THE POTENTIAL OF PNP, TCS AND THPS
AS UNCOUPLERS TO REDUCE WASTE
ACTIVATED SLUDGE (WAS)

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

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DECLARATION

The work contained in this thesis has not been previously submitted for a degree or diploma at any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature:

Date:
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# TABLE OF CONTENT

DECLARATION ............................................................................................................. i

ACKNOWLEDGEMENT ................................................................................................. ii

TABLE OF CONTENT .................................................................................................. iii

ABSTRACT .................................................................................................................... viii

CHAPTER ONE

INTRODUCTION ............................................................................................................ 1

1.1 Background .............................................................................................................. 1

1.2 Objectives and Scope .............................................................................................. 3

1.3 Layout of Thesis ..................................................................................................... 4

CHAPTER TWO

LITERATURE REVIEW ................................................................................................. 5

2.1 Overview ............................................................................................................... 5

2.2 Sludge Production ................................................................................................ 8

2.2 Waste Activated Sludge (WAS) Production in Victoria ............................................ 9

2.3 Sludge Treatment Processes .................................................................................. 10

2.3.1 Sludge Thickening ............................................................................................ 10
2.3.2 Sludge Stabilization ................................................................. 10
2.3.3 Sludge Dewatering ................................................................. 11
2.3.4 Final Disposal of Waste Activated Sludge .............................. 12
2.4 Methods to Reduce WAS .......................................................... 12
   2.4.1 WAS Pre-treatment .............................................................. 13
   2.4.2 Sludge Predation Method ...................................................... 16
   2.4.3 Modified AS process ............................................................ 16
   2.4.4 Energy Uncoupling ............................................................... 18
2.5 Sequencing Batch Reactor System .............................................. 23

CHAPTER THREE

MATERIALS AND METHODS .......................................................... 26

3.1 Start-up of SBRs .................................................................. 26
3.2 Effect of Uncouplers on AS Yield Using Batch Tests ................. 27
3.3 Effect of Uncouplers on AS Yield Using SBR Tests ................... 29
3.4 Analytical ........................................................................... 31
   3.4.1 Measurements of MLSS, MLVSS and SVI ......................... 31
   3.4.2 Measurements of COD ....................................................... 32
   3.4.3 Measurements of uncouplers concentration .................... 32
   3.4.4 Determinations of $Y_{obs}$ ............................................... 33
3.4.5 Measurements of Oxygen Uptake Rate (OUR) ........................................34
3.4.6 Measurement of SOUR ........................................................................35
3.4.6 Microbiology of AS Flocs Analysis.........................................................36
3.5 Uncouplers Characteristics.......................................................................38
  3.5.1 Para-nitrophenol (PNP)........................................................................38
  3.5.2 3',3',4,5-tetrachlorosalicylanilide (TCS) .................................................39
  3.5.3 Tetrakis Hydroxymethyl Phosphonium Sulphate (THPS) .................40

CHAPTER FOUR

RESULTS AND DISCUSSION ........................................................................42

4.1 Batch Test in the Absence of Uncouplers..............................................42
4.2 Effect of Uncouplers on AS OUR ...............................................................43
  4.2.1 Effect of TCS......................................................................................45
  4.2.2 Effect of PNP....................................................................................49
  4.2.3 Effect of THPS .................................................................................52
  4.2.4 Effect of Combined Dosing of PNP and TCS ......................................53
4.3 Effect of PNP and TCS on AS Using SBRs .............................................56
4.4 Effect of Combined Dosing of TCS And PNP On AS Using SBRs ...........63
4.5 Effect of THPS on AS using SBRs...............................................................69
4.6 Cost Analysis.............................................................................................73
CHAPTER FIVE

CONCLUSIONS

REFERENCES
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>Activated Sludge Process</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<tr>
<td>DAF</td>
<td>Dissolved Air Flotation</td>
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<tr>
<td>DO</td>
<td>Dissolve Oxygen</td>
</tr>
<tr>
<td>F/M</td>
<td>Food to Microorganism Ratio</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed Liquor Suspended Solids</td>
</tr>
<tr>
<td>PNP</td>
<td>Para-Nitrophenol</td>
</tr>
<tr>
<td>OSA</td>
<td>Oxic-Settling-Anaerobic</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen Uptake Rate</td>
</tr>
<tr>
<td>SBR</td>
<td>Sequence Batch Reactors</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge Retention Time</td>
</tr>
<tr>
<td>SVI</td>
<td>Sludge Volume Index</td>
</tr>
<tr>
<td>TCS</td>
<td>3’,3’,4,5-Tetrachlorosalicylanilide</td>
</tr>
<tr>
<td>THPS</td>
<td>Tetrakis Hydroxymethyl Phosphonium Sulphate</td>
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<td>WAS</td>
<td>Waste Activated Sludge</td>
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<tr>
<td>WTP</td>
<td>Western Treatment Plant</td>
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<td>WWTP</td>
<td>Wastewater Treatment Plant</td>
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ABSTRACT

Activated sludge processes are widely used by municipal wastewater treatment plants. Although these processes are very efficient they generate large quantities of waste activated sludge (WAS). The cost of treatment of excess sludge can be 60% of the total operation costs of wastewater treatment plants.

This study focused on a recently developed technique for minimising WAS production by adding chemicals which can slow activated sludge growth. These chemicals, also known as uncouplers, can create deviation between the energy generated from catabolism and the energy required for anabolism, hence imbalance the growth process. The net reduction in energy utilization will result in lower sludge production. The chemicals reported with uncoupling characteristics include para-nitrophenol (PNP), 3',3',4,5-tetrachlorosalicylanilide (TCS) and Tetrakis hydroxymethyl phosphonium sulphate (THPS). According to the literature, these chemicals have the potential for reducing WAS production without causing negative impacts on the efficiency of the biological wastewater treatment process. However, the published literatures in this field did not look into the effect of different dosing methods and the use of combined uncouplers to synergise their effect on WAS production. Therefore, the purpose of this study was to investigate the feasibility of using these chemicals to reduce WAS production. The scope of this study is as follows:
(i) Determination of the optimum dosages of three uncouplers: para-nitrophenol (PNP), 3',3',4,5-tetrachlorosalicylanilide (TCS), and tetrakis hydroxymethyl phosphonium sulphate (THPS) both using batch and continuous flow conditions. Batch tests were employed as screening tests to assess sludge production at different concentrations of each of the uncouplers.

(ii) Assessing the effects of the addition of the three uncouplers, into the feed to an activated sludge reactor, on WAS production, as well as, on the process performance measured in terms of COD removal. The uncouplers will be applied at the concentrations determined from batch screening tests

The results obtained using the sequencing batch reactors for TCS, PNP and THPS at initial concentrations of 0.5, 20 and 0.02 mg/L showed 18%, 28% and 30% reduction in WAS production, respectively. TCS and PNP showed only 5% reduction in COD removal, whereas, THPS reduced COD removal by 13%. For all uncouplers tested, no acclimatisation to the uncoupler was observed during the 30-day SBR tests where reduction in activated sludge growth continued for the duration of the test.

Spiking the feed with the two uncouplers, TCS and PNP at 0.5 mg/L and 5 mg/L, respectively, showed higher sludge reduction compared to the reduction obtained using each uncoupler individually. The intermittent dosing
of the uncouplers into the feed to the SBRs showed that the dosing frequency had no significant effect on activated sludge yield, where dosing once a day had almost the same effect compared to dosing of the uncoupler into the feed three times a day, i.e. in the feed to each cycle. At these conditions, WAS production dropped 12% for both dosing frequency.

The batch OUR tests showed that TCS and PNP can enhance the activity of sludge, as the activated sludge OUR increased 64% and 22% using batch OUR tests for 0.2 and 20 mg/L TCS and PNP, respectively. On the other hand, THPS inhibited activated sludge activity by 35% at a concentration of 0.02 mg/L, which could be due to the inhibition to activated sludge substrate removal capability.

The microscopic analyses of activated sludge flocs collected from the SBRs at day 1 and day 30 of the test, showed that spiking the feed with uncouplers had insignificant effect on activated sludge floc sizes. Increased filamentous bacteria numbers were observed in the reactors that received uncouplers into the feed three times per day, but reducing dosing frequency into the feed to one of the three cycles reduced the filamentous bacteria population.

The results obtained indicated that TCS and PNP have the potential to be used in activated sludge processes to reduce WAS production. The use of combinations of the uncouplers tested also indicated potential for WAS
reduction which exceeded reduction measured for each uncoupler when applied individually.
CHAPTER ONE
INTRODUCTION

1.1 Background

Many methods have been developed for wastewater treatment but activated sludge (AS) process is by far the most widely used method in the world. In this method, wastewater which contains organic matters and nutrient is mixed with bacteria groups, which one also called sludge, and converted into carbon dioxide and insoluble biomass. As the basic theory for the system is using bacteria to consume nutrients and convert it into bacteria cells, the amount of sludge will grow as process goes. A limited amount of sludge will recycle to the beginning of the system as “seed” to mix with new raw waste and the rest of the sludge has to be removed from the system and treated to make it harmless to environment. Treatment and disposal of the excess sludge is time and cost consuming. Handling of the excess sludge can take up to 60% of the total capital and operational cost in an activated sludge treatment plant. Ultimate disposal is becoming more difficult as a result of stringent environmental regulations and lack of suitable landfill sites. In order to solve the problem, many approaches have been proposed to reduce the quantity of excess sludge produced in plant.
Varies of approaches have been proposed to reduce the excess sludge production, such as excess sludge pre-treatment and applying metabolic uncouplers to create energy dissipation. The former approach includes the use of thermal treatment (Paul et al., 2006, Pérez-Elvira et al., 2006a) and ozonation (Campos et al., 2009, Egemen et al., 1999). This approach increases the sludge biodegradability using physical or chemical forces and converts the sludge to secondary substrate. The overall sludge production can be reduced by up to 50% as a result. However, this approach requires extra facility and energy to create the forces to dissolve the sludge and the water product has to return to the main process.

The second approach of using metabolic uncouplers to generate energy dissipation is a recently developed method. This method creates dissociation in the energy transfer from catabolism and anabolism (Low and Chase, 1999). Anabolism and catabolism are coupled under normal conditions, which mean all the energy generated from catabolism will be used by anabolism to sustain the life of bacteria and reproduce itself. However, under some conditions, such as high temperature, existence of heavy mental, or some chemicals called metabolism uncouplers, the energy transfer will be uncoupled (Strand et al., 1999, Chen et al., 2001, Liu and Tay, 2001). In engineering aspect, the futile cycle will lead to a reduction in sludge production.

The chemicals reported as efficient uncouplers, are: 3,3’,4,5-tetrachlorosalicylanilide (TCS), para-nitrophenol (PNP) and Tetrakis
(hydroxymethyl) phosphonium sulphate (THPS) (Strand et al., 1999, Chen et al., 2002, Ye and Li, 2005, Zheng et al., 2008, Aragón et al., 2009, Kusçu and Sponza, 2009). However, there is limited literature on the effect of these uncouplers under different dosing methods and at different operation conditions. Therefore, this study was focused on the use of uncouplers under different conditions to reduce waste activated sludge production.

1.2 Objectives and Scope

The main aim of this was to investigate the feasibility of applying uncouplers to reduce sludge production. TCS, PNP and THPS were selected. The scope of this study comprised:

1. Investigation of the effect of TCS, PNP and THPS on sludge yield and substrate removal.
2. Investigation of the synergistic effects of simultaneous dosing two uncouplers, e.g. TCS and PNP.
3. Investigation of the frequency of dosing the uncoupler(s) into the reactor.

The study was carried out into two phases. The first phase used batch tests to determine the optimum concentrations for each uncoupler. The second phase assessed the effect of the uncoupler at optimum concentrations
determined in the first phase under continuous operation conditions, for this purpose 5L SBRs were operated and monitored, at least over 30-days.

1.3 Layout of Thesis

Chapter 2 gives an introduction on activated sludge treatment process, excess sludge handling process and methods used to reduce excess sludge production. The concept of energy spilling and the researches have been carried out on uncouplers are also included in Chapter 2. The experimental methods used in this study to investigate the impact of uncouplers and the effect of different dosing methods are discussed in Chapter 3. Chapter 4 investigates the effectiveness of three uncouplers in both batch reactors and SBRs. It also covers the cost analysis and recommendations for future work. Chapter 5 is the conclusion.
2.1 Overview

Wastewater treatment methods can be divided into three stages, primary, secondary and tertiary treatment. Secondary treatment is mainly biological processes such as lagoons, trickling filters and activated sludge. Biological processes are the most effective way to remove organic compounds from wastewater. It uses micro-organisms, mostly bacteria, to convert organics to carbon dioxide, water and bacterial cells (Horan, 1990).

Organic pollutions in wastewater can be converted to carbon dioxide, water and cell materials for self-produce purpose. As a result of continuously feeding, the biomass keeps growing during the process. After been separated from the water in secondary clarifier, part of the sludge has to be wasted in order to keep the total biomass in a certain level, and the others will be recycled.
In a conventional activated sludge plant, as illustrated in Fig 2.1, excess sludge is produced from the primary clarifier and the secondary clarifier. The latter contribute more than 60% of the total amount of excess sludge generated from the AS process (An, 2004).

The most commonly parameters used to control the AS system are the sludge retention time (SRT) or sludge age and the food to microorganism ratio (F/M) (Horan, 1990). Sludge age can be calculated by the following equation:

\[
\frac{1}{SRT} = Y \cdot \left(\frac{F}{M}\right) \cdot \frac{E}{100} - K_d
\]  
(eq 2.1)

Where \( Y \): sludge yield, (mg/L*d\(^{-1}\)), E: COD removal (%), \( K_d \): endogenous decay coefficient, (d\(^{-1}\))
The endogenous decay coefficient, $K_{d}$, accounts for the loss in cell mass due to oxidation for energy for self-maintenance, cell death, and predation by organisms higher in the food chain.

The SRT represents the length of time of which the sludge is contained in the system. Long SRT blamed for low nitrogen removal and too short SRT will bring settling problems. F/M indicates the “food” fed to the microorganisms and high F/M in long term will affect effluent quality while low F/M will cause high growth rate of filamentous bacteria.

Extra sludge contains 98% of water so the sludge treatment has two main objectives, dewatering and destabilisation. Some typical methods include thickening, digestion with or without heat, drying by nature, conditioning with chemicals, elutriation and vacuum filtration. The increasing amount of excess sludge is becoming a problem for treatment plants. Statistics from Victoria Water shows in 70000 tonnes of dry sludge was produced state wide in 2006, and by the end of 2005, excess sludge production at European Union is 10.1 million tonnes of dry solids per year. Main methods for sludge disposal in EU are landfill and incineration, which are restricted to prevent health risks to human health and livestock due to potentially toxic elements in the sewage sludge. Moreover, incineration is not appropriate most of the time because of toxic substances and heavy metals content.

Concerning above sludge handling and management issues, several methods have been proposed to reduce the activated sludge production, such as
the use of uncouplers to reduce activated sludge microorganisms growth, hence yield, and changes to activated sludge configurations, e.g. use of extended aeration and integrated fixed-film activated sludge (IFAS).

2.2 Sludge Production

Chemical oxygen demand (COD) is used to determine the organic matters content. As shown in eq 2.2, a portion of the influent organic waste is oxidized into carbon dioxide and water by bacteria and the others is converted into cell materials to achieve self-produce. The first reaction is called catabolism and the latter one is called anabolism.

\[
C_xH_yO_z + \left( x - \frac{11n + z}{2} + \frac{y}{4} \right)O_2 + nNH_4 \rightarrow (x - 5n)C_2O_4 + \left( \frac{y}{2} - 3n \right)H_2O + (C_5H_7O_2N)n
\]  

(eq 2.2)

Where \( C_xH_yO_z \) stands for organic source and \( C_5H_7O_2N \) stands for the composition of biomass

In addition to the biomass growth from anabolism, the effect of endogenous respiration, which is also known as sludge decay, should also be considered. The definition of endogenous respiration is the phenomenon that part of the biomass is oxidized in the purpose of cell maintenance. The body of dead bacteria is solubilised into substrates and mixed with the nutrient from influent for the utilization by microbes. Long sludge age can enhance the effect of sludge decay and therefore reduce the sludge production. Meanwhile, old
sludge population will also reduce effluent quality. A correlation between sludge production and sludge age has been found. According to a research under substrate-sufficient culture (Liu, 1996), sludge production decreased when the sludge age increased. Since long sludge age is related with low F/M ratio, as shown in eq 2.1, operating at low F/M has been used as a practical method to reduce sludge production.

2.2 Waste Activated Sludge (WAS) Production in Victoria

According to a report prepared by the Department of Sustainability and Environment June 2003, the annual WAS production in 150 Vic regional small plants was 19,000 dry tonnes/year excluding the two major metropolitan plants in Melbourne, Western Treatment Plant (WTP) and Eastern Treatment Plant (ETP). In addition, the Victorian Water in 2006 reported that biosolids production is 70,000 dry tonnes, which also brought the stockpiled amount to 1.7M dry tonnes. From the annual report of 2009, there was 27,152 m$^3$ of harvested biosolids at ETP and 26,000 dry tonnes at the WTP.

Under Victoria’s EPA guideline, most of the excess sludge is banned from being used on human food. The common method for final disposal is landfilling. The report also suggests that the stockpiling and heat drying are the most cost efficient methods to treat the digested WAS and incineration is suitable for undigested sludge.
2.3 Sludge Treatment Processes

2.3.1 Sludge Thickening

Activated sludge from the second clarifier contains large percentage of water (more than 98%). Thickening of the sludge is a critical step to reduce the volume of sludge entering the further processes. Dissolved air flotation units (DAFs) and gravity thickeners are used in most of cases (Merlo et al., 2007).

DAF units are more expensive to build and run, but have a better overall performance compared to the gravity thickener (Arora et al., 1995). Gravity thickeners are cheap and easy to run but their performance largely depends on the plant operating conditions and sludge settling abilities. The sludge retention time is so long that there are must be at least two thickeners working together and taking the inflow alternatively.

2.3.2 Sludge Stabilisation

The WAS is rich in pathogens, microorganisms and has a bad smell. Therefore, so as the regulation requires, all WAS to be stabilized before it can be released to the environment (European Commission, 2010). The three common methods for stabilisation are anaerobic digestion, aerobic digestion and composting.
Anaerobic digestion is a well-established process which can produce biogas as a by-product. But according to a report from a WWTP, the major problem is large amount of nitrogen and phosphorus that are released in liquors’ phase and contaminate the effluent (Pitman, 1999). To beneficially use the biogas which usually contains 60% to 70% methane, a combined heat and power system is needed. These systems are of significant capital cost with a long payback period (Van Oorschot et al., 2000).

Aerobic digestion is the biological degradation of the organic substances under aerobic conditions. The supernatant produced is low in BOD, SS and ammonia.

Composting has been recognized as a common method to eliminate the pathogens and smell, especially in undeveloped countries. But this method takes much longer than the other two to stabilize the activated sludge therefore large area will be needed and the method is less suitable in cold area.

2.3.3 Sludge Dewatering

The purpose of dewatering is to reduce the water content so the volume of activated sludge can be minimised, so do the costs associated with transport and landfill. In Victoria, belt filter presses and sand drying beds are the most common ones (Van Oorschot et al., 2000). Polyelectrolytes are added to sludge to enhance the dewatering characteristics of sludge in this process. The
process time is minimised to prevent P and N release to the liquid (Pitman, 1999).

2.3.4 Final Disposal of WAS

Dewatered activated sludge is disposed in three major ways: 1. Land filling or other land application; 2. Agriculture use; 3. Incineration. Among these three, land filling is the most commonly used method by far. However, as the capacities of appropriate locations are exhausted and due to tighter environmental regulations, alternative methods are urgently needed, especially for densely populated cities such as Melbourne. Agricultural use is also limited by the uncertainty of final product as we have no control on hazard materials in the raw wastewater such as heavy metals. Those materials may accumulate in soil and ultimately danger the human body so the method is only suitable for plants that have tight control on influent quality and do not treat industrial wastewater. Incineration is a costly method and the gas emission has gained series concern and greater public scrutiny makes the method not suitable in most cases.

2.4 Methods to Reduce WAS

Given the importance of reducing the amount of excess sludge production, several methods have been developed. They can be catalogued into three strategies: 1. Excess sludge pre-treatment; 2. Sludge predation; 3. Modified AS process; 4. Energy uncoupling (Abbassi et al., 2000, Chen et al., 2001, Liu and Tay, 2001, Low and Chase, 1999, Paul et al., 2006). Among the
first strategy, sludge ozonation and sludge thermal treatment are included (Yasui and Shibata, 1994, Kamiya and Hirotsuji, 1998, Campos et al., 2009, Järvik et al., 2011, Bougrier et al., 2006, Paul et al., 2006). For the second strategy, some researchers (Elissen et al., 2006, Liang et al., 2006, Buys et al., 2008) have suggested a modified AS process with two steps where sludge predators such as protozoa and metazoan are kept in limited substrate environment to enhance the predation effect. Some technologies such as oxic-settling-anaerobic (OSA) and high dissolve oxygen (DO) have been used to the conventional AS process for excess sludge reduction purpose. Metabolic uncouplers have been found to efficiently create the energy dissociation in cells and ultimately reduce the sludge yield (Strand et al., 1999, Aragón et al., 2009, Kusçu and Sponza, 2009).

2.4.1 WAS Pre-treatment

The aim of sludge pre-treatment is to use physical or chemical methods to disintegrate or “dissolve” the sludge to liquid substrates. When treated sludge return to biological reactor, the microorganism will degrade the substrate to water and carbon dioxide. Thus, an overall reduction in sludge production will be achieved.

2.4.1.1 Ozonation combined process

Ozone is a strong chemical oxidant which has been widely used in the disinfection step of AS process and drinking water plant. Activated sludge can be partly mineralized to CO2 and water as the sludge is solubilised to
biodegradable organics. Based on this theory, recently some researches have been trying to reduce the sludge production by ozonation combined process (Sakai et al., 1997, Yasui and Shibata, 1994, Campos et al., 2009). In the modified system, sludge from the secondary clarifier was pumped to the ozonation process before entering the recycle line. Experimental results showed that up to 50% excess sludge reduction was achieved when 10 mg/g MLSS d-1 ozone was present in the aeration tank, and even no excess sludge was produced when the dosage was 20 mg/g MLSS per day (He et al., 2006).

The concept of this method is (Egemen et al., 1999, Kamiya and Hirotsuji, 1998): part of the excess sludge from the secondary clarifier goes into an ozone tank where most of microorganism killed before been oxidized to organic substances. Those organic substances go back to the biological process together with activated sludge and are degraded to organic carbon. Yasui (Yasui et al., 1996) has found that total organic carbon in effluent was higher than the conventional activated sludge process, due to degraded excess sludge. It should also be mentioned that introduction of ozonation has highly improved the sludge settleability (Kamiya and Hirotsuji, 1998).

This method requires high operational and capital cost because of the ozone generator and ozonation tank. Moreover, as a strong oxidant, ozone can react with other materials other than microorganisms from activated sludge and in some cases, toxic matters, which can inhibit the followed activated process, can be generated. Is has also been reported that the effectiveness of this method
is strongly depended upon sludge structures and the operation conditions in plant (Liu, 2003). Further research is needed to investigate the optimal ozone dosage and dosing mode.

2.4.1.2 Thermal treatment of WAS

Thermal treatment aims to improve the hydrolysis of biodegradable organics in activated sludge. The recycle activated sludge is hold in a heating tank at 130-180°C and kept for 30 to 60 minutes in this method to reduce the production of excess sludge.

Bougrier (2006) investigated thermal treatment of WAS. He showed that the method can reduce 50% of excess sludge production compared to conventional process. Temperature and time can affect the efficiency of this method significantly. The increase of hydrolysis begins at around 100°C, whereas a temperature higher than 200°C is not recommended as it can reduce the production of sugar and amino acids, and sequencely reduce the biodegradability of treated sludge. In addition, it has been reported that this method can improve sludge settling characteristics. A review by Paul et al., (2006) suggested that thermal treatment requires large investment for energy and maintenance costs, which make it less attractive than the ozonation method. The related odour problem also limited the use of thermal pre-treatment method.
2.4.2 Sludge Predation Method

Liang et al., (2006) found that considerable biomass reduction could be achieved if bacteria were cultured with protozoa. However, in activated sludge process, the bacteria exist in the form of floc. The structure protects them from the predator, makes the method not efficient in conventional AS process. The effect of protozoa is therefore to capture the dissociative microorganisms in supernatants to improve the sludge settleability and effluent quality.

A two-stage AS process was proposed by Lee and Welander (1996) to improve the method. The sludge retention time (SRT) is short in the first stage to create small and unstable flocs. The sludge age in the second stage is long to maximise the effect of predation. An overall of 60% to 70% reduction in excess sludge production was achieved as a result. Poor effluent quality was obtained due to the short SRT in the first stage and filamentous bacteria, which may further lower the effluent quantity and endanger the AS process, is likely to grow in the second stage due to the long sludge age.

2.4.3 Modified AS process

2.4.3.1 Oxic-settling-anaerobic (OSA) process

The OSA process has a modified conventional AS process in the sludge recycle line to enhance the endogenous respiration. The thickened sludge from secondary settling tank is returned to the aeration tank via a completely mixed
aerated sludge holding tank. The principle of this design is to create an anaerobic- aerobic alternate cycle for activated sludge to stimulate catabolism in the holding tank and make catabolism disassociated with anabolism, which will lead to a reduction in sludge growth yield as mentioned.

The method was first reported by Westgarth (1963) when the excess sludge rate was reduced by half compared to the conventional AS process. Other researchers (Chudoba et al., 1992, Sun et al., 2009, Wang et al., 2007) further studied the method since then and a successful application of this method using a lab-scale system has reduced sludge production by 20-65% while no effect on effluent quality and settling ability have been observed (Chudoba et al., 1992).

The modified process provides a promising technology for effectively reducing the sludge production but further research is needed to establish the optimum conditions in particular the sludge holding time and aeration level. The method also requires high investment if applies to exist plants.

### 2.4.3.2 High dissolve oxygen (DO) process

It has been recognised that level of DO places an important role in the AS process and the supply of oxygen is the limitation of higher organic loading for the treatment facilities (Chen et al., 2001, Pérez-elvira et al., 2006b). In order to increase the oxygen transfer efficiency, purified oxygen is used for aeration instead of air. Although the mechanism of reduction in sludge growth
is still unknown, McWhirter (McWhirter, 1978) reported only 56% of sludge was produced in the purified oxygen aerated system compare to conventional AS process. Abbassi (Abbassi et al., 2000) also observed a 25% reduction in the amount of biomass when the DO in the reactor increased from 2 to 6 mg/L.

Sludge bulking is one of the most common operation problems seriously affect the AS process. It has been found that low level of DO in aeration tank is one of the causes of the overgrowth of filamentous bacteria which severely affect sludge settling (Wilén and Balmér, 1999). So apart from the reduction in sludge produced, high DO content can also repress the growth of filamentous. The high cost of oxygen is the barrier keeps the method from largely application.

2.4.4 Energy Uncoupling

2.4.4.1 Background

The discovery of energy uncoupling provides another way to reduce sludge growth yield in AS process. In normal condition, energy generated from biodegradation, or catabolism, will be used to meet the requirements of maintaining life and achieve self-production. However, under some circumstances such as high temperature, existence of heavy metal, or presence of certain chemicals, a discrepancy between the energy generated and the needs. This low efficiency on energy utilization was first observed by Walter (Walter,
1976) and since then, many methods have been originated based on this phenomenon to reduce activated sludge reduction.

2.4.4.2 Concept of energy spilling

Metabolism is a sum of catabolism and anabolism transformations occur in organism cells. Catabolism breaks down nutrients and carbon sources and generates energy while anabolism will use the energy generated to maintain life and reproduce cells. The energy transfer is completed by the formation of adenosine triphosphate (ATP) and adenosine diphosphate (ADP). ATP has three phosphate groups attached to it, which are held together by high energy bonds. A great amount of energy is released when one of these bonds is broken, which also turns the ATP to ADP. The ATP is generated by oxidative phosphorylation, during which process, according to Mitchell (Mitchell, 1961), electrons are transported through the electron transport system (ETS) from substrate to oxygen.

The energy released in oxidate keep pumps intracellular H⁺ out of membrane reverse concentration gradient. Because of the selective membrane, H⁺ cannot freely through the cell. Thus a membrane gradient was formed, which forces H⁺ to go through a specific and only proton channel to enter the cell. By the movement of H⁺, ATP can be generated by combination of ADP and PO₄³⁻ (Liu, 2000). Under some circumstances, the permeability of cell membranes for H⁺ can be increased so the proton channel for H⁺ to enter cell is not unique. Production of ATP will decrease because H⁺ can go through membrane to cells to eliminate the H⁺ gradient between the inside and outside
membrane. So less energy will be used for anabolism when the same amount of substance was consumed. The energy that not used on cell growth and life maintenance is called “spilling energy”. (Liu, 2000).

Based on this theory, the energy balance in cells under the energy uncoupled situation is as follows:

\[ Ec = Eg + Em + Es \]

Where \( Ec \): total energy generated from catabolism, \( Eg \) = energy used for reproduce cells or sludge growth, \( Em \) = energy used to sustain life, \( Es \) = spilled energy

From the equitation, the increasing in \( Es \) will lead to a decrease on \( Eg \).

2.4.4.3 Energy uncoupling under nutrient sufficient condition

Some researchers have observed an over-consume of energy by bacteria under substrate-sufficient cultures (Chen et al., 2000, Liu, 1996). They also reported that a reduction in growth yield was observed under high substrate content. This phenomenon indicates energy spilling occurred in cells as a result of excess energy generated from catabolism. The excess energy dissipates through futile cycles.

In a pure culture cultivation of Streptococcus bovis bacteria, Cook and Russell (1994) observed that glucose consumption rate was much faster than the growth needs. The substrate over-consumption of the substrate was
associated with excess energy which was dissipated in the form of heat. Ultimately, the excessive energy was dissipated in form of heat and less energy was used for anabolism.

2.4.4.4 Energy uncouple caused by chemical uncouplers

It has been found that the energy spilling can be triggered under conditions such as existence of organic protonophores, heavy metals, abnormal temperature and alternative aerobic anaerobic cycle, and some chemicals, which named uncouplers (Pérez-elvira et al., 2006b, Liu and Tay, 2001). There are some reports about microorganisms can over consume substrate at a higher rate under the energy spilling condition (Cook and Russell, 1994, Low and Chase, 1999, Paul et al., 2006). In environmental engineering, the over consuming of substrate will lead to a higher catabolism rate than it required for maintenance and growth, and ultimately, a reduced activated sludge growth yield.

As mentioned in section 2.2 the membrane gradient plays an important part in the formation of ATP. Protonophores can increase proto permeability of bacteria membrane, and collapse of such an electrochemical gradient will make a short-cut for the H+, which will ultimately reduce the efficiency of ATP formation. It has been found protonophores have phenolic or anilinic structure (Walter, 1976). Most of the uncouplers can be classified into two groups: chlorophenols and nitrophenols. The difference on structure can lead to difference in acidity constant (pKa) value. Stronger acid has a lower pKa value.
and based on Xuefu Fang’s comparison experiments uncouplers with low pKa are more effective in dissociating the energy couple (Yang et al., 2003). Most of metabolic uncouplers are protonophores, which can travel freely through the cell membrane and release a proton inside of the cell (Bering, 1989). As mentioned before, membrane gradient is the element to synthesize ATP, the proton motive force (pmf) can be seen as a representation of membrane gradient and can be calculated by this equation:

$$\text{pmf} = \Delta M - 2.3RT\Delta pH/F,$$

Where $\Delta M$ stands for membrane potential, $\Delta pH$ is the difference of pH between inside and outside of the cell. In environment without uncoupler, $\Delta pH$ is a negative value, but by the proton metabolic uncoupler released inside the cell, the value will become positive. Depend on the equation; the shift can lead to lower pmf so less ATP will be produced.

It has been reported that 3,3',4,5-tetrachlorosalicylanilide (TCS), para-nitrophenol (PNP) and Tetrakis (hydroxymethyl) phosphonium sulphate (THPS) can stimulate reduce the amount of extra sludge (Chen et al., 2002, Strand et al., 1999, Aragón et al., 2009, Abbassi et al., 2000, Ye and Li, 2005, Chen et al., 2004). From those previous researches, TCS can reduce the sludge yield by more than 40% with the concentration of 0.8mg/l, and Low even achieved a zero sludge growth in batch culture when the concentration of PNP reaches 120mg/l (Low et al., 2000).
From the previous researches, it has been confirmed the uncouplers have a potential of reducing activated sludge production without changing the activated sludge process. But it is still unknown the amount of uncouplers is degraded during the activated sludge process, nor the concentration of residue uncouplers remains in the effluent, which is critical to apply these uncouplers to plants. Also there is no research has been done to examine the effect of combined use of these uncouplers. It should be mentioned that some researchers claim that uncouplers have minor effect on activated sludge species (Aragón et al., 2009, Chen et al., 2007, Low et al., 2000, Chen et al., 2004) while some believe the microbial acclimation will lead to a switch in sludge population in long-term (Strand et al., 1999).

### 2.5 Sequencing Batch Reactor System

SBR is a well-developed activated sludge treatment system operated according to the designed time sequence. A conventional SBR cycle consists of five periods of filling, reacting, settling, drawing and idling. Nitrogen and phosphors removal can be achieved by the control of aeration time in the settling period instead of setting up anaerobic and anoxic zones in other activated sludge system. By this, SBR is considered to be a simpler and less space required system compare to the others. This character also makes it especially suitable for small treatment plant or plants with inconstant quantity influent. Considering each SBR can only take influent in a certain time in one
cycle, an adjustment pond is needed or several SBRs will work at different sequences so at least one reactor is taking influent at any given time.

Fig 2.2: Typical five phases from an operational cycle

Previous researchers have concluded the advantages of SBR system as follows (Ni and Yu, 2007, Sun, 1996):

1. Separation of biomass is achieved in the main reactor other than a separate tank. The sludge settles at ideal quiescent conditions and no short circuiting or other disturbances.

2. Food to microorganism ratio (F/M) is high on the filling period, which makes it unlikely to have filamentous to grow.

3. Because operating mode is easily to control (in particular the aeration) during the process, nitrogen and phosphorus removal can be achieved depending on needs.

4. No sludge recycling is needed and all the functions are achieved in the same reaction tank, which leads to saving in both capital and operational cost.
5. It has higher tolerance to shock loading on both quantity and quality.

The above advantages make SBR a very attractive research system especially in a lab scale because of its versatility and flexibility.
CHAPTER THREE
MATERIALS AND METHODS

This study was divided into two phases. The first phase comprised batch tests using varying concentrations of TCS, PNP, THPS as well as combined TCS and PNP. Three hours batch tests were carried out to determine the effectiveness of uncouplers and the optimum dosing. A lab-scale pilot SBR was used to provide activated sludge for this phase. The second phase comprised SBRs tests. The SBRs were operated at conditions typically used at large wastewater treatment plants to assess effect of uncouplers at these conditions. Certain concentrations of uncouplers including combined uncouplers were tested to investigate the effectiveness in long-term.

3.1 Start-up of SBRs

Activated sludge was collected from the Eastern Treatment Plant in Melbourne. Then it was cultivated in a sequencing batch reactor (SBR) with working volume of 35 L. Synthetic wastewater, which has a composition shown in Table 3.1, was used to feed the SBRs. The amount of glucose was added to get the feed strength around 500 mg/L COD. Sodium bicarbonate was
also added as a buffer to keep the pH around 7.0, whereas dissolved oxygen (DO) level was kept above 2 mg/L. The reactors were operated at three cycles per day and 20 L of synthetic wastewater was fed every cycle, which makes the total F/M around 0.45 mg COD/mg SS. Sludge was wasted daily to maintain the MLSS concentration at 2500 mg/L.

Table 3.1: Synthetic wastewater composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>500</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>120</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>40</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>20</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>40</td>
</tr>
<tr>
<td>FeCl₃.6H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>ZnSO₄.7H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>0.02</td>
</tr>
<tr>
<td>MnCl₂.6H₂O</td>
<td>0.04</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>Na₂MoO₄.2H₂O</td>
<td>0.044</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.1</td>
</tr>
<tr>
<td>KI</td>
<td>0.02</td>
</tr>
</tbody>
</table>

3.2 Effect of Uncouplers on AS Yield Using Batch Tests

Batch tests using activated sludge from the pilot scale SBR were conducted for different initial concentrations of each of the uncouplers TCS, PNP and THPS or of combinations of two uncouplers. The range of
concentrations used were, 0.2 – 1.2 mg/L, 5 – 45 mg/L and 0.02 – 0.1 mg/L for TCS, PNP and THPS, respectively. Sludge was collected from the pilot scale SBR (described in Chapter 3.1) and aerated for one hour to stabilize biological activities before being washed with distilled water twice. Sludge was then left settling for one hour after which the concentrated sludge was collected from the bottom of the flask. A set of 6 flasks each received a designated volume of the concentrated sludge and fed with synthetic wastewater contains 1000 mg/L COD to provide a substrate sufficient environment to enhance the effect of energy spill triggered by uncouplers (Liu, 1996) and prevent sludge decay. The initial biomass concentration in each flask was around 1500 mg/L. The flasks were continuously aerated and mixed for three hours. DO was kept above 2 mg/L and the pH maintained between 6.5 and 7.5.

In order to ensure the accuracy and consistency of the amount of uncoupler introduced into the system, pre-prepared uncoupler solution was used. Stock solutions of 1000 mg/L PNP and 500 mg/L TCS were prepared. As TCS is slightly soluble in water (0.161 mg/L), the stock solution was subjected to ultrasonic until the TCS particles disappeared. The solution was then shacked thoroughly before use. The uncouplers were mixed with the feed first before being added to the sludge to avoid the high-concentration shock.

At the end of the tests, 30 mL sample was taken from each flask, while the stirrers were still rotating to determine the biomass concentration. Then the bottles were left to settle for around 15 minutes before a sample from the
supernatant was collected for measurement of soluble COD and concentration of the uncoupler. All the tests were repeated at least twice.

### 3.3 Effect of Uncouplers on AS Yiled Using SBR Tests

Three SBRs were used to examine the effect of uncouplers on activated sludge yield. Each has a working volume of 4 L and was fed with 2 L synthetic wastewater (described in Chapter 3.1) three times a day. To start-up the SBRs, wasted activated sludge from the pilot plant was added to SBRs and mixed with synthetic wastewater. Wasting of sludge did not begin until the MLSS reached 3000 mg/L. The biomass concentration for each SBR was then measured daily and used to determine the exact amount of sludge to be wasted to maintain the MLSS at 3000 mg/L. The COD concentration in effluent was measured twice a week, whereas ammonia and nitrate concentration in the effluent were monitored once a week. Operation conditions of the SBRs are given in Table 3.2.

The injection of uncouplers didn’t begin till two weeks after the reactors reached steady state conditions. One of the SBRs was used as a control whereas the other two received the uncoupler at different concentrations and frequency. Samples from the effluent were taken twice a week for COD, ammonia and nitrate analysis; once a week for microbiology analysis including microscopy, floc size. To determine uncoupler’s effect on sludge activity, specific oxygen uptake rate (SOUR) tests were carried out at day 1, 15 and 30. Microscopy analysis was performed once a week to examine possible shifts in the activated
sludge micro-organisms community. In the meantime floc projected area analysis was also performed using specific software, Image J. Details of the techniques are given in Chapter 3.4.6.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>20-25 °C</td>
</tr>
<tr>
<td>MLSS</td>
<td>3000 mg/L</td>
</tr>
<tr>
<td>COD in feed</td>
<td>500 mg/L</td>
</tr>
<tr>
<td>Length of cycle</td>
<td>8 hours</td>
</tr>
<tr>
<td>Volume of feed/cycle</td>
<td>2 L</td>
</tr>
<tr>
<td>F/M</td>
<td>0.33</td>
</tr>
<tr>
<td>HRT</td>
<td>6 hours</td>
</tr>
<tr>
<td>Sludge age</td>
<td>7-10 days</td>
</tr>
</tbody>
</table>

Similar to real treatment plants, the SBRs were operated at substrate-limited condition. The hydraulic retention time (HRT) was long enough to utilize most of the organic matters so the COD removal abilities in three reactors were expected to be similar.

Daily wasted activated sludge for each SBR was recorded and cumulative excess sludge production was calculated as the sum of total daily sludge wasted during the testing period. Low excess sludge accumulation reflects a low sludge yield, which indicates the effectiveness of uncouplers. The accumulation rate also reflects the sludge growth rate in each reactor.
SOUR was used to determine the effect of uncouplers on sludge activity. As catabolism is an oxygen consumption process, the SOUR is considered indicative of the microbial activity. SOUR measurement was performed before dosing, then on day1, day15 and day30 after the dosing of the uncouplers begun.

3.4 Analytical

3.4.1 Measurements of MLSS, MLVSS and SVI

The measurement of MLSS, MLVSS and SVI were carried out according to the standard method (APHA et al., 2005) section 2540 D, 2540 E and 2710 D, respectively. The procedure used is as follows:

1) Take 25 mL of sample from the reactor using measuring cylinder
2) Note down the level of sludge after 15 minuets’ settling
3) Filter the sample through a pre-weighted filter paper
4) Dry the filter paper with the solids on top at 105°C for one and half hour and cool down in a desiccator to room temperature then weigh the dry filter paper
5) calculate the required parameters as follows:

\[
\text{MLSS} \left( \frac{mg}{L} \right) = \frac{(A-B)}{\text{sample volume (mL)}} \times 1000 \quad \text{(eq.3.1)}
\]

\[
\text{SVI} \left( \frac{mL}{g} \right) = \frac{V1}{A-B} \times 1000 \quad \text{(eq.3.2)}
\]
Where A: weight of filter paper + dried residue (mg), B: weight of filter paper (mg), V₁: volume of settled sludge after 15 minutes

6) Burn the dry filter paper and residue in the furnace at 550°C for 20 minutes. Then cool it down in a desicator to room temperature before measuring the residue remaining in the crucible

7) calculation of MLVSS:

\[
\text{MLVSS} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{(A - C)}{\text{sample volume (mL)}} \times 1000
\]

(eq.3.3)

Where C: weight of filter paper + dried residue after 550°C (mg)

### 3.4.2 Measurements of COD

Sufficient volume of sample was taken from the reactor and filtered through a membrane filter paper (Advantec, pore size 0.45µm). A 2 mL filtered sample was then added to the COD test tube of appropriate range. The COD vials were kept in a digester at temperature of 120°C for 2 hours. After cooling down to room temperature, the test vials were measured by a DR 5000 UV-Vis spectrophotometer.

### 3.4.3 Measurements of uncouplers concentration

As PNP solution will be degraded in the sun, a series of standard PNP solutions of 1, 2, 5, 10, 15, 20, 30, 40 and 50 mg/L were prepared fresh and analysed before analysis of samples collected from the SBRs. The UV-vis
spectrometer (Therom, Helios Delta mode) was first used at scan mode with wavelength from 300 - 630 µm, using the 50 mg/L solution, to identify the peak of absorption. The observed peak of UV absorption occurred at wavelength between 310 - 350 µm. Therefore, a wavelength of 320 µm was selected. This result is similar to that reported by Tomei et al., (2004). A standard curve was then established and used to interpret the absorptions of samples collected.

3.4.4 Determinations of \( Y_{obs} \)

The sludge growth yield was calculated as the increase in the mass of SS per g COD consumed according to the following equation:

\[
Y_{obs} = \frac{X_t - X_0}{S_0 - S_t} \times 1000 \quad \text{(eq.3.4)}
\]

Where \( X_0 \) and \( X_t \) are the MLSS value before and after the tests, \( S_0 \) and \( S_t \) are the COD measured before and after the tests.

The reduction in \( Y_{obs} \) observed in batch test is a combined effect caused by energy spill and the decrease in COD removal. However, the lower COD removal only occurred in batch test which is under substrate-sufficient environment. In long-term test and real plants, the reactors will be running at substrate-short condition, under which decrease in COD removal is minor. Then the \( Y_{obs} \) can be seen as an indicator of energy spilling.
3.4.5 Measurements of Oxygen Uptake Rate (OUR)

The OUR test uses activated sludge cultivated in an uncoupler-free environment. The procedure of OUR measuring as follows:

1. Certain amount of activated sludge was collected from the pilot plant as described in 3.1
2. The sludge was then washed with distilled water twice and left to settle
3. Measure the MLSS for the concentrated sludge after settling. Then mix the concentrated sludge with appropriate amount of distilled water to adjust the MLSS to 10.5 g/L.
4. Into a 500 mL volume flask, 300 mL synthetic wastewater was mixed with 50 mL activated sludge, which made the MLSS concentration 1.5 g/L.
5. The uncoupler was then added to each flask at the designed concentration. Then the flasks were put in a shaker at 100 rpm. Aerate the flasks contents ensuring DO level is more than 2 mg/L.
6. After 30 minutes, the flasks were taken out of the shaker and the air supply stopped. A magnetic stirrer was used to keep the liquid well mixed. A DO probe (YSI 5905) and DO meter (YSI, Model 58) were used to measure the initial DO.
7. The final DO was read after 5 minutes. OUR was then determined as the oxygen consumed per unit mass of MLSS in the bottle, according to the following equation:
Where \( \text{OUR} \): oxygen uptake rate (mg O\(_2\)/gSS.min);

\( \Delta \text{DO} \): drop in DO during the test (mg/L)

3.4.6 Measurement of SOUR

Based on previous research carried out by Chen et al., (2002), Maas et al., (2008), the SOUR was measured according to the following procedure:

1) The MLSS in each SBR was measured according to the standard method;

2) A 10 mL sample was taken from each SBR, diluted with 200 mL distilled water. Leave the mixed liquid settling for 5 minutes before pouring out the supernatant

3) The sample was then diluted with pre aerated feed in a headspace-free bottles (330 mL BOD bottle) containing a magnetic stirrer

4) A DO probe (YSI 5905) and DO meter (YSI, Model 58) were used to measure the DO continuously during the test

5) To assure the accuracy of results, initial DO and final DO were kept greater than 8.0 and 2.0 mg/L, respectively

5) SOUR was then determined as the oxygen consumed per unit mass of MLSS in the bottle, equation as follows:
Where \( \text{SOUR} \): specific oxygen uptake rate (mg O\(_2\)/g SS hr); \( \Delta \text{DO} \): DO drop during the test (mg/L); \( \Delta t \): Time elapsed (hr)

3.4.6 Microbiology of AS Flocs Analysis

Fresh sample was taken from the reactor using a 1 mL pipette. Put 1 drop of sample on a glass slide, and then use a glass coverslip to cover the sample carefully, make sure no air bubble between the glass slide and cover. Set the glass slide under a microscope equipped with a camera PC connected camera. Photos were taken. For each drop of sample, around 8 to 10 pictures were taken under 25 and 100 magnify times, and two drops were taken from each reactor for analysis to get representative results.

ImageJ (version 1.37v, http://rsb.info.nih.gov/ij/index.html) was used for floc projected area analysis. To ensure the results were statistically useful, only pictures contain more than 100 flocs were used for analysis. Learning from Mesquita’s work (2011), the pictures were pre-processed in order to substrate the background light and enhance the aggregates. Next, the Colour Threshold tool was used to boundary the flocs. Fig 3.1 represents an example of picture before and after pre-process.

Owing the fact that flocs are mostly fractal shape, the Feret diameter, which is the maximum distance between two parallel tangents touching
opposite borders of the object, is a better dimension parameter than diameter (Wilén et al., 2003). A micrometer was used to calibrate the distance from pixel unit to micrometer (µm).

To determine potential presence of filamentous bacteria, analysis was carried out according to the Filamentous Index (Eikelboom and Van Buijsen, 1981). The 5-graded classification scheme rates filamentous present in picture from 1 to 5, where 1 corresponds to absence of filaments and 5 corresponds to excessive numbers.

(a)
3.5 Uncouplers Characteristics

3.5.1 Para-nitrophenol (PNP)

Para-nitrophenol (PNP), also known as 4-nitrophenol, is commonly used to manufacture drugs, fungicides, insecticides, and dyes and to darken leather. (Chen et al., 2001, Sponza and Kusçu, 2005, Tomei et al., 2004). Moreover, PNP can be degraded during the activated sludge process which means less PNP will be left in the effluent. The chemical structure of PNP is shown in Fig 3.2.
3.5.2 3',3',4,5-tetrachlorosalicylanilide (TCS)

3',3',4,5-tetrachlorosalicylanilide (TCS) is a kind of protonophores, commonly used in products such as soap and shampoo (Aragón et al., 2009). According to Cook’s research (Cook and Russell, 1994), TCS can efficiently enhance the energy spilling in Streptococcus bovis. The structure of TCS is shown in Fig 3.3 and the characteristics are shown in Table 3.4.
Table 3.3: Characteristics of TCS

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>( \text{C}<em>{13}\text{H}</em>{7}\text{Cl}_4\text{NO}_2 )</td>
</tr>
<tr>
<td>CAS number</td>
<td>1154-59-2</td>
</tr>
<tr>
<td>Solubility</td>
<td>Negligible in water</td>
</tr>
<tr>
<td></td>
<td>Soluble in alkaline aq. solution and organic solvents</td>
</tr>
<tr>
<td>Aquatic Ecotoxicity</td>
<td>No data available</td>
</tr>
</tbody>
</table>

3.5.3 Tetrakis Hydroxymethyl Phosphonium Sulphate (THPS)

THPS is a popular biocide especially to control sulphate reducing bacteria growth in oilfield. It was registered in 1995 by US EPA as an industrial use biocide. It is also classified as non-hazard and low toxic to the environment (Thomas, 1997). It can be oxidized to trashy droxymethyl phosphate oxide (THPO) which is even more environmentally friendly.

![Chemical structure of THPS](image)

Fig 3.4: Chemical structure of THPS
THPS is an effective biocide when the concentration is above 15 mg/L (Zhao et al., 2009). Table 3.5 shows a time/kill profile for THPS on Enterobacter Aerogenes bacteria (Thomas, 1997). The chemical structure of THPS is shown in Fig 3.4 and its characteristics are listed in Table 3.5.

Table 3.4: Effect of THPS as biocide (Thomas, 1997)

<table>
<thead>
<tr>
<th>THPS concentration (mg/L)</th>
<th>Survived Bacteria per ml after exposed time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 hours</td>
</tr>
<tr>
<td>0</td>
<td>2.3×10^5</td>
</tr>
<tr>
<td>15</td>
<td>4×10^4</td>
</tr>
<tr>
<td>37.5</td>
<td>1.0×100</td>
</tr>
</tbody>
</table>

Unlike the effect observed with the bacteria, THPS showed no strong inhibition to activated sludge. Thomas, (1997) performed a trial experiment to access the effect of THPS on activated sludge system and concluded that THPS can be dosed to wastewater plants at concentrations up to 200 mg/L, without seriously inhibiting the bacteria activity.

Table 3.5: Characteristics of THPS

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>[(CH$_2$OH)$_4$P]$_2$SO$_4$</td>
</tr>
<tr>
<td>CAS number</td>
<td>55566-30-8</td>
</tr>
<tr>
<td>Density</td>
<td>1.4 g/mL</td>
</tr>
<tr>
<td>Aquatic Ecotoxicity</td>
<td>Slightly Toxic</td>
</tr>
</tbody>
</table>
As oxygen take an essential participation in metabolism of activated sludge, oxygen uptake rate (OUR) is used to determine its respiratory activity. The effect of uncouplers on activated sludge is therefore measured in terms of OUR. The first test was performed for activated sludge receiving synthetic wastewater free of uncouplers.

4.1 Batch Test in the Absence of Uncouplers

The results of COD removal and activated sludge growth in a batch reactor are shown in Fig 4.1. The test was performed over 24 hours in continuously aerated reactors. The results showed that the substrate was utilized efficiently during the first six hours, as indicated by the 86.7% removal of COD. This removal corresponded with 900 mg/L growth in activated sludge (900 mg/L corresponds to growth of 144 mg sludge in 160 mL activated sludge slurry). After 6 hours, as the COD dropped to 100 mg/L, the substrate concentration became a limiting factor and as a result, the sludge growth began to slow down. This is indicated the drop in activated sludge production from
900 mg/L to 510 mg/L at the end of test. This trend indicated that sludge entered endogenous respiration phase where the microorganism break down themselves in order to survive at the low substrate environment. The observed trends of simultaneous substrate removal and activated sludge growth indicate that substrate utilization and biomass growth were coupled.

This test also indicated the conditions at which the endogenous respiration prevails. Therefore, batch tests adopted 3 hours as the testing period to ensure that activated sludge are in the lag phase during the whole time.

4.2 Effect of Uncouplers on AS OUR

Table 4.1 (a-c) shows the OUR of activated sludge in the presence of different concentrations of THPS, TCS and PNP. The results in Table 4.1a and
Table 4.1b show that THPS had an inhibitory effect on activated sludge, whereas, TCS and PNP enhanced the respiratory activity of activated sludge.

Similar effect was observed for 2,4-dinitrophenol on activated sludge growth (Mayhew and Stephenson, 1998). The results in Table 4.1b and c show that OUR more than doubled in the presence of 0.6 mg/L TCS whereas a 48% increase in AS OUR was observed in the presence of 60 mg/L PNP. The increase in oxygen consumption represents a high level of dissipation of energy transfer between metabolic and anabolic reactions, which ultimately lead to reduction in sludge production.

On the other hand, the high oxygen consumption level can cause unexpected anoxic period, especially during the feeding phase where fresh wastewater entered the reactor just finishes drawing phase and contains low DO sludge. Enhanced aeration will be needed to minimise the anoxic period and in industry, this will cause an increased operational cost. In the lab scale experiments, where the aeration is not auto-adjustable, extended feeding period is needed to solve the problem.
Table 4.1 a: OURs in the activated sludge reactors that received THPS

<table>
<thead>
<tr>
<th>THPS mg/L</th>
<th>0.02</th>
<th>0.05</th>
<th>0.08</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1</td>
<td>-34.3%</td>
<td>-67.3%</td>
<td>-78.8%</td>
<td>-86.5%</td>
</tr>
<tr>
<td>Changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1-2</td>
<td>-33.6%</td>
<td>-56.5%</td>
<td>-88.8%</td>
<td>-80.7%</td>
</tr>
<tr>
<td>Average</td>
<td>-34.0%</td>
<td>-61.9%</td>
<td>-83.8%</td>
<td>-83.6%</td>
</tr>
<tr>
<td>Standard deviation (STD)</td>
<td>0.004</td>
<td>0.054</td>
<td>0.050</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 4.1 b: OURs in the activated sludge reactors that received TCS

<table>
<thead>
<tr>
<th>TCS mg/L</th>
<th>0.2</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 2-1</td>
<td>+67.0%</td>
<td>+147.8%</td>
<td>+192.0%</td>
<td>+215.7%</td>
</tr>
<tr>
<td>Test 2-2</td>
<td>+56.2%</td>
<td>+161.8%</td>
<td>+196.9%</td>
<td>+206.0%</td>
</tr>
<tr>
<td>Test 2-3</td>
<td>+69.9%</td>
<td>+153.7%</td>
<td>+204.7%</td>
<td>+208.4%</td>
</tr>
<tr>
<td>Average</td>
<td>+64.4%</td>
<td>+154.4%</td>
<td>+197.8%</td>
<td>+210.1%</td>
</tr>
<tr>
<td>STD</td>
<td>0.059</td>
<td>0.058</td>
<td>0.052</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Table 4.1 (c): OURs in the activated sludge reactors that received PNP

<table>
<thead>
<tr>
<th>PNP mg/L</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 3-1</td>
<td>+12.6%</td>
<td>+20.9%</td>
<td>+33.5%</td>
<td>+48.2%</td>
</tr>
<tr>
<td>Test 3-2</td>
<td>+10.8%</td>
<td>+18.5%</td>
<td>+34.8%</td>
<td>+46.4%</td>
</tr>
<tr>
<td>Test 3-3</td>
<td>+16.6%</td>
<td>+25.9%</td>
<td>+36.8%</td>
<td>+50.8%</td>
</tr>
<tr>
<td>Average</td>
<td>+13.3%</td>
<td>+21.7%</td>
<td>+35.0%</td>
<td>+48.5%</td>
</tr>
<tr>
<td>STD</td>
<td>0.024</td>
<td>0.031</td>
<td>0.014</td>
<td>0.018</td>
</tr>
</tbody>
</table>

4.2.1 Effect of TCS

Figure 4.2(a) shows the results of TCS at concentrations from 0.0 mg/L to 1.2 mg/L on COD removal and activated sludge MLSS concentration. The
initial MLSS was around 1500 mg/L, and in order to eliminate the effect of sludge decay, the initial COD was around 1500 mg/L to ensure that the final COD does not drop below 100 mg/L at the end of test.

The results show that activated sludge production yield ($Y_{obs}$), which was determined by Eq.3.4, decreased for all TCS concentrations tested. Compared with activated sludge yield in the absence of uncouplers, $Y_{obs}$ decreased by 16% at a TCS concentration of 0.4 mg/L and almost 45% at TCS concentration of 0.8 mg /L. However, there was noticeable decrease in $Y_{obs}$ when TCS concentrations increased from 0.4 mg/L to 0.6 mg/L.

Along with the reduction in yield, the COD removal was also affected by TCS. As shown in Figure 4.2(b), COD removal decreased as the TCS concentration increased. The removal of COD decreased by 12% at 0.4 mg/L TCS added. It should be noticed that the batch tests were carried out at substrate-rich environment and for a duration of 3 hours. This means the substrate up take process could not have been completed by the end of the test, based on the results in Fig 4.1. In wastewater treatment activated sludge systems the hydraulic retention time is longer than 3 hours, therefore the activated sludge will have longer time for removal of COD. Therefore, in WWTPs the actual COD removal is expected to exceed that observed in batch tests.
Energy spill triggered by presence of uncouplers and associated substrate removal can together have a net effect in the forms of reduction in $Y_{\text{obs}}$. In order to compare the effect of the uncoupler on growth rate and substrate removal, relative specific COD removal rate ($q$) and relative specific sludge growth rate ($u$) are defined, where $u$ is for specific growth rate in the presence of TCS; $u_0$ is for specific growth rate in the absence of TCS; $q$ is for specific COD removal rate in the presence of TCS and $q_0$ is for specific COD removal rate in the absence of TCS. The results are shown in Figure 4.2(c).

From the graph, it can be seen that both $u/u_0$ and $q/q_0$ decreased as TCS concentration increased, which means TCS caused reduction in both sludge production and substrate removal. However, higher reductions in $u/u_0$ were observed for all TCS concentrations, indicating TCS caused higher reductions
in sludge growth than in COD removal. This suggests that although part of the sludge growth was reduced due to lower COD consumption, the predominately cause of reduction in sludge growth is the lower sludge growth rate. For instance, when 0.5 mg/L TCS was added to the system, the COD removal declined by 10% while the associated reduction in sludge growth was more than 20%. But the reduction in activated sludge growth was double than that observed for substrate removal for TCS concentration higher than 0.4 mg/L.

A discrepancy was also observed when the concentration was over 0.4 mg/L. This indicates an enhancement of uncoupling effect when the dose amount is higher than 0.4 mg/L. The finding suggests optimum dosing of TCS should be between 0.4 to 0.6 mg/L, considering that higher concentration may lower the substrate consumption removal as well as increase operation cost.
4.2.2 Effect of PNP

The effect of PNP on activated sludge production, measured in terms of yield, is shown in Fig 4.3. The drop in yield was proportional to PNP concentrations. The drop in yield at PNP less than 10 mg/L was less severe compared to that for observed for PNP concentrations in the range 10 to 25 mg/L. Large drop in yield at PNP of 25 mg/L suggest that this concentration is highly toxic or inhibiting to activated sludge. Ultimately up to 57% reduction in yield was observed at PNP concentration of 40 mg/L. Similar to TCS, PNP also had a negative effect on COD removal. As shown in Figure 4.3, COD removal decreased by 23% at 30 mg/L PNP compared to that in the absence of PNP.
The results in Fig 4.2 and Fig 4.3 show that 0.6 mg/L TCS can induce a drop in activated sludge growth of almost the same magnitude as that induced by 30 mg/L PNP. Both uncouplers caused similar level of inhibition to activated sludge capacity for COD removal but the dosage of PNP was 50 fold of TCS.

The results suggested that TCS is more effective than PNP as uncoupler, where reduction in sludge production and effect on substrate removal are concerned.
Fig 4.4 shows the concentrations of PNP remaining at the end of the three-hour batch tests. The results indicated that around 60% of the PNP dosage was not utilised, hence remained in the reactor. This removal was consistent regardless of the initial PNP concentrations.

The high concentration of PNP remaining at the end of the tests includes that the use of PNP may impose negative impact on the environment if high concentrations of PNP reaches receiving waters. The US EPA prohibits the discharge of effluent contains PNP at concentration higher than 516 µg/L (EPA, 1992). Tomei et al., (2004), Wan et al., (2007), Sponza and Kusçu, (2005), reported that aeration conditions, feeding period and biomass concentrations can all affect PNP removal. They proposed that adjusting feeding rate and the
switch of aerobic and anaerobic conditions, up to 300 mg/L PNP can be removed in AS processes.

### 4.2.3 Effect of THPS

THPS is a bactericide, widely used around the world. Recently, researchers reported that it has potential for reducing activated sludge production. Using a 500 L continuous flow AS pilot plant, Stephanie et al., 2006) achieved 28% reduction in activated sludge growth by dosing 2 ml/L/day THPS into the feed to the plant.

The sludge yield and COD removal in the presence of 0.0-0.2 mg/L THPS are shown in Fig 4.5. THPS showed inhibition to substrate removal at 0.02 mg/L, where 12% reduction in yield was observed as well. Compare with TCS and PNP, THPS achieved the same impact on COD removal at much lower concentration. The OUR tests found THPS can reduce the microbial respiratory activity. It was observed that COD removal declined by 14% at 0.02 mg/L THPS, exceeding the reduction in sludge yield. The differences became larger as the THPS concentration increased and ultimately, COD removal was reduced by 34% while sludge yield declined by 20%. The decreased COD removal and OUR indicate that different from TCS and PNP, where sludge yield were reduced as a result of energy spilling, THPS reduced sludge production by inhibiting activated sludge microorganism activities, or in other words, toxic to the bacteria.
**Fig 4.5**: Changes in yield (MLSS) and COD removal at different PNP concentrations

### 4.2.4 Effect of Dosing PNP and TCS Combined

Although the long-term tests have confirmed that PNP and TCS can efficiently reduce the WAS production with minor reduce in substrate removal, it is still unknown whether the ability of reducing sludge production can be synergized by combined dosing of these two uncouplers. Some researches also reported uncouplers cause some variations in the sludge microbial population (Strand et al., 1999, Zheng et al., 2007). Thus, it is needed to investigate whether the selective effect on microorganism can be offsetted by combined dosing of two different uncouplers.

Based on the previous results, TCS is more effective than PNP regarding to the dosing amount and COD removal caused. So TCS was chosen
to be the primary uncoupler tested range from 0.2 to 0.6 mg/L, and PNP was added at a fixed amount of 5 mg/L. Each test includes two groups of batch reactors, one group was fed with pure TCS at various concentrations and the other one was fed with mixed uncouplers. Results of observed sludge yield and COD removal are shown in Fig 4.6 and 4.7.

Figure 4.6: Changes in yield for different TCS/PNP
The results show a general trend of lower sludge yield from the mixed dosed group compared to the pure TCS dosed group. 39% of sludge yield was reduced when 0.6 mg/L pure TCS was added while 49% of reduction was observed when 5 mg/L PNP plus the same amount of TCS were added. From the previous tests, PNP can reduce the sludge yield by 6% at this dosage. Thus, the combined dosing increased the efficiency of energy spilling and ultimately, lead to a higher sludge yield reduction than the pure uncoupler dosing.

The combined dosing also improved the substrate removal. Fig 4.7 shows the COD left in the effluent after the 3-hour tests. The batch reactors received mixed dosing showed slightly lower COD strength than the pure TCS dosed group.
4.3 Effect of PNP and TCS on AS Using SBRs

Based on results from batch screening tests, the lowest effective doses for TCS and PNP, in terms of reduction in yield and effect on COD removal, are 0.2 and 10 mg/L, respectively. Considering the potential impact on environment and to minimise the operational cost, the effect of the two uncouplers under continuous flow condition was carried out using SBRs for the 0.5 mg/L TCS and 20 mg/L PNP.

Fig 4.8 shows the accumulated excess sludge production at different environments. 1.01±0.16 g SS was wasted from the control reactor, 0.83±0.20 and 0.72±0.17 g SS were wasted from the SBRs that received TCS and PNP into their feed, respectively. Overall, the observed WAS reduction was 28.6% for 0.5 mg/L TCS dosing and 17.8% for 20 mg/L PNP dosing. The results showed a reduction in the activated sludge both for TCS and PNP, which was related in the mass of WAS need to be wasted daily. The results suggested that sludge did not develop resistances to uncouplers during the 30-day test.

Compared to the batch test, where 35% and 22% reduction in activated sludge growth was observed at the dosage of 0.5 mg/L TCS and 20 mg/L PNP, the reduction in activated sludge growth during the SBR tests was 28.6% and 17.8% for TCS and PNP, respectively.
Figure 4.8: Cumulative sludge production for TCS and PNP during 30-day SBR test

The results in Fig 4.8 showed that the reduction in sludge growth increased over the duration of test for both TCS and PNP. But reduction in yield was higher in the presence of TCS for all concentrations tested.

Table 4.2: COD removal during SBR test

<table>
<thead>
<tr>
<th>Day</th>
<th>COD removal uncoupler free</th>
<th>COD removal 10 mg/L PNP</th>
<th>COD removal 0.25 mg/L TCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>97.4%</td>
<td>96.4%</td>
<td>96.8%</td>
</tr>
<tr>
<td>Day 8</td>
<td>97.0%</td>
<td>95.3%</td>
<td>94.3%</td>
</tr>
<tr>
<td>Day 15</td>
<td>96.3%</td>
<td>95.9%</td>
<td>93.9%</td>
</tr>
<tr>
<td>Day 22</td>
<td>96.5%</td>
<td>96.3%</td>
<td>96.1%</td>
</tr>
<tr>
<td>Day 29</td>
<td>98.4%</td>
<td>98.0%</td>
<td>97.9%</td>
</tr>
</tbody>
</table>
Table 4.2 shows COD removal results during the test. It is clear that the presence of both uncouplers had minor effect on COD removal in the SBRs. Lower COD removal rates were observed in the batch test where the reactors were operated under a substrate-rich environment at all times. This can be explained in terms of the longer time available for COD degradation during the SBR tests. This finding indicate a feasibility for using TCS and PNP to reduce biological sludge production without adversely affecting effluent COD levels.

It is controversial how the draw-and-fill process in SBR can affect the uncoupler concentration. No previous research showed the changes in uncoupler concentration during AS process so the PNP content in effluent from the reactor was measured in a weekly basis and the results are shown in Table 4.3.

Table 4.3: PNP content in feed and effluent

<table>
<thead>
<tr>
<th></th>
<th>PNP in feed (mg/L)</th>
<th>PNP in effluent (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>20.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Day 8</td>
<td>20.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Day 15</td>
<td>20.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Day 22</td>
<td>19.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Day 29</td>
<td>19.2</td>
<td>6.3</td>
</tr>
</tbody>
</table>
The result shows a constant PNP content in the effluent during the test period. As the PNP content kept consistent during the test, it has been proven that there was no uncoupler accumulates effect in the reactor.

Microscopic analysis was also performed to monitor changes in microorganism in the AS reactor. The images in Fig 4.9 (a-d) show the change in the microorganism population during the SBR test. Before dosing with the uncoupler, the activated sludge contained a combination of bacilli and cocci in both of the reactors. After the 30-day operation, though the dense of the flocs were still unchanged, no protozoa have been observed in the reactors. Instead, filamentous bacteria were found in the culture which received the two uncouplers. The finding was compatible with the previous reports that protozoa ceased growing and filamentous proliferated when uncouplers were added. Zheng et al., (2008), in microscopic analysis, found that the number of filamentous bacteria increased in reactors received 2,4,6-Trichlorophenol (another uncoupler) addition. The PCR analysis he performed also confirmed that some species in the activated sludge were replaced by new microbes after the uncoupler was added. Low et al., (2000) also found that ciliated protozoa were disappeared after the adding PNP and filamentous began to grow in the reactor.
Fig 4.9 (a): Image of Reactor2 on Day1 (X100)

Fig 4.9 (b): Image of Reactor3 on Day1 (X100)
Fig 4.9 (c): Image of Reactor2 on Day30(X100)

Fig 4.9 (d): Image of Reactor3 on Day30(X100)
The increase of OUR may be one of the reasons caused the shift on activated sludge species. From the results in 4.2, TCS and PNP have been found can increase the sludge activity, which then lead to a high oxygen uptake rate (OUR), by 190% and 22% when 0.5 mg/L TCS and 20 mg/L PNP was added. As the air supply to the three reactors is constant, the reactors received TCS and PNP dosing were consuming oxygen faster than the control one. Thus, the dosed reactors were stay at anoxic condition longer than the control one. As a result, anoxic bacteria such as filamentous would begin to grow. To avoid this, technologies such as pure oxygen aeration and improved aeration systems are needed to increase the oxygen transfer rate. For the lab scale reactors, reducing the influent flow rate to extend the feeding phase is a feasible option.

Table 4.4: SOUR measurements for SBR test

<table>
<thead>
<tr>
<th>Day</th>
<th>SOUR (mg O₂/g MLSS/h)</th>
<th>Control</th>
<th>20 mg/L PNP</th>
<th>0.5 mg/L TCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day1</td>
<td>77.5</td>
<td>92.6</td>
<td>92.5</td>
<td></td>
</tr>
<tr>
<td>Day15</td>
<td>72.6</td>
<td>89.2</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>Day30</td>
<td>74.6</td>
<td>89.4</td>
<td>95.9</td>
<td></td>
</tr>
</tbody>
</table>

SOUR values for AS collected from the three reactors are measured on day1, day15 and day30. The results are shown on Table 4.4. Similar to the OUR test in chapter 4.2, an increase on sludge respiratory was observed in the TCS and PNP dosed reactors through the testing period. Previous research has
reported that the over-consumption of oxygen is a representation of energy spilling (Mayhew and Stephenson, 1998). Therefore, the higher oxygen uptake rate in TCS dosed reactor can be seen as a sign of greater metabolism disassociation than the PNP dosed one, which corroborates with the lower sludge yield observed in the TCS dosed reactor.

4.4 Effect of Combined Dosing of TCS And PNP On AS Using SBRs

As mentioned in 4.3, the importance of long term SBR tests is to investigate the potential effect on microorganism species and to exam if there is any resistance grown in the long term. The SBR tests also adopted two different dosing methods: reactor two was dosed three times a day, while reactor one received the same amount of dosing but one time a day. The aim of using these two methods is to investigate the effect of intermittent dosing. Reducing the dosing frequency from three times a day to one time a day not only will reduce the chemical cost when the uncoupler applied to real plants, but also can minimise possible negative effect the uncouplers bring to the AS process.
Figure 4.10: Changed Cumulative sludge production during SBR test
SBR1: Control, SBR2: 0.5 mg/L TCS+5 mg/L PNP (dosed three times per day), SBR3: 0.5 mg/L TCS+5 mg/L PNP (dosed once per day)

Fig4.10 shows accumulated excess sludge production during the SBR test. Average excess sludge production in the control reactor was 1.131 ± 0.114 g MLSS/day, compared to 0.803 ± 0.148 and 0.855 ± 0.163 g MLSS/day in SBR2 and SBR3, respectively. This represents a reduction of 29% and 24% in sludge production. The results showed that dosing the uncoupler once per day into the feed had almost the same impact on yield compare to dosing the uncouplers three times. The results indicated that under continuous operation conditions, the effect of dosing the uncoupler, on activated sludge
microorganisms, dosed intermittently may only induce the same effect of dosing at a lower dose, eg 1/3 under the conditions tested. One of the benefits of the intermittent dosing is the variation in the concentration of the uncoupler over the day because the shift between high and low uncoupler concentrations will minimise the potential effect on microorganism species as discussed in Chapter 4.3.

The microscopic analysis confirmed the conclusion. The four pictures in Fig 4.11 show that the sludge flocs in SBR2 and SBR3 were similar on day1, but at the end of the test, sludge flocs showed a filamentous growth in SBR2, which was dosed three times a day. On the other hand, less filamentous bacteria were observed in SBR3, which received uncouplers into the feed to one of the three cycles. Filamentous, a bacterium that is considered responsible for bulking problems in WWTPs, has been reported to grow faster in the presence of uncouplers (Ye and Li, 2005, Chen et al., 2002). Both research teams observed filamentous growth in lab scale SBRs when TCS and 2,4-dinitrophenol (used as uncouplers) were added. Dosing of the uncouplers 3 times per day would increase the F/M, the high F/M may explain the growth of filamentous bacteria observed in SBR2. Thus, the results suggest that concentrations as well as dosing frequency are important factors and should be optimised to minimise or eliminate the potential effects of uncouplers on the activated sludge process operation.
Fig 4.11 (a): Image of the flocs collected from SBR2 on Day 1 (100X)

Fig 4.11 (b): Image of the flocs collected from SBR2 on Day 30 (100X)
Fig 4.11 (c): Image of the flocs collected from SBR3 on Day 1 (100X)

Fig 4.11 (d): Image of the flocs collected from SBR3 on Day 30 (100X)

Floc projected area analysis was also performed to investigate the potential relationship between sludge settleability and changes to sludge flocs
characteristics. Table 4.5 (a,b) show the summary of floc projected area. As described in section 3.4.6, two samples were taken for each analysis and at least 12 pictures were taken from the microscope for each sample. The pictures were then analysed using the software Image J 1.33 (http://rsb.info.nih.gov/ij/). Compared with traditional microscopic analysis, analysis of flocs using Image J does not require staining or complex preparation. The size selection was set to measure flocs at 10 µm - infinities to exclude inorganic matters in activated sludge.

The results showed the addition of uncouplers did not have significant impact on floc projected area. But the final Feret diameter was larger than that measured on Day 1. As the Feret measures the longest length between any two points on the floc, the change showed the average length of flocs has increased. This could be because of the growth in the number of filamentous bacteria in the reactors during the period of dosing of uncouplers.

Table 4.5 (a): Analysis of flocs collected on Day1 using Image J

<table>
<thead>
<tr>
<th></th>
<th>SBR1 (control)</th>
<th>SBR2</th>
<th>SBR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average floc projected area (mm²)</td>
<td>Feret (mm)</td>
<td>Average floc projected area (mm²)</td>
</tr>
<tr>
<td>sample 1</td>
<td>0.093</td>
<td>0.052</td>
<td>0.085</td>
</tr>
<tr>
<td>sample 2</td>
<td>0.098</td>
<td>0.046</td>
<td>0.093</td>
</tr>
<tr>
<td>Ave</td>
<td>0.095</td>
<td>0.049</td>
<td>0.089</td>
</tr>
</tbody>
</table>
4.5 Effect of THPS on AS using SBRs

The batch tests suggested the activated sludge is more sensitive to THPS than the other two uncouplers TCS and PNP in terms of high on COD removal reduction at low dosage, so 0.02 and 0.05 mg/L were chosen for the SBR test.

Fig 4.12 shows the accumulative excess sludge production during the 30-day test. At day30, 30% and 72% of sludge production were reduced in the reactors that received 0.02 and 0.05 mg/L THPS, respectively. Compared to the batch test, THPS caused higher reduction in yield in the SBR than batch test. The same phenomenon has been found for TCS and PNP receiving SBRs and could be due to the longer hydraulic retention time in SBR tests. The result also
showed consistent sludge growth rate for all the three reactors during the testing period, indicating the impact of dosed chemicals remained the same during the test period, in other words, there was no resistance effect was developed in the activated sludge systems.

From the previous experiments on TCS and PNP, it’s understood that the decrease in COD removal in batch test, which was set at substrate rich environment, may not occur in the SBR test. The reaction time in the SBR test is long enough for the microorganisms to completely utilize substrate.

![Fig 4.12: Cumulative sludge production in the SBRs receiving THPS](image)

COD removal result, which is present in Fig 4.13, shows THPS inhibited substrate removal in SBR test as well. High COD removal reduction was observed in the SBR test. The inhibition was greater in the first two days after dosing, this was due to the disturb THPS brought to the microorganisms.
Unlike TCS and PNP, which had less than 5% drop in COD removal in the SBR test, THPS showed a much severe effect on COD removal and microorganism activity.

Fig 4.13: COD removal in the SBRs receiving THPS uncoupler

Floc projected area analysis results in Table 4.6 shows the THPS did not cause significant change to activated sludge flocs shape. Similar as been observed at floc projected area analysis on SBR test on combined dosing, Feret perimeter showed a slightly increase during the 30-day operation. This is believed to because of the growth of filamentous bacteria.

SOUR values were measured once a week during the test period. From the results shown in Table 4.7 it is clear that THPS can reduce the sludge
activity, which confirms the same observation in the OUR test in chapter 4.2. The results indicate that no resistance has grown in the AS system during the 30-day operation.

Table 4.6 (a): Analysis of flocs collected on Day1 from SBRs receiving THPS using Image J

<table>
<thead>
<tr>
<th></th>
<th>SBR1</th>
<th>SBR2</th>
<th>SBR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average floc</td>
<td>Average floc</td>
<td>Average floc</td>
</tr>
<tr>
<td></td>
<td>projected area</td>
<td>projected area</td>
<td>projected area</td>
</tr>
<tr>
<td></td>
<td>(mm²)</td>
<td>(mm²)</td>
<td>(mm²)</td>
</tr>
<tr>
<td>sample 1</td>
<td>0.087</td>
<td>0.095</td>
<td>0.088</td>
</tr>
<tr>
<td>sample 2</td>
<td>0.098</td>
<td>0.097</td>
<td>0.092</td>
</tr>
<tr>
<td>Ave</td>
<td>0.093</td>
<td>0.096</td>
<td>0.090</td>
</tr>
</tbody>
</table>

Table 4.6 (b): Analysis of flocs collected on Day30 from SBRs receiving THPS using Image J

<table>
<thead>
<tr>
<th></th>
<th>SBR1</th>
<th>SBR2</th>
<th>SBR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average floc</td>
<td>Average floc</td>
<td>Average floc</td>
</tr>
<tr>
<td></td>
<td>projected area</td>
<td>projected area</td>
<td>projected area</td>
</tr>
<tr>
<td></td>
<td>(mm²)</td>
<td>(mm²)</td>
<td>(mm²)</td>
</tr>
<tr>
<td>sample 1</td>
<td>0.108</td>
<td>0.115</td>
<td>0.098</td>
</tr>
<tr>
<td>sample 2</td>
<td>0.099</td>
<td>0.106</td>
<td>0.089</td>
</tr>
<tr>
<td>Ave</td>
<td>0.104</td>
<td>0.111</td>
<td>0.094</td>
</tr>
</tbody>
</table>

72
Table 4.7: SOUR measurements for the SBR receiving THPS using Image J

<table>
<thead>
<tr>
<th></th>
<th>SOUR (mg O₂/g MLSS/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Day1</td>
<td>47.6</td>
</tr>
<tr>
<td>Day7</td>
<td>51.2</td>
</tr>
<tr>
<td>Day14</td>
<td>53.5</td>
</tr>
<tr>
<td>Day21</td>
<td>48.7</td>
</tr>
<tr>
<td>Day30</td>
<td>55.6</td>
</tr>
</tbody>
</table>

4.6 Cost Analysis

The study showed that when using an uncoupler it is possible to reduce sludge yield without significantly affecting substrate removal. It has also been found that the AS microorganisms do not acclimatize to the uncoupler the 30-day test against uncouplers. Therefore, there is an indication that it is feasible to utilize uncouplers to reduce excess sludge production.

Combined dosing of TCS and PNP was effective, where yield reduction increased compared to that for each uncoupler individually but had less effect on COD removal. This means that combined dosing has the potential to reduce operational costs. Intermittent dosing was adopted in the SBR test. The use of the two uncouplers TCS and PNP, simultaneously, 0.5 and 5 mg/L, respectively, reduced sludge production by 24%. Dose frequency was once a day whereas the reactors were fed three times a day.
According to the sustainability report 2007/08 prepared by Melbourne water, an average of 415 ML/day of sewage is treated at the Western Treatment Plant (WTP) and 26,000 dry tonnes of biosolids are generated per year. As summarised in Table 4.8, the cost of sludge treatment and disposal vary across countries. Therefore, the average values were used for this cost efficiency analysis. The total cost for sludge treatment and disposal is $244+264 ± 210 /ton of dry sludge, so it would cost $13.2 ± 5.5 million/year to dispose off all sludge to landfill.

Table 4.8: Summary of sludge treatment and disposal costs

<table>
<thead>
<tr>
<th>Cost of sludge treatment ($/ton)</th>
<th>Cost of final disposal ($/ton)</th>
<th>Resource/Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>343 ± 140</td>
<td>(Frost and Sullivan, 2003) / Europe</td>
</tr>
<tr>
<td>252</td>
<td>-</td>
<td>(Huysmans et al., 2001) / USA</td>
</tr>
<tr>
<td>-</td>
<td>440</td>
<td>(Bode and Gruenebaum, 2000) / UK</td>
</tr>
<tr>
<td>-</td>
<td>154</td>
<td>(Nowak, 2000) / Austrian</td>
</tr>
<tr>
<td>417 ± 133</td>
<td>186 ± 77</td>
<td>(Paul et al., 2006) / France</td>
</tr>
<tr>
<td>277 ± 118</td>
<td>269 ± 89</td>
<td>(Labaquere, 1998) / France</td>
</tr>
<tr>
<td>118</td>
<td>-</td>
<td>(Nowak, 2006) / UK</td>
</tr>
<tr>
<td>-</td>
<td>319</td>
<td>(European Commission, 2010) / Europe</td>
</tr>
<tr>
<td>105</td>
<td>209</td>
<td>(Yasui and Shibata, 1994) / Japan</td>
</tr>
<tr>
<td>230 ± 70</td>
<td>279 ± 53</td>
<td>(Stephanie et al., 2006)</td>
</tr>
<tr>
<td>244 ± 106</td>
<td>264 ± 104</td>
<td>Average ± standard deviation</td>
</tr>
</tbody>
</table>

Assuming that dosing with TCS and PNP as discussed above 24% sludge reduction would be achieved. This will lead to a reduction in sludge
handling fees of $1.8 – $4.5 million. While the market price for TCS and PNP is $3000 and $200 per ton, that brings the chemical cost to $0.13 million. Thus, a potential of $1.7 – $4.4 million will be saved in sludge handling costs.

For smaller plants such as Echuca treatment plant in Victoria, where 4.3 million liters wastewater was treated per day, and 17 ton dry maters of activated sludge was produced per month. The unit ton cost of sludge treatment alone is around $1200/ton, excluding cost for land filling. The total cost for sludge handling will be around $244,800. By using uncouplers, $58,752 per year can be saved in sludge handling fees, while chemical cost is $1,300.

Although the cost estimate does not include the cost of dosing system and additional operational cost, a noticeable saving on sludge treatment and handling fee can be saved by using proposed uncouplers.

Unlike the other methods which require external reactors (OSA process) and energy (Ozonation process), dosing with uncouplers provides a potentially easy and low-cost method to reduce sludge quantities produced by a treatment plant.
This study investigated the feasibility of using Para-nitrophenol (PNP), 3',3',4,5-tetrachlorosalicylanilide (TCS), and Tetrakis hydroxymethyl phosphonium sulphate (THPS) to minimize excess sludge production in AS process. The results obtained showed that TCS and PNP can reduce activated sludge production without affecting substrate removal whereas THPS showed noticeable inhibition to sludge activity and ability for COD removal. The main findings from this research are summarised below:

1. The OUR tests indicated that PNP and TCS increased activated sludge activity by 22% and 65% at concentrations of 20 mg/L and 0.2 mg/L, respectively. Activated sludge OUR increased in the presence of higher concentrations of TCS and PNP, which was observed both for batch and SBR tests. On the other hand, whereas THPS inhibited activated sludge activity by 34% at concentration of 0.02 mg/L in batch tests. The trend observed for OUR suggested that TCS and PNP reduced growth through energy spilling whereas THPS inhibited growth. The latter explains the large drop in COD removal compared to that in the presence of TCS and PNP.
2. The 3-hour batch tests showed that 20% and 36% sludge yield reductions can be obtained using PNP and TCS dosed at 20 mg/L and 0.5 mg/L, respectively. In comparison, 26% of reduction in sludge yield was achieved using 0.1 mg/L THPS. Associated COD substrate removal was 18%, 20% and 27%, respectively. The tests were performed under substrate-sufficient environment.

3. Dosing activated sludge at 0.5 mg/L of TCS and 20 mg/L of PNP caused 18% and 28% reduction in WAS production, during 30-day SBR test, respectively. Both TCS and PNP had minor effect on COD removal. On the other hand, 0.02 mg/L THPS caused 30% reduction in WAS production accompanied by 13% reduction in COD removal compared to that by the control SBR, i.e. absence of uncoupler.

During the 30 days test, i.e. more than 3 sludge ages, no acclimatisation to the uncoupler was observed, where reduction in activated sludge growth continued for the duration of the test.

4. Combined dosing of TCS and PNP synergised the effect on sludge production, where 39% of reduction was observed for feed dosing with 0.5 and 5 mg/L of TCS and PNP. This is 5% more than the total of 28% and 6% when doing the uncouplers individually.
5. The introduction of the uncouplers into the feed to the SBRs at different frequencies indicated that 2/3 of the uncoupler could be saved by reducing the dosing frequency from three times a day to one time a day, whereas the effect on sludge reduction dropped by 5%.

Based on the results above, it can be concluded that TCS and PNP can be used as uncoupler to reduce WAS production. The mechanism of this reduction could be by creating energy spilling in cells. THPS, on the other hand, showed is an inhibitor to the activated sludge. Although it reduced WAS production at low concentration but the COD removal dropped significantly.
REFERENCES


AN, K. J. 2004. Reduction of excess sludge in an oxic-settling-anaerobic (OSA) system: A modified activated sludge process. Hong Kong University of Science and Technology (Hong Kong) Ph.D., Hong Kong University of Science and Technology (Hong Kong).


ARORA, H., DEWOLFE, J. R., LEE, R. G. & GRUBB, T. P. Year. Evaluation of dissolved air flotation process for water clarification and sludge thickening. In, 1995 1995. I W A PUBLISHING, Alliance House, 12 Caxton St, London, SW1H 0QS, UK, [mailto:publications@iwap.co.uk], [URL, 137-137-147.


BODE, H. & GRUENEBAUM, T. 2000. The cost of municipal sewage treatment - Structure, origin, minimization - Methods of fair cost comparison and allocation, Elsevier Science Ltd., Pergamon, P.O. Box 800 Kidlington Oxford OX5 1DX UK.

BOUGRIER, C., DELGENÈS, J. P. & CARRÈRE, H. 2006. Combination of Thermal Treatments and Anaerobic Digestion to Reduce Sewage Sludge


EUROPEAN COMMISSION 2010. Evaluating and comparing different disposal and recycling options for sewage sludge.


LABAQUERE, H. 1998. *Quelques couts d'exploitation de filières de valorisation des boues*.


NOWAK, O. 2000. Expenditure on the operation of municipal wastewater treatment plants for nutrient removal, Elsevier Science Ltd., Pergamon, P.O. Box 800 Kidlington Oxford OX5 1DX UK.


85
*Under Alkaline Conditions*, Houston, TX, ETATS-UNIS, National Association of Corrosion Engineers.
