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Anaerobic Digestion Foaming Causes

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ABSTRACT

Anaerobic digestion foaming has been encountered in several sewage treatment plants in the UK. Foaming has raised major concerns for the water utilities due to significant impacts on process efficiency and operational costs. Several foaming causes have been suggested over the past few years by researchers. However, the supporting experimental information is limited and in some cases site specific. The present report aimed to provide a better understanding of the anaerobic digestion foaming problem and to identify the underlying mechanisms of foaming.

Field and laboratory investigation identified organic loading as a cause of foaming. Bench scale batch digestion studies in sludge showed that the critical organic loading for foaming was at 2.5 kg VS.m^{-3} while the 5 kg VS.m^{-3} resulted in persistent foaming. Moreover, full scale foaming digesters exhibited higher foaming potential in digested sludge under aeration in the laboratory than the full scale non-foaming digesters indicating that the concentration of surface active agents was higher. Further investigation of the effect of the surface active compounds, BSA and n-valeric acid on foaming showed that both compounds induced persistent foaming at all the examined concentrations. Filamentous bacteria contribution to foam initiation and stabilization was considered insignificant, apart from one occasion (FI:5), due to the abundance of filaments in foaming sludge ($FI \leq 3$) and their partitioning in foam ($FI \leq 3$).

Part of the current work also assessed the cost implications of a foaming incident at the full scale. The antifoam cost was found to be of major concern for the water utilities costing between £1.30 and £13.00 per 1000 m^3 of digester volume per day. However, there was no information on biogas and energy loss whereas the information provided on cleaning, maintenance costs and manpower working hours was poor. Thus, the overall cost of a foaming incident at the full scale could not be estimated at this stage.

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Abbreviations

(t)VFA: (total) Volatile Fatty Acids

AA: Acetic Acid

AD: Anaerobic Digestion

AEOs: Alcohol Ethoxylates

AS: Activated Sludge

BSA: Bovine Serum Albumin

CMC: Critical Micelle Concentration

COSHH: Control of Substances Hazardous to Health

DOC: Dissolved Organic Content

EI: Emulsification Index

EPS: Extracellular Polymeric Substances

F/M: Food to Microorganism

iB: iso-butyric

IC: Inorganic Carbon

iV: iso-valeric

LAS: Linear Alkylbenzene Sulphonates

MW: Molecular Weight

nB: n-butyric

NPEOs: Nonylphenol Ethoxylates

nV: n-valeric

OLR: Organic Loading Rate

PA: Propionic Acid

SAS: Surplus Activated Sludge

SCOD: Soluble Chemical Oxygen Demand

SD: Standard Deviation

SDS: Sodium Dodecyl Sulphate

SE: Standard Error

SMPs: Soluble Microbial Products

SRT: Solids Retention Time
STWs: Sewage Treatment Works
TC: Total Carbon
TS: Total Solids
TSS: Total Suspended Solids
UK: United Kingdom
VS: Volatile Solids

Notations

%TS: dry matter content of the feed sludge in kg.kg^{-1}
%VS: volatile matter content of the feed sludge kg.kg^{-1}
A: ml standard acid used
 EI_{24} : Emulsification Index measured 24 hours after mixing
foam stability: the amount of foam (cm^3) per ml of air per minute remaining 1 hour after aeration of the sample was stopped
foaming propensity: the amount of foam generated (mm) from a sample after 10 minutes of aeration over the solids content of the sample
foaming tendency: the amount of foam (cm^3) generated from a sample after 10 minutes of aeration per ml of air per minute
h: tube calibration in cm
M: molecular weight
N: normality of standard acid
n: population number
P: Sugden's parachor (a function of molecular bonding)
V: volume of feed sludge in ml
 W_1 : Dish Weight, g
 W_2 : Weight of dish + wet sludge sample, g
 W_3 : Weight of dish + dry sludge, g and
 W_4 : Weight of dish + ignited sludge sample, g

X: arithmetic mean

x: measured value

ρ : density (L for liquid, V for vapor)

σ : surface tension ($\text{mN}\cdot\text{m}^{-1}$)

Chapter 1: Introduction

1 Introduction

1.1 Project background

Anaerobic digestion (AD) has become increasingly popular as a stabilization process due to the significant benefits of the utilization of biogas for on-site energy production (Batstone et al. 2002, Gerardi 2003). However, one of the most important operational problems of AD in the wastewater industry having a direct impact on the production of renewable energy is foaming (Pagilla *et al.* 1997). AD foaming can result in inefficient gas recovery and hence reduced energy production, poor digestion efficiency, equipment failure and additional costs arising from imported energy, cleaning, maintenance and workforce overtime (Pagilla *et al.* 1997, Westlund *et al.* 1998, Barjenbruch *et al.* 2000, Barber 2005).

A number of researchers have investigated the foaming problem in AD in order to identify the foaming causes. Initially, Ross and Ellis (1992) suggested that AD foaming was related to overloading and the accumulation of acetic acid in digesters. According to a study conducted by Pagilla *et al.* (1997), *Gordonia* filamentous bacteria were identified as the cause of foaming in two full scale anaerobic digesters at the Sacramento Regional sewage treatment works (STWs). A following study by Westlund *et al.* (1998) reported that *Microthrix* filamentous bacteria were the foaming cause at a full scale anaerobic digester in Stockholm. Recent suggestions, according to Barber (2005) and Barjenbruch *et al.* (2000), have identified parameters, such as inadequate mixing, temperature fluctuations, shock loads, extracellular polymeric compounds and hydrophobic substances as foaming causes. However, the above reports do not represent a systematic investigation of a complex industrial-scale problem that requires fundamental

understanding of the biological AD process and substantial insight of the foaming mechanisms as the information provided is either site specific or lacks supporting experimental evidence.

1.2 Project development

The poor understanding of AD foaming along with the numerous foaming incidents encountered in a number of STWs in the United Kingdom (UK) led to the need of a detailed investigation of AD foaming at both the full and laboratory scale for a number of STWs across the UK region. For that reason, the current project was developed by 6 UK Water Companies including Anglian Water Limited, Northumbrian Water Limited, Severn Trent Water Limited, Thames Water Utilities Limited, United Utilities Plc, Yorkshire Water Services Limited, the Engineering and Physical Sciences Research Council (EPSRC) and Cranfield University. Full funding of the current project was provided by the 6 UK Water Companies and the EPSRC.

1.3 Aims and Objectives

The aim of the present thesis is to identify the underlying causes of AD foaming and provide a better understanding of the mechanisms of foam generation.

The objectives of the current work involved to:

- examine the effect of the type of mixing on foaming,
- investigate the relationship between maintenance of digesters and foaming,
- quantify the critical organic loading thresholds for foam initiation and stabilization and

- examine the overall digestion efficiency at the full scale and its relation to foaming,
- identify suitable examples of surface active agents in the laboratory and further investigate their behavior and effect on foaming in sludge under aeration (foaming tests) and during digestion (batch anaerobic digestion studies),
- examine the association of the presence of surface active agents in anaerobic digesters at the full scale with foaming by assessing the sludges foaming potential and quality characteristics,
- identify the filamentous bacterial species present in foaming and non-foaming digesters at multiple sites
- longer-term monitoring of the filamentous bacterial species present in a selected foaming and a non-foaming digester
- examine the filaments contribution to foaming during bench scale batch AD.

1.4 Thesis plan

The present thesis comprises of nine chapters. Chapter 1 introduces the AD foaming problem and explains the rationale of this work. Chapter 2 provides a critical review of the current knowledge on foam kinetics and AD foaming causes, and evaluates the applicability of a number of established foam control methods on AD foaming. Additionally, Chapter 2 reviews wider knowledge of wastewater foams through the well studied example of activated sludge (AS) foaming, in order to identify similarities regarding the foam kinetics and causes and provide useful information on understanding the mechanisms of foaming in AD. Chapter 3 includes a detailed overview of the experimental designs used in this work and the analytical procedures that were followed. Chapter 4 examines the effect of the operational characteristics of AD on foaming at both the full and laboratory scale. The hypothesis developed in Chapter 4 supports that there are critical operational

aspects in AD that contribute to or result in foaming. Chapter 5 examines the relationship between surface active agents and foaming under aeration and during anaerobic digestion, also at full and bench scale. The hypothesis in this chapter emphasizes that sludge and sludge digestion modifies the behavior of surface active agents and states that there are critical concentrations of surface active agents that can induce foaming in sludge under aeration and during batch AD. Chapter 6 examines the association of filamentous bacteria to foaming at both the full and bench scale. The hypothesis in Chapter 6 supports that filamentous bacteria are not the cause of foaming but contribute to foam stabilization. Chapter 7 presents information collected from the full scale on the implications for the water utilities arising from foaming incidents. Chapter 8 discusses the findings of the current work, identifies AD foaming causes through experimental evidence and suggests mechanisms of foam generation. Problems and limitations found in this work are also stated in Chapter 8. Finally, Chapter 9 summarizes the conclusions of the thesis and identifies areas for further research in order to fully comprehend the AD foaming problem.

Chapter 2: Literature Review

2 Literature Review

2.1 Introduction

Anaerobic digestion foaming is considered to be one of the most important problems in the wastewater industry (Pagilla *et al.* 1997). AD foaming has been recorded in many STWs for over a decade with severe impacts on the overall process (Barjenbruch *et al.* 2000, Barber 2005). Oerther *et al.* (2001) have characterized microbial foams generated on the surface of activated sludge as a viscous, deep brown– colored layer. Varley *et al.* (2004) have also characterized foam of a culture medium as ‘a gas-liquid dispersion with gas content of more than 95%, produced due to intense agitation, aeration and the presence of surfactants’. Based on the above statements, foams created in anaerobic digesters could be characterized as an accumulation of gas bubbles surrounded by a liquid film on the surface of sludge.

AD foaming can have severe impacts on the digester’s operation. Foaming results in inefficient gas recovery from the digesters creating additional costs for electricity production. Foaming can also result in an inverse solids profile having higher solids concentrations at the top of a digester and lower solids concentrations at the bottom. The inverse solids profile creates dead zones and reduces the active volume of the digester. This results in a digested sludge stream, which has not received the same degree of stabilization. Other problems caused by foaming incidents are blockages of gas mixing devices, foam binding of sludge recirculation pumps, fouling of gas collection pipes due to entrapped foam solids, foam penetration between floating covers and digester walls and tipping of floating covers during foam expansion and collapse. The economical issues that arise from energy loss, manpower overtime and cleaning costs are of major concern to the

wastewater industries (Pagilla *et al.* 1997, Westlund *et al.* 1998, Barjenbruch *et al.* 2000, Barber 2005). Westlund *et al.* (1998) reported that a STWs in Sweden suffered in 1996 from 40% biogas loss after a 10-week foaming incident. The total cost of suppressing foaming, which included the additional oil consumption for energy production, the usage of polymer for improved dewatering and other costs reached the amount of 150,000 USD. However, this is the only reference found in the literature on costs arising from foaming events in anaerobic digestion.

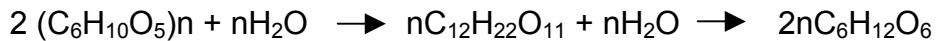
The present report aims to review the foaming problem in mesophilic AD. Extensive investigation of the theory behind foaming in AD is conducted in the following paragraphs and limitations in knowledge are identified. The key parameters in foam kinetics are examined in relation to foaming in anaerobic digesters in order to provide a better understanding of the foaming mechanisms during AD. Additionally, the current paper reviews wider knowledge and understanding of foams of a well studied example, the activated sludge (AS) foaming, in order to identify similarities regarding the mechanisms of foam formation and stabilization and provide useful information on understanding the mechanisms of foaming in AD. Prior to the in depth examination of foaming, the next few paragraphs will provide a brief summary of AD fundamental principles for the reader's better understanding.

2.1.1 Background information

Anaerobic digestion involves the degradation of organic matter of sludge by microorganisms in the absence of oxygen (Mosey 1983). The main advantages of anaerobic digestion over other sludge biological stabilization processes, such as composting, liquid storage and chemical stabilization are a) the reduced sludge production after digestion (total solids reduction between 30 to 35% of input load for mesophilic AD) b) the improved dewaterability of sludge, which subsequently

leads to minimization of sludge transportation costs and c) the utilization of biogas produced during digestion for on-site energy production (0.8 – 1.1 m³ of gas per kg volatile solids destroyed in mesophilic AD). Composting and chemical stabilization, on the other hand, result in increased sludge volumes after treatment due to the bulking agent and chemical added in each case, respectively and do not offer the use of biogas for subsequent energy production. Finally, liquid storage is less frequently used and is not suitable for treatment of large volumes of sludge due to the large area required and long storage retention times (minimum of 3 months) (Handbooks of UK Wastewater Practice 1996, Batstone et al. 2002, Gerardi 2003, Metcalf and Eddy 2003). AD comprises of four successive degradation pathways, as explained below:

Stage 1 - Hydrolysis: Initially, when sludge enters a digester, the polymeric compounds of sludge, such as proteins, carbohydrates, lipids, fats and grease, are hydrolyzed by extra-cellular enzymes to simpler and smaller soluble compounds that can penetrate the micro-organisms cell membrane. The hydrolysis products are amino acids, sugars, fatty acids and alcohols. The equation below is an example of the hydrolysis of starch under mesophilic AD (Kiely 1997, Gerardi 2003, Hoyland 2006).

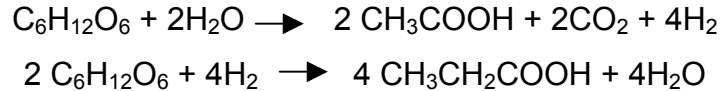


Equation 2-1: Hydrolysis of starch to glucose

Hydrolytic acidogenic bacteria are responsible for the hydrolysis of the compounds in AD by producing the exoenzymes that directly hydrolyze the polymeric compounds. The majority of acidogenic bacteria are obligate anaerobes with populations found to be around 10⁸-10⁹ cells per ml in sludge (Handbooks of UK Wastewater Practise 1996).

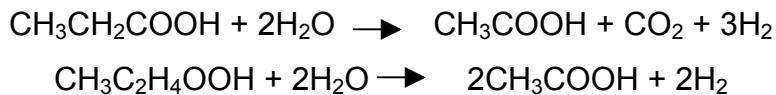
Stage 2 - Acidification: The hydrolysis products of stage 1 are subsequently fermented to short – chain fatty acids, alcohols, ammonia, hydrogen and carbon

dioxide by acidogenic bacteria. An example is given below for the fermentation of the hydrolyzed products to simpler compounds (Kiely 1997, Gerardi 2003, Hoyland 2006).



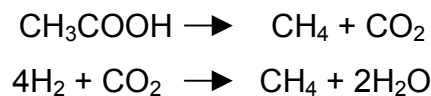
Equation 2-2: Acidification of glucose to acetic and propionic acid

Stage 3 - Acetogenesis: The short – chain fatty acids produced in stage 2 are converted to acetate, hydrogen and carbon dioxide. The main bacterial population responsible for the degradation of these compounds is the acetogenic bacteria, which however, live in close symbiosis with the methanogenic bacteria, another type of bacteria involved in a subsequent stage of digestion. The symbiosis of the two groups of bacteria is important for the survival of the first because acetogenic bacteria can only survive under low concentrations of hydrogen, which is constantly removed by methanogenic bacteria. The equations below show the conversion of propionic and butyric acid to acetic acid followed by the release of hydrogen (Handbooks of UK Wastewater Practise 1996, Kiely 1997, Gerardi 2003, Hoyland 2006).



Equation 2-3: Conversion of propionic and butyric acid to acetic acid

Stage 4 - Methanogenesis: During the final digestion stage, described as methanogenesis stage, methanogenic bacteria utilize acetic acid, carbon dioxide and hydrogen to produce methane and water (Kiely 1997, Gerardi 2003, Hoyland 2006). An example is provided below.



Equation 2-4: Acetic acid, H₂ and CO₂ converted to methane

Methanogenic bacteria are the only microorganisms that reduce simple carbon compounds to methane and most of them can directly use carbon dioxide as a carbon source (Handbooks of UK Wastewater Practise 1996). There is a variety of species that can grow in a wide range of temperatures ranging from 25°C to 70°C, but the majority of species has an optimum temperature in the mesophilic range (30-40°C). Methanogenic bacteria are obligate anaerobes and therefore sensitive to oxygen and they can reproduce within 3 days at 35°C but at lower temperatures their reproduction rate decreases, i.e. up to 50 days at 10°C. Due to their low reproduction rates and sensitivity to oxygen they are the most susceptible group of microorganisms in the digesters and a long retention time of at least 12 days is required in anaerobic digesters in order to ensure sufficient degradation (Handbooks of UK Wastewater Practise 1996, Gerardi 2003).

AD can be distinguished as psychrophilic, mesophilic and thermophilic anaerobic digestion according to the temperature range the digester is operated at. Psychrophilic digestion takes place at temperatures of about 5-25°C. Due to the low temperature range, the rate of digestion is low thus setting the residence time of the influent to be treated into the digesters in several months. Consequently, psychrophilic AD is not capable of treating large amounts of sludge and is usually applied to small treatment works. Mesophilic digestion occurs at 30-35°C. It has been found that mesophilic digestion enhances the degradation processes in shorter retention times than psychrophilic AD and therefore the retention time can drop from several months to 10 - 20 days. Thermophilic AD occurs in temperature ranges from 50° to 70°C. The major concern in thermophilic AD is the maintenance of the high temperature. Thermophilic AD can be highly affected by temperature fluctuations even by 1°C, high endogenous death rates of microorganisms, low bacterial growth and lack of diversity and sensitivity in toxic compounds. However, it has been reported by Song *et al.* (2003) and Halalsheh *et al.* (2005) that thermophilic digestion can achieve the same reduction levels of organic matter as

mesophilic AD in shorter retention times. Another advantage of thermophilic AD is the efficiency in pathogens' reduction resulting in an enhanced end-product (Gray 2004, Mosey 1983, Metcalf and Eddy 2003, Gerardi 2003, Song *et al.* 2004, Halalsheh *et al.* 2005).

AD can also be distinguished in single – stage digestion or two – stage digestion. The single – stage digesters are batch digesters with no recycling of sludge. Raw sludge is usually fed near the top of the digesters and digested sludge is abstracted from the bottom. In the two – stage digesters, the digestion process is taking place in two different reactors. In the first reactor, continuous stirring and heating of sludge at a constant temperature are maintained. The retention time of sludge is about 15 days or less. In the second reactor, psychrophilic conditions are predominant and the reactor can be used either for sludge separation where stirring is stopped or for psychrophilic digestion where stirring is maintained (Gray 2004, Mosey 1983, Metcalf and Eddy 2003).

However, in recent years, more complex configurations dominated the wastewater industry in an effort to enhance the digestion efficiency and produce better product (Class A digested sludge). These alternative configurations involve a pre-digestion treatment stage, such as acid-phase digestion, thermal pre-treatment of sludge and enzymatic hydrolysis digestion (Water Environment Federation 2004).

2.2 Key parameters in foams

This section reviews the current state of knowledge on foam rheology and behavior with the aim of understanding the foam properties in AD. The following paragraphs address the key features that characterize foam and model foam behavior as provided by research conducted in both the microscale and macroscale of aqueous foams (Durand and Langevin 2002, Koehler *et al.* 2004).

2.2.1 Foam types

Foam, present in wastewater and sludge, has been categorized by other researchers in two principal types: 1) unstable or transient foam and 2) metastable foam. Unstable foam is usually caused by fat or filamentous microorganisms that float to the surface of sludge or wastewater by attachment to the gas bubbles. Unstable foams tend to reach equilibrium but continuously break down due to drainage of the liquid film surrounding the bubbles and usually have a lifetime of seconds. Metastable foams, on the other hand, cannot be easily destroyed by mechanical means but can collapse due to an irregular disturbance, such as vibrations, radiant heat and temperature differences. Metastable foams have a lifetime of a few days and usually occur when the process in a bioreactor is unstable or if hydrophobic matter is present in wastewater or sludge (Westlund *et al.* 1998, Vardar-Sukan 1998, Barjenbruch *et al.* 2000, Barber 2005).

It is clear from the above that the key parameter that distinguishes between the two foam types is stability. Information on the causes of unstable and metastable foams is also provided above. However, there are no quantitative data (critical concentrations of each 'cause' required for the creation of each foam type) and accurate differentiation between the causes of unstable and metastable foams, as fat, filamentous bacteria and hydrophobic matter are all hydrophobic substances, yet, some hydrophobic substances (fat and filamentous bacteria) result in formation of unstable foams whereas others (hydrophobic matter) result only in metastable foams. Moreover, there is no qualitative information regarding the 'hydrophobic matter'. The 'unstable bioreactor process' is also not clearly explained.

In foaming anaerobic digesters differentiation between unstable and metastable foams has not been made in the literature. Part of this work investigates the frequency of the two types of foaming in AD as this could provide significant information about the mechanisms of foaming and better understanding of its destruction. The following paragraphs review current knowledge on key parameters for foaming (foam drainage and film thickness and elasticity) and discuss the differences between the two types of foams.

2.2.2 Foam drainage

The term foam drainage describes the flow of a liquid through the bubble lamellae interface, known as Plateau borders, in the foam matrix. The Plateau borders are created when three liquid films that surround adjacent bubbles are in contact. The Plateau borders are connected via nodes and form a network through which the liquid flows. The main forces controlling drainage are gravity and capillary action. Drainage influences foam stability by thinning the liquid films between adjacent bubbles. The bubbles can then easily coalesce leading to foam destruction (Vardar-Sukan 1998, Durand and Langevin 2002, Koehler *et al.* 2004, Barber 2005).

A number of researchers have stated that the rate of drainage depends on the viscosity of the solution under the foam layer (Takesono *et al.* 1993, Pugh 1996, Morey *et al.* 1999, Moen 2003, Barber 2005). Bramforth (2004), for instance, reported that a change in the viscosity of beer from 0.0016 to 0.0018 Pa.s would increase the time to reach a certain film thickness by 12%. Vardar-Sukan (1998) also stated that film elasticity, defined as the ability of liquid films to resist localized thinning while general thinning proceeds, can affect the drainage rate. Film elasticity is studied in following paragraphs as a separate key parameter in foams.

Surface viscosity depends on the composition of the solution. Substances that can increase the viscosity of a solution are generally proteins, or mixed surfactants, polymers and particles with high contact angle attached to the air/surface interface (Vardar-Sukan 1998, Barber 2005). However, there is no further information about what the latter group of compounds consists of and how it can affect surface viscosity. Yet, there are numerous reports in the literature assessing the relationship of surface viscosity of aqueous solutions with concentrations of one or a mixture of proteins, surfactants and polymers (Ogino. and Takigami 1979, Merta and Stenius 1997, Hong 2003, Piculell et al. 2003, Takesono et al. 2006). For the purposes of this work though, the viscosity changes in relation to concentrations of the above compounds will only be investigated in sludge. Goel *et al.* (2004) studied the changes in sludge viscosity and other sludge quality characteristics during anaerobic digestion. The report showed that an increase in the total solids content in digesting sludge from 2.7% to 6.3% resulted in about 2.5 fold increase in sludge viscosity. The researchers demonstrated a clear association of sludge viscosity to the total volatile solid concentration of digesting sludge rather than the total solid content indicating that viscosity was affected by the organic compounds such as proteins, surfactants etc. that make up the volatile solids content in sludge. The total volatile solids ranged from approximately 1.2 to 3.2% with viscosity varying from approximately 300 to 4000 mpa.s⁻¹.

In conclusion, it is clear from the above that foam drainage and surface viscosity are key factors for foam stability. In sludges, according to findings of Goel *et al.* (2004), viscosity increases with increasing total volatile solids content. The information found in the literature indicated that there are critical thresholds of sludge viscosity in foaming sludges, which however, remain unknown, that reduce the foam drainage rate and potentially result in the creation of metastable foams. Additionally, the above evidence suggests that reduction of the surface viscosity by reducing the organic content would increase the drainage rate and prevent the creation of metastable foams in AD.

2.2.3 Surface tension

Surface tension is defined as ‘a property of liquids arising from unbalanced molecular cohesive forces at or near the surface, as a result of which the surface tends to contract and exhibit properties resembling those of a stretched elastic membrane’ (Dictionary of the English Language 2000). According to Barber (2005), surface tension is given by the following equation and is a function of the liquid and vapor densities and the molecular weight of the compound involved.

$$\sigma = \left(\frac{P (p_L - p_V)}{M} \right) \times 10^{-12}$$

Where: σ : surface tension ($\text{mN}\cdot\text{m}^{-1}$)

P : Sugden’s parachor (a function of molecular bonding)

ρ : density (L for liquid, V for vapor)

M : molecular weight

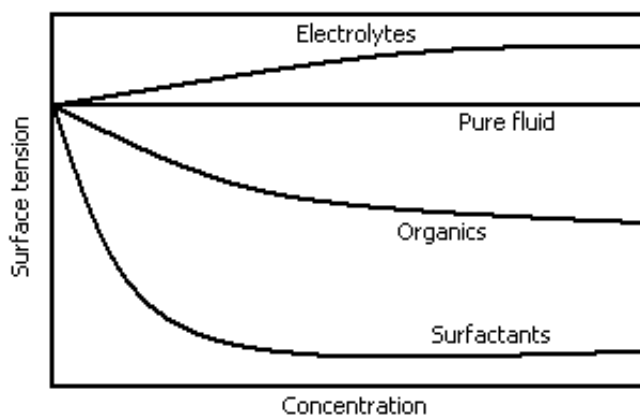


Figure 2.1: Effect of various compounds to the surface tension of a pure fluid at 20°C

(Source: Barber 2005)

The surface tension of pure water is approximately $72 \text{ mN}\cdot\text{m}^{-1}$ at $20 \text{ }^\circ\text{C}$ (Vardar-Sukan 1998). Different compounds in a solution tend to increase or decrease the surface tension of the solution due to their molecular weight and physical and chemical properties. It can be seen from Figure 2.1 that organics and surfactants tend to lower the surface tension of a solution, increasing the surface activity, which facilitates the initiation of foaming (Barber 2005). However, it is not clear whether the above graph was generated based on experimental values. In addition, it is not clear as to what the critical concentrations are for each group of compounds in order to have an impact on the surface tension of a solution and what kind of solutions this graph applies to. Yet, the information in Figure 2.1 matches experimental data found in the literature on surface tension measurements of individual compounds, such as the amino-acid l-leucine and the proteins lysozyme, pepsin, bovine serum albumin (BSA), yeast alcohol dehydrogenase (YADH), human immunoglobulin G (IgG), and catalase as provided by Glinski *et al.* (2000) and Clarkson *et al.* (1999), respectively. Even trivial amounts of 0.001mM of lysozyme reduced the surface tension of the aqueous solution to below $58 \text{ mN}\cdot\text{m}^{-1}$, as it was demonstrated by Clarkson *et al.* (1999). The examination of surface tension against the concentration of a surface active compound identifies its critical micelle concentration (cmc). That is the concentration of the compound at which the aggregation of molecules into clusters (micelles) starts by orientation of the hydrophobic ends of the molecules towards the centre and the hydrophilic ends towards the solution. At concentrations lower than the cmc, the molecules of the compound exist as monomers, whereas at concentrations higher than the cmc as micelles (Elmitwalli *et al.* 2001, Ying 2006). According to Schramm (2000), the effect of the compound is greatest at concentrations higher than the cmc where a significant number of micelles are present. Simply, the cmc of a compound determines the concentration beyond which surface activity increases and foaming would appear if air bubbles were introduced into solution. Surface tension is greatly influenced by parameters such

as viscosity, alkalinity and temperature, which are addressed in the following paragraphs.

Barber (2005) states that increases in viscosity follow increases in surface tension reducing the foaming potential. However, where foam is present, there are potentially critical thresholds of viscosity that lead to the creation of metastable foams, as discussed earlier. The relationship between viscosity and surface tension is better understood if the temperature effect is taken into consideration. Hayta *et al.* (2001) demonstrated experimentally by measuring the consistency and flow behavior index of a mixture containing corn, rice and wheat flour that viscosity decreased as temperature increased. According to Barber (2005), when the surface tension of a solution in mesophilic anaerobic digestion is about $70 \text{ mN}\cdot\text{m}^{-1}$, in thermophilic anaerobic digestion it will drop to about $66 \text{ mN}\cdot\text{m}^{-1}$. Thus, both surface tension and viscosity are adversely influenced by the temperature of the solution. The significance of a $4 \text{ mN}\cdot\text{m}^{-1}$ decrease of surface tension in anaerobic digesters for foam initiation, taking into consideration that sludge surface tension is lower than that of pure water due to the numerous compounds found in sludge, has not been experimentally assessed. The association of sludges' surface tension with foam initiation in anaerobic digesters would provide useful information about the critical thresholds for foam initiation.

Gerardi (2003) reports that alkalinity is inversely proportional to the surface tension of sludge. An increase in the alkalinity in sludge out of the normal range would result in a decrease in the surface tension and thus higher foaming potential. Within anaerobic digesters, where the normal range of alkalinity is around 2000-3000 $\text{mg}\cdot\text{l}^{-1}$, increased levels of alkalinity can be caused by increased loading of ammonium ions, amino acids, proteins, cationic polymers, death of large numbers of strict aerobic bacteria that release large quantities of amines and decreased alkalinity destruction within the digesters (Gerardi 2003). Alkalinity has not been

identified as a foaming cause in the literature and the relationship between alkalinity values, surface tension and foaming in AD has not been investigated.

In conclusion, the determination of surface tension is an indirect measurement of the foaming potential of a solution. The identification of the critical threshold of surface tension for foam initiation in AD would provide useful information for the prediction of foaming incidents. However, complications involved with surface tension determination in sludge samples are attributed to the fact that surface tension measurements can only be carried out in solids free samples. So far, a number of researchers have carried out surface tension measurements in soil samples and bacterial cultures for the determination of biosurfactants production (Rahman *et al.* 2003, Verma *et al.* 2006, Nitschke Pastore 2006). Yet, a suitable method that would allow the determination of surface tension in sludges has not been developed. Additionally, the investigation of the effect of compounds, such as surfactants and organics, which are commonly found in sludge, on surface tension of sludge could potentially identify a link between these compounds and foaming in sludge.

2.2.4 Film thickness and elasticity

As mentioned earlier, film elasticity is 'the ability of liquid films to resist localized thinning while general thinning proceeds' (Vardar-Sukan 1998). This phenomenon is also known as the Gibbs-Marangoni effect. When a particular area of the surface thins due to disturbances the surfactant concentration at the surface decreases, causing the localized surface tension to rise. In order to re-establish stability on the surface of the liquid, the surrounding area moves towards the thinned spot to equalize the surface tensions. The movement of the surface layer drags along layers of the underlying bulk liquid until equilibrium is reached. Under these conditions, foam can either thrive or collapse, depending on the strength of the

Gibbs-Marangoni effect. Film rupture and thus foam collapse can also occur at high concentrations of surfactants (above the cmc). The Gibbs-Marangoni effect is suppressed at excess surfactant concentrations due to the high diffusive migration of surfactants from the bulk to the surface. Equilibrium is re-established in the thinned area by the movement of surfactants from the bulk solution, which is not followed by movement of the surrounding area. Therefore, the thinned spot cannot be restored and further thinning and eventual rupture will occur. Film restoration will occur only if the rate of attaining equilibrium surface tension by surfactant adsorption from the bulk phase is slower than surface migration. (Vardar-Sukan 1998, Barber 2005, Buzzacchi *et al.* 2006).

The above information could suggest that a substantial difference between unstable and metastable foams can be attributed to the Gibbs-Marangoni effect. According to the above, metastable foams exhibit film elasticity to such extent that stability is always re-established at the thinned spot whereas unstable foams are characterized by film rupture due to either weak Gibbs-Marangoni effect or excess of surfactants in solution.

Film elasticity measurements or the Gibbs-Marangoni effect has been studied in aqueous surfactant solutions by the thin film pressure balance technique (Wang and Yoon 2006). The Gibbs-Marangoni effect in the matrix of foam from anaerobic digesters has not been studied so far. The number of compounds found in sludge, such as proteins, surfactants and by-products from the metabolic activity of microorganisms that could potentially promote foam generation, the interactions between these compounds and the degradation processes in digesters that constantly change the quality characteristics of sludge would potentially not provide a representative evaluation of the film elasticity of digester foams.

2.3 Activated sludge foaming – The best studied example

The activated sludge (AS) process is being extensively used in wastewater treatment and involves the degradation of organic matter in wastewater by microorganisms under diffused or mechanical aeration (Metcalf and Eddy 2003). Foaming is a widespread problem in AS plants and there is extensive information in the literature on the foaming causes and control. The consequences of foaming in AS involve foam binding and blockages of mechanical equipment, maintenance costs and poor effluent quality. This section aims at reviewing in brief the well studied foaming problem of AS plants in order to gain knowledge from the literature on wastewater foams and recognize potential similarities between the causes of AS and AD foaming.

Foaming in activated sludge plants is described as floating biomass and has been attributed by many researchers to the combination of the presence of surfactants (detergents), biosurfactants (substances produced during the metabolic activity of microorganisms) and the presence of two groups of filamentous bacteria, *Gordonia spp.* (formerly known as *Nocardia sp.*) and *Microthrix parvicella*. The filamentous microorganisms are generally bacteria, fungi and algae whose cells do not become detached from one another after cell division and therefore tend to grow in the form of 'filaments'. *Gordonia spp.* comprise of filamentous microorganisms, known as *Actinomycetes*, which are extremely hydrophobic due to the presence of mycolic acids on their cell walls (Stainsby *et al.* 2002, de los Reyes and Raskin 2002). *Microthrix parvicella* is also hydrophobic and utilizes long chain fatty acids as carbon source. It can store excess long chain fatty acids in large globules and has an advantage over other bacteria for water-insoluble fats and lipids due to its hydrophobicity (Mamais *et al.* 1998). The mycolic acids in their cell walls make them sufficiently hydrophobic and along with the morphological characteristics of filamentous bacteria they become attached on the gas bubbles present in activated sludge and rise to the surface of the liquid increasing the surface activity and

promoting stable foams (Mamais *et al.* 1998, Eikelboom 2000, Oerther *et al.* 2001, de los Reyes and Raskin 2002, Davenport and Curtis 2002, Torregrossa *et al.* 2005, Carr *et al.* 2006, Hug 2006, Kragelund *et al.* 2007).



Figure 2.2: Foam covering an activated sludge plant

(Source: Hug 2006)

De los Reyes and Raskin (2002) carried out batch tests involving the addition of *Gordonia amarae* cells to AS and found that the threshold of *Gordonia* levels for foam formation and foam stability were approximately $2 \times 10^8 \mu\text{m} \cdot \text{ml}^{-1}$ and $1 \times 10^9 \mu\text{m} \cdot \text{ml}^{-1}$ (filament length), respectively. The results were verified by full scale and laboratory scale measurements.

Davenport and Curtis (2002) found that large rod and coccoid mycolata numbers (mycolic-acid containing bacteria) varying from approximately 8×10^6 to 30×10^6 per ml of AS and accounting for more than 79% of the mycolata population were highly associated with foaming events at three full-scale AS plants. However, branched filamentous mycolata presence in foaming periods was insignificant, accounting for less than 21% of the mycolata population in the mixed liquor and foam samples examined. Furthermore, filamentous mycolata did not contribute to any of the significant differences in mycolata concentration observed between foaming and non-foaming periods. These findings indicated that filamentous microorganisms were not the cause of foaming on this occasion.

De los Reyes *et al.* (2002) reported that large numbers of *M. parvicella* and even inactive *M. parvicella* cells were linked with foaming in AS. The length of *M. parvicella* in the monitored foaming AS plants varied from just above 0 μm per ml to 2.6×10^9 μm per ml of AS. Hwang and Tanaka (1998) also stated that seasonal foaming at an activated sludge plant was attributed to increased levels of *M. parvicella* with persistent foaming corresponding to filament length between 200 and 500 μm .

Foaming in AS plants is regarded as a 3-phase system, comprising of gas bubbles, liquid (wastewater) and solid particles (hydrophobic bacteria) (Davenport and Curtis 2002). Hug (2006) stated that the onset of foaming could be due to high surfactants and biosurfactants loads in wastewater, which is then stabilized by the mycolic-acid containing microorganisms. Therefore, the key parameter for foam control in AS plants is the treatment of mycolata. Another study investigated the effect of three strains of the filamentous bacterium *Gordonia amarae* on foam initiation and stabilization. Pure cultures of the three strains after isolation of the microorganisms from foam or mixed liquor samples from full scale showed that the agent responsible for foam initiation was the biosurfactant produced during the exponential growth phase of the *G. amarae* strains and not the *G. amarae* bacteria. It was also found that each strain produced a different biosurfactant or at different quantities as the filtrates of each culture had different foaming behaviour. Although the biosurfactants were not quantified in this study, their concentrations were measured indirectly through surface tension and the foaming potential and surface tension values below $60 \text{ mN}\cdot\text{m}^{-1}$ were necessary for foam initiation. The stabilization of foam was attributed to the presence of *G. amarae* as $\geq 55\%$ of the strains was partitioned into the foam resulting in reduction of the foam drainage rates. The partitioning of the bacteria in the foam was not associated with the origin of the strains (foam or mixed liquor sample) and did not change greatly with the life cycle (Heard *et al.* 2008).

Foam control methods, according to Hug (2006), focus mainly on the inhibition of foam inducing bacteria, on changing their surface properties, on the control of foam formation and stabilization and on the reduction of the foaming impacts on the plant. Removal of foam layers from foaming plants is an approach commonly applied, offering removal of the bacteria that cause foaming. Yet, treatment/disposal of the foam volume removed is still required. Reducing the sludge retention time (SRT) has been found to inhibit *Nocardioform actinomyces* and *Microthrix* growth but can wash out nitrifying bacteria. In addition, the SRT in a stable foam layer is higher than that in the mixed liquor, therefore it has to be removed first to prevent inoculation. Contact or selector zones have also been reported to suppress growth of *Nocardioform actinomyces* and *Microthrix*, although reports of ineffective treatment exist. The key operational aspects of the selector zones are to promote selective growth of floc-forming bacteria for a short period of time (10-30 minutes) at the initial stage of the process and provide high food-to-microorganism ratio at controlled dissolved oxygen levels. The organic matter remaining after the contact zone is insufficient to encourage subsequent filamentous bacteria growth. Oxidants (i.e. chlorine, peroxide), poly-electrolytes and (poly-) aluminum salts aiming at damaging the bacteria or changing the floc structure have also been used but downstream effects and inhibition of other groups of bacteria are likely to occur (Metcalf and Eddy 2003, Hug 2006). Oerther *et al.* (2001) suggested high dissolved oxygen concentrations, high food to microorganism ratios (F/M), and low solids retention times (SRTs) that do not create favorable conditions for the growth of filamentous microorganisms as efficient control methods regarding AS foaming.

Hwang and Tanaka (1998) reported that biological control of *M.parvicella* and hence foam suppression, such as the control of the mean cell residence time, the sludge age and the F/M ratio, have been proved ineffective due to the special characteristics of *M.parvicella* (slow growth rates and diversity in metabolic abilities). However, three chemical agents were proved to be efficient on foam

control with quaternary ammonium-based anti-filament polymer to be the most efficient.

De los Reyes and Raskin (2002) stated that an increase in the concentrations of *Gordonia spp.* and *G. amarae* coincided with a temperature increase showing that *Gordonia spp.* and *G. amarae* growth was favored during warmer periods.

In conclusion, foaming in AS plants is a well studied problem by many researchers with significant impacts on the process efficiency. Several studies by various researchers have demonstrated a clear link between the AS foaming and the presence of surfactants, biosurfactants and the mycolic-acid containing microorganisms. In detail, recent studies (Hug 2006, Heard *et al.* 2008) have showed that initiation of AS foaming is due to surfactants and biosurfactants, although critical concentrations for foam initiation have not been quantified due to the numerous compounds involved and their variability between different sludges. Foam stabilization is mainly due to the filamentous *Gordonia* and *M.parvicella* but there is evidence suggesting that non filamentous mycolic-acid containing microorganisms, of which specific species have not yet been identified, also act as stabilizing agents. Additional information on the exact mechanisms of foam generation and stabilization in AS plants has not been provided potentially due to the complexity of the process (degradation pathways and numerous surface active compounds present in wastewater). Effective foam control methods have been put forward by a number of researchers, have been tested at full, pilot and laboratory scale and involve the inhibition of *Gordonia* and *M.parvicella* microorganisms.

2.4 Anaerobic digestion foaming

The following paragraphs look at operational parameters as well as the chemical and microbiological composition of sludge that have been reported in the literature

as foam inducing agents in AD. Each of them will be examined individually and the contribution to foam formation will be assessed.

2.4.1 Surface active agents

The term 'surface active agents' refers to substances that are either surfactants or bio-surfactants. The surfactants include oil, grease, volatile fatty acids, detergents, proteins and particulate matter (Vardar-Sukan 1998, Westlund et al. 1998, Barber 2005). However, the term 'particulate matter' as found in the literature is not clearly stated and can lead to confusion and misinterpretations. The particulate matter involves potentially the inorganic components of sludge, often referred to as grit, such as metals, sand and generally indigestible material that accumulates at the bottom of digesters. The term biosurfactants refers to substances produced during the metabolic activity of microorganisms found in sludge, such as hydroxylated and cross-linked fatty acids, glycolipids, proteins, lipoproteins, phospholipids and polysaccharide-lipid complexes (Kosaric 1992, Ron and Rosenberg 2002, Nitschke and Pastore 2006).

Surface active agents have both hydrophilic and hydrophobic properties. The hydrophobic ends of surface active agents tend to move towards the air phase, being forced out of the solution due to their hydrophobicity. The hydrophilic ends, on the contrary, tend to move towards the liquid phase. The orientation of hydrophobic and hydrophilic ends of surface agents in solution is schematically described in Figure 2.3. The accumulation of surface active agents' hydrophobic ends at the air-liquid interface increases the surface activity and lowers the surface tension of the solution. Foam initiation in aqueous solutions containing a surface active agent and providing air bubbles are introduced in solution can be predicted through determination of its critical micelle concentration, as explained in paragraph 2.2.3.

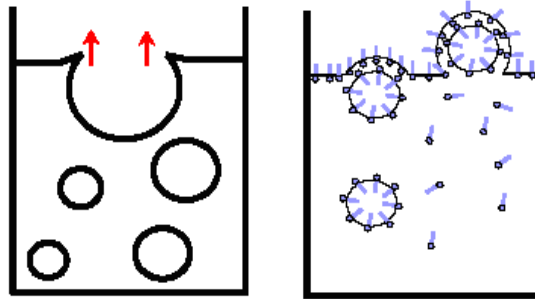


Figure 2.3: The impact of surface active agents' presence in an aqueous solution

(Source: North American Surface Specialties Coatings Additives business website)

All the above mentioned surface active agents, i.e. oil, grease, volatile fatty acids (VFAs), detergents, proteins and products from the metabolic activity of microorganisms are largely present in anaerobic digesters (Gerardi 2003). However, it is known that these substances break down in a digester into smaller and simpler compounds. A better look into the degradation pathways and the by-products of these compounds during anaerobic digestion could provide fundamental understanding of the impact of surface active agents on foaming during AD.

Proteins are complex, high molecular weight compounds with a relatively large surface area that do not dissolve or settle in wastewater. In sludge, they are found in solution as soluble microbial products but also attached to the solid particles as extracellular polymeric substances. Due to the size of proteins, microorganisms produce exoenzymes (proteases or peptidases) to break down the proteins into smaller compounds (amino acids) and subsequently absorb them into their cells to utilize the carbon source. Amino acids are converted to organic acids once inside the cells, which are then released along with ammonia into the bulk phase. Organic acids are the substrate for methane forming bacteria and as digestion proceeds CH_4 and CO_2 are produced (Gerardi 2003).

Proteins have been recognized as foam forming agents by many researchers and the cmcs' of many proteins are available in the literature, as explained earlier in paragraph 2.2.3 (Khan and Forster 1990, Rouimi *et al.* 2005, Foegeding *et al.* 2006, Glaser *et al.* 2007). Khan and Forster (1990) conducted aeration tests with a non-foaming AS to determine the impact of a protein in the foaming potential of AS. The protein used in the experiments was bovine serum albumen (BSA) at concentration of 2 g.l^{-1} . Khan and Forster (1990) reported that BSA induced foam in AS under aeration, however, with low stability. Vardar-Sukan (1998) stated that proteins exhibit their lowest solubility and highest foaming potential at their isoelectric point, which is highly dependant on the pH of the medium. So far no information is available in the literature on how different proteins affect the foaming potential in anaerobic digesters and what concentrations are critical above which foaming is induced. There is indication that BSA would induce foaming in digester feed sludge under aeration as it is a mixture of primary and SAS and according to Khan and Foster (1990), BSA induced foaming in AS under aeration. However, proteins are broken down to amino acids in anaerobic digesters by exoenzymes. Gonzales *et al.* (2003) found that the protein content in AD was less biodegradable than fiber and lipids and that there was a final equilibrium concentration value of 8.41 mg.g^{-1} for each non-foaming sludge that was independent of the initial protein concentration. The maximum initial protein concentration tested in this study was 44.8% of dry matter of sludge. Accumulation of proteins at the air/liquid interface could be facilitated during AD due to their surface active properties, which could then lead to enhanced foaming potential. On the other side, the interaction of proteins with other proteins, solids and other compounds in solution could also affect the behavior of proteins, such as the electrostatic interactions reported by Glaser *et al.* (2007) between BSA and protamine resulting in a molecular double layer entrapping liquid, which reduced drainage thus increasing foam stability. Other types of interaction include the affinity of proteins to fat, as described by Eisner *et al.* (2007), in the protein – fat mixture containing 9.75% molten butter

(82% fat content), 11.3% spray dried skim milk powder (low heat), 12% sugar, 4% glucose syrup solids, 0.1% locust bean gum and 0.1% guar gum by weight, which resulted in bridging between adjacent foam bubbles and between bubbles and the bulk solution resulting in reduced foam drainage and hence the creation of more stable foams. However, it was demonstrated in the same report that the presence of nonionic emulsions of monolaurate (0.9 μM), monooleate (0.7 μM) and trioleate of sorbitan (0.3 μM) in the protein – fat matrix reduced the foaming potential and stability. Further investigation on the effect of proteins, the proteins by-products and potentially the production of exoenzymes that could affect the foaming potential in anaerobic digesters is considered necessary.

Volatile acids are a group of organic acids, often described as volatile fatty acids (VFAs). They can vary in length but generally are low molecular weight compounds, soluble in water and sludge. Seven of the commonest fatty acids found in anaerobic digesters are formic acid (HCOOH), acetic acid (CH_3COOH), propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$), butyric acid ($\text{CH}_3(\text{CH}_2)_2\text{COOH}$), valeric acid ($\text{CH}_3(\text{CH}_2)_3\text{COOH}$), iso-valeric acid ($(\text{CH}_3)_2\text{CHCH}_2\text{COOH}$) and caproic acid ($\text{CH}_3(\text{CH}_2)_4\text{COOH}$). The suggested ranges of the above acids in digesters vary between 50 and 300 mg.l^{-1} as total VFAs concentration. Acetic acid is the predominant acid and accounts for approximately 85% of the volatile acids content in an anaerobic digester (Metcalf and Eddy 2003, Gerardi 2003). Accumulation of acetic acid has been identified in the literature as a foaming cause by many researchers (Pagilla *et al.* 1997, Westlund *et al.* 1998, Barjenbruch *et al.* 2000). This is understandable as methanogenic bacteria are the only bacteria that utilize acetic acid and, as described in paragraph 2.1.1, they are characterized by slow growth rates, which indicates that fluctuations resulting in excess acetic acid concentrations in an anaerobic digester would result in degradation of only the maximum uptake of acetic acid by the methanogens with the remaining acetic acid lowering the pH of the digester and inhibiting the digestion process. However, there is no experimental or quantitative evidence in the literature to support the above

interpretation that accumulation of acetic acid leads to foaming in AD and the critical concentration of acetic acid in sludge beyond which digestion inhibition and potentially foaming occurs is unknown.

Lipids are extremely hydrophobic organic molecules that do not dissolve in water. Due to their hydrophobicity, lipids are attached to the solid particles in sludge. The most common lipids in municipal and industrial wastewater, and subsequently in sludge are fats and oils. Fats and oils that enter a digester, although surface active agents as previously stated, are hydrolyzed to simpler compounds (glycerol and fatty acids) to give ultimately organic acids (Gerardi 2003). Fats and oils are mainly present in primary sludge at concentrations between 6.4 to 14.8% of dry matter but can also be detected in digesters and SAS in smaller concentrations (digesters: 2.4 – 9.0%, SAS: 0.8 – 2.52% of dry matter) (Gonzales *et al.* 2003). Gonzales *et al.* (2003) found that lipids were utilized by microorganisms in AD faster than proteins and similarly, there was a final equilibrium concentration value of 1.07 mg.g^{-1} for each sludge tested that was independent of the initial lipid concentration. Given the hydrophobicity of lipids but also their degradability during AD, it is not clear whether lipids would potentially accumulate on the surface of the bulk phase in an anaerobic digester, losing contact with the majority of bacteria found in the bulk phase and hence leading to partial degradation of fats and oils and increased surface activity. The biogas bubbles could become entrapped due to the surface active properties of the lipids and potentially induce foaming. However, additional experimental data demonstrating a clear contribution of lipids to the sludge's foaming potential during AD were not found in the literature. There is indication that lipids contribution to foaming in AD is potentially smaller than the proteins contribution due to the low degradability of proteins and accumulation of lipids at the air/liquid interface resulting in increases surface activity could be eliminated by maintaining a homogenous digester.

Detergents are another group of compounds recognized as surface active agents, as mentioned earlier in this section. Detergents present in wastewater derive from industrial effluents, such as breweries, dairies, paper and textile industries but also the municipal wastewater. Industrial effluents can significantly increase the concentrations of detergents that enter a STWs to such extent where they can inhibit biological treatment processes (Leitao *et al.* 2006). The most important group of detergents is the linear alkylbenzene sulphonates (LAS). LAS are characterized as anionic surfactants and are the most frequently used world wide in both domestic and industrial applications. It has been found by Jensen (1999) that a large amount of LAS is adsorbed onto the particles and organic matter of sludge and is removed from the wastewater via primary sludge. Due to the high degradability of LAS under aerobic conditions, primary sludge is the only stream that will contain substantial detergent concentrations. However, the amount of LAS in the final sludge (mixture of primary and secondary sludge) is highly dependant on the site processes. (Table 2.1 shows the concentrations of LAS found in sludge derived from different STWs)

Table 2.1: Concentrations of LAS in sewage sludge from different STWs

Country	Number of STWs	Sludge description	LAS concentration (mg/kg dry wt)
Germany	8	Anaerobically digested	1600-11800
Germany	10	Aerobically digested	182-432
Italy	1	Anaerobically digested	11500-14000
Spain	2	Non-treated	400-700
Spain	2	Aerobically digested	100-500
Spain	5	Anaerobically digested	7000-30200
Switzerland	10	Anaerobically digested	2900-11900
UK	5	Anaerobically digested	9300-18800

(Source: Jensen 1999)

Prats *et al.* (1997) examined the removal of anionic (LAS) and nonionic detergents in wastewater treatment plants. The findings from this study revealed that during sludge settling and subsequently anaerobic digestion of sludge, the degradation of the nonionic detergents was 27% and only 7% for LAS. Jensen's (1999) finding that detergents are adsorbed onto the solids and organic matter is also supported by Prats *et al.* (1997) who showed that most of the detergent was removed by attachment to the suspended solids. According to Petrovic and Barcelo (2004), LAS concentrations in sewage sludge can range from 100 mg/kg to 30 g/kg and are highly dependant of the site processes. In the same report, it is also stated that LAS concentrations in sludges obtained from three STWs in Spain were in the range of 8.4–14.0 mg.g⁻¹ (average 12.6 mg/g) and 12.1–18.8 mg.g⁻¹ (average 15.8 mg.g⁻¹) before and after digestion, respectively. However, it is not stated in the report whether foaming was recorded in the digesters of the STWs in Spain. Along with LAS, Petrovic and Barcelo (2004) examined other groups of detergents such as NPEOs_n (nonylphenol ethoxylates, n: 1 – 15) and AEOs (alcohol ethoxylates) and found that significant amounts of short-chain NPEOs and AEOs are also retained during anaerobic digestion. Typically, they mentioned that NPEOs concentrations range from a few mg/kg to over 500 mg.kg⁻¹, and for AEOs, which are the second most widely used surfactants worldwide, maximum concentrations can reach 300 mg.kg⁻¹ and removal efficiencies range from 33% to 86% during AD. The low removal of detergents during AD, especially for the anionic detergents, along with their properties as surface active agents results in increased surface activity in sludge that could potentially contribute to foaming events in AD.

The information found in the literature for biosurfactants in sewage sludge, such as glycolipids, lipoproteins, phospholipids, polysaccharide-lipid complexes and their association to foaming is limited, potentially due to the numerous and complex compounds present and the variability of these compounds between different sludges. Indirect biosurfactants measurements have been conducted by researchers in wastewater, soil or other bacterial culture media samples (Pirog *et*

al. 2003, Verma *et al.* 2006, Nitschke and Pastore 2006). Nitschke and Pastore (2006) conducted surface tension measurements in a wastewater based culture medium of a bacterial species to monitor the biosurfactants production. The biosurfactant, which was identified as a lipopeptide, reduced the surface tension of the culture medium to 26 mN.m^{-1} at concentration of 3g.l^{-1} while its cmc was 33 mg.l^{-1} . The large and diverse microbial population in anaerobic digesters would suggest that the production of biosurfactants in digesters is significant. However, biosurfactants are present in AD under non-foaming conditions. It is not clear whether an upset in the metabolic activity of microorganisms in AD is necessary to result in higher production of biosurfactants that would facilitate foaming. Therefore, biosurfactants might not be a direct AD foaming cause but an effect of an underlying cause that triggers the production of biosurfactants. Additionally, the likelihood of these compounds to induce foaming in a digester would probably depend on the type of biosurfactants present and their concentrations. No conclusion can be made at this stage for the contribution of biosurfactants in AD foaming due to lack of experimental evidence.

In summary, a large number of compounds commonly found in anaerobic digesters are surface active. The impact of surface active agents on AD foaming depends on the properties of each compound. The literature has suggested that the effect of proteins in a digester is greater as they are less biodegradable than lipids and fiber. Accumulation of acetic acid has been identified as a foaming cause and anionic detergents presence in AD is significant due to their low degradability under anaerobic conditions. During digestion, however, two major factors need to be taken into consideration, a) interactions between these compounds could enhance or reduce their foaming potential, which would result to either unstable or metastable foams and b) the fact that the surface active agents are ideally broken down to simpler compounds (organic acids) and are utilized by the bacteria. Unstable digestion, however, such as accumulation of acetic acid due to increased acetic acid in the feed and its partial utilization by the methanogens, as explained

earlier, or accumulation of proteins and detergents due to their low degradability during AD, could initiate or contribute to foaming. Therefore, it is necessary to determine the critical concentrations of surface active agents necessary to induce and / or stabilize foaming in AD along with the impact of the metabolic activity that alters the sludge quality characteristics.

2.4.2 Filamentous microorganisms

A number of reports in the literature have identified *Gordonia* species and *Microthrix parvicella* as the cause of foaming in AD (Pagilla *et al.* 1997, Westlund *et al.* 1998, Moen 2003, Barber 2005). However, there is no differentiation between foam initiation causes and foam stabilization causes when referring to the above filamentous species. *Gordonia* species and *Microthrix parvicella* are present in anaerobic digesters via surplus activated sludge (SAS). They can be present in the liquid phase but also bound to the flocs. Although, they are primarily aerobic organisms, literature has shown that they can survive under anaerobic conditions, as discussed in following paragraphs. Their hydrophobic properties tend to drive the filamentous microorganisms towards the air/liquid interface as the microorganisms become attached to the biogas bubbles. The accumulation of filamentous microorganisms on the air/liquid interface of anaerobic digesters along with the potential of biosurfactants production, results in lower surface tension of sludge and enhanced foaming potential (Eikelboom 2000, Barber 2005).

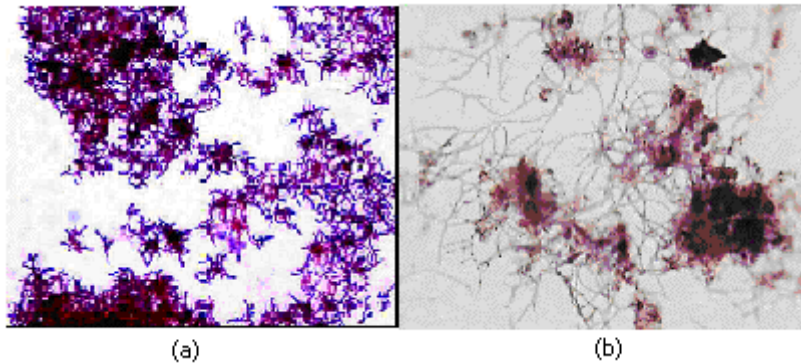


Figure 2.4: (a) Actinomycetes after staining in an activated sludge sample, (b) Filamentous microorganisms after staining in sludge samples

(Source: Environmental Leverage website)

Hernandez and Jenkins (1994) studied the fate of *Gordonia* during mesophilic anaerobic digestion of sludge. Severe foaming was induced at laboratory scale batch digestion experiments at concentrations of *Gordonia spp.* between 0.05-0.1 gram *Gordonia* per gram total solids. That concentration matched the range of gram *Gordonia* per gram total solids observed at full scale digesters that experienced foaming. Hernandez and Jenkins (1994) reported that, although *Gordonia spp.* are known to be obligate aerobes, they survived under anaerobic conditions with only 37% filament reduction at a 14-day SRT and 60% of filaments capable of respiration after 14 days. Another interesting finding was that viability of *Gordonia spp.* decayed more slowly in single-phase digestion than in two-phase with a first order rate coefficient of $0.02.\text{day}^{-1}$. Mamais *et al.* (1998) supported these findings by stating that *Microthrix* is capable of surviving under anoxic and / or anaerobic conditions.

Westlund *et al.* (1998) monitored the presence and abundance of filaments during an anaerobic digester foaming incident at the full scale. Filaments were identified microscopically in the foam and bulk phase of sludge. The dominant species of filaments was recognized as *Microthrix parvicella*. Table 2.2 lists the characteristics

of the sludge and foam samples obtained from the digester during foaming and compares them with sludge characteristics obtained from the same digester when foaming was not recorded. Westlund *et al.* (1998) carried out laboratory tests with samples obtained from the foam phase of the digester. The foam samples were collected in a glass vessel and the potential to force the foam sample to foam again was tested by shaking the glass vessel. The foam sample collapsed, when shaking the glass vessel, to produce sludge and it was not possible to foam again. *Microthrix* filaments were found attached to the gas bubbles in the foam samples after microscopic examination. According to Westlund *et al.* (1998) it was concluded that the foam in the digester was produced by *Microthrix*, which was bound to the gas bubbles during digestion. The binding between the gas bubbles and the filaments was strong in order not to release the gas during digestion and only by shaking the foam the gas bubbles could be released and foam could be destroyed.

Table 2.2: Operational data and filament abundance of a full scale anaerobic digester during foaming

Monitored parameter	Foam	Sludge¹	Sludge²
pH	-	7.3	7.2
Tot. Alkalinity (g/l)	-	3.3	3.5
Total Solids (%)	6.0	2.2	2.4
Volatile solids (%)	70	60	55
Filament abundance	5	0-1	0-1

¹ Sludge from the digester during a foaming period

² Sludge from the digester during a non-foaming period

(Source: Westlund *et al.* 1998)

Pagilla *et al.* (1997) also monitored the levels of filaments along with other operational data of two full scale anaerobic digesters for a period of 10 months. Foaming was recorded in the digesters during the period of sampling. The two

digesters were run under the same operational conditions but one of them was mechanically mixed and the other gas mixed. The presence of excessive levels of *Gordonia* in the feed sludge (up to 10^7 numbers per gram VSS) coincided with an increase of the foam layer with a more pronounced effect in the gas mixed digester.

Soddell and Seviour (1995) determined the ability of mainly *Nocardia* and other filament species to grow in a wide temperature range. The filaments were isolated from foaming activated sludge and cultivated in laboratory conditions at different temperatures. The majority of the filaments species examined could grow in cultures in the mesophilic range of 30-35°C, same as the temperature range in mesophilic digesters, indicating that the temperature in mesophilic AD has no adverse impacts in filaments growth. A major factor that needs to be taken into consideration is that in cases of foaming in mesophilic AD, the temperature in the foam matrix is lower than the temperature in the bulk phase, which, according to the study of Soddell and Seviour (1995), favors the growth of more species once found in the foam matrix.

According to the information provided above, *Gordonia* and *Microthrix* are the species that have been found to induce foaming in AD. The same species were found responsible for AS foaming. The findings of Hernandez and Jenkins (1994) clearly demonstrated at both full and laboratory scale that concentrations between 0.05-0.1 gram *Gordonia* gram total solids resulted in severe foaming during AD. However, two different species of filaments were identified as the causative foaming agent at full scale digesters in the reports by Pagilla *et al.* (1997) and Westlund *et al.* (1998). Earlier in this section, knowledge on AS foaming was reviewed in order to identify potential similarities between AS and AD foaming. Recent publications on AS foaming have shown that it is the biosurfactants production by *Gordonia spp* and *Microthrix* and potentially other mycolata that initiate foaming but the bacteria that stabilize it. So far, only the presence and

abundance of these species has been investigated in relation to AD foaming and not the biosurfactants production. There is evidence that the reduction in filament numbers in AD is small and hence the production of biosurfactants by these species could still occur during AD indicating the same foaming mechanisms in both AS and AD. Investigation of the impact of other filament species with similar morphological characteristics (i.e. hydrophobicity due to mycolic acids) on foaming in AD would provide useful information about the extent filaments are responsible for foaming.

2.4.3 Temperature

Dohanyos *et al.* (2004) reported that foam generation is facilitated more in mesophilic digestion than in thermophilic digestion. This could be attributed to the effect of higher temperatures on surface tension and viscosity of sludge and hence foam drainage, as explained in previous paragraphs. Gerardi (2003) states that temperature fluctuations of 2°C can significantly affect the bacterial activity, lead to accumulation of surface active agents and promote foaming, although no experimental evidence is being provided. Taking into consideration Gerardi's statement, thermophilic digestion can possibly be effective in foam destruction when foam is present and the temperature is constantly stable. Maintaining the temperature up to the thermophilic range of 50-70°C though is more difficult than maintaining it in the mesophilic range (30-35°C) and thermophilic digestion is more likely to be subjected to temperature fluctuations, accumulation of non-degraded substances and potentially foaming. Chae *et al.* (2008), on the other hand, provided experimental evidence on the effect of temperature fluctuations during anaerobic digestion of swine manure and showed that a temperature decrease and subsequent increase from 35°C to 30°C to 32°C affected only the biogas yield, which however, returned to initial values quickly after the temperature was

stabilized. No reference was made to foaming in the report during the digestion period.

This section aimed to examine the relationship between the type of digestion in terms of operational temperature range (mesophilic versus thermophilic) to foaming but also investigate the impact of temperature fluctuations during AD on foaming. It was evident that for foaming digesters temperatures in the mesophilic range facilitate mestable foam generation more than temperatures in the thermophilic range of AD due to the impact of temperature on the foam kinetics and properties such as viscosity and drainage. Yet, the effect of temperature fluctuations to the metabolic activity of microorganisms in AD, potential accumulation of surface active agents due to the microbial upset and ultimately foaming is not clear as the only experimental information found in the literature shows no impact of temperature fluctuations during AD on foaming. It is however, an area of further investigation as temperature fluctuations can potentially be a common problem at full scale digesters due to technical problems frequently arising from digester operation, such as equipment failure and poor monitoring.

2.4.4 Mixing and digester shape

Mixing aims to achieve optimum process performance by keeping the bulk phase in a digester in suspension and in full contact with the bacterial population (Metcalf and Eddy 2003). Mixing is therefore critical to avoid the creation of dead zones and a corresponding reduction of the active volume of the digester (short-circuiting of sludge). Pagilla *et al.* (1997) studied foaming in a gas-mixed and a mechanically-mixed digester receiving the same feed and operated under similar conditions (loading, temperature etc.) and stated that the gas-mixed digester accumulated more foam than the mechanically-mixed digester. It is believed that gas mixing provides favorable conditions for foam generation due to the presence of bubbles

in the bulk phase that promote attachment of the surface active and hydrophobic compounds found in sludge onto the bubbles. As the bubbles rise to the surface of the liquid in digesters, the surface active and hydrophobic compounds form a liquid film around the bubbles that prohibits the bubbles from bursting, increases the surface activity and results in higher foaming potential. Barber (2005) also identifies gas mixing systems in anaerobic digesters as an operational cause of foaming. Moen (2003) also reports that fine bubble gas mixing systems along with excessive mixing are considered as causes of foaming for anaerobic digesters.

In addition, several advantages and disadvantages have been identified between different digester shapes, according to the literature. Cylindrical digesters have a relatively big surface area compared to egg-shaped digesters allowing large volumes of gas to be stored and facilitating the accumulation of scum and foam. On the other hand, egg-shaped digesters have a very limited surface area above the bulk phase of the digester reducing the scum and foam accumulation potential. Poor mixing and grit accumulation has been observed in cylindrical digesters creating dead spaces and short circuit of sludge whereas no such reports were found for egg-shaped digesters. Clearly, cylindrical digesters are more commonly used due to the cost implications of egg-shaped digesters but no information so far has suggested that egg-shaped digesters can prevent foaming occurrence (Metcalf and Eddy 2003).

In summary, mixing along with digester shape play an important role in anaerobic digestion efficiency. There is evidence suggesting that gas mixing contributes to foaming but there will have to be critical concentrations of surface active material and filamentous bacteria in a gas mixed digester that would attach to the gas bubbles, prolong the bubbles life and hence result in foaming. On the other hand, failure to maintain sufficient mixing during digestion results in stratification and short-circuiting of sludge potentially affecting the microbial activity due to substrate availability. Under these conditions surface active agents and other non-degraded

hydrophobic material could rise to the surface of the bulk phase in a digester and potentially induce foaming. It is crucial, therefore, to monitor the mixing efficiency in full scale digesters and investigate any relation of foaming occurrence to inadequate mixing. Lastly, digester shape should allow efficient digestion to take place in a homogenous bulk phase.

2.5 Foam control

Due to the significant impacts of foaming in AD, as addressed in the introduction, foam destruction and control is highly important for the water utilities. Great interest has been given by a number of researchers in the identification of effective foam destruction mechanisms. Control measures for foaming destruction and prevention in industrial applications are generally categorized in three groups, mechanical methods, physical methods and chemical methods. In the following paragraphs, each of these methods is addressed in detail (Vardar-Sukan 1998, Barber 2005, Riera *et al.* 2006).

2.5.1 Mechanical foam control methods

Mechanical methods for foam destruction generally involve the application of shear stress on the foam matrix. There have been a number of devices used for mechanical foam destruction in many industrial applications. Each one of them is described in the following paragraphs. The main advantage of mechanical foam destruction is that there are no downstream effects when applied to the sludge stream, since no chemicals are used. Therefore, in industrial applications there are no additional costs for downstream treatment before discharge. However, the industries have to consider the energy demand of operation and most importantly that mechanical treatment is only a responsive and not a preventative method.

Another limiting factor in the mechanical treatment of foams is the likelihood of shear damage to the microorganisms population in a bioreactor (Vardar-Sukan 1998, Barber 2005).

One of the types of mechanical treatment devices includes injectors, ejectors, orifices and vacuum systems. The principle in the operation of these devices is the sudden pressure drop within the foam matrix that forces the bubbles to burst (Vardar-Sukan 1998, Barber 2005, Riera *et al.* 2006). Another configuration of foam destruction mechanisms are the revolving disks, impellers and stirrers, which are placed on the top section of a reactor so that a part of them is submerged into the foam. The pressure within the foam matrix is altered as the submerged parts move and foam collapses (Vardar-Sukan 1998, Barber 2005). Centrifuges and cyclones have also been in use for foam destruction. Their operation is based on centrifugal forces. The foam passes through the device and under the influence of the centrifugal forces the bubbles coalesce onto the walls of the tube. The gas in the bubbles is released and collected at the centre of the tube and subsequently discharged out of the device. The remaining liquid phase from the foam is returned into the reactor (Vardar-Sukan 1998, Barber 2005).

Mechanical vibrations are another effective foam destruction mechanism and are widely used in the confectionery and building industries to increase the fluidity of soft solids and viscous fluids, such as chocolate, and enhance their flow. The exact mechanisms of mechanical vibrations on soft solids and viscous liquids have not yet been fully understood and information on the effects of mechanical vibrations on foam destruction is limited (Morey *et al.* 1999).

Research has been conducted on the effectiveness of mechanical vibrations at breaking static foams generated from non-Newtonian shear thinning liquids (Morey *et al.* 1999). Sharman (1969) defines non-Newtonian liquids as all liquids, which when subjected to a shearing stress, the rate of shear is not proportional to the

shearing stress. Alternatively, in Newtonian liquids the ratio of the shearing stress to the rate of shear is a constant known as the coefficient of viscosity or viscosity of a liquid. As an example for the reader's better understanding, Metcalf and Eddy (2003) classify water, oil and unthickened activated and trickling filter sludges as Newtonian liquids whereas thickened sludges are recognized as non-Newtonian liquids. Morey *et al.* (1999) used an experimental test rig to investigate the effects of mechanical vibrations on a surfactants solution. The experimental trials showed that mechanical vibrations were effective at destabilizing static foams produced from non-Newtonian solutions by enhancing foam film collapse and liquid drainage. Morey *et al.* reported that the exact mechanism of mechanical vibrations for foam destruction in the experimental trials was based on the shear thinning mechanism resulting in a reduction in yield stress and shear viscosity of polymer base liquids. However, mechanical vibrations were not effective in Newtonian liquids. The effectiveness of mechanical vibrations was found to be highly governed by the optimum vibration amplitude and frequency (Morey *et al.* 1999).

The effectiveness of mechanical vibrations on foams in anaerobic digesters has not been investigated. As sludge in digesters is recognized as non-Newtonian liquid, mechanical vibrations would be expected to be effective on foam destruction. However, the applicability of mechanical vibrations as a foam control method in foaming digesters is not clear due to space requirements and operational complications that might arise.

Another effective method of foam destruction, as found in the literature, is the liquid sprays. Moen (2003) and Barber (2005) reported that water sprays have been used in digesters headspace for foam suppression. Yet, the volume of water added in the digesters active volume has a significant impact on the hydraulic retention time. An alternative to that approach was digested sludge sprays over the foam layer at the top of the digesters through large bore nozzles. This method was

successful when applied at a STWs but generated operational concerns due to blockages of the nozzles (Moen 2003).

2.5.2 Physical foam control methods

Physical methods for the destruction of foams that have been in use are the application of ultrasound, thermal disintegration and electrical treatment. Similarly to the mechanical methods, physical methods are considered to have no downstream effects regarding the chemical composition of the treated effluent since no chemicals are added. Therefore, physical methods are highly recommended in processes where contamination is to be avoided. However, these methods may not be appropriate for use in bioreactors due to their impact on microorganisms (Vardar-Sukan 1998, Morey *et al.* 1999, Barber 2005).

- **Ultrasound vibrations**

There is an extensive literature on ultrasound vibrations and their effect on foam destruction. The first applications of ultrasound to control foam formation were made in the 1950s in fermenters, in jet fuel tanks during pumping and in many degassing systems. The majority of the first of these applications were based on aerodynamic acoustic sources of various types. The most well known ones are the Hartmann whistle and the rotatory siren. However, these systems had to deal with noise, the need for high air generation capacity, the control of the air-flow and the high energy consumption (Riera *et al.* 2006).

The exact mechanism of foam destruction due to ultrasound vibrations is not yet well understood. However, the ultrasound vibrations are generally associated with acoustic pressure, undirected radiation pressure, induced resonant vibrations in the bubbles, high internal pressure in foam bubbles as compared to that in the

surrounding area, vacuum caused by sonic energy and turbulence produced by sonic waves (Sandor and Stein 1993, Morey *et al.* 1999, Barber 2005, Riera *et al.* 2006)

The main principle of ultrasound foam destruction is based on the deliberately induced vibrations that enhance film drainage resulting in the destabilization of foam. Ultrasound vibrations can produce surface waves on liquids. Surface waves, when applied on liquids films in foams, can induce drainage only when they are accompanied by waves on other surfaces of the films. Ultrasound vibrations can also stimulate the liquid flow in the foam matrix. It should be noted though that the orientation of the liquid films in a foam matrix is random and therefore, under ultrasound vibrations, the induction of liquid motion without systematic connection with surface waves would result in liquid transportation from the Plateau borders into the films and the opposite. When surface waves and liquid motion occur simultaneously foam drainage is accelerated destabilizing foam (Sandor and Stein 1993, Morey *et al.* 1999)

Ultrasound and its effects on foam destabilization and destruction have been extensively tested, experimentally, in different solutions. Sandor and Stein (1993) studied the effects of ultrasound in destabilizing foams created by a surfactants solution (0.0025 M of sodium dodecyl sulphate in distilled water). According to the findings of the experiments, Sandor and Stein concluded that ultrasound vibrations can effectively destroy foam. They also proved that when a broad vibrating tip is used for the generation of ultrasound waves and at a small distance from the foam, foam destruction is significantly accelerated. It is stated, however, that particular consideration needs to be taken to the determination of the optimum frequency and amplitude of ultrasound (Sandor and Stein 1993).

Morey *et al.* (1999) reported the effects of ultrasound in destabilizing unstable and metastable foams in surfactants solutions. The solution contained 0.5 g/l NaDBS in

distilled water. A sonicator was placed at the top end of the apparatus containing the solution. Foam was generated and the sonicator was operated at a fixed frequency and varied amplitude. The series of experiments showed that ultrasound vibrations were efficient at destabilizing metastable foams but were also effective at controlling unstable foams produced by either Newtonian or non-Newtonian liquids. Ultrasound vibrations were proved to be, in this report, highly suitable for processes that require continuous defoaming. It was also highlighted that the effectiveness of ultrasound was greatly related to the frequency and amplitude of vibrations (Morey *et al.* 1999).

Sandor and Stein (1993) and Morey *et al.* (1999) findings suggest that ultrasound could potentially be effective as a foam destruction mechanism in anaerobic digesters where continuous defoaming is necessary. The great advantage of the method is the effective destruction of both unstable and metastable foams, which appear in anaerobic digesters. The only limitation on its application to anaerobic digesters is to ensure that the microbial population in the bulk phase remains unaffected.

- **Thermal disintegration**

Thermal methods are based on the effect of temperature on the foam matrix. Higher temperature can result in intensive expansion of bubbles and evaporation of the liquid films that facilitates rupturing of the bubble lamellae. Surface viscosity is reduced and freezing and / or reduction in surface tension is noticed. Some of the applications of thermal treatment include hot weirs or heating coil placed over the surface of a solution or to the upper part of a reactor, respectively (Vardar-Sukan 1998, Riera *et al.* 2006).

Thermal disintegration methods have not been applied yet to anaerobic digesters as a foam control method. The effect of temperature rise in the foam matrix on the overall digestion efficiency needs to be considered.

- **Electrical treatment**

Electrical treatment of foam is based on the generation of an electric current passing through the foam matrix. However, the mechanisms of foam destruction due to the electric current are not well understood (Vardar-Sukan 1998, Riera *et al.* 2006). Riera *et al.* (2006) have suggested that the effect of electric current is possibly due to the forces created acting differently on the liquid and on the gas. However, due to limited information in the literature on electrical foam destruction, it is difficult to assess its applicability and effectiveness in AD.

2.5.3 Chemical foam control methods

Chemical methods for foam destruction involve the use of chemical compounds, known as antifoam agents, with the ability to change the interfacial properties of a liquid. Chemical foam destruction is widely used in many industries but its use is based on an empirical approach both with regards to the type of chemical used and the amount added. The main problem of the use of chemicals is the adverse downstream effects in processes and the possibility of contamination. Moreover, in many processes the potential mass transfer limitations might need to be taken into consideration. However, the major advantage of chemical treatment is that it is a preventative method and simple in application and operation. Currently, there are more than 700 commercially available anti-foam agents with a wide range of applications. Yet, the anti-foaming characteristics and the mechanisms of foam destruction are not well known (Vardar-Sukan 1998, Barber 2005, Riera *et al.* 2006)

Antifoam agents are generally surface active substances that lower the viscosity of liquids and prevent metastable foam formation. Foam collapses as a result of the tendency of the liquid containing anti-foam agents to attain the equilibrium between the surface elasticity of the liquid and the anti-foam agents (Vardar-Sukan 1998, Riera *et al.* 2006). The main compounds of anti-foam agents are usually oils, esters, fatty acids, polyglycols, siloxanes, alcohols, sulfites and sulfonates. Their mode of action is highly dependant on the nature of the agent, the type of foam and the nature of the substances causing foaming (Vardar-Sukan 1998).

There was only one reference found in the literature on antifoam dosing in AD. Westlund *et al.* (1998) reported that the antifoam polyaluminium salt (PAX-21, 3-6g Al per kg TSS per day) was used at three STWs but was successful only at one of the three. The failure of the antifoam in controlling foaming in the digesters at the other two plants was attributed to poor mixing of the antifoam with the sludge stream.

The relationship between the nature of the agent and the effects on foam can be seen in Figure 2.5 below. The performance of a number of antifoam agents in the destruction of synthetic foams is illustrated. The solution used in this experiment contained 60mM SDS (surfactant) and 200 mg.l⁻¹ of antifoam agent. Five different antifoam agents were tested in the same solution and equal amounts were added each time. It can be seen from Figure 2.5 that the majority of the antifoam agents had a significant effect on foam within 2 to 10 minutes from addition. After that period, foam destruction was much slower. In addition, different antifoam agents had different destruction rates (Barber 2005).

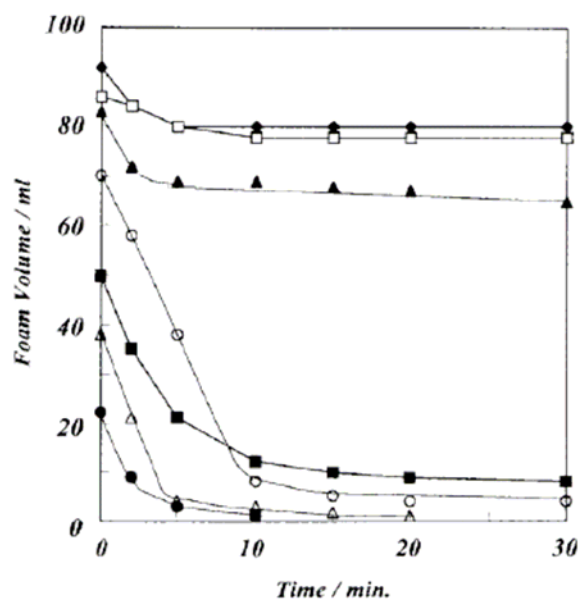


Figure 2.5: Performance characteristics of different antifoam agents on foam destruction. (◆) Blank, (▲) Silicone oil KF96, (□) Hydrophobic silica H51, (Δ) mixed type anti-foam agent F-16, (■) Silicone resin particle, (○) H51/KF96 : 5/95, (●) Silicone resin particle/KF96 : 5.95.

(Source: Barber 2005)

The performance of antifoam agents is also related to the pH of a liquid. Zhang *et al.* (2003) found that oil anti-foam agents did not destabilize synthetic foams when tested but when calcium and alkalinity were added, foam was destroyed.

The fact that different antifoams can have different results when applied on the same solution indicates that the selection of specific antifoams to be applied on a bioprocess such as AD, should be based on experimental results including the antifoam efficiency, the antifoam's destruction rate and the amount of antifoam required. The selection of the right antifoam is crucial in industrial applications to provide a sustainable solution. Further consideration needs to be given at the downstream effects of antifoams when applied in industrial applications and the

cost implications involved. So far, there are no reports found on the evaluation of different antifoams effectiveness on foam control in AD and more importantly a broad comparison of the cost implications of the use of different antifoams.

2.6 Conclusions

AD foaming is currently a recognized operational problem with severe impacts on the performance of STWs. This chapter aimed at providing a critical review of the current state of knowledge on AD foaming. Gaps in knowledge were underlined in order to identify limitations and areas of further research.

A review of the foam properties in anaerobic digesters was carried out in order to provide information about the nature of foam in AD. A differentiation between two foam types, the unstable foams with a lifetime of seconds and the metastable foams with a lifetime of days, was made based on the foams stability. The generation mechanisms of both foam types were attributed to the presence of hydrophobic material in sludge but the critical concentrations for the creation of either unstable or metastable foams are unknown, whilst the identification of the two foam types during AD was poor. The stability of foams and hence the differentiation between the two foam types was highly associated with foam drainage. Foam drainage rates however, were found to depend on surface viscosity. References in the literature demonstrated that an increase in surface viscosity reduces the foam drainage from the foam matrix resulting in metastable foams, such as the data provided by Bramforth (2004) where a 0.0002 Pa.s increase in the surface viscosity of beer reduced foam drainage and increased the time to reach a certain film thickness by 12%. Yet, the critical viscosity thresholds for the creation of metastable foams in sludge are unknown at this stage. According to data presented by Goel *et al.* (2004), an increase in volatile solids in digesting sludge from 1.2 to 3.2% increases viscosity from approximately 300 to

4000 mpa.s⁻¹ indicating that reduction of the organic matter in sludge would reduce viscosity and subsequently increase the foam drainage rates resulting in the destruction of metastable foams. Surface tension was also identified as a critical parameter in AD and was highly linked to temperature, viscosity and alkalinity. The correlation of surface tension and the foaming potential in AD is not yet fully investigated due to the lack of an established method for surface tension determination in sludge matrices. Lastly, the investigation of the film thickness and elasticity of digester foams, although a key parameter in foaming, would potentially not provide a representative evaluation due to the complexity of the process and the number of compounds found in sludge that can alter the foam properties during digestion.

Part of this chapter reviewed knowledge on foaming of other biological systems in order to gain knowledge from extensively studied areas, to apply the current understanding of biological-systems foaming to AD foaming and recognize potential similarities. AS foaming was chosen as the best studied example. The onset of foaming in AS plants has been attributed to surfactants and biosurfactants, although their critical concentrations for foam initiation have not been quantified. Foam stabilization has been attributed to the filamentous *Gordonia species* and *Microthrix parvicella*, yet experimental evidence suggests that the presence of mycolic acid-containing microorganisms is also associated with foam stabilization. Critical concentrations for foam initiation and stabilization for *Gordonia species* were approximately $2 \times 10^8 \mu\text{m} \cdot \text{ml}^{-1}$ and $1 \times 10^9 \mu\text{m}$ filament length per ml of AS, respectively, whereas persistent foaming was recorded at AS plants containing approximately 8×10^6 to 30×10^6 numbers of rod and coccoid mycolata per ml of AS and between 200 and 500 μm filament length for *M. parvicella* (Hwang and Tanaka 1998, Davenport and Curtis 2002, de los Reyes *et al.* 2002). However, detailed information on the exact foaming mechanisms in AS has not been found due to the complexity of the process.

The literature review suggested that the foaming causes have been found to be linked with either operational parameters (gas mixing, temperature fluctuations, digester shape) or the quality of feed sludge in terms of critical concentrations of surface active agents and filamentous bacteria for foaming. There was only one reference in the literature demonstrating that temperature fluctuations of 5°C during mesophilic AD did not result in foaming. There was also evidence that gas mixing potentially contributes to AD foaming only under critical concentrations of surface active agents and / or filamentous bacteria. Although the critical concentrations of surface active agents for foam initiation and stabilization in AD are not known due to the large number of compounds involved, the variability between sludges and the degradation processes of AD that change the quality characteristics of sludge, it is known that persistent (metastable) foaming is induced at concentrations of 0.05-0.1 gram *Gordonia* per gram total solids and *Microthrix* filament abundance of 5, according to Hernandez and Jenkins (1994) and Westlund *et al.* (1998). In conclusion, it is evident that further research on AD foaming causes is crucial so as to understand the underlying mechanisms of foaming in anaerobic digesters.

Part of the present chapter also aimed to present the current state of knowledge on foam control in AD. The literature review identified that mechanical foam control using spray nozzles inside the digesters was successful with however, operational problems due to blockages of the nozzles, whereas experiences of chemical control of AD foams showed that the selection of antifoam should be based on the antifoam's efficiency for a particular sludge type, as experimental findings demonstrated that different antifoams have a different effect when applied on the same solution (Moen 2003, Barber 2005). Additionally, a number of reports studying the destruction and control of synthetic foams were reviewed for the potential of application and effectiveness of other foam control methods on AD foaming. However, due to limited information on the operation of such methods on biological systems such as AD, this section was limited to recommendations on areas for further research involving the capital, operational and maintenance costs

of the methods reviewed, downstream effects and the effects on the microbial population in digesters.

Chapter 3: Materials and Methods

3 Materials and Methods

The work carried out for the purposes of this study involved both full scale and laboratory scale investigation of AD foaming. 17 STWs were visited in total during the 3-year period of the project. Initial experimental work involved a broad full scale examination of AD foaming through single visits at 15 STWs, of which 9 presented foaming in AD, for the completion of a site survey questionnaire and for collection of sludges which were subsequently analyzed in the laboratory. The laboratory work during the broad full scale investigation of AD foaming involved standard analysis of sludge including solids, VFAs, alkalinity, pH, filamentous bacteria and dissolved organics in order to identify differences between the foaming and non-foaming digesters regarding the sludges quality characteristics. Subsequent work involved a site specific long-term monitoring of a consistently foaming and a consistently non-foaming digester investigating the potential differences between the two digesters in terms of digester operation and digestion efficiency that would lead to the identification of the foaming cause(s) through sludge collection and subsequent advanced analysis in the laboratory (further determination of proteins and carbohydrates as soluble microbial products and extracellular polymeric substances, surface tension and chemical oxygen demand). The long term monitoring included 9 site visits for Site 12 with the first visit in 2006 and the following 8 visits a year later during a 6-month monitoring period (April '07 to October '07) and 15 site visits during 15 months (February '07 to May '08) for Site 16. However, it was also considered necessary to investigate foaming during AD at controlled laboratory environment due to limitations associated with on-site work, as explained in following paragraphs. Further laboratory investigation of AD foaming involved examination of the sludges foaming potential through foaming tests and batch anaerobic digestion experiments. Detailed information on the materials and methods used in this work is provided in the following paragraphs.

3.1 Site survey – Questionnaire

The site survey questionnaire involved systematic investigation of foaming at a number of UK's STWs aiming to define and understand the foaming problem in the UK. The objectives of the site survey were to record foaming occurrence at the full scale, identify potential links of foaming with digester and overall plant operation, review current practices employed at full scale for foam control and provide information on cost implications arising from foaming where possible. The objectives of this part of work were met through a questionnaire comprising of 35 questions, which was developed for the present work based on a previous survey study carried out by an MSc student at Cranfield University (Lamelot 2004). The questionnaire involved questions on the site's processes and performance, the digesters description (construction and operational characteristics), the digesters performance and potential technical problems/failures, foaming frequency, severity and duration, the operators experiences of foaming in terms of identified foaming causes by the operators and control actions and costs arising from foaming incidents. The questionnaire along with the answers are provided in Appendix A (Tables 1 – 6) and was completed for every STWs visited. The information was collected during the site visits by direct observation, interviewing the sites operators and managers and in cases, where additional information was required by phone conversations / interviews.

The selection of STWs was not geographically restricted as all catchment areas of the 6 UK water companies sponsoring the project were visited and included both small scale works of a population equivalent of around 43,000 (Internet source: Aker Solutions) and large scale works of population equivalent of around 1.2 million (Internet source: United Utilities Water Plc). As a result, there was no regional correlation with foaming at the full scale. The digesters were all operating

at the mesophilic range and were all treating municipal sewage sludge. In some cases, the digesters were receiving municipal sewage sludge imports and industrial imports. Where possible, a record of these imports was kept (i.e. type of import, type of industry, amount of import, frequency). Digesters that had regular foaming (foaming that was lasting for weeks or months and was reappearing) for over a year of operation prior to the initiation of this study were classed as foaming digesters and digesters that had no foaming for over a year of operation were classed as non-foaming. It was assumed that one year was sufficient time for this study to establish accurate differentiation between foaming and non-foaming digesters. The questionnaire was completed for 16 STWs of which 9 were foaming digesters (Site 7 – Site 15), all conventional mesophilic digesters but Site 11, which was characterized as alternative configuration digester as a pre-treatment stage such as pasteurization, enzymatic hydrolysis or acid phase digestion existed upstream of the mesophilic digestion. All foaming digesters were visited when foaming was present, according to the operators of the STWs. Similarly, for the non-foaming digesters (Site 1 – Site 6 and Site 16), two sites (Sites 5 & 6) out of the 7 had alternative configuration mesophilic digesters. The investigation of the effect of pre-treatment of sludge on digester foaming was considered necessary as several pre-treatment technologies have become increasingly popular for the water industry aiming at AD optimization in terms of biogas production. A brief description of the STWs visited is provided in Table 3.1.

Additional information obtained during the site survey involved the type of mixing (gas / mechanical) and frequency of maintenance at the digesters. A 6 – year period was set as the limit to differentiate between good and average maintenance as there was evidence, according to the operators and site managers experiences that a reduction in the effective digester volume could occur after 6 to 7 years of operation, although not quantified, and mixing systems recommended maintenance period, according to the literature, is after 8 to 10 years of operation (Biogas – Energy Inc.). Hence, digesters where maintenance work took place

within the last 6 years of operation were classed as good and digesters where maintenance work took place more than 7 years ago were classed as average. The digestion efficiency and organic loadings were assessed by sampling and subsequent analysis of sludges in order to calculate the full scale organic loading rates and the sludges quality characteristics. The detailed protocols of sampling and the analytical procedures are given in following paragraphs.

Table 3.1: Description of the 16 STWs visited in this study

Site / STWs		Pre-treatment	Antifoam dosing	Severity of foaming at the time of visit (scale from 1:low to 10:high)
Non-foaming	1	No	-	-
	2	No	-	-
	3	No	-	-
	4	No	-	-
	5	Pasteurization	-	-
	6	Acid phase digestion	-	-
	16	No	-	-
Foaming	7	No	Yes	9 – 10
	8	No	No	3 – 4
	9	No	Yes	1
	10	No	Yes	1 – 3
	11	Enzymatic hydrolysis	No	8
	12	No	Yes	1
	13	No	No	8 – 9
	14	No	No	9
	15	No	Yes	1 – 2

Complications during the site survey work involved difficulties collecting information, despite repeated attempts, and accuracy of data. Usually, at smaller

STWs the lack of monitoring systems on site and manpower was the main hindrance on information acquisition. At the larger STWs, information acquisition was becoming difficult due to the work load of operators and managers that in cases did not allow effective collaboration or information was passed on under the pressure of time.

3.2 Samples collection and storage

Unlike the site survey work, where several visits were made to STWs across the UK, the experimental work took place at Cranfield University laboratories. For the purposes of the experimental work, samples were obtained from the full scale of the STWs visited in all cases. Some STWs were visited more than once for samples collection and others only one time. This was due to the different experimental protocols followed in this study. Further details on single and multiple sampling from each site are given in following paragraphs. In general, four different sludge streams were sampled during the 3-year period of the project for the purposes of the experimental work and included primary sludge, surplus activated sludge, feed and digested sludge. Grab samples were obtained on all occasions. When the sampling point was a pipe outlet, acquisition of a fresh sample was ensured by discarding at least the first 10-15 litres of sludge to avoid collecting aged sludge. In addition to sludge sampling, foam sampling was carried out after generation of foam in the laboratory during bench scale batch anaerobic digestion, as explained in following paragraphs. Details of the sampling procedures are given below.

Samples had to be stored in suitable conditions that would not affect their quality characteristics until analysis was completed. Several reports were reviewed evaluating different sludge storage methods including storage at room temperature, refrigeration, freezing and freeze drying. Freezing and freeze drying

were found to have an effect on the sludges dewaterability and microbial population levels, whereas storage at room temperature and refrigeration had minimum effects on the sludges quality characteristics (Chu *et al.* 1999, Omerci and Vesilind 2001, Castro *et al.* 2002). For the purposes of this study, all samples were kept in plastic containers in a cold room at 4°C for no more than 4 days, with exception to digested sludge collected specifically for batch digestion in the laboratory which was placed in a water bath at 35°C immediately after collection to ensure viability of bacteria.

3.2.1 Primary sludge sampling

Primary sludge was sampled from a pipe outlet of the thickening process on site and before the blending tank.

3.2.2 Surplus activated sludge (SAS) sampling

Similarly, SAS was sampled from the outlet of the thickening process and before the blending tank.

3.2.3 Feed sludge sampling

Feed sludge samples were collected from a pipe outlet after the blending tank and upstream of the digester. The feed sludge contained in most cases a mixture of primary and SAS along with any sludge imports each site would receive. Site 14 was the only digester receiving primary sludge alone and not a mixture of SAS and primary. Generally, the ratio of primary to SAS in all feed sludges obtained was around 70% / 30% to 60% / 40%, according to the operators.

3.2.4 Digested sludge sampling

Digested sludge samples were collected either from an overflow pipe on top of the digesters or from a pipe outlet before the secondary digestion tanks, depending on each site's available sampling point.

3.2.5 Foam sampling

Foam samples were collected during the bench scale batch digestion studies of which details are given later in this section. Digestion bottles were removed from the water bath and foam samples were collected with a spoon by scraping the foam layer off from the bottles. The foam was then kept in plastic containers in a cold room at 4⁰ C until analysis was completed and for no more than 4 days. Foam samples collection at full scale was not carried out due to on-site limitations of accessing foam but also due to the absence of foam in most cases because of the antifoam dosing.



Figure 3.1: Foam sampling from digestion bottles

3.3 Analytical work

Analysis of sludge, foam and aqueous samples involved the determination of solids, pH, alkalinity, individual and total volatile fatty acids (VFAs), soluble chemical oxygen demand (SCOD), dissolved organic content (DOC), proteins and carbohydrates as extracellular polymeric substances and soluble microbial products. Analysis of biogas samples involved the determination of methane composition. All analysis was carried out in duplicate or triplicate unless stated otherwise. Part of the analysis was carried out in solids free sludge samples which derived after centrifugation (Rotanta 96 R centrifuge, Hettich Zentrifugen, Tuttlingen, Germany) and subsequent filtration through 0.45µm glass-fibre filter papers (70 mm Schleicher & Schuell Grade GF 52, Patterson Scientific, UK). The speed and duration of centrifugation varied depending on the type of analysis and more detailed information is provided on the paragraphs below.

3.3.1 Solids determination

Samples were analyzed for total solids (TS) and volatile solids (VS) according to the Standard Methods for the Examination of Water and Wastewater (APHA, Greenberg *et al.* 1998).

$$\% \text{ Total Solids} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \text{ (as a \% of the wet sludge)} \quad \text{Equation 3-1}$$

$$\% \text{ Volatile solids} = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \text{ (as \% of the total solids)} \quad \text{Equation 3-2}$$

Where: W_1 = Dish Weight, g
 W_2 = Weight of dish + wet sludge sample, g

W_3 = Weight of dish + dry sludge, g and

W_4 = Weight of dish + ignited sludge sample, g

Alternatively, the TS and VS concentrations were calculated as g/l based on the following equations:

$$TS \text{ (g.l}^{-1}\text{)} = \frac{(W_3 - W_1) \times 1000}{A} \quad \text{Equation 3-3}$$

$$VS \text{ (g.l}^{-1}\text{)} = \frac{(W_3 - W_4) \times 1000}{A} \quad \text{Equation 3-4}$$

3.3.2 Determination of full scale organic loading rate

Full scale digesters organic loading rate (OLR) was calculated based on the following formula:

$$\text{Organic loading rate (kgVS.m}^{-3}\text{d}^{-1}\text{)} = \frac{VS \text{ (kg.m}^{-3}\text{)} \times \text{feed (m}^3\text{.d}^{-1}\text{)}}{\text{digester volume (m}^3\text{)}} \quad \text{Equation 3-5}$$

3.3.3 pH determination

pH was measured in sludge and solids free samples by a Jenway 3540 pH meter. The pH meter was calibrated before use with standard buffers (VWR, UK) and could measure a pH range of -2 to 20 with an accuracy of ± 0.003 .

3.3.4 Alkalinity determination

Solid-free samples were analyzed for alkalinity after centrifugation at 2000g for 15 minutes and filtration. Alkalinity was determined by titration according to the Standard Methods for the Examination of Water and Wastewater (APHA, Greenberg *et al.* 1998). The titrate was 0.02M hydrochloric acid. The pH was constantly monitored during titration to determine the end point of the reaction, which was at pH of 4.5. The formula for the calculation of alkalinity as $\text{mg.l}^{-1} \text{CaCO}_3$ is given below.

$$\text{Alkalinity } (\text{mg } \text{CaCO}_3 \text{ .L}^{-1}) = \frac{A \times N \times 50,000}{\text{ml sample}} \quad \text{Equation 3-6}$$

Where A = ml standard acid used
N = normality of standard acid

3.3.5 Volatile fatty acids determination

Individual volatile fatty acids (VFAs) were determined by high performance liquid chromatography (HPLC) (Shimadzu VP Series, Shimadzu, UK). Sludge samples were centrifuged at 3000g for 3 minutes. The supernatant was acidified with concentrated sulphuric acid to stop microbial activity and stored at -20°C until analysis. Prior to analysis, the samples were filtered through $0.45 \mu\text{m}$ glass-fibre filter papers. No preparation was necessary for aqueous samples. A Biorad fermentation column (Cat 125-0115) was used for separation of VFAs. The column temperature was set at 65°C and 1mM sulfuric acid was used as mobile phase at 0.8 ml.min^{-1} flow. VFAs detection was performed by a UV detector at 208nm (Galanos *et al.* 1995, Sanford *et al.* 2002, Parawira *et al.* 2004). Calibration was carried out each time the column was used for the 6 VFAs, acetic acid (AA),

propionic acid (PA), n- and iso-butyric acid (nB, iB), n- and iso-valeric acid (nV, iV). The calibration curve was obtained by duplicate or triplicate analytical-grade standards in ultra-pure water (Fisher Scientific, UK) of 4 different calibration levels at 50 mg.l^{-1} , 100 mg.l^{-1} , 500 mg.l^{-1} and 1000 mg.l^{-1} . A typical chromatogram of absorbance corresponding to the second level of calibration (100 mg.l^{-1}) against time (minutes) is shown in Figure 3.2. The three peaks appearing before acetic acid correspond to water (retention time: 2.4 min), lactic acid 1 (retention time: 4.4 min) and lactic acid 2 (retention time: 4.9 min). Although originally lactic acid was included in the analysis, the amount of lactic acid found in sludge samples was so small that no further detection of lactic acid took place during this work.

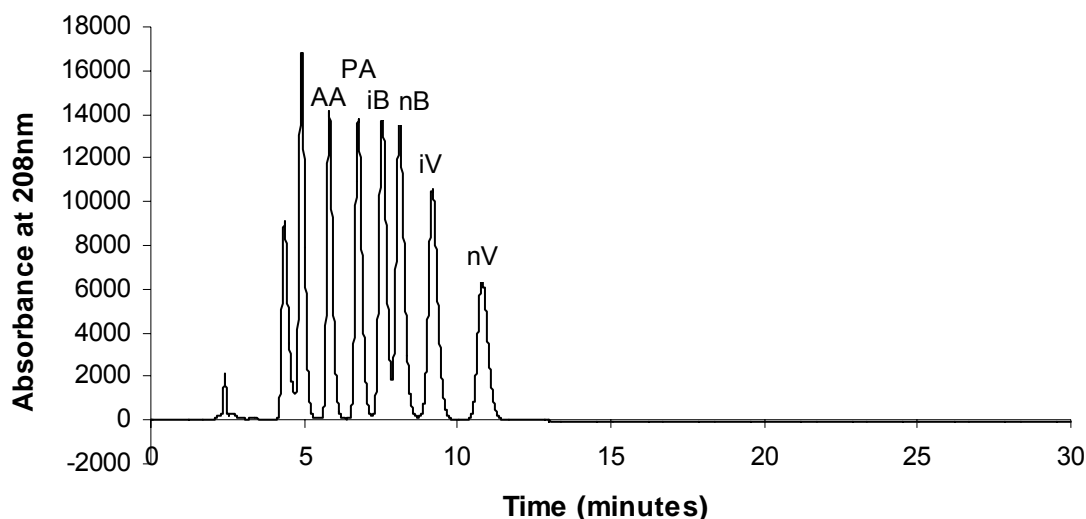


Figure 3.2: Absorbance chromatogram of individual VFAs provided by HPLC

Total VFAs were also determined according to the esterification method (Zhang and Zhu 2006, Hach, 1993) in solid-free samples. The method is based on esterification of the carboxylic acids present in a sample followed by colorimetric determination of the esters produced by the ferric hydroxamate reaction. VFAs are reported as their equivalent mg.l^{-1} acetic acid. 0.5 ml of solid free sample was added to a 25 ml HACH cell with 1.5 ml ethylene glycol and 0.2 ml of 19.2 N sulphuric acid. The sample was heated at 100°C for 3 minutes in a water bath and

0.5 ml hydroxylamine hydrochloride, 0.2 ml of 4.5 N sodium hydroxide, 10 ml ferric chloride sulphuric acid and 10 ml deionised water were added after the heated mixture had cooled at room temperature. After a 3 minute reaction time, tVFAs were determined colorimetrically with a HACH DR 2010 spectrophotometer against a blank (deionised water). All reagents were provided by Camlab Ltd (Cat.no HH/22447-00). Where necessary samples were diluted with deionised water to ensure the measured values were within the calibration range.

3.3.6 Soluble COD (SCOD) determination

Soluble COD (SCOD) was determined in samples after centrifugation at 2000g for 15 minutes and filtration by a COD kit (VWR, UK). The absorbance was measured by a Spectroquant Nova 60 Spectrophotometer (VWR, UK).

3.3.7 Dissolved organic content (DOC)

Dissolved organic content (DOC) was determined with a Shimadzu TOC – 5000A analyzer in samples after centrifugation and filtration as described previously. Total carbon (TC) and inorganic carbon (IC) were measured in the solid free samples. The catalyst was running at 680°C. The dissolved organic content concentration was found by subtracting the TC concentration from the IC concentration. Calibration of TC and IC was carried out for every 10 – 15 samples with standards supplied by Cranfield University. TC was calibrated for the range of 0 to 100ppm and IC for the range of 0 to 50ppm. Where necessary samples were diluted with deionised water to ensure the measured values were within the calibration range.

3.3.8 Proteins and carbohydrates determination as EPS and SMPs

Extracellular polymeric substances (EPS) were extracted from sludge samples following the heating extraction method of Zhang et al. (1999). 200ml of sludge were centrifuged at 2000g for 15 minutes, the supernatant was removed and replaced by de-ionized water and the sludge pellets were re-suspended. The re-suspended material was left at 80°C for 10 minutes (equivalent to 60 minutes in an oven at 105°C) and subsequently centrifuged while still hot at 8000g for 10 minutes. The supernatant was then filtered through 0.45 µm glass-fibre filter papers and the filtrate was used for the determination of proteins and carbohydrates.

Soluble Microbial Products (SMPs) were obtained by centrifuging the sludge samples at 2000g for 15 minutes and subsequently filtering the supernatant.

Proteins were determined following the Ohnishi and Barr's modification of micro Lowry method (Sigma Assay Kit Cat.no.TP0200). 0.2ml of the solid-free sample were mixed with 2.2ml Biuret reagent and kept at room temperature for 10 min. then, 0.1ml of Folin and Ciocalteu's reagent were added and mixed well. Colour was allowed to develop for 30 minutes and the UV absorbance was measured against a blank at 750nm by a Jenway 6505 UV / Visible Spectrophotometer. The concentration was calculated from a calibration curve obtained from bovine serum albumin (P0914 Sigma – Aldrich, Gillingham, UK). The calibration curve is provided in Appendix A (Figure 1)

Carbohydrates were determined by the phenol – sulphuric acid method (Dubois et al. 1956). 0.4 ml of solid-free sample was added to 0.4 ml of 5% (w/w) phenol solution (Sigma – Aldrich, UK) and subsequently mixed with 2 ml concentrated sulphuric acid (98%, Fisher Chemicals, UK). Colour was allowed to develop for 10 minutes and the UV absorbance of samples was measured against a blank at

480nm by a Jenway 6505 UV / Visible Spectrophotometer. The concentration was calculated from a calibration curve obtained from D-glucose. The calibration curve is provided in Appendix A (Figure 2).

3.3.9 Biogas collection and methane composition determination

Biogas was collected during the laboratory batch digestion tests, as explained in subsequent paragraphs, from the top part of the digestion bottles by loosening the rubber bungs and sampling about 20ml of biogas with a syringe. Sampling had to be done carefully and quickly to prevent loss of biogas and ensure anaerobic conditions in the digestion bottle. For that reason, only three measurements were taken during the batch digestion studies, on Day 3, Day 6 and Day 10 of digestion. The biogas sample was subsequently injected to a gas analyzer. CH₄ was measured as a percentage by a Servomex gas analyzer (Model 1440, Servomex Group Ltd, UK). Calibration of the instrument was carried out by the company and was subsequently checked by all users at regular intervals. All users were also responsible for flushing the analyzer with nitrogen gas upon completion of analysis, as advised by Servomex Group Ltd.

3.4 *Methods development*

Further analytical work involved the microscopic investigation of bacteria, the determination of surface tension in aqueous and sludge samples and the determination of hydrophobicity of sludge samples. These methods were reviewed and developed for the purposes of the current work, as described below.

3.4.1 Filamentous bacteria identification

Filamentous bacteria identification was carried out according to Eikelboom (2000). Gram and Neisser stains were used for staining of filaments in sludge. Gram stains were provided by HD Supplies, UK (Cat.no. Z2HS802) with safranin as the counter stain. The staining procedure followed the protocol as provided by the kit.

Neisser stains were prepared in the laboratory from stock powdered dyes provided by Fisher Scientific (UK) including methylene blue (Cat.no. 41424-0250), chrysoidin (Cat.no. 20074-0500), crystal violet (Cat.no. 40583-0250) and glacial acetic acid (Cat.no. A/0360/PB08) as described by Eikelboom (2000). The solutions used for Neisser staining involved: 1) solution A containing methylene blue (0.1g), glacial acetic acid (5ml), 96% ethanol (5ml) and deionised water (100ml), 2) solution B containing crystal violet, 10% in 96% ethanol (3.3ml), 96% ethanol (6.7ml) and deionised water (100ml), 3) solution C containing chrysoidin, 1% in aqueous solution (33.3ml) and deionised water (100ml). a freshly made mixture containing 2 parts of solution A and 1 part of solution B was applied to a fixed smear for 10 – 15 seconds. The smear was subsequently rinsed with solution C and excess of the solution was allowed to stay on the slide for about 45 seconds. Then the slide was rinsed with deionised water and dried at room temperature.

The identification of filamentous bacteria was carried out by light microscopy at the highest magnification (x100). The microscope used was a student's light microscope (BHB, Olympus). A large area of the slide was examined prior to identifying the filament species and abundance. Identification keys and description tables provided by Eikelboom (2000) assisted in the identification of filamentous bacteria. The filament index (FI) was used to describe the abundance of each species on a scale from 1 to 5 with 1 being the lowest and 5 being the highest abundance. Part of the results was validated by Anglian Water and United Utilities

laboratories as both laboratories have been carrying out the same analysis for activated sludge samples.

3.4.2 Determination of dynamic surface tension

- Dynamic surface tension in aqueous solutions

Dynamic surface tension measurements in aqueous solutions were carried out based on the Wilhelmy plate method as described by Glinski et al. (2000) and Elmitwalli et al. (2001). A ST500man tensiometer was provided by Nima Technology Ltd (UK) together with Wilhelmy paper plates which were used only once per type of solution. The tensiometer was connected to a computer which was monitoring changes in the surface tension per second during immersion and lifting of the paper plate into and out of solution and a final reading of the surface tension of the solution was given in $\text{mN}\cdot\text{m}^{-1}$.



Figure 3.3: ST500man tensiometer

In order to identify suitable examples of surface active agents and select the appropriate / most surface active compounds for further investigation in sludge and during AD according to the purposes of this work, a number of compounds reported in the literature as surface active, were examined for their surface activity

through dynamic surface tension measurements of aqueous solutions containing a range of concentrations for each compound. The compounds involved proteins, VFAs and carbohydrates, as listed below:

- bovine serum albumin (BSA) (protein) with molecular weight (MW) of 66.43kDa (P 9369, Sigma-Aldrich) containing 100 mg/ml of bovine serum albumin in 0.85% sodium chloride solution with 0.05% sodium azide added as preservative,
- gelatine (protein) derived from lime-cured bovine skin with a Bloom number of 225 (medium bloom) and average molecular weight of 40-50 kDa (G9382, Sigma-Aldrich)
- casein (protein) (C5890, Sigma-Aldrich) derived from bovine milk. The main constituents of casein are presented in the table below along with their molecular weights

Table 3.2: Casein subunits and MW

Subunit	Molecular weight (kDa)
$\alpha - s1$	22 – 23.7
$\alpha - s2$	25
β	24
κ	19

- acetic acid (analytical grade, Fisher Scientific UK) (VFA) with molecular weight of 60.05 g.mol⁻¹
- n-valeric acid (analytical grade, Fisher Scientific UK) (VFA) with molecular weight of 102.13g.mol⁻¹
- the monosaccharide D-glucose (C₆H₁₂O₆, MW: 180.16) (carbohydrate) (Sigma-Aldrich),

- the disaccharide sucrose ($C_{12}H_{22}O_{11}$, MW: 342.30) (carbohydrate) (Sigma-Aldrich) and
- the polysaccharide starch ($(C_6H_{10}O_5)_n$) (carbohydrate) (Sigma-Aldrich).

Although carbohydrates have not been reported in the literature as surface active, considerable amounts of carbohydrates were found in sludge both as EPS and SMPs in this work with measured values of up to 500 mg.l^{-1} as SMPs and up to 1000 mg.l^{-1} as EPS (Figures 15, 16, 21, 22, Appendix B). Thus, further investigation of their surface activity and potential contribution to foaming was important. The selection of the above compounds was also based on their molecular weights. Clarkson *et al.* (1999) reported that the surface activity of proteins is affected by their molecular weight and that cmc decreases with increasing molecular weight. According to Kordialik-Bogacka and Ambroziak (2007), the literature supports the theory that polypeptides (proteins) of certain sizes such as 43 or 9.7 kDa have the greatest foaming potential. Therefore, compounds with a range of molecular weights were selected at this stage for examination of their surface activity. Table 3.3 below lists the compounds examined and the concentration ranges for which surface tension was determined.

Table 3.3: Dynamic surface tension determination in aqueous solutions

Compound	Concentration range tested for surface tension
BSA (g.l ⁻¹)	0.0 – 0.2
Gelatin (g.l ⁻¹)	0.0 – 0.9
Casein (g.l ⁻¹)	0.0 – 0.6
0.1g.l ⁻¹ BSA+gelatin	0.0 – 0.3
0.5g.l ⁻¹ Gelatin+BSA	0.0 – 0.2
Acetic acid (g.l ⁻¹)	0.0 – 4.0
n-Valeric acid (g.l ⁻¹)	0.0 – 4.0
D-glucose (g.l ⁻¹)	0.0 – 2.0
Sucrose (g.l ⁻¹)	0.0 – 2.0
Starch (g.l ⁻¹)	0.0 – 2.0

Limitations associated with surface tension determination involved the low solubility of casein in water and a buffered aqueous solution of 0.01M sodium hydroxide had to be used containing the protein for surface tension determination.

- Dynamic surface tension in sludge samples

A number of papers were reviewed in order to establish a method for the determination of surface tension in sludge samples. Rahman *et al.* (2003) carried out surface tension measurements in soil extracts (1:10 soil to water ratio) following the du Nuoy ring method to monitor the production of biosurfactants by microbial activity. Verma *et al.* (2006) and Nitschke and Pastore (2006) carried out surface tension measurements in cell free solutions derived from bacteria culture media to monitor the production of biosurfactants. The two methods, as described by Verma *et al.* (2006) and Nitschke and Pastore (2006), were followed for the determination of surface tension in sludge. The first method involved centrifugation

at 4500g for 10 minutes and collection of the supernatant for surface tension determination and the second method centrifugation at 8000g for 20 minutes and similarly collection of the supernatant for surface tension determination following the Wilhelmy plate method, as described above (ST500man, Nima Technology Ltd). All measurements were replicated 4 to 5 times and the two methods were assessed after surface tension determination in a number of samples.

The results were reproducible for each sample with very small standard deviations (≤ 2.97) and statistical analysis of the data obtained showed that there was no statistical difference ($P = 0.37$, $\alpha = 0.05$) between the two methods. For this reason, subsequent determination of surface tension followed the method as described by Verma *et al.* (2006).

3.4.3 Hydrophobicity determination

The Emulsification Index (EI) method has been used to determine the hydrophobicity of samples due to the presence of biosurfactants by a number of researchers (Fleck *et al.* 2000, Bodour *et al.* 2004, Pirog *et al.* 2004, Bonilla *et al.* 2005). The method was applied to sludge samples in order to quantify the hydrophobic material found in a sample. The emulsifier used in this work was diesel oil. Samples were centrifuged at 7000rpm for 20 minutes and the supernatant was used for the determination of the EI. 2 ml of diesel oil were added to 2 ml of centrate sample in a glass tube and the contents were mixed with a vortex for 2 minutes. The tubes were then allowed to stand at room temperature for 24 hours and the height of emulsified layer was measured. The EI_{24} index was calculated as shown below:

$$EI_{24} = \frac{\text{height of emulsified layer (mm)}}{\text{total height of liquid (mm)}} \times 100 \quad \text{Equation 3-7}$$

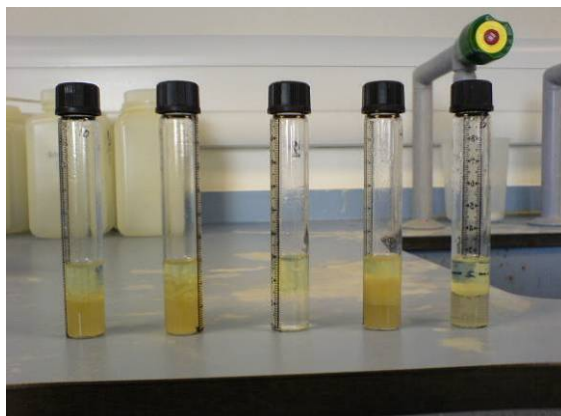


Figure 3.4: Emulsified layer in centrate sludge samples for the determination of hydrophobicity

However, reproducibility of the data was poor and it was decided that the method was not suitable for sludge samples.

3.5 Foaming tests apparatus and methodology

Part of the experimental work aimed at foam generation by mechanical means (aeration). The foaming tests used in this study were an indirect method of determination of the amount of surface active agents in a sample. In following paragraphs, the ability of a sample to generate foam is described as foaming potential. The foaming potential of aqueous solutions or wastewater samples containing one or more surface active agents has been studied by several researchers. Yet, similar information regarding the foaming potential of sludges was not found. A number of experimental procedures on foaming potential measurements followed by many researchers were reviewed in order to establish a method for the determination of sludges foaming potential (Khan and Forster 1990, Sandor and Stein 1993, Morey *et al.* 1999, Desphande and Barigou 2000, Desphande and Barigou 2001, Dedhia *et al.* 2004, Nakajima and Mishima 2005).

Details of each of the methods reviewed are provided in Appendix A (Table 7). In general, all researchers followed aeration of a given volume of sample at specified gas flow rate and at given duration for the determination of the foaming propensity. A similar approach was followed in this work by examining the foaming potential of all samples under a specified flow rate of air and given duration of aeration.

The apparatus used for the foaming tests (Figure 3.5) comprised of a column with a diffuser placed at the bottom. The column was 1m high with a diameter of 5.2cm. The pressure of air was controlled with a pressure gauge at 1 bar for all experiments and the flow rate at $0.5 \text{ l}\cdot\text{min}^{-1}$. Each sample was aerated for 10 minutes. The air was then stopped and the foam height generated in the column was measured.

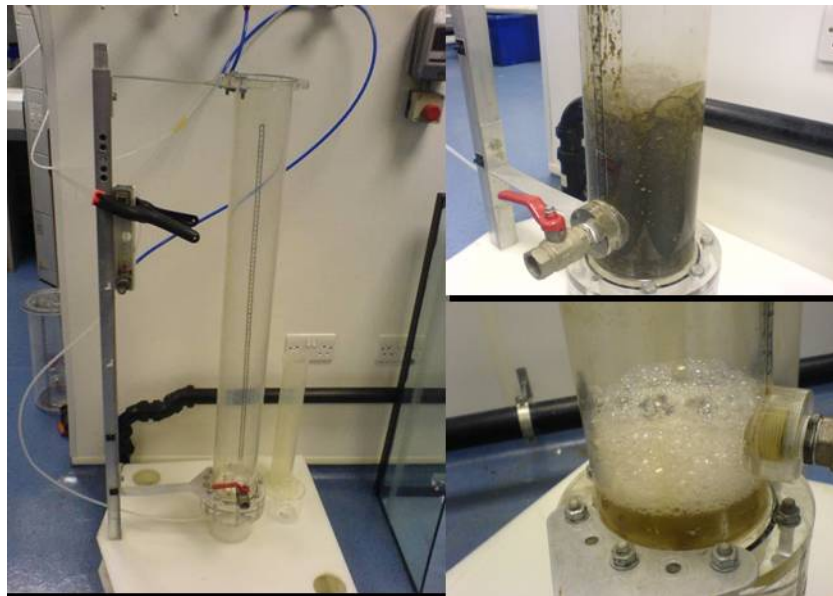


Figure 3.5: Foaming apparatus and foaming tests in sludge and sludge centrate samples

Three parameters were measured during the foaming tests, the foaming propensity of the sample, the foaming tendency and the foam stability. The foaming tendency measured the amount of foam generated from a sample after 10 minutes of

aeration while the stability of foam was monitored indirectly by measuring the foam height 1 hour after aeration of the sample was stopped. Both parameters were previously used by NG et al. (1977) in order to determine the foaming potential of aqueous samples. The foaming propensity was generated for the purposes of this work in order to allow comparison of the foaming potential of different sludge samples. Therefore, the foaming propensity was calculated based on the amount of foam generated from a sample after 10 minutes of aeration normalized over the solids content of the sample. Normalization over other parameters such as alkalinity, volatile fatty acids and dissolved organics was also examined but no strong correlation was found between these parameters and the foaming potential. All measurements were carried out at least in duplicate. The foaming propensity, foaming tendency and foam stability were calculated as shown below:

$$\text{foaming propensity} = \frac{\text{mm of foam after aeration}}{\text{gram total solids}} \quad \text{Equation 3-8}$$

$$\text{foaming tendency} = \frac{\text{foam volume produced, cm}^3}{\text{air flow rate, ml} \cdot \text{min}^{-1}} \quad \text{Equation 3-9}^1$$

$$\text{foam stability} = \frac{\text{foam volume after 1hr settling, cm}^3}{\text{air flow rate, ml} \cdot \text{min}^{-1}} \quad \text{Equation 3-10}^1$$

Cleaning of the column was carried out after every experiment. Prior to cleaning, the column was filled with water and air was introduced at a max flow rate of 2 l.m⁻¹ for about 45-60 minutes. Between different samples tested, the air diffuser was either removed and replaced by a new one or cleaned by soaking in up to 5% hydrochloric acid solution and then put back on the column.

¹ NG et al. (1977)

3.5.1 Laboratory investigation of the foaming potential

The foaming tests were initially carried out in water in order to determine the effect of individual proteins (BSA, gelatine and casein), VFAs (acetic and n-valeric acid) and carbohydrates (d-glucose, sucrose and starch) and two protein mixtures containing a) 0.1 g.l⁻¹ BSA and varying concentrations of gelatin and b) 0.5 g.l⁻¹ gelatin and varying concentrations of BSA on the foaming potential of water and the effect of the interactions between the two proteins, gelatin and BSA on the foaming potential of the mixtures. The mixtures of BSA and gelatin were examined as BSA was the most surface active protein and gelatin the only examined protein that generated stable foams, as seen from the data obtained in this work (Chapter 5). Subsequently, the foaming tests were carried out in sludge in order to understand the interactions between the examined compounds (either individual compounds or mixtures) and the solids and organics in sludge and their effect on the foaming potential of sludge. The sludge samples were obtained during a single visit to a non-foaming digester (Site 16) and all tests were carried out on the same sludge. The foaming tests were carried out in 200ml of aqueous or sludge sample to allow measurements of the foam height of a broad concentration range of the studied compounds. The concentration ranges used for the foaming tests were selected based on the effect of a range of concentrations for each compound on surface tension but were also above and below values of proteins, VFAs and carbohydrates commonly found in sludge samples according to measurements carried out in this work (Chapter 4). Detailed information on the concentrations of each compound and of the protein mixtures tested can be seen in Table 3.4. The sludge samples were kept in a cold room at 4°C for not more than 4 days prior to testing. All other samples were made fresh. Casein was only tested in water. The foaming tests in sludge were not carried out for casein due to the low solubility of the protein in water, the lower surface activity compared to the other two proteins and time limitations.

Table 3.4: Concentration ranges tested for surface tension and foaming tests

Compound	Concentration range for foaming tests (g.l⁻¹)	
	In water	In sludge
BSA	0.0 – 0.3	0.0 – 0.3
Gelatin	0.0 – 0.7	0.0 – 0.7
Casein	0.0 – 0.8	-
0.1g.l ⁻¹ BSA+gelatin	0.0 – 0.3	0.0 – 0.7
0.5g.l ⁻¹ Gelatin+BSA	0.0 – 0.04	0.0 – 0.3
Acetic acid	0.0 – 5.0	0.0 – 5.0
n-Valeric acid	0.0 – 5.0	0.0 – 5.0
D-glucose	0.0 – 2.0	0.0 – 2.0
Sucrose	0.0 – 2.0	0.0 – 2.0
Starch (g.l ⁻¹)	0.0 – 2.0	0.0 – 2.0

The foaming tests were also carried out for sludge samples obtained from the batch digestion studies after addition of the studied surface active agents, BSA, n-valeric and acetic acid, on Days 0, 3 and 10 of digestion in order to assess the effect of the digestion process on the foaming potential. The foaming potential was measured in 200ml of sludge and sludge centrate samples after centrifugation at 2000g for 15 minutes (Rotanta 96 R centrifuge, Hettich Zentrifugen, Tuttlingen, Germany).

Limitations during this part of experimental work involved the high foaming potential of BSA, the low solubility of casein and the hazardous nature of mainly n-valeric acid but also acetic acid. Although the aeration column used for the foaming tests was 1 meter high (capacity of 2125ml) and a minimum of 200ml of sample was used, BSA produced high foaming with the foam height exceeding the column height at concentrations of 0.3 g.l⁻¹ and further investigation of higher BSA concentrations was not possible. The limitations associated with casein were due to the low solubility of the protein in water and the foaming tests were carried out

for the buffered solution (0.01M sodium hydroxide) containing casein. In addition, a COSHH (Control of Substances Hazardous to Health) assessment was completed for the particular activities undertaken in this part of work involving VFAs as it was found that VFAs and especially longer-chain VFAs such as n-valeric acid are related with high health risk and any activity should be contained in a well ventilated area. Hence, the foaming tests for aqueous and sludge samples with added concentrations of acetic and valeric acids were completed in open space at temperatures lower than the room temperature (20°C).

3.5.2 Field investigation of the foaming potential

The foaming tests were carried out for sludge samples from full scale foaming and non-foaming digesters as an indirect method of determination of the amount of surface active agents found in anaerobic digesters. Initially, the experimental work involved single site visits and collection of grab sludge samples from the same previously studied 9 foaming and 6 non-foaming digesters. Digester inlet, primary, SAS and digested sludge were collected at full scale and their foaming potential was assessed in order to identify links between the foaming potential and hence the amount of surface active agents contained and foaming in digesters. Further work examined the presence of surface active agents through the foaming tests in feed and digested sludge of a non-foaming digester (Site 16) for a period of 10 months. In order to promote a better understanding of the origin and characteristics of surface active agents (i.e. whether the surface active compounds were predominantly in solution in sludge or adsorbed to the solids due to their hydrophobicity) and the effect of anaerobic digestion on these compounds the foaming potential in samples from Site 16 was measured in sludge and sludge centrate samples after centrifugation at 2000g for 15 minutes (Rotanta 96 R centrifuge, Hettich Zentrifugen, Tuttlingen, Germany). All foaming tests were carried out in 1-litre of sample.

To ensure that the quality characteristics of centrate samples had not been changed by centrifugation of the whole sludge sample, digested sludge samples were obtained from two different sites (Site 16 & 17) and the foaming propensity was assessed in 1 litre of digested sludge, digested-centrate and digested sludge sample after re-suspension of the solids in the centrate by manual mixing. Further work examined the effect of different concentrations of solids on the foaming tendency of the digested centrate by addition of different amounts of sludge to the same sludge centrate sample.

3.6 Batch anaerobic digestion rig operation

The WRc design anaerobic digestion rig was used for batch digestion of sludge. Digestion took place in 1-litre bottles placed in a thermostatically-controlled water bath. The temperature in the water bath was set at 35°C. The digestion bottles were all sealed with bungs. The gas collection columns were fixed upright in a separate water bath, which contained acidified water. The columns were 110 cm in length with an internal diameter of 5 cm. The columns were calibrated in millimetres with the zero mark at the top of the tube. The gas was collected by displacement over the acidified water containing hydrochloric acid to a pH lower than 4 to prevent dissolution of carbon dioxide. The open bottoms of the columns were connected to the digestion bottles through reinforced tubing. The tops of the columns were sealed with bungs through which clamped reinforced tubing led to a vacuum pump, which filled the columns in with water at the initiation of every experiment. Magnetic stirrers were placed underneath the water baths to keep the sludge in suspension.

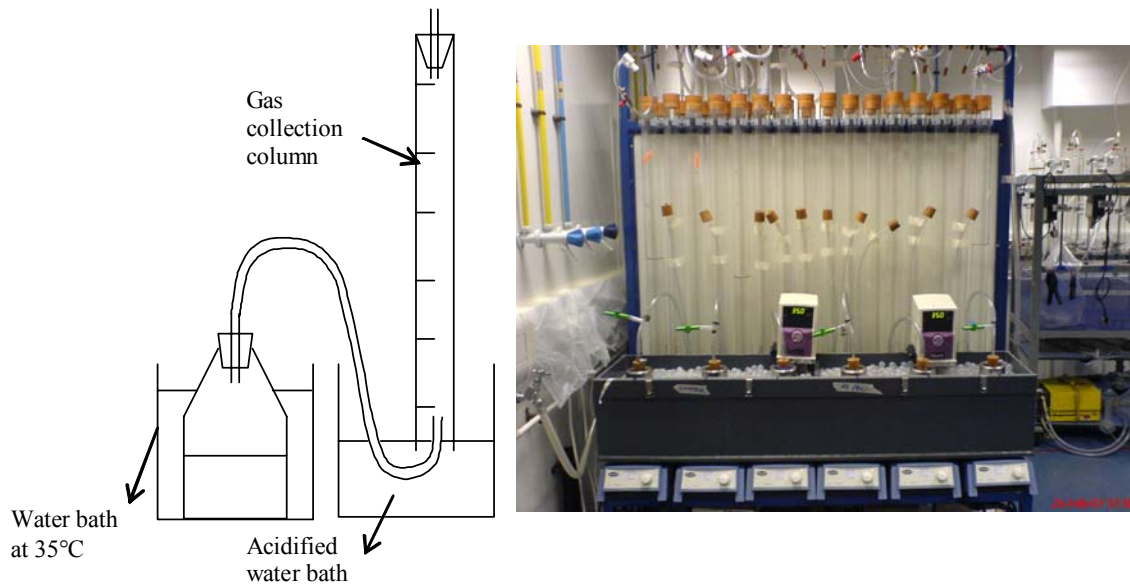


Figure 3.6: Batch anaerobic digestion rig

Sludge samples were collected fresh on initiation of each experiment from one selected non-foaming conventional anaerobic digester (Site 16) with the greatest proximity to Cranfield University to ensure viability of bacteria in sludge prior to batch digestion. The water baths could take up to 12 1-litre digestion bottles with 12 stirrers placed underneath the water baths. Therefore, four different conditions/tests could be examined during each batch digestion experiment in triplicate with one of them as the control. The incubation period in the bottles was 10 days as the biogas production was found to be small ($<27.4 \text{ cm}^3 \cdot \text{day}^{-1}$) after Day 10 of digestion. The height of foam in the bottles was measured daily by direct reading with a measuring tape on the side of each bottle. Foam was subsequently destructed daily by stirring. The organic loading for each bottle was given by the following equation (Lamelot 2004).

$$\text{Organic load } (\text{kgVS} \cdot \text{m}^{-1}) = \frac{\%TS \times \%VS \times V \times 10^6}{(100 \times 100) \times 1000 \times 500} \quad \text{Equation 3-11}$$

where $\%TS$ = dry matter content of the feed sludge in $\text{kg} \cdot \text{kg}^{-1}$

%VS = volatile matter content of the feed sludge kg.kg^{-1}

V = volume of feed sludge in ml

500 = working volume of the digester in ml

10^6 = correction factor between ml and m^3

100×100 = correction factor due to percentages

1000 = estimated density of sludge (kg.m^{-3})

The gas production was measured every 24 hours. The equation to convert the height of acidified water measured in each column into volume of gas produced was given by:

$$\text{Gas volume (ml) at atmospheric pressure} = 19.87 \times h \times \frac{1019.7 - 83.8 + h}{1019.7}$$

Equation 3-12

where: h= tube calibration in cm

1019.7 = standard atmospheric pressure in cm water gauge

83.8 = the working length of the gas collection column (cm)

(Lamelot 2004)

Total and volatile solids reduction (%) was calculated from average total and volatile solids values according to the mass balance formula given below (Tillman G.M. 1996).

$$\% \text{ reduction} = \frac{(\text{mass solids in} - \text{mass solids out})}{\text{mass solids in}} \times 100$$

Equation 3-13

3.6.1 Batch digestion experiments on organic loading

The controlled-laboratory batch digestion of sludge investigated solely the effect of organic loading on foaming. Three different organic loading rates plus the control containing only digesting sludge were tested. The loading rates were chosen based on full scale findings from the site survey work and information found in the literature on recommended organic loading rates for anaerobic digesters, as listed in Table 3.5. Consequently, the organic loadings examined during the batch digestion tests were 1.25, 2.5 and 5kg VS.m⁻³. The batch anaerobic digestion experiment was repeated three times in order to demonstrate reproducibility of the results obtained.

Table 3.5: Common organic loading rates for anaerobic digesters

Source	Organic loading rates (kg VS. m³ d⁻¹)
Handbooks of UK Wastewater Practice (1996)	0.8 – 1.6
Metcalf and Eddy (2003)	1.6 – 4.8
Brown (2002)	< 4.5
Gerardi (2003)	designed: 3.2 – 7.2 (usually 0.5 – 0.6)
Lamelot (2004)	<2.5
Braguglia <i>et al.</i>	0.7 – 1.4
Bolzonella (2005)	~ 1

3.6.2 Batch digestion experiments on surface active agents

The batch digestion tests investigated the behavior of three of the surface active agents studied in this work, BSA, n-valeric and acetic acid, during AD and their effect on foaming. Fresh sludge samples were collected from a full scale non-

foaming digester (Site 16) prior to initiation of each batch experiment. The batch studies were carried out only for the lowest loading of 1.25kg VS.m⁻³, at which no foaming occurs according to findings from this work (Chapter 4), in order to determine whether the tested surface active compounds have the potential to induce foaming under non-foaming AD conditions. BSA, n-valeric acid and acetic acid were added in sludge at the start of each batch anaerobic digestion experiment at concentrations of 0.1, 0.3 and 1g.l⁻¹ for BSA and 0.5, 1.5 and 5 g.l⁻¹ for the two acids. The selection of the examined concentrations was based on the following criteria: i) a low concentration of the compound beyond its CMC and hence capable of producing foams in water ii) a three-times higher than the low concentration that would also double the concentration of the compound in sludge and iii) an extreme value, 10 times higher than the low concentration in order to assess the effect on foaming. The selection of the above concentrations was also based on experimental data obtained in this work that determined the concentrations of proteins and VFAs commonly found in sludge and thus the values for examination in the batch studies.

3.7 Statistical analysis

Descriptive statistics were carried out for all data involving the calculation of mean values, standard deviations and standard errors as given by the formulas below. Further statistical analysis of the data involved examination of the normality of the data and subsequently one-way analysis of variance (ANOVA). The software used for the purposes of this work was Statistica.

$$\text{arithmetic mean (mean)} = \frac{x_1 + \dots + x_n}{n} \quad \text{Equation 3-14}$$

$$\text{standard deviation}(SD) = \sqrt{\frac{\sum (x - X)^2}{(n - 1)}} \quad \text{Equation 3-15}$$

$$\text{standard error (SE)} = \frac{SD}{\sqrt{n}} \quad \text{Equation 3-16}$$

Where x: measured value
 X: arithmetic mean
 n: population number

Chapter 4:
Effect of anaerobic digester
operation on foaming

4 Effect of anaerobic digester operation on foaming

4.1 Introduction

A number of operational parameters have been reported in the literature as foaming causes. The literature has suggested that gas mixing in anaerobic digesters has been found to contribute to foaming by promoting attachment of the hydrophobic and surface active compounds found in sludge onto the gas bubbles. As the bubbles rise to the surface of the liquid in digesters, the surface active and hydrophobic compounds form a liquid film around the bubbles that prohibits the bubbles from bursting, increases the surface activity and results in higher foaming potential. Furthermore, a number of researchers have stated that overloading of digesters that can occur in the form of AS content in the feed sludge, which is the main source of filamentous bacteria and proteins, organic loading resulting in accumulation of surface active organic substances, concentrations of individual compounds in the feed such as lipids or other hydrophobic substances, or polymer overdose during dewatering that generally can affect the microbial activity and result in accumulation of substances are potential causes of foaming. However, the reports provided limited experimental evidence to support the above statements (Pagilla *et al.* 1997, Barjenbrugh *et al.* 2000, Moen 2003, Barber 2005). In addition, maintenance of digesters could potentially be linked to foaming. Maintenance prevents grit accumulation and insufficient mixing, which could result in poor digestion efficiency, accumulation of substances including surface active agents and potentially foaming.

4.1.1 Hypothesis

This chapter was based on the hypothesis that there are critical operational aspects of AD that contribute to or result in foaming and that sludge from non-foaming digesters can foam under these critical conditions.

4.1.2 Aims and objectives

This chapter aimed to understand the effect of full scale anaerobic digester operation on foaming.

The objectives were to:

- examine the effect of the type of mixing on foaming,
- investigate the relationship between maintenance of digesters and foaming,
- quantify the critical organic loading thresholds for foam initiation and stabilization and
- examine the overall digestion efficiency at the full scale and its relation to foaming.

4.2 Results

4.2.1 Mixing

Overall, three types of mixing were observed at the visited full scale digesters, (a) gas, (b) mechanical and (c) gas combined with re-circulation pumps. All three types of mixing were found at the non-foaming digesters examined. Of the foaming digesters, 4 digesters had gas mixing, 3 digesters had mechanical mixing and 1 digester had gas mixing combined with re-circulation pumps. These findings

suggested that gas mixing alone did not lead to foaming in the examined digesters. The contribution of gas mixing to foaming could not be assessed at this stage as the work was carried out at the full scale and it was impossible to predict the digesters performance without gas mixing.

4.2.2 Maintenance

The relationship of the digesters condition in terms of maintenance to foaming was investigated at full scale. However, random technical problems, such as pumping failures, temperature fluctuations or operation of digesters at temperatures lower than recommended and insufficient mixing were recorded at some of the full scale digesters that were irrelevant of the digesters scheduled maintenance work and had an impact on digestion efficiency. In detail, 5 out of the 7 non-foaming digesters received good maintenance (maintenance took place not more than 6 years ago) of which 1 digester (Site 5) had operational failures during the site visit (the pre-treatment stage was not in use resulting in different quality feed sludge to the digester and digested sludge was not discharged from the digester). Looking at the foaming digesters, maintenance work had been carried out at 5 foaming digesters not more than 6 years ago, of which 2 digesters had operational failures during the site visits (Site 13: digester feeding problems and temperature fluctuations, Site 15: digester pumping system failure) and 4 digesters where maintenance work took place more than 6 years ago (average maintenance), of which 1 had operational failures (Site 10: no mixing in digesters). Foaming digesters with good maintenance and operational failures could have had reduced effective volumes due to insufficient mixing and hence grit accumulation or inhibited microbial activity due to temperature fluctuations or under-loading (Sites 13 and 15), which although were not the causes of foaming as digesters were foaming without the failures for over a year of operation, could have contributed to foaming. Also, according to the above data, foaming was present in digesters with

good maintenance and no operational failures. Arguably, a poorly maintained non-foaming digester (maintained 7 years ago) could have had the same amount of grit accumulation with a well maintained foaming digester (maintained 6 years ago) depending on the sludge quality characteristics and the up-stream processes since the exact effective digester volumes were not known at this stage. Therefore, it can only be concluded from the above data that operational failures or poor maintenance alone did not result in foaming on the occasions of Sites 3, 5 and 6.

4.2.3 Organic loading

The relationship between organic loading and foaming was investigated at both the full and bench scale. Below are the calculated organic loading rates from single sampling visits at foaming and non-foaming digesters.

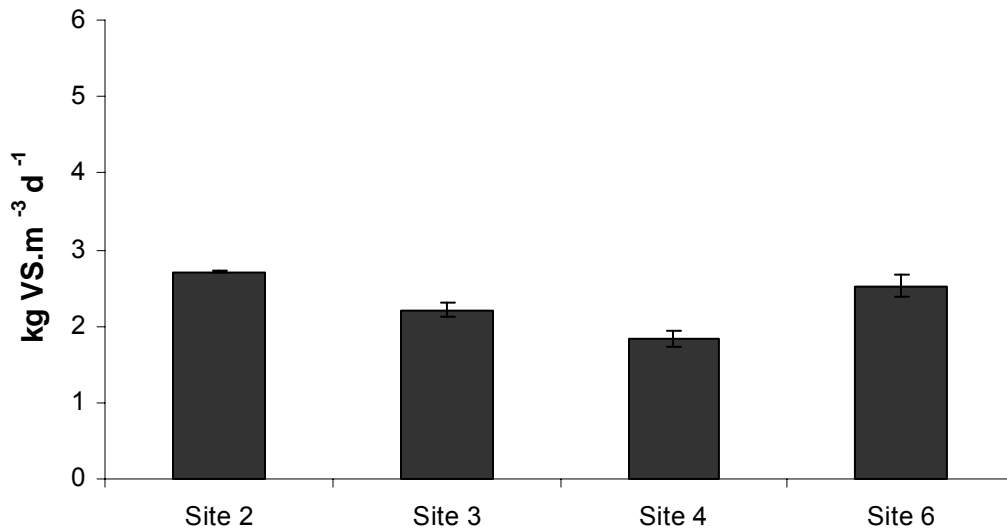


Figure 4.1: Volatile solids loading rate (kg VS.m⁻³ d⁻¹ ±SD) at non-foaming digesters

According to Figure 4.1, the volatile solids loading at the non-foaming digesters did not exceed $2.7 \text{ kg VS.m}^{-3} \text{ d}^{-1}$. According to the literature, these values were within the suggested organic loading rates (OLR) for anaerobic digesters (Chapter 3, Table 3.5).

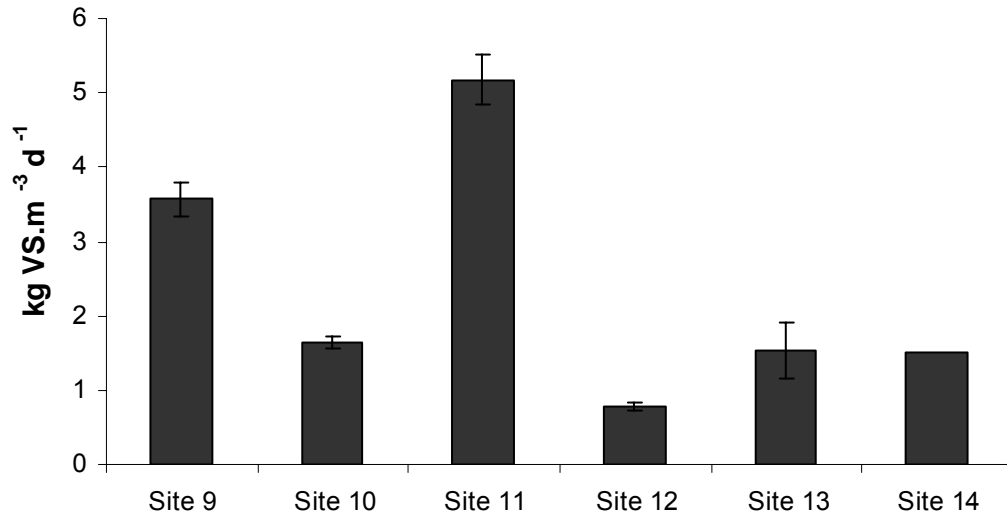


Figure 4.2: Volatile solids loading rate ($\text{kg VS.m}^{-3} \text{ d}^{-1} \pm \text{SD}$) at foaming digesters

The organic loading rates obtained for the foaming digesters demonstrated higher variability with values ranging from 0.78 to $5.17 \text{ kg VS.m}^{-3} \text{ d}^{-1}$. Site 9 loading was near the upper loading limit of the suggested OLR ($1.6 - 4.8 \text{ kg VS.m}^{-3} \text{ d}^{-1}$) according to Metcalf and Eddy (2003) and Site 11 loading exceeded that range. Yet, there were a number of foaming digesters with OLR within the suggested range. In detail, Sites 10 and 13 digesters OLR were within the suggested range but the digesters were experiencing operational failures during the site visit. However, no problems or high loadings were encountered in digesters from Sites 12 and 14.

Following the broad investigation of OLR and foaming in a number of digesters across the UK, a site-specific long term monitoring of OLR in relation to foaming

was carried out. 9 visits followed by sampling took place at a foaming digester (Site 12) between April 2006 and October 2007 and 15 visits to a non-foaming digester (Site 16) between February 2007 and May 2008 and the OLR was calculated as previously. The following graphs illustrate the values of OLR obtained during the monitoring period.

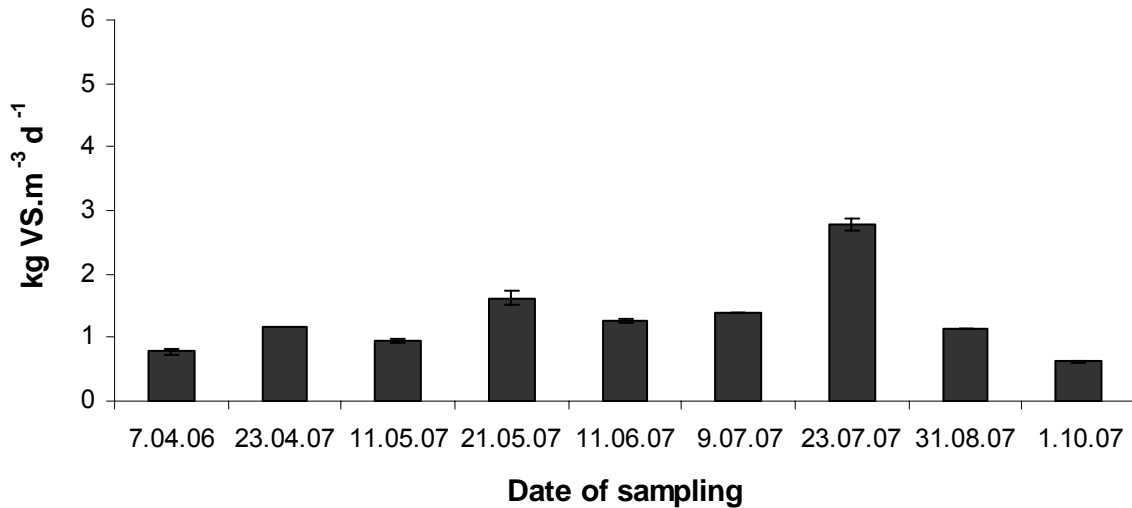


Figure 4.3: Volatile solids loading ($\text{kg VS.m}^{-3} \text{d}^{-1} \pm \text{SD}$) of a foaming digester (Site 12) at different sampling occasions

Site 12 digester had reportedly experienced foaming for over a year and antifoam was being dosed daily to the digester to suppress foaming. The OLR values obtained showed that the digester loading was in accordance with the suggested loading rates found in the literature. The highest OLR recorded during the monitoring period was $2.77 \text{ kg VS.m}^{-3} \text{d}^{-1}$ (23.07.07), which however is comparable to OLR values obtained from the non-foaming digesters during the site survey (Figure 4.1 above). Due to the daily antifoam dosing, it was impossible to know whether foaming was regular at the digester or occasional. The data obtained on the OLR for Site 12 were similar to the OLR data obtained from the site survey on

the non-foaming digesters and similar to OLR values of some of the foaming digesters.

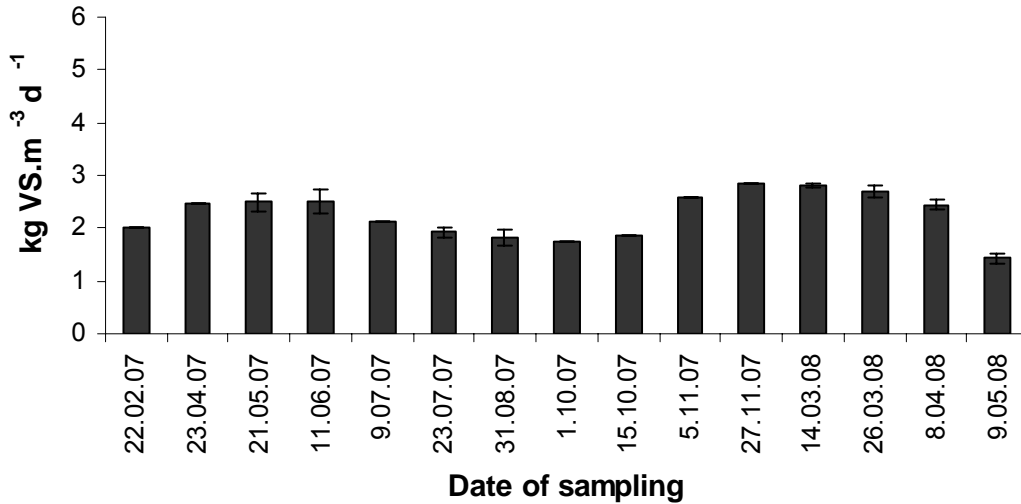


Figure 4.4: Volatile solids loading ($\text{kg VS.m}^{-3} \text{d}^{-1} \pm \text{SD}$) of a non-foaming digester (Site 16) at different sampling occasions

According to Figure 4.4, there was seasonal variation of the OLR in the non-foaming digester varying from 1.44 to 2.84 $\text{kg VS.m}^{-3} \text{d}^{-1}$ during the 15-month monitoring period with an average value during this period of 2.25 $\text{kg VS.m}^{-3} \text{d}^{-1}$ (SD: 0.43). A foaming event was recorded in the digester between two sampling occasions, the 26.03.08 and the 8.04.08, which exhibited characteristics of unstable foaming, as explained in the literature (Chapter 2), as it collapsed and disappeared after a few days without any control action taken. The foaming incident followed loadings of 2.81 and 2.68 $\text{kg VS.m}^{-3} \text{d}^{-1}$.

Due to the number of parameters affecting a digester's performance at the full scale, such as temperature fluctuations, mixing and the quality of feed sludge, it was impossible to find a clear correlation of foaming and OLR. Controlled-laboratory batch digestion of sludge was important at this stage to investigate

solely the effect of organic loading on foaming. The data obtained from the batch digestion experiments on organic loading are presented below.

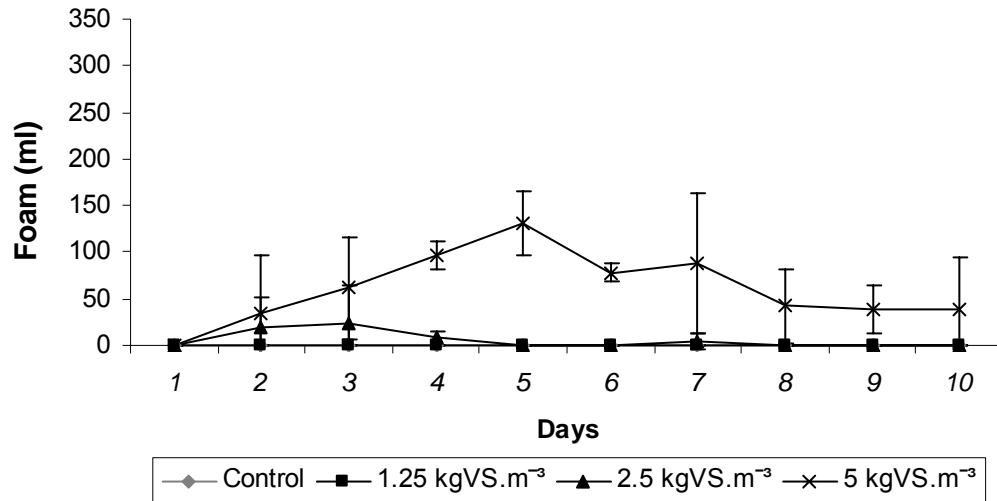


Figure 4.5: Daily foam production (ml \pm SD) during batch anaerobic digestion of Experiment 1 on organic loading

During experiment 1 on organic loading, the 5 kg VS.m⁻³ loading produced consistent (metastable) foaming (35 – 131 ml foam per day) throughout the batch digestion period. The 2.5 kg VS.m⁻³ produced less foam (<24 ml foam per day) only within the first 4 days of digestion. It is important to highlight at this stage that foam was destroyed daily by mechanical means (stirring) after each measurement and was reappearing within a day. No foaming was recorded in either the control or the 1.25 kg VS.m⁻³ loading digestion bottles.

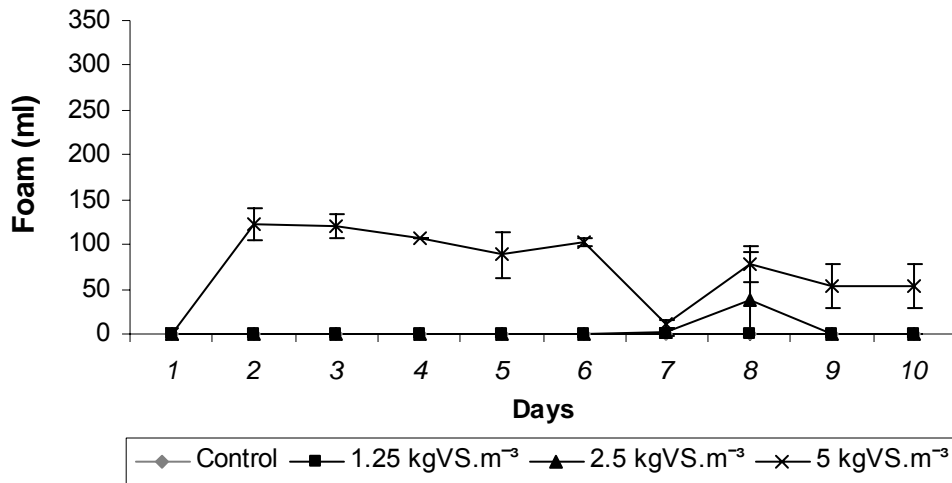


Figure 4.6: Daily foam production (ml \pm SD) during batch anaerobic digestion of Experiment 2 on organic loading

Experiment 2 on loading demonstrated similar patterns with experiment 1 with the 5 kg VS.m⁻³ loading resulting in daily (metastable) foaming. A reduction in the foam volume on Day 7 (from 103 to 10 to 78 ml foam per day) from the highest loading was noticed. Possible explanations that caused that reduction in foam could be the daily destruction of foam or the reduced digestion activity as the biogas production from Day 6 until Day 10 was very small. However, foam was recreated within a day of its destruction in all cases. Foaming was recorded from the 2.5 kg VS.m⁻³ loading only on Day 8 of digestion. No foaming was recorded in either the control or the 1.25 kg VS.m⁻³ loading digestion bottles.

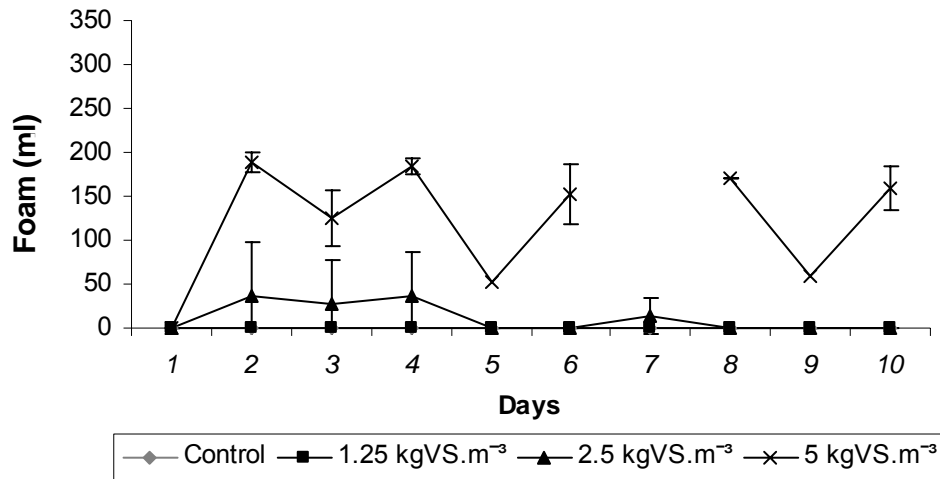


Figure 4.7: Daily foam production (ml ±SD) during batch anaerobic digestion of Experiment 3 on organic loading

Experiment 3 on loading showed similar patterns with the previous two experiments. The foam generation of the highest loading had greater variability this time (increases and decreases) that were not seen in the previous two experiments. Foam was also generated from the 2.5 kg VS.m⁻³ loading on the first 4 days of digestion and again on Day 7.

Additional information on methane and daily biogas production, solids reduction, VFAs and alkalinity for the three experiments on loading showed that the digestion process was not inhibited in any case for all the loadings tested. The 5 kg VS.m⁻³ loading was consistently producing more methane than the other loadings in all three experiments. More importantly, there was no accumulation of VFAs from the 5 kg VS.m⁻³ loading. Individual VFAs concentrations were 0 mg.l⁻¹ on Day 3 for all loadings tested for the three batch experiments apart from one occasion on Day 3 of the third experiment with 57 and 266 mg.l⁻¹ as tVFAs of the 2.5 and 5 kg VS.m⁻³ loading, respectively, which however were within the suggested range (50 – 300 mg.l⁻¹) according to Metcalf and Eddy (2003). It was also noticed that alkalinity values from all three loadings and all three batch experiments ranged from 2950 to

3325 mg.l⁻¹ with the 5 kg VS.m⁻³ loading not exhibiting consistently higher alkalinity values than the 1.25 kg VS.m⁻³ loading. However, the SCOD and DOC values for digested sludge at the end of batch digestion (Day 10) were consistently higher in the digestion bottles from the 5 kg VS.m⁻³ loading than the 1.25 kg VS.m⁻³ loading demonstrating a statistically significant difference for both SCOD and DOC (SCOD P=0.004, DOC P=0.002, $\alpha=95\%$). Further information can be found in Appendix B (Figures 1 – 10, Tables 1 – 3). Yet, the SCOD and DOC values for the 2.5 kg VS.m⁻³ loading were lower than the values of 1.25 kg VS.m⁻³ loading in 2 out of the three batch experiments at the end of batch digestion (experiment 2: 604 and 578 mg.l⁻¹, experiment 3: 1073 and 615 mg.l⁻¹ for the 1.25 and 2.5 kg VS.m⁻³ loading, respectively). These findings demonstrated that typical monitoring and analysis for AD including TS reduction, methane production, tVFAs and alkalinity measurements did not demonstrate any differences regarding the sludges quality characteristics between the non-foaming 1.25 kg VS.m⁻³ loading and the foaming 2.5 and 5 kg VS.m⁻³ loadings. Further analysis of sludges (SCOD and DOC) did not demonstrate any differences regarding the sludges quality characteristics between the 1.25 kg and the 2.5 VS.m⁻³ loading. However, there was a statistically significant difference in the organic content of sludges from the 5 kg VS.m⁻³ loading than the 1.25 kg VS.m⁻³ loading.

During the batch digestion tests on organic loading, foam samples were also collected and analyzed. Solids, SCOD and DOC were determined in foam samples obtained from the highest loading (5kg VS.m⁻³) for the three batch experiments.

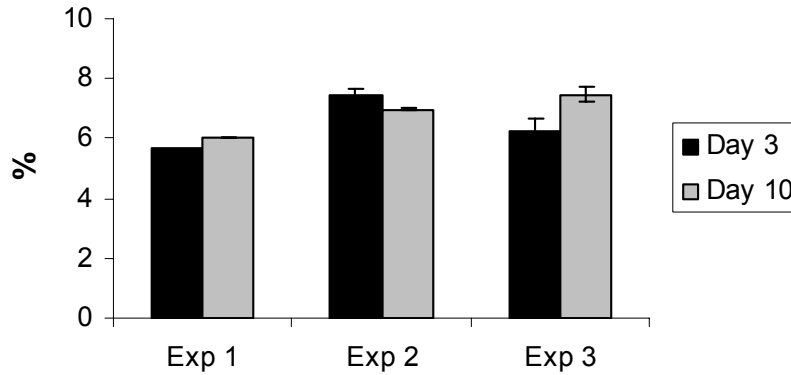


Figure 4.8: Total solids (%±SD) in foam samples obtained from the 5kg VS.m⁻³ organic loading digestion bottles

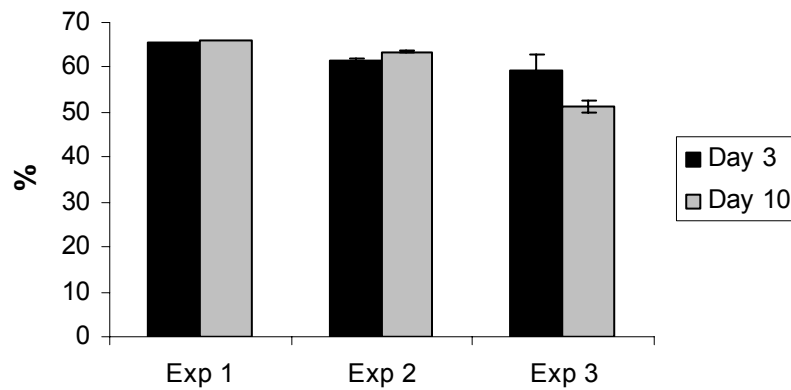


Figure 4.9: Volatile solids (%±SD) of foam samples obtained from the 5kg VS.m⁻³ organic loading digestion bottles

The total solids content in the foam samples was significantly higher than that in sludge. In detail, total solids in foam were between 5.7 and 7.5% for experiments 1, 2 and 3, whereas in sludge the total solids were 2.5% for experiment 1 & 2 and 4.5% for experiment 3. Volatile solids, on the contrary, did not vary between sludge and foam with foam VS content from 51 to 66% and sludge VS content between 50 and 63% for all experiments. Although there was a total solids reduction in sludge during batch AD (Table 2, Appendix B), the mass of solids in both sludge and foam at the end of batch AD was higher than the mass of solids at the start of batch AD.

For, instance, 14.3 g total solids were initially in each digestion bottle of the 5 kg VS.m⁻³ loading and 14.4 g at the end of batch digestion (12 g in sludge, 2.4 g in foam) for experiment 1. Similar patterns were observed for total solids of the third experiment. The second experiment had lower total solids mass for both sludge and foam at the end of batch AD (by 1.3 g) due to better solids reduction in sludge during digestion. The increase in total solids could probably indicate an increase and partitioning of microbial mass due to the increased substrate (5 kg VS.m⁻³ organic loading). Ross and Ellis (1992) reported that analysis of foam samples obtained from full scale foaming digesters had total solids between 7.6% and 13.2% and 65 – 69% volatile solids. Additionally, Westlund *et al.* (1998) reported that foam collected from a full scale foaming digester contained 6% total solids and 70% volatile solids. The values obtained here for total and volatile solids were similar to the TS and VS values for foams from full scale digesters indicating that the foam generated during the batch studies was not different to foams occurring at the full scale.

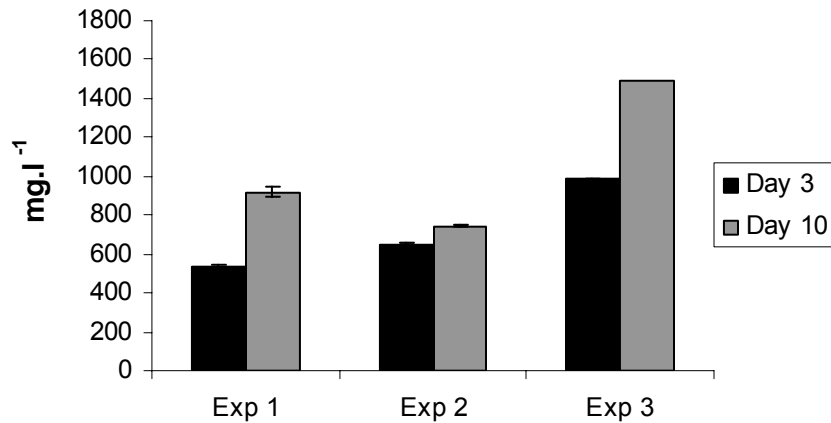


Figure 4.10: Soluble SCOD in foam samples of the 5kg VS.m⁻³ loading digestion bottles

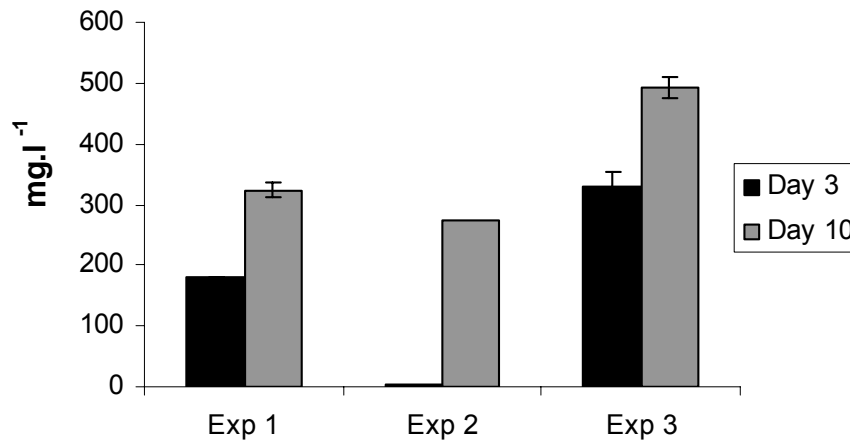


Figure 4.11: Dissolved organic content in foam samples of the 5kg VS.m⁻³ loading digestion bottles

Both SCOD and DOC in foam increased on Day 10 compared to Day 3 in all three experiments. According to Imai *et al.* (2002), DOC contains hydrophilic and hydrophobic substances that can be differentiated to acids, bases and neutrals. The accumulation of dissolved organic material in the foam during batch AD could be attributed to the hydrophobic substances presence. Also the increase in DOC could have been caused by cell lysis due to the limited substrate for acidogenic and perhaps acetogenic bacteria at Day 10 of digestion and release of the intracellular organic material.

In conclusion, there was an indication from the full scale data that organic loading might have been related to foaming as during the broad investigation of the full scale digesters it was found that the organic loading at 2 foaming digesters was 3.5 kg VS.m⁻³ d⁻¹ (Site 9) and 5.17 kg VS.m⁻³ d⁻¹ (Site 11) while the OLR for the non-foaming digesters varied from 1.84 to 2.7 kg VS.m⁻³ d⁻¹. Additionally, during the long-term monitoring of Site 16 a foaming incident followed loadings of 2.81 and 2.68 kg VS.m⁻³ d⁻¹. However, the loading threshold for foaming was not clear during neither the site survey nor the long term monitoring of the two full scale digesters. The batch anaerobic digestion studies showed a clear correlation of loading with

foaming with the 2.5 kg VS.m⁻³ loading resulting in unstable foaming during batch digestion and the 5 kg VS.m⁻³ loading resulting in metastable foaming. Although an OLR over 2.5 kg VS.m⁻³ d⁻¹ was recorded in one non-foaming digester (Site 2, 2.7 kg VS.m⁻³ d⁻¹), a possible explanation of the non-foaming digestion of Site 2 at OLR over 2.5 kg VS.m⁻³ d⁻¹ could be attributed to the uninhibited digestion efficiency, which is addressed in the following paragraphs in addition to the potential effect of the recent maintenance that was carried out 12 months before the site visit and the lack of operational failures that provided the optimum conditions for digestion.

4.2.4 Full scale digester performance

Further digester monitoring involved the determination of the sludges quality characteristics in order to identify correlations between the digestion efficiency at the full scale and foaming. The digestion efficiency was assessed at the foaming and non-foaming digesters (broad investigation) previously examined in this chapter (Sites 1 – 15) but also during long – term monitoring of a foaming (Site 12) and a non-foaming digester (Site 16) to ensure fundamental investigation of the relationship of digestion efficiency to foaming. The broad investigation involved single visits and sampling of the number of foaming and non-foaming digesters. Site 12 was initially included in the broad investigation study and then a year later long – term monitoring of the digesters took place. 9 visits in total followed by sampling took place at Site 12 (April 2006 – October 2007) and 15 visits to Site 16 (February 2007 – May 2008).

The parameters monitored involved total solids reduction, total volatile fatty acids (tVFAs), dissolved organic compounds (DOC) and alkalinity. Below are schematic illustrations of the data obtained.

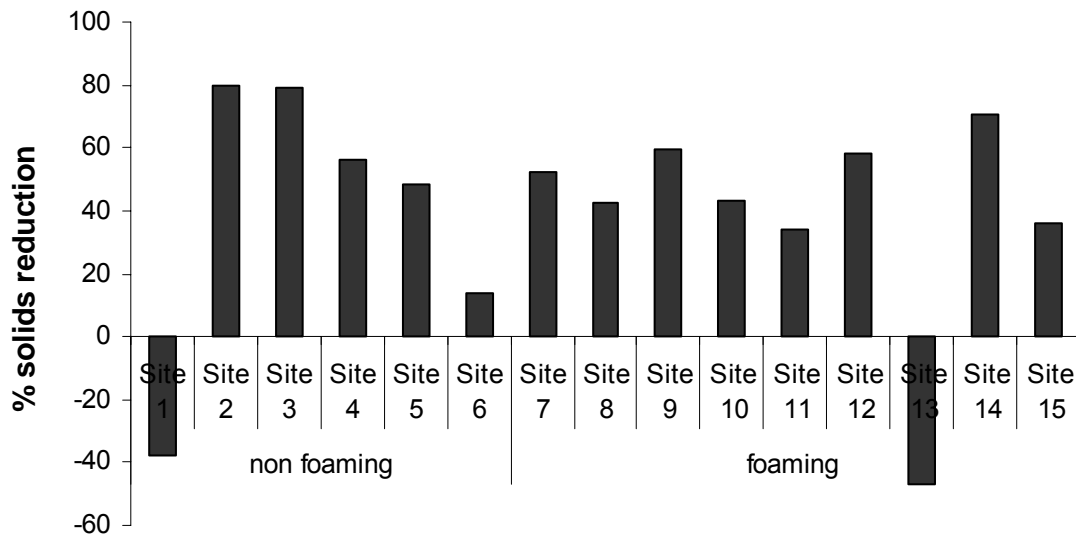


Figure 4.12: Total solids reduction (%) in foaming and non-foaming digesters

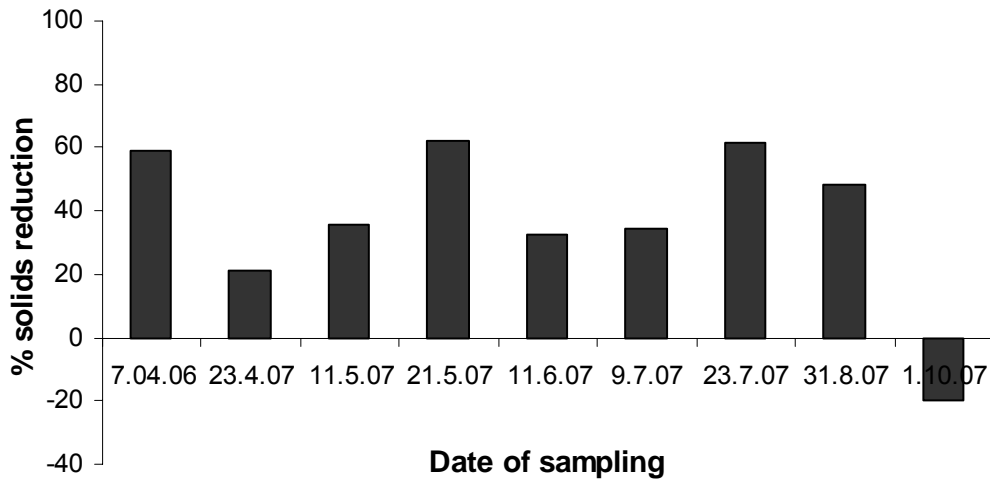


Figure 4.13: Total solids reduction (% \pm SD) for Site 12 digester

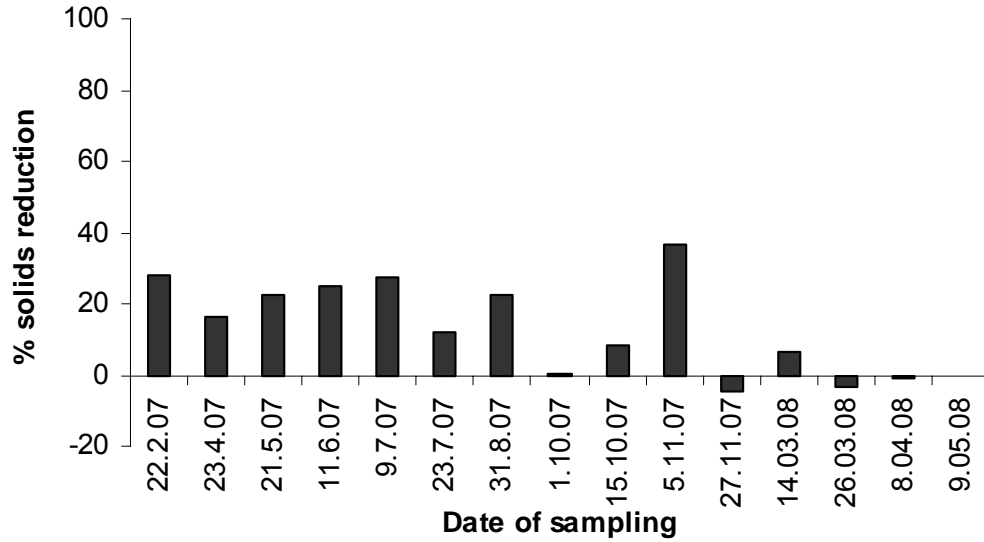


Figure 4.14: Total solids reduction (% \pm SD) for Site 16 digester

According to Handbooks of UK Wastewater Practice (1996) total solids reduction for anaerobic digestion vary between 30-35% of input load. In general, the foaming and non-foaming digesters showed solids reduction (Figure 4.12) within and above the suggested range with the exceptions of Sites 1, 6 and 13. Sites 1 and 13 showed negative values of solids reduction due to the dilute feed sludge and not due to poor digester performance. Site 6 showed very low solids reduction of 14% as feed and digested sludge solids content was similar (36.2 and 31.2%, respectively). The TS reduction of the foaming digester at Site 12, as seen in Figure 4.13, was within or above the suggested ranges with one exception (23.04.07, TS red.: 21%). The negative value on 1.10.07 was due to dilute feed sludge. Yet, the non-foaming digester of Site 16 achieved 30 to 35% solids reduction only in one occasion (5.11.07) with all the other values being below that range indicating poor solids reduction and potentially poor digestion efficiency, which however, did not coincide with foaming apart from one occasion. The values obtained on the 1.10.07 and 15.10.07 corresponded to the effect of dilute feed and not poor digestion efficiency.

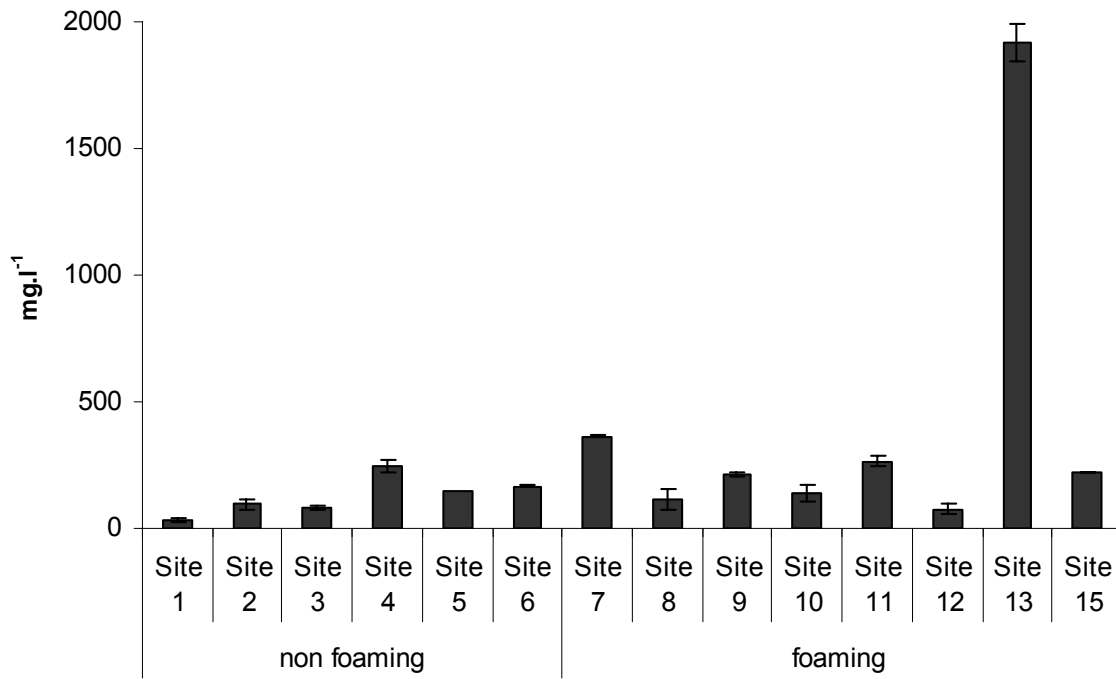


Figure 4.15: tVFAs (mg l⁻¹ ±SD) in digested sludge of foaming and non-foaming sites

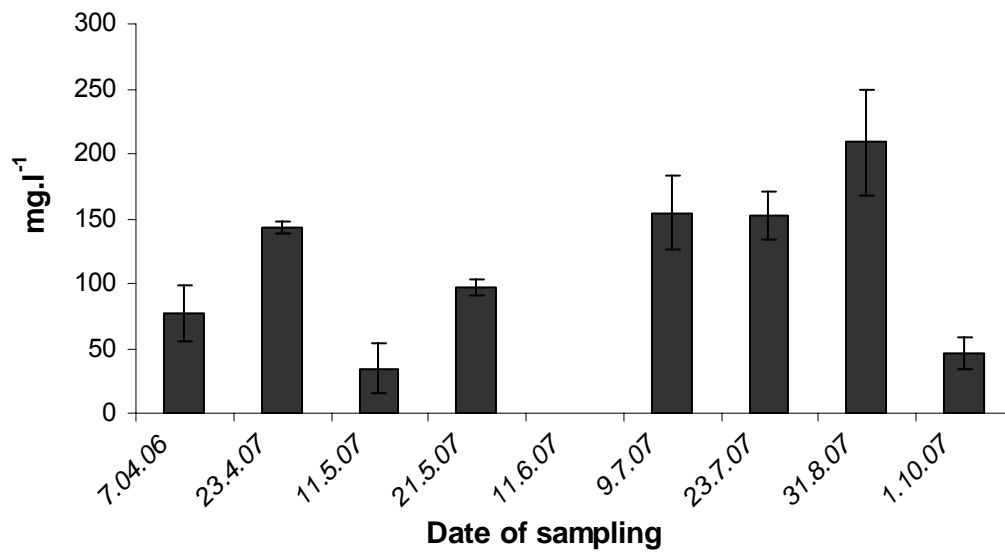


Figure 4.16: tVFAs (mg.l⁻¹ ±SD) in digested sludge of Site 12

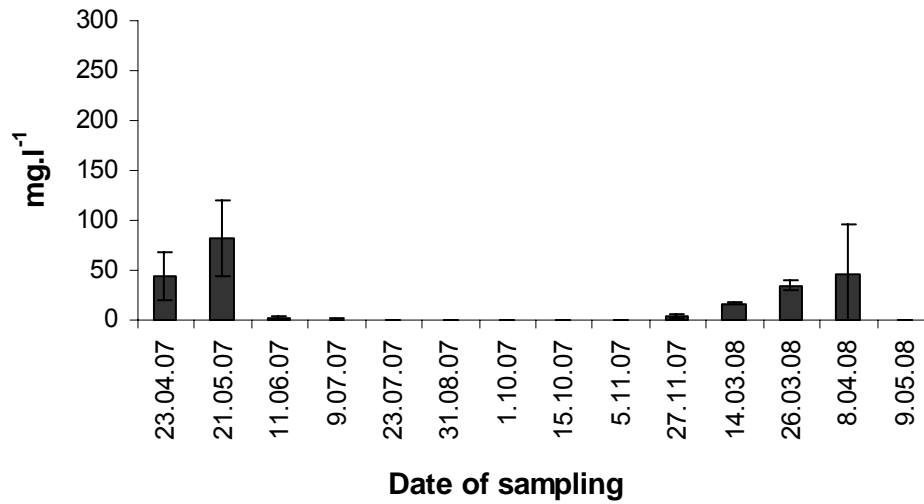


Figure 4.17: tVFAs (mg.l⁻¹ ±SD) in digested sludge of Site 16

Recommended ranges for total VFAs concentrations in digested sludge vary between 50 to 300 mg.l⁻¹ (Metcalf and Eddy 2003). All the non-foaming digesters presented tVFAs values within the above range. tVFAs in the foaming digesters exceeded the above range in only 2 (Sites 7 and 13) out of the 8 digesters. No value was obtained for Site 14 foaming digester due to analytical equipment related difficulties. An interesting finding was that the feed tVFAs of Site 13 did not vary significantly from the values obtained for the other digesters (feed VFAs: 1942 mg.l⁻¹, range of VFAs for non-foaming and foaming digesters: 614 – 7072 mg.l⁻¹). However, very high tVFAs (1918 mg.l⁻¹) were measured in digested sludge. The accumulation of tVFAs in the digester indicated unstable digestion process which could potentially have resulted in foaming. The cause of the VFAs accumulation, however, was not known. Site 12 and Site 16 long term monitoring showed that both digesters did not exhibit VFAs values out of the normal range.

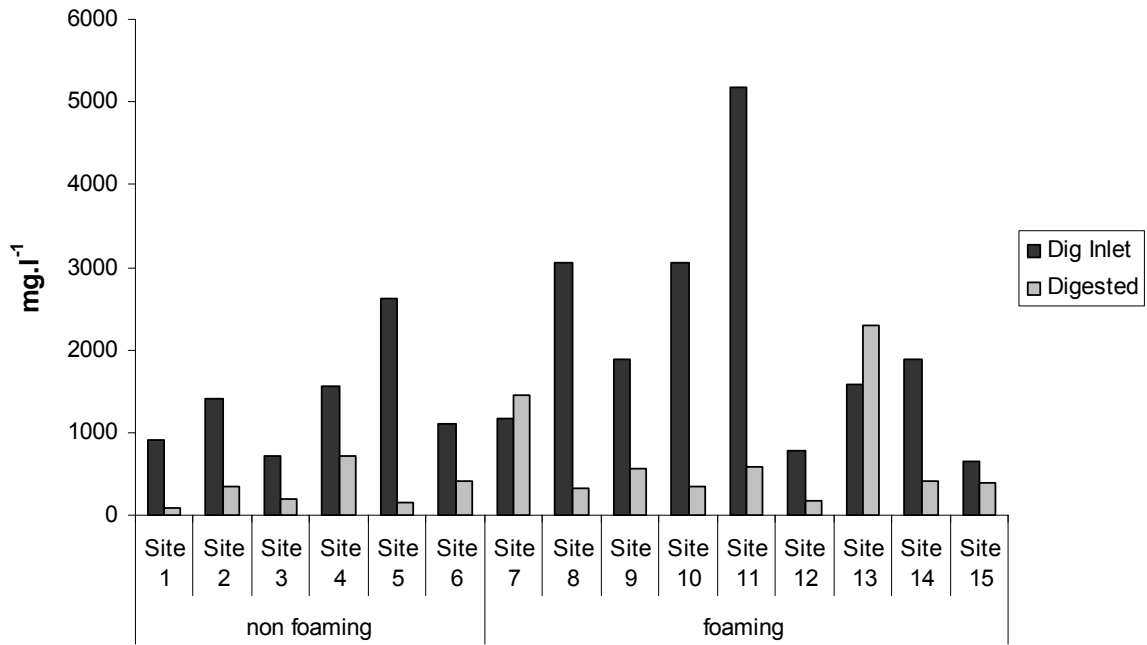


Figure 4.18: Dissolved organics (mg l⁻¹ ±SD) in digester inlet sludge and digested sludge of foaming and non-foaming sites

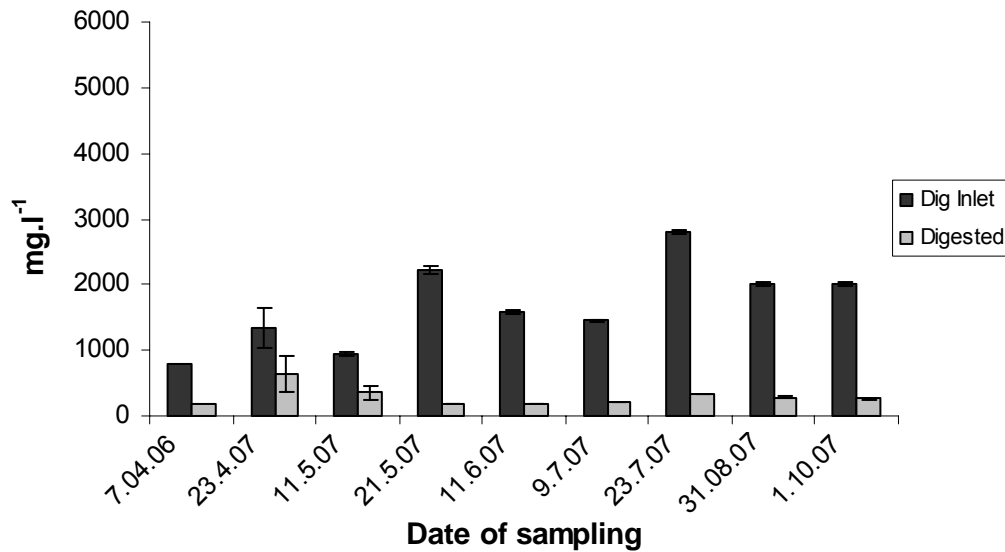


Figure 4.19: Dissolved organics (mg l⁻¹ ±SD) in digester inlet sludge and digested sludge of Site 12 digester

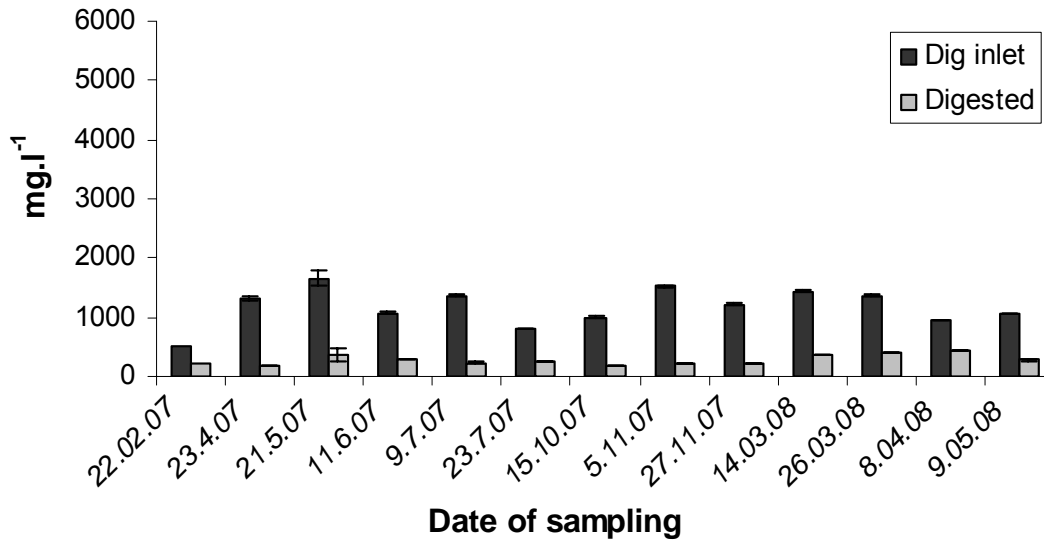


Figure 4.20: Dissolved organics ($\text{mg l}^{-1} \pm \text{SD}$) in digester inlet sludge and digested sludge of Site 16 digester

The concentrations of dissolved organics were variable for both feed and digested sludge for foaming and non-foaming digesters. Yet, it is clear from Figure 4.18 that of the 6 highest DOC values in digester inlet, 5 were found in foaming digesters. The same was not observed in digested sludge. Additionally, it was noticed that digested sludge from Sites 7 and 13 had higher DOC content than that in feed sludge. This indicated that digestion was very poor at the two sites or not taking place at all as no organic matter was degraded by the bacteria but was accumulating instead. However, as there is no information in the literature regarding DOC levels in AD, it is not possible to further critically assess the data obtained. Statistical analysis of the data obtained from the foaming and the non-foaming digesters showed that there was no statistical difference in the DOC concentrations between foaming and non-foaming digesters ($P = 0.08$, $\alpha = 95\%$) (Figures 11 and 12, Appendix B). Long-term monitoring of Site 12 foaming digester showed that the DOC values were similar to the ones obtained for the non-foaming digesters (Site 12 max feed and digested DOC values were 2793 and 634 mg l^{-1} , respectively whereas max feed and digested DOC values of the non-foaming digesters were 2622 and 721 mg l^{-1} , respectively). Similarly, for Site 16 digester,

maximum feed and digested DOC values were 1662 and 440 mg l⁻¹, respectively, matching the data obtained from the non-foaming digesters.

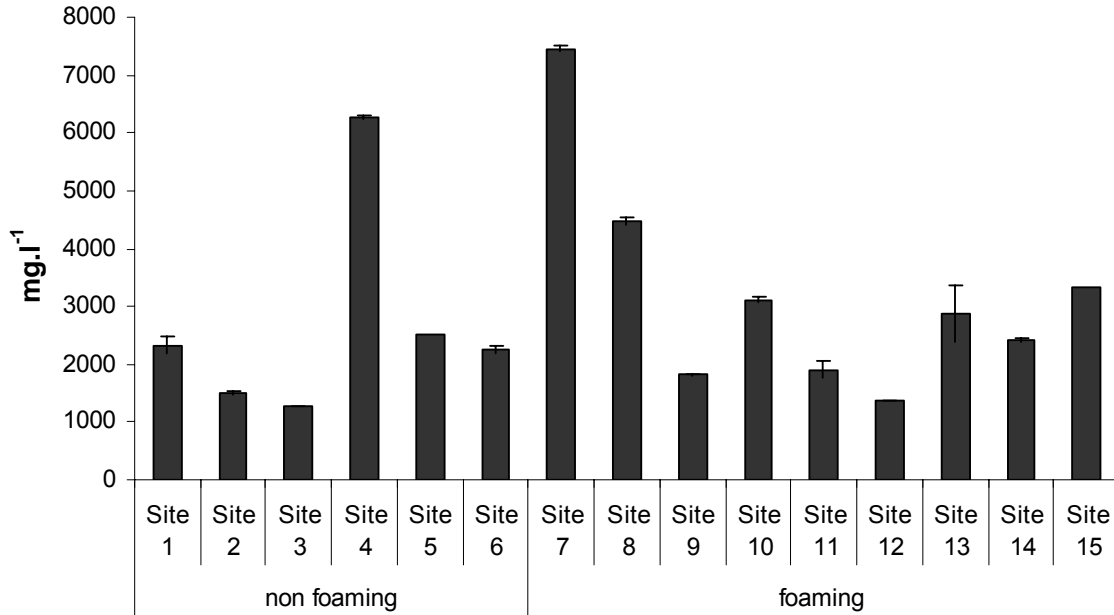


Figure 4.21: Alkalinity (mg l⁻¹ ±SD) in digested sludge of foaming and non-foaming sites

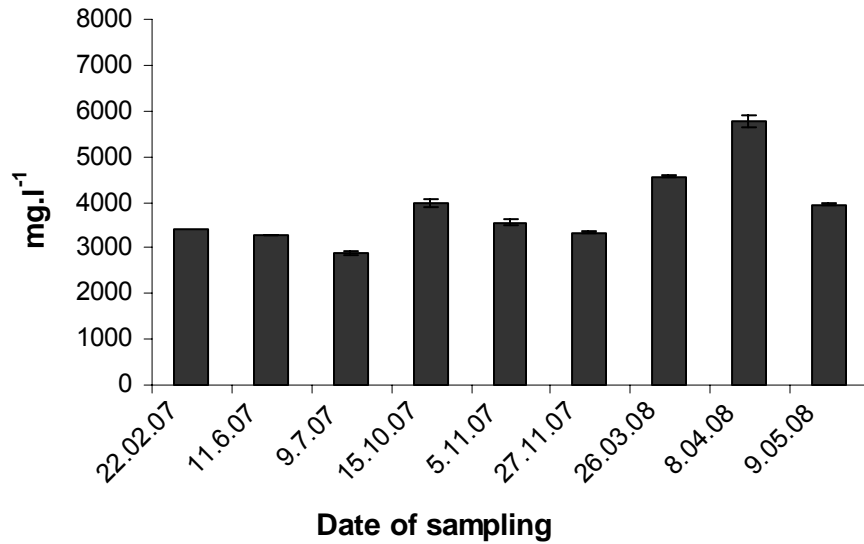


Figure 4.22: Alkalinity (mg l⁻¹ ±SD) in digested sludge of Site 16

The suggested alkalinity range in a digester varies between 2000 mg l⁻¹ and 3000 mg l⁻¹ (Gerardi 2003). Only one of the non-foaming digesters had alkalinity higher than the normal range (Site 4: 6267 mg l⁻¹). Four out of the nine foaming digesters had alkalinity levels over 3000 mg l⁻¹ (Site 7: 7450 mg l⁻¹, Site 8: 4479 mg l⁻¹, Site 10: 3112 mg l⁻¹, Site 15: 3330 mg l⁻¹). Additionally, Site 12 digester alkalinity was lower than the recommended values. Yet, statistical analysis of the data obtained from the foaming and the non-foaming digesters showed that there was no statistical difference in the alkalinity concentrations between foaming and non-foaming digesters ($P = 0.3$, $\alpha = 95\%$) (Figures 13 and 14, Appendix B). Subsequent monitoring of Site 12 was limited and only three alkalinity values were obtained for the foaming digester at Site 12. All three values were between 4010 and 4645 mg l⁻¹ and hence over the suggested range. Similarly, Site 16 digested sludge exhibited alkalinity values over the suggested range.

At this stage it is necessary to mention that during the long term monitoring of the foaming and the non-foaming digester advanced sludge analysis was carried out in order to identify differences in the sludges quality characteristics and hence the digesters performance that would lead to the identification of the foaming cause(s). The advanced analysis involved additional monitoring of surface tension changes in digested sludge, concentrations of proteins and carbohydrates as EPS and SMPs, SCOD and individual VFAs. The ranges of the measured values for digester feed and digested sludge during the monitoring period of the two digesters are presented in Table 4.1 (detailed information on the additional parameters for the two digesters can be found in Appendix B, Figures 15 – 30). The solids reduction was lower in the non-foaming digester (Site 16) and the DOC values from the foaming digester were similar to the ones obtained for the non-foaming digester, as shown earlier in this paragraph (Figure 4.13, Figure 4.14, Figure 4.19, Figure 4.20). The individual VFAs showed that substantial amounts of propionic acid between 40 and 165 mg.l⁻¹ were present in 4 sampling occasions at Site 12 digester when

propionic acid was present in only 2 sampling occasions at Site 16 at concentrations of 14 and 44 mg.l⁻¹. However, as previously stated, all tVFAs values were within the suggested range for AD showing that there was no inhibition or accumulation of VFAs in both digesters. No statistically significant difference was found in the EPS concentrations as proteins (P=0.11, a=95%) and carbohydrates (P=0.06, a=95%) between Site 12 and Site 16 digester. SMPs as proteins were also not significantly different (P=0.99, a=95%) between the two digesters. Carbohydrates in digested sludge, however, were significantly lower at Site 16 than Site 12 (P=0.03, a=95%). Surface tension and COD removal were in the same ranges, as shown in Table 4.1 and statistical analysis of the data was not necessary. In conclusion, apart from the carbohydrates content as SMPs, no other statistically significant differences were found during the monitoring period of the two digesters to indicate possible causes of foaming. Continuous foaming had been observed in the past (2006 – 2007) for the Site 12 digester and since then antifoam has been dosed daily to the digester to prevent foaming. Therefore, it is unknown whether foaming was continuous or not during the monitoring period. Evidence from the data obtained from the advanced analysis of sludges suggested that foaming might had been occasional during the monitoring period and a number of sampling occasions could potentially refer to the digester's normal operation. After collaboration with the site's operators, antifoam dosing was stopped and there were no incidents of foaming on site for the following 9 months (until July 2008), when the work presented here was completed.

Table 4.1: Ranges of measured values of proteins and carbohydrates as EPS and SMPs and surface tension for digester feed and digested sludge and COD removal from the Sites 12 and 16

	EPS		SMPs		Surface tension ($mN.m^{-1}$)	COD removal (%)
	Proteins ($mg.g^{-1}$ VS)	Carbohydrates ($mg.g^{-1}$ VS)	Proteins ($mg.l^{-1}$)	Carbohydrates ($mg.l^{-1}$)		
<i>Feed</i>						
Site 12	20 – 38	20 – 73	220 – 732	172 – 1216	30.6 – 54.4	-
Site 16	20 – 42	9 – 53	213 – 423	196 – 475	32.7 – 57.1	-
<i>Digested</i>						
Site 12	22 – 82	20 – 88	168 – 291	202 – 439	37.1 – 51.8	69 – 89
Site 16	15 – 38	2 – 51	184 – 298	148 – 348	38.6 – 53.7	70 – 87

Table 4.2 summarizes the foaming and non-foaming digesters that were found to operate outside the suggested ranges, as found in the literature, in terms of sludge quality characteristics.

Table 4.2: Anaerobic digestion performance data for foaming and non-foaming digesters				
		<i>TS reduction (%)</i>	<i>tVFAs (mg.l⁻¹)</i>	<i>Alkalinity (mg.l⁻¹)</i>
Suggested range		30 – 35	50 – 300	2000 – 2500
Non-foaming digesters	Site 1	n/a	+	+
	Site 2	+	+	+
	Site 3	+	+	+
	Site 4	+	+	-
	Site 5	+	+	+
	Site 6	-	+	+
Foaming digesters	Site 7	+	-	-
	Site 8	+	+	-
	Site 9	+	+	+
	Site 10	+	+	-
	Site 11	+	+	+
	Site 12	-	+	-
	Site 13	n/a	-	+
	Site 14	+	n/a	+
	Site 15	+	+	-

+ : Digesters operating within the suggested ranges

- : Digesters operating outside the suggested ranges

n/a: not applicable, negative values due to dilute feed sludge or value was not obtained

The summarized representation of digesters efficiency in Table 4.2 shows that 6 out of the 9 foaming (67%) and 2 out of the 6 non-foaming (33%) digesters did not comply with the suggested operational ranges of solids reduction, VFAs and alkalinity indicating that poor digestion efficiency was more common in foaming than in non-foaming digesters. However, long term monitoring of Site 16 non-foaming digester showed that the digester's performance varied throughout the monitoring period and was not always in accordance with the above suggested ranges. Yet, Site 16 digester did not have foaming (apart from one occasion where foaming coincided with OLR over $2.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$).

4.3 Discussion

Earlier in this work (paragraph 3.1), it was mentioned that three of the STWs involved in the site survey had alternative digester configurations, meaning that a pre-treatment stage was placed before the digester. Site 5 (non-foaming) pre-treatment stage was a pasteurization unit, Site 6 (non-foaming) acid phase digestion and Site 11 (foaming) enzymic hydrolysis. The sites were selected in order to investigate the effect of pre-treatment on AD foaming. Clearly, there was no link between pasteurization and acid phase digestion and foaming as both digesters had not had foaming for over a year of operation. The pre-treatment stage at Site 11 had only been commissioned on site 3 weeks before the site visit and sampling and according to the operators foaming was due to the commissioning phase. This was understandable as there is normally a transition period of acclimation for the microbial population due to the change in the feed quality characteristics, which potentially led to foaming on this occasion. However, the experimental work showed that apart from the high organic loading, the solids reduction, tVFAs and alkalinity were within the suggested ranges for AD. In conclusion, the information obtained here was not considered sufficient to promote an understanding of the effect of pre-treatment on AD foaming.

Surface tension of solutions was identified as a key parameter in foams in the literature review (paragraph 2.2.3) and one of the limitations in knowledge was the identification of the critical threshold of surface tension in sludge for foam initiation in AD. Surface tension values were obtained during the long term monitoring of Sites 12 and 16 for both feed and digested sludge. However, as previously explained in this chapter, it is unknown whether Site 12 digester was actually foaming or not and it was not possible at this stage to identify surface tension values in digested sludge corresponding to foaming at the full scale. Additionally, the literature review suggested that the investigation of the effect of compounds commonly found in sludge, such as surfactants and organics, on surface tension could potentially identify a link between these compounds and their importance in foaming in sludge. The data obtained in this work allowed investigation of the effect of proteins and carbohydrates as SMPs, tVFAs and dissolved organics on surface tension. Table 4.3 and Table 4.4 provide the correlation matrices for surface tensions values and the monitored parameters in digested sludge from Site 12 (9 sampling occasions) and Site 16 (15 sampling occasions).

Table 4.3: Correlation matrix for digested sludge from Site 12 digester

	Surface Tension ($mN.m^{-1}$)
tVFAs ($mg.l^{-1}$)	0.02
Proteins as SMPs ($mg.l^{-1}$)	-0.29
DOC ($mg.l^{-1}$)	-0.81
Carbohydrates as SMPs ($mg.l^{-1}$)	0.28

Table 4.4: Correlation matrix for digested sludge from Site 16 digester

	Surface Tension($mN.m^{-1}$)
tVFAs ($mg.l^{-1}$)	0.04
Proteins as SMPs ($mg.l^{-1}$)	0.47
DOC ($mg.l^{-1}$)	0.04
Carbohydrates as SMPs ($mg.l^{-1}$)	0.76

According to Table 4.3, a strong negative correlation was seen between surface tension and DOC (-0.81) in digested sludge. No other strong positive or negative correlation was seen between the examined parameters and surface tension for Site 12. Table 4.4 showed that the only strong correlation found in the non-foaming sludge was between surface tension and carbohydrates (0.76) as SMPs. Overall, tVFAs, although identified in the literature as surface active agents, did not affect the surface tension of sludges and hence their foaming potential, whereas dissolved organics and carbohydrates as SMPs had an impact on surface tension of sludge.

The literature has identified gas mixing systems in anaerobic digesters as an operational foaming cause, as explained in paragraph 4.1. According to findings from this work, gas mixing alone was not found to be a foaming cause at the number of digesters examined as mechanically mixed digesters also had foaming. The contribution of gas mixing to foaming could not be assessed at this stage as the work was carried out at the full scale and it was impossible to predict the digesters performance without gas mixing.

During the examination of the relationship between maintenance of the digesters at the full scale and foaming, technical failures that were irrelevant of the digesters scheduled maintenance work and had an impact on digestion efficiency had to be taken into consideration. The data obtained showed that operational (technical)

failures or poor maintenance alone did not result in foaming at the full scale digesters examined.

One of the objectives of the work completed in this chapter was the identification of the critical organic loading thresholds for foam initiation and stabilization. Full scale data showed that the OLR at 2 out of the 9 foaming digesters was $3.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ (Site 9) and $5.17 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ (Site 11) while the OLR for the non-foaming digesters varied from 1.84 to $2.7 \text{ kg VS.m}^{-3} \text{ d}^{-1}$. Additionally, during the long-term monitoring of Site 16, a foaming incident followed loadings of 2.81 and $2.68 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ when the average OLR during the 15-month monitoring period was $2.25 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ (SD: 0.43). Poor solids reduction (0% solids reduction) was also recorded before the foaming incident while alkalinity values before the foaming incident, although higher than the suggested range, were between 2900 and 4555 mg.l^{-1} and went up to 5780 mg.l^{-1} after foaming. tVFAs were below 300 mg.l^{-1} (within the suggested range) in all samples during the monitoring period. The poor solids reduction before foaming and increase in alkalinity after the foaming incident indicated an upset in the digestion process of Site 16, which could be attributed to the increased OLR as there were no other differences or operational failures observed in the period before and after foaming.

At this stage there was evidence that higher organic loading could have been associated with foaming at the full scale. Further work during bench scale batch anaerobic digestion studies of sludge obtained from a non-foaming digester identified the 2.5 kg VS.m^{-3} loading as a critical organic loading for unstable foaming with foam lasting between 1 and 4 days and subsequently disappearing without any foam control action taken, which indicated that these foams could be characterized as unstable, as explained in paragraph 2.2.1, and the 5 kg VS.m^{-3} loading always resulting in persistent metastable foaming. Unstable foams have a lifetime of seconds, as supported by the literature, however, it is possible that foams created by the 2.5 kg VS.m^{-3} loading reached equilibrium therefore lasting

between 1 and 4 days and subsequently collapsed. Their destruction was random and was not attributed to irregular disturbances required for the destruction of metastable foams, which is why they were characterized as unstable. It was also seen that sludge obtained from non-foaming digesters has the potential to foam under critical conditions (increased organic loadings) at bench scale. The bench scale findings on foam initiation were also in accordance with the full scale data obtained from Site 16 as foaming in a normally non-foaming digester coincided with organic loading over $2.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$. The findings in this chapter clearly demonstrated that any full scale digester can foam simply by increasing the loading beyond the $2.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$.

The standard monitoring and analysis during the batch digestion experiments, which involved the determination of gas and methane production during batch digestion in addition to the determination of solids reduction, alkalinity and tVFAs in sludges at the end of batch digestion, showed no difference in sludges obtained from the foaming and non-foaming digestion bottles and hence no indication of foaming and no inhibition of the digestion process due to the higher loadings. In detail, total solids reduction ranged from 0% (2.5 kg VS.m^{-3} , experiment 2) to 28% (5 kg VS.m^{-3} , experiment 2), volatile solids reduction ranged from 8% ($1.25 \text{ kg VS.m}^{-3}$, experiment 3) to 37% (5 kg VS.m^{-3} , experiment 2), gas and methane production increased with increasing organic loading, tVFAs were below 300 mg.l^{-1} on both Day 3 and 10 of batch digestion and alkalinity values, although slightly higher than the suggested 2000 – 3000 mg.l^{-1} range, varied from 2950 to 3325 mg.l^{-1} for all loadings and all three batch experiments. These findings did not match with information found in the literature suggesting that overloading of digesters results in an imbalance, accumulation of acetic acid and subsequently foaming (Barjenbrugh *et al.* 2000). Further determination of SCOD and DOC in digested sludges obtained at the end of the batch experiments showed no difference in sludges obtained from the non-foaming $1.25 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ loading and the $2.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ loading for foam initiation. A statistically significant difference in SCOD

and DOC content was seen only in sludges between the 1.25 and 5 kg VS.m⁻³ d⁻¹ loading. These findings indicated that typical monitoring of the AD process involving gas and methane production, solids reduction, tVFAs and alkalinity is not adequate to identify differences between foaming and non-foaming digesting sludge and hence promote a better understanding of the foaming mechanisms and causes. Moreover, further monitoring of SCOD and DOC in sludges at the end of batch digestion was not sufficient to differentiate between non-foaming and critical conditions for unstable foaming. Taking into consideration that foam at the 2.5 kg VS.m⁻³ d⁻¹ loading was only present for 1 to 4 days, this could be attributed to the fact that sludge from the 2.5 kg VS.m⁻³ d⁻¹ loading had reached equilibrium of substances similar to sludge from the 1.25 kg VS.m⁻³ d⁻¹ loading once foam had disappeared. It was also seen that foam was recreated in the digestion bottles after mechanical daily destruction. Recreation of foam could have been attributed to the increase of dissolved organics in sludge due to the higher loading, which can act as surface active agents, as explained in the literature review and promote foaming. Similar patterns to the findings from the batch digestion studies demonstrating that typical monitoring of the AD process is inadequate to identify differences between foaming and non-foaming digesting sludges were seen at the full scale foaming digesters at Sites 9 and 11 where the sludge quality characteristics did not show any inhibition in the digestion process and were within the suggested ranges for solids reduction, tVFAs and alkalinity, yet the organic loading at both sites was 3.5 kg VS.m⁻³ d⁻¹ and 5.17 kg VS.m⁻³ d⁻¹, respectively. The DOC values in digested sludge from both sites were the second and third highest values out of the 9 values obtained for the foaming digesters (Site 9: 571 mg.l⁻¹, Site 11: 590 mg.l⁻¹). This makes understanding of full scale digester foaming complex as the cause cannot be identified and subsequently prevented from recurring unless detailed monitoring is in place. An OLR over 2.5 kg VS.m⁻³d⁻¹ was recorded in a non-foaming digester (Site 2, 2.7 kg VS.m⁻³ d⁻¹). Nevertheless, there was no indication of inhibited digestion according to the sludge quality characteristics, maintenance work had been carried out at the digester 12 months

before the sampling visit and there were no operational failures on site, which would probably justify that the digester was not foaming due to the optimum conditions. The experimental work in this chapter however, demonstrated that there are critical OLR that result in foam initiation and stabilization supporting the hypothesis as set out at the beginning of this chapter.

The above findings helped develop a model of the foaming mechanisms in batch AD due to the increased organic loading, as shown in Figure 4.23 and Figure 4.24. The model demonstrates the differences between the non-foaming digestion condition ($1.25 \text{ kg VS.m}^{-3}$) and the foaming digestion condition (5 kg VS.m^{-3}) at the start (Day 1) and at the end (Day 10) of batch digestion based on the findings from the analysis of sludge and foam samples.

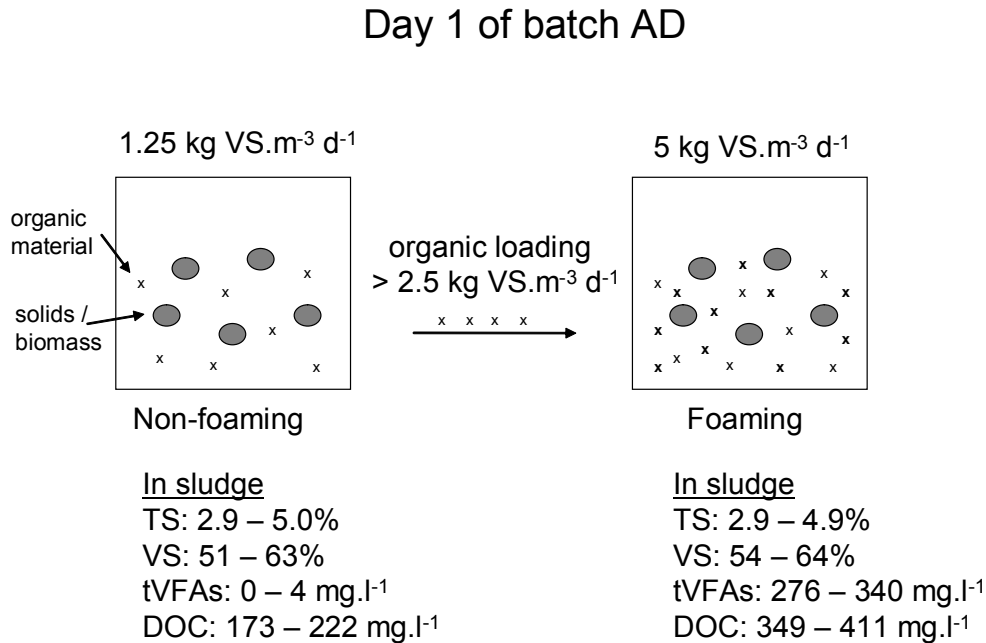


Figure 4.23: Model for foaming mechanisms in batch AD based on organic loading experiments (Day 1)

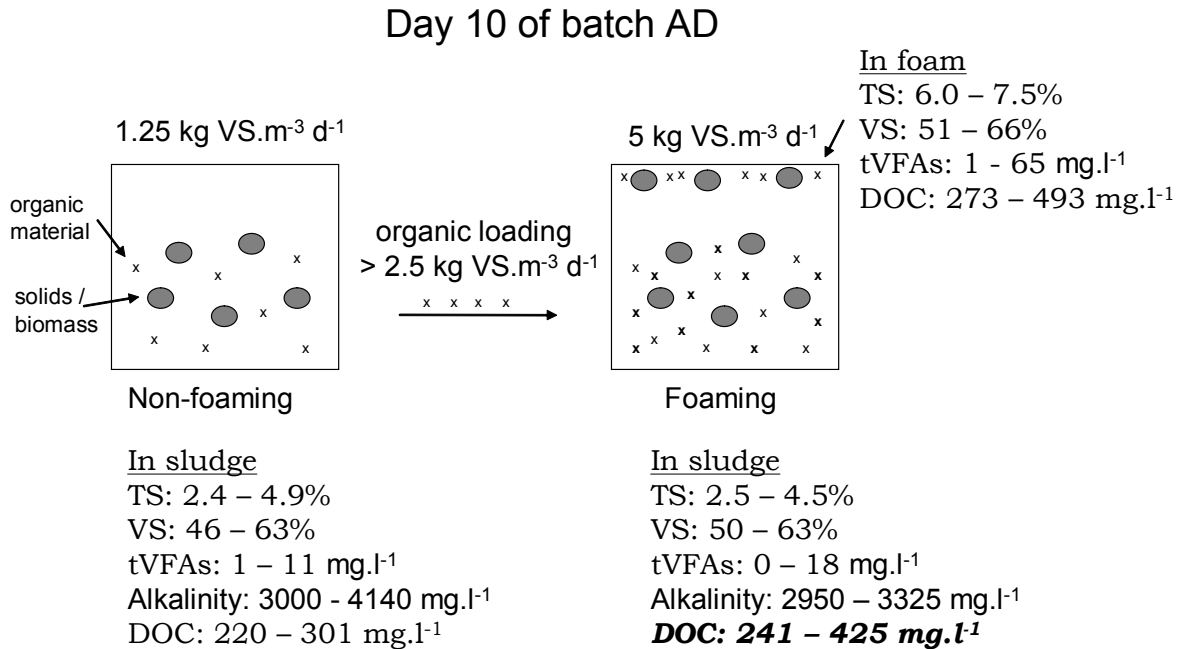


Figure 4.24: Model for foaming mechanisms in batch AD based on organic loading experiments (Day 10)

As shown in Figure 4.24, the increased concentrations of dissolved organics (x) in sludge at Day 10 of batch digestion were the only difference between the foaming (5 kg VS.m⁻³) and non-foaming (control) digestion bottles (ranges refer to average values of the three experiments on loading) while other parameters such as solids reduction, VFAs and alkalinity did not show any digestion inhibition. The total and volatile solids content in foam generated during batch AD was in accordance with information in the literature on solids concentrations for foam samples obtained at the full scale (Ross and Ellis 1992, Westlund *et al.* 1998). The total solids mass balance showed that the solids mass of foam and sludge combined at the end of batch AD from the 5 kg VS.m⁻³ loading was higher than the solids mass at the start for experiments 1 (start: 14.3 g, end: 14.4 g) and 3 (start: 23.6 g, end: 35.1 g). Experiment 2 showed similar concentrations of total solids in the foam (Figure 4.8) but achieved better solids reduction in sludge (Table 2, Appendix B) and overall the mass solids at the end of batch digestion was lower than at the start. The

generation of total solids during batch digestion could be attributed to generation of biomass due to the increased substrate of the 5 kg VS.m⁻³ loading. tVFAs (≤65 mg.l⁻¹), dissolved organics (5 – 493 mg.l⁻¹) and other organic material as volatile solids (51 – 66%) was measured in foam, which could have acted as surface active material for the creation of foam.

The literature has suggested that efficient digestion should correspond to 30 – 35% solids reduction, tVFAs less than 300 mg.l⁻¹ and alkalinity between 2000 mg.l⁻¹ and 3000 mg.l⁻¹ in digested sludge for any full scale conventional mesophilic digester and values outside of the suggested ranges could be indicative of inhibited digestion process. These parameters were monitored during the site survey work in order to identify correlations between the digestion efficiency at the full scale and foaming. The site survey findings of the 9 foaming and 6 non-foaming digesters indicated that poor digester performance could potentially be related to foaming as 6 out of the 9 foaming digesters (67%) demonstrated at least one operational parameter (alkalinity, VFAs or TS reduction) outside of the suggested range and only 2 of the 6 non-foaming digesters (33%) had at least one parameter outside the suggested range. Long term monitoring of the non-foaming, full scale digester at Site 16 did not verify that finding as the digester's performance varied throughout the monitoring period and was not always in accordance with the suggested operational ranges. This, however, agreed with the conclusion that a foaming cause cannot always be identified at the full scale based on standard analysis and routine and detailed monitoring is necessary.

The potential contribution of all parameters examined in this chapter to foaming was also investigated. Digesters, mechanically mixed, with good maintenance and digestion efficiency according to the suggested ranges had foaming (Site 14). Then again, digesters (Site 7) with gas mixing, average maintenance and poor digestion efficiency (tVFAs and alkalinity over the suggested range) had foaming. Although a clear correlation of organic loading with foaming was found at full and bench scale

supporting the hypothesis stated in this chapter that there are critical operational aspects of AD that contribute to or result in foaming and that sludge from non-foaming digesters can foam under these critical conditions, there was no indication that a common cause could be seen at all the foaming digesters examined. This suggested that full scale investigation of AD foaming is complex and further research of other potential causes of AD foaming, as identified in the literature review (Chapter 2), such as the effect of surface active agents and filamentous bacteria on AD foaming, was necessary.

Chapter 5:

Effect of surface active agents on sludge and digester foaming

5 Effect of surface active agents on sludge and digester foaming

5.1 Introduction

As previously mentioned in the literature review (Chapter 2), a large number of compounds commonly found in anaerobic digesters are surface active. Proteins are the most important group of surface active agents for AD as they are less biodegradable than other organic molecules such as lipids and fiber and thus have a greater effect on the digestion process and potentially foaming (Gonzales *et al.* 2003). An association between volatile fatty acids and more importantly accumulation of acetic acid and AD foaming has also been suggested by many researchers in the literature (Pagilla *et al.* 1997, Westlund *et al.* 1998, Barjenbruch *et al.* 2000). However, the critical concentrations for foaming in AD have not been identified. The findings in Chapter 4 demonstrated that tVFAs, although surface active according to the literature, had no impact on surface tension of digested sludge obtained from two full scale digesters (Sites 12 and 16). Additionally, foaming was recorded at both full and bench scale without any accumulation of acetic acid and generally VFAs during AD. The literature also showed that interactions between surface active compounds in a solution can enhance or reduce the foaming potential of the solution and depend on the type of surface active agents present (Glaser *et al.* 2007, Eisner *et al.* 2007). An example provided by Eisner *et al.* (2007), showed that the foaming potential and stability of foams created by the protein – fat mixture containing 9.75% molten butter, 11.3% spray dried skim milk powder, 12% sugar, 4% glucose syrup solids, 0.1% locust bean gum and 0.1% guar gum by weight was reduced when nonionic emulsions (monolaurate (0.9 μM), monooleate (0.7 μM) and trioleate of sorbitan (0.3 μM))

were added. Similar interactions between the surface active compounds in sludge and the sludge solids, or the organic matter or other surface active compounds found in sludge can be responsible for the creation of either unstable or metastable foams. However, understanding of these interactions in sludge and the effect of the degradation processes of AD on the foaming potential of sludges remains limited.

5.1.1 Hypothesis

The hypotheses developed in this section was that sludge and sludge digestion modify the behavior of surface active agents and there are critical concentrations of surface active agents that can induce foaming in sludge under aeration and during batch anaerobic digestion.

5.1.2 Aims and Objectives

This chapter aimed to provide an understanding of the effect of both the interactions between surface active agents in sludge and the impact of the metabolic activity in digesters on the sludges foaming potential and identify the critical concentrations of surface active agents necessary to induce and / or stabilize foaming in AD.

The objectives of this part of work involved:

- identification of suitable examples of surface active agents in the laboratory and further investigation of their behavior and effect on foaming in sludge under aeration (foaming tests) and during digestion (batch anaerobic digestion studies)

- examination of the association of the presence of surface active agents in anaerobic digesters at the full scale with foaming by assessing the sludges foaming potential and quality characteristics.

5.2 Results

5.2.1 Laboratory investigation of the effect of surface active agents on foaming in sludge and under batch anaerobic digestion

5.2.1.1 Examination of surface activity of proteins

The surface tension and foaming potential of the compounds BSA, gelatin, casein and the protein mixtures are given below. The surface tension of deionized water was measured at the start of all experiments and was approximately $72 \text{ mN}\cdot\text{m}^{-1}$, which was in accordance with references in the literature (Vardar-Sukan 1998).

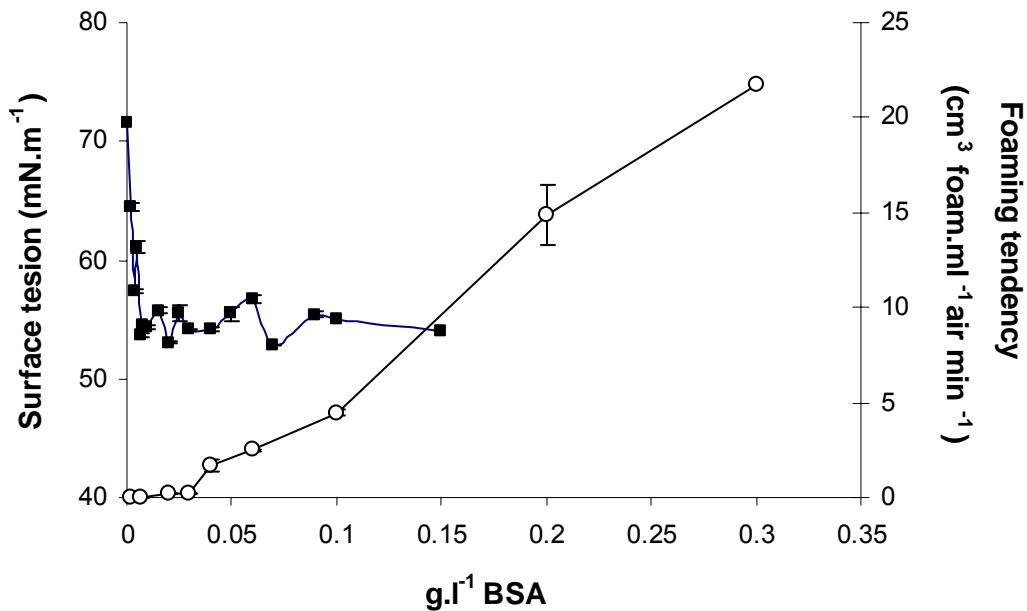


Figure 5.1: Surface tension ($\text{mN}\cdot\text{m}^{-1} \pm \text{SD}$) (■) and foaming tendency ($\text{cm}^3 \text{ foam}\cdot\text{ml}^{-1} \text{ air min}^{-1} \pm \text{SD}$) (○) of BSA in water

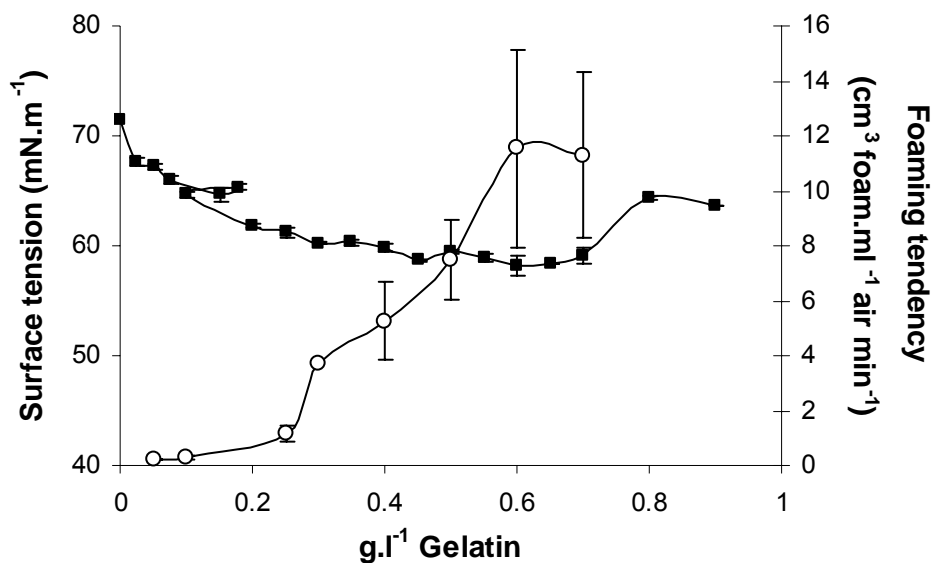


Figure 5.2: Surface tension ($\text{mN}\cdot\text{m}^{-1} \pm\text{SD}$) (■) and foaming tendency ($\text{cm}^3 \text{ foam}\cdot\text{ml}^{-1} \text{ air min}^{-1} \pm\text{SD}$) (○) of Gelatin in water

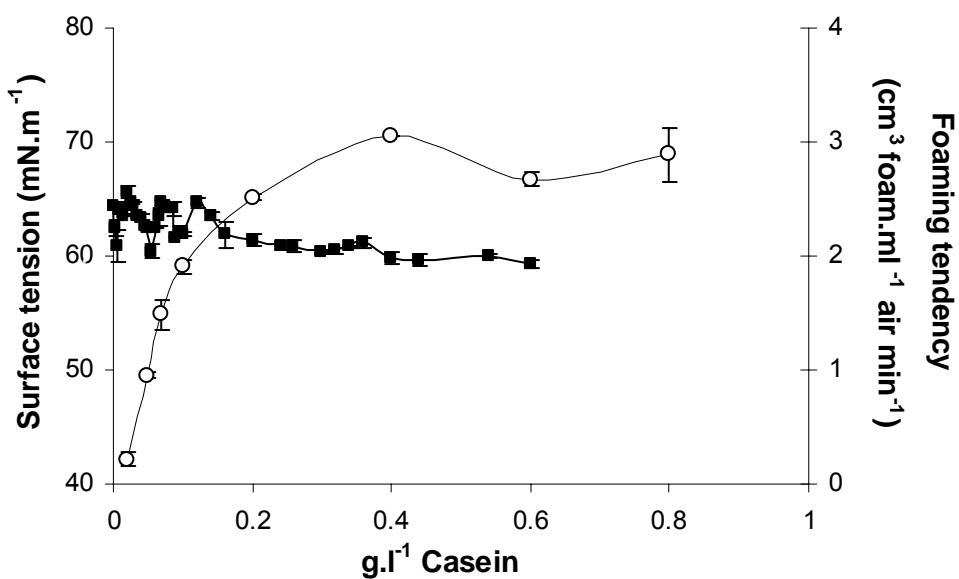


Figure 5.3: Surface tension ($\text{mN}\cdot\text{m}^{-1} \pm\text{SD}$) (■) and foaming tendency ($\text{cm}^3 \text{ foam}\cdot\text{ml}^{-1} \text{ air min}^{-1} \pm\text{SD}$) (○) of Casein in water

All three proteins were surface active as they decreased the surface tension and induced foaming in water which increased with increasing protein concentrations (Figure 5.1, Figure 5.2, Figure 5.3). Specifically, BSA was the most surface active protein lowering the surface tension to about $55 \text{ mN}\cdot\text{m}^{-1}$ at very small concentrations of $0.007 \text{ g}\cdot\text{l}^{-1}$. Surface tension of gelatin also decreased with increasing gelatin concentrations and remained stable at around $60 \text{ mN}\cdot\text{m}^{-1}$ for concentrations over $0.3 \text{ g}\cdot\text{l}^{-1}$. Casein was insoluble in water and was suspended in 0.01 M sodium hydroxide (NaOH) aqueous solution. Surface tension and foaming tendency were determined in the 0.01 M sodium hydroxide solutions. Surface tension increased and decreased inconsistently between concentrations of $0.0035 \text{ g}\cdot\text{l}^{-1}$ and $0.16 \text{ g}\cdot\text{l}^{-1}$ due to the presence of the different subunits of casein. However, at concentrations over $0.2 \text{ g}\cdot\text{l}^{-1}$ surface tension was consistently lowered by the presence of casein with a value of around $60 \text{ mN}\cdot\text{m}^{-1}$.

During the foaming tests, gelatin was found to be the only protein to result in stable foams with foams produced from the other two proteins collapsing within seconds and BSA was the most surface active with only $0.06 \text{ g}\cdot\text{l}^{-1}$ of BSA resulting in the same foaming tendency (2.5 cm^3 of foam per ml air per min) as $0.25 - 0.3 \text{ g}\cdot\text{l}^{-1}$ of gelatin and $0.2 \text{ g}\cdot\text{l}^{-1}$ of casein. Thus, the mixtures of BSA and gelatin were examined for their foaming tendency and stability in order to assess the combined effect of a protein with high surface activity and hence foaming potential and a protein with stability and study their behavior in water and sludge. The following graphs present data on foaming tendency and stability in water for all proteins and proteins mixtures examined.

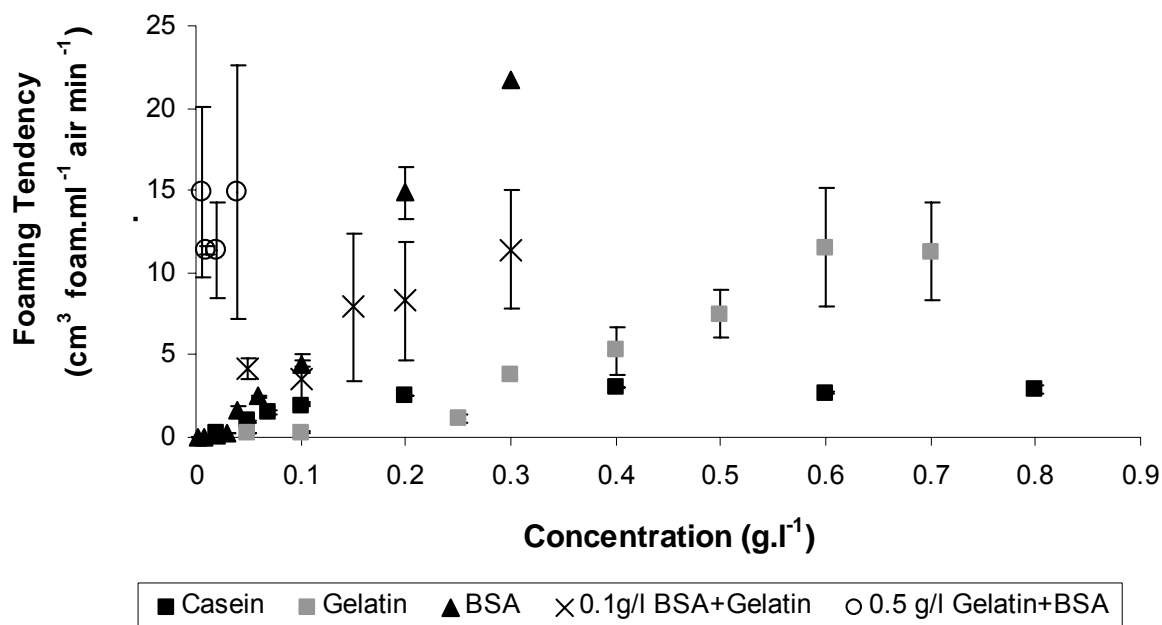


Figure 5.4: Foaming tendency ($\text{cm}^3 \text{ foam.ml}^{-1} \text{ air min}^{-1} \pm \text{SD}$) of proteins and protein mixtures in water

It can be seen from the graph above that BSA alone had the greatest foaming tendency. At BSA concentrations over 0.2 g.l^{-1} foam was exceeding 1 meter of height which was the maximum height of the aeration column. Gelatin and casein foaming tendency was similar for concentrations up to 0.4 g.l^{-1} . Further concentration increase showed that gelatin had higher foaming tendency than casein at the concentrations tested. The mixture of proteins containing 0.1 g.l^{-1} BSA and varying concentrations of gelatin had lower foaming tendency than BSA alone but higher than gelatin alone. The mixture of 0.5 g.l^{-1} gelatin and varying concentrations of BSA had similar or higher foaming tendency than gelatin but the standard deviations were significant and the effect of increasing concentrations on increasing foaming tendency was not seen.

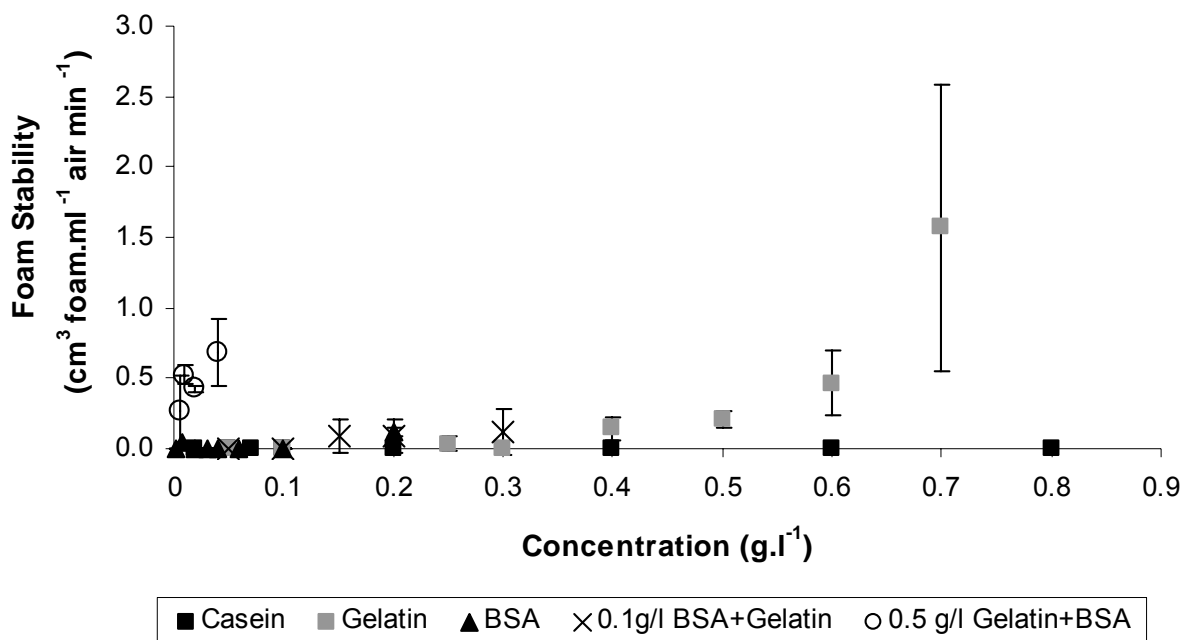


Figure 5.5: Foam stability ($\text{cm}^3 \text{ foam. ml}^{-1} \text{ air min}^{-1} \pm \text{SD}$) of proteins and proteins mixtures in water

Gelatin produced stable foams and stability increased with increasing concentrations. Casein stability was 0 for all concentrations as well as BSA apart from two occasions at concentrations of 0.007 g.l^{-1} and 0.2 g.l^{-1} having 0.05 and $0.12 \text{ cm}^3.\text{ml}^{-1}\text{air min}^{-1}$ stability respectively. The mixture of 0.1g.l^{-1} BSA + Gelatin had little stability only at the two highest concentrations tested (0.08 and $0.12 \text{ cm}^3.\text{ml}^{-1}\text{air min}^{-1}$ at 0.2g.l^{-1} and 0.3g.l^{-1} respectively) which, however, was higher than that of gelatin alone at the same concentrations (<0.03 and $0 \text{ cm}^3.\text{ml}^{-1} \text{ air min}^{-1}$ at 0.2g.l^{-1} and 0.3g.l^{-1} respectively). There was indication therefore that foam stability was slightly improved in the mixture of 0.1g.l^{-1} BSA + Gelatin than that of gelatin alone. The mixture of 0.5 g.l^{-1} gelatin and varying concentrations of BSA produced stable foams and stability increased with increasing concentrations.

5.2.1.2 Effect of proteins on foaming in sludge

The behavior of proteins in sludge was examined under aeration and subsequent determination of the sludges foaming potential.

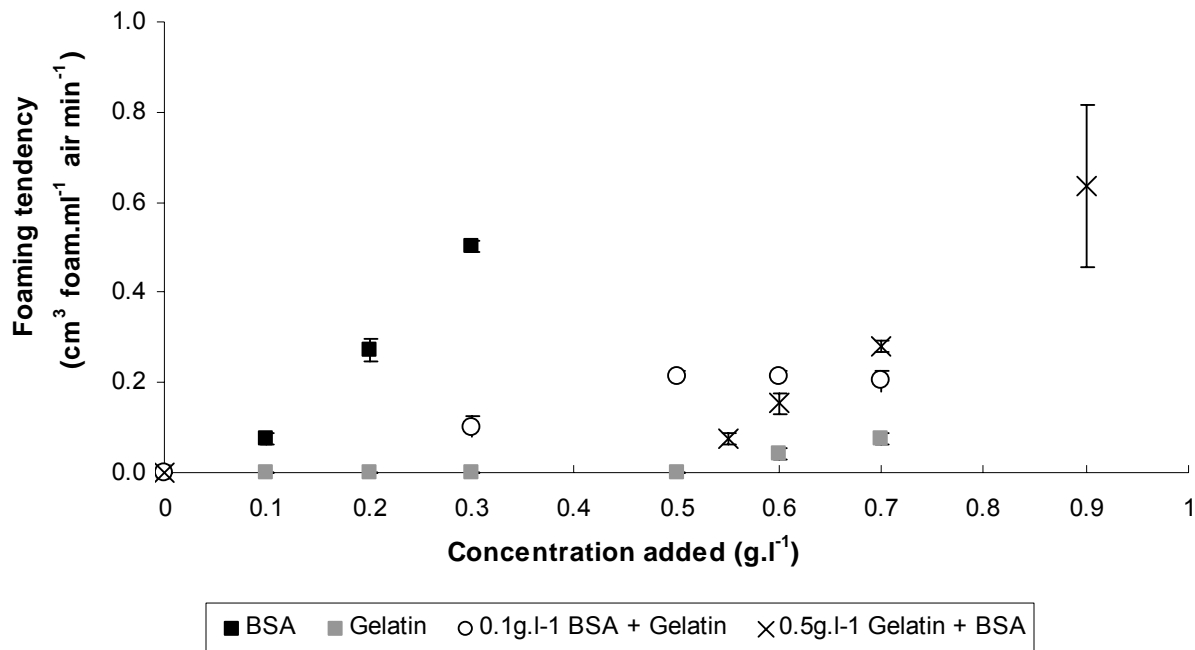


Figure 5.6: Foaming tendency (cm³ foam.ml⁻¹ air min⁻¹ ±SD) of proteins and proteins mixtures in sludge

Sludge had much lower foaming tendency compared to water after addition of the same concentrations of proteins. Specifically for BSA, the foaming tendency in sludge was approximately 56 times lower than the foaming tendency in water at 0.1g.l⁻¹ BSA, which supported the hypothesis that sludge modifies the behavior of surface active agents. BSA was again the most surface active protein in sludge. Gelatin and the mixtures of proteins also induced foaming in sludge at smaller scale compared to foaming in water. However, no stability was recorded for any of the proteins or the protein mixtures in sludge. Table 5.1 summarizes the total protein concentrations (g.l⁻¹) for foam initiation in water and sludge under aeration.

Table 5.1: Total protein concentrations for foam initiation in water and sludge under aeration

Compound / mixture	Foam initiation concentration in water (g.l⁻¹)	Foam initiation concentration in sludge (g.l⁻¹)
BSA	0.007	0.090
Gelatin	0.030	0.600
0.1 g.l ⁻¹ BSA + Gelatin	0.100	0.180
0.5 g.l ⁻¹ Gelatin + BSA	0.500	0.540

According to Table 5.1, the concentrations required for foam initiation in sludge under aeration are much higher than the ones for water. This could be attributed to interactions such as adsorption between the added surface active agents and the solids or the organic matter of sludge. The partitioning of proteins in solution and on the solids was studied through determination of proteins concentrations in sludge as EPS and SMPs after addition of the examined concentrations (Figures 31 – 34, Appendix B). Addition of 0.1 g.l⁻¹ BSA did not have an impact on the protein concentrations as EPS and SMPs (sample without BSA: EPS: 1015±35 mg.l⁻¹, SMPs: 136±40 mg.l⁻¹, sample with 0.1 g.l⁻¹ BSA: EPS: 1010±40 mg.l⁻¹, SMPs: 130±11 mg.l⁻¹) but addition of 0.3 g.l⁻¹ BSA increased the SMPs to 295±41 mg.l⁻¹ (EPS: 901±27 mg.l⁻¹). At this stage it is necessary to mention that the methods used for the determination of proteins as SMPs and EPS could have generated some error in the values obtained, which is however, unavoidable. The concentrations of SMPs and EPS after addition of BSA showed that BSA had the affinity to stay in solution when added in sludge, yet it didn't have the same effect on the foaming tendency in sludge as it had in water indicating that its surface activity and foaming potential was suppressed by the presence of organic compounds and solids in sludge. Similar findings have been found in the literature demonstrating that the presence of the nonionic emulsions of monolaurate (0.9

μM), monooleate ($0.7 \mu\text{M}$) and trioleate of sorbitan ($0.3 \mu\text{M}$) reduced the foaming potential and stability in a protein – fat matrix (Eisner *et al.* 2007). The partitioning of gelatin and the mixtures of BSA and gelatin as SMPs and EPS in sludge exhibited similar patterns with BSA.

5.2.1.3 Effect of proteins as BSA on foaming during batch anaerobic digestion

Subsequently, the effect of added surface active agents as protein in sludge on foam initiation and stabilization during batch anaerobic digestion was examined. Only BSA of the above proteins was studied during batch anaerobic digestion of sludge due to time and rig limitations. Although BSA is not usually found in anaerobic digesters and it would be expected that the majority of surface active agents in AD would be of smaller size due to the degradation processes providing there is no digestion inhibition, further examination of BSA was attributed to the fact that it was the most surface active compound. Addition of BSA in this work represented a change in the feed quality often described in the literature as shock loading of full scale digesters that can result from various sludge or industrial imports at the full scale and induce foaming in AD.

Previous experimental work showed that 0.1 g.l^{-1} was associated with foam initiation in sludge during aeration and any higher concentration than that induced more foaming. Three different concentrations of BSA were tested, 0.1, 0.3 and 1 g.l^{-1} in addition to the control, which contained only seed sludge. The organic loading was 1.25 kgVS.m^{-3} , as it was clearly demonstrated from previous experiments that no foaming was induced at that loading during batch digestion. The data obtained from batch digestion of sludge with added BSA are presented in the following paragraphs.

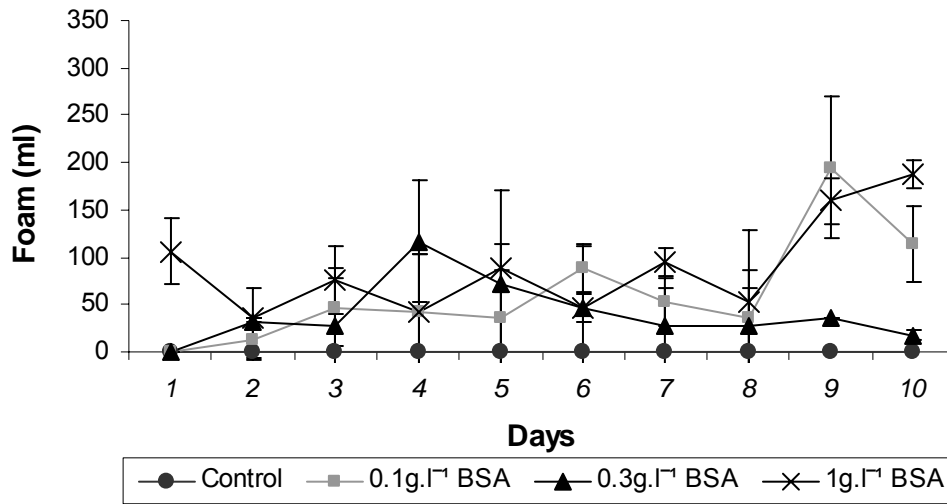


Figure 5.7: Daily foam production (ml \pm SD) during batch anaerobic digestion, experiment on BSA

All three tested concentrations of BSA resulted in foam initiation and stabilization during batch digestion of sludge. There was not a significant difference between the effect of 0.1 g.l⁻¹ BSA and 0.3 g.l⁻¹ BSA on foaming during digestion ($P=0.43$, $\alpha=95\%$) but the addition of 1 g.l⁻¹ BSA during batch digestion resulted in significantly higher foaming production than the lower BSA values tested ($P= 0.035$ for the 0.1 g.l⁻¹ BSA and $P=0.002$ for the 0.3 g.l⁻¹ BSA) (Figures 39-44, Appendix B). The control (seed sludge only) did not present any foaming during the digestion period. At this stage it is important to mention that foam was mechanically destroyed by high stirring on a daily basis and was recreated within 24 hours of its destruction.

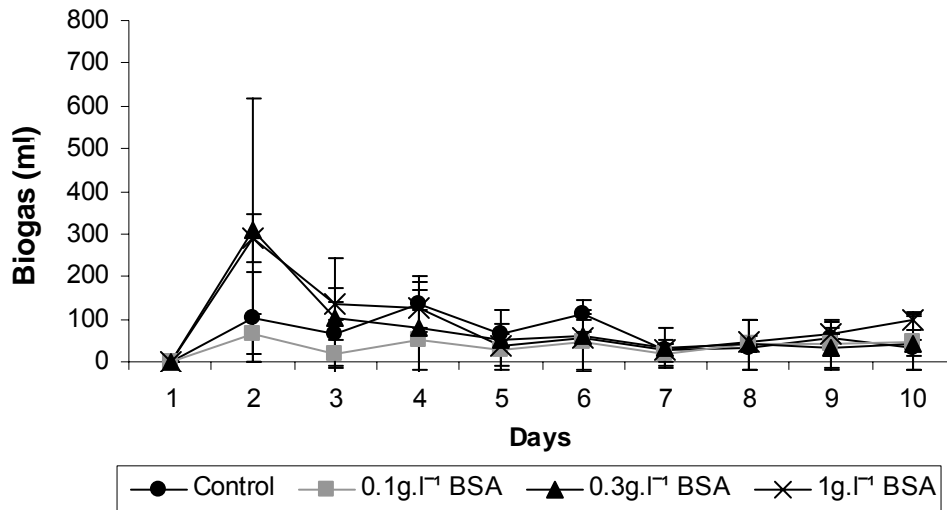


Figure 5.8: Daily biogas production (ml ±SD) during batch anaerobic digestion, experiment on BSA

The biogas production of the control and the digestion bottles containing 0.1g.l⁻¹ BSA was similar potentially suggesting that BSA had not been degraded during digestion and subsequently converted to biogas, which was in accordance with information found in the literature stating that proteins are less biodegradable than other organic molecules (Gonzales *et al.* 2003) or the amount of BSA added was too small to have an impact on the biogas production. Similar gas production was also recorded for the two highest loadings of BSA indicating that the excess BSA in the 1g.l⁻¹ BSA digestion bottles had not been degraded during digestion. Further sludge quality analysis, which is presented in the following paragraphs, could support this suggestion. Additional monitoring parameters are summarized in Table 5.2 including the total and volatile solids reduction and methane composition during the BSA batch digestion experiment.

Table 5.2: Solids reduction and gas composition (as methane) ranges during the batch digestion period, experiment on BSA

	%TS reduction	%VS reduction	%CH ₄
Control	11.5	17.7	20 – 52
0.1g.l ⁻¹ BSA	8.0	11.7	35 – 56
0.3g.l ⁻¹ BSA	5.8	12.1	52 – 58
1g.l ⁻¹ BSA	15.3	17.2	53 – 65

According to the data above, methane was formed during digestion from all the digestion bottles as well as from the control indicating that the methanogenic phase of the digestion process had been reached. The literature suggests that the CH₄ content in biogas at full scale digesters is around 62 – 70% and total solids reduction vary between 30-35% of input load (Handbooks of UK Wastewater Practice 1996). Taking into consideration the small scale of the batch experiments the lower percentages of solids reduction and methane found in these experiments are not unexpected.

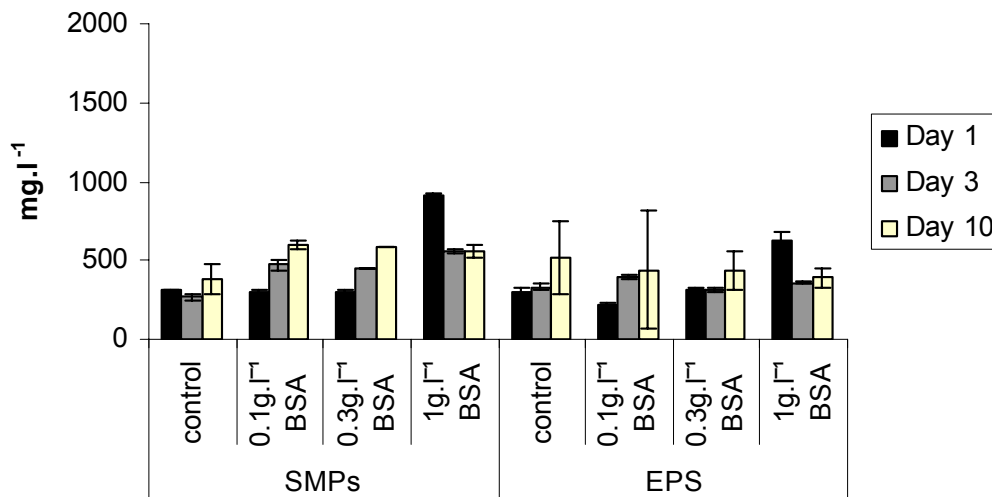


Figure 5.9: Proteins as SMPs and EPS in sludge samples during batch anaerobic digestion, experiment on BSA

According to Figure 5.9, the total protein concentration as SMPs and as EPS of sludge with added 0.1 and 0.3 g.l⁻¹ BSA was similar during batch digestion for both concentrations. The addition of 1g.l⁻¹ BSA to sludge resulted in high protein load both as SMPs (918 mg.l⁻¹) and EPS (624 mg.l⁻¹) at the start of the batch digestion which however decreased as the digestion proceeded and similar values with the two lower BSA concentrations were obtained (Day 10, 0.1 g.l⁻¹ BSA: 602 mg.l⁻¹, 0.3 g.l⁻¹ BSA: 584 mg.l⁻¹, 1 g.l⁻¹ BSA: 558 mg.l⁻¹ as SMPs and 0.1 g.l⁻¹ BSA: 441 mg.l⁻¹, 0.3 g.l⁻¹ BSA: 435 mg.l⁻¹, 1 g.l⁻¹ BSA: 391 mg.l⁻¹ as EPS). This was in accordance with the findings of Gonzales *et al.* (2003) demonstrating that there was a final equilibrium concentration value for each sludge that was independent of the initial protein concentration. Given that sludges with 0.1 and 1 g.l⁻¹ BSA contained similar amount of total protein as SMPs and EPS at the end of batch digestion, the excess protein that was initially added of the highest BSA loading would have been degraded or accumulated in the foam due to the surface active properties of BSA or both. Further analysis of sludges from the three BSA loadings as presented below helped understand the behavior of BSA during batch AD.

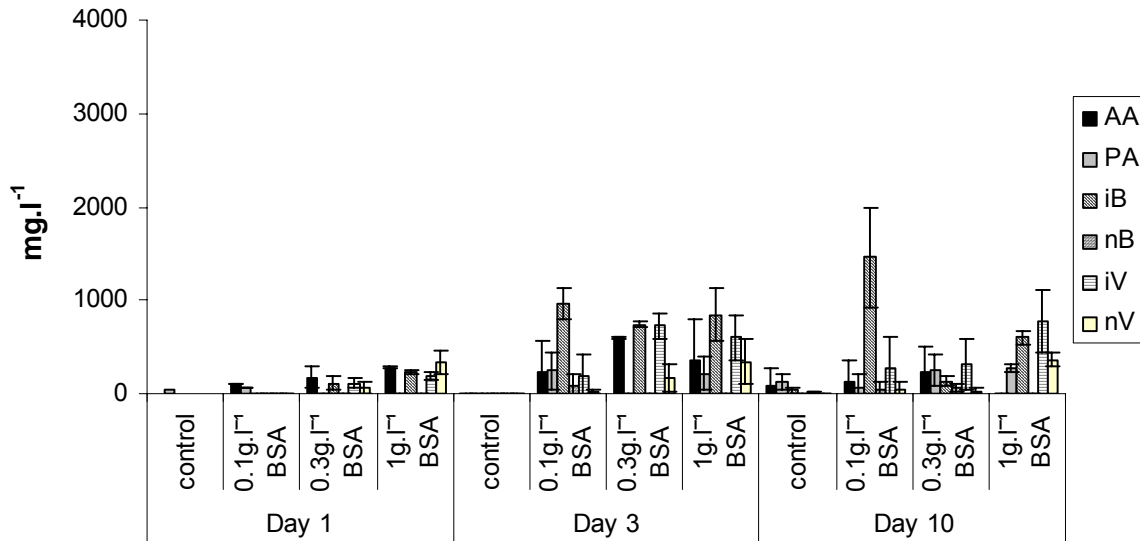


Figure 5.10: Individual VFAs for sludge samples during batch digestion, experiment on BSA

Recommended ranges for total VFAs concentrations in digested sludge vary between 50 to 300 mg.l⁻¹ (Metcalf and Eddy 2003). The values measured in sludge samples at the end of batch digestion (Day 10) exceeded the recommended concentrations for all sludge samples containing BSA. Also, the concentrations as total VFAs measured in sludge from the lowest and highest BSA loadings were almost the same, as sludge from the 0.1 and 1g.l⁻¹ BSA digestion bottles contained 2012 and 2000mg.l⁻¹ total VFAs, respectively. This could have been due to the breakdown of BSA into amino acids and subsequently into organic acids which then increased the concentrations of VFAs. The high VFAs values found in the samples were also an indication of poor digestion performance due to the high protein loading. At this stage however, the effect of the excess protein that was initially added of the highest BSA loading was still not seen as similar tVFAs were measured in sludge from the 0.1 and 1g.l⁻¹ BSA digestion bottles.

Alkalinity values increased from 4840 (SD:14) mg.l⁻¹ for sludge with 0.1 g.l⁻¹ added BSA, to 4930 (SD: 240) mg.l⁻¹ for sludge with 0.3 g.l⁻¹ added BSA and 5250 (SD:

70) mg.l^{-1} for sludge with 1 g.l^{-1} added BSA while alkalinity from the control was 5400 mg.l^{-1} (SD:848). Soluble SCOD and DOC concentrations increased as digestion proceeded (from Day 1 to Day 10 of batch digestion). Sludges with 0.1, 0.3 and 1 g.l^{-1} BSA had SCOD values of 3567, 2707 and 3630 mg.l^{-1} and DOC values of 1231, 862, 1606 mg.l^{-1} , respectively at the end of batch digestion. These values were significantly higher than the SCOD and DOC of the control at the end of batch digestion (SCOD: 1468 mg.l^{-1} , DOC: 503 mg.l^{-1}). Schematic representation of the data can be found in Appendix B (Figures 35 – 38). According to previous findings from Chapter 4 (paragraph 4.3), there was a statistically significant difference in the concentrations of DOC in sludges obtained from the lowest, non-foaming organic loading and sludges from the highest, foaming organic loading. The DOC content in sludge from the highest organic loading, which resulted in persistent foaming ranged between 241 and 354 mg.l^{-1} . The DOC values in sludge samples containing BSA were about 5 times higher than the values obtained from the organic loading experiment and 2 to 3 times higher than the DOC values from the control of the BSA experiment indicating that there was an increase in the dissolved organics content due to addition of BSA and could have contributed to foaming. Furthermore, the difference in SCOD from the addition of 0.1 and 1 g.l^{-1} BSA in sludge was 1472 mg.l^{-1} at the start (Day 1) of batch digestion and only 63 mg.l^{-1} at Day 10 of batch digestion showing almost no difference in SCOD values in sludge between the lowest and highest BSA loading at the end of batch digestion. The difference in DOC values from the addition of 0.1 and 1 g.l^{-1} BSA in sludge was 167 mg.l^{-1} at the start (Day 1) of batch digestion and 375 mg.l^{-1} at Day 10. Hence, a difference (increased DOC) in the sludge quality characteristics during batch digestion between 0.1 and 1 g.l^{-1} BSA loading was recorded due to the effect of the excess protein that was initially added of the 1 g.l^{-1} BSA loading. The effect of the excess BSA induced by the addition of 1 g.l^{-1} BSA in comparison to the 0.1 g.l^{-1} BSA was not seen on all the other measurements obtained on gas and methane production, solids reduction, proteins as EPS and SMPs and tVFAs, as explained in previous paragraphs. At this stage there was

experimental evidence about the fate of BSA during batch AD demonstrating that increased protein load during batch AD increased the dissolved organics content in sludge 2 to 3 times higher than the control at the end of batch digestion and resulted in accumulation of tVFAs out of the normal range for AD showing potentially inhibition of the digestion process. Further increase of the BSA load continued to increase the dissolved organics content but did not alter further other sludge quality characteristics such as proteins as EPS and SMPs and tVFAs and did not affect the gas and methane production and solids reduction.

Foam samples were collected during the experiment on BSA and analyzed from the digestion bottles of 0.1 and 1g.l⁻¹ BSA. The data obtained are presented below.

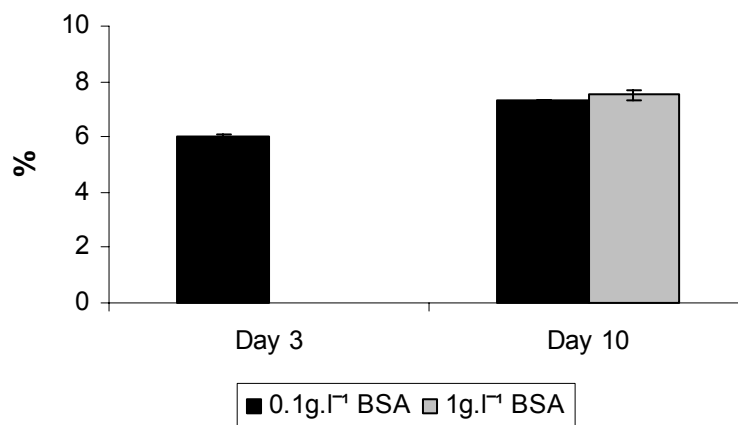


Figure 5.11: Total solids (% \pm SD) in foam samples, experiment on BSA

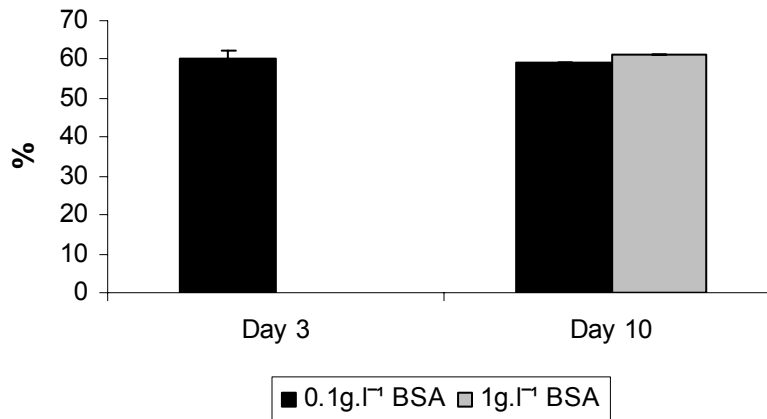


Figure 5.12: Volatile solids (% \pm SD) in foam samples, experiment on BSA

Total solids in foam varied from 6% to 7.5% and volatile solids from 59% to 61%. These values matched the solids concentrations in foam samples obtained from the experiments on organic loading (total: 5.7 – 7.5%, volatile 51-66%), as given in paragraph 4.2.3, indicating that although the cause of foaming was different the foam had very similar solids concentrations. These values were also in accordance with information from the literature as total solids in foam samples obtained from full scale foaming digesters vary between 6% and 13.2% and volatile solids between 65 – 70% (Ross and Ellis 1992, Westlund *et al.* 1998). Calculation of the mass of total and volatile solids of the 0.1 and 1g.l⁻¹ BSA digestion bottles showed that overall the solids (both total and volatile) for sludge and foam combined increased at the end of batch digestion (0.1g.l⁻¹ BSA TS increase: 6.6 g, VS increase: 11.3 g, 1g.l⁻¹ BSA TS increase: 3.5 g, VS increase: 6.8 g). This indicated that biomass was potentially generated during batch digestion. The increase in the total mass of VS (sludge+foam) at the end of batch digestion could be attributed to metabolic by-products or cell lysis of the acetogenic bacteria due to the limited substrate as digestion proceeded.

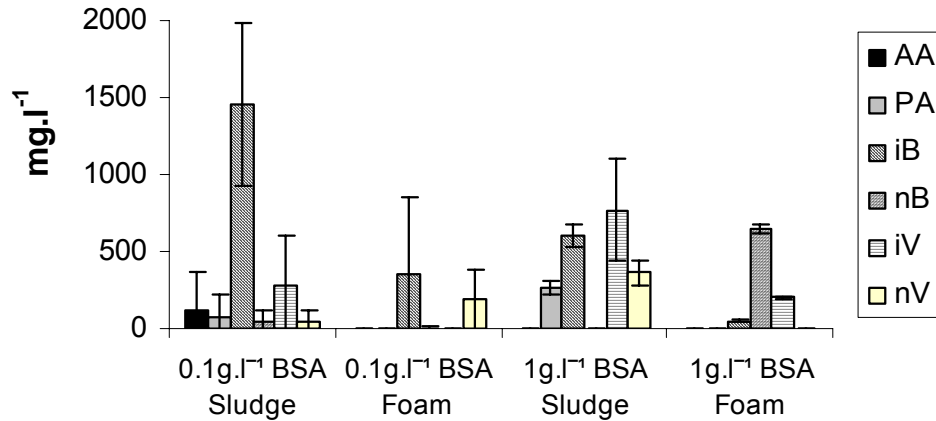


Figure 5.13: Individual VFAs ($\text{mg.l}^{-1} \pm \text{SD}$) in foam and sludge samples on Day 10, experiment on BSA

The analysis of foam and sludge samples on Day 10 of digestion showed that there seemed to be no VFAs partitioning in the foam samples and in most cases lower concentrations of VFAs were found in the foam compared to the ones found in the sludge samples.

Soluble COD in the foam samples was lower than the SCOD in the sludge samples on Day 10 (2125 g.l^{-1} in foam and 3567 g.l^{-1} in sludge of the 0.1 g.l^{-1} BSA, 2933 g.l^{-1} in foam and 3630 g.l^{-1} in sludge of the 1 g.l^{-1} BSA) (Figures 36 & 37, Appendix B). The dissolved organics concentration of the 0.1 g.l^{-1} BSA decreased from Day 3 to Day 10, as represented in the following graph. Only one value was obtained for the 1 g.l^{-1} BSA digestion bottles on Day 10 of digestion due to the small sample volume for analysis.

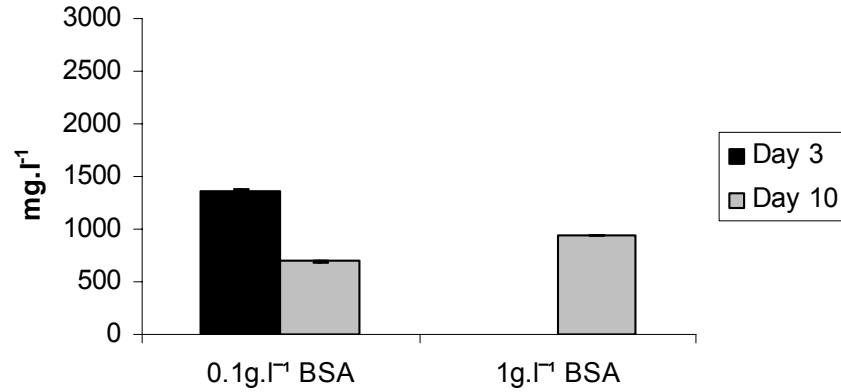


Figure 5.14: Dissolved organic content (mg.l⁻¹ ±SD) in foam samples, experiment on BSA

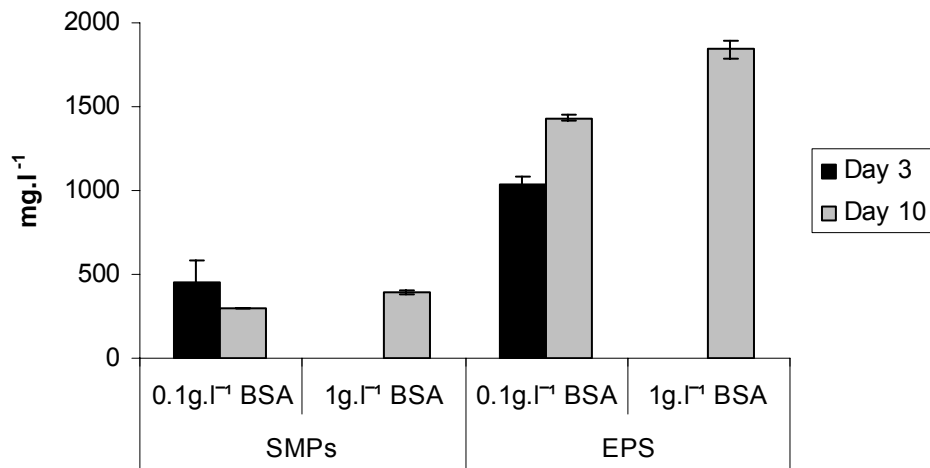


Figure 5.15: Proteins as SMPs and EPS (mg.l⁻¹ ±SD) in foam samples, experiment on BSA

Similar values were observed for proteins as SMPs in foam on Day 10 from the digestion bottles of 0.1 and 1g.l⁻¹ BSA. The proteins measured in foam samples as EPS were double and over the values found as SMPs, but again not too different on Day 10 between foam samples obtained from 0.1 and 1g.l⁻¹ BSA digestion bottles considering the excess BSA added in the latter case. No foam sample was obtained on Day 3 from the highest BSA concentration due to sample volume

limitations. In addition, proteins as SMPs in the foam samples were not higher than protein values found in sludge (for 0.1 g.l⁻¹ BSA: 452 and 301 mg.l⁻¹ in foam and 471 and 602 mg.l⁻¹ in sludge on Day 3 & 10 respectively, for 1g.l⁻¹ BSA: 394 mg.l⁻¹ in foam and 558 mg.l⁻¹ in sludge on Day 10). The proteins concentrations as EPS, however, were much higher in foam than in sludge (for 0.1g.l⁻¹ BSA: 1040 and 1434mg.l⁻¹ in foam and 396 and 441mg.l⁻¹ in sludge on Day 3 & 10 respectively, for 1g.l⁻¹ BSA: 1840 mg.l⁻¹ in foam and 391mg.l⁻¹ in sludge on Day 10). The higher amount of protein found as EPS in the foam indicated potential absorbance of proteins onto solids and the affinity of partitioning in the foam layer due to the higher solids concentration than the ones found in sludge, as previously mentioned.

In summary, the previous paragraphs showed that all three proteins and the two protein mixtures studied above were surface active. Their effect on foam initiation and stabilization under aeration was different in water to the one in sludge and the hypothesis that sludge modifies the behavior of surface active agents was supported by the experimental data. Concentrations higher than the ones used in water were needed in sludge for foam initiation under aeration and the surface activity and foaming potential of proteins was potentially suppressed by the presence of organic compounds and solids in sludge. The effect of added concentrations of BSA on foaming during batch anaerobic digestion showed that although BSA induced foaming under aeration, which increased with increasing concentrations, during digestion there was not a statistically significant difference in foam production between 0.1 and 0.3 g.l⁻¹ BSA and only the 1 g.l⁻¹ BSA resulted in higher foaming. In addition, BSA did not produce stable foams in either water or sludge under aeration. Yet, all foams were stable and were re-created after mechanical destruction during anaerobic digestion. The effect of BSA on AD involved accumulation of VFAs during batch AD, increase of the dissolved organic content in sludge and partitioning of proteins as EPS in the foam. Further increase of the BSA load continued to increase the dissolved organics content in sludge but

did not alter further other sludge quality characteristics such as proteins as EPS and SMPs and tVFAs while partitioning of proteins as EPS in foam increased.

5.2.1.4 Examination of surface activity of n-valeric acid

Below are the data obtained on surface tension and foaming tendency in water for n-valeric acid.

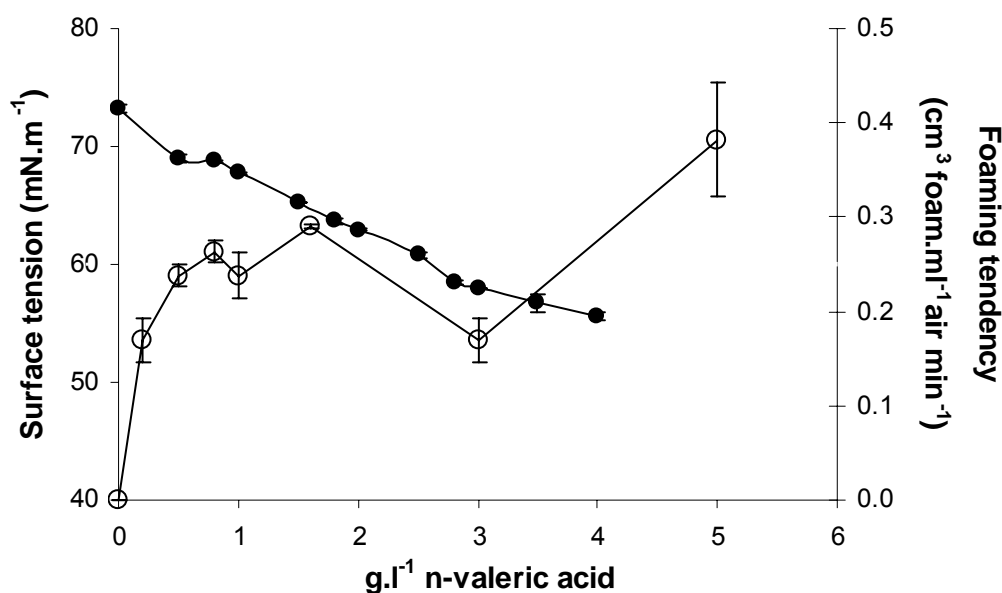


Figure 5.16: Surface tension (mN.m⁻¹ ±SD) (●) and foaming tendency (cm³ foam.ml⁻¹ air min⁻¹ ±SD) (○) of n-valeric acid in water

Although n-valeric acid lowered the surface tension and induced foaming in water at concentrations as low as 0.2g.l⁻¹, no stability was observed in all foams created.

5.2.1.5 Effect of n-valeric acid on foaming in sludge

N-valeric acid had no impact on the sludge's foaming tendency at the examined concentrations (0 – 5 g.l⁻¹). The determination of the concentrations of n-valeric in

solution in sludge after addition of the studied concentrations (Figure 45, Appendix B) showed that approximately 54% of the added nV was found in solution and the reduced effect of nV on the sludge's foaming tendency could have been attributed to adsorbance of nV onto the solids due to its hydrophobicity or interactions with the organic matter of sludge. N-valeric was subsequently studied during batch anaerobic digestion.

5.2.1.6 Effect of n-valeric acid on foaming during batch anaerobic digestion

The daily foam production after addition of the studied concentrations of nV during batch anaerobic digestion of sludge is illustrated below.

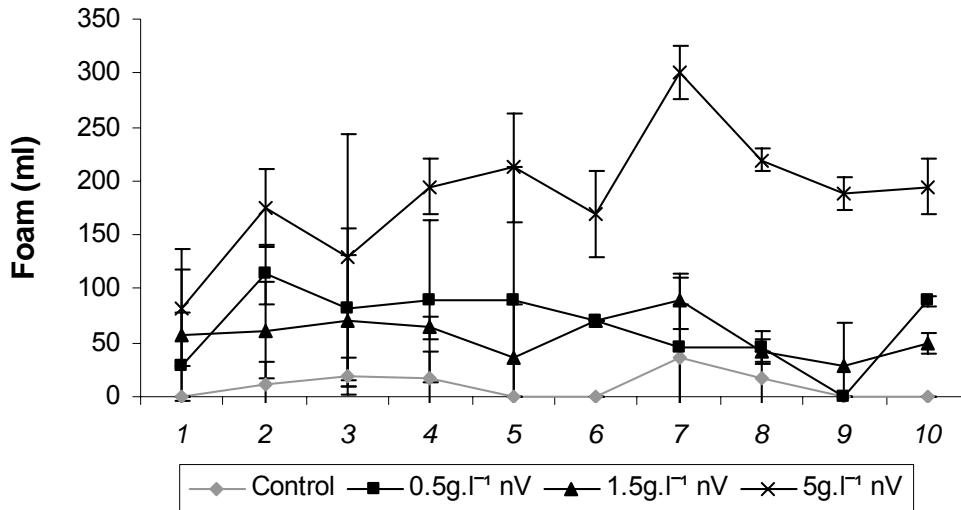


Figure 5.17: Daily foam production (ml ±SD), experiment on n-valeric

The investigation of n-valeric in sludge during digestion showed a link between foaming and n-valeric presence. The foam volume increased with increasing concentrations of nV. Similarly to previous batch experiments on organic loading and BSA, foam was mechanically destroyed by high stirring on a daily basis and

was re-created within 24 hours. Additional data on the digestion efficiency included biogas production, solids reduction and methane composition, as presented below.

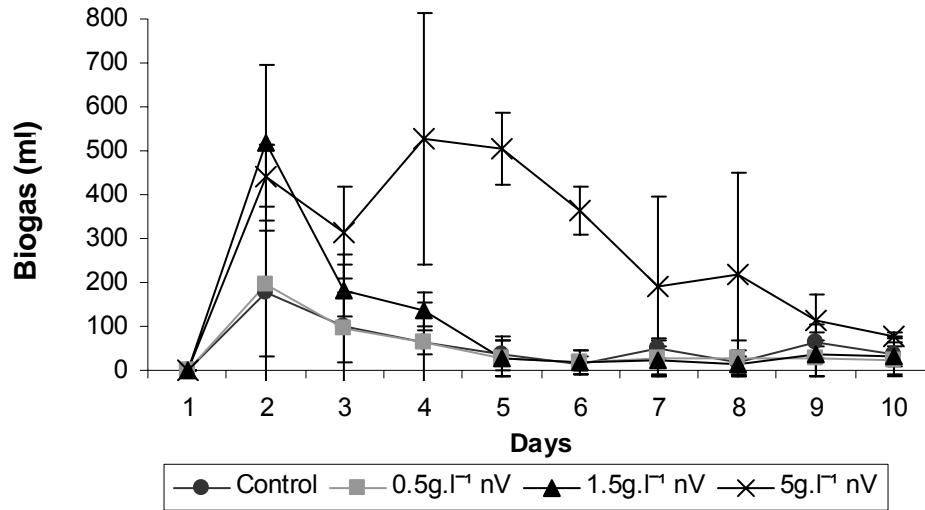


Figure 5.18: Daily biogas production (ml ±SD) during batch anaerobic digestion, experiment on n-valeric

Table 5.3: Solids reduction and gas composition (as methane) ranges during the batch digestion period, experiment on n-valeric

	<i>% TS reduction</i>	<i>% VS reduction</i>	<i>%CH₄</i>
control	27.5	24.1	43 – 58
0.5g.l ⁻¹ nV	30.8	29.1	60 – 64
1.5g.l ⁻¹ nV	30.1	28.4	64 – 66
5g.l ⁻¹ nV	32.8	30.0	64 – 78

Given that methane percentages at full scale digesters vary around 62 – 70% and total solids reduction vary between 30-35% of input load, as previously stated, and taking into consideration the small scale of the batch digestion experiments, the above results are understandable. The higher methane content with increasing n-valeric concentrations in sludge was a result of high amounts of acetic acid

deriving from the degradation of n-valeric and subsequent utilization of acetic acid by methanogenic bacteria.

The alkalinity did not vary significantly between the four conditions examined (control and 3 n-valeric concentrations) with the lowest concentration at 4050 mg.l⁻¹ and highest at 4550 mg.l⁻¹. These data were comparable to alkalinity values from the previous batch experiment on BSA where alkalinity values were between 4840 mg.l⁻¹ and 5400 mg.l⁻¹. Individual VFAs values in sludge samples were measured on Day 3 and Day 10 of digestion. The breakdown of nV was immediate as on Day 3 there was no nV left in the digestion bottles. This indicated that even at extreme n-valeric loadings of 5 g.l⁻¹, the excess n-valeric acid was still utilized by the bacteria. The highest n-valeric concentration increased dramatically the SCOD and DOC at the start of the batch digestion (SCOD: 1818 and 8810 mg.l⁻¹ for 0.5 and 5 g.l⁻¹ nV, DOC: 536 and 3914 mg.l⁻¹ for 0.5 and 5 g.l⁻¹ nV, respectively), however on Day 10 of digestion the values of SCOD and DOC for the 5 g.l⁻¹ nV (1523±117 mg.l⁻¹, 395±48 mg.l⁻¹, respectively) were slightly higher than the values obtained for the lowest n-valeric concentrations (1460±28 mg.l⁻¹, 364±73 mg.l⁻¹) and the control (1403±25 mg.l⁻¹, 348±71 mg.l⁻¹). Schematic representation of the data can be found in Appendix B (Figures 46 – 49). The increased dissolved organic content in sludge from the foaming digestion bottles in comparison to the organic content from the control was previously seen during the batch experiments on organic loading and BSA. However, accumulation of tVFAs was not noticed here, although it was seen for BSA. In addition, the sludge quality characteristics in terms of alkalinity, tVFAs and solids reduction and the gas and methane production during batch AD did not show any inhibition of the digestion process. Similar findings had been obtained from the batch studies on organic loading (Chapter 4).

Similarly to previous batch experiments, foam samples were again collected and analyzed from the 0.5 and 5 g.l⁻¹ nV digestion bottles. The data are presented in the following paragraphs.

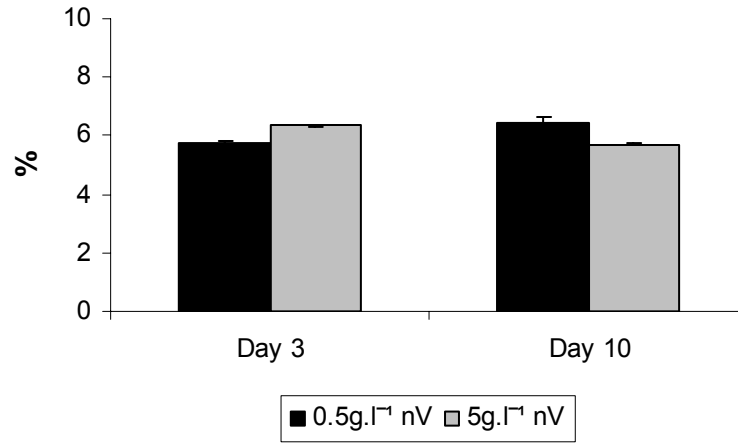


Figure 5.19: Total solids (%±SD) in foam samples, experiment on n-valeric acid

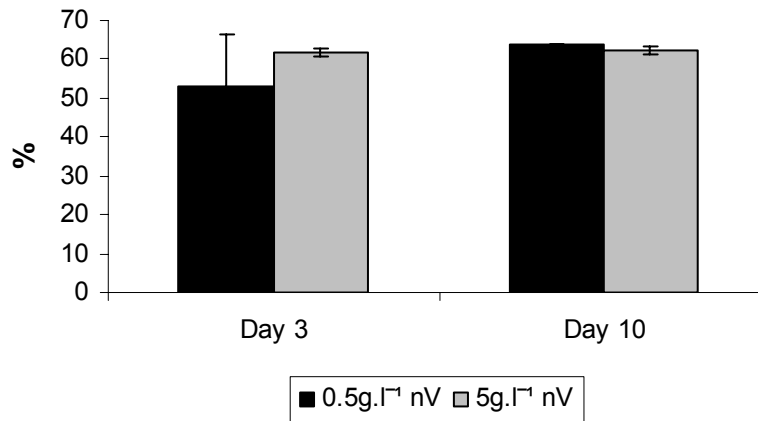


Figure 5.20: Volatile solids (%±SD) in foam samples, experiment on n-valeric acid

The total solids for the nV batch experiment ranged from 5.7 to 6.4% and the volatile solids from 53 to 64%. Again, the solids content in the foam samples from this experiment was similar to solids concentrations from previous experiments on organic loading and BSA and matched the information in the literature referring to solids content of foams obtained from full scale digesters (total: 6% - 13.2%, volatile: 65 – 70%) (Ross and Ellis 1992, Westlund *et al.* 1998). Calculation of the mass of total solids at the start and end of batch digestion showed that overall the

total solids content was reduced in the digestion bottles containing 0.5 g.l⁻¹ nV. However, the mass total and volatile solids at the start of batch digestion from the 5 g.l⁻¹ nV was 18.3 g and 10.8 g, respectively and 24.3 g and 14.9 g at the end of batch digestion indicating that the increase in solids could be attributed to initial generation of biomass due to increased substrate (nV) and potential subsequent cell lysis of the acetogenic bacteria due to limited substrate as digestion proceeded.

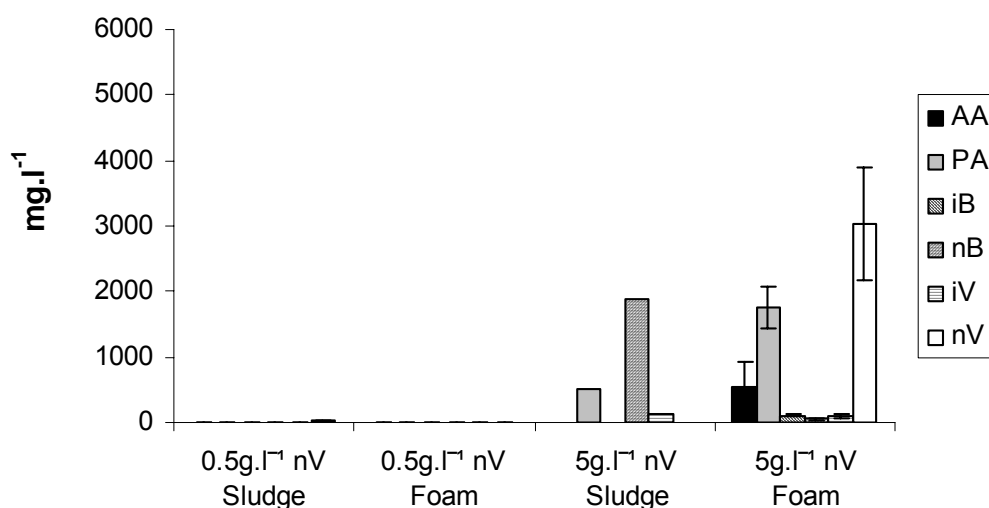


Figure 5.21: Individual VFAs (mg.l⁻¹ ±SD) in sludge and foam samples on Day 3, experiment on n-valeric acid

The concentrations found in sludge and foam samples from the digestion bottles containing 0.5 g.l⁻¹ nV were zero or very close to zero, as it can be seen from Figure 5.21. Sludge and foam samples from the 5 g.l⁻¹ n-valeric digestion bottles did not contain any VFAs on Day 10 of digestion but foam samples had high acetic (528 mg.l⁻¹), propionic (1770 mg.l⁻¹) and n-valeric (3033 mg.l⁻¹) acid concentrations on Day 3 of digestion. Less propionic (268 mg.l⁻¹) and n-valeric acid (23 mg.l⁻¹) were found in sludge and no acetic acid. The presence of the smaller chain fatty acids (acetic, propionic) in the samples was probably due to the breakdown of nV.

Also, the presence of nV and propionic acids in the foam when their concentrations in sludge was considerably lower and did not exceed the suggested range of 300 mg.l⁻¹ as tVFAs indicated affinity for partitioning in the foam due to the surface active properties of nV and potentially propionic acid.

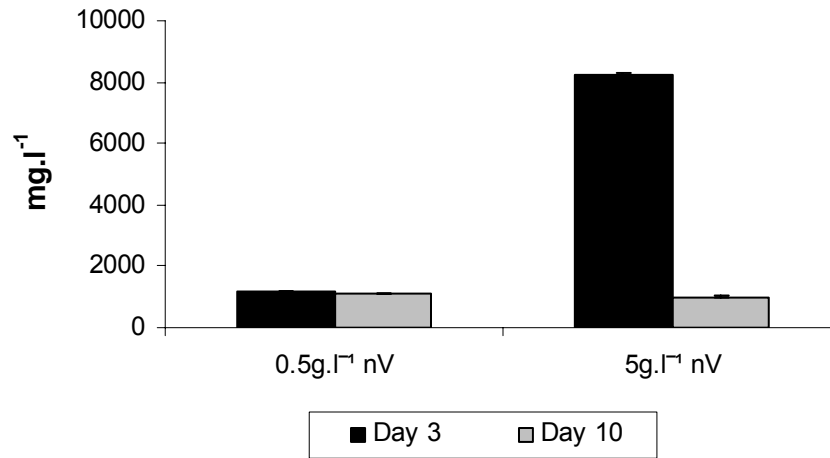


Figure 5.22: Soluble COD (mg.l⁻¹ ±SD) in foam samples, experiment on n-valeric acid

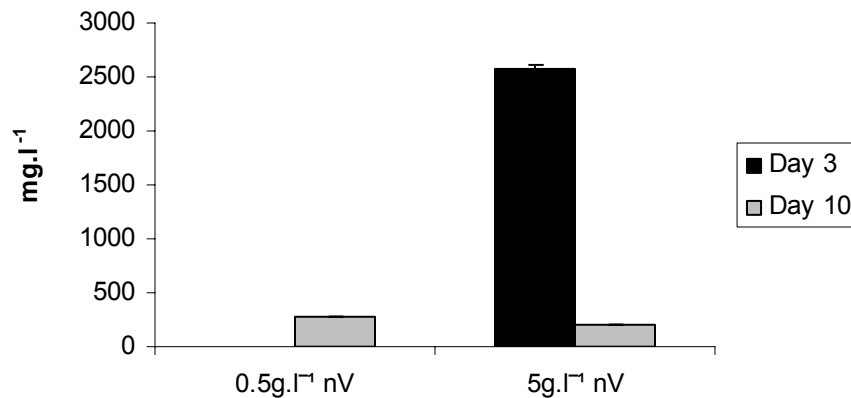


Figure 5.23: DOC (mg.l⁻¹ ±SD) in foam samples, experiment on n-valeric acid

The SCOD values obtained for foam samples from the digestion bottles containing 0.5 g.l⁻¹ n-valeric acid were similar between Day 3 (1163 mg.l⁻¹) and Day 10 (1090

mg.l⁻¹) of digestion. The foam samples from the digestion bottles containing 5 g.l⁻¹ n-valeric acid had about 7 and 10 times higher SCOD (8218 mg.l⁻¹) and DOC (2574 mg.l⁻¹) values, respectively, on Day 3 which, however, decreased by Day 10 of digestion (SCOD: 1005 mg.l⁻¹, DOC: 208 mg.l⁻¹) to values similar to those obtained for foam samples from the 0.5 g.l⁻¹ n-valeric digestion bottles (SCOD: 1090 mg.l⁻¹, DOC: 270 mg.l⁻¹). The corresponding concentrations in sludge were in all cases higher than the ones found in foam (Figures 48 and 49, Appendix B).

In conclusion, n-valeric acid was identified as a surface active agent and was capable of inducing foaming in water under aeration at concentrations as low as 0.2g.l⁻¹, which increased with increasing concentrations but no stability was observed in all foams created. N-valeric, however, did not induce foaming in sludge under aeration and about 54% of the initial n-valeric concentration added was found in solution in sludge. The suppressed surface activity and foaming potential of nV under aeration could be attributed to adsorption on the sludge solids and potentially interactions with the sludge's organic material. During batch anaerobic digestion, all three examined concentrations of nV created metastable foaming, which increased with increasing concentrations without inhibiting the digestion process. Accumulation of nV acid in the foam was seen only from the 5 g.l⁻¹ nV loading on Day 3, which was probably due to the surface active properties of nV but not on Day 10 as all VFAs were 0 in both sludge and foam samples at the end of batch digestion.

5.2.1.7 Examination of surface activity of acetic acid

Acetic acid had no impact on surface tension of water at the examined concentrations, as seen in Figure 5.24. Very small foaming tendency was found for only three of the examined concentrations, which, however, was not attributed to

AA as it was not surface active but was potentially due to water impurities. Therefore, AA was not surface active at the examined concentrations.

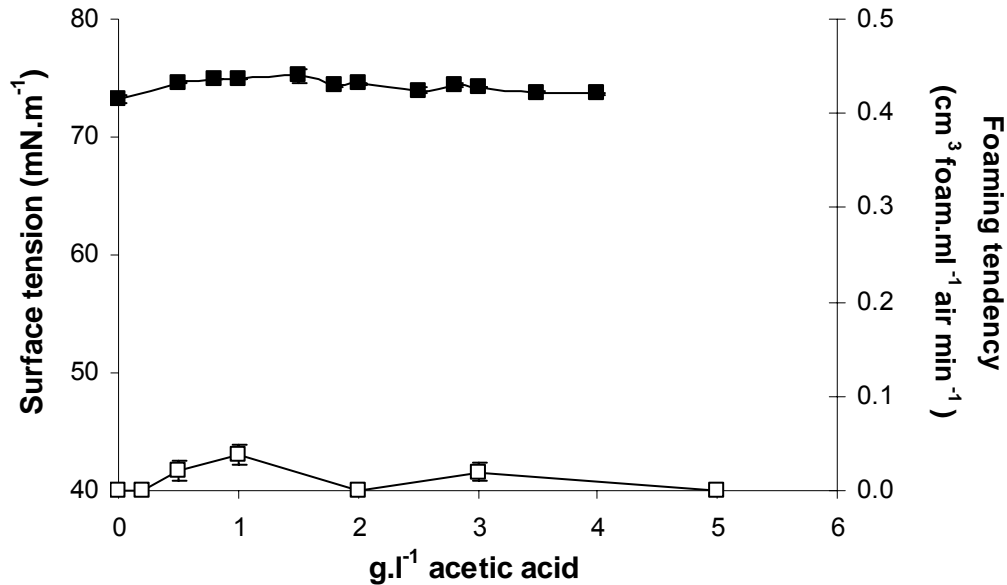


Figure 5.24: Surface tension ($\text{mN.m}^{-1} \pm \text{SD}$) (■) and foaming tendency ($\text{cm}^3 \text{ foam.ml}^{-1} \text{ air min}^{-1} \pm \text{SD}$) (□) of acetic acid in water

5.2.1.8 Effect of acetic acid on foaming in sludge

Acetic acid was also examined for its foaming potential in sludge. No foaming was induced under aeration of sludge at the examined concentrations of acetic acid ($0 - 5 \text{ g.l}^{-1}$).

5.2.1.9 Effect of acetic acid on foaming during batch anaerobic digestion

Batch studies were carried out in order to examine the impact of AA on foaming during digestion of sludge. Three different concentrations of acetic acid, 0.5 , 1.5 , and 5 g.l^{-1} were examined with the control containing a mixture of feed and seed

sludge. AA had no impact on foaming during digestion, as the foam production was 0 in all cases (Figure 50, Appendix B) but was inhibitory for the digestion process as the AA concentration increased. Figure 5.25 shows that the biogas production of the 5 g.l⁻¹ AA digestion bottles dropped to values very close to zero after Day 1 of digestion indicating inhibited digestion process. This finding was supported by the low VS reduction and methane production of the 5 g.l⁻¹ AA digestion bottles (Table 5.4).

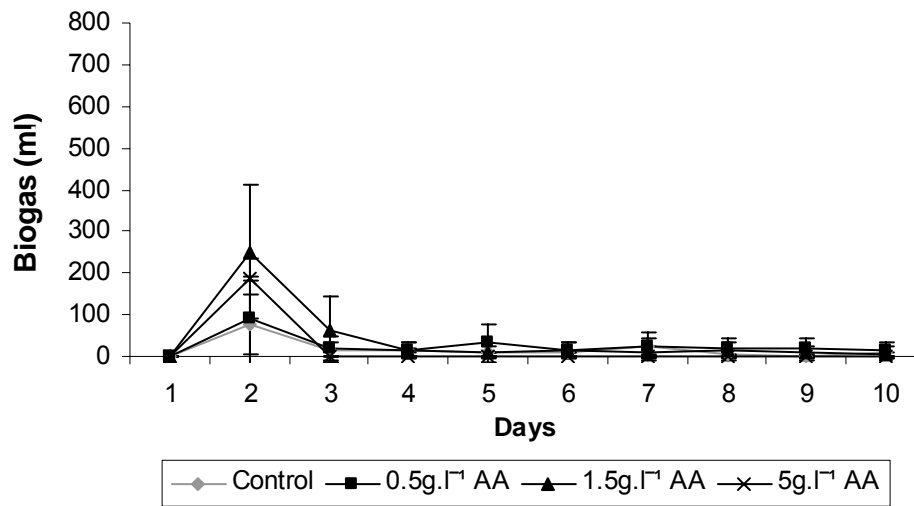


Figure 5.25: Daily biogas production (ml ±SD) during batch anaerobic digestion, experiment on acetic acid

Table 5.4: Solids reduction and gas composition (as methane) ranges during the batch digestion period, experiment on acetic acid

	<i>% TS reduction</i>	<i>% VS reduction</i>	<i>%CH₄</i>
control	14.3	15.2	22 – 31
0.5g.l ⁻¹ AA	15.8	17.9	38 – 47
1.5g.l ⁻¹ AA	15.9	17.0	45 – 49
5g.l ⁻¹ AA	14.7	8.1	3 – 7

Alkalinity did not vary significantly at the end of the digestion exhibiting similar values to the ones obtained from previous batch experiments apart from the 5 g.l⁻¹ acetic acid loading as the high concentration of AA was inhibitory for the digestion process. The VFAs were consumed during batch anaerobic digestion in all digestion bottles apart from the ones containing 5 g.l⁻¹ acetic acid. SCOD and DOC values measured in sludge samples after digestion exhibited similar patterns. The digestion bottles containing the added 5 g.l⁻¹ acetic acid had high SCOD and high DOC values throughout the digestion period. The lower concentrations of added acetic acid increased SCOD and DOC at the beginning of digestion but subsequently reached the same levels as the control. Schematic representation of the data can be found in Appendix B (Figures 53 – 54).

5.2.1.10 Examination of surface activity of carbohydrates

The last group of compounds that was examined for their surface activity and foaming propensity in water and sludge included 3 carbohydrates. As mentioned earlier, carbohydrates have not been reported in the literature as surface active but they are a significant component in sludge found as EPS and SMPs, and according to previous findings in Chapter 4 (paragraph 4.3) there was a strong correlation between carbohydrates as SMPs and surface tension of sludge obtained from a non-foaming digester. Below are the combined data obtained from the examination of the monosaccharide D-glucose, the disaccharide sucrose and the polysaccharide starch.

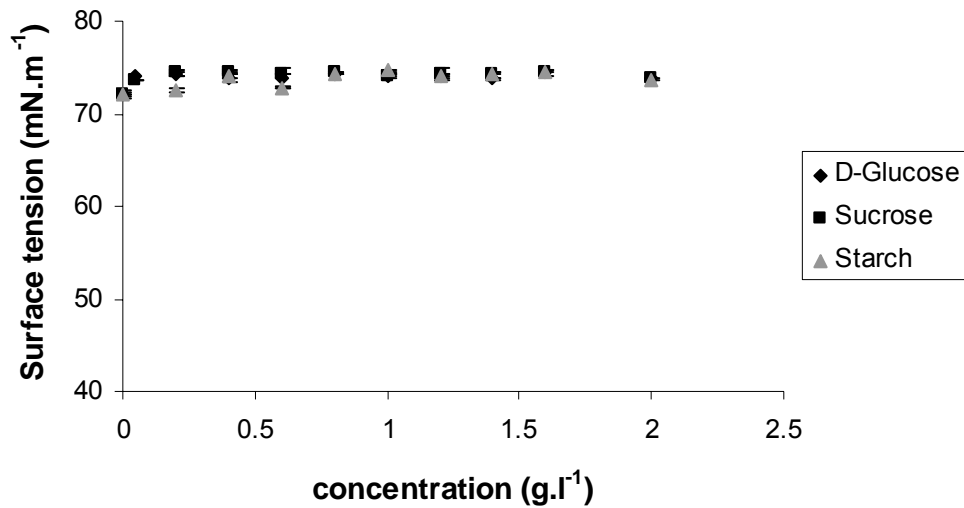


Figure 5.26: Surface tension (mN.m⁻¹ ±SD) of D-Glucose (◆), Sucrose (■) and Starch (▲)

None of the carbohydrates examined lowered the surface tension of water at the examined concentrations indicating that the three carbohydrates were not surface active. In addition, none of the carbohydrates produced foam under aeration in water.

5.2.1.11 Effect of carbohydrates on foaming in sludge

None of the carbohydrates produced foam under aeration in sludge at the examined concentrations.

5.2.2 Field investigation of the effect of surface active agents on foaming in anaerobic digesters

The work in the previous paragraphs of this chapter demonstrated so far that the surface activity and foaming potential under aeration of the studied proteins and n-

valeric acid as surface active agents was suppressed when the compounds were added to sludge. Experimental evidence showed that there was partitioning of the added compounds in the liquid phase of sludge and on the sludge solids, which was potentially responsible for the reduced foaming potential. Yet, addition of BSA and n-valeric in sludge during batch anaerobic digestion resulted in metastable foaming for all the examined concentrations. Hence, the two examined surface active agents, BSA and n-valeric, were identified as foaming causes during batch AD and indicated that the presence of surface active agents could potentially have an impact on foaming at the full scale. Therefore, the objective of this part of experimental work was to examine the association of the presence of surface active agents to foaming in anaerobic digesters at the full scale by assessing the sludges foaming potential and quality characteristics. Initially, a broad investigation of the sludges foaming potential between 9 foaming and 6 non-foaming digesters and the link between the foaming potential and the sludge quality characteristics was carried out, as described in paragraph 3.5.2. Further work examined the presence of surface active agents in a non-foaming digester (Site 16) for a period of 10 months in order to promote a better understanding of the origin and characteristics of surface active agents and the effect of anaerobic digestion on these compounds. The work followed the materials and methods as set out in 3.5.2. The foaming potential of sludge samples was evaluated through calculation of the foaming propensity, which is the foaming potential normalized over the solids content of a sample, as explained in Chapter 3.5, in order to facilitate comparison of the foaming potential between different sludge samples. Below are schematic representations of the average foaming propensity values recorded in feed (mixture of primary and surplus activated sludge) and digested sludge samples obtained from the 9 foaming and 6 non-foaming digesters.

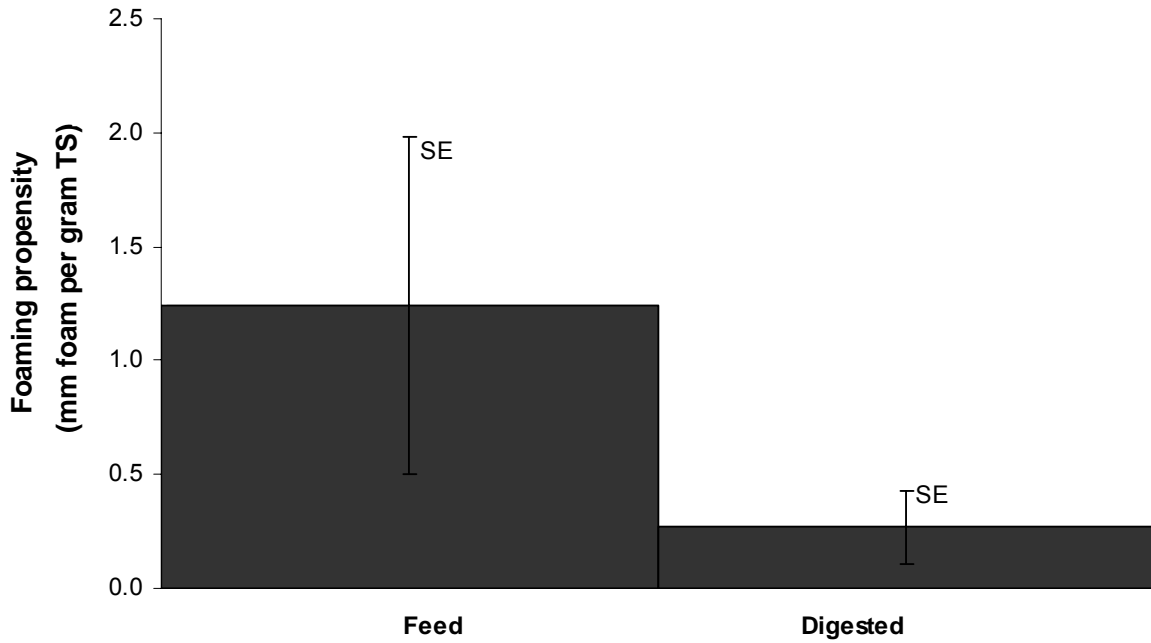


Figure 5.27: Average foaming propensity values (mm foam per gram TS \pm SE) of feed and digested sludge samples obtained from non-foaming digesters

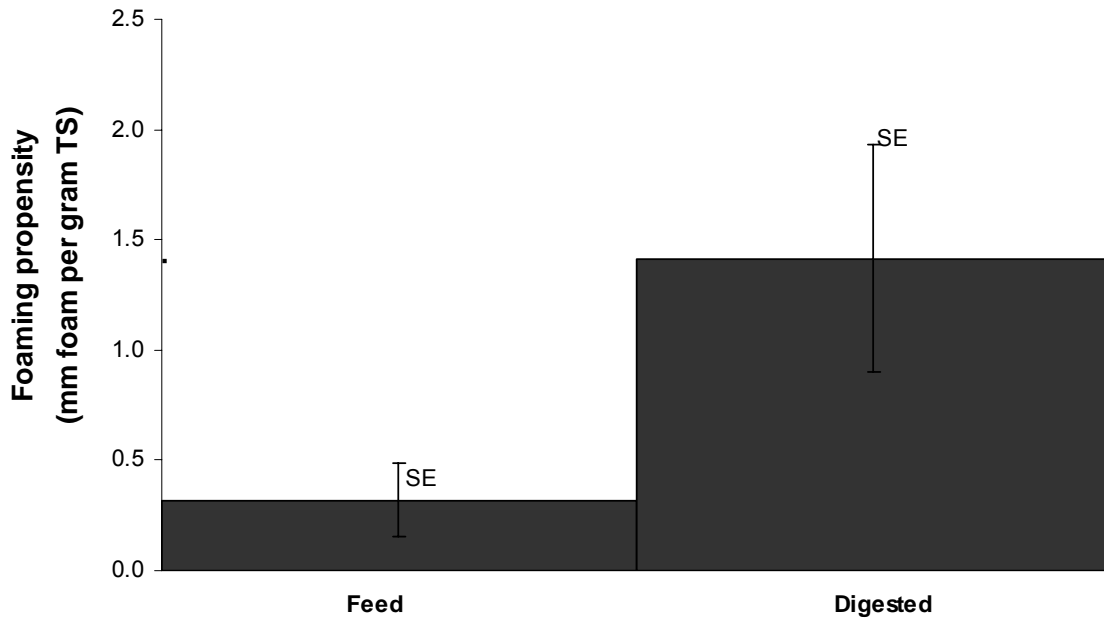


Figure 5.28: Average foaming propensity values (mm foam per gram TS \pm SE) of feed and digested sludge samples obtained from foaming digesters

Lower foaming propensity and hence indication of the presence of less surface active agents was recorded in digester feed samples from foaming sites than in samples from non-foaming sites. However, the standard error of the feed foaming propensity of the non-foaming digesters was significant demonstrating high variability of the foaming propensity of the feed sludge. A major finding from the foaming propensity tests was that digested sludge samples from the foaming digesters exhibiting higher foaming propensity overall than digested sludges from the non-foaming digesters. This indicated that foaming digesters contained higher concentrations of surface active agents that increased the foaming propensity of sludges. Statistical analysis of the data showed that there was a significant difference ($P=0.04$, $\alpha=0.05$) of the foaming propensity of digested sludge between the foaming and the non-foaming digesters.

The foaming tendency of samples presented above was also examined in relation to the sludge quality characteristics, including total VFAs, total solids, alkalinity and DOC values. The correlation matrices below give the relationships between these parameters.

Table 5.5: Correlation values for feed sludge obtained from foaming and non-foaming digesters

	<i>Foaming Tendency</i>
Foaming Tendency	1
tVFAs (mg.l^{-1})	-0.29
TS (g.l^{-1})	-0.59
Alkalinity (mg.l^{-1})	-0.11
DOC (mg.l^{-1})	-0.35

According to Table 5.5 the stronger correlation of these parameters with foaming tendency was for total solids (-0.59) but there was no real strong positive or

negative correlation between any of the examined parameters and the foaming tendency of feed sludge samples.

Table 5.6: Correlation values for digested sludge obtained from foaming and non-foaming digesters

	<i>Foaming Tendency</i>
Foaming Tendency	1
tVFAs (mg.l ⁻¹)	0.12
TS (g.l ⁻¹)	0.17
Alkalinity (mg.l ⁻¹)	0.38
DOC (mg.l ⁻¹)	0.38

Similarly, the correlation matrix for digested sludges showed that there was no strong correlation between foaming tendency and the observed parameters with the stronger correlation between alkalinity and DOC with foaming tendency (0.38 for both values).

In addition to the examination of the foaming propensity of feed and digested sludge samples from the full scale digesters, the foaming propensity of primary and SAS samples from the same digesters was also evaluated. An important observation during the foaming propensity tests was that none of the SAS samples produced foam during aeration apart from one occasion (Site 8). According to the literature, SAS is a source of filamentous bacteria and surface active agents (biosurfactants, proteins etc) that have been recognized as foam causes (See literature review, Chapter 2.3). Of the 11 sites where SAS samples were collected, 3 sites had foaming in the activated sludge plant during sampling (Site 3, non-foaming digester, Site 4, non-foaming digester and Site 7, foaming digester) and one had bulking (Site 8, foaming digester). Higher foaming propensity in SAS samples obtained from the foaming AS plant to SAS obtained from the non-foaming AS plant was not observed from the data collected in this work. Also, there

was no link between foaming in AS plant and foaming in AD. Since foaming propensity was recorded in only one of the SAS samples obtained, feed sludge foaming propensity was potentially due to the quality of primary sludge. However, in some occasions both primary and SAS foaming propensity was zero while feed sludge exhibited foaming propensity greater than zero (Table 4, Appendix B). This could be attributed to limitations of the full scale work involving single site visits and grab samples that do not take into consideration the retention times and potentially instant changes in the quality of SAS and primary sludge that would not be noticeable in feed due to dilution in the blending tanks.

Subsequent work involved a site specific investigation of sludges foaming potential. The foaming propensity, tendency and stability of full scale sludges were assessed in both sludge samples and sludge centrates. A number of sampling visits were carried out and feed and digested samples were collected. The graph below includes the foaming propensity of Site 16 sludge and centrate samples.

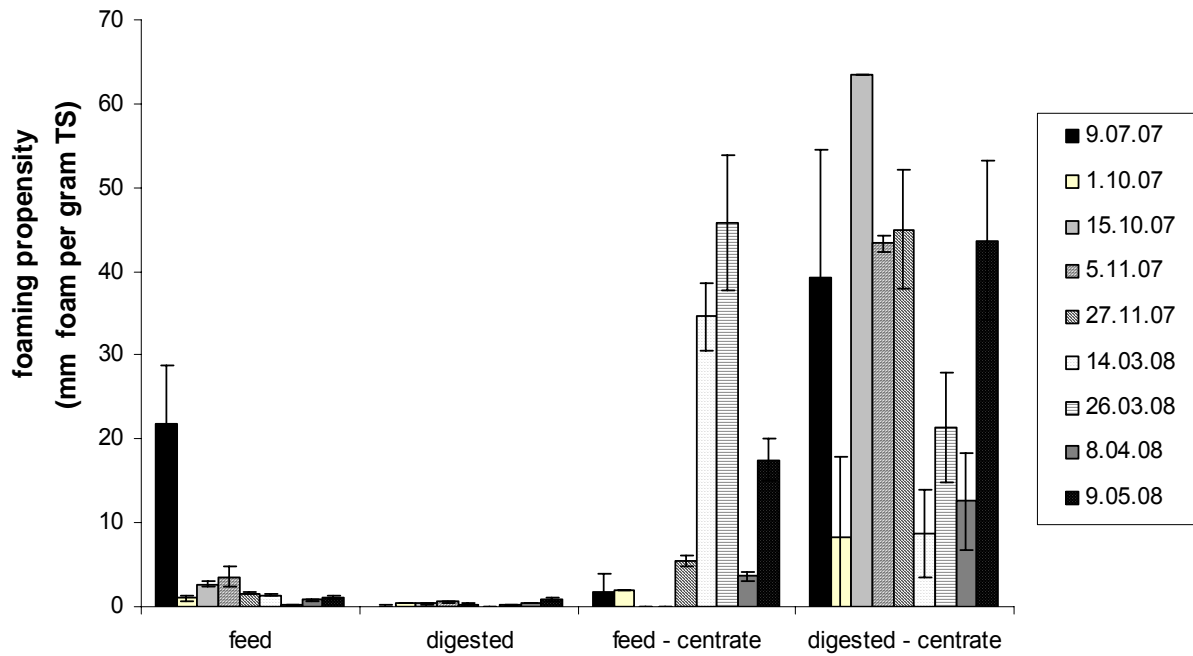


Figure 5.29: Foaming propensity (mm foam per gram total solids \pm SD) of sludges and sludge centrates of Site 16

Feed sludge samples and feed-centrate samples showed a variable foaming propensity during the 10-month monitoring period. Stability was observed in two occasions and only in the whole feed sludge sample (5.11.07, $0.27 \pm 0.03 \text{ cm}^3 \text{ foam.ml}^{-1} \text{ air min}^{-1}$, 9.07.07, $11.76 \pm 1.55 \text{ cm}^3 \text{ foam.ml}^{-1} \text{ air min}^{-1}$), which however had no impact on the digested sludge foam stability and on anaerobic digestion. Previous findings on foaming propensity of feed sludges derived from the site survey work demonstrated that foaming propensity in the feed sludge was variable. This finding was confirmed during the site specific work on foaming propensity of feed sludges as feed and feed-centrate foaming propensity varied. The high foaming propensity of feed and feed-centrate in comparison to the rest of the values obtained (9.07.07: $21.73 \text{ mm foam.gram}^{-1} \text{ TS}$ in sludge, 14.03.08: $34.63 \text{ mm foam.gram}^{-1} \text{ TS}$ in centrate and 26.03.08: $45.71 \text{ mm foam.gram}^{-1} \text{ TS}$ in centrate) did not have an impact on digested sludge foaming propensity or foaming at the full scale digester. Thus, the surface active agents responsible for foaming under

aeration in digested sludge and for foaming in AD are not the ones found in feed sludge and the foaming potential of digested sludge and digesters is irrelevant of the feed sludge foaming potential.

Digested sludge foaming propensity was consistently much lower than digested-centrate foaming propensity. Stability was observed in one occasion and only in the digested-centrate sample (9.07.07, $0.04 \pm 0.06 \text{ cm}^3 \text{ foam.ml}^{-1} \text{ air min}^{-1}$). The foaming propensity of digested sludge from Site 16 non-foaming digester was comparable to the foaming propensity values obtained for digested sludge of the non-foaming digesters during the site survey work (Site 16 digested sludge foaming propensity (\pm SD): 0.36 ± 0.27 , non-foaming digesters digested sludge foaming propensity (\pm SD): 0.26 ± 0.39). Site 16 foaming propensity average, minimum and maximum values were 0.36, 0 and 0.88 mm foam per TS, respectively and the average foaming propensity of non-foaming digesters was 0.27 mm foam per TS (SE=0.16) while the average digested sludge foaming propensity of foaming digesters was 1.42 mm foam per TS (SE: 0.51). The consistent findings of digested centrate's much higher foaming propensity than the whole digested sludge foaming propensity indicated that the centrate contained surface active agents that were able to induce foaming under aeration in the absence of solids but were not able to induce foaming under anaerobic digestion. Previous findings in this chapter on the examination of the foaming potential of sludge under aeration containing BSA and nV have also demonstrated the reduced foaming potential of surface active agents when in sludge due to the interactions with the solids and the organic material in sludge (paragraphs 5.2.1.2, 5.2.1.5). It is possible that under aeration the air bubbles cannot detach the surface active agents from the surface of the solids or other organic molecules due to the adsorption forces but under centrifugation the gravitational forces separate the solids from the surface active agents which then increase the foaming potential in the centrate.

There was also indication that the surface active agents responsible for foaming under aeration in the digested centrate were produced during anaerobic digestion as the digested centrate foaming propensity did not seem to be affected by the feed and feed centrate foaming propensity. Data on foaming propensity of sludges and sludges centrates obtained from the control digestion bottles of the batch studies showed that there was an increase in both sludge and sludge centrate foaming propensities as digestion proceeded, although less consistent for sludge centrate foaming propensity. Figure 5.30 illustrates the foaming propensity of the control for the batch experiments on organic loading (experiment 3), on BSA, on nV and on AA. The control of the organic loading and BSA experiments contained only seed (digested) sludge, whereas the last two experiments contained a mixture of feed and seed sludge at an organic loading of 1.25 kg VS.m⁻³.

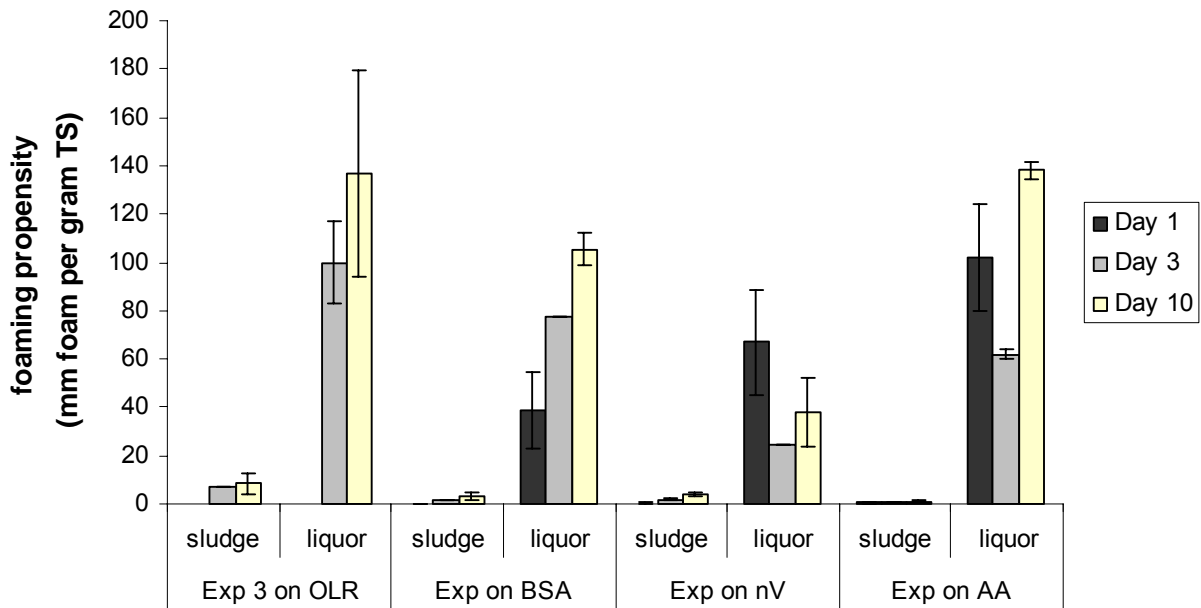


Figure 5.30: Foaming propensity (mm foam per gram TS \pm SD) of sludge and sludge centrate samples from the control digestion bottles

5.3 Discussion

The literature has identified surface active compounds as foaming agents due to their hydrophilic / hydrophobic properties. The laboratory investigation in this chapter identified the proteins BSA, gelatin and casein, the protein mixtures containing 0.1 g.l⁻¹ BSA and varying concentrations of gelatin and 0.5 g.l⁻¹ gelatin and varying concentrations of BSA and n-valeric acid as suitable examples of surface active agents in order to study their behavior in sludge under aeration and during batch anaerobic digestion. It was observed that the concentrations of the studied surface active compounds needed in order to induce foaming in sludge under aeration were higher than the ones required to induce foaming in water, which supported the research hypothesis that sludge modifies the behavior of surface active agents. This was attributed to the sludge solids and organic compounds that facilitate interactions such as adsorption or binding to take place and reduce the foaming potential of the studied surface active compounds. The behavior of the studied surface active agents in sludge during batch anaerobic digestion varied. BSA induced unstable foaming in sludge under aeration, which increased with increasing BSA concentrations but the concentrations of BSA examined during batch anaerobic digestion resulted in metastable foaming in all occasions with the 1 g.l⁻¹ BSA producing more foam than the two lower concentrations (0.1 and 0.3 g.l⁻¹ BSA). N-valeric acid did not induce foaming in sludge under aeration but metastable foaming increased with increasing concentrations during batch digestion. The foams produced by BSA and n-valeric acid during batch digestion were re-created within 24 hours of destruction. The following schematic representations illustrate the foaming mechanisms during batch anaerobic digestion after addition of BSA and n-valeric acid.

Day 1 of batch AD

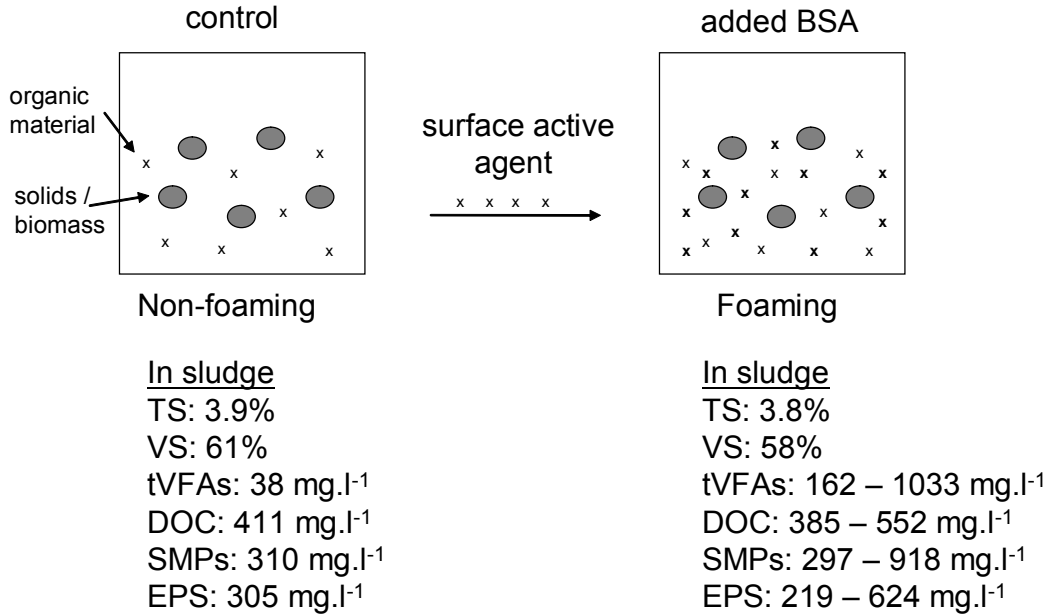


Figure 5.31: Model for foaming mechanisms in batch AD of sludge and added BSA (Day 1)

As shown in Figure 5.31, addition of 0.1, 0.3 and 1 g.l⁻¹ BSA in sludge on Day 1 (start) of batch digestion increased the total protein concentration as EPS and SMPs. An increase in tVFAs in sludge after addition of BSA was recorded, which was attributed to the effect of freezing of sludge samples prior to analysis for VFAs by HPLC and denaturing of BSA as there was no other difference regarding the sludge quality characteristics between the control and the foaming digestion bottles apart from the addition of BSA.

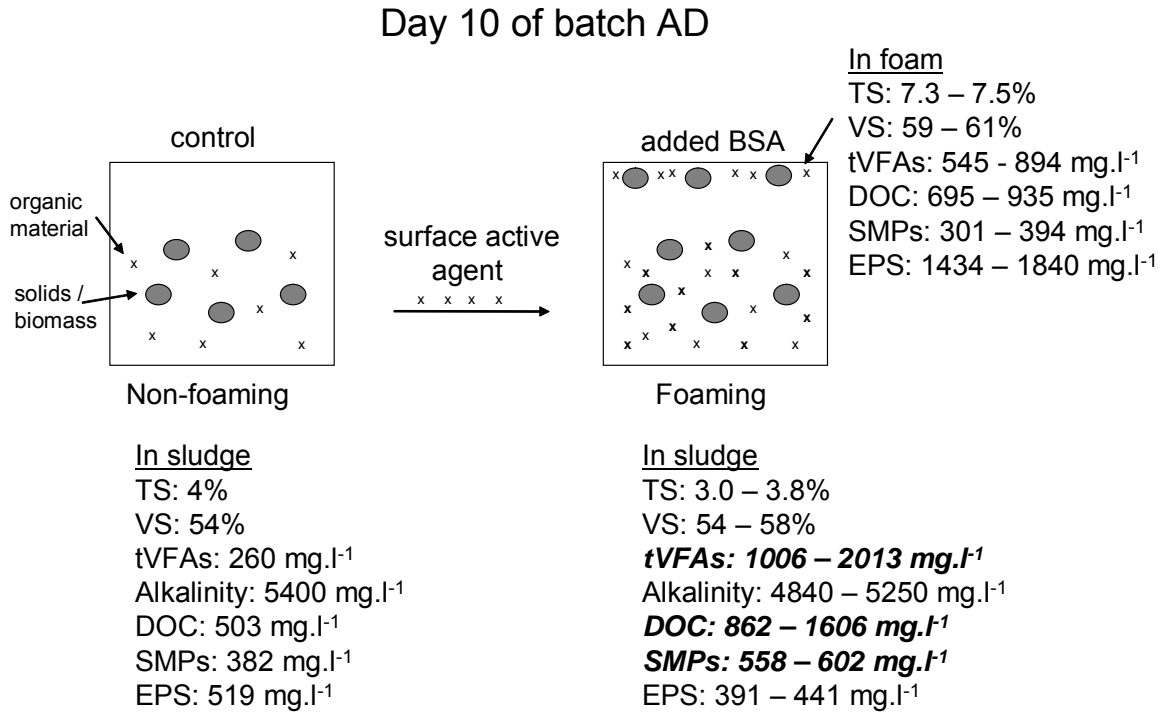


Figure 5.32: Model for foaming mechanisms in batch AD of sludge and added BSA (Day 10)

Figure 5.32 shows the accumulated organic material (x) in sludge measured in this work as tVFAs, DOC and SMPs from the foaming digestion bottles containing the three different concentrations of BSA. Ranges of the parameters monitored (TS, VS, etc.) are given as they refer to the foaming digestion bottles containing the three different concentrations of BSA. There was partitioning of proteins as EPS in the foam whereas significantly higher concentration of proteins as SMPs (558 – 602 mg.l⁻¹) was found in the foaming digestion bottles in comparison to the proteins as SMPs from the control (382 mg.l⁻¹). The presence of EPS in the foam matrix denotes the accumulation of microorganisms, in this work measured as high solids concentrations in the foam and suggests that the stability of the foams could be attributed to the liquid retention in the aggregates formed and sorption of surface active organic molecules measured here as DOC (Flemming and Wingender

2001). Accumulation of tVFAs even at concentrations of 0.1 g.l^{-1} added BSA indicated unstable digestion due to the protein load. This was in accordance with the literature stating that proteins are less degradable than lipids and fiber and have a greater impact on the digestion process (Gonzales *et al.* 2003). Accumulation of tVFAs was seen at two foaming full scale digesters, as previously mentioned in Chapter 4, which also happened to have the two highest DOC concentrations measured amongst the foaming digesters (Site 7 VFAs: 365 mg.l^{-1} , DOC: 1461 mg.l^{-1} and Site 13 VFAs: 1918 mg.l^{-1} , DOC: 2302 mg.l^{-1}). This could potentially indicate a similar foaming pattern to foaming induced by BSA at bench scale.

Day 1 of batch AD

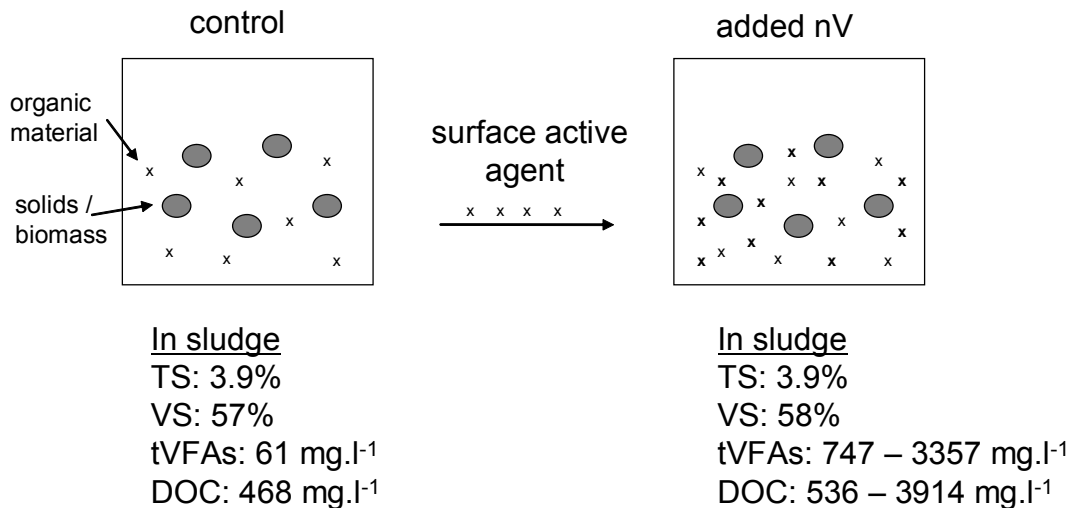


Figure 5.33: Model for foaming mechanisms in batch AD of sludge and added n-valeric acid (nV) (Day 1)

Figure 5.33 illustrates the conditions in the control and the digestion bottles with added 0.5 , 1.5 and 5 g.l^{-1} n-valeric acid at the start of batch digestion. The concentration of n-valeric did not exceed 3357 mg.l^{-1} after addition of 5 g.l^{-1}

potentially due to the hydrophilic / hydrophobic properties of nV that led to adsorption on the solids.

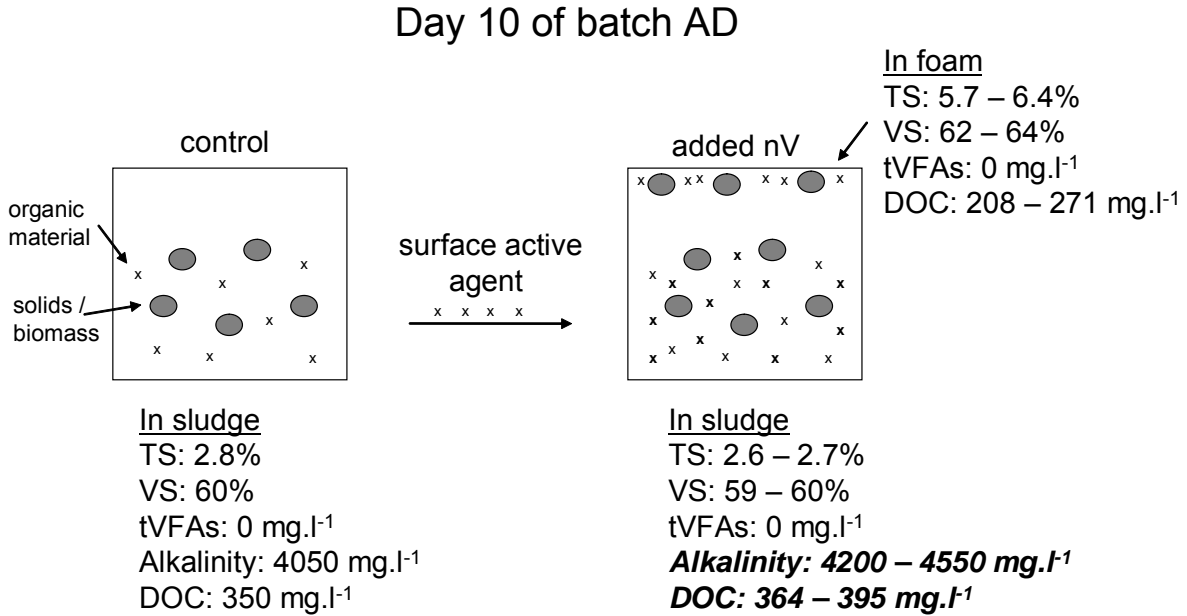


Figure 5.34: Model for foaming mechanisms in batch AD of sludge and added n-valeric acid (nV) (Day 10)

At the end of batch digestion (Day 10), alkalinity and DOC measurements (shown as (x) in Figure 5.34) showed a difference in sludge between the control and the digestion bottles containing the three different concentrations of nV, hence the ranges of the parameters monitored are given. However, it is necessary to highlight that all the nV was utilized by Day 3 of batch digestion, according to individual VFAs measurements (Figure 47, Appendix B), yet metastable foaming continued in the absence of the foaming initiator (nV) until Day 10 of batch digestion and the absence of nV did not result in reduced foam production. On this occasion, therefore, there was indication that foaming was due to instability of the digestion process that could be linked with the production of biosurfactants from the metabolic activity of microorganisms in order to degrade the high concentrations of

nV. However, foaming was recorded in the control during the batch digestion experiment.

At this point it is necessary to bring to mind that sludge for all the batch experiments was collected fresh prior to initiation. Prior to the nV batch digestion experiment, samples collection followed a full scale digester foaming incident (Site 16), which coincided with organic loading rates over $2.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$. To examine the effect of sludge samples collection from Site 16 digester shortly after a foaming incident on foaming during batch digestion, the control this time contained a mixture of feed and seed sludge at 1.25 kgVS.m^{-3} organic loading. The sludge quality characteristics of feed and seed sludge obtained on sampling occasions before (26.03.08) and after (8.04.08) the foaming incident were compared to previous data obtained from the same digester. Higher alkalinity (average: 3860 mg.l^{-1} , 26.03.08: 4555 mg.l^{-1} , 8.04.08: 5780 mg.l^{-1}), SCOD (average: 735 mg.l^{-1} , 26.03.08: 878 mg.l^{-1} , 8.04.08: 1279 mg.l^{-1}) and DOC (average: 282 mg.l^{-1} , 26.03.08: 410 mg.l^{-1} , 8.04.08: 440 mg.l^{-1}) compared to the rest of the values were recorded for digested sludge before and after the foaming incident (Figure 4.20, Figure 4.22). It was not possible to identify the foaming cause at Site 16 digester due to lack of detailed monitoring (samples collection and analysis) during the foaming period. However, based on information from the batch studies on organic loading and surface active agents, it was assumed that foaming was due to either the organic loading of the full scale digester exceeding the critical threshold of 2.5 kgVS.m^{-3} for foam initiation, or one or more surface active compounds in the feed that resulted in foam initiation, which subsequently had an effect on alkalinity, SCOD and DOC similar to the one seen from the batch studies on n-valeric acid or even the contribution of both conditions – organic loading and surface active compounds.

Additionally, findings on acetic acid showed that acetic acid was neither a surface active compound nor an AD foaming cause at the examined concentrations,

contradicting information in the literature stating that VFAs are surface active compounds and accumulation of acetic acid leads to foaming. Carbohydrates were not found to be surface active and foam inducing in water or sludge under aeration. The effect of different concentrations of carbohydrates on batch AD was not investigated in this study due to time limitations.

The field investigation on surface active agents involved the determination of foaming propensity of sludge samples before and after AD. The data obtained demonstrated a statistically significant difference in the foaming propensity of sludge samples between foaming and non-foaming digesters indicating that the foaming digesters contained higher concentrations of surface active agents. Additionally, full scale findings during the long term monitoring of Site 16 non-foaming digester showed that digested sludge and centrate foaming propensity was not affected by the feed sludge and centrate foaming propensity indicating that i) the properties of surface active agents contained in the feed sludge were affected by AD, which supported the hypothesis that AD modifies the behavior of surface active agents and that ii) surface active agents are potentially produced during AD. Bench scale data supported the generation of surface active agents during batch AD by demonstrating that the foaming propensity in digesting and digested sludge and centrate increased as digestion proceeded (Figure 5.30). The consistent foaming propensity of digested centrate showed that the surface active agents responsible for foam generation in anaerobic digesters are found in solution and can induce foaming under aeration in the absence of solids. In solids presence, however, the foaming potential is suppressed verifying the hypothesis that sludge modifies the behavior of surface active agents. The foaming propensity in digesters was found to be suppressed by the solids presence due to potential absorbance of surface active agents onto the solids. Yet, once foam was created during the bench-scale studies the solids presence in foam was significant (5.7 – 7.5% TS, 53 – 66% VS) and could potentially have contributed to foam stabilization.

To ensure that the quality characteristics of centrate samples had not been changed by centrifugation of the whole sludge sample, digested sludge samples were obtained from two different sites (Site 16 & 17) and the foaming propensity was assessed, as described in paragraph 3.5.2, in digested sludge, digested-centrate and the digested sludge sample after re-suspension of the solids in the centrate by manual mixing. The data are presented below.

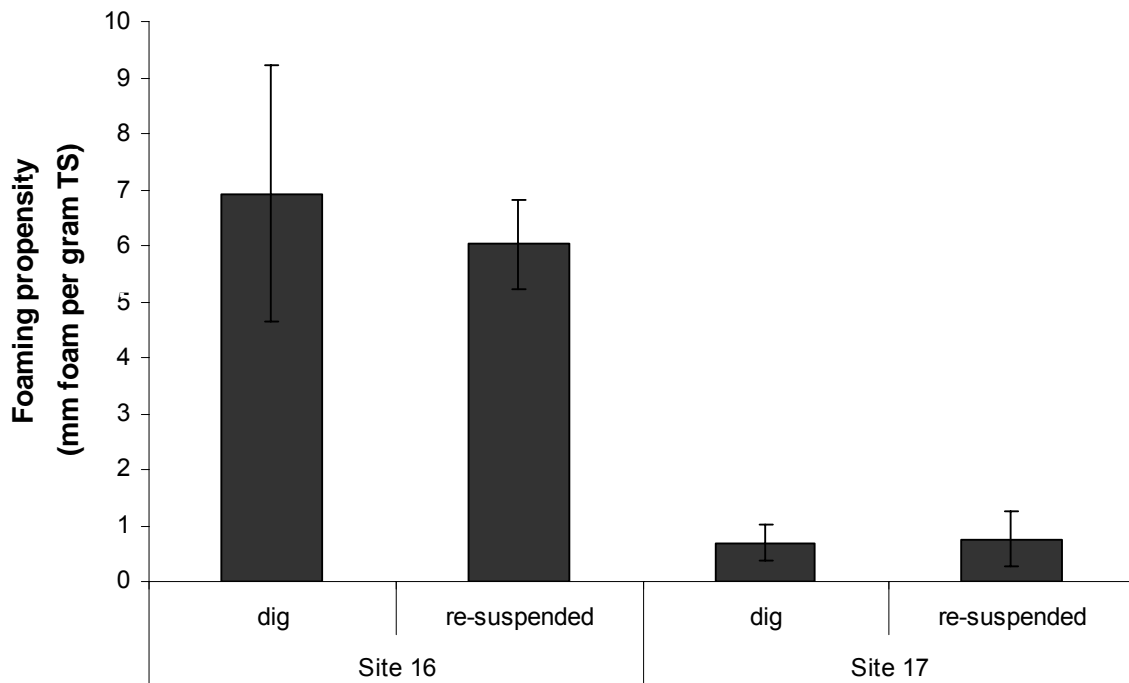


Figure 5.35: Foaming propensity (mm foam per gram TS \pm SD) of digested sludge samples and digested sludge samples after centrifugation and re-suspension of solids

According to Figure 5.35, the foaming propensity of digested sludge and re-suspended digested sludge was very similar from both sites. This indicated that the separation of solids in the sludge by centrifugation did not alter the quality characteristics of sludge samples. Further work examined the effect of different

concentrations of solids on the foaming tendency of the digested centrate. The following graph illustrates the data obtained.

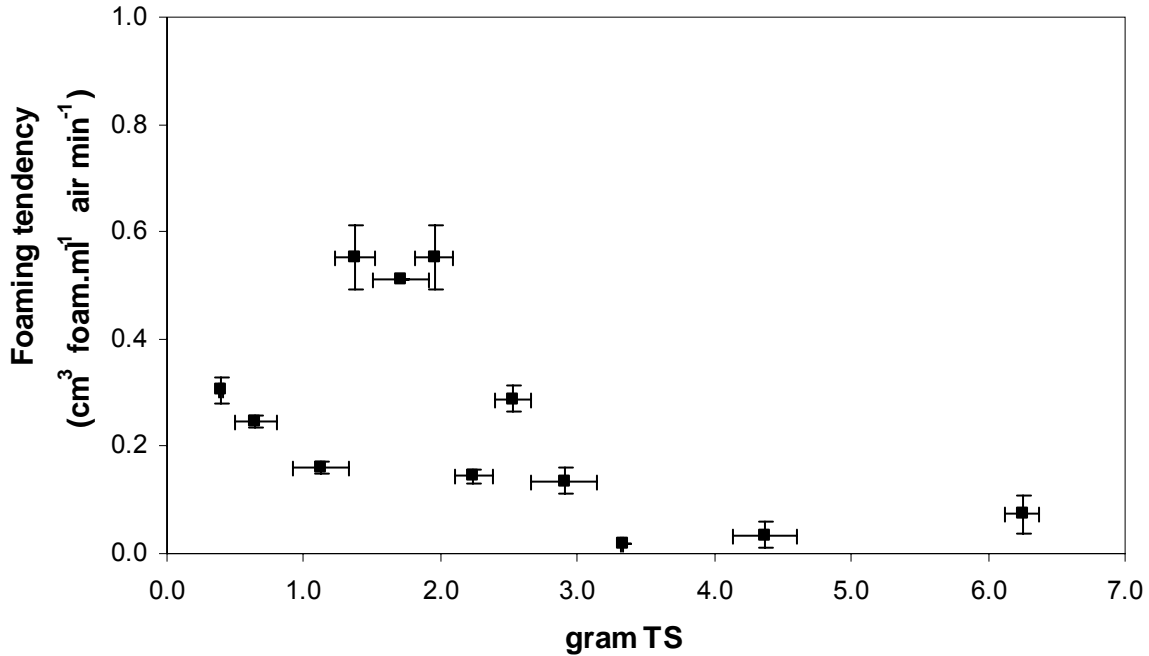


Figure 5.36: Foaming tendency (cm³ foam.ml⁻¹ air min⁻¹ ±SD) of sludge digested-centrate samples after addition of digested sludge

The foaming tendency of the digested centrate obtained from Site 16 was determined in relation to solids concentrations after addition of different volumes of the same whole digested sludge sample. Initially, the foaming tendency decreased with addition of small amounts of sludge and hence small increase of the solids concentration in the sample. Further addition of solids (sludge) increased the foaming tendency of the sample giving values higher than the starting digested centrate foaming tendency. Subsequent increase of solids by addition of sludge to the centrate sample decreased gradually the foaming tendency. No stability was observed in the foam generated on all occasions. It was unclear why there was an increase of the foaming tendency at solids content between 1 and 2 grams. Additional work to investigate this finding was not carried out due to time

limitations. However, the above graph demonstrated the association of solids and foaming tendency of sludges and that at higher solids content (>3), which is similar to the solids content found in sludge, the foaming tendency is reduced.

Chapter 6:
Effect of filamentous bacteria on
AD foaming

6 Effect of filamentous bacteria on foaming

6.1 Introduction

Filamentous bacteria play an important role in AS foams and have been identified in the literature as AD foaming causes. Recent research has shown that the biosurfactants production from filamentous bacteria is responsible for foam initiation in AS but foam stabilization is attributed to filaments due to their morphological characteristics and hydrophobic properties (Hug 2006, Heard *et al.* 2008). Several reports also showed that filaments although predominantly aerobic microorganisms, can survive under anaerobic conditions with only a small reduction in population and respiration (37% reduction and 60% capable of respiration at 14 SRT) (Hernandez and Jenkins 1994, Mamais *et al.* 1998). Earlier, in the literature review, a comparison between AS and AD foaming was made and the same species, *Gordonia* (*Actinomycetes*) and *Microthrix*, were identified by researchers as the foaming causes in both systems (Hernandez and Jenkins 1994, Pagilla *et al.* 1997, Westlund *et al.* 1998, de los Reyes *et al.* 2002, Hug 2006, Heard *et al.* 2008). This is understandable as SAS is the only sludge stream going into a digester that contains filamentous bacteria and a large number of filaments can survive, as mentioned earlier, under anaerobic conditions. However, whether the foaming generation mechanisms are the same in both systems is not clear as the information on the effect of filamentous bacteria on AD foaming is limited and in some cases site specific. Additionally, it remains unknown whether other filaments species would have an impact on AD foaming.

6.1.1 Hypothesis

The hypothesis supported in this chapter was that filamentous bacteria can be a cause of foaming and can contribute to foam stabilization during AD.

6.1.2 Aims and Objectives

This chapter aimed to promote a better understanding of the association of filamentous bacteria to AD foaming.

The objectives of this part of work involved:

- identification of the filamentous bacterial species present in foaming and non-foaming digesters at multiple sites
- longer-term monitoring of the filamentous bacterial species present in a selected foaming and a non-foaming digester
- examination of the filaments contribution to foaming during bench scale batch AD.

6.2 Results

During the site survey work the filamentous species and abundance was recorded in digester inlet and digested sludge, as shown in Table 6.1, obtained from the single site visits to the 9 foaming and 5 non-foaming digesters studied in previous chapters (where FI is the Filament Index, as described in the Materials and Methods chapter). Filamentous bacteria abundance was monitored in both digester inlet and digested sludge samples initially in order to investigate whether there was preferential die off or accumulation of certain filamentous species during AD.

Table 6.1: Filamentous bacteria in digester inlet and digested sludge of foaming and non-foaming digesters

	Species	Digester Inlet FI	Digested sludge FI
Site 1	<i>Streptococcus</i>	1	-
Site 2	<i>N.Limicola I</i>	1	-
	<i>N.Limicola III</i>	2	-
	<i>Microthrix</i>	1	-
	<i>Flexibacter</i>	1	-
Site 3	<i>N.Limicola I</i>	1	0-1
	<i>N.Limicola III</i>	-	0-1
	<i>Actinomyces</i>	2	1
Site 4	1701	0-1	-
	0803	-	1
	0914	-	1
Site 6	021N	-	0-1
	<i>Microthrix</i>	2	-
	<i>N.Limicola I</i>	2	-
	<i>N.Limicola III</i>	2	-
	<i>Microthrix</i>	2	-
	<i>Thiothrix</i>	3	-
	0914	0-1	-
Site 9	<i>Streptococcus</i>	-	1
	<i>Microthrix</i>	2	1
	<i>N.Limicola I</i>	1	1
	<i>N.Limicola III</i>	3	2
	021N	0-1	1
	0411	2	-
	1863	-	1
Site 10	<i>N.Limicola I</i>	1	-
	<i>N.Limicola III</i>	1	0-1
	0914	-	2
	1851	-	2
Site 11	1702	-	1
Site 12	<i>N.Limicola I</i>	3-4	1
	<i>N.Limicola III</i>	2-3	1
	0581	4	3
	<i>Thiothrix</i>	3	2
Site 13	<i>N.Limicola I</i>	1	-
	<i>N.Limicola III</i>	1	-
	021N	0-1	0-1
	0581	-	1

According to Table 6.1, the filament species found in digester inlet were also present in digested sludge at the same or lower abundance involving *N.Limicola I & III*, *Actinomyces*, *Microthrix*, 021N, 0581 and *Thiothrix*. There were also occasions where digester inlet was not containing any filaments, yet digested sludge contained *N.Limicola III*, 0803, 0914, 021N, 1863, 1851, 1702, 0581 with FI not exceeding 2. Additionally, in some cases such as for Sites 2 and 6 digester inlet contained substantial amounts of the filaments *N.Limicola I & III*, *Microthrix*, *Flexibacter*, 021N, 0914, and *Thiothrix* (FI \leq 3) and no filaments were detected in digested sludge. From the data obtained there was no indication that there was preferential die off or accumulation of filaments in AD. The survival of filamentous species, however, was clear and there was not a case where completely different species were found in digester inlet to the ones in digested sludge of the same site. Further monitoring of the filament species and abundance in digester inlet samples was not carried out during this work due to time limitations.

The following graphs illustrate the filamentous species identified in foaming and non-foaming digesters at the full scale.

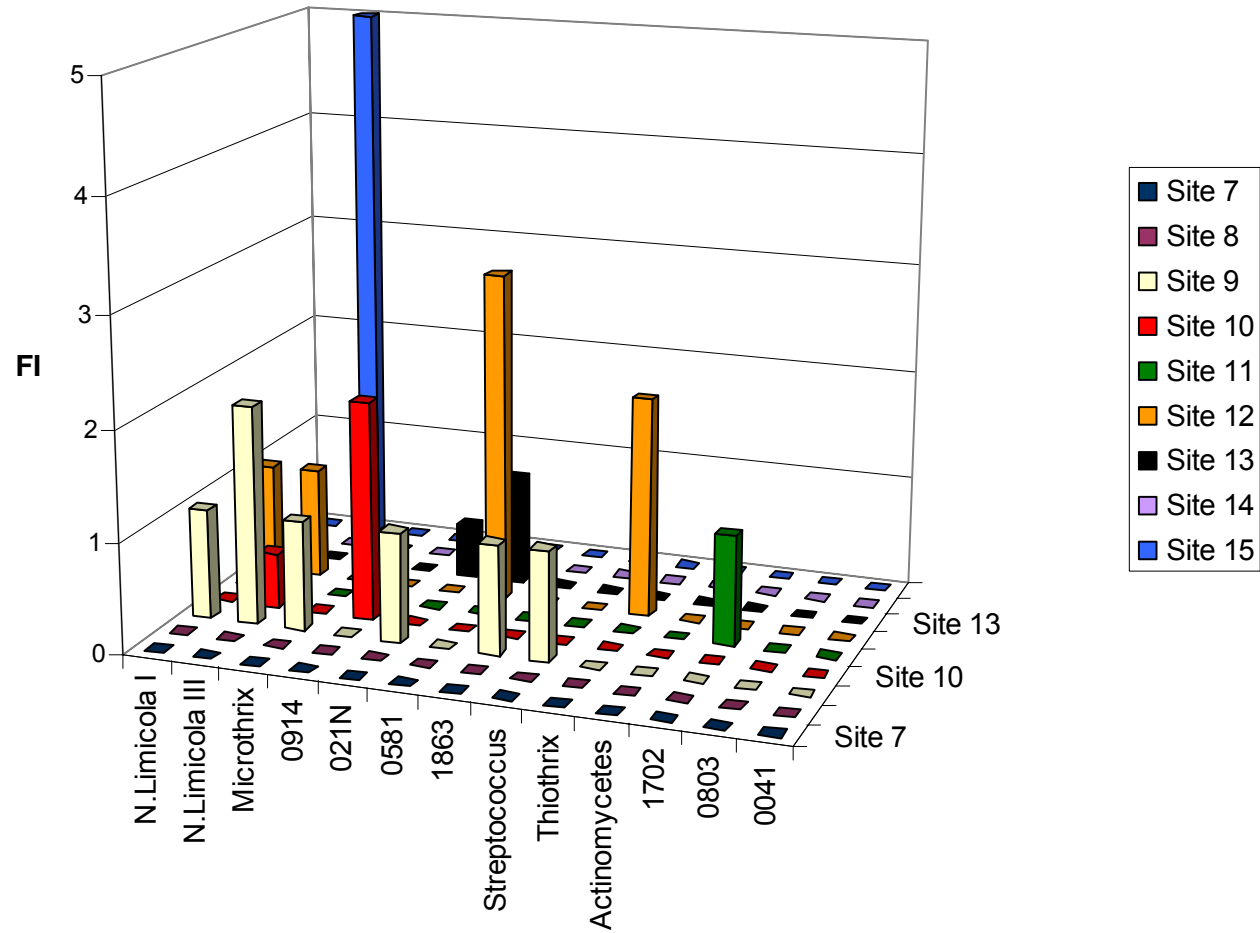


Figure 6.1: Filamentous bacteria abundance in foaming digesters (Filaments species: $n_f=13$, sites: $n_s=9$)

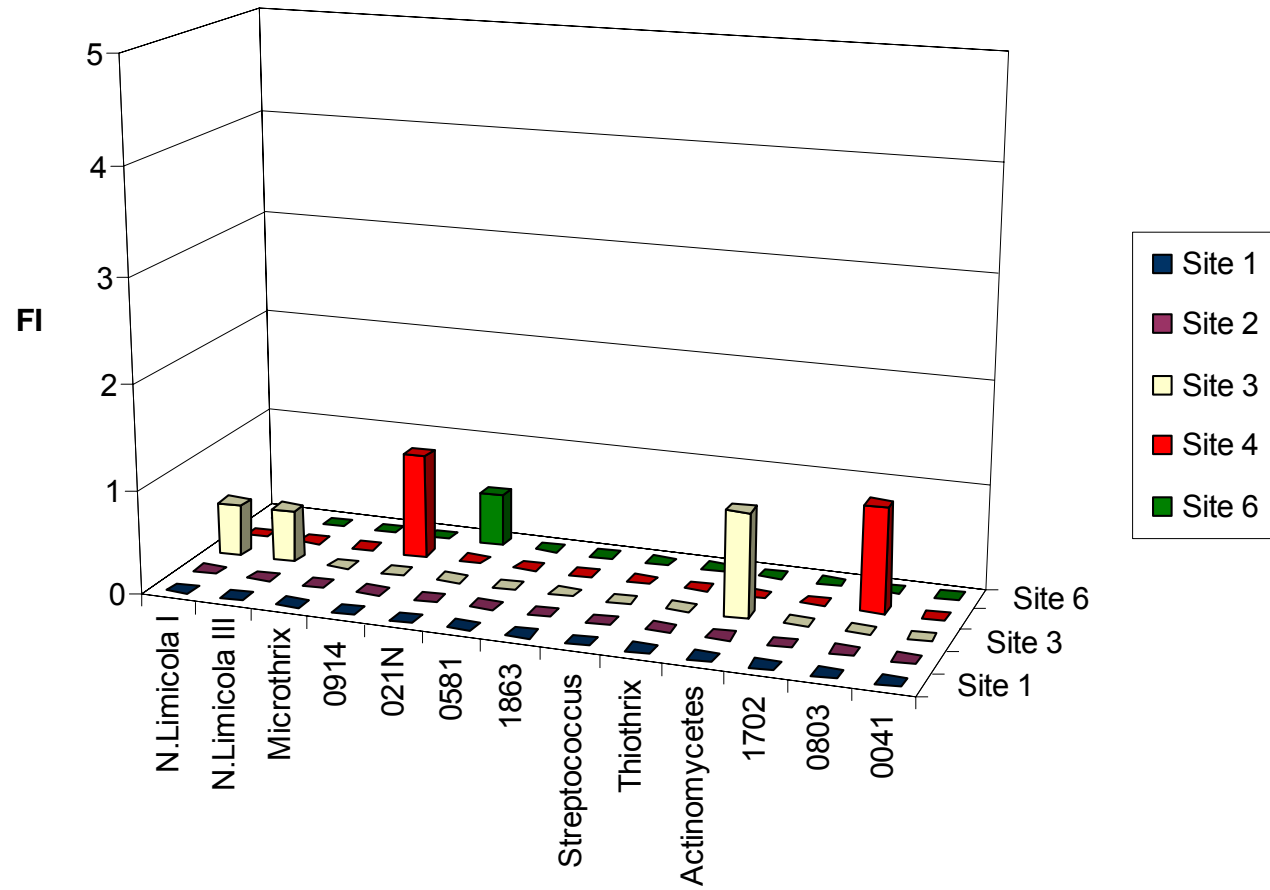


Figure 6.2: Filamentous bacteria abundance in non-foaming digesters (Filaments species: $n_f=13$, sites: $n_s=5$)

Overall, thirteen filamentous species were found in digested sludge samples from both foaming and non-foaming digesters including Nostocoida Limicola I & III, Microthrix, 0914, 021N, 0581, 1863, Streptococci, Thiothrix, Actinomyces, 1702, 0803 and 0041. The abundance of filament species in the non-foaming digesters did not exceed an FI of 1 with cases where no filaments were present in the digesters (Sites 1, 2). The FI of the filament species recorded in the foaming digesters varied between 0 (Sites 7, 8, 14) and 5 (Site 15). Site 15 had foaming in the AS plant during the site visit, which explains the high filament abundance in the digester. The site survey showed less variability of filament species and lower abundance of the filaments in the non-foaming digesters compared to the foaming digesters. However, it was not clear whether the filaments in the foaming digesters with FI of up to 3 (excluding filament abundance of Site 15) could be identified as a foaming cause.

The long term monitoring of Sites 12 and 16 digesters, as shown in Figure 6.3 and Figure 6.4, provided a better understanding of the survival and abundance of filament species in AD.

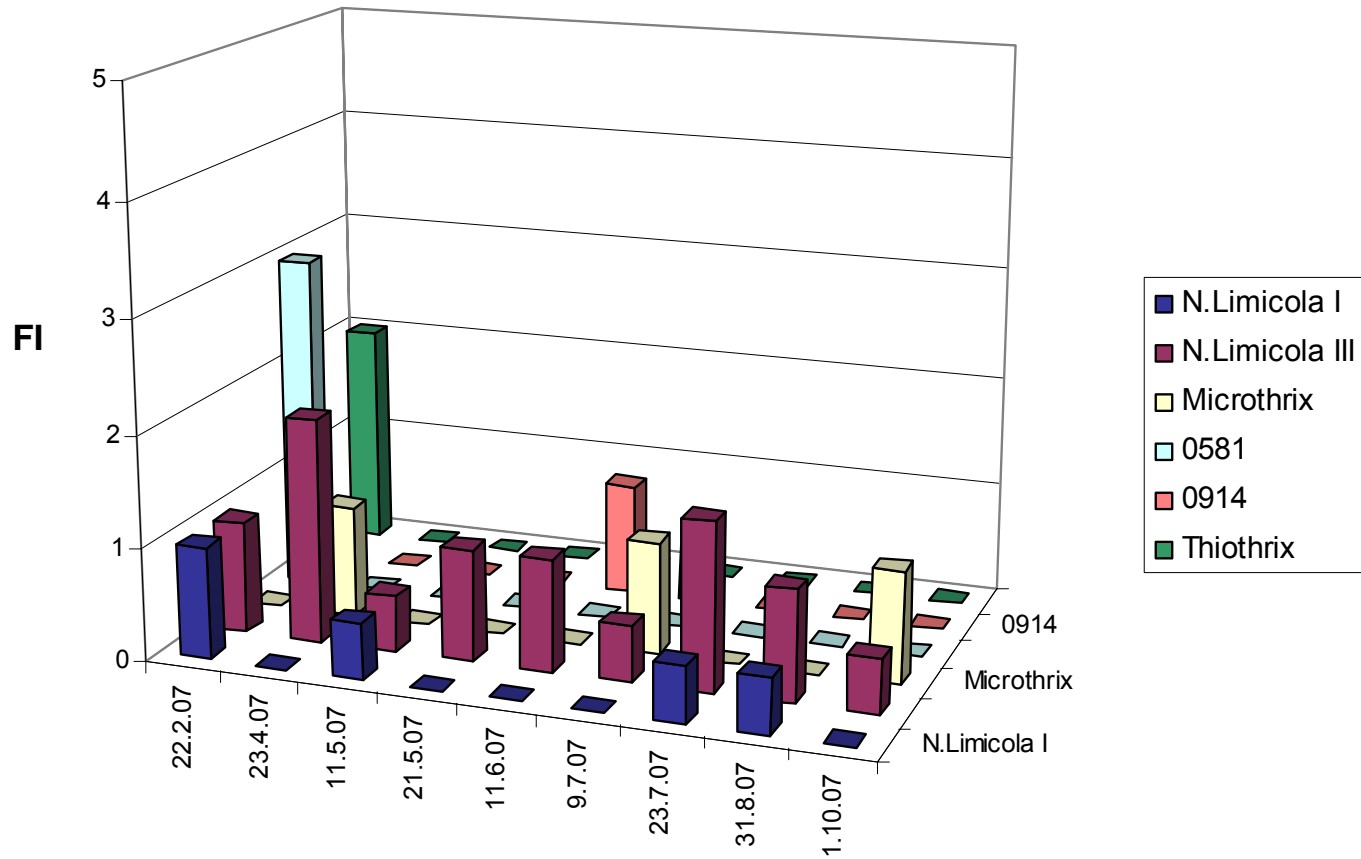


Figure 6.3: Filamentous bacteria abundance in Site 12 digester (Filaments species: $n_f=6$, sampling: $n_s=9$)

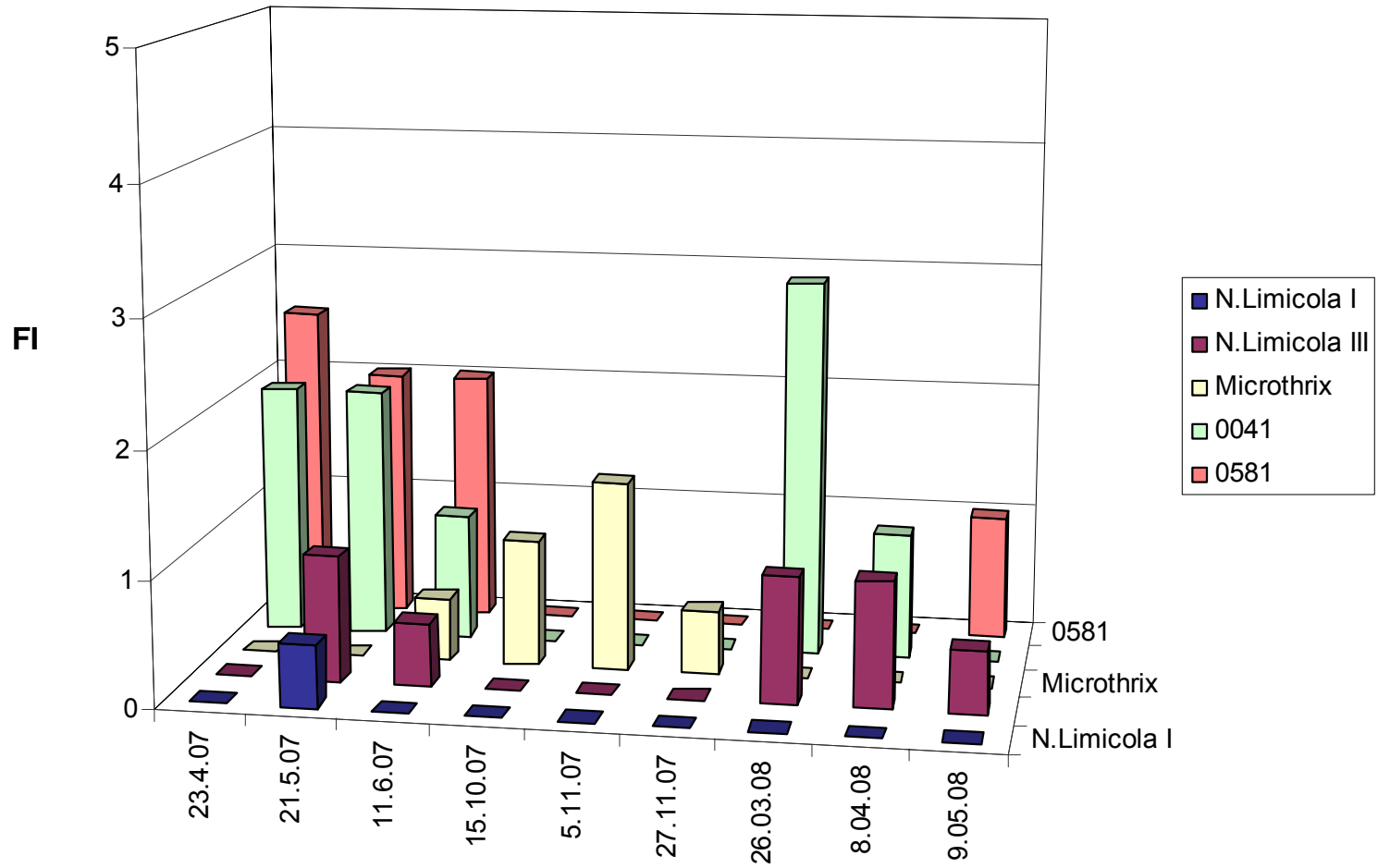


Figure 6.4: Filamentous bacteria abundance in Site 16 digester (Filaments species: $n_f=5$, sampling: $n_s=9$)

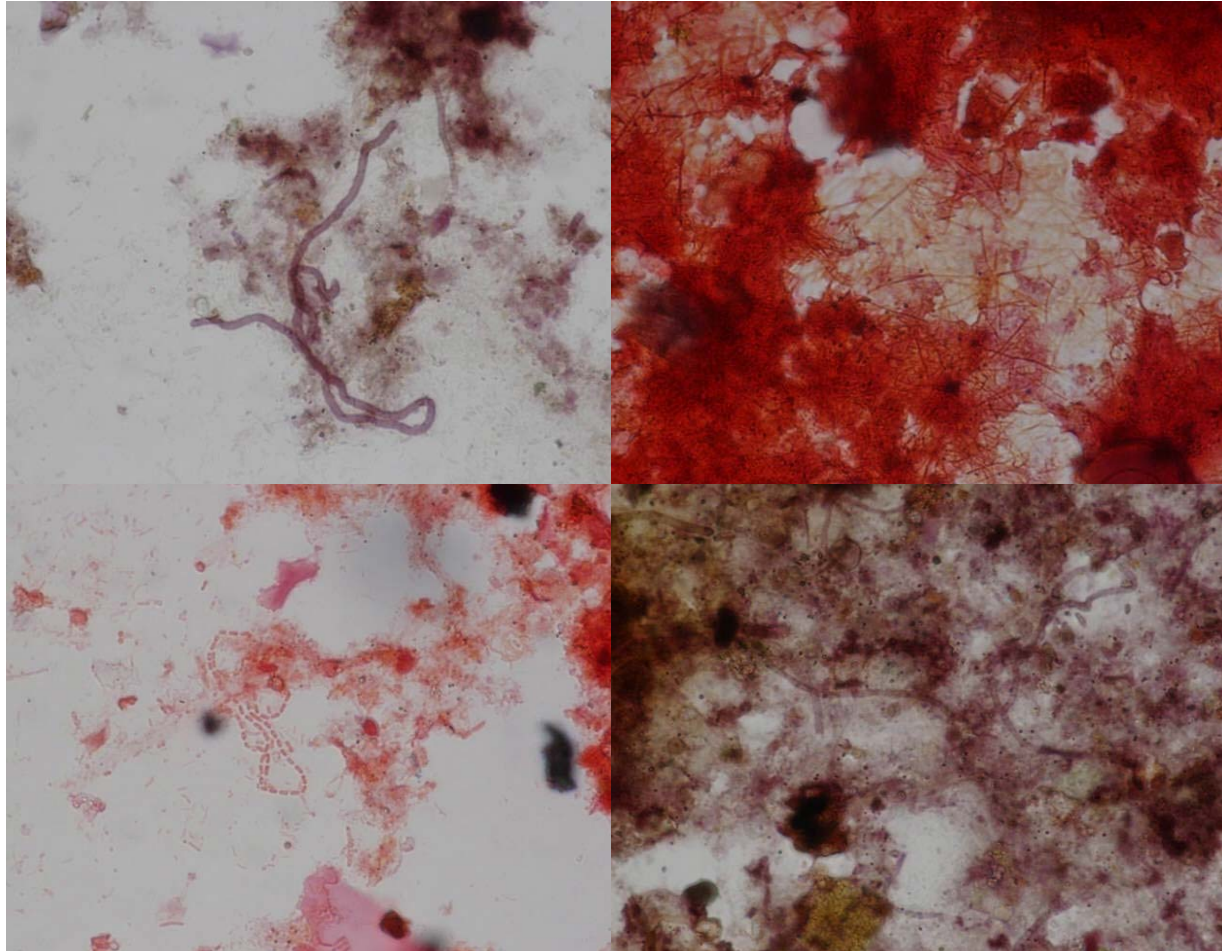


Figure 6.5: Filamentous bacteria in digested sludge samples (from the top left and clockwise: *N.Limicola* I after Neisser staining, stain reaction after Gram staining, *N.Limicola* I & III after Neisser staining and Type 1863 after Gram staining)

During the long-term monitoring of Site 16 non-foaming digester, digested sludge contained at least one filament species on every occasion and the filament abundance and variability was comparable to that found in the 9 foaming digesters. Hence, there was no outstanding evidence that could indicate filaments were the foaming cause in the examined digesters apart from one occasion where Site 15 digester contained large numbers of *N.limicola* III (FI:5) and could have resulted or contributed to foaming. The findings from Site 16 digester indicated, however, that the presence of a single species in a digester with filament abundance equal to $FI \leq 3$ would not result in foaming at the full scale. The data obtained for Site 12 digester showed that 4 of the species identified in Site 16 digester were also present in Site 12 digester and in cases in lower abundance than in the non-foaming digester. 2 more species were seen in Site 12 digester with an $FI < 2$. Therefore, there was no evidence to suggest that filaments were the foaming cause at Site 12.

The filament species and abundance was monitored during the batch digestion studies on organic loading and surface active agents in order to determine their partitioning between the foam and sludge and hence their contribution to foaming. The figures below illustrate the filaments levels found in sludge and foam samples during batch digestion in relation to the control of each experiment.

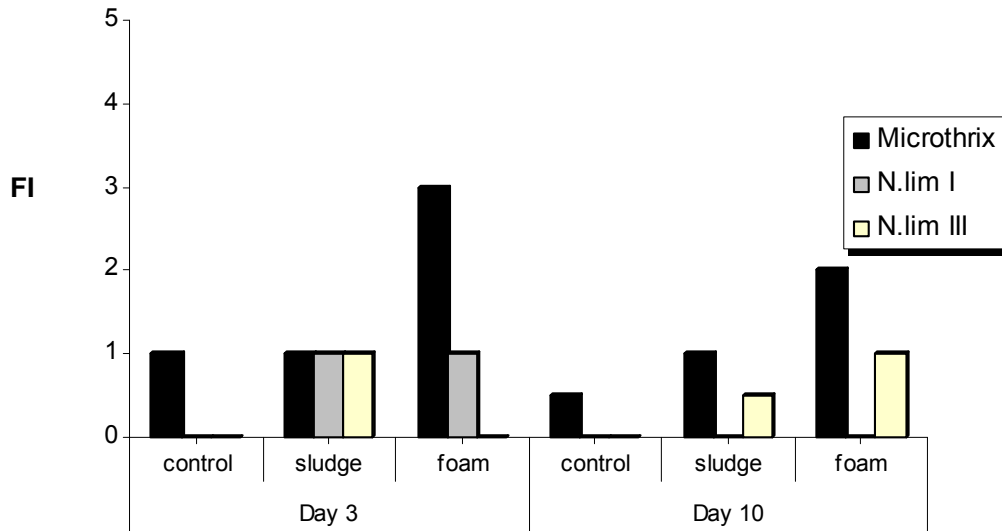


Figure 6.6: Filaments species and abundance of the control and in sludge and foam samples of the 5 kg VS.m⁻³ loading from Experiment 1 on organic loading

Sludge and foam samples were collected on Day 3 and Day 10 of digestion from the control and the digestion bottles of the 5 kg VS.m⁻³ loading. It is important to clarify at this point that the digestion bottles contained different amounts of feed and seed sludge with the control containing only seed sludge and increasing organic loading corresponded to increasing amounts of feed sludge. Two more species were seen in the sludge and foam samples of the 5 kg VS.m⁻³ loading compared to the control, which were present in feed sludge according to microscopic investigation carried out in feed samples and were noticeable in the digestion bottles due to the amount of feed sludge added. The filament abundance in foam was 2 units higher than in sludge for *Microthrix*, the same for *N.limicola* I and there was no *N.limicola* III observed on Day 3 of digestion. On Day 10, foam samples contained slightly higher filament numbers to sludge. The abundance of filaments overall did not exceed FI of 3. There was indication that filaments were probably not the cause of foaming at this experiment as their abundance matched data obtained from the non-foaming digesters earlier in this section and the partitioning in foam was potentially attributed to their morphological characteristics

and hydrophobic properties, as explained in the literature review section. Additionally, after a 10-day digestion period *N.limicola* I disappeared but *Microthrix* and *N.limicola* III were still present in sludge and foam samples. This finding matched the findings of Hernandez and Jenkins (1994) and Mamais *et al.* (1998) stating that only a small reduction in filament numbers can be achieved during AD.

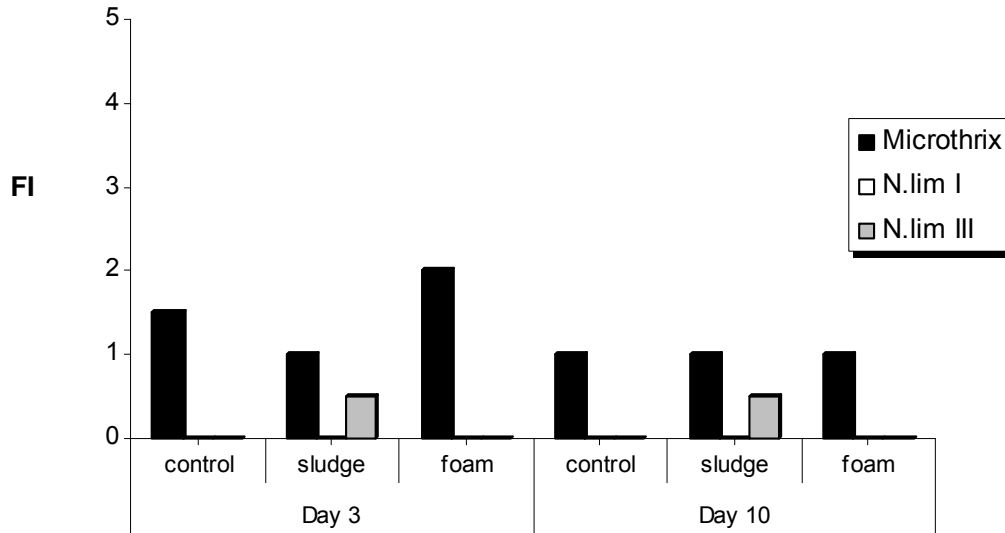


Figure 6.7: Filaments species and abundance of the control and in sludge and foam samples of the 5 kg VS.m⁻³ loading from Experiment 2 on organic loading

Similarly, in experiment 2 on organic loading the abundance of filaments was low in both sludge and foam samples (between 0 and 2 FI) with *Microthrix* being slightly more abundant in foam only on Day 3 of digestion. The reduction of filament numbers again was very small in the control and foam sample and 0 in sludge.

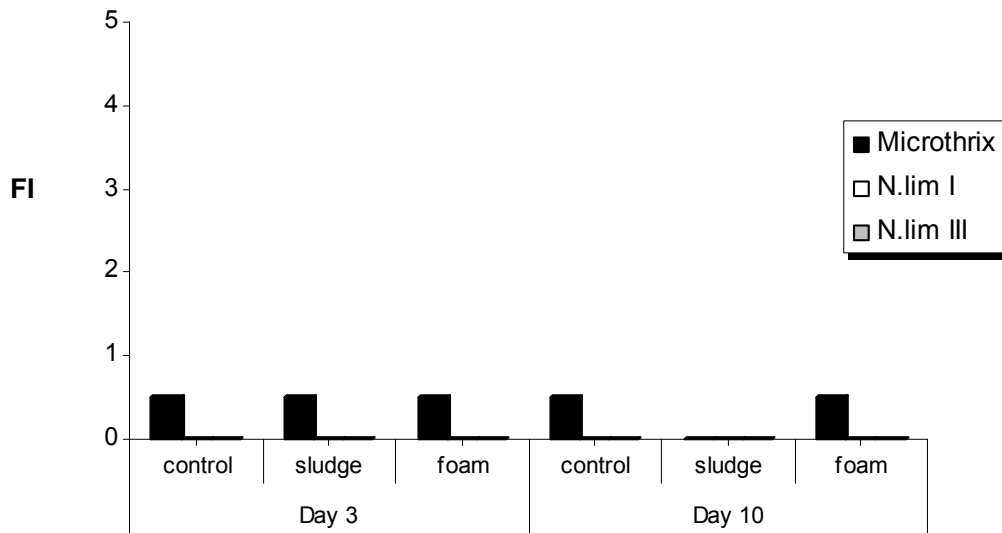


Figure 6.8: Filaments species and abundance of the control and in sludge and foam samples of the 5 kg VS.m⁻³ loading from Experiment 3 on organic loading

During the last experiment on organic loading, the filament numbers were lower than values obtained from the previous experiments on organic loading with FI not more than 1. Yet, persistent foaming was recorded in the bottles of the highest loading. This clearly indicated that foaming was not due to filaments presence in the sludge but due to increased organic loading.

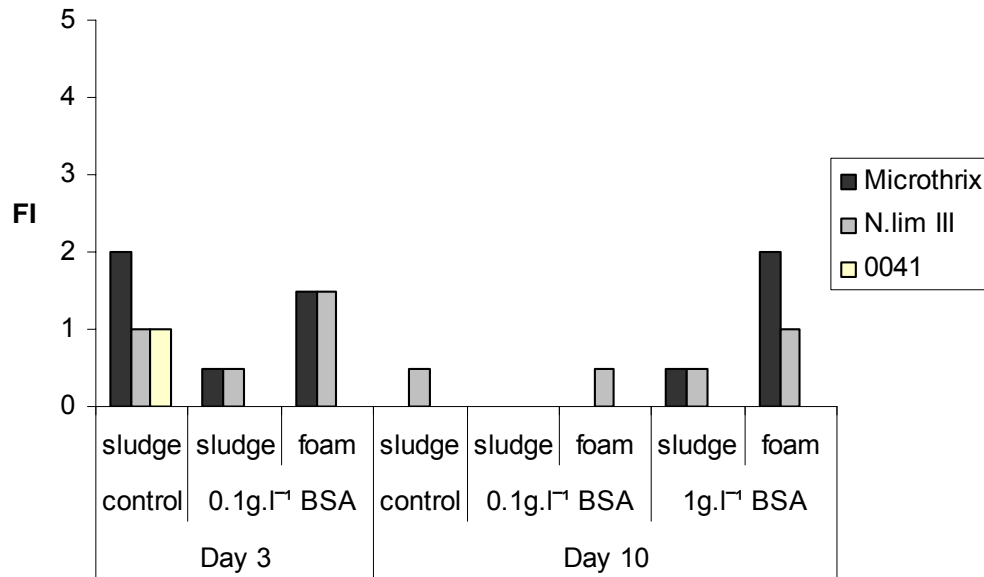


Figure 6.9: Filaments species and abundance of the control and in sludge and foam samples of the digestion bottles from the experiment on BSA

During the experiment on BSA, all digestion bottles contained the same amount of feed and seed apart from the control containing only seed sludge. Foam samples were collected from the digestion bottles containing 0.1 and 1g.l⁻¹ BSA on Day 3 and Day 10 of digestion. The filament numbers were higher in the foam samples on both occasions by one unit on the FI scale but generally low as maximum FI was 2. Some partitioning in the foam was again noticed in all occasions.

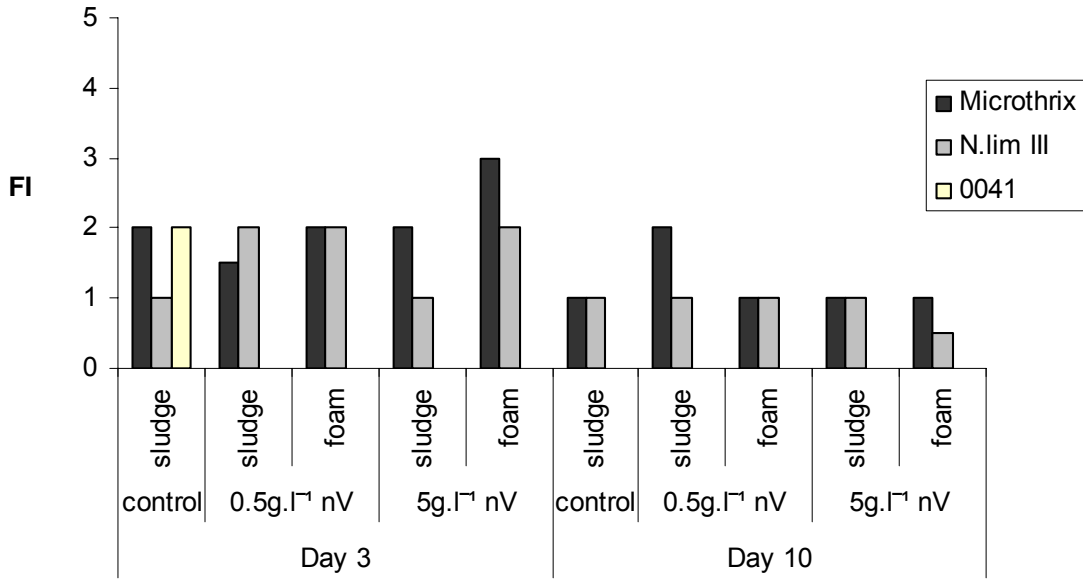


Figure 6.10: Filaments species and abundance of the control and in sludge and foam samples of the digestion bottles from the experiment on n-valeric

The filament levels were also monitored during batch digestion of sludge containing different concentrations of nV. This time all digestion bottles, including the control, contained the same amount of feed and seed sludge and therefore the same number of filaments at the start of digestion. Filaments in foam and sludge samples did not vary significantly during the digestion period. In one occasion only foam contained more filaments than the corresponding sludge sample only by one unit on the FI scale (Day 3, 5 g.l⁻¹ nV). Overall, the FI did not exceed the value of 3.

6.3 Discussion

This chapter aimed to promote a better understanding of the association of filamentous bacteria to AD foaming. Two reports, as discussed in the literature review (paragraph 2.4.2), identified *Gordonia* species, comprising of *Actinomycetes*, as the foaming cause at both full and bench scale digestion (Hernandez and Jenkins 1994, Pagilla *et al.* 1997). During the current work,

Actinomycetes were only found in one sludge sample originating from a non-foaming digester and at low abundance (FI: 1) (Figure 6.2, Site 3). *Actinomycetes* were not seen at any of the foaming digesters and were not present in samples from the batch studies. Therefore, it was not possible to study the contribution of *Actinomycetes* to foaming during the current work.

Additionally, previous reports on filaments and AD foaming have showed that *Microthrix* was identified as the foaming cause and was present in foam and sludge samples of a foaming full scale digester with FI of 5 and 0-1, respectively (Westlund *et al.* 1998). *Microthrix* levels in this work did not exceed an FI of 3 in all foam samples obtained and FI of 2 in all sludge samples obtained. *Microthrix* was more abundant in the foam samples originating from the batch digestion studies by only one unit maximum difference in the FI scale to sludge, which was potentially attributed to the hydrophobic properties of the bacterium. Hence, the affinity of *Microthrix* to partition in the foam and its contribution to foam stabilization was not significant at the examined abundance. It is necessary to clarify at this stage that *Microthrix* was not the cause of foaming during batch digestion as foaming at bench scale was clearly initiated by organic loading or surface active agents, as previously shown in Chapters 4 and 5.

Nostocoida limicola I & III were the next most abundant species after *Microthrix* during the batch digestion studies. Generally, *Nostocoida limicola* was present during batch digestion in both sludge and foam samples at the same abundance (Figure 6.10, Day 3, 0.5g.l⁻¹ nV), present only in sludge (Figure 6.7), present only in foam (Figure 6.9, Day 10, 0.1g.l⁻¹ BSA), or present in foam in higher or lower abundance than in sludge (Figure 6.10, Day 3, 5g.l⁻¹ nV, Day 10, 5g.l⁻¹ nV). The contribution of *Nostocoida limicola* to foam stabilization, therefore, could not be assessed at this stage.

9 more filamentous bacteria were found in sludge samples of the full scale digesters examined including 0914, 021N, 0581, 1863, *Streptococci*, *Thiothrix*, 1702, 0803 and 0041. Slightly higher variability in terms of bacterial species was noticed in the foaming digesters (10 species present in total, including *Microthrix* and *Actinomyctes*) with each bacterium's abundance not exceeding FI of 3 apart from one occasion (Site 15, *N.limicola* III FI: 5). 8 filament species were found in the non-foaming digesters with FI between 2 and 3. Yet, no outstanding differences were observed between foaming and non-foaming digesters in terms of their filamentous microbial population.

In conclusion, so far there was little information in the literature on filaments presence and abundance in AD and their association to AD foaming, as reviewed in paragraph 2.4.2. More importantly, the literature showed that only two species are known to exist in AD and be linked to foaming. The current work identified 13 filamentous species at full scale anaerobic digesters and determined their abundance. These findings were in accordance with the findings of Hernandez and Jenkins (1994) and Sodell and Seviour (1995) that filamentous bacteria can survive during mesophilic AD with only a small reduction in population and are capable of respiration. The presence of filamentous bacteria was also seen at bench-scale indicating only a small reduction and in some cases no reduction in the filament population after a 10-day digestion period. The full scale investigation also indicated that the presence of a single species in a digester with filament abundance of $FI \leq 3$ would not result in foaming at the full scale. Additionally, the current work monitored the contribution of *Microthrix* and *N.limicola* species to foam stabilization during bench scale batch digestion. Some partitioning to foam was seen for *Microthrix* only. However, its contribution to foam stabilization in this work did not match information from the literature as reported by Westlund *et al.* (1998) who found that *Microthrix* abundance in foam corresponded to FI of 5 while in sludge to FI of 0-1 at the full scale, hence *Microthrix* did not have a great effect on foam stabilization in this study. Therefore, the data obtained in this chapter did

not verify the hypothesis that filamentous bacteria can be a cause of foaming and can contribute to foam stabilization during AD.

Chapter 7: Cost implications for the water utilities

7 Cost implications for the water utilities

Anaerobic digestion foaming is of major concern to the water utilities due to its cost implications. Amongst the key advantages of AD is the utilization of biogas produced during digestion for on-site energy production. During foaming, the biogas is entrapped in the foam layer resulting in inefficient gas recovery, which creates additional costs for electricity production. According to Pagilla *et al.* (1997), two full-scale mesophilic anaerobic digesters in the US that reportedly presented foaming in digesters, showed an inverse solids profile during the foaming periods. Heterogeneity reduces the active volume of the digester resulting in poor sludge stabilization. Additional impacts from AD foaming include blockages of gas mixing devices, foam binding of sludge recirculation pumps, foam penetration between floating covers and digester walls and fouling of gas collection pipes. Cleaning and manpower are essential to minimize the impacts of foaming but create further economical issues to the water utilities. There is currently only one reference in the literature assessing the costs arising from foaming incidents at the full scale (Westlund *et al.* 1998). The report stated that the total cost of the 10-week foaming incident at the 7 full scale mesophilic anaerobic digesters ($5 \times 5000\text{m}^3$, $2 \times 7000\text{m}^3$) reached 150,000USD. It is evident therefore, that there is a gap in knowledge of the cost implications arising from foaming digesters, which could lead to the overestimation or underestimation of the problem. This work aimed to carry out an investigation of the cost implications involved at a number of STWs encountering foaming problems in the UK.

Information on the costs of foam control techniques, manpower used during foaming incidents, biogas loss, imported energy, cleaning costs and any other economical issues arising from foaming was gathered from the same 16 STWs studied in the current work. The data presented in

Table 7.1 derived from the site survey work as described in Chapter 3. Additional costs in Table 7.1 include the costs arising from cleaning, working hours / overtime and imported energy.

Table 7.1: Cost implications of foaming at full scale

	<i>Antifoam</i>	<i>Antifoam used (litres per 1000 m3 digester volume per day)</i>	<i>Antifoam cost (£ per 1000 m3 digester volume per day)</i>	<i>Additional costs</i>
Site 3	Burst	2.19	2.20	No
Site 4	DDF900	0.5	1.30	No
Site 6	Burst 5400	3.22	≤8.90*	No
Site 7	Burst	n/a	n/a	£300 cleaning costs
Site 9	Burst	1.88	1.90	n/a
Site 10	Burst	n/a	n/a	n/a
Site 12	Burst 5400	1.14	3.15	£5600 per week on cleaning & working hours
Site 15	Burst	n/a	13.00	n/a

*Assuming product price does not change between different water utilities

n/a: Not applicable, information could not be provided

Earlier in the literature review (Chapter 2.5.3), it was discussed that different antifoams can have different results when applied on the same solution and that the selection of antifoam to be applied on a bioprocess such as AD, should be based on experimental results including the antifoam efficiency, the antifoam's destruction rate and the optimum amount of antifoam for efficient foam destruction. It was also identified that there are currently no reports in the literature on the evaluation of the effectiveness of different antifoams on foam control in AD. The

data obtained from this study, as shown in Table 7.1, provide important information for the use of antifoam as a foam control method in AD and its cost implications. The type and amount of antifoam dosed to a digester per day during a foaming period varied across the STWs studied. Burst (Ciba, UK) seemed to be the most popular antifoam. There was not a considerable difference on the amount of Burst used between Site 3 (2.19 litres per 1000 m³ digester volume per day) and Site 9 (1.88 litres per 1000 m³ digester volume per day) for efficient foam control. Yet, as these were the only two sites for which data regarding Burst were obtained for, there was not enough information to assess the antifoam's efficiency and suitability for each occasion / site. Burst 5400 was used in 2 out of the 8 sites using antifoam but the dosing varied significantly and was nearly three times higher at Site 6 than at Site 12. This could indicate that Burst 5400 was not the best suitable antifoam for Site 6. Arguably, the severity of foaming between the two sites could have been different, which could explain the larger amounts of antifoam used on Site 6. However, as antifoam was being dosed to both digesters on a daily basis during the foaming periods, it was impossible to understand the severity of foaming in each occasion. On the other hand, there was indication that DDF900 (EC Chemical Productions Ltd, UK) might have possibly been the most effective on the particular occasion of Site 4 as the least amount was added to the digester in comparison to the amounts of antifoam added to digesters of the other sites. In some occasions, such as at Sites 7, 10 and 15, a set guideline on antifoam dosing was not followed as the operators varied the daily antifoam dosing either because the product was under trial and optimum dosing had not been found at the time of the visit or because antifoam dosing was avoided unless foaming was persistent and was regarded as the last solution for foam control. Under these circumstances, and due to poor information provided from the on-site records on antifoam dosing and daily / weekly consumption, it was difficult to estimate the amount of antifoam used.

The literature review (Chapter 2.5.3) also identified that there is currently no information on the cost implications of the use of antifoams in AD. A significant

outcome from the survey was the acquisition of information on the daily cost of antifoam. Antifoam dosing was costing the water utilities between £1.30 and £13.00 for 1000m³ of digester volume per day, as obtained from the studied foaming sites. At small scale STWs the economical implications from antifoam dosing are not considerably large, as a site with 2 digesters of 1500m³ each would have costs on antifoam between £3.90 and £39.00 per day based on the above data. However, at large scale STWs, the cost of antifoam becomes significantly high. For instance, a site with 8 digesters of 3000m³ each would require antifoam worth between £31.12 and £312.00 on a daily basis, based on the above data. Again, the costs of antifoam dosing depend highly on selection of the best suitable antifoam and the optimum dosing for efficient foam destruction. When these criteria are not met, then the costs of antifoam dosing are high.

The biogas loss during a foaming incident was investigated based on information provided by the sites operators and managers. It was found that Sites 3, 4, 6 and 12 did not suffer from any biogas loss due to the immediate response (antifoam dosing) to foaming incidents. However, none of the other sites monitored the biogas loss during the foaming events. According to Metcalf and Eddy (2003), digesters can produce 0.75 to 1.12 m³ of gas per kg VS destroyed with an energy content of 22,400kJ.m⁻³ providing 65% of biogas comprises of CH₄. For a specific site and given that the amount of biogas lost due to foaming is known, it would be possible to assess whether it is economically viable to invest on foam control systems (antifoam dosing) and minimize biogas loss. At this stage, however, due to the lack of information it is not possible to carry out a cost – benefit analysis.

The additional costs (cleaning, manpower and imported energy) varied significantly between the STWs. Sites 3,4 and 6 had no additional costs due to the immediate response to foaming. Site 7 would spend £300 per foaming incident for cleaning but no other expenditure was usually made according to the operators. The additional costs for Site 12 were much higher than the rest of the sites investigated

with a total for cleaning and working hours of £5,600 per week. On the other hand, some sites (Sites 11, 13 and 14) would not take any action after a foaming incident minimising the additional costs for cleaning and manpower to 0. The economical issues on energy loss and imported electricity were not known at any of the above sites. It was concluded that the size of the additional costs depended greatly on a number of factors involving the severity of foaming, manpower availability and the availability of technological equipment for foam detection in the digesters (sensors, radars) which enabled immediate response to foaming. In cases of severe foaming in digesters located near or by touristic areas or environmentally sensitive areas, such as Site 7, cleaning of overflowing foam was essential to avoid the adverse effects of contamination of the surrounding environment but also to ensure a safe working environment for the on-site workforce. Consequently, the location of the STWs was another factor controlling additional costs.

An alternative foam control method to antifoam dosing used as common practice at some STWs amongst the water utilities was recognized during this work. Sites 10, 11, 13 and 14 did not use any antifoam during foaming events but altered the digester operation in order to suppress foaming. Operators reduced (Sites 11, 13, 14) or would reduce (Sites 10, 16) the feed to the digester to minimize foaming. The feed volume reduction was totally empirical and depended on the operator's experience and each digester's response with regards to foam suppression. The operators would often alter feed volumes on a daily basis to achieve foam minimization where foaming was persistent. Therefore, it was difficult at this stage to obtain specific information on digester under-loading as a foam control method (i.e. organic loading values, % volume reduction of feed). However, biogas and hence energy loss was still present on those sites. None of the sites was monitoring the biogas loss. The economical complications arising from cleaning and additional working hours for Sites 11, 13 and 14 were 0, as according to the operators no action was taken after a foaming incident.

Another approach for foam control was seen at Site 8. No antifoam or under-loading of digesters was employed on site. During foaming incidents, the foam was collected from the surface of the digester and discharged to the digested sludge holding tanks. Consequently, the biogas was let to the atmosphere. Information on biogas loss, imported electricity during the foaming periods and costs arising from working hours of manpower were not provided due to lack of on site monitoring.

In summary, there was only one reference in the literature, so far, assessing the economical impacts of AD foaming at the full scale. Cost implications at the full scale of a number of STWs in the UK were evaluated here in order to provide a better understanding of the economical impacts of foaming in AD. The chemical control of foaming (antifoam dosing) induced a substantial daily cost of antifoam between £1.30 and £13.00 for 1000m³ of digester volume per day. However, there was evidence that the selection and dosing rate of antifoam was not the optimum in some cases (Sites 3 and 6) and there were occasions where no information on the amount of antifoam used was provided due to poor monitoring (Sites 7, 10, 15). This indicates that there was not always a systematic approach for foam control at full scale and lack of understanding of the complications involved. An alternative foam control method frequently used in industry in an effort to reduce the costs of antifoam dosing, was identified in this work. Foam minimization was achieved by altered digester operation and specifically reduced feed rates. That was in agreement with findings in this work (Chapter 4) supporting that increased organic loading and hence increased amounts of feed sludge to the digester results in foaming. Although, important information was identified on the various costs involved in a foaming incident, the overall cost of a foaming incident at full scale was not estimated due to poor on-site monitoring of parameters such as biogas loss, cleaning and maintenance costs and imported energy. No data were available from the 16 STWs visited on biogas loss and imported energy. The cleaning and maintenance costs and costs arising from manpower working hours varied greatly between different sites from 0 to £5600 per week. Yet, this was found to be greatly

dependant on the severity of foaming and the foam control method employed on site. In conclusion, chemical control was found to induce a daily cost highly associated with the antifoam efficiency and optimum dosing rates, yet minimizing the subsequent effects (biogas loss, etc) due to the immediate response, whereas the operational control of foaming minimized but prolonged the costs associated with the subsequent effects of foaming.

Chapter 8: Discussion

8 Discussion

8.1 Foam types

The differentiation between two foam types, the unstable and the metastable foams, has been stated in the literature (paragraph 2.2.1) by a number of researchers (Westlund *et al.* 1998, Vardar-Sukan 1998, Barjenbruch *et al.* 2000, Barber 2005). Unstable foams, usually caused by fat or filamentous microorganisms that float to the surface of sludge or wastewater by attachment to the gas bubbles, tend to reach equilibrium but continuously break down due to drainage of the liquid film and usually have a lifetime of seconds. Metastable foams cannot be easily destroyed by mechanical means but can collapse due to an irregular disturbance (vibrations, radiant heat, temperature differences), have a lifetime of a few days and usually occur when the process in a bioreactor is unstable or if hydrophobic matter is present in wastewater or sludge (Westlund *et al.* 1998, Vardar-Sukan 1998, Barjenbruch *et al.* 2000, Barber 2005). However, the information on their generation mechanisms in wastewater and sludge was limited to a description of the substances involved (fat, filaments and generally hydrophobic substances) and not quantitative data. Furthermore, AD foams have not been studied in relation to their stability and differentiated as unstable or metastable.

Findings from the current work indicated the presence of unstable foam at the full scale when a random foaming incident occurred at a non-foaming digester (Site 16). Foaming lasted for approximately 4 – 5 days. Foaming on that particular occasion was not considered as metastable since it subsequently collapsed without any foam control action taken and metastable foaming can only collapse

due to an irregular disturbance, as described above. There was also an indication that unstable foams were present at one of the sites characterized as a foaming digester in this work (Site 12). According to the operators the digester had regular foaming; yet, no foaming was recorded in the digester for a period of 10 months after the daily antifoam dosing was stopped. Whether the digester was actually foaming or not is not clear and it could be possible that random unstable foaming events could have led to the perception that the digester had regular (metastable) foaming. The daily antifoam dosing during the monitoring period did not facilitate monitoring the foaming events in order to conclude whether foaming was unstable or metastable at Site 12. However, the same digester did not foam when the antifoam dosing was stopped for a period of 10 months after the initial monitoring period as mentioned earlier. The numbers and species of filamentous bacteria found in both digesters during the monitoring period of 11 months for the non-foaming digester and 8 months for the foaming digester did not show any evidence of significant changes in filament numbers and species and hence filaments were unlikely the cause of foaming on these occasions. The non-foaming digester, however, exhibited high alkalinity, SCOD and DOC values in digested sludge before and after the foaming incident, which was attributed to the increased organic loading at 2.81 and $2.68 \text{ kgVS.m}^{-3} \text{ d}^{-1}$. It is unknown, however, whether the increased organic loading could have resulted in increased fat (lipids) content into the digester that would result in the creation of unstable foams as described by the literature. No such evidence was found for the foaming digester. Indication of the presence of unstable foam was also seen in this work at bench scale. The organic loading of 2.5 kgVS.m^{-3} during the batch anaerobic digestion studies resulted in foaming that lasted between 1 and 4 days and subsequently disappeared with destruction of foam being random. Filamentous bacteria were not the cause of foaming and only 1 species was found with $FI < 2$ on all occasions. The characterization of the foams examined in this paragraph as unstable was mainly based on the fact that the destruction of these foams was not due to irregular disturbances (vibrations, radiant heat, temperature differences), which are

responsible for the destruction of metastable foams, but also on the likelihood that the foams had reached equilibrium for a limited period of time, even though they were continuously breaking down, determined in this work as 1 to 4 days at bench scale rather than seconds, as mentioned in the literature.

Additionally, findings of the current work identified the presence of metastable foams at both full and bench scale. Foaming at Site 13 was an example of metastable foam formation due to unstable digestion process as foaming was present in the digester on a daily basis for a year of operation and subsequent sludge analysis showed that the digestion process was inhibited. It is necessary to mention here that no antifoam had been dosed to the digester during foaming, thus allowing reliable detection of full-scale foaming events. The creation of metastable foams on that occasion was in accordance with information found in the literature stating that metastable foams in a bioreactor occur when the process is unstable (inhibited digestion) or if hydrophobic matter is present (accumulation of hydrophobic substances due to poor degradation). Metastable foams were also created at bench scale by the 5 kgVS.m^{-3} organic loading and added BSA and nV during the batch anaerobic digestion studies. However, analysis of the sludge samples did not show any inhibition of the digestion process for the batch experiments on organic loading and nV. The presence of hydrophobic substances responsible for the creation of metastable foams according to the literature could potentially be attributed to the hydrophobic fraction of the increased DOC concentrations measured in foam and sludge samples during the batch experiments on organic loading and nV.

In conclusion, a clear differentiation between two foam types and their occurrence in AD was made in this work based on the foams stability and ease of destruction. The stability of foaming varied between 1 to 4 days for foams classed in this work as unstable (from the bench scale 2.5 kg VS.m^{-3} organic loading experiment) and at least 10 days for foams characterized here as metastable (from the 5 kg VS.m^{-3}

loading, BSA and nV batch experiments). The unstable foams disappeared without any foam control action taken whereas the metastable foams were persistent throughout batch digestion. The digestion period of the first batch organic loading experiment was extended to 16 days instead of 10 days in order to assess the end of biological activity at bench scale batch studies and the duration of foaming. After 12 days of batch digestion the metastable foams started varying in volume and even disappearing on some days, which indicated that metastable foams exhibited characteristics of unstable foams (Figure 57, Appendix B). Thus, it could be concluded that unstable foams in AD are potentially a transition phase during the destabilization of metastable foams. There was also indication that the causes of unstable and metastable foaming in this work could have been in accordance with information in the literature as for instance, metastable foaming at full scale coincided with unstable digestion.

8.2 Anaerobic digestion foaming causes

8.2.1 Bench scale investigation of AD foaming causes

The bench scale investigation of AD foaming identified organic loading, and the surface active agents BSA and nV as causes of AD foaming and each one of them had a different effect on the digestion process. Organic loading of 1.25 kgVS.m³ did not result in foaming at bench scale. The 2.5 kgVS.m³ organic loading was critical for foam initiation and the 5 kgVS.m³ organic loading resulted in metastable foaming but neither of them inhibited the digestion process according to solids reduction, gas and methane production, VFAs and alkalinity, contradicting information found in the literature stating that overloading of digesters results in an imbalance, accumulation of acetic acid and subsequently foaming (Barjenbruch *et al.* 2000). The only statistically significant difference found in digested sludge

between the 1.25 and the 5 kgVS.m³ loading was the DOC concentrations (Figure 8.1, \bar{x} represents the increase in DOC) ($P=0.002$, $\alpha=95\%$), which could be justified by the higher loading induced by the 5 kgVS.m³. The batch tests showed that organic loading over 2.5 kgVS.m³ was an AD foaming cause but the actual compounds causing foaming and the mechanisms of foaming during digestion were still unclear.

The literature has showed that interactions between surface active compounds in a solution can enhance or reduce the foaming potential of the solution and depend on the type of surface active agents present (Glaser et al 2007, Eisner et al. 2007). Subsequent experimental work on the surface active compounds BSA and nV demonstrated that the concentrations of the studied surface active compounds needed in order to induce foaming in sludge under aeration were higher than the ones required to induce foaming in water (BSA: 0.007 g.l⁻¹ in water 0.09 g.l⁻¹ in sludge, nV: 0.2 g.l⁻¹ in water, no foam in sludge after addition of up to 5 g.l⁻¹ of nV). This was attributed to the sludge solids and organic compounds that facilitated interactions such as adsorption or binding to take place, as seen from measurements of the two compounds in solution and where applicable in the solid phase of sludge (Figures 31 – 34, 45 Appendix B), and ultimately reduced the foaming potential of the studied surface active compounds. The behavior of BSA and nV in sludge during batch anaerobic digestion varied. BSA induced unstable foaming in sludge under aeration, which increased with increasing BSA concentrations but the concentrations of BSA examined during batch anaerobic digestion resulted in metastable foaming in all occasions with the 1 g.l⁻¹ BSA producing more foam than the two lower concentrations (0.1 and 0.3 g.l⁻¹ BSA). N-valeric acid did not induce foaming in sludge under aeration but metastable foaming increased with increasing concentrations during batch digestion.

Addition of BSA during batch AD increased the dissolved organics content in sludge by 2 to 3 times than the control at the end of batch digestion and resulted in

accumulation of tVFAs out of the normal range for AD (1006 – 2013 mg.l⁻¹) showing potentially inhibition of the digestion process. The protein content in sludge as SMPs and EPS at the end of batch digestion was independent of the initial BSA added, which was in accordance with the findings of Gonzales *et al.* (2003) stating that there is a final equilibrium protein concentration value in sludge that is independent of the initial protein concentration. Addition of nV at bench scale batch digestion resulted in increased alkalinity and DOC in sludge and metastable foaming continued in the absence of nV as all the nV was utilized by Day 3 of batch digestion for all concentrations examined. Therefore, signs of digestion inhibition were not always present for the examined foaming causes (organic loading) and in some cases metastable foaming was present in the absence of the foaming cause (nV). This indicated that foaming was a response of the digestion process to the change in the feed quality (increased organic loading or added surface active agents). The digestion bottles containing the foaming cause had higher DOC than the control in all cases, which could have been due to remaining concentrations of the foaming cause added or from faster degradation rates and generation of compounds from the microbial activity as the sludge quality characteristics showed that there was no tVFAs left at the end of batch digestion for the experiments on organic loading and nV.

Additionally, the literature states that sludge foams are 3-phase systems with gas-liquid-solid interactions (Davenport and Curtis 2002). The biogas production was present in all cases during the batch experiments and increased with increasing loading. Increased foaming with increasing loading was also noticed during the batch experiments. Therefore, the increased gas production could have contributed to foaming as increased gas rates can increase foam formation as demonstrated by Varley *et al.* (2004). The liquid phase of the foam matrix in this work comprised of DOC, proteins and tVFAs, as shown by analysis of foams generated at bench scale. According to Imai *et al.* (2002) DOC from effluent wastewater contains hydrophilic and hydrophobic acids, bases and neutrals. This indicated that

hydrophobic compounds are potentially present in DOC found in foam and could have contributed to the generation and perhaps stabilization of foaming. Proteins, which have been identified in the literature as surface active agents, were also recorded in foam samples during the batch experiment on BSA and could have also contributed to foaming. Furthermore, tVFAs were present in foam samples. During this work it was demonstrated that acetic acid was not surface active and did not initiate foaming in sludge at the examined concentrations. However, propionic, n- and iso-butyric and n- and iso-valeric acid were found in foam at concentrations from a few mg.l^{-1} to hundreds of mg.l^{-1} . Valeric acid was found in this work to be surface active and was responsible for foaming. The last component of the 3-phase wastewater foams is the solid content. Recent studies have shown that wastewater foam stabilization is mainly due to the filamentous *Gordonia* and *M.parvicella* but there is evidence suggesting that non filamentous mycolic-acid containing microorganisms, of which specific species have not yet been identified, also act as stabilizing agents (Hug 2006, Heard *et al.* 2008). Calculation of the mass solids content before and after batch digestion in this work showed that although the TS content in sludge was reducing, the TS content of the foam and sludge together at the end of batch digestion was increased indicating that biomass was potentially generated during batch digestion and was accumulated in the foam. Investigation of the contribution of *Microthrix* and *N.limicola* species to foam stabilization during bench scale batch digestion in this work showed a degree of partitioning to foam for *Microthrix* only. However, the contribution to foam stabilization was potentially insignificant as the filament abundance was either the same or higher by only one unit in the filament index in foam compared to sludge samples. Consequently, it was not seen during this work that filamentous bacteria can cause foaming and can contribute to foam stabilization during AD. It is possible that the high solids content in foam at bench scale could have been attributed to the presence of mycolic acid containing microorganisms, which in addition to the filamentous bacteria stabilized the foam at bench scale.

The bench scale investigation of surface active agents raised the argument that the batch experiments on BSA, n-valeric and acetic acid were effectively experiments on loading similar to the organic loading experiments. However, the experimental work this time was studying only the effect of one compound rather than the whole sludge on digestion in order to recreate full scale conditions where a change in the quality of the inlets of STWs could result in high protein or acid loading but also to investigate gaps in knowledge on surface active agents as identified by the literature. To allow comparisons of the loading induced between the different batch digestion experiments carried out in this work, the SCOD loading was calculated (formula given in Table 5, Appendix B) and compared. A clear differentiation of foaming – non-foaming conditions was achieved based on the SCOD loading values from the organic loading batch experiments. Consequently, when the SCOD loading was between 0.11 and 0.17 kg SCOD.m⁻³ there was no foaming in the digestion bottles. Critical conditions for foaming occurred at SCOD loadings between 0.23 – 0.35 kg SCOD.m⁻³ and daily foaming was present at loadings between 0.46 – 0.70 kg SCOD.m⁻³. It would be expected that the SCOD loadings for the surface active compounds would be approximately in the range of 0.46 – 0.70 kg SCOD.m⁻³ or above as daily persistent foaming was present in all occasions. The SCOD loadings, however, ranged from 0.12 to 7.58 kg SCOD.m⁻³. This indicated that the calculation of the SCOD loading as a foaming prediction tool for a digester is not accurate and it is greatly dependant on the cause.

8.2.2 Full scale investigation of AD foaming causes

A similar pattern to the batch organic loading experiments was seen at full scale foaming digesters at Sites 9 and 11 where the sludge quality characteristics did not show any inhibition in the digestion process and were within the suggested ranges for solids reduction, tVFAs and alkalinity, Yet the organic loading at both sites was

3.5 kg VS.m⁻³ d⁻¹ and 5.17 kg VS.m⁻³ d⁻¹, respectively and the DOC values in digested sludge from both sites were the second and third highest values obtained for the foaming digesters. This makes understanding of full scale digester foaming complex as the cause cannot be identified and subsequently prevented from recurring unless detailed monitoring is in place. However, there were also foaming full scale digesters where inhibited digestion process was recorded (Sites 7 and 13) in terms of tVFAs and alkalinity out of the suggested ranges for AD, as seen during the batch studies on surface active agents. The surface active agents were determined at the full scale by indirect measurements of the sludges foaming potential. Sludge obtained from foaming digesters had higher foaming propensity than sludge obtained from non-foaming digesters demonstrating that foaming digesters had higher concentrations of surface active agents and there was indication that part of these compounds was potentially digestion by-products. It was also shown that the foaming propensity was enhanced in the absence of solids (0.3 cm³ of foam per ml air per minute at 0.4 gram of TS which decreased to 0.03 cm³ of foam per ml air per minute at 4.4 gram TS).

Taking into account the above findings on surface active agents and given that sludge foams are 3-phase systems with a gas-liquid-solid interaction, it becomes apparent that sludge solids would suppress the foaming propensity of sludge up to a certain concentration of surface active agents. When the threshold of surface active agents is passed, the foam is generated and the solids act as stabilizing agents. Although this threshold was not identified in this work, there was a clear differentiation between the average foaming propensity of 0.27 mm foam per g TS (SE=0.16) of non-foaming digesters and the average foaming propensity of 1.42 mm foam per g TS (SE: 0.51) of foaming digesters suggesting that all the requirements for foaming, i.e. solids, surface active agents and gas, were present at the foaming digesters.

The association of filamentous bacteria to foaming in the literature refers to *Gordonia* and *Microthrix* species (Hernandes and Jenkins 1994, Pagilla *et al.* 1997, Westlund *et al.* 1998). 11 more species were found in full scale anaerobic digesters in this study including *N. limicola I & III*, 0914, 021N, 0581, 1863, *Streptococci*, *Thiothrix*, 1702, 0803 and 0041. One of the limitations of this work was that full scale sampling of foam was difficult due to the antifoam dosing and the structural characteristics of anaerobic digesters. Consequently, the presence of filamentous bacteria in foam and therefore their contribution to foam initiation and stabilization was not assessed at the full scale. The filament abundance in the foaming digesters examined in this work ranged from 0 to 5 in the filament index scale, as described by Eikelboom (2000). These findings were in accordance with the findings of Hernandez and Jenkins (1994) and Sodell and Seviour (1995) stating that filamentous bacteria can survive during mesophilic AD with only a small reduction in population. The full scale investigation also indicated that the presence of a single species in a digester with filament abundance of $FI \leq 3$ would not result in foaming at the full scale.

Operational characteristics of anaerobic digesters, such as the type of mixing and maintenance, were investigated. According to information in the literature gas mixing has been identified as an operational cause of foaming by promoting attachment of the hydrophobic and surface active compounds found in sludge onto the gas bubbles (Pagilla *et al.* 1997, Moen 2003, Barber 2005). As the bubbles rise to the surface of the liquid in digesters, the surface active and hydrophobic compounds form a liquid film around the bubbles that prohibits the bubbles from bursting, increases the surface activity and results in higher foaming potential. In addition, grit accumulation and poor mixing resulting from equipment failure, infrequent or no maintenance could result in poor digestion efficiency, accumulation of substances including surface active agents and potentially foaming (Moen 2003, Barber 2005). It was highlighted in the literature, however, that these statements were either site specific or poorly supported by experimental

data (paragraph 2.4.4). The findings in this work suggested that gas mixing alone was not the cause of foaming at any of the foaming digesters. As explained in previous paragraphs, the foaming digesters contained higher concentrations of surface active agents compared to the non-foaming digesters (0.27 mm foam per g TS average foaming propensity of non-foaming digesters and 1.42 mm foam per g TS average foaming propensity of foaming digesters) suggesting that gas mixing would only facilitate the foam formation under these circumstances. Technical failures, such as temperature fluctuations, mixing and pumping, although only recorded in this work and not studied, could affect the microbial activity leading to accumulation of substances, increase in surface active agents and potentially foaming but that was not seen at Site 5 non-foaming digester. This could be attributed to the low content of surface active agents in digested sludge, which was demonstrated experimentally through determination of the foaming propensity. Additionally, poor maintenance alone did not result in foaming at the non-foaming digesters of Sites 3 and 6 due to the lower concentrations of surface active agents. It can be concluded, therefore, that critical concentrations of surface active agents, which were here indirectly determined through foaming propensity of 1.42 mm foam per g TS (SE: 0.51), can initiate foaming during AD at the full scale, which is subsequently stabilized by the solids/biomass content and hydrophobic substances of sludge and the gaseous phase in AD only contributes to foam formation.

In conclusion, the knowledge to date put forward disparate theories and observations about the causes of AD foaming with limited experimental information. Overloading of digesters in the form of AS content in the feed sludge, which is the main source of filamentous bacteria and proteins, or increased organic loading resulting in accumulation of surface active organic substances, concentrations of individual compounds in the feed such as lipids or other hydrophobic substances, or polymer overdose during dewatering that generally can affect the microbial activity and result in accumulation of substances was some of the anecdotal information on AD foaming since there was limited experimental

evidence to support the above statements (Pagilla *et al.* 1997, Barjenbrugh *et al.* 2000, Moen 2003, Barber 2005). An association between volatile fatty acids and more importantly accumulation of acetic acid and AD foaming has also been suggested by many researchers in the literature (Pagilla *et al.* 1997, Westlund *et al.* 1998, Barjenbrugh *et al.* 2000). However, the critical concentrations for foaming in AD have not been identified. The current work provided advancement in knowledge from the information found in the literature to demonstrating experimentally that foaming was induced by critical organic loading rates over $2.5 \text{ kg VS.m}^{-3} \text{ d}^{-1}$ and the surface active agents BSA and nV, which resulted in increased concentrations of dissolved organics and / or tVFAs, SMPs, EPS, alkalinity indicating digestion inhibition / instability. The common element amongst the foaming digesters studied in this work was the unstable or inhibited digestion process that led to an increase in surface active agents in sludge and subsequently foaming, which was further stabilized by the solids. Figure 8.1 demonstrates the transition phase of a digester from a non-foaming state (on the left) to foaming (on the right) following the effect of a foaming cause (identified in this work as organic loading or surface active agent) (x represents the increase in surface active agents).

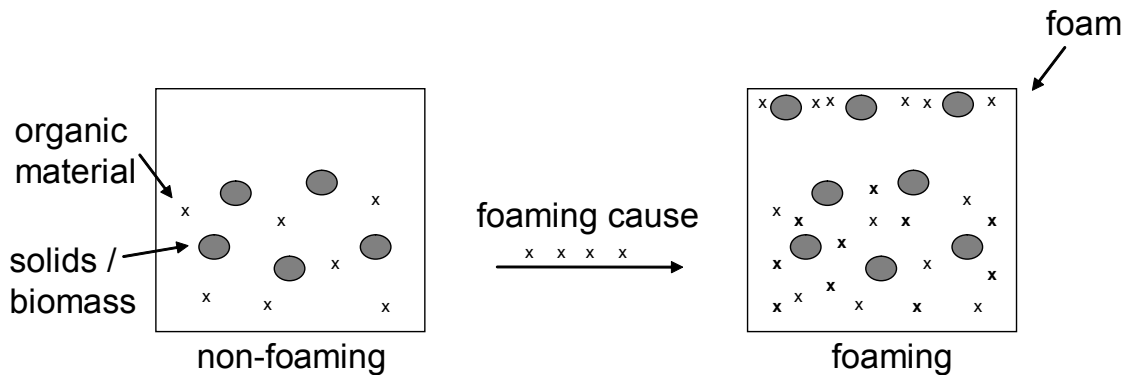


Figure 8.1: Model for foaming mechanisms in batch AD of sludge

Although detailed determination of the surface active agents responsible for foam generation was not provided due to the number of compounds involved and their

variability between different sludges, the foaming propensity tests provided a robust indirect determination of the presence and concentrations of surface active agents in sludge. The literature also suggested that filamentous bacteria are the cause of AD foaming. This work demonstrated that filamentous species are present in anaerobic digesters but do not necessarily cause foaming. There was also indication from the batch digestion studies on nV that accumulation of the longer chain fatty acids, such as butyric, caproic and perhaps propionic acid, is problematic and could potentially result in foaming. This can be of concern when pre-treatments before digestion result in hydrolyzed sludge containing longer chain fatty acids and it is important to ensure conversion to acetic acid during pre-treatment to avoid foaming events.

Anaerobic digesters, therefore, contain all the necessary requirements, 1) gas phase in the form of biogas production and / or gas mixing, 2) surface active agents and 3) solids (biomass) as the stabilizing agents, for foam to occur. Yet, not all digesters foam and it is only when the threshold of surface active agents is exceeded that foam occurs and is subsequently stabilized.

8.3 Limitations

One of the main limitations in this work was the complications arising from the full scale investigation of foaming. Although full scale monitoring provided investigation of the real foaming problem, the uncontrollable environment associated with full scale work had a considerable impact on the data obtained. As an example, Site 13 digester was not running under a constant temperature during the site visit and sampling and the digester was also under-loaded. Temperature fluctuations can affect the microbial activity in a digester, as earlier discussed in the literature (paragraph 2.4.3) and along with under-loading there is a high risk of poor digestion efficiency. As later found by sludge analysis, the digestion process was

inhibited and the operational problems could have been the cause of or contributed to foaming on this particular occasion. Other operational problems commonly found at the full scale, not only digester – related but problems at all of the upstream processes that affect the quality characteristics of sludge, involved passive screens at the inlets resulting in debris in primary sludge that could affect the mixing efficiency of a digester, mixing failure and pumps for feeding and discharging failures. Additionally, in some sites it was recorded that the pre-treatment stage not working or there was an imbalanced SAS to primary ratio in the feed to the digesters resulting in a different quality of feed sludge to the digester that the microbial population had to degrade, there was foaming in the activated sludge plant resulting in high input of filamentous bacteria in digesters and general maintenance of the sludge treatment processes was required. Apart from the operational / equipment failure, the antifoam dosing at the full scale had a major impact on the quality of the data obtained in this work. It was assumed that all the foaming digesters studied in this work were in fact foaming on a regular / daily basis, based on the operators and site managers' long-term experiences. In most cases, antifoam was dosed daily to the digesters, an area on which the author of this work had no control of, and did not allow determination of the foaming existence and severity at the particular time of sampling and investigation. An obvious example was that of Site 12, where the information given by the sites operators and managers suggested that the digesters suffered from regular / daily foaming. However, as explained in paragraph 4.2.4, following evidence from advanced sludge analysis that showed no unusual values for Site 12 digestion efficiency and discussion with the site's operator, the antifoam dosing was stopped and the digesters have not foamed since. The risk of mistakenly identifying non-foaming digesters as foaming due to the antifoam dosing is always present but was treated here as a single case for the following reasons: i) not all foaming digesters were receiving antifoam, as alternative foam control methods were implemented as explained in Chapter 7 and ii) bench scale data showed that it was not necessary for the foaming cause / initiator to be present for regular foaming to exist.

Another limitation of the current work involved foam sampling at the full scale and subsequent analysis. Although foaming has been found in the literature to penetrate between floating covers and digester walls, tipping of floating covers and spreading over and around the digester area at STWs, according to operators' statements, none of the foaming digesters with floating covers visited experienced such severe impacts of foaming due to the foam control methods employed on site, hindering the acquisition of a foam sample. The rest of the foaming digesters visited had fixed roofs and foaming was also controlled. Even in cases of severe foaming, the foam would escape through the gas collection pipes and it was impossible to access the interior of the digester. Therefore, no foam sample was collected from full scale. The analysis of foam was important in this work as it would help understand the foam properties and potentially lead to the identification of destruction mechanisms. For that reason, foam was generated and analyzed at bench scale. The foam generated at bench scale was representative of full scale foaming for the following reasons: i) the quality characteristics of foam samples obtained from the bench scale batch experiments on organic loading were consistent for all three experiments, as addressed in paragraph 4.2.3 ii) different causes of foaming at the bench scale (i.e. organic loading, BSA and n-valeric acid) produced foam with similar solids content in all occasions (TS: 5.7 – 7.5%, VS: 51 – 66%), iii) the solids content in the foam produced at bench scale was comparable to information found in the literature on foam quality characteristics (TS: 6 – 13.2%, VS: 65 – 70%) obtained from a full scale digester (Ross and Ellis 1992, Westlund *et al.* 1998).

Last but not least, the bench scale batch studies were all completed with fresh sludge samples collected prior to initiation of each batch experiment, as stated in paragraph 3.6. Thus, none of the batch studies was carried out with sludge having exactly the same quality characteristics with any other. However, to ensure the minimum variability of the sludges quality characteristics between the batch

studies, all samples were collected from the same non foaming digester and all batch studies were carried out in a period of 7 months. A single visit and sampling could have been carried out and subsequent storage of large amounts of sludge that would enable the completion of all the batch studies. However, as reviewed in paragraph 3.2, all sludge storage methods examined were found to have an effect on the sludges quality characteristics. Therefore, fresh acquisition of samples was most appropriate in this work to ensure the minimum impact mainly on the microbial community but also on the physical and chemical quality characteristics.

Chapter 9: Conclusions and future work

9 Conclusions and future work

9.1 Conclusions

This project investigated organic loading, the surface active agents BSA and nV, and filamentous bacteria as AD foaming causes at full, bench and laboratory scale. The main conclusions arising from this work are listed in the following paragraphs.

9.1.1 Identified AD foaming causes

- Gas mixing alone was not found to be a cause of foaming at the examined foaming digesters
- There was no apparent link between foaming and maintenance of digesters
- There was indication from the full scale data that organic loading could have been correlated with foaming in AD. Bench scale batch digestion identified the 2.5 kgVS.m^{-3} as a critical organic loading for foam initiation (unstable foams) for sludge obtained from a non-foaming full scale digester while the $5 \text{ kgVS.m}^{-3} \text{ d}^{-1}$ resulted in metastable foaming.
- The surface active agents, BSA and n-valeric acid, were the cause of foaming at bench scale batch anaerobic digestion of non-foaming sludge at concentrations of 0.1, 0.3 and 1 g.l^{-1} for BSA and 0.5, 1.5 and 5 g.l^{-1} for n-valeric acid. All examined concentrations of both compounds resulted in metastable foams.
- Acetic acid was not surface active at concentrations between 0 and 5 g.l^{-1} and did not cause foaming during bench scale batch AD at concentrations of 0.5, 1.5 and 5 g.l^{-1} . However, acetic acid inhibited the digestion process at the concentration of 5 g.l^{-1} .

- The carbohydrates, D-glucose, sucrose and starch, were not surface active at concentrations between 0 and 2 g.l⁻¹. Their effect on foaming during AD was not examined in this study due to time limitations.
- 13 filamentous species in total were identified in anaerobic digestion at both full and bench scale studies including *Nostocoida limicola* I & III, *Microthrix*, 0914, 021N, 0581, 1863, *Streptococci*, *Thiothrix*, *Actinomyces*, 1702, 0803 and 0041. Overall, the abundance of filament species in the foaming digesters varied between 0 and 5 in the filament index scale
- *Actinomyces* filamentous bacteria were not present in any of the foaming digesters examined or samples acquired during the bench scale batch AD studies and therefore, their contribution to foam initiation and stabilization could not be assessed in this work.
- *Microthrix* abundance did not exceed a filament index of 2 in all sludge samples obtained from both the full and bench scale. The abundance of *Microthrix* was not considered significant to induce foaming neither at the full scale nor at the bench scale. The partitioning of *Microthrix* in the foam samples at bench scale did not exceeding a filament index of 3 and its contribution to foam stabilization was considered insignificant.
- The abundance of *Nostocoida limicola* I & III was variable in sludge and foam samples at bench scale and did not exceed a filament index of 2 in all sludge samples. Due to the high variability of the presence of *Nostocoida limicola* I & III, it was concluded that their contribution to foam initiation and stabilization was insignificant at bench scale.

9.1.2 Understanding of sludge foaming mechanisms

- Both unstable and metastable foams were found at full and bench scale AD foaming. However, there was not a clear correlation between the foaming mechanisms in this work and the suggested foaming mechanisms by the literature stating that unstable foams tend to reach equilibrium but

continuously break down due to drainage of the liquid film and usually have a lifetime of seconds while metastable foams cannot be easily destroyed by mechanical means but can collapse due to an irregular disturbance (vibrations, radiant heat, temperature differences) and have a lifetime of a few days (Westlund *et al.* 1998, Vardar-Sukan 1998, Barjenbruch *et al.* 2000, Barber 2005).

- The foaming tests showed that digested sludges obtained from full scale digesters had a statistically significant, greater foaming potential under aeration and hence higher concentrations of surface active agents than digested sludges from non-foaming full scale digesters.
- AD foaming was independent of the feed sludge foaming potential determined under aeration and hence irrelevant of the surface active agents in feed responsible for foaming under aeration.
- When the foaming tests were carried out for sludge obtained before, during and after bench scale batch AD, the foaming potential in the whole sludge sample increased as batch digestion proceeded indicating that surface active agents were produced during batch digestion.
- The foaming potential of sludges was greater in the absence of solids (0.3 cm³ of foam per ml air per minute at 0.4 gram of TS as opposed to 0.03 cm³ of foam per ml air per minute at 4.4 gram TS).

9.2 Future work

A significant finding during this study was that the foaming cause / initiator (organic loading or surface active compounds) was necessary at the beginning of batch digestion in order to set off foaming but did not have to be present thereafter for regular / daily foaming to exist. AD foaming is a complex problem that requires a fundamental understanding of the physical, chemical and microbiological processes in an anaerobic digester. The work presented here aimed to identify a

number of foaming causes as set out in the literature and understand their relationship to foaming through experimental evidence. However, further investigation is needed in order to provide a better understanding of the mechanisms of foam generation and stabilization in AD. The proposed areas of further research are listed as follows:

- Findings from the current work showed that organic loading is a cause of batch AD foaming. The data presented here also showed that the two surface active agents examined are a cause of batch AD foaming. However, acetic acid, although it increased the loading, it did not cause foaming at batch AD. Further examination looking at the effect of carbohydrates on foaming at bench scale AD is essential in order to understand whether AD foaming is purely due to the increased concentrations of surface active agents deriving from the increased organic loading and added BSA and n-valeric acid, or foaming is the response of the digestion process due to the increased loading.
- One of the findings of this work supported that it is not necessary for the foaming cause to be present for regular foaming to exist during AD. It is important to identify the exact duration of a foaming incident following a single, instantaneous foaming cause / initiator but also a longer-lasting foaming cause / initiator as this would generate significant implications for the water industry. The duration of a foaming incident following such a foaming cause / initiator would be better studied at semi-continuous bench scale AD as it simulates the full scale process and provides reliable data.
- The semi-continuous studies could then be used to determine the foaming mechanisms of AD by the methods developed in this work. Additional work could look at the profile of the microbial community using molecular techniques such as the phospho-lipid fatty-acid method, to identify microbial population shifts during the foaming incidents.
- The semi-continuous studies could also be used to study the applicability of several cost-effective foam control methods, as addressed in the literature

and provide the water utilities with a more economically viable solution to the foaming problem.

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Appendix A

Table 1: Site Survey Questionnaire

Q1	Are there any operational problems on site at the moment?
Q2	Are there any refurbishment works planned to be done for the next 3 years on site?
Q3	Is there any noticeable difference in operation between the digesters on site?
Q4	Did the site experience foaming in digesters?
Q5	How often does foaming occur? Is it seasonal?
Q6	How long does it last?
Q7	How do operators detect foaming?
Q8	What solutions are employed to deal with the problem?
Q9	What processes are in use at this plant?
Q10	Are there any fluctuations in the influent characteristics?
Q11	Does the digester take in industrial imports? Where does the import come from (type of industry)?
Q12	Does the digester take in sludge imports (type of sludge)?
Q13	Is the import permanent? Is there any relevance to foaming and imported sludge?
Q14	How often does maintenance of digesters take place? (keep a note of the next maintenance date)
Q15	Has the ASP experienced foaming /bulking problems?
Q16	Is there any link between foaming in the AS and foaming in the digesters?
Q17	Have you checked for the presence of Nocardia/Microthrix in sludge?
Q18	How are the digesters build (roof type, headspace above the sludge)?
Q19	How are the digesters mixed? (gas/mechanical)
Q20	How are the digesters fed? (continuous/batch)
Q21	What is the %of SAS in digester feed?
Q22	Other parameters (load, VFAs levels, pH)
Q23	Retention time in digesters and flow
Q24	What is the average temperature in digesters?
Q25	Severity of foaming (scale from 1 to 10)
Q26	What anti-foam chemical is being used on site?
Q27	What is the amount of chemical added?
Q28	What s the cost of it?

(continued)

Q29	How many people are on call in foaming incidents?
Q30	What action is usually taken in foaming incidents?
Q31	What is the biogas loss from foaming occurrence?
Q32	Are there any other economical issues due to foaming?
Q33	Is there any difference in the digested sludge characteristics and the digestion efficiency after foaming occurs?
Q34	What is the overall cost after a foaming incident?
Q35	Who to contact for further information?

Table 2: Questionnaire answers, Sites 1 to 3			
Question	Site 1	Site 2	Site 3
Q1	Controlling the mixed liquors. Also adding more SAS, imbalance between primary and SAS ratios, not helping the digesters	No	Foaming in ASP
Q2	No	No	New thickeners, PFTs, blending tank
Q3	No (2 digesters on site)	No	4 digesters on site, 3&4 half the solids of 1&2
Q4	No	No	Had foaming on site a few years ago, last foaming last year
Q5	-	-	No foaming for years. Digesters were put out of service, when operators put them back on they foamed
Q6	-	-	Varies
Q7	Foaming cannot be detected. Concrete digesters with fixed roof.	View panel	Daily observation
Q8	-	-	Antifoam chemical
Q9	AS	AS	ASP, GBTs
Q10	High solids are reported occasionally	No	No. Has to be noted that STWs receive dairy influents
Q11	No	No	Yes, from the BMW factory
Q12	Yes, primary and SAS	Yes, from other sites	Yes, imported sludge from other sites
Q13	Yes, 150 m ³ /d	Yes. No	Yes. ?
Q14	Hasn't been done for 5 years	12 months ago, every 10 years	6-8 years ago, should be done every 5 years
Q15	No	No	no3 aeration lane foams badly. Since the new oil-fat plant was commissioned ASP foams badly
Q16	-	-	Could be, not sure.

(continued)

Q17	Not lately but due for check	No problems	Yes, normal
Q18	Concrete digesters, fixed roof	Fixed roof, concrete digesters	1&2 are concrete, 3&4 glass coated steel sheets, fixed roof
Q19	Gas mixing	Gas mixed and re-circ pumps	recirc-pumps
Q20	Batch	Feed for 45', rest for 15'	Batch
Q21	It is imbalanced at the moment	Don't know	Don't know
Q22	-	VFAs: 150-160 for no1, 120 for no2, 125 m ³ /d, 1700m ³	Don't know
Q23	Feed to each digester 80m ³ /d, minimum HRT 12days	13.5 days	15-18 d (used to be 25-30) 500-600m ³ /d to all 4 digesters but is proportional so digesters 3&4 half of 1&2, dig vol 1&2: 3454m ³ , 3&4: 1775m ³ (volumes of digesters are given as EDV:effective digester volume)
Q24	32-39	35-36 (at the moment no1@32.8 and no2@34.6)	35-36 in all digesters
Q25	-	-	5 before antifoam
Q26	-	-	Burst
Q27	-	-	2 gallons per digester are enough to suppress it
Q28	-	-	
Q29	1 person standby as usual	Only one person is always on site	Only one person is always on site
Q30	-	Antifoam dosing	Antifoam dosing
Q31	-	-	No loss, it only lasts for minutes till they dose the antifoam chemical
Q32	-	-	No
Q33	-	-	No
Q34	-	-	-
Q35			

Table 3: Questionnaire answers, Sites 4 to 6			
Question	Site 4	Site 5	Site 6
Q1	Currently no issues with foaming in digesters. The volatile acids remain stable. However, like all complex treatment works issues arise on a daily basis.	The digesters are not discharging sludge. The pasteurization units broke down the night before.	One aeration lane is out. High MLSS in lane 5.
Q2	Currently installing 3 CHP engines, a screw PS. No works planned on the digesters, this may be subject to change	Planning to renew the gas mixing system in the digesters. At the moment there is no mixing. The digesters have been running without mixing for 3 months	Yes, an endocrine unit is going to be installed at the end of the works, this will not affect digestion however
Q3	All VFAs are approximately the same. Feed ranges bet 220-280 m ³ /d. No8 digester has recently been out of service due to a blockage, No1 currently has no mixing since Dec'05	Digester no 3 is colder because it is lifted above the ground and so it is difficult to maintain the temperature	No, same in all 3 digesters
Q4	The last extended period was between June 04 to Feb 05	No	In the past, about 12 months ago
Q5	Random	-	More likely to be seasonal, between winter-spring. Not that bad in the summer
Q6	Varies	-	Varies
Q7	Digesters are now fitted with an ultrasonic foam detector, previously through sight glass	View panels on top part of digesters	Visibly
Q8	Antifoam dosing set up to dose in the recirc lines	Avoid overloading the digester, keep the right temperature	Antifoam chemical

(continued)

Q9	6mm screens, CV channels, PST, ASP*2, FST, BAFF, GBT, Digesters, Secondary Digesters	AS, pasteurization units	ASP, APD
Q10	Relatively static	frequently the site experiences high SCOD levels due to factory discharges	No
Q11	Various imports have been treated, some affect gas production others show no affects.	-	Yes, Honda and small chemical works (heavy metals)
Q12	No direct imports as such, sludge imports are brought in raw, thickened and pumped in a homogenous mix to the digesters	Raw sludge from other STWs from rural sites	Yes, other sites
Q13	No	250 m ³ /d	Yes. No
Q14	At 1998, at 2003...	3 years ago with the one before	1990 digesters were constructed, never cleaned but run at 90% capacity at the moment
Q15	ASP2 does experience foaming and filamentous bacteria seasonally	No	No
Q16	No	-	-
Q17	See above	-	It passed
Q18	Fixed roof, 3m head space, concrete, 7500 m ³ each dig, 261.5 m ³ /d feed	Fixed roof, 2m	Steel and fiber glass, fixed roof
Q19	Mechanical	Gas mixing, not on at the moment	Gas mixing
Q20	Continuous	Batch	Batch, every 25' feeding
Q21	Various sludge streams with different ratios that all form the main feed digester sludge		Not sure because of imports

(continued)

Q22	VFA 150	pH: 7.18-7.2, less than 7 before Christmas, load: no more than 233 m ³ /d, VFAs: 100-200	7.2-7.4 pH
Q23	2 phase digestion, 16 days in primary tanks, 12 days in secondary	12d	11.5 days in digesters, 1.5-2 days in APD, dig vol 1710m ³ EDV, max flow 170m ³ /d
Q24	31 C	Mesophilic (Dig 1 & 2-36 C) (Dig 3-34 C)	Just over 35 degrees, 29 in APD
Q25	10	0	-
Q26	Supplied by EC Chemical Productions, DDF900		Used to dose Burst 5400
Q27	Dosed at aprox 20-40 l/h as required		Not excessive, 2 months for 1000 l if continuously dosing
Q28	£2.60 per litre		£2.76 per litre
Q29	None. Site operates a shift pattern		1
Q30	Dose antifoam at agreed level		Antifoam
Q31	Don't know		Don't think they ever had significant biogas loss, only once for short period, always dose antifoam immediately
Q32	Cost of antifoam dosing, no other cost (i.e. cleaning) because action is immediate		No
Q33	No		Don't know
Q34	Don't know		Don't know
Q35	P.J.	N.K.	

Table 4: Questionnaire answers, Sites 7 to 9

Question	Site 7	Site 8	Site 9
Q1	Not at the moment. Mixing problems have occurred in the digesters from time to time	No	High levels of sludge detected in digesters frequently. Antifoam is dosed, it is believed to be foam that causes the increase in sludge levels. Imports from significant trader have high NH ₄ and solids
Q2	Re-introduce coarse screens, aeration lanes will be emptied, refurbish fine screens, cleaning of digester	Major changes. Increase flow to the STW. Extend aeration lanes, sludge production and SAS production will increase	Screens will be refurbished and P removal possibly to be installed on site (chemical or biological)
Q3	There is only one digester on site	3 digesters on site, no difference	no1 doesn't reach temp as easy as no2
Q4	Yes	Seasonally, between autumn-winter and winter-spring when temperature changes	A few months ago. At the moment fluctuations at sludge levels in digesters are detected but operators are not sure if it is foam
Q5	Frequent foaming for the last 4-5 months. Occasionally foaming occurred prior to last summer	Seasonal due to temperature changes in the environment	No history of foaming. It has been occurring for the last 6-12 months
Q6	Months	Few weeks, not more than a couple of months	Few months
Q7	Foam comes out of digesters (visual observation)	IR indicators but cannot detect foam in digesters, foaming is detected when gas supply is cut off to the gas holders due to blockages in pipes	Ultrasound sensors to detect level in digesters. Used to have a glass on roof so operators could look through but not any more
Q8	Anti-foam dosing (BURST)	The operators surplus it off from the digesters	Antifoam BURST
Q9	AS	AS	ASP, thickeners

(continued)

Q10	Influx in the summer due to tourism	No	Only from trader
Q11	Small amounts of industrial imports. Not a big industrial area	No	Y, the surrounding area
Q12	Primary imports every day	Yes	Yes
Q13	Permanent import. It seemed that primary sludge increased odours and was stopped for a while. They also thought that there was a link between foaming and primary feeding but at the moment foaming seems not to be affected by primary feed	Yes, 450 m ³ /d. No relevance	Yes. Don't know
Q14	More than 10 years	Every 12 years	no2 was recently cleaned. Normally every 10years
Q15	A little	Foaming was detected in the past but not great problems any more. Bulking is observed though frequently	No
Q16	Havent looked at that	No	No
Q17	Yes, at the moment there is a good balance	Occasionally, routine monitoring. Nothing unusual	Yes, normal levels
Q18	Concrete digesters, floating roof	Concrete digesters,, fixed	Concrete digesters,, fixed
Q19	Gas mixing	Gas mixing	Gas mixing
Q20	Continuous	Continuous	Continuous
Q21	Varies	Don't know	70:30 (SAS: 5-7% DS)
Q22	The site has always high VFAs ~400 mg/l	90ppm VFAs	Lately 300-500 VFAs but normally around 300 or less
Q23	12d	16-20 d, 330 m ³ feed to each dig,	max 133 m ³ /d in each digester 12days HRT, not feeding that much at the mom, dig volume 1600m3

(continued)

Q24	mesophilic 32-36	28-39 (mesoph)	36
Q25	9 - 10	3-4	Under control at the mom, 1
Q26	BURST	-	Burst
Q27	Still on trial. Trying to find the optimum dose, roughly consume 1000lt every 3-4 months	-	3 l/d to each digester
Q28	£1000 for 1000lt	-	£1000 for 1000lt
Q29	1 person is usually responsible for the whole plant. In cases of extreme foaming a 2nd person is on call however it is rare to happen	1 person standby	1-2
Q30	Anti-foam dosing	Let gas to atmosphere, surplus the foam off the digesters and discharge it to digested sludge holding tanks, import electricity to balance from biogas loss	Stand-by personell on call and cleaning
Q31	No idea	No idea	Don't know
Q32	Tankers to clear the area from foam residues. Costs about £300 for cleaning per time	Only the imported electricity	2 compressors normally on site for cleaning and repairs with 6 guys working on that plus the personell on call
Q33	—	No	No
Q34	Once chemical works and cleaning tankers are not in use, the only cost is the price of the chemical	Don't know	Don't know
Q35	R.B.	V.P.	W.M.

Table 5: Questionnaire answers, Sites 10 to 12			
	Site 10	Site 11	Site 12
Q1	None of the stirrers in the digesters seem to work at the moment. The site regularly foams	Just been commissioned, problems started 3 weeks ago, no foaming in the past	One of the pumps for primary sludge is out. No other major problems. Minor problems however occur every day
Q2	Not major, usual maintenance	The last refurb.works were completed with the installation of EH, now the operators are playing with feed and loading to balance the digesters	Not for the next 5 years
Q3	Y, 8 digesters on site. 1-4 have higher DS conc but 5-8 foam badly.	no1 foams badly, no2 no foam, no3 foams occasionally, all run @35	no2 operates at lower temperature (27 degrees) no1 at 33 degrees, both though perform ok even at 25 degrees with 60% CH4 production. Sample was obtained from digester with higher temperature
Q4	Regular foaming in digesters, especially digesters 5-8	Yes	Severe in the past but under control now due to antifoam dosing
Q5	When gas is too high in digesters more foaming is induced	It has only recently appeared. No1 foams badly constantly but operators change feeding and loading to destruct foam formation. No use of antifoam chemical so far. Only try to destruct it with operational changes	Constantly dosing antifoam now so no major foaming
Q6	Regular foaming		
Q7	Gas production drops	radars in digesters	Visibly, digesters have floating roof
Q8	Antifoam	nothing, the operators play only with operational parameters in dig no1	Antifoam

(continued)

Q9	ASP	AS, GBTs, EH	AS, Fe dosing which goes in the digesters through SAS
Q10	No	No	No, there are spikes in NH4 occasionally derived from the trade effluent
Q11	Y, also biodegradable waste is dosed directly in the digesters, used to get foaming in the past but not anymore	No	Y, industries around the area, major impact has Britvic due to high sugars and carbohydrates conc
Q12	Yes	Yes	Yes, SAS, primary and mixtures
Q13	Yes. No	Yes. Don't know	Not sure, maybe one of imports is affecting foaming. Every time imports from a specific site come to the site digesters foam.
Q14	Not sure	When EH installed, digesters were completely refurbished.	10years, last one 3years ago
Q15	No, the last foaming was at 2000	No	Yes, the site used to have 3 oxidation ditches (AS) in past that used to foam now 4 in use so no foam since the 4th came in use
Q16	No	No	No
Q17	Don't know	Don't know	High Microthrix levels
Q18	Concrete digesters, float roof, central stirrer	Concrete, fixed, 2100m ³ each dig, 3 digesters, 187m ³ feed to each	Concrete, floating roof
Q19	Central stirrer but in 5-8 digesters mixing might be poor, there is a possibility of short circuit	Mechanical, paddles	Gas, re-circ pumps
Q20	Batch	Batch	Continuous
Q21	About 1/3 of SAS, 2/3 of primary roughly	Don't know	Don't know

(continued)

Q22	34.5 degrees	-	Don't know
Q23	HRT 19 days	altering feeds and temperature daily to get foam down, 120m ³ /d 17 days, 2100m ³ each (560 m ³ /d)	Min HRT 14d but can go up to 20d. 100m ³ /d to each dig, 2400m ³ each digester
Q24	Feed is reduced at the moment from 150-190 m ³ /d to 130 m ³ /d, 3400m ³ each dig	Varies	no1: 30-35, no2: 27-30
Q25	Varies because of the antifoam, 1 - 3	8 in both no1 and no3 at the moment	Under control at the moment 1
Q26	Burst	If they had to use they would buy it from EC chemicals	Burst 5400
Q27	Not sure	-	2 RBCs per year, about 1000litres per year, density of Burst 5400 1.05 kg/l
Q28	Don't know		£2.90 per kg
Q29	1		No one since there is no way of detecting it
Q30	Dose antifoam. However, at the moment the operators are varying the feed rates to suppress foam		4-5 people working plus extra people for cleaning
Q31	Don't know		No loss
Q32	Don't know		Basically manpower for cleaning
Q33	Don't know		Sludge always passes all pathogen kill tests
Q34	Don't know		Don't know, assume 4 people working for a week costs around £5600
Q35			M.B.

Table 6: Questionnaire answers, Sites 13 to 16				
Question	Site 13	Site 14	Site 15	Site 16
Q1	Inlet screens are passive and need refurbishment, grit system not working, problems with feeding digesters, maintaining the temperature, heat exchanger blocks every other day, problem with gas mixing in the past	No	PFTs have problems, pumps for digesters have problems	General equipment failure occurs on regular basis
Q2	Yes, in different parts of the works	PFTs, holding tank for pr sludge, mixers	All of sludge treatment processes are to be refurbished	Yes, 2008 CAMBI installed
Q3	No	1 digester on site	Yes. 6 digesters on site, 4 (no 1-4) located together and 2 more (no 5,6) further down the site. No 1 and 4 have worst foaming	yes, no2 always slightly lower temp than no1
Q4	Digesters would only foam when they stop feeding them or when SAS/primary ratio is too high	Yes	Yes regularly. At the moment antifoam keeps it under control	No
Q5	A couple of days before sampling digesters foamed. The operators mixed digested sludge from the 2 digesters on site and seemed to operate well after that	Increase feed and it will foam, when operators keep feed down it operates well	The site has had foaming for 2 years now. They collect the foam and put it through the SAS belts	-

(continued)

Q6	Varies	Varies	From several hours to days	-
Q7	Usually they see it coming out. There is a probe but not quite reliable.	Observation of spills off the outlet pipe of digester which is not covered and located at the top end of digester. Digested sludge is collected at the top and drained out through the pipe	Manpower	Sensors
Q8	Stop feeding for 1-2 days then put the digesters back on	Stop feeding	Antifoam Burst	Reduce feed
Q9	ASP, GBT	No ASP, only primary sludge on site	ASP	AS
Q10	No	Aluminum sulfate is dosed at the inlet of the works / No fluctuations	Yes, nothing unusual though. Mars factory nearby so high organic loading from there (lipids etc)	Yes normal from local small industries
Q11	No	No	Yes, see above	Yes, see above
Q12	No	No but used to	Yes from other sites	Yes from other sites
Q13	-	-	Yes. No	Yes. No
Q14	Last cleaning 6 years ago	5 years ago / every 12 years	During the last 3 years, once every year for no 1,2,3 next year are no 4,5	Due for major refurbishment, not sure when the last one was
Q15	No	-	Yes, especially during my sampling they had high foaming and were dosing bleach to kill filaments	Not recently

(continued)

Q16	Don't know	Don't know	Don't know, don't think so	Don't think so
Q17	Don't know	No	Yes, high levels present which is linked with foaming/bulking in ASP	Yes in AS
Q18	Fiber glass, fixed roof	Fixed fiber glass roof, steel digester	1-4: floating roof, 5,6: fixed	Concrete digesters, fixed roof
Q19	Gas mixing	Pumps, 2 nozzles in the middle of the digester		Gas mixing
Q20	Batch, every hour	Batch, every 3 min sludge is fed, approximately 8 m ³ / hr or 20 m ³ / day	Semi-continuous, 165 m ³ /d, 6 digesters	Batch
Q21	2:1	no SAS	Don't know	Not sure due to sludge imports
Q22	100-140 m ³ /d, 1783 m ³ each digester	537m ³ each dig, 25-30m ³ /d feed	Don't know	300m ³ /d flow, 3700m ³ digester size
Q23	12 days	12-14 days	14 days	12-14
Q24	no1 @38 C, no 2 @ 27	36.4	around 35	no1 ~35
Q25	8-9	9	1 - 2	
Q26	-	-	Burst	
Q27	-	-	At the minute daily feeding, varies	
Q28	-	-	Normally try to run without antifoam but have been dosing for 6 weeks. Cost around 5K per month, digesters volume 2138 m ³ each	
Q29	1 person	-	1	

(continued)

Q30	No action	Stop or reduce feeding	antifoam dosing	Reduce feed
Q31	There is some but don't know, don't have gas flow meters	Don't know	Don't know	
Q32	Possibly cost of cleaning but it doesn't get cleaned it gets left	No	No	
Q33	No	Don't know	Don't know	
Q34	-	-	Don't know	
Q35				F.A.

Reference	Type of sample	Flow rate	Type of gas	Duration of aeration
Khan and Forster (1990)	Activated sludge	0.01 l.min ⁻¹	Air	3 minutes
Sandor and Stein (1993)	Aqueous solution containing sodium dodecyl sulphate	0.045 l.min ⁻¹	Nitrogen	Constant
Morey <i>et al.</i> (1999)	Aqueous solution containing sodium dodecyl benzene sulfonate	0.1 – 0.5 l.min ⁻¹	Nitrogen	To a set foam height
Desphande and Barigou (2000)	Aqueous solution containing sodium dodecyl benzene sulfonate	1 – 10 l.min ⁻¹	Air	Constant
Desphande and Barigou (2001)	Aqueous solution containing sodium dodecyl benzene sulfonate	n/a	Nitrogen	Constant
Dedhia <i>et al.</i> (2004)	Aqueous solution containing sodium lauryl sulphate	0.28 – 0.73 l.min ⁻¹	Air	To a set foam height
Nakajima and Mishima (2005)	Aqueous activated sludge-extracted solution containing albumin and EPS	5 l.min ⁻¹	Air	20 – 30 seconds

- n/a: Not applicable / information was not found

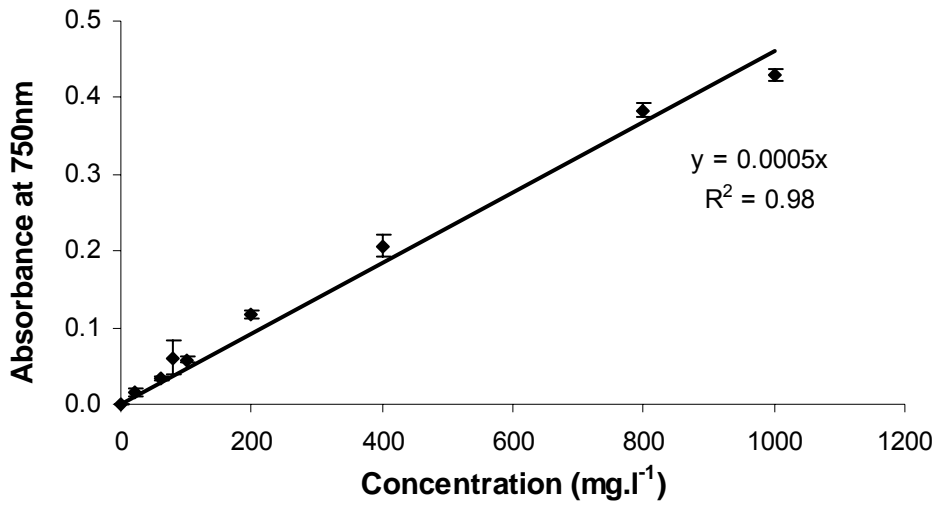


Figure 1: Calibration curve of proteins with BSA as protein standard]

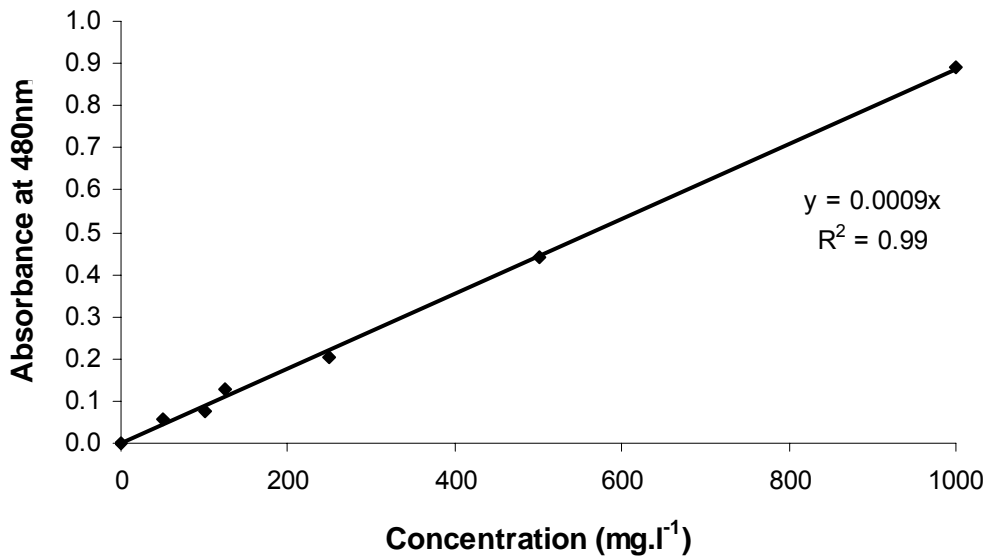


Figure 2: Calibration curve of carbohydrates with glucose as carbohydrate standard

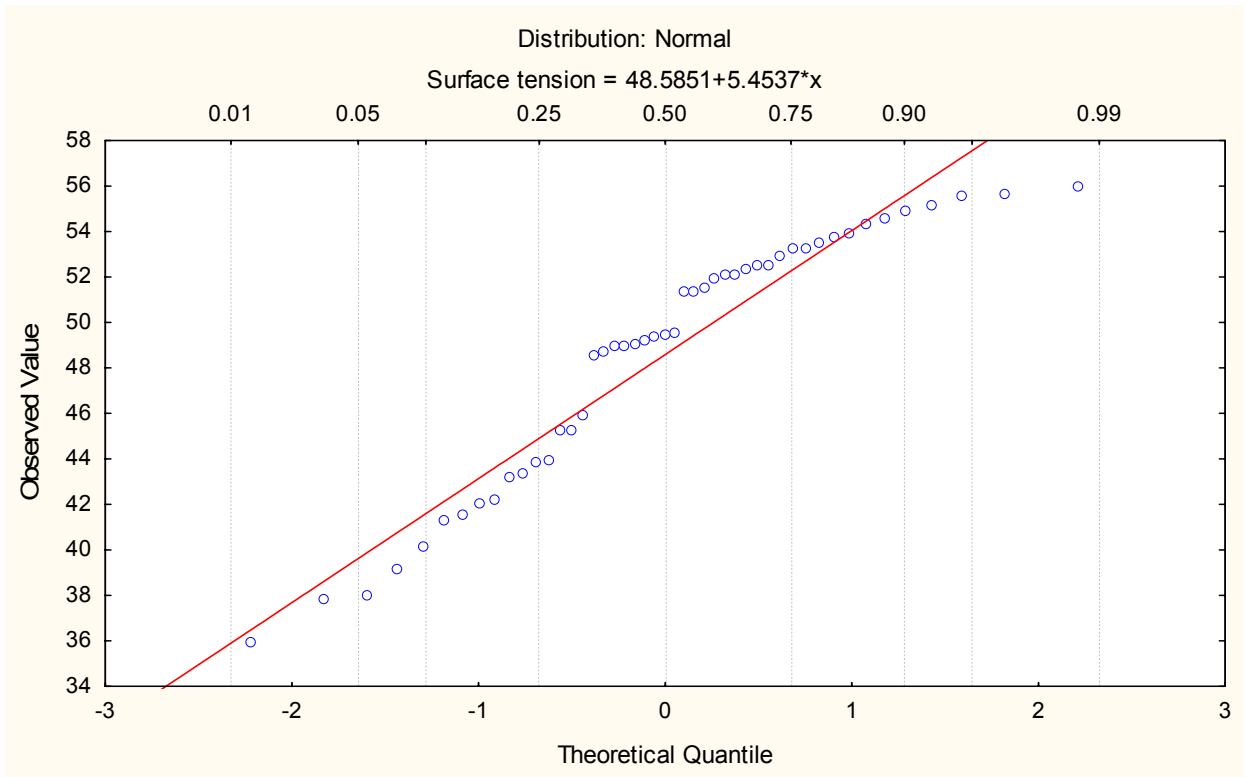


Figure 3: Normal distribution of surface tension ($\text{mN}\cdot\text{m}^{-1}$) in sludge centrate samples

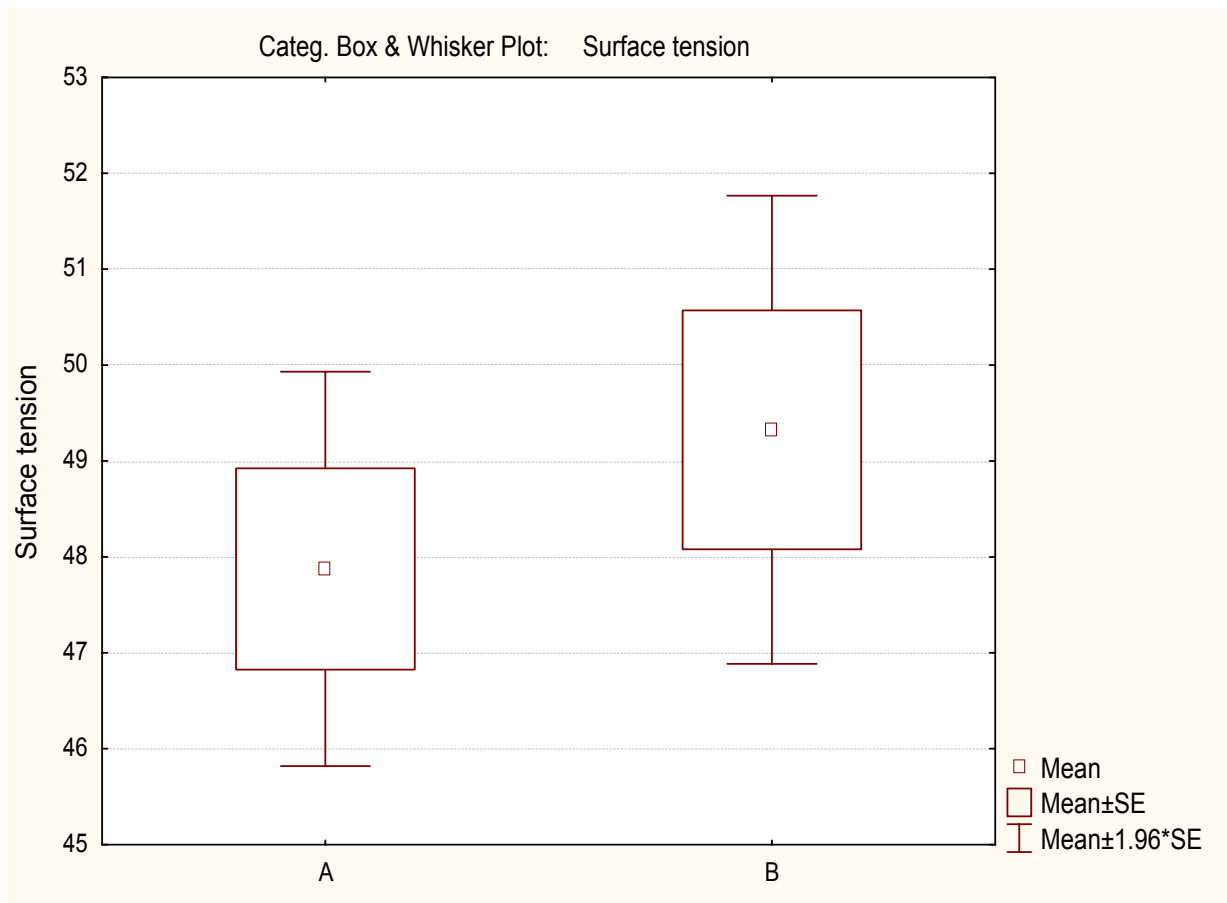


Figure 4: Statistica output for surface tension values after centrifugation at 4500g for 10 minutes (A) and at 8000g for 20 minutes (B)

Appendix B

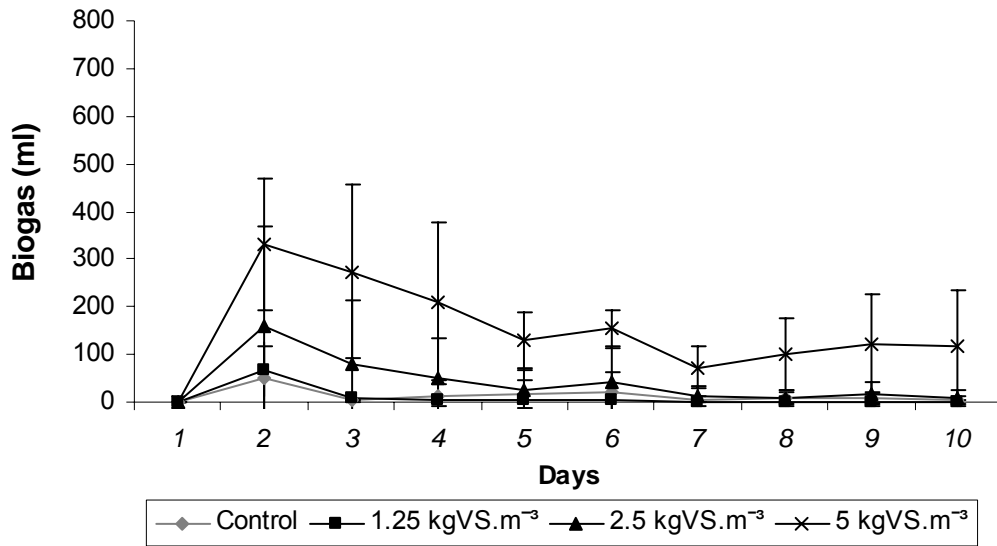


Figure 1: Daily biogas production (ml ±SD) of Experiment 1

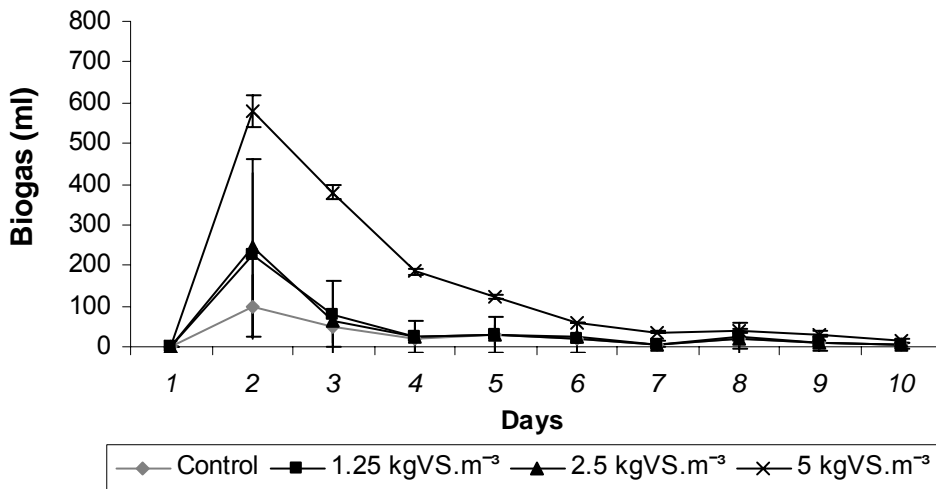


Figure 2: Daily biogas production (ml ±SD) of Experiment 2

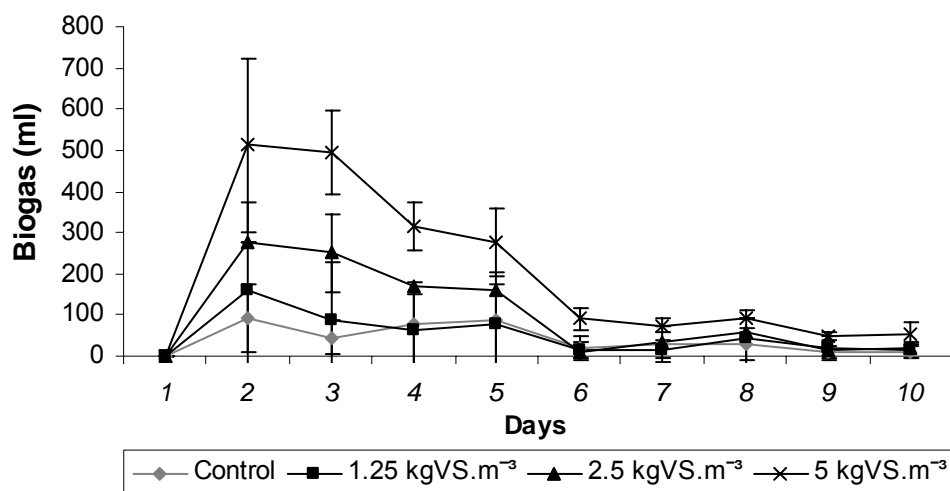


Figure 3: Daily biogas production (ml ±SD) of Experiment 3

Organic loading	Experiment 1	Experiment 2	Experiment 3
Control	36	20 – 39	20 – 52
1.25 kg VS.m ⁻³	43 – 50	40 – 51	35 – 56
2.5 kg VS.m ⁻³	38 – 45	43 – 46	52 – 58
5 kg VS.m ⁻³	52 – 63	59 – 67	53 – 66

	Experiment 1		Experiment 2		Experiment 3	
	%TS red	%VS red	%TS red	%VS red	%TS red	%VS red
control	25	24	26	0	0	4
1.25 kg VS/m ³	9	10	10	12	4	8
2.5 kg VS/m ³	14	19	0	20	24	24
5 kg VS/m ³	16	20	28	37	2	10

	Experiment 1		Experiment 2		Experiment 3	
	Day 3	Day 10	Day 3	Day 10	Day 3	Day 10
control	0	11	0	0	-**	0
1.25 kg VS/m ³	0	0	0	0	-**	28
2.5 kg VS/m ³	0	0	0	0	57	0
5 kg VS/m ³	0	0	0	0	266	17

*The data presented in the table derived from HPLC analysis

**Data were not obtained due to technical problems with the HPLC

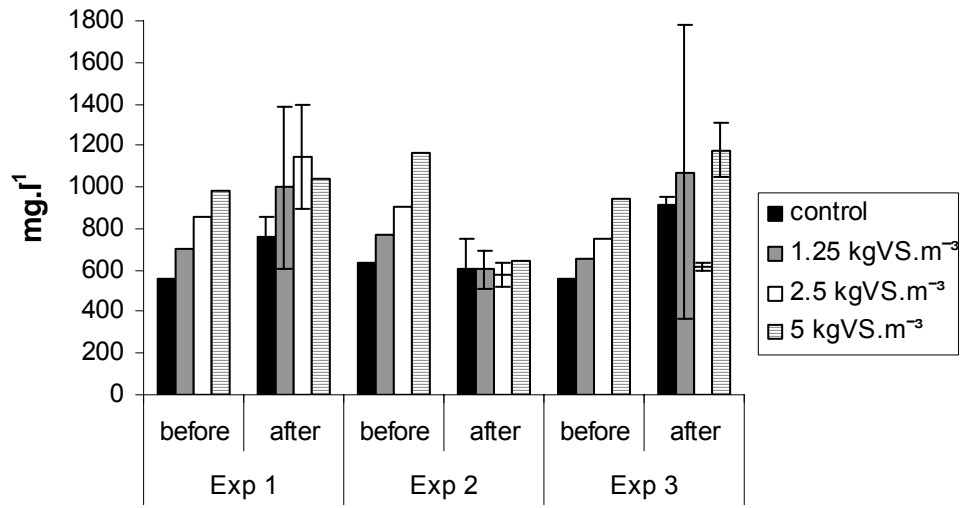


Figure 4: Soluble COD (mg.l⁻¹ ±SD) in sludge samples of organic loading experiments before and after batch digestion

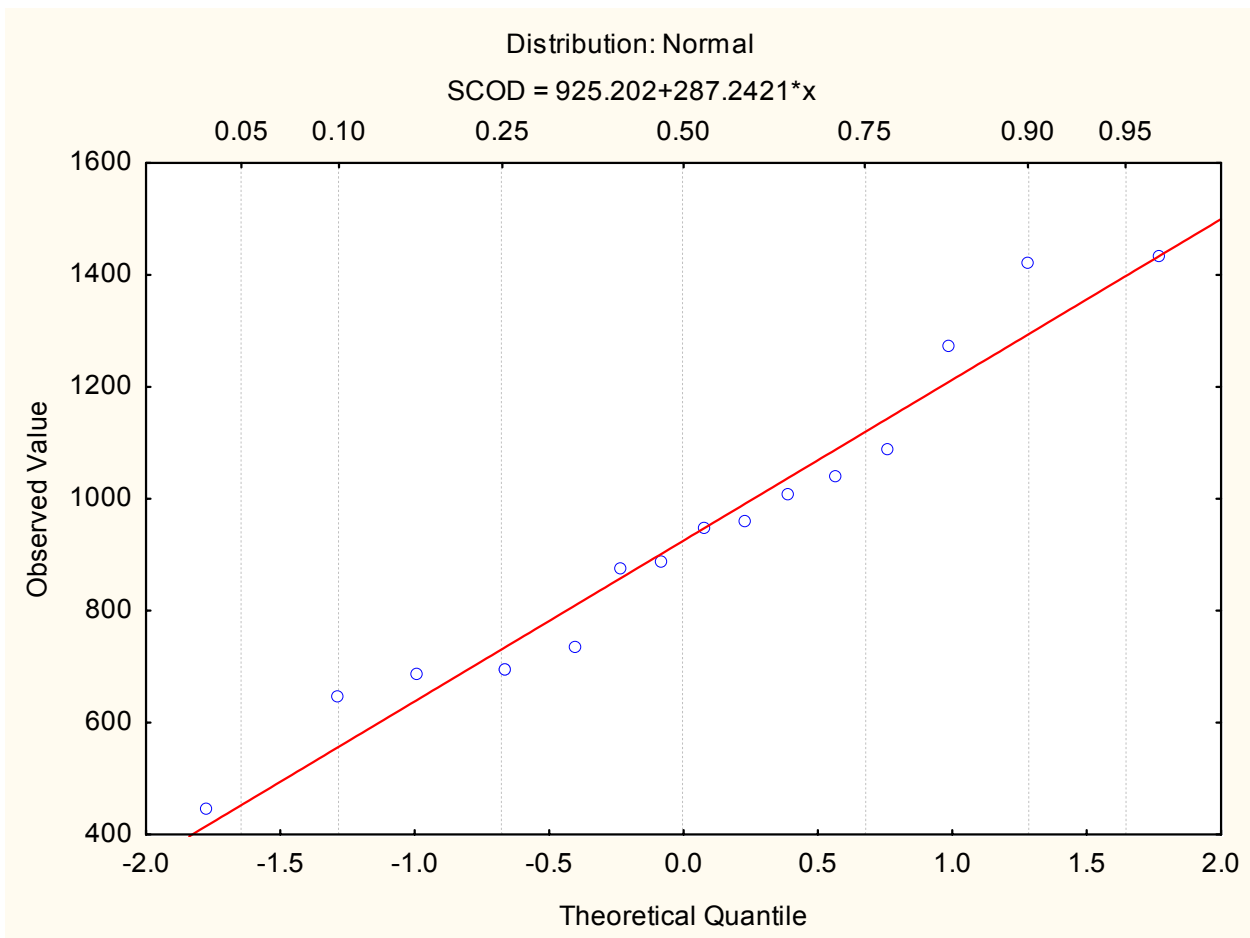


Figure 5: Normal distribution of SCOD concentrations in digested sludge obtained for the 1.25 and 5 kg VS.m⁻³ organic loadings from all three batch digestion experiments

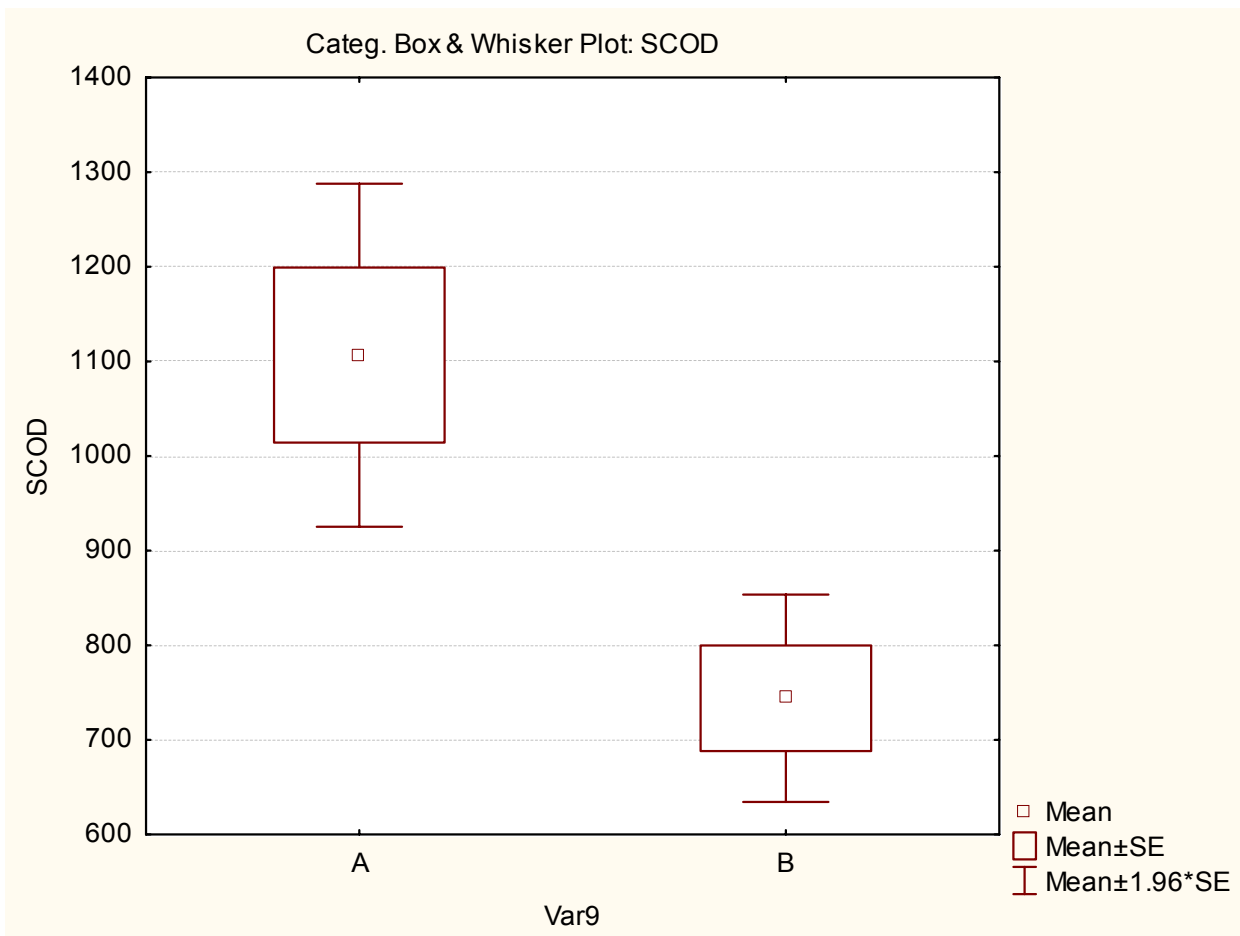


Figure 6: Statistica output for SCOD concentrations in digested sludge obtained for the 1.25 (A) and 5 kg VS.m⁻³ organic loadings (B) from all three batch digestion experiments

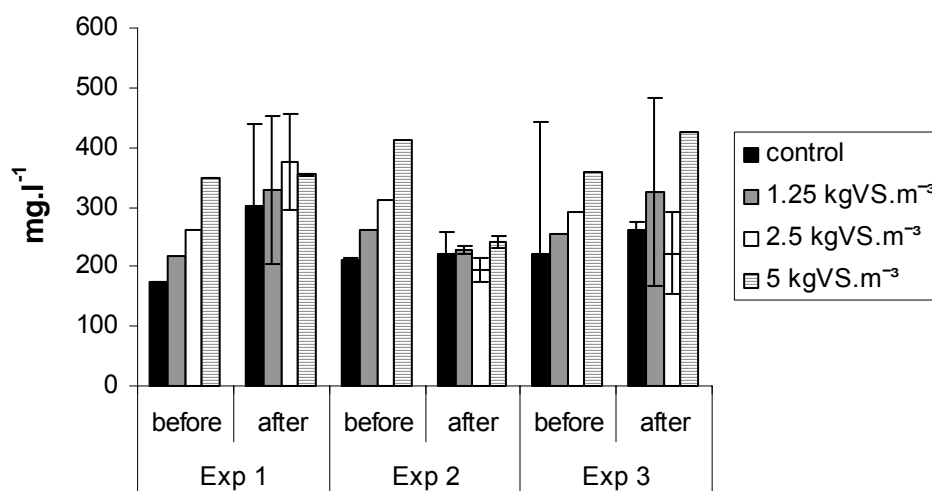


Figure 7: DOC (mg.l⁻¹ ±SD) in sludge samples of organic loading experiments before and after batch digestion

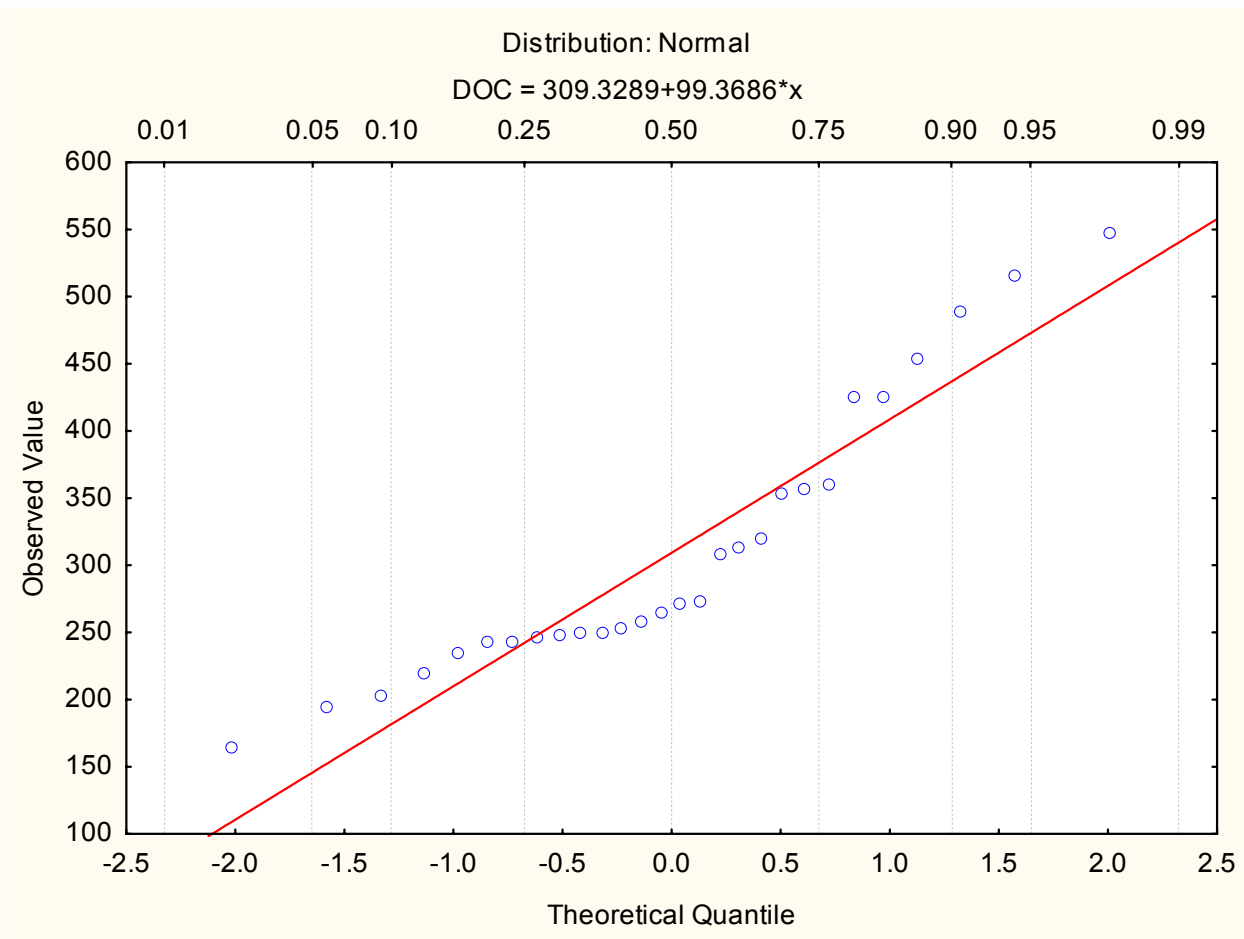


Figure 8: Normal distribution of DOC concentrations in digested sludge obtained for the 1.25 and 5 kg VS.m⁻³ organic loadings from all three batch digestion experiments

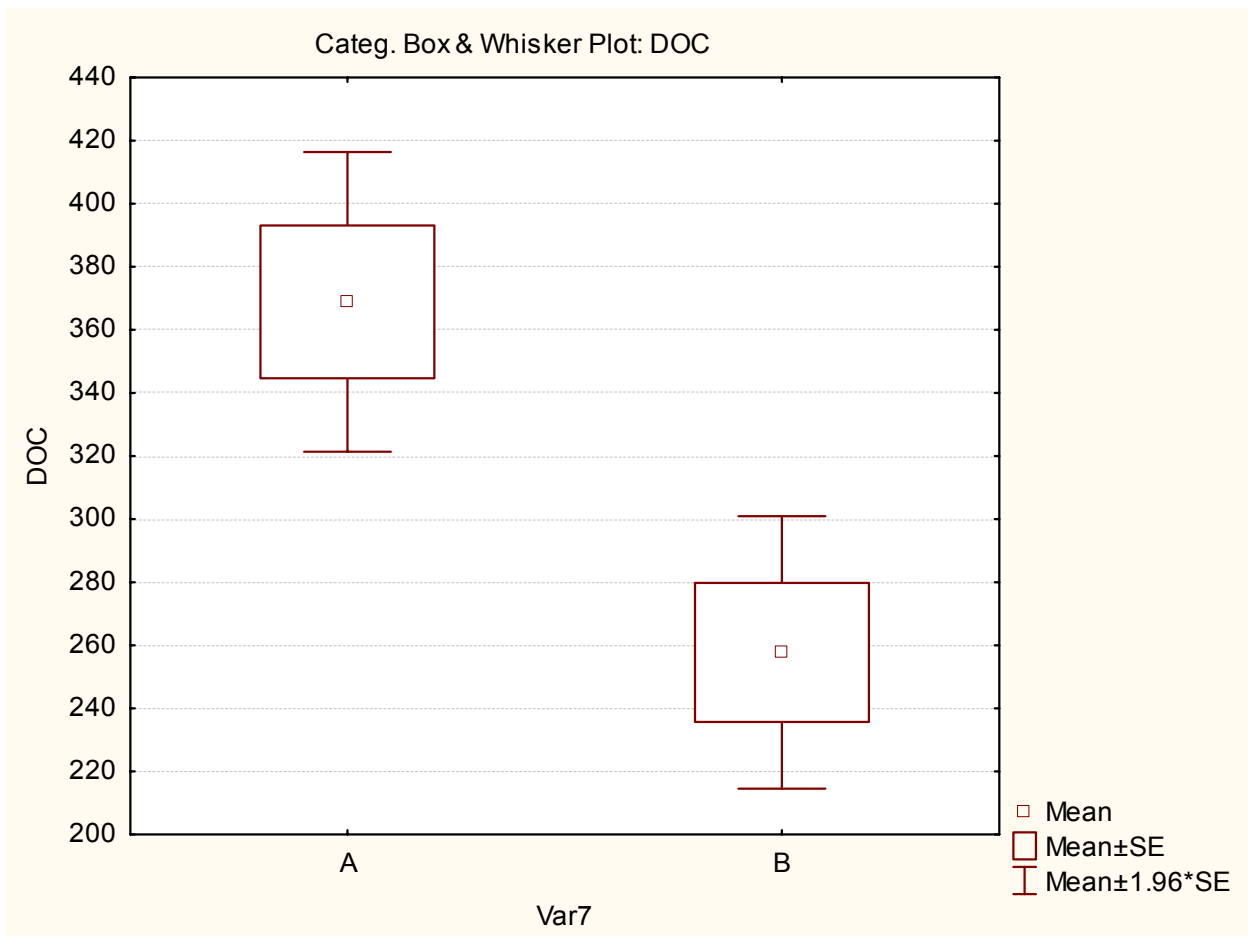


Figure 9: Statistica output for DOC concentrations in digested sludge obtained for the 1.25 (A) and 5 kg VS.m⁻³ organic loadings (B) from all three batch digestion experiments

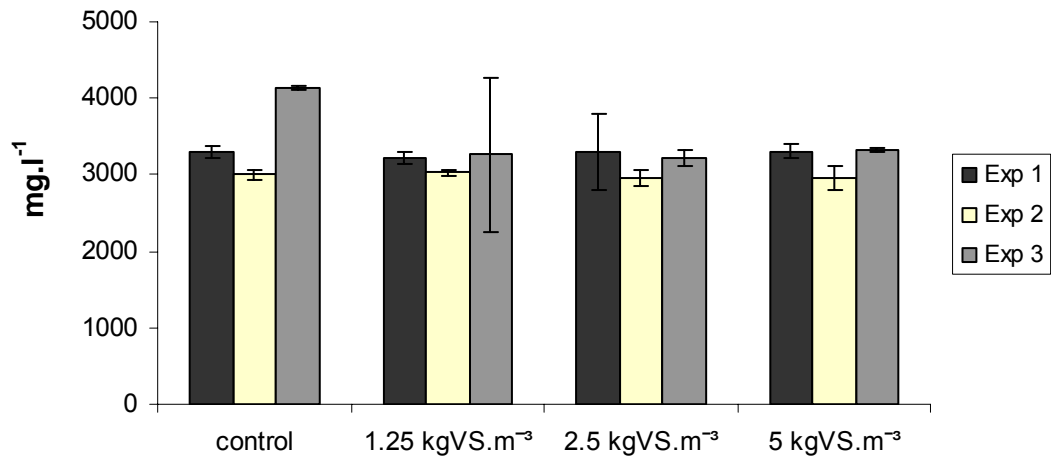


Figure 10: Alkalinity (mg.l⁻¹ ±SD) in sludge samples of organic loading experiments after batch digestion

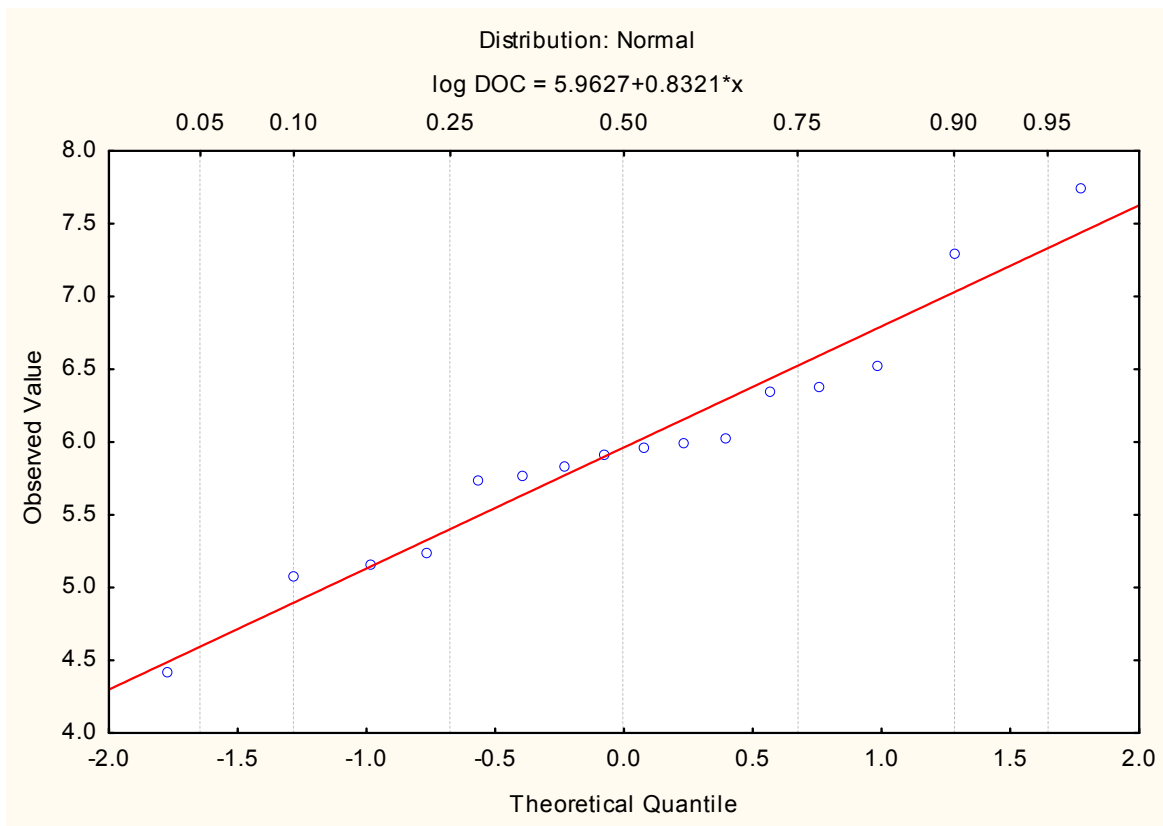


Figure 11: Normal distribution of log DOC concentrations obtained for sites 1 to 15

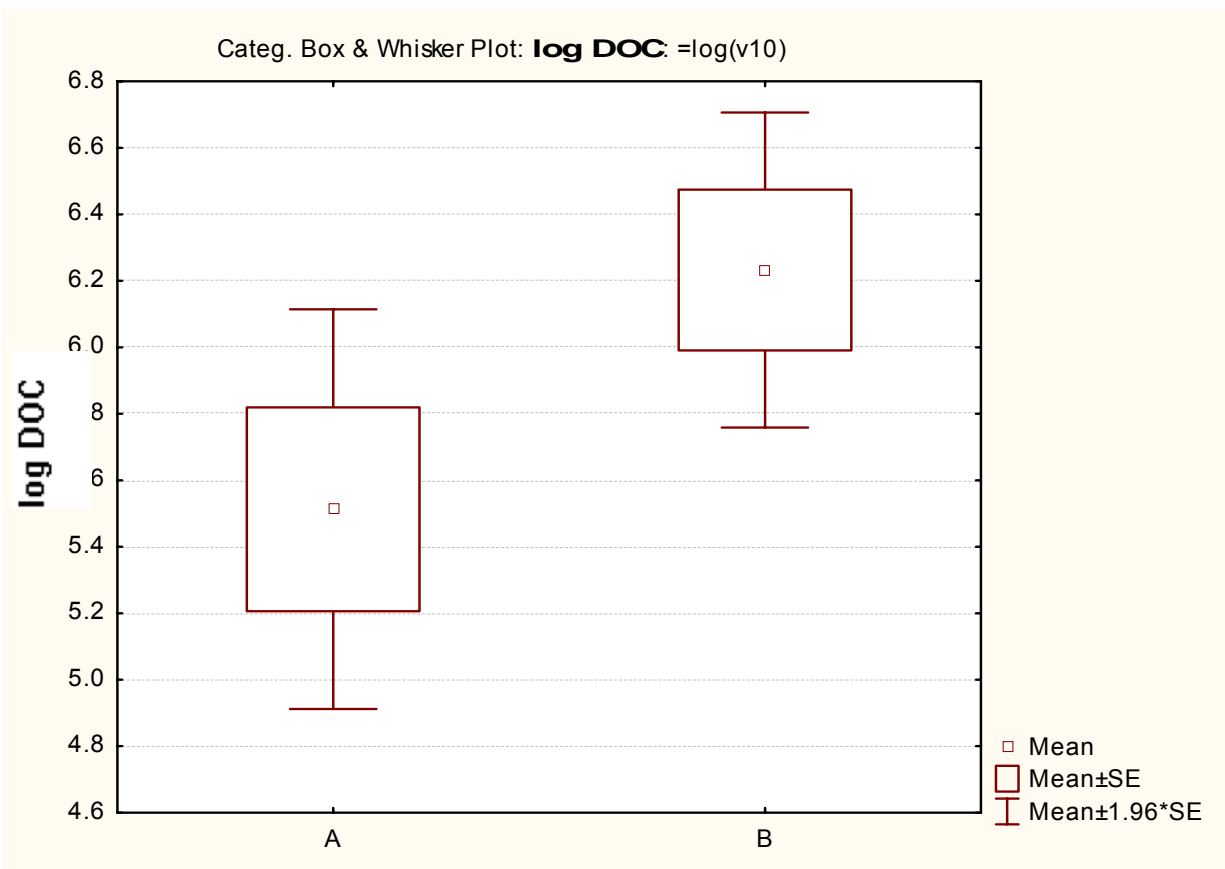


Figure 12 Statistica output for log DOC values from non-foaming digesters (A) and foaming digesters (B)

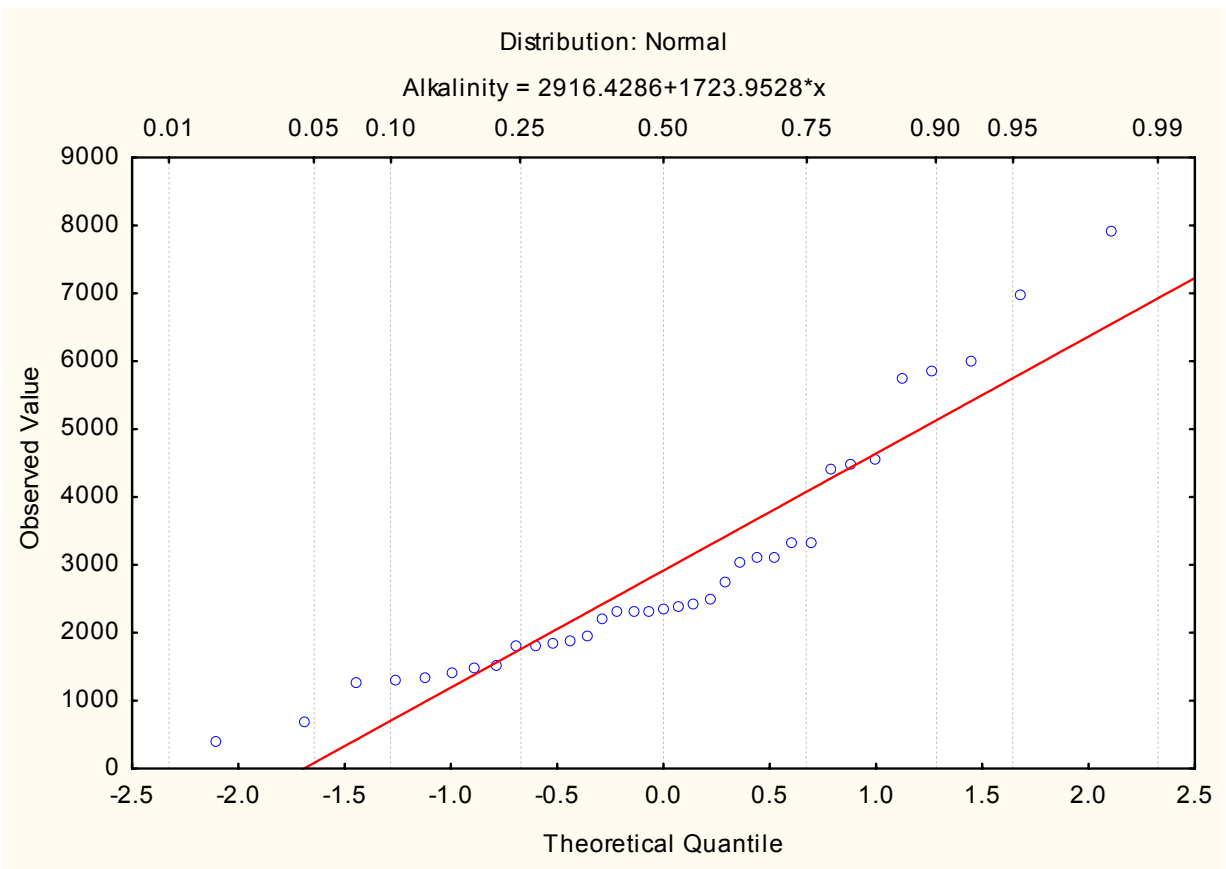


Figure 13: Normal distribution of alkalinity concentrations obtained for sites 1 to 15

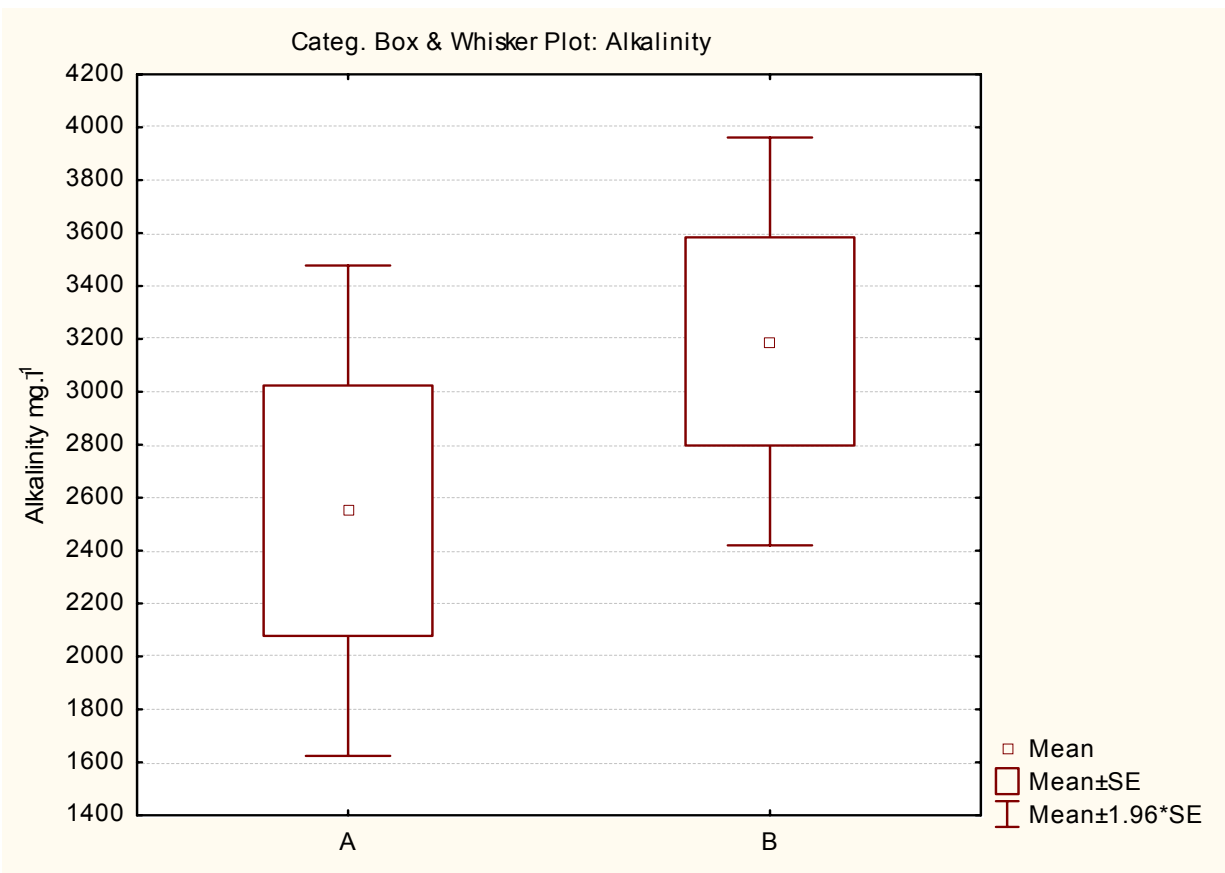


Figure 14: Statistica output for alkalinity values from non-foaming digesters (A) and foaming digesters (B)

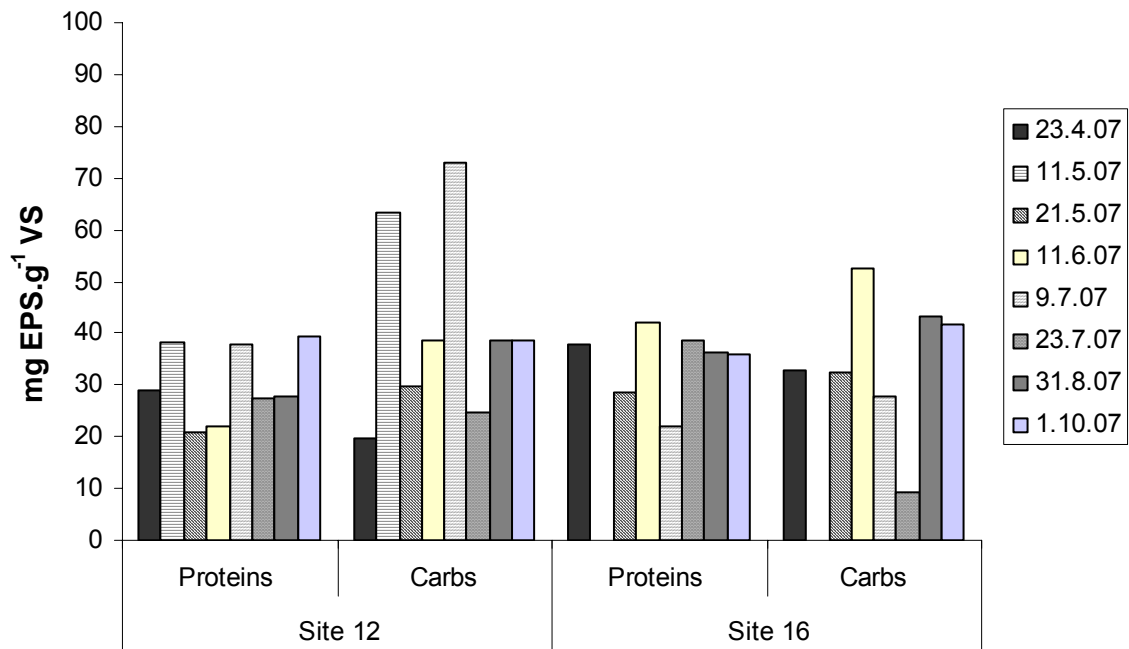


Figure 15: EPS (proteins and carbohydrates) as mg per gram volatile solids in digester inlet sludge from Sites 12 and 16

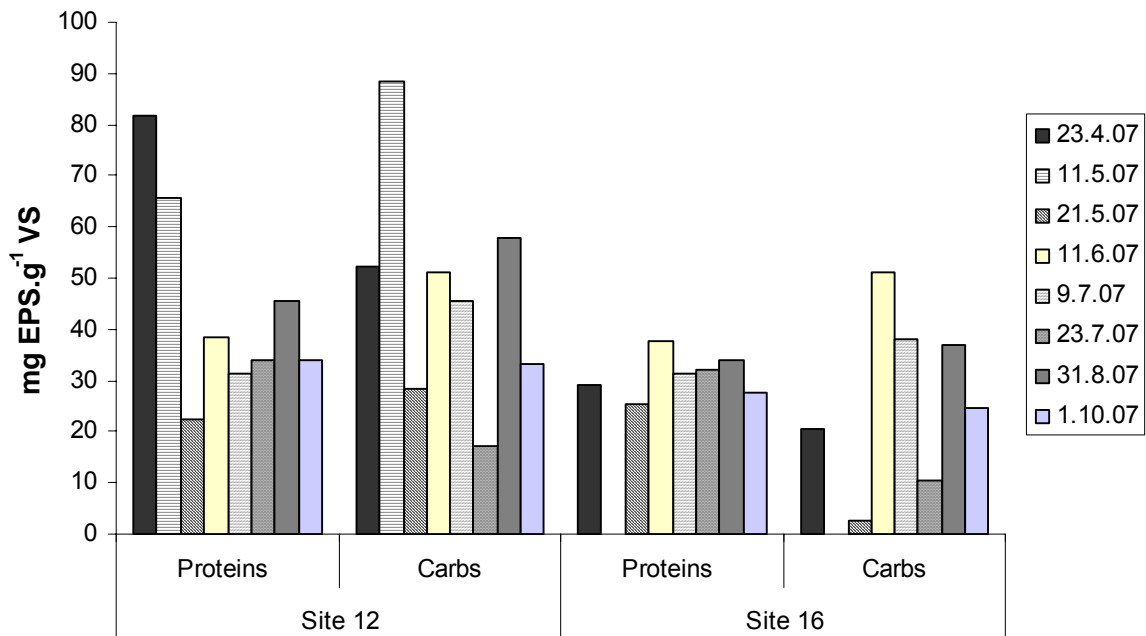


Figure 16: EPS (proteins and carbohydrates) as mg per gram volatile solids in digested sludge from Sites 12 and 16

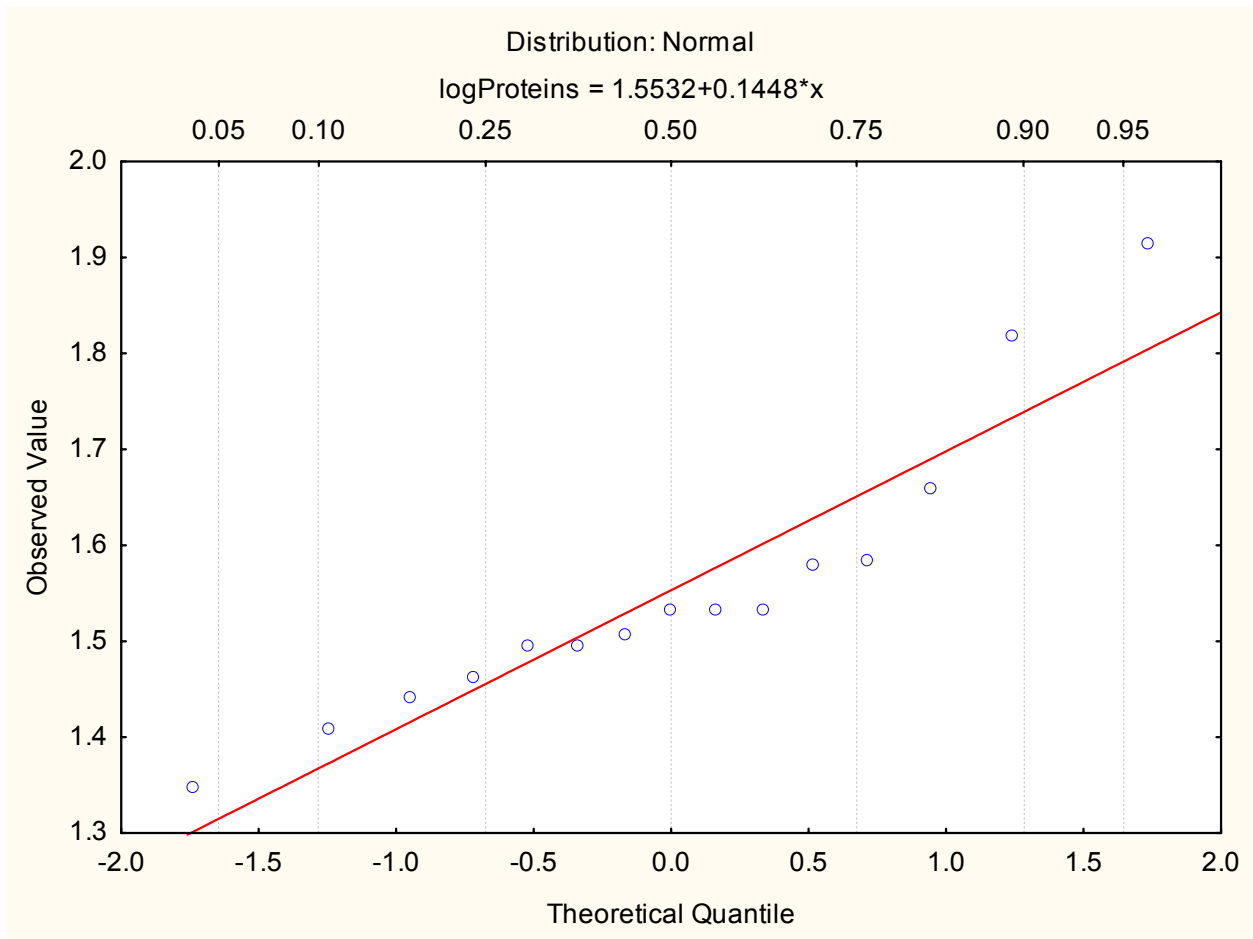


Figure 17: Normal distribution of log proteins as EPS in digested sludge obtained for sites 12 (A) and 16 (B)

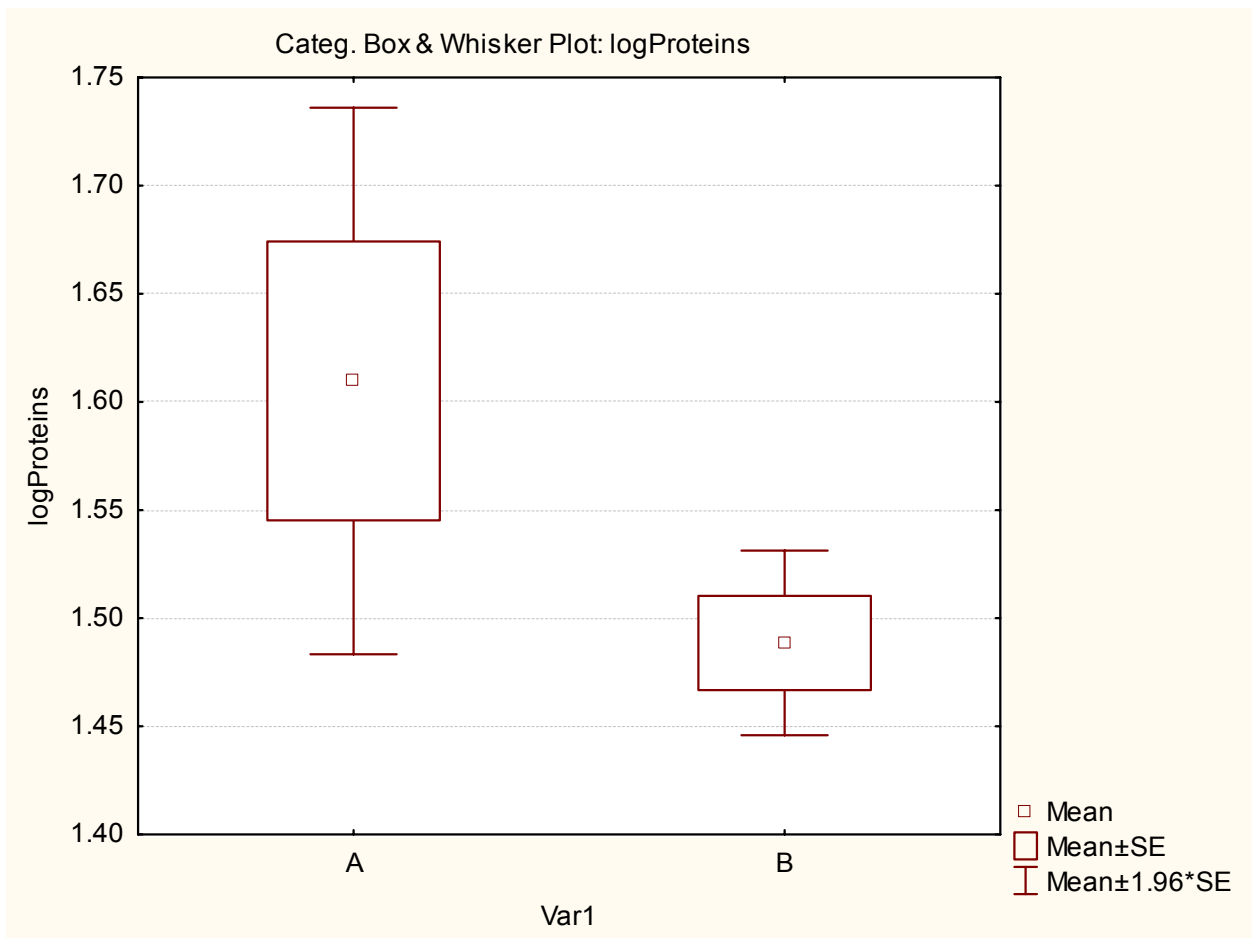


Figure 18: Statistica output for log proteins as EPS in digested sludge obtained for sites 12 (A) and 16 (B)

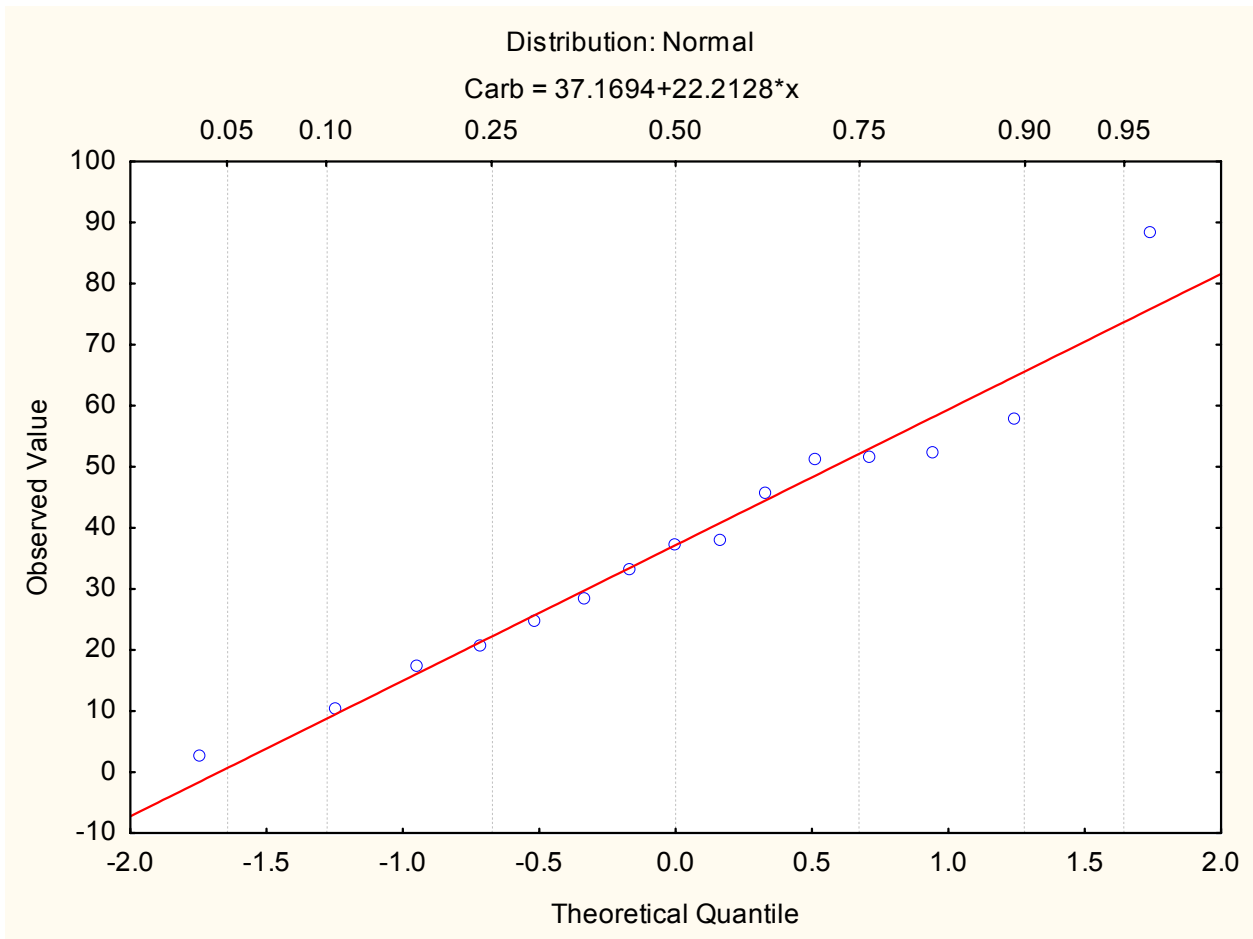


Figure 19: Normal distribution of carbohydrates as EPS in digested sludge obtained for sites 12 (A) and 16 (B)

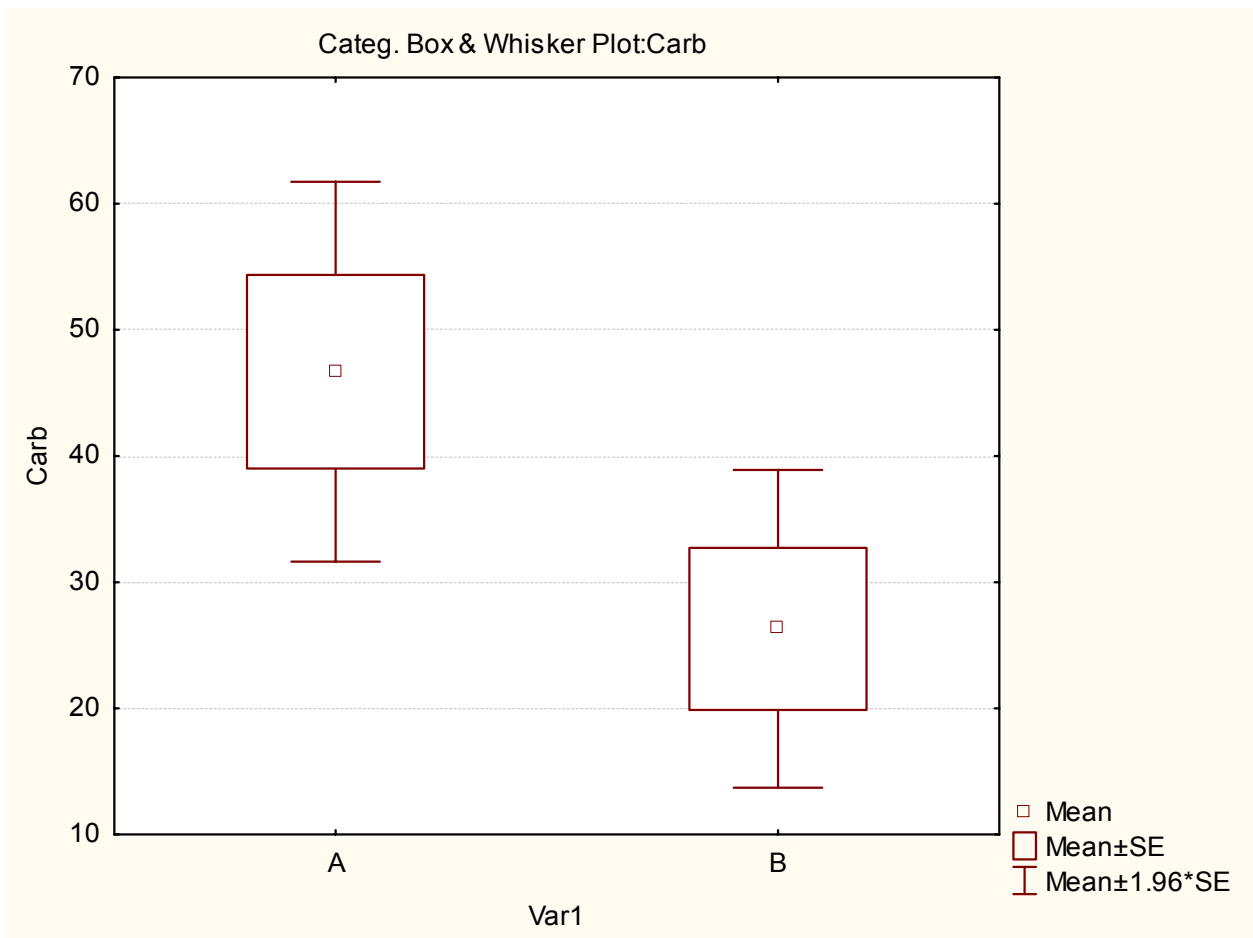


Figure 20: Statistica output for carbohydrates as EPS in digested sludge obtained for sites 12 (A) and 16 (B)

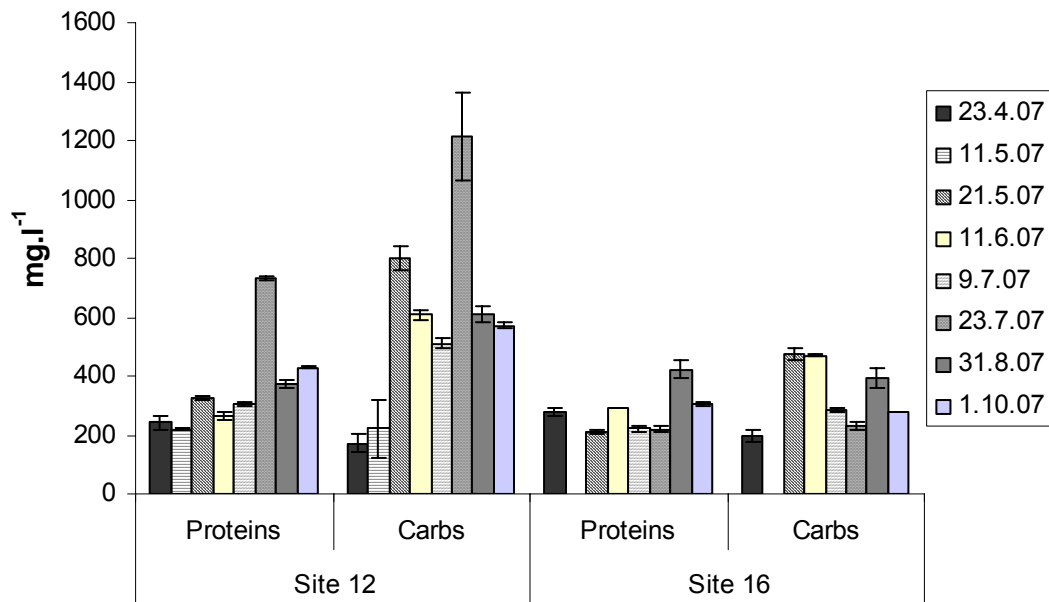


Figure 21: SMPs (mg.l⁻¹ ±SD) as proteins and carbohydrates in digester inlet sludge from Sites 12 and 16

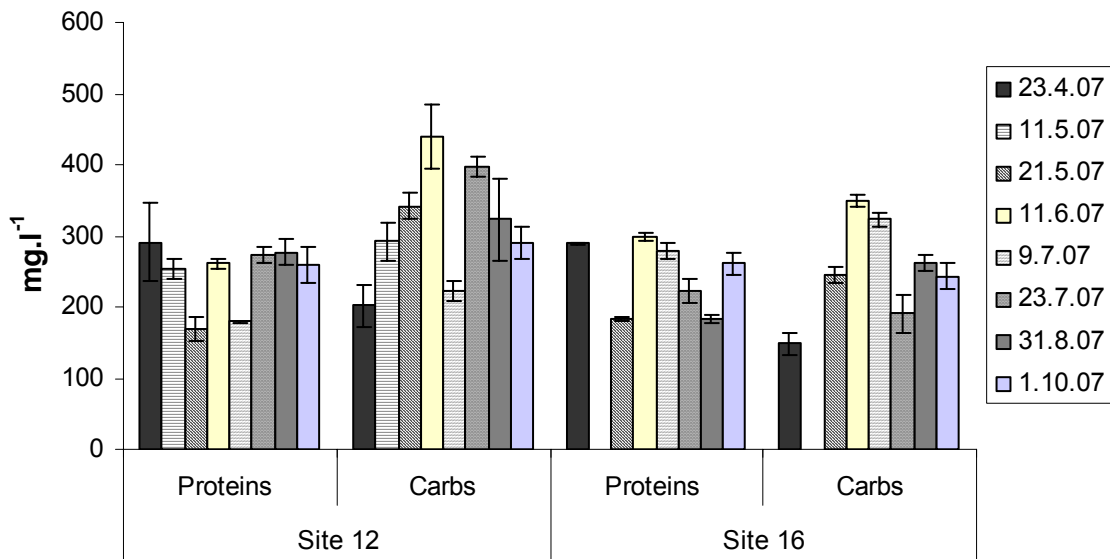


Figure 22: SMPs (mg.l⁻¹ ±SD) as proteins and carbohydrates in digested sludge from Sites 12 and 16

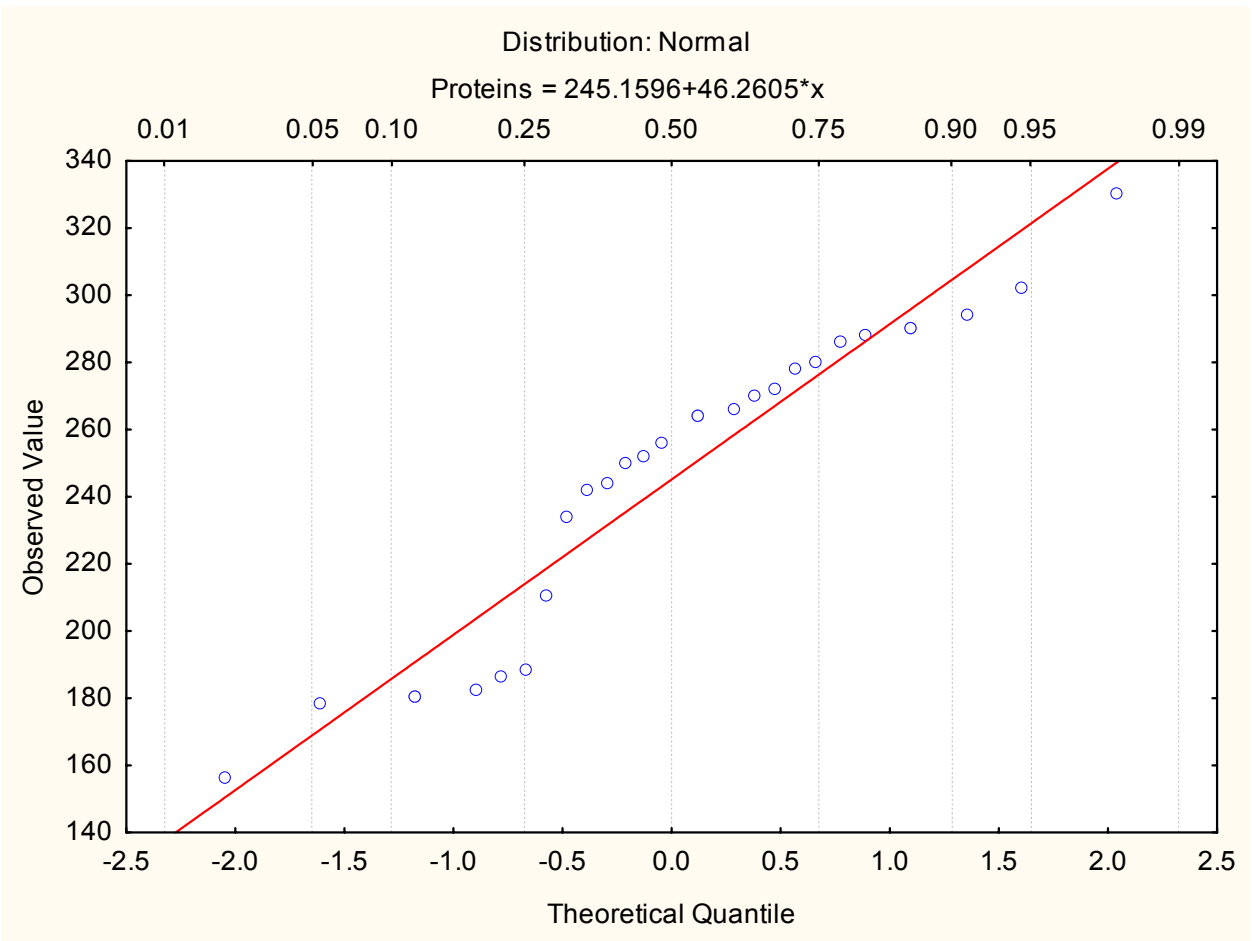


Figure 23: Normal distribution of proteins as SMPs in digested sludge obtained for sites 12 (A) and 16 (B)

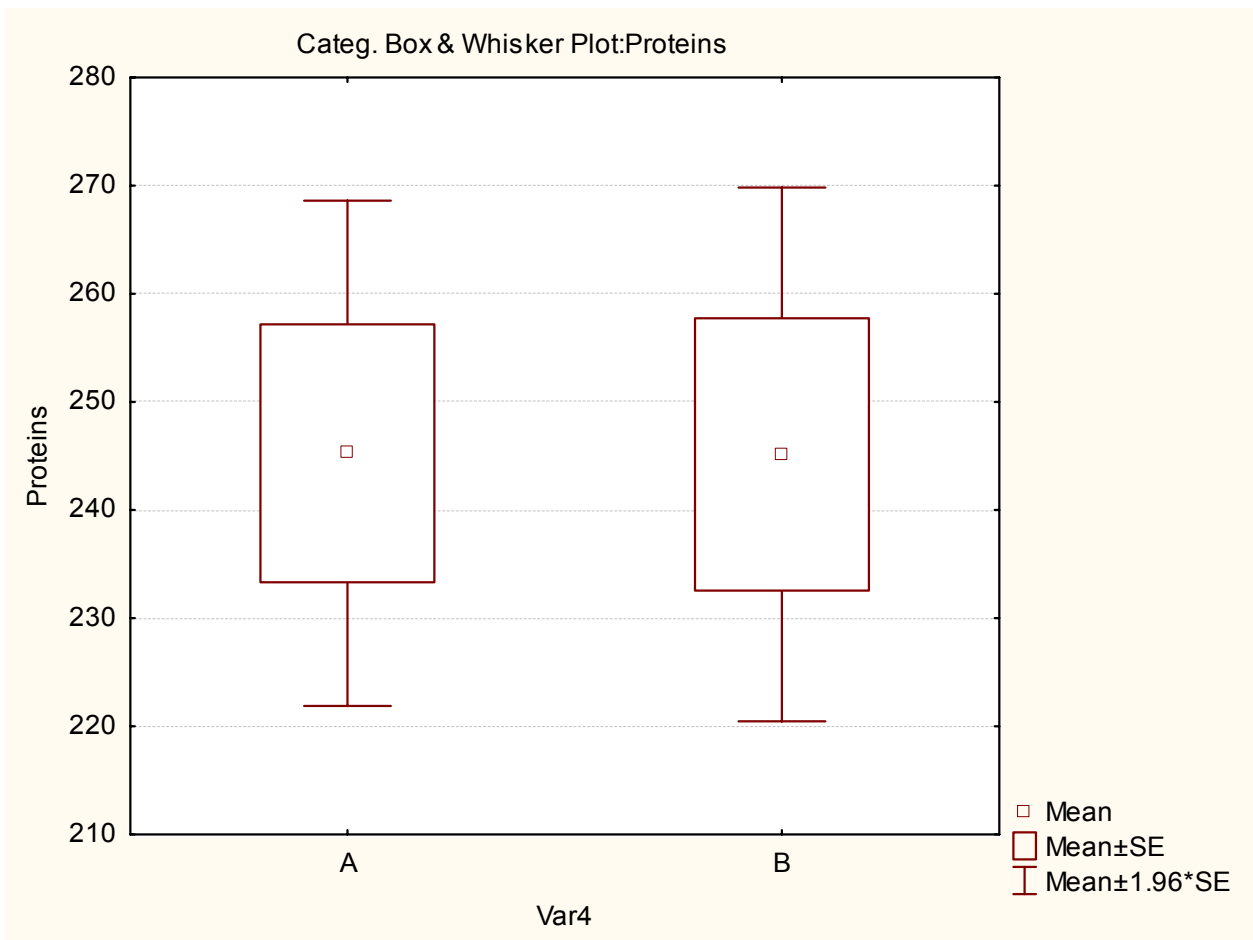


Figure 24: Statistica output for proteins as SMPs in digested sludge obtained for sites 12 (A) and 16 (B)

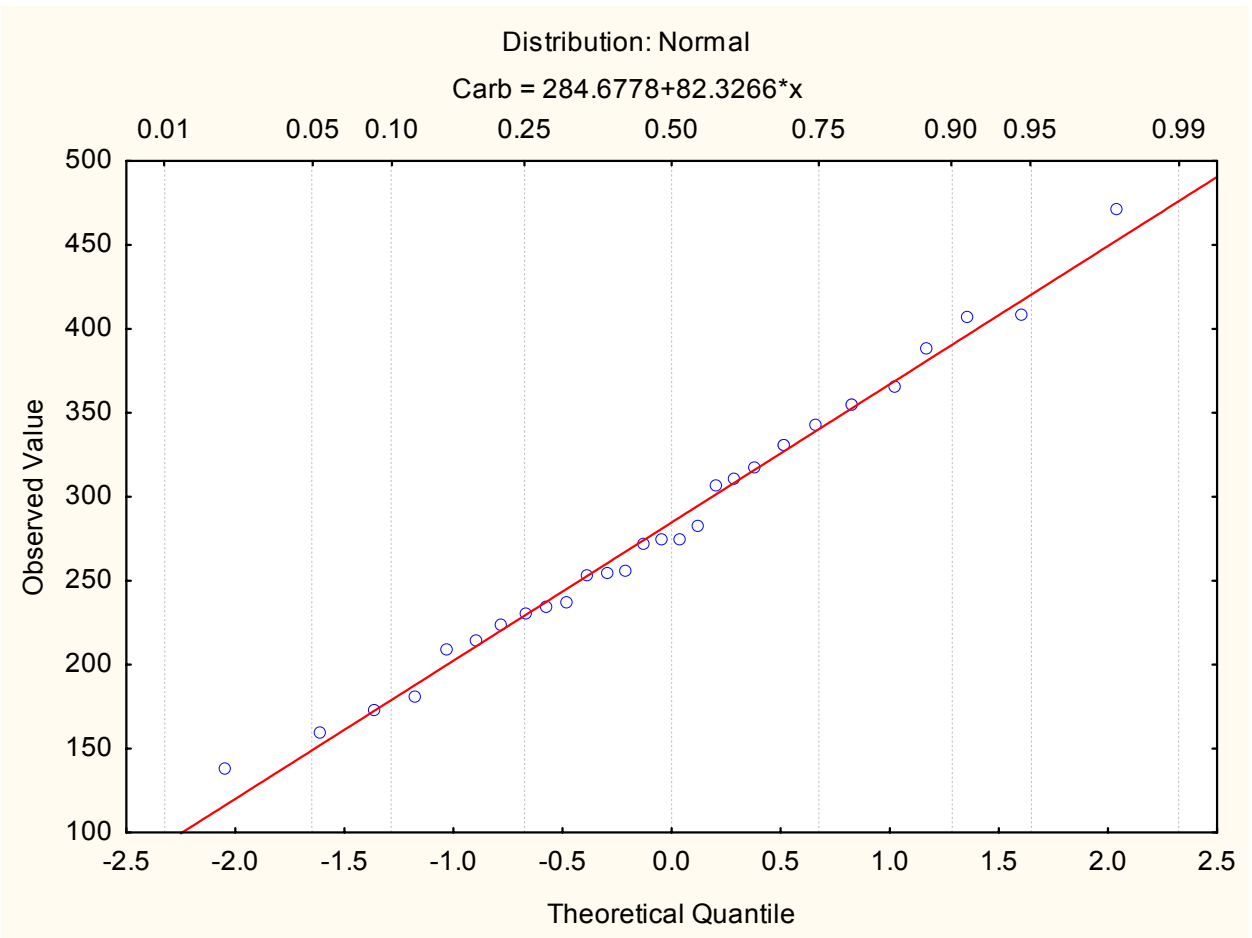


Figure 25: Normal distribution of carbohydrates as SMPs in digested sludge obtained for sites 12 (A) and 16 (B)

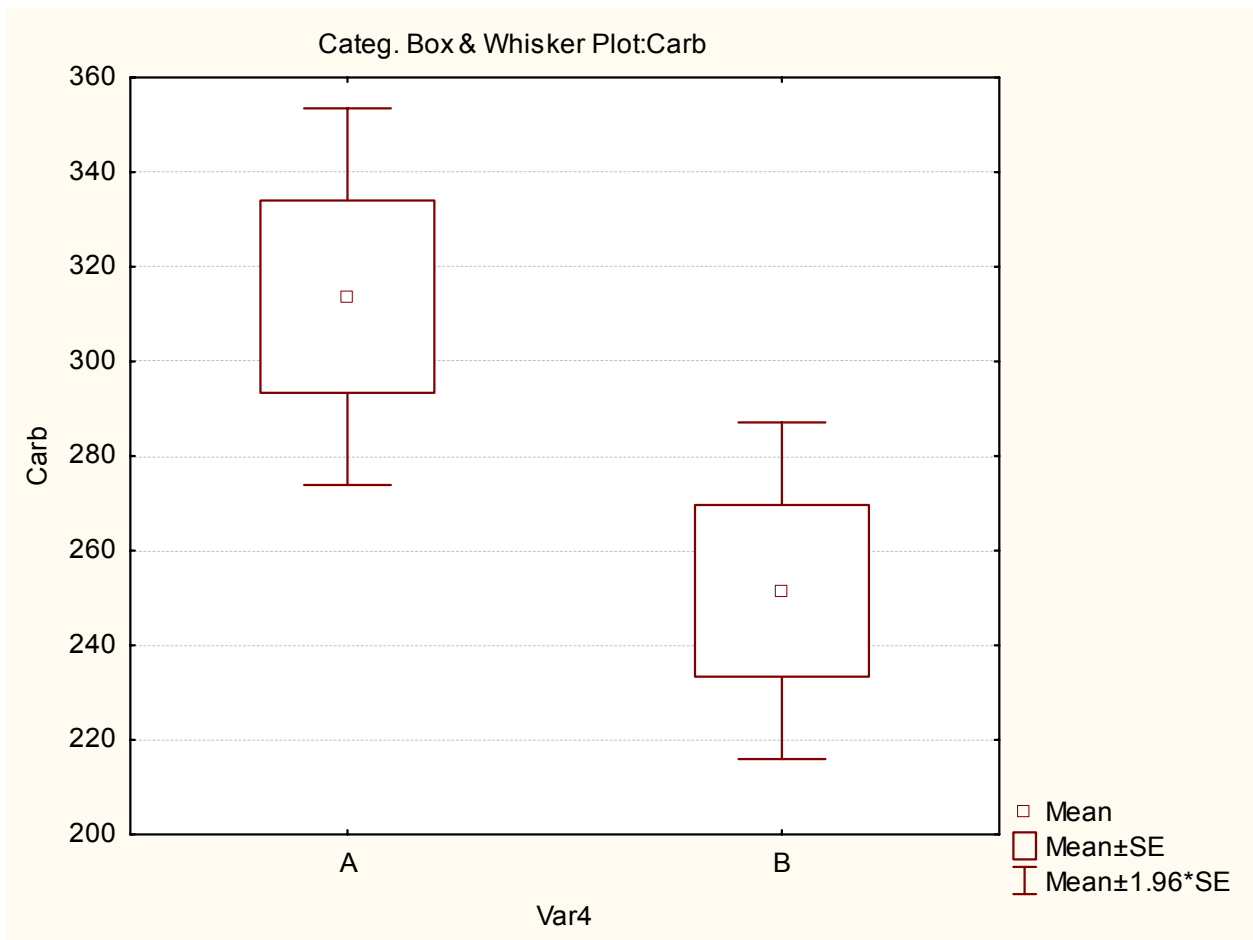


Figure 26: Statistica output for carbohydrates as SMPs in digested sludge obtained for sites 12 (A) and 16 (B)

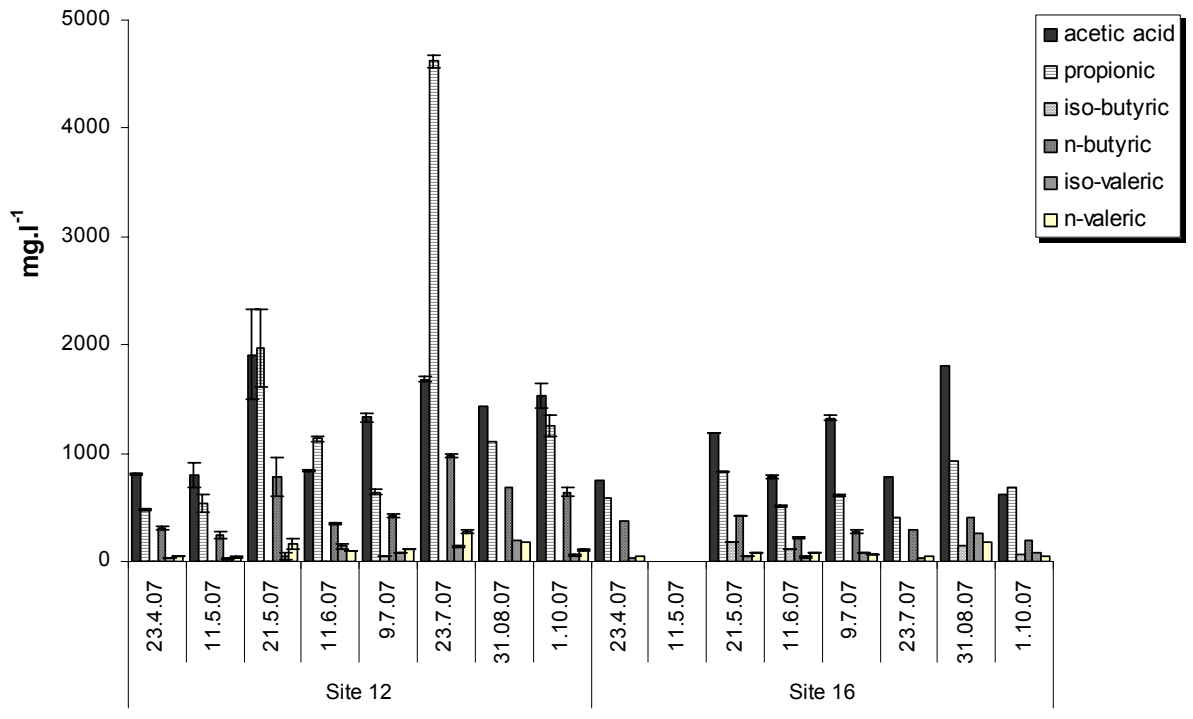


Figure 27: Individual VFAs (mg.l⁻¹) in digester feed sludge from Sites 12 and 16

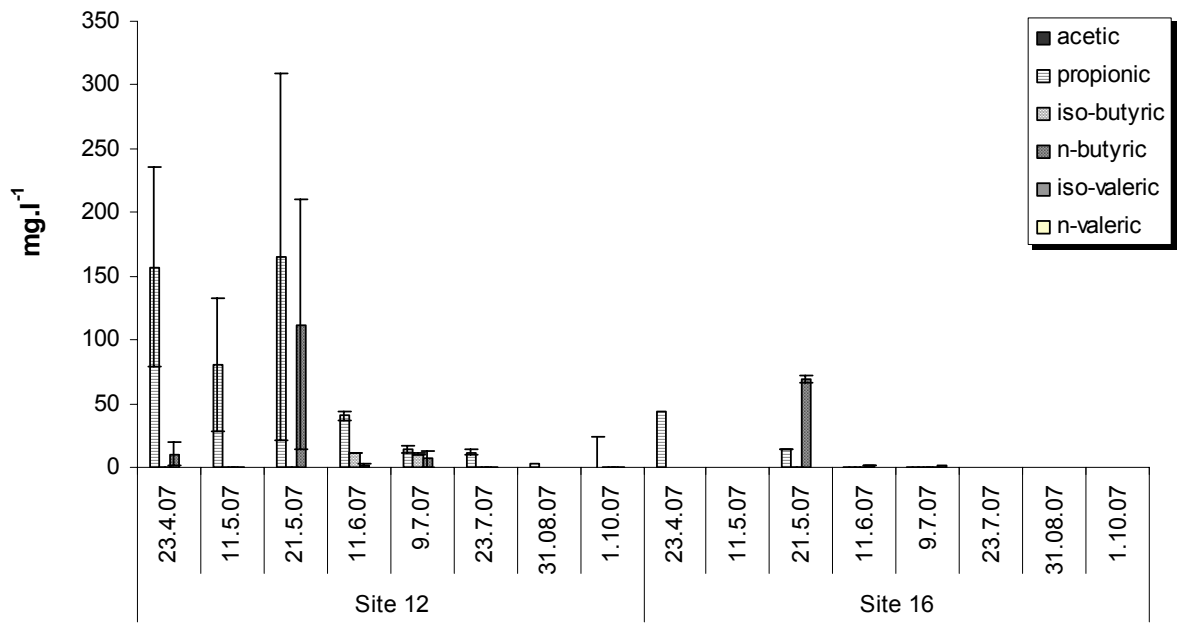


Figure 28: Individual VFAs (mg.l⁻¹) in digested sludge from Sites 12 and 16

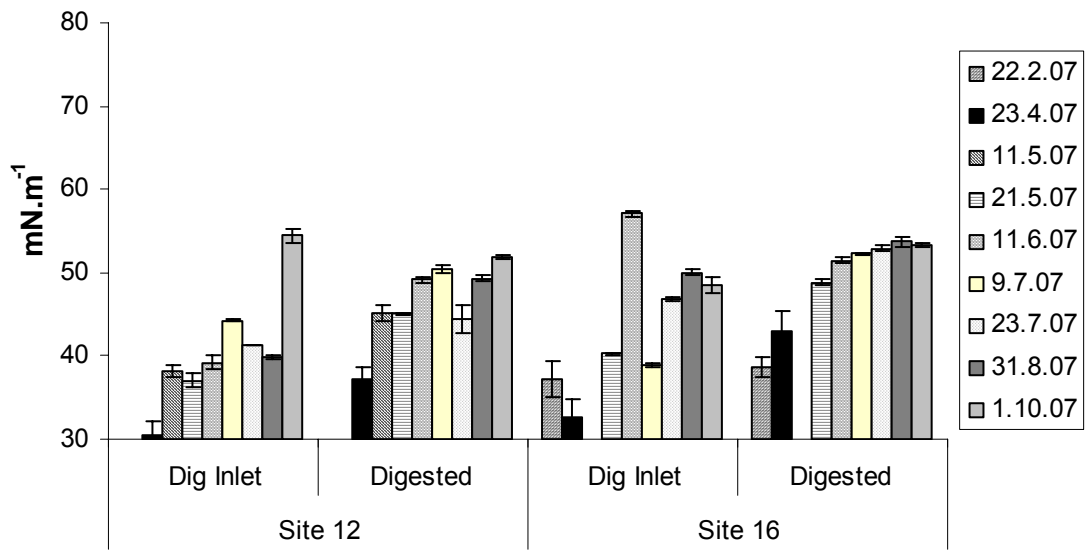


Figure 29: Surface tension (mN.m⁻¹) in digester inlet and digested sludge from Sites 12 and 16

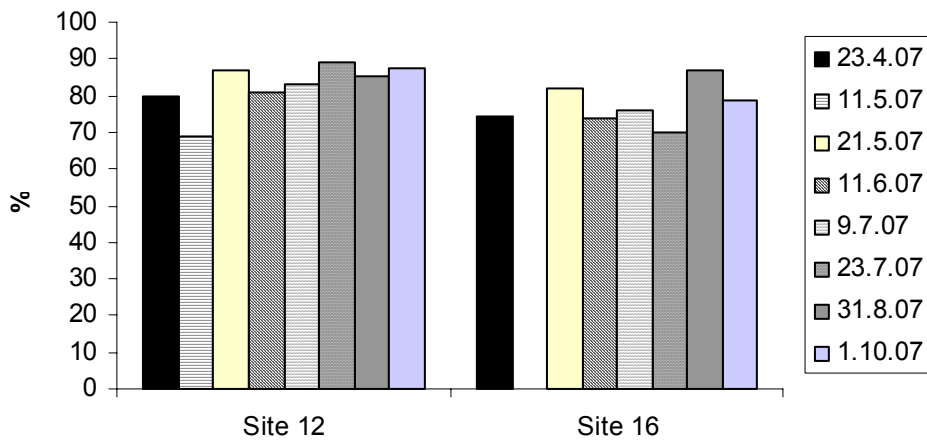


Figure 30: SCOD removal (%) in digesters of Sites 12 and 16

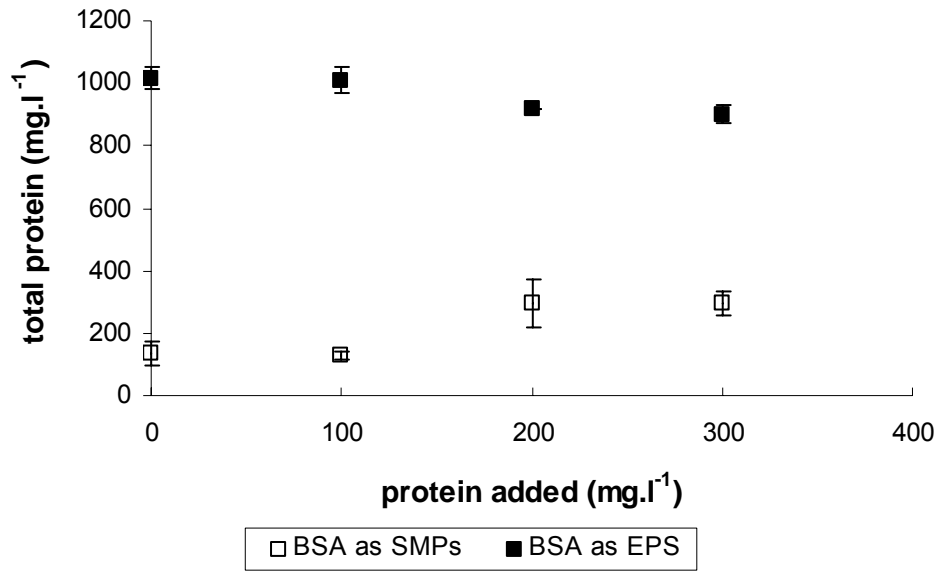


Figure 31: Protein concentrations in sludge as SMPs and EPS (mg.l⁻¹ ±SD) after addition of BSA

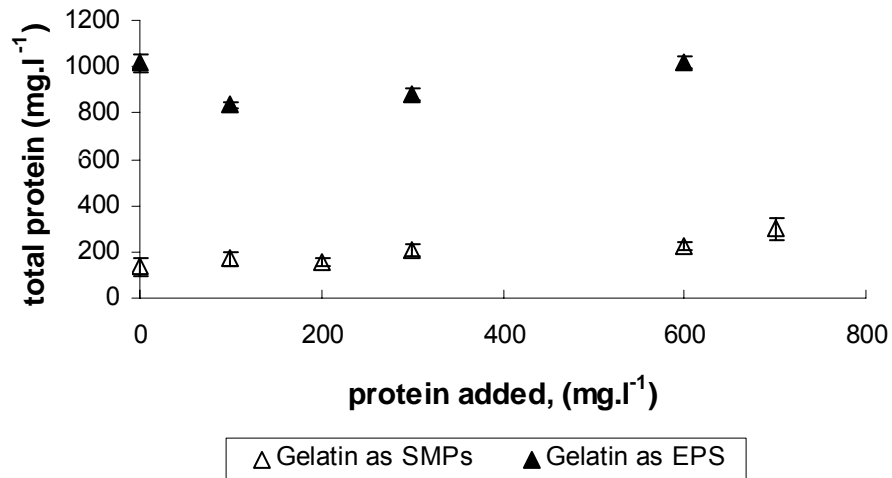


Figure 32: Protein concentrations in sludge as SMPs and EPS (mg.l⁻¹ ±SD) after addition of gelatin

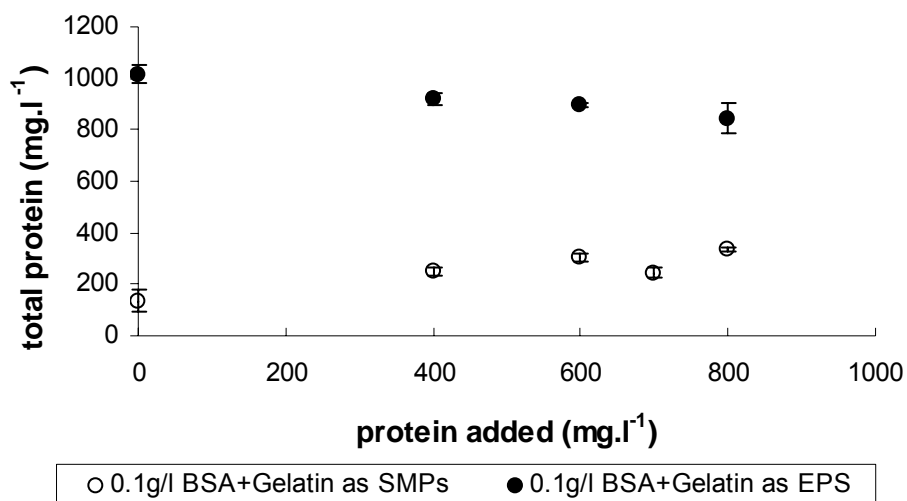


Figure 33: Protein concentrations in sludge as SMPs and EPS ($\text{mg.l}^{-1} \pm \text{SD}$) after addition of 0.1g.l^{-1} BSA and varying concentrations of gelatin

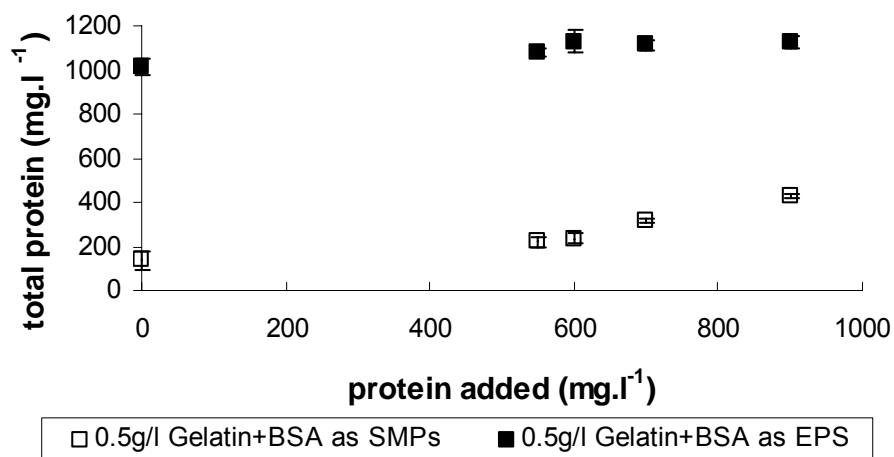


Figure 34: Protein concentrations in sludge as SMPs and EPS ($\text{mg.l}^{-1} \pm \text{SD}$) after addition of 0.5g.l^{-1} gelatin and varying concentrations of BSA

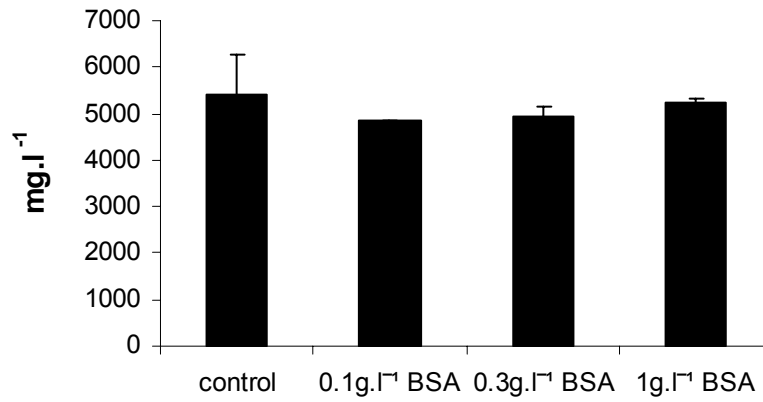


Figure 35: Alkalinity (mg.l⁻¹ ±SD) in sludge samples on Day 10 of BSA experiment

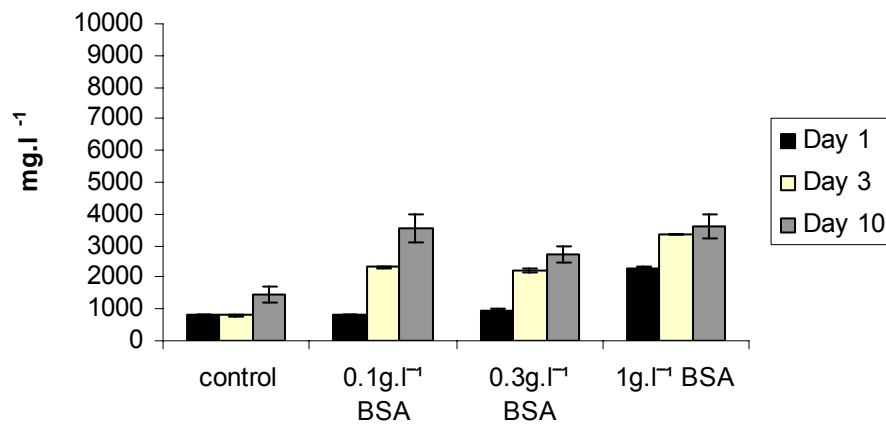


Figure 36: SCOD (mg.l⁻¹ ±SD) in sludge samples during batch digestion, experiment on BSA

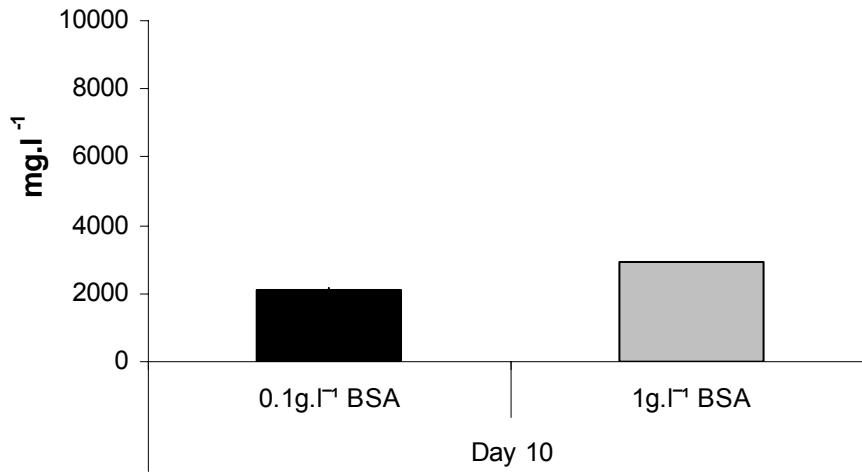


Figure 37: SCOD (mg.l⁻¹ ±SD) in foam samples on Day 10 of batch digestion, experiment on BSA

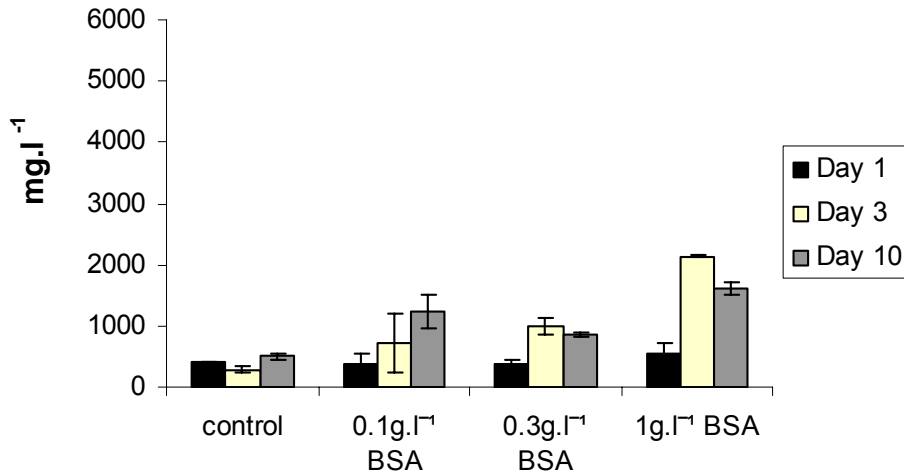


Figure 38: DOC (mg.l⁻¹ ±SD) in sludge samples during batch digestion, experiment on BSA

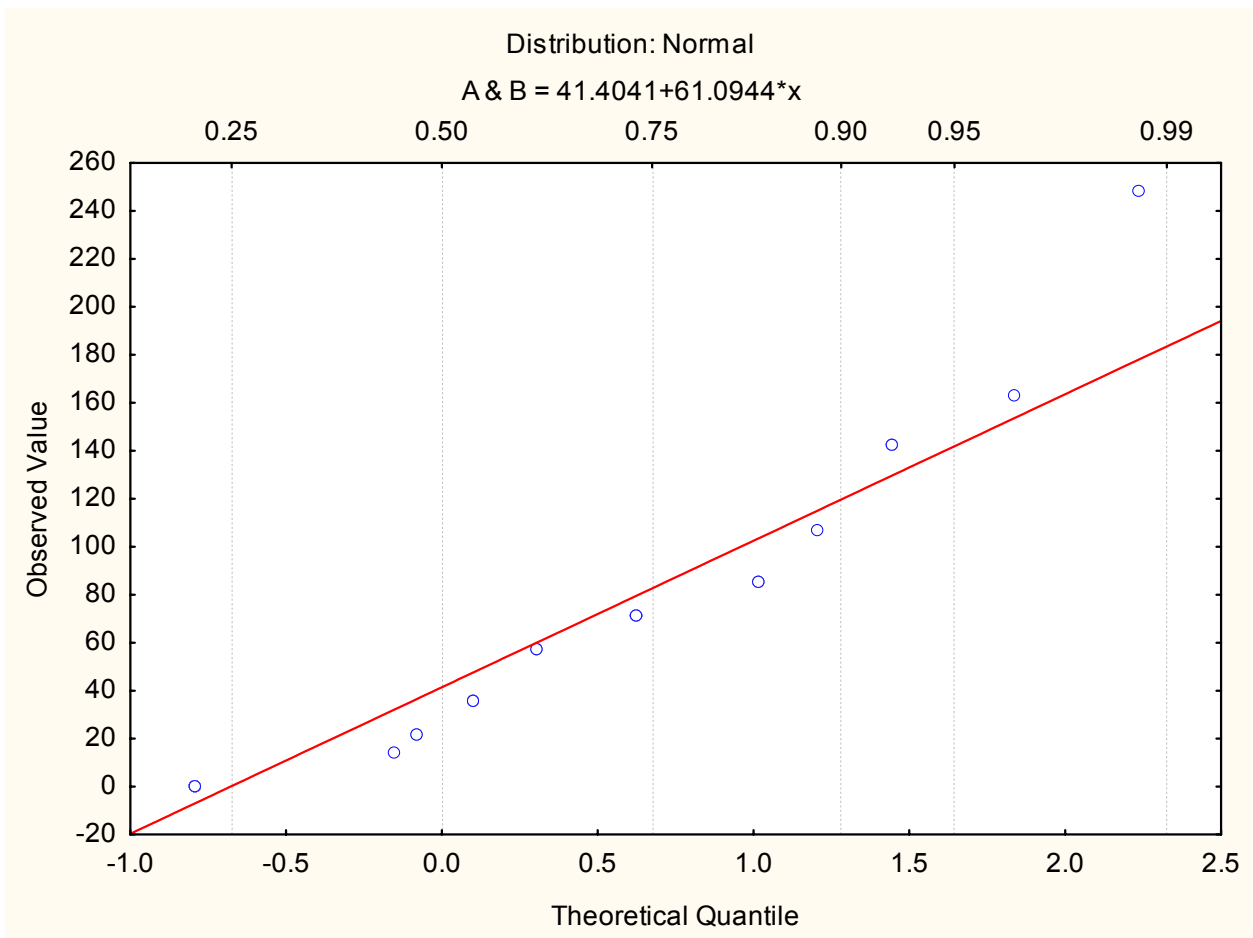


Figure 39: Normal distribution of foam volume data for the 0.1 (A) and 0.3 g.l⁻¹ BSA (B) digestion bottles

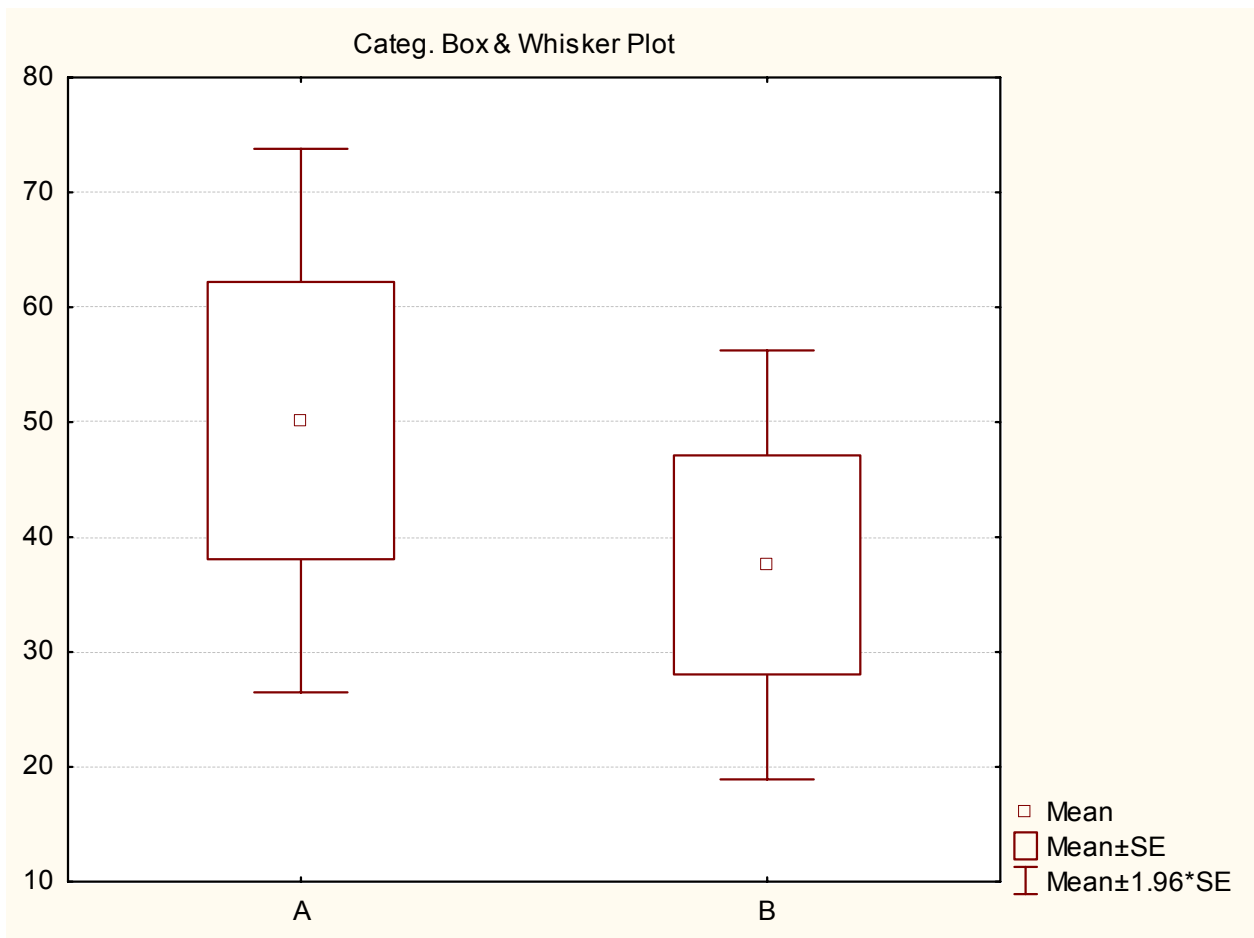


Figure 40: Statistica output for foam volume data for the 0.1 (A) and 0.3 g.l⁻¹ BSA (B) digestion bottles

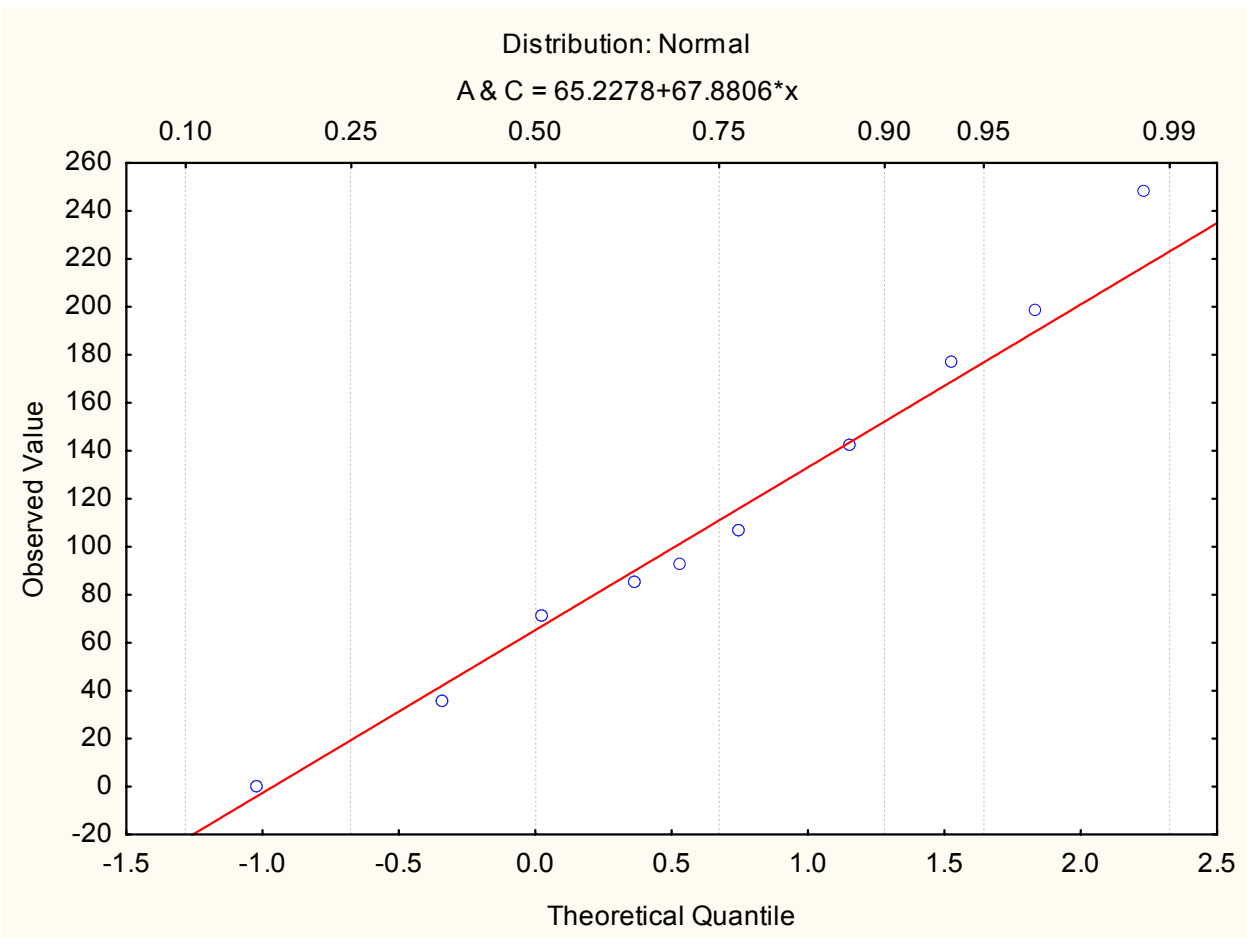


Figure 41: Normal distribution of foam volume data for the 0.1 (A) and 1 g.l⁻¹ BSA (C) digestion bottles

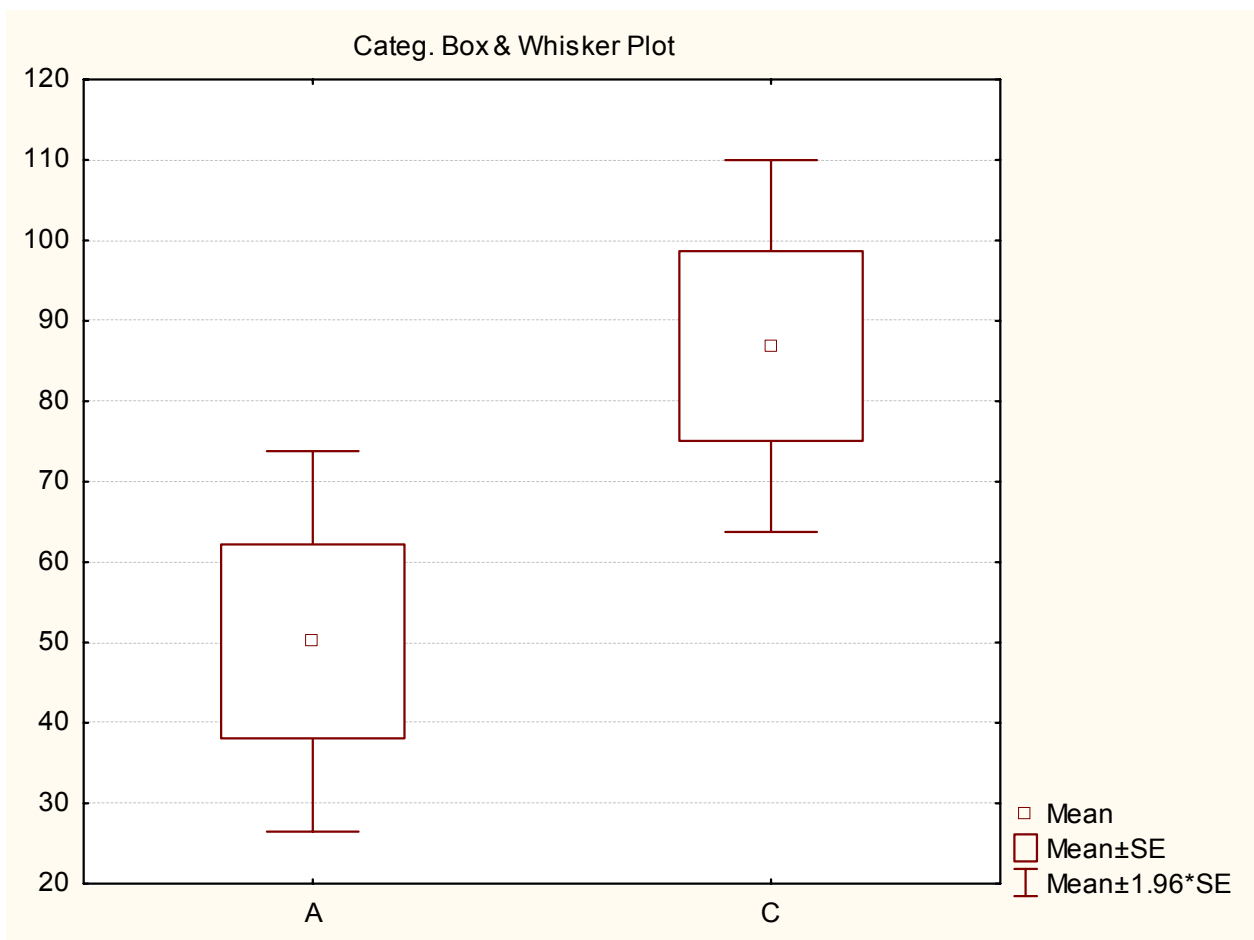


Figure 42: Statistica output for foam volume data for the 0.1 (A) and 1 g.l⁻¹ BSA (C) digestion bottles

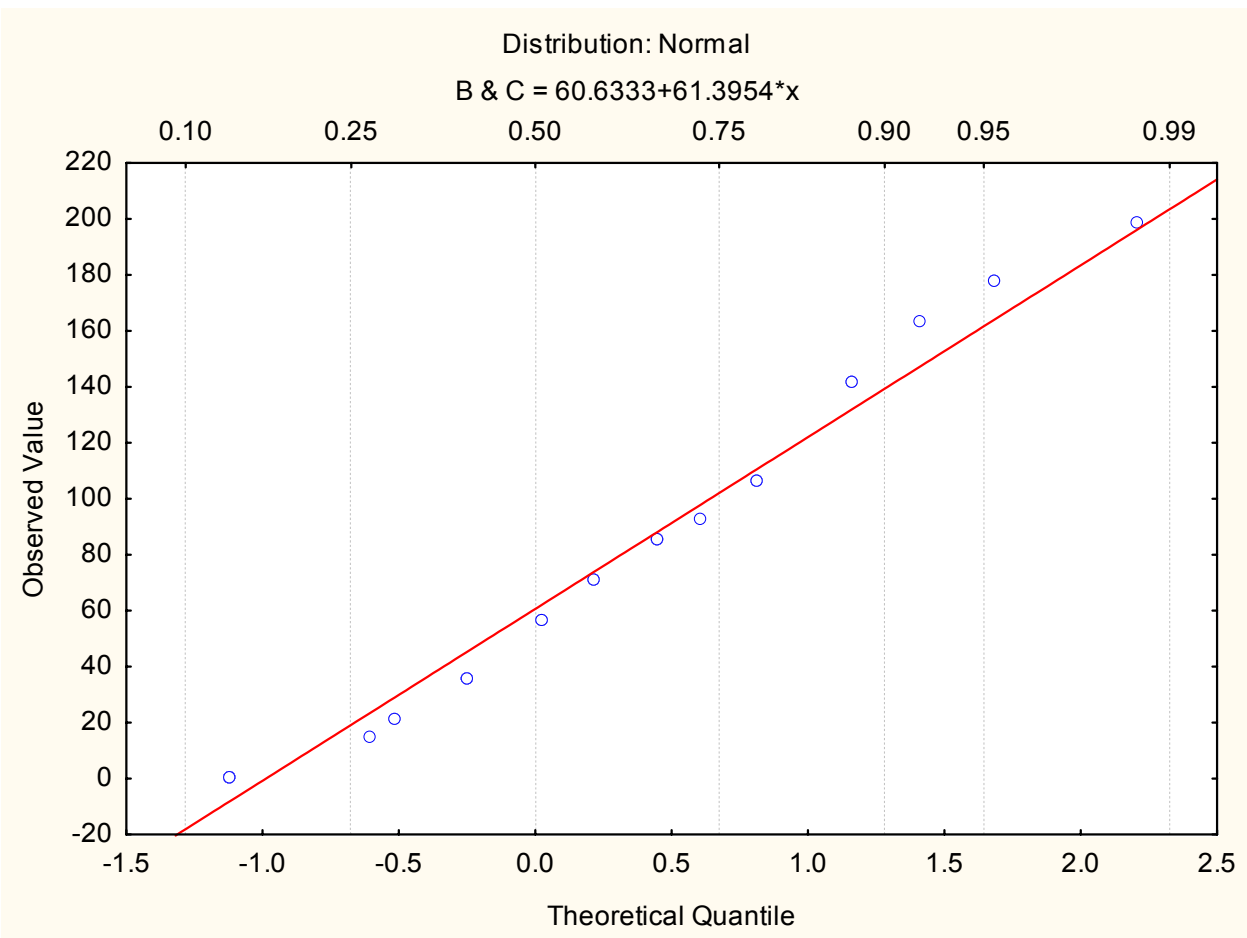


Figure 43: Normal distribution of foam volume data for the 0.3 (B) and 1 g.l⁻¹ BSA (C) digestion bottles

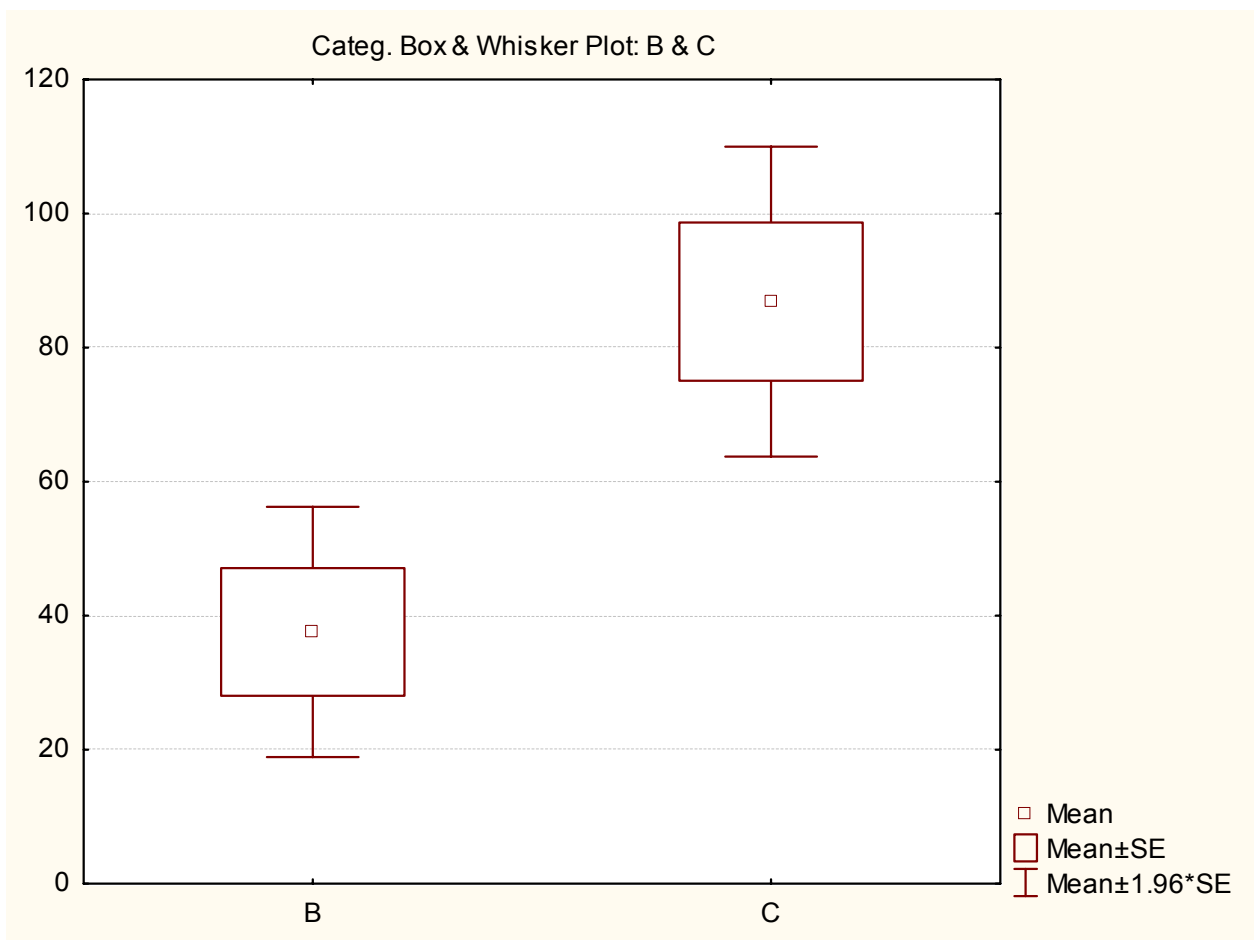


Figure 44: Statistica output for foam volume data for the 0.3 (B) and 1 g.l⁻¹ BSA (C) digestion bottles

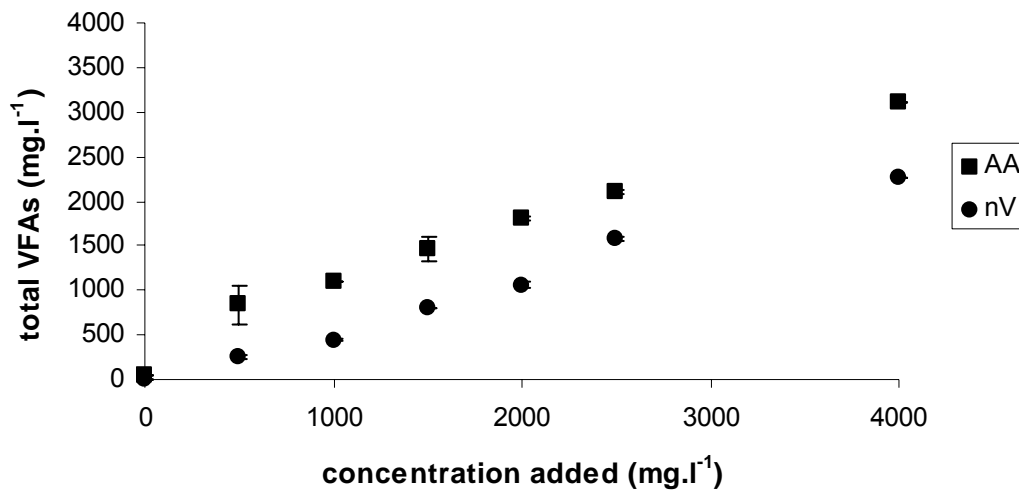


Figure 45: Acetic acid (AA) and n-valeric acid (nV) (mg.l⁻¹ ±SD) in sludge after addition of the studied concentrations of AA and nV

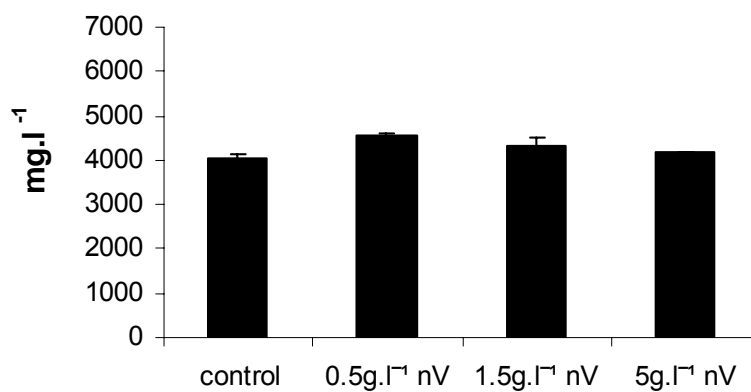


Figure 46: Alkalinity (mg.l⁻¹ ±SD) in sludge samples on Day 10 of n-valeric experiment

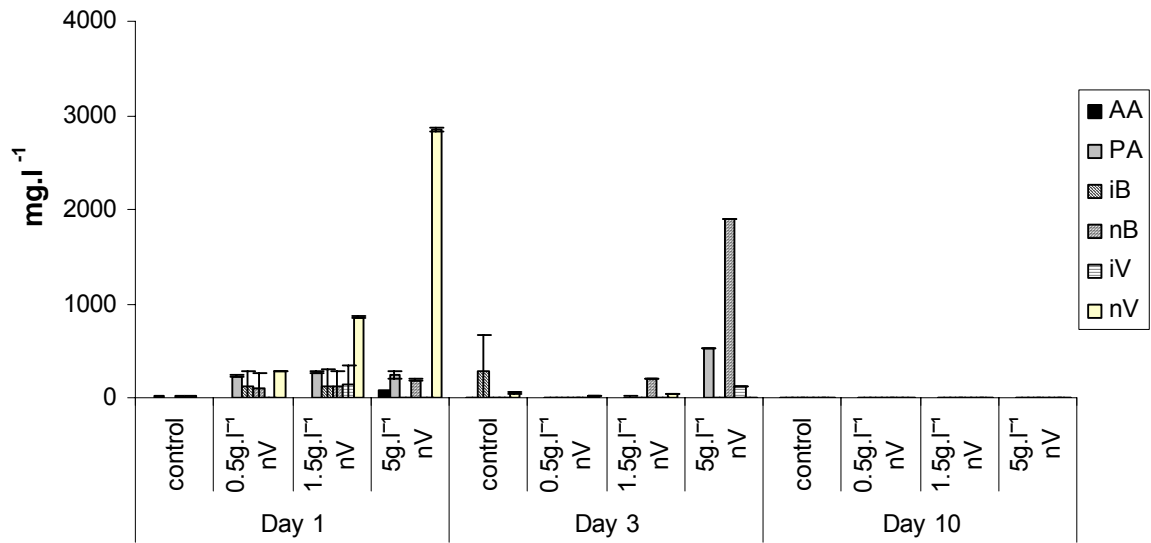


Figure 47: Individual VFAs (mg.l⁻¹ ±SD) for sludge samples during batch digestion, experiment on n-valeric

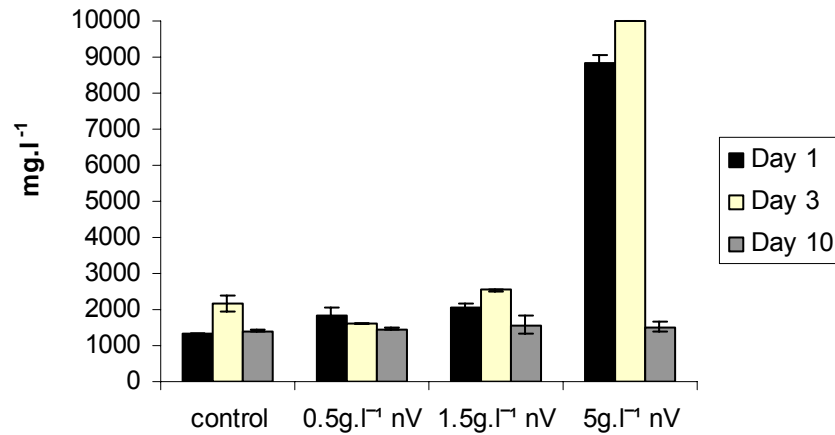


Figure 48: SCOD (mg.l⁻¹ ±SD) in sludge samples during batch digestion, experiment on n-valeric

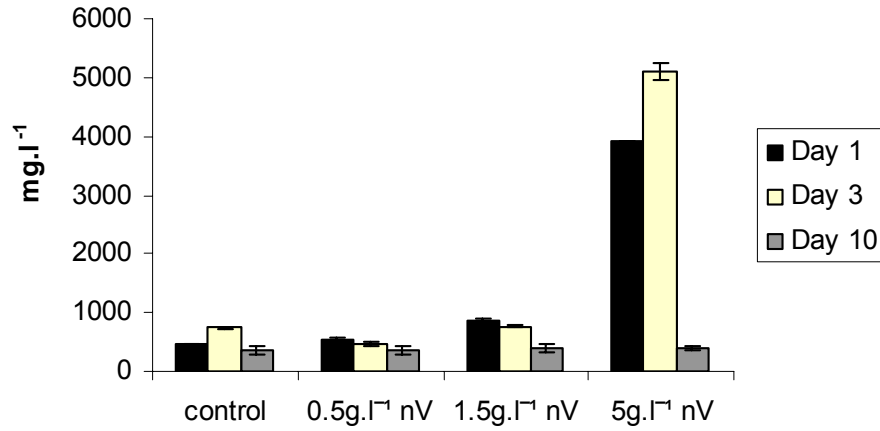


Figure 49: DOC (mg.l⁻¹ ±SD) in sludge samples during batch digestion, experiment on n-valeric

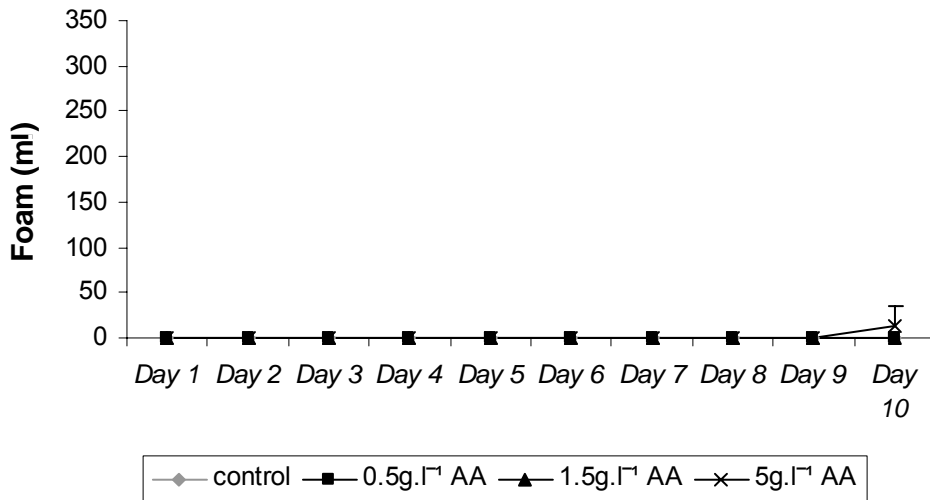


Figure 50: Daily foam production (ml ±SD) during batch anaerobic digestion, experiment on acetic acid

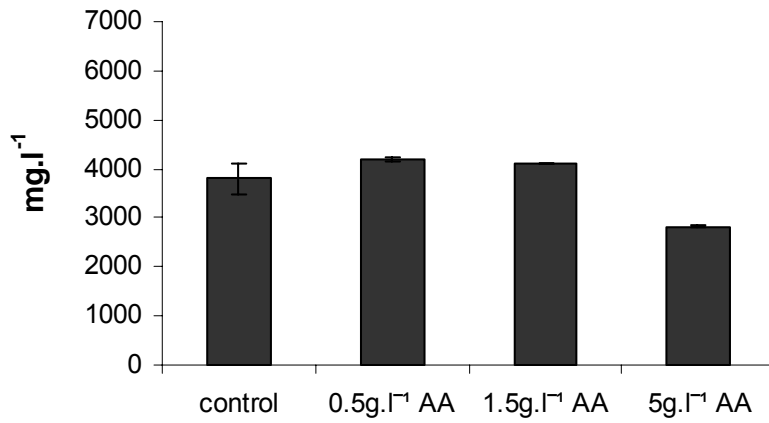


Figure 51: Alkalinity (mg.l⁻¹ ±SD) in sludge samples on Day 10 of acetic acid experiment

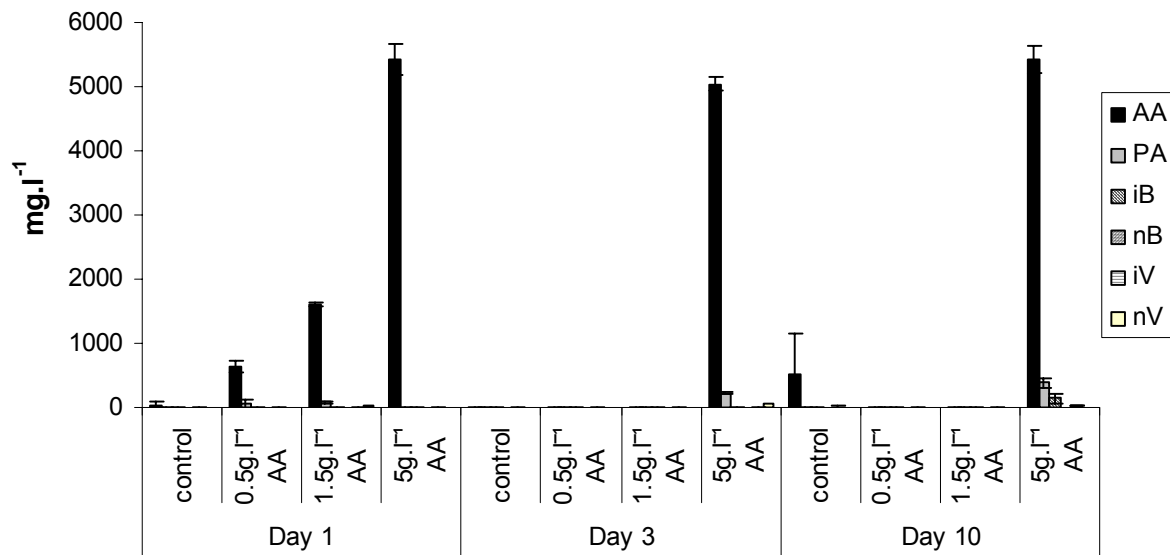


Figure 52: Individual VFAs (mg.l⁻¹ ±SD) for sludge samples during batch digestion, experiment on acetic acid

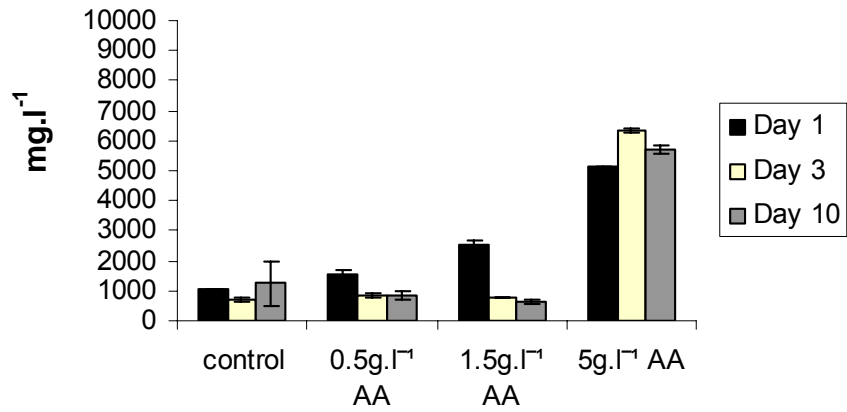


Figure 53: SCOD (mg.l⁻¹ ±SD) in sludge samples during batch digestion, experiment on acetic acid

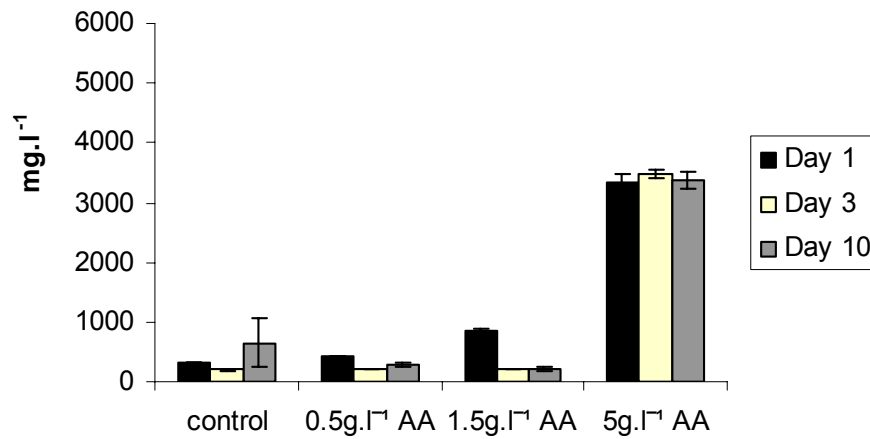


Figure 54: DOC (mg.l⁻¹ ±SD) in sludge samples during batch digestion, experiment on acetic acid

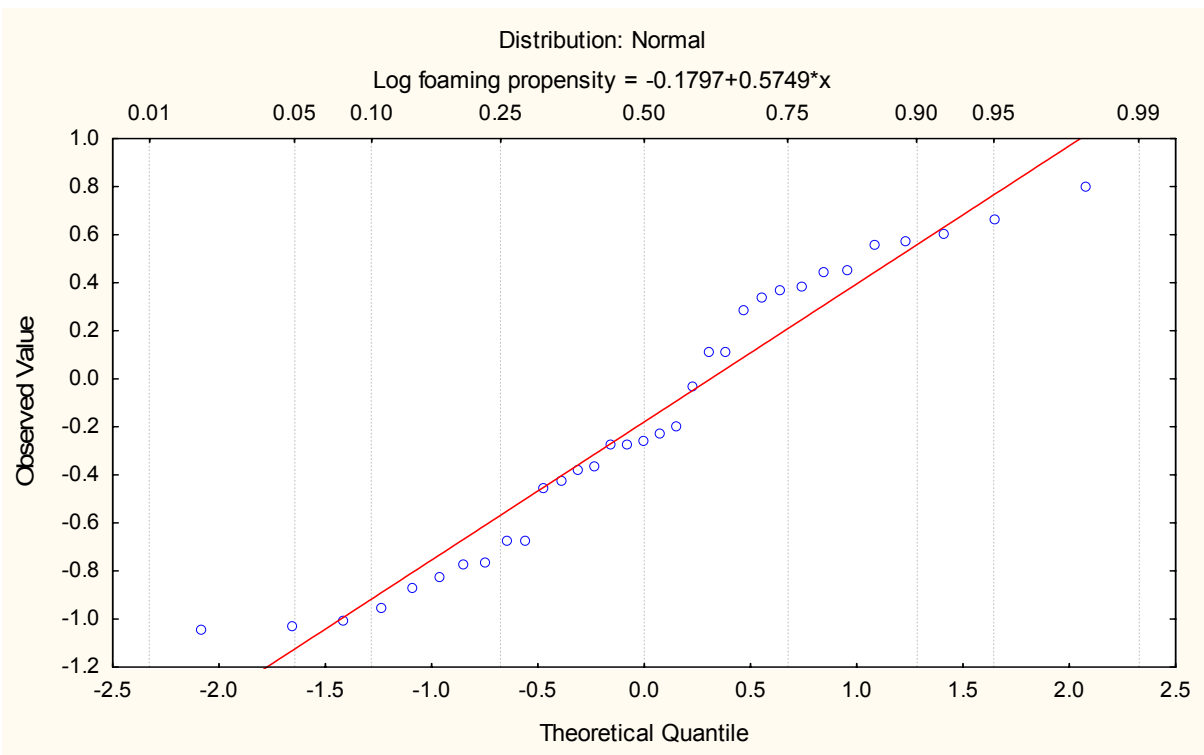


Figure 55: Normal distribution of log values of foaming propensity of digested sludge obtained for foaming and non-foaming digesters

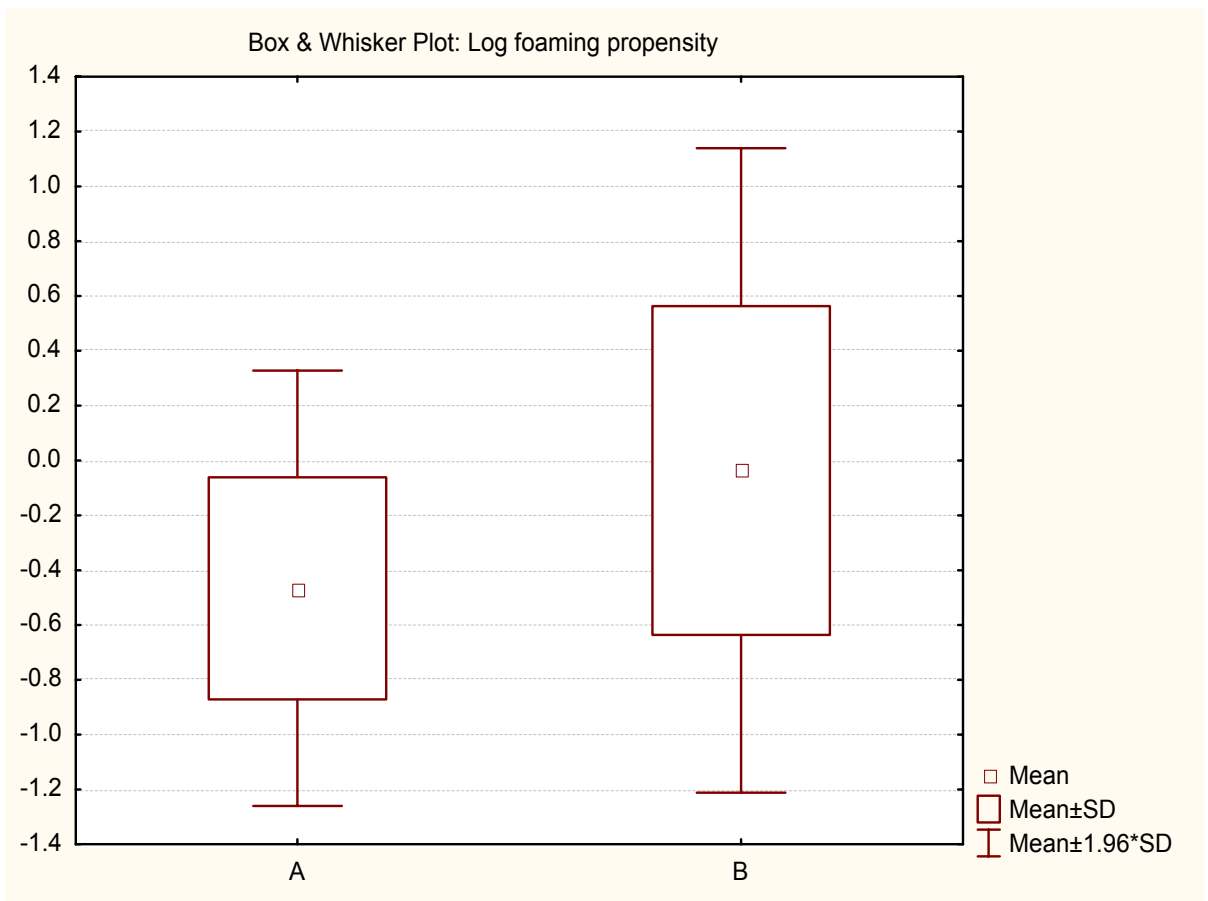


Figure 56: Statistica output for log foaming propensity values from non-foaming digesters (A) and foaming digesters (B)

Table 4: Average foaming propensity values (\pm SD) of primary, SAS and feed sludge samples obtained from foaming and non-foaming digesters				
		<i>Primary</i>	<i>SAS</i>	<i>Feed</i>
non-foaming	Site 1	n/a	0.00 \pm 0.00	4.31 \pm 0.73
	Site 2	0.35 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00
	Site 3	0.027 \pm 0.00	0.00 \pm 0.00	0.14 \pm 0.05
	Site 4	n/a	n/a	0.00 \pm 0.00
	Site 5	1.73 \pm 0.15	0.00 \pm 0.00	0.38 \pm 0.05
	Site 6	0.00 \pm 0.00	0.00 \pm 0.00	2.61 \pm 1.74
	Site 7	0.00 \pm 0.00	n/a	0.00 \pm 0.00
foaming	Site 8	n/a	0.57 \pm 0.00	0.00 \pm 0.00
	Site 9	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	Site 10	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	Site 11	n/a	0.00 \pm 0.00	0.13 \pm 0.03
	Site 12	1.34 \pm 0.74	0.00 \pm 0.00	1.23 \pm 0.10
	Site 13	0.00 \pm 0.00	0.00 \pm 0.00	0.43 \pm 0.06
	Site 14	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	Site 15	n/a	n/a	1.10 \pm 0.10

n/a: not applicable, sample was not obtained

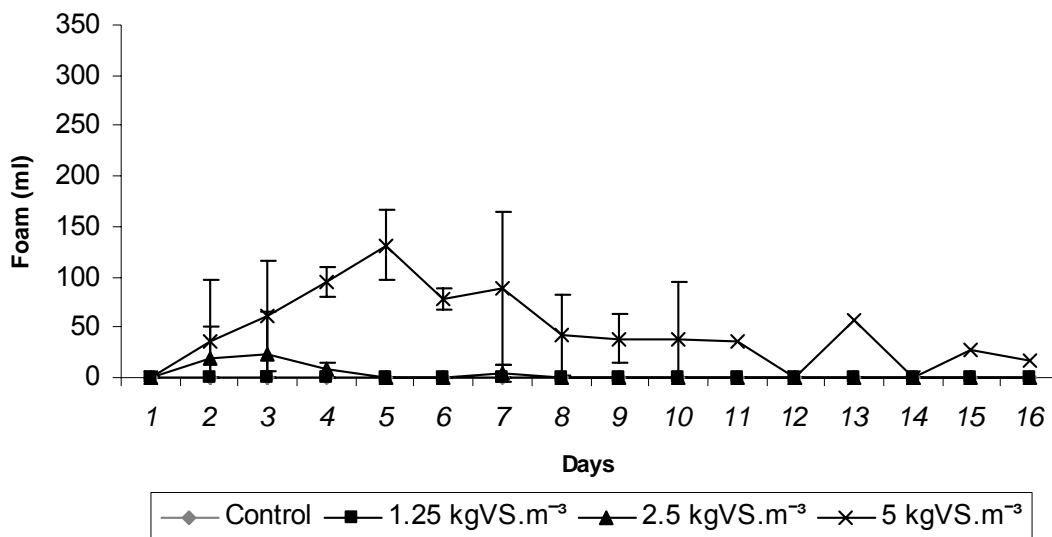


Figure 57: Daily foam volume production (ml) during batch anaerobic digestion, experiment 1 on organic loading

$$COD \text{ loading } (\text{ kgCOD } \cdot \text{ m}^{-3}) = \frac{COD_{\text{feed}} \cdot V}{1000 \times 500} \quad \text{Equation 1}$$

Where COD_{feed} = g.l⁻¹ SCOD in feed sludge
 V = volume of feed sludge
 1000 = correction factor between ml and l
 500 = working volume of digestion

Table 5: SCOD loadings for batch digestion experiments on organic loading and surface active agents		
Experiment	Condition	SCOD loading
Exp 1 on organic loading	1.25 kgVS.m ⁻³	0.18
	2.5 kgVS.m ⁻³	0.35
	5 kgVS.m ⁻³	0.70
Exp 2 on organic loading	1.25 kgVS.m ⁻³	0.16
	2.5 kgVS.m ⁻³	0.32
	5 kgVS.m ⁻³	0.63
Exp 3 on organic loading	1.25 kgVS.m ⁻³	0.12
	2.5 kgVS.m ⁻³	0.23
	5 kgVS.m ⁻³	0.46
Exp on BSA	0.1g.l ⁻¹ BSA	0.12
	0.3g.l ⁻¹ BSA	0.20
	1g.l ⁻¹ BSA	1.53
Exp on nV	0.5g.l ⁻¹ nV	0.58
	1.5g.l ⁻¹ nV	0.81
	5g.l ⁻¹ nV	7.58