstrap procedure. Guidelines for its application can be found in the appendix.

The calculated *mean abundance* for each sample and operator are shown in Table 2-1.

The *subjective uncertainty* ranges, estimated by the operators for each field of view, were found to be symmetrically distributed around the corresponding "best estimate". For all analyzed morphotypes, the subjective uncertainty ranges were smaller or equal plus/minus one category unit ($p = 0.95$).

Due to the *spatial variability*, the uncertainty range of the result depends on the number of analyzed fields as shown in Figure 2-3.

The *repeatability*, measured as two independent quantification series (new hybridization of the same sludge sample), showed no significant differences for any morphotype (operator E1 and E2 in Table 2-1 and Figure 2-3a).

To test the *operator to operator variability*, five operators analyzed exactly the same fields of view (Figure 2-3). The calculated mean values were always within a range of two category units (Table 2-1). This range defines the total uncertainty of the result at an indefinite number of fields (Figure 2-3c).

2.4 Discussion

2.4.1 Microscopy

Characteristic of the presented method is that an operator has to visually judge the sample under the microscope and subjectively assign an abundance category. Even a high quality digital image capturing the whole spatial resolution provided by the microscope exhibits a far lower brightness resolution than a human eye can recognize and it does only show one focus plane of typically about one micrometer. Furthermore, the human brain is very efficient in recognizing and distinguishing shapes. Images of activated sludge flocs taken on an epifluorescence microscope always contain bright blurry parts caused by the sum of out of focus signals (Figure 2-1). As image analysis routines usually set an intensity threshold to distinguish positive from negative signals, they do not provide accurate results for such images due to the bright and variable background intensity. Digital image analysis of activated sludge requires an image quality that is only achievable using a confocal laser-scanning microscope (CLSM). Direct visualization by eye on the other hand allows to easily recognize positively labeled cells inside flocs and to consider a large focus range in order to distinguish non-target fluorescent signals. Moreover, image analysis routines request manual removal of fluorescent debris and non-target cells from the digital image prior to the actual quantification, while an operator at the microscope can simply neglect such signals without losing time. These clear advantages of direct microscopic analysis make the presented method suitable to quantify bacteria in a matrix that contains organisms of very different fluorescence intensity and fluorescent non-target particles such as full-scale activated sludge or probably also sediments or soil.

Table 2-1: Resulting mean abundance of different operators analyzing the same fields of view. Operators A, C and E analyzed additional fields to those analyzed by all operators. Operator E (E2) analyzed also new hybridizations of the same samples.

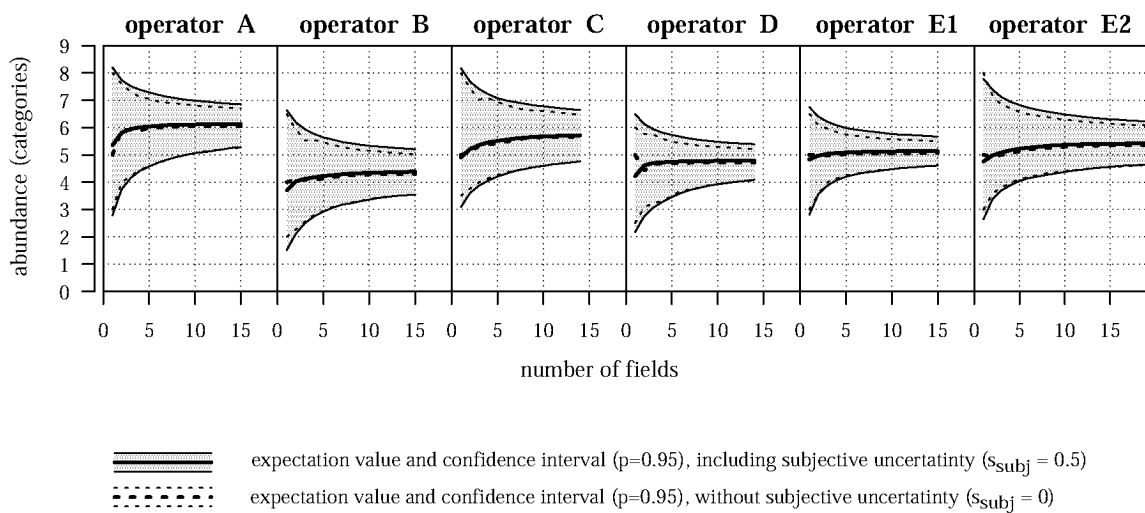
target morphotype, sample	probe	operator						operator				operator	
		n ¹⁾	A	B	C	D	E1	n ¹⁾	A	C	E1	n ¹⁾	E2 ²⁾
long filaments, sample 1	MPA223+645	14	6.1	4.4	5.7	4.7	5.1					100	5.4
long filaments, sample 2	MPA223+645	9	8.1	6.5	6.6	7.2	7.3	19	8.0	6.5	7.3	100	7.2
branched filaments, sample 3	MYC657	6	5.2	4.0	4.8	3.3	3.6	16	5.9	5.8	5.0	100	4.4
branched filaments, sample 4	MYC657	6	3.2	2.9	3.7	1.8	3.0	16	3.7	3.3	3.1	100	2.6
single cells, sample 3	MYC657	6	2.3	1.4	1.0	0.7	1.3	16	2.1	1.0	1.2	100	1.2
single cells, sample 4	MYC657	6	1.7	1.4	0.7	0.6	1.0	16	1.8	0.8	1.2	100	0.9
short filaments, sample 3	MYC657											100	1.8
short filaments, sample 4	MYC657											100	1.4

¹⁾ n = number of analyzed fields

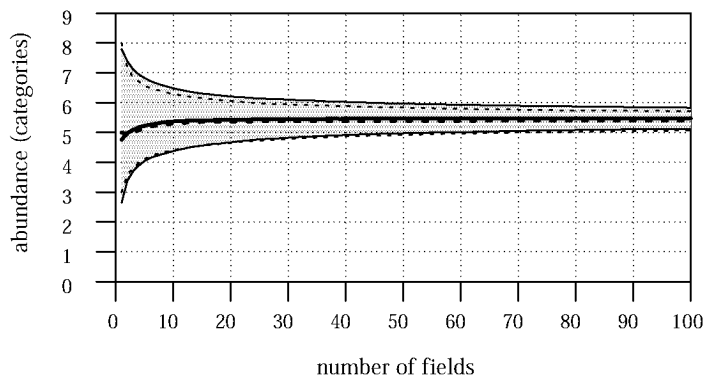
²⁾ E2 = operator E analyzing new hybridizations of the same samples

The time spent for the actual microscopic analysis was less than one minute per field and morpho-type (assigning three values per field: "best estimate" and minimum/maximum values indicating a personal uncertainty range). An operator who is familiar with epifluorescence microscopy of FISH labeled samples needs about ten minutes to quantify a reasonable number of 10-20 fields. This is considerably less than about one hour per sample with semiautomatic analysis of digital CLSM images. For both methods, time for the hybridization has to be added (this depends highly on the number of samples); data handling and analysis can be conducted almost fully automatically.

(a) differences among operators



(b) one operator (E2)



(c) five operators (combined)

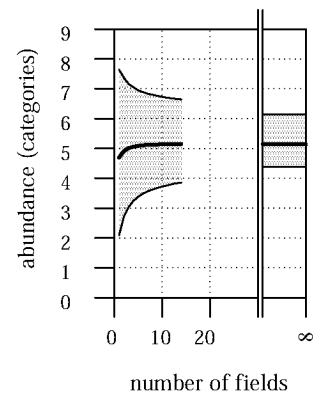


Figure 2-3: The uncertainty range of the mean abundance depends on the number of analyzed fields, due to the spatial variability. The solid lines indicate the median and the 95% confidence interval of the mean abundance including a subjective uncertainty range of $s_{\text{subj}} = 0.5$. The dashed lines indicate the range without subjective uncertainty ($s_{\text{subj}} = 0$). (a) Differences among operators (E2 stands for operator E analyzing a new hybridization of the same sample). (b) Large number of fields analyzed by one operator. (c) The combined results of the five operators.

2.4.2 Rating Systems

Direct visualization by eye offers some important advantages but makes it more difficult to obtain quantitatively comparable results, which was a major reason to develop this new method. The widely used filaments abundance categories (Eikelboom and van Buijsen, 1981; Jenkins *et al.*, 1993; Eikelboom, 2000) are based on microphotographs with arbitrary differences of the concentration from one category to the next and, therefore, do not allow to quantitatively compare the abundance of different categories or even different morphotypes. To overcome these limitations, the presented rating system consists of drawings instead of photographs, designed as quantitatively comparable categories.

The four presented rating systems can be applied not only for the particular bacteria they were designed for (Figure 2-1), but also for other organisms of similar shapes. To make the results quantitatively comparable, the resulting "concentration" must be converted according to the dimensions of the new target organisms. To quantify organisms of a different morphology, new category systems can be designed. The dimensions of the new target cells should be stated, allowing to convert the results for similar morphotypes. For morphologically inhomogeneous groups of bacteria it is probably necessary to create more than one drawing as typical representation of one category. Not suitable, however, is this method for organisms growing in dense spherical agglomerates of very heterogeneous size and inhomogeneous distribution such as nitrifying bacteria (Manser *et al.*, 2005).

2.4.3 Uncertainty analysis

The *target value* of the quantification is the abundance of a particular organism in the sampled system, e.g. the activated sludge tank. The following factors affecting the accuracy of the result were studied:

The influence of the *spatial variability* within the sample is reduced by increasing the number of analyzed fields (Figure 2-3).

Subjective uncertainty is introduced by the operator's choice of a category value. In fact, the operator usually "selects" the value from a certain range that seems reasonable to himself. This subjective uncertainty widens the confidence interval and, due to the logarithmic scale of the categories, increases the median (Figure 2-3). In this study, $s_{\text{subj}} = 0.5$ was found to be appropriate; its effect on the final uncertainty was negligible.

Often, *different operators* are involved in the quantification. If these operators assign systematically different values, the observed "effects" in the studied population might depend on the different operators rather than on the changes in the studied system. Therefore, the operator to operator variability of all involved operators must be determined. According to these differences, the results can either be corrected or the uncertainty range can be widened (Figure 2-3). If the results must be comparable to data obtained by other (unknown) operators, the total uncertainty range has to be enlarged according to the measured differences among an adequate number of operators.

The *calculated uncertainty range* is proportional to the concentration, due to the log-scale rating systems. Without considerable differences among operators, a confidence interval ($p=0.95$) of plus minus one category (i.e. 50% to 200% of the concentration) is achievable with 10-20 fields. This precision proved to be sufficient to clearly recognize typical seasonal variations in the abundance of suspected scum forming bacteria in a full-scale WWTP (Hug *et al.*, 2005c).

A *preliminary uncertainty analysis* should be performed for every new target organism or type of sample and for all involved operators.

The *overall uncertainty* of this or any other quantification method based on FISH and microscopy additionally includes the following aspects, which were not quantitatively analyzed in the presented study:

Only a few flocs of the activated sludge tank are analyzed. To obtain a representative average, the fields must be chosen from different flocs and repetitions of the (sampling and) quantification should be performed. However, selecting these fields cannot be done absolutely randomly. Furthermore, the variable sludge content of the analyzed fields influences the result. To consider this, the assigned values could be weighted; but the necessary estimation of the amount of sludge would introduce further (subjective) uncertainties. The accuracy depends also on the thickness of the analyzed floc and the depth of focus: A thick layer of sludge that is in focus allows to recognize a greater number of target cells and pretends a higher concentration. The small depth of focus of a 100x objective reduces this effect and allows to analyze several thin layers within thick flocs in order to estimate an average concentration of the whole floc depth. Further uncertainty origins from the variable density of activated sludge flocs that is not considered in the rating systems where flocs are drawn as homogeneous gray shapes.

The FISH procedure is another source of possible systematic errors. It involves several centrifugation and washing steps, which may lead to a selective loss of bacteria. A non-optimal hybridization (e.g. due to insufficient permeabilization of Gram-positive cell walls) or bleaching of the fluorochromes under the microscope may lead to low abundance values. High numbers of auto-fluorescent non-target cells or debris makes it more difficult to recognize the target cells.

Other quantification methods using FISH include manual counting of cells or the analysis of digital CLSM images (Kuehn *et al.*, 1998; Bouchez *et al.*, 2000; Daims, 2001). Both do not depend on a logarithmic scale and therefore provide results with a smaller relative uncertainty than the values found in this study. Moreover, they use an apparently more reliable reference system (usually the cells hybridized by a universal probe). However, while cell counts are not applicable to filaments in which the cells often cannot be distinguished, the theoretically quite accurate and objective analysis of CLSM images is seriously limited for the quantification of suspected scum forming Gram-positive bacteria in full-scale activated sludge. The image analysis procedure usually results in a ratio of area of target organisms compared to all biomass hybridized with a universal probe. Unfortunately, this reference system is affected as an unknown fraction of cells is destroyed by the enzymatic pretreatment of the samples that is necessary to permeabilize our target cells. The quantification itself is based on the semi-automatic or manual selection of a fluorescence intensity threshold that distinguishes positive signals from the background. If the sample contains cells of very different fluorescence intensities as it is the case in full-scale activated sludge, the results become additionally inaccurate and subjectively biased (Daims, 2001).

The method presented in this paper compares the fluorescent target cells with the total floc area. This is generally less accurate than using all cells labeled by a universal probe as reference, but it is hardly influenced by the destruction of non-target cells. As this method is based on direct visual analysis by an operator, it can easily handle cells of different fluorescence intensities and samples containing fluorescent non-target cells and debris. Furthermore, it is less time consuming and does not need an expensive CLSM (as discussed above).

The conventional rapid methods like the widely used classification systems for filamentous bacteria (Eikelboom and van Buijsen, 1981; Jenkins *et al.*, 1993; Eikelboom, 2000) are based on arbitrary scales and do not allow the quantitative comparison between different samples and different organisms. Furthermore, these rating systems are limited to filaments outside the flocs.

What is the "*true concentration*" of the quantified microorganisms? Apart from the uncertainty discussion, it has to be clarified what kind of "concentration" is requested. Depending on the research question, total numbers, volume, surface or activity of the target cells is of interest. Possible reference systems are all FISH labeled bacteria, all viable bacteria, total active biomass or mixed liquor suspended solids. This fundamental question has to be discussed further in the field of applied microbiology and engineering (Wilderer *et al.*, 2002), in order to take full advantage of the promising possibilities offered by FISH.

2.5 Conclusions

This paper presents and discusses a new rapid quantification method for bacteria in activated sludge using fluorescence in situ hybridization (FISH) and epifluorescence microscopy. It is based on rating systems of designed log-scale abundance categories and direct microscopic observation. In contrast to quantification methods based on semi-automatic analysis of digital images, the presented method can easily cope with samples containing fluorescent debris and cells of different signal intensities as well as with target organisms that need enzyme treatment prior to FISH. This method is rapid and simple but, nevertheless, provides quantitatively comparable results. These characteristics make it particularly valuable to measure extended time series of the abundance of specific bacteria as it is necessary to investigate bulking and scumming in activated sludge systems.

A thorough uncertainty analysis was performed and discussed. The major factor influencing the uncertainty of the result is the spatial variability within the sample as well as subjective differences among operators. However, finding the most appropriate quantification method does not only depend on the requested accuracy but also on the type of "concentration" that is needed to answer a specific research question.

Guidelines for the application of the method including the rating categories for the four investigated organisms are available in the appendix of this paper.

2.6 Acknowledgements

We thank Elke Hinz, Reto Manser, Kathrin Muche, Justyna Okolowicz and Mandy Ziranke for their experimental contributions and Reto Manser, Marc Neuman, Wouter Pronk and Christoph Ort for their engagement in helpful discussions. This project was performed in the framework of COST action 624.

2.7 Appendix

2.7.1 Guideline for the application of the presented method

To apply the presented quantification method, the following steps should be performed (for details see the methods and discussion sections of this paper:

Rating systems

- 1) This quantification method is based on log-scale rating systems consisting of simplified drawings that represent one field of view (1000 fold magnification, diameter of 200 μm), containing flocs as gray shapes and fluorescent target bacteria as black lines or dots (Figure 2-4 to Figure 2-7).
- 2) For each target organism a rating system representing organisms of the same shape and size (Figure 2-1) must be used.
- 3) To quantify other bacteria of similar morphology, the presented categories can be applied, but the results must be obtained as "pixel concentration" and corrected according to the size of the target organisms.
- 4) To quantify bacteria of a different morphology, new rating systems can be designed, preferably representing the same "pixel concentrations".

Preliminary uncertainty analysis

- 5) A preliminary uncertainty analysis as presented in this paper is recommended. For each morphotype and type of sample (e.g. mixed liquor or scum), a large number of fields should be analyzed by each operator that is involved into the quantification. Plots as shown in Figure 2-3 allow to decide the necessary number of fields to be analyzed in order to achieve the requested accuracy. A program code for the bootstrap calculation is available on request.
- 6) If a preliminary uncertainty analysis cannot be done, we recommend (for activated sludge samples) to chose 10-20 fields per sample and assume a 95% confidence interval of plus minus one category unit. If different operators are involved, a few comparison measurements should be performed.

FISH and quantification

- 7) To perform FISH, the samples are to be applied in random order to the "wells" on microscope slides (at least two "wells" per sample). Positive and negative controls should be included.
- 8) An epifluorescence microscope with a suitable filter and a 100x objective is to be used (the presented rating systems represent fields of view of a diameter of 200 μm).
- 9) The operator selects random fields of view from each well (the total number according to the preliminary uncertainty analysis, see above) covering different flocs and assigns a category value of the rating system fitting to the target organism. The criterion is the ratio of target cells compared to the floc area (concentration of target organisms), not the absolute number of cells per field. During microscopy, the operator should not know what sample is being analyzed.

- 10) The mean abundance is calculated via a transformation of the log-scale categories into "concentrations":

$$\bar{x} = \log_2 \left(\frac{1}{n} \sum_{i=1}^n 2^{x_i} \right)$$

where $\bar{X}$ is the resulting mean abundance in category units, n is the number of analyzed fields per sample and $x_i = (x_1, x_2, \dots, x_n)$ are the assigned category unit values for the n fields.

- 11) If the presented categories are used, "pixel concentrations" as volume ratio of target organisms to the total floc can be calculated as

$$\bar{c}_{\text{pix}} = 0.1\% \cdot 2^{\bar{X}}$$

where $\bar{c}_{\text{pix}}$ is the "pixel concentration" as percent, corresponding to category value $\bar{X}$.

Publishing results

To publish results using this method, the following information should be included:

- the used rating system, including the "pixel concentrations" of its categories
- the size of the target organisms
- the number of analyzed fields
- the results as categories or "pixel concentrations"
- the confidence interval of the results and whether it was calculated for each sample, estimated from prior uncertainty analysis, or assumed

2.7.2 Rating systems

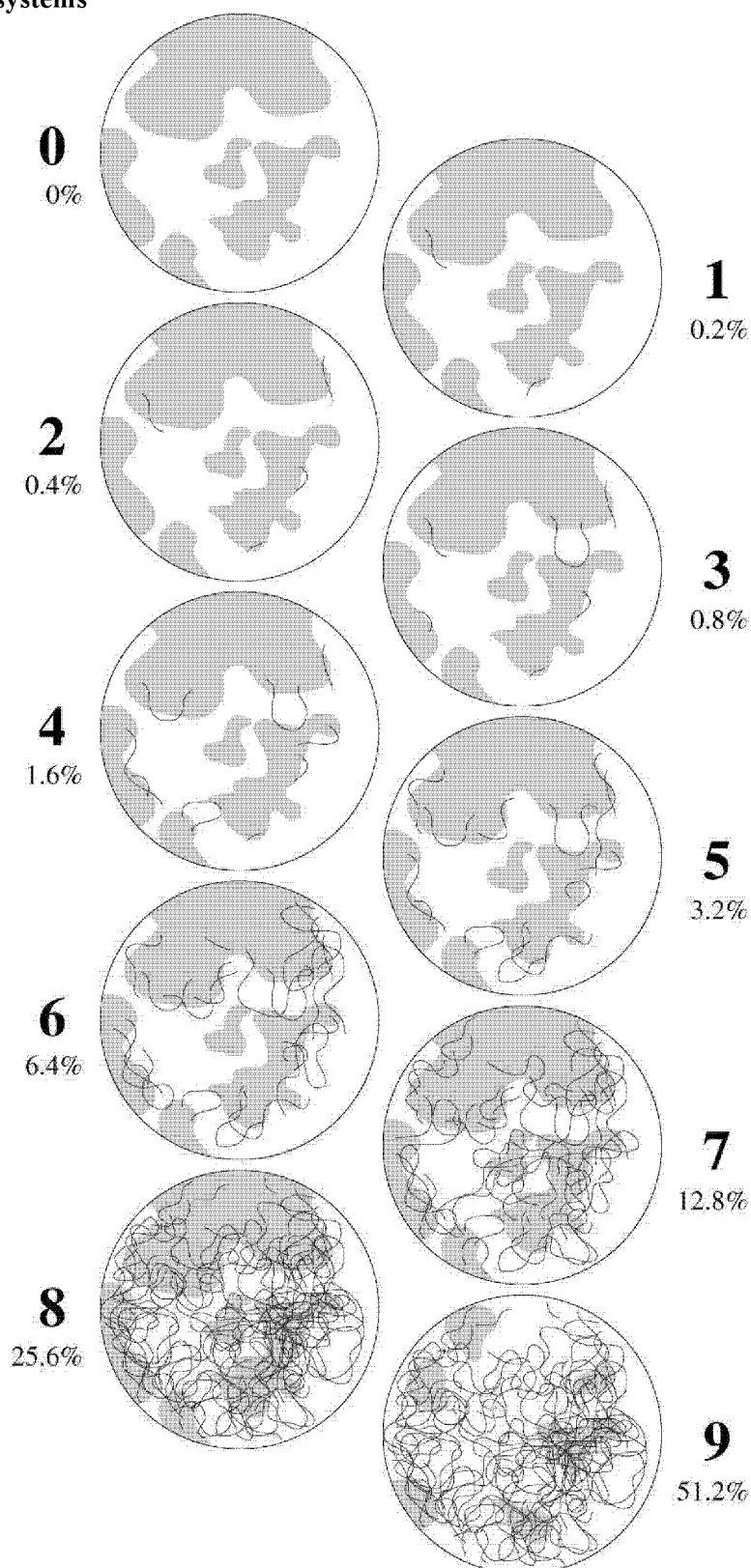


Figure 2-4: Abundance categories for long filaments (*Microthrix parvicella*) with filament width of 0.50-0.67 μm . The circles represent fields of view (1000x magnification, diameter = 200 μm). The figures below the category number indicate the "pixel concentrations", i.e. number of black pixels to sum of black and gray pixels.

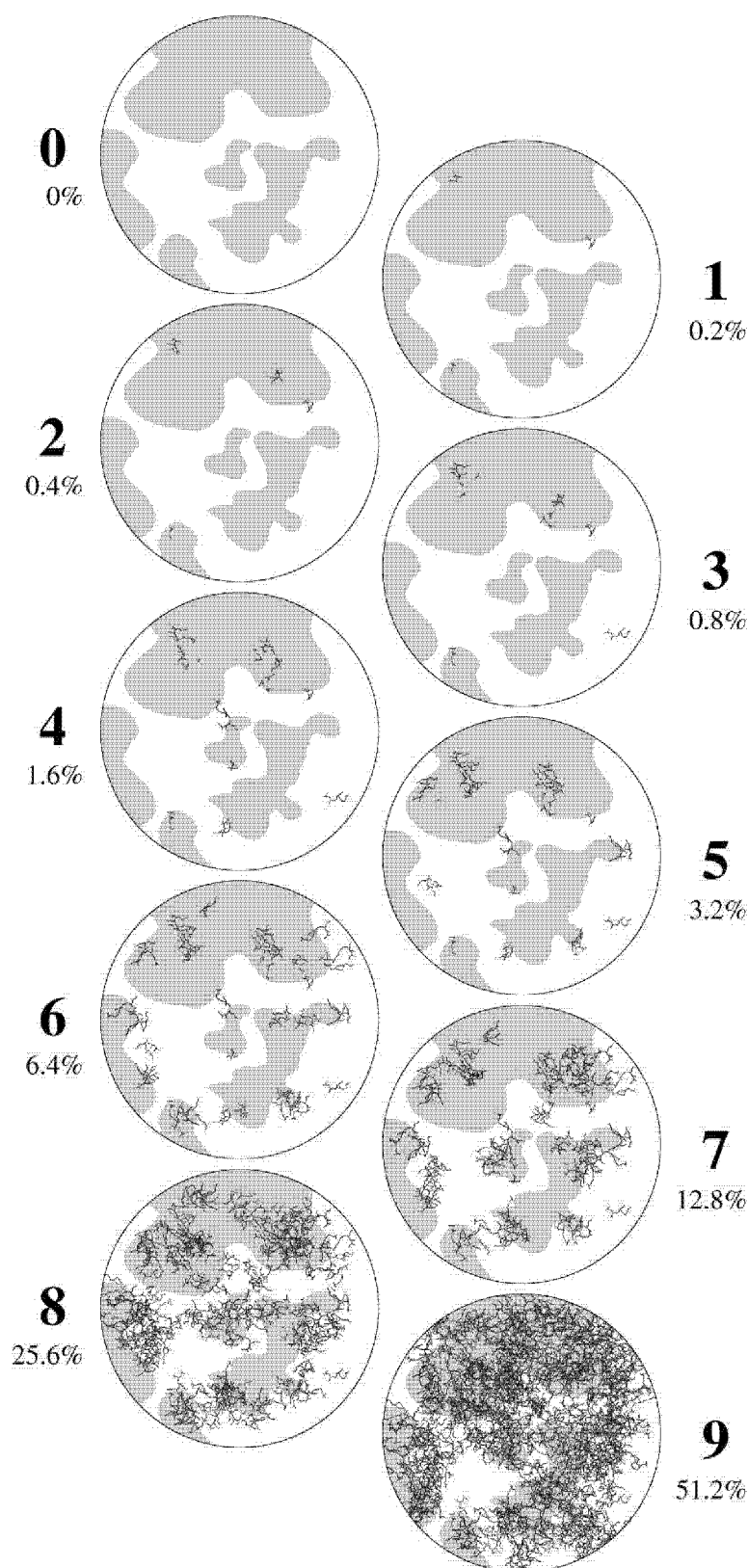


Figure 2-5: Abundance categories for branched filaments (*Gordonia amarae* like organisms) with filament width of 0.50-0.67 μm . The circles represent fields of view (1000x magnification, diameter = 200 μm). The figures below the category number indicate the "pixel concentrations", i.e. number of black pixels to sum of black and gray pixels.

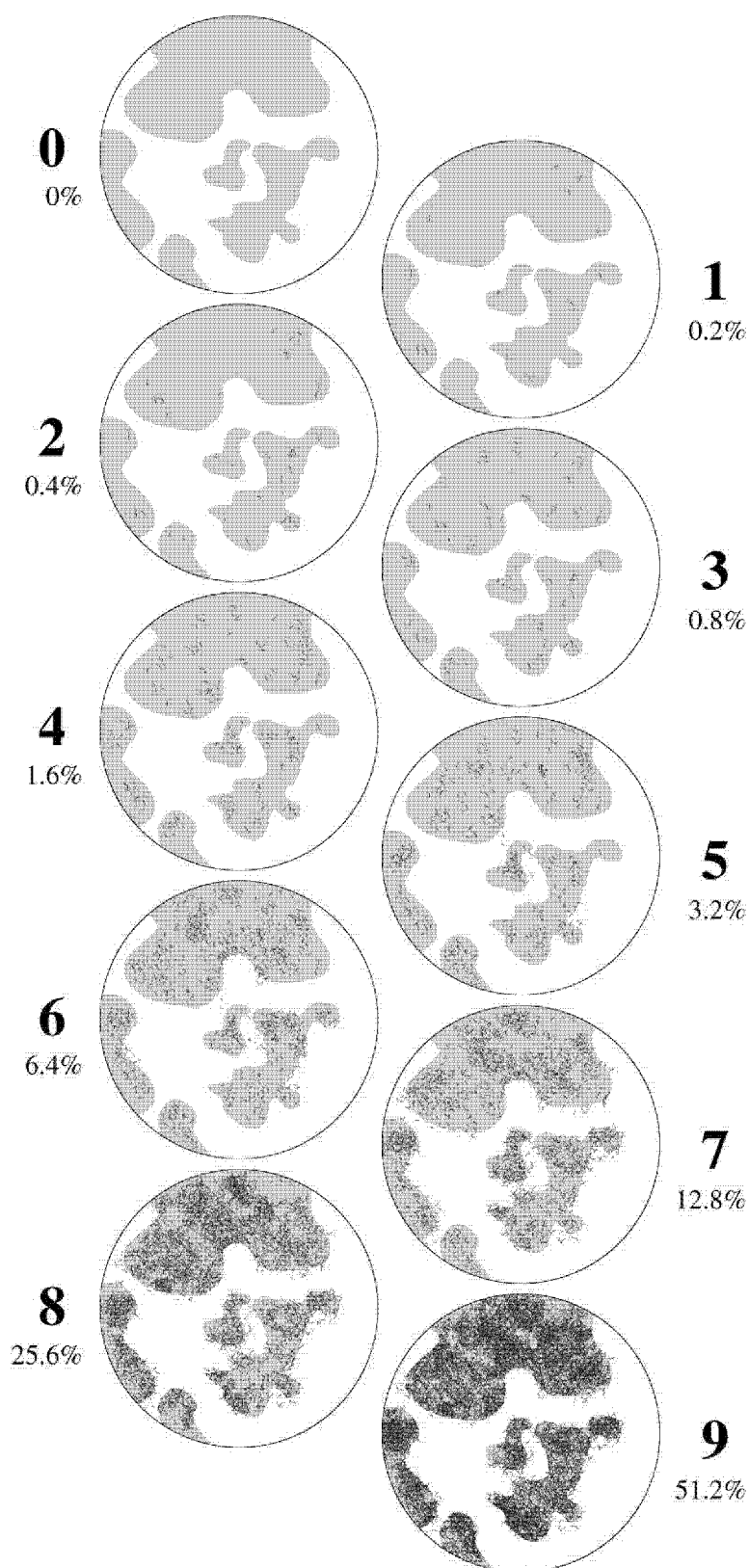


Figure 2-6: Abundance categories for short filaments with filament width of 0.33-0.50 μm . The circles represent fields of view (1000x magnification, diameter = 200 μm). The figures below the category number indicate the "pixel concentrations", i.e. number of black pixels to sum of black and gray pixels.

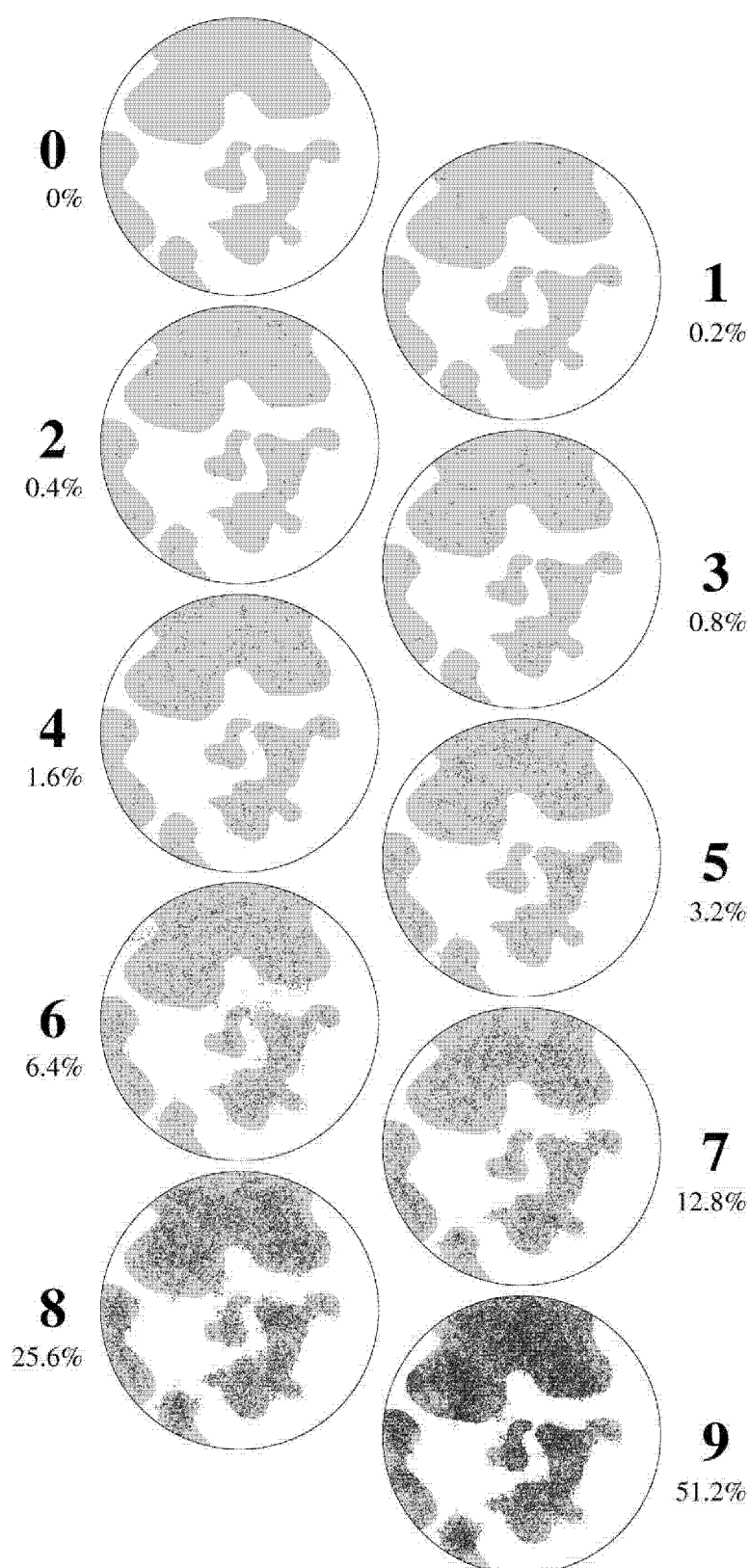


Figure 2-7: Abundance categories for rod shaped single cells (width = 0.50-0.67 μm , length = 1.0-1.2 μm). The circles represent fields of view (1000x magnification, diameter = 200 μm). The figures below the category number indicate the "pixel concentrations", i.e. number of black pixels to sum of black and gray pixels.

Chapter 3

Dynamics of Population and Scumming on a full-scale Wastewater Treatment Plant in Switzerland

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oral presentation at Post-conference Colloquium on Foam and Scum in Biological Wastewater Treatment, Prague Institute of Chemical Technology, Prague, Czech Republic. (2003)

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Abstract

The formation of stable scum (activated sludge foaming) is a serious operational problem in wastewater treatment plants (WWTP) all over the world. Although scumming seems to be related to high numbers of "*Microthrix parvicella*" and nocardioform actinomycetes, it is still unknown whether these organisms are necessary and sufficient for the formation of stable scum. To tackle this question, the abundance of these organisms and the scum coverage of the activated sludge basins were measured on a WWTP in Switzerland for almost two years. Using fluorescence in-situ hybridization we were able to recognize "*M. parvicella*" as well as filamentous and non-filamentous types of nocardioform actinomycetes. A newly developed rapid quantification method allowed us to measure seasonal variations of the population. The highly variable scumming followed only partly the abundance of the filamentous types of nocardioform actinomycetes and was not correlated to "*M. parvicella*". Neither these organisms nor any plant operating condition could fully explain the dynamics of the scumming. The data lead us to suggest that high numbers of "*M. parvicella*" or nocardioform actinomycetes are not sufficient to cause scumming. The measured dynamics of the population and the scum coverage also imply that, to investigate scumming at a particular WWTP, time-series of measurements including different seasons and periods of different scumming intensities are necessary.

3.1 Introduction

The formation of stable scum (activated sludge foaming) is a widespread problem in biological wastewater treatment plants (WWTP), where it causes serious operating problems, increases maintenance and may lead to poor quality effluent. It is widely accepted that the formation and stabilization of foam or scum requires gas bubbles, surfactants and hydrophobic particles. Gas bubbles are present in the system as air bubbles in the aeration tanks or nitrogen gas in the anoxic tank. Surfactants are present in the wastewater, but are also produced by the microorganisms. The hydrophobic

particles are certain microorganisms attached to the bubbles. "*Microthrix parvicella*" and nocardioform actinomycetes are often dominant in scumming WWTP and are enriched in the scum. Therefore, these bacteria are considered to play an important role in the formation and stabilization of scum.

Many studies as well as plenty of empirical knowledge about the occurrence of these organisms and scumming exists. In some WWTPs, the formation of scum was found to be related to the number of "*M. parvicella*" (Hwang and Tanaka, 1998; Miana *et al.*, 2002). In others, however, nocardioform actinomycetes seemed to be responsible (Davenport *et al.*, 2000; de los Reyes III and Raskin, 2002). However, most studies had to acknowledge that other, not measured, factors must contribute to scumming. Despite the large number of investigations, the role of the suspected scum forming microorganisms and the differences among WWTPs is not yet fully understood.

The purpose of the presented study was to analyze the influence of "*M. parvicella*" and nocardioform actinomycetes to the formation of scum on a full-scale WWTP in Switzerland. Additionally, we were asking, which plant operation parameter seem to be important and should, therefore, be further studied. This paper presents and discusses the results of a monitoring program of almost two years.

3.2 Methods

3.2.1 Wastewater treatment plant

The WWTP Kloten-Opfikon (Switzerland) treats the wastewater of 54,000 population equivalents. Between a quarter and a third of the COD-, N- and P-load origins from the airport of Zurich (not containing deicing fluids). The other part of the influent consists of mainly municipal wastewater with effluents from companies in the service sector.

The layout of the plant is shown in Figure 3-1. A partial flow of the primary effluent is led through a first biological stage with two parallel lanes (640 m³ activated sludge volume each). This stage is operated fully aerobic with a sludge retention time (SRT) between 1-2 days. The effluent of the first stage, together with the untreated part of the primary effluent, flows into the main biological stage, which consists of four parallel lanes of 1400 m³ activated sludge volume each. One third is kept anoxic to allow denitrification. The main stage was operated with an aerobic SRT between 8-12 days. Before the main stage, ferric sulfate (for a short period aluminum sulfate) is added to precipitate phosphate. The characterization of the wastewater and the activated sludge of the main stage are summarized in Table 3-1.

In this study, lane four of the main biological stage was investigated.

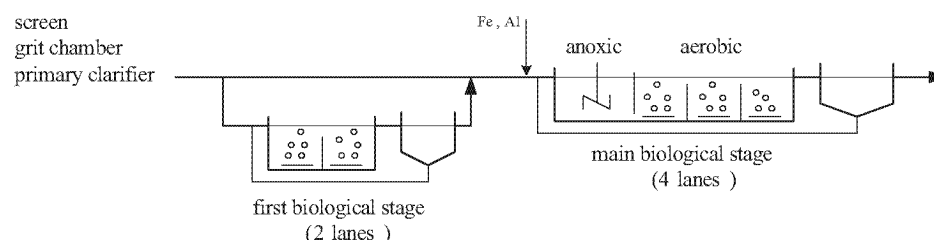


Figure 3-1: Design of WWTP Kloten-Opfikon. A partial flow is pre-treated in the first biological stage. One third of the main stage is operated under anoxic conditions.

Table 3-1: Characterization of the influent (including digester supernatant) and the activated sludge of the main stage: Median and 10%-, 90%-percentiles of the values between 1 July 2001 and 30 June 2003 (total P from 1 Jan 2002 to 30 June 2003).

Influent to main stage				
Inflow	m ³ d ⁻¹	(15000)	17700	(24400)
Temperature	°C	(13)	17	(21)
total COD	mg L ⁻¹	(87)	177	(243)
total BOD	mg L ⁻¹	(47)	86	(127)
total N	mg L ⁻¹	(29)	38	(47)
NH ₄ -N	mg L ⁻¹	(13)	21	(27)
total P	mg L ⁻¹	(3.0)	3.9	(4.8)
activated sludge (main stage)				
aerobic SRT	d	(8)	11	(12)
MLSS	g L ⁻¹	(3.0)	3.5	(4.0)
SVI	mL g ⁻¹	(126)	239	(294)

3.2.2 Samples

For FISH analysis, grab samples from the scum layer on the anoxic tank and from the mixed liquor near the effluent of the aerated compartment were taken. To characterize the load and the plant performance, the staff of the WWTP collected and analyzed 24-h composite samples from different parts of the plant as well as grab samples from the activated sludge tanks.

3.2.3 Scum coverage

The amount of scum on the activated sludge tanks was measured by visually judging the percentage of the surface covered by scum. This was separately done for the anoxic and the aerated compartment and at different times of the day.

3.2.4 Fluorescence in-situ hybridization (FISH)

The following CY3 labeled oligonucleotide probes were used: MPA223 and MPA645 (Erhart *et al.*, 1997), MYC657 (Davenport *et al.*, 2000), GOAM192 (de los Reyes *et al.*, 1997). A FITC labeled mixture of EUB338 (Amann *et al.*, 1990) with EUB338 II and EUB338III (Daims *et al.*, 1999), specific for the most *bacteria*, was used as reference. As negative control, hybridization without probe and NonEUB338 (Wallner *et al.*, 1993), complementary to EUB338, was performed.

Since the cell walls of "*M. parvicella*" and nocardioform actinomycetes are difficult to be penetrated by the oligonucleotide probes, different fixation and permeabilization protocols were tested: Fixation with ethanol (Roller *et al.*, 1994) or paraformaldehyde (de los Reyes *et al.*, 1997); additional treatment as combinations of heat treatment (90°C, 30 min; Jurtshuk *et al.*, 1992) and the application of lysozyme (Beimfohr *et al.*, 1993) and mutanolysin (Erhart *et al.*, 1997) at different concentrations and incubation times. The signal intensity and evenness was judged visually (epifluorescence microscope) for the target organisms shown in Figure 3-2. The following treatment was found to be most appropriate and was therefore applied in this study: Activated sludge samples were fixed in PFA for 1 min according to de los Reyes *et al.* (1997) and stored in Ethanol/PBS (1:1) at -20°C. Prior to hybridization, the samples were applied to Teflon coated slides, air-dried and dehydrated in 50, 80 and 96% ethanol for 2 min each. The samples were then covered with lysozyme (Fluka 62971; 10mg/ml in 100mM Tris, 50mM EDTA, pH 8.0; Beimfohr *et al.*, 1993) for 30 min

at room temperature, followed by another ethanol series. For the hybridization of "*M. parvicella*", the samples were additionally treated with Mutanolysin (Fluka 70017; 5000 U/ml in 0.1 M potassium phosphate buffer at pH 6.8; Erhart *et al.*, 1997) for 15 min at room temperature, followed by a last ethanol series. Hybridization was performed on microscope slides according to Manz *et al.* (1992).

3.2.5 Microscopy and Quantification of bacteria

Microscopy was performed on an Olympus BX50 epifluorescence microscope, equipped with filtersets HQ-CY3 and HQ-FITC (Analysetechnik AG, Tübingen, Germany) and an Olympus Objective (UplanFl 100x, 1.30) under oil immersion.

A rapid procedure to quantify the identified microorganisms was developed; details of the method and a discussion of its advantages and limitations are presented by Hug *et al.* (2005b). For each observed morphotype (Figure 3-2), simplified drawings of a microscopic field of view were made, containing flocs as gray shapes and fluorescent bacteria as black lines or dots. For this study a preliminary rating system was used. It consisted of six categories, where the abundance of target cells approximately doubles from one to the next; category 0 indicates that no target cell was observed. Quantification was done by visually assigning a category to the microscopically observed abundance of fluorescent target organisms. Types of organisms that were fluorescent in the negative controls (no probe or NonEUB338) were not taken into account.

Each sample was applied randomly to two spots ("wells") on the microscope slides. This prevented systematic errors by making sure that the operator did not know what sample was being quantified. From each well, five randomly selected fields of view (100x objective) were quantified; ten analyzed fields per sample and morphotype proved to be sufficient to recognize the seasonal pattern of the observed bacteria. All presented data were quantified by the same operator and are therefore not affected by systematic deviations among different operators.

The mean abundance and its confidence interval of the target organism in one sample were calculated by a non-parametric bootstrap procedure. The principle of the bootstrap is to create many new data sets by sampling (with replacement) from the original data set. We drew 1000 times ten values from the original ten values and calculated 1000 possible mean values. Due to the logarithmic scale of the rating system, each value was transformed into a hypothetical "concentration" ($c = 2^x$, where x is the assigned category value) prior to the calculation of the average; the results were transformed back to category units. From the resulting distribution of 1000 mean abundance values, the median and the 2.5% and 97.5% percentiles were calculated, representing the expectation value and confidence interval ($p = 0.95$) of the mean abundance.

3.3 Results

We were able to distinguish "*M. parvicella*" and three different morphotypes of nocardioform actinomycetes: the typical branched filaments, short filaments thinner than the branched filaments, and small rod-shaped cells (Figure 3-2). Using a specific oligonucleotide probe to further distinguish the nocardioform actinomycetes, the branched filaments were identified as *Gordona amarae*.

The abundance of "*M. parvicella*" and the filamentous nocardioform actinomycetes in the mixed liquor exhibited a clear seasonal variation (Figure 3-3a, b, c). "*M. parvicella*" occurred in high numbers during winter and spring while nocardioform actinomycetes were more abundant in summer and autumn. The data allow the suggestion that the branched filaments were not only highly abundant in autumn 2002 but also one year before and after. The non-filamentous type of nocardio-

form actinomycetes did not show a seasonal variation and was always around category two. Only "*M. parvicella*" and the branched filaments were enriched in the scum.

The severity of scumming is shown in Figure 3-3e as percentage of the activated sludge basins being covered by scum. Several major scumming events occurred: Short peaks occurred in February, June and July 2002. In September 2002 a long period of intensive scumming started. At the end of 2002, the scum coverage collapsed and stayed below fifty percent until the end of April 2003 when it increased again rapidly. Most of the time, however, the amount of scum on the basins seemed to vary randomly with many days of very low coverage.

The typical range of plant operation and environmental parameters are summarized in Table 3-1. The ammonia rich digester supernatant was regularly added at night. The wastewater flow led through the first stage was increased (from 10% to 60% of the total influent) from November 2001 to April 2002 and from January to April 2003. This reduced the COD load to the main stage to about 50% and lead there to a slightly decreased sludge concentration (MLSS) and airflow of the aeration. Trying to control bulking, aluminum sulfate was used instead of ferric sulfate to precipitate phosphate, from 2 to 11 December 2002 and 16 and 17 February 2003. Only the temperature exhibits a distinct seasonal variation. The sludge volume index correlates well with the abundance of "*M. parvicella*". The other parameters were either almost constant or highly variable without showing clear trends.

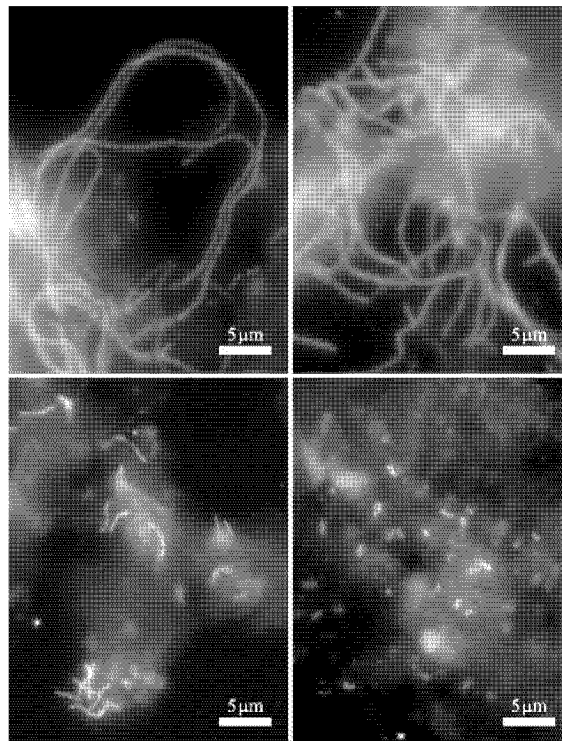


Figure 3-2: The organisms monitored in this study (epifluorescence micrographs after FISH with CY3-labeled probes): The image top left shows the long filaments of "*M. parvicella*". The other images show the three distinguished morphotypes of nocardioform actinomycetes: top right branched filaments (*G. amarae*), bottom left short thin filaments, bottom right small rod-shaped cells.

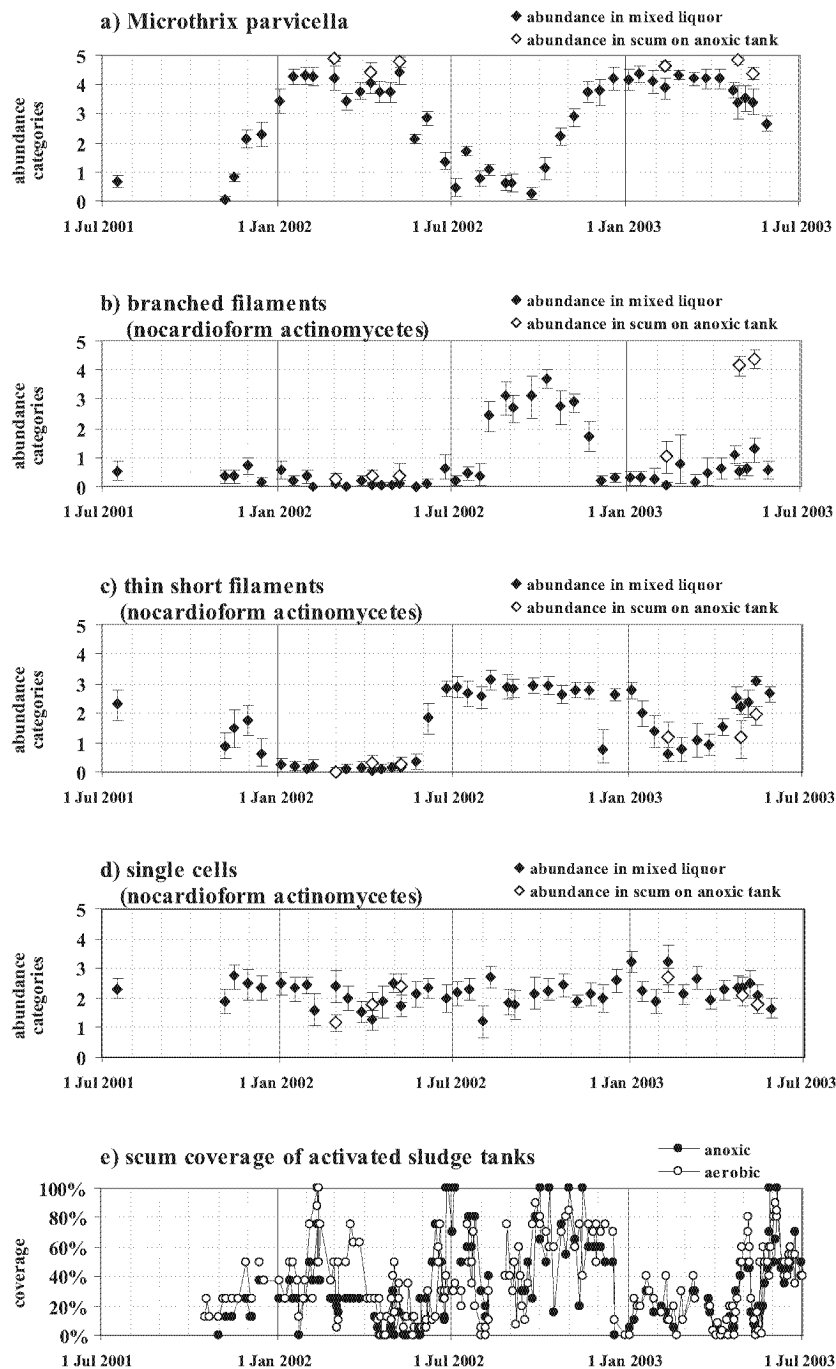


Figure 3-3: The abundance of "*M. parvicella*" (a) and the two observed filamentous types of nocardioform actinomycetes (b, c) followed a distinct seasonal pattern, whereas the non-filamentous type (d) remained rather constant. "*M. parvicella*" and the branched filaments were enriched in the scum. The scale of the abundance is logarithmic: from one category to the other the concentration approximately doubles. The error bars indicate the confidence interval ($p = 0.95$) of the mean values. The scum coverage (e) of the anoxic and aerobic activated sludge basins was highly variable but could partly be correlated to the abundance of nocardioform actinomycetes.

3.4 Discussion

None of the observed bacteria did fully correlate with the scum coverage of the activated sludge basins. However, some patterns seen in Figure 3-3 match together. The steep increase of the scum coverage in early summer 2002 and 2003 can be linked to the growth of the thin short filamentous nocardioform actinomycetes, while the long scumming event in autumn 2002 could, with a certain delay, be related to the branched filaments. The increase of the scumming intensity during winter 2001/2002 occurred in parallel to the accumulation of "*M. parvicella*". Both reached maximum values in February 2002, but "*M. parvicella*" remained in high numbers while the scum coverage decreased again. Apart from that possibility, "*M. parvicella*" seems not to be related to the variations of scumming at the investigated WWTP. This is surprising since it contradicts to the experience of many other WWTP.

Our results correspond generally to the findings by Davenport *et al.* (2000) and de los Reyes III and Raskin (2002) who linked scumming to the concentration of nocardioform actinomycetes in the activated sludge. The authors of those studies considered also non-filamentous organisms to be important. At the WWTP investigated in our study, non-filamentous nocardioform actinomycetes were common but their abundance did not follow the variations of the scum coverage and they were not enriched in the scum. Like the mentioned studies that could not fully explain the observed scumming, we were not able to explain the dynamics of the scum coverage by the abundance of the measured organisms. Our data reveal that none of the observed bacteria is solely responsible for scumming at this particular WWTP.

Nevertheless, at all time at least one of the suspected scum forming organisms occurred in high numbers. That points to the possibility that there is always a certain scumming or floating potential in that activated sludge and that other factors control the actual scumming intensity.

This leads to the second goal of this study, which was to identify plant operation parameters that seem to play a role in the formation of scum. They can either influence the growth of the suspected scum forming organisms or directly the formation and or stabilization of scum.

The only factor that obviously influences the abundance of the monitored organisms is the temperature. All observed filamentous bacteria followed this pattern. "*M. parvicella*" started to accumulate when the wastewater temperature dropped below about 15°C and began to decrease when the temperature exceeded the same value. The short thin filamentous nocardioform actinomycetes grew above about 18°C and the branched filaments preferred more than 20°C.

Trying to control the growth of "*M. parvicella*" that causes serious bulking at this WWTP, the plant operators increased the flow through the first stage during winter. This reduced the COD load but did not influence the abundance of "*M. parvicella*". For the same reason, aluminum sulfate was added at the beginning of December 2002 and in February 2003. While "*M. parvicella*" was not affected, the two types of filamentous nocardioform actinomycetes were possibly reduced. Whether the collapse of the scum at the end of 2002 (Figure 3-3e) was caused by the aluminum dosage is not clear because Christmas holidays started at the same time which could have changed the wastewater composition.

We expected the airflow to directly influence the foaming or scumming intensity in the aerated compartments. In fact, the decrease of the scum coverage at the end of 2002 and the increase in April 2003 correlated with the variations in the airflow. But on the other side, one year before, the two values changed in opposite directions (data not shown).

Similar to the suspected scum forming organisms, we could not find an operation parameter that dominantly controls scumming at this particular WWTP.

3.5 Conclusions

In many scumming WWTPs, "*M. parvicella*" and or nocardioform actinomycetes are abundant in the mixed liquor and enriched in the scum. This leads to the common assumption that high numbers of these organisms necessarily induce severe scumming.

A newly developed rapid quantification method based on logarithmic classification systems allowed us to monitor the seasonal variation of "*M. parvicella*" and different morphotypes of nocardioform actinomycetes. The presented results of almost two years revealed that the scum coverage of the activated sludge basins at the investigated WWTP was partly correlated with the abundance of some types of nocardioform actinomycetes, but not with "*M. parvicella*". Nevertheless, we could not explain the whole variability of the scum formation.

This study confirmed that scumming is not solely controlled by the abundance of one of the suspected organisms or by one dominant plant operation parameter. A still unknown combination of factors is rather controlling the formation and stabilization of scum.

Moreover, our results indicate that, in order to investigate the causes of scumming at a particular WWTP, it is not sufficient to characterize the sludge and the operating conditions only during one single scumming event and to extrapolate the findings to other days and other seasons. In most cases, a monitoring program including periods with different scumming intensities and including different seasons will be necessary.

More research is needed to understand the mechanisms of activated sludge scumming. It is particularly important to search for factors causing a rapid development of scum and, by this, to clarify the role of the suspected scum forming bacteria.

3.6 Acknowledgements

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Chapter 4

Unexpected Growth of “*Microthrix parvicella*” and no Correlation with Floc Structure and Foaming in a Pilot-Scale Activated Sludge Plant

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Abstract

Inhibited sludge settleability (bulking) and the formation of stable foam are still unsolved problems in many wastewater treatment plants (WWTPs) all over the world. The filamentous bacteria “*Microthrix parvicella*” (*Microthrix*) and nocardioform actinomycetes are often thought to play an important role in such solids separation problems. The operation of two pilot-scale plants fed with the same wastewater as a full-scale WWTP where seasonal variations of these organisms were observed revealed surprising findings. While *Microthrix* was usually hardly growing due to the low sludge retention time of ten days (33% anoxic) and the temperature of 20°C, it suddenly steeply increased under apparently the same conditions. Increased nitrite concentration led to the suggestion that incomplete nitrification or denitrification might be responsible. Contradicting to widespread assumptions, *Microthrix* and nocardioform actinomycetes was neither correlating with the poor settleability of the sludge nor with the observed foam formation.

4.1 Introduction

While state of the art wastewater treatment plants (WWTPs) easily fulfill requirements on COD and nutrient removal, floc structure issues leading to inhibited sedimentation (bulking) or the formation of stable foam are still not fully understood. Such solids separation problems are not only a risk for the receiving waters but also increase operational costs due to enhanced maintenance and investment costs because larger tanks are constructed to provide safety.

The filaments bacteria “*Microthrix parvicella*” (in the paper referred as *Microthrix*) cause severe bulking in many plants. *Microthrix* is furthermore, like different types of nocardioform actinomycetes, suspected to produce foam, due to its hydrophobic cell walls (Seviour and Blackall, 1999; Jenkins *et al.*, 2004). Despite decades of research about *Microthrix* growth and its role in the formation of stable foams (Rossetti *et al.*, 2005), there exist still plenty of different hypotheses.

In the Swiss WWTP Kloten-Opfikon (54,000 p.e., pre-denitrification) distinct seasonal variation of *Microthrix* and filamentous types of nocardioform actinomycetes occur (Hug *et al.*, 2005c). During high abundance of *Microthrix* in winter and spring severe bulking occurs, while foam coverage of the activated sludge tanks seems not to be correlated with those bacteria.

These distinct seasonal population shifts and the lack of correlation between foam formation and the abundance of the above mentioned bacteria led to the following questions:

- Is the seasonal population variation caused by the temperature or by seasonal variations of the wastewater composition (e.g. due to biological processes in the sewer)?
- Can bulking and foaming be correlated with particular operating conditions (temperature, oxygen, sludge retention time) and what roles do the filamentous bacteria play?

To tackle these questions a sophisticated experimental design was developed and applied to a pilot-scale activated sludge plant that was fed with the same wastewater as the full-scale plant where these observations were made.

4.2 Methods

4.2.1 Experimental design

An experimental plan was developed (Table 4-1 and Figure 4-2i) to study the influence of the temperature (T), the dissolved oxygen concentration (DO), the sludge retention time (SRT) and the presumable seasonal variation of the wastewater composition. The different conditions include the condition that was expected to favor *Microthrix* (low T and low DO) and favoring nocardioform actinomycetes (high T and high DO), respectively. The latter conditions were used as reference conditions (see below).

A complete 2^n factorial design considers all possible combinations of n factors (T, DO, SRT) at two levels (high/low). This allows to analyze not only the influence of each factor but also of their interactions. For time reasons, we decided to perform a complete factorial design to investigate the influence of T and DO (2^2), and to add one experimental phase to test a higher SRT. In factorial designs it is usually assumed that the experiments are replicable. However, this assumption was not fulfilled as we operated the plants with real wastewater and the effectively measured flow dynamics. Therefore, we operated two parallel plants, one of them always under the same “reference” conditions (REF), and observed the difference to the plant operated under varying conditions (VAR). Observing variations in the REF plant allowed furthermore to study seasonal effects, such as wastewater composition and load, independent of the temperature.

Although both plants were identically constructed, one cannot rule out that slight differences exist and affect the results. To prevent such misinterpretations, not always the same plant was operated as REF, and two experimental phases where both plants were operated under the same (REF) conditions were included into the experimental plan (experimental phases 1 and 5).

To ensure the same initial conditions (sludge composition) in both plants for a particular experimental phase, the following procedure was applied when starting a new experimental phase: The sludge of the VAR plant was wasted, the REF sludge equally distributed to both plants and the reactors filled up with secondary effluent from the full-scale plant (indicated by solid vertical lines in Figure 4-2). The plants were then operated under the new operating conditions, but no sludge was wasted until the expected solids concentration was reached (grey shapes in Figure 4-2).

The plants were inoculated with activated sludge from the parallel full-scale WWTP and operated for five months under REF conditions before the experiments started.

4.2.2 Pilot plant

Each of the of two parallel pilot plants consisted of a 100 liter anoxic and 200 liter aerated reactor (both stirred) followed by a secondary clarifier of 280 liter, with free overflow between the tanks (Figure 4-1). The volumes and hydraulic retention time was chosen according to the design of the full-scale plant. The temperature of the reactors was kept constant and DO was controlled by intermittent aeration with a two-point controller, according the set-points in Table 4-1.

The plant was fed with primary effluent from the parallel full-scale WWTP. To ensure an equal distribution of the influent, a three-way-valve let the wastewater alternate between the two plants, switching every ten minutes. The substrate gradient introduced by this pulse feeding was assumed to be small as the pulse is short compared to the hydraulic retention time in the first reactor (around five hours). The wastewater feeding pump was controlled by the flow measurement of the full-scale plant, so that the same dynamics due to diurnal variations and rain events occurred (Figure 4-2a). Return sludge flow was controlled by the same signal as the wastewater pump; the flow was about equal to the wastewater flow. To control SRT, the waste sludge flow was adjusted every two to four days based on the solid loss in the effluent.

From September 2004, serious foam formation occurred in the VAR plant, leading sometimes to complete coverage of the clarifier. Every one to three days, the floating sludge was manually removed from the clarifier and put into the aerobic reactor. End of November 2004, large parts of sludge in the VAR plant were lost (Figure 4-2b) and the experiments were stopped.

4.2.3 Sampling and measurements

Sampling

Flow proportional 24-hour composite samples were taken every two to ten days from the wastewater (at the effluent of the primary clarifier of the full-scale plant) and the effluent (from just below the surface of the secondary clarifiers of the pilot plants, behind baffles). For sludge analyses, grab samples from the aerobic reactors were taken; prior to sampling the aeration was manually switched on to ensure complete mixing.

Table 4-1: Experimental design (T = Temperature, DO = dissolved oxygen, SRT = sludge retention time)

experimental phase	reference conditions (REF)				variable conditions (VAR)			
	T [°C]	DO gO ₂ m ⁻³	SRT d	plant -	T [°C]	DO gO ₂ m ⁻³	SRT d	plant
1	20	2.0...3.0	10	2	20	2.0...3.0	10	1
2	20	2.0...3.0	10	2	10	0.5...1.0	10	1
3	20	2.0...3.0	10	2	10	2.0...3.0	10	1
4	20	2.0...3.0	10	1	20	0.5...1.0	10	2
5	20	2.0...3.0	10	1	20	2.0...3.0	10	2
6	20	2.0...3.0	10	1	20	2.0...3.0	20	2

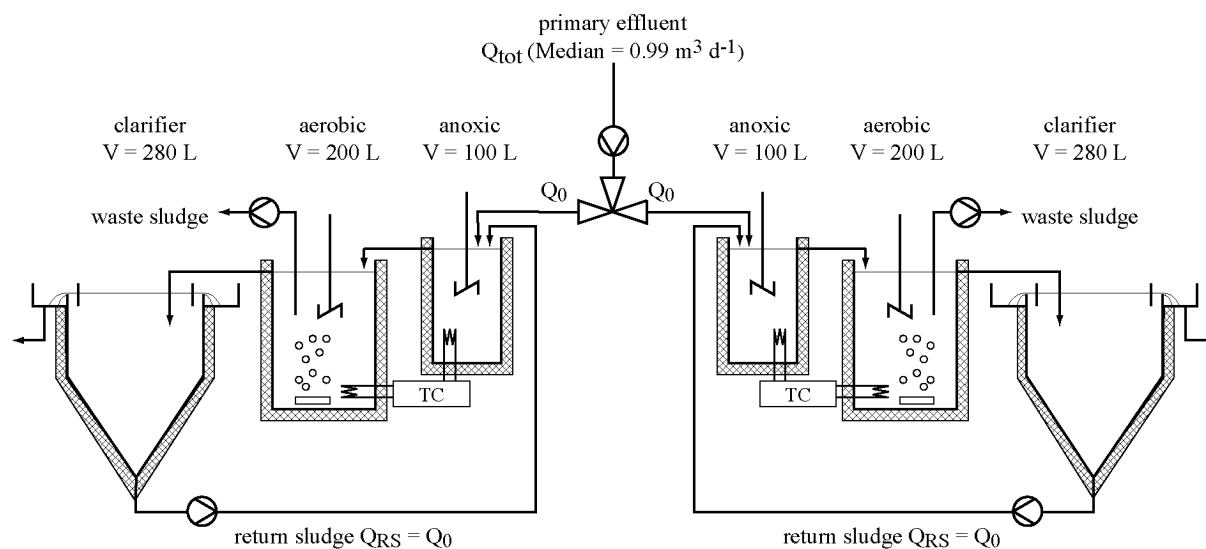


Figure 4-1: Design of the two parallel pilot plants: The reactors are insulated and temperature controlled (TC). The wastewater flow (primary effluent from the full-scale WWTP) is controlled by the flow measurement of the parallel full-scale WWTP and equally distributed to both pilot plants.

Chemical and physical analyses

Chemical analyses (COD, total P, total N, Ammonium, Nitrite, Nitrate) of wastewater, effluent and sludge components were made using photometric LANGE cuvette tests (HACH LANGE GmbH, Düsseldorf, Germany).

Mixed liquor suspended solids (MLSS) were measured by filtration through glass microfibre filters (Whatman GF/F, $0.7 \mu\text{m}$) and drying at 105°C overnight. For the measurement of volatile suspended solids (VSS), the filters were ignited at 550°C .

SVI

The diluted sludge volume index (SVI) was performed according to Jenkins *et al.* (1993) (dilution with effluent until the settled volume after 30 min is less but close to 200 mL L^{-1}).

Floc structure and microbial population

The floc structure was characterized by visual observation under a light microscope with phase contrast. Total filament abundance was determined using the rating system by Jenkins *et al.* (1993), filament identification according to Eikelboom and van Buijsen (1981).

The bacteria abundance was measured by fluorescence in-situ hybridization (FISH), using the probes MPA223 and MPA645 (Erhart *et al.*, 1997) for *Microthrix* and MYC657 (Davenport *et al.*, 2000) for nocardioform actinomycetes. The fixation, pretreatment, hybridization and quantification was done as described by Hug *et al.* (2005b) resulting in "pixel concentration", a measure of the ratio of target organisms to the apparent total floc-volume.

Foaming potential

The foaming potential of the sludge samples was assessed as described by Hug *et al.* (2006). The procedure is based on the separation of sludge solids and liquid fraction of activated sludge, allowing to correct the effect of the solids concentration in the results.

Estimation of *Microthrix* net growth

Net growth of *Microthrix* was estimated by a steady state mass balance including inoculation as

$$\text{net growth} = \frac{\text{biomass loss through waste sludge and effluent}}{\text{inoculation of Microthrix as fraction of suspended solids in the wastewater}}$$

or

$$\mu_{\text{net}} = \frac{1}{\text{SRT}} - \frac{Q_0}{V_{\text{ML}}} \cdot \frac{\text{TSS}_0 \cdot f_{\text{Mic},0}}{\text{TSS}_{\text{ML}} \cdot f_{\text{Mic},\text{ML}}}$$

where μ_{net} is the net growth rate [d^{-1}], SRT the sludge retention time [d], Q_0 the wastewater flow [$\text{m}^3 \text{d}^{-1}$], V_{ML} the mixed liquor or reactor volume [m^3], TSS the suspended solids concentration [kg m^{-3}] and f_{MIC} the fraction of *Microthrix* as pixel concentration [-] with the indices 0 for the wastewater and ML for the mixed liquor.

4.3 Results

4.3.1 Performance of pilot plant

Wastewater flow followed the dynamics of the full-scale plants (Figure 4-2a). The average COD concentration in the wastewater to the plant was steady around 250 gCOD m^{-3} ; from September 2004, it was rising, reaching around 350 gCOD m^{-3} at the end of November. Sludge retention time (SRT) was controlled according to the experimental plan (Table 4-1); consequently, at low temperature, solids concentration (TSS, Figure 4-2b) increased and food to microorganism ratio (F/M ratio) decreased (Figure 4-2c) due to reduced biomass decay. Nitrification was incomplete at the begin of the experimental phases with reduced temperature (Figure 4-2d and e). In June 2004, nitrite and ammonium concentrations in the effluent of the REF plant started to rise. Improving the mixing by increasing the airflow could stabilize nitrification until mid of August, but nitrite concentration in the REF plant remained elevated. Nitrate concentration in the effluent was always around 10 gN m^{-3} , total nitrogen removal was around 60%, considerably influenced by the relatively high solids concentration in the effluent of $10\text{-}20 \text{ gTSS m}^{-3}$. VSS was always around 80% of MLSS.

4.3.2 Microbial population

No type of *nocardioform actinomycetes* was found in the pilot plant at any time. Also in the primary effluent, their abundance was below the detection limit, which is according to the low level in the full-scale plant.

Microthrix abundance in the pilot plants is shown in Figure 4-2g; the confidence interval ($p=0.95$) of the data is around plus minus a factor two (Hug *et al.*, 2005b). Estimated average filament length of *Microthrix* was mostly below $100 \mu\text{m}$, only when *Microthrix* abundance was high, the filaments were longer (VAR in January 2004, REF in September 2004). The temporal variation pattern of *Microthrix* in the pilot plant was similar to that in the full-scale WWTP (Figure 4-2f) but also to the

abundance in the primary effluent (x symbols in Figure 4-2f). This indicates an inoculation of *Microthrix* by the wastewater. The effect of temperature and dissolved oxygen is visible in the difference between the REF and VAR plant, as variations due to the natural dynamics of the substrate load influence both plants equally. *Microthrix* abundance was mostly identical in both plants. Only in the VAR plant during experimental phase 3 (low T, high DO) the abundance was significantly higher than in the REF plant. The estimated net growth rate in the VAR plant was increased compared to the REF plant during experimental phases 2 and 3, i.e. at low temperature. At SRT of 20 days, *Microthrix* was increased as expected (VAR plant in experimental phase 6); inoculation was not recognized during that time.

From September 2004, foaming occurred in the VAR plant. *Microthrix* abundance was two to four times higher in the accumulated foam on top of the clarifier.

Surprisingly, during the last phase, *Microthrix* abundance in the REF (!) plant climbed up to ten times higher values than in the VAR plant where *Microthrix* was already enriched due to the increased SRT. These unexpected results were confirmed by the additional analysis of Gram stained samples.

4.3.3 Foaming

From 31 Aug 2004 foaming occurred in the VAR plant; it started during the steep increase of the MLSS. About 30% of the aerobic reactor was covered with brown foam of large bubbles (up to 10 cm). The clarifier was covered by usually 10 to 30% by a foam that was composed of individual sludge covered bubbles of 0.5 to 2 mm diameter (Figure 1-1c). Mid of September and from begin of November until the end of the operation, the clarifier was completely covered and a considerable fraction of sludge was trapped in the foam layer.

The foaming potential was always low, in the same range as samples from other conventional full and pilot-scale WWTPs (Hug *et al.*, 2006). During the last experimental phase, foaming potential was increasing in the VAR plant where foaming occurred, while it was decreasing in the REF plant where *Microthrix* abundance was high.

4.3.4 Floc structure

The activated sludge flocs in the two plants were of a quite distinct structure (Figure 4-3). Under reference conditions, the flocs were always compact and rounded. Before April 2004 many filaments were protruding from the flocs (Figure 4-3a and b), mainly type 0961 but also type 021N and *Sphaerotilus natans* (Eikelboom and van Buijsen, 1981). In the VAR plant, at low temperature and particularly at low DO, the flocs became open, with filaments inside the flocs but only rarely protruding (Figure 4-3e-g).

Sludge volume index (SVI; Figure 4-2h) in the REF plant decreased from the beginning of the experiments to reach a minimum of 56 ml g⁻¹ in January 2004. Then it slowly rose, reaching a stable value of around 100 ml g⁻¹, until it started to increase again, peaking at 190 ml g⁻¹ in September 2004, before it collapsed to values below 100 ml g⁻¹. SVI was always higher in the VAR plant, particularly at low DO (experimental phases 2 and 4). It did not correspond with *Microthrix* abundance or its estimated filaments length, but was reflected by the floc structure (Figure 4-3e-h)

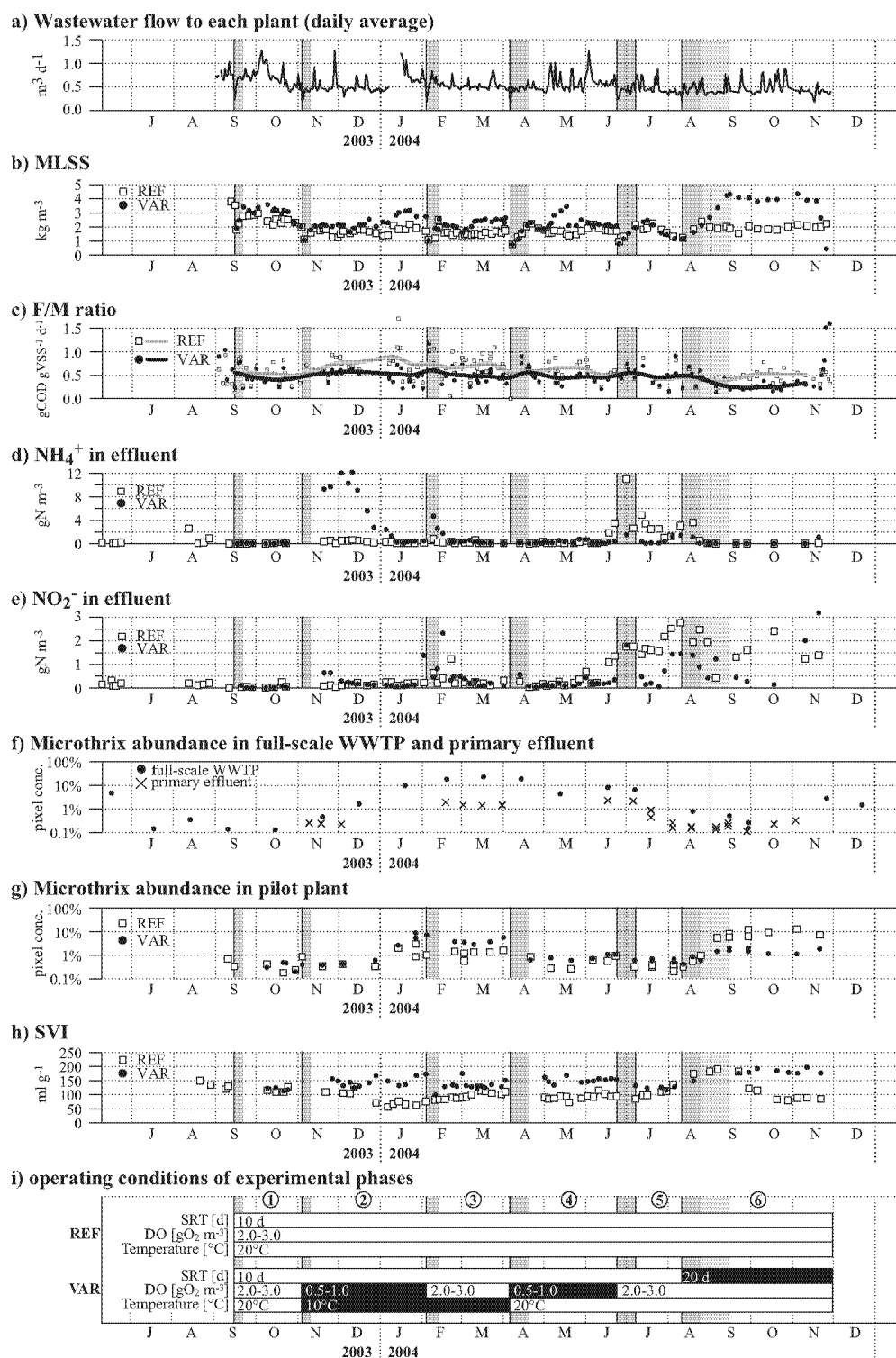


Figure 4-2: Operating conditions and chemical and biological analyses of pilot plant operation. REF stands for the plant operated at reference conditions; the VAR plant was operated at varying conditions. The grey shapes indicate the periods without waste sludge removal at the begin of each experimental phase; at the begin of phase 6 waste sludge withdrawal was switched off longer in the VAR plant (light grey shape). At the begin of each new experimental phase, the VAR sludge was wasted and the REF sludge equally distributed to both plants as indicated by vertical solid lines (in July 2004, due to unstable operation, the sludges were mixed once again when sludge wasting starting).

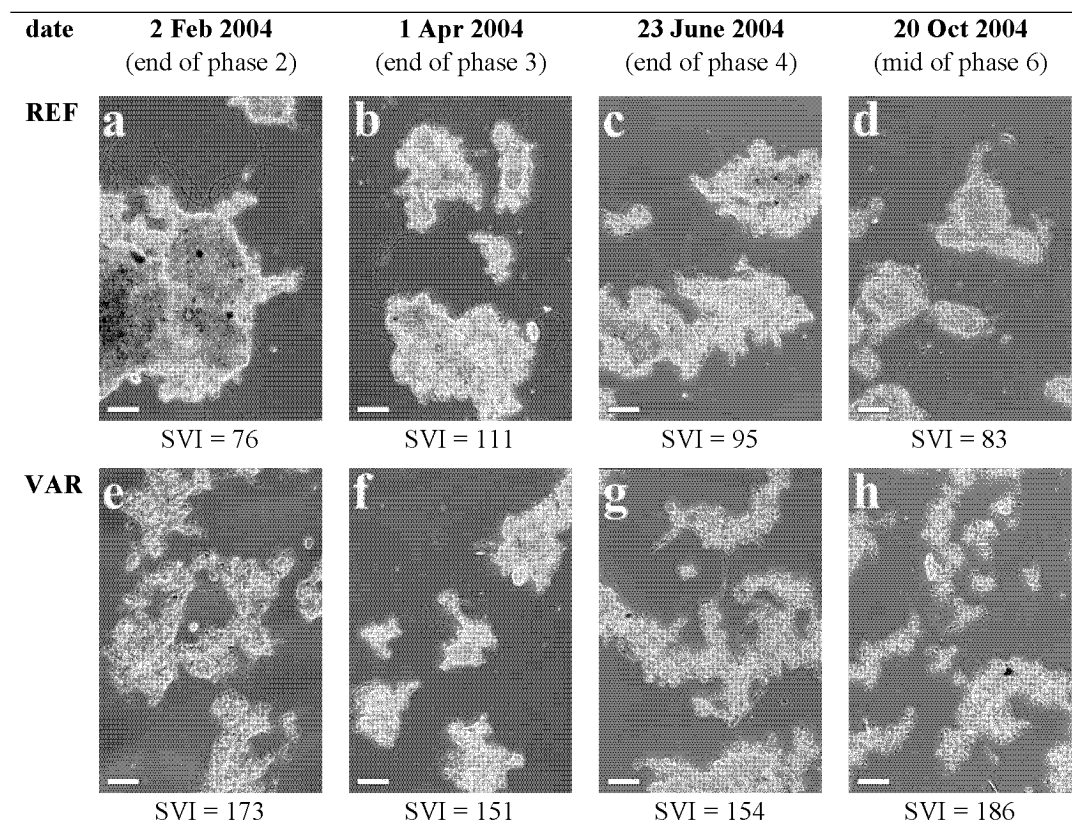


Figure 4-3: Microphotographs of typical flocs (phase contrast, length of bars: 100µm) and diluted sludge volume index (SVI). REF stands for the plant operated at reference conditions; the VAR plant was operated at varying conditions.

4.4 Discussion

4.4.1 Microbial population

The question whether the seasonal variations of the observed microorganisms in the full-scale WWTP Kloten-Opfikon (Hug *et al.*, 2005c) are caused by the temperature itself or by seasonal changes in the wastewater composition could not be answered because nocardioform actinomycetes did not grow in the pilot -scale plants and the total amount of *Microthrix* was influenced by inoculation through the primary effluent. Nevertheless, the influence of the operation conditions is recognizable by the differences between the two pilot plants.

Our results confirm other studies that found *Microthrix* to proliferate at low temperature and high SRT (or low food-to-microorganism ratio, F/M ratio). However, it is surprising that *Microthrix* was able to grow in our experiments with a SRT of 10 days and F/M ratios above 0.4 gCOD gVSS⁻¹ d⁻¹. In the parallel full-scale WWTP *Microthrix* growth was prevented at temperature above 18°C (Hug *et al.*, 2005a; Hug *et al.*, 2005c). The increase of the abundance from 20°C to 10°C was very small compared to the seasonal variations in the full-scale plant over more than two orders of magnitude. This might be due to the generally limited growth because of the low SRT. In contrast to our findings, Knoop and Kunst (1998) washed out *Microthrix* at 10 °C and a F/M ratio of 0.2 gBOD gTSS⁻¹ d⁻¹; at 20°C *Microthrix* changed into short filaments and was finally lost at any F/M ratio. Tsai *et al.* (2003) identified maximum values of 0.30 to 0.54 gCOD gVSS⁻¹ d⁻¹ allowing *Microthrix* growth.

Completely unexpected is the steep increase of *Microthrix* abundance in the REF plant during the last experimental phase (Figure 4-2g) when no inoculation occurred. It shows that *Microthrix* is somehow able to grow extensively at SRT of 10 days, 20°C and a fairly high sludge load of above 0.5 gCOD gVSS⁻¹ d¹ (equivalent to > 0.2 gBOD gTSS⁻¹ d¹). As REF conditions were supposed to be always the same, unknown factors must have changed. It is striking that the increase started after about two months of high nitrite concentrations in the effluent (Figure 4-2e). Incomplete nitrification and denitrification are suspected to favor *Microthrix* growth (Casey *et al.*, 1999; Tsai *et al.*, 2003), as *Microthrix* needs ammonium as nitrogen source and incomplete denitrification inhibit heterotrophic bacteria due to intra-cellular accumulation of the denitrification intermediates NO and N₂O. *Microthrix* is not affected because it is not able to reduce nitrite.

It has to be noted that many results from literature can only qualitatively be compared to our findings due to different wastewater composition and plant design leading to different availability of specific substrate for *Microthrix* and to different substrate gradients in the plants. Furthermore, our quantification method using FISH allows to detect lower concentrations and is able to recognize *Microthrix* inside the flocs, compared to quantification by crystal violet staining (Knoop and Kunst, 1998) or SVI (Casey *et al.*, 1999; Tsai *et al.*, 2003). Accordingly, to investigate the validity of the hypotheses by Casey *et al.* (1999) and Tsai *et al.* (2003) further specific experiments with the same plant design and bacteria quantification methods should be performed.

4.4.2 Foaming

Foaming occurred only during the experimental phase with considerably increased solids concentration. This is in accordance with our findings that the foaming potential of activated sludge is increasing with the solids concentration (Hug *et al.*, 2006). De los Reyes III and Raskin (2002) postulated a threshold concentration of nocardioform actinomycetes that is needed to induce foaming, arguing that the absolute hydrophobic surface controls the foaming potential rather than the fraction of hydrophobic bacteria. However, in our results there were periods without foaming but with higher absolute *Microthrix* concentration than during the foaming period, e.g. in the REF plant during the last experimental phase (Figure 4-2g). This finding as well as the measured foaming potential indicates that *Microthrix* abundance is not necessary or not sufficient for foaming, confirming the observations in the parallel full-scale WWTP (Hug *et al.*, 2005c). Accordingly, other sludge properties must be controlling foam formation.

4.4.3 Floc structure

In literature about bulking sludge and filamentous bacteria it is often implicitly assumed that high SVI is equal to a large number of filaments and sometimes even that it is equal to high *Microthrix* abundance. However, in our experiments, SVI and floc structure were not correlated with *Microthrix* abundance; it rather seems that flocs deteriorate at low temperature and particularly at low DO (Figure 4-2h and Figure 4-3). The fact that under low DO conditions open floc structure developed quickly, leads to the suggestion that the floc is self organizing to optimize diffusion.

In most studies on bulking, SVI is used as quantitative measurement. This is reasonable because it is a simplified measurement of the problem in the plant, i.e. the sedimentation of the sludge in the clarifier. However, SVI represents the effect (quality of sedimentation) but does not differentiate the mechanisms behind; poor sedimentation may be caused by filaments protruding from the flocs but also by open floc structure or by other reasons (Wanner, 1994). Accordingly, our results demonstrate that further information from microscopic examination including filament identification is

essential to clarify the reasons of a high SVI. If possible, filament quantification should be based on fluorescence in-situ hybridization (Wilderer *et al.*, 2002; Hug *et al.*, 2005b).

4.5 Conclusions

To investigate the seasonally alternating occurrence of the filamentous bacteria “*Microthrix parvicella*” (Microthrix) and nocardioform actinomycetes and to study their role in bulking and foaming, two parallel pilot-scale activated sludge plants were operated, fed with the same wastewater as the full-scale plant in which the phenomena were first observed (Hug *et al.*, 2005c).

The results of the pilot-scale studies are in contrast to suggestions by other studies:

- It is generally possible to suppress the suspected foam forming bacteria Microthrix and nocardioform actinomycetes in completely mixed reactors with a total SRT of 10 days and 33% anoxic volume. But in one experiment Microthrix was able to grow to high concentration under just these operating conditions even at 20°C, which is in contrast to reports from other authors, but as discussed above, must be seen in connection with inhibited nitrification or denitrification.
- Foaming was not connected to Microthrix abundance. It occurred when the solids concentration increased.
- Neither floc structure nor sludge volume index (SVI) was correlated with Microthrix but with operating conditions: At low temperature and particularly at low dissolved oxygen concentration flocs of an open structure developed and accordingly SVI increased.

Based on these findings, the following conclusions regarding future research can be drawn:

- Future research efforts on sludge settleability should not only focus on filamentous bacteria but also on other factors affecting floc structure. SVI is a suitable measure for sludge settleability, but to elucidate the reasons for a high SVI or to quantify filamentous bacteria, microscopic analyses are essential.
- In systems exposed to the naturally occurring dynamics of the wastewater flow and composition the knowledge of the actual environmental conditions for the bacteria will always be incomplete. Moreover, in many plants there is the possibility of incomplete mixing that provides ecological niches which are not identified by routine analyses. However, we think that investigations in systems exposed to the natural variability of conditions are necessary to clarify which findings from studies in reproducible systems, such as pure culture studies and experiments with continuously fed artificial wastewater, are valid under the competition in real systems.

4.6 Acknowledgements

We are grateful to the WWTP Kloten-Opfikon, its manager Christoph Liebi and the staff members, for financial, technical and analytical support. We would also like to thank Elke Hinz, Justyna Okolowicz and Mandy Ziranke for their engagement in construction and operation of the pilot plants as well as for contribution in FISH analysis. This project was performed in the framework of COST action 624.

Chapter 5

Modeling Seasonal Dynamics of "*Microthrix parvicella*"

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Abstract

The filamentous bacteria "*Microthrix parvicella*" can cause serious bulking and scumming in wastewater treatment plants (WWTP) all over the world. Decades of research have identified *Microthrix* as specialized lipid consumer but could not clarify the processes that allow this organism to successfully compete in activated sludge systems. In this study we developed a model, based on ASM3, that describes the pronounced seasonal variations of *Microthrix* abundance observed in a full-scale WWTP. We hypothesize that low temperatures reduce the solubility of lipids and inhibit their uptake by non-specialized bacteria. The presented model structure and parameters successfully fit the measured data; however they do not necessarily reflect the only and true selection mechanism for *Microthrix*. This model is not yet to be used for prediction; it is rather a valuable research tool to coordinate the discussion and plan future research activities in order to identify the relevant selection mechanisms favoring *Microthrix* in activated sludge systems.

5.1 Introduction

The filamentous bacterium candidatus "*Microthrix parvicella*" (in the paper referred as *Microthrix*) is associated with serious operational problems in many wastewater treatment plants (WWTP) all over the world. Its long filaments cause serious bulking and its hydrophobic cell surface might cause or support the formation of stable scum.

Microthrix is a slowly growing specialized lipid consumer and is able to store them under aerobic, anoxic and anaerobic conditions (Slijkhuis, 1983; Andreasen and Nielsen, 2000). Lipids seem to be preferentially available for *Microthrix* due to specific sorption onto the hydrophobic cell walls, digestion by cell surface associated extracellular enzymes and released bio-surfactants. Further hypotheses focus on the need for reduced S and N and on the role of incomplete nitrification and denitrification (Tsai *et al.*, 2003). The filamentous morphology might be another advantage in the competition for substrate. Interestingly, *Microthrix* prefers low temperatures, below about 15°C

(Eikelboom *et al.*, 1998; Knoop and Kunst, 1998), and often occurs in distinct seasonal variations (Eikelboom, 1994; Miana *et al.*, 2002; Hug *et al.*, 2005c).

Despite decades of research it is still unknown what mechanisms allow *Microthrix* to successfully compete against faster growing bacteria in activated sludge. In order structure the finding and hypotheses, to coordinate the discussion and to identify further research needs, we propose the use of mathematical modeling. Several approaches have been made to model the competition between floc-forming and filamentous bacteria (reviewed by Martins *et al.*, 2004), but only few attempts were presented to model *Microthrix* growth. To our knowledge, no model structure has been published to investigate the seasonal variations of *Microthrix*. We expect that studying dynamic variations of its abundance, in contrast to its general (quasi steady state) occurrence or absence, will provide new insights into the mechanisms allowing *Microthrix* to successfully compete in activated sludge systems.

In this study we developed a mathematical model that is able to simulate the seasonal pattern of *Microthrix* abundance we observed in a full-scale WWTP (Hug *et al.*, 2005c). To distinguish the influence of the wastewater composition from the substrate affinity, we implemented the hypothesis that low temperature decreases the solubility of lipids and reduce their availability for most bacteria, while *Microthrix* as a specialized lipid consumer is less inhibited. The model is not to be used for prediction; it has rather been developed as research tool to identify key mechanisms leading to excessive *Microthrix* growth. This paper describes the model structure which is based on the activated sludge model no 3 (ASM3; Gujer *et al.*, 1999), presents first results and suggests further developments and applications.

5.2 Methods

5.2.1 Model structure

Allowing the model to be widely used as coordinating research tool, its structure has to be simple, well known and flexible. Therefore, this model is based on ASM3 (Gujer *et al.*, 1999) that reflects the state of the art of modeling activated sludge systems.

ASM3 describes heterotrophic growth (Figure 5-1) as a linear series of processes: hydrolysis of particulate substrate (X_S) leads to soluble substrate (S_S) that is internally stored by the heterotrophic biomass (X_H) which only grows on the stored products. Cell decay is modeled as endogenous respiration of bacteria X_H and stored substrates X_{STO} , where X_H is partially converted to inert particulate COD (X_I). All these processes occur aerobically and anoxically (reduced by a reduction factor η_{NO} , see Table 5-2); anaerobic processes are not part of ASM3.

Microthrix growth was modeled according to the heterotrophic growth in ASM3 (Figure 5-1 and Table 5-1). We introduced as new compounds particulate and soluble lipids (X_{LIP} and S_{LIP}), internal lipid storage X_{STOLIP} and *Microthrix* biomass X_{MIC} , and as new processes lipid hydrolysis, storage of lipids by heterotrophic biomass and by *Microthrix*, *Microthrix* growth and endogenous respiration of *Microthrix* and its stored lipids. *Microthrix* growth on stored lipids was assumed to be obligate aerobic (Slijkhuis, 1983; Rossetti *et al.*, 2002), all other processes occur also under anoxic conditions.

X_S , S_S and X_{LIP} but not S_{LIP} occur in the wastewater as we assumed all lipids to be particulate, adsorbed to particles or immediately adsorbing to the activated sludge when entering the plant.

The kinetic rate expressions of all new processes are according to ASM3. Hydrolysis of X_{LIP} and X_S was modeled dependent on the sum of X_H and X_{MIC} .

The selection mechanism favoring Microthrix in winter was introduced as temperature dependent substrate affinity in the lipid uptake (storage of S_{LIP} by X_H and X_{MIC} , processes $j = 14...17$) as shown in Figure 5-2. This is according to the above-stated hypothesis that low temperature reduces the solubility of lipids and therefore reduces their availability for X_H , while Microthrix as a specialized lipid consumer is less affected.

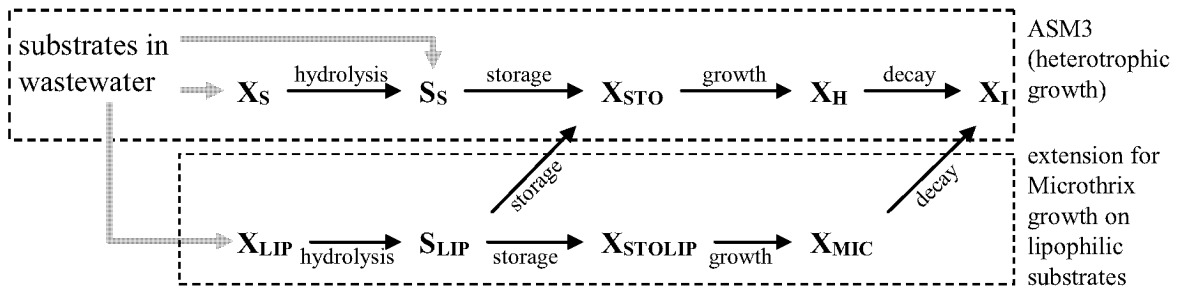


Figure 5-1: The heterotrophic growth model of the ASM3 (upper part) was extended with lipids as substrates X_{LIP} and S_{LIP} that are utilized by Microthrix X_{MIC} and the heterotrophic biomass X_H (bottom part). X_i indicate particulate and S_i soluble compounds. Endogenous respiration of storage products is not shown.

Table 5-1: Stoichiometric matrix: coefficients $v_{j,i}$ describing the model extension to ASM3. The numbers of the new compounds ($i = 14...17$) and processes ($j = 13...22$) are continuing those of ASM3. Compounds of ASM3 are only shown if they are part of the new processes for lipid utilization.

compounds i	new processes j	units	compounds in ASM3								new compounds			
			1 S_{O_2} O_2	2 S_I COD	4 $S_{NH_4^+}$ N	5 S_{N_2} N	6 S_{NOX} N	7 S_{HCO^+} Mole	8 X_I COD	11 X_{STO} COD	14 X_{LIP} COD	15 S_{LIP} COD	16 X_{STOLIP} COD	17 X_{MIC} COD
	13 hydrolysis X_{LIP}			$f_{S_{LIP}}$	$v_{13,4}$			$v_{13,7}$			-1	$1-f_{S_{LIP}}$		
	14 aerobic storage S_{LIP} by X_H		$-(1-Y_{STOLIP,H,O_2})$		$v_{14,4}$			$v_{14,7}$		Y_{STOLIP,H,O_2}		-1		
	15 anoxic storage S_{LIP} by X_H				$v_{15,4}$	$-v_{15,6}$	$-\frac{(1-Y_{STOLIP,H,NO})}{2.86}$	$v_{15,7}$		$Y_{STOLIP,H,NO}$		-1		
	16 aerobic storage S_{LIP} by X_{MIC}		$-(1-Y_{STOLIP,MIC,O_2})$		$v_{16,4}$			$v_{16,7}$				-1	Y_{STOLIP,MIC,O_2}	
	17 anoxic storage S_{LIP} by X_{MIC}				$v_{17,4}$	$-v_{17,6}$	$-\frac{(1-Y_{STOLIP,MIC,NO})}{2.86}$	$v_{17,7}$				-1	$Y_{STOLIP,MIC,NO}$	
	18 aerobic growth X_{MIC}		$-\frac{(1-Y_{MIC})}{Y_{MIC}}$		$v_{18,4}$			$v_{18,7}$					$-\frac{1}{Y_{MIC}}$	1
	19 aerobic endog. resp. X_{MIC}		$-(1-f_{XI})$		$v_{19,4}$			$v_{19,7}$	f_{XI}					-1
	20 anoxic endog. resp. X_{MIC}				$v_{20,4}$	$-v_{20,6}$	$-\frac{(1-f_{XI})}{2.86}$	$v_{20,7}$	f_{XI}					-1
	21 aerobic endog. resp. X_{STOLIP}		-1										-1	
	22 anoxic endog. resp. X_{STOLIP}					$-v_{22,6}$	$-\frac{1}{2.86}$	$v_{22,7}$					-1	

^{a)} stoichiometric coefficients for ammonium S_{NH_4} : $v_{j,4} = -\sum_i i_{N,i} \cdot v_{j,i}$, where $i_{N,i}$ is the nitrogen content of the compound i (values in Table 5-2)

^{b)} stoichiometric coefficients for alkalinity S_{HCO} : $v_{j,7} = (v_{j,4} - v_{j,6})/14$

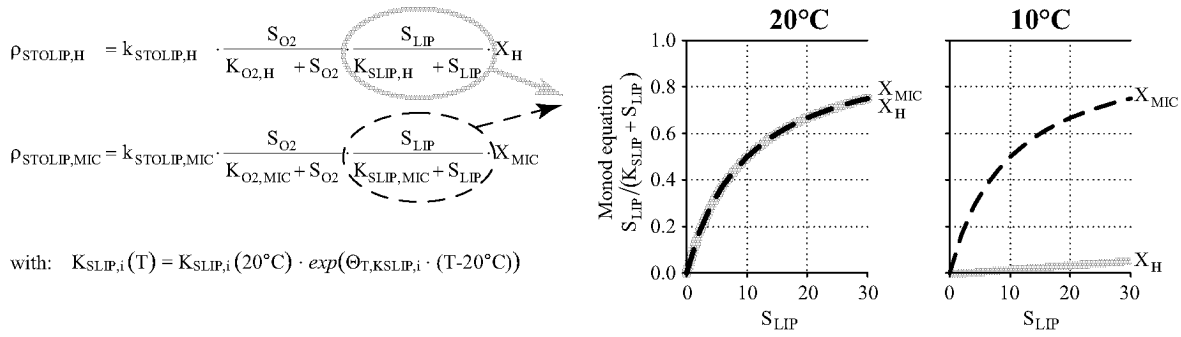


Figure 5-2: Seasonal variation of the lipid affinity is expressed by temperature dependent saturation coefficients $K_{SLIP,i}$ (processes $j=14\dots17$; the expressions for the anoxic storage are corresponding to the shown equations). The parameters in Table 5-2 lead to a highly reduced substrate affinity of the heterotrophic biomass compared to *Microthrix* in winter but no difference in summer.

5.2.2 Model parameters

The stoichiometric and kinetic parameters for the ASM3 part of the model were taken from the calibration for Swiss municipal wastewater (ASM3swiss) by Koch *et al.* (2000). The parameters of the presented model extension for *Microthrix* growth are shown in Table 5-2. Parameters that could not be assumed from literature about *Microthrix* growth were set according to the corresponding parameters in ASM3swiss.

The newly introduced compounds X_{LIP} , S_{LIP} and X_{STOLIP} were modeled as oleic acid ($C_{18}H_{34}O_2$, molecular weight: 282 g mol^{-1} , COD content: $816 \text{ gCOD mol}^{-1}$), since this was mainly used for growth experiments with *Microthrix*.

The maximum growth rate of *Microthrix* μ_{MIC} and its temperature coefficient Θ_T were obtained from data published by Rossetti *et al.* (2002). The yield coefficient for *Microthrix* growth on stored lipids (Y_{MIC}) was derived from chemostat experiments by Slijkhuys (1983) who found a maximum yield of $1.41 \text{ g biomass g}^{-1}$ oleic acid. Transferred to the presented model structure, those experiments represent the combination of storage and growth ($j = 16$ and 18). Extrapolating from the published data a lipid content of 30% (as dry mass) or 55% (as COD) and assuming an aerobic yield for storage $Y_{STOLIP,MIC,O_2} = 0.85 X_{STOLIP} S_{LIP}^{-1}$ (as COD), we calculated the yield of *Microthrix* growth to $Y_{MIC,O_2} = 0.80 X_{MIC} X_{STOLIP}^{-1}$ in COD units. This value is identical to Y_{H,O_2} in ASM3swiss. The anoxic yield $Y_{STOLIP,MIC,NO}$ was set corresponding to the ratio of anoxic to aerobic storage yield of the heterotrophic biomass (Table 5-2).

During manual calibration (see below), the parameters defining the lipid affinity of the bacteria ($K_{SLIP,H}$, $K_{SLIP,MIC}$ and the corresponding temperature coefficients Θ_T) were set to the values in Table 5-2.

5.2.3 Wastewater composition

The wastewater composition used in the presented simulation (Table 5-3) is typical for the investigated WWTP. The COD fractions were chosen according to ASM3swiss (Koch *et al.*, 2000). In the studied wastewater we found $12\text{--}14 \text{ g m}^{-3}$ lipids, corresponding to 13–27% of the total COD (pentane extraction, gravimetric analysis; three 24h composite samples in March 2004). Thus, we set the lipids X_{LIP} to 20% of the total influent COD. The new compound X_{LIP} is a fraction of X_S in ASM3; consequently, in the new model X_S is reduced accordingly to keep the total COD in the

wastewater unchanged. As total COD concentration in the wastewater measured data were used (24h composite samples). The chemical phosphorous precipitation in the activated sludge tank was modeled by introducing precipitation products (as mixture of FePO_4 and $\text{Fe}(\text{OH})_3$) as separate inert compound in the wastewater (X_{Precip}).

Table 5-2: Model parameters for stoichiometry and kinetics of the model extension to ASM3 for Microthrix growth. All parameters of the ASM3 part and most of the extension were set according to the calibration of ASM3 (Koch *et al.*, 2000) performed for Swiss municipal wastewater (ASM3swiss).

description	symbol	unit	value 20°C ^{a)}	Θ_T ^{a)}	comment
stoichiometric parameters					
production of S_I in hydrolysis	f_{SI}	$\text{g} S_I \text{ g}^{-1} X_{LIP}$	0.00		ASM3swiss: $f_{SI} = 0$
aerobic yield of storage of S_{LIP} by X_H	$Y_{STOLIP,H,O2}$	$\text{g} X_{STO} \text{ g}^{-1} S_{LIP}$	0.80		ASM3swiss: $Y_{STO,O2} = 0.8$
anoxic yield of storage of S_{LIP} by X_H	$Y_{STOLIP,H,NO}$	$\text{g} X_{STO} \text{ g}^{-1} S_{LIP}$	0.70		ASM3swiss: $Y_{STO,O2} = 0.7$
aerobic yield of storage of S_{LIP} by X_{MIC}	$Y_{STOLIP,MIC,O2}$	$\text{g} X_{STOLIP} \text{ g}^{-1} S_{LIP}$	0.85		see text, ASM3swiss: $Y_{STO,O2} = 0.8$
anoxic yield of storage of S_{LIP} by X_{MIC}	$Y_{STOLIP,MIC,NO}$	$\text{g} X_{STOLIP} \text{ g}^{-1} S_{LIP}$	0.74		see text, ASM3swiss: $Y_{STO,O2} = 0.7$
aerobic yield of Microthrix growth on X_{STOLIP}	Y_{MIC}	$\text{g} X_{MIC} \text{ g}^{-1} X_{STOLIP}$	0.80		derived from Slijkhuys (1983)
production of X_I in endogenous biomass respiration	$f_{XI,MIC}$	$\text{g} X_I \text{ g}^{-1} X_{MIC}$	0.20		ASM3swiss: $f_{XI} = 0.20$
nitrogen content of X_S	$i_{N,XS}$	$\text{g} N \text{ g}^{-1} \text{COD}$	0.05 ^{b)}		ASM3swiss: $i_{N,XS} = 0.03$
nitrogen content of X_{LIP} , S_{LIP} and X_{STOLIP}	$i_{N,LIP}$	$\text{g} N \text{ g}^{-1} \text{COD}$	0.00		as oleic acid
TSS to COD ratio of X_{LIP} and X_{STOLIP}	$i_{TS,XLIP}$	$\text{g} TS \text{ g}^{-1} \text{COD}$	0.346		as oleic acid
TSS to COD ratio of X_I	$i_{TS,XI}$	$\text{g} TS \text{ g}^{-1} \text{COD}$	0.75		equal to ASM3swiss
TSS to COD ratio of X_S	$i_{TS,XS}$	$\text{g} TS \text{ g}^{-1} \text{COD}$	0.98 ^{b)}		ASM3swiss: $i_{TS,XS} = 0.75$
TSS to COD ratio of X_H , X_A , X_{MIC}	$i_{TS,XBM}$	$\text{g} TS \text{ g}^{-1} \text{COD}$	0.90		equal to ASM3swiss
kinetic parameters					
Hydrolysis rate constant of X_{LIP}	k_{HLIP}	d^{-1}	9.00	0.04	ASM3swiss: $k_H = 9.0$ ($\Theta_T = 0.04$)
Hydrolysis saturation constant of X_{LIP}	K_{XLIP}	$\text{g} X_{LIP} \text{ g}^{-1} (X_H + X_{MIC})$	1.00		ASM3swiss: $K_X = 1.0$
reduction factor for anoxic storage and growth	η_{NO}	-	0.50		equal to ASM3swiss
aerobic lipid storage rate constant X_H ($S_{LIP} \rightarrow X_{STO}$)	$k_{STOLIP,H}$	$\text{g} S_{LIP} \text{ g}^{-1} X_H \text{ d}^{-1}$	12.00	0.07	ASM3swiss: $k_{STO} = 12$ ($\Theta_T = 0.07$)
aerobic lipid storage rate constant X_{MIC} ($S_{LIP} \rightarrow X_{STOLIP}$)	$k_{STOLIP,MIC}$	$\text{g} S_{LIP} \text{ g}^{-1} X_{MIC} \text{ d}^{-1}$	12.00	0.07	ASM3swiss: $k_{STO} = 12$ ($\Theta_T = 0.07$)
substrate saturation constant for storage $S_{LIP} \rightarrow X_{STO}$	$K_{SLIP,H}$	$\text{g} \text{COD m}^{-3}$	10.00	-0.4	calibration , ASM3swiss: $K_S = 10$ (0.00)
substrate saturation constant for storage $S_{LIP} \rightarrow X_{STOLIP}$	$K_{SLIP,MIC}$	$\text{g} \text{COD m}^{-3}$	10.00	0.0	calibration , Rossetti <i>et al.</i> (2002): $K_S = 4$
oxygen saturation/inhibition constant for Microthrix	$K_{O2,MIC}$	$\text{g} O_2 \text{ m}^{-3}$	0.016		Slijkhuys (1983)
nitrate saturation constant for Microthrix	$K_{NO,MIC}$	$\text{g} N \text{ m}^{-3}$	0.04		$K_{NO,MIC} = K_{NO,H} \cdot K_{O2,MIC}/K_{O2,H}$
maximum growth rate Microthrix	μ_{MIC}	d^{-1}	0.42	0.105	derived from Rossetti <i>et al.</i> (2002)
stored lipids saturation constant for growth of X_{MIC}	K_{STOLIP}	$\text{g} X_{STO} \text{ m}^{-3}$	0.10		ASM3swiss: $K_{STO} = 0.10$
ammonium saturation constant for growth of X_{MIC}	$K_{NH,MIC}$	$\text{g} N \text{ m}^{-3}$	0.01		ASM3swiss: $K_{NH} = 0.01$
alkalinity saturation constant for growth of X_{MIC}	$K_{HCO,MIC}$	mole m^{-3}	0.10		ASM3swiss: $K_{HCO} = 0.10$
aerobic endogenous respiration rate of Microthrix X_{MIC}	$b_{MIC,O2}$	d^{-1}	0.042	0.07	$b_{MIC,O2} = b_{H,O2} \cdot \mu_{MIC} / \mu_H$
anoxic endogenous respiration rate of Microthrix X_{MIC}	$b_{MIC,NO}$	d^{-1}	0.021	0.07	$b_{MIC,NO} = b_{H,NO} \cdot \mu_{MIC} / \mu_H$
aerobic respiration rate of stored lipids X_{STOLIP}	$b_{STOLIP,O2}$	d^{-1}	0.042	0.07	$b_{STOLIP,O2} = b_{STO,O2} \cdot \mu_{MIC} / \mu_H$
anoxic respiration rate of stored lipids X_{STOLIP}	$b_{STOLIP,NO}$	d^{-1}	0.021	0.07	$b_{STOLIP,NO} = b_{STO,NO} \cdot \mu_{MIC} / \mu_H$

^{a)} exponential temperature dependence: $\text{value}(T) = \text{value}(20^\circ\text{C}) \cdot \exp(\Theta_T \cdot (T - 20^\circ\text{C}))$

^{b)} corresponding to the ratio X_{LIP}/X_S in the wastewater = 0.57 (X_{LIP} = 20% of total COD, Table 5-3)

^{c)} $X_{BM} = X_H + X_{MIC}$ (as COD)

Table 5-3: Composition of the modeled wastewater: (a) COD fractionation, (b) concentrations.

a) fractions of total COD			b) concentrations		
	ASM3swiss	this model	total COD	data, average=160	gCOD m ⁻³
S _I	6%	6%	X _{Precip}	10	gTS m ⁻³
S _S	10%	10%	NH ₄ ⁺	22	gN m ⁻³
X _I	20%	20%	NO ₃ ⁻ + NO ₂ ⁻	5	gN m ⁻³
X _S	55%	35%	N ₂	0	gN m ⁻³
X _{STO}	0%	0%	Alkalinity	7	mole m ⁻³
X _H	9%	9%			
X _A	0%	0%			
X _{LIP}	-	20%			
X _{STOLIP}	-	0%			
X _{MIC}	-	0%			

5.2.4 Plant layout

The model was applied to the WWTP Kloten-Opfikon in Switzerland, treating the wastewater of 54,000 population equivalents. The plant was modeled as one anoxic tank of 1867 m³ followed by three aerobic tanks of 1244 m³ each. The sludge blanket in the secondary clarifier was considered as anoxic compartment of 560 m³. The plant was operated at a total sludge residence time of about 15 days; for the simulation, measured data were used. Dissolved oxygen concentration in the aerobic compartments was kept constant at 3 gO₂ m⁻³. The wastewater flow was set constant at 18,000 m³ d⁻¹. The measured temperature (moving average over three days) at the influent to the biological stage was used for the simulation (Figure 5-3a).

5.2.5 Quantification of Microthrix

The abundance of Microthrix was measured by in-situ fluorescence hybridization (FISH), using the probes MPA223 and MPA645 (Erhart *et al.*, 1997). The quantification was done as described by Hug *et al.* (2005b) resulting in "pixel concentration", a measure of the ratio of Microthrix bio-volume to the apparent total floc-volume. The values are quantitatively comparable among themselves although the absolute numbers do not represent a real concentration.

As target model results to be compared with the measured Microthrix abundance, the ratio of Microthrix solids to total suspended solids ($X_{TS,MIC} X_{TS}^{-1}$) was calculated according to equations 1 and 2, where $i_{TS,i}$ are the conversion factors from COD to total suspended solids as gTS gCOD⁻¹ and X_j the concentrations of the particulate COD compounds as gCOD m⁻³ (Table 5-2).

$$X_{TS,MIC} = i_{TS,XLIP} \cdot X_{STOLIP} + i_{TS,BM} \cdot X_{MIC} \quad (1)$$

$$X_{TS} = i_{TS,XS} \cdot X_S + i_{TS,XSTO} \cdot X_{STO} + i_{TS,XLIP} \cdot (X_{LIP} + X_{STOLIP}) + i_{TS,BM} \cdot (X_H + X_A + X_{MIC}) + i_{TS,XI} \cdot X_I + X_{Precip} \quad (2)$$

5.2.6 Calibration

The goal of the manual calibration was to simulate a population variation of the same shape and relative amplitude as measured (the absolute numbers of the two quantification scales are not directly comparable). In a first step, TSS was adjusted by choosing a reasonable phosphorous precipitation (X_{Precip}). In order to test the hypothesis, we kept the lipid fraction in the wastewater constant and calibrated the model by changing $K_{SLIP,MIC}(20^\circ\text{C})$ and $\Theta_{T,SLIP,H}$. In order to changed the relative difference between the lipid affinity of Microthrix and the other heterotrophic organisms, we set $\Theta_{T,SLIP,MIC} = 0$ and $K_{SLIP,H}(20^\circ\text{C}) = K_{S,H}(20^\circ\text{C})$ (Table 5-2).

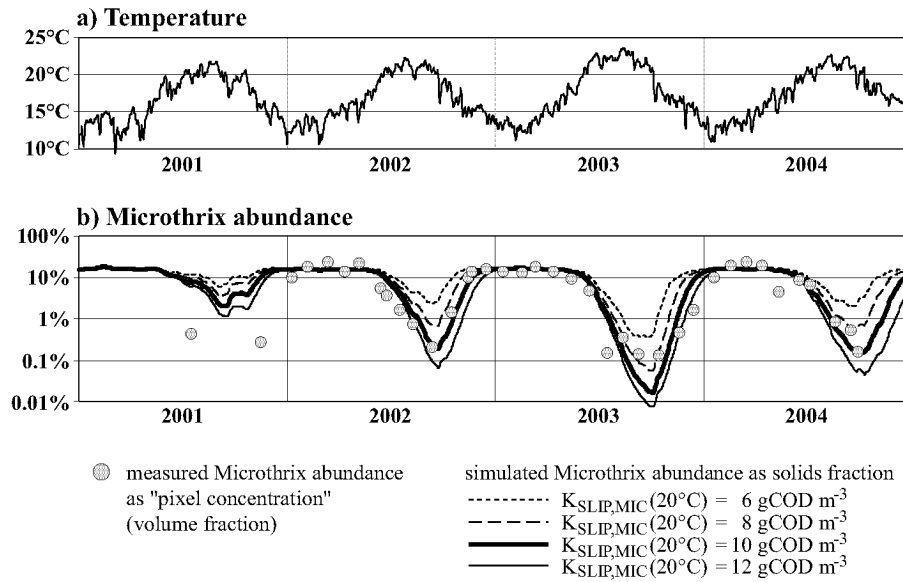


Figure 5-3: The simulation with the recorded temperature course at the inlet to the activated sludge tank (a) resulted in a good approximation of the measured Microthrix abundance (b). The circles represent measured Microthrix abundance as "pixel concentration" (Hug *et al.*, 2005b), the lines stand for the calculated proportion of Microthrix solids ($X_{TS,MIC}$) compared to total solids (X_{TS}). Both scales are quantitative, but are not numerically comparable. The simulation results react sensitive to the substrate affinity of Microthrix.

5.3 Results and Discussion

Figure 5-3 shows the simulated seasonal variation of Microthrix compared to the measured "pixel concentration". The model fitted the measured data well. However, in two summers the minimum concentration was not met satisfactorily. The difference in summer 2001 cannot yet be explained; it might be caused by changing wastewater composition or operation condition that has not been considered in the simulation. The deviation in summer 2003 can be explained by the fact that the applied quantification method has a bottom limit of 0.1%. Consequently, it is well possible that the actual concentration was in the range of the simulated values. The modeled Microthrix abundance reacted sensitively to the saturation constant $K_{SLIP,MIC}$ which was used as calibration parameter (Figure 5-3b) and to the lipid content X_{LIP} of the wastewater (not shown). The temperature coefficient $\Theta_{T,SLIP,H}$ defines the slope of the increase or decrease of the Microthrix fraction. Without temperature dependency of the lipid affinity of the heterotrophic biomass ($\Theta_{T,SLIP,H} = 0$), Microthrix is slowly being washed out of the system (not shown).

As mathematical "switch" to favor Microthrix in winter we chose the storage kinetics. This way, we were able to simulate variations of the Microthrix population over the observed concentration range without varying the influent lipid fraction. Nevertheless, it is probable that other model structures would also have allowed to approximate the measured variations. Therefore, in order to find the important mechanisms allowing Microthrix to successfully compete in activated sludge, it is necessary to apply the model to other data sets, to test other hypotheses, maybe to adapt or extend the model. Already implicitly implemented is the role of surfactants for the availability of lipids; the respective parameters of the hydrolysis and storage processes can be adapted according to experimental results. Similarly, the need of Microthrix for ammonium and its high affinity to oxygen (Slijkhuys, 1983) is implemented in the growth kinetics, ready to be considered for calibration. This

allows to study the role of seasonally incomplete nitrification. The suggested inhibition of *Microthrix* by high long chain fatty acid (LCFA) concentrations (Slijkhuis, 1983) can easily be introduced by an additional Monod term. More complex becomes a model including incomplete denitrification that might also play a role for seasonal variations of *Microthrix* (Tsai *et al.*, 2003). To study the often observed dominance of *Microthrix* in bio-P plants, anaerobic conditions could be modeled with a similar extension to the bio-P module to ASM3 (Rieger *et al.*, 2001). It could also be interesting to introduce nocardioform actinomycetes as other specialized lipid consumer. As the model result is highly sensitive to inoculation of *Microthrix* into the system, the introduction of a scum layer might be another promising way to go.

The fact that a quite extreme temperature dependency of the lipid affinity of the heterotrophic biomass was needed to calibrate the model leads to the suggestion that not mainly the solubility of the lipids is responsible for the variation, as assumed in our primary hypothesis, but rather a population change within the heterotrophic biomass towards bacteria that are less able to utilize lipids. This point should be addressed in future research.

The lipid fraction in the wastewater is relevant for the amount of *Microthrix* in the system. This concentration can not easily be measured as it is not representing one particular substance. Choosing X_{LIP} is part of the calibration procedure for a particular wastewater. Nevertheless, to estimate the concentration X_{LIP} , a standardized and simple method for measuring lipids should be found. Suitable techniques could be the extraction with a hydrocarbon or measuring the ratio of particulate COD to total suspended solids.

5.4 Conclusions

Despite decades of research, it is still unclear how *Microthrix* successfully competes in activated sludge systems. Investigating the observed seasonal variations of its abundance will provide new insights and is therefore a promising way to identify the relevant mechanisms.

In the presented study, we developed a model extension to ASM3 which is able to simulate the measured seasonal variations of *Microthrix* abundance over more than two orders of magnitude. The selection mechanisms favoring *Microthrix* in winter was modeled only by a reduced affinity of the heterotrophic biomass to lipids, while the wastewater composition was kept constant.

Of course, this model is not yet to be used for prediction; it has rather been developed as a tool to structure the findings and hypotheses in the literature, to coordinate the discussion and to plan further research activities. As innovation often happens through regular alternation between experimenting and modeling, we recommend to apply (and adapt) this model to further data sets in order to identify key processes that cause excessive *Microthrix* growth.

5.5 Acknowledgements

We thank Leiv Rieger for his engagement in helpful discussions. This project was performed in the framework of COST action 624.

Chapter 6

The Foaming Potential of Activated Sludge

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Abstract

Activated sludge foaming is a serious operational problem in wastewater treatment plants (WWTP) all over the world. To investigate the actual foam formation independently from the hydraulic and operational conditions in a particular WWTP, we developed a robust foam test procedure. It is based on two different principles of gas bubble production and on the separation of the solid and liquid fraction of the sample. The different measuring scales of these tests were well correlated. Applying the procedure to samples from several pilot and full scale WWTPs in Switzerland revealed a general increase of the foaming potential with the solids concentration. Interestingly, mixed liquor from conventional activated sludge was hardly foaming, while resuspended foam samples from conventional plants and samples from membrane bioreactors (MBR) exhibited a high foaming potential. Furthermore, the critical role of the solids, in contrast to the sludge liquid, could be demonstrated. In contrast to our expectations, the foaming potential was not correlated with the abundance of the filamentous bacteria “*Microthrix parvicella*”, with the sludge volume index or with the foam coverage of the activated sludge tanks. The uncertainty and suitability of the tests and the consequences of our findings for foam control are discussed.

6.1 Introduction

The formation of a stable foam layer on activated sludge basins, secondary clarifiers or anaerobic digesters has become a widespread nuisance in wastewater treatment plants (WWTPs) all over the world. It causes serious operating problems, increases maintenance and may lead to poor effluent quality. Although this phenomenon has been known for decades and is gaining further importance in nutrient removal plants, the actual mechanisms producing and stabilizing the foam are not yet fully clarified. There are still no reliable control strategies.

It is generally assumed that the formation and stabilization of foam requires gas bubbles, surfactants and hydrophobic particles. Gas bubbles are present in the system as air bubbles in the aeration tanks or as nitrogen gas in the anoxic tank. Surfactants are present in wastewater, but are also produced by the microorganisms. The hydrophobic particles are usually thought to be microorganisms

with hydrophobic cell surfaces. The filamentous bacteria “*Microthrix parvicella*” (in the paper referred to as *Microthrix*) and different types of nocardioform actinomycetes are often suspected to play an important role in foaming. However, the problem is highly dynamic and seems to be plant specific. This led to many confusing and partially contradicting findings in the literature and plenty of different anecdotal knowledge.

The observable foam coverage on activated sludge tanks is not solely a result of the sludge properties but highly influenced by the plant layout and operation (e.g. trapping of the foam in certain parts of the plant). Therefore, different types of “foam tests” have been developed in the past to measure the foaming potential of a particular sludge sample under reproducible laboratory conditions. The test procedures basically differ in the type of gas production and measurement scales. Most procedures are based on one of the following references: Aerating the sample with fine bubble diffusers, Pretorius and Laubscher (1987) separated all floatable solids, Goddard and Forster (1987) measured the decay of the foam after stopping aeration, Khan and Forster (1990) measured the development and decay of the foam, and Blackall *et al.* (1991) developed an arbitrary rating system based on foam height, appearance and stability. Ho and Jenkins (1991) used Alka-Seltzer effervescent tablets to produce fine gas bubbles and measured the maximum foam height of surfactant solutions; this procedure was later applied to activated sludge samples by Oerther *et al.* (2001).

Results obtained with such tests are usually varying in a wide range. It is often unclear, whether the variation is caused by the test procedure or by unknown sludge characteristics. Therefore, we developed a robust test procedure that is based on two different test principles derived from Blackall *et al.* (1991) and Ho and Jenkins (1991). This should simulate the different types of foaming in anoxic and aerated reactors and allow to assess the comparability of different measuring scales representing the foaming potential. We applied the new method to numerous samples from different conventional WWTPs and membrane bioreactors (MBR), with particular focus on clarifying the role of the solid and liquid fraction of the activated sludge for foaming.

6.2 Methods

6.2.1 Samples

Grab samples from mixed liquor (near the end of the aeration basins) and from compact foam layers on the anoxic tank were taken from several conventional activated sludge plants (CAS) and two membrane bioreactors (MBR) as shown in Table 6-1. All plants were fed with municipal or partly industrial wastewater in the naturally occurring dynamics. In all of them, periods with and without foam coverage of the reactors occurred.

Table 6-1: Wastewater treatment plants from where samples were analyzed.

name ¹⁾	design	scale	PE ²⁾	SRT _{tot} ³⁾
CAS A1	pre-denitrification	full	54,000	12 - 18 d
CAS A2	pre-denitrification, two parallel lanes	pilot	2 each	10 / 20 d
MBR A	denitrification, partial EBPR ⁴⁾	pilot	100	30 - 70 d
CAS B	EBPR ⁴⁾ (AAO design)	full	130,000	20 - 50 d
CAS C	pre-denitrification	pilot	200	20 d
MBR C	pre-denitrification, two parallel plants	pilot	2 each	20 / 40 d
¹⁾ CAS: conventional activated sludge plant, MBR: membrane bioreactor. The plants with the same letter were operated with the same wastewater. ²⁾ population equivalents ³⁾ solids retention time ⁴⁾ enhanced biological phosphorous removal				

6.2.2 Sample preparation

From the original sample, total suspended solids (TSS) were determined according to the Standard Methods (Clesceri *et al.*, 1998). The samples were then centrifuged (5 min at 4300 rpm) and the supernatant filtered through a coarse pore paper filter (coffee filter). The solids were resuspended in 250 ml aliquots with sludge liquid or tap water, at defined solids concentrations (Table 6-2). Additionally, pure sludge liquid and tap water were prepared to test. The prepared samples were brought to about 20°C; the exact temperature was recorded immediately before the test.

Because the foam samples were very concentrated in TSS, they were diluted prior to the above described preparation. The liquid for dilution was recovered from the same sample by manually destroying the foam structure and subsequent centrifugation.

Table 6-2: The sample preparation resulted in eight test samples for each original sample. Depending on the TSS of the original sample, other appropriate test TSS concentrations were chosen.

liquid type	sludge liquid	tap water
TSS	0	0
	$0.5 \cdot \text{TSS}_{\text{Sample}}$	$0.5 \cdot \text{TSS}_{\text{Sample}}$
	$1.0 \cdot \text{TSS}_{\text{Sample}}$	$1.0 \cdot \text{TSS}_{\text{Sample}}$
	$2.0 \cdot \text{TSS}_{\text{Sample}}$	$2.0 \cdot \text{TSS}_{\text{Sample}}$

6.2.3 Foam tests

The tests were performed in a 1000 ml graduated cylinder made of Plexiglas, with 60 mm inner diameter. The bottom consisted of a perforated rubber membrane as it is used as fine-bubble diffuser in full-scale WWTPs with 12 slits cm^{-2} (ELASTOX-T, Gesellschaft für Verfahren der Abwassertechnik mbH & Co. KG, 42489 Wülfrath, Germany).

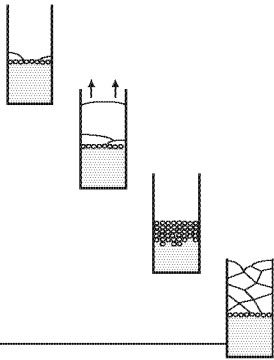
For the *aeration test*, 250 ml prepared sample was aerated for three minutes with 4 l min^{-1} , the foam volume measured and the foam appearance rated according to the rating scale in Table 6-3a. This arbitrary scale was adapted from Blackall *et al.* (1991); it consists of three parts: the formation of large brown bubbles at the liquid surface developing into films that cover the cylinder cross-section and rise upwards (categories 0-4), the accumulation of small bubbles below the surface (categories 5 and higher) and the formation of loose surfactant-like foam structures (white or brown, category X). The stability was assessed 30 s after cessation of the aeration; the stability scale is based on the foam thickness (Table 6-3b).

For the subsequent *Alka-Seltzer test*, the sample was stirred to destroy the foam that was formed during the aeration test, and two Alka-Seltzer effervescent tablets (Bayer Switzerland AG, Zurich; each containing 1650 mg bicarbonate, 324 mg acetylsalicylic acid, 1000 mg citric acid) were simultaneously thrown into the cylinder. The maximum foam volume and the time when it was reached were recorded. In pure liquid the foam immediately collapses after complete dissolution of the tablets. In samples containing solids, the foam only partially collapses and usually all sludge was floating and stayed stable over more than 30 min. Therefore, the stability after the Alka-Seltzer test was not assessed.

This resulted in five values for each sample: foam volume, arbitrary rating and stability for the aeration test and maximum foam volume and time for the Alka-Seltzer test. The foam volume is expressed as relative volume of the foam compared to the original sample volume (equation 1); i.e. no foam results in $V_{\text{rel}} = 0$.

$$V_{\text{rel}} = V_{\text{foam,measured}} / 250 \text{ ml} \quad (1)$$

Table 6-3: Rating scales for aeration test (derived from Blackall *et al.*, 1991), half categories were allowed.

a) rating		
0	small instable bubbles, like pure water	
1	on top of thin foam layer, large instable bubbles (diameter up to 30mm)	
2	Some bubbles cover whole surface, rising films are formed rarely (one of ten attempts)	
3	like 2, films formed regularly (one of three attempts)	
4	like 2, films formed every few seconds (each attempt successful)	
5	large stable bubbles cover surface; small bubbles accumulate below, foam volume <20 ml	
6	like 5, foam volume 20 - 40 ml	
7	like 5, foam volume 50 - 70 ml	
...	...	
X	loose surfactant foam of large bubbles (d=30...60mm, white or brown)	
b) stability		
0	foam collapses, no bubbles remain	
1	foam collapses, few small bubbles remain along cylinder wall	
2	foam collapses, many small bubbles remain along cylinder wall	
3	stable foam, thickness in centre <20 ml	
4	like 3, thickness in centre 20 - 40 ml	
5	like 3, thickness in centre 50 - 70 ml	
...	...	

6.2.4 Transformation of results: TSS and temperature

As the foaming potential increases with the *TSS*, time-series of samples can only be analyzed if they represent the same *TSS*. Therefore, we estimated the values at $TSS=4 \text{ g l}^{-1}$ and 8 g l^{-1} by interpolation with linear or power regression lines through all measured points of the respective sample.

The maximum foam height in the Alka-Seltzer test is proportional to the gas production rate which is increased at high *temperature*, due to faster dissolution and reduced CO_2 solubility. To correct this effect, the relative foam volume at 20°C was estimated with the following procedure: Three samples with high and three samples with low foaming potential were tested at temperatures between 5 and 40°C (Figure 6-1). The foaming potential (V_{rel}) was found to be linearly dependent from the temperature, but with different slopes for both groups of samples. The two lines meet at $T_0 = -20.87^\circ\text{C}$ and $V_{\text{rel},0} = -0.025 \text{ ml}$. The estimated relative volume at 20°C ($V_{\text{rel},20}$) is the value at $T=20^\circ\text{C}$ on a straight line trough the point $(T_0, V_{\text{rel},0})$ and the measured point $(T_{\text{meas}}, V_{\text{rel},\text{meas}})$.

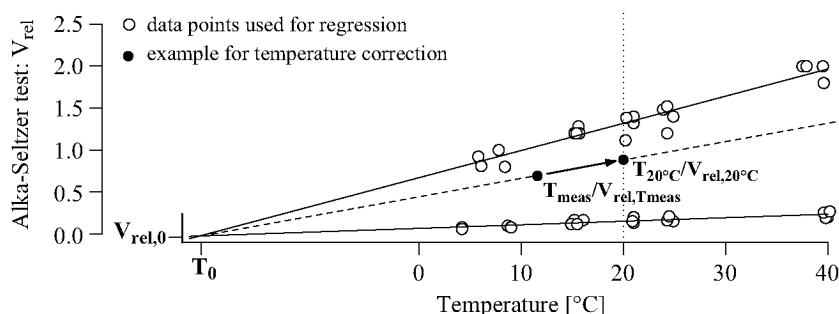


Figure 6-1: Temperature dependency of the Alka-Seltzer test: Testing samples of high and low foaming potential at different temperatures revealed a linear dependency of the results from the temperature, allowing to estimate the values at 20°C .

6.2.5 Bacteria quantification

The suspected foam forming bacteria *Microthrix* and different types of nocardioform actinomycetes were identified and quantified with fluorescence in-situ hybridization (FISH) using the probes and quantification protocol described by Hug *et al.* (2005b). The resulting “pixel concentration” represent a volume fraction of the target organisms compared to the floc volume.

6.2.6 Tests to study the role of the solid and liquid fraction

To study the role of the solid and liquid fraction of the sludge, the following tests and measurements were performed:

- Series of different dilutions from each sample (Table 6-2)
- Sludge liquid or tap water as liquid phase (Table 6-2)
- Repeated washing of solids in tap water
- Exchange of solids and sludge liquid from differently foaming samples
- Addition of chemical additives to improve sedimentation: aluminum chloride was added to plant CAS B, a cationic poly acryl amide (TF31, VTA Engineering und Umwelttechnik GmbH, Weibern, Austria) to the plants CAS A1 and MBR A
- Correlation of foaming potential with foam coverage on reactors
- Correlation of foaming potential with floc structure (sludge volume index, SVI)
- Correlation of foaming potential with the abundance of the suspected foam producing bacteria *Microthrix* and nocardioform actinomycetes

6.3 Results

6.3.1 Method development

The foam tests produced foams of very different appearance: The aeration test generates a loose foam structure of large bubbles (>10 mm) or an accumulation of medium size bubbles (2-5 mm) (Table 6-3); the Alka-Seltzer test produces a dense layer of floating sludge containing small bubbles (< 2 mm). According to these different types of foam, different scales were used to measure the foaming potential. Figure 6-2 shows that the arbitrary rating and the stability measure for the aeration test and the maximum foam volume of the Alka-Seltzer test were well correlated, while the maximum foam volume in the aeration test is not a suitable measure.

As part of the method development and to be able to interpret the results, the uncertainties of the two tests were analyzed: The measured repeatability of particular samples is shown in Table 6-4. It covers a range that is smaller or equal to the estimated uncertainty to assign the value to the respective scale. This assignment is the most important source of uncertainty, particularly due to the uneven foam surface. Compared to that, the measurement uncertainties of the sample volume and TSS play a minor role.

In the Alka-Seltzer tests, two additional crucial factors of uncertainty were identified that have not been discussed in former studies applying this protocol: (1) The temperature affected the results from the Alka-Seltzer test as high temperature increases the gas production rate due to higher dissolution rate and lower solubility of CO₂; this effect was corrected in our data (Figure 6-1). (2) Critical is the fact that Alka-Seltzer tablets sometimes start to float during dissolution and therefore uncontrollably reduce the effective gas production rate.

Table 6-4: Repeatability of the tests (standard deviation in brackets)

Sample origin	TSS of test sample [g l ⁻¹]	n	aeration test rating	aeration test stability	Alka-Seltzer test V _{rel,20°C}
tap water (all tests)	0.00	68	0.65 (0.52)	0.01 (0.06)	0.18 (0.05)
tap water (tests at T = 18-20 °C)	0.00	31	0.60 (0.58)	0.00 (0.00)	0.18 (0.04)
CAS ¹⁾ mixed liquor	3.43	11	1.32 (0.40)	0.82 (0.25)	0.34 (0.05)
CAS ¹⁾ foam	2.47	10	3.10 (0.46)	2.60 (0.57)	0.48 (0.09)
MBR ²⁾ mixed liquor	5.53	10	6.35 (0.24)	4.35 (0.58)	1.14 (0.07)

¹⁾ CAS: conventional activated sludge plant

²⁾ MBR: membrane bioreactor

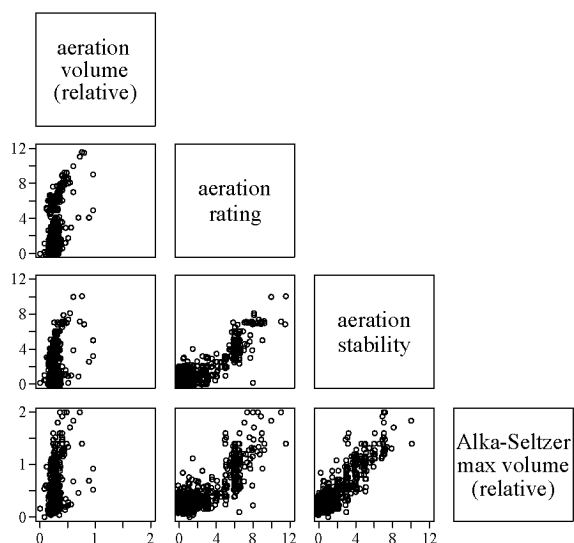


Figure 6-2: All rating scales are well correlated, except the foam height measured in the aeration foam test. (To make all categorized rating and stability values visible in these figures they were numerically dispersed.)

6.3.2 Role of solid and liquid fraction

The foaming potential of different dilutions of samples from different plants (Table 6-1) over four years was measured with the above described protocols.

The resulting values of all measuring scales were widely varying (Figure 6-3). Mixed liquor (ML) samples from conventional activated sludge (CAS) plants exhibited a low foaming potential in all rating scales with only a slight increase with the solids concentration (TSS). Resuspended foam from CAS and samples from membrane bioreactors (MBR) showed a generally higher foaming potential and a strong increase with rising TSS. At very high solids concentrations (TSS > 10 g l⁻¹), however, the measured values stabilized or even decreased, due to the high viscosity that hinders the bubbles from rising.

To differentiate the influence of the liquid from the solids, liquid and solids from differently foaming samples were exchanged (Figure 6-4). The comparison between samples from the plants CAS C and MBR C, both fed with the same wastewater, and the comparison between mixed liquor and foam from CAS A1 led to almost exactly the same results: The mixed samples had the same foam-

ing potential as the sample from where the solids origin, e.g. the mixture of MBR solids and CAS ML liquid had the same foaming potential as the original MBR sample, which was much higher than the respective CAS sample.

To investigate the role of the liquid phase, the separated solids were resuspended in their sludge liquid or in tap water, respectively. The resulting differences were widely scattered, without a significant trend. Sometimes, the sludge liquid lead to a higher foaming potential, sometimes tap water. These effects were not correlated with the time of the day, indicating that the diurnal variation of wastewater composition did not influence the foaming potential near the effluent of the activated sludge tanks. Repeated washing of the separated solids in tap water did not change the foaming potential compared to simply resuspending the solids in tap water.

Pure liquid gained from thick foam layers on anoxic reactors and pure wastewater (primary effluent) produced white surfactant foam in the aeration test (category X in the aeration test rating scale: Table 6-3, Figure 6-3 and Figure 6-4). This effect was not detected in samples containing solids.

Chemical additives to alter the sludge characteristics in order to improve sedimentation were added to the plant CAS B, CAS A1 and MBR A (see list in paragraph 6.2.6). In both conventional plants, the foaming potential and the SVI collapsed immediately. In the MBR, the foaming potential was significantly but not dramatically reduced, while the SVI remained on a very high level. The foam on the surface of the reactors in plant CAS A1 disappeared; in the other plants the effect was unclear.

Analyzing time series of the foaming potential of different plants (Table 6-1) over up to four years could not reveal reproducible seasonal patterns. Accordingly, the foaming potential was neither correlated with the foam coverage of the reactors, nor with the sludge volume index (SVI), the solids retention time (SRT) or the mixed liquor suspended solids (MLSS). E. g. samples from both lanes of the plant MBR C, operated with SRT of 20 and 40 days, had the same foaming potential which was significantly higher than that of samples from the plant CAS C, operated with an SRT of 20 days and fed with the same wastewater (Figure 6-4b). However, no systems with SRT below ten days were studied. Furthermore, the foaming potential was not correlated with the abundance of the suspected foam forming bacteria *Microthrix* (Figure 6-5) which occur in a distinct seasonal pattern in the plants CAS A1 (Hug *et al.*, 2005c) and CAS B. The fact that nocardioform actinomycetes were hardly found indicates that these organisms were not necessary for foaming in the observed plants.

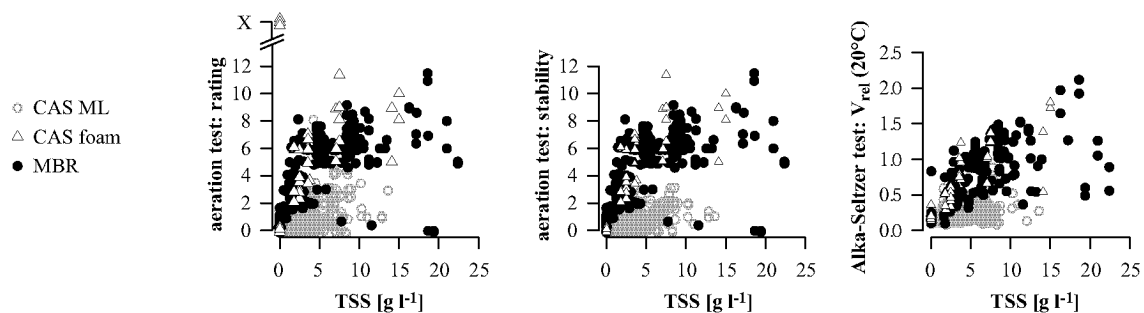
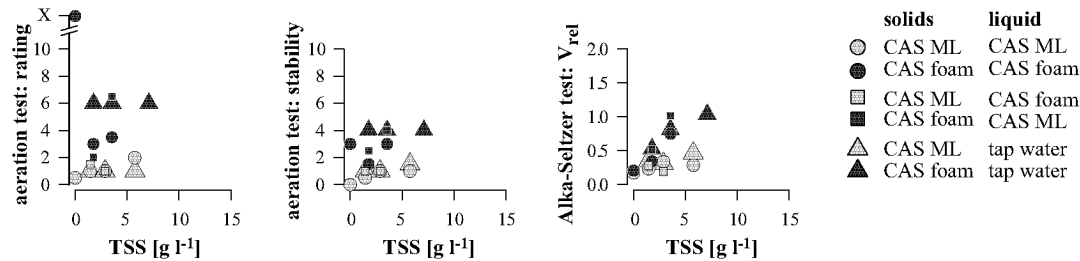


Figure 6-3: The foaming potential increases with the solids concentration of the sample. The effect is furthermore depending on the origin of the sample: Samples from membrane bioreactors (MBR) or resuspended foam samples from conventional activated sludge plants (CAS) have a significantly higher foaming potential than mixed liquor (ML) samples from CAS. (To make all categorized rating and stability values visible in these figures they were numerically dispersed.)

a) foam - Mixed liquor
(CAS)



b) MBR - CAS
(Mixed liquor)

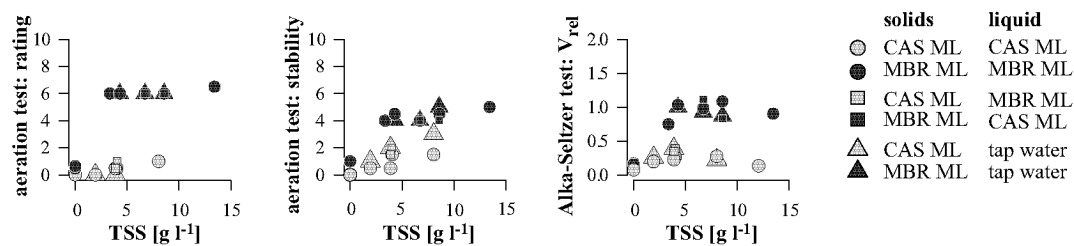
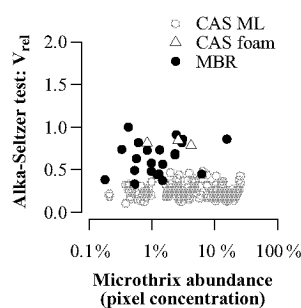


Figure 6-4: Exchanging the solids and liquid from differently foaming samples reveal that the solids determine the foaming potential. (a) Foam and mixed liquor (ML) from the same conventional (CAS) plant CAS A1. (b) ML from plant CAS C and the membrane bioreactor MBR C (both fed with the same wastewater).

a) foaming potential at TSS = 4 $g\ l^{-1}$



b) foaming potential at TSS = 8 $g\ l^{-1}$

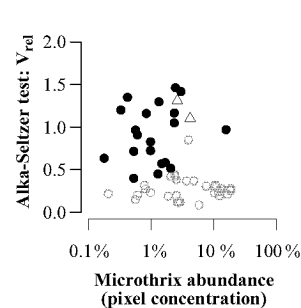


Figure 6-5: The abundance of *Microthrix parvicella* did not define the foaming potential in the observed plants; other factors must lead to the increased foaming potential of MBR and foam samples. The data represent the interpolated foaming potential at TSS = 4 and 8 $g\ l^{-1}$.

6.4 Discussion

6.4.1 Test development and uncertainty analysis

In this study, we further developed and applied two foam test protocols with different types of gas production in order to simulate the rather coarse bubbles in the aeration basins and the small bubbles in the anoxic tank that lead to completely different foam structures in full-scale plants. The correlation of the two test protocols is good (Figure 6-2), despite the different gas sources and the different types of measurement scales.

As in most other protocols in the literature, the uncertainty of our proposed procedure is dependent on the subjective assignment of the observation to the respective scales and by the pronounced variation of the results due to unknown sludge properties. Nevertheless, a careful consideration of the influence factors, particularly the role of the solids concentration and for the Alka-Seltzer test the temperature allows to obtain reproducible results and to draw meaningful conclusions.

6.4.2 Suitability of foam tests

The potential of foam tests lies in the possibility to study the foam formation under comparable laboratory conditions, not influenced by the hydraulic conditions in a particular WWTP.

Can such tests represent adequately enough foam formation in full-scale plants? The water column through which a bubble rises is about fifty times smaller than in an activated sludge tank; therefore, the tests cannot be very sensitive (Jenkins *et al.*, 1993). Furthermore, due to wall effects and different hydraulic conditions in the test equipment and the plant, the detailed characteristics of the particular foam are most likely different. Nevertheless, it can be assumed that the general principles of foam formation and stabilization are the same and can be investigated with such tests.

There are few publications that found a correlation between variation of the foaming potential and the foam coverage in a particular plant (Pretorius and Laubscher, 1987; Paris *et al.*, 2005; Torregrossa *et al.*, 2005). Oerther *et al.* (2001) found an increased foaming potential during a seasonal foaming period in a full-scale WWTP, and de los Reyes III and Raskin (2002) identified a threshold foaming potential that was necessary to start foaming in the full-scale plant. However, similarly to our results, other studies did not find an unambiguous correlation between foaming potential and foam occurrence in the plant (Blackall *et al.*, 1991; Hladikova *et al.*, 2002; Torregrossa *et al.*, 2005). Obviously, the actual foam distribution in a particular full-scale WWTP is strongly influenced by the plant design. Therefore, it is not meaningful to apply such tests to identify foaming problems in a plant (as it was suggested in early literature, Pretorius and Laubscher, 1987). However, as some plants have problems with even a low foaming potential but others can deal with strongly foaming sludge, a standardized test protocol can be a valuable benchmark tool in order to design foam tolerant plants. This is particularly important because there is still no reliable strategy to prevent activated sludge foaming and it is becoming accepted that we have to live with foam.

6.4.3 Role of solid and liquid fraction

The goal of the application of this newly developed protocol was to investigate the role of the solid and the liquid fraction for the formation and stabilization of foam.

Our results demonstrate the important role of the solids for foaming. The foaming potential of different dilutions of a particular sample is increasing with its solids concentration (Figure 6-3 and Figure 6-4). The pronounced differences between mixed liquor from conventional activated sludge plants (CAS ML) and CAS foam or samples from membrane bioreactors (MBR) indicate that not only the solids concentration, but also the solids properties control the foaming potential. This confirms the results from other studies that the foaming potential is increasing with the solids concentration but pronounced differences exist among different types of samples (Goddard and Forster, 1987; Khan and Forster, 1990; Blackall *et al.*, 1991; Oerther *et al.*, 2001).

What could cause the high foaming potential we found in MBR and foam samples? Neither the high SRT, nor the different floc structure in MBRs were found to have an influence. We hypothesize that large organic molecules, originating from microbial activity and cell decay, increase the foaming potential. In MBRs these molecules can be enriched because they are held back by the membrane. Such colloidal compounds are known to be held back by membranes that are typically

used in MBRs (Lee *et al.*, 2005; Rosenberger *et al.*, 2006). In stable foam layers they can accumulate due to the lack of mixing and the high solids concentration.

The liquid phase did not control the foaming potential. Only pure liquid recovered from thick foam layers produced surfactant-like foams, suggesting the local accumulation of surface active substances. Several suspected foam producing bacteria are able to produce bio-surfactants (Desai and Banat, 1997; Lemmer *et al.*, 2000; Pagilla *et al.*, 2002). Supernatant from pure cultures of *Gordonia amarae* have found to produce instable foam that was stabilized by the addition of the respective cells (Blackall and Marshall, 1989; Iwahori *et al.*, 2001; de los Reyes III and Raskin, 2002). However, as pure cultures grow under different conditions than in the competitive environment of activated sludge, it is unclear whether the same organisms and compounds play a crucial role in full-scale activated sludge foaming.

In contrast to widespread assumptions, our results indicate that neither *Microthrix* nor nocardioform actinomycetes are necessary for foaming. This confirms the findings of our previous study where neither *Microthrix* nor nocardioform actinomycetes correlated with the foam coverage of the full-scale WWTP CAS A1 (Hug *et al.*, 2005c). Moreover, we could not confirm the common assumption that high SRT intensifies foaming by increasing the cell surface hydrophobicity and allowing the growth of slowly growing suspected foam forming bacteria. While the TSS in the test sample had a strong influence on the measured foaming potential, the MLSS i.e. TSS of the original sample did not. Furthermore, the foaming potential was considerably reduced by the addition of chemical additives that were added to improve the sludge settleability. As we found that the SVI did not correlate with the foaming potential, this reduction indicates that floc surface characteristics which are altered by those chemicals are important rather than the floc structure.

The surface of sludge flocs is extremely complex; there is no unambiguous borderline between the foam relevant surface and the liquid. Therefore, further research is needed to investigate the foaming potential of activated sludge but including hypotheses from pure culture studies and from other fields such as biotechnology where foaming is a common nuisance (Vardar-Sukan, 1998), mining industries where “froth flotation” of ore particles is achieved by controlled addition of specific surfactants (Rao, 2004) or food technology where foams are stabilized by surfactants, fat globules and proteins (Brooker, 1993; Bos and van Vliet, 2001). For such studies foam tests will be a critical measurement method.

6.5 Conclusions

In order to identify mechanisms behind activated sludge foaming, the foaming potential of sludge samples have to be measured independently from the hydraulic conditions in the wastewater treatment plant. Therefore, we developed a robust foam test protocol, combining two test approaches and based on the separation of solid and liquid fraction. The test was applied to study the role of the solid and liquid phase.

Our results allow the following conclusions:

- We recommend to apply both test approaches (aeration test and Alka-Seltzer test) simultaneously. This leads to more confident findings, as it allows to check whether different scales indicate the same outcomes.
- To produce meaningful results, it is crucial to consider the strong influence of the solids concentration and, for the Alka-Seltzer test, of the temperature.

- The foaming potential is controlled by the concentration and surface properties of the solids, i.e. the activated sludge flocs.
- Foam samples and sludge from membrane bioreactors (MBRs) exhibit a significantly higher foaming potential than mixed liquor from conventional plants.
- The suspected foam producing bacteria “*Microthrix parvicella*” and nocardioform actinomycetes did not play an important role in the formation of stable foams.
- The sludge retention time (SRT) did not influence the foaming potential (in plants with SRT > 10 days).
- The fact that the foam coverage of the activated sludge tanks was not correlated with the measured foaming potential demonstrates the crucial role of the hydraulics in the plant. This emphasizes the importance of such foam tests which allow to study the actual foam formation independent from the conditions in the plant.
- For future research, it will be important to cooperate with other disciplines dealing with stable foams, either as nuisance or as intended product.

To control activated sludge foaming, the following conclusions can be drawn from our results:

- Activated sludge plants designed to operate with smaller solids concentration could reduce the problem.
- Floating sludge has a higher foaming potential than mixed liquor. Accordingly, it should be removed selectively (e.g. by continuously removing foam from the surface) to prevent inoculation back into the mixed liquor.
- Chemical additives to improve the sludge settleability are useful as emergency measure to control severe foaming. Because the exact mechanisms behind foaming are not yet clarified, the product and dosage has to be evaluated with the manufacturer.
- A standardized foam test is valuable to identify foam tolerant WWTP designs.

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Chapter 7

Conclusions

7.1 Products of this thesis

The goal of this thesis was to systematically investigate factors controlling the formation of stable foam in biological wastewater treatment plants (WWTPs). Particularly important findings are the demonstrated importance of dynamics of the microbial population and the foaming intensity as well as the fact that widespread assumptions about causes of foaming were not found to be universally valid, particularly about the role of “*Microthrix parvicella*” (Microthrix). Considering the conclusions from this thesis might help to reduce confusion in future research and suggest new research directions, and therefore provide new insights in order to further elucidate the critical mechanisms in activated sludge foaming.

Based on the questions that were formulated in the introduction to this thesis, paragraph 1.8, the following conclusions can be drawn:

- Despite decades of research, the mechanisms leading to stable foams in activated sludge systems are still not fully clarified. As discussed in the Introduction (chapter 1), this is due to the multifaceted topic and the complex system with its inherent dynamics, combined with inaccurate measuring methods and confusion of terms and phenomena. In order to structure the research on this often confused topic, three major sub-processes of foaming were identified (Figure 1-2 in the introduction section of this thesis): (1) Production of activated sludge of a certain foaming potential. This includes surface active compounds and hydrophobic surfaces, often assumed to be bacteria with hydrophobic cell surface, e.g. Microthrix or nocardioform actinomycetes. (2) The formation and stabilization of foam and (3) the distribution of foam in the wastewater treatment plant.
- A novel method was developed to quantify bacteria in activated sludge (chapter 2). Compared to the traditional morphological identification of filamentous bacteria in activated sludge (Eikelboom and van Buijsen, 1981), this method is based on fluorescence in-situ hybridization (FISH) which allows unambiguous identification, independent of the morphology, and enables

to recognize organisms inside dense flocs. In contrast to semi-automated image analysis procedures, the new method is rapid enough to be used to measure long time-series of bacteria abundance, and it is suitable for samples containing fluorescent debris and cells of different signal intensities and for difficultly accessible target organisms like *Microthrix* and nocardioform actinomycetes.

- A protocol was developed, to measure the foaming potential of activated sludge under reproducible laboratory conditions, independent from the plant design (chapter 6). It is derived from two methods from literature, based on different principles and includes the separation of the solid and liquid fraction of the sample. These tests are valuable to semi-quantitatively identify major influence factors on the foam formation and stabilization.
- Combining the observations of the sub-processes (Figure 1-2 in the introduction of this thesis), it is surprising that no general correlation was found between the abundance of bacteria that are generally assumed to produce foam, the foaming potential and the foam coverage of the activated sludge tanks (chapters 3, 4 and 6). Although these findings might be influenced by the performance of the foam-tests and the fact that nocardioform actinomycetes were mostly absent, they indicate that *Microthrix* does not necessarily play a critical role in foaming and that unknown further factors are important. The findings of this thesis clearly demonstrate the importance to take into account the seasonal dynamics and to separately study the sub-processes.
- Distinct seasonal variation of the suspected foam producing bacteria *Microthrix* and nocardioform actinomycetes were found (chapter 3). Experiments in a pilot-scale plant revealed that operating conditions are defining floc structure and growth of *Microthrix*, rather than the wastewater composition (chapter 4). A mathematical model was developed in chapter 5 as research tool to further elucidate the mechanisms leading to the observed seasonal variations of *Microthrix*.
- The application of the foam tests to samples from all investigated plants (Table 1-1) revealed the dominant role of the concentration and surface properties of the sludge solids. Furthermore, the foaming potential of membrane bioreactor (MBR) sludge and resuspended foam samples was found to be considerably higher than of mixed liquor from conventional plants. This might be caused by an accumulation of macro molecules originating from microbial activity and cell decay (chapter 6). Tackling the reasons for these findings will be a promising way towards understanding the fundamental mechanisms involved in activated sludge foaming.

7.2 Conclusions regarding foam control strategies

The results and conclusions from the analysis of the sub-processes lead to the following recommendations for effective foam control:

- Based on the findings of this thesis it can be concluded that probably the most successful method to control foaming is to continuously collect and remove floating sludge. This does not only keep the problem small but selectively eliminates floating organism and prevents re-inoculation from the foam layer. Consequently the floating ability of certain bacteria becomes a disadvantage (lower effective SRT) while it is a selective advantage when a stable foam layer exists (higher effective SRT). It should be noted that large amounts of removed foam should not be added to an anaerobic digester at the same time as it may cause foaming there.

- To reduce the foaming potential of the removed waste sludge prior to its transfer to an anaerobic digester, only thermal treatment has found to be successful (Westlund *et al.*, 1998; Barjenbruch and Kopplow, 2003).
- The foaming potential of activated sludge can be considerably reduced by the addition of polyelectrolyte as it is often used to improve sedimentation. Although such chemical additives are expensive, they are valuable as an emergency measure when excessive bulking or foaming occurs. Because each sludge has different properties, the product composition and dosage has often to be adjusted to the particular sludge.
- The role of the suspected foam producing bacteria *Microthrix* and nocardioform actinomycetes is not fully clarified; in the investigated plants they did not play a critical role (chapters 3 and 6). Furthermore, there is still no reliable measure known to prevent their growth without putting a risk on nitrification. Accordingly, to prevent or control foaming it is not recommendable to concentrate only on those bacteria - unless they cause severe bulking at the same time. Nevertheless, valuable information for research can be provided if these organisms are identified and quantified throughout the year, during changes of the operation or while trying to suppress their growth.
- Last but not least, we probably have to learn to live with foam, particularly in modern nutrient removing WWTPs and in the emerging MBR technology. Consequently, the focus should be turned to implement measures to increase the foam tolerance of existing and future WWTPs, e.g. high freeboard of the reactors, construction allowing easy access for cleaning, free overflow between reactors, excess sludge withdrawal from the surface, planned option for the installation of water sprays and to dose chemical additives.

7.3 Recommendations for further research

The following suggestions for future research emerge from the findings of this thesis:

- A detailed classification system of different types of foam should be developed in order to reduce confusion and to coordinate research. Similarly, the classification of filamentous bacteria by Eikelboom (1975) and the distinction of different sludge separation phenomena by Wanner (1994) introduced a common language that improved the communication and allowed more efficient cooperative research. A classification of foam types could be based on the types distinguished by Jenkins *et al.* (2004) but should be more detailed, including the foam appearance, distribution in the plant, origin, composition and size of gas bubbles, water content, redox condition, floc structure, dominant organisms, biological activity, sludge surface properties, surface tension, foaming potential and characterization of EPS.
- The sub-processes of foaming in WWTPs (Figure 1-2 in the Introduction of this thesis) have to be clearly distinguished in the classification of foam types, monitoring programs and specific research efforts.
- Measuring protocols should not only be further improved but sound uncertainty analyses should also be provided, also for current procedures. This allows to estimate their accuracy, will prevent inappropriate use, and may even lead to new findings, e.g. by investigation of outlying data.
- It is crucial to consider the inherent dynamics of the investigated systems. Accordingly, single measurements are often not valuable because they may characterize non-representative conditions (chapter 3). To search for bacterial populations that play an important role in the forma-

tion and stabilization of foam, it is crucial to analyze time-series including different seasons and periods with different foaming intensities. Focusing on seasonal variations will not only reduce uncertainty but will certainly provide new insights.

- Mathematical models, as unambiguous formulation of hypotheses, will support communication and help to coordinate future efforts to identify the important mechanisms allowing suspected foam producing bacteria to successfully compete in activated sludge. A suitable model is presented in chapter 5.
- Findings from simplified systems (e.g. pure cultures or lab-scale experiments with artificial wastewater) provide important knowledge about bacterial growth and foaming ability. However, more research is required to check how far those findings are valid for the complex and dynamic system of activated sludge in full-scale WWTPs.
- A promising way to improve the understanding of mechanisms leading to stable foams is to focus on the causes of the increased foaming potential in MBR sludge and resuspended foam (chapter 6). A starting point could be to test the hypothesis that macro molecules from microbial activity and cell decay accumulate in these systems.
- As the system (activated sludge) and the processes behind the formation of stable foam are extremely complex, communication and cooperation among different disciplines have to be improved. This has a long tradition between engineers and plant operators and is currently being intensified between engineers and microbiologists. However, a substantial step further will be made if theories and know-how from different fields that are either suffering from foaming or intending to produce stable foams are combined. Promising disciplines to cooperate with are biotechnology where foaming is a common nuisance (Vardar-Sukan, 1998), mining industries where “froth flotation” of ore particles is achieved by controlled addition of specific surfactants (Rao, 2004) or food processing industries where stable foams are achieved in multiphase systems containing proteins and fat particles (e.g. Brooker, 1993; Goff, 1997; Bos and van Vliet, 2001).

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Curriculum Vitae

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