Assessing the effect of surfactants on activated sludge processes using sequencing batch reactors

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

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DECLARATION

I certify that except where due acknowledgement had 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.

Tsz Kwan Kwok

March 2011
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Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.
LIST OF ABBREVIATIONS

AP  Alkylphenols
APEO Alkylphenol ethoxylates
BOD  Biological oxygen demand
COD  Chemical oxygen demand
EPA  Environmental Protection Agency
HRT  Hydraulic retention time
Kow  Octanol-water partition coefficients
LAB  Linear alkylbenzene
LAS  Linear alkylbenzene sulphonate
MBAS  Methylene blue active substances
MLSS  Mixed liquor suspend solid
NPEO  Nonylphenol ethoxylates
NP1EO Nonylphenol monoethoxylate
NP2EO Nonylphenol diethoxylate
OPEO  Octylphenol ethoxylates
OUR  Oxygen uptake rate
SBR  Sequencing batch reactor
SDBS  Sodium dodecylbenzene sulphonate
SPC  Sulphophenyl carboxylate
SRT  Sludge retention time
SVI  Sludge volume index
WAS  Waste activated sludge
WWTP Wastewater treatment plant
CONTENTS

DECLARATION .................................................................................................. ii
ACKNOWLEDGEMENTS .................................................................................. iii
LIST OF ABBREVIATIONS ............................................................................... iv
CONTENTS ........................................................................................................ v
LIST OF TABLES ............................................................................................. viii
List of Figures .................................................................................................... ix
ABSTRACT ....................................................................................................... xii

1. INTRODUCTION .......................................................................................... 1
   1.1 OUR and Nitrification Inhibition Tests .................................................. 4
   1.2 Sequencing Batch Reactor Technology ............................................. 4

2. LITERATURE REVIEW ................................................................................ 6
   2.1 Overview of Surfactants ......................................................................... 6
      2.1.1 Anionic and Non-ionic Surfactants .............................................. 7
      2.1.2 Linear Alkylbenzene Sulphonate .............................................. 9
         2.1.2.1 Biodegradability of LAS .................................................. 13
         2.1.2.2 Sorption of LAS ........................................................... 14
      2.1.3 Alkylphenol Ethoxylates .............................................................. 15
         2.1.3.1 Biodegradability of APEO/NPEO .................................. 16
         2.1.3.2 Sorption of APEO .......................................................... 19
   2.2 Overview of Sequencing Batch Reactors ............................................ 19
      2.2.1 Use of SBRs in Research Studies ................................................. 22
   2.3 Nitrification and Denitrification Reactions .......................................... 24
      2.3.1 Factors affecting nitrification ....................................................... 27
         2.3.1.1 Ammonia and Nitrite Concentration ................................... 27
         2.3.1.2 pH .............................................................................. 28
         2.3.1.3 Dissolved Oxygen Concentration ..................................... 28
         2.3.1.4 Temperature .................................................. 28
         2.3.1.5 BOD5/TKN Ratio ...................................................... 29
         2.3.1.6 Toxic Compounds ........................................................ 29
2.4 Effect of Surfactants on Activated Sludge

2.4.1 Effect of Surfactants on Activated Sludge Respiration

2.4.2 Effect of Surfactants on Nitrification

2.4.3 Effect of Surfactants on Substrate Consumption

2.4.4 Effect of Surfactants on Activated Sludge Floc Morphology

2.4.5 Effect of Surfactants on Aquatic Organisms' Toxicity

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Activated Sludge

3.1.2 Surfactants

3.2 Methods

3.2.1 Batch Experiments

3.2.1.1 Oxygen Uptake Rate (OUR)

3.2.1.2 Nitrification Inhibition Tests

3.2.2 Sequencing Batch Reactors (SBR)

3.2.2.1 SBR Test 1

3.2.2.2 SBR Test 2

3.2.3 Analytical Techniques

3.2.3.1 Dissolved Oxygen Measurements

3.2.3.2 COD, Ammonia, Nitrate and Nitrite Measurements

3.2.3.3 Anionic Surfactant Measurement

3.2.3.4 Non-Ionic Surfactant Measurement

3.2.3.5 Activated Sludge Characterisation

4. RESULTS AND DISCUSSION

4.1 Batch Tests

4.1.1 OUR Tests

4.1.1.1 Sensitivity of Activated Sludge Samples Check

4.1.1.2 Effect of SDBS on Activated Sludge OUR

4.1.1.3 Removal of SDBS in OUR Tests after 180mins

4.1.1.4 Effect of NPEO on Activated Sludge OUR

4.1.1.5 Inhibition of Mixtures of SDBS and NPEO to OUR

4.1.1.6 Sludge from Sunbury WWTP and 30L SBR
LIST OF TABLES

Table 2-1: US surfactant market 2007 demand (adapted from Rust & Wildes, 2008) ................................................................. 9
Table 3-1: Details of surfactants used in the experiments ..................... 39
Table 3-2: OUR and inhibition nitrification test .................................. 44
Table 3-3: Composition of synthetic wastewater in SBR test 1 .................. 45
Table 3-4: Operating condition in the SBR experiment 1 ....................... 47
Table 3-5: Feed composition for 1.5L SBRs ....................................... 48
Table 3-6: Surfactant compositions in the feed for SBRs ....................... 48
Table 3-7: Operating condition in the SBR experiment 2 ....................... 49
Table 4-1: Effect of sensitivity of sludge to inhibitions ........................... 53
List of Figures

Figure 2-1: Surfactant consumption in household detergents in Europe 1998 (ECOSOL, 2007) ................................................................. 10

Figure 2-2: General chemical structure of LAS, where x and y corresponds with the number of CH2 on each side of the benzene sulphonate group (7x+10y) (Liwarska-Bizukojc, Drews & Kraume, 2008) ......................................................................... 12

Figure 2-3: Sodium dodecylbenzene sulphonate (Alrich-2525) ......................... 12

Figure 2-4: Ultimate biodegradation of LAS (Huddleston, 1979; Swisher, 1963) ................................................................................ 14

Figure 2-5: Breakdown processes of long-chain alkylphenol polyethoxylates (APEOs) under aerobic and anaerobic conditions, and the formation of their halogenated derivatives (Vega Morales, Torres Padrón, Sosa Ferrer & Santana Rodríguez, 2009) ................................................................ 18

Figure 3-1: SBR experiment 1 cycle definition .................................................. 46

Figure 3-2: SBR cycle definition ........................................................................ 49

Figure 4-1: Effect of 3,5 - dichlorophenol on activated sludge OUR ................. 53

Figure 4-2: Activated sludge OUR for SDBS concentrations of 10–60 mg/L ................................................................................................ 55

Figure 4-3: Inhibition to OUR at different concentrations of SDBS after 30 and 180 mins exposure ........................................................... 55

Figure 4-4: Removal of SDBS after OUR test ................................................... 56

Figure 4-5: Activated sludge OUR for NPEO concentrations of 10–60 mg/L ................................................................................................ 57

Figure 4-6: Inhibition to OUR at different concentrations of NPEO after 30 and 180 mins exposure .............................................................. 58

Figure 4-7: OUR test 1 of mixture of SDBS and NPEO .................................... 60

Figure 4-8: OUR test 2 of mixture of SDBS and NPEO .................................... 60
Figure 4-9: Inhibition of SDBS and NPEO (total concentration 60 mg/L) to OUR, average of tests 1 and 2 ...........................................61

Figure 4-10: Effect of 3,5-dichlorophenol on activated sludge OUR for sludge from the large laboratory SBR ........................................ 61

Figure 4-11: OUR for activated sludge collected from a large laboratory size SBR for SDBS concentration of 10–60 mg/L .................................................................63

Figure 4-12: Inhibition of SDBS to OUR for activated sludge from 30L laboratory SBR ................................................................................ 63

Figure 4-13: OUR for activated sludge collected from a large laboratory size SBR for NPEO concentration of 10–60 mg/L ........................................................................ 65

Figure 4-14: Inhibition of NPEO to OUR on SBR activated sludge .............. 65

Figure 4-15: Concentration of nitrite, nitrate and ammonia for SDBS concentration from 0 to 60 mg/L .................................................................67

Figure 4-16: Inhibition to nitrification for SDBS ............................................. 67

Figure 4-17: Removal of SDBS after nitrification test ..................................... 68

Figure 4-18: Concentration of nitrite, nitrate and ammonia for NPEO concentration from 0 to 60 mg/L .................................................................69

Figure 4-19: NPEO inhibition to nitrification .................................................. 69

Figure 4-20: Inhibition of mixture of surfactants to nitrification ..................... 70

Figure 4-21: MLSS profile in the three SBRs receiving 5, 10 and 20 mg/L SDBS ........................................................................................................ 71

Figure 4-22: SVI in the SBRs that received feed spiked with SDBS ............... 72

Figure 4-23: COD removal in the SBRs that received feed spiked with SDBS ................................................................................................. 73

Figure 4-24: Inhibition to ammonia removal .................................................. 73

Figure 4-25: MLSS in the SBRs received feed spiked with surfactants .......... 75

Figure 4-26: SVI in the SBRs that received SDBS, SDBS + NPEO and NPEO ........................................................................................................ 76
Figure 4-27: COD removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO ............................................................. 76

Figure 4-28: Average COD removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO ............................................................. 77

Figure 4-29: Ammonia removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO ............................................................. 77

Figure 4-30: Average ammonia removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO ................................................... 78

Figure 4-31: Oxidised nitrogen formation .............................................................................. 78

Figure 4-32: Removal of surfactants ................................................................................. 79

Figure 4-33: Day 1 nitrate profile ...................................................................................... 80

Figure 4-34: Day 1 dissolved oxygen profile ..................................................................... 80

Figure 4-35: Day 10 ammonia Profile .................................................................................. 82

Figure 4-36: Day 10 nitrate profile ...................................................................................... 82
ABSTRACT

Anionic and non-ionic surfactants have been detected in the influents to and effluents from wastewater treatment plants (WWTPs). Linear alkylbenzene sulphonates (LAS) and alkylphenol ethoxylates (APEO) are the most frequently detected anionic and non-ionic surfactants in urban wastewater. The aim of this study was to assess the effect of the presence of anionic and non-ionic surfactants in the influent to WWTPs on activated sludge processes.

The results obtained from batch tests conducted according to ISO standard methods indicated that both anionic and non-ionic surfactant, Sodium dodecylbenzene sulphonate (SDBS) and Nonylphenol ethoxylates (NPEO), can have adverse effects on activated sludge OUR and nitrification reactions. The inhibition to oxygen uptake rate (OUR) increased from 16.7% to 28.8% SDBS initial concentrations of 10–60 mg/L, measured after 30 mins of exposure. Increasing the exposure time to 180 mins, the inhibition to OUR increased from 17.5% to 48.6% for SDBS concentrations of 10–60 mg/L. The batch tests showed that NPEO inhibition to activated sludge OUR follows a similar trend to that observed for SDBS but was around 8% to 12% less for all concentrations tested and duration of exposure. The inhibition measured was 6.3% to 16.6%, and 19.2% to 40.4% for 10–60 mg/L respectively after 30 and 180 mins of exposure. Inhibition of a mixture of SDBS and NPEO at a total
concentration of 60 mg/L showed lower inhibition to OUR compared with those measured for SDBS and NPEO as a single surfactant in the reactor.

The above inhibition tests were conducted using activated sludge samples collected from the Sunbury wastewater treatment plant (WWTP). In addition, OUR inhibition tests were performed using sludge collected from a 30L lab-scale SBR fed with synthetic wastewater. Inhibition to OUR obtained using these two sources of sludge showed that activated sludge from the lab-scale SBR was more susceptible to inhibition than the activated sludge from Sunbury WWTP was. However, the same trends were observed in both lab scale and WWTP, that is, inhibition was proportional to the initial concentration of the surfactant. The SBRs were fed with synthetic wastewater free of surfactants, so this suggests that the higher inhibition obtained using sludge from the SBR could be due to an acclimatisation effect.

SDBS and NPEO showed varying levels of inhibition to activated sludge capacity for nitrification. The trend observed was in agreement with that observed for SDBS and NPEO inhibition to OUR. Inhibition to nitrification was measured both in terms of reduction to oxidation of ammonia to nitrate and reduction in the production of oxidised nitrogen (nitrite + nitrate), compared with that measured for the control. The inhibition to nitrification measured in the reactors that
received 10–60 mg/L SDBS and NPEO ranged from 4.7% to 26.2% and 4.5% to 26.9%, respectively.

Further, the study examined the effect of the presence of both SDBS and NPEO in the influent to SBRs on their sludge volume index (SVI), chemical oxygen demand (COD) and NH₄ removal. Four bench-top laboratory SBRs were operated as a part of this research study. The SBRs were fed with synthetic wastewater for a few months until they reached a steady state in terms of COD and NH₄ removal. In addition, sludge volume index (SVI), mixed liquor suspended solid (MLSS), dissolved oxygen (DO) and pH were measured on a regular basis. Three of the SBRs received feed spiked with increased concentrations of SDBS, 5, 10 and 20 mg/L. The fourth SBR was used as a control, i.e. it received synthetic wastewater with no surfactants. The amount of sludge wastes (WAS) remained the same after commencement of spiking.

The presence of 5, 10 and 20 mg/L SDBS in the influent to bench scale SBRs showed an adverse effect on the concentration of MLSS and sludge quality measured in terms of SVI. The concentrations of MLSS decreased with time especially in the SBRs that received feed spiked with 10 and 20 mg/L SDBS where 26% reduction in MLSS was measured at the end of the first week after SDBS was introduced into the feed. Similarly, the SVI in these SBRs decreased compared to the control, indicating poor sludge settling properties.
The three SBRs that received feed spiked with SDBS showed deterioration in COD and ammonia removal. The results were in agreement with the reduction in MLSS. Further, they indicated that SDBS may interfere with oxygen transfer that ultimately causes reduction in COD removal. To examine this effect, the fine bubble air diffusers in the SBRs were replaced with coarse bubble air diffusers in the following experiment.

The effect of presence of 30 mg/L SDBS, 15 mg/L SDBS+ 15 mg/L NPEO and 30 mg/L NPEO in the influent to SBRs 2, 3 and 4, respectively was investigated. The performance of the SBRs in terms of MLSS, SVI, COD and NH₄ removal was also examined. The SBRs were aerated using coarse bubble air diffusers. The results showed that the MLSS in all reactors decreased with time, which indicates that the effect of surfactants in terms of saponification and the tendency to reduce floc sizes. Consequently, poor sludge settling was not improved with increased air bubble sizes. However, the removal of COD and NH₄ was not inhibited and remained comparable with the control, which suggests that increased air bubble sizes may have improved transfer of substrate and oxygen to the biomass.
1. INTRODUCTION

Surfactants are a diverse group of chemicals that are designed to have cleaning or solubilisation properties. They generally consist of a polar head group (either charged or uncharged), which is well solvated in water, and a non-polar hydrocarbon tail, which is not easily dissolved in water. Hence, surfactants combine hydrophobic and hydrophilic properties in one molecule. Surfactants are broadly defined as organic compounds that can enhance cleaning efficiency, emulsifying, wetting, dispersing, solvency, foaming/defoaming and lubricity of water-based compositions. Surfactants are classified into four categories: anionic, cationic, non-ionic and amphoteric. Due to their use in households and industries, they have been detected in wastewater treatment plants where they are removed by adsorption to biomass and/or biodegradation, which results in the loss of their tensioactive properties. LAS concentrations of 21 mg/L were detected in raw wastewater (Mungray & Kumar, 2009), whereas nonylphenol ethoxylates (NPEO) concentrations of 3.2–33.7 mg/L were reported (Naylor, 1995; Ying et al., 2002). According to literature, surfactants in WWTPs may be completely or partially removed, depending on many factors including how well they were designed, temperature and characteristics of the raw wastewater. After treatment, surfactants and their metabolites (breakdown products) that remain in the effluent can exert adverse effects on the aquatic life in the receiving water bodies, for example, the presence of LAS in the aquatic environment can damage fish gills, cause excess mucus secretion, decrease respiration in the common goby, and damage swimming patterns in blue mussel larva (Mungray & Kumar, 2009).
Surfactants have been reported to be an inhibitor to nitrification and OUR. A local wastewater treatment plant has been experiencing problems achieving nitrogen discharge limits, mainly due to poor nitrification, where either high ammonia concentration was detected in the effluent or, on many occasions, the concentration of nitrites was high. The problem was associated with poor settling in the secondary clarifier, which would result in washout of activated sludge.

The aim of this project is to evaluate the effects of surfactants on the performance of activated sludge processes (e.g. COD removal, nitrification efficiency) under continuous flow conditions. To achieve this, the effect of the presence of anionic surfactant (SDBS) and non-ionic surfactant (NPEO) on activated sludge OUR and nitrification was assessed in a batch system according to International Organization for Standardization (ISO) standard methods. The OUR method facilitates estimation of the effects of surfactants on activated sludge micro-organisms in aerobic biological treatment systems. The nitrification inhibition test has been used by many researchers to assess the inhibitory effects of surfactants on nitrifying micro-organisms in activated sludge. For a continuous flow system, continuous flow SBR will be used to simulate a conventional activated sludge process. The main concern in this research is to discover: 1) the efficiency of the activated sludge process for surfactants removal and the variation with the initial surfactant concentration; 2) the effect of nitrification with the presence of surfactants; 3) the concentration at which surfactants significantly affect the activated sludge population dynamics; 4) if
there is a change in floc characteristics with the presence of surfactant; and 5) if the level of inhibition measured using batch tests according to ISO standard methods correlate with the level of inhibition measured under continuous flow conditions using an SBR. The research methods will be summarised below.
1.1 OUR and Nitrification Inhibition Tests

Many researchers have used a respiration inhibition test to examine the potential toxicity of certain chemicals in terms of their effect on activated sludge growth rates (Elnabarawy, 1988; Gendig, 1999; Gutiérrez 2002; Liwarska-Bizukojc 2005; Youshioka, 1986). These tests were established based on ISO 8192 (1986), which mentions that the OUR of activated sludge micro-organisms can be reduced in the presence of toxicants. For nitrification inhibitions, the ISO 9509 (1989) test was employed to examine the potential effect of surfactants on nitrification reactions in activated sludge aeration tanks. The effect on nitrification is measured in terms of changes to ammonia oxidation and to oxidised nitrogen (nitrite and nitrate) production.

1.2 Sequencing Batch Reactor Technology

SBR is a fill-and-draw activated sludge process for wastewater treatment. While in continuous systems the reaction and settling occur in different reactors, in the SBR process unit, all processes occur in a single reactor following a sequence of fill, reaction, settling and draw phases. SBR technology was first used in 1914 and became popular because of its operating advantages, such as high flexibility, flowrate independence, easy to control, and direct measurement. Reaction rates can be measured directly by monitoring concentration changes in the reactor tank by manual sampling or with an on-line probe. In a conventional continuous flow reactor, continuous mass transfer associated to
the continuous flow regime prevents in-situ measurements of process kinetics. They must be performed in a separate bench-scale batch reactor.
2. LITERATURE REVIEW

2.1 Overview of Surfactants

Surfactants are widely used in many different market segments, which include household detergents, personal care, industrial and institutional cleaning products, food processing, oilfield chemicals, agricultural chemicals, textiles, emulsion polymerisation, paints and coatings, construction and lubricant and fuel additives. All surfactants have the same basic chemical structure: a hydrophilic (water-loving) ‘head’ and a hydrophobic (oil-loving) ‘tail’, which is always a long (linear) chain of carbon atoms. Surfactants are made from oleochemical (natural) and/or petrochemical (synthetic) raw materials.

The primary function of a surfactant is to enhance the surface activity of water-based formulations composed of a range of ingredients such as solvents, thickeners, alkalis/salts, chelating agents, foamers/defoamers and fragrances.

Surfactants are classified according to their ionic (electrical charge) properties in water into four groups as follows:

- Anionic: Negative
- Non-ionic: No charge
- Cationic: Positive
- Amphoteric: Positive/Negative
2.1.1 Anionic and Non-ionic Surfactants

Anionic surfactants are the largest group, accounting for approximately 40% of the world’s production of surfactants. These products exhibit superior wetting and emulsifying properties and tend to be higher-foaming materials. The following are the main anionic surfactants classes:

- Linear alkylbenzene sulphonate (LAS)
- Fatty acids
- Sulfosuccinates
- Lauryl sulphates
- Lignosulfonates

Non-ionic surfactants are the second largest group by volume, accounting about 35% of the world’s surfactants production. Demand for these sugar-based products is escalating due to their low toxicity. The following are the main non-ionic surfactant classes:

- Alkylphenol ethoxylates
- Alcohol ethoxylates
- Alkanolamides fatty amine ethoxylates
- Polyglucosides sucrose esters
- Sorbian esters
The largest end use market for surfactants is household cleaning detergents (see Table 2–1). The major two surfactants used in household detergents are LAS, which is one of the most widely used type of surfactants in industrial and institutional (I&I) cleaning products, and alkylphenol ethoxylates (APEO), which come under anionic and non-ionic classes respectively. SDBS and nonylphenol ethoxylates (NPEO) are derivatives of LAS and APEO respectively. Therefore, these two surfactants will be used in this research to investigate the effect of surfactants on activated sludge due to their wide use and potential inhibition/toxicity.
Table 2-1: US surfactant market 2007 demand (adapted from Rust & Wildes, 2008)

<table>
<thead>
<tr>
<th>Us Surfactant Market 2007 Demand</th>
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</thead>
<tbody>
<tr>
<td><strong>Market Segment</strong></td>
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<tr>
<td><strong>Key Markets</strong></td>
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<tr>
<td>Household Detergents</td>
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<tr>
<td>Personal Care</td>
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<tr>
<td>Industrial &amp; Institutional Cleaners</td>
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<tr>
<td>Food Processing</td>
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<td>Oilfield Chemicals</td>
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<tr>
<td>Agricultural Chemicals</td>
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<tr>
<td>Textiles</td>
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<tr>
<td>Emulsion Polymerisation (Plastics)</td>
</tr>
<tr>
<td>Paints &amp; Coatings</td>
</tr>
<tr>
<td>Construction</td>
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<tr>
<td><strong>Other Markets</strong></td>
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<tr>
<td>Lubricant and Fuel Additives</td>
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<tr>
<td>Metal Working</td>
</tr>
<tr>
<td>Mining Chemicals</td>
</tr>
<tr>
<td>Pulp &amp; Paper</td>
</tr>
<tr>
<td>Leather Processing</td>
</tr>
<tr>
<td>Other</td>
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<td><strong>Total</strong></td>
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</tbody>
</table>

2.1.2 Linear Alkylbenzene Sulphonate

LAS surfactants were introduced in the 1960s because they are biodegradable. Their introduction was aimed at solving environmental problems such as foaming in rivers and streams caused by poorly degradable surfactants, such as branched alkylbenzene sulphonate, which was the main surfactant used in the production of detergents before the discovery of LAS (Leon et al., 1990).
Today, the LAS group is the most widely used group of surfactants in all detergents and cleaning products. With a contribution of around 27% of the total surfactants consumed in household detergents (see Figure 2-1), LAS has been the most single surfactant used in detergents for more than thirty years and continues to represent a substantial portion of the surfactant market today. Further, LAS is the most efficient (cost-to-performance ratio), versatile and the least harmful surfactant from an impact on environment and human health point of view (ECOSOL, 2007). The average LAS content in typical European detergent formulations ranges between 5% up to 27% by weight depending on the type of detergent (e.g. hand dishwashing liquids, laundry powders etc.) (ECOSOL, 2007).

![Figure 2-1: Surfactant consumption in household detergents in Europe 1998 (ECOSOL, 2007)](image-url)
LAS is a synthetic anionic surfactant developed from Linear Alkylbenzene (LAB). Approximately 99% of the LAB produced worldwide is transformed into LAS through a sulphonation process. In turn, LAS is almost exclusively used as a surfactant ingredient in detergents. In most cases, LAS is used as a sodium derivative. For some special applications, other derivatives are also produced, such as magnesium derivative.

LAS is a non-volatile compound produced by alkylation and sulphonation of benzene. It is a mixture of homologues and phenyl positional isomers, each containing an aromatic ring sulphonated at the para-position and attached to a linear alkyl chain at any position except the terminal one (see Figure 2-2).

LAS can be represented in formula R-C₆H₄-SO₃Na, where R represents an alkyl linear chain with C atoms in the range of C₁⁰-C₁₃ and SDBS, which will be used in this experiment, is a C₁₂ LAS (see Figure 2-3).
Figure 2-2: General chemical structure of LAS, where $x$ and $y$ corresponds with the number of CH2 on each side of the benzene sulphonate group $(7x+10y)$ (Liwarska-Bizukojc, Drews & Kraume, 2008)

Figure 2-3: Sodium dodecylbenzene sulphonate (Alrich-2525)
2.1.2.1 Biodegradability of LAS

LAS concentrations in raw wastewater have been reported to range from 3 to 21 mg/L (Mungray & Kumar, 2009). In general, sewage treatment plants mainly use activated sludge as the secondary treatment process. Trickling filters, stabilisation ponds, upflow anaerobic sludge blanket reactors, and SBRs are alternative secondary treatment technologies. In sewage treatment plants employing activated sludge processes in the US, LAS removal has been found mostly in the range of 95% to 99.9% (Brunner et al., 1988). The LAS removal in activated sludge processes measured in five European countries averaged 99.2% (Waters & Feijtel, 1995) and 99.4% (the range was 98.9–99.9%) (Holt et al., 2003).

Figure 2-4 shows the process of aerobic biodegradation of LAS. It starts with the transformation induced by micro-organisms with formation of sulphophenyl carboxylates (SPC). This biodegradation stage corresponds to the disappearance of the parent molecule and the loss of interfacial activity as well as the toxicity to aquatic organisms.

LC50 is a standard measure of the toxicity of the surrounding medium that will kill half of the sample population of a specific test-animal in a specified period through exposure via inhalation (or respiration). The toxicity of SPC had LC50 values 120% to 240% higher than that of LAS (Kimerle & Swisher, 1977; Ying, 2006). Biodegradation proceeds further with the cleavage of the aromatic ring and the complete conversion of LAS and SPC into water, carbon dioxide,
inorganic sulphates and biomass. This step is also known as ‘Ultimate Biodegradation’ or mineralisation.

![Diagram of ultimate biodegradation of LAS](image)

Figure 2-4: Ultimate biodegradation of LAS (Huddleston, 1979; Swisher, 1963)

Degradation of LAS in anaerobic systems was also reported. In this process, sulphate, nitrate or carbonate act as alternative acceptors yielding, ultimately, hydrogen sulphide (H₂S), molecular nitrogen (N₂), methane (CH₄) and/or ammonia (NH₃). LAS mineralisation under anoxic conditions has not been documented and the known enzymatic steps involved in aerobic mineralisation require molecular oxygen (Gejlsbjerg et al., 2004).

### 2.1.2.2 Sorption of LAS

LAS removal from wastewater by aerobic processes in well-designed municipal wastewater treatment plants was above 90%. However, many researchers reported that the surfactant load into a treatment facility might be removed by
sorption to suspended solids, rather than through direct biodegradation by aerobic microorganisms (Mösche et al. 2002; Rittmann et al. 2001; Rodezno, 2004). For example, Rittmann et al. (2001) and Mösche et al. (2002) reported that when wastewater that contains a surfactant is fed to a bioreactor, the surfactant concentration will initially decrease due to adsorption onto biomass, but slow desorption kinetics were keeping the adsorbed LAS in an unavailable state.

The amount of LAS present in the final sludge is highly dependent on the biological processes running at the WWTP. The most important parameter in controlling the LAS content of final sludge is the aerobic conditions during digesting. Typically, LAS levels in aerobically digested sludge are found in the range of 100–500mg kg/dry weight (Jensen, 1999; Ying, 2006).

2.1.3 Alkylphenol Ethoxylates

APEOs are a class of non-ionic surfactants that are produced by reacting alkylphenols with ethylene oxide. An APEO molecule consists of two parts: the alkylphenols and the ethoxylates moiety. This structure makes APEOs soluble in water and helps disperse dirt and grease from soiled surfaces into water (Ying et al., 2002). The major use for APEOs is as surfactants that can function as detergents, wetting agents, dispersants, emulsifiers, solubilises and foaming agents. APEOs are important to a number of industrial applications, including pulp and paper, textiles, coatings, agricultural pesticides, lube oils and fuels, metals and plastics. Industrial applications comprise 55% of the APEO market.
The remaining uses include I&I cleaning products (30%), household cleaning products (15%) and other uses (<1%).

APEOs are among the most widely used classes of non-ionic surfactants, with an annual worldwide production of about 650,000 tonnes (Guenther et al., 2002). The usage of APEOs has declined since the discovery in 1984 that one of their breakdown products, alkylphenols (APs), are more toxic to the aquatic organisms than APEOs themselves. Alkylphenols that have been detected in the environment due to the discharge of sewage effluents into surface waters have attracted a great deal of scientific attention because of their estrogenic effects and ability to bio-accumulate in aquatic organisms (Ying, 2006). The most significant commercial APEOs are octylphenol ethoxylates (OPEO) and nonylphenol ethoxylates (NPEO). NPEOs account for about 80% of total APEO use and thus NPEO will be used in the experiments as a representative of non-ionic surfactants.

2.1.3.1 Biodegradability of APEO/NPEO

According to the literature, the concentration of APEO in the influents to the WWTP in US can be up to 33.7 mg/L (Naylor, 1995; Ying et al., 2002). APEOs are considered degradable under aerobic conditions and partially degradable or persistent under anaerobic conditions. The measured removal of NPEOs through sewage treatment plants in the US varied from 93% to 99% compared with 66% to 99% in Japan, 74% to 98% in Italy, and 47% to 89% in Switzerland.
These results suggest that often only partial degradation takes place (Ying, 2006).

The biodegradation of APEOs in conventional sewage treatment plants is generally believed to start with a shortening of the ethoxylate chain, leading to short-chain APEOs containing one or two ethoxylate units under the aerobic condition. Further transformation proceeds via oxidation of the ethoxylate chain, producing mainly alkylphenoxy ethoxy acetic acid and alkylphenoxy acetic acid (see Figure 2-5). The three most common groups of intermediates reported were as follows: 1) Alkylphenols (APs), which can be formed only in anaerobic conditions (e.g. nonylphenol and octylphenol); 2) short-chain APEOs having one to four ethoxylate units; and 3) a series of ether carboxylates including alkylphenoxy acetic acid and alkylphenoxy ethoxy acetic acid. Decarboxylated NPEO biotransformation products with the alkyl chain carboxylated were also detected in a sewage treatment plant effluent (Ying, 2006).
Figure 2-5: Breakdown processes of long-chain alkylphenol polyethoxylates (APEOs) under aerobic and anaerobic conditions, and the formation of their halogenated derivatives (Vega Morales et al., 2009)
2.1.3.2 Sorption of APEO

NPEO was found to have higher sorption than LAS on sludge, sediment and soil (Ying, 2006). The octanol-water partition coefficient (log Kow) measures the partitioning of a given compound between two phases, water and octanol. A compound with a high Kow, i.e. low solubility in water, will have limited or slow transport to water bodies. Studies have shown that calculated log Kow is well-correlated with acute and chronic ecotoxicity (Dow, 2011). Measured values of Kow for organic chemicals have been found as low as 10-3 and as high as 107, thus encompassing a range of ten orders of magnitude.

The commercial APEOs have low octanol-water coefficients (log Kow ~3.0) and higher for the low molecular APEOs and alkylphenols (APs) (log Kow = 3.3 to 4.4). The higher mole APEOs will tend to stay in water as opposed to becoming associated with sediments. In contrast, lower mole APEOs and APs are more likely to partition into organic phases (Melcer et al., 2006). Therefore, many studies show that NPEO biodegradation products are more lipophilic than their parent compounds and tend to be adsorbed on sludge and sediments (Hung et al., 2004; Thiele et al., 1997).

2.2 Overview of Sequencing Batch Reactors

SBR refers to one physical unit where the complete aerobic biological treatment process is performed. These reactors have found many applications for the treatment of industrial and domestic wastewater. In general, the reactors are
used for the treatment of medium strength wastewater to comply with discharge limits or as a pre-treatment stage preceding tertiary processes to produce water of quality fit for recycling.

A SBR is a specific fill-and-draw version of the activated sludge process. Metabolic reactions and solid-liquid separation are performed in one tank in a well-defined and continuously repeated time sequence (Wilderer et al., 1993). The tank is filled and then operated as a batch reactor. At the end of the cycle, activated sludge and the liquid phase in the reactor is allowed to settle and clarified supernatant is drawn from the tank followed by a new fill-and draw cycle.

The complete SBR cycle consists of four steps: 1) reactor filling; 2) reaction; 3) biomass settling; and 4) effluent decanting and discharge (see Figure 2-6).
During the fill period, the influent wastewater is added to the biomass (activated sludge) retained in the system after the previous cycle. The influent volume added can be as little as 25% of the total volume of the reservoir or as great as 70%; it depends on the desired food-to-microorganism (F/M) (Irvine & Ketchum, 1989; Woodard & Curran, 2006). The degradation of the organic compounds may start during this period and may be completed during the reaction period, depending on the type of organic compounds present in the wastewater. The reactor may be mixed only or mixed and aerated to promote biological reactions with the influent wastewater (Tchobanoglous et al., 2002).
During the reaction period, the biomass consumes the substrates (this refers to the organics consumed by the micro-organisms) under controlled environmental conditions. Aerobic and anoxic periods can be combined within this reaction phase. The duration of reaction period is usually dictated by the time necessary for the target compound to reach a designated concentration. Time dedicated to reaction can take more than 50% of the total cycle time (Irvine & Ketchum, 1989; Morgenroth & Wilderer, 1998; Tchobanoglous et al., 2002).

During the settling stage, the sludge formed by the floculated bacteria (i.e. growth of micro-organisms as a result of substrate degradation) is allowed to settle to the bottom of the tank under quiescent conditions, resulting in a clarified supernatant and settled sludge.

During the draw period, the supernatant is decanted (supernatant is the reactor effluent) and the system is ready for a new cycle. In some cases, idle period, which is after decanting period, is used when time is needed for a multi-tank system. One reactor has to complete the fill phase before switching to another unit.

2.2.1 Use of SBRs in Research Studies

SBRs are often used in research studies where continuous processes are required instead of using bigger multi-tank set-up for aeration and settling of sludge, due to their advantages over conventional continuous flow reactor, for example:
High flexibility: The operation of SBR can be changed between nitrifying only (aerobic), nitrifying/denitrifying (anoxic/aerobic), or full biological nutrient removal (anoxic/anaerobic/aerobic) by changing the process control parameters of the feed and reaction sequences alone.

Flowrate independent: In SBR, feed, wastage and effluent volumes are correctly identified, perfectly repeatable between cycles, and can be easily modified by changing level set-points as they are controlled by level only.

Easy to control sludge retention time (SRT): For research applications, the SRT of the pilot plant must be easily recognised and controlled to a set value. In an SBR where the sludge is contained in one single tank, the determination of the average total sludge mass in the system is straightforward through regular monitoring of the MLSS concentration during mixed sequences.

Direct measurement during reaction: SBR operates like a closed batch reactor during the reaction sequence. Thus, reaction rates can be measured directly by monitoring concentration changes in the reactor tank by manual sampling or with an on-line probe. Environmental conditions affecting the reaction rate such as temperature, dissolved oxygen (DO), pH, substrate concentrations and mixing rate are truly representative of the process (Stricker & Béland, 2006).

Moreover, some researchers reported that SBRs have a better Biological oxygen demand (BOD) and ammonia removal. Abdel-Kader (2009) simulated the SBR and activated sludge process using a GPS-X (version 5.0) simulation.
2.3 Nitrification and Denitrification Reactions

Wastewater treatment plants are required to remove nitrogen compounds from wastewater before discharge to receiving water bodies, to minimize their impact on the environment such as ammonia toxicity to aquatic life, depletion of oxygen levels in the presence of high concentrations of nitrates and nitrites and eutrophication (Tchobanoglous et al., 2002). Wastewater treatment plants are therefore designed to incorporate nitrification and denitrification biological reactions into the biological stage of wastewater treatment to reduce effluent’s nitrogen concentration to the designated level.

It is well accepted now that nitrification occurs in two steps. The first step is the aerobic oxidation of ammonium to nitrite (Eq 1), followed by the oxidation of nitrite to nitrate (Eq 2) by specific autotrophic bacteria. The genera of nitrifying bacteria that oxidise ammonium ions to nitrite ions are prefixed Nitroso- (such as Nitrosomonas), and the genera of nitrifying bacteria that oxidise nitrite ions to nitrate ions are prefixed Nitro- (such as Nitrobacter).

\[ \text{NH}_4^+ + 1.5 \text{O}_2 \rightarrow 2 \text{H}^+ + 2 \text{H}_2\text{O} + \text{NO}_2^- \]  
(Eq 3-1)
In a subsequent denitrification step, heterotrophic bacteria (denitrifiers) use the chemically bound oxygen of nitrates to degrade carbonaceous organic compounds in the wastewater under anoxic conditions according to the reaction given below (Eq 3) when the DO concentration is less than 0.5 mg/L, ideally less than 0.2.

\[
6\text{NO}_3^- + 5\text{CH}_3\text{OH} \rightarrow 3\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O} + 6\text{OH}^-
\]  \hspace{1cm} (Eq 3-3)

In this process (denitrification), molecular nitrogen is produced. The combination of both processes reduces the level of total nitrogen, ammonia and nitrates in the final effluent to concentrations compliant with discharge license permit. A carbon source (shown in the above equation as CH\(_3\)OH) is required for denitrification to occur. Recently, WWTPs invested into modifying the configuration of their activated sludge process such that organics in the influent are used as a source of carbon for the denitrification process (e.g. Modified Ludzak-Ettinger [MLE] process).

The growth rate of Nitrosomanas is higher than that of Nitrobacter. Thus, the rate-limiting step in nitrification is the conversion of ammonia to nitrite by Nitrosomanas (Gerardi, 2002). Nitrifiers need high concentrations of oxygen and low concentrations of organic material (Wang et al., 2009). However, the conditions for denitrification are high concentrations of readily biodegradable
organic material and the absence of free molecular oxygen. The growth rate of autotrophic ammonia oxidising bacteria is lower than that of heterotrophic bacteria, so without long retention times, the suspended nitrifiers will be easily washed out of the reactor, especially if temperature and oxygen concentration in the biological system are low (Campos et al., 1999; Gujer, 2010; Henze & van Loosdrecht, 2008; Kos, 1998). In addition, nitrifiers washout (evidenced by reduce ammonia removal and increased nitrate concentrations in the secondary clarifier effluent) were observed when WWTPs received high hydraulic loading rates during heavy rain (Environmental Leverage, 2003). Othman et al. (2010) also suggested that high concentrations of surfactants in the influent might have inhibitory effects on nitrifiers leading to their washout and high concentration of ammonia in the final effluent. Therefore, in this study the focus is on nitrification.
2.3.1 Factors affecting nitrification

In general, nitrification is affected by a number of factors including ammonia, nitrite, and oxygen concentrations, pH, temperature, BOD$_5$/TKN ratio and the presence of toxic chemicals (Dincer & Kargi, 2000; Gerardi, 2002; Tchobanoglous et al., 2002).

2.3.1.1 Ammonia and Nitrite Concentration

Dincer and Kargi (2000) reported that the removal of ammonia was inversely proportional to the concentration of ammonia in the influent. Nitrifying bacteria was also inhibited by relatively low concentrations of free ammonia and free nitrous acid. Free ammonia is produced from ammonium ions under a high pH in the aeration tank whereas free nitrous acid is produced from nitrite ions under low pH levels in the aeration tank. This inhibition or toxicity to free ammonia and free nitrous acid is known as substrate inhibition or toxicity (Bitton, 2005; Gerardi, 2002; Wang et al., 2009). Torà et al. (2010) also mentioned that nitrification was partially inhibited by the presence of free ammonia and free nitrous acid. At 20°C and pH 7.0, NH$_4$-N concentration at 100 mg/L and 20 mg/L may initiate inhibition of NH$_4$-N and NO$_3$-N oxidation respectively, and NO$_2$-N concentration at 280 mg/L may initiate inhibition to NO$_2$-N oxidation (Tchobanoglous et al., 2002).
2.3.1.2 pH

According to Gerardi (2002), the optimum pH value for the growth of Nitrosomonas and Nitrobacter lies between 7.5 and 8.5, whereas nitrification stops at or below pH 6.0. Jiao (2009) reported that the optimum OUR of activated sludge occurred in the range of pH 7.5 and 8.0 and that the pH drop that results from nitrification can be improved by aeration to remove CO₂ and the addition of lime.

2.3.1.3 Dissolved Oxygen Concentration

DO concentration is one of the most important factors controlling nitrification. For nitrification to proceed, the oxygen should be well distributed and should not be lower than 2 mg/L (Gerardi, 2002; Wang et al., 2009).

2.3.1.4 Temperature

The growth rate of nitrifiers is reported to occur at temperatures in the range of 8–30°C (Gerardi, 2002; Wang et al. 2009), but no growth of Nitrosomonas or Nitrobacter was reported below 4°C or above 45°C. The optimum temperature range is 28–32°C (Gerardi, 2002; Morling, 2008). Wanner et al. (2005) used simulations with a dynamic model, calibrated for the Zurich WWTP in Switzerland. A quantitative relationship between the wastewater temperature and the ammonium effluent concentration was established. They found that a permanent temperature decrease of 1°C leads to a 10% reduction of the
maximum net specific growth rate of the nitrifiers and of the safety factor for washout of these microorganisms.

2.3.1.5 BOD₅/TKN Ratio

As the BOD₅:TKN ratio increased, the nitrification rate decreased. At low BOD₅/TKN ratios (0.5 to 3), the population of nitrifying bacteria is high and nitrification should not be influenced by heterotrophic oxidation of cBOD (Tchobanoglous et al., 2002). Morling (2008) also found that the increased nitrification rate occurred at the lower ratio of COD/TKN from the results of modelling a SBR plant. However, the BOD₅/TKN ratio in the wastewater influent is typically at least 3, and for denitrification the BOD₅/TKN ratio has to be greater than 5. Okabe et al. (1996) investigated the effects of different C/N ratios on time-dependent population dynamics of nitrifiers and heterotrophs in undefined mixed-population biofilms as well as on nitrification efficiency. The results showed that the population dynamics and nitrification efficiency were strongly related to the initial microbial composition in the biofilms and C/N ratio. It seems that a higher C/N ratio would retard the accumulation of nitrifying bacteria, especially NO₂-oxidisers.

2.3.1.6 Toxic Compounds

The most toxic compounds to nitrifiers are cyanide, thiourea, phenol, anilines and heavy metals at certain concentrations, and many of those compounds are more toxic to Nitrosomonas than to Nitrobacter (Gerardi, 2002). Few published
research studies reported that surfactants showed toxic effects on microorganisms (Dalzell et al., 2002; Gutiérrez et al., 2002; Liwarska-Bizukojc et al., 2005) and the toxicity of the surfactants will be discussed in next sections.

2.4 Effect of Surfactants on Activated Sludge

Surfactant accumulates on gas-liquid interfaces and reduces oxygen transfer rates in water (Bolles, 2010; Rosso et al., 2006). This may cause inhibition on the OUR and affect the nitrification in the WWTP.

2.4.1 Effect of Surfactants on Activated Sludge Respiration

Respirometry tests have been used to assess the potential toxicity of a wastewater stream or a specific compound on both heterotrophic and nitrifying bacteria (Archibald et al., 2001; Dutta, 2002; Pernetti et al., 2003).

Respirometry tests and their use for assessing inhibition or toxicity to activated sludge microorganisms have been well developed and published by several organisations such as the Organization for Economic Co-operation and Development 209 (1993), Environmental Protection Agency 712-C96-168 (1996) and International Organization for Standardization (ISO) 8192 (1986).

Dalzell et al.’s (2002) research showed that LAS was not inhibiting in the respirometry test that was developed according to ISO 8492(86) and OECD (2010). However, LAS showed a toxic effect when the Microtox® method was
used, which utilised the *vibrio fiscgeri*. Dalzell *et al.* (2002) mention that an increase of the concentration of the LAS produced an increment of values of accumulated oxygen up to a given concentration (1000mg LAS/l) corresponding to substrate inhibition, which is typical for biodegradable substrates. Moreover, the reason for non-toxic to activated sludge was that LAS was considered reference biodegradability material (DR. 73/405/CEE modified 31/March/ 82, and Standard Methods 5540C). Any surfactant was considered biodegradable for use when its biodegradability was over 80% with respect to LAS, taking LAS as 100% of biodegradability.

In addition, Jiao (2009) mentioned that Painter (1986) followed the OECD test for assessing inhibition to activated sludge reparation and reported that LAS did not show inhibition to activated sludge oxygen uptake at concentration up to 100 mg/L.

### 2.4.2 Effect of Surfactants on Nitrification

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 surfactant effect on nitrification reactions.

ISO 9509 is a standard method used to test inhibition due to the presence of a certain substance in wastewater. Pagga *et al.* (2006) compared two inhibition testing methods, ISO 9509 and ISO 8192, and reported that inhibition of
nitrification depends on the biodegradability of the potential inhibitory compound.

Dalzell et al. (2002) also showed that LAS had IC50 of 300 mg/L and the experiment’s method was slightly modified to follow the steps described in the Swedish Environmental Protection Agency (EPA) Report No. 4424 (1995). They explained that inhibition of surfactants leads to accumulation of ammonium (and possibly nitrite) and a reduction in nitrate levels within sludge. In their experiments, parallel aeration (by mixing) of a nitrifying activated sludge in the presence or absence of test substances was made over a 2 hour period and the difference in concentration of oxidised nitrogen (nitrite-N plus nitrate- N) produced by the oxidation of ammonium was assessed.

Tomczak-Wandzel et al. (2009) had used batch experiments in 20L volume reactors to observe the inhibition of SDBS to nitrate formation on the activated sludge process. Starch and Urea were used as a carbon and nitrogen source and they found that if the SDBS content does not exceed 100 mg/L. The effect of its concentration is practically unobservable (Tomczak-Wandzel et al., 2009).

Dokianakis et al. (2006) reported that LAS and nonylphenol ethoxylates affected the ammonium oxidisation rate at the initial concentration of 0.5, 1, 2, 6 and 10 mg/L on the isolated ammonium-oxidising bacteria and the nitrifying activated sludge. The isolation of these bacteria was based on feeding the inoculum with a selective culture medium, which supported only the growth of autotrophic bacteria (excluded the growth of heterotrophic bacteria). They also mentioned
that the inhibition by LAS and nonylphenol ethoxylates were higher on isolated ammonium-oxidising bacteria than on activated sludge because of the degradation of surfactants by heterotrophic and the high adsorption on the sludge. All batch experiments were conducted in a shaking bath at 25°C under fully aerobic conditions, except in the experiment with LAS. In that case, no air was supplied to the system in order to avoid any loss of LAS caused by bubbling.

Baillod & Boyle (1968) stated that in their experiment using a SBR with 24 hrs per cycle, concentration up to about 10 mg/L of LAS stimulated both nitrite and nitrate formation, but concentration of above 10 mg/L produced an inhibition. This was due to the effects of the surfactant on cell permeability. Surfactants may increase cell permeability by solubilisation of the lipid material in the cell membrane. Since increased permeability will affect a higher rate of substrate transfer into the cell, it may produce a higher rate of metabolism. However, as the concentration of detergent increases, it is possible that the permeability of the cell membrane is increased to a point that it could no longer function to retain the vital protoplasmic constituents when the concentration of surfactant is high enough to go beyond this point. Cell metabolism would be impaired as vital constituents would be lost to the medium. This hypothesis is supported by reported stimulatory effects of low concentrations of anionic detergents (Glassman, 1948) and is further reinforced by the well-known inhibitory effects of very high concentrations (Dalzell et al., 2002; Tomczak-Wandzel et al., 2009).
2.4.3 Effect of Surfactants on Substrate Consumption

LAS and APEO were found to influence aerobic heterotrophic biodegradation of organic matter in some industrial wastewater. Liwarska-Bizukojc et al. (2008) reported that with a concentration of 50 mg/L, LAS is most likely to decrease the affinity of substrate to biomass compared to four other tested surfactants. However, the non-ionic surfactant alkyl phenol ethoxylates inhibits biomass growth and decreases maximum specific growth rates (μmax) the most among the tested surfactants. They also concluded that surfactants that contained a benzene ring were most likely to deteriorate wastewater treatment processes in the activated sludge systems. Comparing the efficiency of wastewater treatment (in terms of COD removal) in the presence of NPEO and LAS surfactants at the same concentration of 50 mg/L, in spite of higher biomass activity in the NPEO runs in comparison to the LAS ones, the degree of organic pollutant removal was higher for LAS by 10%. This means that organic pollutants were more easily removed from wastewater containing LAS than NPEO. Despite the higher concentration of LAS in municipal as well as industrial wastewater in comparison to NPEO, they are usually more easily biodegraded than NPEO.
2.4.4 Effect of Surfactants on Activated Sludge Floc Morphology

According to Liwarska-Bizukojc and Bizukojc (2006), LAS strongly influenced the activated sludge floc morphology and activity. As a result of saponification processes, the sludge floc became smaller and more circular in comparison to floc that had not been exposed to anionic surfactants. Further, the dehydrogenisation activity of the activated sludge decreased with an increase of the dilution rate in all surfactant runs. Among the tested anionics, SDBS, which belongs to LAS, exerted the strongest saponificative effect on sludge floc and dehydrogenisation activity of microorganisms (Liwarska-Bizukojc et al., 2005). For non-ionic, APEO caused a decrease in the size of activated sludge floc but they did not affect the shape of the floc. The circularity index and convexity that describe the shape of activated sludge floc remained similar to the control run, containing no surfactant. The presence of APEO within the tested concentrations range (5, 50 and 500 mg/L) caused a decrease in biomass activity. In spite of morphological changes of activated sludge floc and a decrease in microbial activity, only higher concentrations of non-ionic in wastewater starting with the level of 50 mg/L can induce pinpoint floc and decrease wastewater treatment efficiency. Melcer et al. (2006) also stated that high doses of APEO (80-100 mg/L) have been shown to lead to floc destabilisation and sludge bulking.
2.4.5 Effect of Surfactants on Aquatic Organisms’ Toxicity

According to Liwarska-Bizukojc (2005), based on toxicity tests to aquatic organisms, all tested anionic surfactants were harmful (LC50 between 10 and 100 mg/L), whereas non-ionic ones were toxic (LC50 between 1 and 10 mg/L) or even highly toxic (LC50 below 1 mg/L). In addition, in the papers reviewed, anionics are usually believed to be more toxic than non-ionic towards aquatic organisms excluding some products of APEO breakdown, i.e. nonylphenols.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Activated Sludge

Activated sludge was obtained from a local domestic WWTP, Sunbury WWTP. Samples were collected early in the morning and the standard tests, OUR and nitrification inhibition, were performed on the same day. After receiving the activated sludge, it was sieved to remove coarse particles and washed three times with deionised water. Finally, the sludge was resuspended at about 3g/L and the dry mass of the sludge was determined by gravimetric analysis according to standard methods (Clescerl et al., 1971). The sensitivity of the sludge was checked according to the method described in the OUR test ISO 8192 and nitrification inhibition test ISO 9509. For the ISO 8192 OUR test, the mixed liquor suspend solid (MLSS) at 1500 mg/L must have EC 50 of 3.5—dichlorophenol in the range of 5–30 mg/L. EC 50 is calculated as the
concentration of surfactant that provokes a response half way between the minimum and maximum inhibition. For ISO 9509 nitrification inhibition test, nitrification rate for the MLSS at 1500 mg/L should be in the ranged from 2 to 6.5 mg N/g biomass.h. During this study, the samples collected from Sunbury WWTP showed sensitivities within the recommended range.

The 30L pilot scale SBR was operated by feeding the synthetic wastewater at 0.5 L/min for 30 mins that contained: 600 mg/L glucose, 120 mg/L NH₄Cl, 40 mg/L CaCl₂·2H₂O, 20 mg/L MgSO₄·7H₂O, 40 mg/L K₂HPO₄ and trace metals which include FeCl₃·6H₂O, ZnSO₄·7H₂O and CuSO₄·5H₂O for improving the growth of bacteria, in three cycles a day in the hydraulic retention time (HRT, the average time of the wastewater stays in the system) = 24hrs and solid retention time (SRT, the average time of the sludge stays in the system) = 15 days where MLSS was at about 3000mg/L. The activated sludge from this reactor was also collect to compare the difference to the activated sludge from WWTP.

3.1.2 Surfactants

Two surfactants were used in the experiments: SDBS (Aldrich D-2525) and Nonylphenol Ethoxylates (NPEO) (Huntsman TERIC N8) (detail in Table 3-1). Stock solutions of each of the surfactants with concentration 1g/L were prepared using Milli-Q water. Since the concentration of SDBS (Aldrich D-2525) was approximately 80% pure, which included all homologues, and the
remainder of the 20% was sodium chloride and water, 1.25g of SDBS was added to 1L of water.
Table 3-1: Details of surfactants used in the experiments

<table>
<thead>
<tr>
<th>Surfactant name</th>
<th>Group of surfactant</th>
<th>Molecular weight (g/mol)</th>
<th>Molecular formula</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium dodecylbenzene sulphonate (LAS)</td>
<td>Linear alkylbenzene sulphonate</td>
<td>348</td>
<td>CH₃(CH₂)₁₁C₆H₄SO₃Na</td>
<td>80%</td>
</tr>
<tr>
<td>Nonylphenol ethylene oxide (NPEO)</td>
<td>Alkylphenol Ethyloxylate (APEO)</td>
<td>572</td>
<td>(C₂H₄O)₈C₁₅H₂₄O</td>
<td>100%</td>
</tr>
</tbody>
</table>

3.2 Methods

3.2.1 Batch Experiments

3.2.1.1 Oxygen Uptake Rate (OUR)

The respiration inhibition test was conducted according to the standard test, ISO 8192 (1986), procedure described in method B (higher sludge concentration: 1500mg/L). The activated sludge used in the test was collected from Sunbury WWTP and a large SBR of 40L operated at the school of Civil Engineering laboratories and fed with synthetic wastewater. The method started with preparing the synthetic wastewater which was made by dissolving the following amounts of substances in one litre of water: 16g peptone, 11g meat extract, 3g urea, 0.7g NaCl, 0.4g CaCl₂·2H₂O, 0.2g MgSO₄·7H₂O and 2.8g K₂HPO₄. The pH of the activated sludge was adjusted to 7.5±0.1 by adding 1M HCL or 1M NaOH, as needed, prior to analysis. In each test, a series of inoculums was prepared by adding a defined concentration of activated sludge to obtain the 1500 mg/L of MLSS, 9.6mL synthetic medium, 0.16g of HACH
nitrification inhibitor (Formula 2533™) and varying concentrations of the surfactant. Finally, the deionised water was added to give a final volume of 300mL. The nitrification inhibitor was used to eliminate the DO consumption through ammonia removal (nitrification).

During the test, inoculums were aerated at the same flow rate, measured using airflow meters and the DO was maintained above 2.5 mg/L. The inoculums were shacked at 130rpm throughout the duration experiment using a water bath shaker (Wish Bath®, Model: WSB–30) at 20°C.

After 30mins of reaction time, the inoculum was transferred into another bottle, which allowed the DO meter probe to fit tightly into the neck of the bottle. The DO was measured for 5mins, while the stirrer was on, and then the inoculum was transferred back to the beaker where shaking and aeration resumed. This procedure was repeated after 180mins reaction time. Each test included two controls and duplicate beakers for each concentration were used. Only tests in which controls and duplicates were within 15% of each other were considered valid (OECD 2010). The sensitivity of the activated sludge used in the test was verified to have an EC50 for 3,5-dichlorophenol in the range of 5–30 mg/L before its use.

The OUR (R) can be calculated from the linear part of the record oxygen concentration versus time graph according to Equation 3-1.

\[
R = \frac{Q_1 - Q_2}{\Delta t}
\]  

Equation 3-1
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; and

$\Delta t$ is the time interval, in mins between these two measurements.

The inhibitory effect (I) of a test chemical on the respiration rate (OUR) of activated sludge, expressed as %, at each concentration is given by Equation 3-2.

$\text{Error! Bookmark not defined. } I = \frac{R_B - (R_T - R_{PC})}{R_B} \times 100$

Equation 3-2

Where

$R_T$ is the oxygen consumption rate in the flasks with surfactant;

$R_B$ is the oxygen consumption rate in blank control; and

$R_{PC}$ is the OUR by physico-chemical control.

3.2.1.2 Nitrification Inhibition Tests

The nitrification inhibition test was conducted according to the procedure described in Method B of ISO 9509 (1989). The activated sludge was prepared as mentioned above and was aerated before use. The activated sludge was adjusted to pH 7.5±0.1 by adding 1M HCL or 1M NaOH prior to analysis. The
synthetic sewage feed is made by dissolving 2.65g (NH₄)₂SO₄ and 5.04g NaHCO₃ in 1L of water. In each test, a series of inoculums was prepared by adding a defined concentration of activated sludge to obtain the 1500 mg/L of MLSS, 30mL of synthetic medium and varying concentrations of surfactant. Finally, the deionised water was added to give a final volume of 300mL. Each test included two control flasks (with sludge, medium but no test substance), a series of flasks with adding varying concentrations of surfactants and a reference flask with adding 2.5mL of reference inhibitor (Allylthiourea (ATU)).

During the test, inoculums were aerated at the same flow rate measured by the airflow meter and the DO was measured to ensure that the DO did not fall below 2.5 mg/L. The inoculums were mixed in a water bath (Wish Bath®, Model: WSB–30) at 20°C and shaken at 130rpm throughout the experiment.

After 240 mins of reaction time, a suitable volume of sample was taken and filtered through a 0.45µm filter paper to analysis NO₂, NO₃ and NH₄.

To test the toxicity of surfactants to the sludge in nitrification, another nitrification test was attempted in which air was not supplied to the batch reactors. The procedure was the same as above, but the inoculums were aerated by shaking in the water bath at 140rpm and the DO was observed above 4 mg/L. This step was suggested by Dokianakis et al. (2006) and OECD/OCDE (2010) for the no foaming condition while testing surfactants as foaming would cause a great deal of sludge solids from the test mixture, which will result in artificially lowered respiration rates that could mistakenly be
interpreted as a result of inhibition. In addition, the aeration of surfactant solution concentrates the surfactant in the foam layer. Loss of foam from the test system will lower the exposure concentrations.

The level of nitrification inhibition induced by each surfactant was assessed according to ISO 9509, which is based on measurements of production of nitrite and nitrate after the addition of an ammonia-containing substrate.

The percentage inhibition of the formation of oxidised nitrogen – N (Nitrite + nitrate) is calculated using the Equation 3-3.

\[
I\% = \frac{C_c - C_t}{C_c - C_b} \times 100
\]  

Equation 3-3

Where

- Cc is the concentration of oxidised nitrogen – N, in the control flask without inhibitor after incubation in mg/L;
- Ct is the concentration of oxidised nitrogen – N, in the control flask containing test substance after incubation in mg/L; and
- Cb is the concentration of oxidised nitrogen – N, in the control flask reference inhibitor (Allylthiourea (ATU)) after incubation in mg/L;

In addition, the nitrification rate during the 4 hrs reaction period was determined based on the removal of ammonium during that period using Equation 3-4.
\[ I\% = \frac{C_i - C_e}{C_o - C_e} \times 100 \]  

Equation 3-4

Where

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

A summary of all OUR and inhibition nitrification test is given in Table 3-2: OUR and inhibition nitrification test.

Table 3-2: OUR and inhibition nitrification test

<table>
<thead>
<tr>
<th>Test</th>
<th>Inoculum</th>
<th>Inoculum concentration (mg MLSS/L)</th>
<th>Surfactant type</th>
<th>Surfactant concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUR</td>
<td>Sunbury WWTP</td>
<td>1500</td>
<td>SDBS</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>Nitrification inhibition</td>
<td>Sunbury WWTP</td>
<td>1500</td>
<td>SDBS</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>OUR</td>
<td>Sunbury WWTP</td>
<td>1500</td>
<td>NPEO</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>Nitrification inhibition</td>
<td>Sunbury WWTP</td>
<td>1500</td>
<td>NPEO</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>OUR</td>
<td>Sunbury WWTP</td>
<td>1500</td>
<td>SDBS and NPEO mixture</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>Nitrification inhibition</td>
<td>Sunbury WWTP</td>
<td>1500</td>
<td>SDBS and NPEO mixture</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>OUR</td>
<td>SBR</td>
<td>1500</td>
<td>SDBS</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>Nitrification inhibition</td>
<td>SBR</td>
<td>1500</td>
<td>SDBS</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>OUR</td>
<td>SBR</td>
<td>1500</td>
<td>NPEO</td>
<td>10, 20, 40, 60</td>
</tr>
<tr>
<td>Nitrification inhibition</td>
<td>SBR</td>
<td>1500</td>
<td>NPEO</td>
<td>10, 20, 40, 60</td>
</tr>
</tbody>
</table>

3.2.2 Sequencing Batch Reactors (SBR)

3.2.2.1 SBR Test 1
Four 2 L SBRs were used in this experimental program to investigate the effect of surfactants under conditions simulating continuous flow processes. The four SBRs each had 1.5L working volume and were fed with synthetic wastewater of the following composition (Table 3-2).

Table 3-3: Composition of synthetic wastewater in SBR test 1

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose,</td>
<td>600 mg/L</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>120 mg/L</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>40 mg/L</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>20 mg/L</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>40 mg/L</td>
</tr>
<tr>
<td>Trace metals</td>
<td>&lt;0.1 mg/L</td>
</tr>
</tbody>
</table>

The reactor operated three cycles per day in the hydraulic retention time (HRT) = 16hrs and SRT = 15 days. The SBRs operated for one month treating 0.75L of wastewater per cycle and using a cycle of 8hrs. The cycle started with aerobic feeding for 30 mins, followed by a 4 hr aerobic reaction and then air pump stopped for anoxic zone for 2 hrs. At the end of the cycle, 1hr was for settling and 30 mins for discharging (see Figure 3-1). The operating conditions are summarised in Table 3-4. Air diffusers that allowed for fine air bubbles used in this SBRs.
Figure 3-1: SBR experiment 1 cycle definition

- Reaction (anoxic), 2hrs
- Discharge, 0.5hr
- Settling, 1hr
- Reaction (aerobic), 4hrs
- Feed (anoxic), 0.5hr

Total cycle time: 8hrs
<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent flow</td>
<td>1.5</td>
<td>L/d</td>
</tr>
<tr>
<td>Total cycle time</td>
<td>8</td>
<td>hrs</td>
</tr>
<tr>
<td>Volumetric exchange ratio</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Minimum volume</td>
<td>0.75</td>
<td>L</td>
</tr>
<tr>
<td>Reaction time</td>
<td>6.5</td>
<td>hrs</td>
</tr>
<tr>
<td>Anoxic reaction time</td>
<td>2</td>
<td>hrs</td>
</tr>
<tr>
<td>Aerobic reaction time</td>
<td>4.5</td>
<td>hrs</td>
</tr>
<tr>
<td>Hydraulic retention time</td>
<td>16</td>
<td>hrs</td>
</tr>
<tr>
<td>SRT</td>
<td>15</td>
<td>days</td>
</tr>
<tr>
<td>Temperature</td>
<td>20</td>
<td>°C</td>
</tr>
</tbody>
</table>

3.2.2.2 SBR Test 2

Four of the same 2 L SBRs were used for the experiment to investigate the effect of surfactants in a continuous process. Each SBRs had 1.5L working volume and were fed with the synthetic wastewater that contained the composition as below (see Table 3-5). The activated sludge was mixed with magnetic stirrers at 350rpm for suspension and aerated to ensure the DO was above 2.0 mg/L in the aerobic period. One SBR worked as a control (no surfactant added), whereas the other three SBRs received varying concentrations of the surfactant being investigated (see Table 3-6).
Table 3-5: Feed composition for 1.5L SBRs

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Formula</th>
<th>Name</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 mg/L</td>
<td>CH₃COONa</td>
<td>Sodium acetate</td>
<td>Carbon source (~650mg COD/L)</td>
</tr>
<tr>
<td>1.9 mg/L</td>
<td>CH₃CH₂COONa</td>
<td>Sodium propionate</td>
<td></td>
</tr>
<tr>
<td>1.9 mg/L</td>
<td>(C₆H₁₀O₅)n</td>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>1.9 mg/L</td>
<td>Tryptone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.14mL/L</td>
<td>CH₃CH₂OH</td>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>0.84g</td>
<td></td>
<td>Dehydrated Meat Extract</td>
<td></td>
</tr>
<tr>
<td>183 mg/L</td>
<td>NH₄Cl</td>
<td>Ammonium chloride</td>
<td>~50 mg NH₄-N/L</td>
</tr>
<tr>
<td>280 mg/L</td>
<td>NaHCO₃</td>
<td>Sodium bicarbonate</td>
<td>Alkalinity</td>
</tr>
<tr>
<td>0.19 mg/L</td>
<td>MnCl₂.4H₂O</td>
<td>Manganese(II) chloride tetrahydrate</td>
<td>Microelements solution</td>
</tr>
<tr>
<td>0.0018 mg/L</td>
<td>ZnCl₂.2H₂O</td>
<td>Zinc chloride dehydrate</td>
<td></td>
</tr>
<tr>
<td>0.022 mg/L</td>
<td>CuCl₂.2H₂O</td>
<td>Magnesium sulphate heptahydate</td>
<td></td>
</tr>
<tr>
<td>5.6 mg/L</td>
<td>MgSO₄.7H₂O</td>
<td>Calcium chloridedehydate</td>
<td></td>
</tr>
<tr>
<td>0.88 mg/L</td>
<td>FeCl₃.5H₂O</td>
<td>Potassium dihydrogen phosphate</td>
<td></td>
</tr>
<tr>
<td>1.3 mg/L</td>
<td>CaCl₂.2H₂O</td>
<td>Dipotassium hydrogen phosphate</td>
<td></td>
</tr>
<tr>
<td>7.0 mg/L</td>
<td>KH₂PO₄</td>
<td>Disodium hydrogen phosphate heptahydate</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>18 mg/L</td>
<td>K₂HPO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 mg/L</td>
<td>NaHPO₄.7H₂O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Corominas, 2006

Table 3-6: Surfactant compositions in the feed for SBRs

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Surfactant added</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor 1 (R1)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Reactor 2 (R2)</td>
<td>SDBS</td>
<td>30 mg/L</td>
</tr>
<tr>
<td>Reactor 3 (R3)</td>
<td>SDBS</td>
<td>15 mg/L</td>
</tr>
<tr>
<td>Reactor 4 (R4)</td>
<td>NPEO</td>
<td>15 mg/L</td>
</tr>
</tbody>
</table>

The SBRs were operated for one month treating 0.75L of wastewater per cycle and using a cycle of 12 hrs. Anoxic feeding for 30 mins was used followed by a 1 hr anoxic reaction phase and then air pumped in for aerobic reaction for 9 hrs. At the end of the cycle, 1 hr was for settling and 30 mins for discharging. The operating conditions are summarised in Table 3-7. Air diffusers that produced relatively larger bubbles was used for the reactors, compared to the SBR experiment 1.
Total cycle time: 12 hrs

Feed (anoxic), 0.5hr
Settling, 1hr
Reaction (anoxic), 1hr
Reaction (aerobic), 9hrs
Discharge, 0.5hr

**Figure 3-2: SBR cycle definition**

**Table 3-7: Operating condition in the SBR experiment 2**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent flow</td>
<td>1.5</td>
<td>L/d</td>
</tr>
<tr>
<td>Total cycle time</td>
<td>12</td>
<td>hrs</td>
</tr>
<tr>
<td>Volumetric exchange ratio</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Minimum volume</td>
<td>0.75</td>
<td>L</td>
</tr>
<tr>
<td>Reaction time</td>
<td>10.5</td>
<td>hrs</td>
</tr>
<tr>
<td>Anoxic reaction time</td>
<td>1.5</td>
<td>hrs</td>
</tr>
<tr>
<td>Aerobic reaction time</td>
<td>9</td>
<td>hrs</td>
</tr>
<tr>
<td>Hydraulic retention time</td>
<td>24</td>
<td>hrs</td>
</tr>
<tr>
<td>SRT</td>
<td>20</td>
<td>days</td>
</tr>
<tr>
<td>Temperature</td>
<td>20</td>
<td>°C</td>
</tr>
</tbody>
</table>
3.2.3 Analytical Techniques

Before measurements, all samples were filtered using a 0.45µm cellulose acetate filter paper (Whatman Cat No. 6874–2504).

3.2.3.1 Dissolved Oxygen Measurements

For the respirometer experiments, DO was measured using YSI 5100 (YSI, Australia) with a 5010 BOD probe oxygen sensor (YSI, Australia).

3.2.3.2 COD, Ammonia, Nitrate and Nitrite Measurements

HACH test kits were used to measure the levels of COD, NH₄–N, NO₂–N and NO₃–N. COD was measured using HACH method 8000 (TNTplus™ 822). Ammonia was measured using HACH method 10031 (Reagent Set, High Range Test ‘N Tube™ AmVer™ Nitrogen Ammonia). Nitrite was measured using HACH method 8507 (NitrIVer3 Nitrite Reagent Powder Pillows, Low range). Nitrate was measured using HACH method 8039 (NitraVer5 Nitrate Reagent Powder Pillows, high range).

3.2.3.3 Anionic Surfactant Measurement

The standard methylene blue active substance (MBAS) analysis, method 5540C (APHA, 1998) was used. 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 4°C until analysis.

3.2.3.4 Non-Ionic Surfactant Measurement

The concentration of the non-ionic surfactant NPEO was measured using 101787 Spectroquant Surfactants (non-ionic) Cell Test. The non-ionic surfactants react with an indicator Tetrabromphenolphthaleinethylester (TBPE) to form a complex that is then extracted with dichloromethane.

3.2.3.5 Activated Sludge Characterisation

The concentrations of MLSS and SVI were measured according to the standard methods 2540D and 2710D respectively (APHA, 1998). Due to the small scale of reactors, the volumes of liquor taken from the reactors were 50mL.
4. RESULTS AND DISCUSSION

4.1 Batch Tests

4.1.1 OUR Tests

4.1.1.1 Sensitivity of Activated Sludge Samples Check

The ISO 8192 OUR inhibition test includes a check of the sensitivity of activated sludge to 3,5 dichlorophenol in terms of 50% inhibition to OUR (EC50). The sludge is considered suitable if EC50 was observed for 3.5 dichlorophenol concentration in the range 5–30 mg/L.

Figure 4-1 shows the inhibition to OUR by 3,5 dichlorophenol. The EC50 values were found in the range of 18–22 mg/L, which met the ISO 8192 standard requirement.

The OUR tests had to be repeated a number of times by several times, in some instances more than five times, to verify accuracy of results and check reproducibility. For example, the range of inhibitions of a certain concentration was too high to obtain reproducible results even if different strategies were established to obtain a unified condition for batch tests, such as using airflow meters to control the airflow for every sample. The variables could be related to the different sensitivity of the sludge (see Table 4–1) which was collected from Sunbury WWTP. As the sensitivity of sludge increased, the inhibition increased.
Figure 4-1: Effect of 3,5-dichlorophenol on activated sludge OUR

Table 4-1: Effect of sensitivity of sludge to inhibitions

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Sludge</th>
<th>IC50 of 3,5 dichlorophenol</th>
<th>OUR Inhibition of 20 mg/L surfactant at 180 mins</th>
<th>Inhibition of 20 mg/L surfactant to ammonia removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDBS</td>
<td>WWTP</td>
<td>24.3</td>
<td>10</td>
<td>17.7</td>
</tr>
<tr>
<td>SDBS</td>
<td>WWTP</td>
<td>19.7</td>
<td>35</td>
<td>20.9</td>
</tr>
<tr>
<td>SDBS</td>
<td>SBR</td>
<td>24.8</td>
<td>30</td>
<td>2.5</td>
</tr>
<tr>
<td>SDBS</td>
<td>SBR</td>
<td>22.2</td>
<td>56</td>
<td>2.5</td>
</tr>
<tr>
<td>NPEO</td>
<td>WWTP</td>
<td>16.1</td>
<td>25</td>
<td>24.5</td>
</tr>
<tr>
<td>NPEO</td>
<td>WWTP</td>
<td>22.7</td>
<td>19</td>
<td>15.4</td>
</tr>
<tr>
<td>NPEO</td>
<td>SBR</td>
<td>23.9</td>
<td>55</td>
<td>41.2</td>
</tr>
<tr>
<td>NPEO</td>
<td>SBR</td>
<td>21.6</td>
<td>34</td>
<td>49.6</td>
</tr>
<tr>
<td><strong>Surfactant</strong></td>
<td><strong>Sludge</strong></td>
<td><strong>IC50 of 3,5 dichlorophenol</strong></td>
<td><strong>OUR inhibition of 50% NPEO and SDBS</strong></td>
<td><strong>Ammonia removal inhibition of 50% NPEO and SDBS</strong></td>
</tr>
<tr>
<td>Mix</td>
<td>WWTP</td>
<td>22.1</td>
<td>22</td>
<td>14.1</td>
</tr>
<tr>
<td>Mix</td>
<td>WWTP</td>
<td>24.8</td>
<td>40</td>
<td>2.6</td>
</tr>
</tbody>
</table>

4.1.1.2 Effect of SDBS on Activated Sludge OUR

The results in Figure 4-2 and 4-3 show the effect of SDBS on activated sludge OUR. OUR decreased with increased SDBS concentration. The inhibition to OUR ranged from 16.7% to 28.8%, after 30 mins exposure for concentrations of 10 to 60 mg/L. It was also noticed that the inhibition to OUR increased from
17.5% to 48.6% with increased exposure time to 180 mins as SDBS concentration increased from 10 to 60 mg/L. Another approach for reporting the effect of SDBS on activated sludge OUR is in terms of IC20 and IC50 (i.e. the surfactant concentration at which 20% and 50% inhibition is measured respectively). The IC20 for SDBS was at about 20 and 16 mg/L, after 30 and 180 mins exposure time respectively. However, there was no IC50 for SDBS, i.e. inhibition for all concentrations used in the test did not reach 50%. The other findings from these OUR tests were that SDBS inhibition increased with increased exposure from 30 to 180 mins and that inhibition was proportional to SDBS concentration. The increase in inhibition with time was more severe at the high concentrations tested, 40 and 60 mg/L. For example, inhibition increased by 1% and 3% for 10 and 20 mg/L respectively, compared with 13 mg/L and 20 mg/L at 40 and 60 mg/L SDBS respectively (see Figure 4-2). This could be attributed to two mechanisms likely to occur in the aeration phase, the first is rapid adsorption of SDBS onto the sludge followed by slow desorption of SDBS into the surrounding activated sludge (Mösche & Meyer, 2002; Rittmann et al., 2001; Rodezno, 2004). The high level of surfactant at 40 and 60 mg/L would cause higher inhibition to OUR. The second possible mechanism is hinder transfer of oxygen in the presence of surfactants, at an extent proportional to the surfactant concentration.
Figure 4-2: Activated sludge OUR for SDBS concentrations of 10–60 mg/L

Figure 4-3: Inhibition to OUR at different concentrations of SDBS after 30 and 180 mins exposure

To compare with a similar experiment set up, Jiao (2009) mentioned that the OUR at 30 mins and 180 mins was 15 and 18 mg/L.min respectively. They were very different from what this study, above 60 mg/L.min was observed. The reason could be the higher air supply to the samples in her study. The different OUR could alter the inhibitions of SDBS at the same concentration to OUR as the oxygen transfer of the bubble would be different which cause the shorter
residence time of the bubble and the smaller effect on activated sludge by surfactants (Rosso & Stenstrom, 2006).

4.1.1.3 Removal of SDBS in OUR Tests after 180mins

The concentrations of SDBS were determined before and after the 180mins OUR tests. Figure 4-4 shows that the removal of SDBS decreased with the increased initial concentration of SDBS. The removal of SDBS in the reactor that received SDBS at an initial concentration of 10 mg/L was 71.8%, which was almost twice the removal measured in the reactor that received 60 mg/L SDBS, at 36.5%. The removal measured at 20 and 40 mg/L SDBS was at 66.4% and 44.6% respectively. This showed that the biodegradation of SDBS was faster in lower initial concentration than in higher initial concentration.

![Figure 4-4: Removal of SDBS after OUR test](image)

Jiao (2009) reported that the removal of SDBS measured at the end of the OUR tests after 180 mins exposure was 38.8% and 24.4% for 25 mg/L and 75 mg/L
SDBS respectively. This shows that the SDBS removal reported by Jiao (2009) was lower than the SDBS removal measured in this study. Considering that inhibition to OUR reported by Jiao (2009) was higher than that measured in this study, it can be concluded that inhibition to OUR seems to be proportional to the concentration of SDBS in the activated sludge aeration reactors.

4.1.1.4 Effect of NPEO on Activated Sludge OUR

The results shown in Figure 4-5 and Figure 4-6 demonstrate the effect of NPEO on activated sludge OUR. The results indicate that activated sludge OUR decreased as the concentration of NPEO increased. After 30 mins exposure, NPEO inhibition to OUR increased from 6.3% to 16.6% for concentrations of 10–60 mg/L. The inhibition increased from 19.2% to 40.4% respectively, after 180 mins of exposure to NPEO. There was no IC50 for NPEO inhibition to OUR for all the concentrations tested. However, IC20 for NPEO was found to be 13.3 mg/L after 180 mins exposure.

Figure 4-5: Activated sludge OUR for NPEO concentrations of 10–60 mg/L
A comparison of the inhibition of SDBS and NPEO to OUR (Figure 4-3 and 4-6) show that SDBS inhibition to OUR was slightly higher than NPEO’s inhibition, measured after 30 mins and 180 mins reaction time. This could be attributed to the fact that SDBS had a stronger effect on decreasing the affinity of substrate to biomass than NPEO did (Liwarska-Bizukojc et al., 2008) and higher adsorption of NPEO on sludge that cause limited bioavailability (Ying, 2006). Liwarska-Bizukojc et al (2008) measured the half-saturation constant for heterotrophic biomass (Ks), which is an indication of affinity of substrate to biomass in respirometric tests showing that the surfactant in wastewater can decrease the affinity of substrate to biomass and SDBS had a higher decrease than NPEO. Adsorption of NPEO on sludge may also play an important role in decreasing the amount of inhibition, as Ying (2006) mentioned that the adsorption of NPEO on sewage sludge was very strong. Moreover, the higher inhibition of SDBS could be attributed to partial degradation occurred by NPEO, such as conversion from nonylphenol polyethoxylates (NP8EO used in the
experiment) to nonylphenol diethoxylate (NP2EO), nonylphenol monoethoxylate (NP1EO), which is more likely to adsorb on the biomass, and so the bioavailability was limited.

4.1.1.5 Inhibition of Mixtures of SDBS and NPEO to OUR

Figure 4-7 and Figure 4-8 showed the data of inhibition of the mixture of SDBS/NPEO of 60 mg/L on activated sludge OUR and Figure 4-9 depicts the average of the two tests. The most striking feature is that the inhibition to OUR by the combined surfactants (SDBS+NPEO) was higher than that measured at the same concentration of pure SDBS (0% of NPEO). The concentration with the highest inhibition in the test was 17% (NPEO / SDBS ratio 1:5), inhibiting averagely at about 30.5% (see Figure 4-9). The inhibition however decreased with the increased concentration of NPEO in the mixture. The inhibition of 83% of NPEO (NPEO / SDBS ratio 5:1) decreased to 23.4% and 19.9% after 30 and 180 mins of exposure (see Figure 4-9). Comparing to the inhibitions of 60 mg/L SDBS or NPEO in the experiments with increasing surfactants concentrations, the inhibitions were less in the experiment with mixture surfactants. This may be because the sludge was collected a month after that of the experiment above. This may also cause the variation in the inhibitions by the pure surfactants.

The lower inhibition to OUR observed for the combined SDBS and NPEO compared when each surfactant was used individually could be due to the variation in the activated sludge samples used in the test, especially those that were conducted several months apart, i.e. the Sudbury WWTP sludge was
collected after an event that affected the sludge diversity (heavy rain) and the laboratory SBR may have just recovered from a problem in the diffusers.

Figure 4-7: OUR test 1 of mixture of SDBS and NPEO

Figure 4-8: OUR test 2 of mixture of SDBS and NPEO
4.1.1.6 Sludge from Sunbury WWTP and 30L SBR

Figure 4-10 showed inhibition to OUR by 3,5-dichlorophenol test. The EC50 values were found in the range of 20–30 mg/L. Although the EC50 met the ISO 8192 standard requirement being in the range 5–30 mg/L, the sensitivity of the sludge from the laboratory SBR was lower than sludge from Sunbury WWTP.

Figure 4-10: Effect of 3,5-dichlorophenol on activated sludge OUR for sludge from the large laboratory SBR
The results in Figure 4-11 and 4-12 show the effect of SDBS on the OUR of activated sludge collected from a 30L laboratory SBR. OUR decreased with increased SDBS concentration, with the majority of reduction occurring at the low concentration end, where 90% of OUR reduction occurred with increased concentration from 10 to 20 mg/L. It was observed that the inhibition increased from 25% to 37% with increased concentration from 10 to 20 mg/L, whereas inhibition measured at 40 and 60 mg/L was slightly higher at about 40%. Extending exposure time to 180 min, it was observed that inhibition increased from 31.7% to 55.9% for concentrations from 10 to 20 mg/L then stabilised at about 56% for 40 and 60 mg/L. The severe effect of SDBS on activated sludge OUR is clearly shown considering the IC values. The IC20 for SDBS were 6.3 mg/L and 8.0 mg/L measured after 30 and 180 mins contact time respectively. However, IC50 was only observed after 180 mins exposure time measured at 17.6 mg/L SDBS. The higher inhibition observed with extended exposure to 180 mins can be explained in terms of rapid adsorption: slow desorption that led to high concentrations of the surfactant in the reactor. Alternatively, it could be due to interference with oxygen transfer, which seems to be at a rate proportional to the surfactant concentration. The higher inhibition to OUR observed using sludge from the laboratory SBR compared with that measured using sludge from the Sunbury WWTP sludge could be due to the acclimatisation effect which also cause the loss of diversity of the sludge, especially as the sludge in the SBR had not been exposed to surfactants because it was fed by a synthetic wastewater. On the other hand, real wastewater is rich in all micronutrient and trazas, and conserve or improve the activity of the WWTP sludge.
Figure 4-11: OUR for activated sludge collected from a large laboratory size SBR for SDBS concentration of 10–60 mg/L

Figure 4-12: Inhibition of SDBS to OUR for activated sludge from 30L laboratory SBR

The results in Figure 4-13 and Figure 4-14 show the effect of NPEO on the OUR of activated sludge collected from the laboratory SBR. OUR decreased with increased NPEO concentration and the difference of the inhibition between 30 mins and 180 mins exposure increased with the concentration of NPEO. The
inhibition observed ranged from 23.0% to 41.5% after 30 mins exposure for concentrations from 10 to 60 mg/L. The inhibition increased with extended exposure from 30 to 180 mins reaching 27.3% and 61.1% at 10 and 60 mg/L respectively. It was noticed that the effect of increased contact time was the same for all concentrations tested, i.e. 20, 40 and 60 mg/L, where approximately 20% increase inhibition was measured. Further, the inhibition was almost the same for these concentrations. A similar trend was observed for the SDBS effect on activated sludge from the laboratory SBR. These results suggest that the sludge from SBR has a threshold of 20 mg/L and that the micro-organisms population dynamics seem to have the same response to concentrations in the range 20–60 mg/L. The reason for the inhibition suppressed after 20 mg/L could be due to the small differences of the effect on cell permeability of 20 – 60mg/L. The effect of NPEO on activated sludge OUR can also be reported in terms of the IC values. The IC20 values for NPEO were 7.3 and 8.7 mg/L measured after 30 and 180 mins contact time respectively. There was no IC50 for NPEO after 30 mins but the IC50 was 30.3 mg/L for 180 mins exposure. Comparing the sludge from SBR and from Sunbury WWTP, the inhibition of NPEO was higher in the experiment in which SBR sludge was used. These results suggest that the higher inhibition observed using sludge from the laboratory SBR could be due to the acclimatisation effect to surfactants in which SBR sludge is fed with synthetic wastewater free of surfactants. Also, SBR technology generate a selection and for ended a reduction of the number of microorganism.
In summary, Figure 4-3, Figure 4-6, Figure 4-12 and Figure 4-14 exemplify the inhibition of SDBS and NPEO to OUR for activated sludge from two different sources. The results show that both surfactants exert higher inhibition to OUR for activated sludge from the laboratory SBR than for the sludge from the Sunbury WWTP, for concentrations of 10 to 60 mg/L. This was attributed to acclimatisation effects, because activated sludge from the SBR had no previous contact with surfactants whereas activated sludge from the Sunbury WWTP is
exposed to a wide range of surfactants in its influent reaching it from different sources, domestic, commercial and industrial usage.

### 4.1.2 Nitrification Inhibition Test

#### 4.1.2.1 SDBS Inhibition to Nitrification

The effect of SDBS of 10, 20, 40 and 60 mg/L on activated sludge nitrification capacity was examined. The effect on nitrification was measured in terms of the concentrations of ammonia, nitrite and nitrate after 4 hrs reaction according to the standard tests (Figure 4-15). The concentration of ammonia measured at the end of the test increased, compared to that measured in the control (the test sample without SDBS added), with increased SDBS. But the formation of nitrite and nitrate decreased slightly with increased concentration of SDBS, compared with that measured in the control. The results showed that inhibition to nitrification was proportional to the concentration of SDBS. Figure 4-16 showed that the inhibition to ammonia removal was higher than the inhibition to oxidised nitrogen (nitrite + nitrate). For example, at 60 mg/L SDBS, the inhibition to ammonia removal was 51.8% but the inhibition to formation of oxidised nitrogen was 21.4%. The higher ammonia removal can be attributed to the utilisation of ammonia for growth of activated sludge micro-organisms. The results also suggest that the nitrifiers known to oxidise ammonia to nitrite, i.e. Nitrosomonas (Eq. 3-1), were more inhibited than Nitrobacter, the microorganisms known to be responsible for oxidising nitrites to nitrates (Eq. 3-2).
Figure 4-15: Concentration of N-NH₄, N-NO₃ and N-NO₂ for SDBS concentration from 0 to 60 mg/L

Figure 4-16: Inhibition to nitrification for SDBS

The removal of SDBS at the end of the nitrification inhibition test decreased with increased SDBS initial concentration (see Figure 4-17). There was a relatively large drop in SDBS removal at the highest concentration tested, i.e. 60 mg/L.
Figure 4-17: Removal of SDBS after nitrification test

4.1.2.2 NPEO Inhibition to Nitrification

The effect of the presence of the non-ionic surfactant, NPEO, at a total concentration of 10 - 60 mg/L, on activated sludge nitrification was examined (Figure 4-18). NPEO caused lower inhibition on activated sludge than SDBS (see Figure 4-20). The inhibition to ammonia removal of 10 mg/L NPEO was about 17.1% and 44.5% at 60mg/L NEPO. Inhibition to oxidised nitrogen followed the same trend, but was almost 6.6% to 17.3% less than inhibition to ammonia (Figure 4-19).
Figure 4-18: Concentration of nitrite, nitrate and ammonia for NPEO concentration from 0 to 60 mg/L

Figure 4-19: NPEO inhibition to nitrification

The mixture of surfactants caused higher inhibition on activated sludge (see Figure 4-20). The inhibition of 17% NPEO (10 mg/L SDBS + 50 mg/L NPEO) was the highest to ammonia removal but 80% SDBS had a slightly higher inhibition on oxidised nitrogen. The differences for the pure and mixed surfactants were not as high as the OUR inhibition test. The lowest inhibition to ammonia removal was 14% with pure NPEO and the highest inhibition was 19.6% with 17% NPEO.
4.2 SBR Test

4.2.1 Effect of SDBS on the SBRs Performance

Four bench top laboratory SBRs were operated as a part of this research study. The SBRs were fed with synthetic wastewater for a few months until they reached a steady state in terms of COD and NH₄ removal. In addition, SVI, MLSS, DO and pH were measured on a regular basis. Three of the SBRs received feed spiked with increased concentrations of SDBS, 5, 10 and 20 mg/L, whereas the fourth SBR was used as a control, i.e. it received synthetic wastewater with no surfactants. The amount of WAS remained the same after the commencement of spiking.

Monitoring of the SBRs on a daily basis showed that the MLSS in the SBRs that received feed spiked with 10 and 20 mg/L (SDBS) decreased slowly (see Figure 4-21). In the meantime, a slight increase in MLSS was observed in the SBR that received feed spiked with 5 mg/L SDBS. These results indicate that activated
sludge growth (or yield) in the SBRs was typical of that expected with increase in the concentration of organics in the influent, i.e. increased rate of growth of MLSS to a certain extent. Conversely, increased concentration of SDBS had an inhibitory effect on growth rate, which led to a reduction in the MLSS concentration. These results could be due to loss of MLSS in the effluent because of sludge poor settling, which developed due to presence of the surfactant. The presence of SDBS encouraged saponification, which has been reported to reduce sludge floc’s size. It could also be caused by loss of sludge and inhibition of growth of microorganism due to increased formation of foaming with increased exposure of the biomass in the SBR to SDBS.

Figure 4-21: MLSS profile in the three SBRs receiving 5, 10 and 20 mg/L SDBS

The results in Figure 4-22 show that activated sludge SVI decreased in three SBRs that received SDBS. The SVI decreased with time, whereas in the control SBR, it remained between 100 and 150mL/g. The drop in SVI could be due to the decrement of activated sludge floc dimensions as a result of saponification,
which was reported to occur in the presence of surfactants (Liwarska-Bizukojc, 2005).

![Figure 4-22: SVI in the SBRs that received feed spiked with SDBS](image)

Figure 4-22 shows that COD removal was adversely affected by the presence of SDBS in the influent, but there was no inhibition (on Day 7) and even stimulation by low (5mg/L) concentration of SDBS (on Day 1 &5). On Day 1, COD removal in the SBRs receiving 10 and 20 mg/L SDBS was about 2.2% and 9.6% respectively, less than the control. However, the deterioration in performance showed improvement until Day 5. Thereafter, a sudden drop in COD removal was observed on Day 7. The recovery of the SBRs’ capacity for COD removal, observed over Days 2 to 5 could be attributed to the acclimatisation of the sludge to SDBS. On Day 7, the foaming problem had intensified, especially in the SBRs receiving 10 and 20 mg/L SDBS causing loss of MLSS.
Figure 4-24 shows the ammonia removal in all SBRs in operation. The removal of ammonia dropped on Day 1 in SBR 3 and 4, and then almost fully recovered over the first five days. In the meantime, as mentioned for COD removal, foaming due to the continual exposure to SDBS led to a large drop in ammonia removal on Day 7.

Figure 4-23: COD removal in the SBRs that received feed spiked with SDBS

Figure 4-24: Inhibition to ammonia removal
4.2.2 Effect of SDBS and NPEO on SBR Performance

In this phase of experimental work, the effect of SDBS and NPEO on activated sludge under continuous exposure to surfactants was examined for the same loading, i.e. 30 mg/L. Similar to experiment 1, one SBR was used as a control, whereas SBRs 2, 3, and 4 received 30 mg/L SDBS; 15 mg/L SDBS + 15 mg/L NPEO and 30 mg/L NPEO respectively. Figure 4-25 shows the concentration of MLSS in the all SBRs over 14 days. The results show that MLSS concentration decreased with time. It was also observed that the MLSS drop was higher in the SBRs that received NPEO. The drop in MLSS could be due to poor settling and foaming, as discussed in the previous section (effect of SDBS on the SBRs performance). The presence surfactants in the influent could cause floc saponification, which decreases floc dimensions and increases floc circularity, resulting in poor sludge settling and washout of biomass in the system (Liwarska-Bizukojc & Bizukojc, 2006).
Figure 4-25: MLSS in the SBRs received feed spiked with surfactants

Figure 4-26 shows the SVI in the four SBRs over 14 days of operation. The SVI in the control ranged from 100 to 120 mL/g, whereas the SVI in the SBRs that received feed spiked with SDBS and/or NPEO decreased during the first week and fluctuated between 65 and 95 mL/g during the second week. These trends are in agreement with the changes in MLSS observed in the SBRs during this period. The SVI of the sludge collected from SBR2 (the SBR that received an influent spiked with 30 mg/L SDBS) was lower than that measured for sludge from SBR3 and SBR4. The larger drop in SVI for the sludge that was exposed to SDBS than that which was exposed to NPEO is in agreement with the results obtained from the batch inhibition to OUR standard tests. Liwarska-Biziojc (2008) also mentioned that raw wastewater that contain NPEO in at concentrations higher than 50 mg/L can induce pinpoint floc leading to a decrease in the wastewater treatment efficiency. In addition, they mentioned that the presence of NPEO at these high concentrations could induce changes to the morphology of activated sludge floc, which ultimately decreases its microbial activity.
Figure 4-26: SVI in the SBRs that received SDBS, SDBS + NPEO and NPEO

Figure 4-27 shows the COD removal in the four SBRs over 14 days of operation after introducing the surfactant into the feed to SBRs 2, 3 and 4. The results showed that COD removal in all SBRs was maintained between 94% and 98%, i.e. no inhibition was observed during this period (see Figure 4-28).

Figure 4-27: COD removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO
Figure 4-28: Average COD removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO

Figure 4-29 and Figure 4-30 show the ammonia removal over 13 days of operation after introducing the surfactant into the feed to SBRs 2, 3 and 4. The results show that ammonia removal in the SBRs that received SDBS and/or NPEO was not affected and remained above 98% during this period.

Figure 4-29: Ammonia removal in the SBRs that received SDBS, SDBS+ NPEO and NPEO
The concentration of oxidised nitrogen over the same period showed no inhibition to nitrification in any of the reactors that received SDBS and/or NPEO (see Figure 4-31). In all SBRs, the oxidised nitrogen formed were almost 100% nitrate as the nitrite measured was nearly 0.0 mg/L.
Figure 4-32 illustrated the removal of surfactants in SBR2, SBR3 and SBR4. Removal of SDBS decreased from 95.7% to 80.7% during the experiment but removal of NPEO remained in the range of 82% and 89%. The reducing SDBS removal could be due to desorption and increase of SDBS in the liquid (Rodezno, 2004).

Figure 4-32: Removal of surfactants

On Day 1, the nitrate formation was not inhibited by surfactants as shown in Figure 4-33, the nitrate concentrations in reactor 2, 3, 4 had a high nitrate production rate. The 0 mg/L of nitrate at the time 1.5 hrs showed that the denitrification functioned well in the system. Figure 4-34 shows that DO profile on Day 1, the DO was above 2.0 mg/L for nitrification.
Figure 4-33: Day 1 nitrate profile

Figure 4-34: Day 1 dissolved oxygen profile

Figure 4-35 shows the ammonia profile in Day 10. The oxidation of ammonia was almost finished at 4.5 hrs as the differences of the ammonia concentration between 4.5 hrs and the effluent was so small. Moreover, Figure 4-36 showed the nitrate production that confirmed the observation as the nitrate
concentrations were stable at 4.5 hrs and 12 hrs. The ammonia and nitrate were not inhibited by surfactants.

In SBR experiment 2, the air diffuser was changed to produce relatively larger bubbles and there was almost no inhibition even at the higher concentration of SDBS (30 mg/L) and when mixed surfactant was added into the reactors. This confirmed that surfactant accumulates on gas-liquid interfaces and reduces mass transfer rates and the reduction in general is larger for fine bubble aerators. Fine bubble diffusers have greater mass transfer depression than coarse bubble aerators do. The high turbulence associated with coarse bubble aerators allows them to achieve better oxygen transfer rates (Rosso & Stenstrom, 2006). Moreover, Tomczak-Wandzel et al. (2009) used a cycle deviation that was similar to SBR experiment 2 and reported that the inhibition was not obvious if the concentration of surfactants in the feed was lower than 100 mg/L.
Figure 4-35: Day 10 ammonia Profile

Figure 4-36: Day 10 nitrate profile
5. CONCLUSIONS

5.1 Batch Tests

- The effect of SDBS and NPEO on activated sludge was examined according to the ISO test 8192 using activated sludge from Sunbury WWTP. Both anionic surfactants SDBS and NPEO showed inhibitory effects on activated sludge OUR. Inhibition to OUR was proportional to the initial concentration of the surfactant, for concentrations of 10–60 mg/L, for both SDBS and NPEO. The results showed that SDBS had more severe inhibitory effects on activated sludge compared with NPEO. This trend was attributed to the affinity of SDBS to rapidly adsorb onto activated sludge then slowly desorb, and consequently be at a higher concentration than NPEO, which desorbs at a lower rate.

- The presence of both SDBS and NPEO in one reactor showed inhibitory effects to activated sludge OUR. Inhibition to OUR correlated with the inhibition trend observed for SDBS and NPEO when present as a single compound in the test. The highest inhibition observed was 17% for the mixture of 1:5 NPEO / SDBS ratio after 30 mins exposure. However, this inhibition level is lower than that observed for SDBS at 60 mg/L. The results were not in agreement with batch tests, which was attributed to the variation in sludge diversity that may have been caused by the long period between the collection of the samples used in the single and combined surfactants OUR tests.
The effect of SDBS and NPEO was also examined according to the ISO 8192 standard test, using activated sludge from a large laboratory SBR. The results obtained showed a similar trend to that observed using sludge from Sunbury WWTP, but the level of inhibition for both SDBS and NPEO using the SBR activated sludge was higher than that measured using activated sludge from Sunbury WWTP. This could be due to the acclimatisation effect to the surfactants, especially since the sludge in the SBR was not exposed to surfactants because it is fed by synthetic wastewater free of surfactants. Also, there was loss of biodiversity of the sludge from the SBR due to the acclimation.

The standard method for assessing the potential inhibition of a test compound to OUR recommends that measurements of OUR should be performed either after 30 mins or 180 mins 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 (SDBS and NPEO in this case).

SDBS and NPEO showed an inhibitory effect 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 for both SDBS and NPEO. SDBS had a stronger nitrification inhibition on activated sludge than NPEO.
The mixture of surfactants also showed higher inhibition than the pure surfactants to nitrification. Similar to the OUR test, 17% SDBS had the highest ammonia removal inhibition while 80% NPEO had the highest inhibition to oxidising nitrogen production. The differences for the pure and mixed surfactants were not as high as the OUR inhibition test.

5.2 SBR Experiments

- The presence of 5, 10 and 20 mg/L SDBS in the influent to bench scale SBRs showed an adverse effect on the concentration of MLSS and sludge quality measured in terms of sludge volume index (SVI). The concentrations of MLSS decreased with time, especially in the SBRs that received feed spiked with 10 and 20 mg/L SDBS; where a 26% reduction in MLSS was measured at the end of the first week, after SDBS was introduced into the feed. Similarly, the SVI in these SBRs decreased compared to the control, indicating poor sludge settling properties. These results were attributed to the saponification effect. That is, the presence of surfactants in the influent could cause floc saponification, which decrease floc dimensions and increase of floc circularity. This results in poor sludge settling and washout of biomass in the system. Further, the presence of SDBS in the feed on a continuous basis intensified foaming formations, which also contributed to loss of biomass with the foams.
The three SBRs that received feed spiked with SDBS showed deterioration in COD and ammonia removal. The results were in agreement with the reduction in MLSS. Further, they indicated that SDBS might interfere with oxygen transfer, which ultimately causes reduction in COD removal. To examine this effect, the fine bubble air diffusers in the SBRs were replaced with coarse bubble air diffusers in the following experiment.

The presence of 30 mg/L SDBS, 15 mg/L SDBS+ 15 mg/L NPEO and 30 mg/L NPEO in the influent to SBR2, 3 and 4 on MLSS, SVI, COD and NH4 removal was also examined. The SBRs were aerated using coarse bubble air diffusers. The results showed that the MLSS in all reactors decreased, which indicates that the effect of surfactants in terms of saponification and tendency to reduce floc sizes, and consequently poor sludge settling, was not improved with increased air bubble sizes. However, the removal of COD and NH4 was not inhibited and remained comparable with the control, which suggests that increased air bubble sizes may have improved the transfer of substrate and oxygen to the biomass.


94


