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Foaming control in the activated sludge process using metabolic control



A dissertation submitted to the University of Dublin

for the Degree of Doctor of Philosophy

Martin Fryer

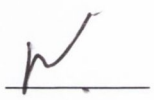
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I thank GOD for Jesus and the love he has always given me

"*For I can do everything through Christ, who gives me strength*". **Philippians 4:13**

Summary

Foam production is an inherent phenomenon within the activated sludge wastewater treatment process. Whilst several different types of foams have been observed it is the presence of thick, viscous brown foams on the surface of aeration basins and final settlement tanks which causes operating problems and the deterioration in treatment efficiency.

This research project examines what triggers activated sludge systems to produce nuisance foam and proposes a way to reduce the risk of foaming through the application of a fuzzy logic model.

Field studies were conducted, primarily focusing on assessing the severity of activated sludge foaming observed within different activated sludge wastewater treatment plants located within the Greater Dublin area in Ireland. A total of fourteen plants were selected which differed in size and the design. Microbial analysis using microscopy determined that there was an association between the presence and enrichment in numbers of specific filamentous bacteria and the on-set and formation of stable foams.

The most widely used technique for assessing the propensity of activated sludge to suffer from biological foaming is to measure 'foam potential'. The Alka-Seltzer test method and experiments using a specially designed sintered disc aeration column represent the two most common approaches for measuring this parameter. Both techniques where applied using samples of activated sludge taken from three different plants. Each method was scrutinised in terms of both accuracy and repeatability. Examination of each was also conducted under a range of different environmental conditions. Overall, better precision was obtained using the sintered disc method but this method has limitations in that the equipment needed to conduct this test is specialised, therefore in most cases the simpler Alka-Seltzer test will be employed by most practitioners.

Whilst foam potential represents a practical means to predict the risk of foaming within activated sludge plants, it was recognised through this work that there was lacking a robust method which could be applied to assess and rank the extent of foaming in within full-scale activated sludge plants. In order to address this deficiency, a new tool called the 'Foaming Scum Index' was devised. Using physical foam characteristics of colour, bubble

size and solids content and incorporating information gained from a number of individual tests normally employed to investigate foaming such as foam potential, foam stability and filament abundance a means to rank and characterise foams has been formulated.

Bench-scale studies were conducted in order to determine the role that wastewater influent has on foaming within activated sludge systems and to establish the key factors that are required for stable activated sludge foam formation. Batch reactors were filled with mixed-liquor samples taken from both foaming and non-foaming sites. The influent from each site was then alternatively fed in a range of combinations to each reactor in order to determine the principle role of the influent and the sludge in activated sludge foaming. A key finding was that the foam potential of the influent was actually found to be higher in the influent samples taken from sites with no history of foaming.

The final part of this work is the production of a fuzzy logic model, which uses information taken from the previous field studies as well as information obtained through an extensive questionnaire survey aimed at activated sludge plants across Republic of Ireland and UK. This model demonstrates an approach which can be used to predict and prevent favourable foaming conditions from developing within aeration basins.

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ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CV	Coefficient of variance
F/M	Food to biomass ratio
FSI	Foaming scum index
MCRT	Mean cell retention time
MLSS	Mixed liquor suspended solids
SBR	Sequencing batch reactor
SRT	Sludge retention time
SSVI	Specific sludge volume index
WwTP	Wastewater Treatment Plant

Chapter 1

The role of filamentous bacteria in the formation of stable activated sludge foam: a literature review

1.1. The activated sludge treatment process and associated foaming problems – An overview

Activated sludge is now the most widely applied technology used to treat domestic and industrial wastewaters (Caravelli et al., 2004; Martins et al., 2004; Seviour et al., 2008). The treatment process involves the oxidation of organic matter by an aerated consortium of microbes followed by a secondary settlement stage (Davenport et al., 1998). This microbial of community is very complex, whose composition is changing continuously under the influence of factors such as influent flow and composition (Comas et al., 2008). The combination of wastewater and activated sludge solids in the aeration tank is called mixed liquor and is maintained in suspension either by mechanical stirrers or through air diffused into the aeration tank (Droste, 1997). The mixed liquor contains discrete aggregates of microbial cells known as 'flocs' (Seviour et al., 2008) (Figure 1.1). The formation of large and compact flocs is essential to the success of activated sludge treatment processes as it allows for the rapid and efficient separation of sludge from the treated wastewater in the final settlement tank (Eckenfelder and Grau, 1998, Tandoi et al., 2006). Activated sludge flocs comprise biological entities consisting of a wide variety of bacteria, fungi and protozoa and a non-biological component of non-organic and organic particulates (Elliot, 2001). Out of the all the microbial entities, filamentous bacteria (filaments) play the most important role in the compaction of flocs by providing a 'backbone' for growth of the floc structure (Sezgin et al., 1978; Motta et al., 2003; Aonofriesei and Petrosanu, 2007; Kragelund et al., 2011). Despite this fact, two of the most common problems encountered in the operation of an activated sludge plant, bulking and foaming sludge are associated with the abundance of excess numbers of filaments (Gerardi, 2002).

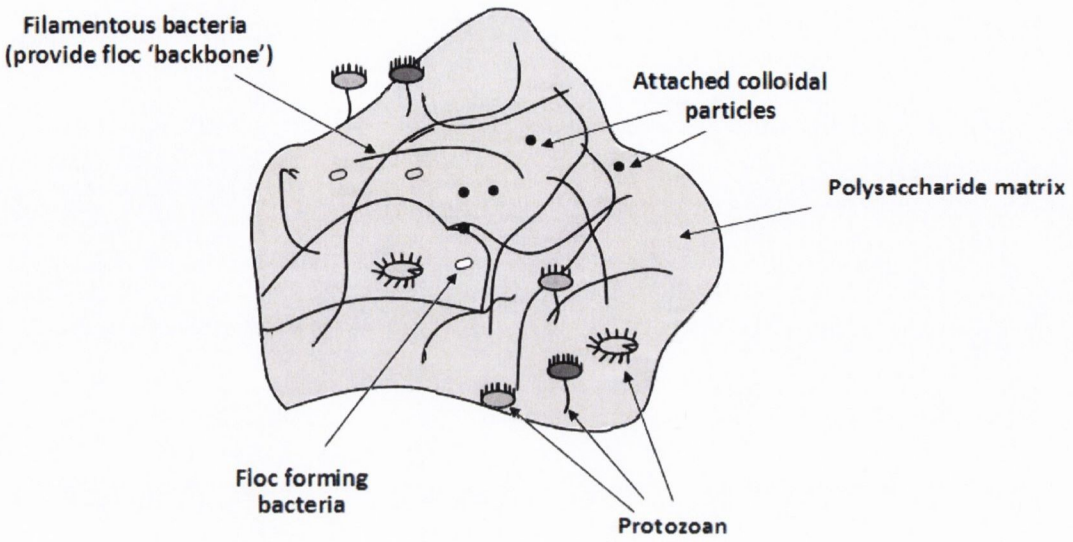


Figure 1.1 – Diagrammatic representation of activated sludge floc (von Sperling, 2007)

Several different types of foams been reported to occur within aeration basins (Table 1.1). The formation of dark-brown viscous foams, which are often very stable, receive most attention as they pose greatest risk to treatment efficiency (Davenport et al., 2000; Torregrossa et al., 2005; Hug, 2006). The microscopic techniques outlined by Eikelboom and van Buijenson (1981) and Jenkins et al., (2004), have led to initial observation of the presence of filamentous bacteria species now linked to the occurrence of stable foams in activated sludge plants (Richard et al., 2003; Horan et al., 2004; Hug, 2006). Recent advancements in technology commonly utilised in other applications, such as fluorescence *in-situ* hybridization (FISH), have provided information regarding the genetic makeup of the bacteria involved, and have been used to identify individual strains of filaments and aid in their enumeration (Davenport et al., 2000; Eales et al., 2005; Levantesi et al., 2006).

Table 1.1: Types of foams found in activated sludge plants (based on Jenkins et al., 2004).

Description - White frothy foam	"Young" foam observed during start-up of plant (usually after 3 to 4 days).
Potential Cause:	Presence of un-degraded surface active organic matter. Also may be due to filament Type 1863.
Comments:	Now less common due to the reduction in the use of non-degradable detergents.
Description - Sticky viscous foam	Foam covering the surface of activated sludge plants.
Potential Cause:	The activated sludge is nutrient limited.
Comments:	Under nutrient limited conditions, surface active exo-cellular polymeric material is formed due to activated sludge micro-organisms, leading to foaming.
Description - Pumice like grey foam	Foam production along with the production of a turbid effluent.
Potential Cause:	Excessive recycling of fine solids from anaerobically digested sludge solids.
Comments:	Large amounts of amorphous inorganic and organic particles lead to foaming.
Description – Sludge blanket foam	Rising sludge leading to foaming on final clarifiers.
Potential Cause:	Poor management of final settlement tanks with poor de-sludging routines and excessive detention times can cause the excessive production of nitrogen gas.
Comments:	Small bubbles of nitrogen gas attach to the activated sludge causing solid particles to be lifted to the air/water interface.
Description – Stable, thick brown foam	Foams formed which are rich in filaments. Foams are unique and problem-causing due to their stability and persistence.
Potential Cause	The formation of foams has strongly been linked with the presence of *nocardioforms* such as *G. amarae* and *Microthrix Parvicella* (Davenport et al., 2008; Frigon et al., 2006; Hug et al., 2006; Jenkins et al., 2004).
Comments:	These filaments are commonly known as *actinomycetes* containing mycolic acids in their cells (Davenport et al., 2008). Mycolic acids are hydrophobic in nature and this along with lipids and other surface active compounds present in the wastewater have been known to lead to foam production and stability (Iwahori et al.,2001; Nielsen et al., 2002; Pagilla et al., 2002; Xie et al., 2007)

1.1.1. The mechanism of foam production and stabilisation

Foams form when air (or an equivalent gas) is introduced beneath the surface of a liquid (Blackall et al., 1991). A collection of bubbles results once the gas becomes dispersed within the continuous liquid phase (Figure 1.2) with each vapour (dispersed) phase being separated from the external phase by a thin film commonly referred to as lamellae (Schramm, 2005; Heard et al., 2008).

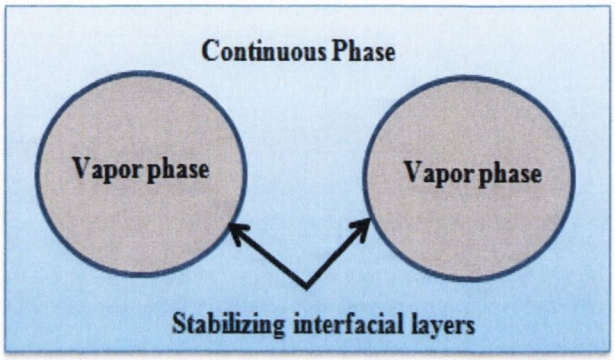


Figure 1.2 – Isolated Foam bubbles (Myres, 2006).

The interfacial liquid layer formed between bubbles as the result of three or more bubbles being in contact with each other is commonly referred to as the Plateau-border region (Iwahori et al., 2001; Myres, 2006) (Figure 1.3).

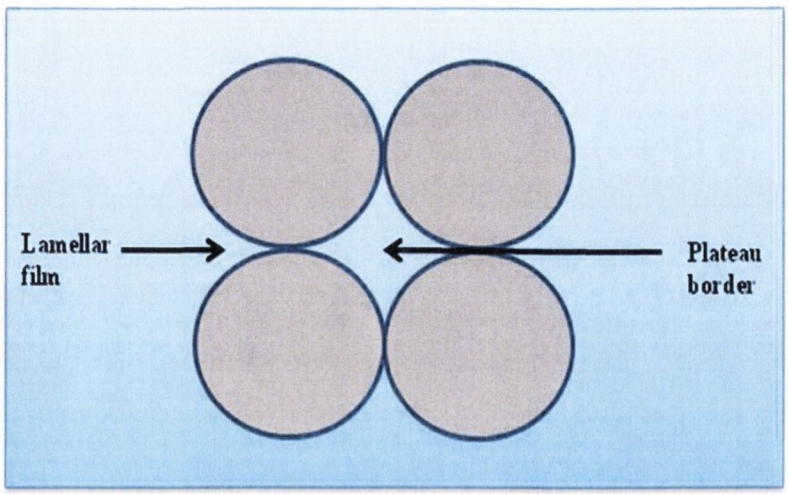


Figure 1.3 – Illustration showing the formation of a Plateau-border when three or more bubbles become in contact with each other (Myres, 2006).

Foam instability occurs by thin film drainage, coalescence and rupture (Pugh, 2005). When discussing film drainage, the stability of the foam matrix is subject to the rate at which liquid is removed from it (Iwahori et al., 2001; Pugh, 2005; Myres, 2006). The actions of gravity and capillary forces have the influence of draining liquid from the thin film lamellae (Pugh, 2005) (Figure 1.4). The Plateau borders connecting the foam films are curved in such a way that the liquid pressure is smaller in these regions than in the flatter parts of the foam films (Langevin, 2000) resulting in a capillary suction effect causing a flux of liquid being drawn from the lamellae into the Plateau-border region (Pugh, 1996). When the liquid in the lamellae films is sucked into the Plateau border channel they become thinner. (Bhakta and Ruckenstein, 1997). If there is nothing to oppose this suction force, the lamella rapidly thins until it becomes unstable and breaks (Bhakta and Ruckenstein, 1997; Breward and Howell, 2004).

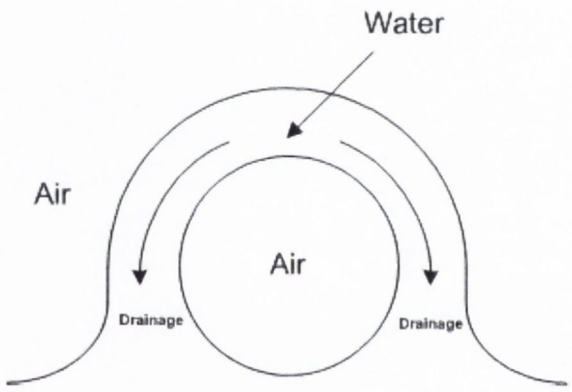


Figure 1.4 – Drainage of liquid from the lamellae into the Plateau-border leads to a decrease in foam stability (Pugh, 1996).

Coalescence and rupture, which are the other processes which result in foam instability, involves the irreversible binding of two or more foam bubbles (Weire and Hurtzer, 1999; Pugh, 2005; Hunter et al., 2008). The process is initiated by the interfacial film shared between these bubbles draining leading to eventual rupture and the formation of a single significantly less stable much larger bubble (Bhakta and Ruckenstein, 1997) (Figure 1.5).

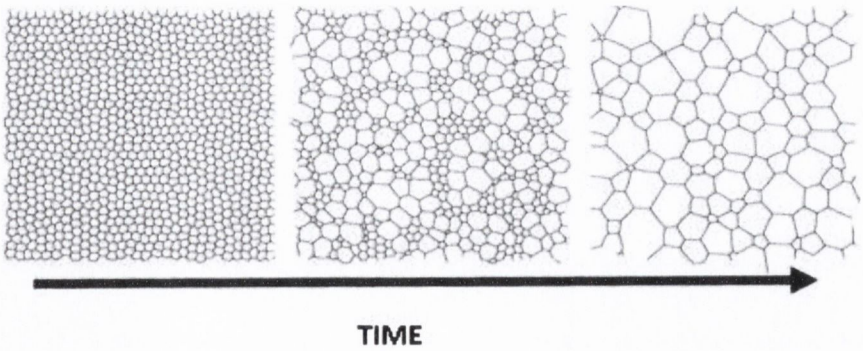


Figure 1.5 – Illustration of the process of Coalescence whereby larger bubbles are formed at the expense of smaller bubbles (Jenkins et al., 2004).

Stable foams cannot be created in pure liquids as they have a strong tendency to spontaneously minimise surface area leading to the collapse of any bubbles formed (Prud'homme and Khan 1995). The presence of surface active compounds within the wastewater is essential to the stabilisation process (Davenport et al., 2008; Heard et al., 2008; Iwahori et al., 2001; Pagilla et al., 2002). The ability of a substrate to perform as a foaming agent is dependent on its effectiveness at reducing surface tension of the solution (Myres, 2006). Molecular surfactants generally contain a polar (hydrophilic) head group and a non-polar (hydrophobic) chain tail which enable them to preferentially adsorb to the air/oil–water interface (Hunter et al., 2008).

In reality, any molecule that has the ability to preferentially adsorb between the fluid–air interface, thus decreasing the interfacial free energy and allowing the surface to expand, acts as a surface-active agent (Heard et al., 2008).

The amount of thermodynamically reversible work required to create an interface is:-

$$W=\sigma \times \Delta A \qquad (1)$$

Where: W is the work in mJ

σ is surface tension in mJ m^{-2} and A is m^2 (Myres, 2006)

As the surface tension of the solution decreases, the surface area that can be developed by a given input of work increases (Myres, 2006). Both synthetic and biological surface

active materials may be considered surfactants when they reach the lowest surface tension of less than 40 dynes cm^{-1} (Pagilla et al., 2002).

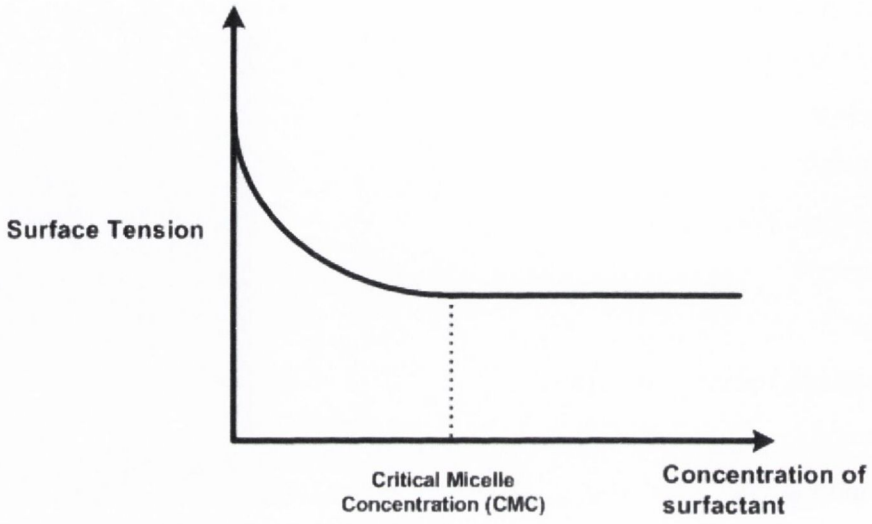


Figure 1.6 – Characteristic change in surface tension as surfactant concentration increases (Weire and Hurtzer, 1999).

The influence of increasing concentration levels of surfactant on measured surface tension is illustrated in Figure 1.6. On addition of surfactant the surface tension decreases until a point is reached whereby the surface tension remained constant. The value for this point is known as the Critical Micelle Concentration (CMC) (Weire and Hurtzer, 1999). Micelles typically contain between 50-100 molecules with the hydrophobic end of each molecule becoming hidden once the CMC point as been reached (Weire and Hurtzer, 1999). Surfactants can be produced either synthetically using chemicals or microbiologically using bacteria, yeast or fungi, the latter products being termed 'bio-surfactants' (Uysal and Turkman, 2005).

Whilst surfactant molecules essentially add to the strength of the water film layer between air bubbles (Jenkins et al., 2004), in the long run if foam is to persist the loss of liquid through drainage must be retarded and this is achieved through the presence of solid particles (Soddell and Seviour, 1990). Particles provide a significant contribution to foam stabilisation by introducing a physical steric barrier to coalescence (Figure 1.7).

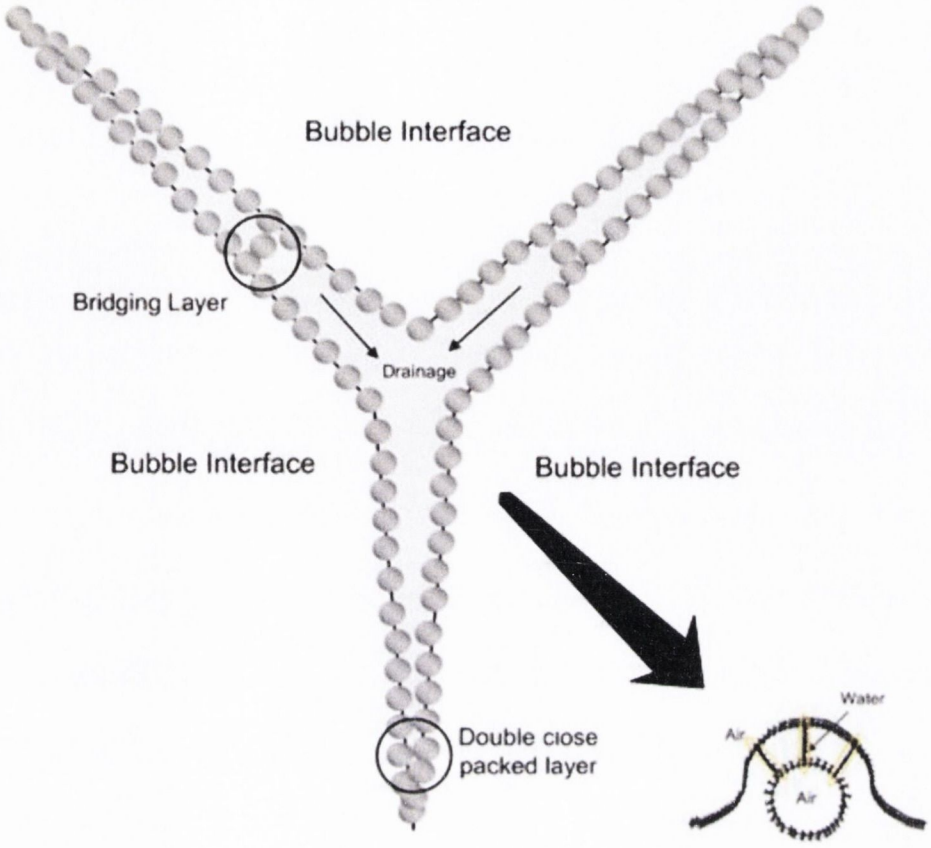


Figure 1.7 – Illustration of particle stabilised foam showing essential bridging of particles preventing drainage of liquid through the process of coalescence (Hunter et al., 2008).

There are now many recognised situations under which stable foams, even in absence of surfactants, have formed such as in rivers, oil-well drilling, and the pulping within the paper industry (Hunter et al., 2008).

1.1.2. The role of filamentous bacteria in stable activated sludge foam formation

Filamentous mycolic acid containing actinomycetes, along with members of *Microthrix* spp. have been strongly implicated as playing a major role in causing foaming within the activated sludge process (Iwahori et al., 2001; Fignon et al., 2006; Lee et al., 2008). The mycolic-acid-containing Mycolata found in foams have been assigned to members of the genera *Dietzia*, *Corynebacterium*, *Gordonia*, *Mycobacterium*, *Rhodococcus*, *Skermania* and *Tsukamurella* (Soddell and Seviour, 1990) out of which, members of *Gordonia* spp.

(formally known as *Nocardia* spp.) have been most strongly implicated with foaming (Iwahori et al., 2001; Frignon et al., 2006; Lee et al., 2008) (Table 1.2).

There has already been much debate regarding the exact mechanism involved in the formation of activated sludge foams (del los Reyes, 2010). Biological foaming is a complex phenomenon based on different physico-chemical and bio-chemical processes and is not just an inherent feature of the filamentous bacteria themselves, but is the end result of various complex interactions occurring under different environmental conditions (Cloete and Muyima, 1997; Petrovski et al., 2011)

Table 1.2: Dominant filamentous microorganisms found in foaming activated sludge (taken from Madoni et al., 2000)

Country	Ranking			Reference
	1	2	3	
Australia	*M. parvicella*	GALOs	Type 0092	Soddell and Seviour, 1990
Czech Republic	*M. parvicella*	GALOs	*N.limicola*	Wanner et al., 1998
France	*M. parvicella*	Type 0675	GALOs	Pujol et al., 1991
South Africa	Type 0092	*M. parvicella*	GALOs	Blackbeard et al., 1986
The Netherlands	*M. parvicella*	GALOs	*N.limicola*	Eikelboom, 1991
UK	*M. parvicella*	*N.limicola*	GALOs	Foot, 1992
Italy	*M. Parvicella*	GALOs	Type 0675	Madoni et al., 2000

**Gordonia amarae*-like organisms (GALO)

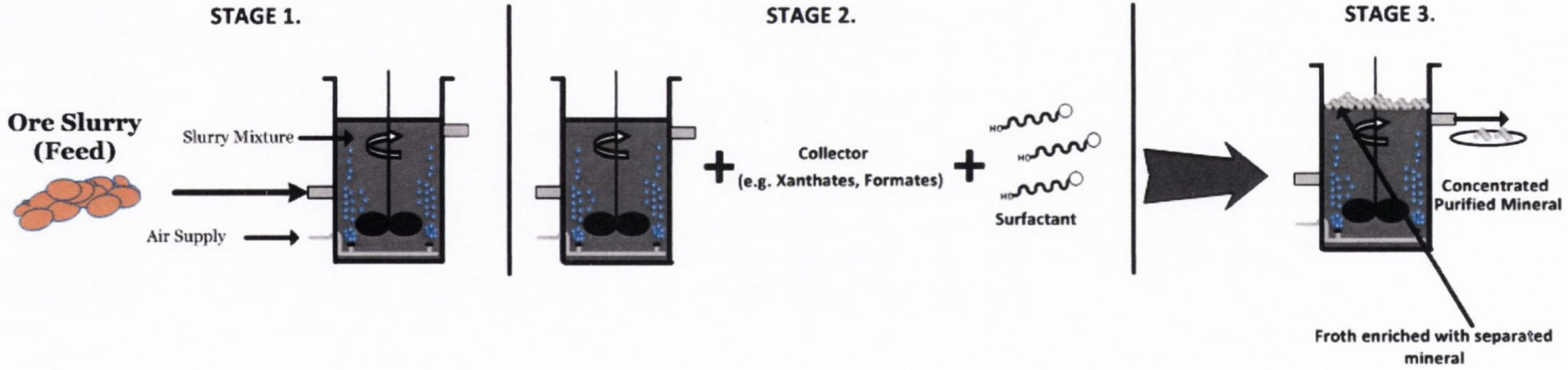
Pipes (1978), one of the first to investigate the phenomenon of activated sludge foaming, proposed that the branched morphology of nocardioforms was responsible for trapping grease and gas droplets causing sludge to float to the surface. It was also suggested that the high lipid content of *M. parvicella* and Mycolata cells (as well as the foam itself) leads to the trapping and floatation of sludge along with foam bubbles (Hao et al., 1988; Soddell and Seviour, 1990). These theories, whilst plausible were never been formally proven (de los Reyes, 2010).

Solids in foaming sludges have been found to possess different properties to non-foaming sludges with most noticeable increases in measured hydrophobicity recorded for activated sludge obtained from foaming aeration basins (Khan et al., 1991). Whilst the influent entering a plant may contain significant quantities of hydrophobic ingredients and

synthetic surfactants (Lemmer et al., 2000; Heard et al., 2008), bacteria also playing a key role in foaming by being themselves possessing hydrophobic properties (Jenkins, 2007).

The current consensus towards an understanding of foaming is now based on a flotation mechanism (Blackall and Marshall, 1989; Soddell and Seviour, 1990; Jenkins et al., 2004; Jenkins, 2007; de los Reyes, 2010). An analogy has been made between those mechanisms involved in filamentous activated sludge foaming and froth flotation theory applied in the industrial processes of mineral separation (Blackall and Marshall, 1989; Jenkins 2007; del los Reyes, 2010) (Figure 1.8). In mineral separation, an additive substance known universally as a 'collector' is dispersed into a mineral – impurities slurry mixture (also known as 'pulp') (del los Reyes, 2010). A surfactant is then added producing a stable foam (referred to in this case as 'froth') from which the purified and concentrated mineral is floated and skimmed off (Leja, 1982; Blackall and Marshall, 1989; del los Reyes, 2010). In comparison to froth flotation theory, certain filamentous bacteria act as collectors by imparting charge properties, rendering them hydrophobic and causing them to adhere to gas bubbles, floating them to the water surface (del los Reyes, 2010). At the top water layer, the bubbles eventually rupture leaving behind an enriched layer of solids containing the hydrophobic filaments (Jenkins, 2007).

FROTH FLOTATION – "Production of valuable metal concentrate and separating off impurities which cover each metal ore" (Leja, 1982)



BIOLOGICALLY MEDIATED FOAM PRODUCTION

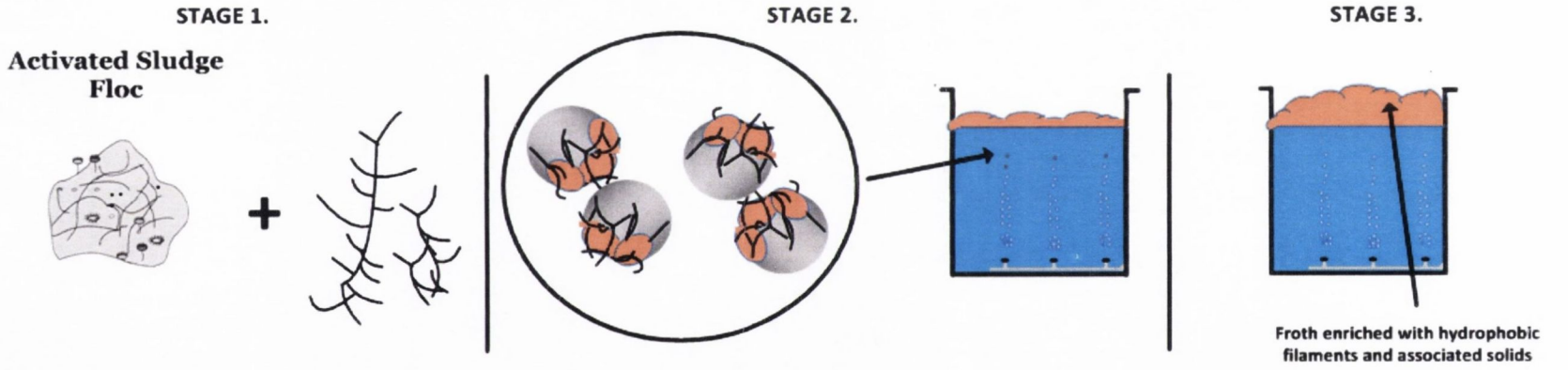


Figure 1.8 – Comparison between mechanisms involved in froth flotation and activated sludge foaming

1.1.3. Problems associated with foaming

Foaming in activated sludge plants is a long established problem causing a range of difficulties, from odour problems, failure of consents through reduction in plant performance and, more seriously, risk to public health (Kim and Pagilla, 2003; Pujol et al., 1991; Tsang et al., 2008; YU, 2007). The phenomenon was first recorded as early as 1969 (anonymous) and has since been recognised as a global phenomenon with 20-60% of global wastewater treatment plants experiencing foaming at any one time (Frigon et al., 2006). Blackall et al. (1988) investigated foaming in activated sludge plants within Queensland, Australia and discovered that more than 90% of plants had experienced sporadic biological foaming episodes and that it remained a constant problem in 38% of the activated sludge plants in the region.

Table 1.3: Operational and other issues associated with foaming within the activated sludge process

Operational/Process Issues		
Problems	Cause	Documented Author(s)
Solids Consent Failure	Foam initially formed on the aeration tank becomes transferred to the final settlement tank where there is the potential for excessive concentrations of solids to be discharged into the final effluent.	Davenport et al., (2000) Kerley and Forster (1995) Hug (2006)
Phosphorus Consent Failure	Solid-liquid separation in the final clarifier is critical to the effectiveness of the enhanced biological phosphorus removal (EPBR) process. An increase in effluent solids (as described above) can result in particulate phosphorus concentrations increasing in the final effluent.	Kim and Pagilla, (2003)
Reduced oxygen transfer	Foam reduces the ability of oxygen transfer within the activated sludge mixed liquor (specifically surface aerators) leading to increased running costs through an increase in oxygen demand for treatment and risk to compliance where this demand cannot be met.	Heard et al., (2008)
Plant monitoring and management	Foam (particularly if sticky) often leads to the malfunctioning of electrodes which are used to monitor and assist in the running of plants, consequently in the running of plants (e.g. DO probes, MLSS)	Hug (2006)
Other Associated Issues		
Hazardous slippery surfaces	Foams can accumulate to such high concentrations that sludge overflows the aeration basins and covers walkways, handrails and surrounding areas.	Jenkins et al., (2004)
Risk to health through contamination	Foams increase the potential risk of airborne pathogenic bacteria which accumulate in the foam.	Heard et al., (2008) Hug (2006)
Odour	Stable foams can putrefy and cause odour problems during periods in the summer	Hug (2006)

1.2. Foaming organisms

1.2.1. Foaming organisms – Microthrix Parvicella

Morphology and structure

Microthrix parvicella is the one of the most commonly observed filamentous taxa seen in foams (Jenkins et al., 2004). This filament is described as a being 'spaghetti-like' in physical nature (Davenport *et al.*, 2008), long and thin (0.6-0.8 μm) and non-branched (Rossetti et al., 2005). The presence of polyphosphate granules allows this filament to be distinguished from other bacteria present in activated sludge (Soddell and Seviour 1990). Research into the kinetics of this filament in both pure and mixed cultures, have shown it to be to be a slow growing micro-organism with a maximum specific growth rate (μ_{max}) of between 0.3 and 0.67 ld^{-1} (Rossetti et al., 2005; Noutsopolous et al., 2006).

Nutrition requirements

M. parvicella can degrade lipids using associated extracellular enzymes, known commonly as 'lipases' (Nielsen et al, 2002). In pure cultures (grown in a non-competitive environment) isolates of *M. parvicella* are able to use a range of simple carbon sources (Rossetti et al., 2005). In activated sludge plants, where the competition for easily biodegradable substrates is more intense, *M. parvicella* is likely to grow on more complex compounds leading to a competitive advantage by virtue of the fact that other bacteria in the activated sludge prefer to utilise biodegradable short chain substrates (Rossetti et al., 2005). The increased hydrophobicity of *M. parvicella* in relation to other activated sludge bacteria ensures that substrates such as lipids and long-chain fatty acids which are also hydrophobic in nature will preferably adsorb to it (Xie et al., 2007).

1.2.2. Foaming organisms – Gordonia amarae

Morphology and structure

The species *Gordonia amarae* is a member of the species *Gordonia* originally referred to as *Nocardia* spp.) and is another major contributor to foaming within the activated sludge process (Jenkins et al., 2004; Marrengane et al., 2011; Petrovski et al., 2011). The number of filaments belonging to *G. amarae* is higher in most foaming plants compared to non-foaming systems (de los Reyes and Raskin, 2002). It is suggested that 40% of all global activated sludge plants have experienced foaming due to *Gordonia* spp. (Richard et al., 2003). The half-life of foam was found to increase steadily as *Gordonia* levels increased from 2.5% to 5.9% of volatile suspended solids (VSS) indicating that increased abundance indicating that increased abundance of *G. amarae* is essential to maintain the stability of any foaming (Nielsen et al., 2002). Furthermore, the analysis of foams of this species typically contain 10-100 fold more *Gordonia* spp. than the underlying corresponding mixed liquor (Richard et al., 2003). Previous batch experiments carried out on pure strains of *G. amarae*, suggest this filament is slow growing with a maximum specific growth rate (μ_{max}) of only 2.3 day^{-1} (Kim and Pagilla, 2003). Unlike *M. parvicella*, this filament exhibits branched morphology which leads to it being very distinctive and easily identifiable (Kragelund et al., 2007).

Nutrition requirements

Chau et al., (1996) investigated the influence of fatty acids containing different carbon chains lengths on the growth of pure strains of *Gordonia* spp. Although each fatty acid could support the growth of this species, growth was noticeably less pronounced with the longer-chain fatty acid acids (Figure 1.9).

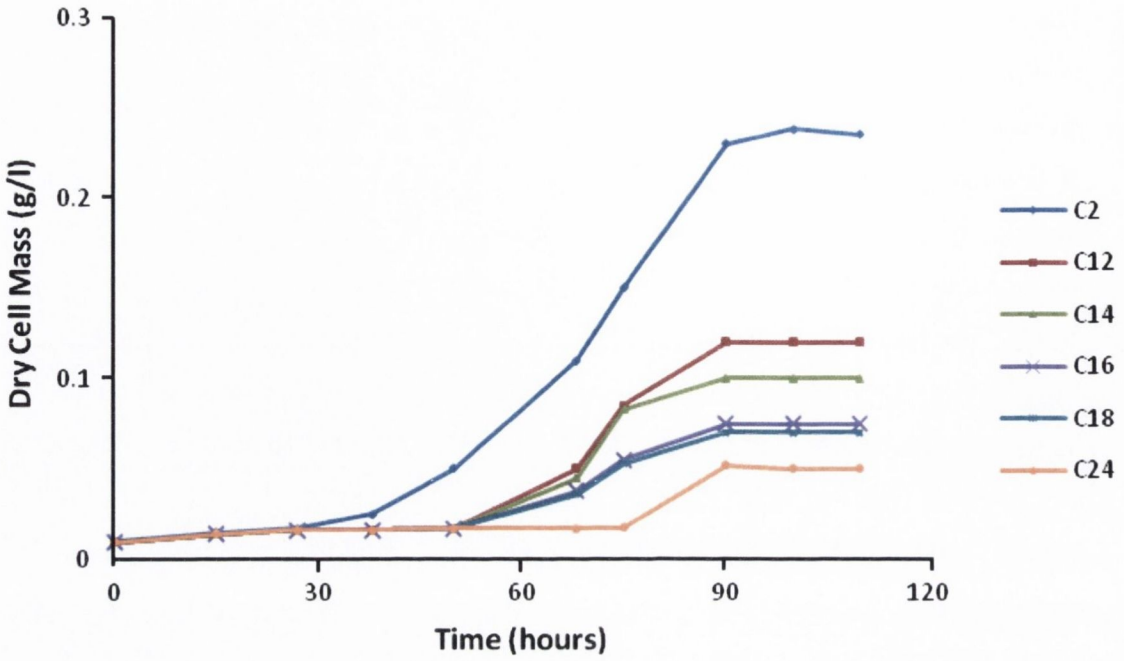


Figure 1.9 – Influence of fatty acid carbon-chain length on *G. amarae* spp. growth (Chau et al., 1996)

Like *M. parvicella*, the growth of this species has been found to be favoured by the presence of slowly degradable organic compounds such as lipids (Richard et al., 2003; Jenkins et al., 2004). Lipids are generally hydrophobic in nature (Duelholm et al., 2002) and in order to degrade and emulsify them, *G. amarae* produces emulsifying agents in the form of bio-surfactants (Pagilla et al., 2002). The production of bio-surfactants enhances the stability of foams by preventing drainage from the liquid films of air bubbles (Tsang al., 2008).

When strains of *G. amarae* were fed on a solution of 1% v/v pure hexadecane and a mixture of 1% v/v pure hexadecane and 0.5% w/v sodium acetate tri-hydrate; maximum biomass concentration was accomplished within four days. In comparison, it took eight days for the stationary phase to be reached when hexadecane was used as a sole carbon source (Pagilla et al., 2002). Acetate enhances initial biomass growth and acclimation of this species by providing a readily degradable substrate (Tsang et al., 2008), whilst the presence of a hydrophobic carbon source enhances bio-surfactant production (Pagilla et al., 2002). The bio-surfactant may itself also be serving as a carbon substrate for growth after the biomass concentration has reached its peak (Pagilla et al., 2002).

1.2.3. Foaming organisms – Rhodococcus

Another filamentous species that can cause operational problems within the activated sludge plants by contributing to the formation of thick surface foams is *Rhodococcus* spp. (Pujol et al., 1991; Davenport et al., 1998; Frignon et al., 2006).

Comparable to the other *Acinomycetes*, *Rhodococcus* spp., as well as *G. amarae* and *Tsukamurella* spp., contain mycolic acids (Stratton et al., 1999) (Figure 1.10). Like *G. amarae*, the hydrophobicity of the cell wall together with the long shape of the hyphae enable these bacteria to link together (Sunairi et al., 1997). Activated sludge flocs which are made hydrophobic by *Rhoddocci* spp. may contribute to the problem of foaming. Particularly important is that the linking of bacteria might cause bubbles to become trapped within a rigid matrix, producing stable foam (Bell et al., 1998).

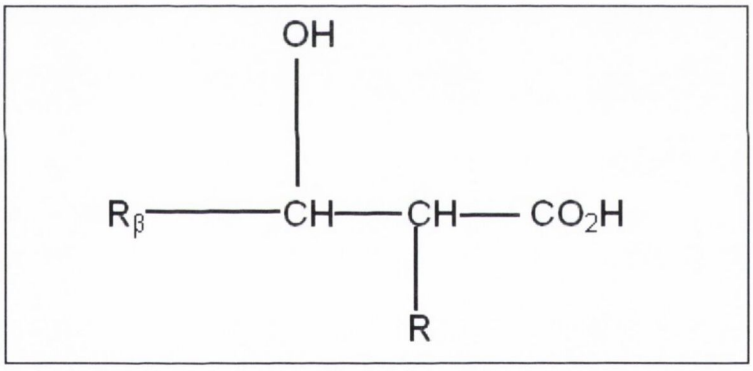


Figure 1.10 – General structure of a mycolic acid (adapted from Stratton et al., 1998) Where **R** groups represent the hydrocarbon chains and $\mathbf{R}_{\beta}$ represents the unsaturated region of the molecule.

Unlike other filamentous bacteria associated with foaming, investigations involving different strains of *Rhodococcus* spp. show that this bacterium is rarely found enriched within the actual foams formed themselves (Sunairi et al., 1996).

Nutrition requirements

The genera *Rhodococcus* spp. use hydrocarbons in a similar manner to *G. amarae* to produce bio-surfactants (Iwahori et al., 1995). The bio-surfactants produced within the

first 24 hours of substrate assimilation are believed to play a key role in solubilising the remaining hexadecane and turning this insoluble material into a form which is more accessible for subsequent uptake (Khan et al., 2000). Experimental studies on *R. rhodochrous* and *R. erythropolis* show both these strains to have a higher affinity than *G. amarae* for the long chain hydrocarbon, octadecane (C_{18}), both producing greater specific growth rates than *G. amarae* (μ_{max}) (Iwahori et al., 1995). Likewise, pure cultures of *R. rubra have* been found to exhibit enhanced growth when grown on straight chain alkanes such as hexadecane, with increased growth rates occurring when the substrate carbon chain length was increased (Khan et al., 2000). When provided with the shorter chain molecule glucose, the same strain of *Rhodococcus* spp. still displayed an increase in biomass production and growth rate alongside a decrease in surface tension of the experimental broth to 42 dynes cm^{-1} compared to 55 dynes cm^{-1} shown in control samples (Khan et al., 2000).

Table 1.4: Example of various strains of foam causing filaments

Gordonia amarae strains		
Filament	Morphological/Metabolically Distinguishing Properties	Source
G.*amarae* SF3	Foam causing (μ_{max} = 2.8 d^{-1})	Jenkins et al., (2004)
G.*amarae* ASAC1	Foam causing (μ_{max} = 3.0 d^{-1})	Jenkins et al., (2004)
G.*amarae* DSM 43392	Found to be able to use substrates d-Galactose, d-Ribose, d-Sucrose (Linos et al., 2002)	Lechevalier, M. P and H. A. Lechevalier (1974) cited by Arenskotter (2004)
G.*amarae* ML1 G.*amarae* ML2 G.*amarae* F1	Significant differences found in the foaming behaviour of filtrates of the different strains during stationary phase, suggesting that the different strains produced surfactants in different amounts or of different nature	Heard et al., (2008)
Eikelboom Type 1863 Strains		
Type 1863	Diameter 0.8-1.0 µm, Length 10-50 µm Present as oval rods usually free in bulk solution	Jenkins et al., (2004)
Type 1863	Non-branched (= unbranched:?) Immobile Diameter 0.5-0.8 µm No sheath Round cells Length <200 µm	Eikelboom, (2000)
Type 1863- Strain RT2	Cell diameter 0.8-1.0 µm Coccus-coccobacillus rod Members of the genus Acinetobacter and strain of A. johnson11	Rossetti et al., (1997)
Candidatus Microthrix Strains		
12 Different strains analysed belonging to the genera *Microthrix*	Some strains belonged to the *Candidatus Microthrix calida* indicated by only having a slightly thinner diameter of 0.3-0.7 µm than the filaments belonging to the *Candidatus Microthrix parvicella*. The former had a weaker reaction to Gram-staining and was able to grow under higher temperatures compared with *M. parvicella*. Additionally both grew on different agar substrates.	Levantesi et al., (2006)
R. rhodochrous R-2 *R. rhodochrous S-2* *R. rhodochrous S-1* *R. rhodochrous R-1*	*R. rhodochrous* R-2 produced thick foams, unlike *R. rhodochrous* S-2 and S-1. The order of the cell surface hydrophobicity of these four strains was determined as R-2 >> R-1 >S-1 = S-2	Sunairi et al., (1997)

1.3. Environmental Influences on foaming

1.3.1. Abiotic factors

1.3.1.1. Temperature

The growth of foam-causing filaments is temperature dependant (Rossetti et al., 2005) with optimum growth of *M. parvicella* between 12 and 15°C (Knoop and Kunst, 1998). In a study investigating foaming and bulking incidents caused by large quantities of *M. parvicella* within a triple oxidation ditch at full-scale works, it was found that foaming events mostly only occurred in the cold winter and spring; this filament also became shorter (less than 100 μm), in length during summer periods compared to winter periods (500 μm) (Slijkhuis, 1983). These effects were attributed to the higher concentrations of degradable hydrophobic substances at lower temperatures (Xie et al., 2007). During winter months, high values for sludge volume index (SVI) (indicating poor settlement of sludge) were obtained along with the occurrence of intense foaming occurring episodes due to the presence of the *M. parvicella* (Hwang and Tanaka 1998). The proliferation of *Microthrix* spp. at low temperatures has been linked to competitive psychrophilic growth utilising greater concentrations of degradable hydrophilic substrates (Xie et al., 2007). At temperatures equal to or greater than 20°C, it is unable to multiply even if the loading rate is as low as 0.01 kg BOD/kg MLSS (Knoop and Kunst, 1998). Under warmer conditions *M. parvicella* may produce enzymes which could actually destroy its cell wall particularly in temperatures greater than 20°C (Xie et al., 2007). Yu (2007) states that growth of *Gordonia* spp. is increased in a similar manner to *M. parvicella* during the colder months (from October to April), although Pitt and Jenkins, (1990) and Frignon et al., (2006) associate increased growth of *Gordonia* spp. and *S. piniformis* spp. strongly with warmer waste water temperatures.

1.3.1.2. Dissolved Oxygen (DO)

Under critical conditions, for example, low temperatures and low dissolved oxygen (DO) concentrations, kinetic selection mechanisms play an important role in how a species will proliferate based upon organism growth rate characteristics (Rossetti et al., 2005). Conditions of low DO have been shown to promote the growth of several types of

filamentous organisms within activated sludge (Hao et al., 1983: Jenkins et al., 2004) with deficiency in dissolved oxygen suggested as one of the major causes of filamentous growth (Lui and Lui, 2006). For example, DO levels of 0.1 mg O_2 l^{-1} were found to provide optimum growth conditions for Type 1863 (Scruggs and Randall, 1998). A clear link between an increase in filament numbers with sludge settleability and levels of oxygen has been determined (Martins et al., 2004).

The outcome of competition between two or more species sharing a single limiting resource can be predicted based upon knowledge of Monod growth kinetics (Wanner, 1998). The DO concentration needed to control filaments is not a constant figure, but is in fact a function of the organic loading rate of a system (Palm et al., 1980). The half-life saturation constant (K_o) represents the DO concentration required for a particular species to growth at half its highest maximum rate. In experiments conducted by Hao et al., (1983), the half-life saturation constant for two *Sphaerotilus* filament species was determined. Lower K_o values were determined for the filament species than floc-forming bacteria demonstrating that these filamentous microorganisms can outgrow and compete against floc-forming microorganisms in activated sludge when DO in the aeration basin is low. The filamentous species *M. pavicella* has been described as a 'micro-aerophile' as its proliferation has been found to be greater in conditions of low DO, with a concentration of DO above 6 mg O_2 l^{-1} being toxic (Rossetti et al., (2005). At a low DO concentration of around 0.4 mg l^{-1}, *M. parvicella* produces long and regular filaments with none of the number of deformed cells observed at higher DO concentration. However, the specific maximum growth rate of *M. parvicella* has been found to be less under conditions of anoxia μ_{max}= 0.57 l d^{-1} compared to aerobic conditions (μ_{max}= 0.67 l d^{-1}) (Xie et al., 2007). In common with both phosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs), *M. parvicella* can take up and store organic matter for growth under anaerobic conditions, utilising nitrate (NO_3^-) as a terminal electron acceptor (Nielsen et al., 2002; Xie et al., 2007). In contrast to *M. parvicella*, the proliferation of *Gordonia* spp. is greatest under DO concentrations of 1.0 mg O_2 or higher with a near linear relationship up to 5 mg O_2 l^{-1} (Scruggs and Randall, 1998). The uptake rate of acetate by *Gordonia* spp. is optimal under saturated DO conditions (Blackall et al., 1991).

Anaerobic and anoxic selectors work on the basis that certain filamentous bacteria cannot use nitrate or nitrite as an electron acceptor (Metcalf and Eddy, 2003). This observation

indicates that properly designed anaerobic and anoxic selectors could be effective in controlling the growth of *Gordonia* spp. in activated sludge plants.

1.3.1.3. pH

It is generally understood that at low pH levels (<5.0) fungal growth is favoured over bacterial growth (Jenkins et al., 2004; Wanner, 1998). In the work of Sezgin and Karr (1986), a decrease in foam coverage resulted primarily from a decrease in pH caused by the on-set of nitrification. When investigating the influence of pH on *G. amarae*, at pH 6.5 the growth of the filament was found to be approximately 66% of that at pH 7.0. Likewise, at pH 5.0, growth was less than 1% of that at pH 7.0 (Hiraoka and Tsumura, 1984). Similar work conducted by Cha et al., (1992), showed the maximum (optimum) growth of this species occurs at pH 6.5 and in contrast to previous findings, a change in pH from 7.0 to 6.5 resulted in a 20% increase in *G. amarae* growth rates. Slijkhuis (1983) studied the effect of pH on *M. parvicella*. The rate of proliferation increased with pH and was zero at an initial pH value 7.1 (Figure 1.11). It has been suggested that pH could influence the charge on the molecules specifically the electrostatic reaction between negatively charged bacteria and charges on organic molecules, therefore acting upon the attraction of substrate and subsequent uptake (Hao et al., 1988).

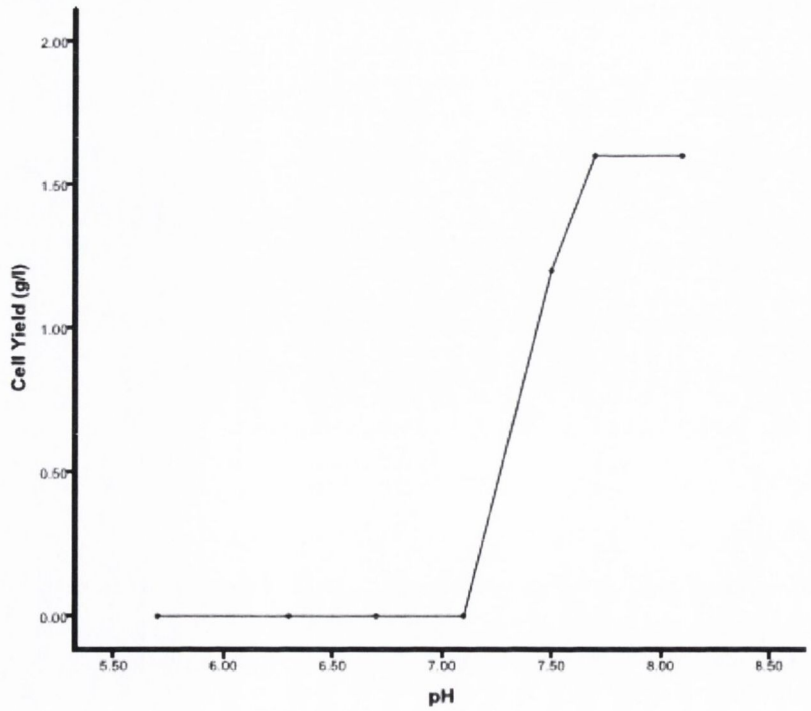


Figure 1.11 – Effect of pH on growth of *M. parvicella* (adapted from Slijkhuis, 1983)

1.4. Effect of plant configuration and design on foaming

Nutrient removing activated sludge configurations provide ideal conditions for foaming as well as membrane bio-reactors (Hug, 2006). Systems that lack primary clarification (the main grease and oil removal mechanism) also suffer more foaming problems (Richard et al., 2003). *Gordonia* spp. foams have been found to occur in all plants with no particular association with specific modes of operation or aeration configuration (Richard et al., 2003), whereas *M. parvicella* is the most common filamentous species responsible for bulking and foaming problems in biological nutrient removal systems (Knoop and Kunst., 1998; Noutsopoulos et al., 2006). In one case, *M. parvicella* appeared abruptly in a plant in which one lane had been converted to anaerobic/oxic system which was reflected in high values of SVI being recorded (above 400 ml g^{-1}) (Hwang and Tanaka 1998). High nitrate concentrations inhibit aerobic growth of heterotrophic floc-forming micro-organisms, promoting the on-set of low F/M bulking thus enabling foam-producing filaments to proliferate in biological nutrient-removal based systems where nitrite levels increase through either nitrification or de-nitrification (Casey et al., 1992). In bench and pilot-scale studies comparing various complete mix systems (with and without selectors), showed that plug-flow reactors produced the best quality settling sludge with the greatest influence in controlling the growth of *M. parvicella* (Mamais et al., 1998). Many Type 1863 foaming episodes have been attributed to a reduction in primary clarification when units were placed following service for repair or cleaning, and grease and oil has entered the aeration system (Richard et al., 2003). In a survey of over 6000 treatment plants in France, 87% of foaming incidents were associated with sites operating under an extended aeration operation (without primary settlement) (Pujol et al., 1991).

Completely mixed aerobic systems (i.e. without anoxic zones) are also susceptible to bulking and scumming problems, due to the high solids retention time required for nitrification (Kappeler and Gujer, 1994). The presence of baffling within an activated sludge plant can play a significant role in the formation of stable foams (Davenport et al., 2008). Mechanical stress (e.g. that typical in surface aerated systems) could lead to increased levels of *M. parvicella*. When mechanical stress was applied to activated sludge the subsequent break up of flocs is believed to have led to an increased release of

surfactants and lipids released from the destroyed cells, allowing increased growth of *M. parvicella* (Kappeler and Gujer, 1994). *Gordonia* spp. on the other hand, is more likely to be severe in plants employing fine bubble or jet aeration (Richard, 2003).

1.5. Foam control

1.5.1. Introduction

Unlike detergents activated sludge foams are much more difficult to remove (Jolis et al., 2007; Pujol et al., 1991; Wanner, 1998). Several techniques have been applied to reduce foam once formed on the surface of plants. The use of chemicals such as biocides (ozone, hydrogen peroxide and chlorine) and anti-foaming agents acting as a control and preventative measure against the onset of foaming within the activated sludge process have been widely documented (Caravelli et al., 2004; Soddell and Seviour, 1990; Nielsen et al., 2002; Lansky et al., 2005; Paris et al., 2005; Ovez et al., 2006; YU, 2007). The degree of success has been variable with the use of such chemical technology in most cases only presents a temporary solution to the foaming problem (Wanner, 1998). Alternative methodologies based upon biological control methods in order to create an environment whereby the *Microthrix* spp. and *Gordonia* spp. are out-competed by other bacteria have been suggested as being more favourable (Kim and Pagilla, 2003). Whatever method is used, it is paramount to apply specific methods of foam control that are selective for the target microorganism and that do not damage the remaining biomass (Rossetti et al., 2005).

The presence of excess filaments can be controlled by either metabolic selection or kinetic selection or by a mixture of the two (Liu et al., 2006; Jolis et al., 2007). Metabolic selection is based on the environmental conditions in which the filaments are able to take up substrate whilst kinetic selection is based on differences in growth and substrate utilization rates (Eckenfelder and Grau, 1998). Within the activated sludge process, the constituents of the biomass have the opportunity to adopt two strategies when competing for substrate. Most filamentous bacteria (K strategists) achieve the highest affinity for substrate under low substrate conditions whilst most floc-forming bacteria (μ strategists) demonstrate maximum specific growth and substrate utilization rates occur under conditions of high substrate availability (Wanner, 1998; Martins et al., 2004) (Table 1.5). Under high readily biodegradable COD (RbCOD) concentrations, the floc-formers develop

a higher rate of RbCOD utilisation and are able to remove available RbCOD in preference to filamentous organism (Ekama et al., 1996).

Among the activated sludge microbes, kinetic selection is an alternative to controlling filamentous overgrowth specifically under low F/M (Food/Biomass) conditions, as the substrate gradient favours the growth of floc-formers instead of filamentous bacteria (Martins et al., 2003). When considering the main filaments associated with activated sludge process, *Gordonia* spp. represents a metabolically limited fraction of the biomass seen in foaming plants (Oerther and de los Reyes, 2001), whilst pure cultures of *M. parvicella* have been shown to be able to use a range of both simple carbon sources and more complex organic substrates (Rosetti et al., 2005).

Table 1.5: General differences between floc-forming bacteria and filamentous bacteria under different metabolic constraints (Wanner, 1998)

Distinguishing quality	Bacteria	
	Floc-Formers	Filamentous
Low substrate concentration causes decrease in specific growth rate	Significant	Moderate
Resistance to starvation	Low	High
Low DO causes decrease in specific growth rate	Significant	Moderate
Potential to assimilate organic substrate when excess is available	High	Low
Capacity to use nitrate as an electron acceptor	Yes	No
Ability to perform luxury uptake of phosphorus	Yes	No

1.5.2. Physio-Chemical control

Chlorination of return activated sludge (RAS) or the foam surface is considered by many to be the most successful way to control foaming (Richard et al., 2003; Soddell et al., 1990). In pure cultures floc-forming bacteria have been found to be more vulnerable to the application of chlorine, however, within activated sludge, the location of the filaments protruding from the flocs makes the filaments more susceptible to the addition of chlorine (Carvelli et al., 2004). The result of chlorine addition was effective when applied to activated sludge samples at a rate of 3 g Cl^{-1} per kg MLSS. day for 12 days (Ovez et al., 2006). The chlorine destroyed the filament material located between flocs, leaving only

loose and chopped filament fragments which were unable to sustain foaming. One disadvantage with using chemicals such as chlorine is that they are non-selective towards what they attack (Ovez et al., 2006; Soddell and Seviour, 1990). When using chlorine to control filament numbers, those centralised within the floc structure often remain protected leaving a potential risk of being available for future excessive growth (Ovez et al., 2006). In a detailed survey of plants in Italy suffering from foaming and bulking issues, the use of chlorine in the form of sodium hypohlorite (NaOCl) was successful in 63% of plants (Madoni et al., 2000). Some filaments are more resilient than others to the disinfection effect that chlorine creates. For example, *M. parvicella* has been observed as being highly tolerant against the addition of chlorine (Hwang and Tanaka, 1998; Richard et al., 2004; Xie et al., 2007). It is reported that a 10-100 times greater dosage of chlorine is required to reduce the viability of this species in comparison to any other filament within activated sludge (Hwang and Tanaka, 1998). The disinfecting efficiency of chlorine is dependent on the rate of penetration through the filament cell wall (White, 1982 cited by Hwang and Tanaka, 1998). The extreme hydrophobicity of the tri-chromes within the cell wall of *M. parvicella* is believed to prevent the penetration of chlorine into the cytoplasm thus ensuring that the full disinfection process does not readily take place (Hwang and Tanaka, 1998).

The use of ozone as a potential chemical agent to control foaming has also been researched by Caravelli et al., (2006), who found that for an applied ozone dose of 34 μg O_3/g VSS and a contact time of 20 mins, the respiratory rate for the filaments was recorded as decreasing by up to 91%. This is compared to a reduction in respiratory activity of 64-70% recorded for the whole floc. Like chlorine, the influence of ozone is non-specific; in addition further research is needed to quantify the suitability of the use of ozone to control foaming in full-scale plants.

Whilst ozone has been found to reduce the numbers of filaments associated with bulking and foaming, its prime influence is to reduce the size of the filaments (without directly damaging them). The remaining filaments are located shielded within the flocs (Saayman et al., 1996).

An applied dose of ozone equivalent to 6 kg $O_3/10^3$ kg MLSS.d not only improved sludge settlability but also effluent quality (Jenkins et al., 2004).

Cationic polymers are used extensively in the wastewater industry to control foams associated with *Gordonia* ssp. (Jenkins et al., 2004). The cationic polymer potentially eliminates foam by re-incorporating the free-floating nocadioforms into the flocs within the mixed liquid (Shao et al., 1997). In the research performed by Hwang and Tanaka, (1998), foams produced by *M. pavicella* were found to disappear with the addition of polyacrylamide, but after 2-3 weeks *M. pavicella* levels steadily rose again.

Anti-filament polymer (AFP), based on poly-quaternary ammonium flocculant, is another potential controller of filaments (Hwang and Tanaka, 1998). In the activated sludge process, its addition to the RAS line caused *M. parvicella* filaments to start to break down and be destroyed within 3 days. The effect was attributed to a general disinfection function that the poly-quaternary ammonium cation has on bacteria. The AFP was found to be able to penetrate deep within the flocs and produce significant inactivation of *M. pavicella* (Hwang and Tanaka, 1998).

When aluminium chloride was added to control *M. pavicella,* the overall hydrophobicity of the activated sludge was found to decrease with the relationship between hydrophobicity and aluminium levels directly inversely proportional to each other (Paris et al., 2005). The attraction that the positively charged aluminium species has to the surface *M. pavicella* cells produces a 'masking' effect to the hydrophobic end of the filament which reduces its hydrophobicity (Nielsen et al., 2005; Paris et al., 2005). The change in hydrophobicity is unlikely to be the main reason for the observed reduction of foaming, rather the flocculant nature of chemicals such as poly-aluminium chloride, cause the *M. pavicella* filaments to become more integrated within flocs reducing their foaming propensity (Nielsen et al., 2005).

Once deeper inside the flocs, the availability of substrate becomes less and the proliferation of *M. pavicella* is then reduced. A novel chemical process investigated by Yu (2005), involves the use of a chemical dosing system and a designated reaction zone which is able to provide a minimum mean hydraulic retention time of 1 hour. The process has been named Foaming Inhibitor on Nocardia (FIONA) and uses the application of metallic salts of nitrates e.g. sodium nitrate to create anoxic conditions in order to promote the removal of readily biodegradable substrates in the sewage prior to the main reactor stage. The aim is to create an environment whereby the growth of facultative micro-organisms is

encouraged which can then outcompete with *Nocardia* (Yu, 2005). Some disadvantages have been reported such as increased primary sludge production and increases in recorded values of SVI.

All the above processes have been found to contribute to different degrees of success in controlling foaming within the activated sludge process, however, what is apparent is that all these procedures only represent short-term measures to manage this problem.

1.5.3. Abundance of foam-filaments and the concept of threshold limits in foaming

There is seemingly a relationship in the abundance of foam-causing organisms and the commencement of the foam process in activated sludge plants (Bitton, 2011). It is therefore reasonable to expect that a threshold in numbers of foam-causing organisms exists whereby, foaming can only occur once this value is exceeded (del los Reyes, 2010). Intense foaming was found to occur when Mycolata numbers were higher than 10^6 cells ml^{-1} in mixed liquor samples (Mori et al., 1992). Using FISH and oligonucleotide probes designed specifically to target the rRNA members of the Mycolata though correlating the numbers of foaming filaments to foaming events in a pilot-plant, a threshold concentration of $2x10^6$ cells/ml was proposed for the abundance in Mycolata required to induce foaming (Davenport et al., 2000). After reviewing results obtained from 14 full-scale plants, this threshold value was assigned to be universally applicable to all activated sludge foaming plants but only applicable to the most hydrophobic members of the Mycolata including *Gordonia*, *Rhodococcus*, *Skermania* and *Tsukamurella* (Davenport et al., 2008). Two foaming threshold values have been proposed for *Gordonia* spp. by de los Reyes and Raskin (2002). The first a "formation" threshold corresponds to total filament length of $2x10^8$ µm/ml reflecting numbers of this species required to produce a sudden increase in measured foaming potential, while the second higher "stability" threshold of $1x10^9$ µm/ml was considered to be the population level at which a stable foam developed. The idea of assigning a threshold to groups of bacteria or specific species when considering the formation of stable activated sludge foams comes with conditions which need to be considered. Firstly, the influence of cell surface hydrophobicity (CSH) on foaming has been well documented (Sunairi et al., 1997; Stratton et al., 2002; Eales et al., 2005; Noutsopoulos et al., 2006 ; Kragelund et al., 2007 ; Xie et al., 2007 ; Petrovski et al.,

2011). Not all Mycolata cells possess the same cell surface hydrophobicity and therefore the same propensity to foam (Petrovski et al., 2011). Secondly, the threshold values proposed do not consider dead cells or metabolically dormant filaments which may not be detected by such techniques as FISH (del los Reyes, 2010) but still may lead to foaming through still retaining hydrophobic properties (del los Reyes et al., 1998; del los Reyes, 2010; Petrovski et al., 2011). Other factors must be taken into consideration when assigning threshold values to foaming such as the presence of surfactants in plants (Petrovski et al., 2011) and the influence of temperature (Frignon et al., 2006).

1.5.4. Operational factors - Kinetic control

Sludge age

Actinomycetes found in scum are considered as being a group of slow growing micro-organisms, therefore the running of plants at low solids retention times (SRTs), can create a detrimental selective advantage for the growth of filamentous organisms (Comas et al., 2008; Oerther and de los Reyes, 2001). Low sludge ages can result in the wash out of slow-growing filament species whose growth is lower than the dilution rate (Wanner, 1998),

$$D = 1/\Theta_X \qquad (2)$$

Where: D = dilution rate

Θ_X = sludge age

The actual sludge age at which actinomycetes are wasted out from the activated sludge process is lower than which they first appear (Sezgin and Karr, 1986) and the necessary SRT required to wash out these organisms is also a function of temperature (Cha et al,. 2002).

Operation at reduced sludge ages has also been found to exert a physiological influence over known foam-causing filaments. It was discovered when using bench-scale continuous flow experiments operating at temperatures between 14 and 18 °C, and SRTs less than 5.7 days, that *M. pavicella* was reduced in size to less than 150 μm and the growth of this filament become significantly hindered (Noutsopoulos et al., 2006). In addition, at sludge ages less than six days, *M. pavicella* starts to lose its hydrophobicity and hence its foaming potential. In moderate wastewater temperatures the proliferation of *M. parvicella* can be controlled at a sludge age less than 8-10 days (Richard et al., 2003) and numbers of *Gordonia* spp. have been controlled in sites operating with mean cell retention times (MCRT) of less than three days (Nielsen et al., 2002). In the study of pure oxygen based activated sludge plants, Jolis et al., (2007) discovered that an SRT of 0.3 days resulted in the complete washout of Nocardioform bacteria from the activated sludge system. Once this species was initially removed, operating the system at an SRT of three days kept it free of this bacterium. Despite these observations, there is substantial conflict regarding the

correct sludge age needed to prevent foaming and some authors state that there are limitations in using sludge retention time as a basis for foaming control. The manipulation of SRT under normal conditions may not be completely successful in removing foam present in foaming activated sludge plants because most of the cells become entrapped in the foam layer and are not removed normally through wasting procedures (del los Reyes and Raskin, 2002). Secondly, once accumulation occurs of significant amounts of biomass into the foam, this fraction of the activated sludge then becomes unavailable and the possibility of controlling the sludge age becomes increasingly difficult (Wanner, 1998; Hug 2006). Thirdly, the process of using SRT as a sole method to control foaming is not suitable in sites that need to nitrify (Blackall et al., 1991; Rossetti et al., 2005; Xie et al., 2007). Just reducing the sludge age does not seem to automatically guarantee the removal of copious amounts of Mycolata. For example, control of foaming through adjustment of MCRT is dependent on the actual strain of *Gordonia* spp, involved in foam production (Soddell and Seviour 1990). Other factors have been highlighted in playing a crucial part in foam stability along with SRT and these include both lipid concentrations in the influent (Richard et al., 2003; Xie et al., 2007) and temperature (Richard et al., 2003; Xie et al., 2007). An important issue which cannot be overlooked and is now given quite a large amount of consideration is that of the process of foam trapping and recycling. Richard et al., (2003) explains that when plants trap foam produced from *Gordonia* spp. in their aeration basins, there ends up being what could be referred to as a Nocardia "foam age". The new SRT of the *Gordonia* spp. is significantly higher than that of the system mixed liquor providing this species with opportunity to proliferate and grow (Jolis et al., 2007). The foam, which is much richer in *Gordonia* spp. than the mixed liquor, can provide a reservoir of these filaments which may then reflux back and forth between the foam layer and the mixed liquor (Blackall et al., 1991).

High SRTs create a selective advantage for the growth of filamentous organisms (Oerther and de los Reyes, 2001; Comas et al., 2008). Bench-scale continuous flow experiments on *M. pavicella* found that at temperatures of between 14 and 18°C and SRTs less than 5.7 days reduced the size of this filament to less than 150 μm hindering its growth. In addition, at sludge ages less than 6 days, *M. pavicella* starts to lose its hydrophobicity and hence its foaming potential starts to become minimised (Noutsopoulos et al., 2006). A

summary of solid retention times (SRT) suggested to prevent foaming are shown in Table 1.6.

Table 1.6: Summary of suggested solids retention time (SRT) to prevent foaming

SRT	COMMENTS	AUTHOR
0.3days	An SRT of 0.3days was found to completely wash-out *Gordonia* spp.	Jolis et al., (2007)
1.3days	At an SRT of 1.3days, quantities of *Gordonia* spp. were kept low enough to prevent stable foam formation from occurring.	Albertson and Hendricks (1992)
5.7days	Bench-scale continuous flow experiments showed that *M. parvicella* growth can be significantly suppressed with SRT less than 5.7days.	Noutsopoulos et al., (2006)
6-8days	*Gordonia* spp. can normally be controlled at a sludge age of less than 6-8days.	Richard et al., (2003)
8-10days	Under normal moderate waste-water temperatures, *Microthrix* spp. can normally be controlled with between 8-10days SRT.	Richard et al., (2003)

1.5.5. Operational factors - Metabolic control

F/M (Food/Biomass ratio)

F/M and sludge age are both useful indicators for detecting conditions favourable for filamentous foaming (Comas et al., 2008). High substrate gradient conditions, such as those used within selectors operating under high F/M loading, have been shown to control both the growth of *Microthrix* spp. and *Gordonia* spp. An increase in F/M ratio supplies more substrates to the floc-forming bacteria which then have higher growth rates than filamentous bacteria under higher soluble substrate concentrations (Xie et al., 2007; Al-Mutairi 2009). It is recognised that the running of convention activated sludge plants under 'nominal loading conditions' of between 0.2- 0.6 kg BOD / kg MLSS.day, can result in a significant increase in overgrowth of filamentous bacteria, including those known to cause stable foams (Tsang et al., 2008). Furthermore, survey data showed that a number of plants run under loading conditions between 0.1 to 0.2 kg BOD / kg MLSS.d experienced operational issues associated to bulking and foaming (Madoni et al., 2000) (Table 1.7).

Table 1.7: Results from surveys of French Wastewater Treatment Plants WWTPs comparing F/M to foaming events (Tandoi et al., 2006)

	Sludge F/M (kg BOD/kg MLVSS.d)	% of cases
Extended aeration	<0.1	87
Low loading	<0.2	10
Middle loading	0.2-0.5	2
High loading	>0.5	1

Several authors have reported different contrasting loading rates under which foaming was found to either be reduced or eradicated as a result of the change in numbers of the filament species *Microthrix* spp. and *Gordonia* spp. (Westlund et al., 1996; Madoni et al., 1997; Parker et al., 2004; Tsang et al., 2008) (Table 1.8). In general an organic load of < 0.2 kg BOD/ kg MLSS.d has been recommended to be most favourable conditions for the growth of *Microthrix* spp. Regarding Type 1863, conditions of high organic loading (between 0.3 - 0.6 kg BOD/kg MLSS.d) with corresponding mean cell residence times of less than four days, leads to the greatest prevalence of this filament species (Eikelboom, 2000; Lemmer et al., 2005). A set of experiments were carried out under feast-famine conditions to establish a way of controlling foaming under average common F/M conditions of 0.5 kg BOD/kg MLSS.d. Under 'feed' and 'starvation' conditions the microbes that proliferate are primarily those which are able to take up soluble substrate and share it internally for use during the starvation period (Jenkins et al., 2004); these are predominantly the floc- forming bacteria.

Using a dual-reactor system consisting of two 3 L-aeration columns and a shared final sedimentation tank, a feasting column was operated at a higher F/M ratio of 0.75 g BOD/g MLSS/d, followed by a fasting column was run at a lower F/M ratio of 0.16 g BOD/g MLSS/d (Tsang et al., 2008). The process controlled numbers of *G. amarae* ensuring that the abundance of this species never became large enough to produce stable foam. The decrease in filament numbers compared to those noted when the system was operated at a fixed F/M ratio of 0.4g BOD/g MLSS/d was reflected by a rapid decrease in measured values of sludge volume index (SVI) from 300 to 80 ml g^{-1} and BOD removal efficiency remained at 95% despite these changes to operating conditions (Tsang et al., 2008).

When investigating the influence of anoxic conditions on the growth of Mycolata, a complete cessation in the growth of *Gordonia* spp. was possible when return activated sludge (RAS) was returned under both anoxic conditions and F/M of 11.48 kg BOD/kg MLSS.d prior to aeration treatment (Madoni et al., 1997) (Table 1.8). A substantial suspension in the growth of *M. parvicella* was also found when RAS was mixed with wastewater under high loading conditions of 24 kg BOD/kg MLSS.d in an oxic environment. Operating the activated sludge process under higher loading may have additional benefits. As well as reducing the risk of foaming and problems relating to sludge settlement through the onset of bulking of activated sludge in the final clarifiers, the phenomenon of pin-flocs leading to further issues with settlement can be resolved by operating at higher F/M ratios and low sludge ages (Richard, et al., 2003).

Table 1.8: Summary of observations from different authors regarding F/M

F/M	COMMENTS	AUTHOR
0.1 kg BOD/kg MLSS/d	Total cessation of foaming reported at this loading.	Parker et al., (2004)
0.2 - 0.6 kg BOD/kg MLSS/d	Under these conditions of normal operation, filamentous growth will be in abundance.	Tsang et al., (2008)
0.7 - 0.05 kg BOD/kg MLSS/d (Corresponding to sludge ages of 50days and 2.2days)	*Gordonia* spp. growth will occur within this range of F/M.	Jenkins et al., (2004)
0.2 - 0.05 kg BOD/kg MLSS/d (Corresponding to sludge ages of 8days and 2.2days)	*M. parvicella* growth will occur within this range of F/M.	Jenkins et al., (2004)
0.3 kg BOD/kg MLSS/d	Below this value of F/M and operating at a sludge age greater than 3days, *M. parvicella* growth was reported to be significant.	Westlund et al., (1996)
11.48 kg BOD/kg MLSS/d	*Gordonia* spp. growth was found to be completely retarded at this ratio of F/M.	Madoni et al., (1997)
24 kg BOD/kg MLSS/d	In experiments where RAS was mixed with wastewater under aerated conditions, this loading led to a substantial suspension of the *M. parvicella.*	Madoni et al., (1997)

1.6. Utilising Metabolic and Kinetic Control Measures – Selector Technology

1.6.1. Overview

A selector is a small tank which has a short hydraulic retention time (20 - 60 mins) and is located directly prior to the main aeration tanks (Forster and Foot., 1997; Metcalf and Eddy, 2003); terms such as 'pre-mixing zone' or 'initial contact zone' are additionally used to describe this part of the treatment process (Wanner, 1998). It may be used to describe as a form of a filamentous control strategy, being based on either kinetic or metabolic selection theory (Liu et al., 2006). These contact zones have been reported to suppress the growth of nocardioforms and *Microthrix* although numerous reports of unsuccessful trials also exist (Hug, 2006). The goal of this technology is to provide a short term, high substrate environment which favours certain floc-formers but which discourages filaments (Richard et al., 2003). The end result of operating selectors prior to the main aeration process is the removal of readily biodegradable BOD (up to 80%) from solution, which reduces the oxygen demand in the aeration zone immediately after the selector. This encourages the production of floc-forming bacteria in two main ways: 1) the substrate is now unavailable for filamentous organisms in the aerobic zone; and 2) higher dissolved oxygen levels can be more easily maintained (Parker et al., 2004). The process of a high F/M in the selector zone followed by a low F/M in the aeration basin can be likened to a feast- famine scenario and such cycles have been shown to favour the growth of well-settling micro-organisms (Droste, 1997). Successful application of sectors relies on detailed knowledge of the following (Al-Mutairi, 2009):-

- Physiology and knowledge of the substrate requirement of the filamentous micro-organisms present in the system
- Influent waste-water composition
- The substrate removal kinetics occurring within the selector

Kinetic-based selector designs are F/M selectors, essentially aerobic selectors, whilst metabolic-based selectors are either anoxic or anaerobic processes (Metcalf and Eddy, 2003). Anaerobic selectors are assigned as being 'kinetic' in nature since most activated sludge filaments are aerobes, and thus under these conditions selection occurs because of

the relative abilities of the micro-organisms to rapidly take up and store substrate (Jenkins *et al.*, 2004). In anoxic or anaerobic selectors other factors play a part, such as the ability of bacteria to be able to take up and store intracellular glycogen or to be able to de-nitrify; hence the term 'metabolic' selectors are applied to these systems (Jenkins et al., 2004). Selection pressure can also be physically applied resulting in a change in the growth kinetics of foam-causing filaments in the overall process (Metcalf and Eddy, 2003). In classifying selectors, the foam that is enriched in foam-causing organisms is wasted prior to any main contact with the activated sludge, rendering the population levels of Mycolata in the mixed liquor too low to cause the production of stable foam (Parker et al., 2004).

The design of selectors is primarily still based on empirical knowledge (Richard et al., 2003). The design of selectors operating under oxic conditions should be based on food/biomass (F/M) ratios with the initial contact zone operating under an F/M between 10 to 12 kg COD/kg MLSS/d and the overall selector having an F/M between 3 to 4 kg COD/kg MLSS/d (Jenkins et al., 2004). Previous findings have shown that a hydraulic retention time (HRT) of about 90 mins to be suitable for sizing both anaerobic and anoxic selectors whilst HRTs of 11 mins are appropriate for aerobic selectors (Droste, 1997). Attempts to use selectors for control of *Gordonia* spp. have not been universally successful (Shao et al., 1997), despite anoxic or anaerobic selectors potentially being able to control *G. amarae* spp. that this species does not take up and utilise acetate under anoxic or anaerobic conditions (Blackall et al., 1991). A laboratory-scale anaerobic selector was able to control nocardioforms to below detectable levels by operating at a MCRT of 2.4 days (@20°C) and an HRT of 50 mins (Fainsod et al., 1999). In full scale trials, an anaerobic selector working under an average MCRT of 3.5 days with a HRT between 17 and 24 minutes was able to significantly reduce levels of *Gordonia* spp. from a count of 4.3×10^6 intesections/g VSS to 1.4×10^6 intesections/g VSS (Fainsod et al., 1999). Al-Mutairi et al., (2009) looked into substrate removal efficiency in an aerobic contact zone prior to main aeration as a function of floc-loading (mg COD/g VSS). It was demonstrated that maintaining a contact loading of around 150 mg COD/g VSS, resulted in the best removal efficiencies along with reduced numbers of filaments and lower recorded values of SVI.

1.6.1.1. Aerobic Selectors

Aerobic selectors work by utilising differential growth kinetics to promote the growth of floc forming bacteria rather than filamentous bacteria (Al-Mutairi, 2009). Such selectors control the growth of filaments by creating substrate concentrations that result in faster uptake by floc-forming bacteria which have higher growth rates under high soluble conditions (Metcalf and Eddy, 2003).

The aerobic selector configuration should consist of a series of reactors at relatively low hydraulic retention times (minutes) (Metcalf and Eddy, 2003). An effective arrangement to provide loadings between 3 to 4 kg COD/kg MLSS/d, comprises, firstly to equal-sized compartments followed by a third compartment that is double the size of the first two (Jenkins et al., 2004). The process goal of an aerobic selector is to provide an environment in which floc-forming bacteria assimilate as much of the soluble BOD as quickly as possible, therefore denying the filamentous bacteria this food source (Al-Mutariai, 2009). Previous studies show that aerobic selectors are able to control bulking sludge attributed to excessive growth of the filaments *S. natans*, *Thiothrix* spp. and Type 021N, whereas numbers of *M. parvicella* can be reduced but to a lesser extent (Martins et al., 2004). The correct design of aerobic selectors is critical in reducing filament numbers. When the contact time is insufficient, soluble substrate is not consumed in the contact zone, and may enter into the main aeration leading to the growth of filamentous microorganisms. On the other hand, when the contact time is even slightly too long, the concentration of substrate becomes too low, approaching the level typical of completely mixed tanks, which also favours the growth of filamentous microorganisms (Martins et al., 2004). Aerobic selectors were effective in controlling *Nocardia* at an MCRT of 5 days but not when the MCRT was higher at 10 days (Cha et al., 1992).

1.6.1.2. Anoxic Selectors

A schematic representation of an anoxic selector is shown in Figure 1.12. Anoxic selectors utilise both kinetic and metabolic selection (ability to denitrify) to control filamentous growth (Jenkins et al., 2004). Under anoxic and anaerobic conditions, pure strains of *G. amarae* were shown to be unable to take up and utilise acetate (Blackwell et al., 1991). Like aerobic selectors, anoxic selectors work by ensuring high uptake of readily

biodegradable COD (selector effect). In this scenario, nitrate nitrogen rather than oxygen is used as the electron acceptor, thus favouring the growth of floc-forming bacteria at the expense of filamentous bacteria. (Soddell and Seviour, 1990). For effective control of excess filamentous growth, soluble COD needs to be reduced to approximately 60 mg/l (Jenkins et al., 2004). In the analysis of five full-scale plants a target anoxic selector F/M ratio of 0.7 to 1.2 kg BOD/kg MLSS/d and aerobic SRT of 8 to 12 days were found to produce the best selector performance (Xie et al., 2007). When the F/M or system aerobic SRT were increased to greater than these ranges it was found to result in persistent filamentous bulking (Marten and Daigger, 1997). Anoxic selectors controlled *Gordonia* spp. in a nitrifying activated sludge at an MCRT of 12 days (Cha et al., 1992). In contrast, this process technology may not be appropriate to remove *M. pavicella* as it can grow under anaerobic and anoxic conditions (Tandoi et al., 2006) utilising nitrate as a terminal electron acceptor, making it a highly competitive bacterial species within both anaerobic and aerobic conditions (Xie *et al.*, 2007). In the work of Mamais et al., (1998), bench and pilot-scale conditions the use of anoxic selectors had no influence on the growth of *M. pavicella.* The combined uses of both aerobic and anoxic selectors have been suggested as the most appropriate approach to control the growth of *M. pavicella.* According to Forster and Foot, (1997) troublesome filaments such as *M. pavicella* can be controlled by using a selector which incorporates an initial anoxic selector followed by an aerated zone operated for 9- 12 hours per a day. In comparison to aerobic selectors, anoxic selectors are advantageous because nitrate is used instead of oxygen, saving on energy input into the system (Parker et al., 2004).

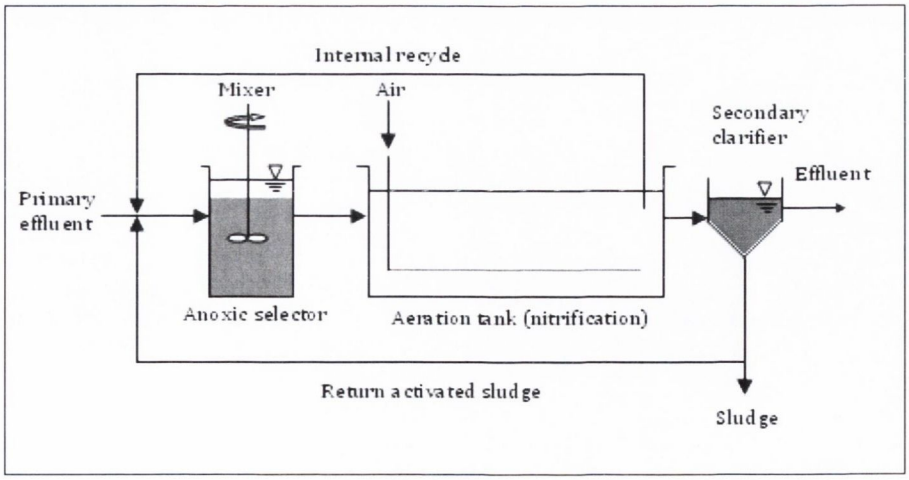


Figure 1.12 – Anoxic selector (adapted from Metcalf and Eddy, 2003)

1.6.1.3. Anaerobic Selectors

It has been demonstrated that anaerobic selectors promote the growth of phosphorus-accumulating organisms (PAOs) that achieve enhanced biological phosphorus removal (EBPR); these organisms are able to grow faster and therefore can out-compete foam-causing filamentous bacteria thus reducing the risk of foaming occurring (Jolis et al., 2007). An anaerobic zone prior to an aerobic zone reduced the populations of *Gordonia* spp. responsible for foaming (Jenkins et al., 2004). The design of anaerobic selectors is reliant upon the concentration of readily biodegradable COD (rbCOD) entering the selector being appropriate for phosphorus removal, ensuring no rbCOD enters the main aeration basin, hence preventing re-growth of filaments (Martins et al., 2004). In a basin / pilot / full-scale study, it was recorded that effective foam control was only achieved once the anaerobic zone had a hydraulic retention time of at least 55 mins; this time is required to allow adequate removal of COD in the anaerobic phase (Jolis et al., 2007). It has been proposed that anaerobic selectors are more effective in filament control over anoxic selectors (Parker et al., 2004). Incomplete de-nitrification in anoxic selectors can result in the passage of de-nitrification intermediates into the downstream aerobic zone, thereby inhibiting floc forming organisms (but not filamentous organisms), resulting in filament growth (Parker et al., 2004). Therefore when using anaerobic selectors, foam trapping and recycling back from the aeration basin to the selector zone must be avoided (Jolis et al., 2007). The application of anaerobic selectors is not recommended for waste-waters which are rich in sulphur compounds that could be used as a source of oxygen by filaments (Martins et al., 2004). When anaerobic selectors were utilised to control the growth of *M. parvicella*; its growth was not hindered and settleability was not improved (Madoni and Davoli, 1993).

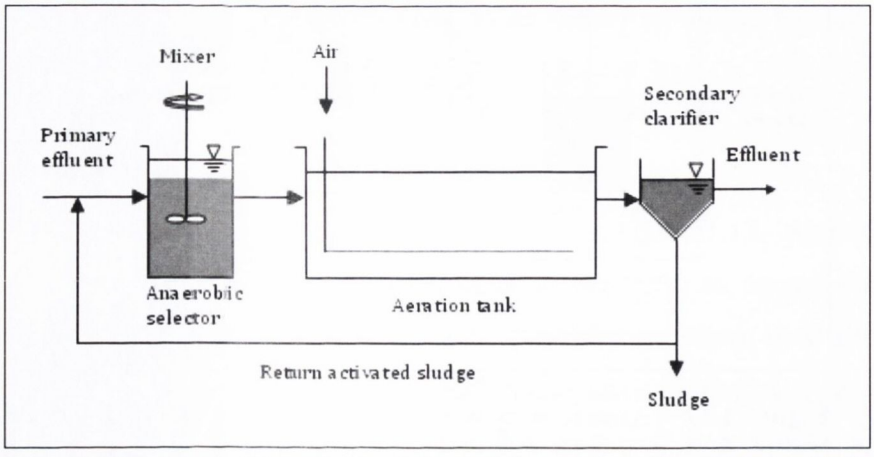


Figure 1.13 – Anaerobic selector (adapted from Metcalf and Eddy, 2003)

1.7 Other applications suffering foaming – Foaming in Anaerobic Digestion (AD)

Conventional mesophilic digestion of surplus activated sludge (SAS), like activated sludge, is prone to foam production (Han et al., 1997) (Table 1.9). In similarity to secondary aeration treatment processes, filamentous bacteria have been implicated as a primary cause of digester foam production (Pagilla et al., 1997; Westlund et al., 1998; Ganidi et al., 2011). Excessive numbers *G. amarae* and *M. parvicella* have been linked to digester foaming (Marneri et al., 2009). It has been suggested that digester foaming due to *G. amarae* can be controlled and potentially eradicated by preventing growth of this filamentous species in activated sludge, thus eliminating it in the SAS digester feed (Tetreault and Diemer, 1987; Hernandez and Jenkins, 1994). In the absence of dissolved surfactants, measured foam potential readings taken using digested sludge were found to be in proportion to the abundance of filaments belonging to *Gordonia* spp. (Hermandez and Jenkins, 1994). Anaerobic digester foaming is most prominent during process start up and is worse after the digesters have been fed (Gerardi, 2003). Whilst foaming has been observed in digesters during periods of nominal organic loading (Mara and Horan, 2003) several authors have proposed that over-loading and variable (shock) loading conditions can be a significant trigger of digester foaming (Tetreault and Diemer, 1987; Ross and Ellis 1992; Ghosh et al., 1995; Gerardi, 2003; Ganidi et al., 2011). Bench-scale trials carried out to establish a critical organic loading threshold (measured in terms of volatile solids (VS) for foam formation and stabilisation), indicated that an initial threshold value existed for applied organic loading of 2.5 kg VS m^{-3} above which the foaming process would be initiated and a second stability threshold of 5kg VS m^{-3} which beyond this level of organic loading, persistent foams would be produced in anaerobic digesters (Ganidi et al., (2011).

Table 1.9: Operational and other issues associated with foaming within anaerobic digestion

Operational/Process Issues		
Problems	Cause	Documented Author(s)
Blockage of gas mixing devices	Along with gas collection pipes and sludge recirculation pumps, gas mixing devices can become blocked with solids from digester foam.	Pagilla et al., (1997) Comas et al., (2009) Ganidi et al., (2009) Dalmau et al., (2010)
Inversion of digester solids profiles	Foaming can also result in an inverse solids profile having higher solids concentrations at the top of a digester, leading to the creation of dead zones and reduction of the active volume of the digester, resulting in sludge, which has not received the same degree of stabilization.	Hernandez and Jenkins (1994) Pagilla et al., (1997) Comas et al., (2009) Ganidi et al., (2009)
Decrease of the digestion efficiency.	The foam produced reduces the active volume of the digesters, resulting in interruption of biogas collection and reduced performance in both the digestion an d thickening processes.	Van Neikerk et al., (1987) Pagilla et al., (1997) Ganidi et al., (2009)
Odour Production	The anaerobic break- down of digester foam produces significant odours.	Water Environment Federation (WEF) (1995) Ganidi et al., (2009)

High solid loading leads to an increase in biological solids breakdown, which in turn as by-products of the digestion process produces increased alkalinity levels, pH and ammonia (Tetreault and Diemer, 1987). When digesters become significantly overloaded as a result of poor operation management or shock loading, the digestion process becomes unstable (Dalmau et al., 2010; Ganidi et al., 2011). Connected with unstable digesters is the accumulation of proteins and detergents as the result of partial sludge degradation (Pagilla et al., 1997; Westlund et al., 1998; Ganidi et al., 2009; Geradi, 2003; Ganidi et al., 2011). Such intermediate compounds therefore have the ability to initiate or contribute to foaming (Ganidi et al., 2009).

Controlling foaming in anaerobic digestion

The most effective method to control foam is to physically remove the foam from the digester through either the use of water sprays or scum-skimming systems (WEF, 1995). Foaming is significantly reduced in digesters operating under thermophilic temperatures

(50°C -70°C) (Rimkus et al., 1982; Han et al., 1997). M. Marneri et al., (2009) set up batch trials involving three reactors operating at mesophilic temperatures (around 35°C) and three operating under thermophilic conditions (around 55°C). Whilst significant reduction in the viability of G. *amarae* and *M. parvicella* was noted in the mesophilic reactors, greatest degradation (over 90% for both species) was noted applying thermophilic (or a combination of mesophilic followed by thermophilic) digestion (M. Marneri et al., 2009).

1.8 Recent developments in foam control within activated sludge systems

The use of chemical technology as a measure to prevent stable foam from developing is still being researched. Kragelund et al., (2010) investigated the use of FEX-120, a blend of calcium chloride and polymer. After only 8-17 weeks of applying this chemical at one site suffering from intense Mycolata foaming, the foam was found to be completely de-stabilised (Kragelund et al., 2010). FEX-120 was also applied to 11 further sites suffering from foaming due to excess *Gordonia* spp and *Skermania* spp. with good results achieved.

The use of swine waste as an external carbon source for enhanced biological nitrogen removal has been found to be effective in controlling the foaming (Chen et al., 2010). This could be an alternative carbon source for nutrient removal plants that are prone to foaming.

Other researchers are exploring the use of bacteriophages to infect and lyse filamentous bacteria responsible for activated sludge foaming (Thomas et al., 2002; Withey et al., 2005; Petrovski et al., 2011). This foam control method is based on reducing down the number of foam-causing filaments in the mixed liquor below the foaming threshold value by applying suitable lytic phages (Thomas et al., 2002). This approach is still in the early stages (Petrovski et al., 2011) but experiments with *Lecane Inermis* to control *M. parvicella* responsible for bulking as well as foaming in activated sludge plants, have provided promising results (Fialkovska et al., 2008). In activated sludge samples taken from plants in Poland, it was observed that *Lecane* rotifiers reduced numbers of *M. parvicella* with a statically significant negative correlation being found between numbers of this bulking filament and population density of the rotifiers (Fialkovska et al., 2008).

Modelling filamentous foaming using kinetic and metabolic control

Advanced engineering applications, such as activated sludge, requires the input of suitable mathematical models (Sotomayor et al., 2003); when applied to wastewater treatment plants (WWTPs), they can be used to predict influent load, and estimate biomass activities and effluent quality parameters (Gernaey et al., 2004). Complex microbiology-related activated sludge solids separation problems, including foaming, can be investigated using different modelling techniques (Comas et al., 2008). Attempts have been made to produce intelligent control systems to modify and optimise wastewater processes based upon mathematical models (Ohtsuki et al., 1998; Fiter et al., 2005; Baroni et al., 2006), however, developing suitable models can be difficult processes within WWTPs are very complex and data is often incomplete and imprecise (Belanche et al., 1999). Sotomayor et al., (2003) used a linear-time invariant dynamic model to identify and illustrate the processes occurring with activated sludge. Using this approach it was possible to produce algorithms which are numerically robust, non-iterative (and therefore able to quickly carry out calculations) and accurate (Sotomayor et al., 2003). The disadvantage of such model techniques is that they require a large amount of input data and it is not possible to understand how a particular process variable which is not directly included in the model affects the process. One solution to these problems is the production of mathematical models based upon fuzzy logic (Comas et al., 2008). Controllers using fuzzy logic are most suitable controlling time-varying, ill-defined or non-linear systems and such models can work with inexact and vague data (Fiter et al., 2005). Fuzzy logic also provides plausible judgement based upon qualitative knowledge of on-line and observed data.

Using a fuzzy logic model it has been shown that it is possible to optimise an activated sludge system process, primarily because of its ability to work using information directly provided by a WWTP operator; significant amounts of energy were able to be saved by improving air usage (Baroni et al., 2006). It follows that such simplicity in using fuzzy logic control has resulted in this approach being now widely used.

The mechanism of how fuzzy logic works is illustrated in Figure 1.14. Each fuzzy module usually consists of the following components: fuzzifier, rule based, interference and de-fuzzifier (Baroni et al., 2006). The first stage of the process is known as fuzzification. During this operation, input data provided by sensors (crisp values) are converted into

fuzzy values (Fiter et al., 2005). These values are then grouped into sets of corresponding membership functions (Comas et al., 2008). In determining the risk of activated sludge foaming, Comas et al., (2008), found the following indicators/variables to be useful for inclusion into each membership function: - F/M, SRT, DO and levels of influent biodegradable substrate. The processing part of the information then occurs within the fuzzy interface which uses a decision matrix based on definitive rules. An example in the case of activated sludge foaming might be, for example, if SRT is high and DO is low, risk of foaming is high. The final de-fuzzification process converts all information back into crisp numerical values. A decision is then made as to the likelihood of foaming based on probability and risk.

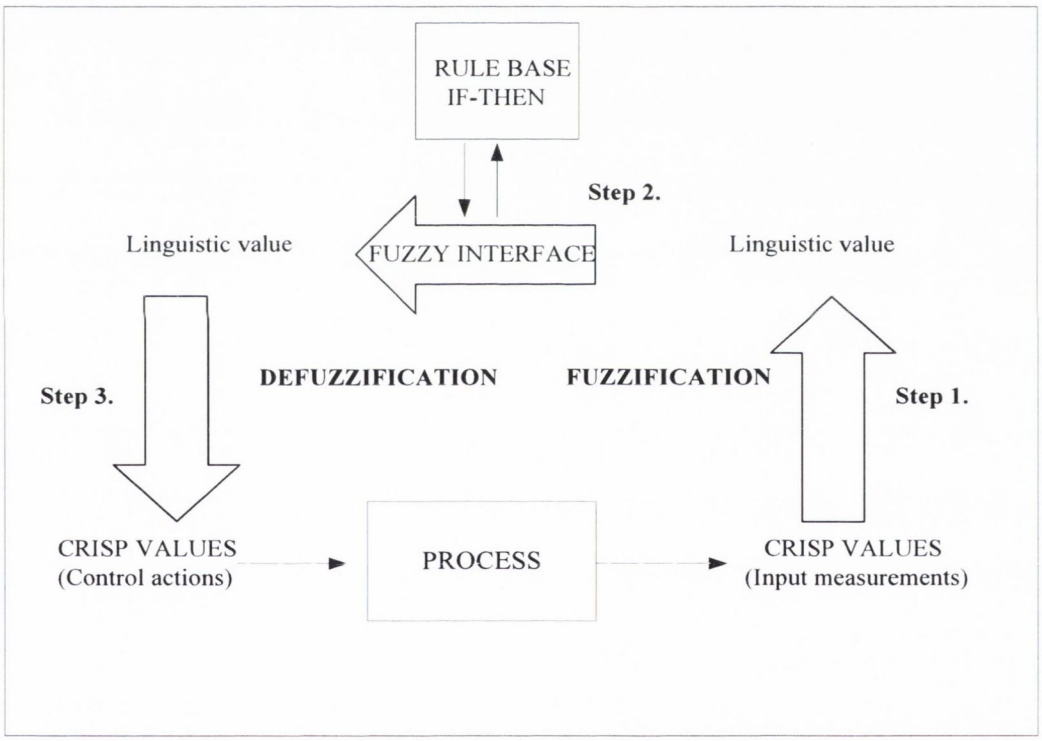


Figure 1.14 – Fuzzy logic control system (adapted from Fiter et al., 2005)

Although fuzzy logic has been used to form the basis of controllers to optimise activated sludge aeration processes (Baroni et al., 2006; Fiter et al., 2005; Kalker et al., 1999), there has been little research carried out in using this technology to assess the risk of foaming within the activated sludge process. The research of Comas et al., (2008), showed however, that it is possible to quantify the parameters influencing the risk of foaming within activated sludge. Whilst this work was based on a limited number of operational

parameters it was complemented by inclusion of cost benefit analysis along with final effluent quality assessment.

1.9 Aims and objectives

The aim of this project is to (1) investigate the underlying environmental and metabolic factors which lead to the formation of thick dark-brown viscous foams on the surface of activated sludge treatment plants and (2) devise a methodology that can be used to limit foaming through better control of environmental and metabolic factors. The research hypothesis is "The risk of foaming within activated sludge plants can be reduced through applying metabolic control".

1.9.1. Key Objectives

A series of objectives were identified which needed to be addressed by the work.

1. To investigate the role that filamentous micro-organisms play in foam formation.
2. To establish the role of the solid and liquid fraction components of the activated sludge on foam formation and stabilisation.
3. To examine the robustness of existing methodology used to determine the propensity of activated sludge samples to produce stable foam.
4. To develop a tool for comparing severity of activated sludge foaming among different wastewater treatment plants.
5. To develop a fuzzy logic and rule-based decision model that can be used to limit the risk of occurrence of activated sludge in activated sludge plants.

1.9.2. Thesis structure

A schematic outline of the different steps conducted in this research study is shown in Figure 1.15. The thesis is presented in paper format, with two of the chapters having already been published (Chapters 2 and 3). The first chapter puts into context problems associated with activated sludge foaming and discusses the constituents of foam and the

current held theories towards how foams develop within activated sludge basins. Attention was paid to the role of filamentous bacteria in activated sludge foam development.

An evaluation has been conducted on the methods currently employed to assess the propensity of mixed liquor samples to produce foam (Chapter 2). Suggestions were made regarding how to ensure optimum repeatability throughout the testing procedure.

As a consequence of this work, there was found to be a clear lack in suitable methodology which could be used to rank foam severity. The foam potential method only assesses the likelihood of sludge samples to foam and is unable to accurately reflect what is occurring at plant level in terms of foam severity. Therefore, to address this deficiency a tool was developed which could then be used to rank and classify foam severity for those plants where foam has already developed (Chapter 3).

In the next two chapters (Chapters 4 and 5), effort was concentrated towards trying to establish the key factors associated with activated sludge foaming. Extensive microscopy was carried out in order to identify and enumerate filamentous bacteria and this was complemented with design, operation and influent evaluation. The actual components of activated sludge (including solids and liquid) were also investigated to determine their role in contributing towards measured foaming potential.

In the final stage of the thesis project, a novel approach was developed to predict the risk of activated sludge foaming occurring (Chapter 6). The end product of this work was a model based on fuzzy logic which employs a rule-based decision matrix to determine foaming risk.

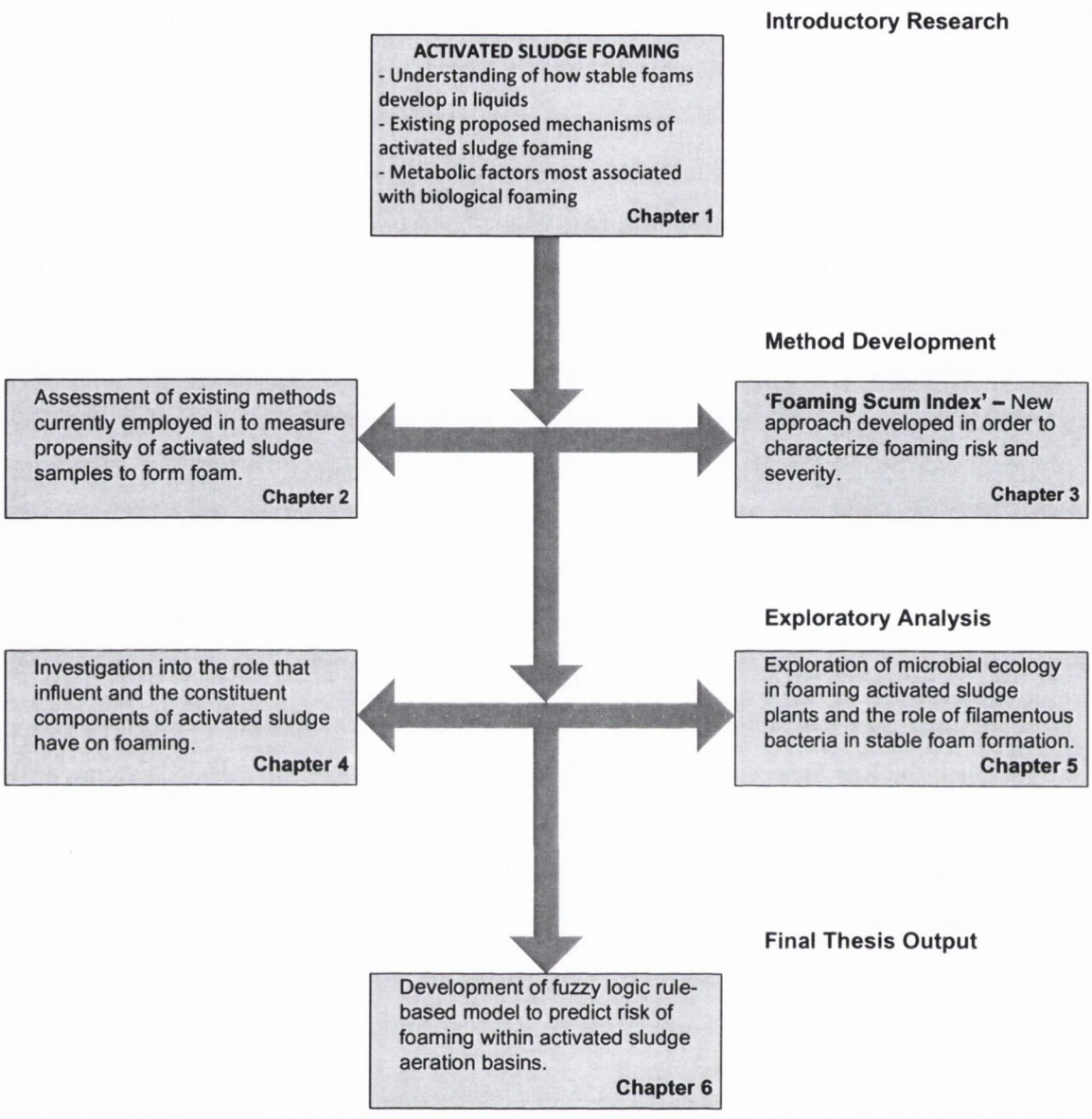


Figure 1.15 – The thesis as a flow chart

Chapter 2

Evaluating the Measurement of Activated Sludge Foam Potential

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2.1. Introduction

Biologically mediated foaming continues to be a significant problem for activated sludge operation and management. It is the stability and persistence of these foams which differentiates them from other bubble entities seen on the surface of aeration tanks (Pipes, 1978). In large volumes foam can result in a multitude of operational problems including: reduction in plant performance, physical hazard to operators from exposure to pathogens and walkway obstruction (Hug, 2006) (Kragelund et al., 2010, Nakajima and Mishima, 2005, Ovez et al., 2006), blockage of pipes (Hug, 2006), reduction in oxygen transfer (Heard et al., 2008), interference with essential plant monitoring equipment (Hug, 2006), and risk to consent failure thorough solids loss from the final settlement stage of the treatment process (Davenport et al., 2000; Kerley and Forster, 1995; Nam et al., 2004). Recently, the presence of foam has also been recognised in systems which employ advanced wastewater treatment such as membrane bioreactors (MBR) (Nakajima and Mishima, 2005). The exact mechanism of how foaming is initiated and then stabilized still remains unanswered (Heard et al., 2008) (Nakajima and Mishima, 2005, Lemmer et al., 2000). In general terms, the formation of foam initially involves the dispersion of a gas in a liquid (Heard et al., 2008). In the presence of surface active agents, thin films of water surrounding the gas bubbles are prevented from draining back into the bulk liquid rendering the foam partially stable (Guitián and Joseph, 1996, Pugh, 1996, Soddell and Seviour, 1990). Activated sludge foam production also involves the addition of hydrophobic material (Müller et al., 2005). Studies have shown that sites suffering from activated sludge foaming are often complemented by the presence of large quantities of the filamentous species *Microthrix parvicella* and/or nocardioform actinomycetes which are found in large amounts within the foam and mixed liquor (Hug, 2006). These filamentous species are strongly hydrophobic due to having cell walls composed of mycolic acid (Davenport et al., 2000, Nielsen et al., 2002, Stratton et al., 2002, Wanner, 1998). In the

absence of such species, non-stable foams are created (Blackall and Marshall, 1989). One widely recognized theory behind activated sludge foaming is that the hydrophobic filaments attach themselves to surfaces of solid particles to create what is described as 'three-phase foams' (Pugh, 2005). Following this, the floc particles then have the ability to adhere to the surface of gas bubbles, causing them to rise and form a stable froth on the surface of aeration basins (Nakajima and Mishima, 2005, Soddell and Seviour, 1990) . These hydrophobic filaments are also believed to perform an additional role of producing extra bio-surfactants (Uysal and Türkman, 2005) as well as stabilizing foam bubbles by bridging the interspatial water layer, forming a dam which then prevents liquid drainage and film thinning (Blackall and Marshall, 1989, Jenkins et al., 2004). Within MBRs the occurrence of foams has been linked to the presence of extracellular polymeric substances (EPS) in the form of extracellular proteins (Nakajima and Mishima, 2005). These have the characteristics of surface active agents and can come from sources other than filamentous bacteria (Di Bella et al., 2011). The key problem facing plant operators is that unlike other foams associated with the activated sludge process, the removal of biological foams are not straightforward as they are neither disrupted nor broken down to any degree by any inherent mixing or aeration processes (Pujol et al., 1991) and are generally resistant to dispersion by water sprays (Pipes, 1978). To add to the problem of activated sludge foaming, there are currently only limited tools available that can be used to predict the onset of foaming events (Comas et al., 2008).

2.1.1. Common Methods Used to Determine Foam Stability and Foam Potential

Historically, the production of surfactants for detergent applications necessitated the development of methods to assess their performance. When evaluating foaming, two of the most important factors to be taken into consideration are how easily a foam forms and its stability (Wilson, 1995). Traditionally the ease of foaming, or foam potential, is the foam-generating power of a liquid or the tendency for a liquid to form foam (Wilson, 1995, Weaire and Hutzler, 1999). Foaming propensity of solutions (characterised by volume of foam generated during testing) depends on composition of the liquid being tested, temperature and method of foam generation (Ekserova and Kruglëiiakov, 1998). Stability, in turn, is determined by the volume of liquid which is drained from the foam within a fixed time period (Sebba, 1987) and represented by rate of collapse or decay of the whole

foam column or part of it (Ekserova and Kruglëiiakov, 1998). There are several different ways in which foams can be formed, including whipping, stirring, plunging, shaking, oscillating, pouring, and bubbling (Kelly and Borza, 1966). Probably the simplest way to produce foam is by shaking a fixed volume of a mixture of liquid/gas within a container for a set period of time. The collapse time of the foam after this test is then an indication of the stability of the foam (Guitián and Joseph, 1996). Such procedures are, however, difficult to standardise due to variations in bubble size which can differ between different experimental tests; therefore, this approach can only be used as a reference guide towards determining foam formation capability of different foaming systems (Guitián and Joseph, 1996). One of the most commonly used procedures for the assessment of the foam potential and stability of foam solutions (which has been adopted by the ASTM as a standard test protocol) is the Ross-Miles pouring test (Lunkenheimer and Malysa, 2003, Pinazo et al., 2001). During this test, a fixed quantity of foaming solution is released from a specially designed pipette which is positioned at a set height above a container of the same dilute testing solution (Ross and Miles, 1941). The volume of foam that is produced immediately upon the draining of the pipette is then recorded as the capacity of the solution to form foam, and the stability of the resultant foam is evaluated from the degree of decay in foam volume measured within a designated time period (Schramm, 2005). Variations on this test have been developed including temperature-controlled conditions (Kelly and Borza, 1966). Pinazo et al., 2001 adapted the original Ross-Miles test so that the volume of liquid in the dispensing vessel was kept constant by continuously pumping the previously released solution back into the dispensing container at a constant rate so that the time period over which the test was conducted could be controlled. Despite the simplicity and wide use of this test as a means to assess foaming potential, its fundamental disadvantage is the lack of control over the amount of gas being introduced into the liquid matrix (Lunkenheimer and Malysa, 2003). For test solutions which possess greater foaming propensity and hence tend to foam more easily, bubbling a gas through the liquid represents a milder method of treatment which is better suited to such solutions (Weaire and Hutzler, 1999). Early experiments passed air at a fixed flow-rate through a porous glass disc at the bottom of a glass column (Bikerman, 1938), and this method (*i.e.*, the Bikerman test) was adopted to assess the stability of dynamic foams under conditions of continuous aeration. Whilst modifications have been made to this test, such as the use of video technology and optical detection to accurately record changes in foam height (Baniel

et al., 1997, Malysa et al., 2005) and measuring foam stability through detecting liquid draining rates using conductivity (Wilson, 1995, Kato et al., 1983)]; the basic practice that originally formed the Bikerman test is still one of the main procedures used today. One widely-used test, the Rudin test for beer foam quality assessment, measures foam stability using a similar gas bubbling/sparging technique (Weaire and Hutzler, 1999). Activated sludge foam is often described as being both viscous and stable in nature (Soddell and Seviour, 1990). Once removed from the aeration zone, activated sludge foam quickly loses its structure and stability. To evaluate the extent of foaming within activated sludge plants, only tests which produce foam that remains stable long enough to be accurately measured and generated under conditions that mimic as closely as possible those experienced within the aeration process should be considered. Therefore, whilst all the tests described have been successively integrated for use within the food, manufacturing and surfactant industries, their use with activated sludge is limited.

2.1.2. Assessing Severity of Activated Sludge Foaming and Use of Foam Potential

Microscopic techniques are now established as one of the first steps in evaluating biological problems within activated sludge. For example, the relationship between the density of filamentous bacteria and reduced sludge settleability leading to bulking (Sezgin et al., 1978). Likewise, an enrichment of *Nocardia* (*Gordonia*) spp. and the filamentous species *Microthrix parvicella* have been associated with activated sludge foaming (Jenkins et al., 2004). Such work has lead to the principle of threshold numbers of mycolata being required for the formation of stable activated sludge foams (Davenport et al., 2000, De los Reyes and Raskin, 2002). A subjective approach to assessing activated sludge foaming has been to simply classify foams using the degree of aeration basin foam coverage (Kocianova et al., 1992), although it has not always been possible to directly correlate foam coverage with the abundance of suspected foam-causing bacteria (Hug, 2006).

Attempts have been made to simulate the portion of biomass that becomes entrained within the foam matrix by sequential factional flotation methods. Gravimetric analysis is then used to compare the mass of biomass in the foam to that of the biomass recorded in the mixed liquor to give rise to what has been termed 'scum index' (Pretorius and Laubscher, 1987). The classification of foams has also been extended to ranking each according to

stability and bubble size and assigning a 'foam rating' accordingly on this basis (Blackall and Marshall, 1989).

Other methods have focused on looking into the properties of foam and mixed liquor rheology through measurements of surface tension and viscosity. Decreases in the surface tension of mixed liquor samples measured during foaming events have been reported (Goddard and Forster, 1987), while foaming sludge samples can possess higher degrees of hydrophobicity compared to non-foaming sludge samples (Khan et al., 1991). This has prompted other authors to attempt to measure changes in the level of hydrophobicity within the activated sludge biomass (De los Reyes and Raskin, 2002, Hladikova et al., 2002). Scum index and foam rating have been correlated with the hydrophobicity of the mixed liquor but not with foam coverage (Torregrossa et al., 2005) which is subject to the individual hydraulic regime within each plant (Hug, 2006). Foam quality tests have been applied to the substrates themselves within the activated sludge. Foaming power is a test which concentrates on what happens during foam production rather than the volume or stability of the foam produced. Albumin and EPS were extracted from mixed liquor samples taken from the MBR process and the foaming power due to these components was determined as the sample volume consumed during foam production (Nakajima and Mishima, 2005). Foaming power was found to increase proportionally to the protein content within the EPS. There is a lack of similar research when considering foaming within the conventional activated sludge process. Whilst these techniques represent potentially valid approaches for investigating foaming within activated sludge, a major drawback is the complexity and cost of analysis particularly when considering the actual extraction of constituents of the mixed liquor itself.

In order to determine the likelihood of activated sludge samples to foam, the most commonly used method is to directly measure the foam propensity of mixed liquor and return activated sludge (RAS) samples. Within the context of wastewater research, the term 'foam potential' is specifically used to reflect foaming propensity and can be viewed as the propensity of mixed liquor samples to foam under conditions of aeration. Ho and Jenkins (1989) proposed a simple method to measure foam potential in activated sludge that has been widely adopted, by dropping two effervescent tablets of Alka-Seltzer into a 500 mL graduated cylinder containing a fixed volume of mixed liquor. The bubbles rise up

through the sample, producing foam similar to that encountered in a wastewater treatment plant (WWTP). The maximum height of the foam generated was then used as a measure of the foaming power of the sludge. Earlier methods assessed foam potential by introducing a set volume of compressed gas through a sintered sand diffuser directly into a sample of mixed liquor and recorded the amount of foam generated in this way (Vega-Rodriquez, 1983). This test is similar to the Bikerman test described above. Despite the range of methods which have already been used to evaluate foaming characteristics, to date there is no standard method for assessing the foam potential or stability of activated sludge (Hug, 2006) (Table 2.1). Due to its simplicity and ease of operation, the Alka-Seltzer test remains the most popular method for both researchers and operators (De los Reyes and Raskin, 2002, Jolis et al., 2007, Lee et al., 2008, Oerther and De Los Reyes, 2001, Paris et al., 2005). Some significant relationships have been identified using this test. For example, seasonal foaming events were directly linked with increased values of measured foam potential (Oerther and De Los Reyes, 2001, Paris et al., 2005) and using this method a threshold level of foam potential that was necessary to start foaming was proposed (De los Reyes and Raskin, 2002). Unlike many of the tests detailed in Table 2.1, the Alka-Seltzer test is widely employed by plant operators. The aim of this study is to compare two contrasting approaches for the assessment of activated sludge foam potential. This was achieved through measuring within-test variation for the commonly-used Alka-Seltzer (Ho and Jenkins, 1989) and sintered disc tests (Hug, 2006).

Table 2.1. Brief descriptions of alternative methods to the Alka-Seltzer test used to measure foam potential of activated sludge.

Summary of method	Description of key apparatus	Ref.
500 mL of activated sludge at specific mixed liquor suspended solids (MLSS) of 3,340 mg SS/L was aerated in a graduated cylinder and aerated with a flow-rate of 2 L min^{-1} through sintered sand diffuser for 60 seconds. Foam height was recorded every 15secs throughout this period.	Graduated cylinder with sintered sand diffuser.	Tsang et al., 2008
20 mL of sample (filament cell culture) was placed in a glass column with sintered glass disc through which samples were aerated with industrial grade air at 100 mL min^{-1} for 1 min. Test used to measure foam stability assessed by the time taken after the cessation of air.	Specially blown cylinder of diameter 21 mm and length 210 mm.	Heard et al., 2008
250 mL of activated sludge sample taken from different sources with contrasting solid concentrations. Samples were aerated for three minutes at an air flow-rate of 4 L min^{-1} and foam produced was rated on an arbitrary scale adapted from (Blackall et al., 1991)	1 litre graduated cylinder of 60 mm diameter. Air purged through an Elastox-T© rubber diffuser membrane.	Hug, 2006
Identical aeration conditions to Heard et al., (2008) performed on cell culture broth. Foam generation assessed on scale rating from 0 (no foam formed) to 7 (dense stable foam, stable for more than 5 minutes after aeration ceased).	250 mL measuring cylinder with sintered disc in base	Stratton et al., 2002
50 mL of MLSS liquid sample was placed into a glass cylinder. Gas was passed through a sintered disc and the foam produced was then assessed using an arbitrary rating system.	Specially blown cylinder of diameter 40 mm and length 500 mm. Sintered glass disc had a pore size of 40–90 μm	Hug, 2006
Test performed on combined surfactant and *Gordonia* spp. containing activated sludge sample. Instantaneous foam heights were recorded every 10 seconds for 10 mins and average foam height determined.	1 litre graduated cylinder and aerated by compressed at rate of 0.11 $m^3 h^{-1}$ through a sintered silica sand diffuser.	Vega-Rodriquez, 1983

2.2. Materials and Methods

2.2.1. Samples of Activated Sludge

Samples of mixed liquor were collected from four different activated sludge wastewater treatment plants (WWTPs) situated within south-east Ireland. The basic type and configurations of each plant are summarised in Table 2.2. The plants at Greystones, Swords and Leixlip (industrial) suffer from regular foaming events while Leixlip (domestic) is largely free from foam although has suffered from severe foaming in the past. In all cases mixed liquor was collected directly before the combined effluent discharge weir, prior to secondary settlement. The samples obtained were all stored at ambient temperature and transported to the laboratory where they were continuously aerated and analysed within 24 hours.

Table 2.2. Brief descriptions of wastewater plants included in study.

	Greystones WWTP (30,000 pe)	Swords WWTP (60,000 pe)	Leixlip Industrial WWTP (35,000 pe)	Leixlip Domestic WWTP (45,000 pe)
System	Conventional Plug-flow	Extended Aeration	Conventional Plug-flow	Completely Mixed
Primary Treatment	Yes	Yes	No	Yes
Aeration type	Fine bubble diffused aeration	Fine bubble diffused aeration	Fine bubble diffused aeration	Mechanical
Aerobic reactor	Yes	Yes	Yes	Yes
Anoxic reactor	No	Yes	Yes	No
Industrial wastes treated	10%	5%	70%	20%

2.2.2. Alka-Seltzer Foam Potential and Stability Test

Using the method described by Ho and Jenkins (1989) two tablets of Alka-Seltzer™ (acetylsalicylic acid 324 mg, sodium hydrogen carbonate, 1,625 mg, citric acid anhydrous, 965 mg, Bayer Corporation: Berkshire, UK) were dropped into a 250 mL aliquot of mixed liquor in a 500 mL graduated cylinder. The foam potential was recorded as the maximum volume of the foam generated during the test. Foam stability was calculated by noting the foam half-life, *i.e.*, the time elapsed for half the volume of foam generated in the foam potential test to dissipate. Initial results using this method proved unreliable as in all cases a persistent layer of scum was formed once the foam had collapsed. Therefore stability data was not included as part of this study. When measuring the effect of environmental variables on the sample, an ice bath or hot water bath was used to adjust the sample to the required temperature. Initially, replicate tests were conducted to determine the effects of storage time and conditions (e.g. aeration of samples) on foam potential using this test. Ten replicate tests of aerated and non-aerated mixed liquor were tested at time intervals of 0, 4, 8 and 12 hours from the time the samples had reached the laboratory (*i.e.*, normally within 1 h of sampling). For testing whether sample volume has an effect, graduated cylinders of 1,000 mL and 2,000 mL were used. It was observed through the course of the experiments that substantial variation could appear in the results due to the movement of the individual Alka-Seltzer tablets within the samples (e.g., tablets could float to the surface and subsequently sink again in an unpredictable manner). T his phenomenon has been reported by other authors (Jenkins et al., 2004; Hug 2006). In order to reduce this source of potential variation between replicate samples, a galvanised wire cage with 10 mm square holes was constructed to contain the tablets and reduce their movements within the sample (Figure 2.1). Replicates were compared with and without the cage. All total suspended solid concentrations (TSS) were measured in accordance to *Standard Methods* (APHA, 1998)

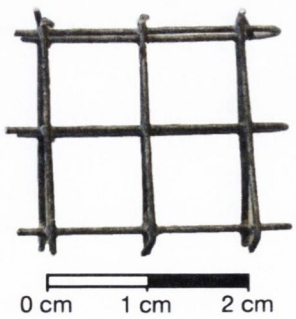


Figure 2.1. Galvanised wire cage used in modifications to the Alka-Seltzer test.

2.2.3. Foam Potential Aeration Apparatus

The bubble column apparatus used to measure foam potential was constructed from a 60 mm diameter 1 litre glass cylinder containing a borosilicate sintered disc embedded 20 mm from its base (Figure 2.2). Air was supplied at the base to pass through the disc by an air pump (Rena Air 200, Rena SA Ltd., France). Air flow rate was measured and controlled using appropriately-sized rotameters with scales of 0.1 – 1.0 L min^{-1} (GAP, England) and 0.5 – 10 L min^{-1} (Stabillmento Ltd., Italy). The test aeration period was 60 seconds. Three different sintered disc porosities were tested. Porosity 0 corresponds to a pore size between 160 to 250 µm and pore size 1 and 2 to pore sizes of 100 to 160 µm and 40 to 100 µm respectively. Each disc complied with ISO 4793. Prior to each experimental period, the sintered glass disc and cylinder were cleaned with 70% v/v nitric acid. The foam potential was evaluated as the maximum level of foam achieved within the aeration period. A sample volume of 150 mL was used in all disc porosity and flow-rate experiments. Subsequent testing was used to confirm the correct volume of sample which should be used when using this apparatus.

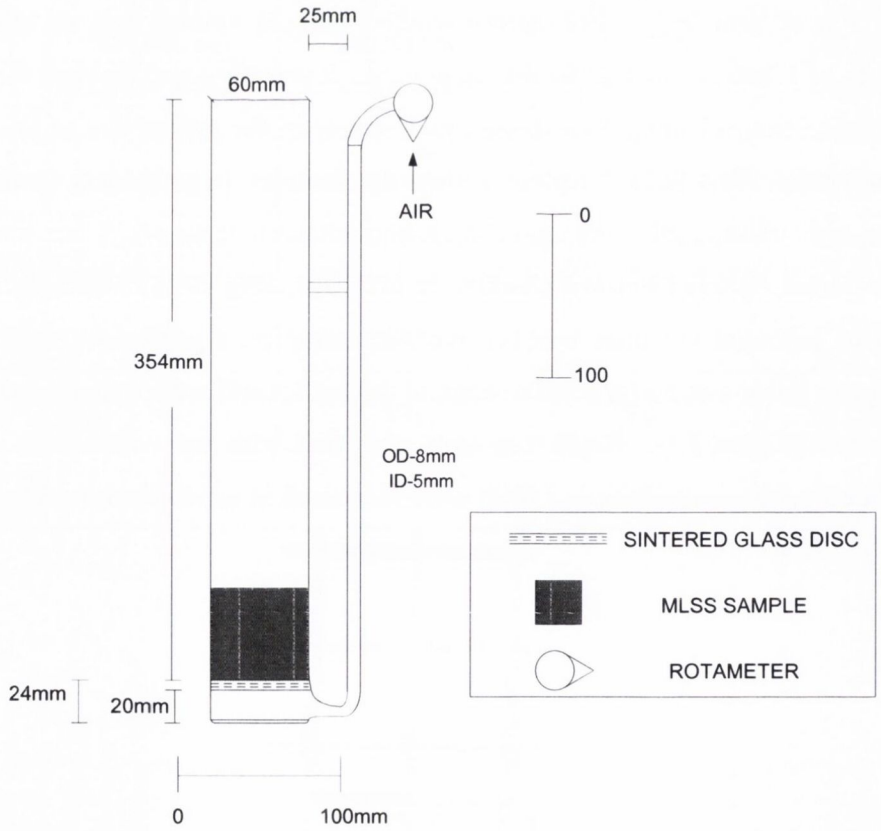


Figure 2.2. Glass column sintered disc foam potential testing apparatus.

2.3.4. Statistical Analysis

Analysis of variance (ANOVA) at the 95% confidence level was used to determine the significance of air flow-rate, volume and disc porosity on the results obtained using the different sludges when assessing the sintered disc method. Replicate numbers were validated using power analysis (Cohen, 2004) conducted with G*Power (version 3) package software (Faul et al., 2007). The coefficient of variation (CV) allowed for comparison of the variability in data obtained between the different experiments.
When comparing the difference between the Alka-Seltzer and the sintered disc methods a two sample unpaired t-test was utilised.

The degree of precision was by assessing calculating the repeatability (r) using the equation:

$$r = t\sqrt{2s} \tag{1}$$

Where r is the repeatability of test method, t is taken from a two-sided t-table based on the degrees of freedom (n-1) at the 95% confidence level where n is the total number of replicates and s is the sample standard deviation of repeat determinations (Caulcutt and Boddy, 1983) The repeatability value (r) is the value below which the absolute difference between two single results obtained under repeatability conditions may be expected to lie with a probability of 95 %. Hence the smaller the value of r the more precise and repeatable the data is.

The number of replicates (n) required for a particular given variation (δ) in the assessment of the wire cage was determined using the following equation:

$$n = \left(\frac{\sigma}{\delta}\right)^2 (Z_\alpha - Z_\beta) \tag{2}$$

where Z_α and Z_β refer to significance level of Type I and Type II errors [60].

To achieve the appropriate power to minimise variances associated with type II error, an optimum of value of 0.80 is necessary (Cohen, 2004). Based on a large effect size (f2 =

0.40, $\alpha = 0.05$) to approximate to this power level a minimum number of 20 replicates (n) (total sample size of 60) was performed.

2.3. Results and Discussion

2.3.1. Assessing Optimum Conditions for Sintered Disc Foam Potential Testing apparatus

To ensure that foam potential testing using the sintered disc method was performed within an environment that provided maximum repeatability, the propensity of mixed liquor samples to produce foam was first evaluated under varying operating conditions of disc porosity (Kragelund et al., 2007) applied air flow-rate (Q_{air}), and sample volume (V).

2.3.1.1. Disc porosity

No significant difference was observed in mean foam potential obtained when tests were conducted using different porosity discs on mixed liquor samples taken from either Leixlip (domestic) or Leixlip (industrial) WWTPs, although foam potential results showed significant differences for all porosities investigated using the mixed liquor from Greystones WWTP (ANOVA, $p < 0.05$) (Table 2.3). During the experiments the bubbles produced were observed to become more uniform in size distribution as the porosity was reduced and for all the samples tested, maximum repeatability was obtained using the smallest porosity of sintered disc examined (*i.e.*, porosity 2: pore size range 40 to 100 μm).

Table 2.3. Influence of disc porosity on repeatability of foam potential results obtained using three different activated sludges (V = 150 mL, Qair = 0.5 L min^{-1}, n = 20).

WWTP	Porosity	Min (mm)	Mean (mm)	Max (mm)	Standard Deviation (SD)	Coefficient of variation (%)	Repeatability (*r*)
Greystones	0	295	317	350	20.2	6.4	56.0
	1	285	306	330	11.5	3.8	31.9
	2	290	306	320	7.76	2.5	21.5
Leixlip (Domestic)	0	210	261	330	23.5	9.0	56.0
	1	240	253	275	8.2	3.2	31.1
	2	250	261	270	6.2	2.8	21.5
Leixlip (Industrial)	0	220	251	270	15.5	6.2	65.1
	1	230	252	300	18.4	7.3	22.7
	2	230	244	270	8.4	3.5	17.2

2.3.1.2. Air flow-rate

The influence of the rate of air flow through the disc on foam potential was then assessed using the column apparatus with the porosity 2 disc. Initially using the mixed liquor from the Greystones WWTP, a range of flow rates were tested (0.5–1.5 L min^{-1}). The higher flow rates produced excessive foaming (Table 2.4). The test was then repeated using the two Leixlip mixed liquors using similar flow rates (0.1–0.5 L min^{-1}). The results showed that a greater precision was obtained operating under the low flow-rate conditions. At these flow rates only approximately 40% of the surface of the disc allowed bubbles through, however, all the bubbles were produced uniformly from within the centre of the disc. At the higher flow-rates of 1.0 L min^{-1} and 1.5 L min^{-1} the air bubbles were produced unevenly causing a swirl effect within the column. Analysis of variance shows that flow rate caused significant differences in measured foam potential (ANOVA, $p < 0.05$). Using mixed liquor samples from Leixlip (domestic) WWTP, a rise in flow rate over the range of 0.1–0.5 L min^{-1} brought about an overall improvement in repeatability (and decrease in standard deviation).

When flow rates >0.5 L min^{-1} were tested using mixed liquor samples from Greystones WWTP repeatability became progressively worse. These trends were less clearly observed with the Leixlip Industrial WWTP samples (Table 2.4).

Table 2.4. Influence of applied air flow-rate (Qair) on repeatability of foam potential results obtained using three different activated sludges (V = 150 mL, ø = 2, n = 20).

WWTP	Flow rate (L min^{-1})	Min (mm)	Mean (mm)	Max (mm)	Standard Deviation (SD)	Coefficient of variation (%)	Repeatability (*r*)
Greystones	0.5	290	306	320	7.76	2.5	23.0
	1.0	480	574	700	57.4	10.0	170.0
	1.5	620	720	820	65.1	9.0	193.9
Leixlip (Domestic)	0.1	230	250	300	13.6	5.4	40.4
	0.3	230	250	270	9.03	3.6	26.7
	0.5	250	261	270	6.20	2.4	18.3
Leixlip (Industrial)	0.1	230	247	265	8.90	3.6	26.4
	0.3	250	268	300	13.3	5.0	39.4
	0.5	230	244	270	8.44	3.5	25.0
	1.0	270	282	300	8.49	3.0	25.1

2.3.1.3. Sample volume

A change in sample volume had a significant influence on foam potential observed from each site ($p < 0.05$) (Table 2.5). For Greystones WWTP and Leixlip (Domestic) WWTP, optimum conditions of repeatability were achieved when conducting tests using 150 mL sample volumes whilst the difference in repeatability was marginal in results obtained using sample volumes of 150 mL and 200 mL for Leixlip (Industrial) samples. The smallest volume of 100 mL consistently produced the highest coefficient of variation (CV). Also, when testing using the smallest volume of 100 mL, large 'flat' films were produced which immediately separated away from the bulk of the rest of the foam and then were forced up the column. These bubbles interfered with the reading of results and made it difficult to determine the true foam potential. At increased volumes, the distance between the surface of the solution and the disc becomes greater. This leads to a dramatic change in the bubble size distribution and the transfer efficiency of the air into the solution matrix. Additionally, a greater volume of test solution will impart an increase in the pressure exerted on the sintered disc at the bottom of the cylinder which in turn possibly influences the ease of bubble release and the size of bubbles produced. Therefore the best volume, whereby the transfer of air is such that the size of bubbles are produced in a reproducible manner and that the release of bubbles is not hindered to any degree, is achieved using a minimum volume of sample of 150 mL.

Variability between the optimum operating parameters of porosity ($ø = 2$), air flow-rate ($Q_{air} = 0.5$ L min^{-1}) and sample volume ($V = 150$ mL) determined here on the basis of best repeatability in each case, has been shown to be very small when comparing the corresponding values of CV.

Table 2.5: Influence of sample volume (V) on repeatability of foam potential results obtained using three different activated sludges (Qair = 0.5 L min−1, ø = 2, n = 20).

WWTP	Volume (mL)	Min (mm)	Mean (mm)	Max (mm)	Standard Deviation (SD)	Coefficient of variation (%)	Repeatability (*r*)
Greystones	100	220	296	420	55.7	20.7	164.8
	150	295	308	320	7.02	2.3	20.8
	200	420	455	525	37.4	8.2	111.0
Leixlip (Domestic)	100	140	199	215	17.8	9.0	52.8
	150	250	260	270	6.11	2.4	18.0
	200	285	300	320	8.55	2.9	25.3
Leixlip (Industrial)	100	170	191	210	12.1	6.3	35.9
	150	230	244	270	9.30	3.8	27.6
	200	250	272	280	7.72	2.8	22.9

2.3.2. Assessing Optimum Conditions for Alka-Seltzer Foaming Method

To evaluate the usefulness of Alka-Seltzer foaming test (Ho and Jenkins, 1991) a program of testing was undertaken to first determine the repeatability by identifying potential environmental and experimental sources of variation, and finally testing for repeatability under identified optimized conditions. The variables investigated included sample storage time, sample temperature, tablet movement, sample volume and MLSS concentration.

2.3.2.1. Use of wire cage

Efficacy of the wire cage in ensuring that a consistently steady stream of bubbles was generated during the Alka-Seltzer test by preventing the tablets from rising to the surface was evaluated. The data produced showed that the use of the wire cage made a significant difference ($p < 0.05$) in the level of repeatability (r) obtained when the cage was in use (mean = 96.1 mm, SD = 5.19, r = 14.4, n = 10) compared to when it was not implemented (mean = 136.0 mm, SD = 13.86, r = 38.4, n = 10). The minimum number of replicates (sample size) required to maintain 10% variance between mean results decreased from 35 to 7 (Equation 2). The floating effect that sometimes occurs when Alka-Seltzer tablets are added to the sample can reduce the effective gas production rate (Hug, 2006). The application of the cage prevented this from happening by ensuring the rate of the gas released by the tablets was constant over time. However, when the cage was used this

reduced the rate of gas release as the total surface area of the tablets exposed was reduced which resulted in a lower expansion of the foam and hence foaming potential.

2.3.2.2. Effect of storage

Values of foam potential were found to differ for all samples when stored, regardless of the influence of aeration (ANOVA, $p < 0.05$). In general, for both aerated and un-aerated samples an increase in foam volume was generated when the time period between collection and testing increased. A paired sample t-test was performed to determine the level of significance in the variation of foam potential under conditions of aeration and non-aeration during storage. No significant difference ($p > 0.05$) was found between samples which had been oxygenated and those left in the absence of air for up to 4 hours, however, beyond this period when storage was extended to 8 hours and 12 hours average values of foam potential recorded were found to differ significantly depending on the storage conditions of the sludge (Table 2.6). A negligible change in TSS was recorded in both aerated samples and non- aerated samples after 12 hours on standing (7% mean increase in MLSS for non-aerated sample and 5% mean increase for aerated sample). The fact that the solids concentration did not significantly change (specifically decrease to any degree) would reflect that an endogenous state of biological activity had not been reached. Additionally, the foam potential increasing over time in the aerated sample suggests both the production and release of additional bio-surfactants, increased filaments or both.

Table 2.6. The influence of storage time on obtained results of foam potential demonstrated using samples maintained under conditions of aeration and non-aeration.

Time Period (Hours)	Number of replicates	Foam potential (mm) (AERATED)	Foam Potential (mm) (NON-AERATED)	Significance ($\alpha = 0.05$)
0	10	*211 ± 12.7 (17.8)		
4	10	258 ± 9.7 (13.8)	253 ± 14.0 (19.6)	$p > 0.05$
8	10	262 ± 7.6 (10.6)	294 ± 17.5 (24.3)	$P < 0.05$
12	10	310 ± 10.9 (15.2)	271 ± 6.0 (8.3)	$P < 0.01$

* Foam potential values are means ± 95% confidence limits. The standard deviation is shown in brackets.

2.3.2.3. Effect of temperature

The effect of temperature on foam potential was examined by performing ten replicates of the Alka-Seltzer test at temperatures of 4, 8, 12, 16, and 20 °C using activated sludge obtained from Swords WWTP. This temperature range was considered as being most representative of typical ambient temperatures experienced in Ireland. The temperature was controlled to ±1 °C over the testing period. A significant linear correlation was found to exist between foam potential and temperature ($n = 50$; $R = 0.98$; $p < 0.05$) (Figure 2.3). ANOVA analysis showed 90% of the variation in data could be explained by linear regression. Whilst the slope produced was found to be significant at the 95% confidence level, the calculated intercept was not found to significantly differ from zero ($p > 0.05$) demonstrating that temperature plays a key role in foam potential measurements when using this test. This is most likely due to the rate of gas release as the tablets dissolve at different temperatures. Ideally foam potential should be carried out at the same temperature as the mixed liquor within the aeration basin.

However, for comparative purposes the test should be performed at a single controlled temperature.

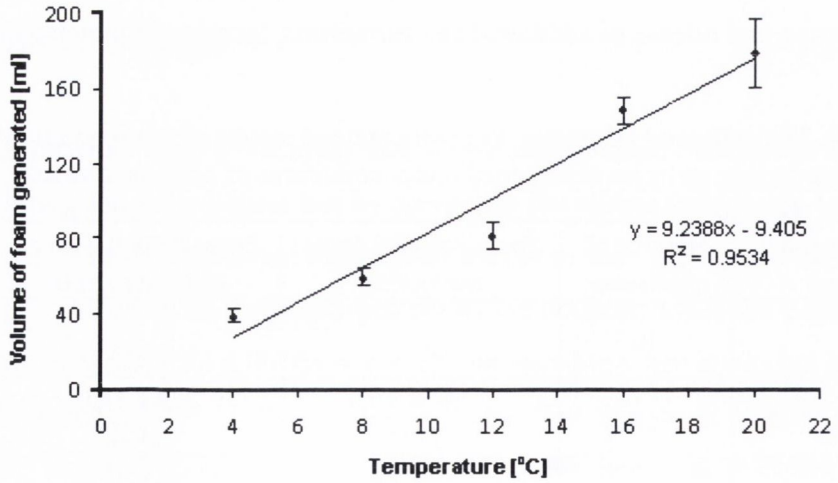


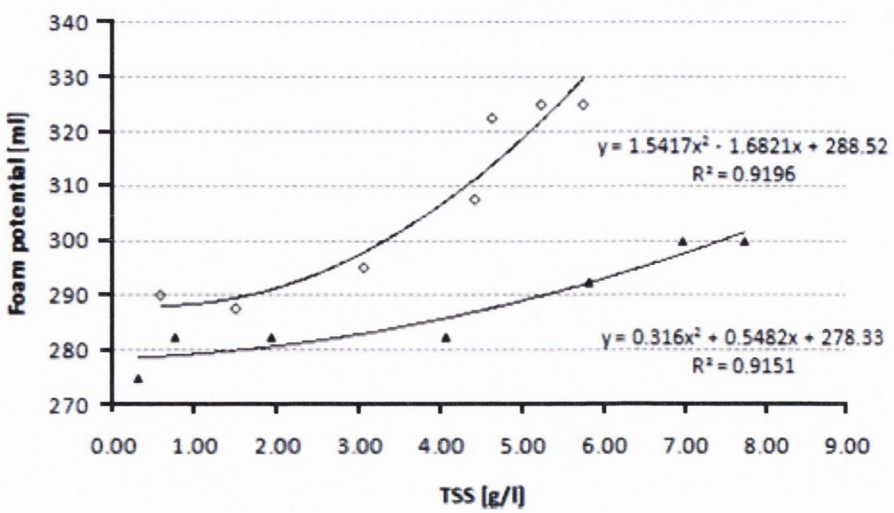
Figure 2.3. – Relationship between temperature and volume of foam produced (i.e. foam potential) for Alka-Seltzer foaming test (Error bars represent 95% confidence intervals of mean values at each specific temperature).

2.3.2.4. Sample volume

Based on the original test methodology a working volume of 250 mL is consistently used as the benchmark sample size chosen by researchers performing the Alka-Seltzer foaming test. When experiments were conducted using sample volumes of 500, 1,000 and 2,000 mL, the addition of two Alka-Seltzer tablets caused the production of a thick layer of floating sludge (approximately 5–7 mm in height) rather than measurable volume of foam. Therefore, increasing the volume of sample in order to conduct this test is not feasible.

2.3.3. Influence of MLSS concentration

Samples of RAS were taken from both Leixlip (domestic) WWTP and Leixlip (industrial) WWTPs. The foam potential of each sample was then tested at a controlled temperature of 20 °C using a water bath. Subsequent tests were performed with samples diluted with the settled supernatant. Dilution was carried out to produce solutions which contained the following percentage of RAS: 10%, 25%, 50%, 75%, 80%, 90%, and 100%. For each site tested, foam potential increased with TSS (Figure 2.4). The relationship in each case was found to be nonlinear, confirming similar observations (Oerther and de Los Reyes, 2001).



▲Leixlip Domestic WWTP ◊ Leixlip Industrial WWTP.

Figure 2.4. Influence of total suspended solids concentration on foam potential measured using Alka-Seltzer test.

For routine plant monitoring this test should be performed at the operational MLSS. As a research tool, however, where there is the need for accurate comparison of data obtained from different plants, over prolonged operational periods or from other studies, it is recommended that this test is carried out on sludge at the same standard concentration of MLSS. In order to establish the ideal solid concentration for this purpose, replicate testing was performed at three contrasting solid concentrations (approx 2.0 g L^{-1}, 3.5 g L^{-1}, and 5.0 g L^{-1}) (Table 7). While analysis of variance shows that the foam potential for each solid concentration differs significantly (ANOVA, $p < 0.05$), values obtained for repeatability at each solids concentration are comparable. It is therefore recommended the test should be performed on sludge samples with a MLSS of 3.5 g L^{-1}. This concentration is appropriate as it is the same as used to express the specific stirred volume index ($SSVI_{3.5}$) test and represents the conventional operational MLSS concentration in activated sludge (von Sperling, 2007).

Table 2.7: Repeatability of foam potential measurement using the Alka-Seltzer test at three different mixed liquor suspended solids (MLSS) concentrations.

Foam Potential					
MLSS (g L^{-1})	Number of replicates (*n*)	Mean Foam potential (mm) (± 95% CI)	Standard Deviation	Coefficient of variation (%)	Repeatability (*r*)
1.84	10	293 ± 3.0	4.22	1.4	13.8
3.57	10	300 ± 3.6	4.97	1.7	15.9
4.95	10	305 ± 2.6	3.69	1.2	11.8

2.3.4. Comparison of Foam Potential between Alka-Seltzer and Sintered Disc Tests

Once operating parameters had been established to ensure maximum precision in results from both the sintered disc and the Alka-Seltzer methods, the two tests were directly compared in terms of precision based on repeatability. The Alka-Seltzer test was performed using 250 mL of sludge. For the sintered disc method, using 250 mL led to the foam approaching the maximum height of the test cylinder apparatus; therefore the optimum volume of 150 mL of sludge was used with this apparatus. In order to be able to compare the results obtained from each method, foam potential is expressed as a

percentage of the maximum expansion of foam compared to the volume of mixed liquor used. When analysing Leixlip (Domestic) WWTP samples, both methods produced comparable results of mean foam potential whilst sample results from Leixlip (Industrial) WWTP differed significantly ($p < 0.05$). In both circumstances the sintered glass disc method produced the best repeatability of the two methods investigated (Table 2.8). In all cases, the variability of data measured remained minimal as indicated by the small values of CV.

Table 2.8: Comparison of repeatability obtained when measuring foam potential (reported as a percentage of sample starting volume) using the standard Alka-Seltzer test (without the cage) and sintered disc test under pre-determined optimum operating conditions at ambient temperature of 18 °C.

Test Method	Sample Size (*n*)	Min (%)	Mean (%) ± 95% CI	Max (%)	Standard Deviation (SD)	Coefficient of variation (%)	Repeatability (*r*)
Leixlip Domestic WWTP							
Air test	20	66.7	74.0 ± 1.8	80.0	4.13	5.6	12.2
Alka-Seltzer	20	44.0	73.3 ± 4.1	84.0	9.27	12.6	27.4
Leixlip Industrial WWTP							
Air test	20	53.3	62.3 ± 2.6	80.0	5.63	9.0	16.7
Alka-Seltzer	20	32.0	49.9 ± 4.2	64.0	9.63	15.0	28.5

2.4. Conclusions

- The optimum operating conditions for the sintered disc method are a porosity of 40 to 100 μm (*i.e.*, porosity disc size 2), an air flow-rate of 0.5 L min^{-1} and a sludge sample volume of 150 mL.

- The application of the wire cage greatly improved the level of precision obtained when performing the Alka-Seltzer test, although the volume of foam produced was reduced by the more controlled release of gas.

- A strong positive linear correlation was found between foam potential results obtained from the Alka-Seltzer test and temperature in the range investigated (4–20 °C). Therefore for comparative purposes the test should be carried out at a prescribed

temperature, while for operational use it should be carried out at the same temperature within the aeration basin.

- The Alka-Seltzer method is also affected by mixed liquor solids concentration with non-linear relationships recorded for different sludges. For comparative research then consideration should be given to expressing foam potential at a fixed MLSS concentration (e.g., 3.5 g L^{-1}).

- Based on the mixed liquors used in this work, when measuring foam potential the sintered disc method produces results with better repeatability than the Alka-Seltzer test.

- The Alka-seltzer test also has inherent problems related to the rate of gas released from the tablets as they dissolve which controls the ultimate volume of gas produced.

- The characteristics of the sintered disc test, involving more complex and specialised equipment renders it inappropriate in most cases for use at plant level. Therefore, for routine operational monitoring the Alka-Seltzer test is more appropriate at the operational MLSS and temperature.

Chapter 3

Foaming Scum Index (FSI) – A new tool for the assessment and characterisation of biological mediated activated sludge foams

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3.1. Introduction

Activated sludge foaming was first recorded in the late 1960s (Anonymous, 1969) and since then has been recognised as a common world-wide phenomenon. The stability and persistence of activated sludge foam makes it especially difficult to remove from the surface of aeration basins (Richard et al., 2003). In severe cases of activated sludge foaming a significant proportion of the biomass becomes entrained within the actual foam itself leading to loss of treatment capacity (Pujol et al., 1991).

The exact mechanism of foam formation and stabilisation has yet to be confirmed and may involve several steps (Davenport et al., 1998; Hug, 2006). In many cases microscopic examination has shown these foams to be enriched with Gram-positive filamentous bacteria, in particular *Microthrix* spp. and members of the branched actinomycetes including *Gordonia* and *Rhodococcus rhodochrous* (Frigon et al., 2006; Kragelund et al., 2007; Petrovski et al., 2011). The mycolic acid contained within the cell walls of these species of filamentous bacteria renders them hydrophobic in nature meaning that they are then able to play a key role in foam stabilisation by adhering to the surface of air bubbles, preventing film drainage and rupture from occurring (Blackall et al., 1991, Iwahori et al., 2001, Petrovski et al., 2011). The solvent partitioning technique discussed in the original work of Rosenberg et al., (1980) has been adopted in order to directly measure the actual cell surface hydrophobicity (CSH) of the activated sludge biomass (Kerley and Forster, 1995; Khan et al., 1991; Torregrossa et al., 2005). This has then been correlated with the capacity of the sludge samples to foam (Torregrossa et al., 2005). The normal approach when investigating activated sludge foaming is to carry out a series of foam propensity tests (known as foam potential) which simulate aeration conditions within a plant and provides an indication of the propensity of sludge samples to foam. Microscopic analysis of the biomass is often carried out alongside such foam potential tests (Ho and Jenkins,

1989; Oerther and de Los Reyes, 2001; de los Reyes and Raskin, 2002; Jolis et al., 2007). The combination of these analytical techniques has led to the concept of threshold numbers being assigned to known foam causing filaments above which foaming is most likely to occur (Davenport et al., 2000; De los Reyes and Raskin, 2002). A summary of the advantages and disadvantages of different techniques that have been implemented to measure foaming is given in Table 3.1. Currently, the only applicable test for both on-site and in the laboratory, is the Alka-Seltzer™ foam potential test (Oerther and de los Reyes, 2001). The simplicity of this test and the fact that it requires limited equipment means that to date it is the most favourable approach (Fryer et al., 2011).

A standard procedure to assess the potential risk posed from the presence of stable foams established on the surface of aeration basins has still yet to be developed. Previously foam surface coverage has been used to illustrate the severity of foaming between different plants (Hladikova et al., 2002) and in some cases foam coverage has been found to correlate well with readings of foam potential (Torregrossa et al., 2005). Since the apparent degree of foam coverage on activated sludge tanks is likely to be influenced by plant layout and configuration as well as operation (e.g. trapping, recycling and building up of the foam in certain places within the process) this variable must be used with extreme caution when compared on its own to other parameters such as foam thickness and stability (Hug, 2006). A more viable option is to consider using foaming coverage along with other criteria to rank the extent of foaming on aeration basins.

In this work a new method of rating the severity of activated sludge foaming is proposed. The Foaming Scum Index (FSI) uses on-site measurements to include organoleptic characteristics of foam colour, bubble size and coverage, the physical foam properties of solid content and finally biological composition relating to the enrichment in numbers of dominant filaments species within the foam. To increase robustness of the model, foam potential results have also been included. The FSI is a quick and simple scale ranking system which has been formulated specifically to quantify activated sludge foaming. Using this method, activated sludge plants can be evaluated and compared in terms of foaming severity. The monitoring of sites prone to activated sludge foaming is achievable through assessing changes in calculated values of FSI. High values of FSI indicate that the site is enduring the presence of stable, thick persistent foams which may be covering the whole aeration basin. Low values of FSI typically infer the occurrence of foams which can

be easily destabilised and only occupy a small proportion of the aeration surface area. By quantifying foaming in activated sludge systems, it will now be possible to accurately model this phenomenon, leading to a better understanding of the mechanisms involved in stable foam production.

Table 3.1: The advantages and disadvantages of contrasting approaches for assessing activated sludge foaming

Method	Brief summary of method	Advantages	Disadvantages	Reference
Foam potential (air method)	Assessment is carried out using a graduated cylinder with a sintered glass diffuser in its base. Air is supplied to aerate the sludge sample and the maximum increase of air bubbles within a fixed time period is then measured.	Relatively easy method to carry out and produces comparative results in a short period of time.	Specially manufactured (blown) equipment required. Equipment does not necessarily represent full-scale plant environment conditions.	Blackall et al., (1991) De los Reyes and Raskin. (2002) Heard et al., (2008)
Filament identification and enumeration (traditional microscopy)	Gram and Neisser stained slides are evaluated using phase contrast microscopy under oil immersion. Using a lined graticule the number of intersections with each filament being enumerated is recorded and expressed as counts/gVSS.	Produces comparative data and can be used for both identification and enumeration of filamentous bacteria.	Time consuming and only applicable to laboratories with phase-contrast microscopy.	Kerley and Forster (1995) Kim and Pagilla (2000) Paris et al., (2005) Ovez et al., (2006)
Fluorescent in-situ hybridization (FISH)	Specific fluorescently labelled rRNA-targeted oligonucleotide probes are used in the identification and enumeration of mycolata and *M. parvicella.*	Allows for the accurate detection and enumeration of target organisms in-situ without prior culture and regardless of their morphology.	Requires specialised equipment along with good expertise in advanced micro-biological techniques. Only applicable to well-equipped micro-laboratories.	De los Reyes et al., (1998) Davenport et al., (2000) Oerther and de los Reyes. (2001) Eales et al., (2005)
Cell surface hydrophobicity (CSH)	Method involves partitioning the mixed liquor biomass in a hydrophobic organic solvent. Absorbance of the water layer is measured before and after treatment and the difference is used to calculate the hydrophobicity.	Well understood theory	Time consuming method requiring the use of a UV spectrophotometer.	Khan et al., (1991) Kocianova et al., (1992) Hladikova et al., (2002)
Surface Tension	Surface tension measurements on mixed-liquor samples from foaming and non-foaming sites and pure filament cultures are performed normally using Wilhelmy plate method.	Has been applied to monitor and demonstrate the production of bio-surfactants by foam foaming bacteria observed as a lowering in surface tension.	Reproducibility of surface tension measurements has is affected by the presence of solid particles within samples.	Pagilla et al., (2002) Stratton et al., (2002) Heard et al., (2008)
Scum Index	Factionary flotation is performed on the mixed liquor biomass at a standard aeration rate. The flotation is repeated during which foam-forming micro-organisms are transferred into the scum. The mass of suspended solids (to approximate for mass of biomass) is then recorded as a percentage to that of the started suspended solids mass.	Scum index has previously been shown to increase well with foam coverage.	In some cases, no clear correlation has been found between that of hydrophobicity and scum index. Test has still not been widely adopted with surface coverage more often used to indicate foam severity.	Pretorius and Laubscher (1987) Torregrossa et al., (2005)

3.2. Materials and Methods

3.2.1. Sample sites

Ten different wastewater treatment plants (WWTPs) which were actively experiencing the production of activated sludge foams at the time of this study were chosen for inclusion in the formation of the FSI. The 12 WWTPs represented a range of different operating regimes including extended aeration, biological nutrient removal, completely mixed and plug-flow configuration (Table 3.2).

To obtain adequate data to perform structural equation modelling (SEM) (Olobatuyi,2006) data was collected from each site during Feb/March 2010 and a year later during March/April 2011, allowing for the necessary collection of additional data obtained under the same seasonal conditions.

3.2.2. Activated sludge foam and mixed liquor samples

Grab samples (10 litres) of mixed liquor were obtained from the final stage of the aeration process prior to settlement in each plant. Multiple grab samples of foam (representing 2-3 grams of foam) were collected from the surface of the aeration contact zones using a 1mm mesh sieve, allowing for the drainage of surplus water during sampling. All samples were maintained at ambient temperature after sampling and analysed within three hours of collection.

Table 3.2: Wastewater treatment plants used in this study

WWTP	Location	Population Served	Secondary Treatment	Aeration Type	Anoxic Reactor	Industrial wastes treat
Baltinglass	Co. Wicklow	2,700	Completely Mixed	Mechanical	No	10%
Enniskerry	Co. Wicklow	6,000	Completely Mixed	Mechanical	No	0%
Greystones	Co. Wicklow	30,000	Conventional Plug-flow	Fine bubble diffused aeration	No	10%
Laragh	Co. Wicklow	1,000	Completely Mixed (rotating contactor)	Mechanical	No	0%
Leixlip Domestic	Co. Kildare	45,000	Completely Mixed	Mechanical	No	30%
Leixlip Industrial	Co. Kildare	35,000	Conventional Plug-flow	Fine bubble diffused aeration	Yes	70%
Navan	Co. Meath	40,000	Completely Mixed	Fine bubble diffused aeration	No	10%
Newcastle	Co. Wicklow	1,500	Extended Aeration (Carousel)	Fine bubble diffused aeration	No	0%
Swords (New) Plant	Co. Fingal	35,000	Extended Aeration (Carousel)	Fine bubble diffused aeration	Yes	5%
Swords (Old) Plant	Co. Fingal	25,000	Extended Aeration	Fine bubble diffused aeration	Yes	5%

3.2.3. Parameters used for Foaming Sludge index (FSI)

Seven parameters were used in the formulation of the FSI. Each variable was selected on the basis of:

a) Showing only a weak extent of multi-collinearity between each other; b) Being easy to interpret and relate to foaming; and c) Requiring minimal expertise to measure, using equipment found on-site in the laboratories of most wastewater treatment plants.

Each parameter was assigned a severity score based on how its properties were indicative of the on-set of problematic foaming (Figure 3.1). For example, small bubbles measured within foams would imply that the observed foam samples were particularly stable. Therefore bubble sizes in the size range a 1-2 mm were assigned the most severe rating of 5.

1	Colour that most represents the closest to your foam	Yellow 1	Light Brown 2	Brown 3	Green/Brown 4	Dark Brown 5
2	Bubble Size	>5mm 1	4mm 2	3mm 3	2mm 4	1-2mm 5
3	Stability	0-10secs 1	10-15secs 2	15-20secs 3	20-60 4	>60secs 5
4	Foam coverage on-site	10% 1	25% 2	50% 3	75% 4	100% 5
5	Filament Index of Foam	1 (negligible) 1	2 (minor) 2	3 (moderate) 3	4 (considerate) 4	5/6 (excessive) 5
6	Foam Potential	0-25% 1	25-50% 2	50-75% 3	75-100% 4	>100% 5
7	Total Suspended Solids Content (TSS)	<10g/l 1	10-20g/l 2	20-30g/l 3	30-40g/l 4	>40g/l 5

Figure 3.1 – Illustration of Foam Scum Index with Scoring

(Lower scores reflect smaller contribution of each parameter to activated sludge foaming)

Foam potential and stability

Foam potential was measured using the glass column sintered disc method using equipment and procedures as described by Fryer et al., (2011). In the present study a sample of mixed liquor (150 cm^3) was aerated for a period of 1 min at a controlled flow rate of 0.5 L min^{-1} during which time the foam potential was determined as a percentage of the maximum expansion of foam achieved in relation to the starting height. Foam stability was recorded as the time taken for all bubbles to collapse on the cessation of air after the 1min period. All tests were conducted under ambient temperature conditions (20°C) using activated sludge samples at a pre-determined solids concentration of 3 g L^{-1}.

Microscopic Analysis

Filamentous bacteria were identified according to the dichotomous identification key described by Jenkins et al., (2004), which is based on modifications to the key originally proposed by Eikelboom and van Buijsen (1981). The relative density of dominant foaming filaments was estimated by subjectively ranking them on a scale rating from 0 (none) to 6 (excessive) (Jenkins et al., 2004). These methods follow a Gram staining procedure with subsequent observation under oil immersion using phase contrast microscopy at 1000x magnification.

Foam thickness and coverage

Foam thickness was evaluated using a novel technique based on observed differences in conductivity to identify the interface between the foam layer (low conductivity) and the underlying mixed liquor (high conductivity). Measurements were carried out using a WTW LF91 conductivity meter (WTW, Weilheim, Germany). The probe was secured to a metre long pole with a calibrated rule along its side. Initially the conductivity of the mixed liquor was noted. The rod was then lowered directly into the foam within each aeration basin. The point at which a sudden increase in conductivity was detected reflected the interface between the foam and underlying water layer, from which the thickness of the foam could then be measured using the rule on the pole.

Aeration basin coverage was expressed as an average percentage of the lane/pocket surface area based on the work of Kocianova et al., (1992). To be able to assess foam coverage in fine bubble diffused air systems a new set of descriptive criteria was added (Table 3.3).

Table 3.3: Subjective rating for aeration basin foam surface coverage

Cover (%)	Mechanical Aeration	Diffused air aeration
0	No foam	No foam
10	Foam in corners extending to one quarter length of pockets	Foam in corners and/or discrete patches of foam
20	Foam extending from corners meeting in the centre of side walls	Foam extending from corners meeting in the centre of side walls
30	Foam forming circle around aerator drawing away from the side wall	Foam extending from corners meeting in the centre of side walls with additional discrete patches of foam being present elsewhere
50	Foam extending towards aerator by one third the radius of the tank	Foam present in each corner of basin and also in the middle of the tank
70	Foam extending towards aerator by one half the radius of the tank	Foam covers each corner and side wall. Most of aeration zone covered, only patches where there is turbulence are not covered
100	Total foam coverage	Total foam coverage

Foam bubble size and colour

Bubble size was visually assessed and an average value was assigned based on a subjective rating scale (Figure 3.1).

The Munsell chart system was used to identify colour of activated sludge. Its application for comparison of colour in soils has long been documented (Torent, 1983; Konen et al., 2003). The Munsell colour system separates colour on the basis of hue (actual perceived colour e.g. brown), lightness (tone) and intensity of colour. Upon comparison, a unique identifiable number is generated on the basis of these parameters which can then be used to compare colour data from other sites.

For activated sludge foams, five colours were chosen which most closely represent the colours of foam samples that had been observed in the WWTPs included in this work (Table 3.4).

Table 3.4: Application of the Munster colour scale for a quick reference guide for foam samples

Colour description	Example	Munsell Colour	Munsell Ref ID	SS (g/l)
Yellow			**2.5Y7/6**	**4.1g/l**
Light Brown			**10YR7/6**	**5.8g/l**
Brown			**10YR2/6**	**8.6g/l**
Green/Brown			**10YR1/2**	**13.27g/l**
Dark Brown			**5Y1/2**	**17.17g/l**

Total Suspended Solids (TSS)

Total suspended solid concentrations (TSS) were measured gravimetrically after drying foam and mixed liquor samples at 105°C and cooling until constant weight was achieved according to *Standard Methods* (APHA, 1998).

3.2.4. FSI Development process

Background

A multi-step strategy was used in the formulation of the FSI (Figure 3.2). Based on literature and previous field studies, seven parameters were chosen which have previously been found to relate to the formation of stable foam. These were; foam colour, bubble size, foam stability, foam potential, coverage, and filament abundance within each foam. Next a series of hypotheses were formulated regarding the assumed influence that each variable has on each other. Field studies were then conducted at 12 different wastewater treatment plants which employed activated sludge as the main treatment process, and a series of measurements were taken for each parameter mentioned in the hypotheses. Once data had been collected and analysed, a preliminary weighting scheme was proposed in order to convert measured values into standardized scores (Figure 3.2) so that parameters were comparable on the same scale. This also took into consideration colour and bubble-size which cannot be readily assigned absolute values. Multivariate correlation analysis was then carried out to ascertain correlation coefficients for both parameter values and standardized scores from which each hypothesis could then be tested in turn. A structural equation model (SEM) was subsequently constructed to represent those hypotheses that had been accepted. Path analysis was to adjust the final weighting of each parameter in the FSI.

Hypotheses used to formulate the FSI

A series of hypotheses were formulated to investigate the strength of inter-relationships between parameters within the FSI. The robustness of each hypothesis was checked using multivariate correlation analysis and the strength of dependency of each variable on one another determined. This led to the formulation of a Path diagram based on the variables which were linked together through associated dependency. The key hypotheses were:

1. *Foam colour reflects the solid content of each foam, with darker samples representing foams of higher solids concentration.*
2. *Foam potential is positively correlated with foam coverage.*
3. *Foam stability is positively related to both bubble size and solids content.*
4. *Foam potential is a function of the number of known foam causing filaments within the foam.*

5. *Foam coverage is directly related to stability and the number of known foam causing filaments within the foam.*
6. *Foam thickness is related to foam coverage with conditions which lead to plants exhibiting greater coverage also exhibiting greater thickness.*

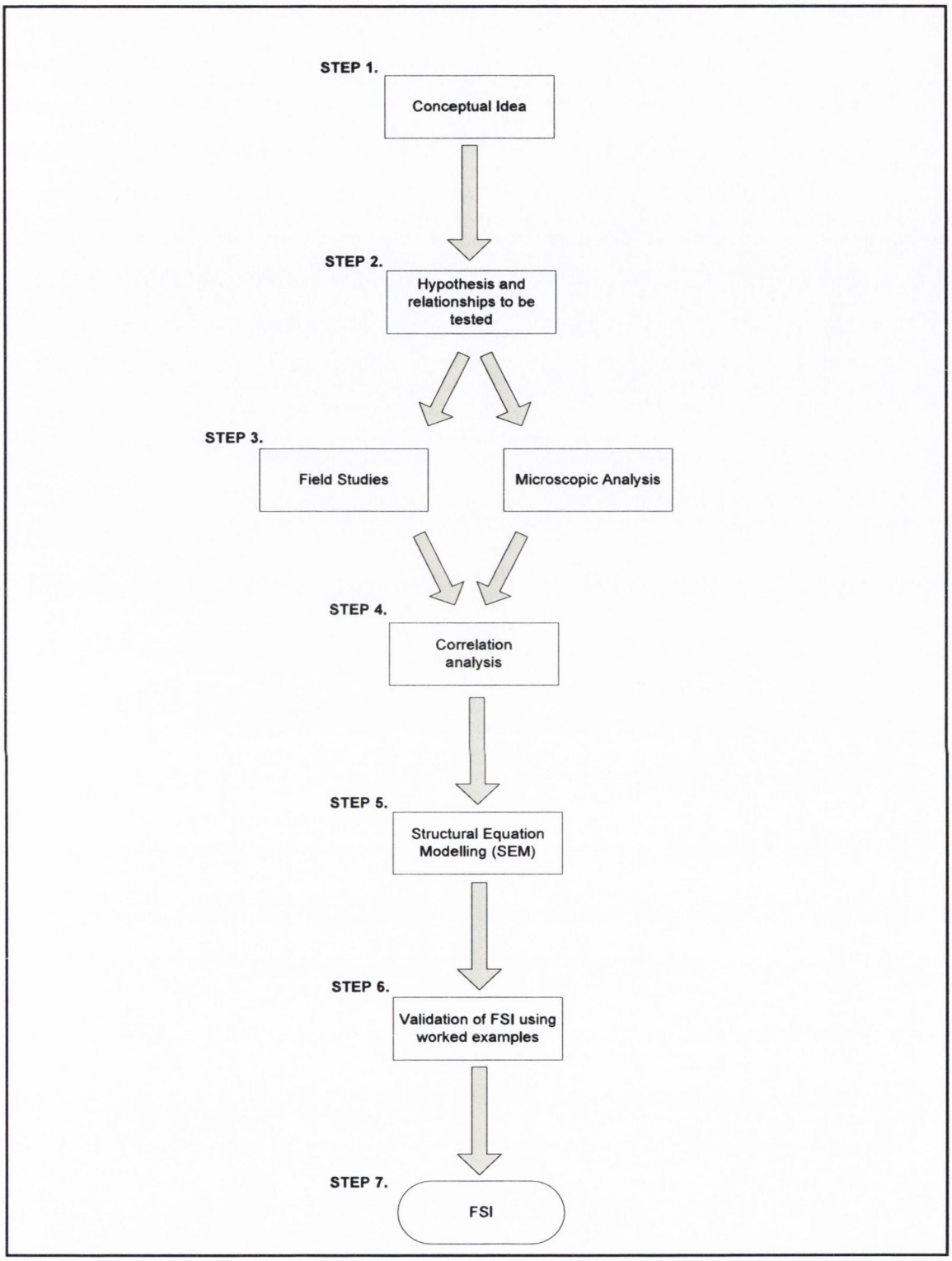


Figure 3.2 – The development of the weighted foam scum index (FSI)

Path Analysis

The FSI was weighted using IBM SPSS® version 19.0 (Statistical Package for the Social Sciences) along with IBM SPSS® AMOS version 19.0 structural equation modelling (SEM) software. Variables from the original FSI model were inserted into a Path diagram in order for them to be correctively weighted (Figure 3.3). Path Analysis has been previously used to determine if specific operating conditions (such as temperature) had either an indirect on direct effect on activated sludge foam production (Frigon et al., 2006). The aim of Path Analysis is to provide estimates of the magnitude and significance of hypothesised causal connections between sets of variables (Stage et al., 2004). The influence of each variable on a dependant parameter is taken into consideration by calculating both indirect and direct influence effects (Olobatuyi, 2006). The weighting of each parameter within the FSI was carried out against values of foam thickness obtained from 12 different sites (Figure 3.3). The IBM SPSS® software produces standardized beta coefficients (β) which were used to finalise the weighting of each parameter in the FSI.

Calculation of the FSI

Values were assigned to the FSI by using the following calculation:-

$$FSI = \left(\sum_{n=7}^{i=1} p_i w_i \right)^2 \qquad (1)$$

Where FSI is Foaming Scum Index (from 0 to 25)

pi is the score value of the variable from 1 to 5 (Figure 3.1)

wi is the weighting attributed to the ith parameter

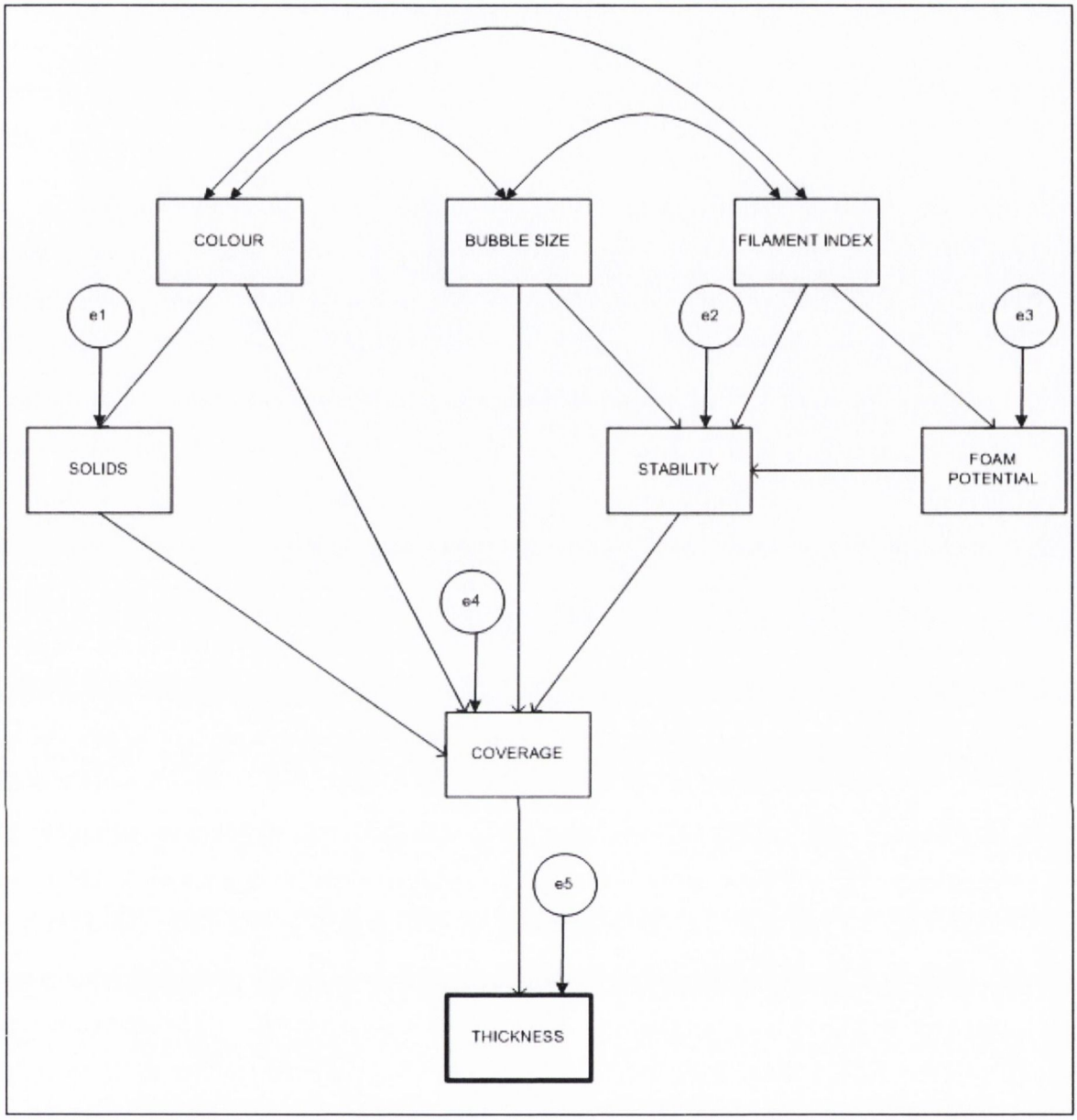


Figure 3.3 – Path diagram used for corrective weighting of the FSI to provide estimates of the magnitude and significance of hypothesised casual relationships between different sets of variables. The total effect of one variable on another can be divided into direct effects (no intervening variables involved) and indirect effects (through one or more intervening variables). The total effect is the sum of direct and indirect effects and the labelling e1, e2… represents that of the unexplained variance in each linear regression.

3.3. Results and Discussion

3.3.1. Correlation between variables within the FSI

The results show that not all the null hypotheses were supported (Table 3.5). No significant correlation was found between foam coverage and measured values of foaming potential ($r = 0.245$, $p>0.05$). This is noteworthy since foam potential is widely accepted as the benchmark tool to assess foaming and foam coverage would be the most apparent and simplest procedure to characterise foaming. Other studies have also been unable to identify a clear correlation between foam potential and extent of foam surface coverage (Hug et al., 2006) suggesting that factors such as hydraulic regime may play an important role when considering the true extent of foam coverage within a plant (Torregrossa et al., 2005). A significant positive relationship ($p<0.05$) was found between foam colour and suspended solids content (Table 3.5). Filaments entrained within the foam are subject to longer sludge ages than those present within the mixed liquor (Jenkins et al., 2004). It therefore follows that those foams which are darker in colour have had longer times to accumulate greater quantities of solids. Bubble size was found to significantly correlate ($p<0.01$) with the stability of foam generated in the foam potential test in each plant. The foam bubble size distribution determines the rigidity of the foam (smaller bubbles make "stiffer" foam) and this in turn has a bearing on the stability of foam (Ekserova, D.R and Kruglëiìakov, 1998). When values were converted to scores (following the rating scheme proposed in Figure 3.2) a significant positive correlation ($p<0.05$) was identified between foam potential score and the density of foam causing filaments within the foam (Table 3.6). This supports the concept that there are density thresholds of mycolata above which the foaming potential is found to significantly increase (de los Reyes and Raskin, 2002).

3.3.2. Results of Path Analysis

Results of foam thickness were used to weight the parameters in the FSI through the process of structural equation modelling (Path Analysis). The total influence (both direct and indirect effects) exerted by the individual variables which form the FSI are summarized in Table 3.7. The results from the Path Analysis show that foam coverage maintained the highest total (indirect + direct) effect on foam thickness ($\beta= 0.33$). In

contrast, the density of foam causing filaments (filament index) exerted the least influence on values of foam thickness within the model ($\beta = 0.03$). The beta coefficient values were rounded to form an approximate percentage to the overall contribution of each parameter in the model from which a corrective weighting could then be assigned.

Table 3.5: Correlation coefficients (Pearson two-tailed) between different parameter values used to evaluate foaming in activated sludge plants and significance.

Parameter	COLOUR	BUBBLE SIZE	COVERAGE (%)	THICKNESS (mm)	FILAMENT INDEX	FOAM TSS (mg/l)	STABILITY (Secs)	FOAM POTENTIAL (mm)
COLOUR	1	0.131	+	-	-	+	-	-
BUBBLE SIZE	0.131	1	-	-	-	-	++	-
COVERAGE	0.515*	-0.264	1	++	-	-	+	-
THICKNESS	0.362	-0.012	0.634**	1	-	-	-	-
FILAMENT INDEX	-0.010	0.120	-0.076	0.132	1	-	-	-
FOAM TSS	0.522*	0.240	0.371	0.273	-0.061	1	-	-
STABILITY	0.030	0.629**	-0.501*	-0.295	0.378	0.133	1	-
FOAM POTENTIAL	-0.063	0.130	0.245	0.012	0.340	0.024	0.174	1

* The level of significance is $p<0.05$.
** The level of significance is $p<0.01$.

Table 3.6: Correlation coefficients (Pearson two-tailed) between different parameter FSI scores (using Figure 3.2) to evaluate foaming in activated sludge plants and significance.

Parameter	COLOUR	BUBBLE SIZE	COVERAGE (%)	THICKNESS (mm)	FILAMENT INDEX	FOAM TSS (mg/l)	STABILITY (Secs)	FOAM POTENTIAL (mm)
COLOUR	1	0.131	+	-	-	-	-	-
BUBBLE SIZE	0.131	1	-	-	-	-	++	-
COVERAGE	0.515*	-0.264	1	++	-	+	-	-
THICKNESS	0.153	-0.012	0.291	1	-	-	-	-
FILAMENT INDEX	-0.014	0.120	-0.092	0.404	1	-	+	++
FOAM TSS	0.493	0.195	0.511*	0.116	-0.136	1	-	-
STABILITY	0.037	0.581**	-0.300	0.110	0.522*	0.028	1	-
FOAM POTENTIAL	-0.080	0.132	0.245	0.406	-0.572	0.001	-0.406	1

* The level of significance is p<0.05.
** The level of significance is p<0.01.

Table 3.7: Results of multivariate analysis conducted through Path Analysis. The default model fits well with high values for both goodness of fit and the normal fit index. Lower Chi Square (χ^2) values per degree of freedom (χ^2/degrees of freedom) and NFI/GFI values close to 1 indicate better a fitting model.

	Colour	Filament Index	Bubble Size	Foam Potential	Solids	Stability	Coverage
Standardized beta coefficients (β)	0.17	0.026	0.136	0.103	0.126	0.057	0.328
MODEL FIT RESULTS							
Test	**Degrees of freedom (*df*)**	**Chi-Square (χ^2)**	**Root Mean Square of Approximation (RMSEA)**	**Goodness of Fit (GFI)**		**Normal Fit Index (NFI)**	
DEFAULT MODEL	14	8.914	0.000	0.914		0.842	
INDEPENDENT MODEL	n/a	n/a	0.220	0.612		0.000	

Validation of FSI results using worked examples

The final stage of the development process for the FSI (Figure 3.1) involved reviewing the values of FSI produced from foam characteristics obtained from a number of familiar foaming sites. A summary of FSI values calculated from the mean of parameter scores obtained from the total numbers of times that each site had been separately visited are shown in Table 3.8. A description of the foaming condition nominally experienced at each site is also provided in this table. The magnitude in FSI figures has been carefully checked against each description to ensure that the FSI development process has been accurate. The rationale behind the development and application of the FSI is demonstrated in further detail for specific sites in Figures 3.4 and 3.5. The value of FSI calculated in each case reflects the nature and extent of foaming. In the first example, a partial layer of non-stable foam covers each aeration basin. This does not normally lead to operational difficulties but the extent of foaming within the second site is problematic due to the presence of a thick stable bubble layer which is hard to remove and entrains a significant amount of the mixed liquor solid inventory. The FSI has also been applied to additional sites not used in its development (Figure 3.5). The variation of FSI within a site has also been compared to other parameters such as SSVI, foam potential and MLSS which are often used to assess sludge separation problems in activated sludge systems. A drop in values of FSI calculated

for Kilcoole WWTP was found to be associated with an improvement in sludge settleability (reduction in measured SSVI), along with a decrease in foam potential and overall reduction in MLSS (Figure 3.6).

Table 3.8: Comparison of mean values of FSI calculated for sites used in the development process along with brief description of foam

SITE	AVERAGE DETERMINED SCORES							MEAN FSI $\sum_{n=7}^{n=1}(p_i w_i)^2$	DESCRIPTION OF FOAM
	COLOUR	BUBBLE	COVERAGE	FOAM POTENTIAL	FILAMENT INDEX	TSS	STABILITY		
BALTINGLASS WWTP	2.0	2.5	2.0	2.0	5.0	1.5	2.5	5.3	Thick, light coloured foam containing significant quantities of entrained foaming filaments.
ENNISKERRY WWTP	3.0	5.0	3.0	4.0	5.0	3.0	5.0	13.6	Very thick, dark coloured stable foam. Normally covers all aeration basin surface area and seen to disrupt surface aerators.
GREYSTONES WWTP	3.0	1.0	4.0	2.8	4.5	2.0	2.5	9.0	Foam is often light in colour and normally occupies total surface of aeration tanks as well as migrating to final settlement tanks.
LARRAGH WWTP	1.0	2.0	2.0	5.0	5.0	1.0	2.0	4.5	Light coloured, unstable foam (can be dispersed with water spray) which can occupy surface of final settlement tank.
LEIXLIP (DOM) WWTP	1.0	5.0	1.0	2.5	4.5	1.0	5.0	4.3	Foam normally occupies only corner sections of aeration zone and is typically light in colour and consists of small bubbles.
LEIXLIP (IND) WWTP	4.0	4.0	4.0	2.0	4.0	2.3	2.8	12.9	Foam is often thick in places and occupies most of the surface of this conventional plug-flow plant. Older foam is seen to develop as a crust layer on the water surface.
NAVAN WWTP	3.0	5.0	3.0	2.0	4.0	2.0	1.0	8.7	The foam at this site can be dispersed under operator intervention. Significant portion of the biomass is found entrained within foam samples from this site.
NEWCASTLE WWTP	3.0	4.5	1.5	1.5	4.5	2.5	5.0	6.9	The foam layer is fairly stable but nominally only found near the single surface aerator, adjacent to the feed pipe.
SWORDS (OLD) WWTP	3.0	1.0	3.0	1.5	3.0	2.5	1.0	6.0	The foam at this site is unstable, typically consisting of large bubbles.
SWORDS (NEW) WWTP	3.5	2.5	4.0	2.5	2.5	2.5	2.5	9.4	A thin layer of foam is often found covering the majority of the surface area of the aeration and anoxic zones of this plant.

	Ratings (unadjusted)						
Site	Colour	Filament Index	Bubble Size	Foam Potential	Solids	Stability	Coverage
Swords WWTP	3	3	4	3	3	4	4

Foaming Frequency

Foam occurs once/twice per year

Description of foam:-

- **Foam is generally light in colour and does not overflow basin.**
- **Stability of the foam is questionable as it can be dispersed by spraying with water.**
- **Generally only a portion of the surface of the aeration tanks is covered with foam.**
- **Foam tends to be not very thick.**

$$\text{FSI} - \left(\sum_{n=7}^{n=1} p_i W_i\right)^2 = 12.60$$

	Ratings (unadjusted)						
Site	Colour	Filament Index	Bubble Size	Foam Potential	Solids	Stability	Coverage
Enniskerry WWTP	4	5	5	5	4	5	5

Foaming Frequency

Foam occurs most of the year

Description of foam:-

- **Foam is thick (at least 30mm) and dark in appearance.**
- **Foam is fairly stable (mixed liquor forms bubbles which are stable >2mins).**
- **The foam consists of small bubbles (average 1mm diameter)**
- **At least ¾ of aeration basin is covered.**
- **The foam is highly enriched in *M. parvicella*.**

$$\text{FSI} - \left(\sum_{n=7}^{n=1} p_i W_i\right)^2 = 22.00$$

Figure 3.4 – Examples of the application of the FSI applied to two contrasting sites (Swords and Enniskerry WWTPs) experiencing different severity of activated sludge foaming

	Ratings (unadjusted)						
Site	Colour	Filament Index	Bubble Size	Foam Potential	Solids	Stability	Cover
Kilcoole WWTP	2	2	5	3	1	4	2

Foaming Frequency

Foam occurs once per year (Summer months)

Description of foam:-

- **Foam is generally light in colour and does not overflow basin.**
- **Stability of the foam is questionable as it can be dispersed by spraying with water.**
- **General only a portion of the surface of the aeration tanks is covered (typically the corner wals).**

$$\text{FSI} - \left(\sum_{n=7}^{n=1} p_i W_i\right)^2 = 6.4$$

	Ratings (unadjusted)						
Site	Colour	Filament Index	Bubble Size	Foam Potential	Solids	Stability	Cov
Malahide WwTP	3	3	5	2	2	4	

Foaming Frequency

Foam 2x per year

Description of foam:-

- **Foam is light in colour and covers majority of aeration surface area**
- **Foam is fairly stable and cannot be easily removed by water spray**
- **The foam consists of small bubbles (average 1mm diameter)**
- **The foam is highly enriched in *G. amarae* spp.**

$$\text{FSI} - \left(\sum_{n=7}^{n=1} p_i W_i\right)^2 = 14.4$$

Figure 3.5 – Examples of the application of the FSI applied to two sites (Kilcoole and Malahide WWTPs) both of which were not used in development of FSI

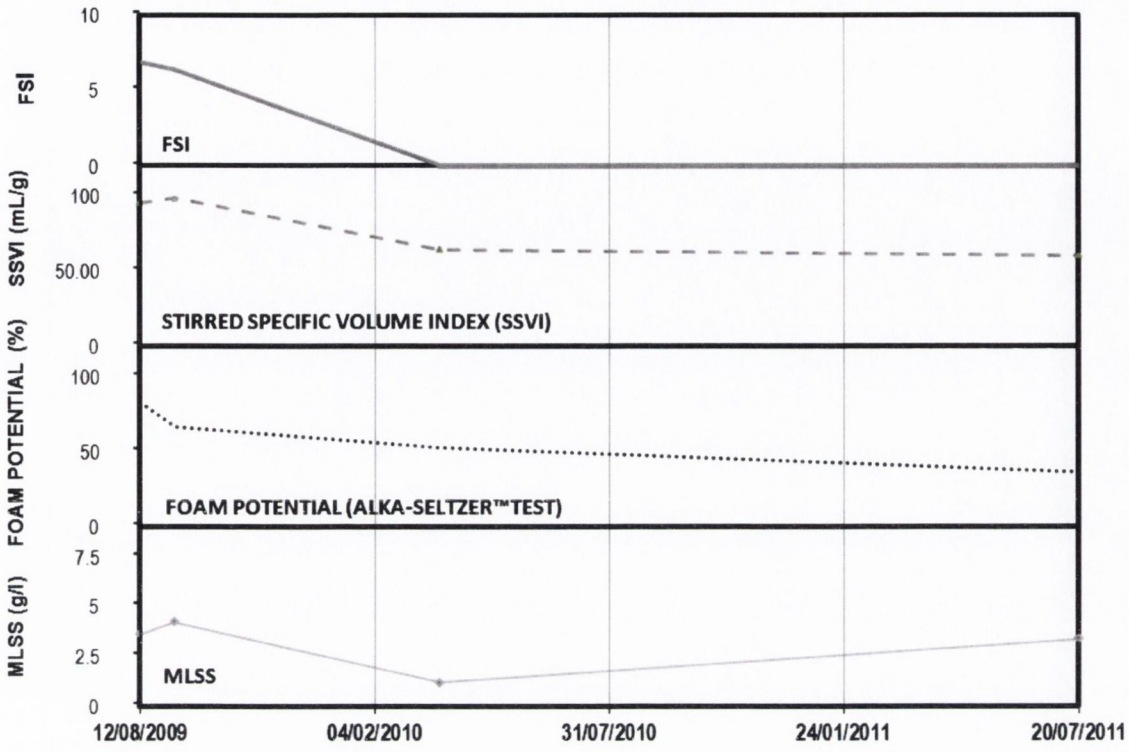


Figure 3.6 – Comparison in variation of FSI values determined for Kilcoole WWTP shown alongside other measured parameters normally used to assess sludge separation problems

Assignment of a class ranking system for FSI

A ranking scheme has been proposed for values of FSI under which sites can readily be classified in terms of foam severity. FSI figures were firstly segregated from each other on the basis of the operating regime that each of the plants that they were determined for operated under. A plot was then made between values of FSI and corresponding foam thickness for each configuration (Figure 3.7). Based on these relationships, values of FSI corresponding to foam thicknesses of 5, 10 and 15 cm were then extrapolated for each type of activated sludge plant (Figures 3.8, 3.9, 3.10).

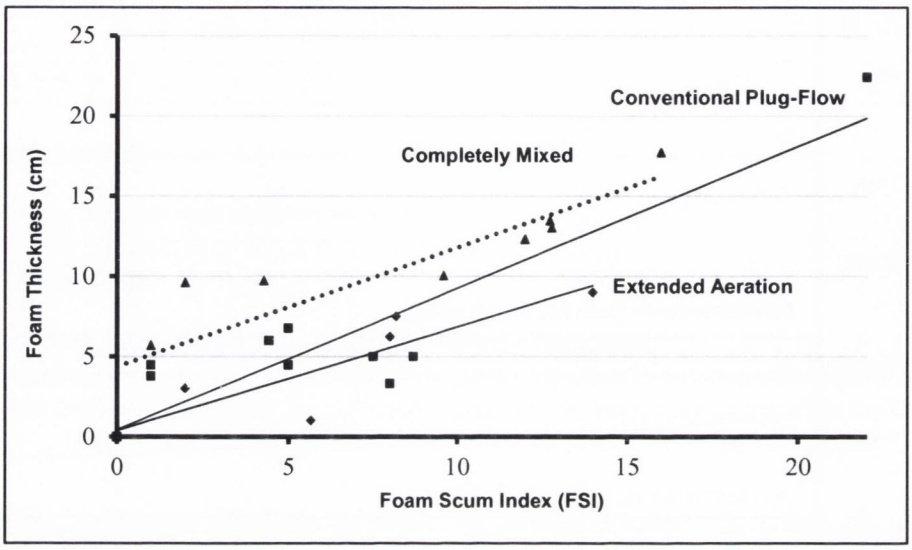


Figure 3.7 – Comparison of the relationship between measured foam thickness and FSI for different configurations of the activated sludge treatment process

From the overall results obtained for values of FSI a class rating system has been proposed in terms of severity of foaming on WWTPs (Table 3.9). From this it can be seen that Swords WWTP suffers from moderate foaming implying there is no immediate risk posed to the activated sludge process, whilst foaming at Enniskerry WWTP is classified severe, posing a current risk to operability of the secondary treatment process.

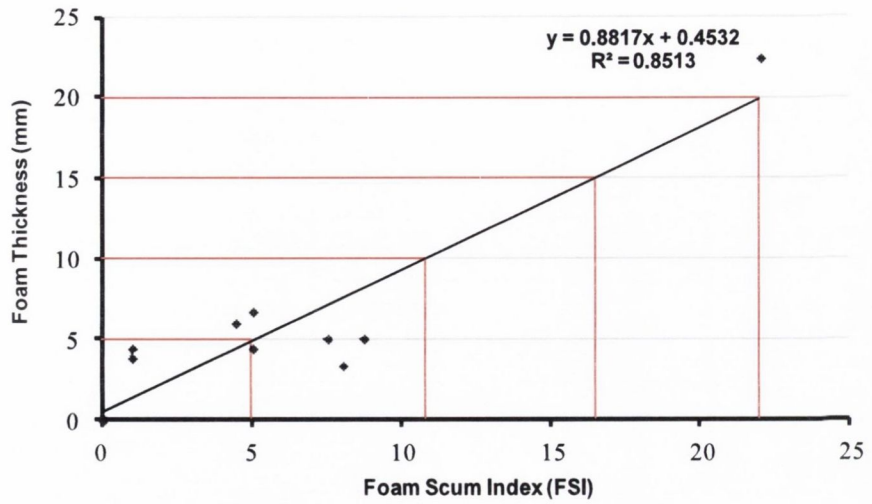


Figure 3.8 – Relationship between foam thickness and FSI for Plug-flow aeration

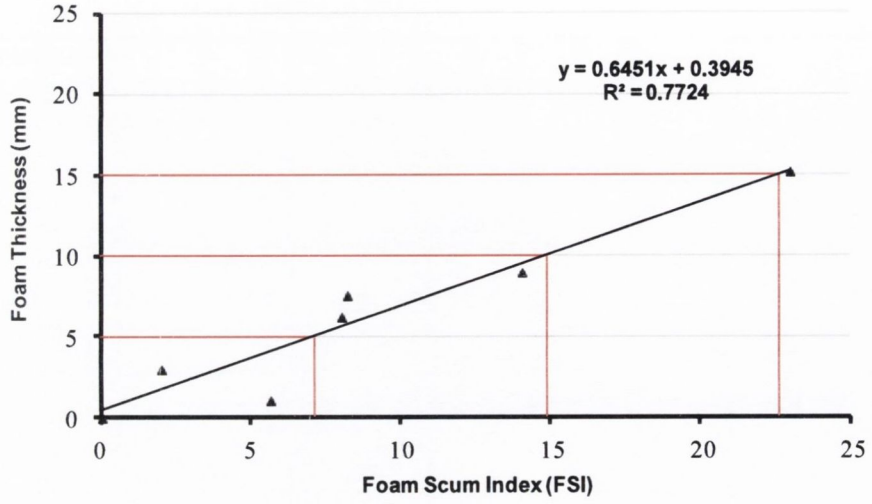


Figure 3.9 – Relationship between foam thickness and FSI for Extended

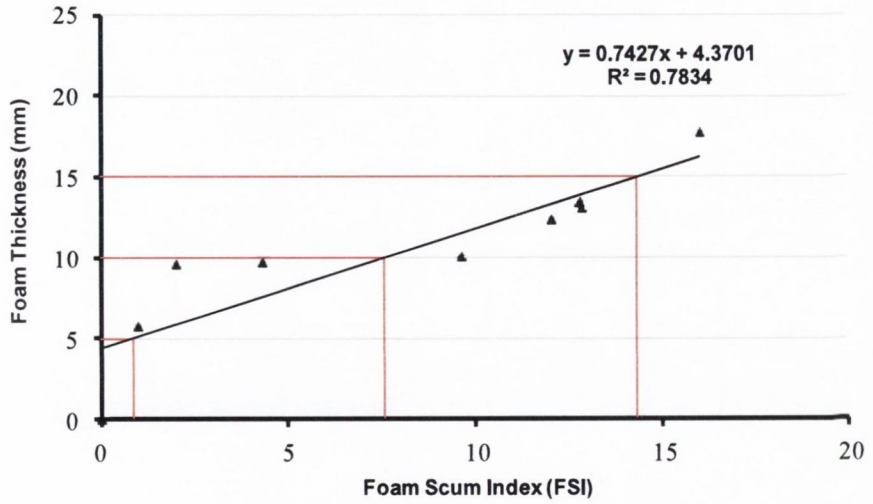


Figure 3.10 – Relationship between foam thickness and FSI for Completely-mixed

Table 3.9: Guide for characterising foam severity based on calculated values of FSI.

Foam Thickness	FSI RANGE FOR DIFFERENT CONFIGURATIONS			Description of foaming conditions	Classification
	Completely Mixed	Plug-Flow	Extended Aeration		
0-5	0-1	0-5	0-7	I. Site suffering from minimal foam coverage. II. Foam has low quantities of *M. parvicella*/mycolata entrained within it. III. Foam is of a light colour in appearance and/or is fairly unstable. IV. Bubble size within foam is likely to be greater 1mm.	CLASS 1 (Low severity)
5-10	1-8	5-11	7-15	I. Foam coverage is likely to be minimal II. Foam has low quantities of entrained *M. parvicella*/ mycolata and/or solids content. III. The foam is likely to be fairly unstable.	CLASS 2 (Minimal severity)
10-15	8-14	11-17	15-23	I. Foam coverage is likely to be minimal along with stability and/or low solids content.	CLASS 3 (Moderate)
15-20	14-21	17-22	23-30	I. Foams are likely to cover most of the aeration basin surface area. II. Foams have significant (FI= 4 +) quantities of entrained known foam causing filaments. III. The stability of the foam may be questionable. IV. The foam may take on a light colour in appearance indicating low solids content.	CLASS 4 (Problematic)
20-25	>21	>22	>30	I. Foams cover most of the aeration basin surface area. II. Foam is dark and thick in appearance. III. The bubbles which make up the foams are small in diameter leading to potentially greatest stability IV. Large quantities of M. *parvicella*/ mycolata are entrained within the foam.	CLASS 5 (Severe)

3.4. Conclusions

The foaming scum index (FSI) is a new powerful tool providing a means to rank and characterise foams which are already established on the surface of aeration basins. This index has been formulated using physical foam characteristics of colour, bubble size and solids content along with data produced from a number of individual tests normally employed to investigate foaming such as foam potential, foam stability and filament abundance. Multivariate analysis and structural equation modelling (SEM) has been used to weight each parameter within the FSI, taking into account the relevance of each to activated sludge foaming. The combined approach of using various data sources ensures greater robustness than any singular test method to assess foaming. The path analysis model was calibrated against measurements of foam thickness. This parameter was specifically chosen on the basis of not exhibiting any degree of multi-collinearity with any of the other variables within the FSI.

The FSI has been designed to further co-ordinate work between different researchers in order to establish patterns in the origin and distribution of foams within different activated sludge plants. Since the development process of the FSI involved using results only collected from the Republic of Ireland, suggested further work would involve applying the FSI to activated sludge plants based in other countries.

Chapter 4

Investigation into the mechanisms involved in stable foam formation and the role of wastewater influent

4.1. Introduction

The presence of viscous dark biologically enriched foams within activated sludge plants is now a widely observed phenomenon (del los Reyes, 2010; Petrovski et al., 2011). Several mechanisms have been proposed for the formation and stabilisation of these foams which typically start to form on the surface of aeration tanks (Jenkins et al., 2004; de los Reyes, 2010). The latest theories are based upon the foam becoming stabilised by the presence of hydrophobic filamentous bacteria which are selectively entrained in significant large numbers within the close packed matrix of most activated sludge foam samples (Lemmer et al., 2005; Kragelund et al., 2007; Seviour et al., 2008; de los Reyes, 2010; Petrovski et al., 2011). The most well-known and familiar foaming filamentous bacteria associated with the foaming phenomenon are Gram-positive members of Candidatus 'Microthrix parvicella' and short branched filaments belonging to *Gordonia* spp. (Kragelund et al., 2007; Seviour et al., 2008; de los Reyes, 2010). According to Lemmer et al., (2005) any filament which has a hydrophobic cell is a potential foam stabiliser.

The growth of filaments in activated sludge is primarily dictated by metabolic or kinetic selection or a mixture of the two (Liu and Liu. 2006; Jolis et al., 2007). Metabolic selection relates to the environmental conditions under which the filaments are able to assimilate substrate, whilst kinetic selection focuses on differences in growth and substrate utilization rates (Eckenfelder and Grau, 1998). Most filamentous bacteria are adapt as *K*-strategists, achieving highest attraction for substrate under low substrate concentrations, whilst most floc-forming bacteria (μ-strategists) demonstrate maximum specific growth and substrate utilization rates under conditions of high substrate affinity (Eckenfelder and Grau, 1998; Wanner, 1998) (Figure 4.1). The strategies adopted by competing floc-forming and filamentous micro-organisms are a function of wastewater composition (Wanner, 1998). Along with plant configuration and operating conditions, wastewater composition has been implicated as playing a fundamental role in foam production within activated sludge systems (Kappeler and Gujer, 1994; Marrengane et al., 2011).

Foam-forming actinomycetes have an advantage over other bacteria within activated sludge by having the ability to switch between being *K* and μ-strategies (Lemmer et al., 1986). Under normal activated sludge conditions, these filaments apply a *K*-strategy of substrate uptake, avoiding direct competition for biodegradable compounds with other activated sludge biota but when this substrate is in excess they switch, becoming μ-strategists, temporarily obtaining high biomass levels by assimilating the readily biodegradable fraction (Lemmer, 1986).

The composition of wastewater entering a plant is dynamic and constantly changing over time (Hug, 2006). To gain a better understanding simplifying the properties of sewage, the carbonaceous substrate, expressed in the form of chemical oxygen demand (COD), is separated into: (1) Readily biodegradable substrate (2) Slowly biodegradable substrate (3) Inert suspended organic matter (4) Inert soluble matter (Kappler and Gujer, 1994; Tandoi et al., 2006). When comparing the growth of filamentous bacteria against that of floc-forming bacteria, only readily biodegradable substrate and slowly biodegradable substrate are generally considered (Comas et al., 2008).

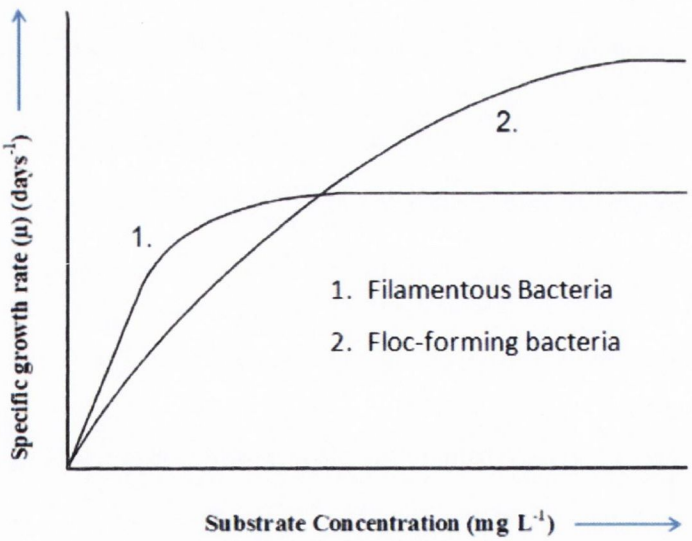


Figure 4.1 – Specific growth rates of filamentous and floc-forming bacteria as a function of substrate concentration (adapted from Lui and Lui, 2006)

The readily biodegradable portion of wastewater COD (RBCOD) generally consists of simple molecular compounds such as mono-saccharides, alcohols, volatile acids and amino acids representing around 10-20% of the total COD in domestic sewage (Wanner, 1998).

Readily bio-degradable substances favour the growth of specific selective filaments (Gulez and de Los Reyes, 2008) and these are often those associated with low organic loading (F/M) conditions (Comas et al., 2008). It has been suggested that the filamentous species, Type 021N along with *S.natans*, *H.hydrossis* and Types 1701 and 0041 are able to proliferate in plants where there are significant inputs of RBCOD (Wanner, 1998; Horan, 2004; Jenkins et al., 2004).

Slowly biodegradable COD, consist of generally large complex molecules, requiring enzymatic breakdown (often referred to as hydrolysis) before being able to be transferred through the cell wall and used for subsequent metabolism (Kappeler and Gujer, 1994). Most organic compounds entering municipal treatment plants are particulate in nature and fall under this category of being slowly biodegradable (del los Reyes 2010). Types 0041, 0675 and 0092, are able to proliferate using slowly biodegradable material (Jenkins et al., 2004; Horan et al., 2004). The slow breakdown of these compounds is due to the kinetics of hydrolysis compared to utilization kinetics, making the hydrolysis stage being the rate-limiting step of the total biodegradation process (Tandoi et al., 2006).

Surface-active agents in the form of surfactants generally enter the wastewater through synthetic washing powders and cleansing products (Lemmer et al., 2000). Surfactant molecules contain both strongly hydrophilic and strongly hydrophobic groups (Bell et al., 1998). Foams generated in solutions containing surfactants normally have a longer lifetime than those not containing surfactants and this is because in solution, surfactants lower water surface tension and provide stabilization of the liquid-film layer between air bubbles (Stypka, 1998). The input of synthetic surfactants into activated sludge plants is one of the foremost causes of foam production (Stypka, 1998; Lemmer et al., 2000; Mara and Horan, 2003). Previously, the presence of fluffy-white billowing foam in plants was a common phenomenon, associated with the input of non-biodegradable surfactants, but a change in recent years to biodegradable surfactants means that this is now no longer the situation (Richard, 2003). Foaming issues connected with the manifestation in excess numbers of *Gordonia* spp. are intensified in the presence of surfactants (Ho and Jenkins, 1991; Horan et al., 2004). An investigation into the influence of surfactants on foaming by Ho and Jenkins (1991) revealed that the addition of non-ionic surfactants promoted foaming when bacterial filaments belonging to *Gordonia* spp. were present but the surfactants on their own applied to samples of mixed liquor were unable to produce stable

foam. A source of long-chain fatty acids (LCFA) within wastewater is potentially via synthetic surfactants, whether it is the parent substances themselves or their intermediates (Lemmer et al., 2002). Members of Candidatus 'Microthrix parvicella' utilize fatty long-chain fatty acids as principle source of energy and carbon (Slijkhuis, 1983; Stypka, 1998; Horan et al., 2004; Levantesi et al., 2006).

Since wastewater concentrations of LCFA produced from surfactant degradation are normally below 10 mg L^{-1}, these substrates are not in sufficient quantities as a source for excess biomass production in *M. parvicella*, however, they may be used by this species for initial growth and establishment (Lemmer et al., 2002). Effective control of *G. amarae* and *M. parvicella* has been achieved through the application of anti-foams which act directly on the chemical surfactants in the wastewater rather than the foams themselves (Richard, 2003).

Mechanical stress in the activated sludge process can lead to the release of lipids along with surfactants through the breakdown and decay of dead cells (Kappeler and Gujer, 1995). Bacteria have also been found to exacerbate foaming by contributing to the total surfactant load by producing bio-surfactants (Heard et al., 2008). Generally bio-surfactants comprise polysaccharides along with lower molecular weight compounds such as glycolipids (Arenskotter et al., 2004). The quantity and composition of surfactants produced by bacteria is dependent on the substrate which they utilize (Goddard and Forster, 1986; Khan and Foster, 1990). There are several reported findings pertaining to the production of bio-surfactant by different strains of *G. amarae* (Cairns et al., 1982; Soddell and Seviour, 1990; Pagilla et al., 2002; Heard et al., 2008). Variations in suspended solid concentrations and surface tension have been observed by Pagilla et al., (2002) when isolates of *G. amarae* were grown on either hexadecane and acetate or a mixture of the two. Greatest bio-surfactant production was achieved when hexadecane was present as one of the substrates (Pagilla et al., 2002). Bacterial production of surface active material has the property of ensuring the emulsification of hydrophobic material making it readily available for assimilation (Kappeler and Gujer, 1994; Lemmer et al., 2000). Pagilla et al., (2002) was able to demonstrate that the production of bio-surfactants was found to exert a synergistic effect by emulsifying the hydrophobic hexadecane substrate. Bio-surfactants can also be formed as a by-product of the autolysis of dead cellular material (Geradi, 2004). Bacterial produced bio-surfactants play an important role in the degradation of water insoluble and hydrophobic material through either promoting

the incorporation of hydrophobic compounds into microbial cells or by physically dispersing them, providing a greater surface over which microbes can then utilize them (Bell et al., 1998; Arenskotter et al., 2004).

This study principally sets out to determine the role that influent has on foaming within the activated sludge systems and to establish the key components that are needed in stable activated sludge foam formation. In order to achieve these goals the work has been conducted in two key stages. Firstly, lab-scale experiments have been performed using batch reactors filled with mixed liquor samples taken from both foaming and non-foaming sites. The influent from these sites has then been alternately fed in a range of combinations to different reactors containing each type of sludge to determine the affect of each type of sewage. In the second stage of this work, foam potential and stability tests have been carried out on the various fractions that make up each activated sludge sample and results of foam stability and potential have been compared to water and sewage samples used as control samples within each experiment. The hypotheses that were tested are listed as follows:

First Stage - Bench-reactor studies:

1) The foam potential and foam stability of the influent feed statistically differs from that of the mixed liquor taken from the same plant.

2) The foam potential (propensity of sludge to form foam) of a non foaming sludge (NFs) is increased when fed on influent from a plant currently experiencing foaming problems ($F_{INFLUENT}$).

3) The numbers of known foam causing filaments is increased by supplementation of influent from the foaming site.

4) It is possible to induce foaming on activated sludge taken from a site with no history of foaming using influent taken from a site suffering from persistent activated sludge foaming.

Second Stage - Experimentation on constituents of activated sludge:

1) The addition of sludge solids, taken from a foaming site, when added to water leads to a significant increase in foam potential.

2) The supernatant of mixed samples has increased foam stability compared to water alone which statistically is greater in samples taken from foaming sites due to the assumed presence of a greater concentration of bio-surfactant.

3) Wastewater Influent has a fundamental role in activated sludge foaming, reflected by higher foam potential readings being measured for influent taken from foaming sites when compared to that taken from non-foaming sites.

4) The stability of foam bubbles measured on aerated influent samples (regardless of nature of plant source from which they were taken from) is significantly greater than that of water alone.

4.2. Materials and Methods

4.2.1. Microscopic analysis

Microscopic identification and enumeration of *M. parvicella* was carried out using Gram staining under phase contrast at 1000x magnification (Jenkins *et al.*, 2004). To quantify the presence of *M. parvicella* the microscopic counting method originally developed by Pitt and Jenkins (1990) for *G. amarae* was applied and results were expressed as number of intersections/g MLSS.

4.2.2. Site survey – Questionnaire

A detailed site survey was conducted investigating foaming at a number of wastewater treatment works located in both the Republic of Ireland and United Kingdom. The primary objectives of the survey were to record the regularity and persistence of full-scale foaming events and to determine if any potential relationship exists between the occurrence of scum production and running conditions of each plant. Questionnaires were sent to all County Councils (29 total) within the Republic of Ireland. In the UK, wastewater plants are the responsibility of 12 main water companies, each of which were approached to try and obtain information regarding their experience of activated sludge foaming. Operational and plant management staff were contacted who had active knowledge of sites which were either suffering from foaming events at the time of questioning or had a prior history of activated sludge foam production. The questionnaire involved specific questions pertaining to the operating environment of each activated sludge process (e.g. sludge age and organic loading, control strategies for problems) as well as the type of influent normally treated (e.g. composition, sources of wastewater, fluctuations, septicity) and the actual design of the plant itself. Additional questions were asked in regard to foaming problems experienced within each plant (e.g. frequency of occurrence, time of year foaming normally occurs, extent of coverage). In total, 41 specific questions were presented with survey details being requested only once in each case. The selection of treatment works for inclusion in the survey was not restricted either by population equivalent served, size or by plant configuration and geographic location.

4.2.3 Foam potential and stability

Foaming potential tests were conducted throughout the period of the trial prior to feeding each reactor by adding two tablets of Alka-Seltzer (containing 650 mg sodium acetylsalicylate, 3832 mg heat-treated sodium bicarbonate, and 2000 mg citric acid; Bayer Corp Elkhart, IN) to 250 ml of well-mixed samples in a 500 ml graduated measuring cylinder. Foaming potential was assessed as the maximum expansion in volume of foam achieved in relation to the starting volume of 250 ml. Foaming potential was also determined using the glass column sintered disc apparatus previously described in Fryer et al., (2011). A portion of mixed liquor (150 cm^3) was aerated for a period of 1 min at a constant flow rate of 0.5 L min^{-1} during which time the foam potential was determined as a percentage of the maximum expansion of foam achieved in relation to the starting height. Foam stability was acknowledged as the time required for all bubbles to collapse following the retardation of air after the 1 min period.

4.2.4. Bench-scale reactor experiments

Bench-scale continuously mixed batch reactors were used in order to investigate the influence of wastewater composition on *M. parvicella* growth. The studies were conducted using sixteen 5L HDPE containers placed within a temperature controlled greenhouse environment maintained at a constant temperature of 20°C ± 2 °C throughout testing. Air was supplied by eight air pumps (Rena Air 200, Rena SA Ltd., France) diffused through fine glass hollow rods (diameter 5mm) in order to ensure adequate mixing within each reactor and to maintain a dissolved oxygen (DO) level between 3.0 and 4.0 mg/l. Experiments were run for 12 days in order to monitor the growth of *M. parvicella*.

All reactors were filled with sludge with a final MLSS of 2 g L^{-1}. The concentration of solids in each reactor, as well as influent COD, was determined immediately prior to feeding of each reactor vessel. By adjusting (dilution of influent) an attempt was made to maintain a F/M of 0.1 kg BOD/kg MLSS in each vessel throughout the testing period (12 days). The experimental protocol followed in this study is illustrated in Figure 4.2. Mixed liquor and settled influent samples were taken from two sites actively experiencing foaming (Portlaoise WWTP and Swords WWTP) and two contrasting non-foaming sites (Athy WWTP and Ringsend WWTP). Four scenarios were tested:-

(1) Non-Foaming Sludge (NF) fed with Foaming Influent ($F_{INFLUENT}$)

(2) Foaming Sludge (F) + Foaming Influent ($F_{INFLUENT}$)

(3) Non-Foaming Sludge (NF) + Non-Foaming Influent ($NF_{INFLUENT}$)

(4) Foaming Sludge (F) + Non-Foaming Influent ($NF_{INFLUENT}$)

Experimental controls were carried out using sludge samples taken from each site and feeding them with influent taken from the same site.

SLUDGE	$NF_{INFLUENT}$ 1. (Athy WWTP)	$NF_{INFLUENT}$ 2. (Ringsend WWTP)	$F_{INFLUENT}$ 1. (Portlaoise WWTP)	$F_{INFLUENT}$ 2. (Swords WWTP)
Non Foaming Sludge (NF_1) (Athy WWTP)	**CONTROL**	CONDITION 3	CONDITION 1	CONDITION 1
Non Foaming Sludge (NF_2) (Ringsend WWTP)	CONDITION 3	**CONTROL**	CONDITION 1	CONDITION 1
Foaming Sludge (F_1) (Portlaoise WWTP)	CONDITION 4	CONDITION 4	**CONTROL**	CONDITION 2
Foaming Sludge (F_2) (Swords WWTP)	CONDITION 4	CONDITION 4	CONDITION 2	**CONTROL**

Figure 4.2 – Experimental protocol adopted within batch experiments investigating the influence of alternating feed and sludge type

4.2.5. Mixed liquor fractionation tests

Mixed liquor samples obtained from both non-foaming and foaming site were first subject to being centrifuged (3000g for 5mins). The supernatant obtained from centrifugation was then batched together and held within a 1000 ml volumetric flask for subsequent foam potential testing. Suspended solids were obtained from each mixed liquor sample by filtering approximately 500 ml of sludge (in each case) through coarse pore paper filter (coffee filter) paper. The solids were then left to dry at room temperature for approximately 2 hours after which approximately 2 grams of solid material was weighed out and quantitatively dissolved into 600 ml of de-ionised water held within a 1000 ml volumetric flask. The samples were then vigorously shaken after which they were made

up to 1000 ml with de-ionised water. The concentration of suspended solids within these samples was then verified applying test methods as stated according to *Standard Methods* (APHA, 1998).

4.3. Results and Discussion

4.3.1. Results from Bench-Scale Studies

Throughout this pilot study, *M. parvicella* was found to be dominant filamentous species observed in all activated sludge samples. Maintaining the growth of this species is especially difficult under laboratory conditions (Rossetti et al., 2005). This could account for some of the results obtained through the bench-scale studies where no significant increase in numbers of *M. parvicella* and foam potential were observed. Changes in numbers of *M. parvicella* were only observed in the tests conducted with sludge taken from Swords WWTP and its associated influent. A significant ($p<0.05$) increase in numbers of *M. parvicella* was observed when Ringsend WWTP (NF) sludge was supplemented with $F_{INFLUENT}$ feed taken from Swords WWTP and this in turn was reflected in an increase in measured foam potential (Figure 4.3).

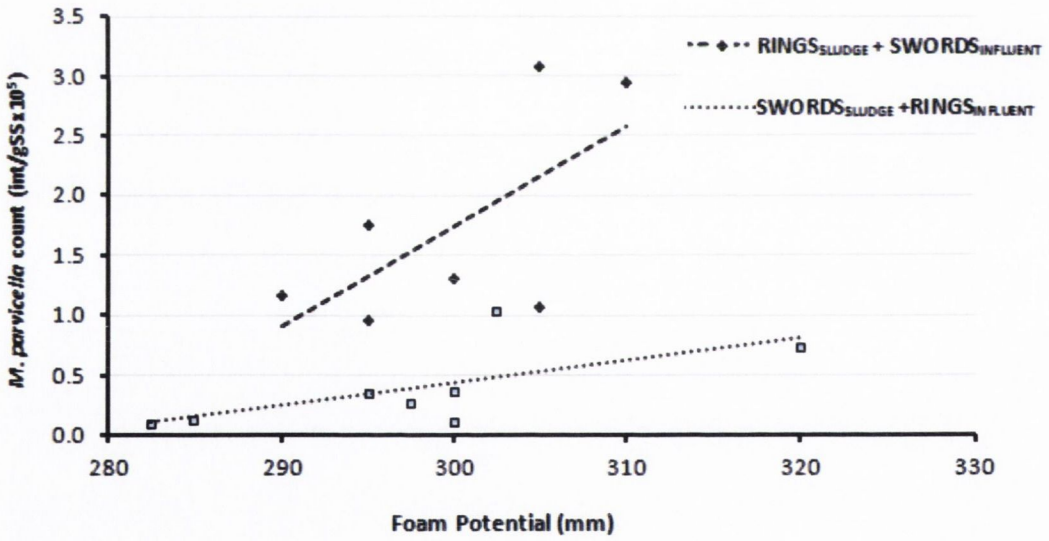


Figure 4.3 – Relationship between numbers of *M. parvicella* and foam potential measured during influent tests

During testing, the increase observed in *M. parvicella* occurred within the first days (Figure 4.4), after which the growth of this species could not be maintained in any of the reaction vessels.

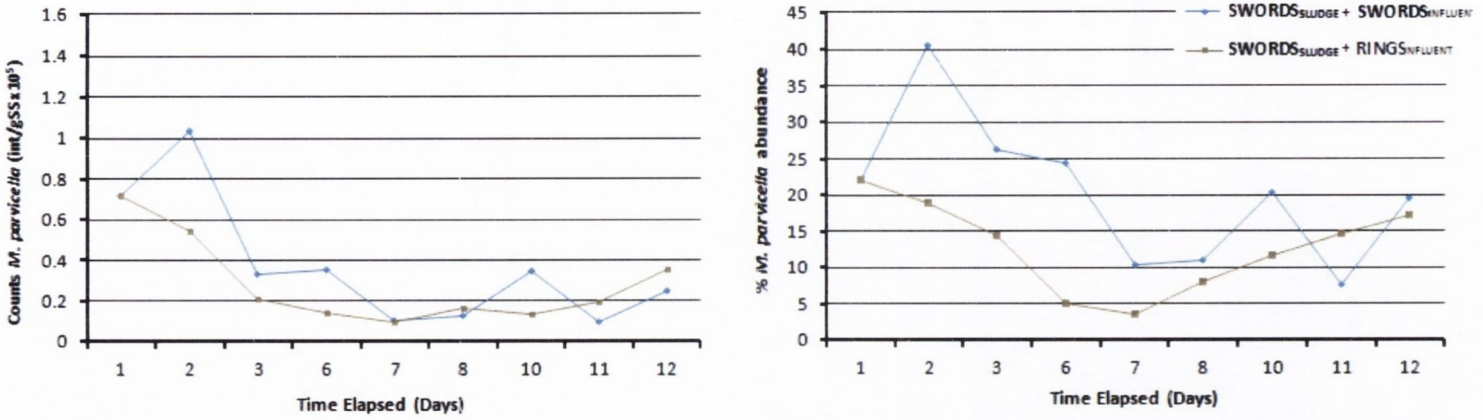


Figure 4.4 – Change in abundance of *M. parvicella* when Swords Sludge (F) was fed with $NF_{INFLUENT}$ taken from Ringsend WWTP

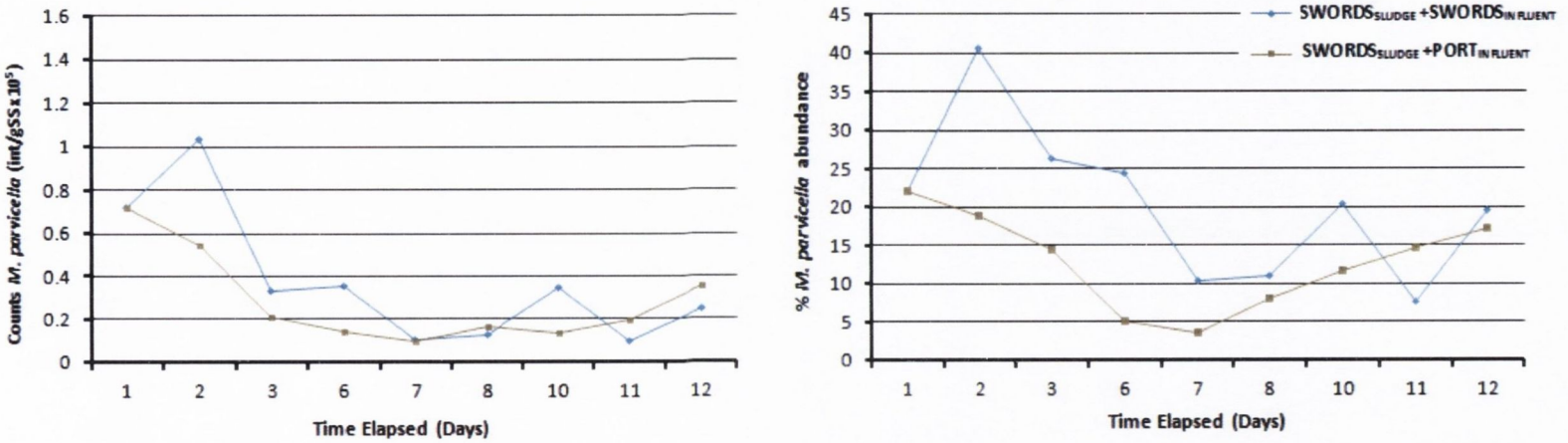
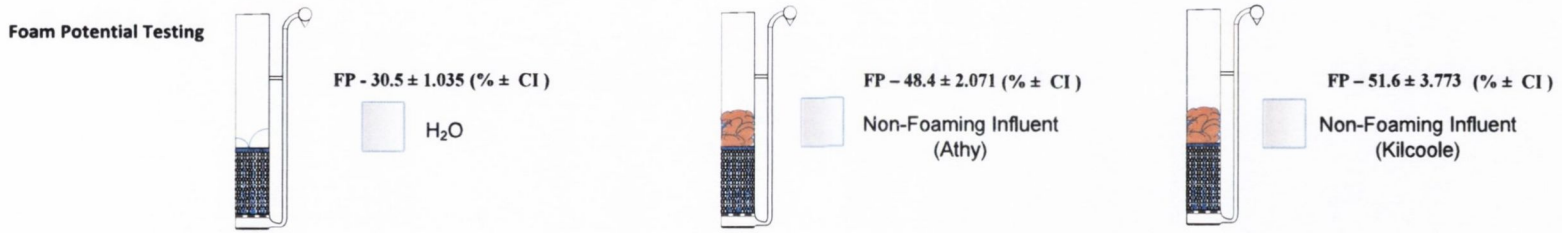


Figure 4.5 – Change in abundance of *M. parvicella* when Swords Sludge (F) was fed with $F_{INFLUENT}$ taken from Swords and Portloaise WWTP

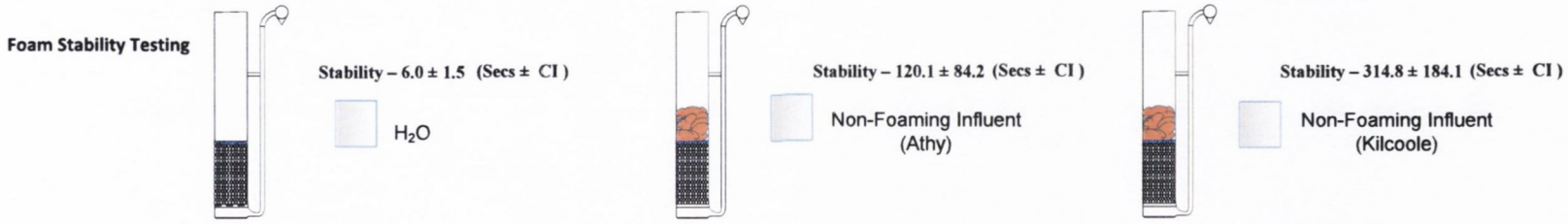
4.3.2. Results obtained through experimentation on individual constituents of activated sludge

A significant increase in both foam potential and foam stability was observed when influent taken from two non-foaming plants (Athy and Kilcoole WWTPs) was compared to water alone (Figure 4.6). A similar pattern of results was noted when foaming influent samples were tested against water alone, however, surprisingly in all cases measured values for foam potential and stability were noted to be higher in the influent taken from sites with no history of experiencing foaming. This potentially suggests that the nature of wastewater influent does not have overriding influence over the final foaming condition within each aeration basin. The addition of solids (obtained from both non-foaming and foaming sludges) made no noticeable difference to the stability of each foam (Figure 4.7). These results therefore do not support the froth flotation theory analogy in relation to activated sludge foaming (Chapter 1). In similar experiments conducted by Hug (2006), no confirmation could be made as to the role that mixed suspended solids play in activated foaming. It was noted in the testing conducted as part of this work that the addition of solids made a significant difference to measured foam potential. Within the matrix of the suspended solids lie bound associated hydrophobic filaments which assist with the buoyancy of the sludge particles, rendering them hydrophobic so they can readily attach themselves to each bubble and be floated to the surface (Chapter 1). It is proposed that the hydrophobic filaments may actually have a role in initial foam production rather than attributing to the overall stability of each foam. Tests were conducted on the supernatant obtained from each mixed liquor sample (Figure 4.8). No significant change was observed in foam stability or foam potential. A significant difference could be inferred as being the result of the supernatant having surface active properties but such was not the case. Mycolata and other foaming filaments have been observed to produce a bio-surfactant (Chapter 1) which may have contributed to the stability of foams produced within each supernatant sample.



Significance: Difference between Non-Foaming Influent (Athy) and Water $p<0.01$
Difference between Non-Foaming Influent (Kilcoole) and Water $p<0.01$

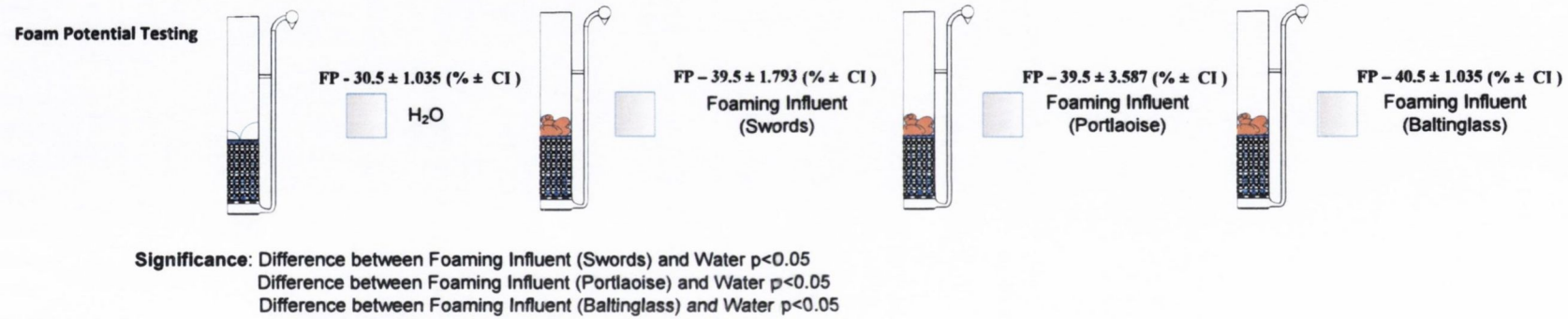
Statistical Inferences: Foam Potential measured in influent to non-foaming works is statistically different to that of water alone



Significance: Difference between Non-Foaming Influent (Athy) and Water $p>0.05$
Difference between Non-Foaming Influent (Kilcoole) and Water $p>0.05$

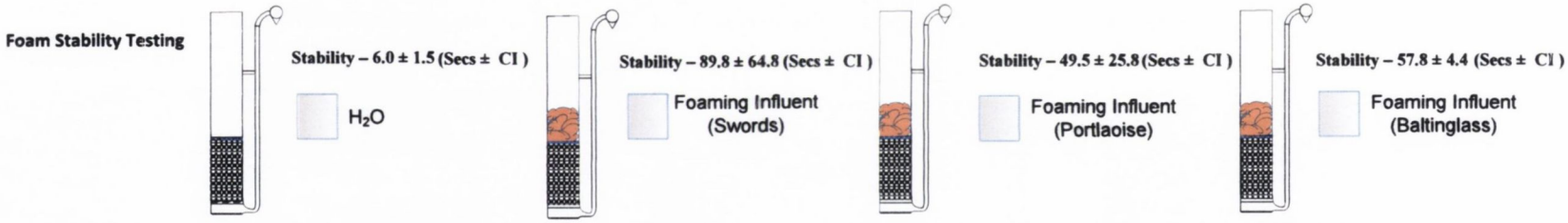
Statistical Inferences: The stability of influent samples was found to statistically not differ to that of water. On comparison, however, the stability of influent samples is notably much greater than water alone which produces only unstable bubbles that readily collapse on the cessation of air.

Figure 4.6 – Comparison of Foam Potential (FP) and Stability for Non-Foaming Influent to that of water alone



Significance: Difference between Foaming Influent (Swords) and Water $p<0.05$
Difference between Foaming Influent (Portlaoise) and Water $p<0.05$
Difference between Foaming Influent (Baltinglass) and Water $p<0.05$

Statistical Inferences: Foam Potential measured in influent to Foaming plants is statistically different to that of water alone



Significance: Difference between Non-Foaming Influent (Swords) and Water $p>0.05$
Difference between Non-Foaming Influent (Porlaoise) and Water $p>0.05$
Difference between Non-Foaming Influent (Baltinglass) and Water $p<0.01$

Statistical Inferences: Whilst the stability of foaming influent samples was greater than water, in only one case (Baltinglass) was this difference proved to be significantly different.

Figure 4.7 – Comparison of Foam Potential (FP) and Stability for Foaming Influent to that of water alone

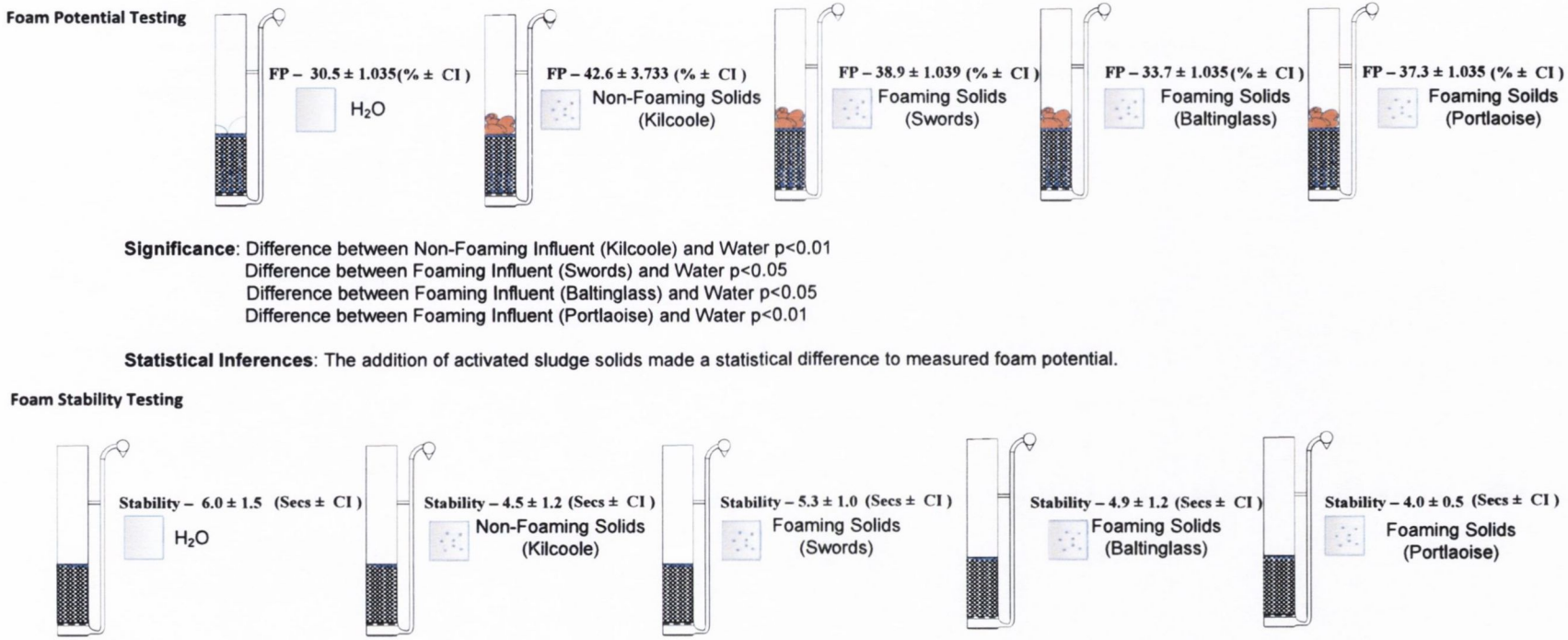


Figure 4.8 – Comparison of Foam Potential (FP) and Stability measured for water and water with added activated sludge solids taken from non-foaming sites and foaming sites

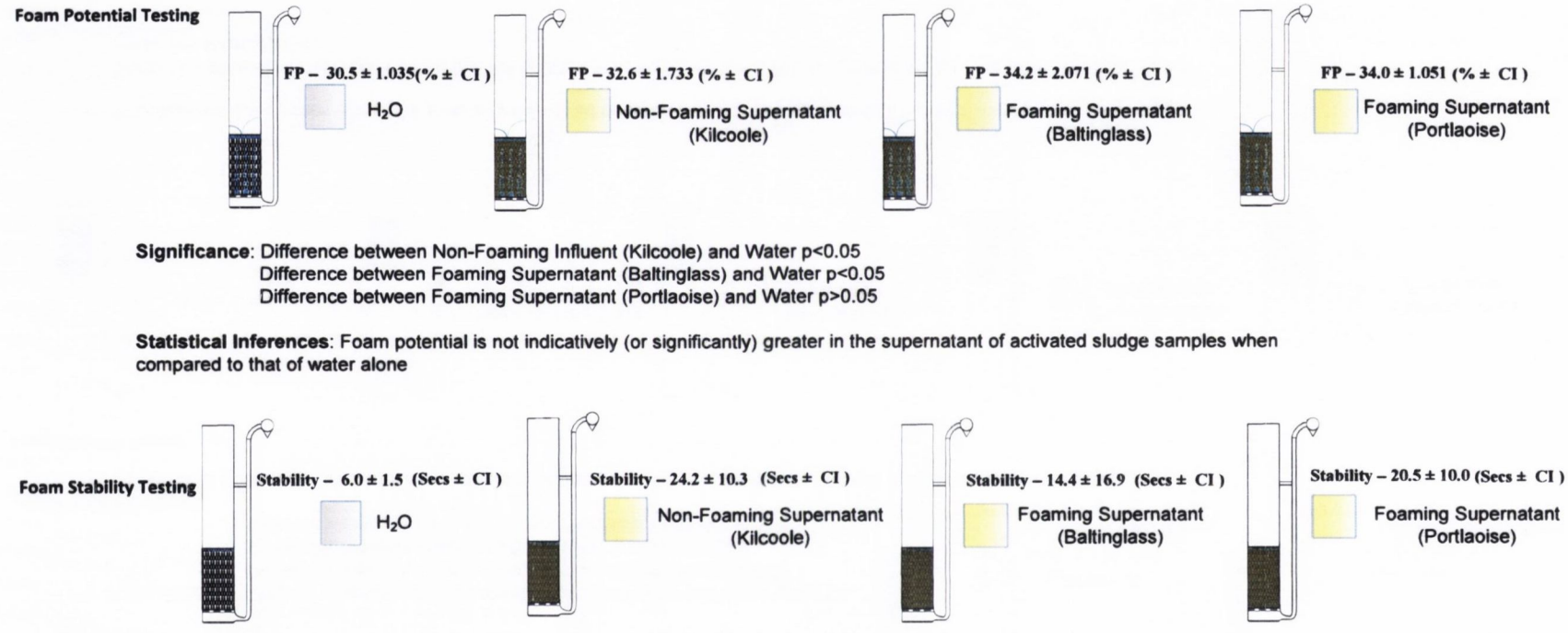


Figure 4.9 – Comparison of Foam Potential (FP) and Stability measured for water alone and supernatant from activated sludge samples

4.3.3. Observations from full- scale plants – Survey results

The survey responses detailed a total of 35 sites which had either suffered from activated sludge foaming in the past or were currently experiencing foaming at the time of questioning responded to the study. Of these sites, nine were based in main-land UK whilst the remaining 26 were located within the Irish Republic. The size of plants surveyed varied considerably with the largest plant, located in the UK (Crossness WWTP) serving a population equivalence of over 2million and the smallest Irish site serving a PE of just 950. Within this random survey, the response showed that the greatest proportion of sites suffering foaming were configured to an extended-aeration regime (46%), followed by plug-flow (26%), tapered aeration (11%), and other configurations including SBR (17%). Extended-aeration plants operate under plants operate long sludge ages under low organic loading and these lead to favourable conditions for foaming (Kappeler and Gujer, 1994; Nielsen et al., 2002; Richard, 2003; Jenkins et al., 2004; Jolis et al., 2007). A survey of 6000 activated sludge plants in France showed that of the 1200 plants that reported a history of foaming, either chronically or periodically, 87% of these plants operated to an extended aeration treatment regime (Pujol et al., 1991). Subdividing the surveyed plants into different categories on the basis of applied food-to-biomass load (F/M) showed the greatest number of plants suffering foaming corresponded to those sites experiencing an organic loading between 0.10 – 0.15 kg BOD/ kg MLSS.d^{-1} (46.9%) followed by sites that were fed at an even lower organic loading rate <0.01 kg BOD/ kg MLSS.d^{-1} (21.9%) (Figure 4.10). Excessive growth of *G. amarae* and foaming is related to low F/M ratios (Pitt and Jenkins, 1990). The presence of *M. parvicella* is most noticeable in those plants operating low organic loading rates (Jenkins et al., 2004). The responses in the questionnaire revealed that the presence of fats, oils and grease (FOG) in the influent was not a prerequisite for foaming to occur. Despite this, 23 of the 35 sites that responded to the survey, confirmed they had no means of FOG removal, indicating that whilst each catchment may have no registered discharges of lipids, the domestic and municipal input and build-up of FOG may be a factor in activated sludge foaming. The combination of septic tank waste and imports from landfill sites may be a contributing factor to foaming, with over 25% of the sites in the survey accepting regular imports of combined septic tank and landfill waste. Septic conditions, for example, have previously been highlighted as a major cause of bulking and the promotion of associated filaments such as *Thiothrix* spp. (Ramothokang et al., 2003; Comas et al., 2008; Al-Mutairi, 2009). Early studies

investigating the treatability of landfill leachate by aerobic processes, led to serious foaming problems being encountered suggesting that plants treating this type of waste may be more susceptible to foaming (Boyle and Ham, 1974).

4.4. Conclusions

As in the work of Hug (2006), the exact role that each fraction of the sludge supernatant/solids and influent has towards stable foam production could not be defined. Clearly, however, each fraction possesses its own unique foam propensity and stability properties. Further work could involve additional testing, however, since the current foam potential tests that are normally applied to sludge are subject to their own limitations (Chapter 2). It is suggested that other test procedures are adopted when investigating particularly foam stability. These could include, for example, changes in conductivity, sludge rheology and viscosity and/or simpler tests such as hydrophobicity.

Whilst extensive bench-testing was carried out for this part of the work, the results obtained were subject to much variability due to the fact that the filamentous species *M. parvicella* did not proliferate under any condition. It is suggested that bench scale tests are carried out using a higher inoculum of this species and this being best achieved using a mixed liquor concentration of > 4 g L^{-1}.

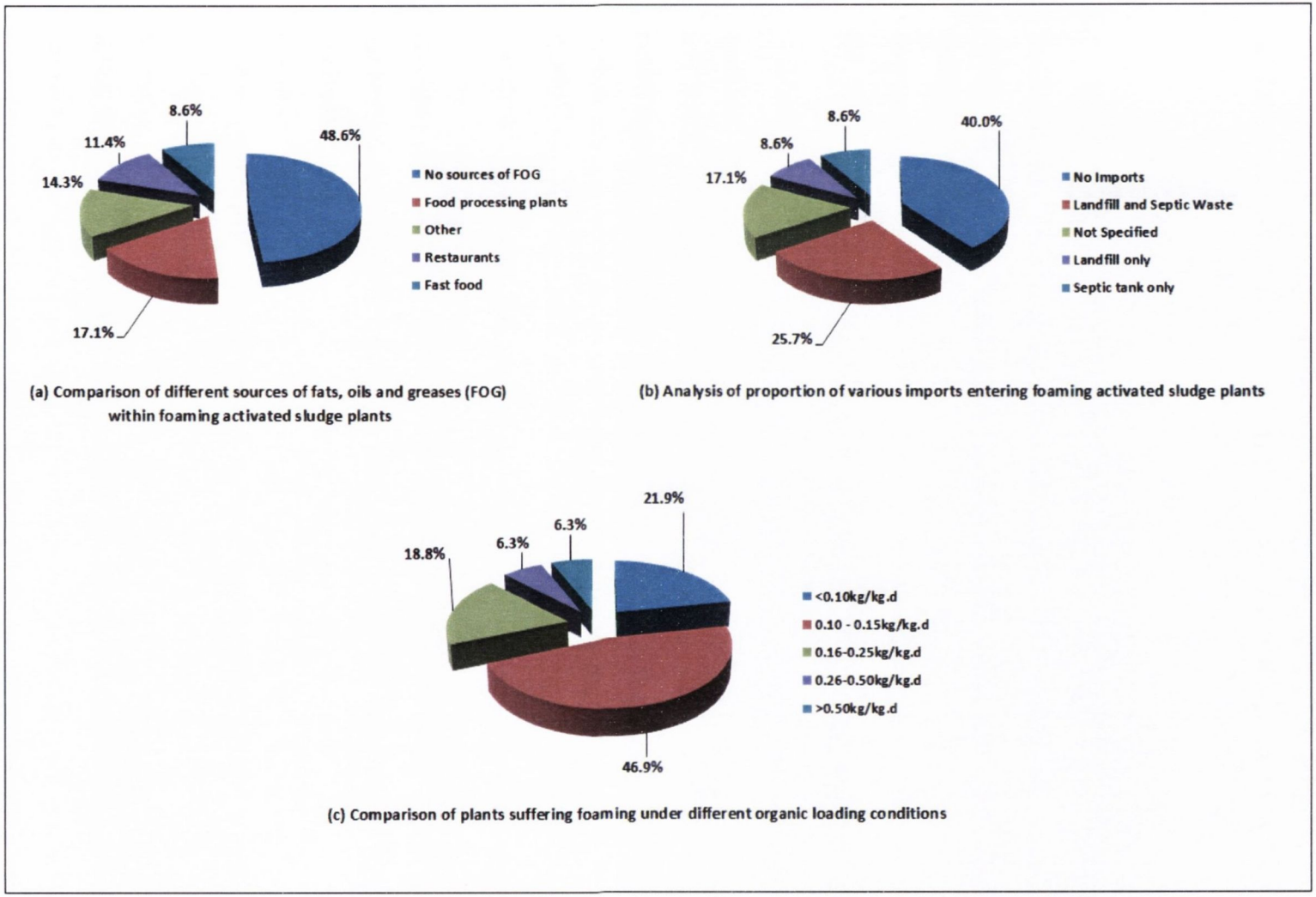


Figure 4.10 – Results pertaining to plant loading and influent characteristics obtained through survey data

Chapter 5

The effect of microbial ecology of foaming filamentous bacteria within Irish activated sludge plants

5.1. Introduction

Activated sludge is established as a widely used process for treating wastewater from both industrial and domestic sources (Seviour et al., 2002; Caravelli et al., 2004). This technology consists of two stages, a biochemical stage (aeration tank) in which organic matter is oxidised by an aerated consortium of microbes (Davenport et al., 1998) followed by a physical settlement stage (secondary clarifier) where solids are concentrated for subsequent recycling (Goodwin and Forster, 1985; Martins et al., 2004).

A prerequisite of this process is the unhindered settlement of flocs in the final treatment stage (Soddell and Seviour, 1990; Foot et al., 1992; Aonofriesei and Petrosanu, 2007). The biomass on average is composed of around 95% bacteria and 5% higher organisms (Horan et al., 2004). Filamentous bacteria provide the necessary backbone to produce stable, compact flocs (Sezgin et al., 1978; Motta et al., 2003; Aonofriesei and Petrosanu, 2007; Kragelund et al., 2010). Whilst under most circumstances, the activated sludge system is a reliable and stable process (Forster and Foot, 1997), performance is dependent on the equilibrium between floc forming bacteria, such as *Zoogloea* spp. and filamentous bacteria like *Microthrix parvicella* (Foot et al., 1992; Chua et al., 2000). Operational difficulties including reduced treatment performance are experienced when an ecological imbalance results in the proliferation of filamentous bacteria over that of the floc-formers (Strom and Jenkins., 1984; Foot and Forster, 1994; Baxter-Plant et al., 1998; Chua et al., 2000).

One of the most widespread and proliferate problems in this process is the formation of stable biologically-induced foams (Davenport et al., 2000; Torregrossa et al., 2005; de los Reyes, 2010; Petrovski et al, 2011). The exact mechanisms for formation of these foams are still poorly understood (Carr et al., 2005; Hug, 2006). Filamentous microorganisms have been identified as playing an important role in the stabilisation of such foams (Chua et al., 2000). Analysis of activated sludge biota has led to over 30 different filamentous bacterial types being characterised on the basis of morphology (Eikelboom, 2002; Jenkins

et al., 2004). Foaming has been attributed to the presence of Type 1863 filaments (Seviour et al., 1997; Rossetti et al., 1997; Lemmer et al., 2005; Comas et al., 2008) which form loose but viscous white-grey foams that collapse easily (Jenkins et al., 2004; Hug, 2006). More common and posing greater risk is foam caused by an excess of *Microthrix parvicella* spp. along with actinomycetes including Gordonia amarae-like organisms (GALO) such as *Rhodococcus* spp. and *Skermania Piniformis* spp. (Goodfellow et al., 1998; Soddell and Seviour, 1998; del los Reyes, 2002; Jenkins et al., 2004; Eales et al., 2005; Kragelund et al., 2007; Seviour et al., 2008; Petrovski et al., 2011). Unlike detergent based foams, these are highly viscous and stable and therefore difficult to remove (Soddell and Seviour, 1990).

The development of chemical foaming is normally attributed to the presence of an excess of surfactants and other surface-active compounds (Foot and Robinson, 2003; Heard et al., 2008). Likewise biological foaming has been correlated with hydrophobic compounds that are synthesised by certain microorganisms (Greenfield et al., 1984; Madoni et al., 2000). Foaming problems in activated sludge have been shown in some instances to be caused by the production of bio-surfactants from *G. amarae* when hydrophobic substrates such as hexadecane are present (Pagilla et al., (2002). Whilst the stabilisation of foam in activated sludge plants is most certainly aided by surface-active compounds excreted from the bacterial cells in the sludge (Khan et al., 1991), the hydrophobic nature of the cell wall of foaming also plays an important role (Davenport et al., 1998; de los Reyes et al., 1998; Carr et al., 2005; Frigon et al., 2006; Davenport et al., 2008). Resembling the process of selective flotation, which is used in the mining industry to purify ores (Hossain, 2004), air bubbles attach themselves to the hydrophobic cell wall and then float the flocs (along with the bacteria) to the water surface where the hydrophobic particles then take on a secondary role by impeding film drainage and subsequent bubble collapse (Jenkins, 2007). Actinomycetes such as *G. amarae* and *R. rhodochrous* contain mycolic acid within their cell wall which provides the necessary hydrophobicity required for foam stabilisation (Soddell and Seviour, 1990; Bell et al., 1998; Davenport et al., 2000, Nam et al., 2003; Eales et al., 2005; Davenport et al., 2008). In contrast, *M. parvicella* is able to utilise certain lipids and then accumulate them in the cell leading to increased hydrophobicity (Khan et al., 1991). This has led to it being referred to as 'lipid-accumulating organism' (LAO) (Nielsen et al., 2009).

The foremost approach to understanding foaming control has been the identification through microscopy, of the bacteria implemented with the problem (Wanner and Grau, 1989, Soddell and Seviour, 1990). Due to the variety of different substrates in most wastewaters a diverse ecosystem develops (Mayhew and Stephenson, 1997). The mere fact that an organism may be selectively enriched in numbers within the foam does not mean that it is a foam-causing organism (Blackall et al., 1986). Foam biocenoses in activated sludge plants often leads to the identification of other noticeable organisms such as Eikelboom types 0041/0675, 0581, 0092, and 1851, as well as *Nostocoida limicola*-like organisms (Lemmer et al., 2005). For example, Type 0092 was found to be only incidentally trapped within foam, and therefore, was not assumed to be a true foaming organism (Blackall et al., 1989). On the other hand, *Nostocoida limicola* and Type 0041 cause foaming since both species produce bio-surfactants (Foster and Goddard, 1987).

Not all filamentous species are able to stabilise foams to the same extent. Mori et al. (1988) reported that foams enriched by *Gordonia* spp. had a half-life of 14-15 hours whilst sludges dominated by *Rhodococcus* spp. were stable for 44-55 hours. Foams attributed to the proliferation of *N. limicola* were found to be less stable than those originating from the dominance of *Gordonia* spp. (Foot et al., 1992). Organisms with Gram negative cell walls, such as filament type 0092 and types 1851, and those with the existence of attached growth, such as types 0041 and 0675, have been found to be much less effective in scum stabilisation (Lemmer et al., 2005).

Varying plant operating conditions have been found to lead to the presence of different activated sludge filaments (Williams and Unz, 1985). In order to prevent the causes of activated sludge foaming, it is essential to be able to recognise the factors which stimulate excessive proliferation of filamentous organisms predominating in foam. (Drzuwiki et al., 2008). Within high organic load plants often bacteria of type 1863 and non-filamentous actinomycetes are found (Lemmer et al., 2005) whilst, *M. parvicella*, *Gordonia* spp. and Type 0041 are indicator organisms of under-loaded conditions (Nowak and Brown, 1990). Low growth rates associated with *M. parvicella* range from 0.3 to 1.4 d^{-1} (Rossetti et al., 2005) and sludge ages greater than 10 d are required to prevent washout of this species (Richard, 2003). Likewise, if the mean cell retention time (MCRT) is long enough (5-10 d), a stable population of actinomycetes can build up (Soddell and Seviour, 1990). A common approach to control filamentous bacteria is to operate plants under reduced

MCRT conditions (Pitt and Jenkins, 1990) but this is not a suitable approach for those plants with the requirement to nitrify as they have to be operated at high MCRT values (Kim and Pagilla, 1999).

Multiple surveys have been conducted to identify and enumerate filaments within activated sludge plants suffering from bulking and foaming (de los Reyes et al., 2010). The results show contrasting findings between different countries (Table 5.1). There is currently a distinct absence of published data regarding the speciation of different filamentous bacteria within Irish-based activated sludge plants. Whilst there are several reports on the common species of filamentous bacteria associated with foaming, such as *M. parvicela* and *G. amarae* there is still a need for additional information regarding the trend in other indicative species of bacteria which can be associated with foaming plants (i.e. less common filaments). Recently the concept of a threshold in numbers of filaments required for stable activated sludge production (Davenport et al. 2002) has been brought into question (Petrovski et al., 2011).

Table 5.1: Comparison of ranking of dominant filaments within different European studies.

Country surveyed	Ranking			Reference
	1	2	3	
Republic of Ireland (1)	*M. parvicella*	GALOs	Type 0041	This Study
Republic of Ireland (2)	Type 0041	*Type 021N*	*M. parvicella*	Byron, (1987)
Italy	*M. parvicella*	Type 0041	*N. Limicola*	Madoni et al., (2000)
United Kingdom (1)	*M. parvicella*	*Type 021N*	*N. Limicola*	Horan et al., (2004)
United Kingdom (2)	*M. parvicella*	*N. Limicola*	GALOs	Foot, (1992)
France (1)	*Type 0092*	*M. parvicella*	*Thiothrix spp.*	Graveleau et al., (2005)
France (2)	*Type 0092*	*M. parvicella*	*Thiothrix spp.*	Pujol et al., (1991)
Denmark (1)	*M. parvicella*	Type 0041	*N. Limicola*	Eikelboom et al., (1998)
Denmark (2)	*M. parvicella*	Type 0041	*Type 021N*	Kristensen et al., (1994)
Czech Republic (1)	*M. parvicella*	*Type 0092*	GALOs	Krhutkova´ et al., (2005)
Czech Republic (2)	*M. parvicella*	GALOs	*N. Limicola*	Wanner et al., (1998)
Netherlands	*M. parvicella*	*Type 021N*	*H. hydrossis*	Eikelboom et al., (1998)
Germany	*Type 0092*	*M. parvicella*	Type 0041	Wagner, (1982)

To summarise, the critical conditions leading to the abundance of foaming filaments and the specific role which these bacteria play in foam formation is still subject to scrutiny and further discussion (Hug, 2006) (Figure 5.1). A general consensus has been reached in that the filamentous bacteria involved rely on the presence of specific substrates for growth

(Richard, 2003; Rossetti et al., 2005). Once present, the propagation and proliferation of filamentous bacteria is subject to the running conditions that each plant operate under, particularly dissolved oxygen (Hao et al., 1983; Richard, 2003; Jenkins et al., 2004; Liu and Liu, 2006), organic loading (Westlund et al., 1996; Chua et al., 2000; Eikelboom, 2002; Xie et al., 2007; Tsang et al., 2008; Al-Mutairi et al., 2009) and temperature (Pitt and Jenkins, 1990; Knoop and Kunst, 1998; Scruggs and Randall, 1998; Mara and Horan, 2003; Rossetti et al., 2005) have all been shown to play important roles. Considerations have also to be given to the physical configuration that each plant functions under, in particular foam trapping and recycling features that have both been observed to increase the severity of foam incidents (Soddell and Seviour, 1990; Blackall et al., 1991; Narayan et al., 2010).

The aim of this research is to provide a better understanding of factors leading to foaming in Irish-based activated sludge plants. The objectives of this study are:

(i) to determine the typical filamentous bacteria inhabiting different Irish activated sludge plants.

(ii) to investigate the relationship between foam severity and operational factors, kinetic and metabolic factors and the association between these and speciation and abundance of known foam forming filaments.

(iii) to establish if contrasting plant designs support different species of filamentous bacteria

(iv) to investigate the existence of a threshold in filaments required for stable foam production.

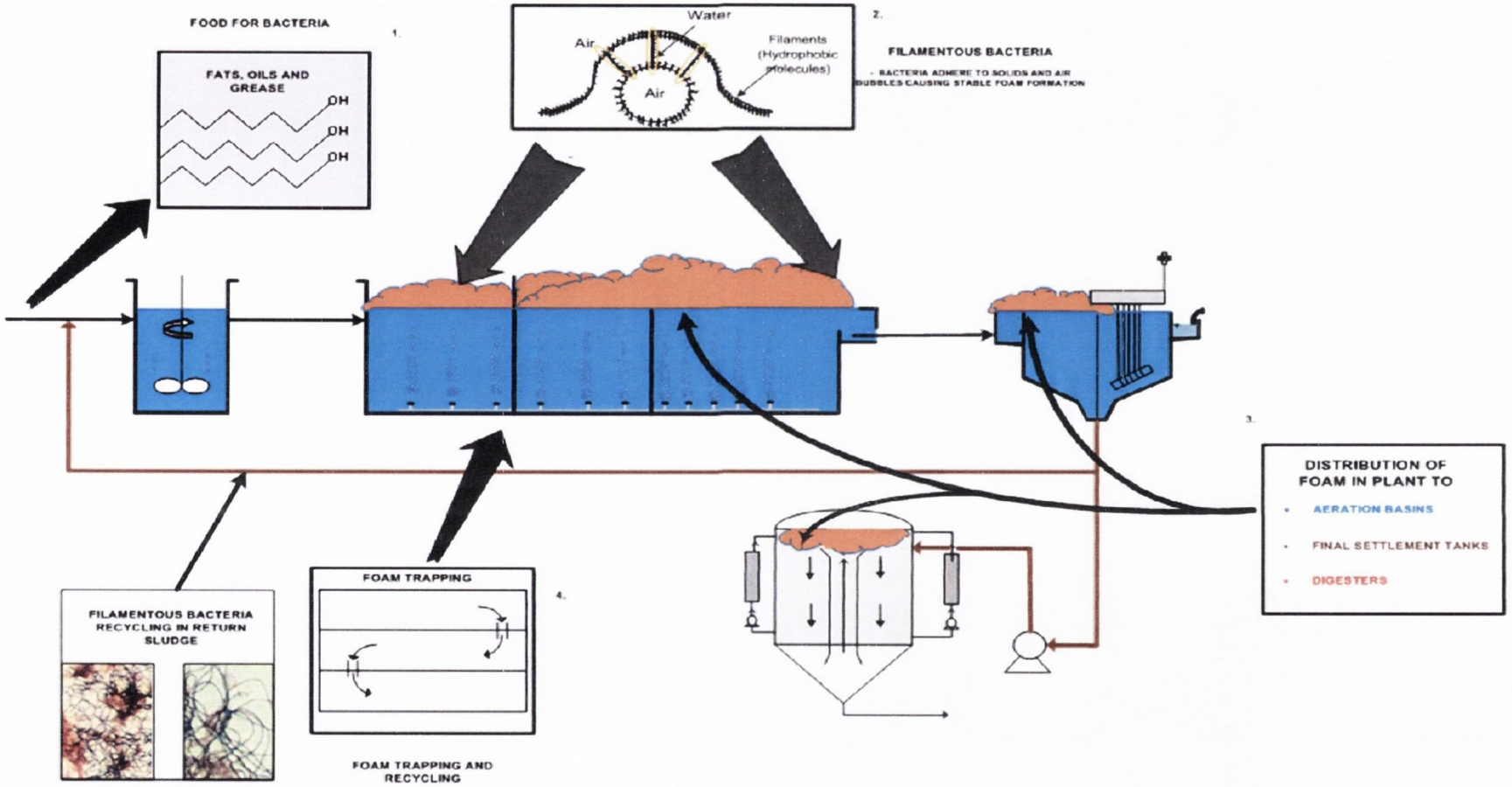


Figure 5.1 – Key processes associated with the establishment of bacterial-induced foaming within activated sludge plants.

5.2. Materials and Methods

5.2.1. Sample sites

Fourteen activated sludge plants were investigated in this study (Figure 5.2) including two separate treatment streams at Swords wastewater treatment plant (WWTP). They were located within the Greater Dublin area and its environs and operated under different regimes of extended aeration, biological nutrient removal, completely mixed and plug-flow configuration. The plants varied in both the degree of treatment attained and the populations they served. The smallest plant, Kilcoole WWTP serves a population equivalent of 2700, whilst the largest and newest plant, Ringsend WWTP provides treatment for a population equivalent of 1.9 million within central Dublin (Table 1). Some of the plants selected had no history of activated sludge foaming whilst others experienced sporadic or constant foaming. A total of 33 samples taken from 14 different activated sludge plants were microscopically analysed.

5.2.2. Mixed liquor samples

Grab samples (10 litres) of mixed liquor were obtained from the final stage of the aeration process. Once samples had been collected, they were kept at ambient temperature and transported to the laboratory where they were continuously aerated and analysed within 24 hours. Samples intended for microscopic examination were refrigerated at 4^{o}C and analysed on the same day (Dubber and Gray, 2009). When present, multiple grab samples of foam (representing 2-3 grams) were collected from the surface of the aeration contact zones using a 1 mm mesh sieve, which allowed for the drainage of surplus water during sampling.

SITE	P.E	CONFIGURATION	DESCRIPTION OF WASTE TREATED			FOAM OCCURENCE	NOMINAL OPERATING CONDITIONS			MICROSCOPIC EVALUATION - COMMON FILAMENTS FOUND
			Domestic Waste	Industrial Waste	Septic Tanks/ Land fill		MCRT (Days)	F/M ($kg\ kg^{-1}d^{-1}$)	DO ($mg\ O_2L^{-1}$)	
ATHY WWTP	17,000	Plug-Flow	✓			NON FOAMING SITE	13	0.15	1.0	*G. amarae*, *M. parvicella*, *H.hydrossis,* Type 0041, Type 021N, Type 0411, Type 1851
BALTINGLASS WWTP	3,000	Extended Aeration	✓		✓	FOAMING SITE (>4x per year)	14	0.10	1.0	*M. parvicella*, *H.hydrossis,* Type 0041, Type 021N,Type 1851
ENNISKERRY WWTP	6,000	Extended Aeration	✓		✓	FOAMING SITE (>4x per year)	16	0.15	0.9	*G. amarae*, *M. parvicella*, Type 0041,Type 021N, Type 0675, Thiothrix, *N.limicola*, Type 1863
GREYSTONES WWTP	30,000	Plug-Flow	✓	✓		FOAMING SITE (>4x per year)	15	0.40	2.5	*G. amarae*, *M. parvicella*, *H.hydrossis,* Type 0041,Type 021N, Type 1851, Type 1863, Thiothrix, *N.limicola*
KILCOOLE WWTP	3,000	Extended Aeration	✓			OCCASIONAL FOAMING (1x per year)	25	0.05	1.3	*G. amarae*, *M. parvicella*, Type 0041, Type 021N, *N. limicola*
LEIXLIP DOMESTIC WWTP	45,000	Completely Mixed	✓	✓		FOAMING SITE (>2x per year)	14	0.12	1.0	*G. amarae*, *M. parvicella*, Type 0041, Type 0411, *S.natans, N.limicola*
LEIXLIP INDUSTRIAL WWTP	35,000	Plug-Flow		✓		FOAMING SITE (>4x per year)	13	0.15	1.0	*G. amarae*, *M. parvicella*, Type 0041, Type 0675,Type 1851, *N.limicola*
MALAHIDE WWTP	20,000	Oxidation Ditch	✓			FOAMING SITE (>2x per year)	19	0.08	1.2	*G. amarae*, *M. parvicella*, Type 1851, *N.limicola,* Thiothrix
NEWCASTLE WWTP	2,700	Oxidation Ditch	✓		✓	FOAMING SITE (>2x per year)	30	0.07	0.8	*M. parvicella,* Type 0041, Type 021N, Type 0675, Type 1851
OSBERSTOWN WWTP	40,000	SBR	✓			NON FOAMING SITE	10	0.06	2.0	*M. parvicella*, Type 0041, Type 0092, *H.hydrossis,*
PORTLAOISE WWTP	35,000	Completely Mixed	✓	✓		FOAMING SITE (>2x per year)	18	0.08	1.8	*M. parvicella*, *H.hydrossis,* Type 0041,Type 0675, Type 021N, Type 1851,Type 1863
RINGSEND WWTP	1900,000	SBR	✓	✓		NON FOAMING SITE	19	0.15	5.5	*G. amarae*, *M. parvicella*, Type 0041, Type 0092, Type 0411, Type 0675,
SWORDS (OLD) + SWORDS (NEW) WWTP	60,000	Plug-Flow/ Oxidation Ditch	✓	✓		FOAMING SITE (>2x per year)	18	0.11	2.0	*G. amarae*, *M. parvicella,*, Type 0041, Type 0092, Type 0675,Type 1851, *N.limicola, H.hydrossis*

Figure 5.2 – Contrasting Irish activated sludge plants investigated in this study and filament diversity found at each site

5.2.3. Microscopic Analysis

Filamentous bacteria were identified applying the dichotomous identification key described by Jenkins et al. (2004). Absolute filament numbers as well as counts of *G. amarae* and *M. parvicella* were determined using the microscopic counting method originally developed by Pitt and Jenkins (1990). Samples of activated sludge mixed liquor and foam were diluted/concentrated to a pre-determined solids concentration of approximately 3 g L^{-1} prior to counting of filaments. Three or more slides which were prepared by placing 80 µl of sample on them and leaving to air dry followed by staining using the Gram (or Neisser) Hücker modification procedure. Three equi-distance scribe marks were then made on each slide and using microscopic eyepiece graticule with a line ruled on it, the number of intersections made between each strain of *G. amarae* (or) *M. parvicella* spp. and the length along each scribe mark was recorded. Results were then determined as the average of the sum of the number of intersections counted and expressed as number of intersections/g MLSS (Pitt and Jenkins, 1990).

The relative density of dominant foaming filaments was estimated by subjectively ranking them on a scale from 0 (none) to 6 (excessive) (Jenkins et al., 2004). These methods followed a Gram staining procedure with subsequent observation under oil immersion using phase contrast microscopy at 1000x magnification. Filaments were assessed both in terms of frequency (% of sites where each filament was found) and dominance based on the total number of sites where each filament was ranked in either first, second or third place.

5.2.4. Plant operational data and process performance information

Nominal running conditions of MCRT (Mean cell retention time) and F/M (Food to Mass ratio) for each plant were provided by on-site treatment personnel. Where this information was not available (particularly for the smaller sites) the following approaches were adopted:

Organic loading (F/M) to each activated sludge plant corresponding for the days in which each site visit was also calculated based on measured crude influent BOD values using the following equation:-

$$\text{F/M (Food to Mass ratio)} = \frac{Q_i \times [BOD_i]}{V_a \times [MLSS]} \quad (1)$$

Where,

Q_i = Daily influent flow (m^3/d)

V_a = Volume of aeration basin (m^3)

[MLSS] = Mixed Liquor Suspended Solids (mg/l)

$[BOD_i]$ = Influent BOD concentration (mg/l)

According to Giorgi (2009), MCRT is calculated using the following standard formula:-

$$\text{MCRT} = \frac{[MLSS] \times [\text{Aeration tank} + \text{secondary clarifier volume}]}{SS_{wasted} \times SS_{effluent}} \quad (2)$$

Where,

[MLSS] = Mixed Liquor Suspended Solids (mg/l)

SS_{wasted} = Suspended solids wasted ($kgSSd^{-1}$)

$SS_{effluent}$ = Suspended solids win effluent ($kgSSd^{-1}$)

In the absence of accurate waste activated sludge rates (WAS) being available, the MCRT corresponding to the days in which samples were taken, was determined using values of observed sludge yield (Y_{obs}), calculated from organic loading values (F/M) related as follows:-

$$Y_{obs} = 1.139 \times (F/M)^{\wedge 0.35} \quad (3)$$

Where Y_{obs} represents the ratio between the amount of TSS produced to the amount of BOD removed (Forster and Robinson, 2003).

The MCRT was then calculated using the following equation:-

$$\text{MCRT} = \frac{[MLSS] \times [\text{Aeration tank} + \text{secondary clarifier volume}]}{Y_{obs} \times [BOD_i - BOD_{eff}]} \quad (4)$$

Where, $[BOD_i]$ = BOD in influent ($kgBODd^{-1}$)

$[BOD_{eff}]$ = BOD in effluent ($kgBODd^{-1}$)

Foam age was calculated as an approximation based on the ratio of suspended solids measured within the foam compared to that to the underlying MLSS:-

A scoring system was used to categorise foam age as follows:-

Foam Age	<10days	10-50days	50-100days	100-150days	>150days
SCORE	**1**	**2**	**3**	**4**	**5**

5.2.5. Physico-chemical parameters

The mixed liquor suspended solids (MLSS) and suspended solids of foam samples were determined following Standard Methods (APHA 1998). Biochemical oxygen demand (BOD) was measured on influent and effluent samples using the OXI-TOP® method (WTW, Weilheim, Germany) whilst chemical oxygen demand (COD) was determining by the LANGE cuvette photometric chemical technique (HACH LANGE GmbH. Dusseldorf, Germany). Dissolved oxygen (DO) and pH were measured on-site at the time of sampling using a multi-meter (Hanna HI 9828) equipped with a galvanised dissolved oxygen (DO) sensor and a gel filled pH/ORP electrode. Water temperature within each basin was recorded using the same equipment.

5.2.6. Foam potential and stability

Foaming potential was determined using the glass column sintered disc apparatus previously described in Fryer et al. (2011). A portion of mixed liquor (150 cm^3) was aerated for a period of 1 min at a constant flow rate of 0.5 L min^{-1} during which time the foam potential was determined as a percentage of the maximum expansion of foam achieved in relation to the starting height. Foam stability was measured as the time required for all bubbles to collapse following the retardation of air after the 1 min period.

Foaming potential tests were also conducted by adding two tablets of Alka-Seltzer (containing 650mg sodium acetylsalicylate, 3832 mg heat-treated sodium bicarbonate, and 2000mg citric acid; Bayer Corp Elkhart, IN) to 250 ml of well-mixed samples in a 500 ml graduated measuring cylinder (Ho and Jenkins, 1991). Foaming potential in this case was recorded as the maximum expansion in volume of foam achieved in relation to the starting volume of 250ml.

All tests were conducted under ambient temperature conditions (20°C) using activated sludge samples at a pre-determined solids concentration of 3 g L^{-1}.

5.2.7 Foaming Scum Index (FSI)

A simple rating scale system was formulated to assess foam severity within each activated sludge plant and was given the name 'Foaming Scum Index' (FSI) (Fryer and Gray, 2012). Seven parameters had been utilised in the composition of the FSI. These were: - foam colour, bubble measurement, stability and foam potential, foaming filament index within each foam and foam suspended solids content with the measured values for each standardised by conversion to scores using on a proposed 1 to 5 unit scale. The absolute contribution of each score to the FSI was then weighted using multivariate analysis and the final index value determined using the product of these values (Fryer and Gray, 2012).

5.2.8 Specific sludge volume index (SSVI)

The SSVI was measured using a special settling column 0.5 m deep and 10cm in diameter (Dixon Glass Ltd, Beckenham, UK), with settlement impeded by a wire stirrer rotating at 1 rpm (Metcalf and Eddy, 2003). The SSVI was calculated by pouring the mixed liquor into the cylinder to the 50 cm level. The stirrer was then connected and the height of the sludge interface in the column was measured (h_0). After 30 minutes the height of the sludge interface was again measured (h_1).

The standard SSVI was obtained by doing a number of SSVI tests over a range of sludge concentrations from 2 to 6 g/l and interpolating the value at 3.5 g/l and the SSVI was calculated as follows:

$$SSVI = [(100\ h_1)/(C_o\ h_o)]\ \ mL\ g^{-1} \qquad (6)$$

Where, C_o is the initial concentration of suspended solids (%w/w)
(i.e. MLSS (g L^{-1})/1000) x 100)

5.2.9. Statistical analysis

Correlations involving different foam and mixed liquor properties (Filament index, SSVI, FSI foam thickness) were determined using a distribution-free statistic (Spearman's coefficient of rank correlation, r_s) (Barber and Veenstra, 1986). The paired Student t-test was used to assess the probabilities of different mean values.

Missing values in the basic dataset (less than 2% of the total numbers of observations) were estimated using the missing values function within SPSS (version 19.0). The procedure used involved first comparing the data-set that contained missing values to that of data within another parameter to which it exhibited a strong linear correlation with it. The next step used the Expectation Maximization (EM) algorithm (Copper et al., 2009) within SPSS which is specifically suited to estimating the missing at random (MAR) values within a dataset (Hutzschenreuter, 2009). The EM algorithm is a two-step iterative process – the first estimation (E) phase step involves the use of multiple regression analyses to approximate for missing values, followed by maximum likelihood procedures (M) which lead to the creation of variance-covariance matrices and regression weights (maximum likelihood estimates) which are then fed back into the first step (Weiner et al, 2003). The process iteratively repeated until the variance within the newly produced dataset is similar to that in the original data-set which had missing values. For robustness the comparison procedure originally applied to the two data-sets was then repeated to check that a similar magnitude of correlation coefficient was obtained.

Multivariate analysis was applied with Varimax rotation (Madoni et al., 1993) using SPSS (version 19.0) to assess the influence of different operational and physico-chemical parameters on activated sludge foaming along with their relationship to foaming filament abundance and interactions between different species of filaments bacteria. Principal component analysis (PCA) is a multivariate linear transformation technique which when used finds and interprets complex hidden relationship by reducing the number of variables into groups which explain most of the variance in the original dataset (Tomita et al., 2002;

Costa et al., 2009). Whilst the inputted process variables are often correlated, the produced principal components are uncorrelated and can be interpreted to explain important trends in a process (Lee et al., 2004).

5.3. Results and Discussion

5.3.1. Observations from plants surveyed

Foaming was recorded in 10 out of the 14 WWTPs analysed on 25 separate occasions whilst bulking, defined as those sites where recorded values of stirred specific volume index ($SSVI_{3.5}$) exceed 120 ml/g (Horan et al., 2004), occurred on seven separate occasions at five of the plants sampled. There was no observed trend between the onset of foaming and sites experiencing settlement problems due to bulking. A two sample t-test confirmed that foaming sites could not be distinguished from non-foaming sites on the basis of the MLSS concentration at which they operated ($p>0.05$).

Diversity of filamentous bacteria found in plants

In all the samples tested (both non-foaming and foaming plants), *Candidatus* '*Microthrix parvicella*' was the most common dominant organism in the plants examined during this study (Table 5.1). This species is found in most activated sludge treatment systems (Blackall et al., 1996) and its frequency is similar to studies carried out in UK activated sludge sites (Horan, 2003). *Microthrix parvicella* has also been found to be prevalent in other European plants (de los Reyes, 2011) for example, in Germany (Kunst and Reins, 1994), Denmark (Kristensen et al., 1994) and Italy (Madoni et al., 1999). Microscopic observations as part of an extensive survey of 6000 plants in France, showed *M. parvicella* to be the commonest filament found in over 45% of the samples analyzed (Pujol et al., 1991). The second most common dominant organisms are Nocardioforms, also known as *G. amarae*-like organisms - GALOs (Kragelund et al., 2007) and Type 0041 (Table 5.1). While *M. parvicella* has been found to dominate mixed liquor samples from European WWTPs (Rossetti et al., 2005) and WWTPs in colder regions of Australia and USA (Seviour et al., 1990), mycolata such as *G. amarae* have been extensively documented in activated sludge plants in the United States (Richard, 2003). Byron (1987) showed both *M. parvicella* and Type 0041 to dominate the biocenoses of activated sludge samples from

WWTPs in Ireland but ranked each species in a different order to the present study, probably because Byron (1987) reviewed a larger number of activated sludge plants over a greater demographic area than this study (Table 5.1).

The six most frequently occurring filaments were: *M. parvicella* (92% of plants), GALOs (82%); Type 0041 (76%), Type 1851 (59%), Type 0675 (52%), and Type 021N (46%) (Figure 5.3).

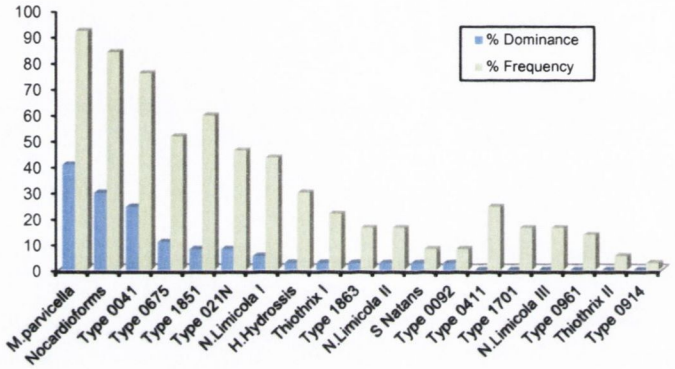


Figure 5.3 – Frequency and dominance (% of plants where the filament index of each filament was ranked 1st in terms of relative abundance, where total number of samples, n= 37) of filamentous bacteria in mixed liquor samples in Irish activated sludge plants

Filamentous bacteria implicated with foaming in Irish activated-sludge plants

Gram-positive bacteria, specifically the distinctively right-angled branched mycolata, along with 'loosely-coiled' *Microthrix* spp., were found to be in significantly high numbers within foam and mixed liquor samples taken from those plants actively experiencing activated sludge foaming (Figure 5.4). Foam samples were generally dominated by either species whilst in some cases, the underlying mixed liquor was dominated by both type of species. The excess in numbers of these filaments and specifically, their increased abundance in relation to other filamentous micro-organisms, led to the initial interpretation that they were the sole causative agents involved in foaming

within the activated sludge plants reviewed. However, previous studies comparing the frequency of filamentous bacteria occurrence within foams and mixed liquor samples has shown that other capricious filaments are present in both foam and samples of mixed liquor (e.g., Blackbeard et al., 1988; Lemmer et al., 2005) and this finding is also reflected in this current study (Figure 5.5).

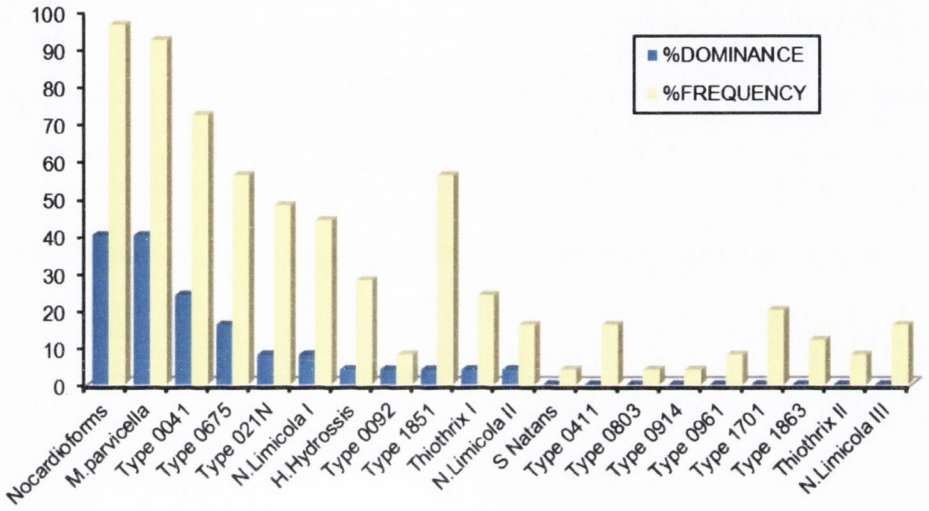


Figure 5.4 – Frequency and dominance of filamentous bacteria in Irish activated sludge plants during foaming events.

The high relative abundance of Type 0041 has previously been noted in mixed liquor samples taken from foaming plants (Marrengane et al., 2011). A survey of 33 biological nutrient plants (BNR) in South Africa showed this species to be nearly as common in foam samples as *M. parvicella* (Blackbeard et al., 1998). Type 0041 has the ability to withstand and adapt to a wide range of environmental conditions including variations in pH, temperature and dissolved oxygen (Jenkins et al., 2004). In this work, the presence of Type 0041 was often found with Type 0675 in mixed liquor samples and there was no significant difference between the mean abundance of the two species in all samples analysed ($p>0.05$). Both Type 0675 and Type 0041 proliferate in aeration tanks with low F/M ratios and both species exhibit similar physical and metabolic properties (Rampersad, 2002). A survey of bulking plants in Italy showed that along with *M. parvicella* and Type 0041, Type 021N was the most common abundant filament species (Madoni et al., 2000). In this work, a significant negative linear correlation was found between numbers of Type

021N and *M. parvicella* in all the sites reviewed ($p<0.05$) indicating that those conditions favourable for the growth of Type 021N are different to those required for *M. parvicella*.

Diversity of filamentous bacteria found within foam samples

Significant quantities of *M. parvicella* and *G. amarae* were found to be present within the foam samples analysed in this work. Both these bacteria types were in general, found to be in greater abundance in foam samples than corresponding mixed liquor samples, indicating that these species are primarily responsible for foam production within the activated sludge plants reviewed. In addition to these bacteria other filaments were found in scum but noticeably in reduced relative abundance e.g. Type 0041 (Figure 5.5). Types 0041, 0675, 1851 and 0092 and *N.limicola* spp. have also been reported to be found entrained within foam samples (Blackbeard et al., 1998; Wanner and Grau, 1989; Lemmer et al., 2000). These filaments may also contribute to foaming through similar mechanisms to that reported for *M. parvicella* and GALOS. For example, *N. limicola* spp. has also been reported to exhibit hydrophobic properties responsible for foaming (Horan, 2004).

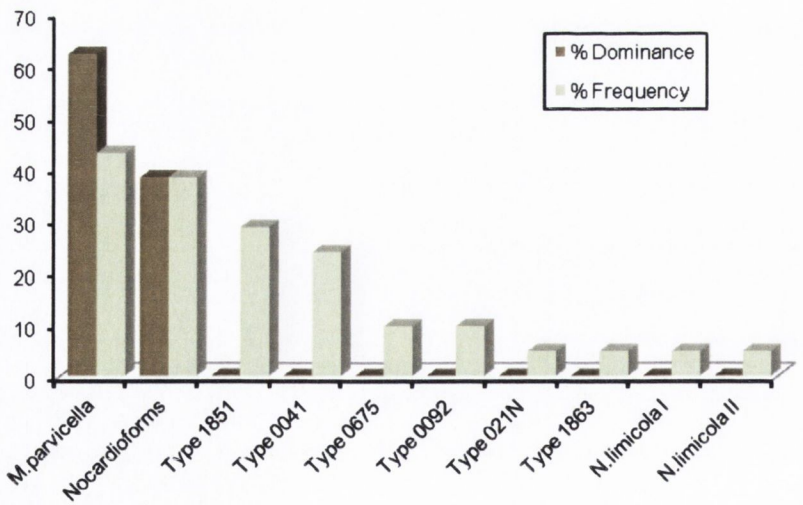


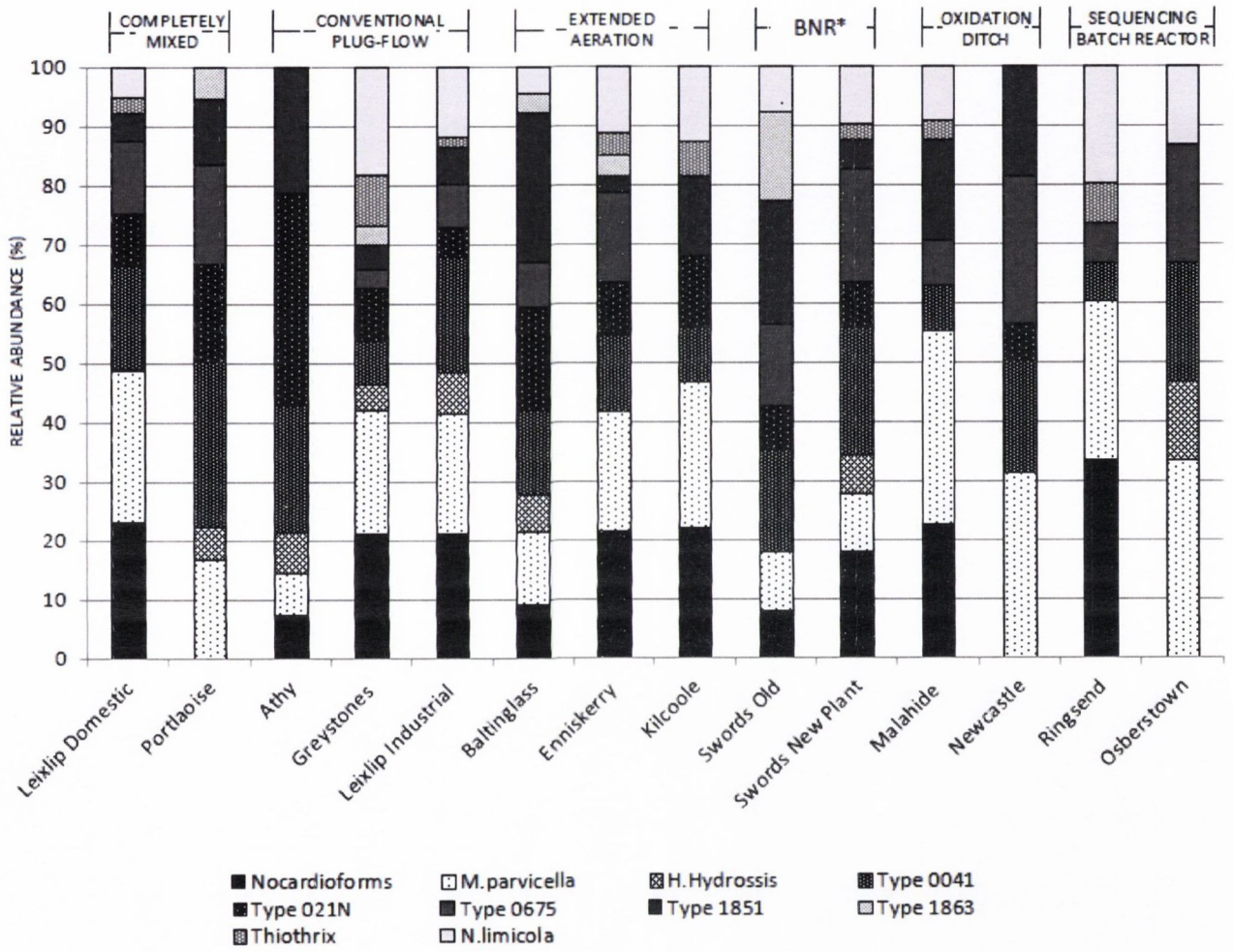
Figure 5.5 – Occurrence of filamentous bacteria in foam samples from Irish activated sludge plants.

5.3.2. Relationship between plant configuration, operational regime and filamentous species composition

The relative abundance of filamentous species found within the contrasting plant configurations in Irish WWTPs is illustrated in Figure 5.6. Filamentous species belonging to *Microthrix* spp. were found to be present in all the plants reviewed. *M. Parvicella* was found to be in greatest abundance in those plants operating under prolonged aeration conditions such as Osberstown WWTP and Kilcoole WWTP, as well as those plants configured to run as oxidation ditches (e.g Swords New WWTP) which are generally operated at a sludge age greater than 10 days in order to ensure nitrification. Previous studies of French WWTPs have shown foaming in such extended aeration plants to be linked to the proliferation of this species (Pujol et al., 1991) which is also reported as the main culprit leading to bulking within biological nutrient removal (BNR) plants (Blackbeard et al., 1998; Knoop and Kunst., 1998; Noutsopoulos et al., 2006). *M. parvicella* is a slow-growing species and can therefore usually be controlled at a sludge age between 8-10 days under moderate wastewater temperatures (Richard, 2003). Those plants operating under plug-flow regime have also been found to control the growth of *M. parvicella* (Mamais et al., 1998). In this study, the conventional plug-flow systems were found to be operated under lowest MCRT conditions. Despite these findings, no significant difference was found (at the 95% confidence limit) between mean values of *M. parvicella* filament index in extended aeration configured plants compared to plug-flow systems ($p>0.05$). In this study Type 021N was most abundant within plants operating a plug-flow regime.

Foam trapping seems to be a prevalent problem at some of the sites investigated. For example, extensive baffling in the pre-aeration zone at Portlaoise WWTP often leads to the build-up of retained air bubbles. In comparison, the operation of an undersized sub-surface treated mixed liquor withdrawal mechanism seems to be the primary cause of foam build up in the main aeration zone in Greystones WWTP. Using chemostats with both surface and sub-surface withdrawal mechanisms, Blackall et al., (1991) was able to demonstrate that operation of each configuration leads to *G. amarae* adopting different growth forms. Surface withdrawal leads to the removal of hydrophobic *G. amarae* filaments which have a tendency to float, whereas sub-surface withdrawal ensures that this species of filamentous bacteria grows in a dispersed form. This results in a greater propensity to cause activated

sludge foaming (Narayanan et al., 2010) because dispersed or free-floating nocardioforms expose an increased hydrophobic surface to the aqueous layer which in a clumped culture are held mostly inside flocs (Jenkins et al., 2004).



**Note - Swords (New) WWTP is configured to a carousel (oxidation ditch) configuration and Swords (Old) WWTP can be regarded as a conventional plug-flow plant however, both plants contain both anoxic and anaerobic zones in order to perform biological nutrient removal (N+P)

Figure 5.6 – Influence of plant configuration and operation regime on filament diversity.

5.3.3. Establishing key factors involved in activated sludge foaming

Metabolic factors and foaming filaments – Substrate utilisation

The level of readily biodegradable carbonaceous material within the influent wastewater was estimated by comparing the ratio of measured COD to BOD with higher COD/BOD values reflecting higher recalcitrant fraction of the organic load. A significant correlation was found between the COD/BOD ratio and the relative abundance of foaming filaments (both *M. parvicella* and mycolata) (Figure 5.7). Whilst no significant relationship could be found between numbers of *Gordonia* spp. and recorded values for COD/BOD ($p>0.05$), a significant linear correlation ($r_s= 0.47$, $p<0.05$) was found between this variable and the relative abundance of *M. parvicella* (Figure 5.7).

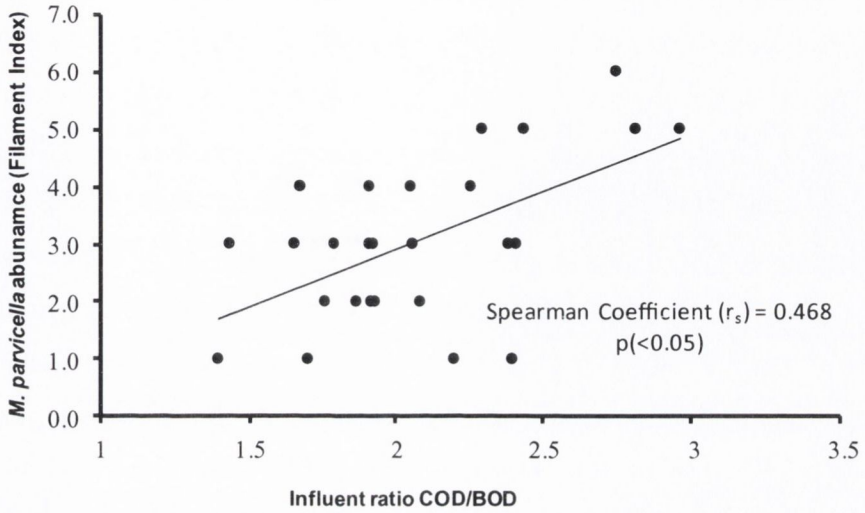


Figure 5.7 – Plot of *M. parvicella* abundance (measured as Filament Index) as a function of COD/BOD ratio

Metabolic factors and foaming filament abundance – Influence of temperature and seasonal variations

It has been suggested that the presence of foam is dependent on the temperature of the wastewater (Pitt and Jenkins, 1990; Cha et al., 1992). The influence of temperature on the growth of pure strains of *Gordonia* spp. was investigated by Soddell and Seviour (1995). The findings from this study showed that overall, different strains of *Gordonia* spp. grew best at higher temperatures (>15°C). Specifically, the growth of this species in bench-scale activated sludge experiments has been observed at 18°C, 20°C and 25°C, but not at 13°C (Pitt and Jenkins, 1990). However, within the mixed population of bacteria observed in the activated sludge plants surveyed, *G. amarae* dominated the sludge biocenosis in some mixed liquor samples taken from sites with wastewater temperatures recorded as low as 9 °C. When the abundance of this species was compared directly to temperature measurements recorded within each aeration basin, no significant correlation trend could be found (α=0.05). In contrast, a significant negative relationship was found between *M. pavicella* spp. density and wastewater temperature (r_s = -0.43, $p<0.05$). Optimum growth of this species has been found to occur at temperatures less than 13°C (Knoop and Kunst, 1998).

The different temperatures to which species of filamentous bacteria are adapted, leads to seasonal shifts being observed in species dominance (de los Reyes, 2010). The ability for *Candidatus* '*M. parvicella*' to survive and proliferate in temperatures as low as 7°C provides this species with a competitive advantage over other bacteria in activated sludge systems during colder months (de los Reyes. 2010). Likewise, it has been reported that in winter months this species replaces *G. amarae* which dominates activated sludge systems during the warmer summer periods (Wanner, 1998: Jenkins et al., 2004). The ranges of temperatures recorded in this study were between 9 and 22°C. The comparison of the influence of seasonal variation of the growth of *M. parvicella* at Greystones WWTP is shown in Figure 5.8. This filament is shown to decrease in numbers as the temperature increased along with subsequent disappearance of foam. In comparison, increase in the abundance of *G. amarae* was found to correspond with an increase in temperature experienced during the summer period at Lexlip Domestic WWTP (Figure 5.9).

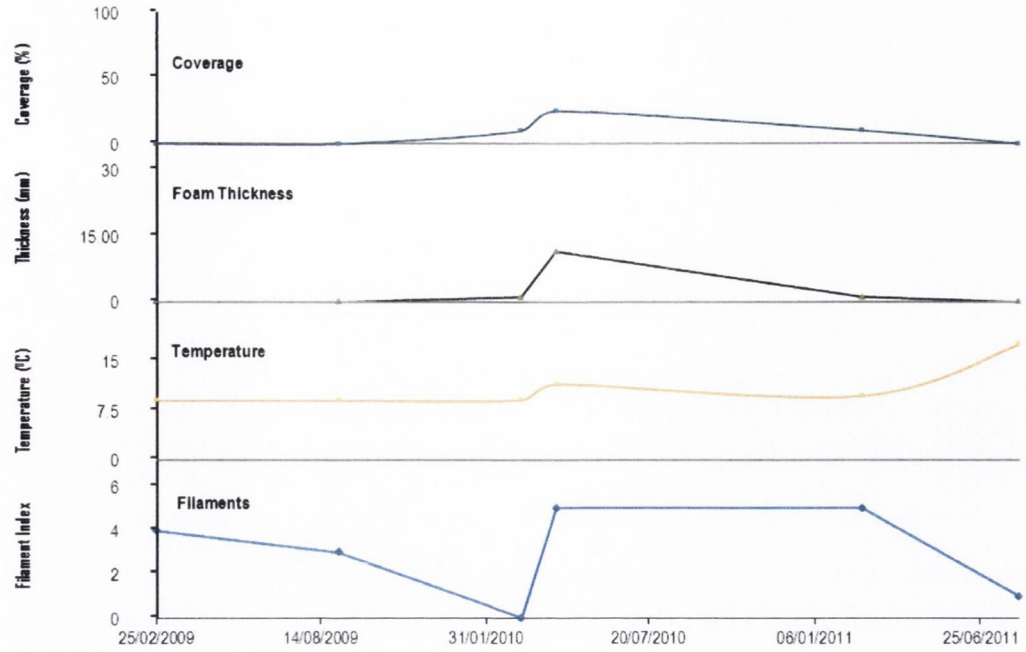


Figure 5.8 – Seasonal changes in foam thickness, foam coverage, and foaming filament abundance of M. parvicella observed in Greystones WWTP

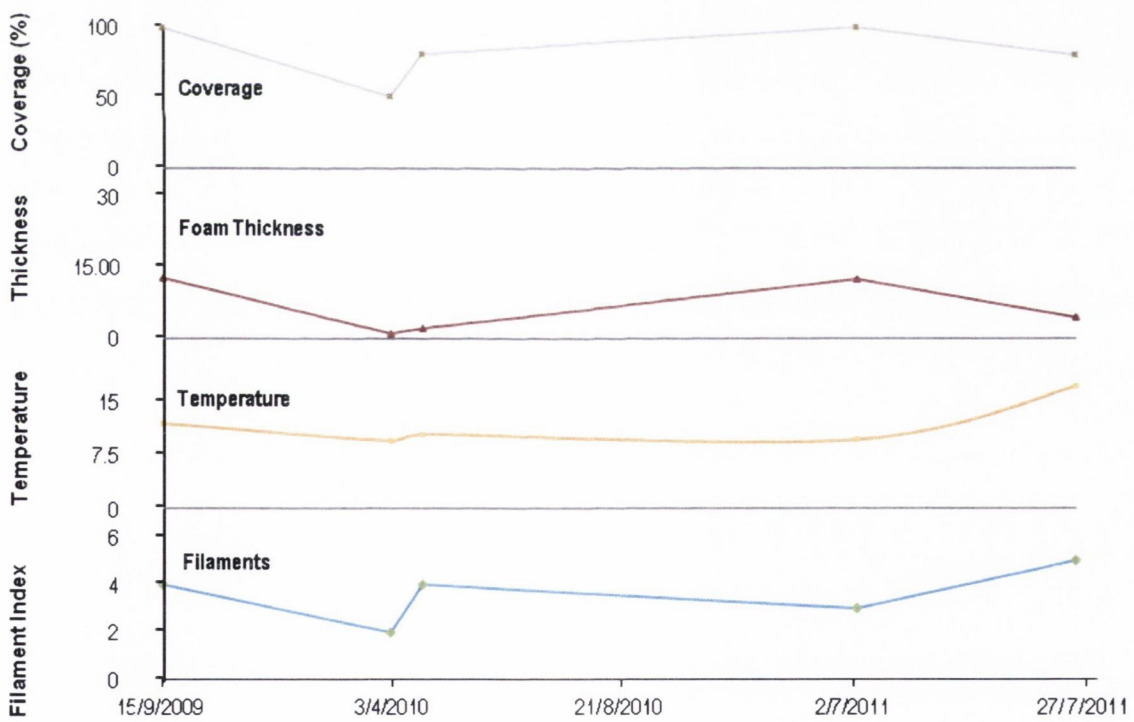


Figure 5.9 – Seasonal changes in foam thickness, foam coverage, and foaming filament abundance of *G. amarae* observed in Leixlip WWTP

Metabolic factors and foaming filaments – Dissolved oxygen levels

Low DO has been observed as being the main pre-requisite of filamentous over-growth leading to bulking within activated sludge systems (Gerardi, 2002). The filamentous microorganisms associated with activated sludge foaming are able to utilize substrate or at least able to survive, under anaerobic and/or anoxic conditions leading to foaming problems being reported in nutrient removal plants (Wanner and Grau, 1989). A wide range of DO values between 0.5 mg O_2/l and 3 mg O_2/l were measured in the activated sludge plants in this study. In contrast to the literature, no statistically significant ($p<0.05$) relationship was found between the abundance of *M. parvicella* or *G. amarae* and dissolved oxygen levels recorded within each aeration basin. Palm et al., (1980), explains that the DO concentration required to control filaments is not a constant figure, but a function of the loading rate of a system as organic loading influences the efficiency of oxygen uptake.

Metabolic factors and foaming filaments – pH

Within the WWPTs studied, the range of pH measured was between 6.42 and 7.85. The optimum growth of *Gordonia* spp. has been recorded at pH 6.5 (Pitt and Jenkins, 1990; Cha et al, 1992; Jenkins et al., 1993). Whilst this study found no significant relationship between the abundance of these species to variations in pH ($p>0.05$), there is a significant relationship between *M. parvicella* abundance and pH (Figure 5.10). In ex-situ pure-culture experiments it was demonstrated that *M. parvicella* proliferates in conditions of pH > 7.0 with optimum growth around pH 8.0 (Slijkhuis and Deinema, 1982).

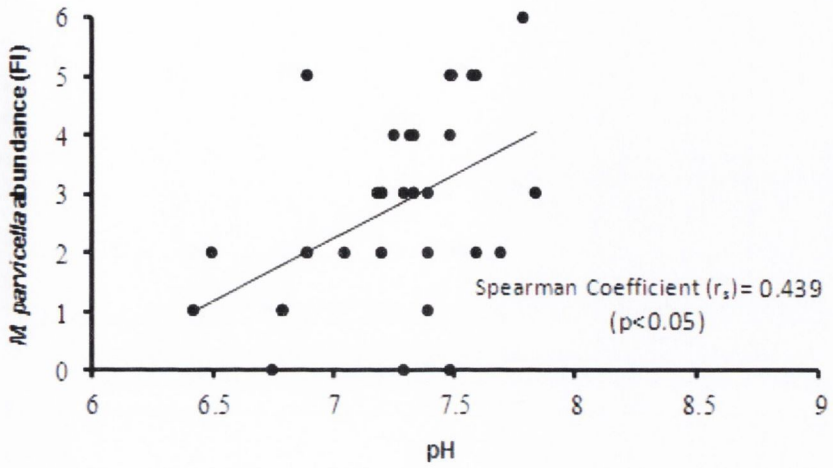


Figure 5.10 – Filament density of *M. parvicella*, expressed as Filament Index, as a function of mixed liquor sample pH.

Metabolic factors and foaming filaments – MCRT Sludge Retention Time ***and 'Foam Age'***

In the original case of recognised activated sludge foaming, the **'Milwaukee Mystery'** (Anon, 1969), the problem of scum (foam) formation was resolved by reducing the sludge age. Under conditions of reduced sludge retention times, slow-growing filaments associated with foaming, such as *M. parvicella* and *G. amarae*, **are subject to 'kinetic washout' therefore preventing their proliferation and** hence foam production (Jenkins et al., 2004; del los Reyes et al., 2010). In the field trials, MCRT data was determined for a range of different plants at different times of the year yet there was no significant correlation ($p>0.05$) between MCRT and the filament index or counts of *mycolata* and *M. parvicella*. The sludge age at which filaments are influenced is also a function of the wastewater temperature (Cha et al., 1992). In this study sites suffering from foaming were associated with almost the full range of MCRT identified (Figure 5.11) whereas non-foaming sites were associated only with lower levels of MCRT. A MCRT threshold was found (around 22 days), above which all plants suffered from foaming.

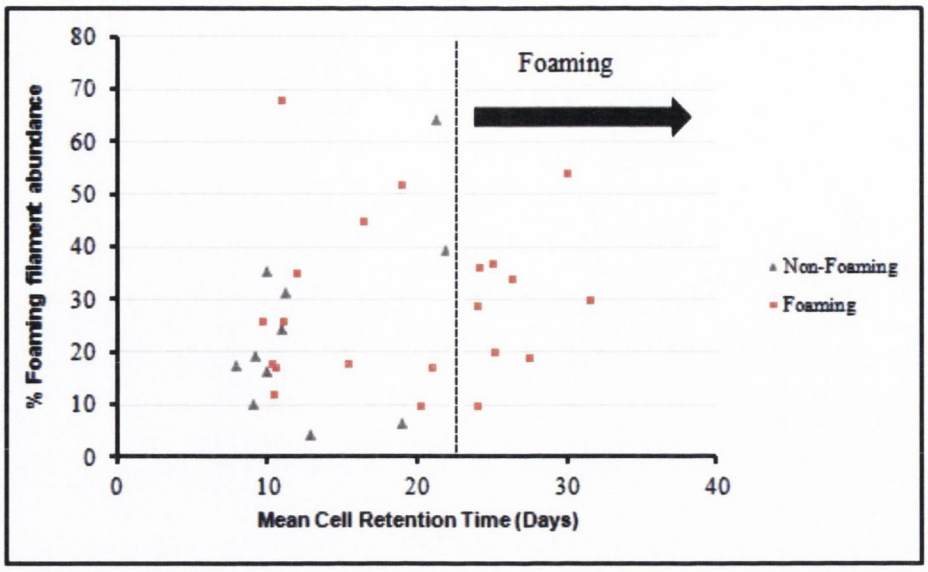


Figure 5.11 – Variation in the abundance of foam forming filaments as a function of average cell residence time (MCRT) determined within each aeration plant. Dotted line indicates threshold of MCRT and the presence / absence of foaming.

When foam thickness and FSI are used to rate the severity of foaming within plants, significant positive correlations between both of these variables and MCRT values are found (Figures 5.12 and 5.13).

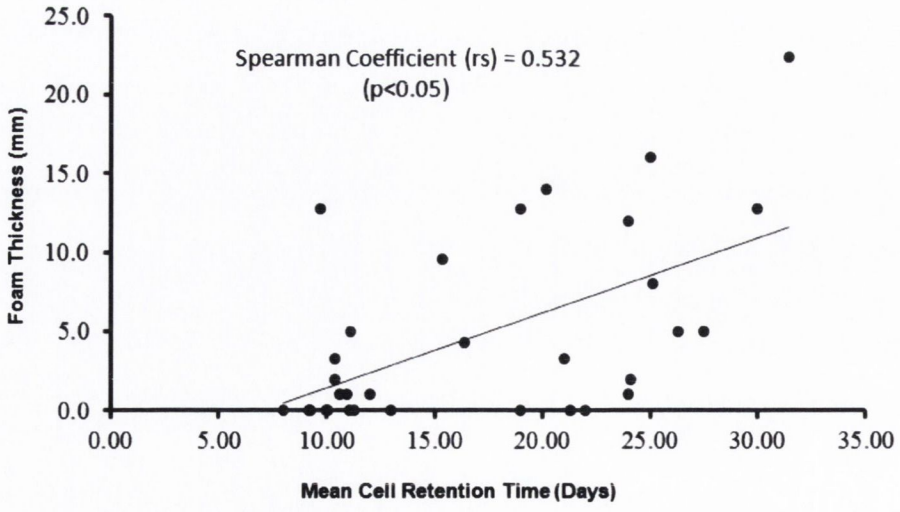


Figure 5.12 – Relationship between MCRT and foam thickness on surface of aeration tanks

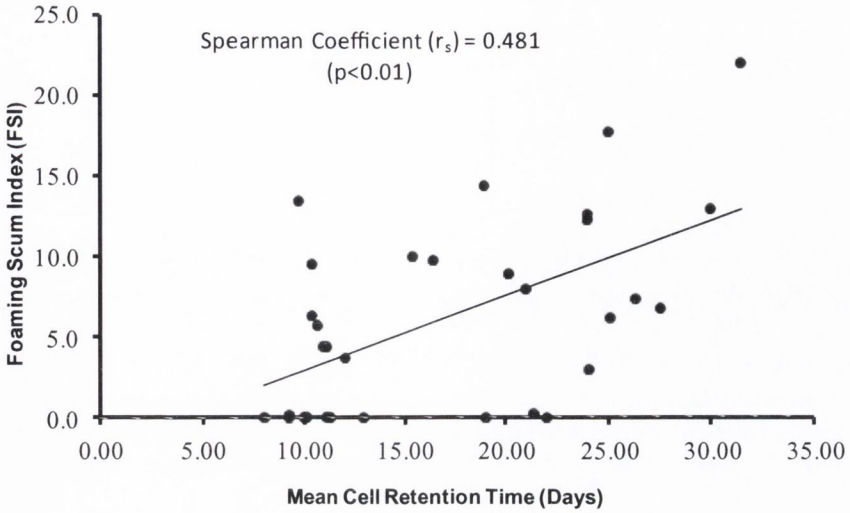


Figure 5.13 – Relationship between MCRT and foam severity measured in terms of FSI

Increased MCRT is also associated with an increased suspended solid content in each foam (Figure 5.14). The MCRT of the biomass within the aeration basin is a common parameter used by process operators to directly assess microbial growth and represents the most logical parameter on which to base the operation of an activated sludge plant (Jenkins and Garrison, 1968).

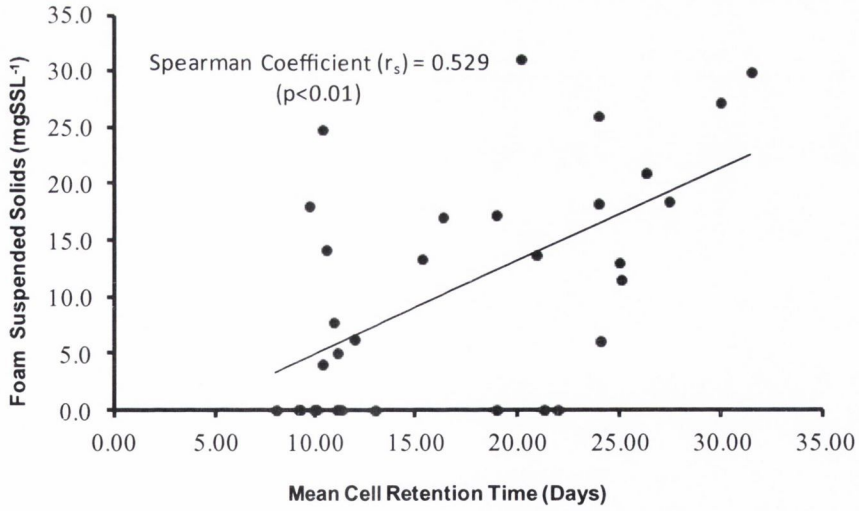


Figure 5.14 – Relationship between MCRT and suspended solids contents of foams

The transfer of significant quantities of the active biomass into the foam reduces the amount of biomass available for treatment purposes (Wanner, 1998). Therefore, the overlying foam has the influence of altering the actual MCRT occurring within activated

sludge plants (Gujer and Kappler, 1992). This leads to the potential concept of 'foam age' reflecting the average time that solid particles are present within the foam layer (Kragelund et al., 2007). In this study, an approximation of foam age, based on the ratio of suspended solids within the foam compared to that of MLSS, was significantly related to the severity of foaming events represented by values calculated for FSI ($p<0.05$)(Figure 5.15). The MCRT at which a plant is operated is often used to as a guide to crudely predict the likelihood of activated sludge foaming occurring (Jenkins et al., 2004). Increased sludge ages lead to a greater opportunity for slow-growing foaming filaments to proliferate and not be washed out of this treatment process (Rossetti et al., 2005; Noutsopolous et al., 2007). The findings of this study highlight the value of also considering the foam age which is relevant in assessing foaming severity once the foam has formed on the surface of aeration basins. The foam acts as a 'reservoir' for bacterial filaments which become more enriched over time (hence with greater 'foam ages'), and the foaming problem then becomes exacerbated by the re-seeding of the activated sludge with these micro-organisms from the overlying foam (Soddell and Seviour, 1990).

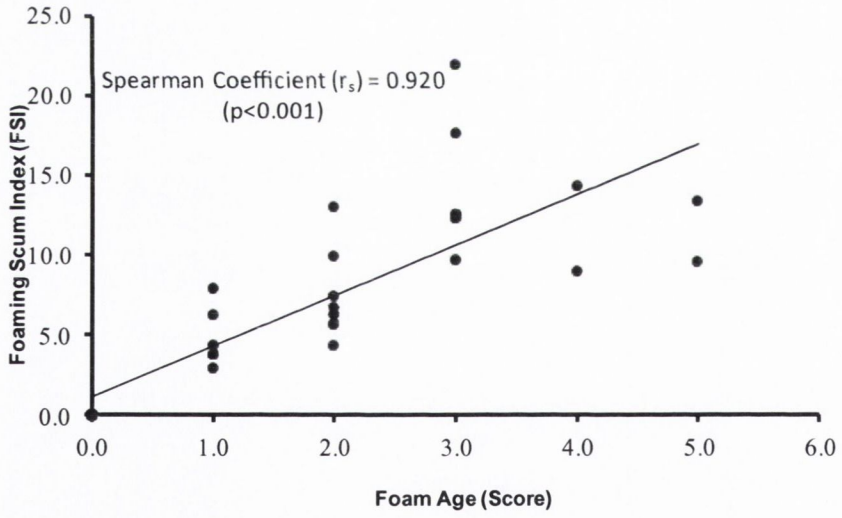


Figure 5.15 – Relationship between MCRT and foam age relating to time that solids reside within the foam structure

5.3.4. Establishing key components involved in activated sludge foaming – A multivariate analysis approach

Principal component analysis (PCA) was used to assess the influence of different operational and physico-chemical parameters on activated sludge foaming, and the

relationship with foaming filament abundance. Three principal components were extracted using varimax rotation (Table 5.2). In order for each factor to be relevant, the percentage explained by variance must be greater than 10% (Lee et al., 2004). The first group consists of components which indicate the severity of activated sludge foaming and accounts for 34.5% of overall variance explained within the model. The second principal component explains 15.9% of the variance in the original dataset and demonstrates the significance of foaming filament abundance (mycolata and *M. parvicella*) and foam stability measured on samples of mixed liquor. This indicates that although foam stability can be strongly linked to other parameters normally used to assess foam severity (such as foam coverage, foam thickness and foam potential) it demonstrates a stronger association with the abundance of foaming filamentous bacteria. A similar amount of variance (13.8%) is explained by the final principal component. This component contains common operational variables (e.g. MCRT and organic loading) which are often strongly linked to foam production through proliferation of foaming filaments. DO and f/m are also included in this group. Organic loading rates influence oxygen uptake and utilisation levels (Palm et al., 1980).

Table 5.2: Factors derived by varimax rotation (PCA) showing relationships between variables reflecting severity of activated sludge scum formation and foaming filamentous bacteria abundance along with key plant physico-chemical and operational variables.

	FACTOR		
	1	2	3
Foaming Scum Index (FSI)	**.966**	.104	-.095
Foam Coverage (%)	**.913**	.037	.164
Foam Suspended Solid Content	**.902**	-.003	-.034
Foam Thickness	**.875**	-.013	-.183
Filaments Foam (FI_{FOAM})	**.737**	.143	.319
Foam (Air) Potential Test	**.612**	.162	-.187
Foaming Filament Count (MLSS)	.107	**.907**	.026
Percentage Foaming Filaments (MLSS)	.008	**.858**	-.181
pH	.093	**.686**	.151
Foam Stability (Air Test)	.362	**.428**	-.388
Foam : Biomass	.137	-.015	**.837**
Mean Cell Residence Time (MCRT)	.279	-.030	**-.645**
Dissolved Oxygen (DO)	-.119	-.091	**.595**
SSVI	.116	.372	**.488**
VARIANCE EXPLAINED (%)	**34.8**	**15.9**	**13.8**
ACCUMULATED VARIANCE (%)	**34.8**	**50.7**	**64.5**

5.3.5. Foam in Activated Sludge Plants: Impact of Non-filamentous and Filamentous Bacteria

In the Irish WWTPs surveyed, distinctively right-angled branched filaments belonging to the species *Gordonia* exhibited the strongest positive correlation with those parameters used to assess severity of a foaming event (i.e. FSI, foam stability and thickness and coverage) (Table 5.3). Like *Gordonia, M. parvicella* also showed a significant ($p<0.05$) correlation with SSVI. Several authors have however linked increased numbers of *M. parvicella* to activated sludge bulking and hence higher recorded values of SSVI (Mamais et al., 1998; Snoop and Kunst, 1998; Andreasen and Nielsen, 2000; Jenkins et al., 2004; Rossetti et al., 2005; Azimi and Zamanzadeh, 2006; Noutsopoulos et al., 2012). Bacteria associated with activated sludge foaming comprise morphotypes other than *Candidatus M. parvicella* and *Gordonia* spp. (Lemmer et al., 2005). Biological foaming has been associated with some hydrophobic compounds that are produced by certain micro-

organisms within activated sludge (Greenfield et al., 1984; Madoni et al., 2000). One such filamentous species which potentially produces bio-surfactants leading to foaming is *N. limicola* (Stypka, 1988). Notably, there was a significant (positive) relationship between numbers of *N. limicola* spp. and FSI ($p<0.05$) recorded in this study. Both *N. limicola* and Type 0041 were blamed for causing the first serious foaming event in Czechoslovakia (Wanner and Grau, 1989) and two laboratory-scale reactors produced foams dominated by *Nostocoida limicola* and Eikelboom Type 0041 respectively (Goddard & Forster, 1987). However, *N. limicola* spp. has been described as producing much less stable foam layers than *G. amarae* or *M. parvicella* (Lemmer et al., 2000), and organisms with attached growth such as Type 0041 are also not effective at enhancing foam stabilisation (Lemmer et al., 2005).

A change from *G. amarae* and *M. parvicella* to ineffective scum stabilisers such as Type 0041 and N. limicola represents a potential control measure to the foaming problem with activated sludge plants (Lemmer et al., 2005). This could in theory be achieved by providing an environment whereby ineffective scum stabilising filaments are able to out-compete *G. amarae* and *M. parvicella* - such as under conditions of reduced oils and grease and general reduced organic loads (Lemmer et al., 2005).

Table 5.3: Spearman rank correlation coefficients for comparison between different operational parameters and the abundance of the most dominant filamentous species found in Irish activated sludge plants.

Species	FSI	Foam Thickness	Foam Stability	Foam Potential	Foam Coverage	SSVI
M. parvicella	0.095	0.151	0.115	0.192	0.172	0.398*
G. amarae	0.594**	0.438*	0.306	0.283	0.567**	0.444*
Type 021N	-0.120	-0.152	-0.152	-0.279	-0.167	-0.218
Type 0041	-0.149	-0.06	-0.438*	-0.205	-0.051	-0.317
Type 0675	0.076	0.192	0.055	0.270	0.134	-0.270
Type 1851	-0.125	0.053	-0.021	-0.081	-0.17	0.015
Thiothrix	0.275	0.198	0.153	0.183	0.299	0.109
N.limicola	0.386*	0.411*	-0.006	0.302	0.529**	0.368*

* The level of significance is $p<0.05$.
** The level of significance is $p<0.01$.

The correlation coefficients obtained from the relative abundance of each of the most common filaments found in the activated sludge plants reviewed in this survey are illustrated in Table 5.4. Most noteworthy, abundance of *G. amarae* and *M. parvicella* were found not strongly correlated with each other. Previous studies have shown growth of *G. amarae* to be optimum at pH 7.0 (Cha et al., 1992), whilst *M. parvicella* prefers more alkaline conditions (pH>7.0) (Slijkhuis, 1983). The filamentous species *M. parvicella* has been described as a 'micro-aerophile' with greatest proliferation observed in environments of low dissolved oxygen (Rossetti et al., (2005), whilst *G. amarae* growth has been found to increase linearly as a function of dissolved oxygen levels (Scruggs and Randall, 1998). Seasonal shifts between the dominance of *M. Parvicella* and *G.amarae* was observed in some of the plants reviewed in this survey (particularly Greystones and Leixlip Domestic WWTPs). During the whole cycle of a year *M. parvicella,* which was proliferating within plants in winter, was often found to be replaced by *Gordonia* spp. over the summer months. The growth of foam-causing filaments is temperature dependant (Rossetti et al., 2005) with optimum growth of M. parvicella between 12 and 15°C (Knoop and Kunst, 1998) and in contrast, *Gordonia* spp. preferer warmer wastewater temperatures (Pitt and Jenkins, 1990; Richard,2003).

A significant negative correlation was found between numbers of Type 021N and *M. parvicella* (r_s= -0.49, P<0.001). Type 021N prefers high organic loading rates (Tandoi et al., 2006) and high sulphide concentrations in the aeration tank can cause excessive growth of this species (Bitton, 2011), as it can oxidise sulphides to elemental sulphur (Gerardi 2002).

Table 5.4: Correlation coefficients (Spearman two-tailed) between the most common filamentous bacteria found in activated sludge plants examined in this study.

Species	*M. parvicella*	*Gordonia* spp.	Type 0041	Type 021N	Type 0675	Type 1851	*Thiothrix* spp.	*N. limicola* spp.
M. parvicella	1	-	-	++	-	-	-	+
Gordonia spp.	0.068	1	++	-	-	-	+	-
Type 0041	-0.730	-0.497*	1	-	++	-	-	-
Type 021N	-0.499**	-0.029	0.204	1	-	-	-	+
Type 0675	0.119	-0.322	0.566**	-0.117	1	-	-	-
Type 1851	-0.159	-0.273	0.096	-0.090	-0.075	1	-	-
Thiothrix spp.	0.150	0.629**	-0.299	-0.058	-0.171	-0.135	1	-
N. limicola spp.	0.350*	0.130	-0.148	-0.386	-0.036	0.110	0.207	1

* The level of significance is p<0.05.
** The level of significance is p<0.01.

Using PCA to predict the Impact of Non-filamentous and Filamentous Bacteria on Activated Sludge foaming

Table 5.5 shows the association between the most commonly observed filaments in this study and parameters of FSI (i.e. foam potential, thickness, and stability as well as SSVI) obtained by PCA. These are used to reflect severity of sludge separation problems in the activated sludge process as the result of either bulking or foaming. PCA identified four main factors which represented 66.7% of the total variance. The first principal component (factor 1) accounted for 27.3% of variation in the data. This factor is associated with variables related to foam severity with particular high loadings for FSI and thickness illustrating the extent to which foam has already taken hold within aeration basins. Factor 2 (16.1% of variation) is most strongly associated with filaments assumed to be responsible for the foam production in the majority of foaming incidents recorded in this study.

Table 5.5: Factors derived by varimax rotation (PCA) and their relationships to variables reflecting sludge separation problems in activated sludge through bulking and foaming and foaming filamentous bacteria abundance.

	FACTOR			
	1	2	3	4
Foaming Scum Index (FSI)	**.894**	.166	.116	.059
Thickness	**.839**	.003	.138	.259
Foam Potential (Alka-Seltzer Test)	**.684**	.086	.296	-.147
Foam Stability	**.677**	.044	-.233	-.394
Type 0041	-0.52	**-.827**	-.070	.224
Type 0675	.304	**-.808**	.134	-.230
Gordonia spp.	.429	**.728**	.069	-.101
Thiothrix spp.	.286	**.474**	.063	-.040
Microthrix spp.	-.005	-.019	**.808**	-.179
Type 021	-.147	-.047	**-.771**	.119
N.limicola spp.	.307	.011	**.593**	.342
SSVI	-.044	.487	**.526**	.324
Type 1851	-.009	-.084	-.131	**.820**
VARIANCE EXPLAINED (%)	**27.3**	**16.1**	**13.6**	**9.7**
ACCUMULATED VARIANCE (%)	**27.3**	**43.4**	**57.0**	**66.7**

The third factor (13.6% of variation) is most strongly associated with filaments such as *M. parvicella* and *N. limicola* spp. which have previously been linked to foam production within other studies (Rossetti et al., 2005; Lemmer et al., 2005; del los Reyes et al., 2010). Out of the filaments in this group, the occurrence of *M. parvicella* and Type 021N has been associated with sites suffering from bulking (Jenkins et al., 2004). This factor additionally contained the highest loadings assigned to SSVI. This group therefore represents the strongest association with bulking sludge separation problems. Factor 4 is associated only with Type 1851 abundance. The separation of this filament from the other filament species demonstrates that Type 1851 plays a less significant role in activated sludge foaming.

5.3.6. Threshold concept for foaming filamentous micro-organisms

The concept of a threshold number in mycolata being required to initiate the onset of activated sludge foaming was first discussed by Mori et al., (1992). Studies using quantitative fluorescence in situ hybridization (FISH) have supported the principle that there are mycolata concentration thresholds, above which foaming is likely to occur (de los Reyes and Raskin, 2002; Davenport et al., 2008; Petrovski et al., 2011). Likewise, two foaming threshold values were proposed for *Gordonia* spp. by de los Reyes and Raskin (2002). The first was associated with the numbers of this filament species required to start the foaming process which corresponded to a sudden increase in measured foam potential. The second, known as the stability threshold, was based on the number of filaments measured when a sudden increase in foam stability was measured (de los Reyes and Raskin (2002). In this work, no absolute value could be assigned to a threshold in the number of filaments observed to cause an increase in each case. A district 'cut-off' in foam potential, however, was observed for the range of foam potential values recorded in foaming and non-foaming plants where foaming occurred in all plants which had measured foam potential values greater than 65% (Figure 5.15).

The presence of foam in aeration basins was also seen in all plants with measured stability >25 seconds (Figure 5.17). The ability to assign threshold values to foaming through foam potential and stability measurements is questionable when considering foaming in more than one plant, since activated sludge plant design and operational factors also have an influence on foaming (Davenport et al., 2008). It therefore can be concluded, whilst it seems viable to evaluate foaming in terms of a sudden increase in foam potential and stability, the use of a distinct threshold value for the abundance of foaming filaments required for stable foam formation, does not exist in practice when considering evaluating foaming in a number of different plants.

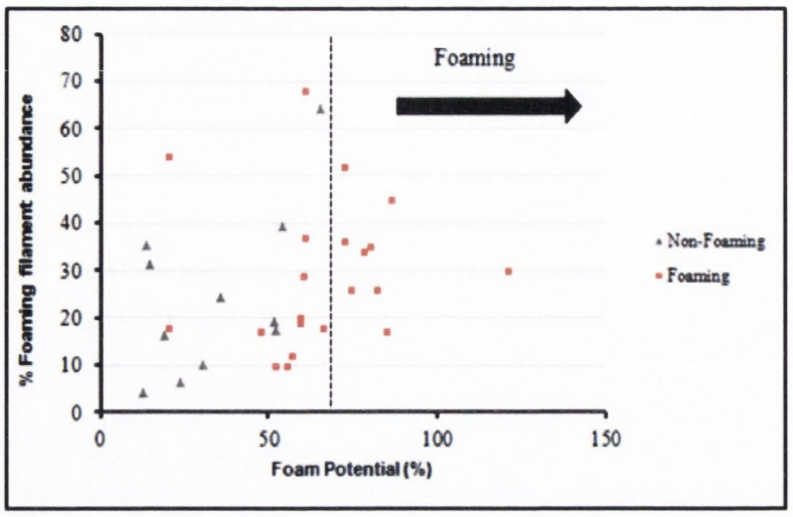


Figure 5.16 – Foaming filament abundance (%) as a function of foam potential (%). Dotted line represents threshold value in foam potential (65 %) above which all sites suffered from foaming.

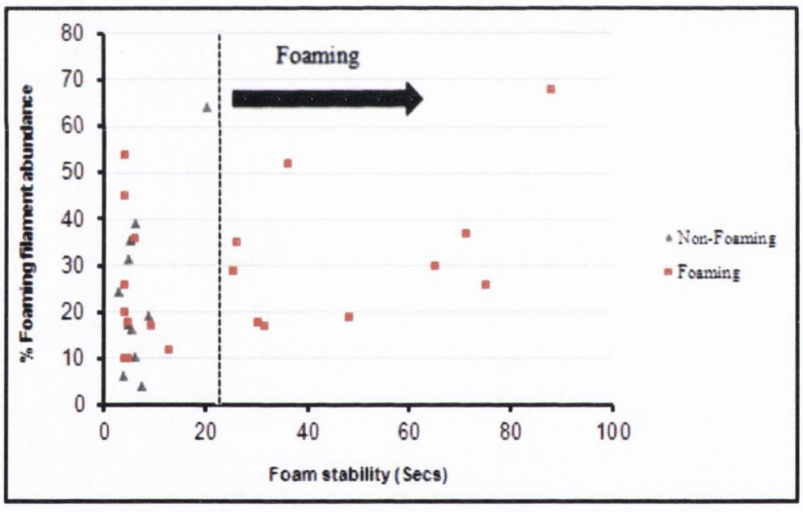


Figure 5.17 – Foaming filament abundance (%) as a function of foam stability (seconds). Dotted line represents threshold value in foam stability (25 seconds) above which all sites suffered from foaming.

5.4. Conclusions

- In all mixed liquor and foam samples taken from both non-foaming and foaming plants, *Candidatus 'Microthrix parvicella'* was the most dominant filamentous organism.

- In addition to M. parvicella and G. amarae-like organisms (GALOs), Eikelboom Type 0041 was found to also dominate biocenoses of activated sludge samples.

- Types 0041, 0675, 1851 and 0092 were also present in samples but never as the dominant filament species.

- Candidatus 'M. parvicella' were in all the WWTPs surveyed. This filamentous species was most abundant in those plants operating under prolonged aeration conditions such as Osberstown WWTP and Kilcoole WWTP.

- Whilst no significant relationship was found between numbers of Gordonia spp., and COD/BOD, a significant (positive) relationship was found between this variable and the relative abundance of M. parvicella indicating that this species flourishes best where there is less bio-degradable substrate within the influent wastewater.

- A greater proportion of foaming plants have high MCRTs.

- A threshold in MCRT of around 22 days was identified, above which all sites s suffer from activated sludge foaming.

- Foam age scores are significantly related to foam severity (measured in terms of FSI - $r_s = 0.92$, $p<0.001$).

- Principal component analysis (PCA) revealed a clear relationship between foaming filaments within mixed liquor and foam stability, whereas foaming filaments were grouped alongside foam potential and other parameters used to represent foam severity.

- The use of a threshold value for the abundance of foaming filaments associated with stable foam formation does not exist in practice across different WWTPs.

- A threshold in foam potential was observed; foaming occurred in all plants which had measured foam potential values greater than 65%.

- *Microthrix* spp. is not primarily responsible for foam production within the Irish plants surveyed; the abundance of G. amarae and other so-called 'non-famous' foaming filaments was associated with foam severity parameters of foam stability and coverage.

Chapter 6

Development of knowledge-based (KB) risk assessment model to predict risk and severity of activated sludge foaming

6.1. Introduction

Bulking and foaming, resulting as a consequence of imbalances between numbers of filamentous and floc-forming bacteria, are two of the biggest operational issues experienced within activated sludge systems (Kappeler and Gujer, 1994; Jenkins et al., 2004; Comas et al., 2008). Activated sludge processes, are complex systems and operational problems which have a biological origin are amongst the most difficult to resolve (Comas et al., 2003; Hug, 2006). Such complexity is a result of the presence of a dynamic multi-species microorganism population whose composition is changing continuously under the influence of factors such as influent flow rate, composition and plant operational parameters (Jeppsson, 1996).

Mathematical models (also referred to as 'mechanistic models') are increasingly gaining use in the understanding of intricate biological interactions occurring within activated sludge (Banadda et al., 2001; Tandoi, 2006). Mechanistic models provide a means of describing all chemical, biological and physical laws that govern complex systems (Banadda et al., 2001). Within activated sludge processes, Monod Kinetics are commonly applied to represent the adsorption, transport, and enzymatic consumption of substrate which takes place during cellular growth (Richard et al., 1985; Soddell and Sevious, 1990; Eckenfelder and Grau, 1992). Whilst the Monod model is basically empirical it can be individually fitted to a single mathematical expression (Chiu et al., 1972):-

$$(1) \qquad \mu = \mu_{max} \frac{C_s}{K_s + C_s}$$

Where,

μ = specific growth rate (l/h)

C_s = substrate concentration (g/l)

μ_{max} = maximum specific growth rate (l/h)

K_s = affinity constant for substrate (g/l)

An early mechanistic approach to modelling activated sludge bulking was carried out by Chudoba et al., (1973) based upon the assumption that the growth of both floc-forming and filamentous bacteria is subject to following Monod kinetics. It was hypothesized in this work that filamentous bacteria are slow-growing organisms, characterised as having lower associated values for μ_{max} and K_s when compared to floc-forming bacteria. Theoretically therefore, it is possible to control the growth of filamentous micro-organisms by out-competing them by floc-forming bacteria under conditions of high substrate concentrations (Cenens et al., 2000; Martins et al., 2004; Lou et al., 2008).

The most widely applied mechanistic representation of the activated sludge process belong to the ASM1 model and its subsequent counterparts, ASM2, ASM2d and ASM3, originally developed by the International Water Association (IWA) (Vanrolleghem et al., 2003; Liwarska-Bizukojc et al., 2011; Keskitalo and Leiviska, 2012). The substrates assimilated by autotrophic and heterotrophic bacteria considered within the ASM family of models are broken down into various different fractions (Figure 6.1) (Gernaey et al., 2004). The first ASM1 model was primarily developed to describe the removal of organic carbon compounds along with the simultaneous consumption of oxygen and nitrate as electron acceptors (Gernay et al., 2004). Since the publication of ASM1 in 1987, the knowledge of bacterial physiology has increased and the complexity of these models has developed significantly (Henze et al., 2000).

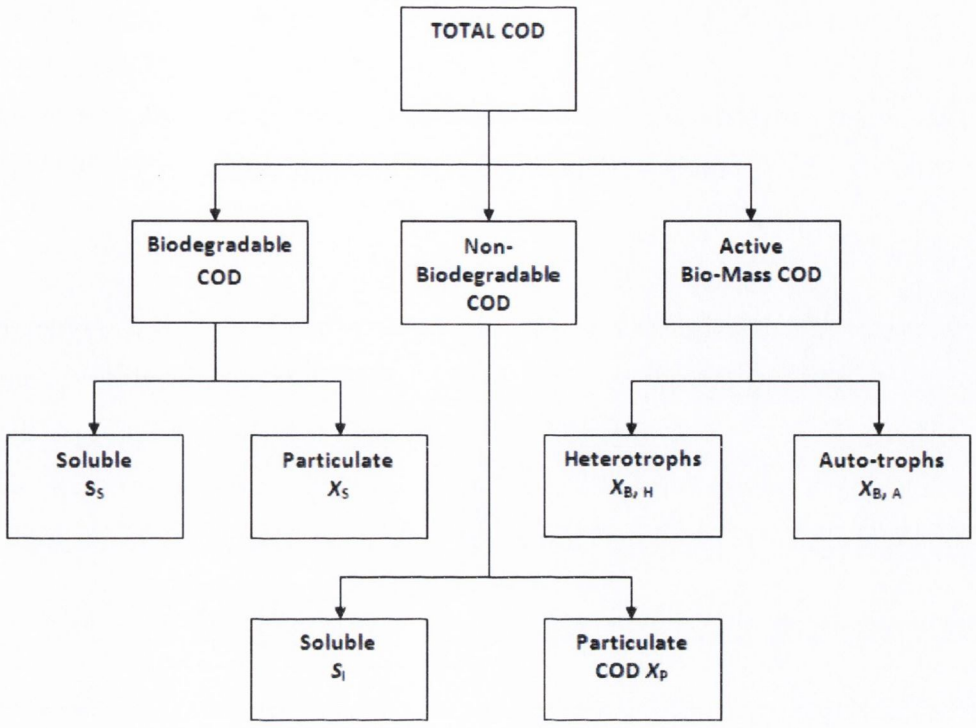


Figure 6.1 – COD fractionation in influent wastewater used to describe metabolic processes within the ASM group of models (adapted from Vanrolleghem et al., 2003)

Most mechanistic activated sludge models are calibrated using an ad-hoc and trial and error approach which leads to a decrease in their overall robustness (Keskitalo and Leiviska, 2012). Currently the kinetic properties of filamentous bacteria are not fully understood, which makes development of a mechanistic description of the growth of filamentous bacteria in a general model challenging (Flores-Alsina et al., 2009). The uncertainty about the exact causes of scum production has hindered the development of a thorough mechanistic model to assess biological foaming (Comas et al., 2009). Whilst mathematical modelling is useful when considering plants under steady state conditions, when investigating capricious events such as activated sludge bulking or activated sludge and digester foaming, it is necessary to include the heuristic knowledge of plant operators (Serra et al., 1997).

The complexity involved in the description of operational problems of microbiological origin can be tackled by Knowledge-Based Systems (KBS), as complementary aids to mechanistic models (Comas et al., 2003; Poch et al., 2004; Dalmau et al., 2010). Knowledge-based systems (KBS) are computational tools that enable the integration of

numerical data with heuristic knowledge to mimic the human decision-making processes in order to solve complex problems (Comas et al., 2009). These systems apply the knowledge of human experts, often acquired through years of experience, to diagnose the state of a process, and to propose solutions to new problems (Comas et al., 2003) (Figure 6.2).

A knowledge-based (KB) approach was developed by Comas et al., (2003) to monitor the de-flocculation problem in activated sludge. The methodology adopted used data from online, offline and heuristic sources which via a decision tree algorithm process and rule-based decision system were able to detect conditions leading to an increase in turbidity in the final effluent as the result of poor settlement through de-flocculation in final settlement stage.

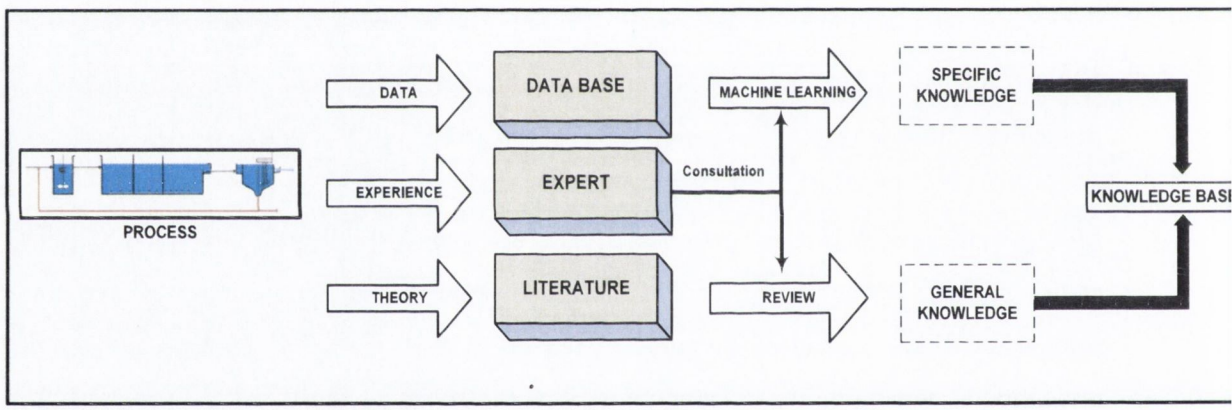


Figure 6.2 – Example of the knowledge base acquisition process in order to be able to model activated sludge (adapted from R-Roda et al., 2001)

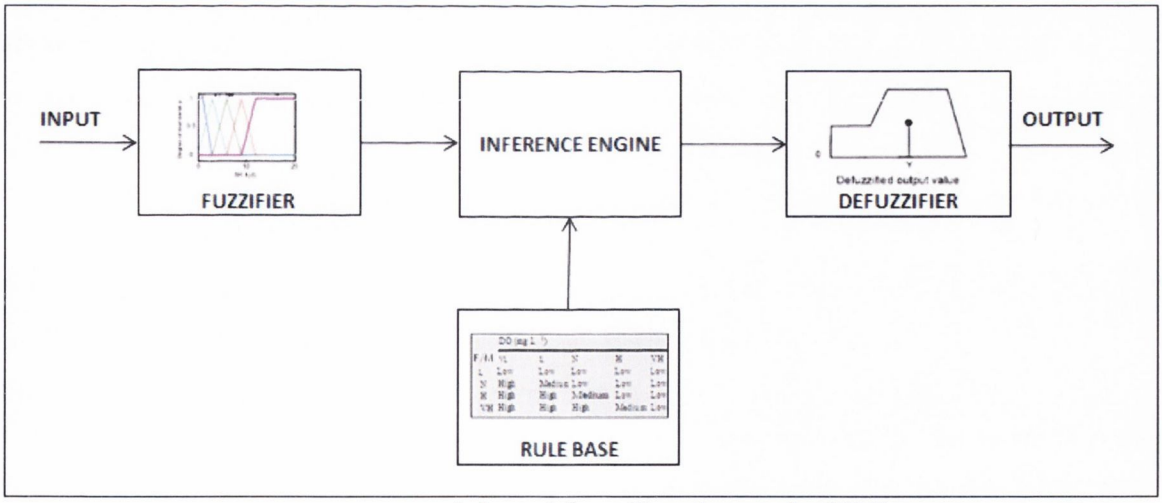
The advantage of applying the KB approach in this case over classical control methods was besides just detecting turbidity, it was able to establish the cause of the problem such as, hydraulic over-loading of the final clarifiers and issues from de-nitrification within the sludge itself (Comas et al., 2003).

There are several examples referred to on the application of knowledge-based tools (expert systems, fuzzy logic or qualitative modelling) and data-based models (neural networks or data-driven models) which have been specifically developed to gain a greater understanding of the design and operation of wastewater treatment systems (Capodaglio et

al., 1991; Ceccaroni et al., 2003; Pires et al., 2006; Comas et al., 2008; Güell et al., 2010). Out of these 'soft computing techniques' fuzzy logic has shown itself to be the most suitable candidate for controlling ill-defined, time-varying and non-linear conditions within wastewater systems (Adriaenssens et al., 2004; Fiter et al., 2005).

Fuzzy logic was first introduced by Lofti A. Zadeh as an extension to Boolean theory (Zadeh, 1965). Boolean logic is traditionally based on providing only two solutions to a problem, being either "true" or "false" (represented by the binary numbers 1 and 0). In contrast, fuzzy logic reasoning employs fuzzy sets that allow for partial membership between the crisp constraints of 0 and 1 therefore allowing for proposed association to be represented by varying degrees of truthfulness and falsehood (Iliadis, 2005; Jinturkar et al., (2010). Fuzzy systems use linguistic descriptions such as 'low', 'high' or 'moderate' to quantify variables and utilise expert knowledge to transform these descriptions into a mathematical structure in which suitable data processing can then be performed (Mouton et al., 2009). For example, when considering the problem of activated sludge foaming, the statement "there is foaming" might be 100% true if the reactor is fully covered, 80% true if there is a lot of foam, 50% true if there is a little foam, and 0% if there is no foam at all (Fiter et al., 2005). An outline of the fuzzy logic procedure is depicted in Figure 6.3.

Figure 6.3 – Block diagram illustrating the key paths involved in the fuzzy logic process (adapted from Jinturkar et al., 2010)



The first phase of the process involves 'fuzzification' where the crisp numerical values from the data are converted into linguistic labels as risk assessment indicators (i.e. low, high, etc.) by means of corresponding membership functions (Comas et al., 2008; Dalmau et al., 2010).

A fuzzy rule-base system is constructed that connects the input variables to the output variable by means of IF–THEN set of commands (Adriaenssens et al., (2004). The inference engine then sorts the mapping from input fuzzy sets into output fuzzy sets by determining the degree to which the antecedent is satisfied for each rule (Jinturkar et al., 2010). The final step in the application of fuzzy logic involves de-fuzzification, whereby linguistic labels assigned in the inference stage are converted back into numerical 'crisp' values (Dalmau et al., 2010).

The primary aim of this work is to produce a detailed knowledge-based model (KBR) using heuristic background information to assess activated sludge foaming. The objectives of this study are:

- To establish the significance of those operating parameters discussed in the literature as having a critical bearing on activated sludge foaming by applying multivariate analysis and principal component analysis (PCA).

- To develop a thorough model based on the principles of fuzzy logic applying fuzzy rules to those principal parameters identified in objective (i) and assigning constraints to corresponding membership functions based on values highlighted in the literature ('The knowledge base').

- To then apply this knowledge-based model to extensive results obtained in the field from fourteen different activated sludge plants which experienced different degrees of foaming on more than one occasion during different times of the year.

- To carry out sensitivity analysis (determination of uncertainty) on the model, comparing the calculated risk of foaming obtained when using those operating conditions recorded in the field and comparing actual findings to those predicted by the model.

- Once the robustness of the model has been verified and any relevant improvements made, results using the model will be then be compared to data obtained from 37 different plants through a survey aimed at various different water companies.

6.2. Materials and Methods

6.2.1. Development of Rule-Based Model

The development of a rule-based risk model was carried out in four fundamental stages (Table 6.1). Previous attempts to mechanistically represent filamentous foaming have been unsuccessful due to lack of information on the basic mechanistic population dynamics pertaining to foaming micro-organisms (Comas et al., 2008). Heuristic and empirical knowledge regarding activated sludge foaming was therefore tackled applying the principles of fuzzy decision theory (Zadeh 1965, Bellman and Zadeh, 1970). Fuzzy logic is an approach well suited to describing ill defined processes, non-linear processes such as those of microbial origin within activated sludge (Comas et al., 2006; Comas et al., 2008).

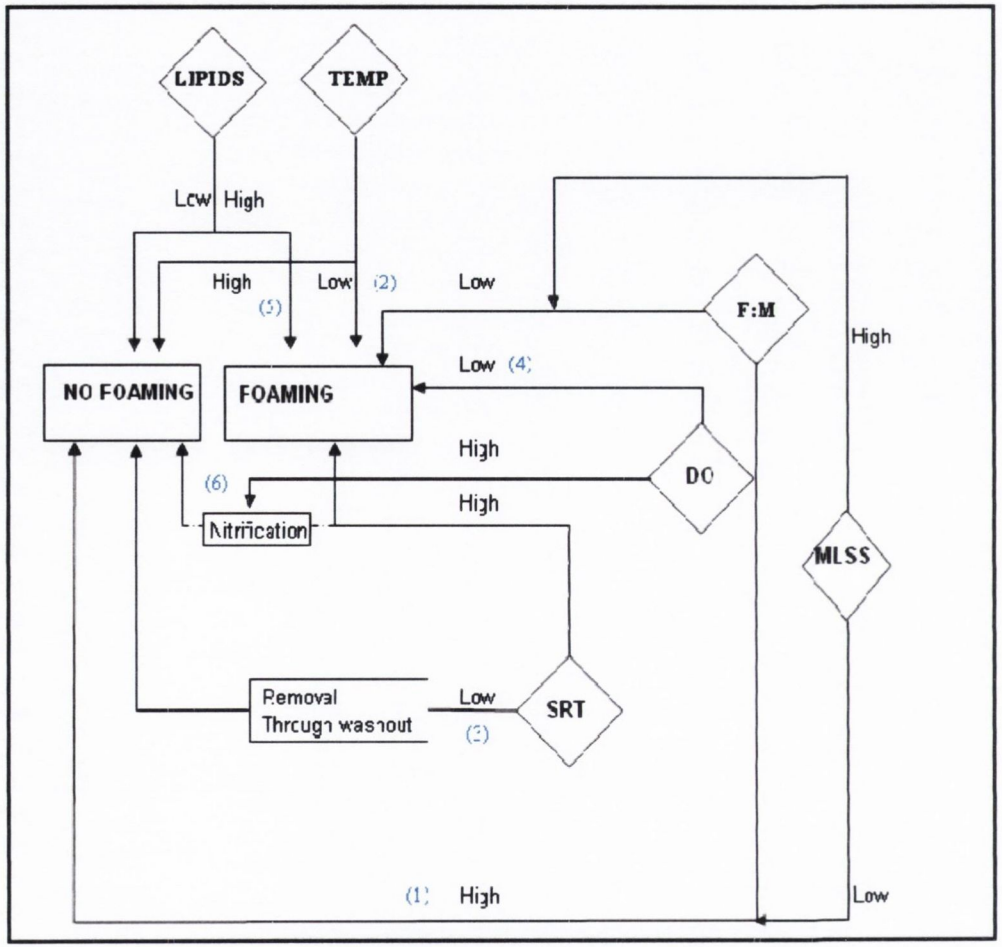
Table 6.1: Different stages adopted in the production of knowledge-based foaming model

STAGE	TASKS INVOLVED
Stage 1 – Knowledge Acquisition:	➢ Assessment and review of current knowledge on probable causes of activated sludge foaming. ➢ In-depth field studies and attainment of data measured from various activated sludge plants subject to various degrees of foam formation.
Stage 2 – Key Variable Selection:	➢ Application of multivariate analysis, including principal component analysis (PCA) to establish key elements related to activated sludge foaming.
Stage 3 – Model Implementation:	➢ Weighting of key membership functions selected to predict risk of foaming based on recorded foam coverage at various plants. ➢ Expert knowledge addressed applying fuzzy-logic theory as described by Beltmann and Zadeh, 1970.
Stage 4 – Sensitivity Analysis:	➢ Calibration of model using data obtained from extensive questionnaire and survey conducted on activated sludge plants based in Republic of Ireland, England and Wales.

6.2.2. Knowledge Acquisition on factors related to foaming – Literature Review

An extensive review of the literature was initially conducted in order to gain a thorough understanding on the current knowledge of the probable causes of foaming within activated sludge aeration basins. Previous research focuses heavily on the causative micro-organisms believed to be responsible for inducing and supporting foaming within activated sludge systems. Much bias has been diverted to gaining a further understanding of two different contrasting types of filamentous bacteria, *Microthrix* spp. (e.g. Foote et al., 1992; Chau et al., 2000; Nielson et al, 2002; Rossetti et al., 2005; Noutsopolous et al., 2006; Xie et al., 2007) and the distinctive branched filaments, *Gordonia* spp. (e.g. Pitt and Jenkins, 1990; Soddell and Seviour, 1995; de los Reyes and Raskin; 2002; Kragelund et al., 2007;

Seviour et al., 2008; Petrovski et al., 2011). The primary environmental conditions which have been associated with the proliferation of *Microthrix* spp. are illustrated in Figure 6.4 and likewise for *Gordonia* spp. in Figure 6.5.



(1) Growth is significantly hindered under conditions of high F:M ratios (Chau *et al.*, 2000; Jenkins *et al.*, 2004; Tsang *et al.*, 2008; Al-Mutairi *et al* 2009).

(2) Cooler temperatures are preferred for the proliferation of *Microthrix* spp. as the reduced solubility of lipids actually leads to a competitive advantage for this species against floc-forming bacteria (Seviour *et al.*, 2008).

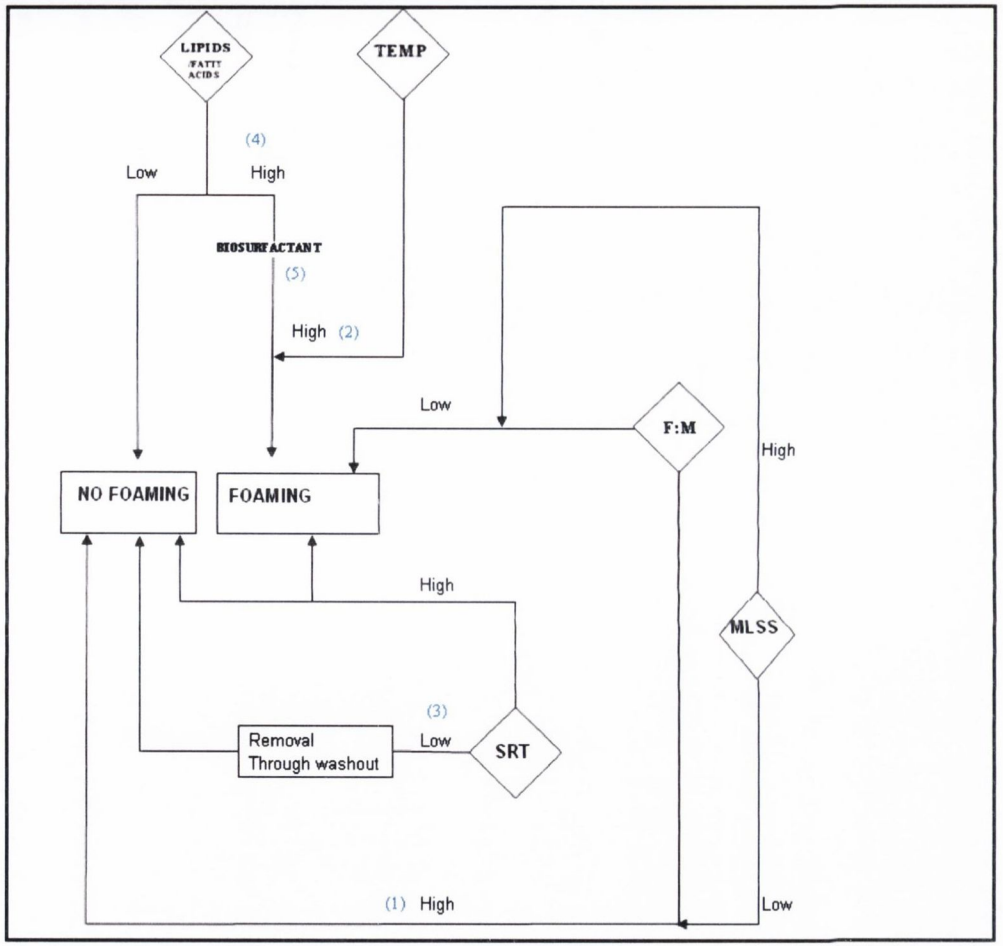
(3) Operation of low solids retention times (SRTs) can washout slow-growing organisms including *Microthrix* spp. and *Gordonia* spp. (Oerther *et al.*, 2001; Lui and Lui 2006; Jolis *et al.*, 2007; Comas *et al.*, 2008).

(4) *Microthrix Parvicella* is a 'micro-aerophile' and its proliferation is greater in conditions of low DO (Rossetti *et al.*, 2005).

(5) *Microthrix* spp. are lipid accumulating organisms (LAO) (Rossetti et al., 2005) the growth of which is dependent on the presence of large quantities of lipids (Andrea and Nielsen et al., 2000; Kragelund *et al.*, 2005; Hug 2006; Xie *et al.*, 2007).

(6) Ammonia nitrogen is an indispensible source of nitrogen for the growth for *Microthrix* spp. (Tsai *et al.*, 2003; Rossetti *et al.*, 2005; Drzewicki *et al.*, 2008).

Figure 6.4 – Environmental conditions associated with the growth of *Microthrix* spp.



(1) *Gordonia* spp. growth is significantly hindered under conditions of high F:M ratios (Chau *et al.*, 2000; Jenkins *et al.*, 2004; Tsang 2008; Al-Mutairi *et al* 2009).

(2) Higher temperatures are preferred for the proliferation of *Gordonia* spp. therefore being present in summer periods (Oerther *et al.*, 2001) aiding the hydrolysis of lipids (Blackall et al., 1991; Wanner 1998).

(3) Operation of low solids retention times (SRTs) can washout slow-growing organisms including *Microthrix* spp. and *Gordonia* spp.
(Oerther *et al.*, 2001; Lui and Lui 2006; Jolis *et al.*, 2007; Comas *et al.*, 2008).

(4) Activated sludge processes receiving high concentrations of hydrophobic substances including lipids are more likely to suffer from Nocardioform foaming (Blackall *et al* 1991; Frigon *et al.*, 2006).

(5) Gordonia spp. produces bio-surfactant which stabilises air bubbles leads to persistent foam
(De los Reyes III *et al*,2002; Liao 2003; Heard *et al.*, 2008).

Figure 6.5 – Environmental conditions associated with the growth of *Gordonia* spp.

6.2.3. Knowledge Acquisition on factors related to foaming – Detailed Questionnaire

A detailed site survey was conducted investigating foaming at a number of wastewater treatment works located in both the Republic of Ireland and United Kingdom. The primary objectives of the survey were to record the regularity and persistence of full-scale foaming events and to determine if any potential relationship exists between the occurrence of scum production and running conditions of each plant. Operational and plant management staff were contacted who had active knowledge of sites which were either suffering from foaming events at the time of questioning or had a prior history of experiencing from activated sludge foam production. The questionnaire involved specific questions pertaining to the operating environment of each activated sludge process (e.g. sludge age and organic loading) as well as the type of influent normally treated and the actual design of the plant itself. The selection of treatment works for inclusion in the survey was not restricted by either population equivalent served, size or by plant configuration and geographic location.

Detailed questionnaire with over 40 specific questions requesting information on:-

- Plant design and running conditions
- Details regarding type of influent treated
- Frequency, time of year and severity of foaming experienced
- Problems experienced in running of plant as well as foaming

A total of 35 wastewater plants, based in England, Wales and Republic of Ireland responded to the survey (Table 6.2). Each was different in both the population equivalent served (PE) and configuration.

6.2.4. Knowledge Acquisition on factors related to foaming – results from field studies

Data obtained from field work studies was principally used in the derivation of a fuzzy logic model to predict activated sludge foaming. Results were obtained from fourteen activated sludge plants situated in and around the Greater Dublin area (see chapter 5). Site visits were made to each plant more than once with overall survey analysis being conducted over a period of three years. Each plant was required to meet different consent limits and therefore was subject to operating under different conditions, applying

contrasting process regimes to meet the required level of treatment in each case. Measurements were made both out in the field (SSVI, pH, Temperature, Foam Thickness) and under controlled laboratory conditions (Microscopy analysis, MLSS, Foam Potential and Stability Testing) (See Chapters 3 + 5 for detailed method statements). In many cases information was also obtained from site operational staff pertaining to specific operating conditions such as plant organic loading, sludge ages and historical sludge wastage rates.

Table 6.2: List of activated sludge plants which responded to the survey suffering from various extents of foaming

WASTEWATER TREATMENT PLANTS (WwTP)	LOCATION	POPULATION SERVED	TYPE OF PLANT
ABERYSTWYTH	WALES	40,000	Plug-flow
BALLINCOLLIG	IRELAND	20,000	Extended Aeration (Oxidation Ditch)
BALLINA	IRELAND	25,000	Plug-flow
BALTINGLASS	IRELAND	3,000	Completely Mixed Extended Aeration
BALLYMOTE	IRELAND	2,311	Extended Aeration
BINLICKY	IRELAND	100,000	Plug-flow
BIRR	IRELAND	7,659	Extended Aeration (Oxidation Ditch)
CASTLETROY	IRELAND	32,000	Tapered Aeration
CLAREMORRIS	IRELAND	3,000	Plug-flow
COLOONEY	IRELAND	950	Extended Aeration
CROSSNESS	ENGLAND	2,000,000	Tapered Aeration
EAST WORTHING	ENGLAND	133,065	Contact Stabilisation
ENNIS NORTH	IRELAND	17,000	Extended Aeration
FORD	ENGLAND	130,937	Tapered Aeration
GARNSWLLT	WALES	33,000	Extended Aeration
GLAMBIA	IRELAND	10,000	Extended Aeration (Oxidation Ditch)
HUTTON ISLAND	IRELAND	91,600	Plug-flow
KNOCKBRIDGE	IRELAND	1,000	Extended Aeration
LISTOWELL	IRELAND	9,000	Extended Aeration
LONGFORD	IRELAND	16,000	Extended Aeration
MACROOM	IRELAND	5,200	Extended Aeration (Oxidation Ditch)
NAVAN	IRELAND	40,000	Extended Aeration
NEWHAVEN	ENGLAND	58,112	Tapered Aeration
OXFORD	ENGLAND	100,000	Plug flow/ Tapered Aeration
PONTHIR	WALES	11,050	Tapered Aeration
PORTSWOOD	ENGLAND	60,882	Plug-flow
RATHCORMAC	IRELAND	3,000	SBR
RATHDRUM	IRELAND	3,500	SBR
SEAFIELD	ENGLAND	900,000	Plug flow/ Tapered Aeration
SLIGO	IRELAND	27,000	Plug-flow
SWANSEA	WALES	500,000	Extended Aeration
TARRENT CRAWFORD	ENGLAND	18,493	Completely Mixed Oxidation Ditch
TUAM	IRELAND	22,000	Extended Aeration
WESTPORT	IRELAND	15,000	Extended Aeration
WIMBOURNE	ENGLAND	22,999	Plug-flow
WOOLSTON	ENGLAND	64,000	Plug-flow

6.2.5. Key Variable Selection – Principal Component Analysis

The foremost operational and environmental variables which have a key bearing on activated sludge foaming were determined through the application of Principal Component Analysis (PCA) using the results obtained through field work. The PCA technique is one of the most powerful multivariate linear transformation techniques used for reducing the dimensionality of large sets of data without loss of information (Al-Mutairi, 2009). This statistical technique organises the number of variables into groups which explain most of the variance in the original dataset (Tomita et al., 2002; Costa et al., 2009). PCA was applied with Varimax rotation (Madoni et al., 1993) using SPSS (version 19.0).

6.2.6 Membership functions and application of fuzzy sets

Membership function can be expressed by various shapes (Comas et al., 2008). Triangular functions are usually applied because of their simplicity and because they can approximate most non-triangular ones (Adriaenssens et al., 2004; Illadis et al., 2005; Comas et al., 2008). Likewise, trapezoidal functions are applied as straightforward extensions to triangular membership functions (Adriaenssens et al., 2004).

The degree of membership to any given parameter for a numerical value, x, using triangular functions is computed using the following rules (Jinturkar et al., 1989; Adriaenssens et al., 2004; Illadis et al., 2005) :-

$$f(x, a, b, c) \begin{cases} 0 & \text{for} < a \\ \dfrac{x-a}{b-a} & \text{for } a \le x < a \\ \dfrac{c-x}{c-b} & \text{for } b \le x \le c \\ 0 & \text{for } x > c \end{cases} \qquad (1)$$

The above formulae used with triangular membership functions are illustrated in Figure 6.6. below.

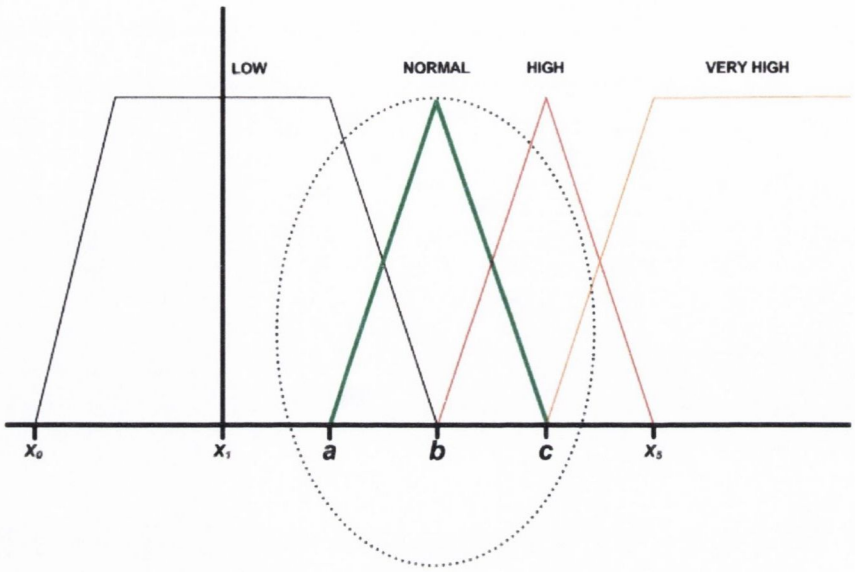


Figure 6.6 – Example of a triangular membership function (circled) showing constituent parameters *a*, *b*, and *c*.

Trapezoidal curves depend in a similar manner on parameters a, b, c and d, as given below Equation (2):-

$$f(x, a, b, c, d) \begin{cases} 0 & \text{for } x < a \\ \dfrac{x-a}{b-a} & \text{for } a \le x < b \\ 1 & \text{for } b \le x < c \\ \dfrac{d-x}{d-c} & \text{for } c \le x < d \\ 0 & \text{for d} \le x \end{cases} \qquad (2)$$

The above formulas used with trapezoidal membership functions are illustrated in Figure 6.7. below.

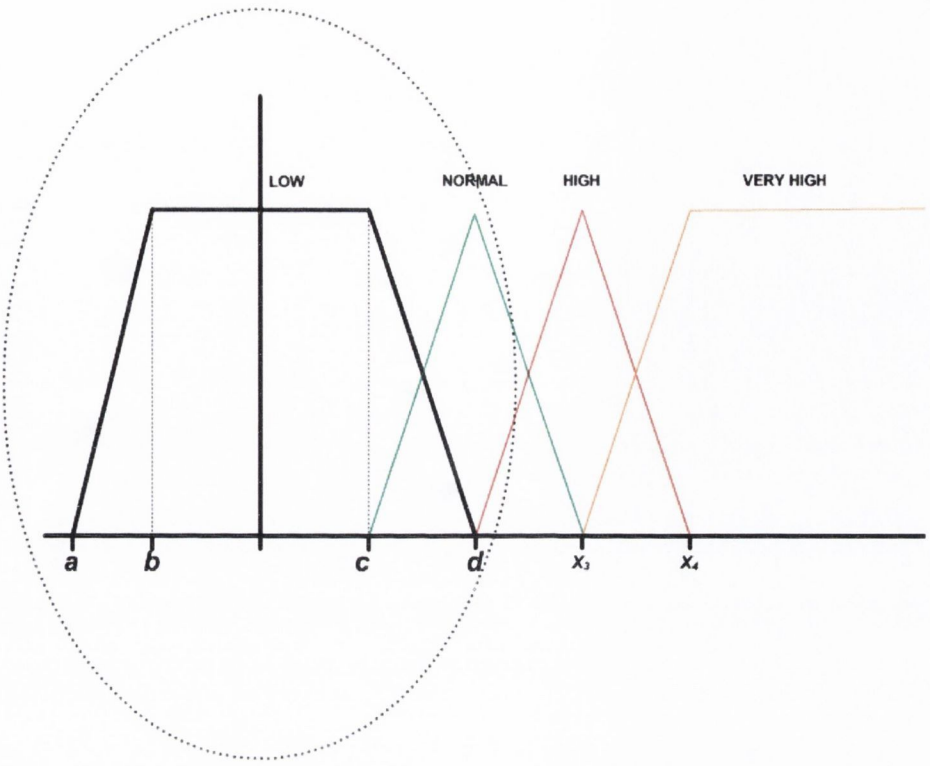


Figure 6.7 – Example of a trapezoidal membership function (circled) showing constituent parameters *a*, *b*, *c* and *d*

The fuzzification approach adopted in this work is explained using the example illustration (Figure 6.8). Let's suppose we have to assess the degree of association of a hypothetical value of F: M (in this case 0.030 kg/kg.d) to each category (low, normal, high, very high). In the scenario below, the chosen value for organic loading could be described as belonging to both 'low' and 'normal' categories for this variable.

The actual chosen value of 0.030 kg/kg.d has an association of 23% (0.23) to the normal category classification and 75% (0.75) to the low class.

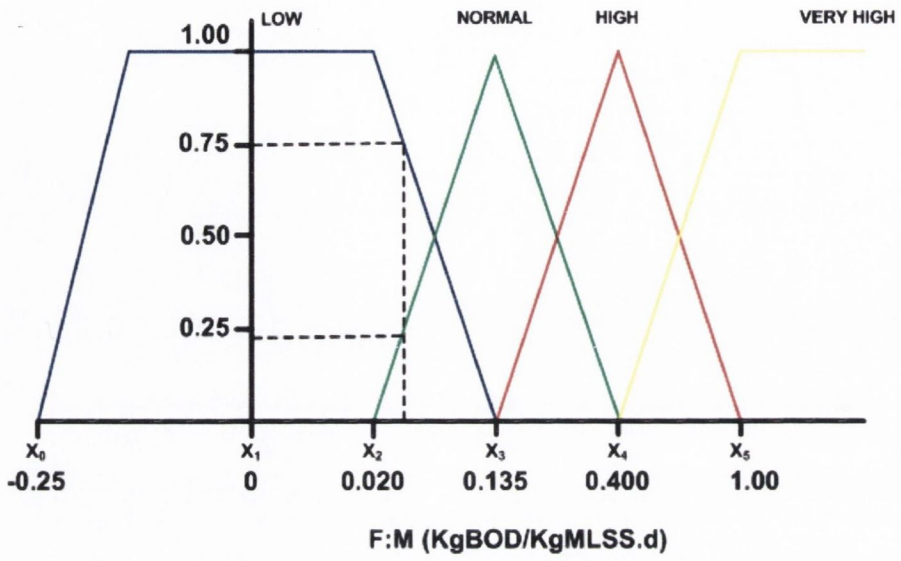


Figure 6.8 – Example of a the fuzzification process and the role of membership functions

The next stage of the modelling process involves defining rules for each variable and group of membership functions. Initial fuzzy sets applied in the model were based on those suggested by other authors in the field (Comas et al., 2008; Dalmau, 2009) as well as the knowledge base on activated foaming and the key metabolic and kinetic environments needed for optimum sustained growth of the two main foaming filamentous species *G. amarae* and *M. parvicella* as highlighted in the literature (Figures 6.4 and 6.5)

A rule-based decision matrix to determine foaming risk was formulated for each of the grouped variables selected (F/M and DO, MCRT and MLSS). Using empirical data obtained from field studies as well as the survey data, the risk of foaming was calculated by the model for different combinations of F/M, DO, MCRT and MLSS. The model was then calibrated against foam coverage which was recorded under each specific condition. The decision matrix was then adjusted in an iterative fashion, producing different sets of fuzzy rules that were applied until good agreement was obtained between both field-based and survey based models as well as significant correlation being obtained in each case between risk of foaming and foam coverage. The final decision matrix determined for foaming due to organic loading under varying dissolved oxygen conditions is shown in

Figure 6.9. Using this decision matrix, for example, it can be seen that under variable DO conditions, the probability of activated sludge foaming arising would be subject to the following rule – "IF **F/M** is **high** *and* **DO** is **low** THEN risk of foaming issues is **moderate**". Likewise, IF **F/M** is **high** *and* **DO** is **normal** THEN risk of foaming issues is **high**".

Foaming due to low DO					
		F/M (kg/kg.d)			
		L	**N**	**H**	**VH**
DO (mg/l)	Very Low (VL)	High	Moderate	Moderate	Low
	Low (L)	Low	Moderate	High	Low
	Normal (N)	Low	Low	High	Moderate
	High (H)	Low	Moderate	High	High
	Very High (VH)	Low	Moderate	Moderate	Low

Figure 6.9– Example of the fuzzy decision matrix adopted for low dissolved oxygen

6.2.7 Defuzzification

The output of the fuzzy inference process needs to be a single scalar quantity rather than in the form of a fuzzy set (Lai et al., 2007). In order to obtain a final crisp value, the output of the fuzzy set must be defuzzified (Adriaenssens et al., 2004). For de-fuzzification purposes the centre-of-gravity (COG) method was applied (Fiter et al., 2005; Comas et al., 2008; Dalmau 2009; Dalmau et al., 2010). As in the fuzzification process, membership functions were used in the de-fuzzification process, In this case, three simplistic equi-distance symmetric triangular functions were used to ascertain the extent of membership of the fuzzy output to different risk scenarios, low, Medium and High (F igure 6.7).

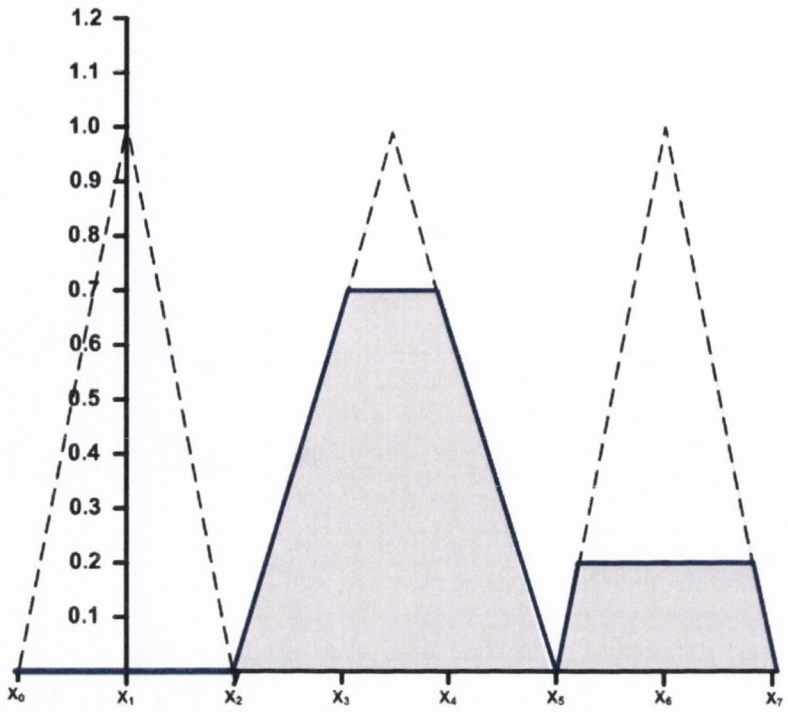


Figure 6.10– Simple membership functions (representing low, medium, high) to obtain the de-fuzzification output

6.3 Results and Discussion

6.3.1 Principal components involved in activated sludge foaming

Principal Components explained 67% of the overall variance when operating and environmental parameters previously linked to activated sludge foaming (Figure 6.8) were subjected to principal component analysis.

OPERATIONAL/ENVIRONMENTAL PARAMETER	FACTOR		
	1	2	3
Hydrophobicity	**.818**	-.097	.075
Temperature	**.821**	.071	.228
Effluent SS	**-.642**	.167	.422
COD:BOD	-.066	**-.872**	-.048
Dissolved Oxygen (DO)	--.053	**.702**	-.203
Food: Biomass (F/M)	-.291	**.519**	-.276
Mean Cell Retention Time (MCRT)	.125	-.070	**.902**
Mixed Liquor Suspended Solids (MLSS)	-.001	-.427	**.738**
VARIANCE EXPLAINED (%)	**23.3**	**21.9**	**21.4**
ACCUMULATED VARIANCE (%)	**23.3**	**45.1**	**66.6**

Figure 6.8 – Results from principal component analysis (PCA) applied to various operating and environmental parameters.

The results from multivariate analysis strongly suggest that mean cell retention time and MLSS have a significant bearing on activated sludge foaming, as cited many times in the literature. The relationship between each environmental parameter (determined using results from field studies is shown in Table 6.3) and the importance of each foaming parameter selected to be part of the principal component analysis has been verified by determining the significance of their association to measured values of FSI (Chapter 3)(Table 6.4). In the construction of the model, DO, F/M, MCRT and MLSS were selected as parameters used to calculate risk of activated sludge foaming primarily on the basis of a) being easily measureable b) parameters which can be readily altered by making adjustments to the running of a plant c) having been determined as being key to activated sludge foaming, as determined through PCA and correlation analysis.

Table 6.3: Pearson Correlation coefficients between various different principal components

Species	pH	Total Filaments	SSVI	Hydro-phobicity	Temp	Effluent TSS	COD:BOD	Dissolved Oxygen	F/M	MCRT	MLSS
pH	1	+	+	-	-	-	-	-	-	-	-
Total Filaments	0.433*	1	++	-	-	-	-	-	-	-	-
SSVI	0.378*	0.518**	1	-	+	-	-	-	-	-	-
Hydrophobicity	0.075	-0.213	-0.265	1	++	-	-	-	-	-	-
Temperature	-0.309	-0.298	-0.394*	0.578**	1	-	-	-	-	-	-
Effluent TSS	0.204	0.215	0.239	-0.301	-0.285	1	-	-	-	-	-
COD:BOD	0.121	-0.036	0.011	0.060	-0.045	-0.076	1	++	-	-	+
DO	0.045	0.077	-0.010	-0.172	-0.153	-0.113	-0.446*	1	-	-	-
F/M	0.076	0.236	0.134	-0.182	-0.116	0.239	-0.236	0.267	1	-	+
MCRT	0.076	-0.021	-0.238	0.118	0.241	0.160	0.054	-0.192	-0.320	1	+
MLSS	-0.510	-0.203	-0.270	0.078	0.093	0.103	0.352*	-0.291	-0.353*	0.608**	1

* The level of significance is $p<0.05$.
** The level of significance is $p<0.01$.

Table 6.4 Linear relationship between different parameters and Foaming Scum Index (FSI) tested using Pearson and Spearman correlation

Species	Pearson (r)	Spearman (r_s)
pH	0.122	0.069
Total Filaments	0.291	0.308
SSVI	0.069	0.086
Hydrophobicity	-0.187	-0.191
Temperature	0.084	0.098
Effluent TSS	0.311	0.418*
COD:BOD	0.012	-0.047
DO	-0.182	-0.173
F/M	0.080	0.012
MCRT	0.588**	0.517**
MLSS	0.344*	0.260

* The level of significance is $p<0.05$.
** The level of significance is $p<0.01$.

6.3.2 Results obtained for risk of foaming using calibrated model

Figure 6.9 illustrates the results obtained for risk of foaming within activated sludge plants against foam coverage.

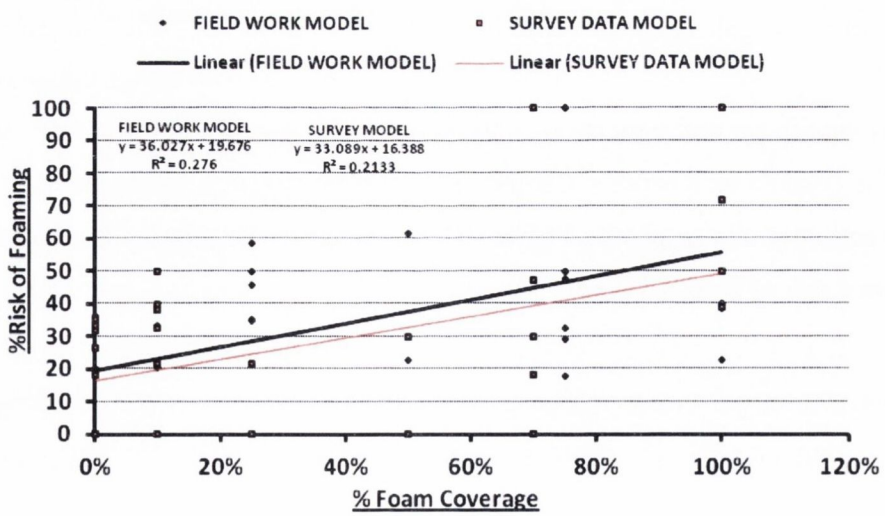


Figure 6.9 – Relationship between risk of foaming (obtained from the fuzzy logic model) and foam coverage

The results illustrated in Figure 6.9 were used in sensitivity analysis and final calibration of the model and its decision matrix components. When field data was entered into the final model a highly significant linear correlation was obtained ($r = 0.62$, $p<0.001$). Likewise the input of survey data produced similar results ($r = 0.46$, $p<0.001$). Values for foam severity were also determined out in the field applying the foaming scum index (FSI) and then relating this back to corresponding values of risk of foaming as calculated by the fuzzy logic model.

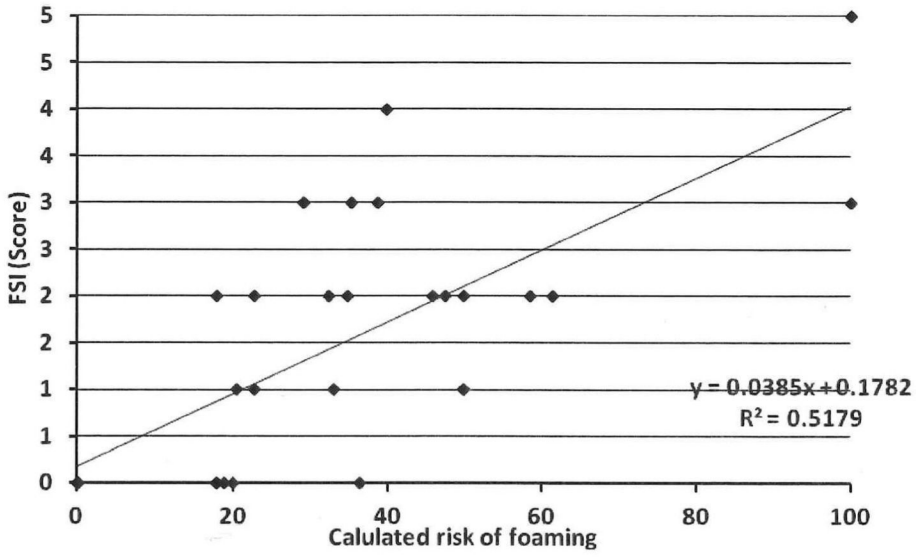


Figure 6.9 – Relationship between risk of foaming (obtained from the fuzzy logic model) and FSI

Highly significant and very strong correlation was found to exist between risk of foaming and FSI (ranked as a score – Chapter 3) ($r= 0.69$, $p=0.00$). This indicates that for any given activated sludge plant a higher risk of foaming occurring is also associated with an increased risk of foam severity.

6.4. Conclusions

In this work, fuzzy logic has been applied as a tool to assess the risk of foaming occurring under different operating conditions. Several other authors have used a similar approach in their methodology to assess similar problems. For example, in the work of Comas et al., (2008), a general examination of sludge settlement problems in activated sludge plants (including foaming and bulking) was tackled using fuzzy logic. The performance of the risk assessment model was evaluated applying a number of control strategies under the IWA/COST simulation benchmark framework model (Comas et al., 2008). A similar fuzzy logic tool (this time for predicting risk of foaming in anaerobic digestion systems) was proposed by Dalmau et al., (2010) and once again the IWA Benchmark Simulation Model was used as a validation approach. This work differs from other previous approaches using fuzzy logic to assess the risk of foaming occurring in wastewater and sludge treatment systems. Firstly, the sole focus of this work has been towards activated sludge foaming using pre-determined principal components associated with the phenomenon to determine the risk of foaming occurring. Secondly, sensitivity analysis (confirmation of model precision) has been applied to the generated model using actual survey data obtained from a detailed questionnaire aimed at a number of independent activated sludge plants across the UK and Republic of Ireland. Such an approach could potentially evoke a great deal of variability due to results having been obtained under much contrasting conditions. Despite this, there is very good agreement overall shown with the risk of foaming compared to foam coverage with results obtained using data from both the field and provided by others through the survey.

Key findings of this work indicate that:-

- Fuzzy logic is a highly favourable tool when used to predict foaming events in activated sludge basins.

- Significant linear relationships were not generally found between the principal components (DO, MLSS, MCRT and F/M) used here to predict foaming.

- Significant dynamic variability is known to continually exist within the activated sludge process due to ill-defined and time-varying environmental conditions. This means that classical attempts based on Boolean logic and represented in the form of

mechanistic models are not recommended approaches to assess this biologically mediated foaming phenomenon.

- The fuzzy logic approach appears to be a good candidate for the capability of fuzzy systems to approach non-linear functions. The model developed within this work and the integral fuzzy decision rules which govern it, have much potential. Firstly, as a starting point on which more complex models could be developed and secondly, as a basic model in its own right.

- The primary aim in building this model was initially to demonstrate how a fairly simple and logical approach using fuzzy logic could be adopted to calculate the risk of foaming on the basis of inputted variables which are those normally used in the running of an activated sludge treatment plant.

- The software used to build this model (Microsoft Excel 2010) is commonly used universally by the computer community. Such an approach was taken using this software on the basis of cost and familiarity. It is suggested that an easier and much quicker modelling approach would involve designated dynamic modelling software such as Math Labs *Fuzzy Toolbox*®.

Chapter 7
Overall discussion and summary

7.1. Introduction

The goal of this thesis was to methodically investigate those principal factors previously highlighted by others as being critical to the production of stable foams within activated sludge systems. This information was then interpreted in order that a mathematical model could then be produced, which could fundamentally predict the risk of foaming occurring on the basis of specific inputted operating variables. The outcome of the work from this thesis confirms the original hypothesis stated for the project as a whole (Chapter 1) that it is possible to reduce the risk of foaming occuring within activated sludge plants through the application of measures based on metabolic control. This was demonstrated by the model produced which, when fed with operational data obtained from full-scale plants suffering different extents of foaming, was able to accurately predict and reflect the risk of foaming occurring for each plant.

The programme of research carried out was designed to follow a set of original objectives (see Chapter 1). The first undertaking was to establish the best methodology to be adopted and used throughout the rest of the work in order to measure and evaluate activated sludge foaming. The most widely used technique for assessing the propensity of activated sludge to suffer from biological foaming is to measure foam potential. This involves measuring the amount of foam produced from sludge under conditions of controlled aeration. Initial work identified flaws in the standard methods widely used to evaluate foam potential and critical weaknesses in the way data and measurements had previously been presented. Two approaches most commonly applied in the testing of foam potential were investigated: (1) air passed through specially designed columns from fine porous diffusers at a fixed rate to form uniform sized bubbles (Blackall and Marshall, 1989; Blackall et al., 1991; Ho and Jenkins., 1991; Oerther and de los Reyes., 2001) and (2) employing Alka-Seltzer™ tablets which effervesce when added to the sludge (Ho an Jenkins, 1991; Oerther and de los Reyes, 2001; Hug 2006; Jolis et al., 2007. Both methods were examined under a range of environmental conditions. Optimum conditions were established for the sintered disc method when a sample of volume 150 ml was aerated at a rate of 0.5 L min^{-1} through a

sintered disc of porosity (ø) = 2. Foam potential measured by the sintered disc method displayed better repeatability compared to the Alka-Seltzer test. The Alka-Seltzer test is much simpler to conduct than the comparative sintered disc test method, therefore rendering itself more practicable for use at a plant level. With this in mind, suggested improvements have been made to maximise precision of this methodology, including use of a wire cage (preventing excessive movement of tablets), expressing foam potential at a fixed MLSS concentration (e.g. 3.5 g L^{-1}) and conducting the test using sludge samples at the same temperature as that of the mixed liquor within the main aeration basin.

Whilst a significant relationship was found to exist between foam potential and the abundance of foaming filaments, no correlation could be found between foam potential and either foam coverage or foam thickness, commonly used in order to assess foam severity on full-scale works. A need was therefore identified for a method to rate foam severity for those works where foam has already established itself on the surface of aeration basins. The Foaming Scum Index (FSI) was developed with this in mind and is formulated using measurements made on various inherent properties of the sludge and mixed liquor itself. Through the application of the FSI it has been possible to quantify foaming for a number of contrasting activated sludge plants. The FSI also produced very significant linear correlations with MCRT, foam thickness and foam age (ratio of suspended solids within the foam compared to that of MLSS), normally associated with foam severity.

Investigative work was carried out into the mechanisms behind stable foam formation and the role played by wastewater influent. Bench-scale reactors were filled with sludge taken from two foaming plants and two plants with no history of suffering from activated sludge foaming. Wastewater influent was also collected from the same sites and fed in different combinations to each reactor. No overall conclusion could be drawn in terms of the impact of supplementing a non-foaming sludge with influent taken from a foaming site. In only one combination scenario (Ringsend sludge and Swords influent) did the application of foaming influent (taken from a site experiencing foaming at the time of experimentation) cause a significant increase in numbers of *M. parvicella*. The enrichment in this species of filamentous bacteria was also accompanied by an increase in measured foam potential. In contrast, when foaming sludge (Swords) was fed with influent taken from the same previous non-foaming site (Ringsend) a proliferation in abundance of *M. parvicella* was accompanied by an increase in foam potential but to a much less extent. Results obtained

from the other reactor combinations were conflicting due to the fact that, in general, it was not possible to maintain the growth of *M. parvicella*. Difficulties with sustaining the growth of this filamentous species, particularly as a pure culture, have been reported by others (Blackall et al., 1996; Rossetti et al., 2005).

During experimentation conducted on the constituents of activated sludge, it was found that measured values for foam potential and stability were observed to be greater in the influent taken from sites with no history of foaming compared to plants that were actively experiencing (and had a history) of foaming. This is a noteworthy observation, since it indicates that biologically mediated activated sludge foaming is not conditional on the nature of the influent going into the treatment process. Historically foams have been produced in aeration basins as the result of high concentrations of fats, oils, greases and surfactants entering a plant. The foams produced often have a shorter lifespan than the foams which are the focus of this thesis project and they have different physical properties (e.g. lack of entrainment of solids). The addition of solids (obtained from both non-foaming and foaming sludge) made no noticeable difference to the stability of each foam. This finding therefore does not support those theories based on froth flotation (Blackall and Marshall, 1989; Jenkins 2007; del los Reyes, 2010) whereby solids are believed to be rendered hydrophobic by filamentous bacteria, allowing them to then attach themselves to air bubbles causing a subsequent steric hindrance to the drainage of liquid from the lamellae, resulting in greater stability of each bubble layer. The addition of activated sludge solids to wastewater influent samples did, however, result in a significant observed increase in foam potential. It is proposed that the hydrophobic filaments present within the sludge matrix have a role in initial foam production and stability rather than attributing to the overall stability of each foam.

Throughout this work, it has been clear that filamentous bacteria play a key role in activated sludge foaming; the fact that foam samples were almost always found to be enriched in numbers of foaming filaments, including members of mycolata, along with 'loosely-coiled' Microthrix spp. is strong evidence for this. Additionally, the underlying mixed liquor samples, contained significant numbers of these filaments but to a much less extent, indicating that they have the ability to migrate into the foam where they then have the opportunity to alter physical properties of the foam bubbles. Whilst *M. parvicella* and G. amarae-like organisms (GALOs) dominated the biocenoses of activated sludge mixed

liquor and foam samples taken from the foaming sites, Types 0675, 1851 and 0092 were also found to be present but in significantly reduced numbers. The enrichment of these hydrophobic filaments within foam samples, in contrast to earlier findings in this thesis, follow the selective froth flotation theory proposed by other authors. In previous studies a universal threshold limit has been assigned to numbers of mycolata, above which the measured values of foam potential increase drastically, reflecting the circumstance under which foaming is most likely to occur (Mori et al., 1992; Davenport et al., 2000; de los Reyes and Raskin 2002). The work in this thesis does not support the existence of such a threshold. The ability to generate foam will not only be dependent on the number of individual filaments, but also on the type of species present and will be influenced by environmental conditions such as the presence of surfactant molecules (Petrovski et al., 2011).

From the findings in this thesis research, a fuzzy logic model was generated which predicts the occurrence of foaming in activated sludge plants. Activated sludge foaming is seemingly a multi-faceted problem, the complexity of which has led to no definite understanding of the complex chemical and biological mechanisms involved. For example, in this work no significant linear relationship was established between the principal metabolic components found to be associated with activated sludge foaming (DO, MLSS, MCRT and F/M). The application of a fuzzy logic in order to produce a rule-based model allows for these inconsistencies, where the data is complex and difficult to interpret, when trying to evaluate a dynamic scenario. Therefore such conditions are not amenable to being resolved via a traditional mechanistic modelling approach. The model produced is unique, particularly in the way that sensitivity analysis was conducted. It is hoped that the work behind the model will form the basis for further development by others, so that a supervisory intelligent system can be created, which can then be integrated into full-scale works.

Suggested Further Work

Since the development process of the FSI involved using results only collected from the Republic of Ireland, suggested further work would involve applying the FSI to activated sludge plants based in other countries. No firm conclusions could be drawn in regard to each of the constituents of the activated sludge and their role in stable foam production and

therefore it is suggested that further work is needed to identify the mechanisms involved in foaming within sludge samples.

The main output of the thesis was a fuzzy logic model. The intention of this part of the project was to demonstrate a novel approach into reducing the risk of foam occurring within aeration plants. Key to this model are the rules constructed based on data collected through extensive field work along with findings obtained from several sites within the UK. It is hoped that this part of the work will be developed further and the rules developed applied to a future model which can then become integrated into the control philosophy of a full-scale works.

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APPENDIX

Summary Survey Results

	PLANT DESIGN				PLANT RUNNING CONDITIONS					OPERATION ISSUES ASSOCIATED W/FILAMENTS		FOAMING SEVERITY		
	CONFIGURATION	ANOXIC ZONE	Baffling	FOG REMOVAL	MCRT	f/m	MLSS	AVE DO	pH	SVI(ml/g)	Settlement Issues	OCCURENCE	Coverage	EASE OF REMOVAL
ABERYSTWYTH WwTW	Plug flow	None	yes	None	10	0.11	2000	-	-		-	4x	70%	No Dispersal
BALLINCOLLIG WwTW	Extended Aeration (Oxi Ditch)	yes	none	yes	27.5	0.05	2900	0.1	7.0	260	Bulking	4x	10%	Partial
BALLINA WwTW	Plug flow	None	yes	None	9	0.1	2400	1.4	7.0	80	All (Rising Sludge/Pin-floc/Bulking	2x	70%	Partial
BALTINGLASS WwTW	Completely Mixed Extended Aeration	None	yes	None	12	0.1	3000	1.5	8.0	270	Rising Sludge, bulking, Pin flocs	4x	50%	Partial
BALLYMOTE WwTW	Extended Aeration	yes	none	None	15	0.1	2800	1.75	7.4	170	Bulking	Rarely	10%	Partial
BINLICKY WwTW	Plug flow	yes	yes	yes	12	0.05	3700	1.5	6.9	100	Bulking, pin-flocs	Rarely	10%	Quick
BIRR WwTW	Extended Aeration (Oxi Ditch)	None	none	None	30	0.1	4000	2	7.3	100	Pin-flocs	2x	100%	No Dispersal
CASTLETROY WwTW	Tappered Aeration	None	yes	None	22	0.1	4000	0.5	8.0	200	Rising Sludge/Bulking	1x	100%	No Dispersal
CLAREMORRIS WwTW	Plug Flow	yes	none	None	12	0.12	3000	2	7.1	170	None	Rarely	100%	No Dispersal
COLOONEY WwTW	Extended Aeration	None	none	None	15	0.1	3200	0.8	7.3	-	Bulking	1x	10%	No Dispersal
CROSSNESS WwTW	Tapered Aeration	yes	yes	None	6	0.16	2600	1.25	7.2	100	Filamentous Bulking (occasional)	4X	10%	Partial
EAST WORTHING WwTW	Contact Stabilisation	None	none	None	3.2	0.54	2800	1.5	-	110	Bulking, Rising sludge	2x	70%	Partial
ENNIS NORTH WwTW	Extended Aeration	None	none	None	10	0.1	2500	0.5	-	200	Rising Sludge	1x	25%	Quick
FORD WwTW	Tappered Aeration	yes	none	None	3	0.7	1200	1.2	-	70	Bulking	4x	70%	Quick
GARNSWLLT WwTW	Extended Aeration	yes	yes	None	10.1	0.1	2200	1.2	-		Pin flocs, Rising Sludge	1x	70%	Partial
GLAMBIA WwTW	Extended Aeration (Ox Ditch)	yes	none	yes	15	0.1	3000	2.5	-	140	None	Rarely	25%	Partial
HUTTON ISLAND WwTW	Plug-flow	yes	-	yes	27	0.229	3750	4	7.0	255	Rising sludge, pin flocs, bulking	1x	100%	No Dispersal
KNOCKBRIDGE WwTW	Extended Aeration	yes	-	None	-	-	2500	-	-	-	-	1X	10%	Quick
LISTOWELL WwTW	Extended Aeration	None	-	None	5	0.13	3500	0.7	6.9	125	-	1x	10%	Quick
LONGFORD WwTW	Extended Aeration	None	yes	None	21	0.06	5000	1.5	-	80	Pin-Flocs	Rarely	10%	Partial
MACROOM WwTW	Extended Aeration (Oxi Ditch)	None	yes	None	50	0.02	8100	1.2	6.6	90	Rising Sludge	4X	70%	Partial
NAVAN WwTW	Plug flow	yes	none	yes	12	0.25	3000	2	7.1	-	Pin flocs	Rarely	50%	Partial
NEWHAVEN WwTW	Tappered Aeration	yes	yes	None	2	0.5	2000	1.2	-	80	Rising sludge	4X	70%	Partial
OXFORD WwTW	Tappered Aeration	yes	yes	None	12	0.1	4000	1.5	7.5	80	None	Rarely	100%	Partial
PONTHIR WwTW	Tapered Aeration	yes	yes	None	13.8	0.13	2500	1.8	7.0	80	Filamentous Bulking/Pin-flocs	Rarely	100%	Partial
PORTSWOOD WwTW	Plug-flow	None	-	None	16	0.11	1300	-	-	-	None	1x	10%	Partial
RATHCORMAC WwTW	SBR	yes	none	Yes	20	0.06	3500	2	-	-	-	Rarely	25%	Partial
RATHDRUM WwTW	SBR	yes	none	yes	12	<0.01	3250	2	7.3	-	-	4X	25%	Partial
SEAFIELD WwTW	Tappered Aeration	None	yes	None	2	0.2	1750	1.75	7.0	100	Bulking, Pin flocs	2x	70%	No Dispersal
SLIGO WwTW	Plug flow	None	none	None	10	0.1	2500	2	7.6	80	Pin-Flocs	Rarely	10%	Partial
SWANSEA WwTW	Extended Aeration	None	yes	Yes	5	0.21	3500	0.85	7.3		None	4X	100%	No Dispersal
TARRENT CRAWFORD WwTW	Extended Aeration (Oxi Ditch)	None	none	None	8	0.3	2750	1.5	7.2	80	Rising Sludge, bulking, Pin flocs	1X	50%	Partial
TUAM WwTW	Extended Aeration	None	-	None	6	0.18	4000	1.75	7.0	250	Filamentous Bulking	4X	10%	Partial
TRAMORE WwTW	Extended Aeration	-	none	yes	8	-	3000	-	-	-	-	4X	25%	Partial
TULLYALLEN WwTW	Extended Aeration	yes	none	None	-	-	2500	-	-	-	-	1X	10%	Quick
WESTPORT WwTW	Extended Aeration	yes	yes	Yes	15	0.08	3000	2	-	250	None	1X	10%	No Dispersal
WIMBOURNE WwTW	Plug-flow	yes	yes	None	9	0.12	2750	1	7.4	80	Rising Sludge, Bulking	Rarely	10%	Partial
WOOLSTON WwTW	Plug-flow	None		None	1.9	0.5	1600	1	-	100	Rising Sludge, bulking, Pin flocs	2X	100%	No Dispersal