THE EFFECT OF SODIUM DODECYL SULPHATE (SDS) AND SODIUM DODECYLBENZENE SULPHONATE (SDBS) ON ACTIVATED SLUDGE OXYGEN UPTAKE RATE (OUR) AND NITRIFICATION

A Thesis submitted in fulfilment of the requirements for the degree of
Master of Engineering

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March 2009
DECLARATION

I certify that except where due acknowledgement has been made; the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

Yi Jiao (Joanna)

9th March
ACKNOWLEDGEMENTS

Firstly I would like to thank my supervisors, Dr Maazuza Othman from Civil, Environmental and Chemical Engineering School, her advice and suggestions help me a lot in the whole study. I also would like to thank my supervisor, Professor Philip Marriott from Applied Science School of RMIT for help me in the study.

Also many thanks go to the Sunbury Wastewater Treatment Plant at Sunbury for their support in this research and for allowing me to take samples and to use all of their facilities. In particular, I wish to thank Gerard Barry and all other staff members in Sunbury WWTP. Also I would like to thank all the staff in Melton Wastewater Treatment Plant for their generous help.

Special thanks must go to colleagues and technicians in Chemical Engineering, Micrology and Applied Science laboratories in RMIT. Those that deserve special mention are: Peg Gee Zhang, Cameron Crombie, Sandro Longano, Philip Francis and Paul Morrison. Other staff members in Civil, Environmental and Chemical Engineering School that I extend my thanks to are: John Buckeridge, Mike Xie, Marlene Mannays and Sharon Taylor.

Finally and most importantly, special thanks must go to my family. I wish to thank my parents for all their ongoing support, encouragement and patience over the years.
LIST OF ABBREVIATIONS

ABS: Branched Alkylbenzene Sulphonate

AE: Alkyl Ethoxylates

AES: Alkyl Ethoxy Sulphate

APE: Alkyl Phenol Ethoxylate

AS: Alkyl Sulphate

LAS: Linear Alkylbenzene Sulphonate

MBAS: methylene blue active substances

MLSS: Mixed Liquor Suspended Solid

OUR: Oxygen Uptake Rate

QAC: Quaternary Ammonium Compounds

SAS: Sodium Alkyltrioxyethylene Sulphate

SDBS: Sodium Dodecylbenzene Sulphonate

SDS: Sodium Dodecyl Sulphate

SPC: Sulfophenyl carboxylates

WWTPs: Wastewater Treatment Plants
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ABSTRACT

Anionic surfactants are used worldwide in detergent and household cleaning products. Due to their extensive use, surfactants can find their way into wastewater treatment plants (WWTPs), where they may be completely or partially removed. Surfactants in WWTPs which incorporate an activated sludge process are removed through a combination of degradation into other by-products and sorption onto activated sludge. After treatment, surfactants and/or their biodegradation by-products (metabolites) that remain in the effluent find their way into the environment through receiving waters. Anionic surfactants not only have adverse effects on aquatic and terrestrial environment, but also can have adverse effects on the WWTP operation and performance.

Surfactants can be classified into three major classes namely, nonionic, cationic and anionic. Anionic surfactants are the major class used in detergent formulations. Linear alkylbenzene sulfonates (LAS) are the most frequently detected anionic surfactants in urban wastewater. They can reach concentrations of up to 20 mg/L in the influent to WWTPs receiving mainly domestic wastewater and up to 300 mg/L in influents to WWTPs receiving domestic and industrial wastewater. Sodium Dodecylbenzene Sulphonate (SDBS) is a member of the LAS group and Sodium Dodecyl Sulphate (SDS) is a member of the Alkyl Sulphate (AS) group. This study selected SDS and SDBS as model anionic surfactants due to their widespread use. Most of the published research studies have focused on the adverse effects that anionic surfactants may have on the aquatic and
terrestrial environment and the biodegradability of surfactants in these environments. There are many studies that have assessed WWTPs performance for the removal of surfactants in general and anionic surfactants in particular but, there is limited literature on how anionic surfactants affect activated sludge processes, especially the nitrification stage of the process.

The aim of this study is to assess the effect of the presence of anionic surfactants in the influent to WWTPs on activated sludge processes. To accomplish this aim, the effect of the presence of SDS and SDBS on activated sludge oxygen uptake rate (OUR) and nitrification was assessed according to the ISO standard methods. The OUR method facilitates estimation of the effects of anionic surfactants on activated sludge microorganisms in aerobic biological treatment systems. A nitrification inhibition test was developed by International Standard Organization and has been used by many researchers for assessing the inhibitory effects of anionic surfactants on nitrifying microorganisms in activated sludge. The morphology of activated sludge flocs was also evaluated to help investigate the effect of anionic surfactants on the settling behaviour of flocs.

The results indicated that the anionic surfactants SDS and SDBS have an adverse effect on the activated sludge OUR and nitrification activities. Inhibition to OUR increased from 12.9% to 44.2% for SDS concentrations from 5 to 100 mg/L, after 30 minutes of incubation. The inhibition to OUR decreased with increased incubation time to 180 minutes reaching 6% at 5 mg/L and 27% at 100 mg/L. SDBS showed a strong inhibitory effect on
activated sludge OUR where an inhibition of 19.8% to 79.1% was measured after 30 minutes, which declined reaching 15% to 69.2%, after 180 minutes, for the same concentration range. The results showed that the extent of inhibition to activated sludge OUR induced by SDBS was higher than that induced by SDS for all concentrations tested. The higher inhibition exerted by SDBS can be attributed to the presence of the benzene ring which has low biodegradability.

SDS and SDBS also showed an inhibitory effect on activated sludge nitrification which followed a trend that was in agreement with that observed for their inhibition to OUR. Inhibition to nitrification was measured in terms of the reduction in the production of nitrites and nitrates (i.e., ammonia oxidation to nitrite and nitrates) compared with that in the absence of SDS and SDBS. SDS inhibition to ammonia oxidation to nitrites, i.e. to *Nitrosomonas* bacteria was higher than that measured for nitrite oxidation to nitrates, i.e. to *Nitrobacter* bacteria. SDS and SDBS inhibition to nitrification was proportional to their initial concentrations. For example, inhibition to nitrification increased from 5.9% to 46.5% with increase SDS concentration from 5 to 100 mg/L compared with 12.9% to 53.6% for the same concentration range of SDBS.

It is very well known that activated sludge activities, especially nitrification slow down in cold weather. The results obtained demonstrated that the inhibitory effects of SDS and SDBS on activated sludge biological activities were intensified at low temperature, as measured in terms of OUR and nitrification. In the presence of SDS, inhibition to OUR measured at 10°C
almost doubled when the temperature increased to 20°C, within 3 hours of incubation. The results obtained showed that both SDS and SDBS have strong inhibitory effects on ammonia oxidation to nitrite and nitrate at 10°C. Inhibition to nitrification almost doubled at SDS concentrations below 25 mg/L and increased by 25% - 50% for SDS concentrations from 25 to 100 mg/L. Inhibition to nitrification decreased significantly with temperature increase from 20°C to 30°C.

Inhibition to nitrification decreased significantly with a temperature increase from 20°C to 30°C. SDBS inhibition to nitrification was higher than that induced by SDS under all conditions tested. In addition, it was also observed that at 30°C, SDS showed no inhibitory effects for concentrations less than 10 mg/L. These results show that care should be taken when reporting inhibition test results and that these tests should be carried out at the temperatures at which the wastewater treatment plant operate.

The concentration of SDS and SDBS at the end of OUR and nitrification inhibition tests indicated that low percentages of the initial concentration of SDS and SDBS were removed. After 180 minute of incubation, 67.4% and 42% of the initial SDS and SDBS concentration were removed.

The results showed that the presence of SDS and SDBS in the activated sludge aeration basins may lead to changes to the morphology of activated sludge flocs measured in terms of the mean projected area and perimeter. The mean projected area and perimeter of the flocs, decreased by approximately 50% and 24%, respectively, in the presence of 100 mg/L
SDS. For SDBS, the flocs perimeter decreased by 46.6% at 10°C and 37.4% at 20°C at 100 mg/L. The result showed that SDBS has a stronger negative effect on activated sludge flocs than SDS indicating that SDBS is more likely to induce problems in the operation of activated sludge process, especially settling properties than SDS. The change to the activated sludge flocs mean projected area and perimeters suggested that the threshold or tolerance of the activated sludge microorganisms to SDS and SDBS decrease with increased temperature.

The results obtained in this research suggested that the inhibition to OUR and nitrification was more likely to be due to interference with the biodegradation mechanism. Interference with the biodegradation process could be due to interference with the availability or transfer of oxygen.
CHAPTER ONE INTRODUCTION

1.1 Introduction

Anionic surfactants are widely used in household cleaning detergents and personal care products as well as industrial processes such as dry-cleaning, electroplating, lubrication, emulsion polymerization and paper manufacturing. Surfactants are classified into three categories, namely anionic, cationic and nonionic. Because of their use in household and industrial processes, surfactants including anionic surfactants have been detected in wastewater collection systems. Surfactants are usually discarded down the drain into WWTPs, where they are completely or partially removed by sorption to biomass and biodegradation which results in the loses of their tensioactive properties. After wastewater treatment, non-degraded surfactants with their biodegradation products (metabolites) are discharged by WWTPs effluents into rivers, lake or sea. Also many studies reported in the literature have examined the fate of Linear Alkylbenzene Sulphonate (LAS) in Wastewater treatment plants and the fate of anionic surfactants in the environment.

The aim of this research was to investigate the potential effects of anionic surfactants on the activated sludge processes. Many wastewater treatment plants have experienced washout problems especially with a drop in weather temperature, i.e. at the beginning of the cold months, which resulted in high concentrations of suspended solids, ammonia-nitrogen and
nitrite-nitrogen in the effluent. Specifically, one of the local wastewater treatment plants detected an increase in the concentration of surfactants in the influent to the treatment plant was associated with the washout event and high concentration of ammonia and nitrates in their effluent (Othman, 2008). Therefore the objective of this research is to examine the potential inhibition of anionic surfactants to nitrification reactions in the activated sludge process. In addition, this research study was concerned with the inhibition to activated sludge at low temperatures. Recently, many wastewater treatment plants in Melbourne have reported 20% - 30% reduction in inflow into wastewater treatment plants, with an associated change to the characteristics of the influent. These changes have been attributed to reduced flow in the sewers because of the drought and water restrictions. Consequently, another objective of this research was to examine the effect of increased concentrations of anionic surfactants on the activated sludge process. To achieve these objectives, this research investigated the effect of anionic surfactants on activated sludge microorganisms OUR and nitrification reactions, and the morphology of activated sludge flocs after exposure to these surfactants.

1.2 OUR Tests

Respiration of activated sludge microorganisms have been used as an indicator of their growth under conditions tested. Many research studies employed respiration inhibition tests (Gengig et al., 2003; Klečka and Landi, 1985; Yoshioka et al., 1986) to examine the potential toxicity of certain
constituents in terms of their effect on activated sludge (Gutiérrez et al., 2001; Verge and Moreno, 1996). Most of these research studies employed the standardized test published by the International Organization for Standardization (ISO 8196, 1986). This test was established based on the fact that the respiration rate of activated sludge or sludge organisms can be reduced in the presence of toxicants. Nitrification inhibition tests, ISO 9509 (1989) was employed to examine the potential effect of anionic surfactants on nitrification reactions in activated sludge systems. The effect on nitrification was measured both in terms of changes to ammonia oxidation and to ammonia oxidation products, i.e. nitrites and nitrates.

1.3 Morphology of activated sludge

Activated sludge is a complex ecosystem constituted mainly of bacteria and protozoa. The bacteria are agglomerated as flocs. Morphology studies of activated sludge flocs can be used to predict the affect anionic surfactants on the settleability of the sludge and level of suspended solids in the effluent. Morphology of activated sludge flocs was assessed using image analysis. This technique provides data on mean projected area, perimeter, equivalent diameter and form factor of the activated sludge which can be used to assess potential relationship between the size of the sludge flocs and their settling properties. Ideally, sludge flocs should be round and firm. However, one reason cause settling failures is bulking sludge which is characteristic as irregular sludge focs, it can cause a washout of the sludge. Another cause is a large amount of very small sludge flocs which not only
cause settling problem, but also causing a very turbid effluent (Grijspeerdt and Verstraete, 1996; Motta et al., 2001; Sezgin, 1982).

1.4 Thesis layout

Chapter Two of this thesis presents a literature review summarizing published research on anionic surfactants fate in the environment, biodegradability, removal in WWTPs, and test methods used in assessing inhibition and toxicity to activated sludge processes. Chapter Three describes the materials and the methodology used in this research study. Chapter Four includes the results obtained and a detailed discussion of these results. Chapter Five presents the conclusions deducted based on the results presented in Chapter Four.
CHAPTER TWO LITERATURE REVIEW

2.1 Surfactants

2.1.1 Definition of surfactants

Surfactants are a diverse group of chemicals that modify chemicals interfacial properties of liquids to which they are added. Structurally, they have two main parts, a soluble polar head group and a non polar hydrocarbon tail which is not easily soluble in water. As a result, surfactants have both hydrophobic and hydrophilic properties.

Surfactants have been extensively used in domestic products. Specifically, they are widely used in household cleaning detergents and personal care products. In addition, many synthetically produced surfactants are used in industrial processes such as dry-cleaning, the manufacture of textiles and paints, the electroplating process, as lubrication and emulsion polymer, and in paper manufacturing (Dirilgen and Ince, 1995; Ying, 2006) (Figure 2.1). The use of surfactants is gradually increasing because of increased consumption of the above products and their useful properties.
Annually, 7.2 million tons of synthetic surfactants are produced worldwide (Di Corcia, 1998). Linear alkylbenzene sulphonates (LAS), alkyl ethoxy sulphates (AES), alkyl sulphates (AS), Alkylphenol ethoxylates (APE), Alkyl ethoxylates (AE), and quaternary ammonium compounds (QAC) are the commonly used commercial surfactants. Figure 2.2 shows the production of the different types of surfactants used in the United States, Japan and Western Europe in 1982.
Surfactants can be classified into three major classes based on their ionizing properties, nonionic, cationic and anionic surfactants (Table 2.1). Nonionic surfactants contain a polyethoxylate hydrophilic group (ROCH$_2$CH$_2$OCH$_2$CH...OCH$_2$CH$_2$OH; abbreviated REO$_n$, where $n$ is the average number of ethoxylate (EO)-OCH$_2$CH$_2$ units in the hydrophilic group) and are uncharged (Stache, 1984). Cationic surfactants usually contain amino or quaternary nitrogen, i.e. (RMe$_3$N)$^+$Cl$^-$ and a positive charge. Amine oxides and monoamines with long chains are the main groups of cationic surfactants. Anionic surfactants are negatively charged on the surface active moiety, i.e. (RSO$_3$)$^-$Na$^+$, where $R$ is a hydrophobic aromatic and/or aliphatic chain. It also contains organic acid salts of sodium and potassium. The major anionic surfactant groups are carboxylates, sulfonates and sulfuric acid esters (Stache, 1984).
<table>
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<th>Common name</th>
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<td>LAS</td>
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<tr>
<td></td>
<td>Alcohol sulphates (alkyl sulphates)</td>
<td>AS</td>
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<tr>
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<td>Alkylphenol ethoxylates</td>
<td>APE (or APEO)</td>
</tr>
<tr>
<td></td>
<td>Nonyl phenol ethoxylates</td>
<td>NPE (or NPEO)</td>
</tr>
<tr>
<td></td>
<td>Octyl phenol ethoxylates</td>
<td>OPE (or OPEO)</td>
</tr>
<tr>
<td></td>
<td>Alcohol ethoxylates</td>
<td>AE (or AEO)</td>
</tr>
<tr>
<td>Cationic surfactants</td>
<td>Quaternary ammonium-based compounds</td>
<td>QAC</td>
</tr>
<tr>
<td></td>
<td>Alkyl trimethyl ammonium halides</td>
<td>TMAC</td>
</tr>
<tr>
<td></td>
<td>Alkyl dimethyl ammonium halides</td>
<td>DMAC</td>
</tr>
</tbody>
</table>
2.1.2 Anionic Surfactants

Anionic surfactants are the major class used in detergent formulations. Scott and Jones (2000) reported that the predominant class of anionic surfactants, after 1960s, is branched alkylbenzene sulphonate (ABS). However, branched alkyl chain of ABS was found to be resistant to biodegradation and caused problems to receiving water bodies. This forced manufacturing companies to do further research work into modifying the surfactants to be more biodegradable. One of the products that were introduced as more biodegradable and with less effect on the environment was LAS. Other commonly used are AS and AES. The surfactants used in this study SDBS and SDBS. SDBS belongs to the LAS group whereas SDS belongs to the AS group (Figure 2.3)

![Figure 2.3: Structural formulae of A) SDBS B) SDS](image-url)
2.1.2.1 Linear alkylbenzene sulphonates (LAS)

The LAS are the most commonly used synthetic anionic surfactants. They consist of a hydrophobic part (alkylbenzene group) and a water-soluble hydrophilic part (sulfonic acid group and other cations) (Stache, 1984). These chemicals have been widely used for the past 30 years, for example 2.8 million tons were produced globally in 1998 (Verge et al., 2000). LAS products produced for commercial purposes are alkyl benzene sulphonate compounds. These can be represented by the formula $\text{R- C}_6\text{H}_4^{-\text{SO}_3}\text{Na}$, where R represents an alkyl linear chain with C atoms in the range of $\text{C}_{10} - \text{C}_{13}$. Since the phenyl group maybe attached to any internal carbon atom of the alkyl chain, each homologue can contains 5-7 positional isomers (Ying, 2006).

LAS are the compounds most frequently found in urban wastewater. They can reach concentrations of up to 20 mg/L in the influent to WWTPs (Oviedo et al., 2004). Table 2.2 shows the concentrations of LAS in WWTPs in different countries.
Table 2.2: Reported concentrations of LAS in the influent and effluent in WWTPs (Petrovic and Barceló, 2004)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Country</th>
<th>Influent (mgL⁻¹)</th>
<th>Effluent (mgL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS</td>
<td>Germany</td>
<td>1.9-8</td>
<td>0.065-0.115</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td>3.14-8.4</td>
<td>0.013-0.115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4-10.7</td>
<td>0.021-0.29</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>1.423-2.17</td>
<td>0.010-0.091</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.049-0.158</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.988-1.309</td>
<td>0.136-0.197</td>
</tr>
<tr>
<td></td>
<td>The Netherlands</td>
<td>3.4-8.9</td>
<td>0.019-0.071</td>
</tr>
<tr>
<td></td>
<td>UK</td>
<td>15.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.85-5.58</td>
<td>0.04-1.09</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>1.8-7.7</td>
<td>&lt;0.001-1.5</td>
</tr>
</tbody>
</table>

As LAS represent more than 40% of all surfactants used worldwide, it is not surprising that a large proportion of the available literature focused on environmental problems associated with LAS.

2.1.2.2 Alkyl Sulphates (AS)

Anionic surfactants such as AS and AES are have diverse properties. They can be modified by adding a base of alcohol to the alkyl chain. Accordingly, AS and AES have a wide variety of scientific, consumer and industrial applications (Stache, 1984).
The applications of AS in consumer products depend on its alkyl chain. AS with alkyl chains C$_8$ – C$_{10}$ are commonly used in consumer products. However, it is more common for alkyl sulphates in the range C$_{10}$ – C$_{18}$ to be used in such formulations, although other surfactants are generally added to enhance their properties (Stache, 1984). SDS is commonly used in bubble baths, shampoos, toothpastes and detergents (Dirilgen and Ince, 1995). This can be obtained with the highest degree of purity. Furthermore, SDS is often used as reference surfactant when comparing with other surfactants in many chemical and physicochemical studies and determinations.

### 2.2 Fate of surfactants

#### 2.2.1 Fate of surfactants in the environment

Surfactants enter the environment through the discharge of sewage effluents into surface waters. Surfactants in WWTPs are either degraded, or adsorbed onto the activated sludge. Many treatment plants store their digested waste activated sludge on land for years to dry it before it is sent for potential use as a component in fertilisers. Surfactants adsorbed onto activate sludge may be desorbed under wet weather conditions and may find their way into surrounding water bodies or infiltrates into groundwater (Scott and Jones, 2000; Ying, 2006). Both aquatic and terrestrial toxicity data from laboratory and field studies are essential for us to assess the
potential of surfactants impact on ecosystem (Kloepper – Sams et al., 1996; Utsunomiya et al., 1997; Verge et al., 2000).

The effect of anionic surfactants on the ecological system was studied by many researchers (Diriligen and Ince, 1995; Lewis, 1990; Singer et al., 1994) who used toxicity tests as an indicator of the potential impact of anionic surfactants on the aquatic life.

The relationship between the alkyl chain length and LAS toxicity on Fathead minnow and daphnia measured as LC$_{50}$ values was studied using pure LAS homologs. The results showed an increase in the toxicity with increased chain lengths for both tests (Table 2.3), the results also show that EC$_{50}$ for acute toxicity is almost 5 – 10 folds that for long term toxicity, which suggests the potential effect of these surfactants on the environment on the long term.
Similarly, AS have been found toxic to *daphniae* and fish. It was found that C_{12}-C_{18} AS and C_{12}-C_{15} AS have an EC_{50} to fish between 3 and 20 mg/L. They also have an EC_{50} to *daphniae* between 5 and 7 mg/L (Stache, 1984). The aquatic toxicity of LAS and AS to different species is shown in Table 2.4.
Table 2.4: Aquatic toxicity of anionic surfactants (Ying, 2006)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Species</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;10&lt;/sub&gt;LAS</td>
<td><em>Daphnia magna</em></td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;-48h, 13.9 mg/L</td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;LAS</td>
<td></td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;-48h, 8.1 mg/L</td>
</tr>
<tr>
<td>C&lt;sub&gt;14&lt;/sub&gt;LAS</td>
<td></td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;-48h, 1.22 mg/L</td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;LAS</td>
<td><em>Dunaliella sp.</em> (green alga)</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt;-24h, 3.5 mg/L</td>
</tr>
<tr>
<td>C&lt;sub&gt;11&lt;/sub&gt;-C&lt;sub&gt;12&lt;/sub&gt;LAS</td>
<td><em>Oncorhynchus mykiss</em> (rainbow trout fry)</td>
<td>NOEC-54days, 0.2 mg/L</td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;LAS (SDBS)</td>
<td><em>Salmo gairdneri</em> (rainbow trout)</td>
<td>Immobilization EC&lt;sub&gt;50&lt;/sub&gt;-48h, 3.63 mg/L</td>
</tr>
<tr>
<td></td>
<td><em>Gammbusia affinis</em> (mosquito fish)</td>
<td>Immobilization EC&lt;sub&gt;50&lt;/sub&gt;-48h, 8.81 mg/L</td>
</tr>
<tr>
<td></td>
<td><em>Carassius auratus</em> (goldfish)</td>
<td>Immobilization EC&lt;sub&gt;50&lt;/sub&gt;-48h, 5.1 mg/L</td>
</tr>
<tr>
<td>C&lt;sub&gt;12&lt;/sub&gt;AS (SDS)</td>
<td><em>Salmo gairdneri</em> (rainbow trout)</td>
<td>Immobilization EC&lt;sub&gt;50&lt;/sub&gt;-48h, 33.61 mg/L</td>
</tr>
<tr>
<td></td>
<td><em>Gammbusia affinis</em> (mosquito fish)</td>
<td>Immobilization EC&lt;sub&gt;50&lt;/sub&gt;-48h, 40.15 mg/L</td>
</tr>
<tr>
<td></td>
<td><em>Carassius auratus</em> (goldfish)</td>
<td>Immobilization EC&lt;sub&gt;50&lt;/sub&gt;-48h, 38.04 mg/L</td>
</tr>
</tbody>
</table>
2.2.2 Removal of surfactants in the WWTPs

Surfactants that enter the WWTPs may completely or partially be removed by a combination of sorption and biodegradation processes (González et al., 2007; Petrovic and Barceló, 2004). After treatment, non-degraded surfactants with their biodegradation products (metabolites) that remain in the WWTP effluent find their way into receiving water bodies (ie., rivers, lakes and sea).

One of the surfactants that were used in the early 1960s was propylene tetramer benzene sulphonate. This surfactant not only was resistant to biodegradation in WWTPs but exhibited excessive foaming which affected WWTP operation and performance (Scott and Jones, 2000). This has forced major changes to surfactants structures and constituents to improve their biodegradability, which lead to the introduction of straight chain alkyl surfactants, such as LAS. Nevertheless, currently, the problem is not poor degradability of the surfactant but incomplete biodegradation which have been found to result in the formation of metabolites resistant to further degradation and more toxic than the parent compounds (ie., APEO breakdown products) (Petrovic and Barceló, 2004). Also surfactants effects on aeration performance have been studied by Martinov et al., (2008).

Efficient treatment in WWTPs will result in discharge of very low levels of surfactants into the environment. The average surfactant concentration in domestic wastewater has been reported by many researchers to be
between 3 to 21 mg/L (Adak et al., 2005) and 1 to 10 mg/L (Zhang et al.,
1999). Also high concentration of about 300 mg/L was detected in the
influent to WWTPs receiving effluent from industries that use surfactants in
their processing (Zhang et al., 1999).

The World Health Organization allow only 0.2 mg/L for discharge into
environmental waters and ANZECC & ARMCANZ set limits for surfactant at
0.2 mg/L for recreational purposes because the surfactants will undergo
further biodegradation in the environment that together with dilution will
reduce their toxicological effects further.

2.2.3 Biodegradation of surfactants

Biodegradation of a surfactant can by definition be a) primary degradation,
i.e. the initial degradation of the structural surfactant compound when it is
transformed to other products, hence losing its surfactant qualities and b) ultimate degradation (or mineralization) to CO₂, CH₄ and water. Generally,
all surfactant classes have been found to undergo primary biodegradation
under aerobic conditions, but not all compounds are amenable to complete
biodegradation.

The mechanism of breakdown of LAS involves the degradation of the
straight alkyl chain, secondly the sulphonate group and finally the benzene
ring (Schleheck, 2003; Scott and Jones, 2000; Ying, 2006). The breakdown
of the alkyl chain starts with the oxidation of the terminal ethyl group
(ω-oxidation) through the alcohol, aldehyde to the carboxylic acid. The
ω-oxidation of the alkyl chain and the cleavage of the benzene ring require molecular oxygen. The second stage in LAS breakdown is the loss of the sulfonate group (β-oxidation) (Hashim et al., 1992). According to Ying (2006), LAS biodegradation intermediates are mono- and dicarboxylic sulfophenyl acids (SPC). The formation of SPC has been identified with six to ten carbon atoms. Then the SPCs are further desuphophonated.

LAS are the most thoroughly examined synthetic compounds with respect to their biodegradability. Comprehensive literature reviews of surfactants biodegradation have been published (Mohan et al., 2006; Scott and Jones, 2000; Swisher, 1987). LAS are biodegradable and have been used by many researchers as the model surfactant (Hons, 1996; Scott and Jones, 2000; Venhuis et al., 2004). Clara et al. (2007) and Petrovic and Barceló (2004) reported generally more than 95% removal of the LAS in the influent to the WWTPs. Also, very high levels of biodegradation (97-99%) have been found reported by some WWTPs that use aerobic processes (Scott and Jones, 2000). LAS can be degraded by aerobic microorganisms and attached biofilm in the environment (Ying, 2006). Although most of the literature describing research carried out to examine degradation of LAS under aerobic conditions has reached a conclusion that LAS is biodegradable, other researchers reported contradictory results. Zhang et al., (1999) reported that the sodium salt of LAS was not biodegraded even after three weeks of incubation with activated sludge from a municipal wastewater treatment plant, also LAS at 500 mg/L did not induce microorganism’s growth which was attributed to resistant to biodegradation due to the benzene ring. Therefore under these conditions the incomplete
removal of LAS in sewage treatment plants may result in LAS residues
together with SPC escaping most sewage treatment plants and be
discharged into waterways (Ying, 2006).

AS are considered as the most rapidly biodegradable in both primary and
ultimate biodegradation. Ester linkage of AS molecules explains the rapid
biodegradation of this substance when exposed to chemical hydrolysis in
acid media. The mechanism of AS biodegradation is found to involve the
enzymatic cleavage of the sulphate ester bonds to give inorganic sulphate
and a fatty alcohol. The alcohol is oxidized to an aldehyde and subsequently
to a fatty acid, to achieving ultimate biodegradation have to with further
oxidation via the β-oxidation pathway. This pathway is further confirmed by
the identification of alkylsulphatase enzymes, which catalyse the initial
desulphation step, and long-chain alcohol dehydrogenases that follow them.
However, surfactant properties can easily be destroyed by the hydrolysis of
linear primary alkyl sulphates with bacterial enzymes. Also due to its more
simple structure (ie., no benzene ring), it can undergo rapid complete
mineralization within 48 hours in well designed wastewater treatment
facilities, and often only need one day for 95% primary biodegradation. AS
have been found to be the fastest to biodegrade compared to other anionic
surfactants, which usually need several days (Stache, 1984). Lee et al.,
(1995) reported that degradation of SDS by riverine biofilms in Antarcti
coastal waters with half-lives of 160 to 460 hours. In summary, the
literatures suggest that well designed and operated WWTPs can sufficiently
remove AS.
2.3 Domestic WWTPs

According to Cervantes et al., (2006), the first attempt to improve the surface water quality due to discharge of wastewaters was to separate the settleable solids. However, this primary treatment has been significantly improved and additional biological treatment was introduced to also remove the non-settleable organic material. Initially, both anaerobic and aerobic methods were used in biological wastewater treatment, but gradually aerobic systems prevailed over anaerobic facilities. Despite the continual development of wastewater treatment systems and processes by researchers and manufacturing companies of wastewater treatment equipments but a different problem gradually became evident. The synthetic surfactants began to be noticeable in wastewaters, treated sewage, and the receiving waters because of the same property which had led to their success – they retain their foaming properties in natural waters at concentrations down to around 1 part per million (ppm), concentrations far below those detected by simple analytical techniques.

2.3.1 Conventional activated sludge processes

The activated sludge process is the most utilized biological process especially for domestic wastewater treatment. The flow chart for a conventional activated sludge process is presented in Figure 2.4. A typical activated sludge system comprises an aeration basin and a clarifier (or settling basin). In the aeration basin microorganisms are mixed with sewage.
This mixture of microorganisms is termed mixed liquor suspended solids (MLSS) and is also referred to as biomass.

![Diagram of Activated Sludge Process]

Figure 2.4: Typical activated sludge process (Source: Cervantes et al., 2006)

Activated sludge is defined as a suspension of both living and dead mixture of different types of micro-organisms (i.e., bacteria, protozoa, fungi, algae) in wastewater. The second stage in the activated sludge process is the separation of the biomass and other suspended solids from the treated wastewater in a clarifier. A portion of the settled sludge is usually returned to the aeration basin from the clarifier underflow, whereas the remainder is discarded for further processing. Biological nutrients removal occurs partially through sludge production and wasting. However, most wastewater treatment plants employ nitrification and denitrification using aerobic and anoxic zones, respectively, in order to achieve the required nitrogen removal (Tchobanoglous and Burton, 1993; Seviour et al., 1999).
2.3.2 Nitrification in activated Sludge processes

Biological removal of nitrogen from wastewater by nitrification and denitrification has been proven an efficient process. Nitrification is known as the first step of the nitrogen removal. Nitrification is accepted to be a two-step process as described below:

The first step is the oxidation of ammonium ions to nitrite. This reaction is generally considered to be catalysed by the genus *Nitrosomonas*:

\[
\text{NH}_4^+ + 1.5 \ O_2 \rightarrow \text{NO}_2^- + 2H^+ + H_2O
\]

In the second step, nitrite is oxidized to nitrate. The genus *Nitrobacter* is considered to be responsible for the second nitrifying reaction:

\[
\text{NO}_2^- + 0.5O_2 \rightarrow \text{NO}_3^-
\]

The overall nitrification reaction shows that the oxidation of ammonium to nitrate requires a high input of oxygen:

\[
\text{NH}_4^+ + 2O_2 \rightarrow \text{NO}_3^- + 2H^+ + H_2O
\]

In a subsequent denitrification step, heterotrophic wastewater bacteria (denitrifiers) use chemically bound oxygen of nitrate and nitrite to degrade the organic compounds of the wastewater under anoxic conditions. In this
process molecular nitrogen is produced. The combination of both processes results in a complete elimination of nitrogen from wastewater.

High concentrations of oxygen and low levels of organic materials provide the ideal conditions for nitrification. Conversely, high levels of biodegradable organic material and the absence of molecular oxygen provide the ideal conditions for denitrification. Nitrification is more sensitive than denitrification. Therefore, most studies have focused on the nitrification process. Nitrification is carried out by autotrophic bacteria which use carbon dioxide as a source of carbon. This autotrophic process is generally accepted to be the slowest step, more sensitive to temperature variations and inhibitory effects by toxic compounds than heterotrophic bacteria responsible for carbon removal.

The processes that occur during biological wastewater treatment are efficient and reliable, but are susceptible to disturbances and toxic loading. Indeed, many inhibitor materials, including a wide variety of organic compounds are present in wastewater. The degree of inhibition by such compounds is also affected by the pH, the concentration of inhibitor, and the concentration of other cations and molecules present (Juliastuti et al., 2003; Pagga et al., 2006). As a result, substances with the potential to inhibit nitrification must be identified to prevent disturbances (Juliastuti et al., 2003). However, there is no clear relationship between bacterial toxicity tests based on different principles or species (Ren, 2004). Many studies have noted the inhibitory effects of various substances during various nitrification processes. The majority of these studies have evaluated the
maximum concentration that can be tolerated and the removal of toxic substances by the primary and the secondary sludge (Brown and Lester, 1979; Rossin et al., 1982).

2.3.3 Activated sludge OUR tests

Respirometry tests have been used to assess potential toxicity of a wastewater stream or a specific compound on both heterotrophic and nitrifying bacteria (Archibald et al., 2001; Pernetti, et al., 2003; Riedel et al., 2002). Review of respirometric tests for nitrification inhibition detection is presented in the next section.

Respirometry tests and their use for assessing inhibition or toxicity to activated sludge microorganisms have been well developed and published by several organizations such as OECD 209 (1993) (Organization for Economic Co-operation and Development); EPA 712-C-96-168 (1996) (Environmental Protection Agency) and ISO 8192 (1986) (International Organization for Standardization).

The use of these tests in their published form or after modification is well-documented in the literature (Mrafkova et al., 2003). The tests are based on the fact that respiration rate of activated sludge or sludge organisms can be reduced in the presence of toxicants. The most common method of measuring activated sludge respiration rate is the oxygen uptake rate. Gendig et al., (2003) developed a modified version of the activated
sludge respiration inhibition test which allowed a prolonged incubation period of 27 hours based on OECD 209 and ISO 8192 protocols.

In addition there many researchers used other methods to quantify activated sludge respiration rate. Liao et al., (2001) described a biosensor for wastewater toxicity based on inhibition to the respiration of oxygen by sensitive bacteria isolated from activated sludge. When the respiration of the bacteria was inhibited due to toxicity, more oxygen was able to cross the membrane in the biosensor, leading to a decrease in the rate of the oxidation – reduction reactions that occurred on the membrane.

Harrison et al., (1976) demonstrated that 30 mg/L of LAS at cell concentration in suspension of 250 mg/L completely inhibited oxygen uptake by pseudomonas s.p. There is limited published literature on anionic surfactants effect on activated sludge respiration. Painter (1986) followed the OECD test for assessing inhibition to activated sludge respiration and reported that LAS did not show inhibition to activated sludge oxygen uptake at concentrations up to 100 mg/L. Verge et al., (1996) reported that the EC$_{50}$ – 3 hours for LAS and the single homologues C$_{10}$ – C$_{14}$ of LAS ranged from 550 mg/L to 760 mg/L whereas EC$_{50}$ – 3 hours for AS was over 1600 mg/L, indicating that AS are less toxic than LAS. Proksová et al. (1998) reported that the anionic surfactant dialkyl sulphosuccinate not only influenced on respiration rate, but also inhibited activity of enzymes in the range of 250 to 300 mg/L and the growth of degrading bacteria was blocked in the whole tested concentration range. Gutiérrez et al. (2002) assessed the toxicity of LAS using two methods, the Microtox® which utilises the vibrio fischeri and
a respirometry test using the bacterial in activated sludge. They reported that LAS showed a toxic effect using the Microtox® method but showed no toxic effects using the respirometry method.

2.3.4 Inhibition to nitrification in activated sludge tests

The most common method applied to study nitrification in activated sludge consist of monitoring the substrate consumption ($NH_{4}^{+} - N$) or product formation ($NO_{2}^{-} - N + NO_{3}^{-} - N$) rate. However, there is very little published literature on anionic surfactant effect on nitrification reactions. The only published article, the author of thesis could find, is that Dalzell et al., (2002). The authors studied in LAS, LAS with metal toxicants mixture and LAS with organic pollutants mixture following Jönsson et al., (2001) nitrification inhibition test and slightly modified following mainly steps described in Swedish EPA Report No. 4424 (1995) with 2 hours period. Result shows that IC$_{50}$ values were 300 mg/L, 200 mg/L and 2 mg/L, respectively.

ISO 9509 is a standard method used to test inhibition to nitrification due to the presence of certain in the wastewater. The inhibition degree is given as a relationship between the concentration of the substance under investigation and the decrease in production of nitrite and nitrates or the decrease in the oxidation of ammonia. Juliastuti et al., (2003) examined the inhibitory effects of heavy metals (Zinc and Copper) and organic compound (Ethyl benzene, Chlorobenzene, Trichloroethylene and Phenol) on nitrification reactions by using ISO 9509 method. Also Pagga et al., (2006)
compared between the two most used laboratory methods for nitrification inhibition, the ISO standard 9509 and the modified ISO 8192. They reported that the inhibition of nitrification depend on the biodegradability of the potential inhibitory compound and concluded. Also ISO 9509 method needed advanced analytical equipment for nitrate and ammonium determination whereas ISO 8192 required low maintenance.

2.3.5 Morphology of activated sludge

The activated sludge process is one of the most frequently used processes for treatment of wastewater. The usual practice by many treatment plants has been the discharge of this secondary clarifier effluent to nearby receiving water. Recently, many treatment plants began to employ water recycling where secondary effluents are further treated using tertiary processes. In both cases, the operation of the secondary clarifier and the settleability of the activated sludge are critical for the operation of the waste water treatment plant (Grijspeerdt and Verstraete, 1997) and for meeting required effluent quality. Activated sludge is a complex ecosystem constituted mainly from bacteria and protozoa. The bacteria are agglomerated as flocs. A good balance between the different species will affect the efficient settleability of the sludge and ensure low level of suspended solids in the effluent (Motta et al., 2001). However, there are many activated sludge treatment plants that have experienced settling failures because of bulking sludge (Grijspeerdt and Verstraete, 1997). One
possible cause of bulking and poor settleability of flocs is the presence of toxic constituents in the influent wastewater

Developing a tool for monitoring activated sludge flocs properties is essential for the operation, control and prediction of the potential or likely occurrence of sludge bulking. An automated procedure for recognizing and characterizing the morphology of activated sludge using image analysis has been developed and used to monitor the biomass in WWTPs. Even the relationship between flocs characteristics and Sludge Volume index (SVI) was ambiguous. Image analysis still can help obtain information on activated sludge composition, size and shape of flocs and filaments and become a reliable tool for warning filamentous bulking problems (Jenné et al., 2004; Motta et al., 2001).

Image analysis has now been used for several years to characterize the morphology of activated sludge. It examines the size distribution and the internal structure of activated sludge flocs by using image analysis. Grijspeerdt and Verstraete (1997) have quantified the size and shape of bacterial flocs using image analysis. They also established the relation between the biomass concentration and the mean projected area. Liwarska – Bizukojc and Bizukojc (2005) showed that SDS affected activated sludge flocs properties even at low concentration. They showed that the mean projected area of flocs decreased by about 30% at SDS concentration in the range from 2.5 mg/L to 25 mg/L. A more severe effect of nearly 60% reduction in the flocs mean projected area was observed for SDS concentrations from 250 mg/L to 2500 mg/L. The authors concluded that the
presence of SDS can lead to deterioration of activated sludge settleability due to its effect on the size of the flocs dimensions. Liwarska – Bizukojc and Bizukojc (2006) examined the effect of three anionic surfactants, SDS, SDBS and sodium alkyltrioxyethylene sulphate (SAS) on activated sludge flocs. The results showed that all tested anionic surfactants strongly influenced the activated sludge morphology and their dehydrogenase activity. Among the tested anionic surfactants, SDBS had the strongest effect on sludge flocs and dehydrogenase activity.

Recently most water treatment plants in Australia have reported a decrease in their inflow and associated increase in the inflow constituents including surfactants. This literature review show that most of the research concerning surfactants was focused either on their removal, ie concentration in the effluent compared with that in the influent, or effect on aquatic life in the receiving waters. Few studies assessed effect of a model surfactant, LAS, on OUR and one recent study on nitrification. Therefore, this study aimed at addressing this gap of knowledge, i.e., effect of increased concentrations of different surfactants on OUR and nitrification at different conditions.
CHAPTER THREE MATERIALS AND METHODS

3.1 Overview

The laboratory studies employed the ISO8192 Water quality – Test for inhibition of oxygen consumption by activated sludge and ISO 9509 Water Quality – Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters. The activated sludge characteristics are described in section 3.2. The surfactants used are described in section 3.3. The OUR test procedure is described in section 3.4 and the nitrification inhibition test procedure is described in section 3.5. Monitoring program of a local WWTP is described in section 3.6. The analytical techniques employed are described in section 3.7.

3.2 Activated sludge preparation

Activated sludge used in this study was obtained from two local domestic WWTP (Sunbury WWTP and Melton WWTP). Samples were collected early morning and the pre-planned tests were performed on the same day of sample collection. For comparison between activated sludge microorganisms activities in the presence of anionic surfactants, the activated sludge sample obtained from WWTP 2 (Melton WWTP) was named AS#2 whereas the sample of activated sludge from Sunbury WWTP
were named AS#1. Upon return to the laboratory, the activated sludge was
allowed to settle and the supernatant was discarded. The solids were then
transferred into a 5 - litre laboratory-scale activated sludge cylinder, diluted
with deionized water, allowed to settle after which the supernatant was
discarded. Next, the activated sludge was washed two times with
appropriate volumes of deionized water. After washing, the concentration of
MLSS was adjusted to contain around 3000 mg/L of MLSS (mixed liquor
suspended solids), as determined by gravimetric analysis. The sample was
then checked for sensitivity of activated sludge, and used in the tests if
within the accepted range. For the nitrification inhibition test, the washed
activated sludge nitrifying activity was determined by following the ISO 9509
(1989) method. The activated sludge was used directly when the nitrification
rate ranged from 2 to 6.5 mg of N/g·h. During this research all samples
collected from the WWTPs showed sensitivities within the recommended
range.

3.3 Surfactants used in this study

Two different surfactants were used in this study, Sodium Dodecyl Sulphate
(Aldrich L-5750) and Sodium Dodecylbenzene Sulphonic Acid (Aldrich
D-2525) (Table 3.1). Stock solutions of each of the surfactants with
concentrations of 1000 mg/L of the surfactants were prepared using Milli-Q
water. Calculations used to determine the amount of surfactant to be added
to the Milli-Q water accounted for the fact that the surfactants obtained from
Aldrich were not 100% pure. For example, the SDS was approximately 95%
based on the total alkyl sulphate content (the composition of alkyl sulphate
is approximately 70% lauryl sulphate with the balance being higher various homologues). Thus, to make a surfactant solution containing 1,000 mg/L SDS, 1,053 mg of SDS was added per 1.0 L of solution. SDBS was approximately 80% surfactant, including all homologues. Specifically, the main homologues are C_{10} – C_{13}, with homologue C_{12} comprising approximately 20%. The remainder of the mixture is sodium sulphate and sodium chloride (approximately 17%) and water (approximately 3%). Thus, to make a 1,000 mg/L SDBS surfactant solution, 1,250 mg of SDBS were added per 1.0 L of solution.

Table 3.1: Chemical name, group of surfactants, molecular weights and structural formulas of the surfactants used

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Group of surfactants</th>
<th>$M_w$ (g/mol)</th>
<th>Structural formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dodecyl sulphate</td>
<td>Alkyl sulphate (AS)</td>
<td>288</td>
<td>$\text{CH}_3(\text{CH}<em>2)</em>{11}-\text{O-SO}_3\text{Na}$</td>
</tr>
<tr>
<td>Sodium dodecyl benzene sulphonate</td>
<td>Linear alkylbenzene sulphonate (LAS)</td>
<td>344</td>
<td>$\text{CH}_3(\text{CH}<em>2)</em>{8-11}-\text{CH}_2\cdot\text{C}_6\text{H}_4\cdot\text{SO}_3\text{Na}$</td>
</tr>
</tbody>
</table>
3.4 OUR tests

The respiration inhibition test was carried out according to the procedure described in ISO 8196. The test was carried out using at least five concentrations in a logarithmic series, blank control and physico-chemical control were included. Physico-chemical control only contains surfactant, synthetic medium and water. For each inhibition test, a series of flasks with reaction mixtures containing a defined concentration of synthetic sewage and activated sludge inoculum but varying concentrations of the anionic surfactant to be tested were prepared. Prior to inhibition testing, the pH in each activated sludge reactor was adjusted to pH 7.5±0.5 by H$_2$SO$_4$ or NaOH. The synthetic sewage stock solution used in the experiment was composed of (per litre): Bacto-Peptone, 16.0 g; Bacto-Beef extract, 11.0 g; Urea, 3.0 g; K$_2$HPO$_4$, 28.0 g; MgSO$_4$·7H$_2$O, 0.2 g; CaCl$_2$·2H$_2$O, 0.4 g; and NaCl, 0.7 g. The final pH of the stock solution was adjusted to pH 7.0 by the addition of H$_3$PO$_4$. Fresh stock solution was prepared as required and stored for not more than seven days at 5°C.

3.4.1 Effect of anionic surfactants on activated sludge OUR using a low concentration of activated sludge

The tests used to evaluate the effect of the selected anionic surfactants on activated sludge OUR were carried out in accordance with the ISO 8192 (1986) Method A. For each inhibition test, a series of reaction flasks containing reaction mixtures were prepared. The reaction mixtures were
prepared by adding 10 mL of synthetic sewage stock solution each containing a different amount of the anionic surfactant to be tested and 10 mL of the activated sludge prepared as described above. The mixture was then diluted to a volume of 300 mL with Milli–Q water, which gave a concentration of activated sludge of approximately 100 to 200 mg of suspended solids per litre. The activated sludge was adjusted to pH 7.5 ± 0.5 by the addition of 0.1M H$_2$SO$_4$ or 0.1M NaOH prior to analysis.

After the addition of activated sludge the mixtures were kept at room temperature. The mixtures were then aerated, after which they were placed in vessels containing a magnetic stirrer and sealed. Next, the samples were thoroughly mixed. The stopper was then removed from the first test vessel and an adapter containing a dissolved oxygen electrode was inserted. The stirrer was then restarted and the concentration of dissolved oxygen was measured. This procedure was continued from 30 minutes to 180 minutes with the oxygen consumption being determined at 10 minute intervals. The data generated from this experiment was then used to calculate the respiration rate. The dissolved oxygen (DO) levels in the vessel were continuously measured using a DO meter with a built in data logger. The DO meter was interfaced to a computer that allowed continuous monitoring of OUR (Figure 3.1).
3.4.2 Effect of anionic surfactants on activated sludge OUR using a high concentration of activated sludge

The tests used to evaluate the effect of the selected anionic surfactants on activated sludge OUR were carried out in accordance with the procedure described in Method B of ISO 8192 (1986). For each inhibition test, a series of reaction mixtures containing a defined concentration of activated sludge, 16 mL synthetic medium and varying concentrations of the anionic surfactant were prepared using Milli–Q water to give final volumes of 500 mL. The activated sludge concentration in the final test mixture was approximately 1500 mg of MLSS per litre. The activated sludge was
adjusted to pH 7.5 ± 0.5 by the addition of 0.1M H₂SO₄ or 0.1M NaOH prior to analysis.

The test mixtures were aerated throughout the test duration at a rate designed to saturate the mixtures with oxygen. All mixtures were incubated in a water bath (Wish Bath®, Model: WSB – 30) at 10°C, 20°C, or 30°C and shake at 50 rpm throughout the experiment. After 30 minutes, an aliquot from the mixture vessel was transferred to a BOD bottle that contained a magnetic stirrer. An adapter with an oxygen electrode was then fitted into the neck of the bottle and the magnetic stirrer was turned on. The concentration of the dissolved oxygen was then measured for 5 minutes, after which the electrode was removed and the aliquot was returned back to the test vessel where shaking and aeration resumed. This procedure was repeated for 180 minutes (Figure 3.2 and Figure 3.3).
Figure 3.2: Experimental set up used to evaluate the activated sludge OUR
Figure 3.3: Photo of (A) Experimental set-up for OUR at controlled temperature (B) DO meters used to measure oxygen consumption for OUR measurements
### 3.4.3 Data analysis

The oxygen uptake rate (referred to in this section as $R$) can be calculated from the linear part of the recorded oxygen concentration versus time graph according to Equation (1)

$$ R = \frac{Q_1 - Q_2}{\Delta t} \times 60 $$  \hspace{1cm} (1)

where $Q_1$ is the oxygen concentration, expressed in mg/L, at the beginning of the linear phase; $Q_2$ is the oxygen concentration, expressed in mg/L, at the end of the linear phase; $\Delta t$ is the time interval, in minutes, between these two measurements.

The inhibitory effect of a test chemical on the respiration rate (oxygen uptake rate) of activated sludge, expressed as %, at each concentration is given by Equation (2)

$$ I = \frac{R_B - (R_T - R_{PC})}{R_B} \times 100 $$  \hspace{1cm} (2)

where $R_T$ is the oxygen consumption rate in the flasks with anionic surfactant; $R_B$ is the oxygen consumption rate in the blank control; $R_{PC}$ is the oxygen uptake rate by the physico-chemical control.
3.5 Nitrification inhibition tests

3.5.1 Effect of anionic surfactants on nitrification in activated sludge

The method used to assess the short–term inhibitory effects of anionic surfactants on nitrifying bacteria in activated sludge was carried out as described in ISO 9509. For each inhibition test, a series of reaction mixtures were prepared. Each of these mixtures contained a defined volume of activated sludge, 25 mL medium which is 5.04 g of sodium hydrogencarbonate (NaHCO₃) and 2.65 g of ammonia sulphate [(NH₄)₂SO₄] in one litre of water and varying concentrations of the anionic surfactant diluted to final volumes of 250 mL with Milli–Q water. The activated sludge concentration in the test mixture was approximately 1500 mg of suspended solid per litre. In addition, a control flask that contained no surfactant and a reference inhibitor (ATU) was included in each experiment to demonstrate the validity of the results. The activated sludge was adjusted to pH 7.5 ± 0.5 by the addition of 0.1 M H₂SO₄ or 0.1 M NaOH prior to analysis.

The test mixtures were aerated throughout the test. All mixtures were incubated in a water bath (Wish Bath®, Model: WSB – 30) at 10°C, 20°C or 30°C throughout the experiment while shaking at 50 rpm (Figure 3.4). Aliquots were collected from each flask at designated time intervals and filtered through a filter paper. The samples were then tested for the
concentrations of nitrite, nitrate and ammonia. This procedure was repeated at 10, 30, 75, 120 and 240 minutes, respectively.

![Experimental set up for inhibition to nitrification in activated sludge tests](image)

Figure 3.4: Experimental set up for inhibition to nitrification in activated sludge tests

### 3.5.2 Data analysis

The level of nitrification inhibition induced by the surfactants was assessed according to ISO 9509 (International Standard ISO 9509, 1989). The ISO 9509 test is based on measurements of the production of nitrite and nitrate after the addition of an ammonia-containing substrate.

The percentage inhibition of the formation of oxidized nitrogen – N (i.e. nitrate and nitrite) is calculated using Equation (3):
\[ %\text{Inhibition} = \frac{C_c - C_t}{C_c - C_b} \times 100 \] (3)

where \( C_c \) is the concentration of oxidized nitrogen - N, in the control flask without inhibitor after incubation in mg/L; \( C_t \) is the concentration of oxidized nitrogen - N, in the flask containing the test substance after incubation in mg/L; \( C_b \) is the concentration of oxidized nitrogen - N, in the flask containing the reference inhibitor (ie., ATU) after incubation in mg/L.

In addition, the nitrification rate during the 4 hours reaction period was determined based on the removal of ammonium during that period using equation (4).

\[ %\text{Inhibition} = \frac{C_i - C_e}{C_o - C_e} \times 100 \] (4)

where \( C_i \) is the concentration of ammonia in the test flask after incubation in mg/L; \( C_e \) is the concentration of ammonia in the control flask after incubation in mg/L; \( C_o \) is the concentration of ammonia at the beginning of the test in mg/L.

A summary of all OUR and inhibition nitrification tests is given in Table 3.2.
<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Test</th>
<th>Duration</th>
<th>Inoculum</th>
<th>Inoculum concentration (mg/L MLSS)</th>
<th>Surfactant type</th>
<th>Surfactant concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>OUR</td>
<td>30 minutes 180 minutes</td>
<td>Activated sludge from domestic wastewater treatment plant I (AS 1#)</td>
<td>1500 mg MLSS/L</td>
<td>SDS, SDBS</td>
<td>5, 10, 20, 25, 30, 40, 50, 75, 100</td>
</tr>
<tr>
<td></td>
<td>Nitrification Inhibition</td>
<td>4 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>OUR</td>
<td>30 minutes 180 minutes</td>
<td>AS 1#</td>
<td>1500 mg MLSS/L</td>
<td>SDS, SDBS</td>
<td>100, 200, 300, 400, 500</td>
</tr>
<tr>
<td></td>
<td>Nitrification Inhibition</td>
<td>4 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>OUR (10ºC and 20ºC)</td>
<td>30 minutes 180 minutes</td>
<td>AS 1#</td>
<td>1500 mg MLSS/L</td>
<td>SDS, SDBS</td>
<td>10, 25, 50, 75, 100</td>
</tr>
<tr>
<td></td>
<td>Nitrification Inhibition (10ºC, 20ºC and 30ºC)</td>
<td>4 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>OUR</td>
<td>30 minutes to 180 minutes in 10 min intervals</td>
<td>AS 1#</td>
<td>100 mg MLSS/L and 1500 mg MLSS/L</td>
<td>SDS, SDBS</td>
<td>5, 10, 15, 20, 25, 50, 100</td>
</tr>
<tr>
<td>V</td>
<td>OUR</td>
<td>30 minutes 180 minutes</td>
<td>Activated sludge from another domestic wastewater treatment plant II (AS 2#)</td>
<td>1500 mg MLSS/L</td>
<td>SDS, SDBS</td>
<td>10, 25, 50, 75, 100</td>
</tr>
<tr>
<td></td>
<td>Nitrification Inhibition</td>
<td>4 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>OUR</td>
<td>30 minutes 180 minutes</td>
<td>AS 1#</td>
<td>1500 mg MLSS/L</td>
<td>SDS and SDBS mixture</td>
<td>20</td>
</tr>
<tr>
<td>VII</td>
<td>OUR</td>
<td>30 minutes to 48 hours</td>
<td>AS 1#</td>
<td>1500 mg MLSS/L</td>
<td>SDS, SDBS</td>
<td>10, 25, 100, 500</td>
</tr>
</tbody>
</table>
3.6 Monitoring of a local WWTP

The occurrence of anionic surfactants concentration in Sunbury WWTP was investigated through a two week monitoring program. The first was in May and the second was in June 2007. Wastewater samples were collected at three locations, influent after primary (influent to biological treatment) and effluent. Samples from the influent were collected using auto sampler VST – 7750 (Manning Environmental Inc, USA). The auto sampler ISCO 3700 (John Morris Scientific Pty Ltd, AUS) was used to collect samples after primary treatment, and auto sampler ISCO 2900 (Instrument specialties Co. Inc, USA) was used for collection of samples from the effluent. The samplers were programmed to collect samples on hourly basis. Each day the 24 individual samples were mixed as a composite sample in proportion with the flow rate. The samples were sent to a commercial lab for analysis for anionic and non-ionic surfactants concentrations. The lab used the MBAS (methylene blue active substances) method (APHA 5540C).

3.7 Analytical techniques

3.7.1 MBAS

SDBS and SDS as single compounds were measured directly according to the methylene blue active substances analysis method 5540C (APHA, 1998). Prior to analysis, samples were filtered through a 0.45 µm syringe
filter of a 25 mm diameter (Whatman Cat. No. 6874 – 2504). MBAS analysis was either performed in duplicate on the same day that samples were collected or else the samples were acidified by 0.1M HCl and refrigerated at 0ºC until analysis. In addition, a calibration curve covering a range of 0 – 1000 mg/L was constructed both for SDS and SDBS. The absorbance was measured at a wavelength of 652 nm using a UV/Vlvis Spectrometer (Lnican, USA) equipped with 10 mm rectangular UV quartz cells (Starna pty ltd, AUS).

3.7.2 Dissolved Oxygen Measurements

For the respirometer experiments, dissolved oxygen was measured using model O₂ 4050e (Mettler toledo, USA) with an InPro 6050/120 oxygen sensor (Mettler toledo, USA).

3.7.3 Activated sludge characterisation

The concentrations of MLSS were measured according to method 2540D (APHA, 1998) in the Standard Method for the Examination of Water and Wastewater.
3.7.4 Ammonia, Nitrate and Nitrite measurements

HACH tests kits (HACH Company, Australia) were used to measure the concentration of NO$_2$-N and NO$_3$-N. Nitrite was measured using HACH method 8507 (NitriVer3 Nitrite Reagent Powder Pills, low range), with a detection limit of 0.004 mg l$^{-1}$ and nitrate was measured using HACH method 8039 (NitraVer5 Nitrate Reagent Powder Pills, high range) with a detection limit 0.3 mg l$^{-1}$. Ammonia was measured using the Nessler method (detection limit 0.1 mg l$^{-1}$) according to the Standard Method for the Examination of Water and Wastewater method (APHA, 1998), which is equivalent to APHA Method 4500-NH$_3$C, using a UNICAM UV/Vis Spectrometer absorbance at 420 nm.

3.7.5 Microscopic analysis

3.7.6.1 Image capture

Digital images were obtained using an Olympus SZ-CTV microscope (Japan) at 100 × magnification. The microscope was equipped with a PixeLINK video camera (Canada) and connected to a PC. The images were stored and processed with the aid of a PixeLINK Capture SE. Version 2.2. The images were then analysed using the Image J software. Samples were viewed directly in a drop of mixed liquid that was carefully deposited on a
slide and covered with a cover slip without staining or fixation. The images were viewed through a blue filter.

**3.7.6.2 Image analysis**

The mean projected area and the perimeters of the flocs were measured using the Image J software. The mean projected area is considered the basic image analysis parameter and is easily found by pixel count multiplication using a scaling factor (Liwerska-Bizukojc and Bizukojc, 2006). The other parameters are derived from the mean projected area. The perimeter is defined to be the length of the boundary of the object.

In addition, the size of the sludge flocs is an important parameter with regard to their settling properties (Grijspeerdt and Verstraete, 1996; Jenné et al., 2004). According to Equation 5, the size of the flocs is expressed as the equivalent diameter (Deq), which is calculated from the actual projected area as follows:

\[ D_{eq} = 2 \sqrt{\frac{\text{area}}{\pi}} \quad (5) \]

In addition, the shape of the sludge flocs is related to their settling properties. The form factor (FF) is a parameter (Grijspeerdt and Verstraete, 1996; Jenné et al., 2004) that describes the deviation of an object from a circle and is particularly sensitive to the “roughness” of the boundaries. The form factor is given by Equation 6.
\[ FF = \frac{4 \cdot \pi \cdot \text{Area}}{\text{Perimeter}^2} \]
A monitoring program of the performance of a local WWTP, Sunbury Treatment Plant, was developed and performed during the period from May to June 2007. The program involved collection of a 24 samples / day from three different locations at the treatment plant, influent, primary treatment effluent and secondary treatment effluent. The samples were collected for one week in May 2007 and repeated in June 2007. For each day the 24 samples were used to prepare a composite sample. The samples were sent for analysis for anionic and nonionic surfactants.

The results for anionic surfactants concentration at these three points in the treatment plant are shown in Table 4.1. The anionic surfactants concentrations in the influent ranged from 6.4 mg/L to 14 mg/L and ranged from 2.8 mg/L to 14 mg/L in the influent to the aeration tank.
### Table 4.1A: Concentration of Anionic Surfactants at Different Locations at a Local Treatment Plant for the Period 30 April to 6 May 2007

<table>
<thead>
<tr>
<th>Date</th>
<th>30/04</th>
<th>01/05</th>
<th>02/05</th>
<th>03/05</th>
<th>04/05</th>
<th>05/05</th>
<th>06/05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent (mg/L)</td>
<td>10</td>
<td>9.3</td>
<td>6.4</td>
<td>9.6</td>
<td>12</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>After primary tank (mg/L)</td>
<td>9</td>
<td>2.8</td>
<td>14</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>After secondary tank (mg/L)</td>
<td>0.24</td>
<td>0.12</td>
<td>0.46</td>
<td>0.51</td>
<td>0.72</td>
<td>0.77</td>
<td>0.44</td>
</tr>
</tbody>
</table>

### Table 4.1B: Concentration of Anionic Surfactants at Different Locations at a Local Treatment Plant for the Period 5 June to 10 June 2007

<table>
<thead>
<tr>
<th>Date</th>
<th>05/06</th>
<th>06/06</th>
<th>07/06</th>
<th>08/06</th>
<th>09/06</th>
<th>10/06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent (mg/L)</td>
<td>9.4</td>
<td>7.6</td>
<td>8.8</td>
<td>0.85</td>
<td>5.5</td>
<td>9</td>
</tr>
<tr>
<td>After primary tank (mg/L)</td>
<td>7.9</td>
<td>7.6</td>
<td>5.1</td>
<td>0.78</td>
<td>6.3</td>
<td>8.6</td>
</tr>
<tr>
<td>After secondary tank (mg/L)</td>
<td>0.1</td>
<td>0.27</td>
<td>0.57</td>
<td>0.62</td>
<td>0.09</td>
<td>0.13</td>
</tr>
</tbody>
</table>
4.2 OUR tests

4.2.1 Effect of pH on activated sludge OUR

During the preliminary stage of this research experiments were conducted to examine the effect of pH on the OUR of activated sludge. The results obtained are shown in Figure 4.1. It is observed that the highest OUR occurred in the range pH = 7.5 to pH = 8. Therefore all experiments carried out as part of this research were conducted at this pH range.

Figure 4.1: Effect of pH on activated sludge OUR
4.2.2 Sensitivity of the activated sludge samples check

Many researchers reported the EC$_{50}$ values obtained with 3, 5 – Dichlorophenol according to the ISO 8192 respiration inhibition test were reported to be 10 mg/L (Strotman et al., 1994), 12 mg/L (Klečka and Landi, 1985) and 20 mg/L (Yoshika et al., 1986).

For all activated sludge samples obtained from the same local domestic wastewater treatment plant but at different days, the EC$_{50}$ values were in the range of 11.0 mg/L to 14.5 mg/L. A sample result is shown in Figure 4.2. The results were found in the range from 5 to 30 mg/L which met the ISO 8192 standard test requirements. All experiments were repeated at least five times to ensure reproducibility.
4.2.3 Effect of SDS on activated sludge OUR

4.2.3.1 Effect of SDS on activated sludge OUR for concentrations from 5 to 500 mg/L.

The effect of the presence of varying concentrations of SDS on OUR was assessed for two distinct ranges, 5 – 100 mg/L and 100 – 500 mg/L. Figure 4.3 A and B shows the OUR and inhibition for increasing concentrations of SDS from 5 mg/L to 100 mg/L. It was observed that the OUR decreased with the increase in the concentration of SDS.
It is also observed that the extent of the effect of the presence SDS on OUR lessened with increasing incubation time from 30 minutes to 180 minutes.

To evaluate the magnitude of the effect of SDS on OUR, it is important to compare OUR in the presence of SDS with that in its absence (or low concentrations), therefore the results were interpreted in terms of inhibition to OUR. As discussed in chapter 3, section 3.4.4, inhibition is a measure of the reduction in OUR due to the presence of SDS relative to that for the control reactor, which does not have any SDS. Applying this analysis, it was found that the inhibitory effects on OUR increased with increased concentrations of SDS. For example, the percentage of inhibition to OUR increased from 12.9% to 44.2% for SDS concentrations from 5 to 100 mg/L, after 30 minutes of incubation (Figure 4.3B). This effect lessened with increased exposure to SDS. In other words, increasing the incubation time to 180 minutes decreased inhibition to activated sludge OUR by 57% and 38% for SDS concentrations of 5 mg/L and 100 mg/L, respectively. These results suggest that the activated sludge microorganisms can acclimate, to an extent, with the presence of SDS and that this acclimations can minimise the inhibitory effect but does not eliminate it.

There was no 50% inhibition (IC$_{50}$) for SDS concentrations in the range from 5 mg/L to 100 mg/L. The concentrations at which 20% inhibition (IC$_{20}$) was observed, were 12.36 mg/L and 58.3 mg/L after 30 minutes and 180 minutes, respectively. These results indicate that activated sludge has the ability to biodegrade SDS and/or use it for growth.
Figure 4.3: Activated sludge OUR (A) and associated percentage of inhibition (B) for SDS concentrations from 5 to 100 mg/L
SDS concentrations of 100 – 500 mg/L, especially those at the high concentration are quite unrealistic, i.e. not likely reach domestic WWTPs, except if through industrial effluents discharged to the plant (Liwarska-Bizukojc and Bizukojc, 2006). It reported that the average concentration of anionic surfactants in industrial wastewater that reached 300 mg/L. These experiments were carried out to investigate the effect of SDS at extreme conditions. Oxygen uptake rates and the percentage of inhibition as functions of SDS concentration are shown in Figure 4.4 A and B.

The OUR of activated sludge for SDS concentrations from 100 mg/L to 500 mg/L are shown in Figure 4.4A. It was observed that the rate of drop in OUR for concentrations from 100 – 500 mg/L was higher than that for concentrations from 5 - 100 mg/L. In terms of inhibition, increasing the concentration to 100 mg/L induced 28.9% inhibition to OUR, which almost doubled reaching 61.9% with the increase in SDS concentration from 100 to 500 mg/L. In addition it was observed that extending the exposure time from 30 to 180 minutes did not cause the same level of recovery in the magnitude of OUR compared with that observed for the concentration range of 5 -100 mg/L (Figure 4.4B). For example, the inhibition to OUR decreased from 45.3% to 28.9% (ie., 36% recovery in OUR) at 100 mg/L, compared to an increase from 71.8% to 61.9% (ie., approximately 14% recovery in OUR) at 500 mg/L, with the increase in exposure time from 30 minutes to 180 minutes, respectively. This indicates that SDS inhibits activated sludge microorganisms leading to a slower respiration, or growth, but does not toxify and kill the microorganisms.
The values of IC$_{50}$ for SDS concentrations from 5 – 500 mg/L were approximately 165 mg/L and 342 mg/L after 30 minutes and 180 minutes, respectively. These IC$_{50}$ concentrations are much lower compare with the IC$_{50}$ of 1600 mg/L. It reported by Verge et al. (1996) for commercial alcohol sulphates inhibition to activated sludge respiration. These results suggest that SDS could have more severe inhibition effect on activated sludge compared with the commercial alcohol sulphates.
Figure 4.4: Activated sludge OUR (A) and associated percentage of inhibition (B) for SDS concentrations from 100 to 500 mg/L
4.2.3.2 Removal of SDS during OUR tests

Although the methylene blue active substances (MBAS) analysis is used to measure the concentration of anionic surfactants as one group, it was utilised to measure the removal of SDS being the only detectable anionic surfactant in the system. Although there may be other anionic surfactants in the sample adsorb to the activated sludge the concentrations that may remain or desorb in the system after the preparation procedure would be much lower than the concentration used in this study. Initial SDS concentrations after 30 minutes and 180 minutes contact during the OUR test are summarized in Table 4.2. The result shown that only 16% and 10.9% of the 10 and 100 mg/L SDS, respectively, were removed after 30 minutes contact with the activated sludge. After 180 minutes the removal increased to approximately 67.4% and 35.3% for the same concentrations.
4.2.3.3 Effect of temperature on activated sludge OUR

The results shown in Figures 4.3 and 4.4 are from experiments performed at room temperature 20°C ± 2. Inhibition to activated sludge OUR was also assessed at 10°C. Decreasing the temperature to 10°C significantly increased SDS inhibition to activated sludge OUR (Figure 4.5). For example, after 3 hours of exposure to 10 mg/L SDS the inhibition increased from 7% to 46% for temperature decrease from 20°C to 10°C. It was also observed that inhibition to OUR at low temperatures did not diminish with increased reaction time (contact between the synthetic wastewater that contains SDS and activated sludge). Inhibition decreased from 32% to 17% at 50 mg/L SDS with increased exposure from 30 minutes to 180 minutes at 20°C, compared to a slight drop from 66% to 60% at 10°C for the same SDS concentration.

<table>
<thead>
<tr>
<th>Initial concentration (mg/L)</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration after 30 minutes (mg/L)</td>
<td>8.4</td>
<td>21.0</td>
<td>42.5</td>
<td>65.4</td>
<td>89.1</td>
<td>476</td>
</tr>
<tr>
<td>SDS removal (%)</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>12.8</td>
<td>10.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Concentration after 180 minutes (mg/L)</td>
<td>3.3</td>
<td>11.2</td>
<td>27.2</td>
<td>48.9</td>
<td>64.7</td>
<td>412</td>
</tr>
<tr>
<td>SDS removal (%)</td>
<td>67.4</td>
<td>55.4</td>
<td>45.6</td>
<td>34.8</td>
<td>35.3</td>
<td>17.6</td>
</tr>
</tbody>
</table>
The significant effect of temperature on activated sludge is also evident considering the values of IC$_{50}$ and IC$_{20}$. As discussed previously there was no IC$_{50}$ for SDS concentrations from 5 – 100 mg/L at 20°C, whereas with the decrease in temperature to 10°C, an IC$_{50}$ was observed at 8.4 mg/L and 17.0 mg/L after 30 minutes and 180 minutes reaction time, respectively. In addition, the IC$_{20}$ dropped from approximately 58 mg/L to 4 mg/L SDS with the temperature drop from 20°C to 10°C, after 180 minutes reaction time.

Figure 4.5: SDS inhibition to activated sludge OUR for different temperatures. The data depicted are the average

4.2.3.4 The morphology of flocs in the presence of SDS

66
The microscopic images of activated sludge flocs collected from the reactors used to measure activated sludge OUR for different SDS initial concentrations are shown in Figures 4.6 and 4.7 for OUR tests at 10°C and 20°C respectively.

The images obtained by using the microscope are plotted in Figures 4.8 and 4.9. These figures show that both the mean projected area and the perimeter of the activated sludge flocs decreased in the presence of SDS compared with those for the control reactor (ie., in the absence of SDS), for all SDS concentrations.

However, the reduction in the mean projected area of the flocs was larger compared with the reduction in the flocs perimeter. For example, the mean projected area for the flocs, collected from the OUR tests at 20°C, decreased from approximately 50,000 µm² for the control to 25,000 µm² in the presence of 100 mg/L SDS concentration (ie., around 50% reduction). Whereas, the perimeter decreased from 850 µm for the control to 650 µm at 100 mg/L SDS (ie., around 24% reduction). This trend was also observed at 10°C. The results indicated that increased SDS concentration caused a decrease in the activated sludge flocs mean projected area and perimeter, as a result, the activated sludge flocs became subsequently smaller which may effects on it settleability.

Furthermore, the mean projected area of flocs decreased with temperature for all SDS concentrations. For example, the flocs mean projected area at
50 mg/L SDS decreased from 30,000 µm^2 to 27,000 µm^2 at 20°C and 10°C respectively.
Figure 4.6: Microscopic images of activated sludge flocs at the end of OUR tests for different SDS concentrations after 3 hours at 10°C (a) control reactor (b) 10 mg/L SDS (c) 25 mg/L SDS (d) 50 mg/L SDS (e) 100 mg/L SDS (images at magnification 100× with blue filter)
Figure 4.7: Microscopic images of activated sludge flocs at the end of OUR tests for different SDS concentrations after 3 hours at 20°C (a) control reactor (b) 10 mg/L SDS (c) 25 mg/L SDS (d) 50 mg/L SDS (e) 100 mg/L SDS (images at magnification 100× with blue filter)
Figure 4.8: The mean projected area for activated sludge flocs in the presence of different concentrations of SDS for OUR tests at 10°C and 20°C.

Figure 4.9: The perimeter values for activated sludge flocs in the presence of different concentrations of SDS for OUR tests at 10 °C and 20°C.
The changes of three parameters associated with flocs size, the mean projected area, the perimeter and the equivalent circle diameter are shown in Tables 4.3 – 4.5 from samples collected after 3 hours OUR tests, it resulted in a fast decrease of mean projected area and perimeter in both SDS and SDBS tests.

The change in the flocs mean projected area compared with the control for selected SDS concentrations, 10, 25, 50 and 100 mg/L is summarised in Table 4.3. The results show that the reduction in the flocs area was larger for SDS concentrations less than 50 mg/L (almost 48%) compared with that from 50 to 100 mg/L SDS (48% to 54.2%). A similar trend was observed at 20°C but the effect was less severe where 32% reduction was observed at 100 mg/L SDS.

Considering the perimeters of the flocs it was observed that the temperature had a less significant effect on this parameter and that the rate of change in the flocs perimeter was faster at 20°C compared with that at 10°C. The perimeter decreased by 11.90% and 16.92% for SDS concentrations of 10 and 25 mg/L, respectively, at 10°C. Similarly, the perimeter decreased by 4.93% and 9.52% at 20°C, for the same concentrations.

The equivalent circle diameter ($D_{eq}$) is an important parameter property of the sludge flocs that has a great impact on the sludge settling properties. The change in the flocs $D_{eq}$ with increased initial concentration in SDS are summarised in Table 4.5. The flocs $D_{eq}$ decreased by about 32% at 10°C.
and by 27% at 20°C. These results are consistent with the change in the flocs perimeter.

The changes of mean projected area, perimeter and equivalent circle diameter parameters indicated that the anionic surfactant SDS can have a severe affect on activated sludge flocs size and area. The temperature effect on the mean projected area was more pronounced compared with the effect on the perimeter and $D_{eq}$ of the flocs. This indicated that SDS effects on activated sludge flocs equivalent circle diameter, as a result, the activated sludge flocs were became smaller.

Table 4.3: The change in the mean projected area of activated sludge flocs at the end of OUR tests for different SDS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDS concentration (mg/L)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>10</td>
<td>22.23%</td>
</tr>
<tr>
<td>25</td>
<td>28.51%</td>
</tr>
<tr>
<td>50</td>
<td>48.78%</td>
</tr>
<tr>
<td>100</td>
<td>54.21%</td>
</tr>
</tbody>
</table>
Table 4.4: The change in the perimeter of activated sludge flocs at the end of OUR tests for different SDS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDS concentration (mg/L)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>10</td>
<td>11.90%</td>
</tr>
<tr>
<td>25</td>
<td>16.92%</td>
</tr>
<tr>
<td>50</td>
<td>28.34%</td>
</tr>
<tr>
<td>100</td>
<td>32.37%</td>
</tr>
</tbody>
</table>

Table 4.5: The equivalent diameter ($D_{eq}$) of activated sludge flocs at the end of OUR tests for different SDS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDS concentration (mg/L)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>Control</td>
<td>261.78</td>
</tr>
<tr>
<td>10</td>
<td>230.86</td>
</tr>
<tr>
<td>25</td>
<td>221.33</td>
</tr>
<tr>
<td>50</td>
<td>187.34</td>
</tr>
<tr>
<td>100</td>
<td>177.15</td>
</tr>
</tbody>
</table>

In addition to the activated sludge dimensions, this study investigated the changes in the shape of flocs. The results are summarized in Table 4.6. The form factor (FF) is a measure of the deviation of an object from a circle. The FF for the flocs in the presence of SDS showed little variation compared to
the FF for the flocs in the in control for all SDS concentrations tested. Also, the results showed that temperature has no strong effect on the flocs shape. These results indicate that the presence of SDS affect the sludge size not shape.

Table 4.6: The Form factor (FF) of activated sludge flocs at the end of OUR tests for different SDS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDS concentration (mg/L)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 °C</td>
</tr>
<tr>
<td>Control</td>
<td>0.778</td>
</tr>
<tr>
<td>10</td>
<td>0.780</td>
</tr>
<tr>
<td>25</td>
<td>0.806</td>
</tr>
<tr>
<td>50</td>
<td>0.776</td>
</tr>
<tr>
<td>100</td>
<td>0.780</td>
</tr>
</tbody>
</table>

The MLSS in the activated sludge respiration reactors were measured at the end of each OUR test (Figure 4.10). The MLSS in the respiration reactors used for OUR decreased with increased SDS concentrations. For OUR tests at 10 °C, the MLSS decreased from 1.46 g/L to 1.23 g/L with the increase in SDS concentration from 10 mg/L to 100 mg/L, compared with 1.73 g/L in the control reactor (i.e., absence of SDS). Similarly, the MLSS decreased from 1.57 g/L to 1.28 g/L with the increase in SDS concentration from 10 mg/L to 100 mg/L, compared with 1.66 g/L in the control run at 20 °C.
(ie., in the absence of SDS). These results indicated that almost 30% and 23% of the MLSS were lost after exposure for 100 mg/L SDS for 3 hours, at 10°C and 20°C, respectively. It is difficult to explain why the MLSS decreased when no sludge was discharged. One possible reason could be due to SDS causes foaming lead to loss of sludge. Another possibility is may be because surfactants inhibit microorganisms growth. It is necessary to carry out further study on the microbial community composition and microbial growth kinetics in order to find the reasons.

Figure 4.10: MLSS at end of OUR tests for different SDS concentrations at 10°C and 20°C
4.2.4 Effect of SDBS on activated sludge OUR

4.2.4.1 Effect of SDBS on Activated Sludge OUR for concentrations from 5 to 500 mg/L

The results in Figure 4.11 show the effect of SDBS on activated sludge OUR. The OUR decreased with increased SDBS concentrations at a higher rate for concentrations from 5 to 20 mg/L than that for concentrations from 20 to 100 mg/L (Figure 4.11A). SDBS inhibition to OUR increased from 19.8% to 79.1% for concentrations from 5 to 100 mg/L, after 30 minutes of exposure. This inhibition declined to 15% and 69.2% with increased exposure time to 180 minutes (Figure 4.11B). The severe effect of SDBS on activated sludge OUR is clearly shown considering the IC values. The IC$_{20}$ for SDBS were 5.1 mg/L and 10.0 mg/L at 30 and 180 minutes, respectively. These values are lower than SDS IC$_{20}$ of 12.3 mg/L and 58.3 mg/L measured after 30 minutes and 180 minutes, respectively. There was no IC$_{50}$ for SDS from 5 – 100 mg/L. However, the inhibitory effect for SDBS reached 50% (i.e., IC$_{50}$) at 18.8 mg/L and 41.3 mg/L after 30 minutes and 180 minutes, respectively. SDBS inhibition to OUR decreased by approximately by 24% at 5 mg/L and by 13% at 100 mg/L.
Figure 4.11: Activated sludge OUR (A) and associated percentage of inhibition (B) for SDBS concentrations from 5 to 100 mg/L.
Figure 4.12 illustrated that the presence of SDBS at concentrations ranging from 100 to 500 mg/L had a strong inhibitory effect on activated sludge OUR as indicated by the 79.0% and 91.1% measured at 100 mg/L and 500 mg/L respectively after 30 minutes exposure. This inhibition slightly decreased to 69.8% and 86.4% after 180 minutes.

Verge et al. (1996) assessed the effect of pure LAS C\textsubscript{10} – C\textsubscript{14} homologues on activated sludge respiration according to the OECD - 209 procedures. They observed 50% inhibition (EC\textsubscript{50}-3 hours) in the range 500 – 723 mg/L for Na LAS-C12 and 1042 – 1200 mg/L for Na LAS-C10. They also found that the EC\textsubscript{50}-3 hours for several LAS homologue mixtures were from 550 – 760 mg/L. These concentrations are much higher than the IC\textsubscript{50} – 3 hrs obtained for SDBS in this research, indicating that SDS and SDBS used in this research have more inhibitory effect on activated sludge compared with LAS used by Verge et al. (1996). This could be attributed to the presence of benzene and its effect on the mechanism of biodegradation of SDBS.

According to Hashim et al. (1992) and Perales et al. (1999) the mechanism of the breakdown of LAS involves the degradation of the straight alkyl chain, the sulphonate group and finally the benzene ring. They explained that breakdown of the branched alkyl group is more complex than the straight chain where degradation can not be through oxidation by microorganisms rather it must be through loss of carbon atoms one at a time. According to Nunes-Halldorson et al. (2004), the toxicity of benzene reached 33% when toxicity assays were performed at 20 ppm concentrations. Therefore, SDBS inhibition to the biological activities can be attributed to the presence of benzene and its toxicity to microorganisms.
Figure 4.12: Activated sludge OUR (A) and associated percentage of inhibition (B) for SDBS concentrations from 100 to 500 mg/L
4.2.4.2 Removal of SDBS during OUR tests

Table 4.7 show the concentration the concentration of the anionic surfactant SDBS as determined by the before and after the OUR tests. The result showed that only 11% to 2% of the initial SDBS concentrations of 10 mg/L and 500 mg/L were removed after 30 minutes. This removal increased to approximately 42% to 9% at 180 minutes. Comparing these inhibition values with those obtained for SDS, it is clear that SDBS has higher inhibitory effect on activated sludge OUR than SDS, which could be related to the structure of SDBS and the presence of the aromatic ring structure.

The low removal of SDS and SDBS indicate that adsorption of these surfactants to activated sludge particles did not occur during the duration of the test, or that only small percentages of these surfactants were adsorbed to activated sludge. The results also suggest that the inhibition is not due to the breakdown of these surfactants into more toxic compounds rather it is more likely due to interference with the biodegradation mechanism. Interference with the biodegradation process could be due the interference with the availability or transfer of oxygen (O₂).
Table 4.7: SDBS removal during OUR tests

<table>
<thead>
<tr>
<th>Initial concentration (mg/L)</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration after 30 minutes (mg/L)</td>
<td>8.9</td>
<td>23.1</td>
<td>45.2</td>
<td>70.1</td>
<td>90.4</td>
<td>489</td>
</tr>
<tr>
<td>SDBS removal (%)</td>
<td>11</td>
<td>7.6</td>
<td>9.6</td>
<td>6.5</td>
<td>9.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Concentration after 180 minutes (mg/L)</td>
<td>5.8</td>
<td>15.3</td>
<td>35.4</td>
<td>56.7</td>
<td>79.1</td>
<td>457</td>
</tr>
<tr>
<td>SDBS removal (%)</td>
<td>42</td>
<td>38.8</td>
<td>29.2</td>
<td>24.4</td>
<td>20.9</td>
<td>8.6</td>
</tr>
</tbody>
</table>

4.2.4.3 Effect of temperature on activated sludge OUR

The results shown in Figure 4.13 show that SDBS inhibition to activated sludge OUR at 10°C was more than 50% for all concentrations from 5 – 100 mg/L. The results also showed that SDBS inhibition to activated sludge OUR with changes in the temperature, especially when concentrations lower than 25 mg/L. The inhibition to OUR in the presence of 10 mg/L SDBS was around 62% at 10°C compared with 29% at 20°C, after 30 minutes. Only a small reduction in inhibition was observed with increased duration of the OUR test (i.e., exposure to SDBS). For example, after 3 hours, inhibition to OUR reduced to 55% at 10°C and 20% at 20°C, for the same concentration of 10 mg/L. In comparison with SDS (Figure 4.5), it was found
that SDBS has much stronger effect on activated sludge OUR than SDS under low or high temperature conditions.

It has been established that temperature affect activated sludge micro-organisms, not only in governing the rate of reaction, but also in giving rise to significant changes in the microorganisms population structure. The effect of the results obtained at different temperatures for anionic surfactants can be explained as being due to the surfactants effect on the rate of growth and rate of oxidation (or substrate utilisation). It has been reported that activated sludge liquors develop less filamentous bacteria at higher temperatures and have been shown to take longer to acclimatise to changes in temperature than to toxic compounds (Gray, 2004).

Figure 4.13: SDBS inhibition to activated sludge OUR for different temperatures. The data depicted are the average
4.2.4.4 The morphology of flocs in the presence of SDBS

The microscopic images of activated sludge flocs collected at the end of OUR tests from the reactors used to measure the effect of SDBS at different initial concentrations on activated sludge OUR are shown in Figures 4.14 and 4.15 for tests at 10°C and 20°C respectively. Three samples were collected at the end of each OUR test, then for each sample 5 – 10 images were captured and analysed for flocs mean projected area, perimeter, $D_{eq}$ and form factor using the software available with the microscope used for taking these images.

The mean projected area and the perimeter of flocs from all images collected were averaged and the values obtained are shown in Figures 4.16 and 4.17. These figures show that both the mean projected area and the perimeter of the activated sludge flocs decreased significantly with the increase in SDBS concentration, compared with those for the control reactor (ie., in the absence of SDBS), for all SDBS concentrations. Furthermore, the mean projected area of flocs decreased with temperature for all SDS concentrations. For example, the flocs mean projected area at 50 mg/L SDS decreased from 30,000 $\mu m^2$ to 27,000 $\mu m^2$ with the decrease in temperature from 20°C to 10°C.
Figure 4.14: Microscopic images of activated sludge flocs at the end of OUR tests for different SDBS concentrations after 3 hours at 10°C (a) control reactor (b) 10 mg/L SDBS (c) 25 mg/L SDBS (d) 50 mg/L SDBS (e) 100 mg/L SDBS (images at magnification 100× with blue filter).
Figure 4.15: Microscopic images of activated sludge flocs at the end of OUR tests for different SDBS concentrations after 3 hours at 20 °C (a) control reactor (b) 10 mg/L SDBS (c) 25 mg/L SDBS (d) 50 mg/L SDBS (e) 100 mg/L SDBS (images at magnification 100× with blue filter).
Figure 4.16: The mean projected area for activated sludge flocs in the presence of different concentrations of SDBS for OUR tests at 10°C and 20°C.

Figure 4.17: The perimeter values for activated sludge flocs in the presence of different concentrations of SDBS for OUR tests at 10°C and 20°C.
The changes in the three parameters related to the flocs size, namely the mean projected area, perimeter and $D_{eq}$ are shown in Tables 4.8 - 4.10. The change is calculated as the percentage of change in the selected parameter for the flocs in the reactor in the presence of SDBS relative to the flocs in the control. The results in Table 4.8 shown that the flocs mean projected area decreased by 42.21% at 10°C compared with 33.13% at 20°C for SDBS concentration of 10 mg/L. The decrease in the mean projected area can reach 72.96% at 10°C and 61.36% at 20°C in the presence of SDBS at 100 mg/L. The same trend was also observed for the activated sludge flocs, where the flocs perimeter decreased by 46.60% at 10°C and 37.35% at 20°C at 100 mg/L SDBS.

The results also showed that SDBS concentrations less than 25 mg/L induce significant change in the flocs projected area but the effect of SDBS concentration on the flocs projected area became less significant at concentrations larger than 50 mg/L. This means that threshold or tolerance of the activated sludge microorganisms decrease with increased temperature. These results are consistent with the trend in $IC_{20}$ and $IC_{50}$ observed at both temperatures.

Furthermore, the equivalent circle diameter ($D_{eq}$) decreased with the increase in the initial concentration of SDBS (Table 4.10).

The results summarised in Tables 4.8 – 4.10 show that SDBS has a stronger negative effect on activated sludge flocs than SDS (Tables 4.3 -4.5) indicating that SDBS is more likely to induce problems in the operation
of activated sludge processes especially settling properties than would SDS. The results also suggest that SDBS would have such an effect at low and room temperatures whereas SDS negative effects would occur at high concentrations and low temperature.

Table 4.8: The change in the mean projected area of activated sludge flocs at the end of OUR tests for different SDBS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDBS concentration (mg/L)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>10</td>
<td>42.21%</td>
</tr>
<tr>
<td>25</td>
<td>63.66%</td>
</tr>
<tr>
<td>50</td>
<td>69.57%</td>
</tr>
<tr>
<td>100</td>
<td>72.96%</td>
</tr>
</tbody>
</table>

Table 4.9: The change in the perimeter of activated sludge flocs at the end of OUR tests for different SDBS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDBS concentration (mg/L)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>10</td>
<td>22.74%</td>
</tr>
<tr>
<td>25</td>
<td>38.80%</td>
</tr>
<tr>
<td>50</td>
<td>43.69%</td>
</tr>
<tr>
<td>100</td>
<td>46.60%</td>
</tr>
</tbody>
</table>
Table 4.10: The equivalent diameter (Deq) of activated sludge flocs at the end of OUR tests for different SDBS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDBS concentration (mg/L)</th>
<th>Temperature</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
<td>20°C</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>326.73</td>
<td>254.73</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>248.37</td>
<td>208.3</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>196.95</td>
<td>193.62</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>180.25</td>
<td>180.7</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>169.88</td>
<td>158.35</td>
<td></td>
</tr>
</tbody>
</table>

In addition to activated sludge dimensions, the effect of SDBS on the shape of flocs was investigated through the values of the form factor (FF). The results showed that SDBS had no significant effect on activated sludge flocs. Similarly the temperature had no measurable effect on the flocs shape.

Table 4.11: The Form factor (FF) of activated sludge flocs at the end of OUR tests for different SDBS concentrations and temperatures

<table>
<thead>
<tr>
<th>SDBS concentration (mg/L)</th>
<th>Temperature</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
<td>20°C</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.814</td>
<td>0.777</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.787</td>
<td>0.783</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.789</td>
<td>0.779</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.782</td>
<td>0.786</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.772</td>
<td>0.766</td>
<td></td>
</tr>
</tbody>
</table>
The MLSS in the activated sludge were measured at the end of each OUR test and the change of MLSS resulted in Figure 4.18. The MLSS in the respiration reactors used for OUR decreased with increased SDBS concentrations. For OUR tests at 10 °C, the MLSS decreased from 1.50 g/L to 1.23 g/L with the increase in SDBS concentration from 10 mg/L to 100 mg/L, compared with 1.83 g/L in the control reactor (ie., in the absence of SDBS). Similarly, the MLSS decreased from 1.52 g/L to 1.27 g/L with the increase in SDBS concentration from 10 mg/L to 100 mg/L, compared with 1.66 g/L in the control run at 20 °C (ie., in the absence of SDBS). These results indicated that almost 33% and 24 % of the MLSS were lost after exposure for 100 mg/L SDS for 3 hours, at 10 °C and 20 °C respectively. SDBS have stronger effect on MLSS lost than SDS at 10 °C. Some fraction of the sludge was likely lost due to its foaming property. However, there is no measurable effect on MLSS lost after exposure for 100 mg/L SDS and SDBS at 20 °C, respectively, it indicated that temperature strongly effect on surfactants foaming under lower temperature. It is necessary to carry out further study on the foaming characteristics.
Figure 4.18: MLSS at end of OUR tests for different SDBS concentrations at 10°C and 20°C
4.2.5 Effect of MLSS concentration on activated sludge

**OUR at different concentration of SDS and SDBS**

Activated sludge systems operate at designated MLSS and food to microorganisms ratio (F/M). Therefore it is expected that the concentration of MLSS will affect the rate of substrate biodegradation among other factors. The OUR test carried out as part of this research were performed according to the standard method. However the effect of MLSS concentration on the test has not been studied before. In this section the effect of MLSS concentration on activated sludge OUR inhibition due to the presence of SDS or SDBS is discussed.

The effect of MLSS on the inhibition to OUR tests was assessed for two MLSS concentrations, a low concentration of 100 mg/L and a concentration of 1500 mg/L (typical concentration of MLSS in OUR tests). The results in Figure 4.19 show that inhibition to activated sludge OUR increased from 4.3% to 68.3% with the increase in SDS concentrations from 5 – 100 mg/L, after 180 minutes. However, for MLSS concentration of 1500 mg/L, inhibition to OUR for the same concentrations, increased from 1.9% to 30.4%, after 180 minutes. Similar trend was observed for SDBS. Inhibition at 100 mg/L SDBS after 180 minutes using low MLSS of 100 mg/L was 85% compared with 54.3% using 1500 mg/L MLSS (Figure 4.20). These results suggest that the concentration of MLSS in the OUR tests is very critical. The optimum concentration varies with the type of substrate and its inhibitory
properties. Therefore care should be taken when performing these tests to ensure adequate MLSS concentration is available in the bottles.
Figure 4.19A: The inhibition of SDS on activated sludge OUR with lower MLSS (100 mg SS/L) recorded by computer at every 10 minutes interval from 30 minutes to 3 hours at concentration 5, 10, 15, 20, 50, 100 mg/L.

Figure 4.19B: The inhibition of SDS on activated sludge OUR with higher MLSS (1500 mg SS/L) recorded by computer at every 10 minutes interval from 30 minutes to 3 hours at concentration 5, 10, 15, 20, 50, 100 mg/L.
Figure 4.20A: The inhibition of SDBS on activated sludge OUR with lower MLSS (100 mg SS/L) recorded by computer at every 10 minutes interval from 30 minutes to 3 hours at concentration 5, 10, 15, 20, 50, 100 mg/L

Figure 4.20B: The inhibition of SDBS on activated sludge OUR with higher MLSS (1500 mg SS/L) recorded by computer at every 10 minutes interval from 30 minutes to 3 hours at concentration 5, 10, 15, 20, 50, 100 mg/L
4.3 Nitrification inhibition tests

For all nitrification inhibition tests performed as part of this study, ATU was used to check the sensitivity of activated sludge, consequently the suitability of the activated sludge sample for the test. The procedure involved measurement of the concentration of ammonia and oxidized nitrogen after 4 hours of aeration, then the use of these concentrations to calculate nitrification rate. If the nitrification rate for the activated sludge sample was between 2 to 6.5 mg of N/ g. h, it will suitable for assessing the potential inhibitory effects of the substrate on nitrification by activated sludge microorganisms. If the rate of nitrification was lower or higher, then the sample should be discarded and another sample/source for activated sludge that meets these criteria must be found. Alternatively, the proportion of nitrifiers in the activated sludge sample could be changed such that the new sample meets the criteria.

To assess the potential inhibitory effects of the selected anionic surfactants SDS and SDBS on activated sludge nitrification, inhibition tests were performed according to the standard tests as described in chapter 3. The tests evaluated the effect of the presence of increased concentrations of the anionic surfactants SDS or SDBS on the oxidation of ammonia to nitrite and nitrate. Therefore, the concentrations of nitrite, nitrate and ammonia in each reactor, after 4 hours of aeration, were measured.
4.3.1 Inhibition to nitrification for different SDS concentrations

The first phase of nitrification inhibition tests looked into the effect of SDS concentrations of 5, 10, 25, 50 and 100 mg/L. The concentrations of nitrite, nitrate and ammonia measured after 4 hrs are shown in Figure 4.21A - Figure 4.21C. The results in Figures 4.21A show that the concentrations of nitrite increased with time, but the rate of production of nitrite in the presence of SDS decreased compared with that produced in the control reactor. For example, after 10 minutes, 0.7 and 0.5 mg/L NO$_2$ were produced in the control and at 100 mg/L SDS, respectively (ie., 18% inhibition to NO$_2$) compared with 2.3 and 1.1 mg/L NO$_2$ produced after 4 hours in the same reactors (ie., 52% inhibition to NO$_2$). On the other hand, inhibition to nitrate increased only from 27% to 30% with increased reaction time from 10 to 240 minutes. The inhibition to nitrate production began to decrease with time as shown in Figure 4.21B. The reduction in nitrate formation with time suggests that the microorganisms responsible for ammonia oxidation, that is *Nitrosomonas*, were more inhibited by the presence of SDS. The results in Figure 4.21C show that ammonia removal increased with time but decreased with increased SDS concentration.

The inhibition to nitrification was evaluated based on reduction in oxidised nitrogen production (ie., nitrite and nitrate production) as shown in Figure 4.21D. Also, the inhibition to nitrification was determined in terms of reduction in ammonia removal as shown in Figure 4.21E. Results show that
inhibition to nitrification decreased with time particularly during the first 2 hours.

Figure 4.21A: Concentration of nitrite for SDS concentration from 0 – 100 mg/L at 10, 30, 75, 120 and 240 minutes
Figure 4.21B: Concentration of nitrate for SDS concentration from 0 – 100 mg/L at 10, 30, 75, 120 and 240 minutes

Figure 4.21C: Concentration of ammonia for SDS concentration from 0 – 100 mg/L at 10, 30, 75, 120 and 240 minutes
Figure 4.21D: Inhibition to nitrification calculated by oxidized nitrogen for SDS concentration from 0 -100 mg/L at 10, 30, 75, 120 and 240 minutes.

Figure 4.21E: Inhibition to nitrification calculated by ammonium nitrogen for SDS concentration from 0 -100 mg/L at 10, 30, 75, 120 and 240 minutes.
The second phase of inhibition to nitrification tests involved evaluation of nitrification for SDS with small increments in concentration, especially in the range 0 – 50 mg/L (ie., 10, 20, 30 up to 100 mg/L) to gain a better insight into the trend of ammonia inhibition observed during the first phase of experiments. The production of nitrite, nitrate and ammonia was measured (Figure 4.22A) and the reduction in the production of these forms of oxidised nitrogen (as per the terminology in the standard test) was plotted as percentage inhibition versus SDS concentration as shown in Figure 4.22B. Inhibition to nitrification was also determined in terms of reduction in ammonia removal (Figure 4.22B). The results in Figure 4.22B show that the percentage of inhibition to nitrification was proportional to the concentration of SDS increased. In addition, the results in Figure 4.22B indicate that inhibition in terms of oxidised nitrogen production was less compared to inhibition in terms of ammonia removal, for concentrations higher than 50 mg/L. For example at 100 mg/L SDS, inhibition to oxidised nitrogen production was 46.5% compared with 56% in terms of ammonia removal. The higher ammonia removal can be attributed to the utilisation of ammonia for activated sludge microorganisms’ growth. The inhibition in terms of ammonia removal for concentrations larger than 50 mg/L were higher than inhibition to nitrite and nitrate formation most likely due to the which may be attributed to the utilisation of ammonia for microorganisms growth whereas at SDS concentrations less than 50 mg/L the effect of SDS on nitrifiers, consequently nitrification, was more than rate of ammonia utilisation by microorganisms (most likely heterotrophic) for growth. These results seem to be in agreement with inhibition to OUR which reached about 17.4% at 50 mg/L and 27.4% at 100 mg/L SDS after 180 minutes (Figure 4.3).
Figure 4.22A: Concentration of nitrite, nitrate and ammonia for SDS concentration from 0 – 100 mg/L

Figure 4.22B: Inhibition to nitrification for SDS concentration from 0 -100 mg/L
The effect of SDS on nitrification was also assessed for high concentrations in the range from 100 – 500 mg/L (Figure 4.23 A and B). The results obtained were consistent with the results for concentrations from 5 – 100 mg/L, where increased concentrations of SDS lead to increased inhibition. However the inhibition seemed to plateau at around 70%.

Inhibition to nitrification for SDS concentrations from 100 – 500 mg/L measured as ammonia removal was higher than oxidised nitrogen indicating utilisation of ammonia for growth of microorganisms (ie., heterotrophic bacteria, responsible for carbon removal) still occurred under these conditions. Inhibition to OUR for concentrations from 100 – 500 mg/L ranged from 29% to 62% (Figure 4.4) whereas inhibition to nitrification (ie., oxidised nitrogen production) ranged from 45% at 100 mg/L to 75% at 500 mg/L. These results suggest that there may be other mechanisms for inhibition to OUR and to nitrification other than inhibition to growth of heterotrophic and nitrifying microorganisms (Paper from UCLA). The results also show that nitrite and nitrate production had an IC$\text{_{20}}$ at 21.30 mg/L and IC$\text{_{50}}$ at 193.58 mg/L SDS.
Figure 4.23A: Concentration of nitrite, nitrate and ammonia for SDS concentration from 100 – 500 mg/L

Figure 4.23B: Inhibition to nitrification for SDS concentration from 100 -500 mg/L
4.3.2 Inhibition to nitrification for different SDBS concentrations

The first phase of experiments that were performed to look into the effect of SDBS on nitrification in activated sludge were carried out at concentrations of 5, 10, 25, 50 and 100 mg/L. The concentrations of nitrite, nitrate and ammonia measured at different time intervals from 10 to 240 minutes are shown in Figure 4.24A to Figure 4.24C, respectively.

The results in Figure 4.24A and 4.24B indicate that inhibition to nitrate production in the presence of SDBS was higher than inhibition to nitrite production. For example, at 5 mg/L nitrite production, after 4 hours, was almost 7% less than that in the control, whereas nitrate production was 22% less. It was also observed that inhibition to nitrite production slightly decreased with time, especially at low SDBS concentrations. These results may explain the decrease in nitrate production with time. In addition these results suggest that SDBS has more inhibitory effect on *Nitrobacter* than on *Nitrosomans*. The drop in inhibition diminished with increased SDBS concentrations. For example, nitrate formation decreased from 22% to 11%, 32% to 23%, and 59% to 46% at 5, 50 and 100 mg/L SDBS, respectively.

Inhibition to nitrification was determined in terms of reduction in production of oxidised nitrogen (Figure 4.24D) and in terms of reduction in ammonia removal (Figure 4.24E).
As shown in Figure 4.24D inhibition to nitrification, measured as oxidised nitrogen formation, decreased with time for all SDBS concentration (ie., from 77% to 46% at 50 mg/L and 83% to 55% at 100 mg/L), within 4 hours. Similarly, inhibition to nitrification in terms of ammonia removal decreased with time almost at the same rate and was proportional to SDBS concentration.

Figure 4.24A: Concentration of nitrite for SDBS concentration from 0 – 100 mg/L at 10, 30, 75, 120 and 240 minutes
Figure 4.24B: Concentration of nitrate for SDBS concentration from 0 – 100 mg/L at 10, 30, 75, 120 and 240 minutes

Figure 4.24C: Concentration of ammonia for SDBS concentration from 0 – 100 mg/L at 10, 30, 75, 120 and 240 minutes
Figure 4.24D: Inhibition to nitrification calculated by Oxidized nitrogen for SDBS concentration from 0 - 100 mg/L at 10, 30, 75, 120 and 240 minutes.

Figure 4.24E: Inhibition to nitrification calculated by ammonium nitrogen for SDBS concentration from 0 - 100 mg/L at 10, 30, 75, 120 and 240 minutes.
The second phase of inhibition to nitrification tests involved evaluation of nitrification for SDBS with small increments in concentration, i.e. 5, 10, 15 up to 100 mg/L, to gain an insight into the trend of ammonia inhibition observed during the first phase of experiments. The production of nitrite and nitrate was measured (Figure 4.25A) and inhibition nitrification in terms of the production of oxidised nitrogen (as per the terminology in the standard test) and to ammonia inhibition is shown in Figures 4.25B. It was observed that reduction in ammonia removal was higher than formation of oxidised nitrogen for SDBS concentrations higher than 50 mg/L. It was also observed that SDBS inhibition to nitrification was higher than that measured in the presence of SDS, which could be contributed to the lower biodegradability of SDBS due to its structure and the presence of benzene. For example, the percentage of inhibition of SDBS reached 18.4%, 46.1%, 50.1% and 53.6% at 10, 50, 75 and 100 mg/L, respectively. The IC$_{20}$ for SDBS was reached at 11.95 mg/L compared with 21.30 mg/L for SDS needs. Higher inhibition, IC$_{50}$ was reached at 74.38 mg/L for SDBS, whereas the 50% inhibition was reached 193.58 mg/L SDS.
Figure 4.25A: Concentration of nitrite, nitrate and ammonia for SDBS concentrations from 0 - 100 mg/L

Figure 4.25B: Inhibition to nitrification for SDBS concentration from 0 – 100 mg/L
Inhibition to nitrification was also assessed for concentrations from 100 – 500 mg/L. SDBS inhibition to nitrification was proportional to SDBS as shown in Figure 4.26B. Increasing the concentration of SDBS from 100 to 500 mg/L lead to increase inhibition to nitrification from 54% to 79% as measured by nitrite and nitrate production (Figure 4.26A) for concentration from 100 – 500 mg/L. The results in Figure 4.26B also show that ammonia inhibition reached 91.8% at SDBS concentration of 500 mg/L. It was also observed that nitrification inhibition in terms of ammonia was higher for all SDBS concentrations. This suggest that growth of microorganisms, hence utilisation of ammonia, continued under these high concentrations of SDBS.

The breakdown of nitrification in wastewater treatment plants may cause severe damages to the microbiological activities of the activated sludge microorganisms, hence activated sludge process. Consequently this will reduce the quality of plant’s effluent significantly. In addition to complying issues this can lead to negative ecological effects on the aquatic environment. Therefore, further research to on the effect of surfactants on nitrification is required for better understanding of the nitrification process.
Figure 4.26A: Concentration of nitrite, nitrate and ammonia for SDBS concentrations from 100 -500 mg/L

Figure 4.26B: Inhibition to nitrification for SDBS concentration from 100 – 500 mg/L
4.3.3 Effect of temperature on nitrification in the presence of varying concentrations of SDS and S DBS

It is well known that the growth of nitrifiers and their activities are greatly affected by temperature. The published literatures that have been researched are in agreement that the optimum temperature for nitrification processes is 30ºC with growth in the range of 8 - 35 ºC (Tchobanoglous and Burton, 1993).

To assess the effect of the presence of SDS and SDBS on nitrification in activated sludge during the cold months, the inhibition tests were conducted at 10ºC. Similarly to assess the effect of SDS and SDBS on nitrification during the warm months (or summer), the inhibition tests were carried out at 30ºC. The results are shown in Figure 27A for SDS and Figure 4.27B for SDBS. The results obtained showed that both SDS and SDBS have strong inhibitory effects on ammonia oxidation to nitrite and nitrate at 10ºC.

Inhibition to nitrification almost doubled at SDS concentrations below 25 mg/L and increased by 25% - 50% for SDS concentrations from 25 to 100 mg/L. Inhibition to nitrification decreased significantly with temperature increase from 20ºC to 30ºC. For example, at 10 mg/L SDS decreased from 10% to no inhibition with temperature increase from 20ºC to 30ºC. A similar effect although slightly lower was observed at 100 mg/L, where inhibition decreased from 46% to 26%, which is about 50% reduction.

Inhibition to nitrification increased by almost 30% for all SDBS concentrations with the temperature drop from 20ºC to 10ºC. But SDBS
inhibition to nitrification dropped significantly with the increase of temperature to 30°C.

The results in Figure 4.27A and 4.27B show that SDBS inhibition to nitrification is higher than that induced by SDS under all conditions tested. Inhibition to nitrification at 10 mg/L and 100 mg/L SDS was 27.6% and 66.9% respectively, compared with 32% and 75.3% for SDBS at the same concentrations at 10°C. In addition it was also observed that at 30°C, SDS showed no inhibitory effects for concentrations less than 10 mg/L. Therefore these results show that care should be taken when reporting inhibition tests results and that these tests should be carried out at the temperatures at which the wastewater treatment plant operate.
Figure 4.27A: Inhibition to nitrification for different concentrations of SDS at 10° C, 20° C and 30° C

Figure 4.27B: Inhibition to nitrification for different concentrations of SDBS at 10° C, 20° C and 30° C
The values of IC$_{20}$ and IC$_{50}$ for SDS and SDBS at the different temperatures tested, 10, 20 and 30°C are shown in Table 4.12. For example, IC$_{20}$ for SDS and SDBS reached 77.41 mg/L and 69.15 mg/L at 30°C, respectively.

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{20}$</td>
<td>IC$_{50}$</td>
<td>IC$_{20}$</td>
</tr>
<tr>
<td>SDS (mg/L)</td>
<td>7.24</td>
<td>59.47</td>
<td>21.3</td>
</tr>
<tr>
<td>SDBS (mg/L)</td>
<td>6.25</td>
<td>52.49</td>
<td>11.95</td>
</tr>
</tbody>
</table>

### 4.4 Comparison of the effect of SDS and SDBS on activated sludge from two WWTPs

#### 4.4.1 Effect on OUR

The results discussed in the previous sections were carried out using activated sludge from the same WWTP. To assess whether the inhibition effect observed for SDS and SDBS was peculiar to this treatment plant OUR tests and nitrification inhibition tests were repeated using activated sludge from a different WWTP receiving similar influent, i.e. mainly domestic wastewater. In this section the activated sludge that has been used in previous experiments (collected from Sunbury WWTP) was marked as AS#1 and second activated sludge sample (collected from Melton WWTP) was marked as AS#2.
The effect of SDS and SDBS on activated sludge AS#1 and AS#2 OUR is shown in Figures 4.28A and 4.28B, respectively. For SDS, it was observed that inhibition to AS#1 OUR was slightly higher than the inhibition to AS#2 for all SDS concentrations tested. SDBS inhibition to AS#1 OUR was higher than to AS#2 OUR indicating that the microorganisms in AS#1 were more sensitive to the presence of SDBS for all SDBS concentrations. This could be attributed to the population nature of the microorganisms in AS#1. It suggests that microorganisms in AS#2 are more acclimated to anionic surfactants or compounds of similar structure.

Furthermore, both activated sludge samples showed the same tendency towards anionic surfactants, where an inhibition to OUR was proportional was proportional to the anionic surfactant concentration.
Figure 4.28A: SDS inhibition to OUR for activated sludge AS#1 and AS#2

Figure 4.28B: SDBS inhibition to OUR for activated sludge AS#1 and AS#2
4.4.2 Nitrification inhibition test

The effect of the presence of SDS and SDBS on AS# 2 was evaluated for concentration from 5 – 100 mg/L (Figure 4.29). The tests for AS#1 were carried out under the same conditions. For example, samples were collected from the treatment plants on the same day, and the tests were carried out using the same solutions, synthetic waste, DO meters, etc. It was observed that both SDS and SDBS have an inhibitory effect on AS#2 nitrification capacity. The inhibitory effect was proportional to the surfactant initial concentration in the test reactor. The percentage of inhibition induced the presence of SDS ranged from 6.5% to 43.2%, while the percentage of inhibition in the presence of SDBS was from 13.1% to 46.3% for concentrations from 10 mg/L to 100 mg/L.

The IC$_{20}$ of SDS is 33.17 mg/L and the IC$_{20}$ of SDBS is reached at 24.7 mg/L. Both of them have no IC$_{50}$ results shown. Also, it was observed that the percentage of SDBS inhibition to AS#1 was higher than that measured for SDS. The results indicate that the sludge from the two WWTPs (Sunbury WWTP and Melton WWTP) both have the biodegradation capability. The selected concentration of SDS and SDBS has been studied in the test. The sensitive to SDS and SDBS were found not to be exactly the same for the two activated sludge.

According to Figure 4.29, activated sludge in Melton WWTP is adapted to SDS and SDBS better than activated sludge in Sunbury WWTP. When these two anionic surfactants were used as the test substance, activated
sludge in Melton WWTP can withstand a higher level of anionic surfactant than activated sludge in Sunbury WWTP.

Figure 4.29: Inhibition to nitrification in the presence of SDS and SDBS for AS#1 and AS#2

4.5 Combined effect of SDS and SDBS on activated sludge

4.5.1 Effect on OUR

The selected concentration in this experiment was 20 mg/L because it simulates the anionic surfactant concentration in domestic wastewater and
also can used as to compare with the pervious result. The anionic mixture studied in this experiment were 20 mg/L SDS, 20 mg/L 25% SDBS + 75% SDS mixture, 20 mg/L 50% SDBS + 50% SDS mixture, 20 mg/L 75% SDBS + 25% SDS mixture, and 20 mg/L SDBS, respectively. Table 4.13 indicated that for the same concentration, the effect in the presence of both SDS and SDBS intensify the inhibition since the effect of the mixture is higher than the combined effect of SDS and SDBS. For example, the inhibition of 25% SDBS + 75% SDS mixture [SDS in mixture (25%)] was 44.5% after 180 minutes incubation, compare with inhibition were only 5.6% and 23.2% of 5 mg/L SDS and 15 mg/L SDBS, respectively.
Table 4.13: SDS and SDBS mixture inhibition to activated sludge OUR

<table>
<thead>
<tr>
<th></th>
<th>SDS 20 mg/L</th>
<th>30 minutes</th>
<th>180 minutes</th>
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<tbody>
<tr>
<td></td>
<td>27.9%</td>
<td>5.5%</td>
<td></td>
</tr>
<tr>
<td>SDS in mixture (25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i.e. 5 mg/L)</td>
<td>48%</td>
<td>44.5%</td>
<td></td>
</tr>
<tr>
<td>5 mg/L SDS</td>
<td>12.9%</td>
<td>5.6%</td>
<td></td>
</tr>
<tr>
<td>15 mg/L SDBS</td>
<td>34.5%</td>
<td>23.2%</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>SDS in mixture (50%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i.e. 10 mg/L)</td>
<td>44.5%</td>
<td>37.5%</td>
<td></td>
</tr>
<tr>
<td>10 mg/L SDS</td>
<td>18.9%</td>
<td>6.5%</td>
<td></td>
</tr>
<tr>
<td>10 mg/L SDBS</td>
<td>29%</td>
<td>20%</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>SDS in mixture (75%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i.e. 15 mg/L)</td>
<td>38.8%</td>
<td>25.4%</td>
<td></td>
</tr>
<tr>
<td>15 mg/L SDS</td>
<td>19.0%</td>
<td>7.2%</td>
<td></td>
</tr>
<tr>
<td>5 mg/L SDBS</td>
<td>19.8%</td>
<td>15%</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDBS 20 mg/L</td>
<td>49.5%</td>
<td>45.1%</td>
<td></td>
</tr>
</tbody>
</table>

4.5.2 Morphology
Figure 4.30: Microscopic images of activated sludge flocs at the end of OUR tests for a mixture of anionic surfactant after 3 hours: (a) control reactor (b) 20 mg/L SDS (c) 20 mg/L 25% SDBS+ 75% SDS (d) 20 mg/L 50% SDBS+ 50% SDS (e) 20 mg/L 75% SDBS+ 25% SDS (f) 20 mg/L SDBS; (images at magnification 100× with blue filter)
The mean projected area of flocs in the control was around 26,000 $\mu \text{m}^2$.

Compared with the flocs area in the control run, the flocs area decreased by 16.1%, 18.4%, 25%, 38.4% and 42.8% in the presence of 20 mg/L SDS, 20 mg/L 75% SDS + 25% SDBS, 20 mg/L 50% SDS+ 50% SDBS, 20 mg/L 25% SDS + 75% SDB and 20 mg/L SDBS, respectively (Figure 4.31). It has been found that the flocs area decreased with the increase in SDBS portion (Table 4.14). Also it seems that the relationship between the flocs and the concentration of the surfactants is almost linear.

The flocs perimeter decreased from around 600 $\mu \text{m}$ to 500 $\mu \text{m}$ for all anionic surfactant mixtures compare with 650$\mu \text{m}$ in control run. The flocs perimeters decreased by 7.4%, 8.4%, 13.5%, 19.5% and 23.4% for 20 mg/L SDS, 20 mg/L SDS + SDBS at SDBS 25%, SDBS 50%, SDBS 75%, and 20 mg/L SDBS, respectively (Table 4.14).

The results in Table 4.15 show that the flocs $D_{eq}$ values decreased with increasing concentrations of SDBS in the SDS + SDBS mixture runs. The $D_{eq}$ was 167.62 $\mu \text{m}$ at in the presence of 20 mg/L SDS and 138.38 $\mu \text{m}$ in the presence of 20 mg/L SDBS. Increasing the concentration of SDBS from 25% to 75% in the mixture, the $D_{eq}$ decreased from 165.36 $\mu \text{m}$ at 25% SDBS to 143.60$\mu \text{m}$ at 75% SDBS. The presence of anionic surfactants showed no effect on the average flocs shape (FF). The results show that the anionic surfactants can affect the activated sludge flocs characteristics measured in terms of the parameters, flocs area, perimeter and $D_{eq}$. The results showed that the flocs are adversely affected by the presence of
anionic surfactants and that SDBS has a stronger negative effect on the flocs compared with SDS.

Figure 4.31: The mean projected area for activated sludge flocs in the presence of different SDS and SDBS mixture for OUR tests
Figure 4.32: The perimeter values for activated sludge flocs in the presence of different SDS and SDBS mixture for OUR tests

Table 4.14: The change in the Mean projected area and perimeter of activated sludge flocs at the end of OUR tests for different SDS and SDBS Mixture

<table>
<thead>
<tr>
<th></th>
<th>SDS</th>
<th>25% SDBS + 75% SDS</th>
<th>50% SDBS + 50% SDS</th>
<th>75% SDBS + 25% SDS</th>
<th>SDBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean projected area</td>
<td>16.1%</td>
<td>18.4%</td>
<td>25%</td>
<td>38.4%</td>
<td>42.8%</td>
</tr>
<tr>
<td>Perimeter</td>
<td>7.4%</td>
<td>8.4%</td>
<td>13.5%</td>
<td>19.5%</td>
<td>23.4%</td>
</tr>
</tbody>
</table>
Table 4.15: SDS and SDBS Mixture - The calculated results of Equivalent diameter (Deq) and Form factor (FF)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SDS</th>
<th>25% SDBS + 75% SDS</th>
<th>50% SDBS + 50% SDS</th>
<th>75% SDBS + 25% SDS</th>
<th>SDBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deq</td>
<td>183.02</td>
<td>167.62</td>
<td>165.36</td>
<td>158.56</td>
<td>143.60</td>
<td>138.38</td>
</tr>
<tr>
<td>FF</td>
<td>0.786</td>
<td>0.769</td>
<td>0.767</td>
<td>0.790</td>
<td>0.749</td>
<td>0.788</td>
</tr>
</tbody>
</table>

Concentration of MLSS in the test reactors correlated with the biomass concentration (Figure 4.33). In the SDS and SDBS mixture run, the MLSS decreased from 1.57 g/L to 1.43 g/L for all different composition mixtures.
Figure 4.33: MLSS at end of OUR tests for different SDS and SDBS mixture concentrations

For the anionic surfactants mixture of SDS and SDBS, all the indicators of flocs size decreased in the presence of anionic surfactants. The mean projected area of flocs in the presence of anionic surfactants relative to the flocs area in the control (absence of surfactants) decreased by 16.1% and 42.8% at 25% SDBS and 75% SDBS, respectively. Similarly the perimeter in the mixture relative to that in the control decreased by 7.4% and 23.4% for 25%SDBS + 75%SDS mixture and 75%SDBS + 25% SDS mixture (Figure 4.32). The concentration of the biomass in the reactors measured as MLSS decreased slightly in the presence of surfactants.
5.1 Inhibition to OUR and Nitrification

1. Both anionic surfactants SDS and SDBS showed inhibitory effects on activated sludge OUR. Inhibition to OUR was increased to the initial concentration, for concentrations up to 100 mg/L, both for SDS and SDBS. Inhibition to OUR in the presence of anionic surfactants at concentrations from 5 to 100 mg/L increased from 12.9% to 44.2% for SDS and from 19.8% to 79.1% for SDBS respectively, after 30 minutes contact time. This inhibition decreased to 5.6% to 27.4% for SDS and 15.0% to 69.2% for SDBS after 3 hours respectively, indicating the favourable effect of acclimation.

2. The standard method for assessing the potential inhibition of a test compound to OUR recommends that measurements of OUR be performed either after 30 minutes or 180 minutes contact time. However, the results obtained in this research study indicated that inhibition to activated sludge OUR could vary significantly with the duration of the test, especially for biodegradable compounds (e.g. SDS in this case). In addition, it was observed that the effect of contact time, that is acclimation, vary with temperature. For example, at room temperature inhibition may decrease with increased contact time but it may not change for tests performed at low temperatures (mainly below 20°C).
3. SDS and SDBS showed inhibitory effects on nitrification in activated sludge reactors, measured in terms of combined nitrite and nitrate production, for all concentrations tested. Inhibition to nitrification was proportional to the initial concentration both for SDS and SDBS.

4. SDBS had a stronger inhibitory effect on activated sludge than SDS. The results showed that 20% of inhibition to nitrification occurred at 21.30 mg/L for SDS, compared with 11.95 mg/L for SDBS, whereas IC_{50} was measured at 193.6 mg/L and 74.4 mg/L for SDS and SDBS respectively.

5. Typically, nitrification in the presence or absence of anionic surfactants decreases with decreased temperature. Also, SDS and SDBS inhibition to activated sludge nitrification reactions was inversely proportional to temperature. The highest inhibition to nitrification exerted by SDS was 69% measured at 10°C compared with 75% for SDBS. Both SDS and SDBS showed no inhibitory effects on nitrifications at concentrations lower than 25 mg/L and temperature of 30°C.

6. The concentration of activate sludge biomass, measured as MLSS, showed a strong effect on the inhibition to OUR, as expected. The results obtained showed that the extension of inhibition to OUR using a low concentration of MLSS, approximately 100 mg/L, almost doubled compared with that using a high concentration around 1500
mg/L. The results suggest that inhibition test should be performed at the MLSS concentration used by the WWTP as well at the concentration recommended in the standard method.

7. The trends observed for the selected anionic surfactants (i.e. SDS and SDBS) inhibition to OUR and nitrification using activated sludge AS#1 were similar to those observed using the activated sludge sample collected from a different WWTP (AS#2). However the magnitude of inhibition measured using AS#2 was slightly lower than that measured using AS#1. The results obtained indicated that activated sludge microorganisms from the two WWTPs have comparable biodegradation capability for SDS but AS#1 was more sensitive to presence of SDBS. Therefore, inhibition to activated sludge tests should be performed using activated sludge samples from the WWTP under investigation and be compared with results using reference microorganisms (i.e. activated sludge growing under ideal conditions in the lab). Laboratory activated sludge reactors should be used to grow ideal activated sludge mixtures where typical population of heterotrophic and autotrophic bacteria is maintained throughout the research duration.

5.2 SDS and SDBS removal

1. The concentration of SDS and SDBS measured at the end of the OUR and nitrification inhibition tests showed a trend in removal consistent with the inhibition measured for OUR or nitrification in their presence.
For example, the concentrations of SDS and SDBS at the end of tests performed using 100 mg/L (i.e., high inhibition) were about 90% of the initial concentration. The results suggest that adsorption onto activated sludge does not play a major role in SDS and SDBS removal.

2. The results also indicated that the two anionic surfactants, SDS and SDBS, demonstrated different extents and patterns of biodegradation, where surfactant molecules with an aromatic ring structure (i.e., SDBS) appear to slow down the biodegradation activities of activated sludge microorganisms significantly.

### 5.3 Morphology of activated sludge flocs

1. The morphological parameters of activated sludge flocs were investigated in this study. The results obtained show that SDS and SDBS can have significant adverse effects on the morphology of activated sludge flocs, measured in terms of mean projected area, perimeters, and equivalent diameter \(D_{eq}\), even at a low concentration. Overall, the presence of both SDS and SDBS resulted in reduction in the sludge flocs size, which means that the presence of SDS and SDBS may lead to poor solids settling in the secondary clarifier.

2. The inhibition trends observed for SDS and SDBS for different temperatures correlated with the changes to activated sludge flocs size measured at these temperatures.
3. The effect of the presence of SDS and SDBS on the activated sludge flocs shape measured in terms of the form factor (FF) was not significant, except for tests performed at 10 °C in the presence of SDBS.

4. The decrease in the activated sludge flocs mean projected area correlated with the decrease in the activated sludge biomass concentration measured as MLSS. Comparing the two anionic surfactants tested, SDBS had stronger influence on the biomass properties and flocs morphology than SDS.
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