



**Specific models to assess the possible use of
alternative external carbon sources for
nitrogen removal in wastewater treatment.**

By

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CERTIFICATE OF ORIGINAL AUTHORSHIP

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Abbreviations

Symbol	Description
bCOD	Biodegradable COD
BOD	Biological Oxygen Demand
C:N	Carbon: Nitrogen Ratio
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
F/M	Food/ Microorganism Ratio
GAOs	Glycogen-Accumulating Organisms
HRT	Hydraulic Retention Time
IR	Internal Recycle/ Internal Return
MLE	Modified Luzack-Ettinger
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NMB	Nitrogen Mass Balance
OUR	Oxygen Uptake Rate
PAOs	Phosphate-Accumulating Organisms
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
rbCOD	Readily Biodegradable Chemical Oxygen Demand
sbCOD	Slowly Biodegradable Chemical Oxygen Demand
SBRs	Sequence Batch Reactors
SDNR	Specific Denitrification Rate
SDNR (t°C)	Specific Denitrification Rate at T°C
SE	Significant Error
SOUR	Soluble Oxygen Uptake Rate
SRT	Sludge Retention Time
SS	Suspended Solids
SVI	Sludge Volume Index

TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TSS	Total Suspended Solids
VA	Volatile Acid
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids
WAS	Wasted Activated Sludge
WWTP	Wastewater Treatment Plant

PhD Dissertation Abstract

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Abstract

Achieving effective denitrification in municipal wastewater treatment is amongst one of the world's biggest environmental challenges to sustainability. The problem is specifically due to the slow denitrification rate in the anoxic zone, which is caused by the lack of readily biodegradable organic carbon. Without effective treatment, the excessive discharge of nitrogen into waterways can cause eutrophication, deterioration of water sources and danger to human health. Various solutions including the construction, expansion and modification of existing wastewater treatment plants (WWTPs) to meet the increasing demand, however, often require the whole treatment plant being redesigned, with high investment cost, more operating expenses, and retraining of existing staffs.

An alternative strategy is adding an external carbon source directly into the anoxic zone. The advantage of this option is: (i) it is easy to implement, (ii) it requires little modification to an existing WWTP (so high costs and treatment plant operations will not be overly affected), and (iii) it can meet both the short-term and long-term treatment standards. The search for a material that is readily

degradable, inexpensive, and preferably to be either a waste material or by-product with favourable C:N ratio from local industries, has been ongoing in the last few decades, and is also the central theme of this research.

The original contribution this research makes to our knowledge of the topic is done by simulating the potential of industrial-grade sucrose and fermented biosolids. These are two less well-studied carbon subgroups that can act as external carbon sources for improving denitrification in municipal wastewater treatment. This task was specifically achieved by establishing a systematic cross-verification of various mathematical, conceptual and physical models, which will not only provide more information about the two carbon subgroups, but also help to identify various flaws and disadvantages each model may carry. Despite both sucrose and various other fermented sludge types being experimented upon in this thesis, the real original research subjects of this study are the fermented and dark fermented biosolids, two substances within fermented sludge subgroup. They were selected based on the results of a series of fermentation batch tests.

Meanwhile the reason why industrial-grade sucrose was also studied despite it not being necessarily new is, firstly, due to sucrose's insignificant nitrogen content, consistency and uniform characteristics; this makes it the perfect subject to test and develop the cross-verification methodology. Secondly and in reference to future research on this topic, sucrose is the product of cellulose hydrolysis, which has very similar optimal operation conditions (in terms of pH and temperature) with sludge fermentation. This indicates the future potential for utilising cellulose hydrolysis in the same sludge fermenter to improve and optimise fermented sludge generation.

The results indicated that while sucrose could be used to improve the denitrification process and treat several treatment scenarios down to below standard, fermented and dark fermented biosolids however, provide a much

better treatment performance, and complete denitrification in almost all simulations. In fact, its maximum potential is exceptional that within the scope of this study, it was only limited by the treatment demand rather anything else.

The results also found that the NMB models and cross-verification methodology being established by the candidate were very successful in simulating the effects of adding sucrose and fermented biosolids on denitrification improvement. It is however recommended to apply these models and methodology into future external carbon source study, not only to detect their flaws and drawbacks, but also to improve them accordingly.

Keywords: Sucrose, fermented sludge, external carbon source, modelling cross-verification, cellulose hydrolysis.



Chapter 1

Introduction

Chapter 1 - INTRODUCTION

1.1. Research background

Like most other modern and technologically developed countries, since the late 1990s, Australia has been challenged by the need to enhance its water recycling and treatment capacity to meet the increasing demands for water from a rising population. One of the six major urban objectives noted in the Australian National Water Initiative (2006) is the need to provide innovation and better water recycling and treatment, which is a sustainable approach to water resource consumption. Carrying that same spirit, The Australian Drinking Water Guidelines (2004) were adopted by the NSW Office of Water for use by local water utilities under Section 60 of the Local Government Act 1993 and s292 of the Water Management Act 2000.

It should be emphasised that this is not an issue unique to Australia. In Europe for example, European Urban Water Directives 91/271/CE and EWFD 2000/60/CE also aimed to protect the environment from domestic wastewater, mixed wastewater and discharges from certain industries. European countries' approaches were also similar in their emphasis on collecting, recycling, treating and monitoring wastewater in wastewater treatment plants to mitigate its impact on the environment. Considering that total water volume in rivers and lakes only account for 0.01% of global water volume, much of it already under pollution, it is understandable that scarcity of clean water is a serious issue for all nation states and has grave implications for their economic development agendas.

Of the three stages of biological wastewater treatment, secondary treatment and the denitrification process have specifically been at the forefront of wastewater treatment innovation in recent decades. Without effective denitrification, the excessive discharge of nitrogen into waterways can cause eutrophication, deterioration of water sources and hazards to people's health (Li et al. 2010; Schindler

2012). The worst problem is the potential and widespread formation of carcinogenic compounds (such as nitrosamides) and the risk of methemoglobinemia to infants (Fernández-Nava et al. 2010; Wang et al. 2009).

On the issue of denitrification, one of the biggest challenges in the municipal wastewater treatment industry is the slow denitrification rate, caused by the lack of readily biodegradable organic carbon (rbCOD) in the anoxic zone (Shi et al. 2015). This is because despite biological denitrification being the most promising method for nitrate removal (Karanasios et al. 2010; Zhao et al. 2012), it requires the consumption of carbon organic compounds for energy. rbCOD, the essential electron donor, generally consists of small, simple, soluble or dissolved organic matter which can pass directly through the denitrifier's cell wall and be consumed quickly (Li, Zhang, et al. 2016; Pan et al. 2015; Tam, Wong & Leung 1992a, 1992b). Without rbCOD, the denitrification process has to depend on the slowly biodegradable COD (sbCOD), which consists of larger particulate or colloidal organic compounds. These have to be hydrolysed by the slimy surrounding layer of microorganisms' cells first before they can be utilised (Henze 2008). The differences in hydrolysis/degradation rates of these two types of carbon have a direct impact on the COD utilisation rates, the rates of energy being released and subsequently the denitrification rates themselves. It is no exaggeration to claim that rbCOD is the key limiting substrate when it comes to effective denitrification (Meyer et al. 2005).

To overcome this, many studies over past several decades have primarily concentrated on employing pre-anoxic denitrification systems like MLE, Badenpho or variations of them. The aim was to introduce influent wastewater, being rich in organic matter, into the pre-anoxic zone to act as a carbon source for denitrification. However, even for pre-anoxic denitrification, due to influent characteristics¹ as well

¹ such as C:N ratio, the fraction of readily degradable organics.

as other operational and design characteristics², the denitrification process of many municipal WWTP systems left much to be desired. A major part of the problem of trying to achieve effective denitrification was the huge expense. For example, Oleszkiewicz (2004) reported that a reduction of 2-4mg NO₃/L at the Crajka WWTP in Warsaw (580,000m³/d) to meet the new regulations cost the plant equivalent of 5500-8200 AUD/d.

Given the trend for stricter regulations throughout the industry, many WWTPs are now facing the task of finding better economic alternatives to reduce their effluent nitrogen levels. One such alternative is adding an external carbon source directly into the anoxic zone. The advantages of this option are: (i) it is easy to implement, (ii) it requires few modifications to an existing WWTP, and (iii) it can meet both the short-term and long-term treatment standards. In past decades, most studies focused on methanol, one of the world's most used and well-documented carbon sources (Swinarski, Makinia, Czerwionka & Chrzanowska 2009; Swinarski, Makinia, Czerwionka, Chrzanowska, et al. 2009). It was recommended by the US EPA for the supplemental substrate due to its availability, low cost, small sludge production, low volatile organic compounds, low emissions potential, and no additional nitrogen or phosphorus being introduced into the system. However, the fact that methanol is a valuable resource with many applications, means that for large WWTPs the cost of methanol can significantly increase operating costs. Apart from this the long acclimation period makes it unsuitable as a short-term or non-continuous application.

This is why finding an alternative cost-effective external carbon substance has been a major priority for the wastewater treatment industry for the past three decades (Sage et al. 2006). It was ranked the most important research topic during

² parameters such as Hydraulic Retention Time (HRT), Internal Recycle Rate (IR).

the Nutrient Challenge Workshop that was held on 3rd March, 2007 (Swinarski, Makinia, Czerwionka, Chrzanowska, et al. 2009).

1.2. Research Aim

The aim of this thesis is to expand our knowledge of denitrification using external carbon in suspended growth wastewater biological treatment processes. This will be done through the following strategies:

- i. Constructing an experimental methodology that can effectively evaluate the denitrification performance of any external carbon. This is done based on the literature theoretical knowledge regarding the denitrification process of biological treatment, and the candidate's hands-on experience gained from various experiments throughout his candidature.
- ii. Using the above established methodology to simulate the performance and assess the potential of the two main groups of carbon sources: sucrose and fermented sludge.
- iii. Based on the results, evaluating the advantages and disadvantages of different simulation models and how they can be used in future studies on external carbon.

1.3. Research Questions

1. Why is it necessary to add an external carbon source, given the availability of a pre-anoxic denitrification system? (Chapter 2)
2. How can we apply the available theoretical knowledge such as computer software, growth rate equation of activated sludge, and mass balanced equation to effectively simulate the denitrification performance of any given external carbon sources? (Chapter 3)

³ The workshop was organized by the Water Environment Research Foundation.

3. Can sucrose improve denitrification? Is sucrose effective and viable as an external carbon source for future industrial applications? (Chapter 4)
4. Can fermented sludge improve denitrification? Is fermented sludge effective and viable as an external carbon source for future industrial applications, especially in with reference to sucrose? (Chapters 5 and 6)
5. Based on the obtained results, what are the advantages and disadvantages of each simulation model? Which one is more suitable for different real-life scenarios and how could they be best utilised for future studies? (Chapter 7)

1.4. Research Scopes

The scopes of this study include the following. Firstly, the literature review and case-study will involve: (i) accessing literature and available second-hand data for the project rationalising, experimental planning and for results interpretation; and (ii) studying the performance of the Sunbury pilot plant and identifying the scope for experimental works. This is followed, secondly, by the physical experimental works, using:

- a) Specific Denitrification Rate (SDNR) tests and Mumax batch tests to determine the denitrification rate, denitrifier yield rate and growth rate, and assess the effect of different independent variables on those above.
- b) Fermentation batch tests to optimise the fermented sludge quality and generating the fermented sludge samples.
- c) Lab-scale Sequencing Batch Reactors to simulate the post-anoxic denitrification performance in lab-scale condition.
- d) Pilot-scale plants to simulate the pre-anoxic denitrification performance.

Thirdly, there will be software and desk-top simulation works and these will involve setting up a basic Nitrogen Mass Balance (NMB) conceptual model that simulates the pre-anoxic and post-anoxic denitrification performance based on the SDNR. Also

required for setting up will be the Biowin simulations to simulate the results of both pre-anoxic and post-anoxic denitrification based on the denitrifier yield rate and growth rate. A cross-verification system will be then established to assess and verify the results of all the above physical experiments and software and desk-top simulations.

1.5. Significance of the study

As suggested by Maude (2014), who emphasised the need to 'structure the thesis around a paradox, not around a gap', the aim of this research originated from one of the paradoxes of external carbon sources: why there is a need for an external carbon source at all? On one hand, in theory, MLE and other variations of the pre-anoxic denitrification system should have championed municipal denitrification treatment, due to its supply of unlimited organic carbon sources for the anoxic zone. However, in reality, finding alternative cost-effective external carbon substances has been a long-term priority of the wastewater treatment industry for a few decades (Oleszkiewicz 2004) with thousands of research papers published on the topic. Talking with operators of pre-anoxic WWTPs and experts on the subject also indicated that ineffective denitrification is a real problem and utilising external carbon to improve both short and long-term denitrification performance is a more viable option. Also, to be considered are the consequential effects caused by incomplete denitrification such as proliferation of foaming filamentous bacteria (Chen et al. 2010). As well, the loss of MLVSS would seriously affect not only the denitrification process but the longstanding performance of the whole treatment plant (Martins et al. 2004). This certainly has not included the external effects of incomplete denitrification and how the excessive discharge of nitrogen into a waterway such as eutrophication and

formation of carcinogenic compounds (Fernández-Nava et al. 2010; Li et al. 2010; Schindler 2012; Wang et al. 2009).

To better understand this paradox, the effectiveness of the two chosen groups of external carbon sources has been examined: sucrose and fermented sludge on denitrification. Sucrose in this case includes both industrial-grade sucrose, a by-product of the sucrose refining process, and the sugar theoretically being generated from cellulose hydrolysis. If sucrose proves to be an effective external carbon source, municipal WWTPs could not only utilise a by-products of local sugar industry (if available), but also realise the potential of using cellulose hydrolysis to generate or improve an external carbon source. Fermented sludge of both Wasted Activated Sludge (WAS) and anaerobic biosolids meanwhile, as an external carbon source, could both reduce the cost of wasted sludge transport and treatment, and the need to transport another external carbon source from elsewhere to the WWTPs. Hence if sucrose and fermented sludge were effective external carbon sources, then they will provide much-needed alternative options for wastewater treatment plant operators worldwide.

Secondly, and more importantly, however, during the process of finding an effective methodology to test these two carbon types, the study has discovered the lack of a singular clear structure when it comes to simulating the effect of a studied carbon sources on a real treatment plant. The most common method used in literature was to compare the specific denitrification rate (SDNR) of the studied carbon with a common/ well-studied external carbon (commonly, methanol) then test performance through lab-scale/ pilot-scale studies. This method, while helpful in providing some idea of what could be expected, such as whether adding the studied external carbon will improve the denitrification performance as much as adding methanol, cannot illustrate the numerical relationship between the SDNR and pilot/ lab-scale/ life-scale performance in the presence of an external carbon source. This

means it will be difficult to accurately simulate the effect of any newly studied carbon sources on a physical biological system without extensive on-site testing, something that is not accessible and viable for a PhD thesis.

It is therefore obligatory for the researcher to construct a systematic methodology that can simulate the performance of an external carbon source based only on mathematical calculations and lab-scale study. This is because without one, assessments of the two chosen groups of external carbon sources (sucrose and fermented sludge) would have been unreliable. Ideally it will be possible to apply the same methodology on similar future analyses of other external carbon sources.

1.6. Thesis outlines

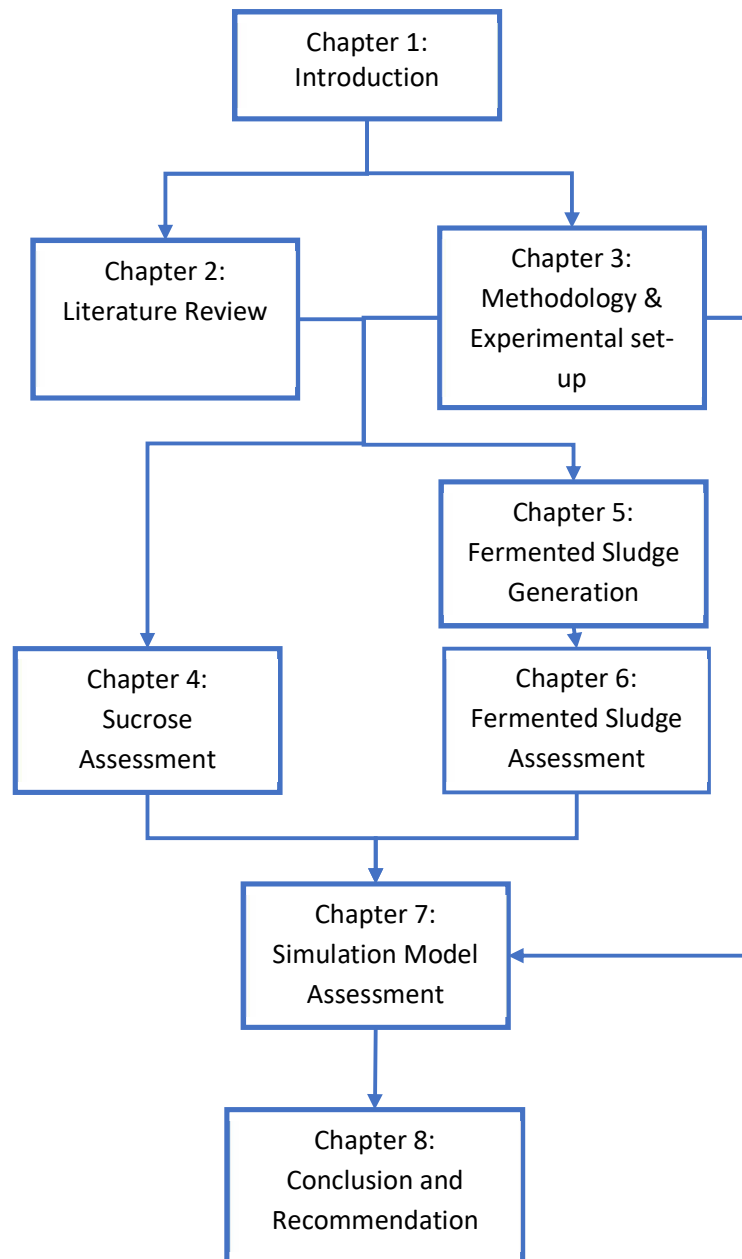


Figure 1.1: The Thesis outline

The content of this thesis is as follows:

Chapter 1 provides an introduction on the topic, including the aims, research questions, scopes, significant and limitation of the study. **Chapter 2** focuses on the literature review, including a brief introduction on activated sludge, denitrification, and why adding external carbon is a good method for improving denitrification. The chapter will also discuss previous external carbon studies in published literature. **Chapter 3** describes the experimental methodology structure, breaking down each model being used in the thesis and their experimental set-up. The experimental results are presented in **Chapters 4 and 6**, specifically on two tested external carbon groups- sucrose and fermented sludge, respectively. Included is a summary of the specific experimental set-up and general information regarding each carbon source). In between is **Chapter 5** which is about the fermented sludge generation and selection process and why the options for fermented and dark fermented biosolids were chosen. **Chapter 7** explains the results documented in **Chapters 4 and 6** and identifies the advantages and disadvantages of each model used in this thesis. Finally, **Chapter 8** presents the conclusion and recommendations.



Chapter 2

Literature review

Chapter 2 LITERATURE REVIEW

This chapter provides general information and a literature review regarding denitrification in wastewater treatment systems and the crucial roles played by both internal and external carbon on a system's denitrification performance. This will be done by assessing issues related to carbon deficiency in practice, then carrying out a literature review on the currently available knowledge and findings regarding external carbon research.

2.1. Introduction to the Activated sludge system

Activated sludge technology was first developed in 1913 in Manchester, United Kingdom and it consists of two main categories. The first is the attached growth (fixed film) treatment technology, which results from a microorganism attaching itself to some merit medium (rocks, slags). Suspended growth treatment technology on the other hand is the state where microbes stay suspended in liquid and all the treatment processes occur within a liquid medium.

It is important to understand that almost all wastewaters can be treated biologically. The objective of biological treatment is essentially to treat particulate and biodegradable matter (carbonaceous organic and ammonia) in wastewater using microorganisms. This will be done by: i) capturing and incorporating suspended solid and non-settleable colloids and microbes into biological floc or biofilm, and ii) breaking and transforming the carbonaceous organic and ammonia into acceptable end products. The autotrophic bacteria will grow by obtaining ammonia and bicarbonate as carbon sources for nitrogen oxidation (nitrification). The produced nitrite and nitrate will then be consumed by facultative autotrophic organisms to produce N^2 and more biomass. Considering that the mass usage of fertiliser results in

nitrogen pollution damaging open water sources, it explains why, from the operational and economic points of view, using biological treatment technology such as the activated sludge system is an ideal solution for municipal wastewater treatment.

Generally, the three key features of the activated sludge technology are: a) aerated reactor with suspended microorganisms; b) separation of solid away from liquid influent; and c) the recycling of RAS, return activated sludge. This is the reason why the main objectives when designing an activated sludge treatment plant always focus on: aeration basin volume, amount of sludge production, amount of oxygen needed and final effluent characteristics. Getting these correct means that the system can remove all soluble, colloidal and particulate organic substances. There are, furthermore, four common types of environmental conditions for the treatment process. Two of them are aerobic and require the use of oxygen, including the obligate aerobic stage, which can use oxygen only, and the facultative aerobic stage. This can also use oxygen in nitrate or nitrite for COD treatment and denitrification. Conversely, the obligate anaerobic stage can only happen in the absence of oxygen (both free and O_2 bound oxygen NO_2) for COD treatment. Meanwhile facultative anaerobic treatment can work whether oxygen is present or not. The anoxic stage, which this thesis primarily focuses on, only happens in the absence of free oxygen, in the presence of nitrite and nitrate as denitrification occurs here.

2.1.1. Microorganisms in the Activated sludge system

Microorganisms in the activated sludge system often work within a certain environmental range, with general optimum pH being between 6.5 and 7.5. The optimum temperature range varies based on microorganism species: Psychrophilic is 12-18°C, Mesophilic is 25-40°C and Thermophilic is 55-65°C. There are also heterotrophic and autotrophic microorganisms: the former comprises organic carbon-consuming species (similar to humans and animals); while the latter can

synthesise carbon from inorganic sources (similar to plants, algae). The synthesis process consumes more energy, so the autotrophs tend to have slower growth rates and cell mass.

To measure or monitor both the existing biomass, biomass yield (grams of biomass generated per grams of substrates) and any other particulate organic matter in wastewater treatment, Volatile Suspended Solids (VSS) measurements are often employed. It is simple and requires minimal time and resources for measurement. Within the biomass yield there are differences between synthesis and observed yields. The observed yield is the actual yield based on physical testing. Meanwhile the synthesis yield is interpreted from the biomass production rate and can be estimated from the produced energy of oxidation-reduction reactions.

2.1.2. Required nutrients for Microorganisms' growth

COD is often being used for monitoring the activated sludge treatment process due to it being easy and simple to perform. For the purpose of classification, the total COD can be divided into active mass, biodegradable and non-biodegradable COD. Biodegradable COD (bCOD) can be divided into readily degradable COD (rbCOD) and slowly degradable COD (sbCOD). Non-biodegradable COD (nbCOD) can be divided into soluble nbCOD (nbsCOD or sCOD_e) and particulate nbCOD (nbpCOD). Some useful fundamental formulations and assumptions include: bCOD equal 1.6 times of total BOD, and inert Total Suspended solid (iTSS), can be calculated by subtracting Total Suspended Solid (TSS) from VSS.

Apart from carbonaceous materials, nutrients for microorganisms' growth include nitrogen-based nutrients, phosphorus-based nutrients, and vitamins. Municipal wastewater often has sufficient nutrients, while industrial wastewater often needs supplements. This study focuses mainly on municipal wastewater treatment, so supplements are not analysed here.

2.1.3. Important operational parameters in the Activated Sludge system

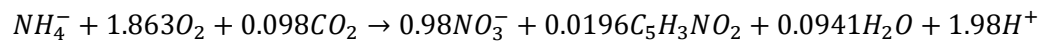
Another crucial element of the activated sludge system is Solid Retention Time (SRT). SRT is a very important factor and must be selected with cautions. For BOD removal, the SRR could be 3 days for (18-25°C), or 5-6 days if the temperature stays constantly at ~10°C. For the removal of nitrogen this number could reach up to 11 days or slightly more. Apart from SRT, there are other important factors such as Food: Microorganism ratio (F/M), loading rate, nutrient requirement (N+P), sludge disposal, oxygen Requirements, alkalinity, DO, and Returned Activated Sludge (RAS).

One of the major issues concerning the activated sludge system is bulking sludge, which is caused by the dispersed Mixed Liquor Suspended Solids (MLSS) with poor settling. If the MLSS cannot settle well, this may lead to loss of sludge, high effluent SS, and subsequently poor quality of the final effluent. Bulking may be caused by the high F: M ratio, leading to the growth of filamentous organisms (filamentous bulking, which is more common). It can also be caused by an excessive amount of extracellular biopolymer (viscous or hydrous bulking), leading to slimy, jelly-like, and water-retentive (absorb) sludge. Both experimental systems (SBRs and pilot plant) used in this thesis have encountered sludge bulking at some points; generally, there was no 'quick fix' other than months of stabilisation, which was very time-consuming and wasted opportunities.

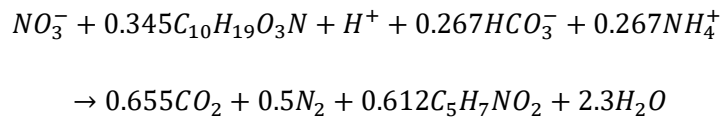
2.2. Denitrification in wastewater treatment

Denitrification is the process of mostly nitrate reduction where nitrate serves as an electron acceptor (in place of oxygen) for the degradation of organics (COD) by facultative heterotrophic organics. This often occurs after the nitrification process where nitrate is generated from the nitrifying of ammonia. The whole chemical reaction can be summarised as shown below:

Nitrification:



Denitrification:



The more technical term for this denitrification process is anoxic heterotrophic denitrification (Meyer et al. 2005) but often it is referred to only as 'denitrification' since it is the most common denitrification process in the activated sludge system. It often can be assumed that the lack of aeration will provide an anoxic stage for denitrification. In real scenarios, however, denitrification does not always work in completely anoxic or anaerobic zones. Due to open denitrification tanks, the DO during denitrification may even be 0.4-2.0 mg/L during winter (13°C) (Tallec et al. 2008).

There are two systems involving denitrification: *post-anoxic denitrification*, and *pre-anoxic denitrification* (or *substrate denitrification*). Sometimes those two are also referred to as post-denitrification and pre-denitrification, but this is simply a variation of terminology. The *post-anoxic denitrification* is the standard system, when anaerobic zone following aerobic and no flow recycling. In *pre-anoxic denitrification*, however, the anaerobic zone occurs prior to the aerobic zone. The difference here is their electron donor source, with *post-anoxic denitrification* using endogenous decay as the electron donor source. Meanwhile the *pre-anoxic denitrification* uses organic substrates (COD) from wastewater, unless external carbon sources are applied.

Organic carbon is often the limiting substrate and main problem for denitrification (Henze 2008; Meyer et al. 2005) as the COD here acts as electron acceptor and crucial for complete denitrification from happening. More specific

details on how organic carbon consumption contribute into the cell growth in anaerobic and aerobic phase is seen in Figure 2.1 below. This especially is true on post-anoxic denitrification, which depends solely on endogenous respiration for energy, and has a much slower rate of reaction than influent COD (Eddy 2003). For example, Dold et al. (2008) stated that an organic carbon level of less than 40 mg COD/L will limit the denitrification rate.

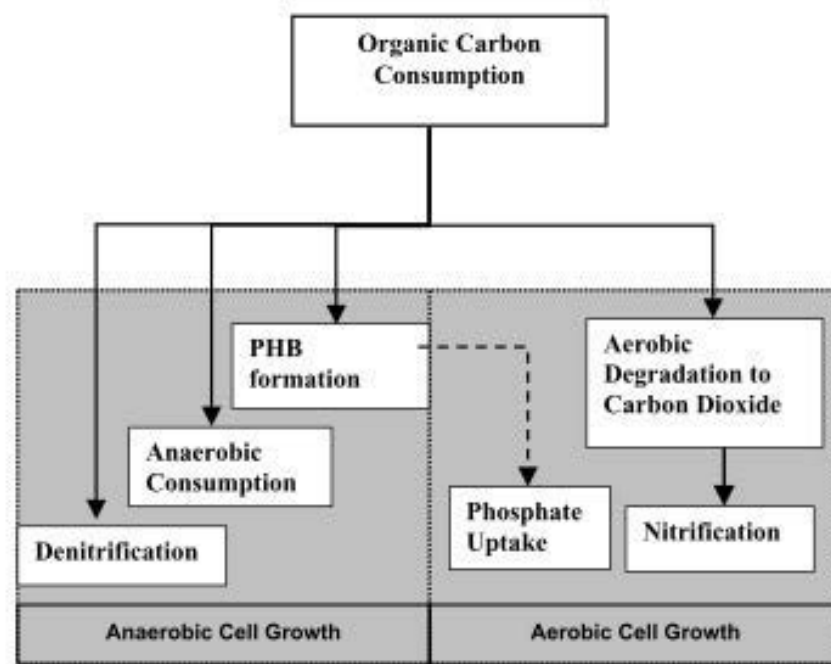


Figure 2.1: Organic carbon consumption in the biological nutrient removal process (adapted from Jang et al. 2004).

To resolve this issue, other than the internal COD sources present in the system itself, exogenous or external carbon sources such as methanol or acetate are often added to provide sufficient bCOD for the purposes of denitrification.

Microbiology

The heterotrophic denitrifiers (bacteria) include the following genera: *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Flavobacterium*, *Hypomicrobium*, *Moraxella*, *Neisseria*, *Paracoccus*, *Propionibacterium*, *Pseudomonas*, *Rhizobium*, *Rhodopseudomonas*, *Spirillum*, and *Vibrio* (Payne 1981, cited in Eddy (2003)), *Halobacterium* and *Methanomonas* (Gayle 1989, cited in Eddy (2003)). Most of these bacteria are facultative aerobic organisms (i.e. they can use oxygen as well as nitrate/nitrite) and some can execute fermentation in the absence of nitrate or oxygen. The most common denitrifiers are *Pseudomonas* species which have been shown to use a wide array of organic compounds including hydrogen, methanol, carbohydrates, organic acids, alcohols, benzoates, and other aromatic compounds (Payne 1981, cited in Eddy (2003)). However, denitrification by *Pseudomonas* culture is generally curtailed by oxygen at a concentration of 0.13-0.2 mg/L, thus making it less reliable.

Stoichiometry

Denitrification is the process of reducing NO_3^- to N_2 through the following enzymes: nitrate reductase ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), nitrite reductase ($\text{NO}_2^- \rightarrow \text{NO}$), nitric oxide reductase ($\text{NO} \rightarrow \text{N}_2\text{O}$) and nitrous oxide reductase ($\text{N}_2\text{O} \rightarrow \text{N}_2$).

Alkalinity produced during denitrification is equivalent to 3.57g of alkalinity (as calcium carbonate, CaCO_3) for every g of $\text{NO}_3\text{-N}$ reduced, which is approximately 50% of the required alkalinity for nitrification. Since denitrification uses nitrite/nitrate as the electron donor instead of oxygen, the oxygen equivalent for nitrite is 1.71 g O_2 /g $\text{NO}_2\text{-N}$ and nitrate is 2.86g $\text{O}_2/\text{NO}_3\text{-N}$. The C:N ratio depends on the system's operating conditions, and Eddy (2003) found as Equation 2.2-1 below:

Equation 2.2-1: the relationship between nitrate consumption and bsCOD

$$\text{g bsCOD/g NO}_3\text{-N} = \frac{2.86}{1-1.42Y_n}$$

Where Y_n is biomass yield for heterotrophic bacteria (0.4 g VSS/ g bsCOD)

Shen et al. (2009) study found that pH plays an important role in denitrification in strong nitrate wastewater. A high pH level - 9.5 for example – demonstrates nitrite accumulation while pH 7.5-8.5 means complete denitrification. A low pH of 6.5 or below that may indicate that insignificant denitrification has occurred.

Simultaneous Nitrification-Denitrification

Nitrification and denitrification can actually happen simultaneously if not much O_2 is present and long HRT. Rittman B E (1985) stated that up to 90% of nitrogen removal can be achieved via denitrification with a DO concentration of less than 0.5 mg/L and sufficient SRT, HRT. This process can also reduce the amount of carbon consumption by 40% (Ge et al. 2014). However, the main problem here is its sensitivity to COD, DO and temperature fluctuation (Kartal, Kuenen & Van Loosdrecht 2010). While this type of denitrification is not covered in this thesis, it is nonetheless important to acknowledge it because simultaneous nitrification-denitrification may affect monitored results. Especially when complete nitrification occurs, however, a much lower nitrate concentration is detected at the start of the anoxic zone.

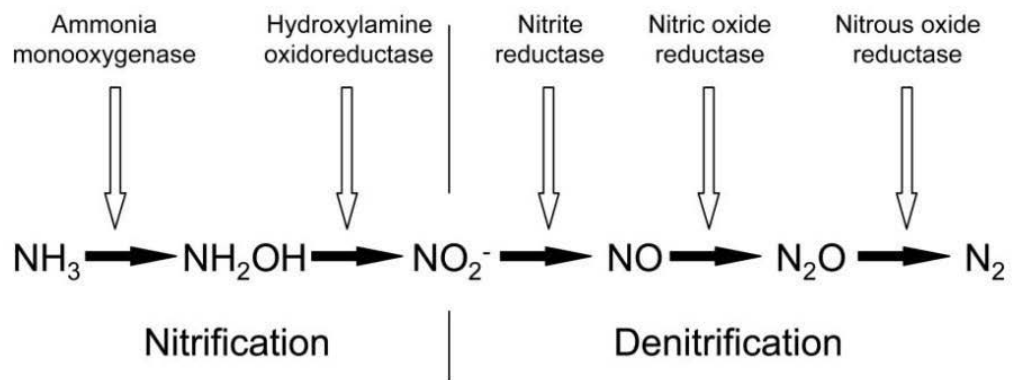


Figure 2.2: Simultaneous Nitrification-Denitrification (adapted from Wrage et al. 2001).

2.3. Denitrification kinetics that corresponds to carbon utilisation

As indicated in the previous chapter, the organic carbon source is one of the most crucial elements in denitrification. In the standard system there are three organic sources available for denitrification; two are available from the substrate (wastewater), the readily biodegradable COD (rbCOD) and slowly biodegradable COD (sbCOD). The rbCOD consists of small simple soluble or dissolved organic matter which can pass directly through the cell wall into the organism, and thence consumed quickly and is very effective for improving denitrification (Li, Shan, et al. 2016; Pan et al. 2015) as it is the best electron donor for denitrifier activities (Tam, Wong & Leung 1992a). sbCOD meanwhile consists of large particulate or colloidal organic compounds, which cannot pass through the organism's cell wall. They have to be hydrolysed by the slime surrounding layer first before being finally consumed (Henze 2008). In the pre-anoxic denitrification system, the sbCOD is not able to be utilised fast enough during its HRT for effective denitrification, while in post-anoxic denitrification, almost all of the sbCOD (and rbCOD) would have already been consumed in previous aerobic states.

The differences in hydrolysis/degradation of these two types of carbon have a direct impact on the COD utilisation rate, and subsequently the denitrification rate itself. According to Henze (2008) the rate could be 7-10 times faster for rbCOD compared to sbCOD. The sbCOD has a much slower hydrolysis rate in the anoxic state compared to aerobic state (only about one-third); this further intensifies denitrification issues regarding COD availability. It should be noted that with the same carbon consumption rate, other factors can still contribute to the denitrification rate. For example Her & Huang (1995) and Akunna, Bizeau & Moletta (1993) found that carbon sources with aromatic rings such as benzoic acid have a better nitrate removal efficiency than those without, for instance glucose, glycerol, acetic, lactic acid and methanol. However, there are limits to compounds with such characteristics, so this

study is still based on the strong correlation/causation between carbon consumption and denitrification rate.

The third organic source is also sbCOD and behaves in a similar way to the sbCOD available in the substrate. However, this sbCOD either comes from the decayed biomass or is stored within the microorganism biomass themselves. When soluble COD is not available in the substrate, the endogenous respiration denitrification through decaying should be fairly consistent, considering the constant decaying rate of the biomass. However, the hydrolysis of stored carbon sources could vary greatly due to both the accumulated amount and its different structure.

The two most commonly stored structures for carbon sources are PHA and PHB. Dobrzynska, Wojnowska-Barylea & Bernat (2004) stated that if a large amount of an organic carbon compound (e.g. acetate) was presented, only 29% of the COD would be oxidised by the microorganisms, 57% would be accumulated in the form of poly (PHB) and 14% for biomass synthesis. A similar study by Beun et al. (2000) claimed that under aerobic and anoxic conditions, 70% of acetate would be used for PHB synthesis, and the rest for biomass yield. According to Dobrzynska, Wojnowska-Barylea & Bernat (2004), under famine conditions, the microorganisms can use the reserved carbon substrate (PHB) as the endogenous carbon and energy source. According to Sun et al. (2000) the increase in C:N ratio from 6 to 20 accelerates the synthesis of organic matter into PHB because the microorganisms that are able to accumulate reserved substances (PHB) grow when there are limiting factors (nitrogen, phosphorus, sulphur, magnesium, oxygen) in the presence of excessive carbon sources.

If carbon was fed into the bioreactor under anaerobic conditions (pre-anoxic denitrification), this allows carbon uptake to be limited to PAOs (phosphate-accumulating organisms) and GAOs (glycogen-accumulating organisms). These

bacteria will use their unusual ability to take up carbon and convert it into intracellular PHA. The use of PHA instead of external carbon sources such as acetate will slow down the carbon oxidation rate, and prolong the time in which carbon is available for denitrification (Meyer et al. 2005). During the anaerobic phase, carbon sources in the form of volatile fatty acids are taken up by PAOs and stored in the form of PHAs. Glycogen-accumulating non-polyphosphate organisms (GAOs) also act in a similar way, via the hydrolysis of glycogen as a source of energy. The difference is that PAOs accumulate phosphate into their cells and are removed through wasting, while GAOs do not contribute to phosphorus removal. In the anoxic and aerobic phases, PAOs grow, accumulate phosphate in the cells, and perform denitrification (Puig et al. 2007). As well, the PAOs generally contribute around 16-28% of the system's denitrification (Swinarski et al. 2012) Makinia et al. 2006, cited in (Swinarski et al. 2012) (Hu, Wentzel & Ekama 2002).

2.4. Denitrification issues regarding internal carbon utilisation

As mentioned previously there are two fundamental denitrification set-ups. The general post-anoxic denitrification set-up is illustrated below.

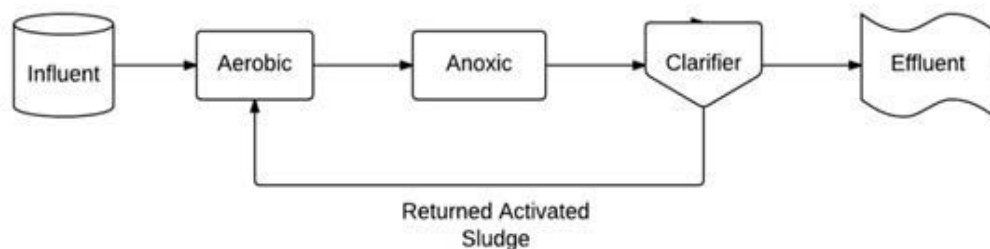


Figure 2.3: Typical post-anoxic denitrification set-up.

Generally, for post-anoxic denitrification, the first reactor is aerobic and then this is followed by an un-aerated anoxic zone. The influent after primary treatment is discharged into the aerobic zone where the heterotrophic and nitrifying organisms' growth happens. Generally, the Hydraulic Retention Time (HRT) will always be long

enough for these heterotrophic and nitrifying organisms to consume the more readily biodegradable components of influent COD.

Denitrification in this case will have to depend on the slowly biodegradable COD (sbCOD) if any is still remaining from the influent, or most of the sbCOD being released by the death of organisms in the anoxic reactor. Due to the fact it can only be slowly biodegraded, consequently the rate of energy released and denitrification rate are expected to be low. Denitrification in that case would have to depend on the slowly biodegradable COD (sbCOD) of either the influent, or from being released by the death of organisms in the anoxic reactor. Since it can only be slowly biodegraded, it only can be slowly consumed by the denitrifier, and consequently the rate of energy released and denitrification rate are also expected to be low. This explains why the availability of readily biodegradable organic carbon is one of the key elements for complete denitrification despite the fact it is often the limiting substrate in this process (Makinia et al. 2011; Meyer et al. 2005; Puig et al. 2007).

According to Henze (2008), this does not mean that it is impossible for an effective denitrification to occur. If the fraction of the anoxic mass is large enough, caused by older sludge or high SRT, a “meaningful reduction of nitrate” can be achieved (Henze 2008; Wang et al. 2015). However, this was considered to be impractical because the anoxic mass or sludge is considered to be a refractory organic, difficult to absorb unlike external carbon sources, and may require pre-treatment for effective denitrification (Doğan & Sanin 2009). Apart from this, a much higher fraction of anoxic mass will seriously affect the system’s ability for nitrification in lower temperature ($< 15^{\circ}\text{C}$) (Henze 2008). Even if nitrification performance will not be affected, there is still another problem with this approach. sbCOD is not the only substance being released from the death of organisms; ammonia and other organic matter are also produced (Zhang et al. 2016). It means that to have a significant enough amount of sbCOD for effective post-denitrification, large amount

of ammonia will have to be introduced back to the liquid state, affecting the effluent performance. Even if an additional aeration zone is located after the anoxic zone to nitrify this ammonia, it will simply increase the nitrate in the effluent instead. Hence post-denitrification with high SRT is not currently widely used in practice unless additional chemical or carbon dosing is used.

The more popular approach to deal with the denitrification problem is pre-anoxic denitrification, with the set-up shown below.

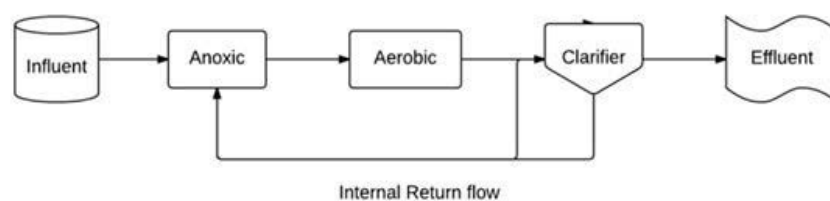


Figure 2.4: Typical pre-anoxic denitrification set-up.

More commonly known as an MLE (Modified Ludzack-Ettinger) approach, credited to its inventors Ludzack and Ettinger in 1962, the MLE provides an alternative approach to the more traditional post-anoxic denitrification method. In this system, the effluent was discharged firstly into an un-aerated anoxic reactor first, before being followed up by an aerobic zone (where the growth of heterotrophic and nitrifying organisms occurs). The nitrified mixed liquor will then be pumped back into the anoxic zone where denitrification takes place. The biggest advantage of this approach is the rbCOD and sbCOD in the effluent will significantly increase denitrification performance in the anoxic zone, especially when compared to its post-denitrification counterpart.

The biggest disadvantage of this approach is the amount of nitrate concentration produced by the aerobic zone, will exit the system in the form of effluent. This means the complete removal of nitrate is not possible, and the system's performance will heavily depend on the Internal Recycle Rate (IR of the system). This

high concentration of nitrate due to incomplete denitrification could generate several secondary outcomes. Firstly, the denitrification of nitrate into nitrogen gas inside the clarifier may lead to the formation of some small air pockets, and cause the floatation of sludge in the clarifier. This in turn leads to the drop in MLSS, rise in effluent suspended solid (SS) concentration, but more importantly reduce the SRT and sludge age of the system (which will in turn affect both nitrification and denitrification performance). A similar finding was documented by Chen et al. (2010), but they argued that the high concentration would cause the proliferation of foaming filamentous bacteria instead, which also leads to sludge floatation. The Sludge Volume Index (SVI) in their study as shown below revealed a correlation to the system's nitrate and ammonia concentration. It also reported incomplete denitrification-nitrification as “the most important factor” causing the sludge to rise.

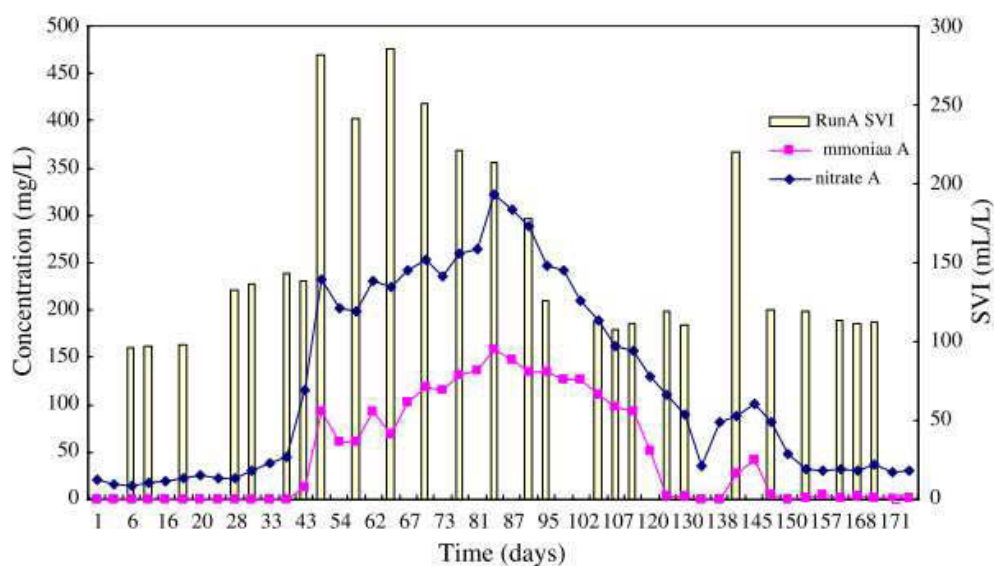


Figure 2.5: Effect of incomplete denitrification on a system SVI (adapted from Chen et al. 2010).

To combat this, a high underflow ratio of recycled activated sludge (RAS) from the clarifier (1:1) to the mean of influent flow was used. This aimed to reduce the

amount of sludge accumulated in the clarifier (Henze 2008), but also means a specially designed clarifier will be needed as the settling of solids in the clarifier will be affected by two equal flows of RAS and discharge.

Denitrification potential of influent rbCOD can also vary greatly, making it difficult to design the required Hydraulic Retention Time for pre-anoxic denitrification accordingly. Swinarski, Makinia, Czerwionka & Chrzanowska (2009), for example, cited various sources where the SDNR (20°C) of domestic wastewater influent rbCOD varied from as low as 1.0 to as high as 6.3. According to Burdick et al.'s (1982) equation cited in Metcalf&Eddy (2003), this is because the SDNR in this case was affected not only by the temperature, but also by the F/M ratio. In turn this was influenced by various other factors. The “food” or amount of organic carbon available in the anoxic tank could be affected by: a) the nature of the wastewater influent and depending on local activities and discharge policy in catchment area; b) variations in time of the day, week, and season; c) dependence on the type of primary treatment, the sludge's age, and treatment plant's internal recycling rate. Those factors also can affect the microorganism ratio but on a different magnitude.

For these reasons, if effective denitrification cannot be achieved, or can be reached but would cause other problems in the system, then using external carbon sources could be a viable alternative. Not only using external carbon sources is a flexible solution in the short-, medium- and even long-term scenarios, it also has fast response, requires less capital investment, especially when compared to redesigning or modifying a treatment plant.

2.5. Studies on external carbon sources in the literature

According to Mokhayeri et al. (2008) the three most common available external carbon sources are methanol, ethanol, acetate. Of these, methanol is the most commonly used, well documented and recommended by the US EPA as the

most appropriate choice for the supplemental substrate. This is because of its availability, low cost, small sludge production, low volatile organic compound, low emissions potential, and no additional nitrogen and phosphorus being introduced into the system (Swinarski, Makinia, Czerwionka & Chrzanowska 2009). However for large-scale wastewater treatment, the cost of methanol may contribute significantly to operating and infrastructure expenses. Oleszkiewicz (2004)) found that to reduce 2-4mg/L at the Crajka WWTP in Warsaw (580,000m³/d) to meet the new government regulation, it will cost equivalent to 5500-8200 AUD per day. Research on the use of variable industrial waste products such as external carbon sources has been going on for the past two decades (Sage, Daufin & Gésan-Guizieu 2006), where potential industrial waste products/ by-products that can be used as external carbon sources are highly recommended especially if one WWTP can has the access (Henze et al. 1995, cited in Swinarski, Makinia, Czerwionka & Chrzanowska (2009). This makes finding an alternative nutrient removal substance a high priority of the wastewater treatment industry (Oleszkiewicz 2004) and was even ranked the highest of all research area priorities during the Nutrient Challenge Workshop organised by the Water Environment Research Foundation (March, 2007).

Other than accessibility and cost issues, the only other concern with adding external carbon substrates is the effect on Phosphorus Accumulating Organisms (PAOs) and Phosphate Uptake Rates (PURs). Studies have shown both a decrease and increase of PURs in the presence of additional substrate during the anoxic period (Czerwionka et al. 2012; Winkler, Coats & Brinkman 2011). Artan et al. (1998) further stated that unlike what has been generally assumed, phosphate was not taken during anoxic process, and the use external carbon sources may cause the release of phosphate. This only happens, however, for some specific types of carbon. For example, phosphate release occurred in the presence of nitrate and acetate, but not for glucose.

Improving denitrification to meet the required standard aside, the removal of accumulated nitrate within the system can also make the system relatively more stable. For example Chen et al. (2010) found the adding external carbon could eliminate the filamentous bacteria, increase the MLSS by 750mg/L, while reduce the effluent SS to 30. This will provide a much more consistent SRT, which is the key to wastewater treatment plant operational stability.

2.5.1. List of external carbon studies in the literature

Many studies have been published on the denitrification of various external carbons sources, and some of the more common substrates are described below.

a) Methanol

As noted in the previous section, methanol is the most commonly used electron donor for the denitrification process in the US. This was due the high availability, generally lower cost and smaller methanol-to-nitrate ratio requirement (Cherchi et al. 2009). However, at the same time, using methanol as an external carbon source also has its disadvantages. Firstly, the current global production of methanol is not renewable. According to Institution for the Analysis of Global Science, most of the methanol produced in the US is from natural gas, and the future potential for methanol is still based on natural gas and coal. This along with its high demand for other essential uses - such as formaldehyde manufacture (for plywood, paints, explosives and permanent-press textiles), MTBE (gasoline additives), DME (fuel blends or alternative fuels), and direct combustion - leads to a non-feasible supply of methanol in the long-term. Increasing demand for methanol has also seen its cost rise, as illustrated in Figure 2.6 below.

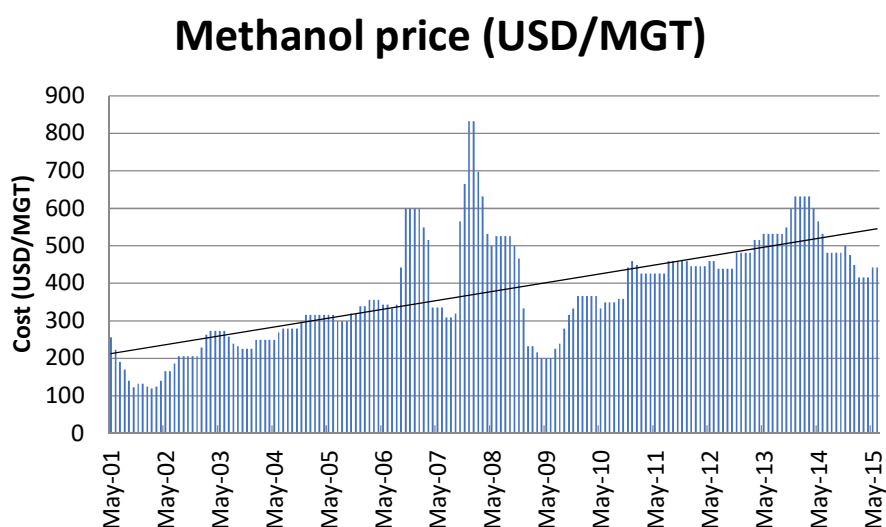


Figure 2.6: Global methanol price from 2001 to 2015, according to Methanex.

As seen above, despite some drawbacks and especially the global financial crisis that erupted in 2007-2008 and fall in US gasoline and crude oil prices in 2015, the general trend has shown rising global methanol prices across the world. This makes the use of methanol only less viable in the long-term. Along with high operating costs, there is also the high capital expense due to safety issues regarding methanol's high flammability. This often leads to a required investment of 25-31% in capital cost just to meet the safety standard for handling, transporting and storage of methanol on-site (Cherchi et al. 2009).

Furthermore, only a specific group of methanol-using denitrifying bacteria can consume methanol effectively, meaning it is necessary to grow and populate the system with this type of denitrifier before reliable nitrate removal can be met. Without doing so, the nitrate's removal of methanol could be less than most other carbon types such as glucose, propionic acid and acetic acid (Liu et al. 2016). At the same time, despite methanol-acclimated biomass can use well some other external carbon sources (such as ethanol and acetate, because they can enter the tricarboxylic

cycle), they cannot utilise other carbon sources well (Cherchi et al. 2009). This problem makes it difficult for the system to adapt if methanol is replaced by another external carbon. In addition, during winter or low temperature, it is also possible for the methanol-using denitrifying bacteria to be washed out of the system, seriously hindering the system's denitrification performance.

Due to its being commonly studied in the past, most of the relevant coefficient and rate related to methanol was found and listed by Dold et al. (2008) based on various sources. The half-saturation coefficient (K_s) was found to be 0.06 -1.6 mg $\text{NO}_3\text{-N}$ (Gaudy and Gaudy, 1980) or 9.1-12.6 g/ m_3 at 20 and 10°C, respectively (Stensel et al. 1973). The cell yield or observed yield (Y) was discovered to be 0.6-1.2 mg/mg $\text{NO}_3\text{-N}$ (US EPA, 1975) or 0.17-0.18 gVSS/gCOD (Stensel et al. 1973). The decay coefficient is constant at 0.04-0.05/day much like that of domestic wastewater. For every 2.6g of methanol consumed, 5.6g of cells were produced for each g of $\text{NO}_3\text{-N}$ reduction (Claus and Kutzner 1985). Methanol could reach a maximum denitrification rate of 320 mg $\text{NO}_3\text{-N}$ /g MLVSS at 25°C.

a) Acetate

There have been many studies on the effect of acetate as an additional carbon source on both denitrification and phosphorus removal. Zhu, Zhang & Miller (2006) tested the effects of additional (external) acetate carbon on an SBR using a raw slurry feed. The reactor with additional acetate increased the TKN and phosphorus removal rate from 96.3% and 96.1% to 98.7% and 98.7%, respectively. Meanwhile Obaja et al. (2003) reported that even for highly concentrated wastewater (COD: 767-1325 mg/L, $\text{NH}_4\text{-N}$: 303-550 mg/L), additional of external carbon sources (acetate of 1275-2125 mg/L) can still increase the phosphate and ammonia removal rate from 94.8% and 96.9% to 97.5% and 98.7%, respectively. The reason for this according to Puig et al. (2007) is that acetate, propionate or fermented sewage provided the advantage for

PAOs growth over GAOs, and can subsequently enhance both phosphorus removal and denitrification of the system.

Similar to methanol, however, acetate-acclimated biomass could use only acetate efficiently, while lower, marginal denitrification rates were obtained with other carbons (Cherchi et al. 2009). Cherchi et al. (2009) stated that the uses of acetate revealed a significant accumulation of nitrite at both 20 °C and 10 °C, with the later indicating the conversion of nitrate to nitrite within 21 hours, yet taking another 1.7 days for nitrite reduction. The incomplete denitrification using acetate was also reported previously by Vanrijin et al. (1996).

b) Biodiesel - Glycerine

Bodík et al. (2009) investigated the potential use of biodiesel waste (glycerine fraction – g- phase) as an organic carbon source for denitrification improvement in both lab-scale and actual WWTP (Vrutky 35,000 PE). g-Phase is a by-product of methylates production by catalysing transesterification natural triacylglycerines with methanol to produce diesel, and contains 56-60% glycerol, 14-16% alkali in form of alkali soup, 18-20% methylate, 10-12% methanol, 2-3% water and other compounds. This compound has a very high COD of approximately 1600g /L, TN of roughly 2 g/L, TP of 0.7 g/L, salinity =21g/L and pH of 10.5. The g-phase substance may need to be mixed well, otherwise “oily eyes” will occur on the wastewater’s surface.

The tested results on an actual WWTP reported a drop in average nitrate from 13.7mg/L to 6-10mg/L (result is influenced by huge amount of rain), or basically a 2-5mg/L increase of nitrate removal per g-phase dose of 100L as external carbon sources.

c) Ethanol

Constantin & Fick (1997) found that ethanol not only needs lengthy sludge acclimation, but its SDNR is also lower than that of acetic acid. The effects of

ethanol on the activated sludge system's nitrogen removal was also studied by Puig et al. (2007). They discovered that the SBR run using ethanol achieved up to 99% P removal, and 97% TN removal (down to below 3.0 mg N/L). The sludge which was used for the test, however, was ethanol-acclimated sludge so non-acclimated sludge may produce worse results.

d) Hydrogen Sulphide (H₂S)

Pérez et al. (2007) made an interesting selection, using sulphides to enhance autotrophic denitrification for reject water during aerobic zone (<1.0 mg DO/L). The reject water had a high COD of 1500-2000 mg/L and NH₄⁺ -N (700-800mg/L). Their study found that both heterotrophic denitrification using methanol and autotrophic denitrification using sulphides achieved >95% nitrogen removal, while having AUR of 22±1 and 35±1, and NUR (nitrite) of 47±2, and 32±2 mg /g VSS/h, respectively. The excess amount of HS⁻, however, could cause aerobic oxidation by the autotrophic sulphide bacteria in the following aerobic period. This in turn can lead to reduced nitrification capacity due to the competition for oxygen between the nitrifier and sulphide-oxidant bacteria.

e) Others

Many different carbon-rich materials that could provide effective denitrification have been studied such as dairy wastewater (Sage, Daufin & Gésan-Guiziou 2006), corn starch (Cappai 2004), reject water (Lee & Welander 1996), syrup from distillery waste products (Czerwionka et al. 2012; Swinarski et al. 2012), polylactic acid and polycaprolactone (Xu et al. 2011), or hydrolysed molasses (Quan et al. 2005). Cherchi et al. (2009) tested a specialised substance specific for denitrification enhancement called Micro™ and found that Micro™ performed similarly with methanol, but did not need bacterial acclimatisation. Micro™ also provides a higher growth rate and functions slightly better with a similar level of COD dosage.

2.5.2. Comparison of carbon sources in the literature

It is generally fairly difficult to judge the effect of an external carbon source by itself. One of the key reasons for this is observed in Table 2.1 where the SDNR of the same or similar types of carbon sources could vary greatly depending on both the used experimental methodology and the sludge types. Hence much of the literature has tested the effects of an external carbon and tried to compare it to a more commonly studied one. Mostly it has been methanol or the wastewater substrate itself.

Table 2.1: SDNR comparison at 20°C

	Cherchi et al. (2009)	Czerwionka et al. (2012)	Dold et al. (2008)	Makinia et al. (2011)	Mokhayeri et al. (2008)_	Peng, Ma & Wang (2007)	Quan et al. (2005)	(Swinarski, Makinia, Czerwionka & Chrzanowska (2009); Swinarski, Makinia, Czerwionka, Chrzanowska, et al. (2009))	Xu et al. (2011)
Acetate	13.6 ± 1.9	-	~3.0	-	31.7	12	-	-	-
Acetate (non-acclimated biomass)	-	-	-	-	4.3 - 18.9	-	-	-	-
Ethanol	-	-	~9.0	-	30.4	9.6	-	-	-
Ethanol (non-acclimated biomass)	-	-	-	1.3 - 1.8	4.7 - 7.2	-	-	-	-
Fused oils	-	2.4 – 2.7	-	1.4 - 1.7	-	-	-	-	-
Methanol	6.1 ± 0.7	-	~10.0	-	9.2	3.2	-	4.2 ± 0.2	-
Methanol (non-acclimated biomass)	-	-	-	-	0.8 - 3.2	-	-	2.7 ± 1.1	-
Micro C TM	6.4 ± 3.6	-	-	-	-	-	-	-	-
Polycaprolactone	-	-	-	-	-	-	-	-	6.34
Polylactic Acid	-	-	-	-	-	-	-	-	6.63
Wastewater	-	4.2 (Distillery)	-	4.8 - 5.6 (Municipal)	-	0.74 (Starch) 2.11 (Starch + waste ethanol)	3.4 - 8.0 (Diary)	3.1 ± 0.9 (Municipal) 2.0 ± 0.9 (Brewery) 4.5 ± 0.2 (Distillery) 3.5 ± 1.1 (Fish-pickling)	-

Unit: mgNO₃-N/ g VSS/h

Apart from SDNR, it is difficult to construct another table that compares all other findings for each tested carbon source due to the differences in definition, methodology and units being utilised. For example, observed yield was regarded by Cherchi et al. (2009) as mgVSS/mgCOD while under US EPA cited by Dold et al. (2008) it is mgVSS/mgNO₃. They cannot be converted to each other based only on published data in respective literature.

Other than the listed SDNR, the section below will list other parameters and key variables that were used to assess and compare different carbon sources in various studies independently. Cherchi et al. (2009) for instance compared the maximum denitrification rate and bacterial observed yield rates of reactors using three different external carbon sources, i.e. methanol, acetate and Micro C[™]. The results are summarised below.

Table 2.2: The denitrification kinetics of MicroC[™], acetate and methanol (adapted from Cherchi et al., 2009).

	Micro C [™]	Acetate	Methanol
observed yield (mgVSS/mg COD)	0.39	0.35	0.29
K _d max (10°C) (mgN/gVss.h)	2.5	3.6	2.3
K _s (mg sCOD/mgN)	38.6±29.2	15.6±11.2	38.1±16.2
Carbon-use-to-nitrate ratio (gCOD/gN)	6.5±3.7	5.7±1.3	4.8±1.5
μ _{max} (20 °C) (day ⁻¹)	3.7		1.3
μ _{max} (10 °C) (day ⁻¹)	1.2		0.3

As seen here the MicroC[™] has a much higher growth rate than methanol, which is partly due to the high observed yield/ and carbon-use-to-nitrate ratio despite having almost the same SDNR. Acetate meanwhile behaves in a very

similar way to MicroC™ except the higher SDNR at 10 °C and the half-saturation Ks. Cherchi et al. (2009) also stated that while methanol utiliser grows only in anoxic zones, the MicroC™ can grow in both reactors. They also found that a reduction from 20 °C to 10 °C in temperature will reduce 60% and 62% of denitrification rates and 67% and 73% in microbial growth rates when employing external carbon sources MicroC™ and methanol, respectively. A more detailed acclimation table is included below.

Table 2.3: The short-term response of each specific carbon acclimated biomass when using other carbon sources (adapted from Cherchi et al.,2009).

	MicroC™ acclimated sludge	Acetate acclimated sludge	Methanol acclimated sludge
Micro C™	+	+	-
Methanol	+	+	-
Acetate	+	+	+
Ethanol	+	+	-
Glucose	+	-	-

Note: + = positive, able to use, - =negative, unable to use.

Dold et al. (2008) conducted an extensive study, mostly on the effects of temperature on different carbon sources. While SDNR increased as the temperature also increased, there was no apparent connection in the samples they tested. The summarised resulted are depicted in Figure 2.7 below.

Specific Denitrification Rates of various carbons

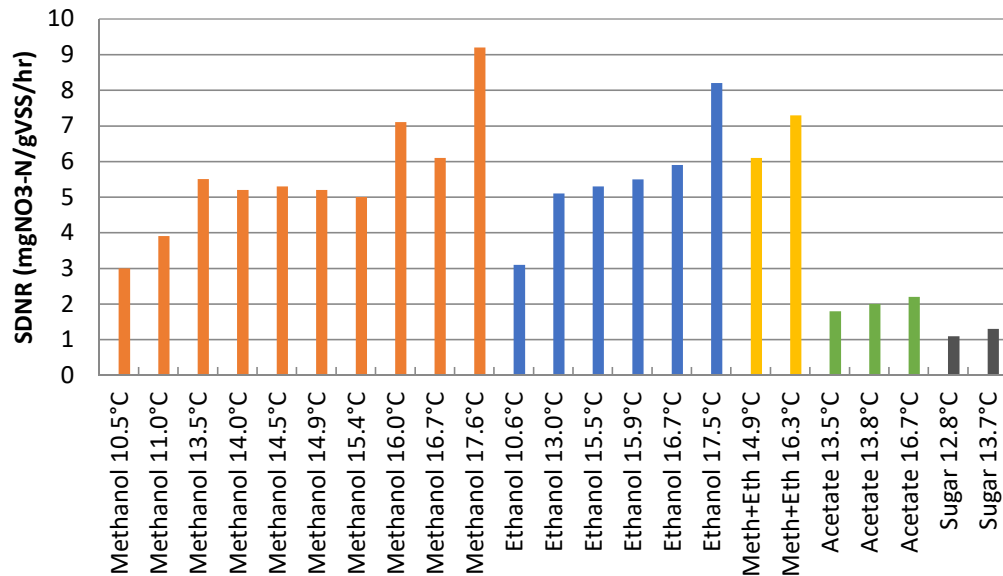


Figure 2.7: SDNR of methanol, ethanol, acetate and sugar at different temperatures (adapted from Dold et al.,2008).

Mokhayeri et al. (2008) evaluated the performance of acetate versus ethanol and found that the former resulted in higher denitrification at 30°C, while ethanol achieved a higher denitrification rate than methanol at 9-16 °C. The lower SDNR of methanol compared to acetate was most likely due to methanol being a single carbon compound. Consequently, it had to undergo a reduction process to form higher carbon intermediates before entering the tricarboxylic cycle (TCA). Acetate, however, can enter it directly.

Peng, Ma & Wang (2007) analysed ethanol, acetate and methanol using NUR batch test, reporting denitrification rates of 9.6, 12 and 3.2 mgN/gVSS/h, respectively, at 20°C and TN of 10 mg/L can be met with all three. The sludge yield for methanol and ethanol was 0.4 gMLSS/g COD. Their study also looked at the denitrification rates and the COD/NO₃ ratios at different temperatures (Figure 2.8).

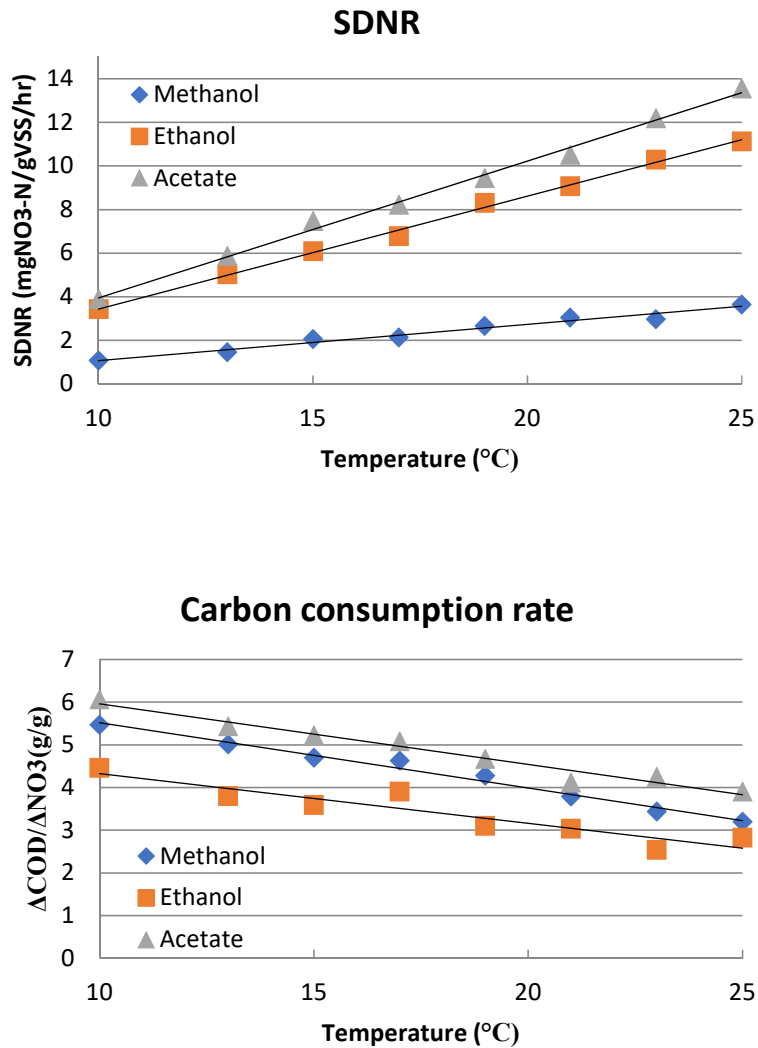


Figure 2.8: SDNR and carbon consumption rate of methanol, ethanol and acetate at different temperatures (adapted from Peng et al., (2007))

Shen et al. (2009) investigated the effect of three external carbon sources - methanol, sodium acetate and sugar - on nitrate and nitrite removal in explosives industry wastewater. Their analysis found that optimum C:N for this wastewater was 1.56 or within the 1.46 – 2.00 range with pH varying from 7.5 to 8.5 and an HRT >22 days. Instead of SDNR, they assessed the performance of each carbon source based on the remaining nitrate concentration. The results are summarised below.

Denitrification of the explosives industry wastewater using different external carbon sources

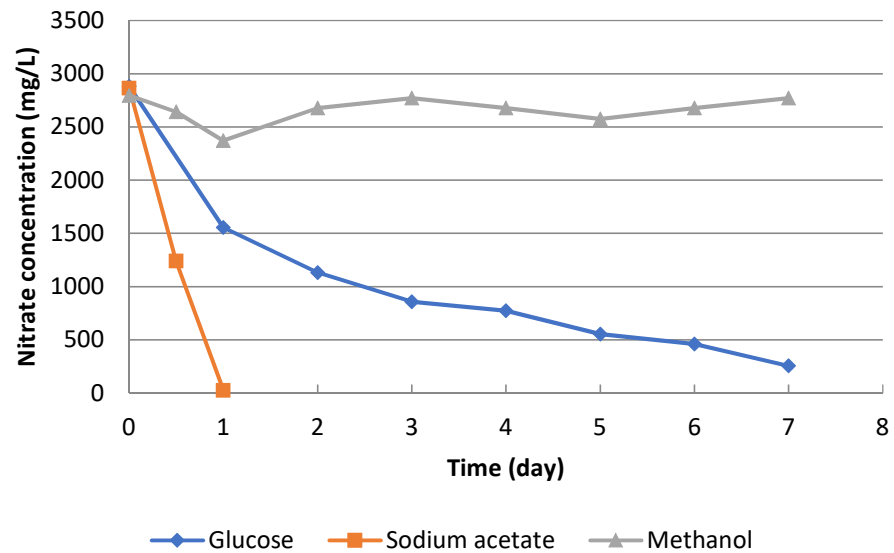


Figure 2.9: Effluent nitrate concentration of explosion wastewater using glucose, sodium acetate, methanol as external carbon sources (adapted from Shen et al., 2009).

The effluent nitrate of methanol in this case was fairly consistent with the long acclimation time of methanol, and confirmed there was no change in effluent nitrate concentration even after a week since it was added.

2.6. Summary

Chapter 2 has provided the basic introduction to denitrification in the activated sludge system, and addressed the many important denitrification kinetics and why carbon utilisation is an important issue when it comes to effective denitrification. With that as its rationale, this chapter explained why adding external carbon is a proven method that improves denitrification, and why searching for alternative carbon sources that are suitable for denitrification improvement and abundant at the local level is necessary.

Another important key point that could be concluded from this chapter is: despite specific denitrification rate (SDNR) having been a crucial part in external carbon studies, it tends to vary for the same substances from study to study. For example, the SDNR (20°C) of wastewater could range from 1.0-5.0 (Henze et al. 1995), 5.7 ± 1.3 at 18.8-21.9°C (Swinarski, Makinia, Czerwionka, Chrzanowska, et al. 2009), or 3.3-5.7 and 1.6-3.6 (Naidoo et al. 1997, cited in Swinarski, Makinia, Czerwionka & Chrzanowska (2009)). Similar variations in denitrification rate were also found with other common external carbon sources such as methanol, ethanol and acetate. This was despite the fact each carbon source should have the same uniform SDNR in controlled conditions. It is evident that SDNR alone is not enough to assess the potential of any carbon source. This is why a proper research methodology is needed to study the effect of external carbon sources and is subsequently addressed in Chapter 3.



Chapter 3

Research Methodology

Chapter 3 EXPERIMENTAL METHODOLOGY

3.1. Definition of model, simulation and system used in this thesis

The definition of terms employed in this thesis is generally based on the GPS-X 6.1 Technical Reference, where a system, experiment, model, simulations can be defined in more detail as shown below.

System

A system in this thesis is defined as an organised and interconnected structure of a set of independent variables (inputs) and a set of dependent variables (outputs). Consequently, the inputs and outputs can be defined and observed, and data can be extracted based on the observations of the system's behaviour.

Experiment

Experimentation in this thesis is defined as the process of extracting data from an experiment through: a) the manipulating of inputs; and b) observing the output experiment. The advantages of an experiment compared to a 'real system' are as follows. Firstly, the outputs of an experiment are influenced by a more limited number of inputs, hence there will be less disturbances and more data or conclusions can be extracted from an experiment (compared to a system). Secondly, due to how an experiment is actually set up for experimentation purposes, some specific outputs can only be accessible for measurement or observation in experiment in opposing to in system (for example denitrification rate)

Model

A model in this thesis is identified as an approximation or an abstraction of a system. Rather than simply indicating a computer program, the model referred to in this thesis could also indicate a physical piece of hardware or an understanding of a system. A similar definition was also used by (Henze 2008). The goal of a model here is to organise and structure knowledge accumulated either through experiments or a literature review. This can provide a better understanding on the cause and effect relationships of input-output, and eventually the system itself.

Simulation

Simulation in this thesis is identified as an experiment performed on a model, and is used for analysis, design, verification or assessing a system's performance. The simulation works defined in this thesis include:

- a. The experiment on a real physical system to extract data,
- b. Establishing a hypothesis or hypotheses,
- c. Simplifying the problem so it can be managed within the scope of the thesis, then verifying findings using various experimental parameters
- d. Analysing the results, verifying the hypotheses and drawing conclusions.

3.2. Experimental Methodology Outline

The main aim of this experimental methodology is to assess the potential of denitrification concerning two selected carbon sources (industrial-grade sucrose and fermented sludge), through a series of experiments, simulations and these being verified with lab-scale and pilot-scale systems. Originally the methodology used is the same one used for general computer simulation, as illustrated below.

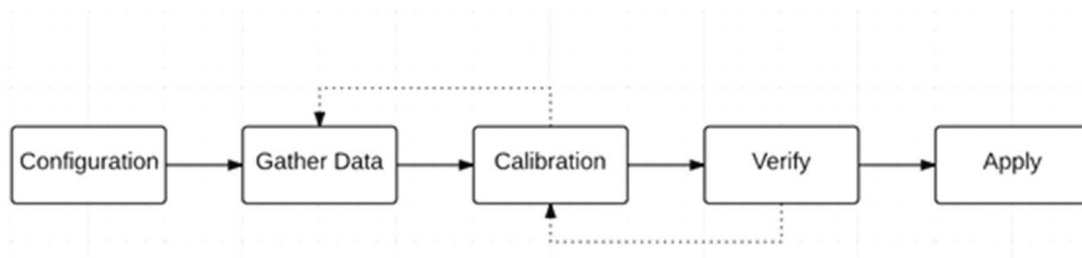


Figure 3.1: Standard mathematical modelling or computer simulation methodology

In the context of this thesis the points in the figure can be interpreted as:

1. CONFIGURATION is the physical configuration of the experiment (size, length, depth, etc.).
2. GATHER DATA includes physical plant data, operational inputs, influent wastewater characterisation, microbiological stoichiometric, kinetics and any associated parameters (physical-chemical process or processes).
3. CALIBRATION: Predicted and observed data generally are varied due to uncertainty in input data. Adjusting or simplifying the unavailable/inaccessible data may be needed, and these adjusted parameters are determined based on experimental/simulation results or based on the literature review.
4. VERIFICATION: test calibrated data with actual performance.

Due to the limited scope, the outlined methodology initially could only be applied in the thesis structure flowchart as shown below. These three steps are the GATHER DATA, CALIBRATION and VERIFICATION phases. The two stages of CONFIGURATION and APPLY are omitted since the physical alteration of the pilot-scale and industrial implementation are beyond the scope of this PhD thesis.

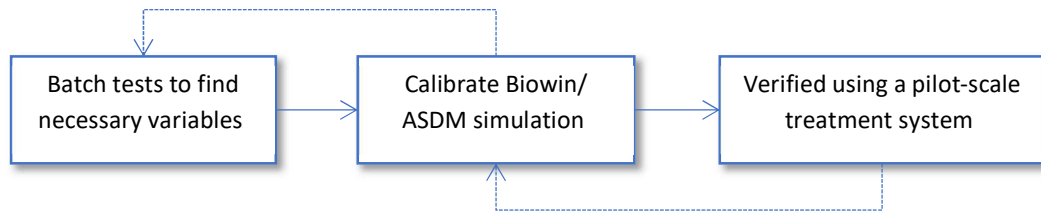


Figure 3.2: Initial thesis structure flowchart.

However, the fundamental issue with this approach is the calibration of the model (as part of the simulation process). Firstly, there is no specific function in the available simulation model (Biowin or ASM) to dedicate to simulating the effects of external carbon sources, apart from methanol. This means that if after the influent characteristics (1), the operating data (2), and some of the stoichiometry value (3) were modified and the simulated results still differed from the results derived from the pilot-scale system, then the only thing that can be done is to dedicate the whole thesis to reprogramming the simulated software to better suit the studied carbon substances. This means adding more inputs, rewrite the equation, simplify, or reduce disturbances in the software model. After consulting with an expert on Biowin software, it is believed that such task will be beyond the scope of this thesis

Even if this was possible to do, it would still be impractical and not viable for future application. Because the reprogramming process will have to be repeated whenever there is a need to study the effect of any new external carbon source. This is especially difficult because simulation software like Biowin or ASM models is perceived as a black-box model by most users (i.e. treatment plant engineers/operators) making it difficult for them to approach the programming task from scratch. It does not stop there, however, as it was highly recommended to use industrial by-products from local industries as alternative external carbon sources. These wastes or by-products almost always have unique characteristics or properties

corresponding to their region. To sum up, this approach to studying potential alternative carbon sources is seriously impractical for the wastewater treatment industry.

Aside from the difficulty related to available software, even the use of an available pilot-scale system has its own limitations when it comes to verifying the effects of denitrification from external carbon sources. This was due to the pilot system characteristics resulting in: a) unremovable/ undetected disturbances that may significantly alter the simulation results (e.g. variance in temperature and influent profiles between days and seasons); b) the restrictions on the number of inputs data that can be manipulated (e.g. physical restrictions such as size of the tanks) ; and c) inaccessible and un-extractable data due to its black-box nature.

Proposed alternative methodology

Due to the reasons above, rather than a single top-down Configuration-Calibration-Verification approach, the experimental methodology used in this thesis will be based on cross-verified results of the four mathematical and physical simulation models:

- The Lab-scale Sequencing Batch Reactors (SBRs) system
- The Pilot-scale wastewater treatment plant system
- The Nitrogen Mass-Balance Conceptual Model
- The Biowin software model

The advantage of this approach is that by cross-verifying various types of simulation models, it is easier to: a) identify whenever the simulated results of one model is significantly affected by disturbances; b) inputs of some models (like those of the SBRs or NMB model) are much easier to manipulate compared to the pilot-plants and hence can assist each other; and c) other than the pilot plant, the other

three are grey-box in nature. This means more data can be extracted and are able to provide a better understanding of the studied carbon sources.

It is also possible for this study to compare the advantages and disadvantages of each simulation model. Then the suitability of each individual simulation model can be assessed and a more practical, viable and economical approach to simulate the performance of a carbon sources can then be established.

A diagram showing the experimental methodology using in this thesis is depicted in Figure 3.3 below.

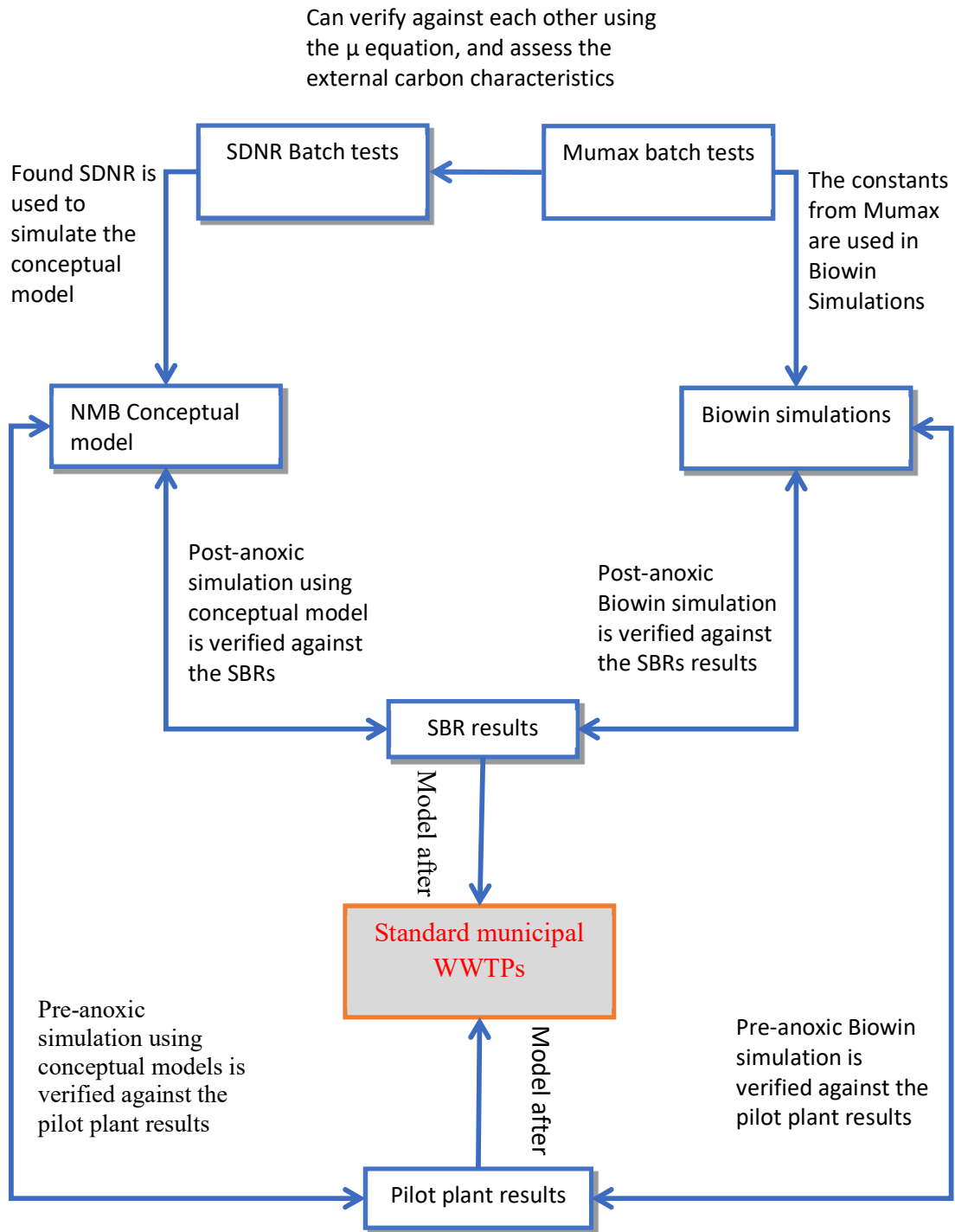


Figure 3.3 Relationships between different simulation models used in this thesis.

The different arrow directions indicate what a simulation method is used for, or to verify another simulation method (one-direction arrow) or whether the two simulation methods can be cross-verified (double direction arrow). Individually the six methodologies have the following purposes:

1. The SDNR batch test: finds the specific denitrification rate (SDNR) of different carbon sources and carbon consumption rates.
2. Nitrogen Mass Balance conceptual model: based on the found SDNR from above, it simulates performance of a system before and after adding an external carbon source (if no accumulation happens between each cycle).
3. Mumax batch test: finds the growth rate and yield rate of the denitrifier.
4. Biowin simulation: using either founded yield rate and growth rate from batch test, or default from the literature to simulate performance of a system before and after adding an external carbon source.
5. Sequencing Batch Reactors (SBRs): simulate the performance of post-anoxic denitrification in lab-scale scenarios.
6. Pilot plant: simulate the performance of pre-anoxic denitrification in pilot-scale scenarios.

3.3. Classifications of methodologies

As stated previously, each of the used methods/ simulation models is unique in principle and can be classified based on several relevant classification features (Henze 2008):

- a) **Model types: divided into *physical*, *conceptual* and *mathematical* model:**
physical model as its name suggests, is a physical scale representation of a system. Unlike the physical model, the mathematical model is one where “the rates of process acting in the system and their stoichiometric interaction with the

compounds are formulated mathematically” (Henze 2008). The mathematical model seldom operates alone, and often is developed along with the conceptual model which meanwhile can be presented as schematic diagrams with a qualitative description, for example a flow diagram of a treatment system.

- b) **Aspects of time and scales: often classified into *dynamic, steady state or frozen model*.** The dynamic model is when the state of the model changes with time. The frozen model is one where an isolated timeframe of the dynamic model (a day, a week) represents its dynamic model. The steady state model is used when most of the variables in the model are kept at constant.
- c) **Level detail of microbial models: varying between *black-, grey- or glass-box model*.** Black-box models for example focus on influent and effluent characteristics and little is known about what happens inside. The pilot plant is a typical black-box model. A lab-scale system like SBRs or batch tests is designed with time-orientation rather than space-orientation in mind and can provide insights on the process that are grey-box in character. The mathematical model or simulation such as ASM1,2,3 Biowin would be the grey- or glass-box model.
- d) **The level of microorganism organisation: individual or surrogate organisms:** a model could simulate a system down to its individual organism level, including the different microorganism species. Or it could be done by grouping certain groups of microorganisms that had a similar function in a wastewater treatment system and identified as a single identity or “surrogate” microorganisms (e.g. denitrifier or nitrifier).

It is crucial to note that while it is possible to build a model with extreme complexity by including micro-biological genetics and genetic changes, it is not recommended to do so. Setting up models foremost depends on the purpose and use of the model. Too many variables and complexities could waste effort and be unpractical (Henze 2008).

As noted in Section 3.1, all of the experiments and methods used to test and verify findings in this thesis will be addressed as “simulation models”. This includes physical models like the pilot plant, laboratory experiments and batch tests in contrast to the more conceptual models like nitrogen balance or simulation software. This also refers to their purpose where it varies between verifying or simulating, completely or partially, a process. All of the methods previously mentioned are classified below in Table 3.3a.

Table 3.1: All of the main methods used in the thesis as classified under modelling classification according to Henze (2008)

Methodology used	Model Types	Time and Scales	Microbial detail	Level of Microorganism organisation	Purpose	Details
SDNR batch tests	Physical Experiment	Steady-state	Grey-box	Down to individual or surrogate level (depending on sludge uses)	Find the denitrification rate of a specific sludge when using a specific carbon source.	- The system is a non-continuous steady-state batch test. - Parameters (such as COD, ammonia and nitrate) can be recorded at any points along the process, hence they are grey-box in nature. - Depends on the sludge used and the need for sludge acclimation so that it can model down to individual or surrogate microorganism levels. - Operational data, Physical plant data, Influent loading data, can be changed. Kinetic and Stoichiometric model parameters depends on the sludge and carbon sources used and can't be changed.
Nitrogen Mass Balance (NMB)conceptual model	Mathematical + Conceptual Model	Steady-state	Glass-box	Down to surrogate level	Model a pre-anoxic or post-anoxic denitrification system using the SDNR batch test results.	- The system is steady-state. - It is mathematically calculated as glass-box, but also based on the schematic diagram of a system; hence it is also conceptual. - The sludge only model down to surrogate microorganism level. - Operational data, Physical plant data, Influent loading data, can be changed. Kinetic and Stoichiometric model parameters are simplified/omitted.

Mumax batch tests/	Physical Experiments + Mathematical Model	Steady-state	Grey-box	Down to individual or surrogate level (depending on sludge uses)	Find denitrifier growth rate and yield rate of sludge using specific carbon sources.	- The system is steady-state and non-continuous. - Both the physical experiment and mathematical equation are based on the key equation below, so it is glass-box: $S_{NO,t} = S_{NO,0} - \eta \cdot \frac{(1 - Y_H)}{2.86} \cdot \frac{S_S}{K_S + S_S} \cdot \frac{\mu_H \cdot X_{BH}}{Y_H \cdot (\mu_H - b_H)} \cdot [e^{(\mu_H - b_H)t} - 1]$ - Depends on the sludge used and the need for sludge acclimation so that it can model down to individual or surrogate microorganism levels. - Operational data, Physical plant data, Influent loading data, can be changed. Kinetic and Stoichiometric model parameters depend on the sludge and carbon sources used and cannot be changed.
Biowin model	Mathematical + Conceptual Model	Frozen state	Grey-box	Down to surrogate level	Model a pre-anoxic or post-anoxic denitrification system based on Mumax batch test results.	- The Biowin could model a system in steady state, or frozen-state. - It's mathematical software so it's glass-box. - The Biowin is set up to model a treatment plant using surrogate level, this can be changed. - Operational data, Physical plant data, Influent loading data, Kinetic and Stoichiometric model parameters can be changed.
Sequencing Batch reactors (SBRs) system/ experiments	Physical System	Steady-state/ Continuous	Grey-box	Down to surrogate level	Model a post-anoxic denitrification system.	- Set-up in laboratory conditions so it can provide a steady-state scenario (same wastewater characteristic every day, temperature does not vary a lot). - Due to its nature (time-based set-up), parameters (such as COD, ammonia and nitrate) can be recorded at any part of the process, hence it is grey-box in nature. - Use synthetic wastewater so the sludge is modelled down to surrogate level. Despite modelling after the MLE system, the SBRs have different hydraulic set-up than the standard MLE system (due to its time-based nature and decant percentage). - Operational data, Physical plant data, Influent loading data, can be changed. Kinetic and Stoichiometric model parameters cannot be changed.

Pilot Plant system	Physical System	Frozen state	Black-box	Down to individual level	Model a pre-anoxic denitrification system.	- The continuous pilot-plant system in this study is set in frozen-state because it is monitored for only a section of time within a day, and does not take seasonal or daily changes in temperature into account. - The system is constantly mixed and each process, denitrification specifically, is not separated so it is black-box in nature. - Use real wastewater so it can model a treatment plant down to the microorganism organisation level. The hydraulic and influent characteristics are different. - Operational data can be changed. Physical plant data, Influent loading data, Kinetic and Stoichiometric model parameters, however, cannot be changed.

Other than the 6 methods mentioned above, there is also a short-bottles fermentation batch test in Chapter 5 that assesses the effect of various invariables on fermented sludge quality and generation of fermented sludge. These fermentation batch tests were not part of the external carbon assessment system, so it could be considered as separate from the set-up methodology.

Table 3.2: Fermentation batch test as classified under modelling classification.

Methodology used	Model Types	Time and Scales	Microbial detail	Level of Microorganism organisation	Purpose	Details
Fermentation batch tests	Physical System	Steady-state	Grey-box	Down to the individual level	- Model the fermentation results of chosen fermentation options. - Generate fermented sludge for other studies.	- The batch test is frozen-state. - Parameters (such as COD, ammonia and volatile acid level) can be recorded at any parts of the process, hence it is grey-box in nature. - Use real anaerobic sludge so it can model a fermentation process down to the microorganism organisation level. - Operational data, Physical plant data, Influent loading data can be changed. Kinetic and Stoichiometric model parameters depend on the sludge used and so cannot be changed.

The detail experimental and conceptual set-ups of all 7 methods are described in sections 3.1 to 3.7. They include their physical or conceptual set-up and how they relate to or support each other.

3.4. Specific Denitrification Rate (SDNR) Batch tests

3.4.1. General introduction to SDNR Batch Tests

The SDNR batch test is constructed based on the basis of denitrification potential. Denitrification potential is identified as the *relatively* maximum amount of nitrate that can be biologically denitrified by the system, or the *relatively* maximum denitrification rate a system can achieve. This denitrification potential is expected to vary depending on the type and composition of an internal or external carbon source. The reason why the term “relatively maximum” was used is because it is only considered within the context of system optimisation. So, for example, while the temperature factor is an important factor when it comes to the actual SDNR value, generally it is not practical to manipulate the maximum temperature of a municipal wastewater treatment system to increase the denitrification rate. So, for assessment purposes, a fixed temperature of 20°C is often used as the standard when comparing or assessing the SDNR of various carbon sources, unless stated otherwise.

3.4.2. SDNR Batch Tests set-up

The SDNR batch test in this thesis was constructed based on the method described in Dold et al. (2008), Cherchi et al. (2009) and Torres Ortiz (2013). The sludge for these experiments was collected from either the nitrification stage of Sunbury WWTP plant, or from laboratory-scale SBRS depending on the purpose of the test (more details in sections 4.2 and 6.2 separately). The idea was to spike the denitrifier biomass with: a) excessive nitrate; and b) a designated, often excessive, dosage of carbon because these two variables are often the two main limiting factors to reach the SDNR of a carbon source. Nitrate concentration was then monitored for up to 4 hours depending on the type of batch test. The SDNR, also referred to as the maximum specific denitrification rate of tested carbon, was calculated from the slope of NO₃-N concentration (Dold et al. 2008). Theoretically, if a straight line was obtained, the SDNR will equal
$$\frac{\Delta(NO_3.N + 0.6NO_2.N)}{MLVSS \times V \times t}$$

(where V = volume of the reactor, and t = reaction time)

Originally nitrite was also tested, but during many sample runs, an insignificant amount was detected due to its instability in wastewater. Therefore nitrite sampling was later omitted from these experiments. Nitrite was hence only tested when an unexpectedly small amount of nitrate was detected.

The batch test used in this paper is a 1L of anoxic reactor, consists of MLVSS of 1000-1500 mg/L. Notably, the MLVSS inside each reactor does not need to be exact, as the difference in mixed liquor biomass affects the overall denitrification removal, but will not affect the calculated SDNR (Peng, Ma & Wang 2007).

Excessive COD of the test carbon (e.g. *wastewater influent, sucrose, fermented sludge...*) was added into each 1L reactor as suggested elsewhere (Dold et al. 2008) and the type of carbon sources used depend on the objective of the experiments.

Potassium nitrate, ammonia chloride, potassium hydrogen orthophosphorus were added into the sludge slurry as required for this particular concentration. For the sucrose study, around 30mgNO₃-N/L, 35mgNH₄-N/L and 9mgPO₄-P/L, should provide excessive nutrient sources for the denitrification process so that the maximum rate is reached (Dold et al. 2008). However, this number may need to be varied depending on the maximum SDNR as documented later in Chapter 6.



Figure 3.4: *One of the SDNR batch tests*

The reactor was semi-sealed and mixed gently and semi-covered to limit contact with the atmosphere, and subsequently minimise the chances of nitrification. DO probe was used to monitor the DO level for each reactor, but generally it will be below the measurement range due to the virtual absence of O_2 inside each reactor. Nitrate and COD samples were mostly taken and analysed every 30 minutes.

Originally, nitrate was measured every 15 minutes during the first hour, and COD was measured every 30 minutes and the experiment only ran for 2 hours. Yet the rate during the first hour was fairly inconsistent even between duplicate reactors. Hence the batch tests were modified to run for up to 4 hours with the first hour's data omitted. The reason for this consistency must be due to the combination of rapid starting nitrate consumption (Peng, Ma & Wang 2007) and the raw sludge taking its time to adapt to batch test conditions. More details are discussed Chapter 4 and 6. Note that experiments such as those documented in Dold et al. (2008) also omitted the first hour of results from the SDNR calculation.

MLVSS and ammonia were tested before and at the end of the experiment, the latter done to ensure insignificant nitrification had occurred.

The batch tests were operated and controlled at room temperature, and the measured temperatures inside each reactor within the 0.5-1.0 degrees range from the room thermometer. The recorded SDNR, however, could be converted to the SDNR at 20°C to eliminate the temperature factor (Metcalf&Eddy 2003) using:

Equation 3.4.1: The equation for temperature conversion (Metcalf&Eddy 2003)

$$SDNR(t) = SDNR(20) \times 1.026^{(t-20^{\circ}C)}$$

Other than to compare the SDNR of different carbon sources, the SDNR batch test in this thesis also served to: firstly, calculate the amount of COD consumed for every mg of nitrate removed; and secondly, set up the Nitrogen Mass-Balance Conceptual Model to simulate and assess the system's denitrification performance.

3.5. Nitrogen Mass-Balance (NMB) Conceptual Models

3.5.1. General introduction to the NMB model

The Nitrogen Mass Balance Model in this case was created to use the known specific denitrification rate from SDNR batch tests for rapid assessment of a system's denitrification performance. As the name suggested, it based on the simple principles of Mass Balance:

$$\text{Input} + \text{Generation} = \text{Output} + \text{Accumulation} + \text{Consumption}$$

This involves the three main nitrogen components in wastewater treatment: soluble ammonia and ammonium (NH_3 and NH_4^+), soluble nitrate (NO_3^-), and organic nitrogen. Nitrite has been evaluated in some lab-scale tests, but the recorded concentration is fairly insignificant, as generally nitrite should be fairly unstable in a wastewater treatment system, so it will be omitted from the

model. The input and output of the Mass-Balance equation can be easily tracked by the concentration of filtered soluble nitrogen (mostly ammonia and nitrate in this case). So once the 'Generation', 'Accumulation' and 'Consumption' are identified or simplified, a Nitrogen Mass Balance Conceptual Model can be structured based on that knowledge.

3.5.2. NMB models of post-anoxic denitrification

Based on existing knowledge on suspended growth wastewater treatment, a typical post-anoxic denitrification system illustration is shown below (Figure 3.5). It consists of an aerobic zone and an anoxic zone with Returned Activated Sludge being recycled back to the first aerobic from the clarifier.

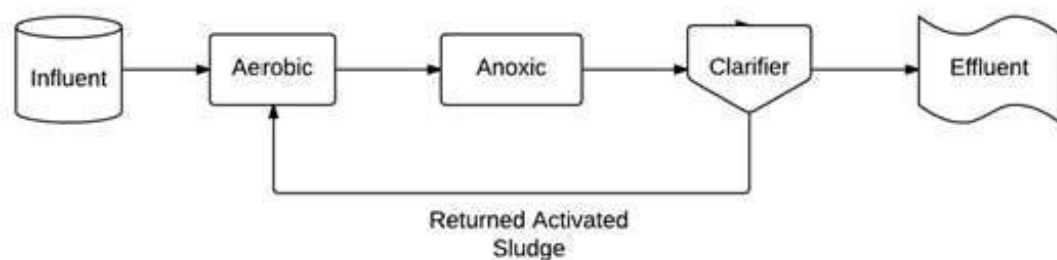


Figure 3.5: A typical post-anoxic denitrification system

Based on the diagram, all of the potential internal nitrogen-generation, accumulation and consumption can be tabulated below (Table 3.3).

Table 3.3: Generation, Accumulation and Consumption of Nitrogen-based matter in wastewater

Components	Process	Description	Equation form
Organic Nitrogen	Generation	Roughly 20% of nitrogen from the influent is incorporated in the sludge mass.	$ON_{G+A} = 20\%TN_{ini}$
	and Accumulation	(Henze 2008) and is trapped within the system when the solid is separated from the liquid phase (e.g. clarifier).	NH_{4A}
	(ON_{G+A0}) Consumption	The remaining organic nitrogen is broken down into NH_4 during aerobic stage.	$ON_C = TN_{ini} - ON_{G+A} - NH_{4ini}$
Ammonia	Generation	Was generated from the breaking down of influent organic nitrogen. The ammonia produced from decaying sludge is omitted in this case.	$NH_{4G} = ON_C = TN_{ini} - ON_{G+A} - NH_{4ini}$
	(NH_{4G}) Accumulation	Along with the influent of organic nitrogen, roughly 20% of influent nitrogen is incorporated in the sludge mass.	$NH_{4A} = 20\%TN_{ini} - ON_{G+A}$
	(NH_{4A}) Consumption	Most will be denitrified during the nitrification stage in the aerobic zone.	$NH_{4C} = NO_{3G} = NH_{4ini} + NH_{4G} - NH_{4A} - NH_{4eff}$
	(NH_{4C})		

Nitrate	Generation (NO _{3G})	Is generated from the nitrification of NH ₄ in aerobic zone.	NO _{3G} = NH _{4C}
	Accumulation (NO _{3A})	Not applicable when there is no internal recycle	NO _{3A} = 0
	Consumption (NO _{3C})	Is denitrified during the denitrification process in the anoxic zone.	NO _{3C} = NO _{3G} - NO _{3eff}

Based on the table above, the NO₃ consumed (removed) can then be simplified as (all units are in mg/L):

$$\begin{aligned}
 \mathbf{NO_{3C}} &= NO_{3G} - NO_{3eff} = NH_{4C} - NO_{3eff} = NH_{4ini} + NH_{4G} - NH_{4A} - NH_{4eff} - NO_{3e} \\
 &= NH_{4ini} + TN_{ini} - ON_{G+A} - NH_{4ini} - NH_{4A} - NH_{4eff} - NO_{3eff} \\
 &= NH_{4ini} + TN_{ini} - ON_{G+A} - NH_{4ini} - 20\%TN_{ini} + ON_{G+A} - NH_{4eff} - NO_{3eff} \\
 &= \mathbf{80\%TN_{ini} - NH_{4eff} - NO_{3eff}}
 \end{aligned}$$

So, when complete nitrification is reached in the earlier aerobic zone, (hence the NH_4 in the effluent is insignificant) the NO_3 expected in the effluent will be:

$$NO_{3\ eff} = 80\%TN_{ini} - NO_{3\ consumed}$$

But considering the SDNR equation shown in section 3.2, if the SDNR for post-denitrification can be reached by adding an external carbon source AND complete nitrification occurs, the NO_3 in the effluent is expected to be:

$$NO_{3\ eff} = 80\%TN_{ini} - SDNR \times MLVSS \times t$$

Or when nitrification is not completed as in Equation 3.5.1 below:

Equation 3.5.1: Nitrogen Mass Balance (NMB) for post-anoxic denitrification:

$$NO_{3\ eff} = 80\%TN_{ini} - SDNR \times MLVSS \times t - NH_{4\ eff}$$

Where t is the anoxic HRT in hours, MLVS is g/L of volatile suspended solids in inside the system, and SDNR is in $mgNO_3/gMLVSS/h$. Note in this case, the assumption is the volume of returned sludge is insignificant when it comes to contributing to the dilution factor of incoming influent (or the initial TN has already taken this into account). In addition, the model cannot simulate the performance of nitrification, so the effluent ammonia $NH_{4\ eff}$ is required to set up the NMB model.

3.5.3. NMB model of pre-anoxic denitrification

The basis of pre-anoxic denitrification is very similar to post-anoxic denitrification, since fundamentally the nitrogen process still starts with nitrification before denitrification begins. The biggest difference is the order of the physical anoxic and aerobic zones, and the large volume of Internal Recycle.

In other words, the key difference here when it comes to the Mass Balance Conceptual Model is its hydro-dynamic. Thus, a typical pre-anoxic denitrification set-up or MLE can be illustrated as shown below.

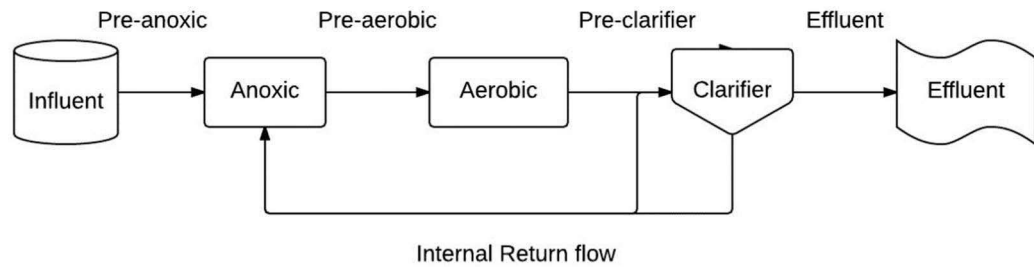


Figure 3.6: A basic pre-anoxic denitrification set-up

Assuming there is complete denitrification the nitrogen profiles of these based on the above diagram are listed below.

Table 3.4: Equations showing concentration of nitrate and ammonia for each stage in pre-anoxic denitrification.

Stages	Equation	Description/ Explanation
Pre-anoxic	• $TN_{ini} = x \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v \text{ (L)}$	This can be regarded as influent characteristics, before any treatment process happens. Note that the nitrate concentration here is assumed to be none as insignificant nitrate is often detected in most municipal WWTP systems.
Pre-aerobic	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v + IR \text{ (mg/L)}$	This stage follows the influent being diluted by the internal return flow and denitrification occurring in the anoxic zone. It is important to assume here that complete denitrification has occurred. 20% of the ammonia was absorbed by the sludge as explained in previous chapter.
Pre-clarifier	• $NH_4 = NH_{4\text{eff}} \text{ (mg/L)}$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} - NH_{4\text{eff}} \text{ (mg/L)}$ • $Q = v + IR \text{ (L)}$	This stage is after nitrification in the aerobic zone. The quality of supernatant here is likely to be the same as in the effluent (assuming no nitrogen removal happens in the clarifier, or during the tertiary process).
Effluent	• $NH_4 = NH_{4\text{eff}} \text{ (mg/L)}$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} - NH_{4\text{eff}} \text{ (mg/L)}$ • $Q = v \text{ (L)}$	The quality of influent is the same as that above but the volume is similar to influent flow rate. Here it assumes that the flow rate of WAS and loss of flow rate to evaporation is negligible. Because the WAS volume often is affected by the wasting method of the treatment plant and the treatment plant volume, it should vary from treatment plant to treatment plant.
Internal Recycle (Internal Return) (IR) flow	• $NH_4 = NH_{4\text{eff}} \text{ (mg/L)}$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} - NH_{4\text{eff}} \text{ (mg/L)}$ • $Q = IR \text{ (L)}$	The internal recycle back to the anoxic zone.
Mixed of pre-anoxic and IR flow	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} \text{ (mg/L)}$ • $NO_3 = \frac{\left(\frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} - NH_{4\text{eff}}\right) \times IR}{v + IR} \text{ (mg/L)}$ • $Q = v + IR \text{ (mg/L)}$	This is a hypothetical stage where the pre-anoxic influent and IR flow were completely mixed but no denitrification had yet occurred. This does not happen in real world as denitrification often start before the two flows are totally mixed

Issues with Nitrate accumulation in pre-anoxic denitrification

Nitrate accumulation cannot be computed into the NMB model if the internal recycle is involved. Firstly this is due to the nature of the NMB as a rapid assessment system. Secondly, to accurately assess the nitrate accumulation of a system, a much more detailed and frequent influent profile will be needed (possibly every hour during the simulation period), which is very impractical during this experiment and future applications.

This actually also happened to the dynamic simulation using Biowin, where the nitrate accumulation can cause a large error in simulation performance (see Chapter 4). However, unlike Biowin where nitrate accumulation will distort the actual result, the NMB simply cannot compute it once the amount of nitrate needed to be removed exceeds the denitrification potential of the system. The reason is because: assuming even if there is a constant nitrate accumulation of x , this will mean the nitrate concentration in the effluent according to the NMB will be:

Equation 3.5.2: Effluent Nitrate based on accumulated nitrate of x

$$\begin{aligned} \text{effluent } NO_3 &= \left(\frac{IR}{v + IR} x + \frac{IR}{(v + IR)^2} x + \dots \frac{IR}{(v + IR)^t} x \right) \\ &+ \frac{0.8x \times v + 0.8NH_{4_{eff}} \times IR}{v + IR} - NH_{4_{eff}} \end{aligned}$$

Where:

- t is the number of cycles =
$$\frac{\text{time where accumulation of } x \text{ occurred}}{HRT_{\text{system}}}$$

This has not even considered the possibility that x may also varied with time. Yet, it does not mean zero nitrate accumulation is allowed to happen in the NMB model. For reliable accuracy, this amount of nitrate accumulation has to be reset every couple of cycles, or days assuming the simulation period lasts up to one month.

3.6. Mumax Batch tests

3.6.1. General introduction to Mumax Batch tests

The Mumax batch test's main objectives are to find some specific denitrification constants: Y_H (Growth yield efficient) and μ_s (Growth rate of substrate). These two kinetic constants are varied depending on different carbon types. The two kinetic are not only applicable to the Biowin program to simulate a system denitrification performance, it is also an essential component in understanding the studied carbon better. For example, the theoretical SDNR of a carbon could be calculated based on the equation using Y_H and μ_s below (Dold et al. 2008):

Equation 3.6.1: SDNR based on Growth yield efficient and Growth rate of substrate

$$\frac{dS_{NO}}{dt} = -\eta \cdot \frac{(1 - Y_H)}{2.86} \cdot \frac{\mu_H}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot X_{BH} \cdot e^{(\mu_H - b_H)t}$$

Another application of Y_H and μ_s includes calculating the C:N consumption rate of a carbon substrate (Metcalf&Eddy 2003):

Equation 3.6.2: C:N consumption rate based on Growth yield efficient and Growth rate of substrate

$$\frac{bCOD}{NO_3N} = \frac{2.86(1 + b_H \cdot SRT)}{1 - 1.42Y_H}$$

Note that Y_H , μ_s , b_H are classified as non-dependent on influent characteristics, and should stay constant under certain temperatures. Denitrifier mass X_s , however, will vary with both carbon source and sludge types. Other constants that related to the Mumax test include: a) maximum specific substrate utilization rate (μ_s / Y gbCOD/gVSS.d); b) half-velocity; c) heterotrophic yield coefficient (0.72 - 0.79 or as low as 0.66 - 0.67 gCOD/gCOD); d) total oxygen uptake ($\int OUR(t)dt$ and $COD_{degraded}$); e) and K_s range for half saturation relatively (5.0 - 40.0 mgBOD/L) (Metcalf&Eddy 2003).

One of the main concerns regarding the Mumax batch test is the need of acclimation to a certain carbon source. The sludge used in the Mumax batch test is often acclimated in a pilot or lab-scale test, sparking another concern that it will potentially change the sludge characteristics. However, Cherchi et al. (2009) compared the Y_H and μ_s of the same carbon source using a lab-scale system (with synthetic wastewater) and larger pilot-scale plant, showing that insignificant differences were evident.

3.6.2. The Mumax Equation

There are two keys component of the Mumax Batch Tests, these being the theoretical equation (in this chapter) and the physical experimental set-up (see section 3.6.3).

The most important theoretical component here is the Mumax Equation as indicated in Equation 3.6.3 below.

Equation 3.6.3: Mumax equation

$$\frac{dS_{NO}}{dt} = -\eta \cdot \frac{(1 - Y_H)}{2.86} \cdot \frac{\mu_H}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot X_{BH} \cdot e^{(\mu_H - b_H)t}$$

The Mumax equation was originally based on the Monod Equation, and the detail formation of Mumax equation is presented in Appendix XI, and was shown to have several variations (Dold et al. 2008; Metcalf&Eddy 2003).

Generally, however, each component can be defined as documented below in Table 3.5.

Table 3.5: Mumax batch tests symbols and units

N	Param	Definition	Unit
o.	eters		
1	T	Time	day
2	S _{NO,t}	Nitrate at time t	mg/L

3	Y_M	Growth yield efficient, the mg of biomass was created for every mg of COD consumed	mgVSS/ mgCOD
4	μ_s	Growth rate of denitrifier when using substrate (e.g. sugar) as the carbon source	N/A
5	$X_{s,t}$	Anoxic mass at time t	mgCOD/ L
6	b_s	Decay rate	N/A
7	η_{Anoxic}	Fraction of denitrifying bacteria	N/A

Note: $X_{s,0}$ is independent of SNO,t and μ_s

3.6.3. The Mumax Test Set-up

The Mumax Test Set up in this case is based on Dold et al. (2008). The reactor used here was a closed, temperature-controlled vessel. At the start of the test, ~50 mL/L of mixed liquor from the anoxic zone was diluted to make approximately 50mgVSS/L. Potassium nitrate (KNO_3), ammonium chloride (NH_4Cl) and potassium dihydrogen phosphate (KH_2PO_4) were added to provide excess concentrations of nearly 100 mg-N/L, 100 mg-N/L and 100 mg-P/L, respectively.

The oxygen input from the atmosphere was originally thought to be a potential problem, hence nitrogen gas was firstly bubbled through the liquid to strip dissolved oxygen and maintain a nitrogen blanket on the liquid surface. The actual trial with sealed container, however, found that no or insignificant nitrification occurred during the experiment's 6 days despite the excessive ammonia concentration. In fact, the bubbled nitrogen gas risked causing gas

exchange in the liquid surface, so this step was omitted from the actual experiment. At time zero, substrate (tested carbon sources) was added to raise the initial carbon concentration to approximately 600 mg-COD/L. The concentration of substrate was saturated relative to the half-saturation coefficient K_s of the tested carbon. This helped to ensure the growth rate was at

its maximum, according to: $\mu = \mu_{MAX} \cdot \frac{S_s}{K_s + S_s} = \mu_{MAX}$.

An air-tight cover was placed over each reactor and the batch test was monitored for a period lasting 144 hours. Samples were withdrawn at intervals of 2-3 hours, filtered immediately, and tested for their nitrate concentration. Sulphuric acid can be added manually, or carbon dioxide gas can be bubbled through the solution to maintain the pH level. The denitrification intermediate (nitrite), however, is insignificant.

3.7. Biowin software simulations

3.7.1. General introduction to Biowin

Biowin is a commercial mathematical software created, developed, and owned by EnviroSim Ltd. It works in the designing, upgrading and optimising of activated sludge wastewater treatment plants. The core of the Biowin program is the biological model that ties together other chemical and physical models (e.g. oxygen mass transfer, gas-liquid interactions). Like many other currently available activated sludge models, Biowin originated in the ASM1 model, which was the first model with a uniform context and standardisation. It was developed by International Water Association (IWA) task group in 1987. However, unlike ASM1, Biowin has addressed several problems associated with ASM1 (Henze 2000):

- a. The lack of kinetic expressions that can deal with nitrogen and alkalinity limitations of heterotrophic organisms.
- b. The inclusion of nitrogen compounds into biodegradable soluble and particulate organic nitrogen as model compounds that cannot be easily measured and thus complicate the simulation studies.
- c. The difficulty in quantifying ammonification.
- d. The separation of inert particulate organic materials depending on their origin (influent or biomass decay) despite it being impossible to differentiate them in real life.
- e. The domination of the hydrolysis process over oxygen consumption and denitrification.
- f. The combination of lysis with hydrolysis and growth to describe endogenous respiration.
- g. Does not take into account the storing of readily biodegradable organic substrates such as PHA or glycogens.
- h. Difference in decay rates of nitrifier under aerobic and anoxic conditions.
- i. Cannot directly predict the frequency of MLSS concentration.
- j. Fixes the high biomass yield of soluble substrates that is caused by inducing a slowly biodegradable substrate.

The only issue with Biowin as reported in the literature was addressed by Sedran, Mehrotra & Pincince (2006) who analysed 'the effects of not calibrating simulation packages of 3 different software models BioWin, GPS-X, and Plan-It STOAT when estimate tank volumes for activated sludge wastewater treatment'. They found that different simulation results for BioWin did not vary much from the relative volume, regardless of set or default influent wastewater fractions and kinetic stoichiometric parameters. However, the other two programs

demonstrated larger changes when parameters were set at different values. This should be taken in account when assessing the Biowin simulation performance.

3.7.2. Biowin simulation in regarding to external carbon

The Biowin set-up employed in this thesis assesses the effect of external carbon is modified based on the 'Methanol as additional carbon' function in Biowin. The version used is the updated licensed version from 2012- 2015. The most important requirement for Biowin software is to calibrate the simulation package according to the wastewater and physical plant characteristics which need to be treated as they are very different from one municipal treatment plant to another. In this study, the characterisation process refers to the pilot plant and SBRs. Parameters such as influent organic matter and total nitrogen components are analysed according to their respective portions using chemical analysis and calculations (Melcer, Dold & Jones 2003). This ensures that the simulation of added external carbon provides similar results as, real system as possible. The specific wastewater characterisation used for Biowin simulation in this study is depicted in Figure 3.7 below.

Parameters		
Name	Default	Value
Fbs - Readily biodegradable (including Acetate) [gCOD/g of total COD]	0.16000	0.13600
Fac - Acetate [gCOD/g of readily biodegradable COD]	0.15000	0.30900
Fxsp - Non-colloidal slowly biodegradable [gCOD/g of slowly degradable COD]	0.75000	0.90000
Fus - Unbiodegradable soluble [gCOD/g of total COD]	0.05000	0.08800
Fup - Unbiodegradable particulate [gCOD/g of total COD]	0.13000	0.13000
Fna - Ammonia [gNH ₃ -N/gTKN]	0.66000	0.59200
Fnox - Particulate organic nitrogen [gN/g Organic N]	0.50000	0.50000
Fnus - Soluble unbiodegradable TKN [gN/gTKN]	0.02000	0.02000
FupN - N:COD ratio for unbiodegradable part. COD [gN/gCOD]	0.03500	0.03500
Fpo4 - Phosphate [gPO ₄ -P/gTP]	0.50000	0.25000
FupP - P:COD ratio for unbiodegradable part. COD [gP/gCOD]	0.01100	0.01100
FZbh - Non-poly-P heterotrophs [gCOD/g of total COD]	1.0000E-4	1.0000E-4
FZbm - Anoxic methanol utilizers [gCOD/g of total COD]	1.0000E-4	1.0000E-4
FZaob - Ammonia oxidizers [gCOD/g of total COD]	1.0000E-4	1.0000E-4
FZnob - Nitrite oxidizers [gCOD/g of total COD]	1.0000E-4	1.0000E-4
FZamob - Anaerobic ammonia oxidizers [aCOD/a of total COD]	1.0000E-4	1.0000E-4
Set typical (Raw)		Set typical (Settled)

Figure 3.7: The characterisation of influent wastewater for Biowin simulation in this experiment.

Further calibration can also be done by carrying out experiments to determine the kinetic stoichiometric parameters. Despite studies having shown that these standard parameters do not vary a lot for different systems that treat municipal wastewater (Melcer, Dold & Jones 2003), in this case, the kinetic stoichiometric parameters of denitrifiers needed to be calibrated. This was due to the impact of utilising different external carbon sources.

Cherchi et al. (2009) for example, used the Biowin model to simulate the effects of external carbon sources such as methanol and MicroC™ on different set-ups and design parameters scenarios. They did this by changing denitrification kinetics and stoichiometric parameters such as maximum specific growth rates and yields for methanol and MicroC™ (determined using Mumax batch tests). Similarly, Dold et al. (2008) stated that simulation allows rapid assessment of

features such as maximum specific methanol-utiliser growth rate μ_{METH} , yield coefficient for methanol-utilising heterotrophs (Y_M), methanol-utiliser decay rate (b_{METH}) and methanol-utiliser half-saturation coefficient ($K_{S, \text{METH}}$). All of these will affect the experiment results and behaviour.

The default setup of the Biowin models used in this thesis is shown in Figure 3.8 below. This was modelled after the set-up of the existing pilot plant, which has two separate aerobic tanks instead of just one.

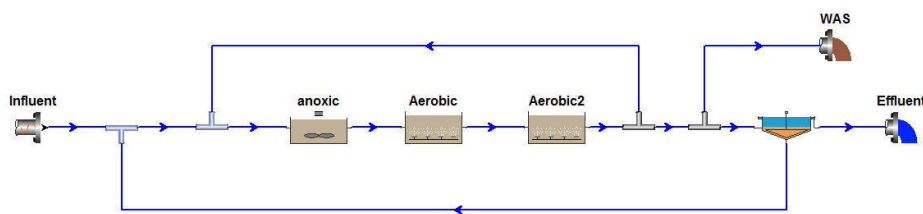


Figure 3.8: The default pilot plant diagram used in this thesis.

3.8. Sequencing Batch Reactors (SBRs)

3.8.1. General Introduction to SBR

SBR is one of the newer activated sludge process technologies that has been made possible by the development of high tech sensors, automatically operated valves and program logic controllers. In SBR, the treatment process is divided into 5 stages that occur in the same tank (fill, react, settle, draw, idle). The SBR (along with oxidation ditch and aerated lagoon) is preferred in hot regions because it can omit the need for primary treatment. The SBR was originally designed in 1914 and proved to be really effective for small communities, and

recently its popularity has increased due to automation. Its advantages include: a) no sludge circulation, b) developed Data Acquisition and Control (DAC) allowing its automation, c) settling occurs during complete quiescent condition, d) it can be adjusted using different time-phase, wastewater load and composition (due to its time-orientated nature rather than space-oriented nature of standard wastewater treatment system), e) favours floc forming bacteria hence minimise bulking, and f) the changes in high and low substrate allow robust bacteria to develop.

The main disadvantage of the SBR is mostly due to the hydraulic limitation (due to its time-orientated nature) which means two or more reactors are required for continuous incoming of influent. Furthermore, due to the need for automation, the equipment and level of maintenance will need to be more sophisticated than standard wastewater treatment systems. That is why various operating conditions were studied using both synthesised and real wastewater, aiming to increase the productivities, reduce the need for personnel and resources (energy, material consumption) and increase its reliability.

The SBRs in this study actually were established under a lab-scale scenario, to simulate the performance of the entire wastewater treatment system. Its major feature of time-orientation rather than space-orientation (standard treatment plant) was taken into account. It means instead of continuous mixing of treating influent as occurs in a standard set-up, the separation by time zone of all processes within the system (fill, anoxic, aerobic, etc.) will allow each process to be monitored independently. This provides the great advantage of monitoring each nitrification and denitrification process separately, and even maintaining a consistent HRT or SRT feature to provide more consistent results. Overall, that is

the reason why the lab-scale SBR is a very useful research tool and hence why it is often used to study different wastewater treatment processes.

Despite already being utilised to assess the effect of additional substances on a system denitrification substance (Bernat & Wojnowska-Baryla 2007; Hingley et al. 2012), the SBRs in this case will not be able to simulate the performance of an external carbon source on a continuous pre-anoxic denitrification system. This is because it is not possible to replicate continuous influent and Internal Recycle with the SBRs when the feeding, anoxic and aerobic stages are completely separated by different time zones (see sections 3.8.4 and 4.4). It explains why SBRs in this study were mostly used to simulate or verify the effect of external carbon sources on the post-denitrification system where internal recycle does not exist. In terms of operation, like the full-scale SBR, the lab-scale SBR system heavily depends on consistency and accuracy of the time-control devices. Most of the issues that arose during the running of the SBRs in this study were due to the failure of these devices.

3.8.2. Lab-scale SBRs parameters used in the literature

As mentioned in the previous chapter, the lab-scale SBRs have been very popular and employed to assess the performance of various wastewater treatment processes. The set-up and performance of the SBRs depended on several factors, including cycle duration, decant percentage, Sludge Retention Time (SRT), Aerobic Duration and others as below

a) Cycle Duration and HRT

The cycle duration has a direct impact on a system HRT, as it provides the contact time for microorganisms, especially the denitrifier in this case, to access the wastewater (Jiang et al. 2016; Moussavi, Jafari & Yaghmaeian 2015; Wang et

al. 2009; Wang, Peng & Stephenson 2009). This is the reason why HRT can directly impact the microbial community, biological treatment (Eker & Kargi 2006; Han et al. 2010) and HRT that is too low does have a direct correlation with ineffective nitrogen removal (He, Wang & Song 2016; Kim et al. 2008; Moussavi, Jafari & Yaghmaeian 2015; Wang et al. 2009).

The cycle duration of lab-scale SBR used especially for municipal wastewater is generally short, ranging from **4 to 12 hours** (Glass & Silverstein 1998; Hajiabadi, Moghaddam & Hashemi 2009; Jang et al. 2004; Kargi & Uygur 2003, 2004; Kargi, Uygur & Baskaya 2005; Keller et al. 1997; Li et al. 2008; Mao et al. 2006; Obaja et al. 2003; Obaja, Macé & Mata-Alvarez 2005; Sun et al. 2017; Zeng et al. 2003; Zhu, Zhang & Miller 2006). However, in some special cases (high salinity, textile dye removal), higher cycle duration of **23h or more** can be used (Fontenot et al. 2007; Kapdan & Oztekin 2006; Obaja, Macé & Mata-Alvarez 2005). This was most likely because higher cycle duration will allow a higher hydraulic contact time and better treatment in those cases. For example, Obaja, Macé & Mata-Alvarez (2005) found that 300, 500, 700, 1300 mgNH₄-N /L can still obtain 99.6% -99.9% if the cycle duration is increased accordingly to 4, 8, 12, and 24 hours, respectively.

b) Decant Percentage

The percentage of decant is generally **25 to 33%** of reactor volume (Alinsafi et al. 2008; Jang et al. 2004; Obaja et al. 2003; Obaja, Macé & Mata-Alvarez 2005), but can be as low as **10%** (Li et al. 2008; Zeng et al. 2003) or as high as **50 to 80%** (Ciggin, Majone & Orhon 2012; Glass & Silverstein 1998; Kapdan & Oztekin 2006; Kargi & Uygur 2004; Kargi, Uygur & Baskaya 2005; Mao et al. 2006; Zeng et al. 2003; Zheng, Chen & Liu 2010). Notably, the decant percentage is quite significant

in this case because it acts similarly to the internal recycle rate in a standard continuous WWTP set-up, where the decant percentage will dictate how much soluble substrates still accumulate within the system. The influent will be diluted by a factor of $\frac{1}{\text{Decant \%}}$ after being fed into the system, means the concentration of influent likely be reduced even without any treatment happens. The decant percentage and cycle duration also make up the Hydraulic Retention Time (HRT) of the system.

c) Sludge Retention Time (SRT)

As previously noted, the SRT is very important for a system because it dictates the sludge age and the time allowed for certain groups of bacteria to grow inside the system. The growth rate of autotrophic AOB is less than those of heterotrophic bacteria, so for nitrogen removal a longer retention time is needed. Otherwise the bacteria may be washed out (Campos et al. 1999; Gujer 2010; Henze & van Loosdrecht 2008; Kos 1998, cited in (Kwok 2011)), especially during high loading rates (or low HRT) (Environment Leverage 2003, cited in (Kwok 2011)).

The SRT of **10 to 15 days** generally showed a good nitrogen removal rate in experimental studies (Kargi & Uygur 2003, 2004; Kargi, Uygur & Baskaya 2005; Li et al. 2008; Obaja et al. 2003; Obaja, Macé & Mata-Alvarez 2005; Zeng et al. 2003). SRTs of 10-12 days are suggested to maintain an effective fraction of denitrification bacteria within the sludge. However, higher SRT of **15 to 21 days** also show good nitrogen removal in some other studies (Jang et al. 2004; Keller et al. 1997; Zhu, Zhang & Miller 2006). One of the effects of higher SRT, is that it increases the Mixed Liquor Suspended Solids of a system. Hajiabadi, Moghaddam & Hashemi (2009) found that for high COD influent (2200 mg/L) with a long cycle duration (24h) and long aerobic zone (22.5h), the higher SRT and MLSS had little

effect on the COD removal rate. The SRT of 5, 10, 15, and 20 days and MLSS of 1824, 2694, 3210, and 3864mg/L have COD removal rates of 95.0, 95.4, 95.9, and 96.2%, respectively.

d) Aerobic/Anoxic Duration

Kargi & Uygur (2004) employed a SBR set up with one initial anaerobic, two anoxic and two aerobic zones for each cycle. They found that an increase of the anaerobic zone (anoxic in the absence of NO_3) from 2 to 2.5 hours, reduced the COD removal rate from 98 to 68% while NH_4^+ showed no definite pattern. An increase in duration of the first anoxic zone from 1 to 2 hours lowered the NH_4^+ removal from 89% to 78%. The first aerobic zone from 6h to 3h to 2h reduced NH_4^+ removal from 92% to 83% to 48%, respectively. An increase in the second anoxic zone from 1 to 2 hours increased the NH_4^+ removal from 82% to 92%. The second aerobic zone from 2.5 to 2h actually enhanced the NH_4^+ removal from 77% to 88%. This is summarised in the table below.

Table 3.6: Effect of multiple anoxic and aerobic stages on COD and NH_4 removal.

	1 st anaerobic	1 st anoxic	1 st aerobic	2 nd anoxic	2 nd aerobic
COD removal rate	-				
NH_4^+ removal rate		-	+	+	-

Note: '+' shows a positive correlated relationship and '-' shows a negative correlated relationship.

Similarly, Zheng, Chen & Liu (2010) also set up the SBR using an 8 hour cycle of Ana/Ae/Ano/Ae/Ano/Ae of 1.5h/1.0h/1.0h/0.5h/1.0h/0.3h cycles and concluded that effective treatment was the result.

e) Temperature

Fontenot et al. (2007) reported that an increase in temperature with very long cycle durations (8 days) did not increase NH_4^+ and COD removal rate because they remained at 99% and 98%, respectively. In fact, at 45°C, the NH_4^+ and COD removal rate actually fell to 91% and 84%, respectively. General room temperature of 18-25°C (Kargi & Uygur 2003; Keller et al. 1997; Sun et al. 2017; Zeng et al. 2003; Zhu, Zhang & Miller 2006) or up to 28-30°C (Fontenot et al. 2007; Obaja et al. 2003; Obaja, Macé & Mata-Alvarez 2005) with the right set up still could still provide 95% or complete nitrogen removal. According to Cherchi et al. (2009) a reduction from 20°C to 10°C in temperature will reduce denitrification rates by 60-62% and microbial growth rates by 67-73% in the presence of external carbon sources.

In summary, effective treatment by the SBR system can be achieved at room temperature and will not be significantly affected by higher temperatures. However, the denitrification rate seemed to be significantly affected when temperature fell to 10°C.

f) C:N ratio

The C:N ratio here is the recorded ratio inside of the reactor rather than the C:N ratio in the feed. It is often measured by dividing COD, soluble COD, BOD_5 with Total Nitrogen (TN) or Total Kjeldahl Nitrogen (TKN).

For Nitrification

Prior studies have reported a good nitrogen removal rate with a C:N ratio of **less than 10** (Fontenot et al. 2007; Jang et al. 2004; Obaja et al. 2003) and **20 or more** (Kargi & Uygur 2003, 2004; Kargi, Uygur & Baskaya 2005; Zheng, Chen & Liu 2010). Fontenot et al. (2007) detected an increase in the C:N ratio higher than 10 (in salinity wastewater with really long cycle durations of 8 days) and having negative effects on NH_4^+ removal rate. The NH_4^+ removal rate dropped from complete ammonia removal for C:N ratio of 5 to 10, to 84% and 81% ammonia removal for C:N ratio of 20 and 30. Finally, Kwok (2011) stated that a BOD_5/TKN of 0.5-3 promoted a high population of nitrifier and should not be influenced by heterotrophic oxidation of BOD.

For Denitrification

The generally required C:N ratios for denitrification ranged from as low as 1.7 to as high as 7.1. Obaja, Macé & Mata-Alvarez (2005) stated that complete denitrification was obtained when C:N was equal or higher than 1.7. Kwok (2011) asserted that denitrification is improved at a BOD_5/TKN of 5. In another study, Bodík et al. (2009), however, stated that if the COD/TN ratio of influent is lower than 3.4, an extra carbon source would be needed for denitrification (Kuab et al. 1996, cited in (Bodík et al. 2009)). WWTP in Pontarlier (used for pig farm manure treatment) also needed to raise the COD/N ratio from 3.7 to 7.1 for good denitrification removal (Montuelle, Goillard & Le Hy 1992).

g) Internal/External Carbon sources

It is not significant whether a carbon source for nitrogen removal is internal or external. As Obaja, Macé & Mata-Alvarez (2005) point out, a ratio between external (acetic acid) and internal (volatile fatty acids) of 3:1, 1:1 and 0:1 showed little difference as almost complete ammonium (99.8%), nitrate (99.8%)

and phosphate (97.8%) removals were achieved. The 100% internal carbon rate indicated a slightly higher ammonium, nitrate and phosphate removal rate.

3.8.3. The chosen SBRs set-up

a) Operational and Physical Reactor parameters

The lab-scale SBRs set-up has been chosen for continuous running experiments 24 hours a day, 7 hours a week, with their cycle (whether mixing, aeration, feeding or decanting) being dictated by a typical commercial timer.



Figure 3.9: Four SBRs set-ups

The four SBRs (named from A-D) are all identical at blank stage, and each have an effective volume of 4L, with a decanting percentage of 50%, including the sludge wasting volume. The SRT is calibrated and controlled to be within the 11.5-12.5 range. The cycle duration is 8 hours, giving a HRT (Hydraulic Retention Time) of 16 hours. These are all chosen based on the literature review in section 3.8.2.

The level of pH is usually not adjusted, but due to short HRT and additional alkalinity, it often falls within the pH 7-8 range of synthetic wastewater. The breakdown of the 8 hours cycle is depicted in Figure 3.10 below:

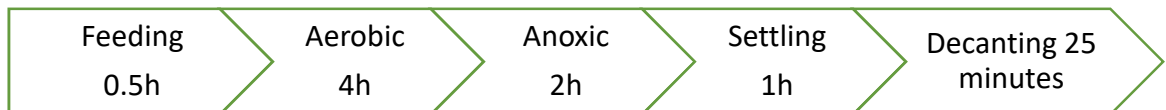


Figure 3.10: Breakdown of a SBR cycle

The 5 minute gap at the end of the cycle serves to prevent problems related to timing device error, where feeding and decanting occur at the same time and subsequently leading to a loss of sludge or influent. The sludge used for all reactors was collected from the nitrification stage of the Sunbury WWTP , sieved, slightly washed, and seeded into each reactor. Then it was left to run in laboratory conditions for at least one month before the experiments started.

b) Influent loading data

Due to the inability to access the required amount of real wastewater for a lengthy amount of time (more than one year), synthetic wastewater was used as feed for the SBRs system. Extensive research is available on carbon sources used (see Reference IV – SBR Feed Selection). To summarise, there are three key components of synthetic wastewater: the trace metal (MgSO_4 , CuSO_4 , FeCl_3 ... etc.), the nutrient (K_2HPO_4 , CaCl_2 ...etc...), and the feed components providing the carbon and nitrogen ratio. The chosen nutrient and trace metal compositions were selected to be the same as suggested by Hu et al. (2010).

The C and N component, however, was much more important and thus selected and tested much more carefully. As previously mentioned in Chapter 2 and section 3.4, the type of carbon used could affect the behaviour of post-anoxic

denitrification. Based on the literature, generally for shorter and smaller tests, peptone was often used with meat extract (ratio 1.5 to 1 w/w) as a supplement for sbCOD in synthetic wastewater (Bajaj, Gallert & Winter 2008; Boeije et al. 1999; Pholchan et al. 2008). This was mostly because the OUR experiment in Insel et al. (2006) found that up to 44% of peptone COD fraction was sbCOD. However, due to the high cost of peptone, it has not been often used for continuous lab-scale experiments. Considering that peptone is derived from animal milk or animal meat, milk powder or meat extract, these substitutions were sometimes instead used as the main components for synthetic wastewater production (Boeije et al. 1999; Bracklow et al. 2007; Hajiabadi, Moghaddam & Hashemi 2009; Tabares 2006).

This explains why a selection of acetate, sugar, milk power, beef extracts and mixtures of some of the above were tested in the SBRs to assess their suitability. Generally, the short-term performance of the system (7-30 days) did not significantly change, so in the end, a mixture of sucrose and beef extract solution was selected. This combination provided overall stable performance for the first 5-6 months (as seen in Figure 3.11 to Figure 3.16). However, due to later sucrose experiments being conducted, the sugar component was completely removed from the feed components, and replaced by entirely beef extract instead. The final carbon components for the SBR feeds were the same as the one used by Tabares (2006).

The final synthetic feeds/wastewater (per litre) components of the SBR consisted of beef extract (the quantity varied depending on the measured COD), 200mg NaHCO₃, 10 mg of CaCl₂, 10 mg of K₂HPO₄, 11mg of KH₂PO₄, 10 mg FeSO₄,

0.15 mg CoCl₂, 0.03 mg of CuSO₄, 0.15 mg H₃BO₃, 0.18 mg KI, 0.12 mg MnCl₂, 0.06 mg NaMoO₄, 0.12 mg of ZnSO₄.

Considering the chosen synthetic feed has most of its TN consisting of organic nitrogen from beef extract (and only 5% is ammonia), there was a concern that the organic nitrogen would not be able to break down completely. This led to either high TN record in the effluent, or varied nitrification and denitrification conditions amongst the 4 SBRs. Consequently, TN, NH₄ and NO₃ at the end of the aerobic stage were tested several times and found to be NH₄ <0.1 mg/L, NO₃ of 17.2 ± 0.9 mg/L and TN of 19.7 ± 2.6 mg/L. Since TN of the effluent seems to consist mostly of ammonia and nitrate, it can be safe to assume that most if not all initial organic nitrogen was broken up into soluble ammonia and underwent nitrification and denitrification just like in real wastewater. The influent loading data is summarised in the following table.

Table 3.7: The synthetic wastewater parameters

Synthetic wastewater	
Soluble COD	
- Before changing feed	660 ± 33 mg/L
- After changing feed	457 ± 27 mg/L
TN	
- Before changing feed	29.7 ± 3.5 mg/L
- After changing feed	55.6 ± 1.4 mg/L
TP	17.3 ± 2.5 mg/L

PO ₄ -P	5.63 ± 0.82 mg/L
Alkalinity	283 ± 29 mg/L
pH	7 – 8

Note that the COD actually dropped dramatically after the beef extract replaced the sucrose. This unfortunately is unavoidable because if more beef extract is used, more TN will be pumped into the system. However, as seen in Figure 3.11 to Figure 3.16, the system's denitrification process - especially developed to operate well with the low COD, with the exception of a slight reduction of denitrification - was probably due to the lower MLSS. Based on the minimum F/M ratio of 0.25, the maximum theoretical MLSS which the system can have would be 2700mg/L. This represents a decline from the earlier 3075 ± 521 mg/L. More on this is presented in the section below.

3.8.4. The SBRs' performance in controlled conditions

The SBRs were run at fairly constant conditions for more than 8 months before the first test and at least one month without any major issues before each experiment. The general performance of the SBRs are illustrated in the figures below.

a) Monitored Parameters of the SBRs

Soluble COD

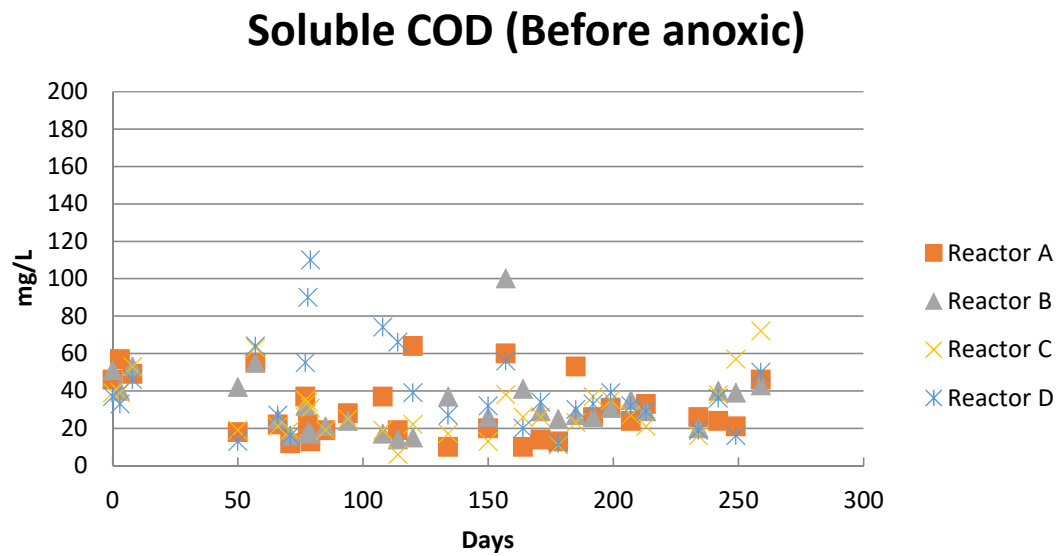


Figure 3.11: Soluble COD of the four SBRs before the start of the anoxic period.

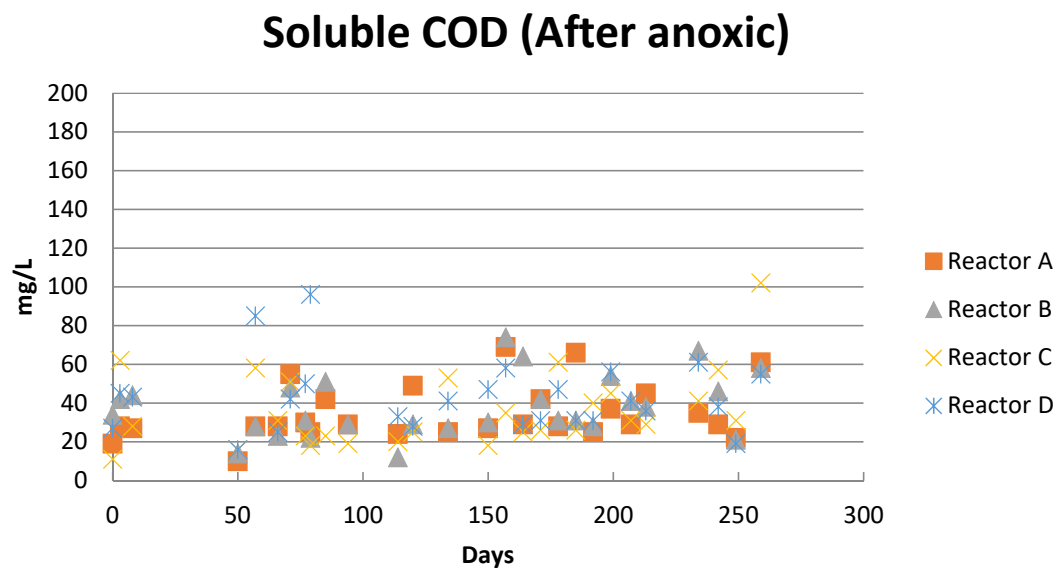


Figure 3.12: Soluble COD of the four SBRS after the start of the anoxic period / before being discharged.

As can be seen in Figure 3.11 and Figure 3.12, other than a few exceptions, the soluble COD of all 4 reactors over the 8-month period were mostly lower than 50mg/, but always higher than 20mg/L. This is true for both before the start of the anoxic period and remained roughly the same after. In fact, most of the soluble COD for post-anoxic denitrification was removed close to this level within 1-2 hours of aerobic period. This indicates it was not because of the length of the aeration zone. Instead this indicated that the remaining soluble COD at the end of aerobic period / start of the anoxic period resulted from non-biodegradable/ slowly biodegradable COD. It could not be utilised well by the activated sludge under both aerobic and anoxic conditions. It should also be noted that the carbon remaining for post-anoxic denitrification did not change in any significant amount regardless of what was used as the main carbon source in the SBR feeds (sugar, acetate, milk power, beef extract, etc.).

Ammonia

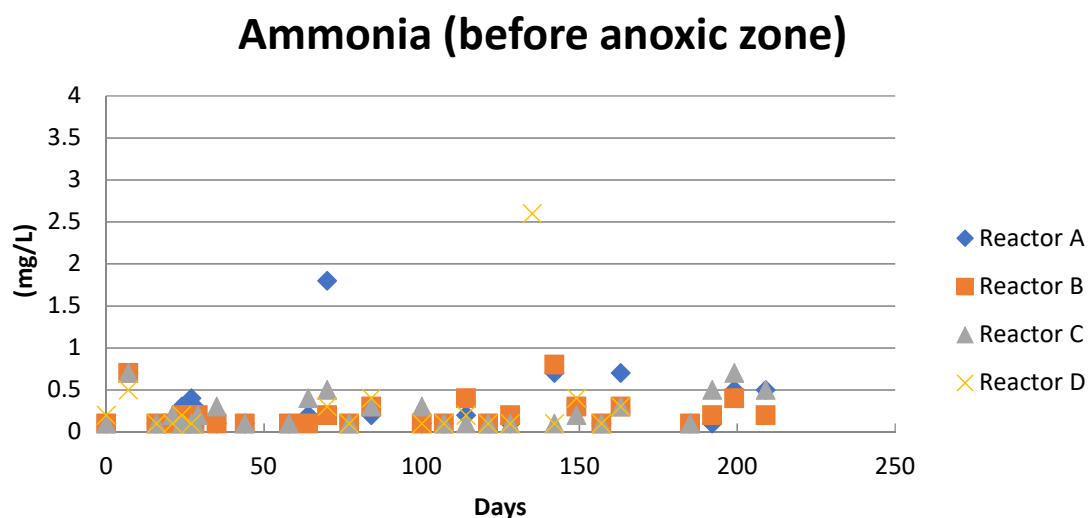


Figure 3.13: Ammonia concentration of the four SBRs before the start of the anoxic period.

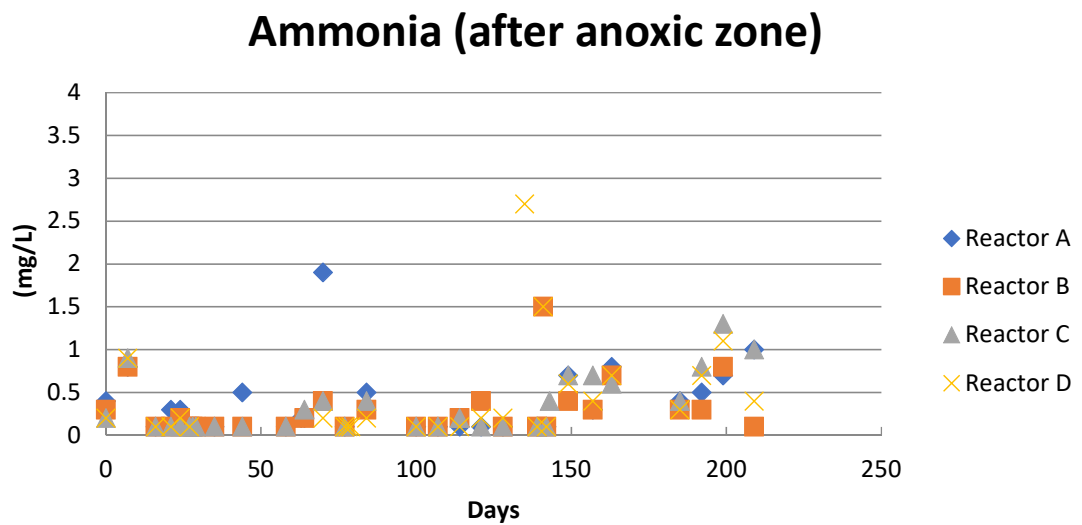


Figure 3.14: Ammonia concentration of the four SBRs after the start of the anoxic zones/ before being discharged.

Similar to the concentration of COD, other than the few exceptions, the ammonia concentrations of all four SBRs were largely unchanged before the anoxic and after anoxic zones as depicted in Figure 3.13 and Figure 3.14. Furthermore, most of the ammonia concentration recorded was lower than 1.0mg/L or even 0.5 mg/L, indicating almost complete nitrification has occurred in the early aerobic stage. An insignificant amount of ammonia was released by the microorganisms during the SBRs' anoxic stages.

Nitrate

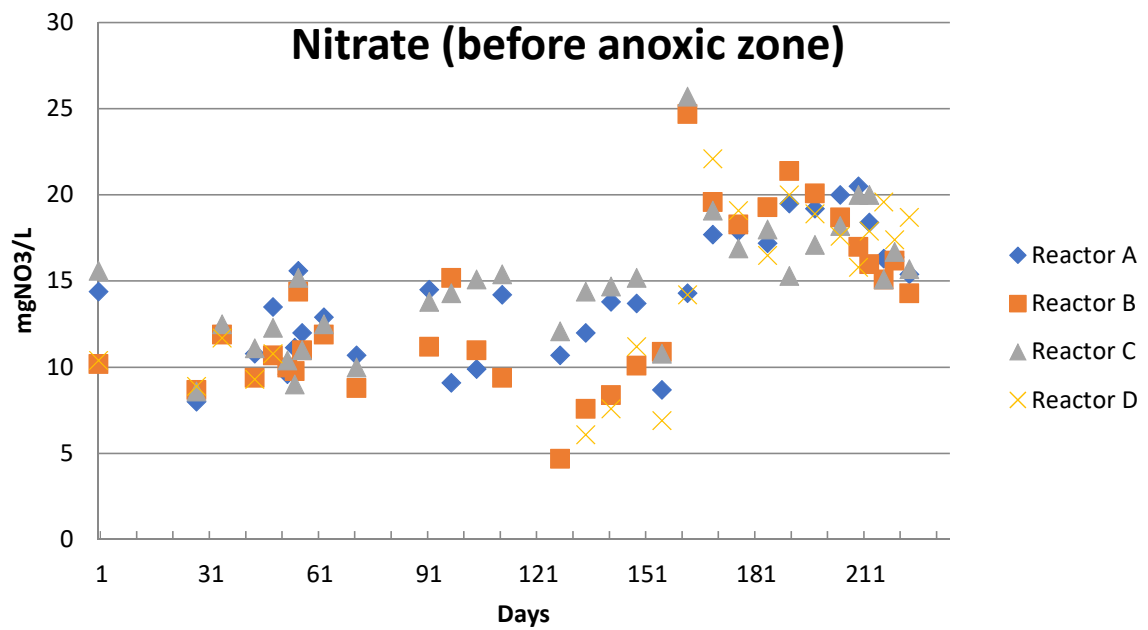


Figure 3.15: Nitrate concentration of the four SBRs before the start of the anoxic zones.

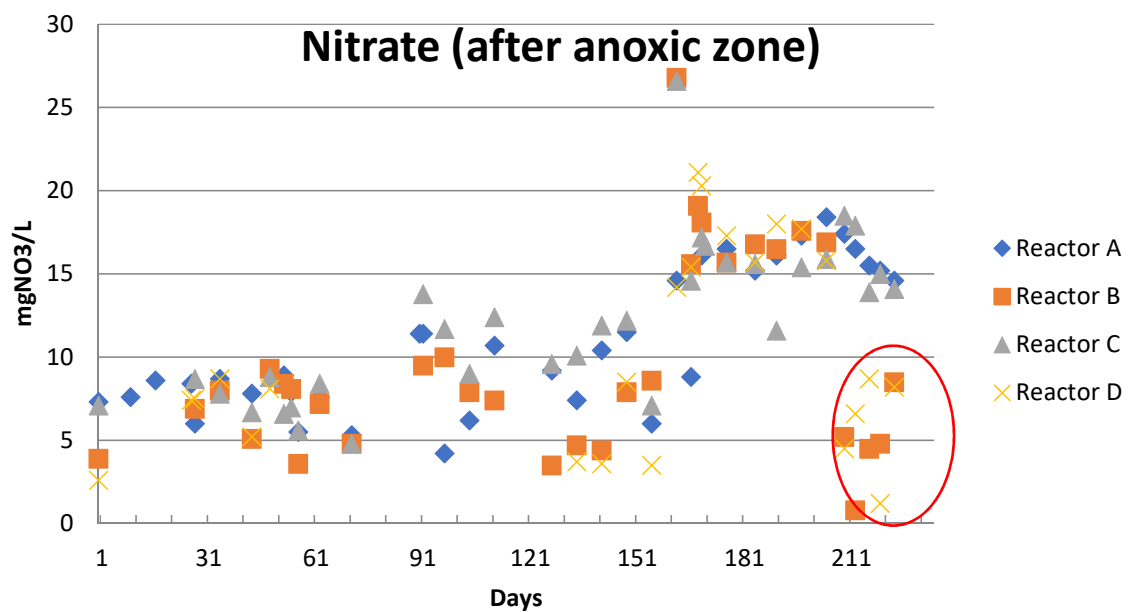


Figure 3.16: Nitrate concentration of the four SBRs after the start of the anoxic zones/ before being discharged.

It is clear that the nitrate concentration increased a lot after the 160th day of the experiment during both pre-anoxic and post-anoxic period. This was due to, as mentioned earlier, the synthetic influent mixture of sucrose with 50% beef extract and 50% sucrose being replaced by full beef extract. The TN of the influent increased from 29.7 ± 3.5 mg/L to almost double (55.6 ± 1.4 mg/L), while COD decreased from 658 ± 33 mg/L to 457 ± 27 mg/L.

Interestingly, it is noticeable that only nitrate (and MLSS presented in next session) was affected by these changes. The ammonia concentration still showed that almost complete nitrification still occurred after the change. The COD concentration also did not change significantly either. Another observation is that no nitrate accumulated in the system after each cycle, indicating that all remaining nitrate at the start of cycle would be denitrified regardless of the presence of sucrose in the feed (without sucrose, influent COD dropped by ~30%). In other words, there was no significant effect on the pre-anoxic/feeding zone of the SBR when additional sucrose was added.

The data in the red circle (Figure 3.17, showing nitrate concentration in Reactor B and Reactor D after the anoxic zone) was affected by adding sucrose as an external carbon source. More details are reported in section 4.4.1.

MLSS/MLVSS

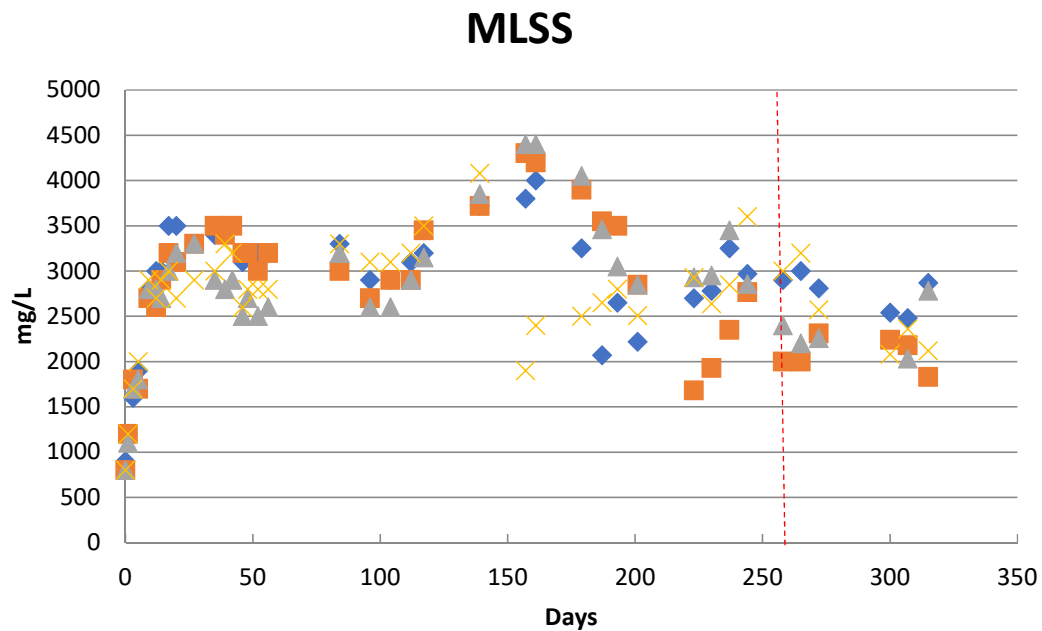


Figure 3.18: MLSS concentration of the four SBRs

The MLSS was monitored 100 days before the concentration of COD, ammonia, nitrate, hence the change of synthetic wastewater happened after the 260th day in Figure 3.18 (marked in red dot line). The change in the MLSS due to the change of synthetic wastewater is not as significant with a drop of around 500 mg/L from the 2500-3000mg/L range to 2000-2500 mg/L range. Also it should be noted that due to the influents are entirely soluble, the MLVSS of the four SBRs was found by SPSS to be not significantly different to the MLSS. Hence the MLSS were used interchangeably with the MLVSS for the SBRs.

Sludge bed/ SVI and turbidity of discharge

SVI of the four SBRs.

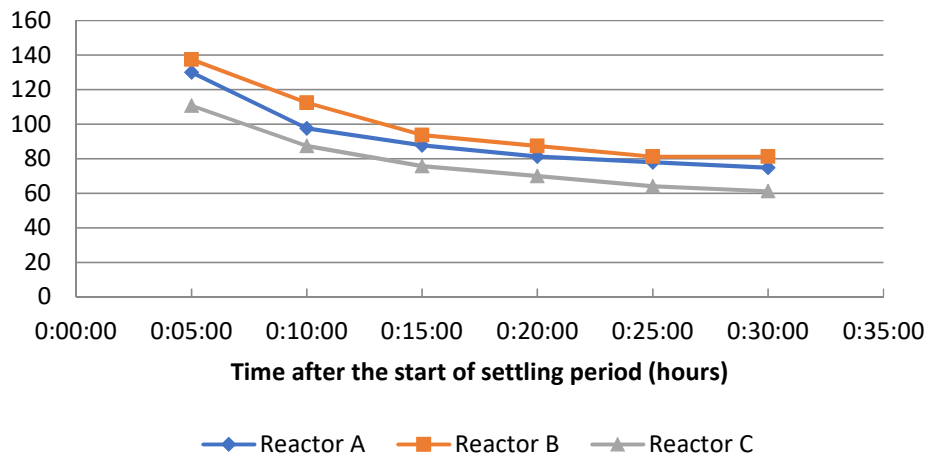


Figure 3.19: The sludge bed and SVI of the four SBRs.

The figure above shows the sludge bed depth of the SBRs systems during the 30 minutes after settling. All the reactors here showed a similar trend. The turbidity of effluents of all SBRs varied from 5-14 NTU.

Soluble Oxygen Uptake rate (SOUR)

Here is the SOUR of the four SBRs during the 2 minutes tested during the aerobic zone. The straight lines show that SOUR can be taken based directly on the slope.

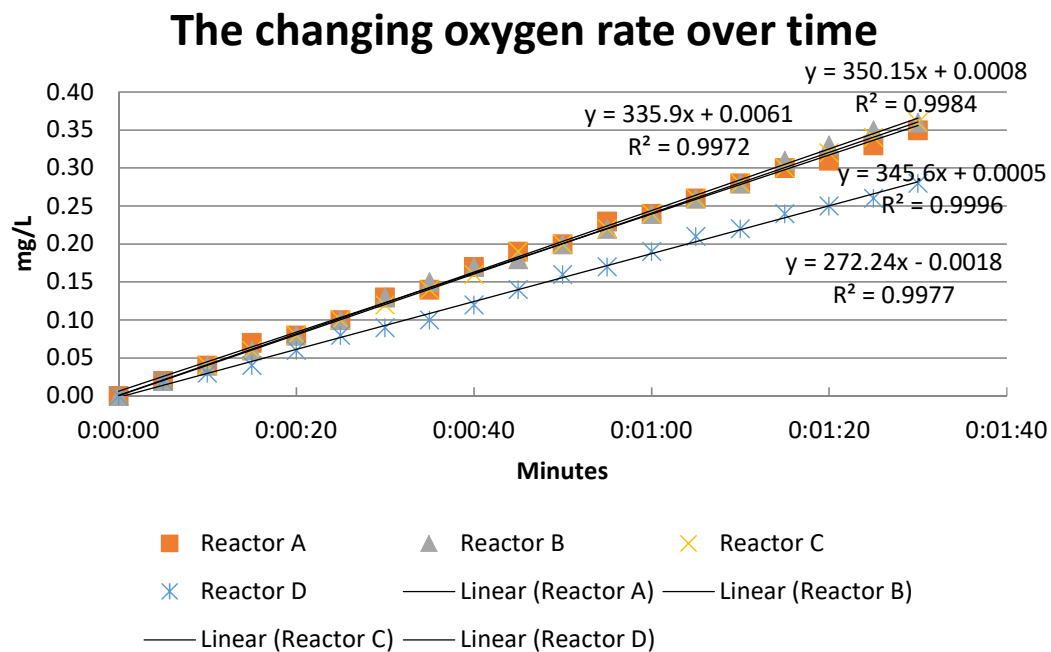


Figure 3.20: The SOUR of the SBRs.

The SOUR hence can be calculated as ranging from 272 – 350 mg O₂/L/day or 11.3 – 14.6 mg O₂/L/h.

b) Summary of SBRs' performance in stable conditions

The effluent characteristics of all SBRs at control condition are summarised in table below.

Table 3.8: The performance of all SBRs in stable conditions.

Effluent Parameters	Before anoxic	After anoxic/ Effluent
Soluble COD	37 ± 33 mg/L	42 ± 37 mg/L
Ammonia	0.24 ± 0.24 mg/L	0.33 ± 0.35 mg/L

Nitrate

- Before changing feed	11.1 ± 2.6 mg/L	7.7 ± 2.4 mg/L
- After changing feed	18.1 ± 2.4 mg/L	16.4 ± 4.3 mg/L*

MLVSS

- Before changing feed	3075 ± 521 mg/L
- After changing feed	2353 ± 290 mg/L

SVI	61 - 81

SOUR	11.3 - 14.6 mg O ₂ /L/h

pH	7.39 ± 0.43

Note: * not counting the data under the effect of sucrose as an additional carbon source (circled in red in Figure 3.8.h).

To sum up, the SBRs have showed almost complete nitrification over the testing period. However as expected, most of soluble biodegradable carbon was also used up during the initial aerobic zone, leading to a deficiency in carbon for effective denitrification in the subsequent anoxic zone. Up to 20 mg/L mostly in the form of nitrate (or up to 36% of influent TN) remained in the effluent. The SBR in this case has quite accurately portrayed the issue of a post-anoxic denitrification system when not enough soluble COD could reach the system's anoxic zone, resulting in overall poor denitrification.

3.9. The pilot plant

3.9.1. Introduction to the pilot plant

The pilot plant was modelled after and located next to a local municipal wastewater treatment plant (Sunbury WWTP) with a capacity of 6 ML/day (recently upgraded to 9 ML per day in 2016). Both the sludge seeded onto the pilot plant and the influent were pumped from the post-primary treatment of the Sunbury WWTP (to avoid large objects in initial sewage), before being further filtered with double layer mesh to remove larger particulates that may clog the pilot plant. Note that the wastewater characteristics of the pilot plant are likely to change compared to the actual treatment plant due to the mesh filtering. However, there is no alternative because the pilot plant is a scaled down version, and its tubes and pipes are not able to handle the same concentration of suspended solids as the treatment plant. Other than the influent characteristics, the biggest issue with the pilot plant (used in this research) is: other than operational parameters (SRT and HRT), other independent variables include physical plant data, influent data and kinetic model parameters of the pilot plant are fixed and could not be changed. This restricted the role of the pilot plant and what could be verified with it.

Disclaimer: The pilot plant was operated and monitored for more than 2 years mostly by another research student working on a different project (Yulian 2014). The author of this thesis only assisted him with the operation when sucrose was added to improve denitrification, and received permission to record and analyse the raw data in this research. No other experiment was carried out in the same period that could affect the results.

3.9.2. Pilot plant set-up

The diagram of the pilot plant is illustrated in Figure 3.21 below. It is a conventional MLE activated sludge system. Due to later modification made since the initial purchase, the pilot plant consists of one anoxic tank followed by 2 aerobic tanks, with the second tank operating at lower DO (2-3mg/L comparing 4-5mg/L of first tank) to reduce the DO concentration being recycled back by the Internal Recycle (IR) stream.

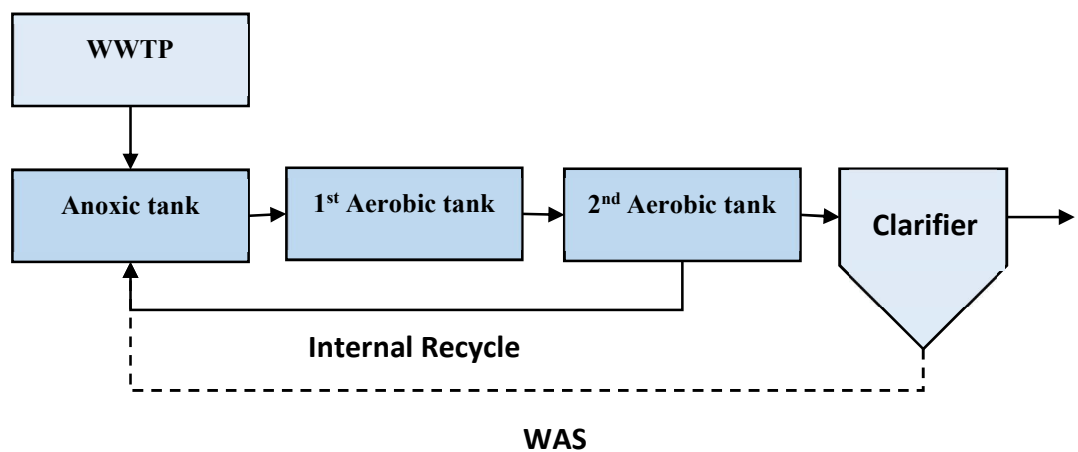


Figure 3.21: Diagram of the pilot plant



Figure 3.22: Photograph of the pilot plant (Yulian 2014).

Overall the pilot plant has a sludge age of approximately 12 days, with a total volume of 113L and influent flow rate of 3.8 - 4.0 L/h (or 91.2 – 96.0 L/d) producing a HRT of ~1.2 day. The internal recycle is roughly 16L /h, four times influent rate, and the Returned Activated Sludge (RAS) is 4 L/h.

3.9.3. Pilot plant influent characteristics and treatment performance

The pilot plant used in this study was monitored for 2 months, one month for the control period, and one for the sucrose testing period. It is, however, crucial to choose the monitoring period wisely (whether for certain hours during each day, or over a period of several weeks, or months), so that it can represent

the pilot plant's performance accurately. What needs to be avoided at all costs is when results of the experiment are affected by changes in the influent wastewater profile (caused by either drought, high rainfall, construction work, weekends, holiday periods, etc.). In this case, selecting a shorter period of 1-2 months that was not affected by external factors and work within that timeframe is better than prolonging monitoring of the pilot plant. The COD profile (both influent and effluent) of the pilot plant during the control period is summarised in Figure 3.23 and Figure 3.24 below.

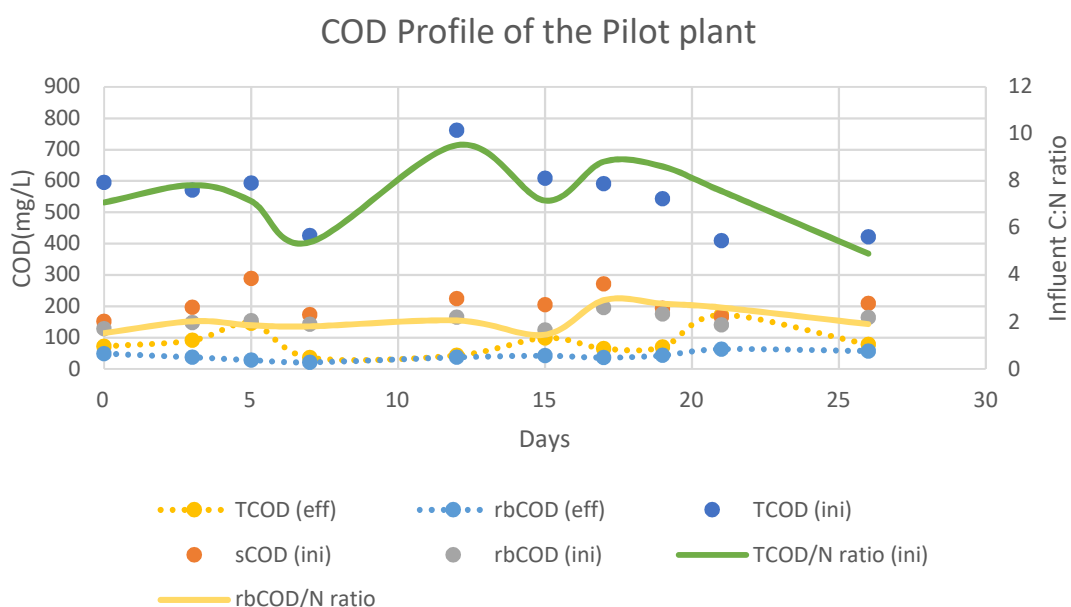


Figure 3.23: COD profile of influent and effluent of the pilot plant.

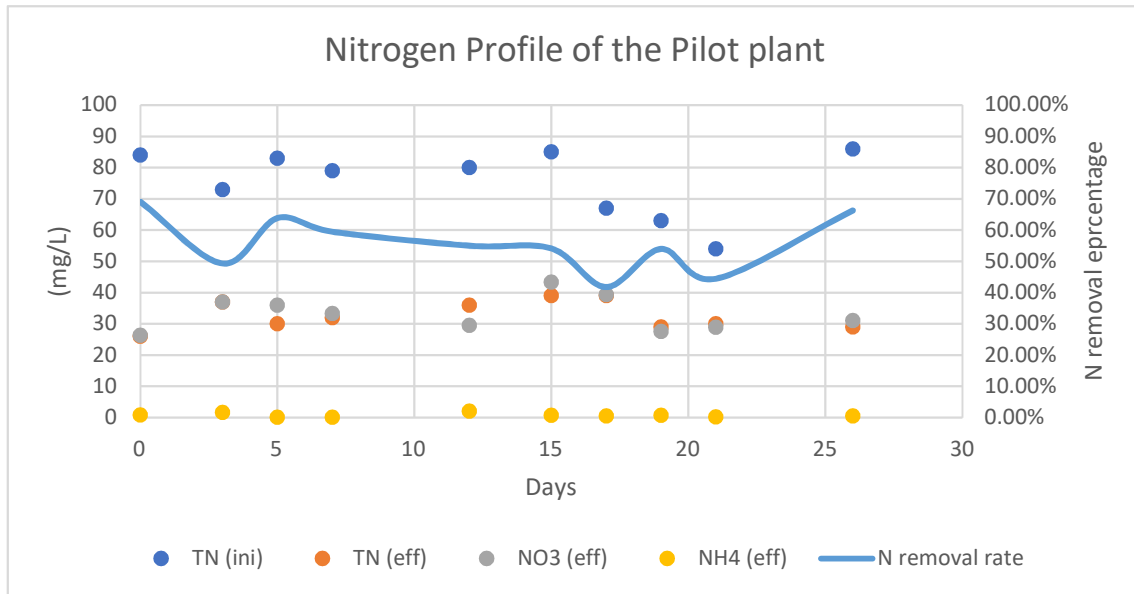


Figure 3.24: N profile of influent and effluent of the pilot plant.

It can be seen that despite the high initial TCOD/N ratio of 5-10 in the influent which should provide complete denitrification for an MLE system such as the pilot plant, the crucial rbCOD/N ratio in the influent was actually less than 2.0 the whole time. This leads to the pilot only producing a nitrogen removal rate as high as 70% but also as low as 41% during the control period. The effluent's total nitrogen level could reach 45mg/L and was much higher than the required standard. In conclusion, the default status of the pilot plant revealed a very poor denitrification performance, thus making it an ideal subject for external carbon testing.

3.10. Fermentation batch test

3.10.1. General Introduction to the fermentation batch tests

Unlike sucrose which is a constant and uniform substance, “fermented sludge” is a collective name referring to various WWTPs’ waste products that could have undergone various fermentation conditions. This has led to the characteristics and properties of fermented sludge being varied according to the literature.

This study examines the fermentation and dark fermentation of Wasted Activated Sludge (WAS) and anaerobic digested biosolids, before assessing the suitability of these fermented sludge types as external carbon sources for denitrification. This was because both of these two substances: firstly, have high VFA which plays a key role in external carbon sources (Wu et al. 2014; Xie et al. 2012; Yang, Wang & Zhou 2012); secondly, the secondary sludge provides consistent microorganism varieties for fermentation (Perot 1989)); thirdly, they are already readily available at every wastewater treatment plant; fourthly, it can be used in existing continuous anaerobic digester system for fermentation and does not significantly affect the biogas production process; and fifthly, utilising even a portion of these two waste products will help to reduce the amount of sludge and biosolids that each WWTP had to dispose of each year. This accounts for >60% of the total investment in a standard WWTP (Ma et al. 2015; Saby, Djafer & Chen 2002). Likewise, it also eliminates the problems associated with increased sludge mass due to added external carbon (Ma et al. 2015; Strong, McDonald & Gapes 2011).

All current WAS and anaerobic digested biosolids treatment methods, include thermal, chemical and advanced oxidation, will cause one or more serious

problems for the environment (Wei et al. 2003). This makes the development of sustainable processes to remove WAS crucial and help to operation costs and reduce damage done to the environment (Jenicek et al. 2014). For example, Guo et al. (2016) used fermented WAS as an external carbon source for denitrification in a partial nitrification system and it reduced the WAS production rate by 21.6%. The fermented sludge batch tests in this thesis will be used to test various fermentation options employing different independent variables. After that, two selected fermented sludge options will be assessed for denitrification potential in Chapter 6 using most of the methodology previously mentioned in this chapter (i.e. SDNR and Mumax Batch Tests, the NMB Models, the Biowin software, and the laboratory-scale SBRs).

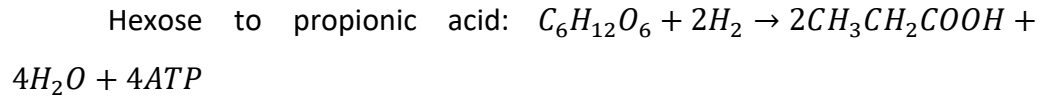
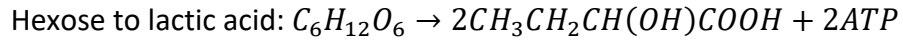
3.10.2. Fermentation Batch Tests set-up

More details concerning the set-up will be documented in Chapter 5. To sum up here, twenty-one fermentation batch tests of 500mL short-bottle were established to analyse various scenarios of fermentation and dark fermentation.

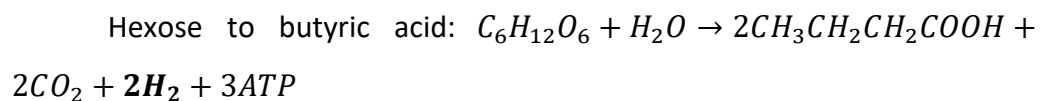
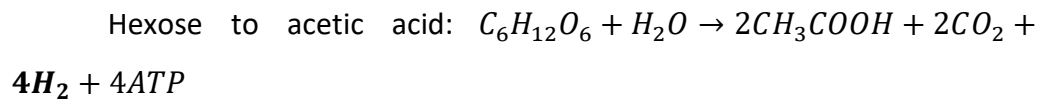
a) The difference in fermentation types

The performance of two fermentation types - standard fermentation and dark fermentation on rbCOD/TN and VFA production - will be assessed based on their optimised pH and temperatures according to literature described in section 5.2.1. The principles of both fermentation and dark fermentation are similar in nature, where the chosen feeds in this case are hydrolysed under their favoured conditions to break down the feed components into simple carbohydrates and short-chained or volatile fatty acids (VFA) (Zhang et al. 2010). The main difference is the objectives of each type of fermentation. Standard fermentation focuses on

generating SCFAs, mostly lactic and propionic acids, to work as an external carbon source for denitrification. The reaction pathway is:



The dark-fermentation's main objective, however, is to produce biohydrogen. It can either be used to improve the combustion efficiency of methane gas (generated from onsite anaerobic digester) and form an alternative automobile engine fuel such as Hythane, or collected separately for other applications. The fermented sludge generated from dark fermentation in this case will be a by-product, making the generation of dark fermented sludge more feasible in many situations when fermented sludge is not. Unlike fermented sludge, dark fermentation aims to break the feeds into acetic acid and butyric acid through the following reaction pathway:



Similarly, Park et al. (2014) stated that lactic acid and propionic acid indicated an unfavourable state for hydrogen production during fermentation. Meanwhile the formation of acetic acid and butyric acid (with butyric acid as major end product) exclusively results in hydrogen production. However, the increased acetic acid production does not signal an improved H₂ yield.

b) The difference in feed types

While the practice of primary sludge fermentation for onsite VFA production is already well established worldwide, the reliability of VFA generation from primary sludge is still not generally adequate for nitrate removal (Yuan, Sparling & Oleszkiewicz 2009). Fermentation of WAS and biosolids is an alternative option, despite many drawbacks such as longer hydrolysis time (Turovskiy & Mathai 2005) and the potential introduction of nitrogen and phosphorus back into the system (Danesh & Oleszkiewicz 1997). This was due to the fact unlike primary sludge, a much larger mass of activated sludge, hence WAS and biosolids can be generated from municipal WWTPs.

While the study of fermented WAS is not new and has been experimented upon since the 1980s (Bi, Li & Hu 2014; Funamizu et al. 1997; Gao et al. 2010; Guo et al. 2016; Liu et al. 2016; Tong & Chen 2009; Wang et al. 2015), only a few analyses have looked at sludge fermentation as part of an existing continuous anaerobic digester process. It is where the inoculum and infrastructure from anaerobic digester can be utilised directly for fermentation. This is what makes the fermented sludge characteristics of this study significantly different from other studies (see Chapter 5). The performance of fermentation and dark fermentation using either municipal WWTPs' WAS or anaerobic digested biosolids is assessed in section 5.2.2.

c) The presence of additional carbohydrates

Finally, the effects of additional carbohydrate sources on fermentation and dark fermentation are examined in section 5.2.3. As previously mentioned, the fermentation of WAS and biosolids can pose the risk of releasing nitrogen back

into the liquid phase. This can become especially significant if the C:N ratio of fermented sludge ends is low. For example, WAS fermentation in Feng, Chen & Zheng (2009) study was found to have a C:N ratio of 7:1, much lower than the minimum suggested C:N ratio of 20:1.

One of the methods suggested in the literature is adding additional carbohydrate substances like rice (Feng, Chen & Zheng 2009) and grass silage (Pakarinen, Lehtomäki & Rintala 2008) to the sludge fermentation process. This may improve both the C:N ratio (for fermented sludge), and H₂ production (for dark-fermentation).

The chosen additional carbohydrate in this case was sucrose, and it is studied in depth in Chapter 4, so any similar findings here can be linked back to the study of sucrose as external carbon source. Secondly, sucrose is a major end-product of soft cellulose hydrolysis, which has the same favourable conditions as sludge fermentation (such as lower pH and temperature). Thus, by looking at the amount of initial added sucrose, and the additional amount of soluble COD generated at the end, how much sucrose will be lost during the fermentation process can be calculated. While the study of 'additional carbohydrates in fermentation' in this thesis will be quite general, it can still provide a simple evaluation for the viability of sludge fermentation and cellulose hydrolysis combination.

3.11. Chapter summary

In summary, this chapter has addressed the standard simulation methodology and how it is unviable and not applicable when it comes to this research. As an alternative, a cross-verification methodology was proposed and consisted of various physical, conceptual and mathematical models, not only to cross-check results, but also to provide a fuller understanding on the study of any external carbon sources. The models include:

- a) The Specific Denitrification Rate (SDNR) Batch tests
- b) The Nitrogen Mass Balance (NMB) Conceptual Model
- c) The Growth Yields and Rates (Mumax) Batch Test
- d) The Biowin Software Simulations Model
- e) The Lab-scale Sequencing Batch Reactors (SBRs) Tests
- f) The pilot-scale Wastewater Treatment Plant

The chapter then classified each model into types, time and scales, microbial level and level of microorganism detail. Each model's set-up and specifications in this dissertation were then explained in detail, include their roles. The set-up of the fermentation batch tests that were used to generate fermentation sludge was also presented here. Finally, the cross-verification methodology was described here and it will be used to test the two external carbon sources: sucrose and fermented sludge in Chapter 4 and Chapter 6 respectively.



Chapter 4

Sucrose as Alternative Carbon Source

Chapter 4 SUCROSE AS AN ALTERNATIVE EXTERNAL CARBON

SOURCE

4.1 Introduction

The issue of slow denitrification rate is one of the biggest challenges facing the municipal wastewater treatment industry. This problem is caused by the lack of readily biodegradable organic carbon (rbCOD) in the anoxic zone (Shi et al., 2015).

To challenge this, one of those alternatives is adding an external carbon source directly into the anoxic zone. The advantages of this option are: firstly, it is easy to implement; secondly, it requires little modifications to an existing WWTP; and thirdly, it can meet both the short-term and long-term treatment standards. Hence this chapter looks at industrial-grade sucrose, a by-product of the table sugar refining process, and examine its potential as an external carbon source. Industrial-grade sucrose was chosen mainly not only due to its availability locally as a by-product rich in carbon, but also for its insignificant nitrogen content. Furthermore, sucrose retains its consistency and uniformity of characteristics regardless of the type and storing method. These characteristics not only make sucrose suitable as a carbon source, but also a very useful subject to research in establishing a consistent methodology for future studies of other external carbon sources.

Another goal of this chapter was to execute the experimental methodology being showed in Chapter 3 (also in Figure 4.1 below) to assess the potential use sucrose for denitrification. If the methodology is proven to be

successful with a stable external carbon source like sucrose, there will be foundation for it to be applied on the other carbon sources.

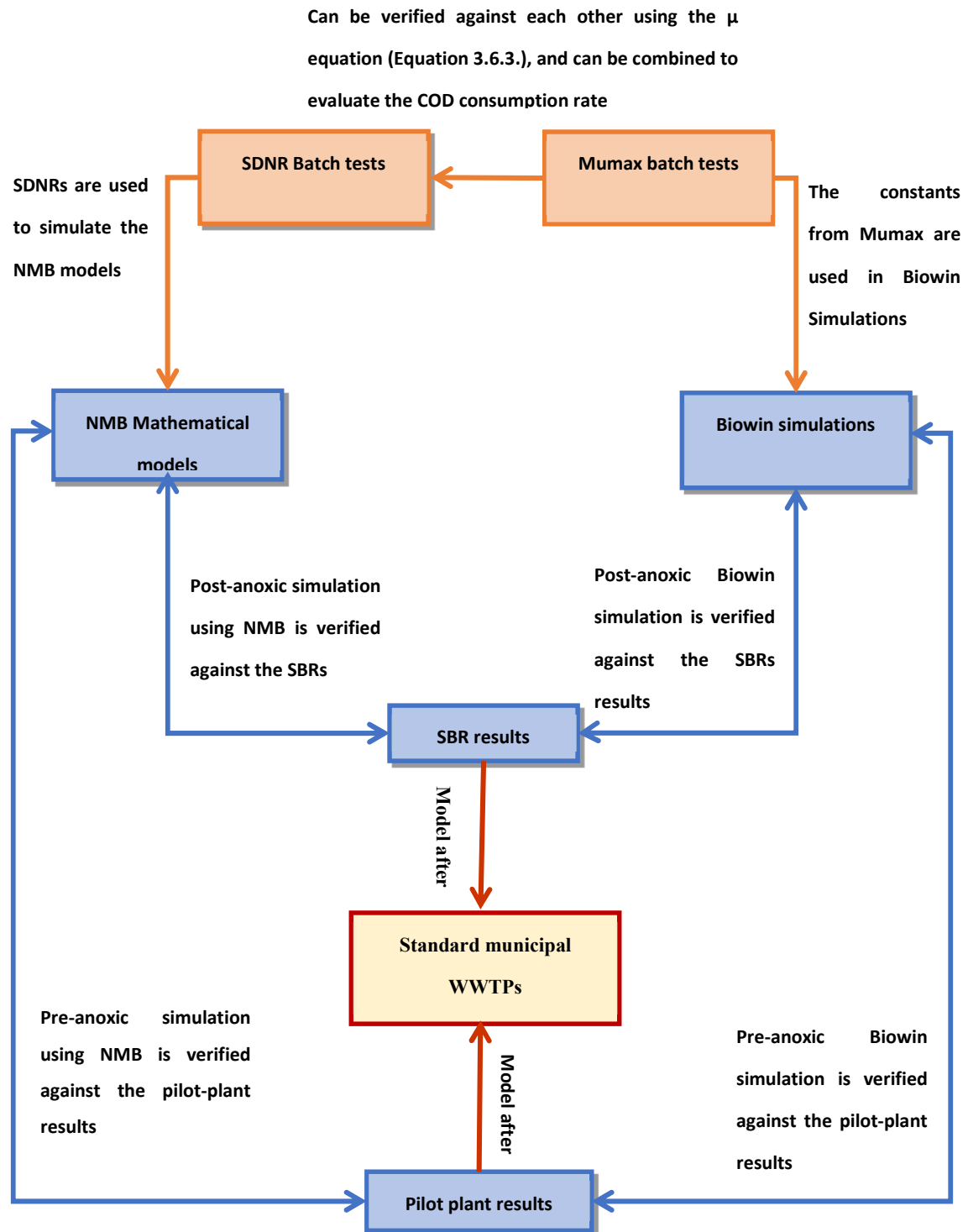


Figure 4.1: The relationships between different simulation models used in this chapter

Note: most of Chapter 4 has been published, or under review of the following papers:

Published

Phung, D.Y., Yulian, J. 2014a, 'The effect of sucrose on denitrification through simulation, lab-scaled batch tests and pilot plants', Science & Technology Development Journal, vol. 17, pp. 22-32.

Phung, D.Y., Yulian, J. 2014b, 'The effects of temperature on specific denitrification rates of sucrose', Science & Technology Development Journal, vol. 17, pp. 86-94.

Phung, D. 2015, 'Effects of sucrose as an external carbon source on denitrification growth rate (μ S) and yield efficiency (YH) through Mumax batch tests.', Journal of Science and Technology, vol. 53, no. 5A, pp. 120-7.

Phung, D.P., Phung, S. 2016, 'Assess the Effects of Sucrose on Post-Anoxic Denitrification through Lab-Scaled Sequence Batch Reactors (SBRs)', Journal of Environmental Science and Engineering B, vol. 5, no. 10, pp. 476-82.

4.2 Materials and Methods

4.2.1 Chemicals and Inoculums

The used sucrose for all experiments stated in this chapter is a by-product of the sugar-refining process. It is a liquid with a very high viscosity, light opaque yellow in colour and remains fairly stable in room condition. It has an average COD constant of 1,125 mg/g despite being stored at room temperature for months (theoretical COD for sucrose is 1,123 mg/g) and insignificant NH_4 and NO_3 concentration (<0.1 mg/L).

The lab-scale SBR and some batch experiments (unless stated otherwise) used synthetic wastewater consisting of commercial beef extract as the main organic carbon and organic nitrogen source. The nutrient and trace metal solutions for this synthetic wastewater were based on Hu et al., 2010 consisting of the following: 10 mg CaCl_2/L , 18 mg $\text{K}_2\text{HPO}_4/\text{L}$, 11 mg $\text{KH}_2\text{PO}_4/\text{L}$, 10 mg MgSO_4/L , 0.15 mg CoCl_2/L , 0.03 mg CuSO_4/L , 10 mg FeSO_4/L , 0.15 mg $\text{H}_3\text{BO}_3/\text{L}$, 0.18 mg KI/L , 0.12 mg MnCl_2/L , 0.06 mg NaMoO_4/L and 0.12 mg ZnSO_4/L . Most of these chemicals were supplied by Science Supply Australia.

4.2.2 Denitrifying biomass

The SBRs and batch tests were seeded with raw sludge taken from the pre-anoxic zone of Sunbury WWTP, transported in an ice box to the laboratory, where they were sieved to remove any large particles before being fed into the SBRs or any batch tests. Referring to the SBRs, the sludge would have to operate under an SBR cycle for at least 3 weeks (2 SRTs) before any experiments can be carried out. For this study, the SBRs were run in stable conditions for roughly 6 months prior to the start of this experiment. For the two tested SBRs to acclimate with sucrose, a sucrose solution with COD concentrations of 150-170 mg/L was added

into the influent tank for 3 weeks before the experiment commenced. A later experiment, however, found that even 24 hours were enough for the sludge to acclimate to sucrose.

For the pilot plant, because it was located right next to the WWTP, the sludge was taken directly from there.

4.2.3 Analytical Method

COD, TN, Ammonia, Nitrate and Volatile Fatty Acids (VFAs) were sampled and analysed using DR 5000 HACH standard methods (Methods 8000, 10072, 10031, 10020 and 8196, respectively). pH and DO were tested by using the Mettler Toledo S20 Seveneasy pH meter and YSI 5100 Dissolved Oxygen Meter. The rbCOD in this case was measured by the filtration of soluble fermented biosolids through a 0.45µm membrane filtration according to Melcer (2003), before being tested with a COD reagent kit. This was done because most of the particulate soluble COD would have been absorbed into the sludge, and the measured COD would be very close to actual rbCOD (difference of <5 mg/L).

Whenever synthetic wastewater is involved (in the SBRs, some Mumax and SDNR batch tests), due to the soluble nature of synthetic wastewater, the Mixed Liquor Volatile Suspended Solid (MLVSS) of the sludge was found (using the SPSS program) to be insignificantly different to the Mixed Liquor Suspended Solid (MLSS). Consequently, these two values were interchanged during those experiments.

The MLSS was measured the same way with Total Solids, where 100 mL samples were filtered and dried overnight in an oven at 105°C. Meanwhile the MLVSS was measured by igniting the MLSS sample at 550°C for 30 minutes to remove volatile solids.

4.3 Experimental set-up

4.3.1 Sequencing Batch Reactors (SBRs)

The operational and synthetic wastewater parameters for the SBR experiment are summarised in Table 1. The nutrient concentration and trace metal solutions are noted in section 2.1.

Table 4.1: Synthetic wastewater characteristics.

No.	Parameters	Characteristics
1	Influent rate	2 L per cycle
2	TN	55.6 ± 1.4 mg/L
3	COD	456.8 ± 26.5 mg/L
4	PO ₄	17.25 mg/L
5	PO ₄ -P	5.63 mg/L
6	Temperature	20-25 °C

Overall, the SBRs set-up followed a standard post-anoxic denitrification system. The main difference of the SBRs compared to the standard wastewater treatment plants was its time-dependent nature (as opposed to space-dependence). In this way it provided a much more consistent HRT and SRT. This gave the SBRs great advantages in monitoring the denitrification process and

allowing the calculation of SDNRs directly from data output which normally is not possible in a standard WWTP.

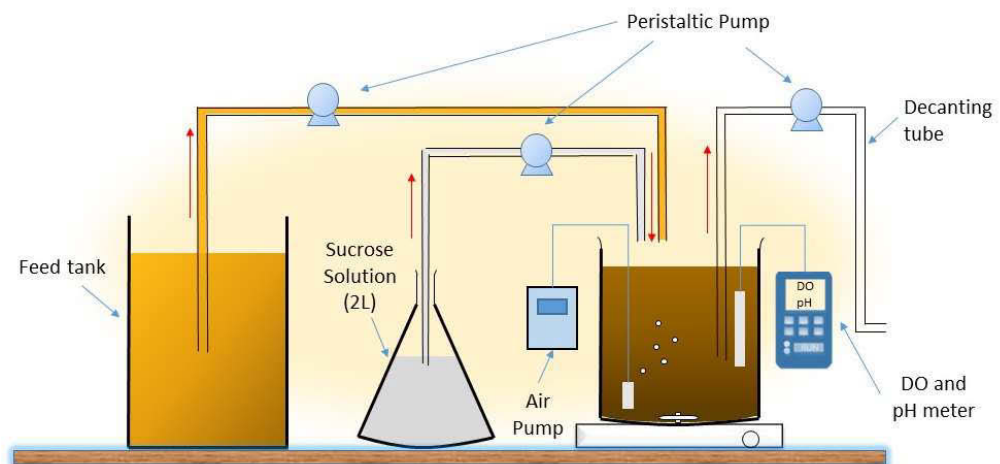


Figure 4.2: Basic diagram of a SBR.

Four SBRs were utilised in this experiment and how they work is shown in Figure 4.2. The SBRs ran continuously 24 hours a day, 7 days a week. One cycle of the SBR was 8 hours long, consisting of 30 minutes of unmixed feeding, 240 minutes of aeration, 110 minutes of anoxic mixing, 70 minutes of settling and 25 minutes of decanting. The 5-minute gap at the end of each cycle helped to prevent issues relating to timing device error, where feeding and decanting occurred at the same time leading to the loss of sludge or influent.

In addition, due to no aeration occurring during the feeding period, this 30-minute duration served as a pre-anoxic zone to remove or 'reset' the accumulated nitrate from the previous cycle. This design was done on purpose

because accumulation of nitrate inside the system is something that a real post-anoxic treatment system does not have to deal with.

Each reactor held an effective volume of approximately 4 L, with the decant volume of 50% or 2 L per cycle. During the aerobic phase, the DO level was constantly kept higher than 3 mg/L to ensure the maximum nitrification occurred. That produced a HRT of 16 hours. The Sludge Retention Time (SRT) was selected to be 11.5 ± 0.5 days based on what has been suggested elsewhere (Kargi et al., 2004; Obaja et al., 2005). It provided an approximate MLVSS of 2,200-2,600 mg/L during the course of the experiment, which was around the range of $2,200 \pm 200$ mg/L being reported in similar study (Wu et al., 2018).

The SBRs ran for more than 6 months before commencing the experiment and to stabilise the denitrification process (Sun et al., 2016). However, the data collected for this specific experiment was only for 7 weeks or roughly 4 SRTs of the SBRs. For the first 3.5 weeks (22-24 days), no external carbon source was used. During the next 3.5 weeks, a sucrose solution was pumped into the post-anoxic cycle of the test SBRs, to increase the rbCOD of the reactors by 150-170 mg/L. The SBRs were monitored twice a week for NO_3^- , NH_4^+ , TN, COD, pH, and DO and the impact of sucrose on the system's denitrification was assessed.

4.3.2 SDNR test and NMB model

The SDNR batch test was set up according to Cherchi et al. (2009) and Dold et al. (2008) and was a 1L of anoxic reactor, consisting of 200mL of settled sludge from the nitrification stage of Sunbury WWTP. After dilution, this provided approximately 1500-2000 mg/L MLVSS in the final reactor. Notably, the MLVSS

inside each reactor did not have to be exact, since the difference in mixed liquor biomass may affect the overall denitrification removal. It will not, however, affect the calculated SDNR (Peng et al., 2007). The sludge collected from the nitrification stage of the WWTP plant was spiked with excessive nitrate (>30mg/L) and a designated dosage of the carbon sources (roughly 300-350mgCOD/L of sucrose, wastewater or a combination of the two). Nitrate concentration was monitored for up to 4 hours depending on when denitrification of the batch tests stopped.

Theoretically, if a straight line was obtained, the SDNR and the maximum specific denitrification rate of tested carbon could be calculated from the slope of removed NO₃-N concentration:

Equation 4.3-1 SDNR calculation equation

$$SDNR = \frac{\Delta(NO_3 \cdot N + 0.6NO_2 \cdot N)}{MLVSS \times V \times t}$$

However, in this case the SDNR was calculated from the slope of line-of-best-fit over all data points using Excel and R-Statistics. Originally nitrite was also tested but an insignificant amount was detected as expected due to its unstable nature. For this reason, nitrite sampling was later omitted from this experiment.

Potassium nitrate, ammonia chloride, and potassium hydrogen orthophosphorus were added into the sludge slurry so that a concentration of 30 mg NO₃-N/L, 35 mg NH₄-N/L and 9 mg PO₄-P/L, respectively, would be created in each reactor. This should provide excessive nutrient sources for the denitrification process reaching its maximum denitrification rate (Dold et al., 2008).

The reactors were mixed gently and covered to limit contact with the atmosphere, hence minimising the likelihood of nitrification occurring. DO probe

was used to monitor the DO level inside each reactor. The results of the first 30 minutes were recorded, but based on duplicates tests, were ignored in the final calculation due to the unstable acclimation period of each batch test. Nitrate and COD samples were taken and analysed every half hour. MLSS and ammonia were tested before and at the end of the experiment, the latter serving to ensure that very limited nitrification occurred.

The batch tests were conducted at different temperatures and converted back to 20°C using the SDNR temperature-converted equation (Metcalf et al., 2003) below:

Equation 4.3-2 SDNR temperature conversion equation

$$SDNR(t) = SDNR(20) \times 1.026^{(t-20^{\circ}C)}$$

Based on the SDNR of the system, the NMB model could then be built to predict the denitrification performance of the pilot-scale system. The detailed calculations for the NMB model and rationale are attached in Appendices VI and VII of this report. In summary, the expected effluent for post-anoxic denitrification based on the NMB model will be:

Equation 4.3-3: Post-anoxic denitrification equation based on the NMB model

$$NO_{3\text{ eff}} = 80\%TN_{ini} - SDNR \times MLVSS - NH_{4\text{ eff}}$$

While the expected effluent for pre-anoxic denitrification based on NMB model will be:

Equation 4.3-4 Expected effluent for post-anoxic denitrification equation

$$NO_{3\text{ effluent}} = \frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}}(mg/L)$$

$$\text{But only when } SDNR \geq \frac{\left(\frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}} \right) \times IR}{v + IR} (mg/L)$$

Where x is the initial TN, v is the flow rate of the influent, IR is the Internal recycle Rate

4.3.3 Mumax Batch test and Biowin model

The Mumax test set-up was also based on Dold et al. (2008) and the rationale was very similar to the SDNR test, except that it increased the amount of excessive nutrients, and reduced the population of denitrifier in the reactors. Thus instead of focusing on the denitrification rate, it focused on the increase in the denitrification rate over time, which is caused by denitrifier growth rate.

The tests were conducted in 1-L cylindrical closed vessels, each with an inlet for carbon dioxide gas to control the pH, and an outlet to relieve pressures. They were mixed gently using a magnetic stirrer plate to prevent aeration by the atmosphere, and temperature controlled at 20 °C. Before beginning the test, a small amount of approximately 50 mg/L MLVSS from the SBR (already set to acclimate to sucrose) was added into the reactor. Sucrose, synthetic wastewater or a mixture of both were added to make an initial rbCOD of 480-550 mg/L. Diluted KNO₃, NH₄Cl and KH₂PO₄ solutions were added to make an initial concentration of approximately 220-250 mg/L, 60 mg/L and 30 mg/L of nitrate, ammonia and phosphorus, respectively. These were deliberately excess amounts to ensure

there was no restriction on anoxic biomass growth. Finally, the reactors were mixed with post-nitrification decanted substrate from the SBR to fill up the 1 L. The filtered decanted substrate will ensure that the denitrifier can quickly adapt to the new environment in the batch tests.

Samples were taken from the batch tests 3 times a day, every 3 hours, with pH being monitored regularly to keep the pH within the 6.9-7.3 range. Carbon dioxide gas and calcium carbonate were added and adjusted in the reactor continuously to control the pH. The reactors were then sealed for the duration of the experiments, except when the samples were taken out. The total number of samples removed from each reactor for the duration of the experiment was less than 100 mL, ensuring at least 90% of initial volume remained at the end of the batch tests. The batch test set-up is shown in Figure 4.3 below.

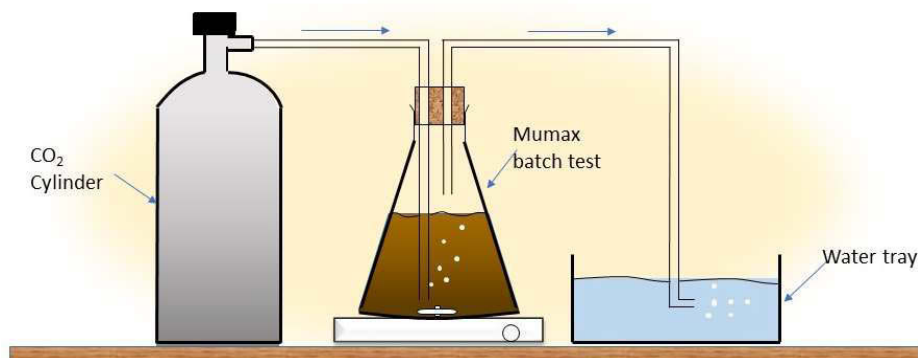


Figure 4.3: The Mumax batch test set up.

The main objective of the Mumax experiment was to record and plot the nitrate reduction over time ($S_{NO_3,t}$ over t) on a graph. Then based on the graph and

known variables, the data could be submitted into a mathematical program like Wolfram Mathematica to best-fit the graph into the Mumax equation below and find the best growth yield (Y_H) and growth rate value (μ_S):

Equation 4.3-5 Mumax equation

$$S_{NOI} = S_{NO0} - \frac{1 - Y_M}{2.86} \times \frac{\mu_S \times X_{S,0}}{Y_M \times (\mu_S - b_S)} \times [e^{((\mu_S - b_S)t)} - 1]$$

The starting Biomass ($X_{S,0}$) was measured based on initial COD of the biomass, while the decay rate was subjected to default literature value of 0.04. Y_H and μ_S could then be input into simulation software like Biowin to simulate the performance of sucrose as an external carbon source.

4.3.4 Pilot-scale plant

A pilot-scale plant modelling a municipal WWTP of 10 ML/day was developed. It was a standard MLE set-up with the internal recycle being pumped from the end of the aerobic zone back to the anoxic zone for denitrification. The pilot-plant set up is illustrated in Figure 4.4.

Each tank held an effective volume of approximately 37L, with the first tank being anoxic, and the second and third tanks being aerobic ($DO > 3.0$ mg/L). The pilot plant's semi-filtered influent was pumped straight from the municipal WWTP equalisation tank at a flow rate of approximately 3.5-3.8L/h, making a HRT of roughly 10 hours for each tank. The influent had to be semi-filtered, otherwise due to the scale of the pilot plant, the larger organic matter from the WWTP would have clogged all of the tubes in the pilot plant. The SRT was about 11.5 days.

Since the temperature inside the reactor at night could drop to as low as 10 °C, this may affect up to 60-70% of denitrifier growth rate and denitrification

rate (Cherchi et al., 2009). A heater served to heat and retain the temperature inside the reactor at $> 15^{\circ}\text{C}$.

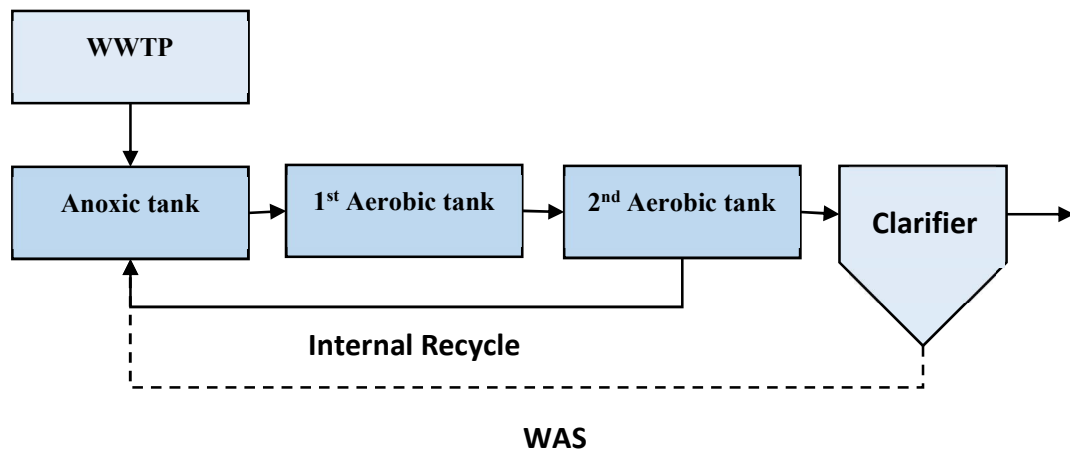


Figure 4.4: Schematic diagram of the pilot plant.

The pilot plant ran for more than one year but the data collected for this specific experiment was only for 2 months. For the first month, no external carbon source was used. During the second month, a sucrose solution was pumped into the anoxic tank to increase the influent rbCOD by 170 mg COD/L. The pilot plant's performance was monitored and the effect of sucrose on the system denitrification was assessed.

4.4 Results and discussion

4.4.1 Results from SDNR Tests

Before NMB models can be set up it was necessary to find the SDNR of sucrose. The denitrification potentials or SDNRs of sucrose and mixture of sucrose and wastewater based on SDNR tests are listed in Table 4.2.

Table 4.2: SDNR of sucrose and sucrose + wastewater mixtures

Sampl es	ΔN H_4	Measured value				Converted value (to 20°C)			$\Delta mgCOD$ / $\Delta mgNO_3$
		SDNR ($mgNO_3/h$) gMLVSS/h)	Adjusted R^2	Standard error	SDNR at 95% Confidence interval	Converted SDNR to 20°C	Standard error	SDNR at 95% Confidence interval	
AS (20)	- 0.06	1.53	0.9550	0.10	1.36-1.70	1.53	0.10	1.36-1.70	4.38±0.27
A50(2 0)	+0.2 8	2.57	0.9573	0.16	2.29-2.85	2.57	0.16	2.29-2.85	5.13±0.31
AS (23)	+0.1 5	1.61	0.9302	0.13	1.37-1.85	1.49	0.12	1.27-1.72	3.86±0.32
A50(2 3)	+0.8 8	2.88	0.9707	0.11	2.69-3.07	2.67	0.10	2.49-2.85	6.17±0.24

AS	-	2.24	0.9771	0.17	1.87-2.60	1.92	0.15	1.61-2.23	3.38±0.26
(26)	0.62								
A50(2	-	3.44	0.9971	0.09	3.25-3.64	2.95	0.08	2.78-3.12	7.00±0.19
6)	0.28								
AS	+0.1	1.69	0.9622	0.17	1.33-2.04	1.69	0.17	1.33-2.04	5.0±0.50
(SBR)	7								

Note:

AS(t): sucrose is the only source of COD at t (°C)

A50(t): sucrose and wastewater each contribute 50% of the COD at t°C

AS (SBR): sucrose is the only source of COD at 20°C, but the used denitrifying biomass was derived from the SBRs

For comparison, the most recent study by Xu et al. (2018) found the SDNR of glucose at 20°C (after being converted) to be 1.43 ± 0.19 .

While the converted SDNRs of sucrose in this study did show some variation, for example ranging from 1.53 to 1.92, they were insignificant comparing to similar studies on other external carbon. For example, the SDNRs of 4.7 - 7.2 for ethanol (Mokhayeri et al., 2008) or 3.4 - 8.0 for dairy wastewater (Quan et al., 2005) were reported in literature. The more surprising finding is with the $\Delta\text{mgCOD} / \Delta\text{mgNO}_3$, where significantly less sucrose was consumed for each mg of NO_3 removed, compared to the sucrose + wastewater mixture. The gap was even larger at higher temperature, possibly suggesting that more wastewater organic carbon was used for, or as catalyst for, cell growth. This will be further discussed in section 4.3 where growth yield (YH) and growth rate value (μS) of the two-tested carbon variations are revealed. Overall, however, the ratio is fairly similar to the 3-7 mgCOD consumed per 1 mg NO_3 removed in other studies (Henze et al., 2002, Choi et al., 2008).

4.4.2 NMB models (based from SDNRs tests) compared to SBRs and pilot plant

Based on the SDNR results in Table 2, the NMB equations from section 3.2 and other influent and operational data, the performance of the post-anoxic denitrification SBRs can be calculated with NMB models as written below.

Control Reactors:

$$\begin{aligned} \text{NO}_3_{\text{pre-anoxi}} &= 80\%TN_{\text{ini}} - \text{NH}_4_{\text{eff}} = 80\%TN_{\text{influent}} \times 50\% - \text{NH}_4_{\text{eff}} \\ &= 80\% \times 44.48_{\pm 1.09} \times 0.5 - 0.76_{\pm 0.30} = 17.03 \pm 0.73 \text{mg/L} \end{aligned}$$

$$\begin{aligned}
NO3_{effluent} &= 80\%TN_{ini} - SDNR_1 \times MLVSS \times t - NH4_{eff} \\
&= 17.03_{\pm 0.73} - SDNR_1 \times MLVSS \times t \\
&= 17.03_{\pm 0.73} - 0.34 \times 2.494_{\pm 0.295} \times \frac{110}{60} = 15.48 \pm 0.91 mg/L
\end{aligned}$$

Tested Reactors:

$$\begin{aligned}
NO3_{effluent} &= 80\%TN_{ini} - SDNR_2 \times MLVSS \times t - NH4_{eff} \\
&= 17.29_{\pm 0.74} - SDNR_2 \times MLVSS \times t \\
&= 17.29_{\pm 0.74} - 2.73_{\pm 0.21} \times 2.212_{\pm 0.205} \times \frac{110}{60} \\
&= 6.22 \pm 2.07 mg/L
\end{aligned}$$

In which the MLVSS, NH_4 eff, TN are operation variables of each respective SBR. t is the anoxic retention time in hours (110 minutes). $SDNR_1$ of the control reactors are based on the general endogenous denitrification rate from literature review. $SDNR_2$ of the tested reactors were sucrose and wastewater SDNR based on the SDNR results.

The calculated results can be compared directly to the results retrieved from the SBRs running for 2 SRTs. The results of the 4 SBRs are represented in Figure 4.5 and Figure 4.6.

The Reactors A and C are duplicates as control reactors. The Reactors B and D are duplicates as tested reactors where sucrose was only added in after the 25th day. The reactor label (A-D) was numbered based on their location on the bench (from left to right), so the tested and control reactors were mixed up (A

with C, B with D) to make sure that their location (and associated variables like distance to the feed tanks, or air flow...) would not have significant effect on the test.

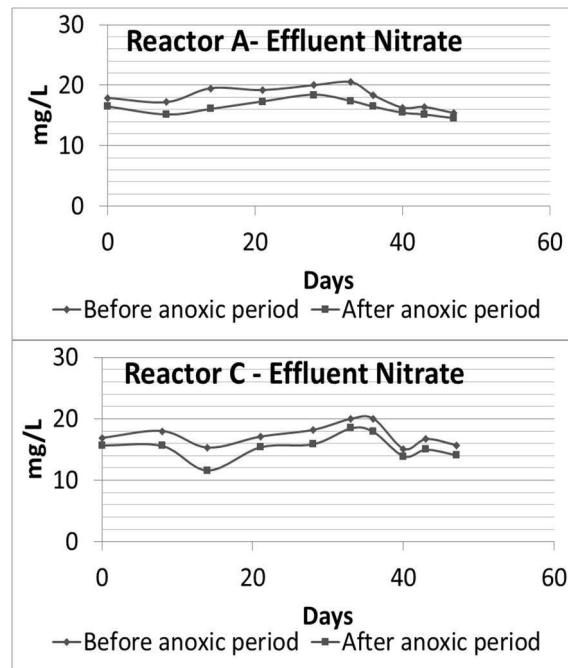


Figure 4.5: Nitrate concentration before and after anoxic zone in Control Reactor (A and C).

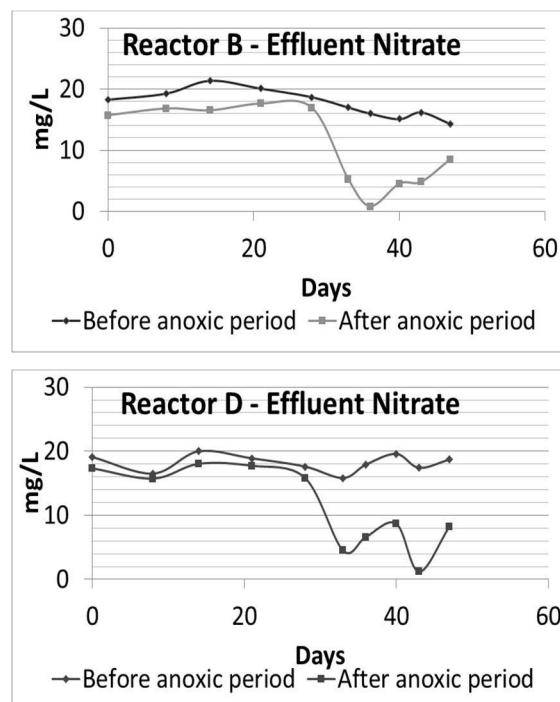


Figure 4.6: Nitrate concentration before and after anoxic zone in Tested Reactors (B and D).

As was showed in the graphs, there was very limited denitrification during the anoxic period in all reactors when sucrose was not added in. This was actually expected as the SBRs faced the same problem with most of post-denitrification wastewater treatment system: the readily biodegradable carbon in the influent was used up during the aerobic zone (stationed previous to the anoxic zone). This can be seen by the average COD of all four SBR reactors during the previous 6 months before operation in Table 2.

It has to be taken into account that the synthetic wastewater soluble COD is 456.8 ± 26.5 mg/L, and the decant rate is 50%. This means the initial soluble COD of each reactor at the beginning of each cycle theoretically always is at least 228.4 ± 13.2 mg/L. The actual figure can't be measured however, as the COD of

synthetic wastewater is quickly consumed, even before it is well mixed with the reactor.

Note in Table 4.3, how the COD after anoxic period of each reactor was almost identical to the COD before anoxic period. This indicated that most of the COD remained before the anoxic zone was either very slowly degradable COD, or non-inorganic COD which can't be utilised by denitrifiers.

The nitrate removal performance of the four SBRs can be summarised in Table 3 above. The results showed a very significant improvement in denitrification in tested reactors (B and D) comparing to control reactors (A and C). The nitrate removal increased from 10% to 68% of total remained nitrate and reduces it down to way below the general worldwide standard of 10 mg/L.

Table 4.3: The measured COD of the SBRs.

No	Reactors	Before anoxic period (mg/L)*	After anoxic period (mg/L)
1	SBR A	34.4 ± 15.9	33.9 ± 12.9
2	SBR B	34.9 ± 15.1	35.5 ± 14.0
3	SBR C	34.4 ± 15.7	34.4 ± 17.1
4	SBR D	51.9 ± 49.6	52.5 ± 56.7

Table 4.4: The measured NO₃ of the SBRs.

No	Nitrate concentration	SBR A	SBR B	SBR C	SBR D
1	Before anoxic period (mg/L)	17.4 ± 1.8	15.7 ± 0.9	17.5 ± 2.1	17.9 ± 1.3
2	After anoxic period (mg/L)	15.8 ± 1.0	4.8 ± 2.4	15.9 ± 1.9	5.8 ± 2.7
3	Nitrate removed (mg/L)	1.6 ± 0.9	11.0 ± 3.0	1.6 ± 0.3	12.0 ± 2.1

The summarised results of the SBRs and the summarised results of the NMB models can then be summarised in Table 4.5 below.

Table 4.5: Comparison of the nitrate concentration results of NMB models and SBR

	Control condition (mg/L)		Tested with sucrose (mg/L)	
NO ₃ concentration	SBRs	NMB	SBR	NMB
Before anoxic	17.45 ±1.97	17.04 ±0.74	16.80 ±1.56	17.30 ± 0.75
After anoxic	15.86 ±1.54	15.49 ± 0.92	5.30 ± 2.66	6.22 ± 2.07

Referring to the simulation of the pilot plant using the NMB model, due to the pilot plant employing pre-anoxic denitrification instead of post-anoxic denitrification, it was necessary to identify whether complete denitrification had

occurred, before the effluent characteristics could be calculated. The required denitrification rate for complete denitrification of the pilot plant using the NMB pre-anoxic denitrification equations (Appendix) is summarised in Table 4.6 and Figure 4.7: Diagram of the pilot plant based on NMB calculations.

Table 4.6: Nitrogen Mass Balance Model of the pilot plant

Each Stage	Used Equations	Calculated value
Pre-anoxic	• $TN_{ini} = x \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v \text{ (L)}$	• $TN_{ini} = 82.26 \pm 12.57 \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = 3.8 \pm 0.1 \text{ (L)}$
Pre-aerobic	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4\text{eff}} \times IR}{v + IR} \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v + IR \text{ (mg/L)}$	• $NH_4 = 13.46 \pm 2.98 \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = 22.8 \pm 0.6 \text{ (L)}$
Pre-clarifier	• $NH_4 = NH_{4\text{eff}} \text{ (mg/L)}$	• $NH_4 = 3.74 \pm 1.95 \text{ (mg/L)}$

	• $NO_3 = \frac{0.8x \times v + 0.8NH_{4\ eff} \times IR}{v + IR} - NH_{4\ eff}(mg/L)$ • $Q = v + IR (L)$	• $NO_3 = 9.72 \pm 4.93(mg/L)$ • $Q = 22.8 \pm 0.6 (L)$
Effluent	• $NH_4 = NH_{4\ eff}(mg/L)$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\ eff} \times IR}{v + IR} - NH_{4\ eff}(mg/L)$ • $Q = v (L)$	• $NH_4 = 3.74 \pm 1.95 (mg/L)$ • $NO_3 = 9.72 \pm 4.93(mg/L)$ • $Q = 3.8 \pm 0.1 (L)$
Internal return flow	• $NH_4 = NH_{4\ eff}(mg/L)$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\ eff} \times IR}{v + IR} - NH_{4\ eff}(mg/L)$	• $NH_4 = 3.74 \pm 1.95 (mg/L)$ • $NO_3 = 9.72 \pm 4.93(mg/L)$ • $Q = 19.0 \pm 0.5 (L)$

- $Q = IR \text{ (L)}$

Mix of pre-anoxic and IR
flow

- $NH_4 = \frac{0.8x \times v + 0.8N_4 \text{ eff} \times IR}{v + IR} \text{ (mg/L)}$

- $NH_4 = 13.46 \pm 3.26 \text{ (mg/L)}$

- $NO_3 =$

- $NO_3 = 8.10 \pm 4.11 \text{ (mg/L)}$

$$\frac{\left(\frac{0.8x \times v + 0.8NH_4 \text{ eff} \times IR}{v + IR} - NH_4 \text{ eff} \right) \times IR}{V + IR} \text{ (mg/L)}$$

- $Q = 22.8 \pm 0.6 \text{ (L)}$

- $Q = v + IR \text{ (mg/L)}$

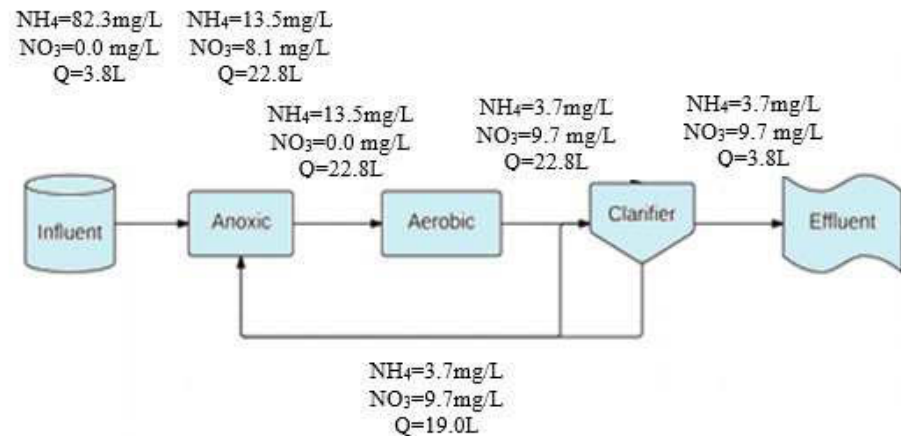


Figure 4.7: Diagram of the pilot plant based on NMB calculations

The important figures in the NMB simulation are summarised in Table 4.7 below. Note the denitrification rates of the pilot plant before and after adding sucrose were calculated based on $\Delta \text{NO}_3 = \text{SDNR} \times \text{MLVSS} \times t$, where SDNR of sucrose and of wastewater were based on the SDNR batch tests, MLVSS referred to the MLVSS of the system, and t was the hydraulic retention time of the anoxic zone.

Table 4.7: Denitrification rate based on NMB models

Denitrification rate of the pilot plant	
(mgNO ₃ -N/L)	
Before adding sucrose	4.21 ± 0.95
After adding sucrose	8.91 ± 1.55
Required for complete denitrification	8.10 ± 4.11

While conclusive results could not be drawn due to the high standard deviation of required denitrification rate¹, the average denitrification rate of 8.91 > 8.10, does reveal that complete denitrification in the anoxic zone could occur most of the time. Results from the NMB models above can also be compared directly to the actual measured data from the pilot plant in Table 4.8, shown below.

Table 4.8: Comparison of the results from NMB models and pilot plant

	Control condition (mg/L)	Tested with sucrose (mg/L)	
NO₃ concentration		Pilot Plant	NMB
Before anoxic	N/A	N/A	8.10 ±4.11
After anoxic	N/A	N/A	0.00
Effluent	33.23 ±5.56	11.21 ±3.32	9.72 ±4.93

The reason why it is not possible to find (nor useful to have) the NO₃ concentration of “before anoxic” or “after anoxic” zone in pre-anoxic system is evident with the NMB results. The concentration of nitrate in the pre-anoxic denitrification system is often affected heavily by Internal Recycle and incoming influent. Consequently, with the constant flow, the effluent nitrate is often higher than the initial nitrate before being treated in the anoxic zone.

In summary, overall the NMB models did show a fairly accurate simulation of the SBRs results. Meanwhile the findings from NMB model of the pilot plant while being largely restrained by the nature of the pre-anoxic system, still indicated fairly similar effluent nitrate with the actual data from the pilot plant.

4.4.3 Mumax Batch Tests

Mumax batch tests for sucrose, sucrose+ww mixtures, and synthetic wastewater were carried out, and the results were fed into the Wolfram Mathematica program. This was done to find the growth yield (Y_H) and growth rate (μ_s) value as seen in Table 4.9.

Table 4.9: Growth yield (Y_H) and growth rate value (μ_S) retrieved from the Mumax batch tests

Experiments	Growth yield (Y_H)					Growth rate (μ_S)			
		Estimate	Std Error	t-statistic	P-value	Estimate	Std Error	t-statistic	P-value
Sucrose only	$\mu(AS)$	0.813	0.068	11.925	1.236	0.707	0.118	6.015	8.732
					$\times 10^{-7}$				$\times 10^{-5}$
Synthetic ww	$\mu(NW)$	0.341	0.114	2.981	1.379	0.288	0.102	2.836	1.767
					$\times 10^{-2}$				$\times 10^{-2}$
Sucrose + ww	$\mu(A50)$	0.644	0.060	10.751	8.154	0.773	0.080	9.632	2.239
					$\times 10^{-7}$				$\times 10^{-6}$
Biowin default		0.50				0.54			

Three conclusions can be drawn from these results. Firstly, the $\mu(\text{A50})$ was close to the average of $\mu(\text{NW})$ and $\mu(\text{AS})$ indicating that when sucrose and wastewater were present, the denitrifier would consume a relatively equal share of sucrose and wastewater as carbon sources to grow its biomass.

Secondly, it was clear that the mixtures of two carbon sources (sucrose and wastewater) provided the highest anoxic growth rate, followed closely by pure sucrose. These two provided more than double the growth rate of synthetic wastewater and were 30-42% higher than growth rate of real wastewater (based on Biowin default value).

Thirdly and finally, this high growth rate was the result of the high growth yield efficient which constituted the rate of mgCOD being consumed for anoxic biomass growth compared to other activities such as denitrification (Akunna et al., 1993, Constantin et al., 1997, Fernandez-Nava et al., 2010). This means that for every 1 mg of COD used for denitrification, 4.3, 0.5 and 1.8 mgCOD/L of $\mu(\text{AS})$, $\mu(\text{NW})$, $\mu(\text{A50})$, respectively, was utilised to grow denitrifier biomass. This led to an initially lower denitrification rates of $\mu(\text{AS})$ and $\mu(\text{A50})$ compared to $\mu(\text{NW})$, but one that nonetheless exponentially increased over time.

Based on the earlier SDNR experiments regarding sucrose, and the yield rate reported for the Mumax batch tests, the amount of carbon consumed as energy per mg of NO_3 removed for sucrose and mixture of sucrose + ww could be calculated and presented in Table 4.10.

Table 4.10: Consumed C: N to produce energies and by-products resulting from denitrification activities (excluding cell growth).

Samp le	Carbon source types (Y _h)	Sludge type ²	ΔmgCO D ³ / mgNO ₃	ΔmCOD/mgNO ₃ consumed for cell growth		ΔmgCOD / mgNO ₃ for other activities (excluding cell growth)	
NS		Non- Acclima ted	6.16±1.3 4	5.01±1. 09	5.01±1. 09	1.16±0. 25	1.16±0. 25
AS(2 0)	Sucrose	Acclima ted	4.38±0.2 7	3.56±0. 22	3.38±0. 28	0.82±0. 05	0.78±0. 07
AS(2 3)	only (SU)		3.86±0.3 2	3.14±0. 26	(Avera ge)⁴	0.72±0. 06	(Avera ge)
AS(2 6)	(0.81)		3.38±0.2 6	2.74±0. 21		0.63±0. 05	
AS(SBR)			5.00±0.5 0	4.06±0. 40		0.94±0. 09	

N50	Mixture of sucrose	Non-Acclimated	6.14±0.29	3.95±0.19	3.95±0.19	2.19±0.10	2.19±0.10
A50(20)	and wastewater	Acclimated	5.13±0.31	3.31±0.30	3.93±0.16	1.83±0.11	2.17±0.09
A50(23)	ater (S&W		6.17±0.2	3.97±0.15	(Average)	2.20±0.09	(Average)
A50(26)	W) (0.64)		7.00±0.19	4.50±0.12		2.49±0.07	
NW	Municipal wastewater (WW) (0.5)	Acclimated	4.67±0.77	2.34±0.39	2.34±0.39	2.34±0.39	2.34±0.39

An interesting conclusion could be drawn in that the amount of rbCOD consumed for cell growth (after acclimation) seemed to be fairly similar regarding whether only sucrose (SU) or a mixture of sucrose and wastewater (S&WW) influent was used, varied from 3.38±0.28 to 3.93±0.16 on average. However, the two were significantly higher than when only wastewater (WW) influent was used 2.34±0.39.

However, the opposite was the case when it came to mgCOD per mg NO₃ being consumed. The $\Delta\text{mgCOD}/\text{mgNO}_3$ figures for WW and S&WW were actually much more similar (2.17 ± 0.09 and 2.34 ± 0.39), and much higher than the one for SU (0.78 ± 0.07). These could indicate that when S&WW was available as carbon, the denitrifier consumed sucrose for cell growth, and the rbCOD of wastewater influent served as energy for denitrification. This is further backed up by the growth rate of SU and S&WW. They proved to be very similar (0.71 ± 0.12 and 0.77 ± 0.08) despite the total COD consumed/ mg NO₃ removed being much smaller in the former.

This may explain why sucrose was found to have a very short acclimation time, which is similar to what being found by Xu et al. (2018). Based on the experiments of this study, the reason is because the denitrifier acclimating to sucrose could be quickly populated inside the system due to its high growth and yield rate. This also means that theoretically, by adding sucrose into a system's anoxic zone, other than to improve denitrification, it could also help regrow the denitrifier especially the case where microorganisms are lost due to the system malfunctioning. This is very important, considering that the denitrifier required the highest SRT to grow. It means they are the most vulnerable when there is a hydraulic fluctuation (high influent flow, high rainfall, flood, etc.).

4.4.4 Biowin simulation (based on Mumax tests) compared to SBRs and pilot plant.

These constants were then fed into Biowin to simulate 13 pre-anoxic denitrifications (for pilot plant) and 5 post-anoxic denitrification

scenarios (and for SBRs). They are listed in Appendices III and IV. The results are then compared to the measured data concerning the SBRs, of the pilot plant and summarised in Table 4.11 and Table 4.12.

Table 4.11: Comparison of the results from SBRs and Biowin simulations

	Biowin Scenario F2		SBR results	
	NO ₃	TN (mg/L)	NO ₃	TN
	(mg/L)		(mg/L)	(mg/L)
Before adding	20.15 ±	31.08 ±	16.80 ±	27.3 ± 7.3
sucrose	6.99	13.70	1.56	
After adding sucrose	4.41 ± 3.51	15.77 ± 8.07	5.30 ± 2.66	8.3 ± 1.5

The Biowin simulation of the SBR showed to be quite accurate, except for two aspects: the high standard error and the effluent TN. The second was more significant because while the TN of the SBRs effluent was showed to consist mostly of just nitrate, the Biowin simulation suggested otherwise. It should be noted the difference could have been caused by the high standard error itself. That difference was intensified when it came to the simulation of the pilot plant as shown below in Table 4.12.

Table 4.12: Pilot plant effluent results compared to Biowin simulations

	Biowin Scenario E1		Pilot Plant results	
	NO ₃	TN (mg/L)	NO ₃	TN (mg/L)
	(mg/L)		(mg/L)	
Before adding	20.15 ±	31.08 ±	33.23 ±	32.70 ±
sucrose	6.99	13.70	5.56	4.67
After adding	16.54 ±	24.23 ±	11.21 ±	16.96 ±
sucrose	6.56	11.42	3.32	4.90

Overall, compared to the NMB model, Biowin was not as accurate when it came to simulate the SBRs and the pilot plant. When tracking through the experiment data, the issue with Biowin simulation seemed to lie on two key issues. The first was inaccuracy when it came to nitrification simulation, which led to the TN value of Biowin being similar to that of the pilot plant. However, the nitrate concentration of Biowin was not.

The second issue with Biowin was the impact caused by the fluctuation of influent parameters on the same day. Only up to 12 different influent data can be inputted into each Biowin simulation, which means for the monitoring period, only 1 influent data can be input for every 2-days. If the influent fluctuated a lot during each day and over a period of days, the nitrate accumulation would be simulated by Biowin. This may have led to a sharp difference in the actual pilot plant data as indicated in Figure 4.8: Biowin simulation versus actual data from pilot plant below.

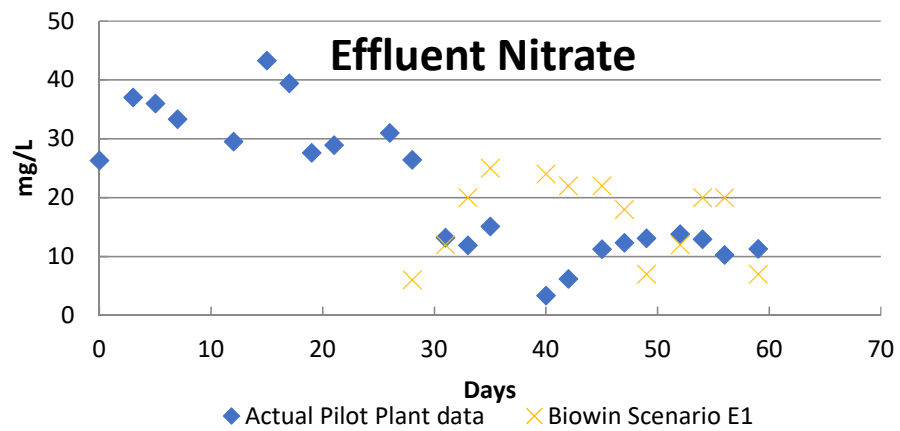


Figure 4.8: Biowin simulation versus actual data from pilot plant

Comparing to Biowin, the NMB is a frozen model, and use the average influent data for the whole 3 weeks for simulation. Hence, it is affected less by the fluctuation of inputs than Biowin.

4.4.5 SDNR calculated from Mumax Batch tests compared to SDNR batch tests

It appears from the aforementioned investigations that few studies have attempted to calculate the denitrification rate of an external carbon source based on the Mumax equation. This was expected because a Mumax batch test is much more difficult to set up, monitor and analyse compared to SDNR tests. However, in our attempt to cross-verify results, it was still intriguing to investigate its potential. Based on the Mumax equation as shown in section 3.3, the denitrification rate when MLVSS was equal 1,000 mg/L could be found by differentiating the Mumax equation:

Equation 4.4-1 Differentiation of Mumax equation

$$\frac{dS_{NO}}{dt} = -\eta_{Anoxic} \cdot \frac{1 - Y_M}{2.86} \cdot \frac{\mu_S}{Y_M} \cdot X_{S,0} e^{(\mu_S - b_S)t}$$

Where t could be calculated using:

Equation 4.4-2 t calculation from Mumax equation

$$t = \frac{\ln\left(\frac{X_{S,t}}{X_{S,0}}\right)}{\mu_S - b_S}$$

By submitting the known variables from experiments documented in the literature, the SDNR calculated from YH + μ_S of Mumax test and SDNR averaged from all SDNR batch tests are summarised below.

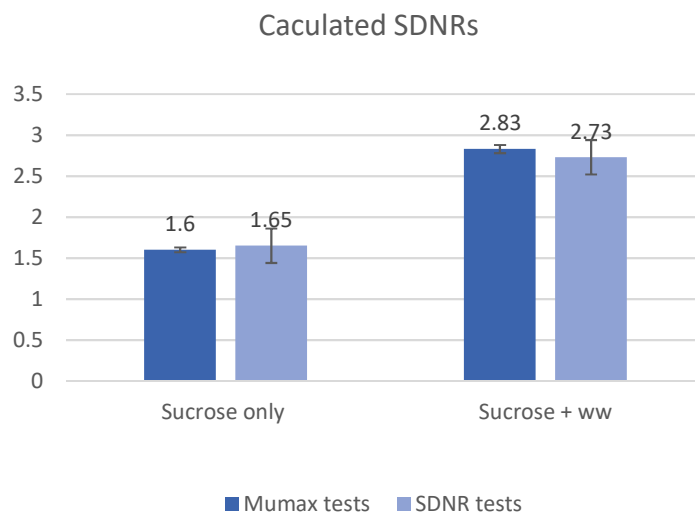


Figure 4.9: SDNR calculated from Mumax tests vs SDNR tests

Based on the data in Figure 8, it was apparent that the calculated results of the tested carbon and carbon mixture based on growth rate and yield rate were very consistent with the results for the SDNR tests in section 4.4.1, especially considering the completely different nature of the two types of batch tests. The finding indicated that the laboratory experiment results were very accurate, and it was possible to use one to verify the results of the other.

4.5 Conclusion

Overall, this study successfully established a workable methodology to study and evaluate the potential use of sucrose as an external carbon source using four different simulation models. The validation of all four used models (NMB, SBRs, Biowin and pilot plant) and the benefit of cross-verifying their results, as each model showed their own advantages. The findings are also consistent with the previous study, in which the SDNR of sucrose was not as high as ethanol and methanol. However, in all four models, the results indicated that adding sucrose could significantly increase denitrification performance in both pre-anoxic and post-anoxic systems and reduce effluent nitrate. Further study on testing the proposed method's applicability with other external carbon sources is necessary.



Chapter 5

Fermented Sludge Generation and Pre- Assessment

Chapter 5 - FERMENTED SLUDGE GENERATION AND PRE-ASSESSMENT

5.1 Introduction

This study examines the fermentation and dark fermentation of Wasted Activated Sludge (WAS) and anaerobic digested biosolids, before assessing the suitability of these fermented sludge types as external carbon sources for denitrification. This was because both of these two substances have high VFA which plays a key role in external carbon sources Xie et al. (2012); Yang et al. (2012); Wu et al. (2014), and generally produce higher denitrification rates than more common substance like acetate Soares et al. (2010).

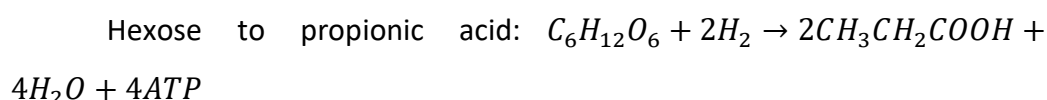
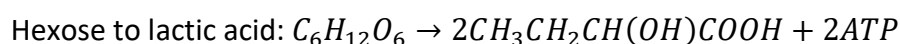
Also, the secondary sludge provides a consistent microorganism variety for fermentation Perot and Amar (1989). They are already readily available at every wastewater treatment plant. The two substances can utilise the existing continuous anaerobic digester system for fermentation and will not significantly affect the biogas production process. Finally, utilising even a portion of these two waste products will help to reduce the amount of sludge and biosolids that each WWTP has to dispose of each year, which accounts for >60% of the total investment in a standard WWTP Saby et al. (2002); Ma et al. (2015). Likewise, it eliminates the problem of increased sludge mass due to added external carbon Ma et al. (2015), Strong et al. (2011).

All current WAS treatment methods including thermal, chemical and advanced oxidation, will cause problems for the environment Wei et al. (2003). This makes the development of a sustainable process to remove WAS a crucial one in saving operational costs and reduce damage done to the environment Jenicek et al. (2014). For example, Guo et al. (2016) used fermented WAS as an

external carbon source for denitrification in a partial nitrification system. They found that it could reduce the WAS production rate by 21.6%

While the study of fermented WAS is not new and experiments have been conducted since the 1980s Funamizu et al (1997), Latimer (2007), Kampas (2007), Tong and Chen (2009), Gao et al (2010), Bi et al (2014), Wang et al (2015), Guo et al (2016), Liu et al (2016), only a few analyses have looked at sludge fermentation as part of an existing continuous anaerobic digester process, where the inoculum and infrastructure from anaerobic digester could be utilised directly for fermentation. Most research focused on primary sludge fermentation instead of WAS or biosolids fermentation because secondary sludge fermentation is a complicated process due to the complex structure of sludge microbial cells Arnaiz et al (2006), Gavala et al (2003), Zheng et al (2009).

The potential of dark fermentation, not only to generate fermented sludge for improving denitrification but also biogas generation has been neglected. The principles of both fermentation and dark fermentation are similar in nature, where the chosen feeds in this case would be hydrolysed under their favoured conditions to break down the complex organic components in influents into simple carbohydrates and short-chained or Volatile Fatty Acids (VFA) Soares et al (2010), Zhang et al (2010). The main difference lies in the objectives of each type of fermentation. Standard fermentation focuses on generating SCFAs, mostly lactic and propionic acids¹, that work as external carbon sources for denitrification. The reaction pathway is:



¹ 90% acetic acid and 10% propionic acid, according to Soares et al. (2014)

The main objective of dark fermentation is to produce biohydrogen. Biohydrogen can be used to either improve the combustion efficiency of methane gas (generated from onsite anaerobic digester) to form an alternative automobile engine fuel such as Hythane, or collected separately for other applications. The fermented sludge generated from dark fermentation in this case is actually a by-product, making the generation of dark fermented sludge more feasible in many situations while fermented sludge does not. Unlike fermented sludge, dark fermentation aims to separate the feeds into acetic acid and butyric acid through the following reaction pathways:

Hexose to acetic acid: $C_6H_{12}O_6 + H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 + 4ATP$

Hexose to butyric acid: $C_6H_{12}O_6 + H_2O \rightarrow 2CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 + 3ATP$

Note: most of Chapter 5 has been published in the following paper:

Published

Phung, D. 2015a, 'Assess the potential of fermented sludge as an external carbon sources for denitrification improvement.', *Journal of Science and Technology*, vol. 53, no. 5A, pp. 242-9.

5.2 Materials and Methods

5.2.1 Chemicals and Inoculums

Similar to one published study on sludge fermentation Park et al. (2014), the inoculum for this fermentation experiment was also taken from a local

anaerobic digestion plant rather than a standard WWTP. This is the best way to simulate the conditions of a continuous fermenter (in oppose to batch fermenter).

The used inoculum has a Total Solid (TS) of $26,861 \pm 693$ mg/L and Volatile Solid (VS) of $19,962 \pm 622$ mg/L.

The Wasted Activated Sludge (WAS) and biosolids were taken from the influent and effluent drains from the same anaerobic digester. Both of the feeds and inoculum were sieved to remove larger particles.

The added sugar used for additional carbohydrate was commercial pure white sugar. Adjusted pH levels for the fermentation and dark fermentation set-ups were controlled using diluted citric acid. Calcium Carbonate and Magnesium Chloride were used for pH adjustment and struvite formation. All were purchased from Science Supply Australia Company.

5.2.2 Analytical Method

COD, TN, Ammonia, Nitrate, and Volatile Fatty Acids were analysed using HACH standard methods for the DR 5000 (Methods 8000, 10072, 10031, 10020, and 8196, respectively). pH and DO were tested with the Mettler Toledo S20 Seveneasy pH meter and YSI 5100 dissolved oxygen meter.

5.2.3 Experimental set-up

For each reactor, roughly 200 mL of inoculum was mixed with 50 mL of feed (either WAS or anaerobic digested biosolids) to produce a ratio of 80:20 inside a 500-mL short-bottle. This allows a head space of roughly 50%. Each reactor's pH was then adjusted using up to 15 mL of diluted citric acid, or Milli-Q

water, or a combination of both to ensure a similar dilution ratio was achieved for each reactor. Each test was done in triplicate.

The pH and temperature of these experimental batch tests were based on a wide range of studies, and varied depending on the type of fermentation test. The control reactor of this experiment was operated under a normal anaerobic digestion state at mesophilic temperature (37 °C) and default pH (7.43 ± 0.02). The fermentation reactors were operated under the reported ideal conditions of thermophilic temperature (50 °C) and pH 6.5 ± 0.1 Lu et al. (2007). This is the scenario where the highest VFAs or soluble COD were recorded, as they are the two most suitable components for denitrification Moser-Engeler et al. (1988). However, it should be noted that other than temperature, VFAs yield is also affected by various other dependent variables (for instance the feeds used for the reactors) Cokgor et al. (2009).

The dark fermentation reactors functioned under the reported ideal temperature of 55 °C and pH 5.7 ± 0.2 as suggested in one study Yuan et al. (2009). However, unlike fermentation, dark fermentation's main aim is hydrogen production, while VFA or soluble COD are just secondary by-products. So, the chosen operating parameters were actually ideal for producing hydrogen rather than VFAs or soluble COD production.

The effect of adding external carbohydrates to improve the C:N ratio of the fermented sludge would also be tested. Notably adding external carbohydrate sources like rice Feng et al. (2009) and grass silage Pakarinen et al. (2008) to fermentation process has already been done before to improve either the C:N ratio or H₂ production for dark fermentation. The idea was to produce similar ideal conditions for the sludge and cellulose fermentation process (low pH and high temperature). This means it is possible for the sludge fermentation process to break down the soft cellulose (in tested substances like rice and grass

silage) into simple carbohydrate like sugar, thus increasing the carbon content of the fermented sludge and its C:N ratio. In this experiment, 2288 ± 17 mgCOD/L of sugar will be directly added into some of the reactors, to see how much rbCOD of sugar remains the final fermented sludge solution compared to the amount added in.

Each fermentation reactor was then purged with nitrogen gas before being placed into shakers for continuous mixing with the temperature adjusted accordingly to the tested fermentation types. Generated gas was released daily from the reactor, except for the first few days where it was released twice a day to prevent pressure building up inside the reactor.

Samples were taken initially, after 5 days and after 10 days to assess the fermentation performance of Sludge Retention Time (SRT) of 5 and 10 days. Choosing the correct SRT is very important because it needs to be long enough for the acidogenesis process to occur, but if SRT is too long, not only it would cost time and money for an unnecessary digestion process, too much VFA will be consumed by methanogenic reaction Zhang et al. (2010). Generally, the SRT of fermentation is only 6-10 days Banister et al. (1988), Grade et al. (1988) and this range was further tested based on other research Yuan et al. (2009), where they operated continuous mixed fermentation reactors with three SRT lasting 5, 7, 10 days. These authors found 5 days to be the optimum SRT for fermentation because SRT of more than 6 days did not significantly improve the VFAs yield, but VFAs decrease with SRT under 4 days.

Note: due to the completely mixed nature, the SRT and HRT are the same for all reactors.

5.3 Results and Discussion

5.3.1 Experimental results

The amount of rbCOD ranged from 6500-14000mg/L in all fermentation and dark fermentation batch tests (using optimum conditions based on the literature) were much higher than the control conditions run at 37°C. The rbCOD and VFAs values in fact were very close to previous similar set ups Liu et al. (2016), Guo et al. (2017). For example, Liu et al. (2016) ran the fermentation batch test with an initial TSS of 19705 mg/L, getting a soluble COD 8070 mg/L, which is in a very similar range to the results of fermentation tests 1-3. C:N and VFAs are the two most important independent variables in sludge fermentation experiments and are shown in Table 1 below.

C:N ratio is calculated from the rbCOD divided by the concentration of soluble NH_4^+ . The rbCOD in this case was measured by the filtration of soluble fermented sludge through 0.45 μm membrane filtration. This was done as according to Melcer et al. (2003), where he stated that the particulate soluble COD in this case should have already been mostly absorbed into the sludge, and the resulted filtered COD should not be significantly lower than actual rbCOD (difference of <5 mg/L). The N component should have been the Total Nitrogen, but soluble organic nitrogen and nitrates were tested and found to be insignificant, especially when compared to the high concentration of soluble NH_4^+ . Considering the method used for NH_4^+ analysis also is much more reliable and produced much less error in comparison to TN measurement, using measured soluble NH_4^+ in this case instead of TN should produce a more reliable C:N ratio.

The reason why C:N ratio was so important in this experiment is due to the ultimate goal of producing fermented sludge. Since they will be returned to the anoxic zone as an external carbon source, a lower C:N ratio would mean more nitrogen would be introduced back into the system to meet the required

amount of organic carbon. An insufficient C:N ratio hence undermines the nitrogen removal capacity of the whole system. As recommended by the literature Feng et al. (2009), to work well as an external carbon source, a minimum C:N ratio of 20:1 or ideally of 30:1 would be needed.

The other important independent variable is the portion of Volatile Fatty Acid (VFA) in fermented sludge Feng et al. (2009); Melcer et al. (2003); Guo et al. (2016); Sun et al. (2016) as VFA in this case is the preferred carbon source for denitrification Tong and Chen (2009), Gao et al. (2011). It means a possibly higher denitrification rate can be achieved when the fermentation process generates higher VFA. This, however, needs to be verified in future studies. The C:N and VFA portion results of the fermentation experiments are summarised in Table 5.1.

Table 5.1: The full set of data for all experiments.

N o.	Test	Feed types	Planned pH	Day 0				Day 5 results						Day 10 results					
				Initial pH	Added sugar	°C	Initial VS	pH	rbCO D	NH ₄	C:N	VF A	VFA %	pH	rbCO D	NH ₄	C:N	VFA	VF A%
1	Control	WAS	7.0	7.43 ±0.02*	N	3	15575	8.11 ±0.14	1193 ± 57	839 ± 54	1.43 ±0.15	252 ±9	21.2 ± 1.2	7.76 ±0.04	940 ±227	887 ±23	1.06 ±0.2	296 ±10	31.5 ±7.7
2	Fermentation 1	WAS	6.5	6.50 ±0.14	N	5	15575	7.19 ±0.05	7100 ± 275	1061 ± 5	6.70 ±0.29	1201 ±18	16.9 ± 0.7	7.15 ±0.02	6440 ±163	1096 ±33	5.88 ±0.2	1340 ±22	20.8 ±0.6
3	Fermentation 2	WAS			Y	5	17613	6.92 ±0.13	7753 ± 238	1015 ± 55	7.65 ±0.24	1401 ±22	18.1 ± 0.6	7.09 ±0.09	8327 ±430	1103 ±25	7.56 ±0.5	1584 ±10	19.0 ±1.0
4	Fermentation 3	Biosolids			N	5	18832	7.26 ±0.04	7633 ± 490	1317 ± 47	5.80 ±0.38	1058 ±19	13.9 ± 0.9	7.29 ±0.02	8367 ±340	1363 ±63	6.14 ±0.0	1195 ±22	14.3 ±0.6

5	Dark	WAS	5.5	5.75	N	5	15575	6.44	11820 ±	1128	10.47	603	5.1 ±	6.28	12427	1073	11.5	814	6.6
	Fermentati			±0.20		5	±469	±0.08	516	± 24	± 0.28	± 13	0.2	±0.07	±802	±54	9	± 19	±0.4
	on 1																± 0.5		
																	4		
6	Dark	WAS			Y	5	17613	5.82	13487 ±	991 ±	13.63	617	4.6 ±	5.70	14067	1039	13.5	769	5.5
	Fermentati					5	±470	±0.07	223	44	± 0.43	± 11	0.1	±0.04	±741	±38	7	± 16	±0.3
	on 2																± 1.1		
																	5		
7	Dark	Biosoli			N	5	18832	6.73	10440 ±	1131	9.27	681	6.5 ±	6.80	14287	1385	10.3	826	5.8
	Fermentati	ds				5	±586	±0.05	508	± 100	± 0.39	± 19	0.4	±0.03	±633	±34	1	± 11	±0.3
	on 3																± 0.2		
																	6		

5.3.2 Results discussion

Many conclusions can be drawn from the results. Firstly, while fermentation for 10 days does show an increase in the amount of VFA and rbCOD generation compared to 5 days, the increase was not as significant (at most 9.6% with the exception of Dark Fermentation 3). Furthermore, the fact that the amount of NH_4^+ released also rose over 10 days means that the C:N ratio did not significantly increase compared to 5 days, and even decreased for some batch tests (for example as seen in Figure 5.1 below). Even the Dark Fermentation 3 experiment only recorded an 11% increase in the C:N ratio.

Furthermore, a twice as long SRT (10 compared to 5 days) means it would take a doubled in size fermenter to generate the same amount of fermented sludge over the same period (10 days), and require twice the amount of energy to maintain the same temperature over the fermentation period. This confirm results documented elsewhere Yuan et al. (2009) that an SRT of 5 days is optimum for fermentation experiments. This makes the 5-day SRT a more ideal option.

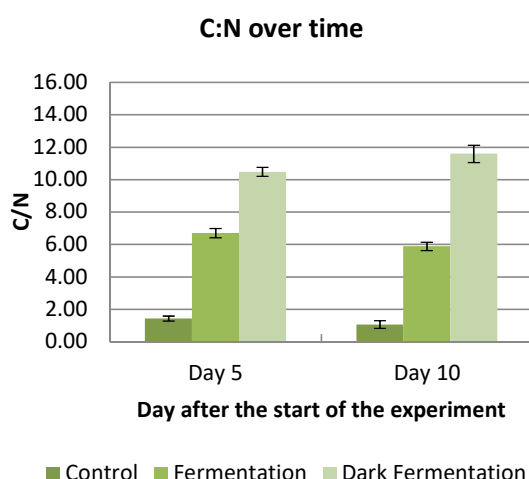


Figure 5.1: The C:N ratio of tested fermented biosolids (Experiments 4 and 7)

Secondly as seen in Table 1, both fermentation and dark fermentation showed a much larger increase in both C:N ratio and amount of VFA generation compared to the control reactors (from 1.43 ± 0.15 to 6.70 ± 0.29 and 10.47 ± 0.28 for C:N of fermentation and dark fermentation, respectively), and much higher than another similar study Soares et al. (2010). The VFA/rbCOD ratio of the fermentation batch tests was actually lower than the control (as seen in Figure 5.2), indicating that the rbCOD generated from fermentation mostly were carbohydrates rather than volatile acids. This is especially true for dark fermentation. The VFA % by fermentation of WAS (31.5%) is almost identical to the figure published in Uccick et al. (2008), where 21mg VFA was obtained for each 1,000 mg of initial COD, or 32.5% in this case.

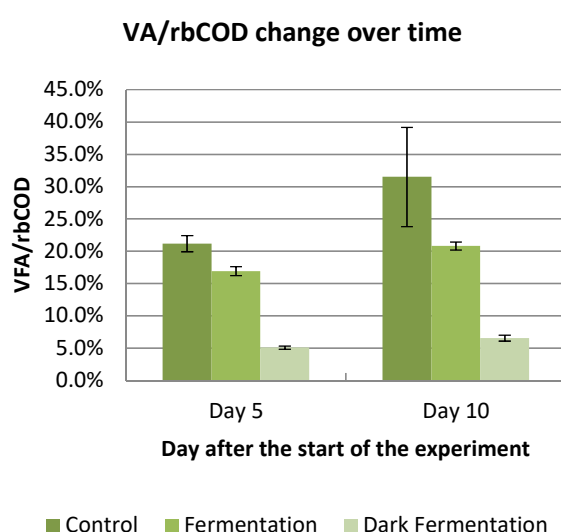


Figure 5.2: VA/rbCOD ratio of the different fermentation set-up

Thirdly, the use of WAS instead of anaerobic digested biosolids does show a better C:N ratio. However, this is not quite significant, with the C:N ratio

increasing from 5.80 ± 0.38 and 9.27 ± 0.39 to 6.70 ± 0.29 and 10.47 ± 0.28 , respectively, for fermentation and dark fermentation as depicted in Figure 3 below.

Fourthly and finally, adding up to 2288 ± 17 mgCOD/L of sugar in each reactor revealed an approximate increase of 653-1660 mgCOD/L (or 29-73% of the added sugar remaining after fermentation and dark fermentation). This helped to increase the C:N ratio from 6.70 ± 0.29 and 7.65 ± 0.24 to 13.63 ± 0.43 and 9.27 ± 0.39 for fermentation and dark fermentation, respectively, as depicted in Figure 5.3 below.

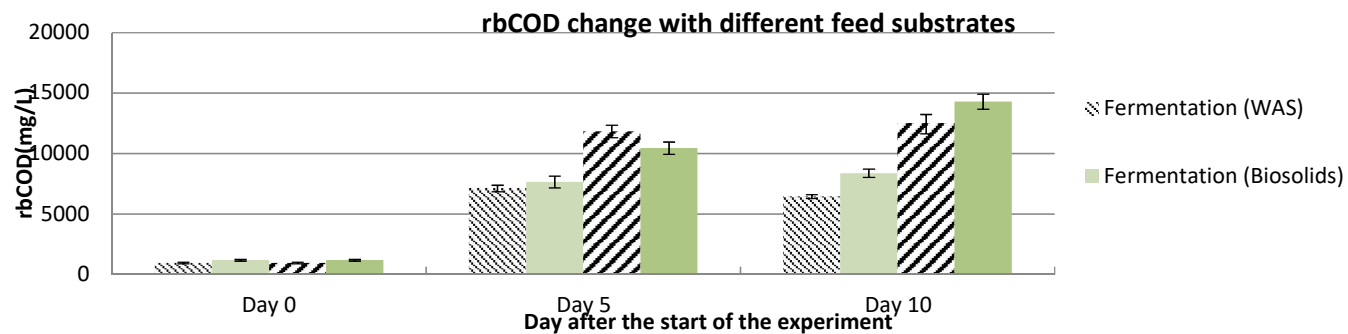


Figure 5.3: rbCOD of the different feed substrates

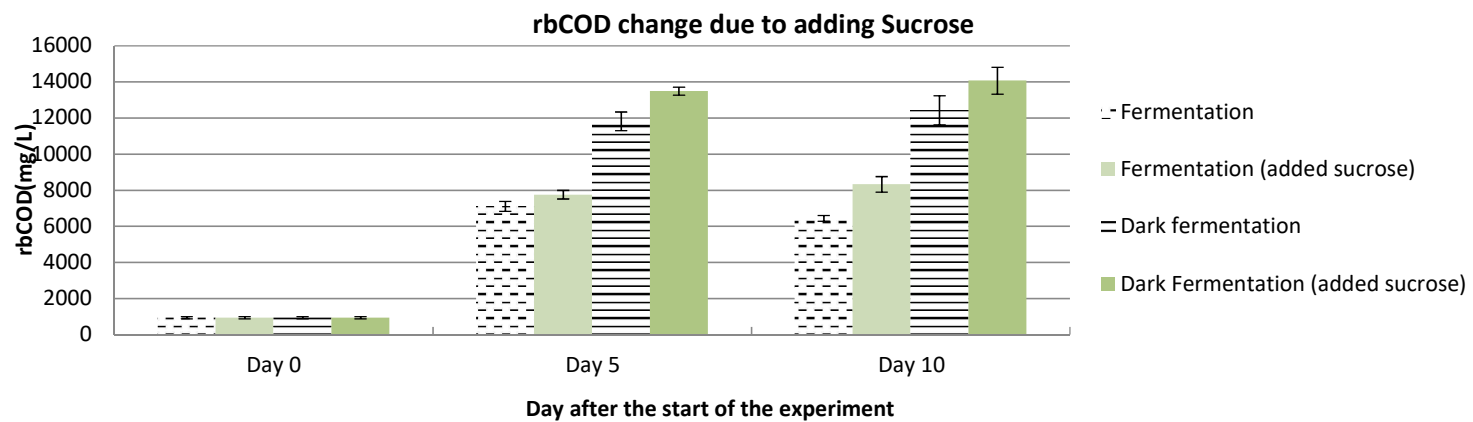


Figure 5.4: Change in rbCOD due to adding sucrose

In summary, the different fermentation types and feed substrates, and adding extra carbohydrate does highlight a change in the C:N ratio for all tested scenarios. Overall a C:N ratio varied from a minimum 5.88 ± 0.26 to a maximum 13.57 ± 1.15 , which is similar in range to the 7.0 reported by Feng et al. (2009) or 11.0-15.0 by Tong and Chen (2009). However, this is still much lower than the suggested minimum C:N ratio of 20:1 and the ideal of 30:1 for external carbon sources. Considering the insignificant change in NH_4^+ concentration in all reactors the low C:N ratio issue is seemingly due to high initial NH_4^+ instead of the fermentation and dark fermentation processes themselves. This proved that all tested options were still far from applicable, despite the fact they already had optimised pH and temperature in a laboratory-controlled situation.

One of the methods used in the literature as mentioned earlier, Pakarinen et al. (2008); Feng et al. (2009), required additional hydrocarbon sources to fix the ratio to 20:1. However, based on earlier results of how only 29-73% of the available sucrose remained in the fermented sludge (which was already higher than the 18-30% reported for VSS by Ubay-Cokgor et al. (2005), a minimum of 14.7 g/L and up to 64.5 g/L of additional sucrose-equivalent will need to be added into/produced inside the fermentation reactors. Unlike sucrose, available carbohydrate substances such as grass, or wood chips would contain hard cellulose and will not be broken down by the fermentation process. For example, in this experiment, only a maximum of 30% of either biosolids or WAS could be broken down by the end of 5 days. This means by adding carbohydrates to fix the C:N ratio to the required 20:1, the theoretical amount of waste TS produced from the fermenter will be at least 4 times bigger than the input biosolids or WAS. This would defeat one of the main purposes of studying fermentation sludge, i.e. reduce the amount of sludge and biosolids that each WWTP has to dispose of each year.

This does not mean the above option is not feasible in every scenario, but may mean it is going to depend on the development of future cellulose degradation technology.

5.3.3 Ammonia stripping and struvite formation as forms of ammonia removal

Another suggested method Feng et al. (2009) was to fix the low C:N ratio by reducing the ammonia concentration through struvite formation. The main goal here is to remove the high ammonia concentration from the samples and this will effectively increase the C:N ratio. This study, however, also looked at ammonia stripping because it is a simpler approach to fixing the low C:N ratio.

5.3.3.1 Literature review and discussion

A further comparison between ammonia stripping and struvite formation is illustrated in Figure 5.5 and Figure 5.6, and Table 5.2 . Figure 5.5 and Figure 5.6 depict the flowchart indicating the minimum amount of infrastructure needed for struvite formation and ammonia stripping, respectively. Table 5.2 documents the advantages and disadvantages of each system in the context of sludge fermentation.

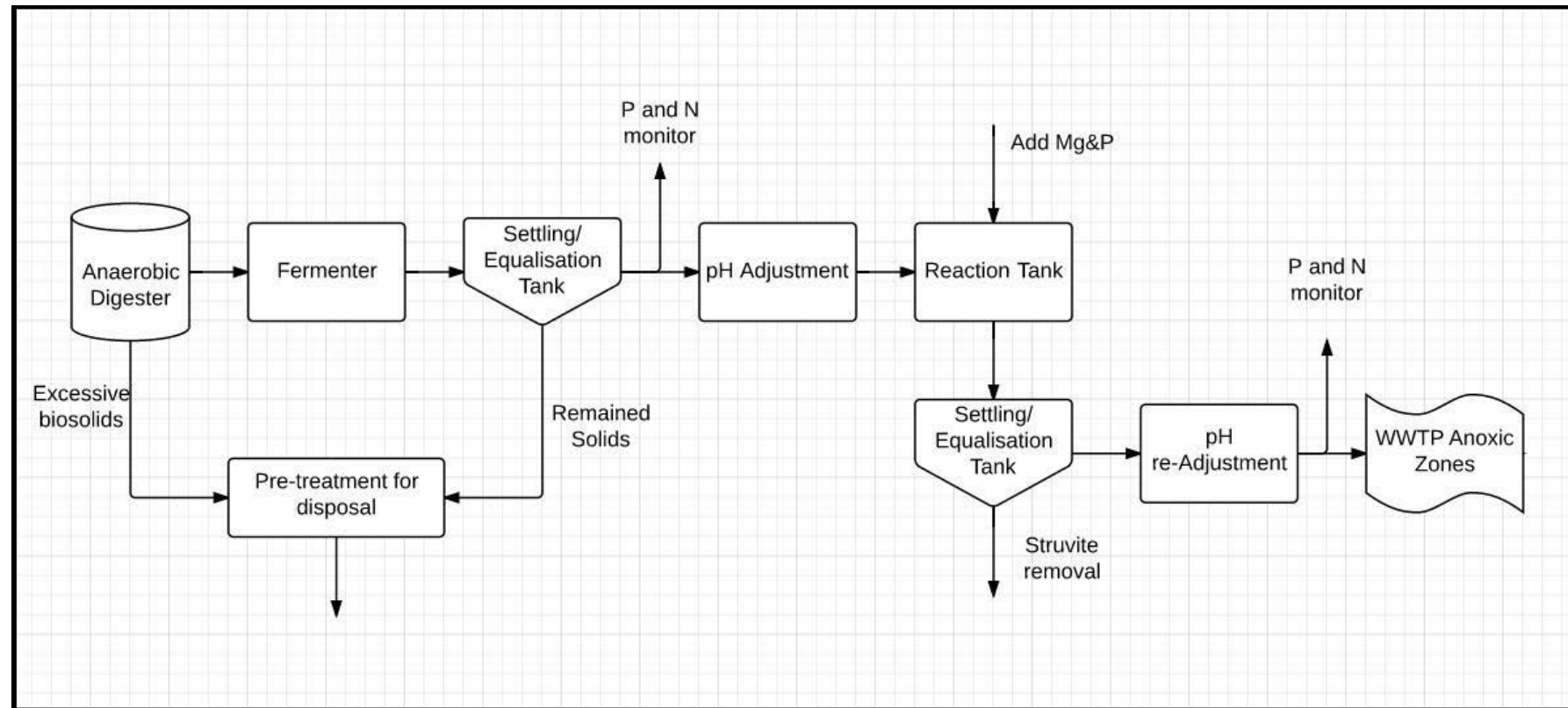


Figure 5.5: Flowchart diagram of Struvite formation set-up

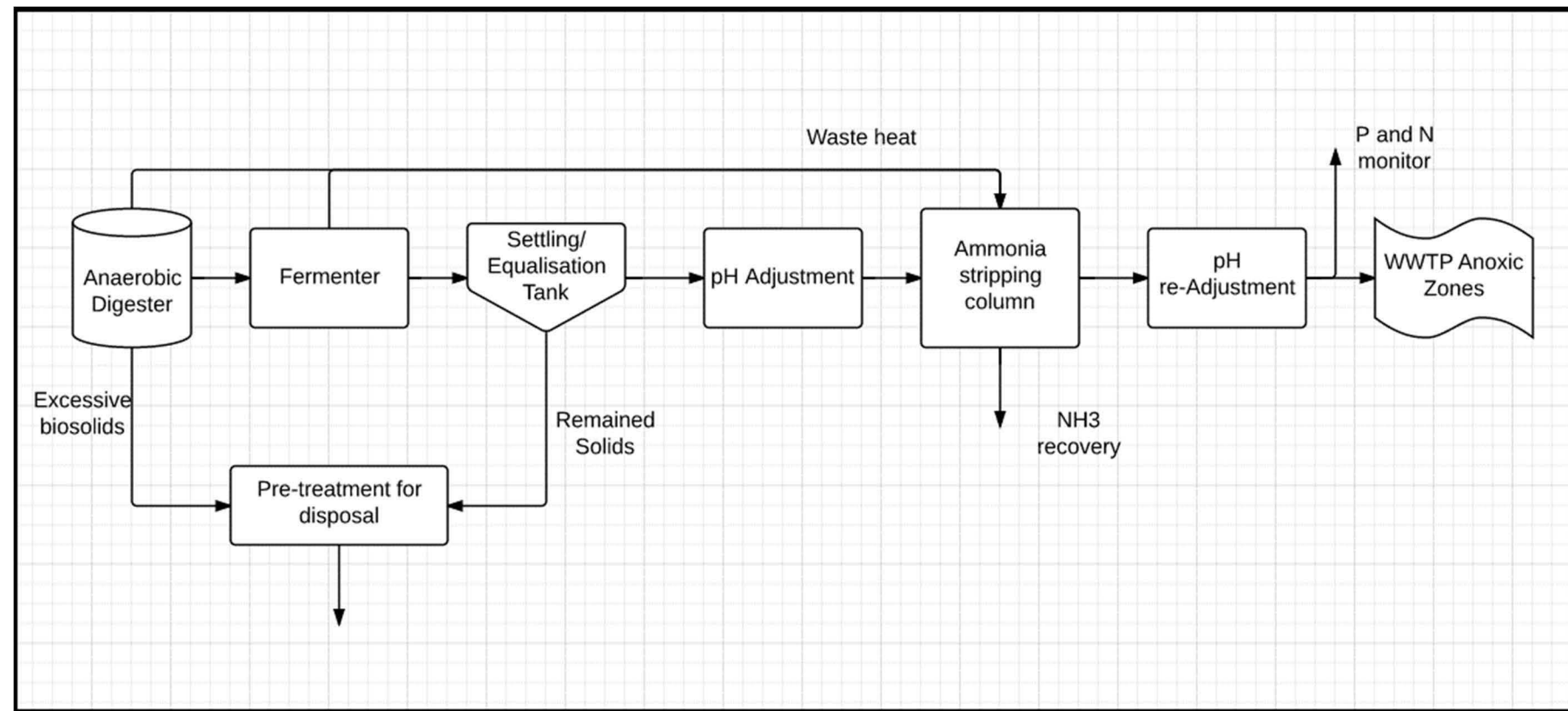


Figure 5.6: Flowchart diagram of Ammonia stripping set-up

Table 5.2: Advantages and disadvantages of Ammonia stripping and Struvite formation

	Struvite Formation	Ammonia stripping	
Advantage	+ Can recover struvite Mg^{2+}, PO_4^{3-} + Can remove phosphorus from fermented sludge + The first pH Adjustment tank may not be needed (according to Uludag et al. (2005))	- Cannot remove phosphorus from fermented sludge - The first pH adjustment tank is required - The volatile ammonia needed to be captured	Disadvantages
Disadvantage	- Requires additional consumable chemicals to provide Mg^{2+}, PO_4^{3-} - If ineffective struvite formation occurs will add phosphorus and magnesium to the anoxic zone - NH_4^+ removal is not as effective - Requires a clarifier tank and fine screening to remove the struvite - The first N&P monitoring is required to dose Mg^{2+}, PO_4^{3-}, followed by second N&P monitoring in case ineffective struvite formation occurs - The amount of Mg^{2+}, PO_4^{3-} dosing will vary depending on the first N&P monitoring - The initial pH in the second pH adjustment tank will be much higher	+ Does not require additional consumable chemicals + If ineffective ammonia stripping occurs, only add more NH_4 back into the anoxic zone + NH_4^+ removal is much more effective + Requires fewer units, especially if the pH adjustment and re-adjustment can be combined into an ammonia stripping column + The first and even the second monitoring state is not required, especially if there is already a monitoring system in place for the anoxic zone + The system can be operated in a stable condition + The initial pH in the second tank will be much lower, especially when waste heat from the anaerobic digester are used + Waste heat from the anaerobic digester can be utilised	Advantages

In summary, ammonia stripping as pre-treatment for the fermented biosolids has some key advantages over struvite formation. Firstly, it is affected by less independent variables and hence is more reliable and effective. Secondly it requires much less space to implement as it only needs an ammonia column, while the struvite formation requires at least a mixing reaction tank, and a clarifier and fine screening to remove crystallised struvite. Most importantly, the ammonia stripping process requires less monitoring, and is less labour intensive as once it works, it will run in a generally stable state. While struvite formation will require constant monitoring and change in Mg^{2+} , PO_4^{3-} dose to meet the required $Mg:NH_4:PO_4$ ratio. This plus the fact that the struvite formation requires consumable chemicals like Mg^{2+} , PO_4^{3-} makes it a much less viable option when it comes to ammonia removal pre-treatment for fermented biosolids.

Based on the previous test, the approximate amount of fermented sludge that needed pre-treatment to remove $\sim 20\text{mg/L NO}_3$ will be around 1.5% of the WWTP flow rate.

5.3.3.2 Lab-scale batch test for struvite formation and ammonia stripping

The lab-scale set-ups for both struvite formation and ammonia stripping in this experiment are simple. For the struvite formation test, magnesium chloride was added into 200mL of a filtered fermented sludge sample to produce a 1.2: 1.0 ratio for $Mg^{2+}: NH_4^+$ Kim et al. (2009). The fermented sludge had to be filtered to ensure that ammonia was not converted to nitrate due to nitrification. Excessive mixing was ensured as a Gtd (produce of mixing intensity and duration, $G \times t$ or Gtd for short) of more than the suggested 106 were achieved Kim et al. (2009).

For the ammonia stripping set-up, nitrogen gas was set to bubble through another 200mL of filtered fermented sludge using an air stone. The sample's pH was then adjusted using calcium carbonate when required. Considering the C:N ratio available in fermentation batch tests, 50-65% ammonia removal would have to be removed. The results of various running scenario are shown in Figure 5.7.

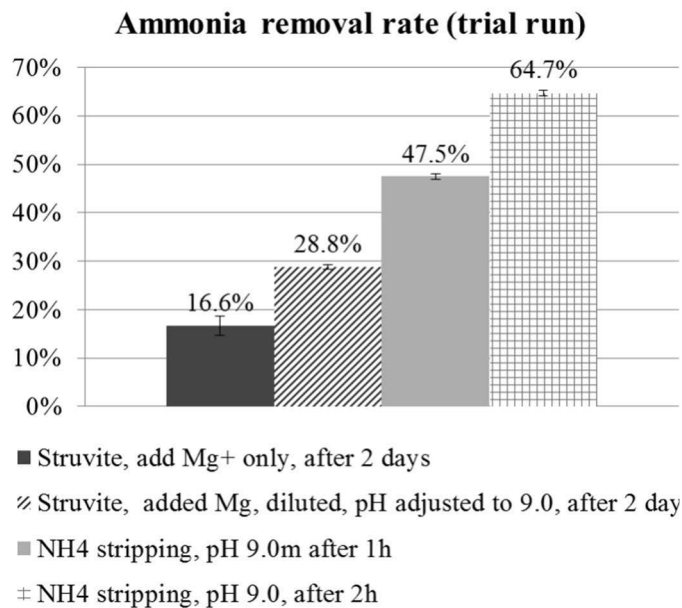


Figure 5.7: The Ammonia removal rate of different set ups.

Regarding struvite formation, while it is definitely possible to further increase the ammonia removal to as high as 75% according to some studies Tong and Chen et al. (2009); Feng et al. (2009), this will require the addition of phosphorus-rich materials into the fermented and dark fermented sludge solutions. However, adding phosphorus here poses the risk that if ineffective struvite formation is achieved, high concentrations of phosphorus will be introduced into the anoxic zone of the WWTP. This will subsequently compromise the WWTP's effluent quality. Ammonia stripping could achieve the 65% ratio within 2 hours, simply through over-aeration of the fermented sludge. The reported commercial ammonia stripping reported that it could achieve as much as 99%.

5.4 Conclusions

The study has assessed the potential of using fermented sludge as an external carbon source for improving denitrification under various independent variables including SRT (5 and 10 days), fermentation type (fermentation and dark fermentation), type of feed (WAS and biosolids) and effect of an added

carbohydrate (sugar). The received C:N ratios for all tested scenarios (varying from a minimum 5.88 ± 0.26 to a maximum 13.57 ± 1.15), however, turned out to be significantly less than the suggested minimum C:N ratio of 20:1 and the ideal of 30:1 for external carbon sources. This is despite the fact it had already optimised pH and temperature conditions. This low C:N ratio was due to the high initial NH_4^+ concentration of inoculum and feeds, meaning further optimising of the fermentation and dark fermentation process would not improve the C:N ratio by much.

While adding more carbohydrate to fix the C:N ratio is possible, this actually defeated one of the main purposes of fermenting sludge: to reduce the amount of sludge and biosolids that each WWTP has to dispose of each year. Instead of reducing the sludge, a theoretical minimum of 4 times of TS will be produced from the fermenter.

To improve these C:N ratios, post-treatment methods including struvite formation and ammonia stripping were reviewed and tested in lab-scale experiments to reduce the high ammonia concentration of fermented sludge samples. While struvite formation did not produce a good outcome, ammonia stripping post-treatment was a success, in that up to 64.7% of ammonia could be removed.

Finally, overall this whole experiment found that a C:N ratio of 20:1 to 30:1 could only be achieved for almost all studied fermentation and dark fermentation scenarios if post-treatment was applied.



Chapter 6

Fermented Sludge as Alternative External Carbon Source

Chapter 6 FERMENTED SLUDGE AS ALTERNATIVE EXTERNAL CARBON SOURCE

6.1 Introduction

Previously in chapter 5, batch tests on various samples of fermented and dark fermented WAS and anaerobic digested biosolids were carried out to: firstly, find the optimum conditions; and secondly, assess their potential as external carbon sources Phung et al (2015). While none of the tested conditions met the minimum required standard due to the initial high ammonia concentration, after being treated by ammonia stripping, their initial characteristics (mostly C:N ratio) all showed them to be viable for further testing in this study.

Two of the fermented sludge amongst seven options - fermented and dark fermented biosolids - were chosen for further study. The decision was based on the simplicity and fewer resources needed to prepare for each batch test as seen in Chapter 5, compared to the others that required a mixed stream and/or addition of cellulose carbons. Biosolids were chosen over WAS as feed simply because the earlier batch test showed the C:N ratio of both to be insignificantly different, means it's likely that fermented and dark fermented of WAS will provide similar results. This selection in practice could be translated to a fermenter set-up (or dark fermenter) being placed directly after an anaerobic digester. This allows the fermenter to utilise the anaerobic digester's inoculum and thermal energy, while improving biogas generation and further reducing the biosolids mass that has to be disposed of.

Note: most of Chapter 6 has been published, or under review of the following papers:

Published

Phung, D. P., Sy, 2016. "Assess the denitrification potential of fermented biosolids based on their specific denitrification rate (SDNR) " Journal of Science and Technology, vol. 54, pp. 112-119.

Phung, D. P., Sy, 2016. "Assess the Denitrification Performance of Fermented and Dark-fermented Biosolids as External Carbon Sources Using Sequence Batch Reactors (SBRs) " Journal of Environmental Science and Engineering B, vol. 5, pp. 425-431.

6.2 Materials and Methods

6.2.1 Methodology

Similar to Chapter 4, except with the absence of the pilot plant due to its unavailability for this study, the study in this chapter is also based on the cross-verification of results obtained from the three mathematical and physical simulation models illustrated in Figure 6.1 below:

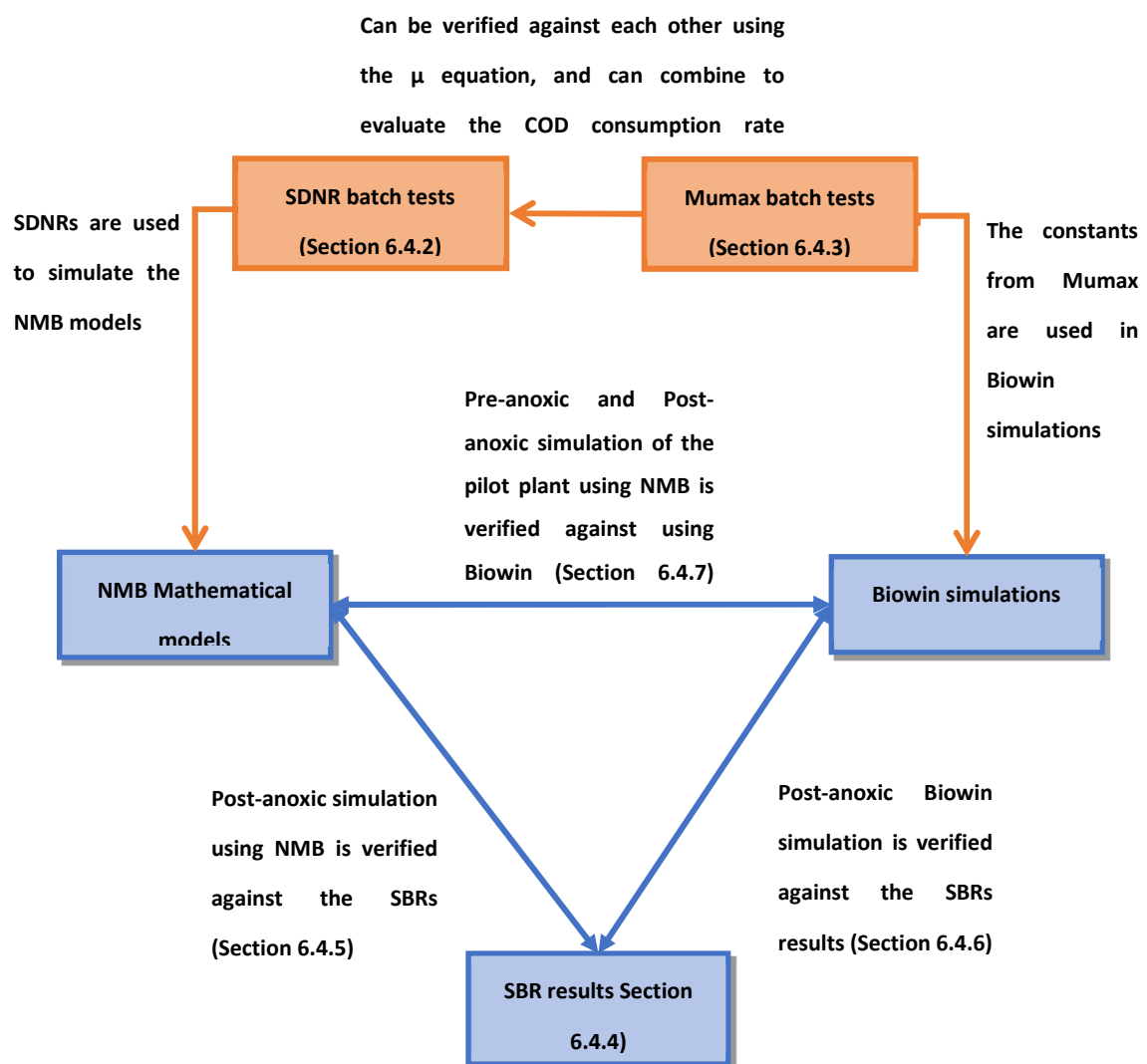


Figure 6.1: The relationships between different simulation models used in this chapter

6.2.2 Chemicals and Inoculums

The synthetic wastewater being used for some of the batch tests consists of commercial beef extract as main organic carbon and nitrogen sources. The real wastewater used in the experiment was collected from the equalisation tank from local wastewater treatment plant.

The nutrient and trace metal solutions for this synthetic wastewater were based on (Hu et al., 2010) consisting of 10 mg CaCl_2/L , 18 mg $\text{K}_2\text{HPO}_4/\text{L}$, 11 mg $\text{KH}_2\text{PO}_4/\text{L}$, 10 mg MgSO_4/L , 0.15 mg CoCl_2/L , 0.03 mg CuSO_4/L , 10 mg FeSO_4/L , 0.15 mg $\text{H}_3\text{BO}_3/\text{L}$, 0.18 mg KI/L , 0.12 mg MnCl_2/L , 0.06 mg NaMoO_4/L and 0.12 mg ZnSO_4/L . pH in this study was controlled using diluted citric acid. Almost all of the chemicals were supplied by Science Supply Australia.

6.2.3 Denitrifying biomass

The SBRs and batch tests were seeded with the raw sludge of the pre-anoxic zone from Sunbury WWTP. The raw sludge was sieved to remove any large particles before being fed into the SBRs or any batch tests.

For this study, the SBRs had been running in stable condition for roughly 10 months before the start of the experiment. For the two tested SBRs to acclimate with the two types of fermented sludge, two tested fermented biosolids solutions with added COD concentrations of 150-170 mg/L were added into anoxic zone for 4 weeks previous to the start of the experiment.

Similar to a recently published study on sludge fermentation (Phung et al., 2015), the inoculum for this fermentation experiment was also taken from a local

anaerobic digestion plant rather than a standard WWTP. This was done because it best simulates the conditions of a continuous running fermenter state. The biosolids were taken from the effluent drains in the same anaerobic digester. Both of the feeds and inoculum were sieved to remove larger particles.

The used biosolids samples had similar characteristic to the one in earlier published papers with Total Solid (TS) of $28,342 \pm 1319$ mg/L and Volatile Solid (VS) of $20,755 \pm 1152$ mg/L. The biggest difference was the high undiluted soluble COD in the inoculum using in this chapter was 4919 ± 392 mg/L. This was generally still low within the expected range due the measured VS of over 20,000 mg/L. However, considering it's higher than the one found in the earlier published paper (1247 ± 79 mg/L) (Phung et al., 2015), this would be taken into account when assessing the final soluble COD.

6.2.4 Analytical Method

COD, TN, Ammonia, Nitrate and Volatile Fatty Acid were sampled and analysed using DR 5000 HACH standard methods (Methods 8000, 10072, 10031, 10020 and 8196 respectively). pH and DO were tested by using the Mettler Toledo S20 Seveneasy pH meter and YSI 5100 Dissolved Oxygen Meter. The rbCOD was measured by filtering with 0.45 μ m membrane filter papers before being tested with COD reagent kit. This is because according to (Melcer et al., 2003), most of the particulate soluble COD would have been absorbed into the sludge, and the measured COD using this method would be very close to actual rbCOD (difference of <5 mg/L).

The MLSS was measured the same way with Total Solids, where 100 mL of sample were filtered and dried overnight in 105-degrees oven. While MLVSS when required, is measured by igniting the MLSS sample at 550°C for 30 minutes to remove volatile solids.

Note: Whenever the synthetic wastewater is involved, due to the soluble nature of the synthetic wastewater, the Mixed Liquor Volatile Suspended Solid (MLVSS) of the sludge was found to be insignificantly different to the Mixed Liquor Suspended Solid (MLSS) (using SPSS program). Hence, these two values are interchanged in those experiments.

6.3 Experimental set-up

6.3.1 Fermentation Sludge Generation

The ratio of fermented and dark fermented biosolids to inoculum were consistent to what has been published (inoculum: feed of 80:20) (Phung et al., 2015) before each reactor pH was then adjusted using diluted citric acid, or/and Milli-Q water. Each fermentation reactor was purged with nitrogen gas before being placed into a shaker subjected to continuous mixing for 5 days. This period time is the ideal one for fermentation according to the literature and earlier experimental results. Generated gas was released daily from the reactor, except for the first few days when it was released twice a day to prevent pressure building up inside the reactor.

The pH for fermentation reactors were set at 6.6 ± 0.1 according to results from earlier batch test findings (Lu et al., 2007), while the pH chosen for dark fermentation was 5.6 ± 0.1 (Yuan et al., 2009). Both sets of reactors operated at a temperature of just below 55 °C, which is reported to be ideal for thermophilic

organisms. The two selected conditions found that a C:N ratio of more than the minimum 20:1 could be attained for both of them after pre-treatment with ammonia stripping.

After the fermentation process, the pH level of the two fermented biosolids was adjusted to pH 10 using calcium carbonate as this pH was reported to be ideal for ammonia stripping. The solid matter was then filtered out of the two fermented biosolids samples to eliminate any chance that ammonia reduction would be caused by nitrification. The ammonia-stripping set-up was simple: air was set to bubble through the 2L beakers of fermented biosolids using an air stone. Ammonia was measured every 2 hours until the target (>65% ammonia removal) was reached.

Because of the need to preserve this sample for the whole duration of the experiment (over 4 weeks), the fermented sludge samples used for the SBRs were adjusted using sulphuric acid to pH < 2 for storage. They were later readjusted to pH 7 before being fed into the SBR reactor.

6.3.2 Sequencing Batch Reactors SBRs

Just like in Chapter 4, overall, the SBRs set-up followed a standard post-anoxic denitrification system. The SBRs ran continuously 24 hours a day, 7 days a week. One cycle of the SBR lasted 8 hours, consisting of 30 minutes of unmixed feeding, 240 minutes of aeration, 110 minutes of anoxic mixing, 70 minutes of settling and 25 minutes of decanting. Also, each reactor held an effective volume of approximately 4 L, with the decanting volume 50% or 2 L per cycle. The Sludge

Retention Time (SRT) was selected to be 11.5 ± 0.5 days based on literature (Kargi et al., 2004; Obaja et al., 2005), providing an approximate MLVSS of 2,200-2,600 mg/L during the course of the experiment.

The SBRs had been running for more than 10 months prior to the experiment commencing, much more than what was required to stabilise the denitrification process (Sun et al., 2016). However, the data collected for this specific experiment was only for 8 weeks or roughly more than four SRTs of the SBRs. For the first 4 weeks, no external carbon source was used. During the next 4 weeks, a fermented biosolids solution was pumped into the post-anoxic cycle of the two tested SBRs. The optimum additional carbon for TN removal is 77-174 mg/L, or 25-56% of influent COD (Park et al., 2014; Lu et al., 2007; Yuan et al., 2009). Regarding the SBRs employed in this experiment, this is equivalent to 130-295 mg/L. An amount of 150-170 mgCOD/L of fermented sludge was chosen because it was consistent with the previous study using sucrose instead of fermented biosolids.

The SBRs were monitored twice a week for NO_3^- , NH_4^+ , TN, COD, pH, DO and the effect of sucrose on the system denitrification was assessed.

6.3.3 SDNR test and NMB model

The SDNR experiments methodology was the same method used in literature (Cherchi et al., 2009; Dold et al., 2008) and earlier published paper (Phung, 2014). The sludge samples used for SDNR tests were taken from the SBRs that were fed with fermented and dark fermented biosolids, respectively, for

more than 2 SRT (22-24 days). The average initial MLVSS of all reactors (include the duplicates) is 908 ± 29 mg/L. The initial COD is 704 ± 2.2 mg/L for fermented biosolids reactors and 607 ± 3.7 mg/L for dark fermented biosolids reactors. The range of 600-700 mg/L was based on the literature to ensure that excessive carbon sources are available during the whole experiment.

COD, NH_4 , Volatile Acid (VA) concentration were measured at the beginning and at the end of each experiment. Nitrate concentration was monitored every 30 minutes for over 2.5 hours. The SDNR (also the theoretical maximum denitrification rate of tested carbons) was calculated from the constant slope of removed $\text{NO}_3\text{-N}$ concentration.

Potassium nitrate, ammonia chloride, potassium hydrogen orthophosphorus were added into the sludge slurry to maintain a concentration of $\sim 30\text{mgNO}_3\text{-N/L}$, $\sim 60\text{mgNH}_4\text{-N/L}$ and $\sim 9\text{mgPO}_4\text{-P/L}$, respectively in each reactor. This should provide excessive nutrient sources for denitrification process to reach the maximum rate (Dold et al. 2008).

Based on the SDNR of the system, the NMB model could then be built to predict the denitrification performance of the pilot-scale system. The detail calculation for NMB model and rationale are attached in Appendix I and II of this report. In summary, the expected effluent for post-anoxic denitrification based on NMB model will be:

$$\text{NO}_{3\text{ eff}} = 80\%TN_{\text{ini}} - \text{SDNR} \times \text{MLVSS} \times t - \text{NH}_{4\text{ eff}} \quad (1)$$

While the expected effluent for pre-anoxic denitrification based on NMB model will be:

$$NO_{3\text{ effluent}} = \frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}}(mg/L) \quad (2)$$

Only when

$$SDNR \geq \frac{\left(\frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}} \right) \times IR}{v + IR} (mg/L) \quad (3)$$

6.3.4 Mumax Batch test and Biowin model

The Mumax test set-up was also based on (Dold et al., 2008) just like the same set up in Chapter 4 of the thesis. The Mumax tests for this experiment were first planned to run for 5 days but later was cancelled halfway through and readjusted to 1.5 days. The rationale for this decision is explained in more detail in the Results and Discussion section of this chapter.

The tests were conducted in 1-L cylindrical closed vessels, each with an inlet for carbon dioxide gas to control the pH, and an outlet to relieve pressures. They were mixed gently using magnetic stirrer plate to prevent aeration by the

atmosphere, and temperature controlled was at 20 °C. Before beginning of the test, a small amount of approximate 50 mg/L MLVSS from the SBR (which already set to acclimate to fermented sludge) was added into the reactor. Fermented sludge was added to make an initial rbCOD of 480-550 mg/L. Diluted KNO₃, NH₄Cl and KH₂PO₄ solution were added in to make an initial concentration of approximate 250 mg/L, 80 mg/L and 30 mg/L of nitrate, ammonia and phosphorus respectively. These are meant to be excessive to ensure there was no restriction on anoxic biomass growth. Finally, the reactors were mixed with post-nitrification decanted substrate from the SBR to make up to 1 L reactor mark. That filtered decanted substrate would ensure that the denitrifier could quickly adapt to the new environment in the batch tests.

The sludge used for this test is the same as the one used in the SDNR tests, where the SBRs sludge was acclimated with fermented and dark fermented biosolids, respectively, for more than 2 SRT (22-24 days). These ensured the sludge had completely acclimated to each fermented biosolid so that a relatively maximum growth rate and yield rate could be achieved.

During the original planning with 5 days running, the samples were taken from the batch tests 3 times a day, every 3 hours, with pH being monitored regularly to keep the pH within the range 6.9-7.3. This however was changed to every 3 hours during the 1.5-day running instead.

Carbon dioxide gas and calcium carbonate were added and adjusted in the reactor to control the pH. The reactors would be sealed entirely with only exception when samples were taken out. The total number of samples being taken out in each reactor for the duration of the experiment was less than 100 mL,

ensuring at least 90 % of initial volume to remain by the end of the batch tests.

The batch test set-up is showed in Figure 6.2 below:

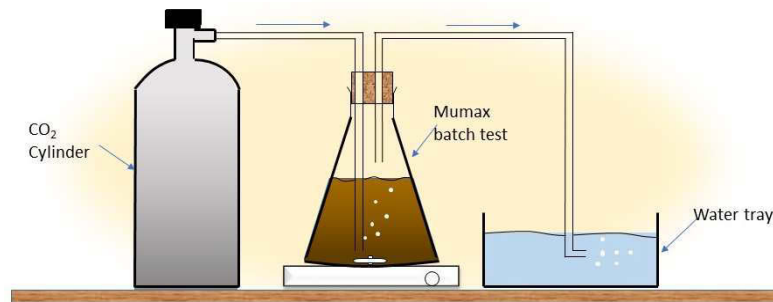


Figure 6.2: The Mumax batch test set up.

The main objective of Mumax experiment was recording and plotting the nitrate reduction over time (SNO.t over t) graph. Then based on the graph and known variables, the data could be submitted into mathematical program like Wolfram Mathematica to best-fit the graph into the Mumax equation and find the best growth yield (YH) and growth rate value (μ_S). YH and μ_S could then be input into simulation software like Biowin to simulate the performance of sucrose as external carbon sources.

6.4 Results and Discussions

6.4.1 Characteristics of Tested Fermented Sludge

The characteristics of the two fermented sludge samples that will be used in this experiment are set out in Table 6.1 below.

Table 6.1: The full set of data for all fermentation experiments.

No.	Types	Feed types	Planned pH	pH	rbCOD	NH ₄	C:N	VF A	VFA %
1	Fermentation	Biosolids	6.5	7.36 ± 0.01	11,730 ± 102	1,135 ± 25	10.34 ± 0.17	1,702 ± 68	14.5 ± 0.6
2	Dark Fermentation		5.5	6.89 ± 0.04	11,257 ± 275	1,110 ± 11	10.14 ± 0.12	1,158 ± 5	10.3 ± 0.3

The full results for the fermentation and dark fermentation batch tests are shown in Table 6.1 above, with the C:N ratio for the two sets of experiments being 10.34 ± 0.17 and 10.14 ± 0.12 , respectively. They are slightly higher than the 5.8–9.3 recorded in an earlier study (Phung et al., 2015) (which is consistent with the analysis by Liu et al. (2016)), most likely due to the initial higher soluble COD in used inoculum as noted in section 6.2.3. However, the difference is not as significant because for both sets, the results were still much lower than the minimum required C:N ratio of 20:1 (or ideally 30:1) to be considered as an effective external carbon source. (Feng et al., 2009)

Note that the C:N ratio or rbCOD/NH₄⁺ is the most important independent variable in this experiment. Because the fermented biosolids will be added to the anoxic zone as an external carbon source, a lower C:N ratio would mean that with the same amount of added carbon, more nitrogen is introduced back into the system. This in turn will undermine the nitrogen removal capacity of the whole system.

The TN component in C:N is theoretically supposed to be the Total Nitrogen instead of NH₄⁺. However, soluble organic nitrogen and nitrates were

tested for the filtered samples and the difference in concentrations of these two were found to be insignificant, especially when comparing the remaining organic nitrogen (after being filtered) to the high concentration of soluble NH_4^+ . The method used for NH_4^+ analysis is also much more reliable and produced far less errors compared to TN measurement. Making use of NH_4^+ measurement ironically proved to be more accurate than the TN measurement in this case.

Note that even in unexpected circumstances where higher organic nitrogen and nitrates were found in the fermented biosolids, the impact it had would still be minimal. This is because once added into the anoxic zone as external carbon sources, the nitrate and/or organic nitrogen would be quickly denitrified and/or absorbed into the sludge mass, respectively. Consequently, these two parameters would be even less relevant.

Just like Chapter 5 mentioned, once the fermented and dark fermented biosolids samples were generated, collected and filtered, ammonia stripping was then carried out as pre-treatment to fix the C:N ratio to the minimum required 20:1 ratio. The results are indicated in Figure 6.3 below.

Table 6.2: Ammonia removal using ammonia stripping

	Duration	Initial NH_4 (mg/L)	Final NH_4 (mg/L)	% NH_4 removed	Final C:N ratio
Fermented biosolids	2 hours	1135±25	673±6	40.6±1.6%	17.4±0.2
	4 hours		392±13	65.5±0.9%	30.0±0.7
Dark Fermented biosolids	2 hours	1110±15	695±15	37.4±2.1%	16.2±0.7
	4 hours		363±2	67.3±0.2%	31.0±0.6

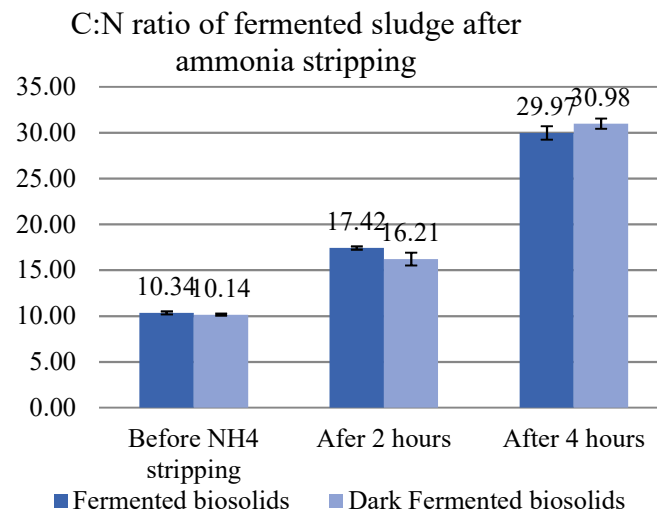


Figure 6.3: Ammonia stripping performance as pre-treatment to fix the fermented biosolids C:N ratio

As seen in Table 6.2 and Figure 6.3, it took 4 hours of constant aeration to reduce the NH₄⁺ of fermentation and dark fermentation by an average of 65.5% and 67.3%, respectively. This results in a C:N ratio of 30.0 ± 0.7 and 31.0 ± 0.6 , which is much higher than the minimum required 20:1 and is around the ideal C:N ratio 30:1 to be considered as an effective external carbon source. Furthermore, it should be noted that this removal efficiency was derived from a simple lab-scale aeration set up and can still be further optimised if needed. Commercial ammonia stripping units were widely reported to reach a value as high as 99%.

6.4.2 Results from SDNR Tests

The C:N ratio of these two fermented biosolids samples show that they are suitable as external carbon sources. The next step is to calculate the specific denitrification rate (SDNR) of these external carbon sources. This was done by setting up two sets of SDNR batch tests with duplicates to theoretically calculate each carbon source's SDNR. The denitrification potentials or SDNRs of fermented

and dark fermented biosolids after adjustment with MLVSS are summarised in Figure 6.4 below.

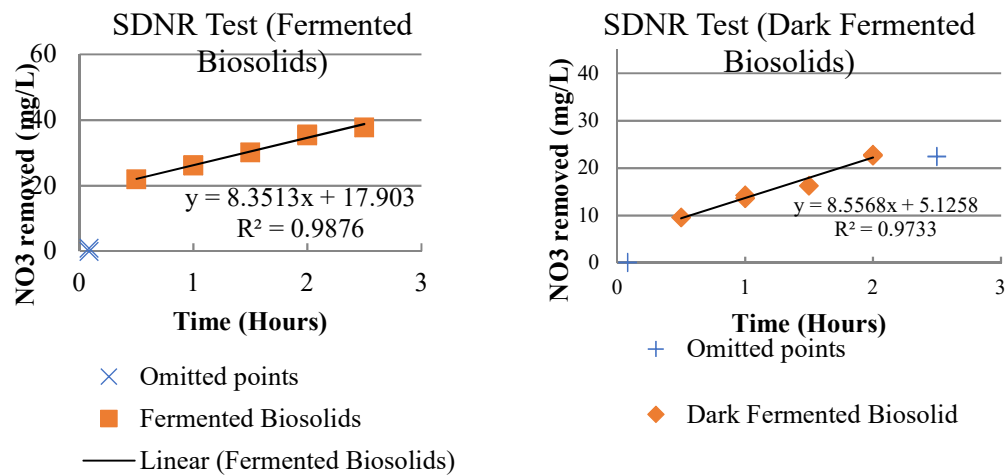


Figure 6.4: Nitrate profile of the two sets of SDNR batch test (including duplicates)

The data for each reactor and its duplicate was then fed into R-Statistics software to calculate the slopes (SDNR), the R^2 , the adjusted R^2 , the Significant Error (SE) and the 95% Confidence Interval (95% CI). The detailed results are presented in Figure 6.5 below.

As can be seen in Figure 6.4, after being omitted for the first 30 minutes due to the microorganisms needing some time to acclimate to the reactors, both reactors demonstrated quite consistent linear trends over most of the 2.5 hours of testing time. The reason why the first 30 minutes were omitted was based on earlier studies finding that even between repeated duplicates with same final SDNR results, the amount of nitrate removed during the first half hour could vary greatly.

For the dark fermented biosolids, the SDNR rate slowed down and in fact almost petered out completely towards the end. This was quite unexpected as the final rbCOD concentration in dark fermented biosolids of the SDNR batch tests was still quite high (460 ± 7.5 mg/L), indicating it was not due to the lack of rbCOD. Upon checking the Volatile Acid (VA) for both SDNR reactors, the VA was found to be almost depleted. This has been explained in other studies (Feng et al., 2009; Melcer et al., 2003; Tong and Chen, 2009; Zheng, Chen & Liu, 2010) , where fermented sludge was used as an external carbon source. VA rather than other non-VA rbCOD is the preferred carbon source for denitrification. If this is true it would mean depletion of VA may ultimately affect the denitrification rate of the system. However, the SDNR results for the fermented sludge actually contradicted that, as the SDNR of fermented biosolids was almost the same as the measured SDNR of dark fermented biosolids. This is despite the former having almost twice the initial VA concentration as the latter sludge, and the higher volatile acid behaving differently than the less volatile one.

SDNR values of 8.35 ± 0.41 for fermented biosolids and 8.56 ± 0.71 for dark fermented biosolids were much higher than the 1.53-2.57 for sucrose and 1.29 ± 0.21 for wastewater that were using the same methodology. The SDNR values for fermented biosolids were also comparable to the 6.1-10.0 SDNR value for methanol (Dold et al., 2008; Mokhayeri et al., 2008). Considering that methanol is the most studied and commonly used external carbon sources in the US, this shows that fermented and dark fermented biosolids have genuine potential as external carbon sources.

Comparing the findings for fermented sludge in other studies, however, leads to mixed conclusions due to the wide range of results. Zheng, Chen & Liu (2010), for example, found the SDNR for fermented sludge to be 1.96, but in the same paper cited various studies where the range varied from 2.0 (Min et al., 2002), to 11.67 (Chae et al., 2004) and 23.75 (Aesoy and Odegaard., 1994). Others include 1.4-1.9 (Soares et al., 2010), 0.5-3.4 (Ahn et al., 2010) and 15.0 (Kampas et al., 2009).

Table 6.3: The SDNR results concerning the two fermented and dark fermented biosolids

	COD (mg/L)		VA (mg/L)		ML VSS	SDNR Results					Δ mg COD / Δ mg NO ₃
	Init ial	Fi nal	Init ial	Fi nal		SD NR	R ²	R ² (adjus ted)	S E	SDN R (95% CI)	
Ferme nted Biosol ids	70 4 ± 2 . 2	44 2 ± 1 . .0	10 2 ± 0 . 3	2.0 ± 1 . .0	930 ± 20	8.3 5	0.9 876	0.985 1	0. 41	7.50- 9.19	7.57 ± 0.13
Dark Ferme nted Biosol ids	60 7 ± 3 . 7	46 0 ± 7 . .5	62 ± 0 . 4	2.0 ± 0 . .5	885 ± 15	8.5 6	0.9 733	0.966 7	0. 71	7.04 - 10.07	7.43 ± 0.58

Not only the similar SDNR, the Δ mgCOD/ Δ mgNO₃ of fermented biosolids and dark fermented biosolids were also very similar to each other. While the consumption of COD per mgN removed often far exceeds the theoretical (Dahab and Lee, 1998; Ge et al., 2012), the figure in this study is higher than the 3.5-4.0 reported by (Zheng et al., 2010) and 4.2-5.7 by (Guo et al., 2017) for other types of fermented sludge. It also much higher than the study by (Winkler et al., 2011)

where they found the SDNR for a mixture of real wastewater and fermented sludge (90-10 ratio) in open and close reactors to be 0.39 and 0.82, respectively. These findings were, however, lower than the 9.0-12.0 documented by (Soares et al., 2010).

6.4.3 Mumax Batch Tests

As previously stated in the Experimental Set-up section, the Mumax tests were established to run over 5 days. However, the tests were immediately halted after the first two days due to the results that are presented in Figure 6.5 below.

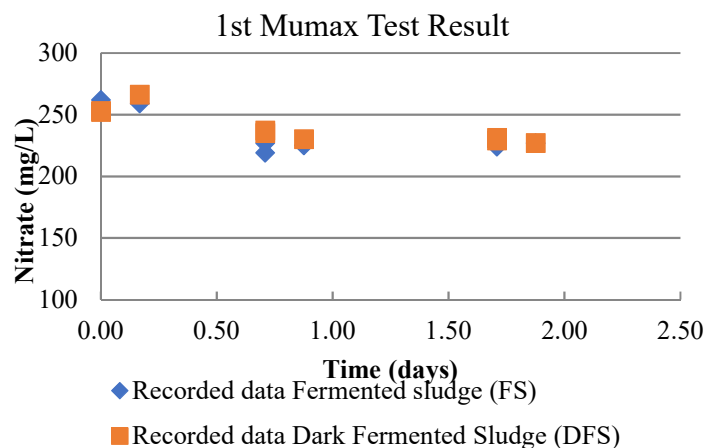


Figure 6.5: The first Mumax batch test performance

It could be observed that the reduction in nitrate concentration during the first day was so unusually high (compared to the author previous study with sucrose). This would mean that if maximum growth rate can be sustained, the experiment would run for much shorter than the 5-days plan. This hypothesis was immediately confirmed as early as the second day of the experiments when no

more denitrification occurred in the second day in all Mumax tests (including the duplicate reactors). With this in mind the experimental plan was revised, where the second Mumax test was run for 1.5 days instead, and the sample taken every 3 hours to better detect the nitrate reduction trend. The results can be seen in Figure 6 below.

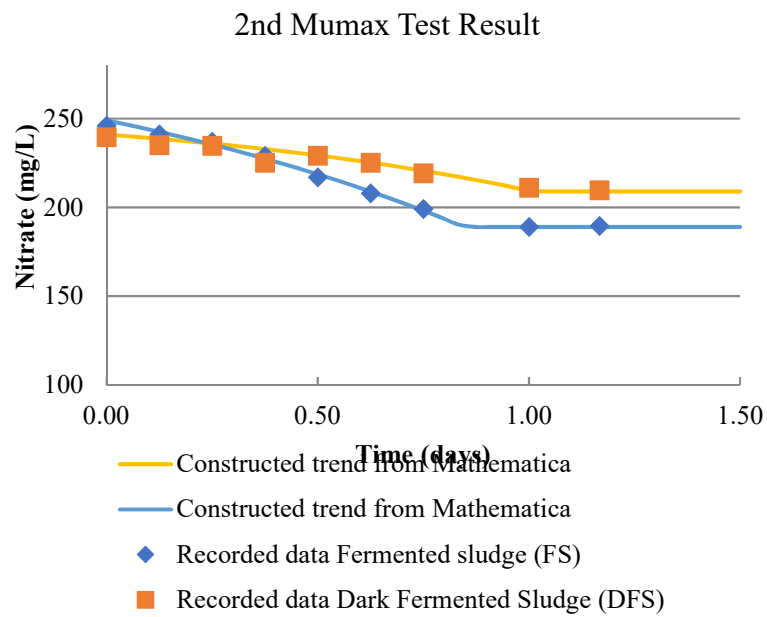


Figure 6.6: The second Mumax batch test performance

The results from the above graphs, along with known parameters, were fed into the Wolfram Mathematica program using $S_{NO,t} = S_{NO,0} - \frac{1-Y_M}{2.86} \times \frac{\mu_s \times X_{S,0}}{Y_M \times (\mu_s - b_s)} \times [e^{(\mu_s - b_s)t} - 1]$ to find the growth yield (YH) and growth rate value (μ_s). These are recorded in Table 6.4 below.

Table 6.4: Results from the Mathematica analysis

Experiments	Growth yield efficient (Y_M)				Growth rate (μ_S)			
	Estimate	Std Error	t-statistic	P-value	Estimate	Std Error	t-statistic	P-value
Fermented biosolids	0.172	0.070	2.465	0.057	0.854	0.324	2.639	0.046
Dark fermented biosolids	0.393	0.207	1.920	0.127	1.099	0.672	1.635	0.177
Biowin default	0.50				0.54			

Table 6.5: Growth yield (Y_H) and growth rate value (μ_S) retrieved from the Mumax batch tests

Experiments	Consumed COD (mg/L)	NH ₄ removed (mg/L)	Nitrate removed (mg/L)	Initial denitrifier mass (Xs.0) (mg COD/L)	Decay rate (default) (b_S)	Growth yield efficient (Y_M)	Growth rate (μ_S)
Fermented biosolids	195 ± 8	7.4 ± 2.0	65 ± 7	34.2 ± 3.3	0.04	0.17 ± 0.07	0.85 ± 0.32
Dark fermented biosolids	409 ± 2	1.1 ± 2.1	30 ± 1	29.9 ± 2.8	0.04	0.39 ± 0.20	1.10 ± 0.67

The growth yield of both fermented biosolids and dark fermented biosolids fell within the 0.06-0.61 range, as reported by Zheng, Chen & Liu (2010).

By combining the carbon consumption rate from the SDNR experiments and the yield rate from the Mumax tests, the amount of carbon consumed by the denitrifier for cell growth (in oppose to carbon consumed for denitrification activities) could be calculated and presented in Table 6.6.

Table 6.6: Carbon used by denitrifiers based on Mumax batch test results

Carbon source types (Y_h)	$\Delta\text{mgCOD}^*/\text{mgNO}_3$	$\Delta\text{mgCOD}/\text{mgNO}_3$ consumed for cell growth	$\Delta\text{mgCOD} / \text{mgNO}_3$ for other activities (exclude cell growth)
Fermented Biosolids (0.17)	7.57 ± 0.13	1.29 ± 0.53	6.29 ± 0.54
Dark fermented Biosolids (0.39)	7.43 ± 0.58	2.90 ± 1.50	4.53 ± 1.61

* The mgCOD consumed in this table is the readily biodegradable COD.

It is evident here that despite having a fairly similar carbon consumption rate for every mg of nitrate removal, dark fermented biosolids actually used more than twice as much carbon for the denitrifier's cell growth when compared to fermented biosolids. This means that despite having a similar level of denitrification performance, the dark fermented biosolids can help more to populate the denitrifier population of a biological treatment system.

6.4.4 The SBRs results

As mentioned previously, the results of the SBRs have been published by (Phung et al., 2016). The results are summarised in Table 6.7 below.

Table 6.7: Summary of the SBRs results

	NO ₃ -N (mg/L)		NH ₄ -N (mg/L)		rbCOD (mg/L)		MLVSS (mg/L)		pH	
	Befo re*	Aft er	Bef ore	Aft er	Bef ore	Aft er	Bef ore	Aft er	Bef ore	Aft er
SBR A (Contr ol)	21.8 ± 1.6	19. 8 ± 1.3	1.2 ± 1.0	1.4 ± 1.0	43.3 ± 9.2	47. 8 ± 18. 3	203 0 ± 110	19 70 ± 13 9	7.03 ± 0.17	7.5 9 ± 0.4 7
SBR B (Ferme nted Biosoli ds)	19.8 ± 1.5	1.4 ± 0.7	0.6 ± 0.4	2.9 ± 1.0	40.6 ± 14.9	11 7.7 ± 35. 3	231 5 ± 45	18 58 ± 65	7.10 ± 0.16	7.8 0 ± 0.3 5
SBR C (Dark- fermen ted Biosoli ds)	20.4 ± 1.2	1.3 ± 0.8	0.6 ± 0.4	2.4 ± 0.9	44.1 ± 8.4	12 0.4 ± 38. 5	230 0 ± 30	19 53 ± 25 8	7.06 ± 0.15	7.5 5 ± 0.3 5

* This is before anoxic zone begin

Overall complete denitrification was achieved in the SBRs when fermented sludge was added. This agrees with another recently published study (Guo et al., 2016) on fermented sludge and revealed its denitrification potential when tested in a lab-scale SBR system. The denitrification rates calculated based on the results are 5.45 ± 0.61 and 5.50 ± 0.77 for fermented biosolids and dark fermented biosolids, respectively. These rates are slightly higher than the 3.32-3.89 for fermented WAS (Guo et al., 2016). Interestingly, despite the ammonia concentration increasing due to the ammonia components in the added fermented sludge (which should be an issue according to Zheng, Chen & Liu

(2010)), however the amount of increased ammonia concentration is less than half of the added amount, indicating some added ammonia was absorbed by the SBRs sludge mass instead of staying soluble.

Since the addition of external carbon source could cause an increase in both the rbCOD and ammonia concentration (Boley et al., 2000), it is recommended that a short additional aerobic zone is retained at the end of the anoxic zone to remove these two components in the effluents. The other alternative is reducing the amount of fermented sludge being added into the system. However, the denitrification rate of the added fermented sludge may be reduced and become more unpredictable because of it.

6.4.5 Post-anoxic denitrification simulation: NMB model vs SBRs

Based on the SDNR results in section 6.4.2, other influent and operation data, and the NMB equations from Appendix, the SBRs performance (post-anoxic denitrification) can be calculated utilising the NMB model written below.

Control Reactor:

$$\begin{aligned}
 NO_3 \text{ previous-anoxic} &= 80\%TN_{ini} - NH_4 \text{ pre-anoxic} \\
 &= 80\%TN_{influent} \times 50\% - NH_4 \text{ pre-anoxic} \\
 &= 80\% \times 56.50_{\pm 2.06} \times 0.5 - 1.18_{\pm 1.02} \\
 &= 21.42 \pm 1.82 \text{ mg/L}
 \end{aligned}$$

$$\begin{aligned}
NO_3 \text{ effluent} &= 80\%TN_{ini} \\
&\quad - SDNR \times MLVSS \times t - NH_4 \text{ pre-anoxic} \\
&= 21.42_{\pm 1.82} - SDNR \times MLVSS \times t \\
&= 21.42_{\pm 1.82} - 0.34 \times 1.970_{\pm 0.139} \times \frac{110}{60} \\
&= 20.19 \pm 1.91 \text{ mg/L}
\end{aligned}$$

Fermented Biosolids Reactor:

$$\begin{aligned}
NO_3 \text{ previous-anoxi} &= 80\%TN_{ini} - NH_4 \text{ pre-anoxi} \\
&= 80\%TN_{influent} \times 50\% - NH_4 \text{ pre-anoxi} \\
&= 80\% \times 56.50_{\pm 2.06} \times 0.5 - 0.59_{\pm 0.42} \\
&= 22.01 \pm 1.24 \text{ mg/L}
\end{aligned}$$

$$\begin{aligned}
NO_3 \text{ effluent} &= 80\%TN_{ini} \\
&\quad - SDNR \times MLVSS \times t - NH_4 \text{ pre-anoxic} \\
&= 22.01_{\pm 1.24} - SDNR \times MLVSS \times t \\
&= 22.01_{\pm 1.24} \\
&\quad - 8.35_{\pm 0.41} \times 1.858_{\pm 0.065} \times \frac{110}{60} \\
&= -11.85 \pm 2.95 \text{ or } < 0.1 \text{ mg/L}
\end{aligned}$$

Dark Fermented Biosolids Reactor:

$$\begin{aligned}
NO_3 \text{ previous-anoxic} &= 80\%TN_{ini} - NH_4 \text{ pre-anoxic} \\
&= 80\%TN_{influent} \times 50\% - NH_4 \text{ pre-anoxic} \\
&= 80\% \times 56.50_{\pm 2.06} \times 0.5 - 0.57_{\pm 0.43} \\
&= 22.03 \pm 1.25 \text{ mg/L}
\end{aligned}$$

$$\begin{aligned}
NO_3 \text{ effluent} &= 80\%TN_{ini} \\
&\quad - SDNR \times MLVSS \times t - NH_4 \text{ pre-anoxic} \\
&= 22.01_{\pm 1.24} - SDNR \times MLVSS \times t \\
&= 22.01_{\pm 1.24} \\
&\quad - 8.56_{\pm 0.71} \times 1.953_{\pm 0.258} \times \frac{110}{60} \\
&= -8.64 \pm 4.78 \text{ or } < 0.1 \text{ mg/L}
\end{aligned}$$

In which the MLVSS, NH_4 eff, and TN are variables measured, respectively, for each SBR. t in this case is the anoxic hydraulic retention time (HRT) in hours. SDNR_1 of the control reactors are based on the endogenous denitrification rate discussed in the literature. Meanwhile the SDNR_2 of the tested reactors refer to fermented biosolids SDNR based on the SDNR results.

Based on the results, because the amount of nitrate removal potential is higher than the remaining nitrate (shown with theoretical NO_3 effluent to be negative in both cases), the NMB model confirms that complete denitrification occurred in both reactors. These calculated theoretical results are comparable to those derived from the SBRs running for two SRTs according to a recent study (Phung et al., 2016). The fermented and dark fermented biosolids that were used as an external carbon source for those SBRs are shown in Table 6.1. The summarised results are listed in Table 6.8 below.

Table 6.8: Results from the NMB model compared to SBR results

NO_3 concentration (mg/L)	Control condition		Tested with fermented biosolids		Tested with dark fermented biosolids	
	SBRs	NMB	SBR	NMB	SBR	NMB
Before anoxic	21.8 ± 1.6	21.3 ± 1.8	19.8 ± 1.5	22.0 ± 1.2	20.4 ± 1.2	22.0 $\pm$
After anoxic	19.8 ± 1.3	20.2 ± 1.9	1.4 ± 0.7	<0.1*	1.3 ± 0.8	<0.1*

*the calculation indicated a higher denitrification potential than the amount of remaining nitrate; hence complete denitrification occurred

While theoretically (see calculations above) the complete denitrification can be achieved due to the denitrification potential being higher than the

available nitrate inside the reactor, this is unlikely to be the case. As mentioned in the SDNR set-up test, one of the key requirements for the SDNR to occur is the excessive availability of both nutrients and available nitrate as electron donor. As nitrate inside the reactor is depleted, the denitrification rate should also significantly decrease and the actual nitrate removal should be much lower than the calculated nitrate removal.

However, the NMB model in this case still indicates that a near-complete denitrification should always occur in the two tested reactors. This is backed up by not only the average SBR performance, but also individual SBR performance over time as seen in Figure 6.7, where almost complete denitrification is recorded in most of the measured results. This is consistent with previous literature (Liu et al., 2016) which also found complete denitrification when using SBRs to test the nitrate removal efficiency of fermented sludge.

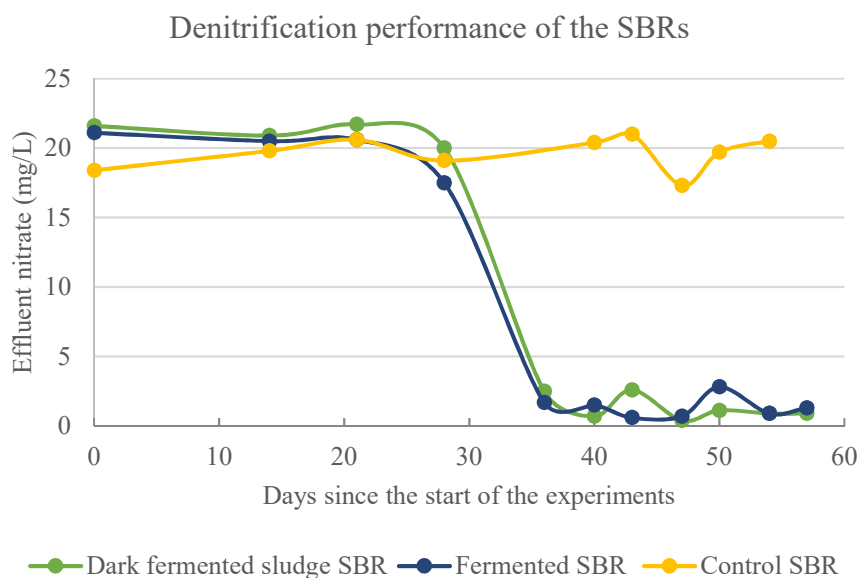


Figure 6.7: The effluent characteristics of the control and tested SBRs

6.4.6 Post-anoxic denitrification simulation: Biowin model vs SBRs

The effect of adding fermented biosolids and dark fermented biosolids into the post-anoxic zones can also be simulated using Biowin. The Biowin simulations are dynamic simulations based on the physical plant and influent characteristics data of the Sunbury pilot plant plus an extra post-anoxic zone: the influent input data recorded once a day, 3 times a week during the period from 21st September 2013 to 21st October 2013. Furthermore, the denitrification stoichiometry and kinetic constants were derived from the Mumax batch tests.

Along with the control condition (before any external carbon being added), four post-anoxic and two-anoxic scenarios were simulated (as listed in Appendix III). In the first two scenarios, fermented biosolids or dark fermented biosolids were added at a constant rate to increase the COD concentration in the anoxic zone by 150mg/L. The remaining nitrate and TN of the simulation are summarised in Figure 6.8 and Figure 6.9 below:

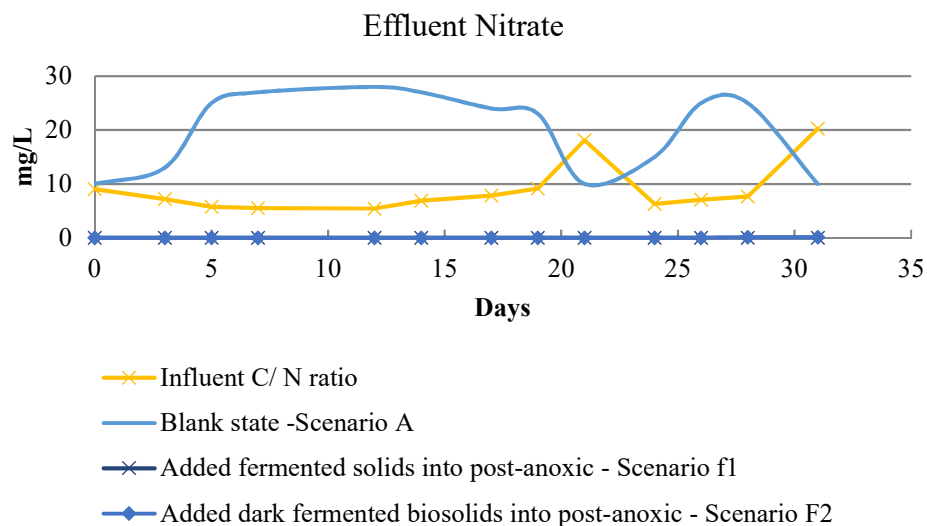


Figure 6.8: The effluent nitrate from Biowin simulation of the pilot plant (control stage versus after added fermented sludge)

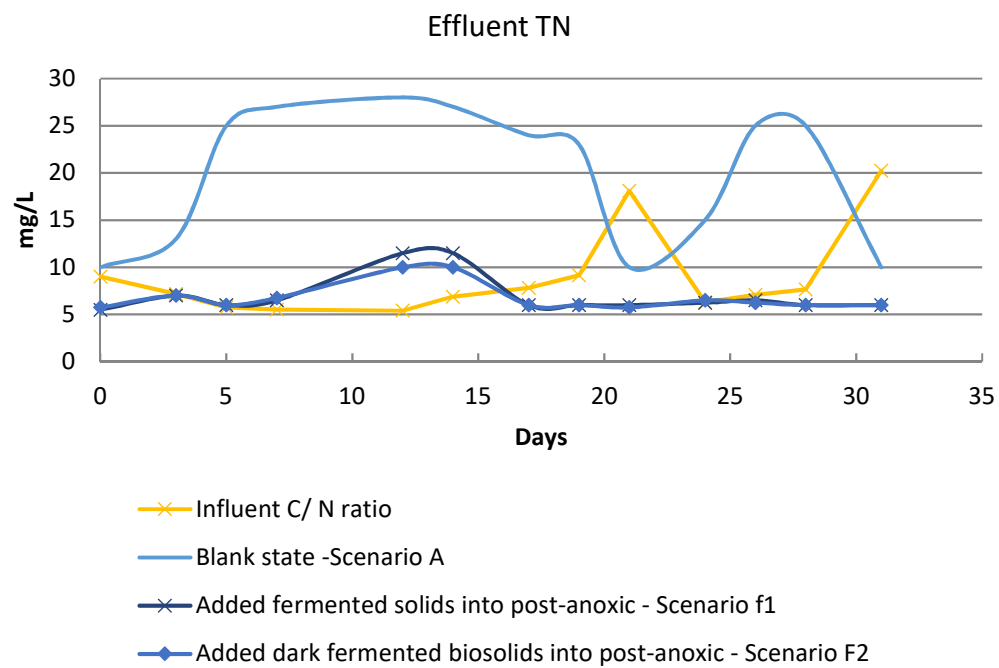


Figure 6.9: The effluent TN from Biowin simulation of the pilot plant (control stage versus after added fermented sludge)

The average results are summarised in Table 6.9 below.

Table 6.9: The effluent NO₃ and TN based on Biowin simulation of the pilot plant compared to the SBRs results

		Effluent NO ₃ (mg/L)	Effluent TN (mg/L)
Biowin simulation	Blank stage	20.2 ± 7.0	31.1 ± 13.7
	Added fermented biosolids into the post-anoxic zone (Scenario f1)	<0.1	6.98 ± 1.96
	Added dark fermented biosolids Into the post-anoxic zone (Scenario f2)	0.02 ± 0.04	6.77 ± 1.42
SBRs results	Blank stage	19.8 ± 1.3	23.5 ± 4.3
	Added fermented biosolids into the post-anoxic zone	1.4 ± 0.7	6.0 ± 1.0
	Added dark fermented biosolids into the post-anoxic zone	1.3 ± 0.8	5.0 ± 1.0

As seen in Figure 6.8 and Figure 6.9, after adding the fermented sludge into a hypothetical post-anoxic zone, despite the fact that not all TN could be removed from the system, complete denitrification was still achieved (even when the influent C:N ratio was as low as 5:1). This result is consistent with those found

in the SBRs where almost complete denitrification also occurred. The remained nitrogen is therefore likely to be a combination of organic nitrogen and ammonia. Another serious problem is how adding the fermented sludge will affect the effluent COD and the system MLVSS. This is why another two simulation scenarios were carried out, where the amount of added fermented sludge (fermented biosolids and dark fermented biosolids) produced an additional 70 mgCOD/L instead of 150 mg/L in the post-anoxic zone (Scenario f1b and f2b). The results are illustrated in Figure 6.10 and Figure 6.11 below.

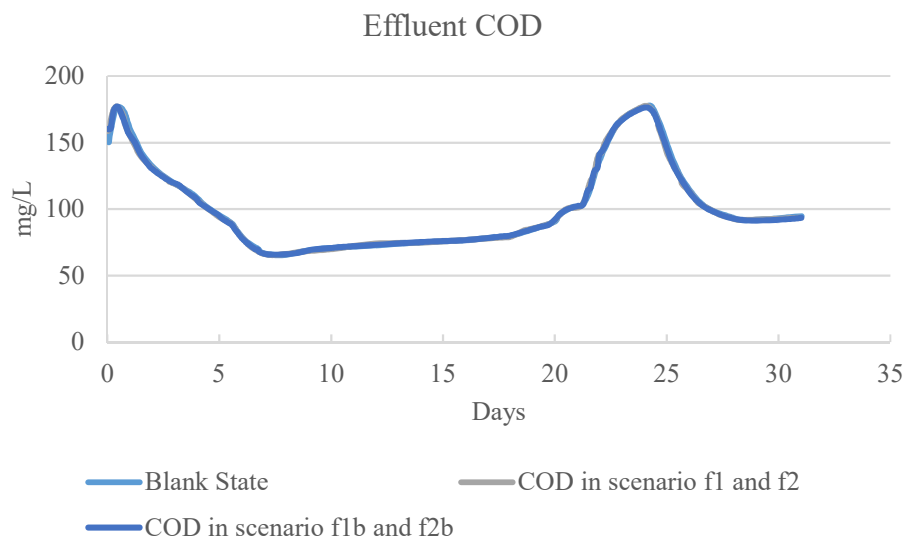


Figure 6.10: The effluent COD from Biowin simulation of the pilot plant (control stage versus after added fermented sludge)

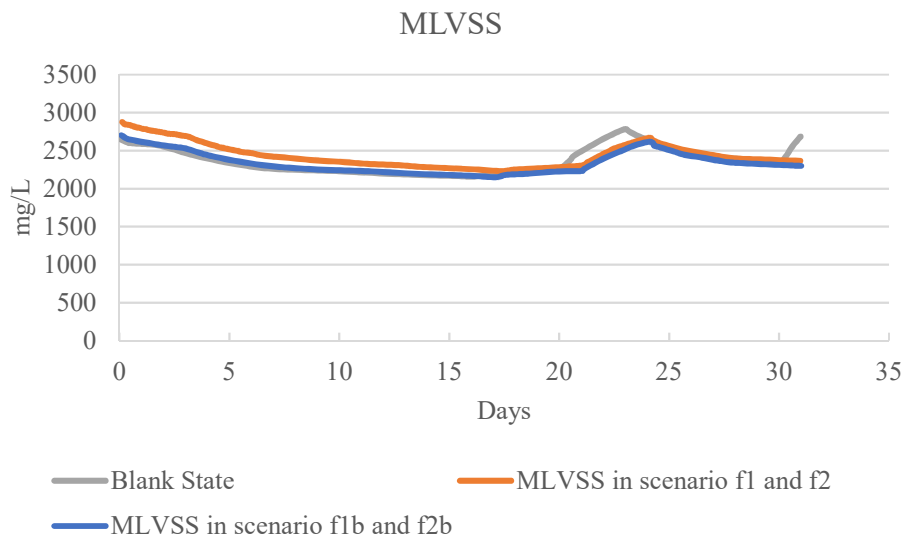


Figure 6.11: The system MLVSS from Biowin simulation of the pilot plant (control stage versus after added fermented sludge)

It should be noted that the effluent COD was identical in both scenarios, where up to 150 mg/L of fermented biosolids was immediately consumed by the post-anoxic zone (HRT of approximately 10 hours). Based on the Mumax test results, only 17-39% of the consumed COD will be used for mass growth of cells. However, that amount did not significantly increase the MLVSS of the system, as the maximum amount of MLVSS increase was less than <100 mg/L compared to the blank state. Furthermore, the effluent nitrate and TN for Scenarios f1b and f2b were simulated and this is depicted below in Figure 6.12 and Figure 6.13.

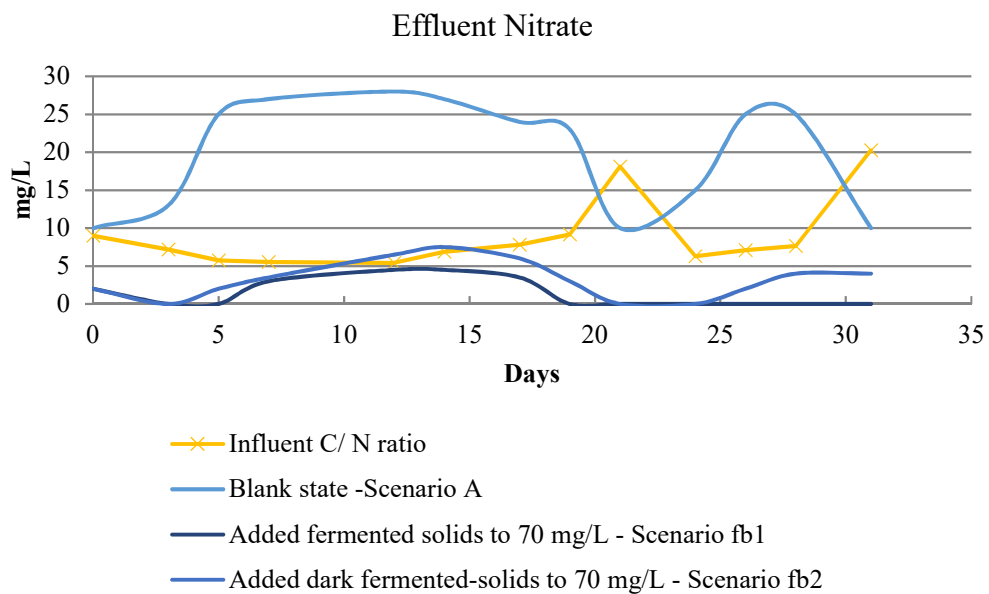


Figure 6.12: the effluent nitrate from Biowin simulation of Scenario fb1 and fb2

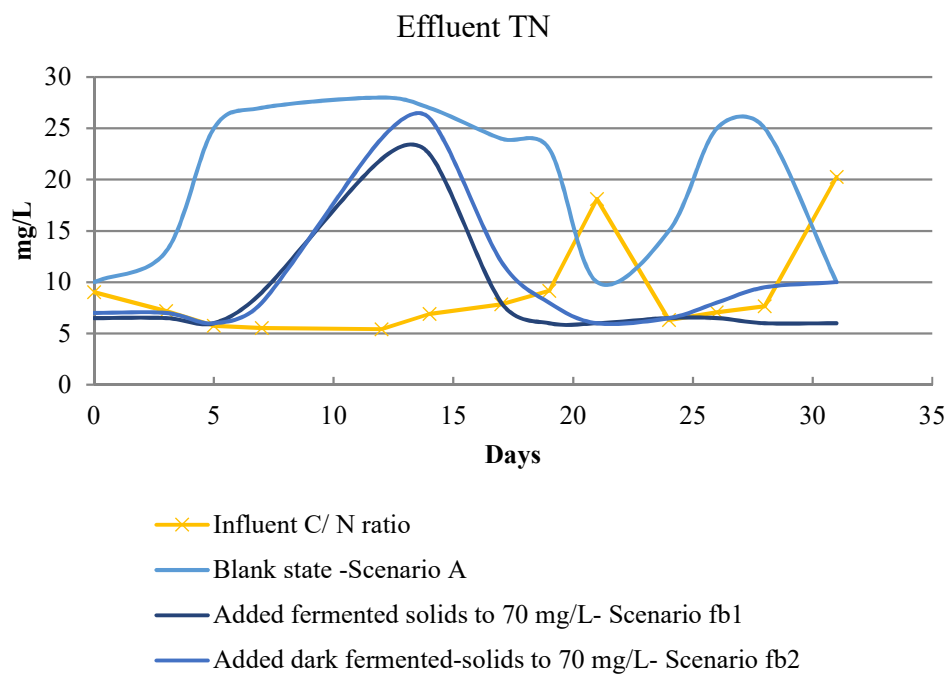


Figure 6.13: The effluent TN from Biowin simulation of Scenario fb1 and fb2

Table 6.10: The effluent NO₃ and TN of Scenarios fb1 and fb2

	Effluent NO ₃ (mg/L)	Effluent TN (mg/L)
Blank stage	20.2 ± 7.0	31.1 ± 13.7
Added fermented biosolids (Scenario f1b)	10.50 ± 1.94	15.27 ± 3.11
Added dark fermented biosolids (Scenario f2b)	14.92 ± 5.03	21.38 ± 8.54

The results documented in Figure 6.12 and Figure 6.13 and Table 6.10 indicate that the reduction in added fermented sludge and dark fermented sludge to 70 mgCOD/L still can provide complete denitrification (with an effluent TN of <10mg/L). This is the case if the influent C:N ratio is higher than 10:1. When the C:N ratio drops below 10:1 and reaches 5:1, however, the accumulation of nitrate and TN in the system will occur and the effluent nitrate and TN will increase to over 7 and 25 mg/L, respectively, within 8 days.

Consequently, based on the Biowin simulation, while overall there is no major disadvantage to adding fermented sludge to produce an additional 150mgCOD/L in post-anoxic zone, it is still possible to reduce that amount to as low as 70 mg/L and still accomplish complete denitrification. However, this will only happen when the influent C:N ratio is higher than 10:1. A dynamic injection of fermented sludge in response to the changing influent C:N ratio would be ideal in practice. But unfortunately, it's outside of the scope of this study due to the

need to develop a responding loop in the pilot plant (require auto-sampler and auto influent TN monitoring).

6.4.7 Pre-anoxic denitrification simulation: Biowin vs NMB model

a) Pre-anoxic denitrification Biowin model

The pre-anoxic denitrification scenario that was used for Biowin simulation are d1 and d2 as shown in Appendix III. The actual performance is documented in Figure 6.14 and Figure 6.15.

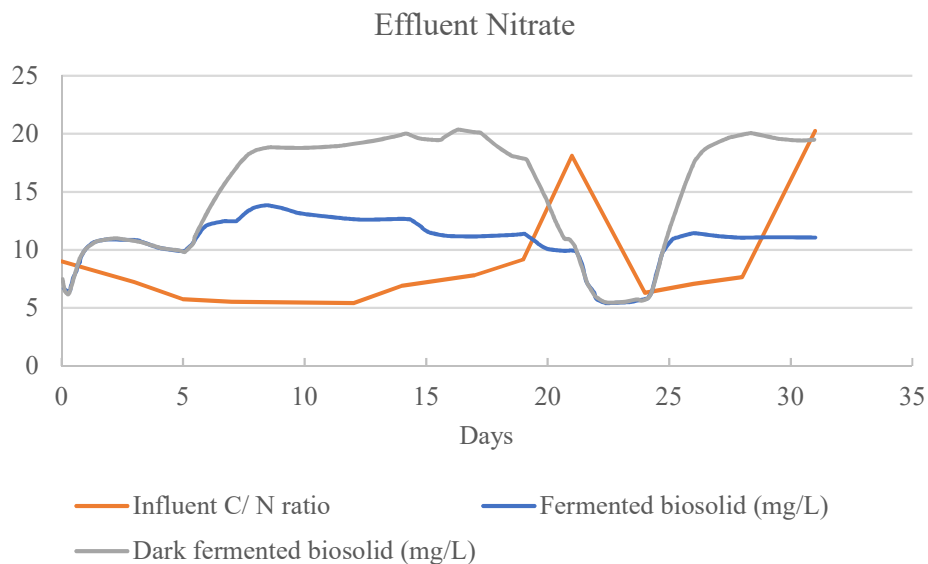


Figure 6.14: The effluent nitrate from Biowin simulation of Scenarios d1 and d2

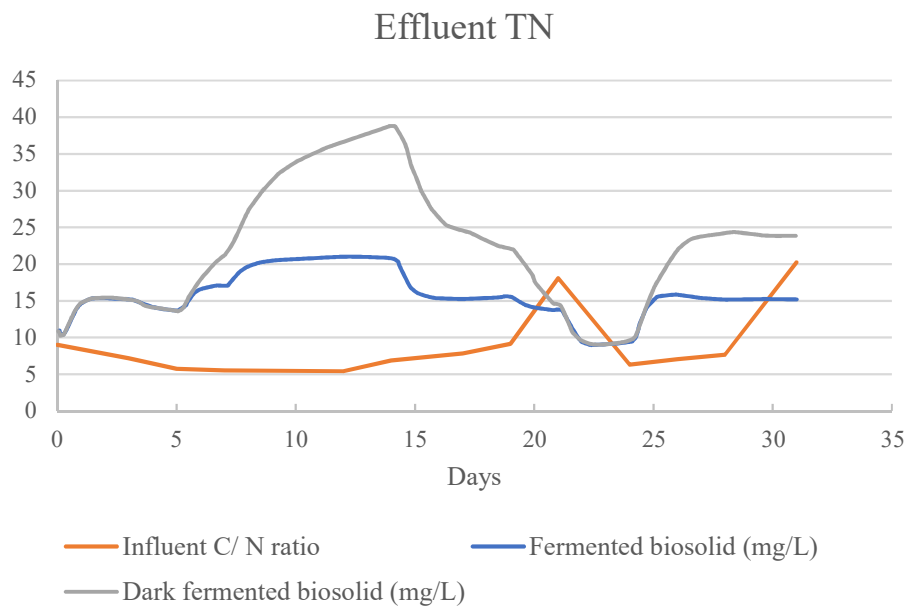


Figure 6.15: The effluent TN from Biowin simulation of Scenarios d1 and d2

Based on simulation data derived from Biowin, it is evident that there will be nitrate and especially TN accumulation inside the system once the influent C:N ratio dips to less than 10:1. In that respect, dark fermented biosolids is actually affected more heavily by the low C:N ratio than fermented biosolids.

b) The Pre-anoxic denitrification NMB model

The simulation of pre-anoxic denitrification can be used based on the physical plant and influent characteristics data of the Sunbury pilot plant. The data was recorded once a day, 3 times a week during the period from 21st September 2013 to 21st October 2013. The NMB simulation is calculated based on the pre-anoxic denitrification equations in Appendix II. It is firstly, however, necessary to identify the denitrification potential of fermented sludge, compared to the required denitrification rate, to see if complete denitrification can occur and no nitrate accumulation occurs within the system. The denitrification potential of the pilot plant before and after adding fermented sludge was calculated based

on $\Delta NO_3 = SDNR \times MLVSS \times t$, where SDNR was based on the SDNR batch test, MLVSS refers to the MLVSS of the system, and t is the hydraulic retention time of the anoxic zone.

Table 6.11: Denitrification rate based on the NMB model

	Denitrification rate of the pilot plant (mgNO ₃ -N/L)
Default	4.21 ± 0.95
Required for complete denitrification	8.10 ± 4.11 *
Added fermented biosolids	13.85 ± 1.65
Added dark fermented biosolids	14.20 ± 1.94

* This is based on item 6 in the calculations documented in Table 6.12.

Because the denitrification rate of the pilot plant after adding the fermented sludge is higher than that required for complete denitrification, it can be concluded that complete denitrification did eventuate in this case. The simulation of the pilot plant when complete denitrification resulted is shown in Table 6.12 and Figure 6.16: The pilot-plant diagram based on NMB calculation below.

Table 6.12: Nitrogen Mass Balance Model for the pilot plant

Each Stage	Used Equations	Calculated value
1 Pre-anoxic	• $TN_{ini} = x \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v \text{ (L)}$	• $TN_{ini} = 82.26 \pm 12.57 \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = 3.8 \pm 0.1 \text{ (L)}$
2 Pre-aerobic	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v + IR \text{ (mg/L)}$	• $NH_4 = 13.46 \pm 2.98 \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = 22.8 \pm 0.6 \text{ (L)}$
3 Pre-clarifier	• $NH_4 = NH_{4\text{ eff}} \text{ (mg/L)}$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}} \text{ (mg/L)}$ • $Q = v + IR \text{ (L)}$	• $NH_4 = 3.74 \pm 1.95 \text{ (mg/L)}$ • $NO_3 = 9.72 \pm 4.93 \text{ (mg/L)}$ • $Q = 22.8 \pm 0.6 \text{ (L)}$
4 Effluent	• $NH_4 = NH_{4\text{ eff}} \text{ (mg/L)}$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}} \text{ (mg/L)}$ • $Q = v \text{ (L)}$	• $NH_4 = 3.74 \pm 1.95 \text{ (mg/L)}$ • $NO_3 = 9.72 \pm 4.93 \text{ (mg/L)}$ • $Q = 3.8 \pm 0.1 \text{ (L)}$

5	Internal return flow	• $NH_4 = NH_{4\ eff}(mg/L)$ • $NO_3 = \frac{0.8x \times v + 0.8N_{4\ eff} \times IR}{v + IR} - NH_{4\ eff}(mg/L)$ • $Q = IR (L)$	• $NH_4 = 3.74 \pm 1.95 (mg/L)$ • $NO_3 = 9.72 \pm 4.93 (mg/L)$ • $Q = 19.0 \pm 0.5 (L)$
6	Mixed of pre-anoxic and IR flow	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4\ eff} \times IR}{v + IR} (mg/L)$ • $NO_3 = \frac{\left(\frac{0.8x \times v + 0.8NH_{4\ eff} \times IR}{v + IR} - NH_{4\ eff} \right) \times IR}{V + IR} (mg/L)$ • $Q = v + IR (mg/L)$	• $NH_4 = 13.46 \pm 3.26 (mg/L)$ • $NO_3 = 8.10 \pm 4.11 (mg/L)$ • $Q = 22.8 \pm 0.6 (L)$

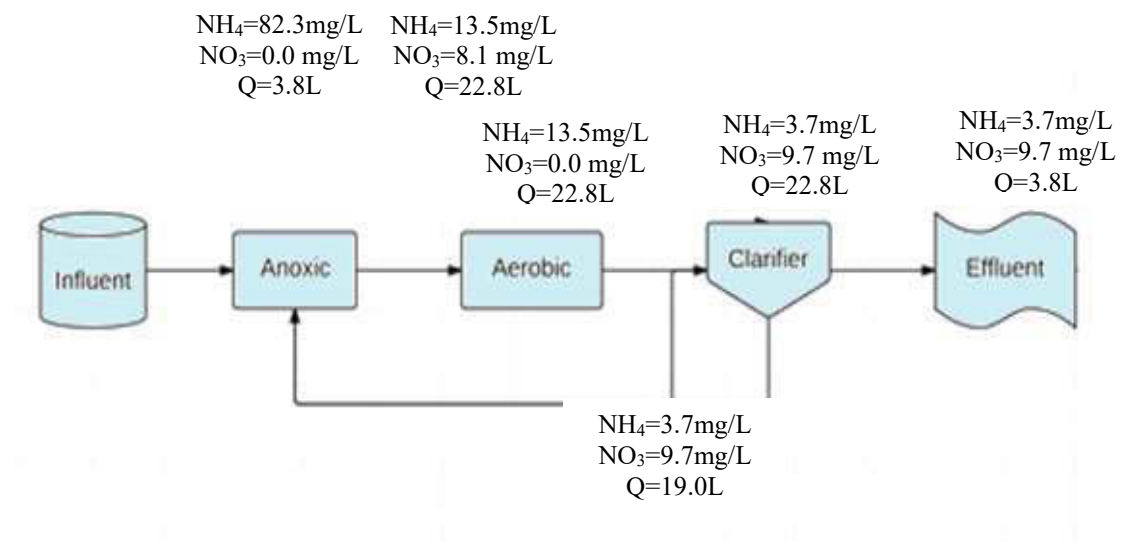


Figure 6.16: The pilot-plant diagram based on NMB calculation

c) Summary

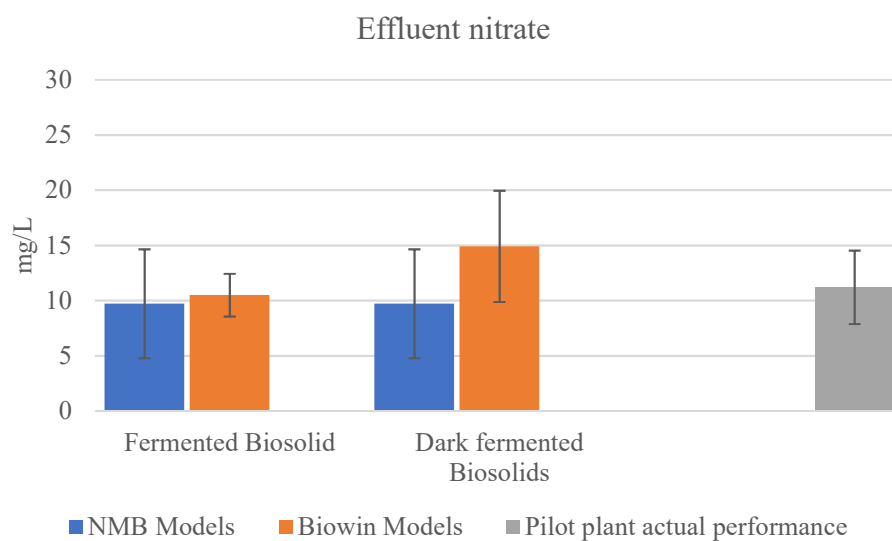


Figure 6.17: The pilot-plant diagram based on NMB calculation

Due to the inability to generate a large enough amount of fermented and dark fermented biosolids from the lab to test to the pilot plant, the pilot plant data above was based on the actual effluent nitrate recorded during the same period (same physical plant and effluent characteristics data but uses a different external carbon source for pre-anoxic denitrification enhancement). While the result cannot verify the performance of the NMB and Biowin models, it still indicates similar results using three completely different simulation models.

Of relevant interest is how the dark fermented biosolid simulation using Biowin was much different to the pilot plant actual performance, especially when compared to the NMB model. It is certainly possible that the unavoidable high standard errors (due to the fluctuation of influent characteristics discharged into Sunbury WWTP) could contribute to this difference. Yet another alternative explanation is the high growth yield of dark fermented sludge (double the amount of sludge) means a lot more of the dark fermented sludge was consumed for biomass growth rather than for denitrification activities. It hence produced a lower denitrification rate than the SDNR for dark fermented sludge. More potential relevant information on this is reported in section 6.4.8 of this chapter.

6.4.8 SDNR calculation: Mumax vs SDNR batch tests

As previously seen in the SDNR tests, the SDNR for fermented biosolids and dark fermented biosolids was found to be 8.35 ± 0.41 and 8.56 ± 0.71 , respectively. Another way to theoretically calculate the SDNR is based growth yield (YH) and growth rate value (μ_S) through the differentiation of Mumax equation over time to produce:

$$\frac{dS_{NO}}{dt} = -\eta_{Anoxic} \cdot \frac{1-Y_M}{2.86} \cdot \frac{\mu_S}{Y_M} \cdot X_{S,0} e^{(\mu_S - b_S)t} \quad (4)$$

Where $\frac{dS_{NO}}{dt}$ is theoretically equal the SDNR at t when the Xs (the system biomass) equal 1000 mg, or $t = \frac{\ln\left(\frac{X_{S,t}}{X_{S,0}}\right)}{\mu_s - b_s} = \frac{\ln\left(\frac{1000}{44}\right)}{\mu_s - 0.04}$. This will result in theoretical SDNRs for fermented biosolids and dark fermented biosolids to be an incredible 97.69 and 136.93, respectively.

These high figures can be further backed up if the SDNR calculation method is carried out using the Mumax results, as presented in Table 6.13 and Figure 6.18: SDNR calculated from the Mumax batch tests below. Note, however, that due to a much longer retention time, and a much lower MLVSS than the typical SDNR tests, the real SDNR figures are expected to be even higher than this.

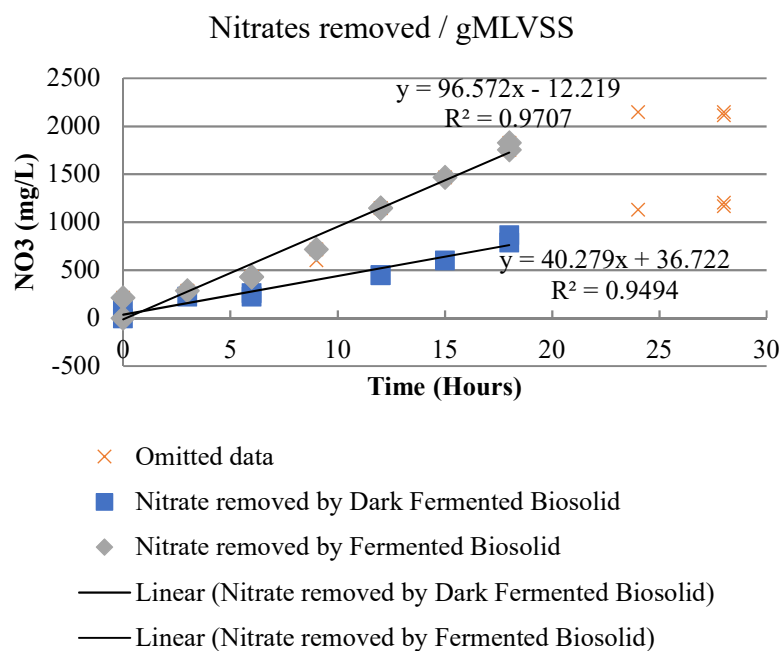


Figure 6.18: SDNR calculated from the Mumax batch tests

Table 6.13: SDNR calculated from the Mumax batch tests

	COD (mg/L)		VA (mg/L)		NH ₄ (mg/L)		M LV SS	Denitrification rates					Δm gC OD / Δm gN O ₃
	Initi al	Fin al	Init ial	Fi nal	Init ial	Fin al		S D N R	R [^] 2	R [^] 2 (a dj)	S E	SD NR (95 %C I)	
Fer men ted Bios olid s	553. 4±1 .7	158. 5±6 .5	80. 2± 0.3	1.5 ±0. 5	79. 4± 3.4	76. 3± 1.3		96 .5 8	0. 97 07	0. 96 75	5. 6 0	86. 33 – 105 .82	6.7 ±0. 3
Dar k Fer men ted Bios olid s	476. 7±2 .9	68.0 ±1. 0	49. 1± 0.3	2.0 ±1. 0	72. 0± 1.4	75. 3± 0.8	43. 67	40 .2 8	0. 94 96	0. 94 32	3. 2 8	34. 17 – 46. 38	8.6 ±0. 4

Generally, all of the SDNR results using various methods are summarised in Figure 6.19: SDNR of the tested fermented sludge samples using various methodologies below.

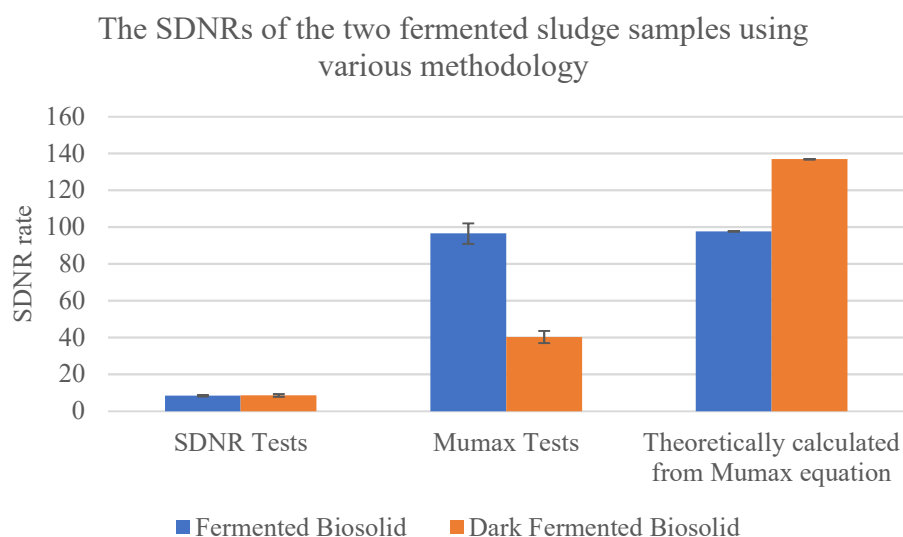


Figure 6.19: SDNR of the tested fermented sludge samples using various methodologies

When based on the results, the maximum denitrification potential for fermented sludge, or more specifically the fermented biosolids and dark fermented biosolids, is exceptionally high. This assessment is fairly consistent with one recent study (Liu et al., 2016) of how fermented sludge can produce a denitrification rate much higher and faster than more commonly studied external carbon sources (these refer to methanol, acetic acid or propionic acid). However, it is unlikely to reach that level in reality because there is no realistic demand for removing such high concentration of mgNO_3 domestic wastewater treatment, not to mention the exceptional amount of fermented sludge needed for such a thing to happen.

Theoretically for example, to reach the maximum SDNR of 97.69 and 136.93 for fermented biosolids and dark fermented biosolids, respectively,

655±26 and 1175±57 mgCOD (of the two carbon types) must be consumed by each g of MLVSS per hour. It is a scenario which is unrealistic, not viable and not applicable.

However, the high demand for excessive available COD may explain why the SDNR figures derived from the SDNR tests were much lower, as the initial COD concentration of SDNR tests were only 600-700 mg/L. The SDNR results were calculated based on the line-of-best-fit from the Mumax tests, however, they are prone to inaccuracy. Nonetheless it is intriguing to see how this figure for fermented biosolids is much closer than the same figure for dark fermented biosolids. The most obvious reason is that the dark fermented sludge has a much higher denitrifier yield rate (0.39 ± 0.20 compared to 0.17 ± 0.07) and growth rate (1.10 ± 0.67 comparing 0.85 ± 0.32). That combines with the really long HRT (24 hours) of the Mumax tests and the excessive available nutrients, meaning the MLVSS would exponentially increase and make the line-of-best-fit method less reliable in this case.

Regardless of the denitrification potentials being 8.35 ± 0.41 and 8.56 ± 0.71 even when they do not reach SDNR level, they still make fermented biosolids and dark fermented biosolids a very effective external carbon source. This was verified by how all three models - NMB, Biowin and SBRs - achieved complete denitrification.

6.4.9 Results summary

This study set out to assess the potential of fermented sludge as an external carbon source. This approach required the use of three different physical and mathematical models - NMB, SBRs and Biowin. Input data was supplied by two batch tests: Mumax and SDNRs. The results of all findings can be read in Table 6.14 below.

Table 6.14: Comparison between different physical and mathematical models

	SDNR batch test	Mumax batch test	NMB Model	SBRs	Biowin Model	Pilot plant (using sucros e)
Denitrification Rates (mgNO ₃ / gMLVSS/h)						
Fermen ted biosolid s	8.35 ± 0.41	97.7 * (96.5 ± 5.6**)	-	>5.45 ± 0.61	-	
Dark ferment ed- biosolid s	8.56 ± 0.71	136.9 * (40.3 ± 3.3**)	-	>5.50 ± 0.77	-	
Effluent Nitrate for post-anoxic denitrification (mg/L)						
Fermen ted biosolid s	-	-	<0.1	1.4 ± 0.7	<0.1	
Dark ferment ed biosolid s	-	-	<0.1	1.3 ± 0.8	0.02 ± 0.04	
Effluent Nitrate for pre-anoxic denitrification (mg/L)						
Fermen ted biosolid s	-	-	9.7 ± 4.9	N/A	10.5 ± 1.9	11.2 ±3.2

Dark ferment ed biosolid s	-	-	9.7 ± 4.9	N/A	14.9 ± 5.0

Note:

* this was calculated based on the Mumax equations, using constants derived from the Mumax tests

** this was based on line-of-best fit of the results from the Mumax tests

6.5 Conclusion

Overall several major findings emerge from this experiment. Firstly, the potential of fermented biosolids and dark fermented biosolids as effective external carbon sources was validated, in that complete denitrification was achieved in all three simulation models, i.e. the NMB mathematical model, the Biowin software model, and the SBR lab-scale physical model. This is especially true for post-anoxic denitrification but pre-anoxic denitrification also proved to be successful. Fermented sludge and dark fermented sludge are fairly similar when it comes to overall denitrification potential.

Secondly, this test once again verified the accuracy of the NMB model, and Biowin model when compared to the lab-scale and pilot-scale experiments. This refers to assessing the effectiveness of new carbon sources. Each model has its own advantages and disadvantages, and further testing is recommended to refine the methodology. However, while the lab-scale and pilot scale physical model take months to set up, maintain and run, the NMB and Biowin models only take weeks or even days to complete (mostly to run the SDNR and/ or Mumax batch tests). Doing so will save a lot of time and resources needed to simulate the performance of a new external carbon sources in a new system.

Thirdly, this studied confirmed the benefits of using different types of models as cross-verification tools for external carbon source study. Not only did it

help to verify the performance of fermented and dark fermented sludge on post-anoxic and pre-anoxic denitrification (through the use of NMB, Biowin and SBRs), it also identified the exceptionally high value of actual SDNR of fermented and dark fermented sludge. The flaw in the SDNR batch tests methodology is that if the initial COD of SDNR batch tests are not sufficient, SDNR of the studied external carbon sources (fermented biosolids in this case) will not occur.

In summary, this study has cross-verified various model types to study and evaluate the potential of two fermented sludge types (fermented biosolids and dark fermented biosolids). Overall it was a success in that it validated the potential of fermented biosolids and dark fermented biosolids as effective carbon sources. As well the application of an applied cross-verification methodology was demonstrated as a reliable method for studying external carbon sources. However, it will be necessary to apply this method to other external carbon sources in the future study to overcome any shortcomings.



Chapter 7

Advantages and Disadvantages of All Models

Chapter 7 - OVERVIEW OF ALL MODELS

As mentioned in Chapter 1, one of the main objectives of this study is to set up a standard methodology that is appropriate for future studies on sucrose, fermented sludge and any other external carbon sources. This chapter describes the advantages and disadvantages of the models used, and also compares the time, resources and labour consumption to maintain and run each system. This will provide a better perspective on why the experiments were done in particular ways, and how future researchers could better utilise their finite resources and time to plan their analyses of external carbon sources.

7.1. Technical advantages and disadvantages of each model

The technical advantages and disadvantages of each model used are summarised below.

Table 7.1: The strategies employed in the thesis and their classification

Methodology used	Model Types	Purpose	Advantages	Disadvantages
SDNR batch tests	- Physical - Frozen state - Grey-box	Find the denitrification rate of a specific sludge when using a specific carbon sources.	- The SDNR is widely utilised and is easier to compare the experiment results with past analyses. SDNR of sucrose and fermented biosolids for example could be assessed in comparison to methanol and other types of fermented sludge. - Provide a very fast (within 7 days) and fairly accurate assessment on the impact of an external carbon source.	- The NMB depends on the assumption that the SDNR is reached by the system. - Only simulate denitrification performance of the system. It is not possible to assess nitrification or phosphate removal. - Unable to simulate efficiently when nitrate is continuously accumulated within the system. - Despite being quite short time-wise, however, the experiment is labour-intensive, so it is hard to carry more than 2-3 experiments at the same time.
Nitrogen Mass Balance (NMB) conceptual model	- Mathematical - Steady-state/non-continuous - Glass-box	Model a pre-anoxic or post-anoxic denitrification system using the SDNR batch test results.	This is especially the case when the characterisation process of the system (that being modelled after) has already been done. - Simple and as a glass-box model and therefore easily modified and improved upon if needed.	- It may be tricky when there is more than one carbon source in a mixture. This is because the denitrifier can store carbon matter in its outer structure layers for later use. For example, the SDNR of sucrose in the SBRs is almost identical to the SDNR of a mixture of sucrose and wastewater. - The model uses an average value (frozen model) rather than a dynamic one, so it is very difficult to model a system according to real time. - The NMB model is a rapid assessment method, so it is possible that it will not be applicable to some special circumstances. It's also created by the author of this dissertation so is limited by the author knowledge on the wastewater treatment.
Mumax batch test	- Physical + Mathematical - Frozen state - Glass -box	Find denitrifier growth rate and yield rate of sludge using specific carbon sources.	- Provide a fast (within a few weeks) assessment on the impact of an external carbon source. - The Mumax tests can calculate the true SDNR of a carbon source, something that a SDNR tests may not be able to	- The growth rate and yield rate are not utilised much in the literature so it is difficult to assess the accuracy of the experimental results.

Biowin model	- Mathematical - Steady-state/ Frozen state - Glass -box	Model a pre-anoxic or post-anoxic denitrification system based on Mumax batch test results.	do. In the fermented sludge study here, the SDNR experiment could not provide excessive nutrients and carbon sources due to the absurdly high consumption rate. - The growth rate and yield rate of an external carbon source by themselves do provide useful information about these sources.	- The model depends on the assumption that the maximum growth rate and yield rate are achieved within the system. - The experiment has to run all day for as long as 6 days. It is difficult to provide a consistent data trend within typical working hours. This problem is especially challenging when the growth rate of the tested carbon is high (like the case with fermented sludge), meaning the data trend has to be plotted within 1-2 days. It means the researcher will have to stay continuously in the laboratory for more than 24 hours. - Software like Biowin is very complicated for users to change, modify or improve upon. Also it does not have a specific function dedicated to external carbon sources. - The Biowin model could provide a dynamic simulation but is not accurate since the wastewater characteristics could vary between weeks, days, and even same time of day. This is why Biowin much more accurately simulates the performance of the SBRs (constant feeds) than the pilot plant (dynamic feeds).
Sequencing Batch reactor (SBR)	- Physical - Steady-state/ continuous - Grey-box	Model a post-anoxic denitrification system	- The system could be modified fairly easily, especially compared to the pilot plant. - The time-based nature of the SBRs means it is possible to stop and sample the characteristics of the wastewater at any point in time. - No internal recycle of wastewater sludge, so the sludge concentration is always very stable. Everything happens in one tank, so all of the liquid body in the system is always mixed very well. - The sludge from the SBRs can be used to seed other SDNR and Mumax tests, especially when carbon-acclimation is needed.	- If new sludge has to be added into the SBRs (start-up or if any issues occur), the time required for the new sludge to adapt and stabilise to the lab conditions could take months. The sludge could also end up being different to real WWTP sludge at the microorganism level. - It is simply not possible for the SBR to simulate a pre-anoxic denitrification system due to its time-orientated nature. It cannot simulate the space-orientated nature of a system with Internal Recycle. - The experiment and monitoring process is not labour-intense but it does consume many resources for setting up and monitoring (lab space, used equipment, monitoring chemical

				reagent kits). It is therefore difficult to run more than 4 SBRs at the same time.
Pilot Plant	- Physical - Frozen state - Black-box	Model a pre-anoxic denitrification system	- It used the real wastewater from onsite treatment plants, so the microorganism level of the pilot plant is the closest to of a real system. - It is the largest system in terms of scale, close to 120L compared to 4L for the SBRs and 0.5-1L for the SDNR and Mumax test. It is also the largest physical model that a typical PhD candidate can access and should be the closest thing to a real WWTP.	- The pilot plant is a very rigid system and consequently very time- and resource-consuming to carry out even the smallest modification. In some cases it is simply impossible to do so such as altering physical characteristics, for example tank size, anoxic zone percentage, etc. It is also very difficult to modify the system to a post-anoxic denitrification system. - Like a real treatment system, the mixing between each tank is not optimum. - Due to it using real wastewater, the influent often tends to fluctuate a lot between days or even within time periods for one day. This means the system is rarely completely stable, and the impact of the added carbon sources could be intensified or lessened by some other variables. - Maintaining the system is very time and labour intensive. It took another fellow researcher a full 4 days a week (including travel time) just to maintain and keep the system running. Almost every issue that the system encountered will need a specialist (electrician, plumber, etc.) to fix when things go wrong. This in turn means more time delays and opportunities lost. - The system itself is a black-box model so it is very difficult to recognise whether the results are affected by unaccountable factors, or what can explain a strange set of results.

7.2. Time and Resources required to operate each model

The approximate time and resources required for each simulation model are listed in detail in Appendix 5. The information is also presented here in Figure 7.1, Figure 7.2 and Figure 7.3 below.

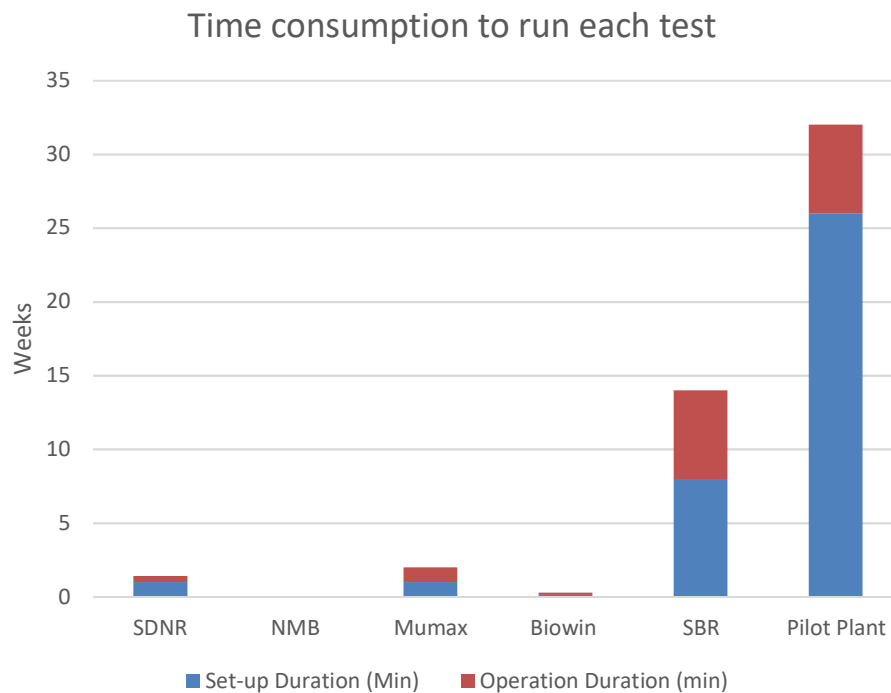


Figure 7.1 Amount of time required to run each tested model.

As expected, a continuous experiment like the SBRs and the pilot plant consumes much time. Batch tests like SDNR and Mumax need much less time in comparison, while conceptual and software models like NMB and Biowin require almost no time at all to set-up and operate. It needs to be noted, however, that the NMB and Biowin operations depend on the characterisation of the objects being modelled and this may take weeks or months to complete.

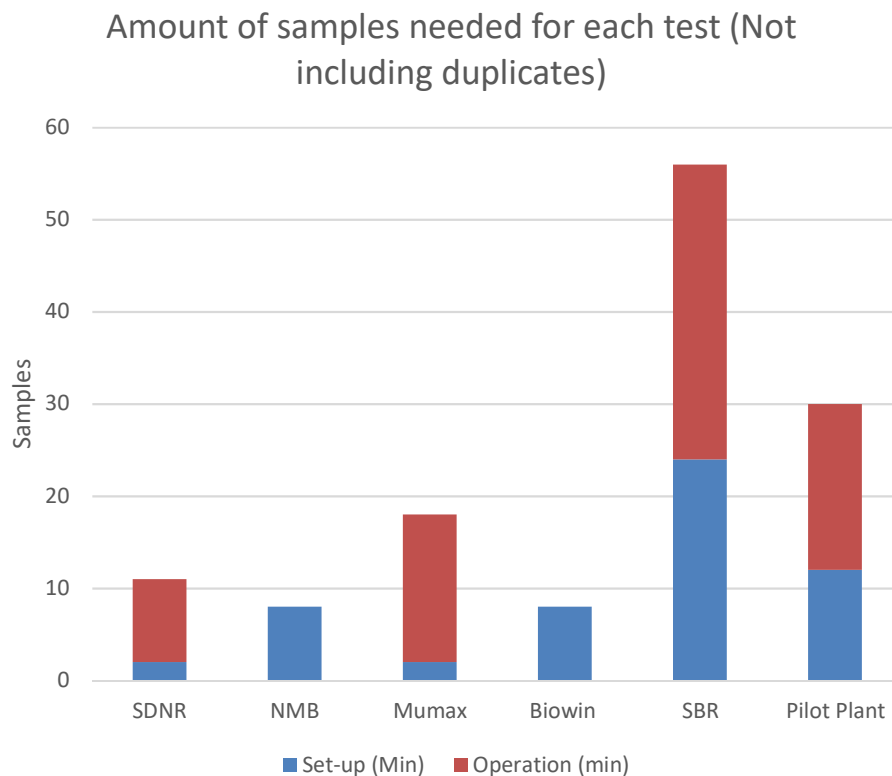


Figure 7.2 Number of samples needed to run each test.

Overall, conceptual and software models like NMB and Biowin require a lot of samples for characterisation and setting up. Batch tests like SDNR and Mumax are different in that twice as much samples are needed to be tested during the operations of the experiment. However, this also could mean that these two tests can provide much more experimental data for each test. Finally, continuous experiments like SBR and the pilot plant require more samples for characterisations before each test is carried out. The reason why the pilot plant requires much fewer samples than SBR is because of its black-box nature where only influent and effluent data is collected, while the data for before-aerobic and before-anoxic zone of the pilot plant cannot be collected (it is heavily affected by the mixing/not-mixing of continuous Internal Recycle). Despite the graph above giving a false impression that the SBRs and the pilot plant can provide more raw data than the other models, what should be noted is the amount of time needed to set up and run each test. The pilot plant for example provided 18 sets of data

for this experiment but took 26 weeks to set up. The Mumax test, however, can provide 16 data points in just 2 weeks with a minimal amount of resources needed. However, this does not indicate that a Mumax batch test can completely replace the job of the pilot plant or vice versa. Nonetheless, it indicates that a Mumax test + Biowin is faster and a less resources-consuming alternative for rapid assessment of an external carbon source if one is required.

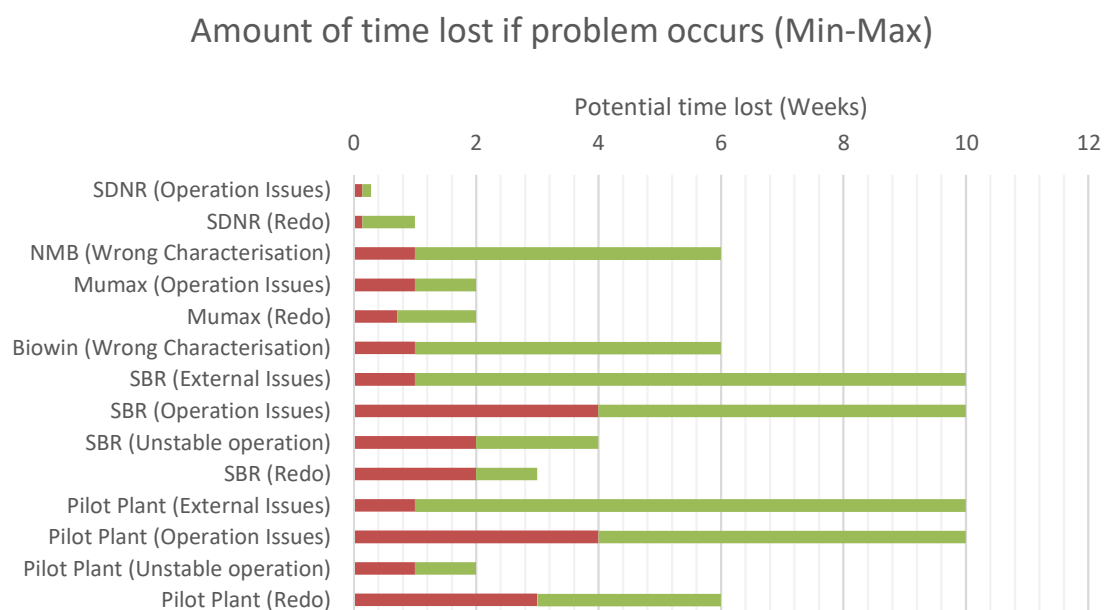


Figure 7.3 Amount of time lost if an issue occurs.

The figure above illustrates the approximate time lost due to all issues encountered in this thesis when each model was run. Firstly, it was quite expected that batch test issues would be resolved within days or a few weeks but the SBR and pilot plant would take longer. Especially in the case of SBRs or the pilot plant if any external issues occur (blackout, air pumps or electronic timers stopped working in the middle of the night), the impact could be so severe that resetting the reactors will be needed. Once this happens, it takes months to reseed the

system and let it acclimate and stabilise to lab conditions and the synthetic wastewater feeds.

Also, despite the wrong characterisations using mathematical, conceptual or software models (NMB model, Biowin) could mean up to six weeks' time lost, however this can be mitigated by a literature review and careful planning. That is why this issue happened only once during the whole four years of the candidature. Similarly, the SDNR and Mumax tests had to be redone, but only 2-3 times in 4 years thank to good planning. As such, operational issues with a continuous system like SBR or pilot plant are much more frequent, especially for inexperienced new operators. During the first 6-12 months of this study, operational issues occurred regularly, i.e. once every month or fortnight, and only became more reliable once the operators could anticipate problems and prevent them beforehand. The longer operations time of SBRs and pilot plant also means it is more likely that external incident (e.g. laboratory blackout) would occur during the middle of the experiment.



Chapter 8

Conclusions and Recommendations

8 - CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

The initial main goal of the research was to find alternative external carbon sources that could enhance denitrification performance in suspended growth wastewater biological treatment processes. However, to do so, firstly it was crucial to develop a cross-verification method based on the results derived from four mathematical and physical simulations (nitrogen mass balance mathematical model, Biowin software model, sequencing batch reactor and large-scaled pilot wastewater treatment plants). Once the methodology was established, the denitrification performance of the two chosen groups of external carbon: industrial-grade sucrose and fermented sludges were assessed. In summary, the specific findings of this dissertation are as follow:

- a) The Nitrogen Mass-Balance (NMB) mathematical model was successfully developed based on the fundamental theory behind the SDNR batch tests and current knowledge regarding system denitrification. Its advantage is its simplicity, in that it allows almost instant simulation of a system regardless of whether it employs pre-anoxic or post-anoxic denitrification. The experimental results using NMB model compared to the lab-scale and pilot-scale experiments indicated a level of reliability and accuracy, sometime even more so than Biowin software.
- b) Using the NMB mathematical model, Biowin software model, lab and pilot-scaled physical models, a workable cross-verification methodology to study and evaluate the potential use of external carbon source was established. This methodology not only helped to verify results from different individual model, but also provided the opportunity to detect whenever one is affected by disturbance or unforeseeable issues (e.g. most significantly in this study was the SDNR of fermented sludge). Furthermore, the different characteristics of each models can cover each other shortcoming (pre-anoxic vs anoxic simulations, black box vs white box), while provide more understanding toward an external carbon source and the tested denitrification system.
- c) Both sucrose and fermented sludge were found to be effective external carbon sources as significant improved (if not 'complete') denitrification were found in all scenarios. Fermented sludges, including fermented

biosolids and dark fermented biosolids, unexpectedly was found to be much more effective than sucrose, and this directly related to its exceptional high Specific Denitrification Rates (SDNR). Similarly, it was also found that external carbon had much more impacts on post-denitrification system than pre-anoxic denitrification system.

- d) Various independent variables were also tested aiming to understand more about the denitrification process and to optimise the denitrification performance using external carbon sources. During that process, some findings have not found in literature were detected. For example, it was firstly found that temperature not only affect the SDNR of external carbon, but also affect different carbon mixtures differently. Whether it is significant or not, further study is needed. Secondly, there are evidences that adding a 50-50 mixture of wastewater influents and sucrose will lead the denitrifiers to specifically utilise sucrose for cell growth. That would explain the reason why sucrose has very short acclimation time comparing to others like methanol or ethanol; and also explain why the SDNR of a real treatment system with mixture of various internal and external carbon sources are different to the SDNR found in SDNR batch tests.
- e) Overall, conceptual and software models like NMB and Biowin although require a lot of samplings for characterisation and setting up, they consume much less time to run and overall is a lot more reliable in face of misstep or accidents. This, however, does not indicate that a NMB and Biowin model can completely replace the job of the SBRs and pilot plant or vice versa. However, depending on the resources, time and expertise available, along with the results demands, it is possible to consider each model advantage or disadvantage and utilise one, or all, or a combination of some accordingly.

8.2 Recommendations

There are several research directions that can be recommended from this research:

- a) While both the NMB models and cross-verification methodology approach were found to be very successful during this study, due to both time and resources limitation, overall, they were only carried out effectively on 4 different type of external carbon: industrial grade sucrose, mixture of

wastewater and sucrose, fermented biosolid and dark fermented biosolid. By applying this same methodology to more carbon source, the shortcoming of NMB models and cross-verification may start to appear. Hence, it is recommended to apply these methods on more external carbon studies, not only to detect their flaws and drawbacks, but also to improve the NMB and cross-verification methodology accordingly.

- b) As previously mentioned about how adding a 50-50 mixture of wastewater influents and sucrose may lead the denitrifiers to specifically utilise sucrose for cell growth. It could be interesting for future study to see effect of not only how one external carbon would have on a denitrification system, but on how a mixture of several internal and external carbon may affect a denitrification system.
- c) As showed in chapter 5, the fact that sludge fermentation has very similar working conditions (pH, temperature) and biological activities to current research on hard cellulose hydrolysis, indicating that there is potential application to combine sludge fermentation and hard cellulose hydrolysis in the same reactor. Not to mention the product of cellulose hydrolysis is glucose, or sucrose, which based on this study showed to be an effective external carbon source. Along with the advance in hard cellulose hydrolysis research, it could make the sludge fermentation option to become more viable than it normally would be. More study could be done on this.
- d) The main goal of dark fermentation is actually to create hydrogen as alternative fuel, or to improve combustion process of methane from anaerobic digester. Dark fermented sludge in this case is more of a by-product, but also a very effective external carbon as proven in this study. Hence, there might be an interest toward future study on the viability of this process, and how to optimise both hydrogen and dark fermented sludge production, especially if working on only one is not financially viable.



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Appendices

APPENDICES

Appendix I – Mathematica's Syntax sample for Mumax experiment analysis

FindFit

```
In[1]:= y=ax+b
Out[1]= ax + b
In[2]:= data={{1,2},{2,3},{3,4},{4,5}}
Out[2]= {{1, 2}, {2, 3}, {3, 4}, {4, 5}}
In[3]:= FindFit[%, a x+b,{a,b},x]
Out[3]= {a -> 1., b -> 1.}
In[4]:= y=245-(1-a)/2.86*(b*43.67)/a/(b-0.03)*(Exp[b*x-0.03*x]-1)
data={{0,245},{0.08,242},{0.17,244},{1,237},{1.08,229},{1.17,227},{2,217},{2.08,217},{2.
17,209},{3,177},{3.08, 172},{3.17,157}}
FindFit[data, {245 - (1 - a)/2.86*(b*43.67)/a/(b - 0.04)*(Exp[b x - 0.04*x] - 1)}, {a, b}, x]
data={{0,223},{0.08,223},{0.17,222},{1,214},{1.08,207},{1.17,214},{2,211},{2.08,208},{2.
17,206},{3,203},{3.08, 197},{3.17,190},{4.0, 160}}
FindFit[data, {223 - (1 - a)/2.86*(b*52)/a/(b - 0.04)*(Exp[b x - 0.04*x] - 1)}, {a, b}, x]
data={{0,219},{0.08,222},{0.17,221},{1,211},{1.08,211},{1.17,204},{2,198},{2.08,198},{2.
17,190},{3,183},{3.08, 178},{3.17,179}}
FindFit[data, {219 - (1 - a)/2.86*(b*43.67)/a/(b - 0.04)*(Exp[b x - 0.04*x] - 1)}, {a, b}, x]
```

NonLinearModelFit

```
In[5]:= nlm=NonlinearModelFit[data, {245 - (1 - a)/2.86*(b*43.67)/a/(b - 0.04)*(Exp[b x  
- 0.04*x] - 1)}, {a, b}, x];
```

```
In[6]:= Normal[nlm](* 245 - (1 - 0.64384a)/2.86*(0.772609*43.67)/0.643841/(0.77260-  
0.04)*(Exp[0.772609 x - 0.04*x] - 1))*
```

```
Out[6]= 245 - 8.90778 (-1 + E^0.732609)
```

```
In[7]:= nlm["ParameterTable"]
```

```

In[5]:= nlm=NonlinearModelFit[data, (245 - (1 - a)/2.86*(b*43.67)/a/(b - 0.04)*(Exp[b x - 0.04*x] - 1)), {a, b}, x];
In[6]:= Normal[nlm] (* 245 - (1 - 0.64384a)/2.86*(0.772609*43.67)/0.643841/(0.77260-0.04)*(Exp[0.772609 x - 0.04*x] - 1))*
Out[6]= 245 - 8.90778 (-1 + E0.732609 x)
In[7]:= nlm["ParameterTable"]
Out[7]=


|   | Estimate | Standard Error | t]Statistic | P]Value                  |
|---|----------|----------------|-------------|--------------------------|
| a | 0.643841 | 0.0598866      | 10.751      | 8.15383 10 <sup>-7</sup> |
| b | 0.772609 | 0.0802117      | 9.63212     | 2.23874 10 <sup>-6</sup> |


In[8]:= |

```

Appendix II – R Statistics analysis for Chapter 4 SDNR tests

```

Call:
lm(formula = Ns ~ time)

Residuals:
    1      2      3      4      5      6
0.113658  0.382346 -0.421034 -0.009225 -0.495315  0.429569

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.173068   0.281251  -0.615   0.572
time         0.019803   0.004301   4.605   0.010 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4377 on 4 degrees of freedom
Multiple R-squared:  0.8413,    Adjusted R-squared:  0.8016
F-statistic: 21.2 on 1 and 4 DF,  p-value: 0.009996

Call:
lm(formula = Nw ~ time)

Residuals:
    1      2      3      4      5      6      7
-0.4902  0.1496  0.3518  0.2780 -0.3474  0.3327 -0.2745

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.420330   0.248453   1.692  0.15147
time        0.023306   0.003843   6.065  0.00176 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3926 on 5 degrees of freedom
Multiple R-squared:  0.8803,    Adjusted R-squared:  0.8564
F-statistic: 36.78 on 1 and 5 DF,  p-value: 0.00176

```

```

Call:
lm(formula = N50 ~ time)

Residuals:
    1      2      3      4      5      6 
0.09145 -0.08473  0.06090  0.01141 -0.13565  0.05663 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.159883   0.064309  -2.486   0.0678 .
time         0.022812   0.001083  21.065   3e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1012 on 4 degrees of freedom
Multiple R-squared:  0.9911,    Adjusted R-squared:  0.9888 
F-statistic: 443.7 on 1 and 4 DF,  p-value: 3.002e-05

\ |

```

```

Call:
lm(formula = AS20 ~ time)

Residuals:
      Min       1Q   Median       3Q      Max 
-0.31820 -0.25056 -0.00255  0.03667  0.66742 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.56820    0.24001  -6.534 2.79e-05 ***
time         0.02552    0.00160  15.953 1.92e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2992 on 12 degrees of freedom
Multiple R-squared:  0.955,    Adjusted R-squared:  0.9512 
F-statistic: 254.5 on 1 and 12 DF,  p-value: 1.918e-09

```

```

Call:
lm(formula = A5020 ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-0.62417 -0.46435  0.08701  0.30405  0.72820

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.774730   0.391379  -7.09 1.27e-05 ***
time         0.042809   0.002609   16.41 1.39e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4879 on 12 degrees of freedom
Multiple R-squared:  0.9573,    Adjusted R-squared:  0.9538
F-statistic: 269.2 on 1 and 12 DF,  p-value: 1.388e-09

```

```

Call:
lm(formula = AS23 ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-0.75428 -0.16031 -0.02748  0.37368  0.85833

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.453284   0.338166  -4.298  0.00126 **
time         0.026893   0.002221  12.111 1.06e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4652 on 11 degrees of freedom
Multiple R-squared:  0.9302,    Adjusted R-squared:  0.9239
F-statistic: 146.7 on 1 and 11 DF,  p-value: 1.058e-07

```



```

Call:
lm(formula = A5023 ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-0.78539 -0.23141  0.08438  0.33291  0.73885

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.965414    0.275810  -10.75 9.23e-10 ***
time         0.048025    0.001864   25.76 < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4723 on 20 degrees of freedom
Multiple R-squared:  0.9707,    Adjusted R-squared:  0.9693
F-statistic: 663.6 on 1 and 20 DF,  p-value: < 2.2e-16

. |

Call:
lm(formula = AS26 ~ time)

Residuals:
    1      2      3      4      5      6
-0.22828 -0.21355  0.62592  0.22828 -0.37555 -0.03682

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.891016    0.356410  -2.50 0.066768 .
time         0.037310    0.002854   13.07 0.000198 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.4135 on 4 degrees of freedom
Multiple R-squared:  0.9771,    Adjusted R-squared:  0.9714
F-statistic: 171 on 1 and 4 DF,  p-value: 0.0001975

```

```

Call:
lm(formula = A5026 ~ time)

Residuals:
    1      2      3      4      5      6 
0.21687 -0.06024 -0.09639 -0.01205 -0.28916  0.24096 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.93976    0.19358  -10.02 0.000558 ***
time          0.05743    0.00155   37.05 3.17e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2246 on 4 degrees of freedom
Multiple R-squared:  0.9971,    Adjusted R-squared:  0.9964 
F-statistic: 1373 on 1 and 4 DF,  p-value: 3.167e-06

```

Appendix III – R Statistics analysis for Chapter 6 SDNR tests

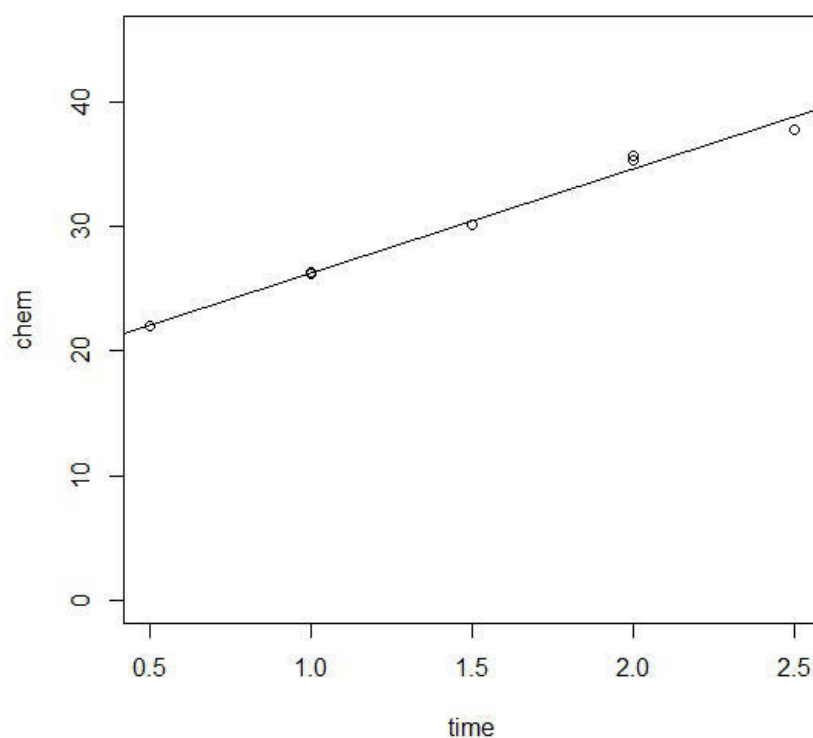
Fermented Biosolids 1

```
Call:
lm(formula = chem ~ time)

Residuals:
    1      2      3      4      5      6      7 
-0.14310 -0.12726 -0.01726 -0.32143  0.98440  0.66440 -1.03976 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)  17.9089     0.6855   26.12 1.54e-06 ***
time         8.3483     0.4189   19.93 5.88e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7255 on 5 degrees of freedom
Multiple R-squared:  0.9876,    Adjusted R-squared:  0.9851 
F-statistic: 397.2 on 1 and 5 DF,  p-value: 5.876e-06
```



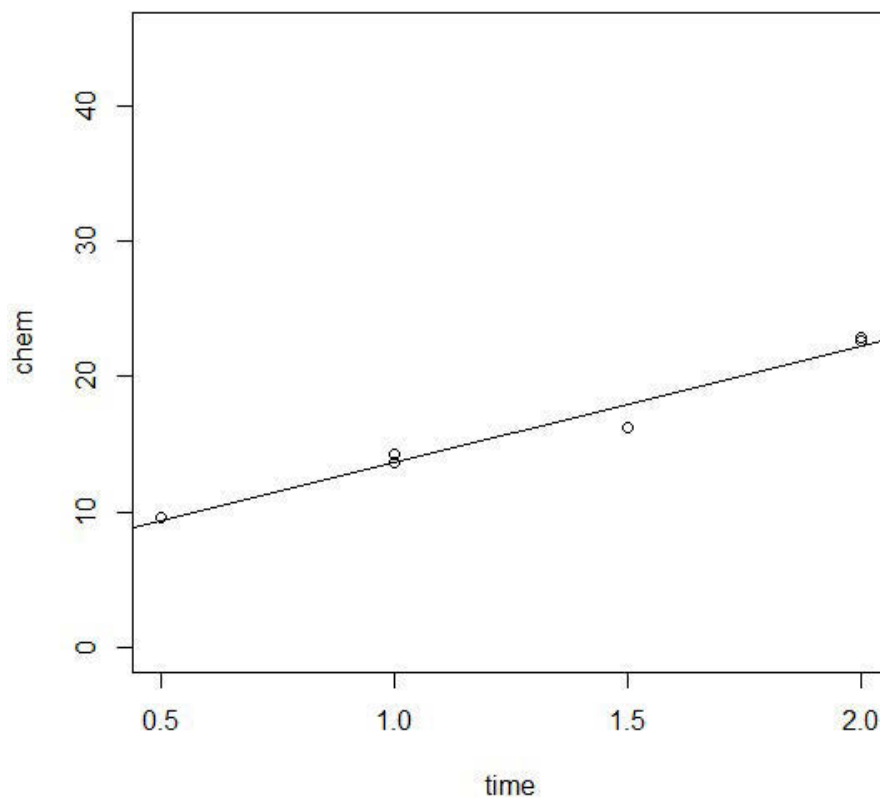
Dark fermented Biosolids 1

```
Call:
lm(formula = chem ~ time)

Residuals:
    1      2      3      4      5      6 
0.19773 0.55909 -0.01091 -1.68955 0.36182 0.58182 

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    5.124      1.022    5.014 0.007417 **
time           8.557      0.708   12.087 0.000269 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9586 on 4 degrees of freedom
Multiple R-squared:  0.9733,    Adjusted R-squared:  0.9667 
F-statistic: 146.1 on 1 and 4 DF,  p-value: 0.0002687
```



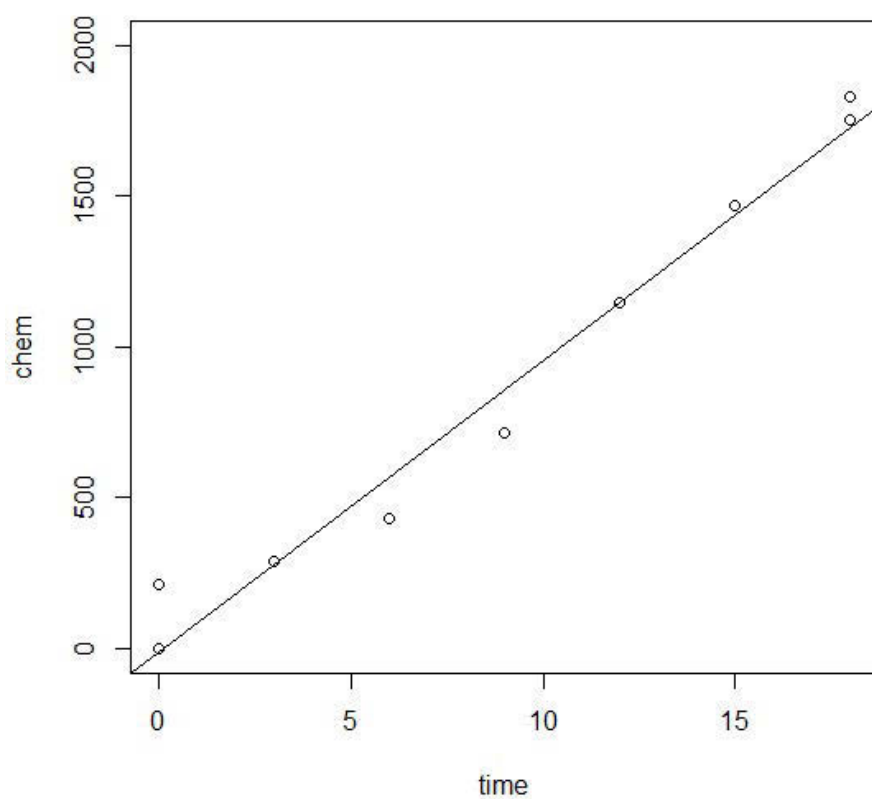
Fermented Biosolids 2

```
Call:
lm(formula = chem ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-140.000  -68.500   9.458   31.677  227.187

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)  -12.188     61.311  -0.199   0.847
time          96.576      5.591  17.275 3.29e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 116.2 on 9 degrees of freedom
Multiple R-squared:  0.9707,    Adjusted R-squared:  0.9675
F-statistic: 298.4 on 1 and 9 DF,  p-value: 3.291e-08
```



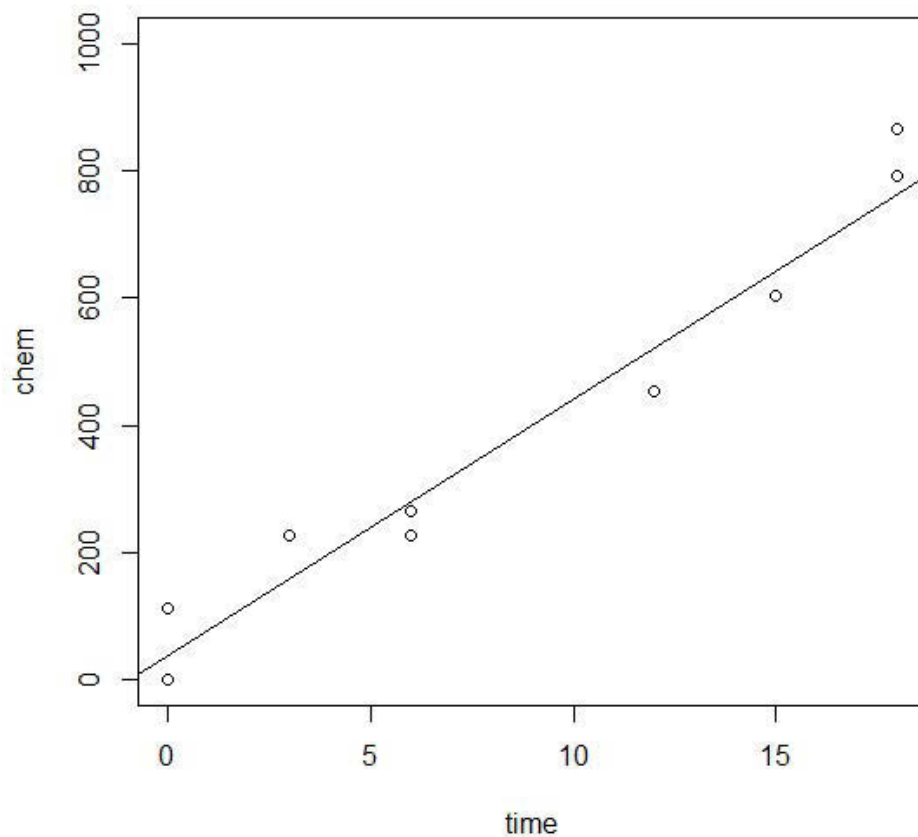
Dark Fermented Biosolids 2

```
Call:
lm(formula = chem ~ time)

Residuals:
    Min       1Q   Median       3Q      Max
-68.13 -48.84 -25.63  58.58 104.20

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   36.800     36.579   1.006   0.344
time          40.278       3.282  12.271 1.81e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 68.22 on 8 degrees of freedom
Multiple R-squared:  0.9496,    Adjusted R-squared:  0.9432
F-statistic: 150.6 on 1 and 8 DF,  p-value: 1.808e-06
```



Appendix IV - SBR feed Selection

COD	1200±30		533±15			300	550	400	747(697 -808)	663(57 1-756)	500	20 00				470-508
BOD		65														
TN	60±3					30			71	101						
TKN			90±10													
NH ₄ -N		7.8		44	65				28	30						
NO ₃				4												
TP	18±2					5			15	12						
oPO ₄ -P		3.5	33±4					2	13	9						
COD/TN/T P	100/50/1.5								100/9.4 /2	100/15 /1.7						100/7/2
	unknown	piggery water	municipal	municipal	municipal	municipal	dairy milk ww	municipal	municipal	municipal					municipal	municipal
	All are in mg/L															
Carbon	Kargi & Uygur (2004)	Jang et al. (2004)	Pholchan et al. (2008)	Mao et al. (2006)	Hu et al. (2010)	Jin et al. (2012)	Hajiabadi, Moghaddam & Hashemi (2009)	Zeng et al. (2003)	Bracklow et al. (2007)	Moreira et al. (2008)			Bajaj, Gallert & Winter (2008)		OECD 2001	Boeije et al. (1999)
CH ₃ COONa	x			1025	195			876.25				20 00				120
butyric acid												31 0				
Uric acid																9
Propionic acid									90	127		17 0				
Methanol												2. 2				
Glucose	x	3200			195	332							3 or 5			

Sucrose											35					
Amide											114					
Cellulose											34					
meat extract			220								208		110		110	15
peptone			320					25	25	28			160		160	15
powdered milk							x		160	185						120
Starch									200	194						(potato)5 0
yeast extract				500					80	83						
Glycerol																40
vegetable oil									35	46	51					
Ammonia																
urea (CO(NH ₂) ₂)			60				x		50	146			30		30	75
NH ₄ Cl	x	400			230	114/ 213/34 2		75	12.8	12.8		22 2				11
(NH ₄)NO ₃														30 00		
(NH ₄) ₂ SO ₄				472												
Ammoniu m Acetate									150	126						

Trace metal+Ph																

osphorus																
CaCl ₂	50	20	8	28	10	75		22.5			4.5	4 7	4	10	4	5
CoCl ₂					0.15			0.045				0. 0 8				0.05
Cr(NO ₃)																0.68
CuCl ₂						40										0.48
CuSO ₄		0.001			0.03			0.009	8	8			0.25			
FeCl ₃	50							0.45				0. 5				
FeSO ₄		0.04			10	44						5	1.34			10
H ₃ BO ₃					0.15	120		0.045								
K ₂ HPO ₄			56	176	18	21.95	x	4.1925				2 1. 7	1		28	
K ₂ MoO ₄																0.02
K ₃ HPO ₄																20
KCl	20															
KH ₂ PO ₄	x				11		x	3.705	26	21		8. 5		50 0		
KI					0.18			0.054								
MgCl ₂											7			20 0		
MgHPO ₄									6	6						25
MgSO ₄	0.1	200	4	180	10	50		50					2		2	
MnCl ₂					0.12			0.036								
MnSO ₄		0.02											1.01			0.1
Na ₂ EDTA		0.011			10			3								0.22

NaCl			14								250		7		7	
NaHCO ₃	0.708				200	100					200	20000				25
NaHPO ₄												33.4				
NaMoO ₄		0.01			0.06			0.018					0.24			
NiCl ₂						48										
NiSO ₄												1	11			0.3
SeOx												0.07				
ZnCl ₂						50										0.18
ZnSO ₄		0.0005			0.12			0.036					0.58			

Appendix V – Time-consumption for each model testing

Methodology used	Minimum Set-up Duration	Minimum Operation Duration (each test)	Potential issues (potential delay time)	Minimum samples that needed to be taken (each test)	Number of tests that were successfully carried out
SDNR batch tests	Short 1 week (non-acclimated sludge) 8-12 weeks (acclimated sludge need SBR)	Very Short 1-day operation + 2 days analysis	External and operation issues (1-2 days) Experimental failure + need to redo (1-7 days)	Setting up: 2 samples Operation: 9 samples	14 tests
Nitrogen balance conceptual model	Instant ^a	Instant	Wrong characterisation of operational and physical parameters of the SBRS or pilot plant (depend, generally 1-6 weeks)	Set-up (characterisation): 8 samples Operation: 0 samples	7 tests
Mumax batch tests	Short 1 week (non-acclimated sludge) 8-12 weeks (acclimated sludge if need SBR)	Short 5 days operation + 2 days analysis	External and operation issues (7-14 days) Experimental failure + need to redo (5-12 days)	Set-up: 2 samples Operation: 16 samples	5 tests
Biowin model	Very Short 1 day ^a	Very Short 1 day	Wrong characterisation of operational and physical parameters of the SBRS or pilot plant (depend, generally 1-6 weeks)	Set-up (characterisation): 8 samples Operation: 0 samples	22 tests
Sequencing Batch reactor (SBR)	Long 8-12 weeks	Long 6 weeks (2 SRT control + 2 SRT testing period)	External issues like blackout, lab incidents (1-10 weeks) Operation issue like bulking sludge, sludge loss due to wasting (4-10 weeks) Unstable operation, need to restabilise (2-4 weeks) Experimental failure + need to redo (2-3 weeks)	Setting up: 24 samples Operation: 32 samples (64 samples if include a control reactor)	4 tests
Pilot Plant	Very Long	Long	External issues like mesh torn up, pipe busted, overflowing	Set-up: 12 samples	1 test

	26 weeks or more	6 weeks (2 SRT control + 2 SRT testing period)	(1-10 weeks) Operation issue like bulking sludge, sludge loss (4-10 weeks) Unstable operation, need to restabilise (1-2 weeks) Experimental failure + need to redo (3-6 weeks)	Operation: 18 samples	
Fermentation batch tests	Short 1 Week	Medium 5-10 days operation + 2 days analysis	External and operation issues (5-10 days) Experimental failure + need to redo (5-10 days)	Set-up: 1 samples Operation: 3 samples	10 tests

Note:

^a: but may depend on the characterisation of the object being modelled after (the pilot plant, the SBR) which may take weeks or months.

Appendix VI – NMB model of a post-anoxic denitrification

A fundamental diagram of a typical post-anoxic denitrification system diagram can be generated in **Error! Reference source not found.** I below consisting of an aerobic zone, an anoxic zone with Returned Activated Sludge being recycled back to the first aerobic from the clarifier. And based on that diagram, all of the potential internal nitrogen-generation, accumulation and consumption can be showed in **Error! Reference source not found.** I below.

As the model cannot simulate the performance of nitrification, the effluent ammonia NH_{4eff} is required to set up NMB model.

Based on the calculation in Table S1, and considering the SDNR equation, the nitrate in the effluent of a post-anoxic denitrification system can be calculated as below:

$$NO3_{eff} = 80\%TN_{ini} - SDNR \times MLVSS \times t - NH4_{eff}$$

Where t is the anoxic HRT in hours. MLVS is g/L of volatile suspended solid in inside the system. And SDNR is in mg NO_3 /gMLVSS/h. Note the volume of returned sludge is assumed to be insignificant when it comes to dilution factor of incoming influent.

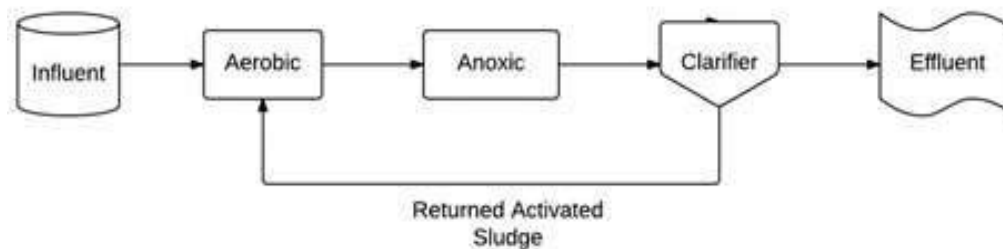


Figure S1 Diagram of a typical post-anoxic denitrification system diagram

Table S1 Generation, accumulation and consumption of nitrogen-based matter in wastewater

Components	Process	Description	Equation form
Organic Nitrogen	Generation	20% of Nitrogen from influent is incorporated in the sludge mass. (Henze 2008). Or being	$ON_{G+A}=20\%TN_{ini}-$
	and Accumulation	trapped within the system when the solid is separated from the liquid phase (e.g. clarifier). (ON_{G+A0})	$NH4_A$
	Consumption	The rest of organic nitrogen is broken down into $NH4$ during aerobic stage. (ON_C)	$ON_C=TN_{ini}- ON_{G+A}-$ $NH4_{ini}$
Ammonia	Generation	Was generated from the breaking down of influent organic nitrogen. The ammonia produced	$NH4_G=ON_C= TN_{ini}-$

	(NH _{4G})	from decaying sludge is omitted in this case.	$ON_{G+A} - NH_{4ini}$
	Accumulation (NH _{4A})	Along with the influent of Organic Nitrogen, 20% of influent Nitrogen is incorporated in the sludge mass.	$NH_{4A} = 20\% TN_{ini} - ON_{G+A}$
	Consumption (NH _{4C})	Most will be denitrified during the nitrification stage in aerobic zone.	$NH_{4C} = NO_{3G} - NH_{4ini} + NH_{4G} - NH_{4A} - NH_{4eff}$
Nitrate	Generation (NO _{3G})	Is generated from the nitrification of NH ₄ in aerobic zone.	$NO_{3G} = NH_{4c}$
	Accumulation (NO _{3A})	Not applicable when there is no internal recycle rate.	
	Consumption (NO _{3C})	Is denitrified during denitrification process in anoxic zone.	$NO_{3C} = NO_{3G} - NO_{3eff}$

Based on these, the NO₃ consumed can then be simplified as (all units are in mg/L):

$$\begin{aligned}
\mathbf{NO3}_C &= \mathbf{NO3}_G - \mathbf{NO3}_{eff} = \mathbf{NH4}_C - \mathbf{NO3}_{eff} = \mathbf{NH4}_{ini} + \mathbf{NH4}_G - \mathbf{NH4}_A - \mathbf{NH4}_{eff} - \mathbf{NO3}_{eff} \\
&= \mathbf{NH4}_{ini} + \mathbf{TN}_{ini} - \mathbf{ON}_{G+A} - \mathbf{NH4}_{ini} - \mathbf{NH4}_A - \mathbf{NH4}_{eff} - \mathbf{NO3}_{eff} \\
&= \mathbf{NH4}_{ini} + \mathbf{TN}_{ini} - \mathbf{ON}_{G+A} - \mathbf{NH4}_{ini} - 20\%\mathbf{TN}_{ini} + \mathbf{ON}_{G+A} - \mathbf{NH4}_{eff} - \mathbf{NO3}_{eff} = \mathbf{80\%TN}_{ini} - \mathbf{NH4}_{eff} - \mathbf{NO3}_{eff}
\end{aligned}$$

Appendix VII – NMB model of a pre-anoxic denitrification

The basis of pre-anoxic denitrification is very similar to the post-anoxic denitrification, since fundamentally the nitrogen process still starts with nitrification before being followed by denitrification. The biggest difference is the order of the ‘physical’ anoxic and aerobic zone, and the large volume of Internal Recycle. In another word, the key difference here is its hydro dynamic.

Based on that knowledge, a typical pre-anoxic denitrification set-up or MLE can be set up as Fig II below. The nitrogen profiles based on the Figure II is as seen in Table II. This is with assumption that the system is completely well-mixed and the volume of returned sludge is insignificant when it comes to dilution factor of incoming influent. Note the NMB model can’t simulate the performance of nitrification, so the effluent ammonia NH_{4eff} is required to set up NMB model.

Unlike the post-anoxic NMB model, the pre-anoxic NMB model only can simulate the effluent nitrate when complete denitrification occurs. Or in another word:

$$NO_{3\text{ effluent}} = \frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - NH_{4\text{ eff}}(mg/L)$$

$$\text{Only when SDNR} \geq \frac{\left(\frac{0.8x \times v + 0.8NH_{4\text{ eff}} \times IR}{v + IR} - N_{4\text{ eff}} \right) \times IR}{v + IR} (mg/L)$$

Where x is the initial total nitrogen, v is the flowrate of influent, IR is the Internal Recycle Rate, NH_{4eff} is effluent ammonia, and SDNR is the denitrification potential of the system.

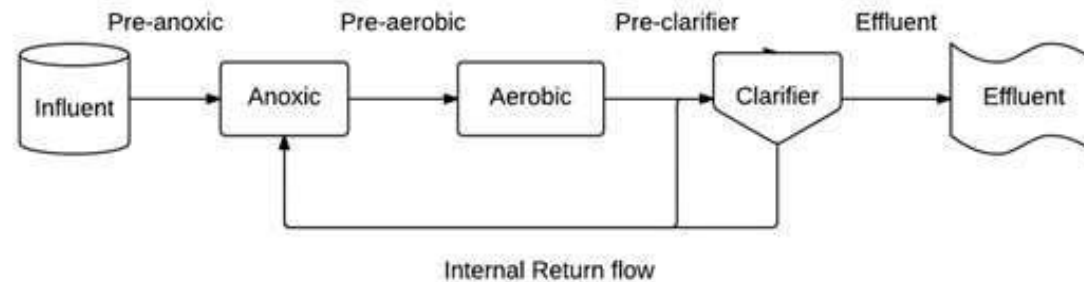


Figure S2 A basic pre-anoxic denitrification set-up

Table S2 Equations showing concentration of nitrate and ammonia for each stage in pre-anoxic denitrification (assume complete denitrification occurred).

No	Stages	Equation	Description
1	Pre-anoxic	• $TN_{ini} = x \text{ (mg/L)}$ • $NO_3 = 0 \text{ (mg/L)}$ • $Q = v \text{ (L)}$	This can be regarded as influent characteristics, before any treatment process happened. Note that the nitrate concentration here is assumed to be none as insignificant nitrate is often detected for most municipal WWTP system.

2	Pre-aerobic	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4eff} \times IR}{v + IR} (mg/L)$ • $NO_3 = 0 (mg/L)$ • $Q = v + IR (mg/L)$	This stage is after the influent was both diluted by the Internal return flow and denitrification was occurred in the anoxic zone. It's important to assume here that complete denitrification has occurred.
3	Pre-clarifier	• $NH_4 = NH_{4eff} (mg/L)$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4eff} \times IR}{v + IR} - NH_{4eff} (mg/L)$ • $Q = v + IR (L)$	This stage is after the nitrification has occurred in aerobic zone. The quality of supernatant here likely to be the same as in effluent (assume no nitrogen removal happens in clarifier, or and tertiary process).
4	Effluent	• $NH_4 = NH_{4eff} (mg/L)$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4eff} \times IR}{v + IR} - NH_{4eff} (mg/L)$ • $Q = v (L)$	The quality of influent is as above but the volume is similar to influent flow ate. Here it assumes that the flow rate of WAS wasting and loss of flow rate to evaporation is negligible. Because WAS volume often is affected by the wasting method of the

			treatment plant and the treatment plant volume. So, it should vary from treatment plant to treatment plant.
5	Internal return (IR) flow	• $NH_4 = NH_{4_{eff}}(mg/L)$ • $NO_3 = \frac{0.8x \times v + 0.8NH_{4_{eff}} \times IR}{v + IR} - NH_{4_{eff}}(mg/L)$ • $Q = IR (L)$	The internal return here is volumetric and also include the Recycled Activated Volume and measure.
6	Mixed of pre-anoxic and IR flow	• $NH_4 = \frac{0.8x \times v + 0.8NH_{4_{eff}} \times IR}{v + IR} (mg/L)$ • $NO_3 = \frac{\left(\frac{0.8x \times v + 0.8NH_{4_{eff}} \times IR}{v + IR} - NH_{4_{eff}} \right) \times IR}{v + IR} (mg/L)$ • $Q = v + IR (mg/L)$	This is an assumption stage where the pre-anoxic influent and IR flow was completely mixed but no denitrification has happened yet.

Appendix VIII – Pre-anoxic denitrification Biowin simulation scenarios

Table S3 All pre-anoxic denitrification Biowin simulation scenarios and results

Scenario	System growth rate and yield rate	Growth rate and yield rate of denitrifiers using external carbon	Note	Simulated Results	
				NO ₃	TN
Scenario A	Municipal Wastewater (default)	N/A (Default)	N/A because no external	20.15 ±	31.08 ±
			carbon was added in	6.99 (mg/L)	13.70 (mg/L)
Scenario B	Synthetic Wastewater	N/A (Default)	N/A because no external	14.92 ±	21.92 ±
			carbon was added in	4.81 (mg/L)	9.64 (mg/L)
Scenario C	Look at Note	N/A (Default)	Scenario A but	19.62 ±	29.69 ±
			modifying growth rate to 0.8	7.36 (mg/L)	14.18 (mg/L)
Scenario	Municipal Wastewater	Sucrose		16.23 ±	24.08 ±

D1	(default)			6.01	11.03
				(mg/L)	(mg/L)
Scenario	Synthetic Wastewater	Sucrose		10.92 ±	16.08 ±
D2				3.58	5.88 (mg/L)
				(mg/L)	
Scenario	Sucrose	Sucrose		19.92 ±	29.62 ±
D3				7.90	13.58
				(mg/L)	(mg/L)
Scenario	Municipal Wastewater	Sucrose	Scenario A, but with	11.46 ±	17.31 ±
Da(3x)	(default)		triple the amount of	4.57	7.65 (mg/L)
			sucrose	(mg/L)	
Scenario	Municipal Wastewater	Sucrose	Scenario A, but with 6	7.38 ± 2.76	13.38 ±

Db(6x)	(default)		times the amount of sucrose	(mg/L)	3.79 (mg/L)
Scenario	Municipal Wastewater	Sucrose	Scenario A, but with 9	4.23 ± 2.33	15.69 ±
Dc(9x)	(default)		times the amount of sucrose	(mg/L)	5.97 (mg/L)
Scenario	Municipal Wastewater	Sucrose	Scenario A, but with 8	6.15 ± 2.82	13.69 ±
Dd(8x)	(default)		times the amount of sucrose	(mg/L)	4.12 (mg/L)
Scenario	Municipal Wastewater	Mix of Sucrose+Synthetic		16.54 ±	24.23 ±
E1	(default)	Wastewater		6.56	11.41
				(mg/L)	(mg/L)
Scenario	Synthetic Wastewater	Mix of Sucrose+Synthetic		11.31 ±	16.69 ±

E2		Wastewater	3.85	6.45 (mg/L)
			(mg/L)	
Scenario	Mix of	Mix of Sucrose+Synthetic	23.92 ±	36.77 ±
E3	Sucrose+Synthetic	Wastewater	8.24	15.70
	Wastewater		(mg/L)	(mg/L)

Appendix IX – Post-anoxic denitrification Biowin simulation scenarios

SBRs

Table S4: All post-anoxic denitrification Biowin simulation scenarios and results

Scenario	System growth rate and yield rate	Growth rate and yield rate of denitrifiers using external carbon	Note	Simulated Results	
				NO ₃	TN
Scenario A	Municipal Wastewater	Default	Default because no external	20.15 ± 6.99	31.08 ± 13.70
	(default)		carbon was added in	(mg/L)	(mg/L)

Scenario B	Synthetic Wastewater	Default	Default because no external carbon was added in	14.92 ± 4.81 (mg/L)	21.92 ± 9.64 (mg/L)
Scenario F1	Municipal Wastewater (default)	Sucrose		2.67 ± 3.39 (mg/L)	9.31 ± 6.41 (mg/L)
Scenario F2a	Municipal Wastewater (default)	Mix of Sucrose+Synthetic Wastewater		4.41 ± 3.51 (mg/L)	15.77 ± 8.07 (mg/L)
Scenario F2b	Municipal Wastewater (default)	Mix of Sucrose+Synthetic Wastewater	Similar to F2a, but only 70mg COD/L is used	8.01 ± 5.49 (mg/L)	17.23 ± 10.81 (mg/L)

Appendix X – Biowin simulation scenarios

Table S5 All Biowin simulation scenarios and results

Scenario	External carbon denitrification type	System growth rate and yield rate	Growth rate and yield rate of denitrifiers using external carbon	Note	Simulated Results	
					TN	NO ₃
Scenario A	N/A	Municipal Wastewater (default)	Municipal Wastewater (default)	The blank/control condition before any external carbon is added	31.08 ± 13.70 (mg/L)	20.15 ± 6.99 (mg/L)
Scenario f1	Post-anoxic denitrification	Municipal Wastewater (default)	Fermented Biosolids	Add fermented sludge to increase COD to 150mg/L	6.98 ± 1.96 (mg/L)	<0.01mg/L (mg/L)
Scenario f2	Post-anoxic denitrification	Municipal Wastewater (default)	Dark Fermented Biosolids	Add fermented sludge to increase COD to 150mg/L	6.77 ± 1.42 (mg/L)	0.02 ± 0.04 (mg/L)
Scenario f1b	Post-anoxic denitrification	Municipal Wastewater (default)	Fermented Biosolids	f1 but only add to 70mg COD/L	9.04 ± 5.70 (mg/L)	1.35 ± 1.80 (mg/L)
Scenario f2b	Post-anoxic denitrification	Municipal Wastewater (default)	Dark Fermented Biosolids	f2 bug only add to 70mg COD/L	10.62 ± 6.36 (mg/L)	3.12 ± 2.38 (mg/L)
Scenario d1	Pre-anoxic denitrification	Municipal Wastewater (default)	Fermented Biosolids	Add fermented sludge to increase COD to 150mg/L	15.27 ± 3.11 (mg/L)	10.50 ± 1.94 (mg/L)-
Scenario d2	Pre-anoxic denitrification	Municipal Wastewater (default)	Dark Fermented Biosolids	Add fermented sludge to increase COD to 150mg/L	21.38 ± 8.54 (mg/L)	14.92 ± 5.03 (mg/L)

Appendix XI – Defining Mumax Equation

The basis of this equation was based on the relationship between Y_h (growth yield) of the aerobic period to COD utilisation. Where for every 1 mg of COD was utilised, Y_h unit of biomass is generated and $(1-Y_h)$ is utilised to produce energy (and other by-products such as CO_2 and H_2O).

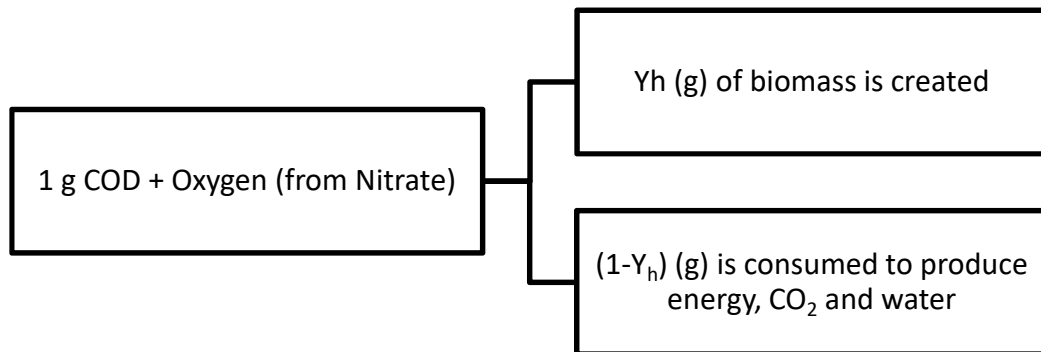


Figure S6: Relationship between Y_h (growth yield) and COD consumed

This means with a growth yield efficiency of Y_h , every 1 g COD will be stabilised by $(1-Y_h)$ g of O_2 . Similarly, to stabilise 1 g of O_2 , a required $\frac{1}{(1-Y_h)}$ g of COD will be needed. So, for example if Y_h equal 0.666, 3g of COD will be consumed for every 1g of COD being used for energy providing as $\frac{1}{1-0.666} = 3\text{g}$.

Or:

$$r_{\text{O}_2,eq} = (1 - Y_H) \times r_{\text{RBCOL}}$$

However, under anoxic conditions, nitrate replaces oxygen as the electron acceptor where every mg $\text{NO}_3\text{-N}$ is equivalent to 2.86 mg of O_2 based on the stoichiometric equation for accepting per mole of electrons. This means to stabilise 1 g of $\text{NO}_3\text{-N}$, $\frac{2.86}{(1-Y_h)}$ g of COD will be needed.

As previously stated, because the Y_h value is determined to vary for different substrates (sugar, methane, wastewater, etc.), the amount of COD needed for each g of $\text{NO}_3\text{-N}$ consumed will also vary, and can be calculated. The default growth rate of municipal wastewater in Biowin is 0.54, for example, means $\frac{2.86}{1 - 0.54} = 6.2 \text{ g}$ g of COD substrate is consumed for every g of $\text{NO}_3\text{-N}$ oxidised.

Growth Rate and denitrification rate

The equation of the growth rate originally based on Monod Equation is as below:

$$r_{GROWTH} = -\eta_{AX,GROWTH} \cdot \mu_{MAX} \cdot \frac{S_S}{K_S + S_S} \cdot X_{BH}$$

However, due to the relationship shown below between growth yield and substrate as seen in Figure , the equation for growth rate can then be converted to:

$$\frac{r_{GROWTH}}{Y_H} = -\frac{\eta_{AX,GROWTH} \cdot \mu_{MAX} \cdot \frac{S_S}{K_S + S_S} \cdot X_S}{Y_H}$$

$$r_{RBCOD} = -\eta_{AX,GROWTH} \cdot \frac{\mu_{MAX}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot X_S$$

As $r_{O_2 \cdot eq} = (1 - Y_H) \times r_{RBCOD}$ The oxygen consumption rate hence will be:

$$r_{RBCOD} \cdot (1 - Y_H) = -\eta_{AX,GROWTH} \cdot \frac{\mu_{MAX}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot X_{BH} \cdot (1 - Y_H)$$

$$r_{O_2 \cdot eq} = -\eta_{AX,GROWTH} \cdot (1 - Y_H) \cdot \frac{\mu_{MAX}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot X_{BH}$$

The nitrate removed or denitrification rate can be presented as:

$$\frac{dS_{NO}}{dt} = \frac{r_{O_2 \cdot eq}}{2.86} = -\eta_{AX, GROWTH} \cdot \frac{(1 - Y_H)}{2.86} \cdot \frac{\mu_{MAX}}{Y_H} \cdot \frac{S_S}{K_S + S_S} \cdot X_{BH} \quad (1)$$

For the Mumax batch test, the SS concentration of substrate had to be saturated relative to the half-saturation coefficient K_S . This was done to ensure the maximum growth rate. Hence it can be assumed that:

$$\mu = \mu_{MAX} \cdot \frac{S_S}{K_S + S_S} = \mu_{MAX} \quad (2)$$

We also can calculate the denitrifier biomass over time as:

$$\begin{aligned} \frac{dX_S}{dt} &= \mu_S \cdot X_S - b_S \cdot X_S = (\mu_S - b_S) \cdot X_S \\ \frac{dX_S}{X_S} &= (\mu_S - b_S) \cdot dt \\ \int_0^t \frac{dX_S}{X_S} &= \int_0^t (\mu_S - b_S) \cdot dt \\ X_{BH} &= X_{S,t} = X_{S,0} e^{(\mu_S - b_S) \cdot t} \end{aligned} \quad (3)$$

Assume the coefficient $\eta_{AX, GROWTH}$ is equal 1 in this case as the experiment is in controlled lab conditions. Based on (1), (2) and (3) above, the denitrification rate equation can then be rearranged with function of time (Dold et al. 2008) as written here:

$$\begin{aligned} \frac{dS_{NO}}{dt} &= -\frac{1 - Y_M}{2.86} \cdot \frac{\mu_S}{Y_M} \cdot X_{S,0} e^{(\mu_S - b_S)t} \\ dS_{NO} &= -\frac{1 - Y_M}{2.86} \cdot \frac{\mu_S \cdot X_{S,0}}{Y_M} e^{(\mu_S - b_S)t} dt \\ \int_0^t S_{NO} &= -\frac{1 - Y_M}{2.86} \cdot \frac{\mu_S \cdot X_{S,0}}{Y_M} e^{(\mu_S - b_S)t} dt \\ S_{NO,t} &= S_{NO,0} - \frac{1 - Y_M}{2.86} \times \frac{\mu_S \times X_{S,0}}{Y_M \times (\mu_S - b_S)} \times e^{((\mu_S - b_S)t)} + \frac{1 - Y_M}{2.86} \times \frac{\mu_S \times X_{S,0}}{Y_M \times (\mu_S - b_S)} \times e^{((\mu_S - b_S) \times 0)} \end{aligned}$$

It is in fact the same equation used for the Mumax batch test:

$$S_{NO,t} = S_{NO,0} - \frac{1 - Y_M}{2.86} \times \frac{\mu_S \times X_{S,0}}{Y_M \times (\mu_S - b_S)} \times \left[e^{((\mu_S - b_S)t)} - 1 \right]$$