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Aerobic Granular Sludge – Study of Applications for Industrial and Domestic Wastewater

Master of Science Thesis in the Master's Programme Infrastructure and Environmental Engineering

TAMARA MOUSTAFA

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Division of Water Environment Technology

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ABSTRACT

The world today and its source of clean water face conflicting challenges. The substantial growth in urbanisation and industrialisation of our society calls for a supreme water treatment technology. A technology that is capable of providing safe, clean water in response to the extensive use and requirements our current generation demands. This thesis particularly focused on the aspect of wastewater treatment by means of aerobic sludge granulation technology.

Aerobic granulation is a phenomenon that transforms activated seed sludge into a substantial granule form in which its self-immobilising properties adsorb and consume polluted and harmful microorganisms. The granulation process is commonly cultivated in a SBR column reactor where an established cycle operation is set to encourage a well-nurturing environment for appropriate granule formation and maintenance as well as adequate treatment efficiency, or total organic carbon (TOC) and total nitrogen (TN) removal efficiency.

This thesis explored the use of the technology in current and future industrial-scale applications, where companies are competing to design innovative models in order to supply adequate treatment as cost-effective and sustainable as possible. In light of the ever-growing stringent standards being placed on the quality of effluent discharged, a laboratory-scale experiment was carried out on three parallel reactors (R1, R2 and R3) investigating the effect various nitrogen loads impart on the granulation process. A COD:N ratio of 100:5, 100:10 and 100:20 were used in R1, R2 and R3, respectively; maintaining a COD load of 5 g/L.d and an ammonium-nitrogen (NH4-N) load of 0.25, 0.50 and 1.0 g/L.d in R1, R2 and R3, respectively.

The various feed compositions were applied to replicate the incoming high-ammonium domestic wastewater at the Gryaab WWTP in Gothenburg, Sweden. This

investigation supplied an assessment of which feed composition led to the most compatible and efficient level of treatment. In conclusion, R3 composed the most desirable granule structure that of which demonstrated excellent settling characteristics and emphasised dense and hydrophobic qualities. It proved to remove the greatest amount of TOC and TN and is deemed most effective in terms of treatment capacity.

Keywords: Wastewater treatment, aerobic granular sludge, aerobic granulation industry, nitrogen removal.

CONTENTS

List of Abbreviations

AOB	Ammonium-oxidising Bacteria
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substances
F/M	Food to Microorganisms Ratio
GAO	Glycogen-Accumulating Organism
HRT	Hydraulic Retention Time
MLSS	Mixed Liquid Suspended Solids concentration
MLVSS	Mixed Liquid Volatile Suspended Solids
NOB	Nitrite-oxidising Bacteria
OLR	Organic Loading Rate
OUR	Oxygen Uptake Rate
PAO	Phosphate-Accumulating Organism
SAV	Superficial Air Velocity
sCOD	Soluble Chemical Oxygen Demand
SBR	Sequencing Batch Reactor
SND	Simultaneous Nitrification Denitrification
SRT	Solids Retention Time
SS	Suspended Solids
SV	Sludge Volume
SVI	Sludge Volume Index
tCOD	Total Chemical Oxygen Demand
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solids
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WET	Water Environment Technology
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

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1 Introduction

An essential element of life is water. Surveys carried out by The World Bank and the World Health Organisation (WHO) illustrates recent trends in the condition of and accessibility to water sources. Globally, the population exposed to improved drinking-water sources rose from seventy-six percent in 1990 to eighty-nine percent in 2011 (WHO, 2014). The population using improved sanitation facilities increased, globally, from forty-nine percent in 1990 to sixty-four percent in 2011. Refer to Appendix A for graphs. Alongside the need for further improvement in the supply of clean water, the substantial growth in urbanisation and industrialisation of our society calls for a supreme water treatment technology. A technology that is capable of providing safe, clean water in response to the extensive use and requirements our current generation demands. This thesis will particularly focus on the aspect of wastewater treatment by means of aerobic sludge granulation technology.

Wastewater can be defined as water streaming from sewage pipes and runoff over ground in which the water has become contaminated due to anthropogenic activity. Urbanisation and industrialisation influence the extent of pollution within surface and ground waters and with an ever-increasing urban population a greater deal of pollution is expected to be treated. The recent upcoming technology of aerobic sludge granulation, that is the central theme in this thesis, is of prime interest in the wastewater treatment realm. It has proven to demonstrate remarkable efficiency in biological wastewater treatment by rapid settleability of granules leading to prompt liquid-solids separation (Adav et al., 2008). Other advantages of aerobic granules include having a highly stable rate of metabolism, ability to withstand high shear forces and toxins, high sludge retention times and self immobilisation within the granule (Ni & Yu, 2012, p.429). The conventional activated sludge system currently has the most prominent presence in wastewater treatment plants (WWTP).

A conventional activated sludge system generally comprises an aeration tank where air activates the biological processes between microorganisms, a settling tank, or clarifier, that helps separate clear effluent from sludge, and a disinfection tank to eradicate pathogenic microorganisms further. The main disadvantage a conventional activated sludge system possesses is the requirement of a large footprint in response to the long settling phase and multiple units required for treatment (de Kreuk, 2006). The granules in this system do not settle promptly due to the slow growth rate of nitrifying microorganisms. Although the activated sludge system in WWTPs is still found to be most common in today's industry, the aerobic granular sludge system is at the cusp of its expansion.

Aerobic granules require certain environmental conditions in order to achieve densely packed microorganisms that promote excellent settling properties and high microbial activity as well as enable treatment of both low and high strength wastewaters (Adav et al., 2008). It is found that optimal operational conditions are achieved within SBRs that are either bubble columns or airlift reactors (de Kreuk & van Loosdrecht, 2004). Processes such as nitrification, denitrification and biological phosphorus removal may be efficiently controlled by inducing high substrate concentrations from which growth of large granules are generated (de Bruin et al., 2004). This contributes to substantial cost savings in energy consumption as biological nitrogen removal consumes the greatest amount of energy in WWTPs.

Previous laboratory-scale aerobic SBR systems, such as one demonstrated by Hamann, 2009, have proven to successfully remove nitrogen and phosphorus, the main nutrients in wastewater, from synthetic wastewater. Further stringent effluent standards are propagating research into obtaining processes that are considerably more efficient, cost effective and sustainable. It has been suggested that promptly adjusting the initial selection pressure by reducing the settling time during the start-up of the SBR propagates a rapid and more stable granulation (Hamann, 2009). It is essential to optimise the feeding regime within the reactor to attain simultaneous nitrification-denitrification. Considerable nitrogen removal is established by feeding through a settled granule bed during anaerobic conditions (de Kreuk & van Loosdrecht, 2004). Field studies have demonstrated that granules are easily formed at various COD:N:P ratios when fed with synthetic wastewater (Beun et al., 1999). This has shown to produce high hydraulic loadings rates that contribute to faster granulation. Several publications, including Beun et al., 2002, de Kreuk et al., 2005 and Lui et al., 2005, have illustrated aerobic granular sludge systems feeding synthetic wastewater containing various carbon sources such as acetate, glucose, ethanol, phenol and sucrose. Intermittent feeding and warm reactor start-up temperatures are claimed to cultivate high treatment efficiency compared to conventional activated sludge. It has been observed that the divalent ions calcium (Ca^{2+}) and magnesium (Mg^{2+}) accelerate granulation (Ren et al., 2008). The aeration phase within an aerobic granular sludge SBR has been hypothesised to compose of a feast period, where high organic content is available within the substrate, and a subsequent starvation period, where the substrate is depleted of nutrients. However, there is a debate on the validity of this theory, as it is not yet clearly understood (Adav et al., 2008). It is certainly noteworthy that both cycle length and feeding strategy play a crucial role in granule formation and stability, as demonstrated by Wang et al., 2006 and McSwain et al., 2004.

To this present day, one full-scale aerobic granular sludge system has been established, the Nereda technology (Royal Haskoning DHV, 2014). One particular phenomenon that is of pinnacle interest within aerobic granulation systems is simultaneous organic and nitrogen removal; of which the Nereda technology achieves. High substrate gradients developing within granules encourages growth of heterotrophic, nitrifying and denitrifying bacteria that enables effective organic and nitrogen removal. By selecting slow-growing microorganisms for the anaerobic feeding phase, granules may be cultivated at low dissolved oxygen (DO) concentrations, which is favourable in supplying more efficient and reduced energy consumption (Adav et al., 2008). Low DO concentrations are applicable to such circumstances as readily biodegradable substrates are converted into storage polymers that enable nourishment to slow-growing microorganisms (de Kreuk & van Loosdrecht, 2004). Aerobic granules contain a greater amount of extracellular polymeric substances (EPS) compared to activated sludge flocs, and this contributes to the more advanced structural stability of granules. Further studies in understanding the different EPS components are recommended to gain better insight into the complex nature and structure of aerobic granules. In addition, further research in the impact start-up periods impart onto the operation of an aerobic granular sludge system should be investigated. This would contribute to a more clarified appreciation of possible initial parameters that may influence the operation of aerobic granulation systems in the long-term.

2 Aims and Objectives

The aims of this thesis are to provide an insight into the current aerobic granulation industry by discussing one avenue of work carried out by a wastewater treatment company and to investigate the effect various nitrogen loads have on aerobic granulation. The wastewater treatment company have requested to conceal their identity; nonetheless, relevant findings are presented and discussed in this thesis. The effect different nitrogen loads impart onto the aerobic granulation of sludge in a sequencing batch reactor holds great interest in developing further appreciation of the technology.

The main objective for exploring the aerobic granulation industry is to further understand a field of wastewater treatment that may be pivotal for present and future demands of sustainable engineering. This study will evaluate the influential parameters of aerobic granulation and discuss the factors that affect the efficiency of its treatment. Nitrogen is a crucial nutrient in wastewater treatment to propagate sludge growth. It is highly present within low-strength domestic wastewater and ammonium-rich digested sludge liquor, or reject water.

The summary of the questions this thesis shall answer include:

1. What type of models is the aerobic granulation industry developing to advance and popularise aerobic granulation technology?

2. What conclusions can be drawn on the structure and composition of the granules during the running of an SBR that treats wastewater of varying nitrogen loads?

3 Literature Review

3.1 Wastewater Treatment

3.1.1 Conventional Wastewater Treatment System

Wastewater, as defined previously, is essentially contaminated water streaming from sewage pipes and runoff over ground. The main source of contamination is caused by anthropogenic activities that stem from industrial and domestic waste. The basic elements involved in treating wastewater include the following, (Metcalf & Eddy, 2004):

- **Preliminary treatment** – where constituents such as rags, floatables, grit and grease are removed via screening in order to prevent maintenance and operational difficulties.

- **Primary treatment** – where a share of suspended solids and organic matter are removed via filtration or chemical addition.

- **Secondary treatment** – where biodegradable organic matter and suspended solids are removed. Removal of biodegradable organic matter is typically achieved by biological processes such as aerobic suspended growth, membrane filtration, chemical oxidation, etc. The addition of a disinfection unit promotes further eradication of pathogenic microorganisms by the addition of chemicals.

Increased industrial activity is the cause to increased amounts of heavy metals and organic compounds found in wastewater (Metcalf & Eddy, 2004). Advancements in technology have encouraged changes in compounds discharged into wastewater streams, altering the wastewater characteristics. This has led to difficult and costly treatment methods that claim disadvantages such as generating large quantities of surplus biomass, limited flexibility regarding loading rates, large footprint and low volumetric conversion capacity (Beun et al., 2002). More sophisticated analytical methods may be required in the near future to gain a greater appreciation of the behaviour of wastewater constituents and their effect on process performance and effluent quality (Metcalf & Eddy, 2004). The imperative reasoning for gaining in-depth analysis of the behaviour of wastewater constituents is to ensure the protection of public health and the environment.

The key concerns involved in wastewater treatment encompass: the changing nature of wastewater to be treated, the impact of new regulations being established, the intricate issue of industrial waste, aging infrastructure and the refurbishment of treatment operations and processes (Metcalf & Eddy, 2004).

3.2 Wastewater Constituents

Wastewater is composed of physical, chemical and biological constituents that require appropriate processes for treatment and removal in order to yield a sufficient quality effluent. The following text illustrates the wastewater constituents of particular importance and the detriment caused if left untreated, (Metcalf & Eddy, 2004):

- **Suspended Solids (physical constituent)** – indicate the potential for wastewater reuse and determine the most suitable operation units and processes for treatment. Suspended solids are of two forms: total suspended solids (TSS) and volatile suspended solids. The TSS is considered to give an indication of the amount of biomass present within a reactor and is also referred to as mixed liquor suspended solids (MLSS). If left untreated or treated insufficiently it can lead to the development of sludge deposits and anaerobic conditions when deposited into an aquatic environment.

- **Biodegradable Organics (chemical constituent)** – essentially comprise proteins, carbohydrates and fats and are most commonly measured in terms of biochemical oxygen demand (BOD) or chemical oxygen demand (COD). If exposed untreated to the environment septic conditions may propagate due to natural oxygen depletion.

- **Pathogens (biological constituent)** – are harmful microorganism commonly in the form of bacteria, virus or protozoa that transmit diseases.

- **Nutrients (chemical constituent)** – are required for growth of microorganisms and are commonly in the form of nitrogen, phosphorus and carbon. If excess nutrients are discharged into the environment undesired aquatic life might be cultivated as well as the instigation of pollution to groundwater sources.

- **Priority Pollutants (chemical constituent)** – consist of organic and inorganic compounds that inherently yield toxic, carcinogenic, mutagenic and teratogenic qualities in wastewater.

- **Refractory Organics (chemical constituent)** – include phenols, agricultural pesticides and surfactants, which are known to resist conventional methods of wastewater treatment.

- **Heavy Metals (chemical constituent)** – commonly discharged from commercial and industrial activities, levels of metals exposed to the environment must be controlled in order to maintain a metabolic balance.

- **Dissolved Inorganics (chemical constituent)** – consist of constituents such as calcium, sodium and sulfate, and are commonly found in domestic water supply, which then require removal if the wastewater is to be reused.

The following sub-sections shall further elaborate on the particular wastewater constituents to be focused in this thesis.

3.2.1 Nitrogen

Nitrogen is one of the main nutrients required for the growth of microorganisms and is important for algal growth that promotes eutrophication in water sources (Henze, 2002). Nitrogen in the form of ammonia, NH_3, can be harmful to the environment and is found to be greatly toxic to fish. There are various forms of nitrogen and the nitrogen cycle is a useful tool in envisaging the conversion processes. Figure 3.1 illustrates the nitrogen cycle.

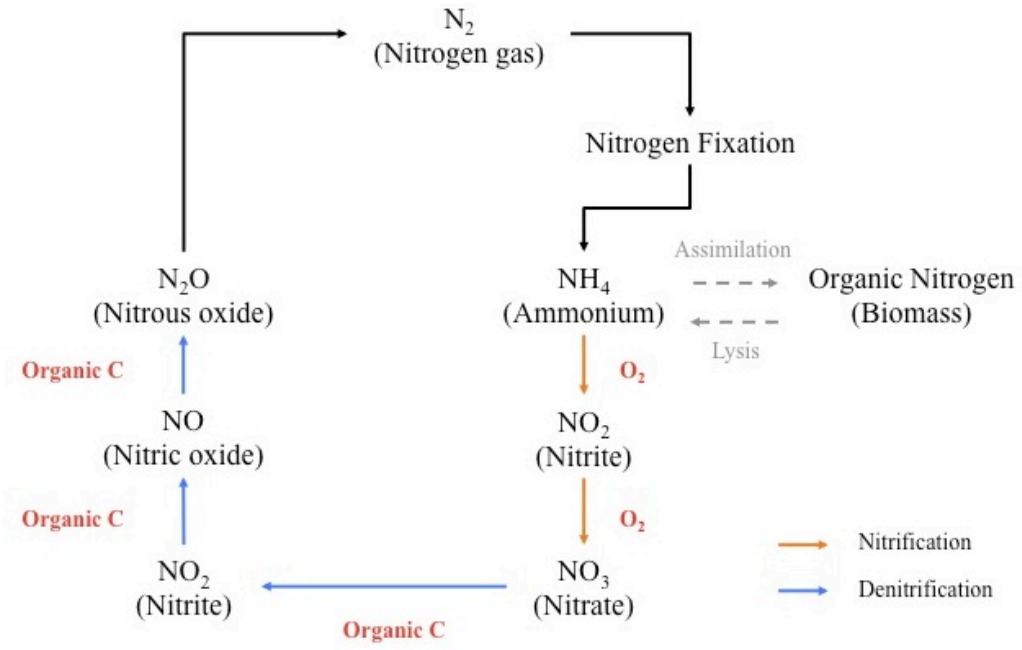


Figure 3.1 **The nitrogen cycle.**

Wastewater is composed of nitrogen fractions, a major inorganic fraction and a minor organic part. Inorganic nitrogen comprises dissolved ammonium (NH_4), nitrite (NO_2) and nitrate (NO_3). Organic nitrogen is composed of suspended degradable organic nitrogen as well as dissolved and suspended inert organic nitrogen. Total nitrogen is defined by the sum of organic nitrogen, ammonia, ammonium, nitrite and nitrate.

As depicted by the orange arrows in Figure 3.1, nitrification is the phenomenon where ammonium is converted to nitrite and then nitrate by means of oxidation. The blue arrows in Figure 3.1 signify denitrification, where nitrate is converted back into nitrite and reduced to nitrogen gas (N_2) by oxidation of organic matter. Organic carbon is

essential for each step of denitrification and is sourced, in wastewater, by substances such as methanol and acetic acid (Henze, 2002). Soft-drink wastewater and residue from a diary plant may also be an alternative carbon source providing an economical means of treating high-nitrate wastewater (Fernandez-Nava et al., 2010).

3.2.2 Organic Constituents

Wastewater is composed of various combinations of organic compounds that include carbon, hydrogen and oxygen and, in some cases, nitrogen. Organic matter itself is a cluster of proteins (40 to 60 percent), carbohydrates (25 to 50 percent) and oils and fats (8 to 12 percent) (Metcalf & Eddy, 2004). As well as the compounds mentioned, wastewater contains a slight proportion of synthetic organic molecules that range in complexity from simple to intricate and complex. Organic matter in wastewater can be classified as either individual organic compounds or aggregate organic constituents. Individual organic compounds encompass volatile organic compounds, haloacetic acids (HAAs) and trihalomethanes (THMs), all of which are extremely detrimental to human and animal health (Metcalf & Eddy, 2004). Aggregate organic materials typically comprise biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC).

3.2.2.1 Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) is the most widely used parameter regarding organic pollution in wastewater and surface water (Metcalf & Eddy, 2004). Although it suffers from a number of limitations such as requiring a high concentration of active seed bacteria, only biodegradable organics are measured and the timely length of its process; the BOD is used to determine: the amount of oxygen required to biologically stabilise organic matter present in the wastewater, to ascertain the capacity of wastewater facilities, to measure the efficiency of certain treatment processes and to establish compliance to wastewater discharge standards (Metcalf & Eddy, 2004).

3.2.2.2 Chemical Oxygen Demand

Chemical oxygen demand (COD) is a measure of the oxygen equivalent within the organic material in wastewater that is able to be oxidised chemically using dichromate in acid solution (Metcalf & Eddy, 2004). The COD test is comparably different to the BOD test as: a great deal of organic substances that are unable to oxidise biologically can be oxidised chemically and numerable organic substances are found to be toxic to microorganisms used in the BOD test. COD can be fractioned into particulate and soluble COD; these assist in assessing wastewater treatability (Metcalf & Eddy, 2004).

3.2.2.3 Total Organic Carbon

Total organic carbon (TOC) is a measure of carbon dioxide that has been converted from organic carbon in an aqueous sample (Metcalf & Eddy, 2004). TOC provides an indication of pollution characteristics within a wastewater. The total carbon (TC) within a sample is simply the sum of the TOC and inorganic carbon (IC), which provides a more abstract indication of organic matter within a wastewater.

3.2.3 pH

The hydrogen-ion concentration, also known as the pH, is a significant quality parameter for wastewater and a suitable range for the presence of biological matter ranges between six and nine (Metcalf & Eddy, 2004). Treated effluents discharged from WWTPs are required to meet an allowable pH range between 6.5 and 8.5. Information regarding optimum pH levels for species selection and aerobic granulation is rather limited. Results have suggested, however, that microbial communities and structural integrity of aerobic granules may be controlled via the alkalinity and pH dosage during feeding (Etienne & Yu-Tung, 2012).

3.2.4 Temperature

Temperature plays an important role in effecting chemical reactions and reaction rates with high temperatures promoting increased biochemical reaction rates that result in a decrease of oxygen within wastewaters, which develops a serious depletion in dissolved oxygen concentrations (Metcalf & Eddy, 2004). Optimum temperatures for bacterial activity range between 25 and 30°C. Once temperatures exceed 50°C aerobic digestion and nitrification cease (Metcalf & Eddy, 2004). It has been found by (de Kreuk et al., 2005) that temperature change may affect the performance of an aerobic granular sludge reactor rather significantly. Concluding remarks state that a preferential start-up during the summer period is recommended while granule stability and treatment efficiency remains unhindered during winter months.

3.3 Biological Wastewater Treatment

Biological treatment processes are considered as one of the most vital parts to wastewater treatment (Etienne & Yu-Tung, 2012). Sludge self-immobilisation purifies wastewater by removing organic matter through heterotrophic microorganisms that consist of mostly bacteria and occasionally fungi. Organic matter is broken down through either biological oxidation or biosynthesis. Oxidation, also known as respiration, of organic matter produces mineralised end products that are disposed of within the treated effluent (Etienne & Yu-Tung, 2012). Biosynthesis combines colloidal and soluble organic matter to form particulate biomass that is then removed during the settling phase of treatment. Microorganisms consume organic and inorganic matter to support their growth. A portion of the matter consumed is oxidised and the energy released from this reaction converts the remaining materials into new cell tissues that aggregate to form particulate biomass (Etienne & Yu-Tung, 2012).

3.3.1 Objective

The main objectives for treating domestic wastewater are defined as, (Metcalf & Eddy, 2004):

1. transforming (oxidising) dissolved and particulate biodegradable constituents into appropriate end products,
2. enabling suspended and colloidal solids to be absorbed into a biological floc or biofilm,

3. removing nutrients such as nitrogen and phosphorus as well as, in some cases, certain trace organic compounds.

For industrial wastewater, the main objective is to remove or reduce the concentration of organic and inorganic compounds, as these may be harmful to microorganisms (Metcalf & Eddy, 2004).

3.3.2 Biological Characteristics

There are a vast array of biological organisms present within surface water and wastewater, as Section 3.2 briefly demonstrated. Pathogenic organisms largely comprise bacteria, protozoa, helminths and viruses. One of the major aims in wastewater treatment is to remove or significantly reduce the presence of pathogenic organisms, as they are responsible for devastating health impacts to humans, animals and the environment. However, biological treatment processes are not always successful in removing the harmful pathogens and, hence, require chemical disinfectants or chlorination to be added to the treatment process for adequate removal. The important constituents to consider when designing a WWTP are the carbonaceous, nitrogenous and phosphorus as these influence biological activity and eutrophication in the receiving water (Etienne & Yu-Tung, 2012).

3.3.3 Role of Microorganisms

Microorganisms are often classified in terms of their trophic levels, meaning by their energy and carbon source and their relationship to oxygen (Etienne & Yu-Tung, 2012, p.4). There are two specific branches of organisms in biological wastewater treatment, named heterotrophs and autotrophs. Heterotrophs consume organic carbon in order to support growth of new cells (biomass) while autotrophs produce cell carbon from carbon dioxide (Metcalf & Eddy, 2004), see Table 3.1. Energy to these organisms is supplied by two means, through oxidation of inorganic or organic chemical compounds (chemotrophs) or through the use of sunlight (phototrophs). Hence, there are two variations of chemotrophs and phototrophs, (Etienne & Yu-Tung, 2012, p.5):

- *Chemoautotrophs* – microbes that use inorganic chemical substances as their energy source and carbon dioxide as their main carbon source.
- *Chemoheterotrophs* – microbes that use organic chemical substances as their energy source and organic compounds as their main carbon source.
- *Photoautotrophs* – microbes that use light as their energy source and carbon dioxide as their main carbon source.
- *Photoheterotrophs* – microbes that use light as their energy source and organic compounds as their main carbon source.

Additionally, microorganisms have two electron sources: those that use reduced organic substances (lithotrophs) or those that use organic compounds to obtain electrons or hydrogen atoms (organotrophs) (Etienne & Yu-Tung, 2012, p.5).

In municipal wastewater treatment, heterotrophic organisms and lithoautotrophic nitrifying organisms are responsible for biological nutrient removal. Nitrifying organisms largely involve ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB). Table 3.1, sourced from (Metcalf & Eddy, 2004), illustrates a selection of microorganisms classified by electron donor, electron acceptor, sources of cell carbon and end products.

Table 3.1 **Microorganisms classified by electron donor, electron acceptor, carbon source and end products.**

Type of Bacteria	Reaction Name	Carbon Source	Electron Donor	Electron Acceptor	End Products
Aerobic heterotrophic	Aerobic oxidation	Organic compounds	Organic compounds	O_2	CO_2, H_2O
Aerobic autotrophic	Nitrification	CO_2	NH_3, NO_2	O_2	NO_2, NO_3
Facultative heterotrophic	Denitrification anoxic reaction	Organic compounds	Organic compounds	NO_2, NO_3	N_2, CO_2, H_2O

Microorganisms are dependant on substrate concentration. There are four phases the growth rate of bacteria undergo, (Metcalf & Eddy, 2004):

- *Lag- phase* – where microorganisms take on a slow rate of growth as they acclimatise to the new environment.
- *Exponential phase* – where microorganisms utilise most of the healthy supply of substrate becoming larger at the greatest rate it will achieve.
- *Stationary phase* – as most of the supply of substrate is consumed the microorganisms are at their peak number and have no fuel to further their population.
- *Endogenous phase* – where there is no longer any substrate supply to the microorganisms and they begin to decay faster than they grow.

The decay of bacteria is essential for the degradation of compounds in the biological treatment process. The degraded bacteria are transformed into slowly degradable substances that eventually become hydrolysed. This hydrolysed material then yields a substrate for other bacteria to utilise (Metcalf & Eddy, 2004).

3.3.4 Environmental Factors

Temperature and pH are regarded as the most signification environmental conditions to configure the selection, survival and growth of microorganisms (Metcalf & Eddy, 2004). For optimal growth of microorganisms a range of temperatures between 20°C and 50°C is required and a pH range between 6.5 and 7.5. In the case of aerobic granular sludge formation, reactor temperatures desire warmer settings as freezing temperatures inhibit inter-granular bioactivity (Adav et al., 2008). The pH is

primarily influenced by the wastewater composition and different biological degradation processes.

3.3.5 Biological Nitrification

Nitrification is the process where ammonium (NH_4-N) is oxidised to nitrite (NO_2-N) and subsequently nitrite is oxidised to nitrate (NO_3-N), as depicted in Figure 3.1. Nitrification is required in wastewater treatment in order to meet water quality standards on ammonia and nitrogen concentration in receiving waters. Nitrogen discharge levels are essential in controlling eutrophication and water reuse applications (Metcalf & Eddy, 2004). It is recommended that a drinking water maximum contaminant level for nitrate nitrogen be 45 mg/L as nitrate or 10 mg/L as nitrogen. The total organic and ammonium nitrogen concentration should typically be in the range 25-45 mg/L for municipal wastewaters (Metcalf & Eddy, 2004, p.611). To accomplish nitrification successfully an aerated tank is required and for the case of aerobic sludge granulation this can be carried out in the same single-sludge process. Aerobic autotrophic bacteria are responsible for nitrification where in the first stage, of NH_4-N being converted to NO_2-N, ammonia-oxidising bacteria (AOB) are put to use and in the second stage, where NO_2-N is converted to NO_3-N, nitrite-oxidising bacteria (NOB) are utilised. The total oxidation process of nitrification can be described by Equation 3.1:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad (3.1)$$

The oxygen required to completely oxidise ammonium is 4.57 gO_2/gN with 3.43 gO_2/g used for nitrite production and 1.14 gO_2/g used for nitrate oxidation. In addition to oxidation, oxygen is obtained from fixation of carbon dioxide and nitrogen into cell mass (Etienne & Yu-Tung, 2012, p.45). The first group of bacteria, AOB, acquire a greater amount of energy compared with the second group, NOB, however, they are equally important in the role they play in nitrification. Nitrous acid may be harmful to the nitrification process as it affects the nitrifying bacteria, hence, adequate alkalinity is required to neutralise the nitrous acid as soon as it is formed. The carbon dioxide produced as an end product may influence a slight decrease in the environmental pH that may then require excess alkalinity to stabilise the pH. Nitrifying bacteria demand carbon dioxide and phosphorus as well as trace elements for cell growth (Etienne & Yu-Tung, 2012). Nitrification is affected by environmental factors such as pH, toxicity, metals and un-ionised ammonia. Carrying out oxygen uptake rate (OUR) tests determines the activity of AOB and NOB within a reactor's contents (Tay et al., 2006, p.173).

3.3.6 Biological Denitrification

Denitrification is the process where nitrate is reduced to nitric oxide (NO) and nitrous oxide (N_2O) and finally converted to nitrogen gas (N_2) for removal from wastewater. Two means of nitrate removal are available in biological processes: assimilating and dissimilating nitrate reduction (Metcalf & Eddy, 2004, p.616). Assimilating nitrate reduction reduces nitrate to ammonia for cell synthesis, where it is independent from

DO concentration and transpires when NH_4-N is not available. On the contrary, dissimilating nitrate reduction, or biological denitrification, uses nitrate or nitrite as an electron acceptor for the oxidation of organic and inorganic electron donors. Denitrification includes a wide variety of both heterotrophic and autotrophic bacteria. A notable aspect to take into consideration is the effect DO concentration has on denitrification; it inhibits nitrate reduction by repressing the nitrate reduction enzyme meaning that higher DO concentrations lead to greater nitrate-reducing enzymes being inhibited (Metcalf & Eddy, 2004, p.622). The end products of denitrification are nitrogen gas, carbon dioxide, water and new cell material. Alkalinity is also a product of denitrification and the pH generally increases, although, pH is not a concerning environmental factor for denitrification (Metcalf & Eddy, 2004).

3.3.7 Simultaneous Nitrification Denitrification

The design of a compact WWTP encourages the phenomenon of simultaneous nitrification denitrification (SND) within aerobic granules. SND can only occur at moderate oxygen concentrations where it requires an aerobic zone in the floc for nitrification and an anoxic substrate-rich interior for denitrification (de Kreuk et al., 2005, p.761). Oxygen penetration depth corresponds to the oxygen concentration within the bulk liquid of the reactor contents. Low oxygen saturation, that of around 40%, is suggested to lead to unstable granules that establish an outgrowth of filamentous structures (de Kreuk et al., 2005). de Kreuk et al. further state that introducing an aerobic feeding period holds certain advantages regarding granule stability, biological phosphate removal and SND. Oxygen concentration is important for SND as the outer layers of aerobic granules, flocs or biofilm oxidise ammonium and the inner layers reduce NO_x. Heterotrophs dominate the outer-most layers as they outcompete the nitrifiers for dissolved oxygen and space (de Kreuk et al., 2005). This, however, is disadvantageous for SND in continuous biofilm systems as COD is consumed at the outer aerobic layers and cannot be used as an electron donor within the core during denitrification. The system is sensitive to oxygen concentration as the nitrifiers are easily outcompeted on growth rate by heterotrophic organisms in the outer aerobic layer (de Kreuk et al., 2005, p.766). The ratio of aerobic layer volume and anoxic core volume are significant for influencing the SND efficiency. Studies have shown that heterotrophic, nitrifying and denitrifying populations can successfully coexist in microbial granules. The N/COD ratio imparts a significant impact to the three populations, where high substrate N/COD ratios enhanced nitrifying and denitrifying activity; however, heterotrophic populations were found to decrease in such circumstances (Liu & Tay, 2004). Studies have also found that DO profoundly affects the efficiency of denitrification, where adequate mixing is required to ensure sufficient mass transfer between the liquid and granules (Liu & Tay, 2004). To achieve SND during heterotrophic breakdown of synthetic wastewater within the aerobic granulation process is a highly acclaimed goal for the industry.

3.4 Sequencing Batch Reactor

The aerobic granulation process is commonly carried out in a sequencing batch reactor (SBR) as it encourages all phases of the technology to be conducted in a single column reactor. All SBR systems share four sequential phases in common, (Metcalf & Eddy, 2004, p.720), and Figure 3.2 provides a depiction:

1. *Filling* – influent is filled into the reactor.
2. *Aeration* – an air blower situated at the bottom of the reactor propagates air bubbles to mix the interior contents.
3. *Settling* – a settling phase promotes the separation of clear effluent, or supernatant, from the reactor sludge.
4. *Decanting* – effluent is discharged containing floc particles and treated wastewater.

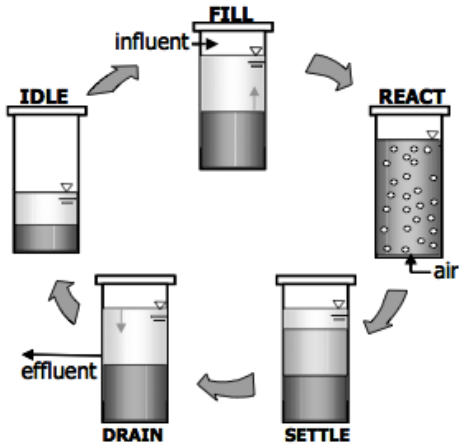


Figure 3.2 **Typical SBR cycle phases (cited in de Kreuk, 2006).**

An additional phase that is regarded as optional for the operation of aerobic sludge granulation is the *idle* phase. The idle phase is where, after the decanting of the effluent, the remaining contents lie still with no aeration or feeding. Note that feeding of the influent may take place at the bottom of the reactor as well as the top. A benefit of using an SBR model is not requiring the need for a return activated-sludge system to maintain an adequate amount of sludge in the reactor. Sludge is not wasted during the aeration phase; hence, none has to be returned to maintain the solids contents. Excess sludge that does accumulate within an SBR is simply removed by scooping the required amount out. A single-reactor system requires the need for new and well-developed control systems to adjust the amount of oxygen supplied to the nitrate that is formed as well as configure a suitable cycle schedule for greatest treatment efficiency (de Kreuk, 2006, p.157).

3.5 Aerobic Granulation

3.5.1 A History

During the late 1990s, the idea of aerobic granulation was birthed from research studies carried out on biofilm structure and formation and the role of storage polymers (de Kreuk et al., 2007). The thought of growing aerobic granules without the use of a carrier material, on which microorganisms settle and layer on, surrounded by readily biodegradable substrates contained within a SBR dawned a new ingenious concept. Readily biodegradable COD convert into substrate that yields slow-growing microorganisms to form substantial granules (de Kreuk et al., 2007, p.75). Primitive

studies proved that the growth of stable granular sludge was made possible alongside the integration of COD and nitrogen removal. At the turn of the millennium, aerobic granulation profusely became a central theme for many research endeavours worldwide. A driven desire to establish theories behind aerobic granulation, important parameters of the process and the appropriate conditions required for its operation were in large demand to enable perfecting the technology. As the wastewater industry currently faces two principal setbacks within the use of conventional activated systems, that of which comprise special footprint and vast sludge production, there is a call for a new and innovative technology.

3.5.2 A Definition

A universal definition of aerobic granular sludge has been identified and is summarised below, (de Kreuk et al., 2007, p.76):

"*Granules making up aerobic granular activated sludge are to be understood as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settle significantly faster than activated sludge flocs.*"

The statement makes the following conclusions and association to activated sludge:

- Aerobic granules require active microorganisms for growth and maintenance, which stem from seeding activated sludge. No carrier material is involved in the process as the granules aggregate without such a requirement.

- The formation of aerobic granular sludge is encouraged during the aeration phase, which induces a hydrodynamic shear force between particles. During the settling phase where hydrodynamic shear is reduced, aerobic granules do not coagulate or settle as separate units such as activated sludge flocs. However, they emerge as a collected combination of sludge flocs and other various microorganisms in a more dense and spherical form. Shear force is an important factor for the formation of dense aggregates (de Kreuk, 2006, p.15)

- Aerobic granules settle significantly faster than activated sludge flocs demonstrating the extent of thickening after settling.

3.5.3 Formation and Morphology of Aerobic Granular Sludge

Aerobic sludge granulation is a relatively new technology in the industry and the paramount features of its formation and stability to this day remain uncertain (de Kreuk et al., 2007). Numerous aspects that are understood to contribute to the granulation process include the use of specific self-aggregating cultures, selection by settling velocity, applied shear stress, growth rate of organisms, substrate gradients within the granules and the formation of extracellular polymeric substances (EPS). Properties that have shown to influence aerobic sludge granulation comprise substrate

composition, organic loading, hydrodynamic shear force, feast-famine regime, feeding strategy, dissolved oxygen, reactor configuration, solids retention time, cycle time, settling time and volume exchange ratio (Etienne & Yu-Tung, 2012, p.430). The organic carbon source found in the substrate composition plays an important role in influencing granule properties through microstructure and species diversity.

A wide variety of organic carbon sources are available for cultivating aerobic granules, such include: glucose, acetate, phenol, starch, ethanol, molasses, sucrose and other synthetic wastewater components (Adav et al., 2008). It has been found that glucose-fed aerobic granules exhibit a filamentous structure, where as acetate-fed aerobic granules demonstrate a more compact bacterial structure in which rod-like species dominate (Liu & Tay, 2004). In addition, N:COD ratios contribute to various effects on granule microbial structure. It was observed that mushroom-like granules developed at high substrate N:COD ratios as well as amplified activity of nitrifying and denitrifying populations (Liu & Tay, 2004). As mentioned previously, aerobic granular sludge provide a high and stable rate of metabolism that is resilient to shocks and toxins due to a protective matrix layer of EPS, they facilitate long biomass retention times and encourage biomass self-immobilisation within granules (Etienne & Yu-Tung, 2012, p.429). A strict selection regime for retaining well settling sludge is accomplished by establishing a short settling time. The potential granule-forming organisms are maintained within the reactor while the lighter suspended flocs are washed out with the effluent (de Kreuk et al., 2007). Applying certain self-aggregating cultures are found to enhance the start-up period of an aerobic granular sludge reactor. Such cultures include the use of phosphate or glycogen accumulating organisms and nitrifying organisms (de Kreuk et al., 2007, p.77). de Kreuk et al. discovered that by sharply decreasing substrate gradients within granules a vastly undesirable consequence was surged upon the granulation process. This phenomenon is avoided by utilising the ability of readily biodegradable substrates to convert into storage polymers and selecting organisms with slow growth rates.

Given that shear stress is an important factor concerning the formation of aerobic granules, a setback to understanding its application involves the difficulty of quantifying its measurement. In many cases, it is often related to superficial gas velocity. A schematic illustration of the layered structure of an aerobic granule is presented in Figure 3.3. Since autotrophic organisms require oxygen, these bacteria exist within the outer aerobic layers of the granule. This is where ammonium is converted to nitrate. The nitrate then penetrates through to the core of the granule where it is able to store substrate that may later come to use as a carbon source for denitrification (de Kreuk, 2006, p.17). For aerobic granulation to take place a combination of physical, chemical and biological forces are required. A model describing the processes of aerobic granulation convey the following steps, (Liu & Tay, 2004):

Step 1. **Physical movement to encourage bacterium-to-bacterium contact**. This step features hydrodynamics, diffusion mass transfer, gravity, thermodynamic effects and cell mobility.

Step 2. **Stabilisation of multi-cell contact**. These initial attractive forces are physical (Van der Waals forces, opposite charge attraction, thermodynamically driven reduction of surface free energy, surface tension, hydrophobicity, filamentous bacteria bridging individual cells), chemical and biochemical (cell surface dehydration, cell membrane fusion, signalling and collective action in bacterial community).

Step 3. **Maturation of cell aggregation**. Achieved through the production of extracellular polymer, growth of cellular clusters, metabolic change, environment-induced genetic effects that facilitate the cell-to-cell interaction resulting in a highly organised microbial structure.

Step 4. **Shaping of the steady-state three-dimensional granule structure.** Conglomeration of microbial aggregates by hydrodynamic shear forces.

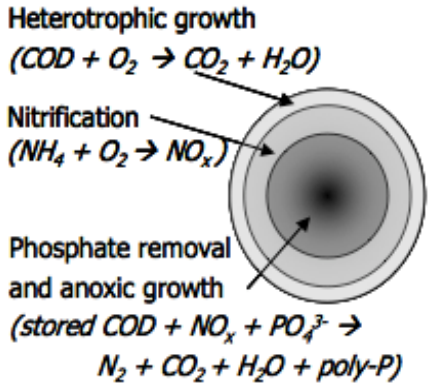


Figure 3.3 **Layered structure of an aerobic granule (cited in de Kreuk, 2006).**

Appreciating the physical characteristics of aerobic granules, they have proved ideal for the absorption and treatment of domestic as well as industrial wastewaters; where a greater content of heavy metals are found in industrial wastewaters (Liu & Tay, 2004). The granules are strong, comprise a large surface area and encompass high porosity for adsorption. Reflecting on the ever-increasing stringent standards being placed on metal concentrations discharged from WWTPs due to high toxicity, aerobic granulation technology is one that is capable of meeting such requirements in an efficient manner.

3.5.4 Hydrodynamic Shear Force

It has been found that high shear force enhances the formation of aerobic granules and granule stability (Liu & Tay, 2004). A higher hydrodynamic shear force provides the composition of a more regular, spherical and compact granule. Observations have implied that the structure of aerobic granules is, to a great extent, determined by the

hydrodynamic shear force within a bioreactor. It is acknowledged that EPS affects the cohesion and adhesion of cells as well as plays an important role in maintaining the structural integrity of immobilised cells. Liu & Tay, 2004 report that the production of EPS is largely correlated to the shear force, where the stability of aerobic granules is significantly associated with the production of EPS.

3.5.5 Cell Surface Hydrophobicity

Cell surface hydrophobicity is an important force in cell self-immobilisation and attachment processes (Liu & Tay, 2004, p.542). The link between cell surface hydrophobicity and the formation of aerobic granulation is not yet clearly understood. However, a connection between the formation of heterotrophic and nitrifying granules to the cell surface hydrophobicity has been established (Liu & Tay, 2004). It can be found that the cell hydrophobicity of a granule is twofold greater than that of a biofloc. Inducing a high shear force or hydraulic selection pressure upon the microorganisms seemed to project a notable increase in cell surface hydrophobicity. In contrast, the cell surface hydrophobicity seemed unaffected by changes in organic concentrations or loading rates ranging between 500 to 3000 mgCOD/L (Liu & Tay, 2004, p.542). A selection of environmental factors has been found to influence cell surface hydrophobicity, which include starvation, oxygen level, selection pressure and the ionic strength of the medium (Liu & Tay, 2004).

3.5.6 Feast/Famine Regime

Aerobic granular sludge is partial to slow-growing organisms in order to establish stable, dense and smooth granules. The feast-famine regime is suggested to promote an environment in which aerobic granules may develop healthily (de Kreuk, 2006, p.16). The feast phase within a cycle corresponds to the abundant availability of readily biodegradable substrate that will be stored by the microorganisms. An aerobic feast promotes the growth of heterotrophic organisms. When the feast phase is anaerobic, phosphate-accumulating organisms (PAO) and glycogen-accumulating organisms (GAO) are cultivated. In either case, the stored substrates are utilised during the famine phase in order to develop and sustain granules. Optimal nitrogen removal occurs when there is an adequate balance between aerobic and anoxic volume during the aeration period; this may be visualised using Figure 3.3. In addition, it has been observed that the starvation period has a profound effect on cell hydrophobicity, which is an essential component affecting aerobic granulation (Liu & Tay, 2004). It was found that cell hydrophobicity is proportionally related to the starvation time in a SBR (Liu & Tay, 2004, p.539). Nevertheless, it is noteworthy to appreciate that the impact of the feast-famine regime on the granulation process is not yet well understood and requires further investigation for clarification of its effects (Adav et al., 2008, p.413).

3.5.7 Sludge Volume Index

The sludge volume index (SVI) is a parameter that provides a representation of the compactness and settling velocity of sludge granules. It has been stated that granules with an SVI of about 100 mL/g seem to indicate flocculated sludge (Beun et al., 2002,

p.703). Aerobic sludge granules are expected to be lower compared to conventional sludge flocs (Etienne & Yu-Tung, 2012, p.444).

3.6 The Aerobic Granulation Industry

3.6.1 Nereda Technology

The Nereda technology is a concept based on aerobic granular sludge developed by Royal Haskoning DHV, Delft University of Technology, the Dutch Foundation for Applied Water Research (STOWA), and six Waterboards, supported by the Ministry of Economical Affairs. It is essentially an intensive applied research program aiming to achieve a full-scale sustainable and cost-effective alternative to conventional activated sludge systems, Nereda®. Applying an understanding of aerobic granular sludge technology into pilot-scale and full-scale installations are the main agendas of this collaboration. A pilot scale program looks into granulation of several types of municipal wastewater and the influence of dosing a carbon source (acetate), stability of granulated sludge over long-term periods, extent of stable nitrogen and phosphorus removal under practical process conditions and low effluent suspended solids concentration. The Nereda technology utilises the following process conditions that have been regarded as important for aerobic granulation, (de Bruin & van Loosdrecht, 2010):

- *Hydraulic selection pressure* – that favours the retainment of biomass with excellent settling properties, while washing out poor settling biomass.
- *Initial high substrate concentration* – to incorporate high gradients within the granules.
- *Conversion of easily biodegradable substrate into slowly biodegradable intermediate products* - to stimulate the growth of slow growing organisms.
- *High shear force* – to stimulate the growth of smooth and dense granules.

Hence, Nereda has been designed with the following cycle operation, as depicted in Figure 3.4.

1. *Fill and Draw phase*: where wastewater is pumped into the reactor, while simultaneously the effluent is drawn.
2. *Aeration phase*: where the biological conversion process takes place. The outer layer of the granules being aerobic as they consist of nitrifying bacteria and the inner core being anoxic as the formed nitrate is denitrified and phosphorus uptake takes place.

3. *Sedimentation phase*: required for the separation of clear treated effluent and sludge. This time is short due to the excellent settling ability of the aerobic sludge.

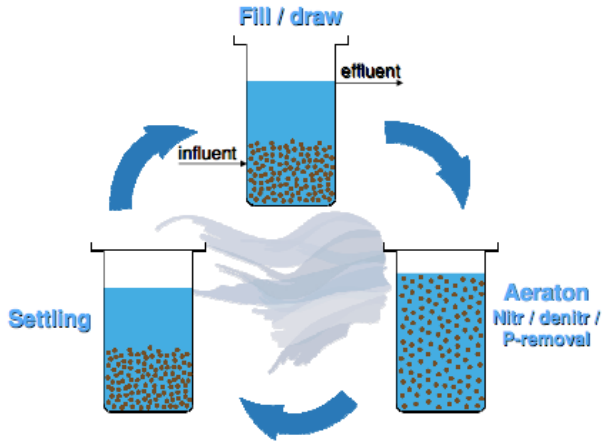


Figure 3.4 **Nereda cycle operation (cited in de Bruin & van Loosdrecht, 2010).**

Since 2003, the Nereda technology has been implemented in five pilot-scale locations in the Netherlands; at Ede, Aalsmeer, Epe, Hoensbroek and Dinxperlo. Several full-scale industrial installations have also been implemented in the Netherlands since 2005. An operational philosophy has been established for growing and nurturing aerobic granular sludge. It preaches a combination of the following to establish desirable conditions: biological phosphorus removal, high sludge loading, higher oxygen concentrations and a gradual increase in hydraulic selection pressure (de Bruin & van Loosdrecht, 2010). After extensive research at the pilot- and full-scale plants between 2003 and 2010, results have proven extremely successful and the technology is expected to expand. Construction of several more full-scale plants in the Netherlands is in planning as well as operating a Nereda-plant in South Africa.

3.6.2 Upcoming Market

Industrial companies are establishing modifications to their standards of nutrient release in the effluent they produce in consideration of the environment and its vulnerability to pollution. Major brand companies have a special role to play in this advancement as they offer the greatest impact in demonstrating change within the current standard of regulations. The World Bank has offered insight into the extent various industries have contributed to water pollution over the past twenty-eight years and is illustrated in Figure 3.5. As demonstrated by the purple line, the total average of all the industries combined depict an overall anchored position at just under 13%. As mentioned previously, BOD is the main indictor of organic pollution that can be oxidised biologically in wastewaters. COD is also a popular indictor of organic pollution, however, it considers contaminants that are unable to be oxidised biologically. The 13% of total BOD emissions corresponding to the average of all industrial wastewaters generated is a substantial amount that certainly requires

reduction. (Furhman, 2012) points out the increasing demands on wastewater treatment facilities and the battle of meeting new regulations. She also draws attention to the fact that each year regulations are becoming more and more stringent, making way for new technologies to provide efficient and sustainable solutions (Furhman, 2011).

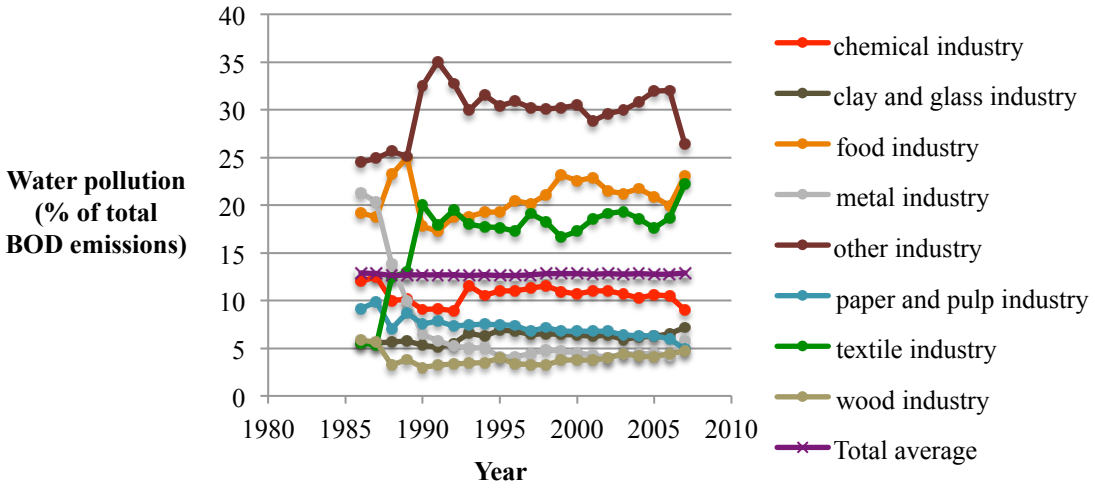


Figure 3.5 **Water pollution correlated to various industries (The World Bank, 2014).**

4 Methodology

4.1 The Aerobic Granulation Industry

In competition with the Nereda technology, wastewater treatment companies are investing in the development of novel designs that may offer an alternative set-up for aerobic granulation to take place. This sub-section shall recount the design, operation and analytical methods of two trial SBR reactors developed by a company who wish to conceal their identity. Although the designs proved unsuccessful, the SBR models described below hold potential for further development in attaining effective results.

4.1.1 Reactor Model One

4.1.1.1 Reactor Design

Reactor model one was a lab-scale attempt at rearranging the traditional configuration of an SBR, with the addition of a new component, to replicate and possibly enhance the formation of aerobic granular sludge. Figure 4.1 illustrates the setup of the model.

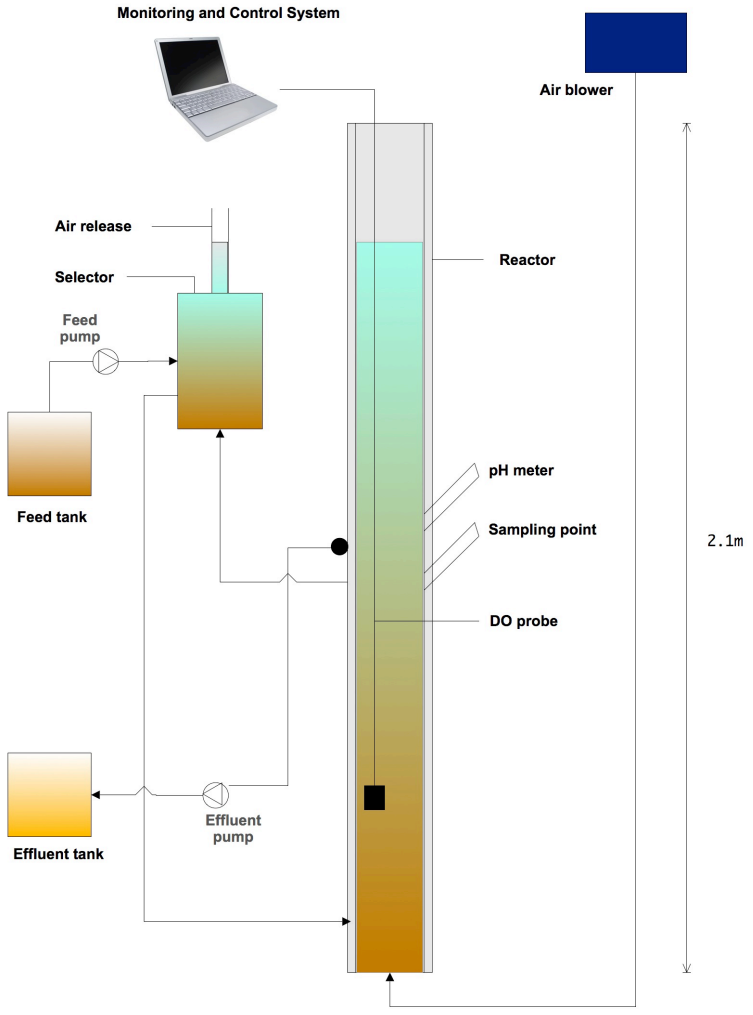
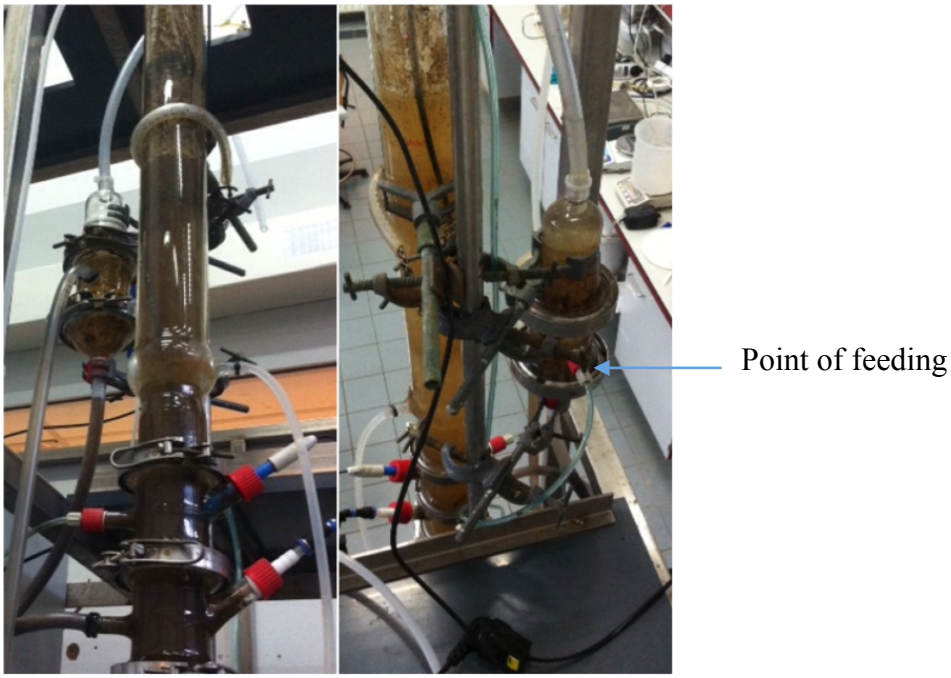


Figure 4.1 **Diagram of reactor model one SBR configuration.**

The addition of the selector, attached to the side of the main SBR, aimed to create anoxic conditions in which the granules would achieve optimal denitrification. The selector was also the point of feeding, as shown in Figure 4.2(b), and was implemented to recycle the reactor contents.



(a) (b)

Figure 4.2 **The selector (a) front view, (b) rear view.**

The main SBR was made of glass and had an internal diameter of 5.6 cm with a total height of 2.1 m. The selector, also made of glass, comprised a diameter of 6.3 cm and a height of 22 cm, where it was attached to the main SBR at a height of 1.3 m from the base. The feed consisted of diluted cola that incorporated a blend of chemicals to replicate the wastewater produced by soft-drink production industries. Table 4.1 presents the mix of chemicals incorporated into one batch of 5L feed. The cola used in the experiment was a low-cost store brand product. It is useful to note that initially the feed recipe called for the use of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) instead of magnesium chloride and it was found that this reacted with the calcium, in the calcium chloride, to form precipitates. Precipitates are undesirable for feeding and heating of the $MgSO_4.7H_2O$ solution did not encourage increased solubility, as would be expected, and so $MgCl_2.6H_2O$ was substituted in its place. Air was supplied to the bottom of the reactor by means of an air blower that was attached to a sparger in order to create fine-sized air bubbles.

Table 4.1 **Reactor model one feed recipe.**

Component	**Amount (L)**
Water	4.68
Cola	0.32
Chemical	**Amount (g)**
Ammonium Chloride (NH_4Cl)	1.52
Calcium Chloride ($CaCl_2.2H_2O$)	1.83
Magnesium Chloride ($MgCl_2.6H_2O$)	2.11
Sodium Phosphate ($HNa_2PO_4.\ 2H_2O$)	1.43
Sodium Bicarbonate ($NaHCO_3$)	3.35

4.1.1.2 Reactor Operation

The cycle scheme for reactor model one consisted of the following regime:

- 8 hour cycle
- 3 cycles per day
- **Feeding** – 4 hours (with aeration on)
- **Aeration** – further 3 hours and 48 minutes
- **Settling** – 5 minutes
- **Decanting** – 7 minutes

The reactor had a working volume of approximately 6L and a water bath maintained the SBR temperature at 36°C. The influent flowrate was 1.5 L/d and a COD concentration of 2 g/L was sustained.

4.1.1.3 Analytical Methods

The investigation prompted laboratory analyses to be undertaken for measurement of the relevant parameters that contribute to granulation of aerobic sludge.

4.1.1.3.1 Chemical Oxygen Demand

The chemical oxygen demand, COD, was carried out using a spectrophotometer with accordance to Standard Methods (APHA, 1998).

4.1.1.3.2 Ammonium Nitrogen

The ammonium nitrogen, NH_4-N, was carried out using a spectrophotometer with accordance to Standard Methods (APHA, 1998).

4.1.1.3.3 Mixed Liquor Suspended Solids

Mixed liquor suspended solids, MLSS, is a measure of the biomass concentration within the reactor. Initially, a sample of the reactor contents was extracted via the sampling point, as depicted in Figure 4.1. A week into the running of the reactor, the sampling method was adjusted to using a syringe attached to a long tube. This contraption was inserted at the top of the reactor and extended near to the bottom in order to attain a more accurate measurement of the reactor biomass concentration. The following procedures for total suspended solids, TSS, and volatile suspended solids, VSS, were carried out according to Standard Methods (APHA, 1998).

4.1.1.3.4 Dissolved Oxygen

The dissolved oxygen, DO, was monitored using a respirometry software that logged DO measurements at one second intervals. Limits were programmed into the software, DO levels between 2 and 5.5 mgO_2/L, in order to control the amount of oxygen the microorganisms were being exposed to. These limits authorised the air blower to only be switched on while the DO probe measured readings that were within 2 and 5.5 mgO_2/L.

4.1.1.3.5 Oxygen Uptake Rate

In theory, oxygen uptake rate, OUR, is classified as the gradient of the DO versus time plot. In the case of reactor model one, the DO probe also enabled the production of OUR logs through the respirometry software.

4.1.1.3.6 pH

pH was logged by means of the pH probe installed within the reactor, as depicted in Figure 4.1, as well as a portable pH probe inserted closer to the bottom of the reactor. The more accurate reading was given by the portable pH probe and this is the ultimate method that was used to record the pH.

4.1.1.3.7 Temperature

The temperature was measured by means of the DO probe, in which the respirometry program enabled temperature readings.

4.1.1.3.8 Solids and Suspended Solids

Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) of the influent and effluent were measured according to the Standard Methods (APHA, 1998).

4.1.2 Reactor Model Two

Alongside the design of reactor model one, another team collaborated in fabricating a contrasting lab-scale prototype that would enable the production of aerobic granules. This model and its operation shall, in essence, be summarised in the following text.

4.1.2.1 Reactor Design

Reactor model two was another attempt at rearranging the traditional SBR configuration and in this trial two parallel SBR reactors were setup to assess the reliability of the design; and to possibly run different operational features simultaneously in the future. Figure 4.3 depicts the basic features of reactor model two.

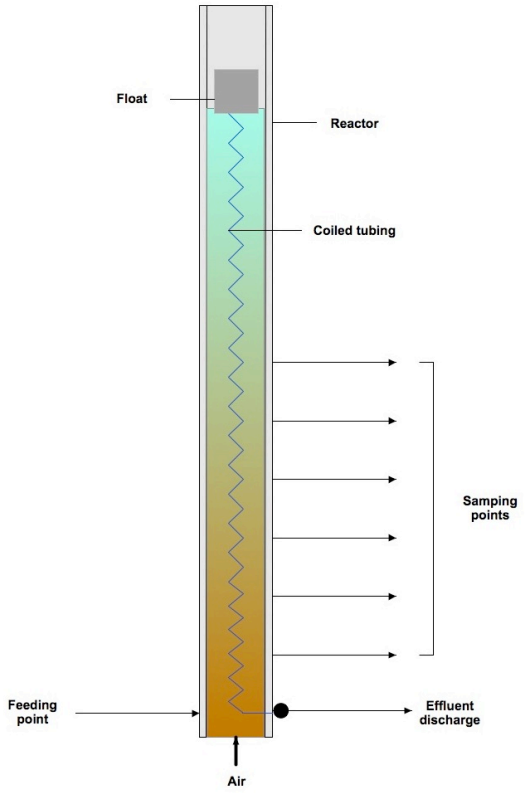


Figure 4.3. **Diagram of reactor model two SBR configuration.**

The float is made out of an empty plastic bottle, in the lab-scale model, and is attached to a coiled tube that withdraws clear effluent from the top of the liquid level, transports it through the tube and discharges it into an effluent tank. The coiled tube contracts and compresses corresponding to feeding and decanting, respectively. This contraption was implemented to provide an experimental concept to extract treated effluent from the system. The float is attached at the top-end of the coiled tube in order to keep the suction point as close as possible to the liquid surface. This is to ensure only clear effluent is withdrawn. Figure 4.4 further depicts the contraption of the float and coiled tube. The sampling points have a spacing of 6 cm between each other, where the lowest sampling point is 2 cm above the base. Air is supplied to the

bottom of reactor by means of an air blower that maintained an air flowrate of 8 L/min and a superficial air velocity (SAV) of 1.7 cm/s.

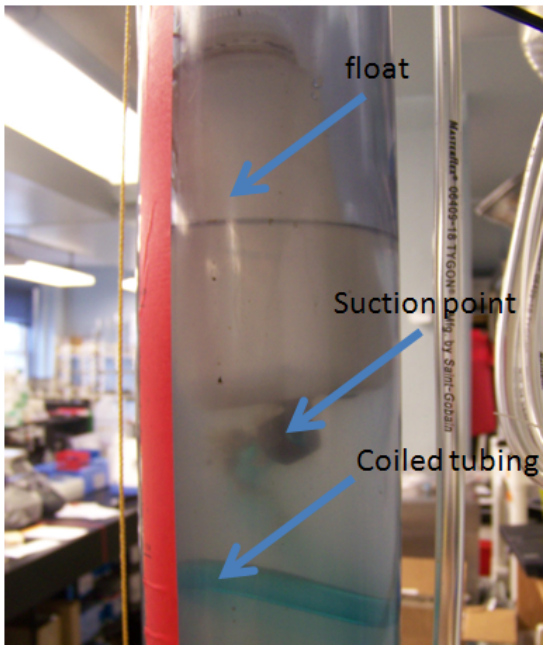


Figure 4.4 **Float and coiled tube contraption.**

The SBR columns in this case were made of plastic and comprised an inner diameter of 10 cm with a height of 2 m. The influent consisted of diluted cola, as in reactor model one, however, concentrated cola syrup was used rather than store bought cola in order to account for storage. One batch of 41.4L of feed, split between the two parallel columns, was prepared using the following recipe, as depicted in Table 4.2.

Table 4.2 **Reactor model two feed recipe.**

Component	**Amount (L)**
Water	0.086
Cola Syrup	41.31
Chemical	**Amount (g)**
Ammonium Chloride (NH_4Cl)	12.66
Calcium Chloride ($CaCl_2$)	11.49
Magnesium Sulfate ($MgSO_4.7H_2O$)	21.22
Dipotassium Phsphate (K_2HPO_4)	11.62
Sodium Bicarbonate ($NaHCO_3$)	27.82

4.1.2.2 Reactor Operation

The cycle scheme for reactor model two consisted of the following regime:

- 8 hour cycle
- 3 cycles per day
- **Feeding** – 15 minutes (with aeration off)
- **Anaerobic phase** – 30 minutes
- **Mixing** – 15 minutes
- **Aeration** –6 hours and 55 minutes
- **Settling** – 1.5 minutes
- **Decanting** – 3.5 minutes

The reactor had a working volume of approximately 14.5L and the SBR temperature was maintained at 23°C. An exchange ratio of 50% was set and a COD concentration of 6 g/L was sustained.

4.1.2.3 Analytical Methods

4.1.2.3.1 Chemical Oxygen Demand

The chemical oxygen demand, COD, was carried out using a spectrophotometer with accordance to Standard Methods (APHA, 1998).

4.1.2.3.2 Mixed Liquor Suspended Solids

The total suspended solids, TSS, and volatile suspended solids, VSS, procedures for the mixed liquor were carried out according to Standard Methods (APHA, 1998).

4.1.2.3.3 Dissolved Oxygen

The dissolved oxygen, DO, was monitored using a respirometry software that logged DO measurements at one second intervals. Limits were programmed into the software, DO levels between 2 and 5.5 mgO_2/L, in order to control the amount of oxygen the microorganisms were being exposed to. These limits authorised the air blower to only be switched on while the DO probe measured readings that were within 2 and 5.5 mgO_2/L.

4.1.2.3.4 Oxygen Uptake Rate

The oxygen uptake rate, OUR, was calculated by the respirometry software enabled by the DO probe.

4.1.2.3.5 pH

The pH was logged by means of a portable pH probe that extended into the dense mixed liquor.

4.1.2.3.6 Temperature

The temperature was measured by means of the DO probe, in which the respirometry program enabled temperature readings.

4.1.2.3.7 Sludge Volume Index

The sludge volume index, SVI, was carried out using a one litre graduated cylinder in which biomass was poured and the sludge height was noted each five minute interval until 30 minutes passed.

4.2 Chalmers Laboratory-Scale Experiment

Shifting focus to the proficiency of a conventional SBR set-up, the effects of varying nitrogen loads on aerobic sludge granulation will be of significant interest within the following chapter. This section shall outline the reactor configuration, operation and analytical methods used for investigating the effect different nitrogen loads have on the running of an aerobic granular sludge SBR. This experiment was carried out within the Water Environment Technology (WET) laboratory at Chalmers University of Technology.

4.2.1 Reactor Design

The Chalmers lab-scale model comprised of three parallel SBR columns that examined the treatment of domestic wastewater with varying nitrogen loads. Table 4.3 presents the COD:N ratios within each reactor as well as the corresponding COD and nitrogen loads. The main design features of the reactors are illustrated in Figure 4.5.

Table 4.3 **Chalmers SBR loading configuration.**

	R1	**R2**	**R3**
COD load (g/L.d)	5	5	5
NH4-N load (g/L.d)	0.25	0.5	1.0
COD:N ratio	100:5	100:10	100:20

The three parallel reactors R1, R2 and R3 are identical in their design features. A 1.5 m tall plastic SBR column with an inner diameter of 6 cm treated a wastewater comprising reject water, synthetic wastewater and acetate buffer solution. Each component of the wastewater in this scenario was added to replicate the mainstream domestic wastewater intake at the Gryaab WWTP. The acetate buffer solution recipe, depicted in Table 4.4, provides the main carbon source within the wastewater to be treated. The reject water, collected from the Gryaab WWTP, was diluted six fold and is the main nitrogen source within the wastewater to be treated. The synthetic wastewater was fabricated using a blend of chemicals, of which the recipes are presented in Table 4.5. Equal amounts of the reject and synthetic wastewaters were

used within each reactor; only the acetate feed was altered regarding the amount and blend of chemicals. It is important to note that plug-flow conditions were observed within the reactor, where the majority of feed was concentrated near the base of the column. This established the sludge at the bottom to receive most contact with the influent.

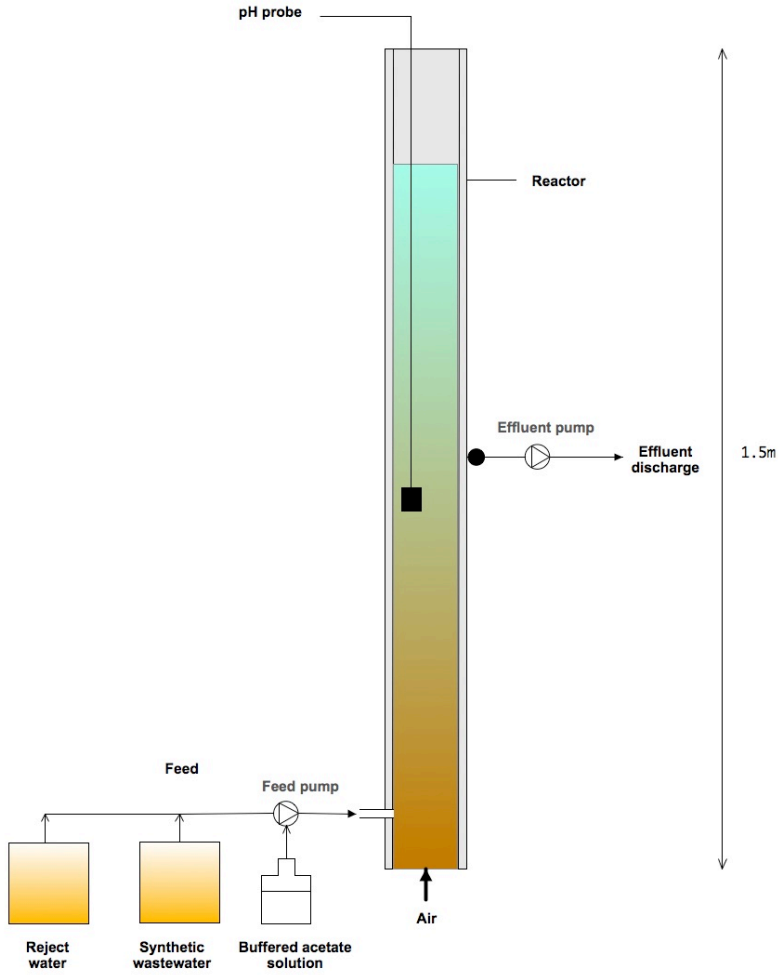


Figure 4.5 **Chalmers reactor configuration.**

The acetate feed recipe is depicted below, in Table 4.4. It comprises a blend of ammonium acetate (CH_3COONH_4) and sodium acetate (CH_3COONa) with each reactor owning different proportions.

Table 4.4 **Acetate feed recipe.**

Final volume of 2 L	**R1**	**R2**	**R3**
CH_3COONH_4 (g)	0	47	140.9
CH_3COONa (g)	150	100	0

The synthetic wastewater recipe is illustrated below, in Table 4.5. It includes a concoction of dipotassium hydrogen phosphate (K_2HPO_4), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4.7H_2O$), iron sulfate heptahydrate ($FeSO_4.7H_2O$) and micronutrients.

Table 4.5 **Synthetic wastewater feed recipe.**

Final volume of 30 L	Amount (mL)
K_2HPO_4	43.6
$CaCl_2$	22.6
$MgSO_4.7H_2O$	25.0
$FeSO_4.7H_2O$	25.0
Micronutrients	30.0

The constituents of the micronutrients are presented below in Table 4.6 and are based on a similar recipe attempted by (Tay et al., 2001).

Table 4.6 **Micronutrient recipe.**

Final volume of 1 L	Amount (mL)
H_3BO_3 stock	1.5
$ZnCl_2$ stock	1.0
$CuCl_2 \times 2H_2O$ stock	1.0
$MnSO_4 \times H_2O$ stock	1.0
$(NH_4)_6Mo_7O_{24} \times 4H_2O$ stock	1.0
$AlCl_3$ stock	1.0
$CoCl_2 \times 6H_2O$ stock	1.0
$NiCl_2$ stock	1.0

4.2.2 Reactor Operation

The cycle scheme for the Chalmers reactor model consisted of the following regime:

- 4 hour cycle
- 6 cycles per day

- **Feeding** – 5 minutes
- **Anaerobic phase** – 55 minutes
- **Aerobic phase** – 2 hours and 51 minutes
- **Settling** – 2 minutes
- **Decanting** – 5 minutes

The reactor had a working volume of approximately 3.11L and remained at a room temperature of around 24°C. The influent flowrate was 9 L/d and a COD concentration of 5 g/L was sustained.

4.2.3 Analytical Methods

4.2.3.1.1 Total Organic Carbon

A Total Organic Carbon Analyzer TOC-V_{CPH} machine connected to an ASI-V carousel unit was used to measure TOC, TC and IC for wastewater and effluent sampled. An additional Shimadzu Total Nitrogen Measuring Unit was used to obtain total nitrogen (TN) measurements.

4.2.3.1.2 Ion Chromatography

A Dionex ICS Series machine with an AS-DV carousel unit and two DCR ICS-900 units for Anode and Cathode was used to carry out tests to obtain NH_4-N, NO_2-N and NO_3-N concentrations within wastewater and effluent samples.

4.2.3.1.3 Mixed Liquor Suspended Solids

The total suspended solids, TSS, and volatile suspended solids, VSS, procedures for the mixed liquor were carried out according to Standard Methods (APHA, 1998).

4.2.3.1.4 pH

The pH was logged by means of a portable pH probe that extended into the dense mixed liquor. The acquisition software PicoLog was used to acquire the pH data in order to export and analyse the data.

4.2.3.1.5 Temperature

The temperature of the reactor contents was based on a room thermometer as no heating was supplied to the reactor and it was assumed that the reactor contents remained at room temperature.

4.2.3.1.6 Sludge Volume Index

The SVI was calculated using the following equation:

$$SVI = \frac{Sludge\ Volume\ (mL/L)}{Suspended\ Solids\ (mg/L)} \quad (4.1)$$

The sludge volume was attained by knowing the sludge height, which was recorded daily, and the suspended solids, in this case, corresponded to the MLSS within the reactor.

4.2.3.1.7 Relative Hydrophobicity

A comparatively modified alternative to the Rosenberg method, (Rosenberg et al., 1980), was used to calculate the relative cell-surface hydrophobicity of the granules within the reactors. The method suggests the following:

1. Extract a 50 mL sample of the granules/flocs.
2. Centrifuge for five minutes at 4000 rpm.
3. Separate the supernatant and add 1xPBS (Phosphate Buffer Solution).
4. Centrifuge once more, separate the supernatant and add another dose of 1xPBS. Resuspend the contents.
5. Homogenise the sample using a sonicator at level 18 for two minutes.
6. Dilute the sample with 1xPBS to an absorbance between 0.6 to 1.2 (about 400 nm of 1xPBS). Measure the initial absorbance.
7. Mix 6 mL of the diluted solution with 1 mL of n-hexadecane.
8. Mix on a rotamixer for two minutes and allow to stand for 15 minutes after mixing.
9. Carefully remove 3 mL of the water layer, beneath the top n-hexadecane layer, using a pipette and measure the absorbance (after emulsification) using a spectrophotometer. In this case, a Shimadzu UV-1800 UV spectrophotometer was used.
10. Calculate the relative hydrophobicity using the following equation:

$$RH\ (\%) = 1 - \frac{ABS\ (e)}{ABS\ (i)} \times 100 \quad (4.2)$$

where RH is the relative hydrophobicity

$ABS\ (i)$ is the initial absorbance

$ABS\ (e)$ is the absorbance after emulsification

4.2.3.1.8 Settling Velocity

The settling velocity of the granules was obtained using a 250 mL cylinder filled with room-temperature water in which the selected granule was dropped into and the time for it to reach a certain distance recorded. The settling velocity was calculated using Equation 4.3.

$$Settling\ Velocity\ (m/h) = \frac{Distance}{Time} \tag{4.3}$$

4.2.3.1.9 AOB, NOB and Heterotrophic Activity

Ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB) are autotrophic organisms that encourage nitrification to take place within the biomass. For the purpose of confirming the presence or absence of nitrification within the reactors a method of measuring the OUR was carried out by means of inserting a DO probe into reactor samples and attaining the DO vs. Time plot; where the OUR is the gradient of the trend line. Allylthiourea (ATU) and sodium chlorate ($NaClO_3$) inhibitors were added to the feed samples to provide DO curves that were then used to calculate the activity of AOBs and NOBs, refer to Equations 4.4 and 4.5. The heterotrophic activity was calculated suing Equation 4.6. The ATU inhibitor provides an indication of the heterotrophic content within a sample whereby inhibiting AOBs as well as NOBs. The $NaClO_3$ is primarily a NOB inhibitor. In addition, DO tests were carried out on feed samples acidified with hydrochloric acid (HCl) in order to lower the pH level. This was in response to the observed high pH within the reactors during the experiment. The method was carried out in triplicates, hence, the final activity of the AOB, NOB and heterotrophs were an average of these values.

$$NOB\ (\%) = \frac{OUR_i - OUR_{NaClO_3}}{OUR_i} \times 100 \tag{4.4}$$

$$AOB\ (\%) = \frac{(OUR_{NaClO_3} - OUR_{ATU})}{OUR_i} \times 100 \tag{4.5}$$

$$Heterotrophs\ (\%) = \frac{OUR_{ATU}}{OUR_i} \times 100 \tag{4.6}$$

where OUR_i is the OUR of the original feed (or the acidified feed in the case of the acidified test)

OUR_{NaClO3} is the OUR of the feed with the addition of NaClO3.

OUR_{ATU} is the OUR of the feed with the addition of ATU.

5 Results

5.1 The Aerobic Granulation Industry

5.1.1 Reactor Model One

Reactor model one was run for a duration of two and a half weeks in total and, hence, the results presented are largely preliminary. The lack of time to complete a full investigation on reactor model one must be considered when evaluating the results. Furthermore, it indicates the monetary disadvantage companies face regarding the competition between various stakeholders in the industry. There is a high demand for new innovative designs for aerobic granulation technology and time is of the essence to achieve success with regards to the economy and future infrastructure of public sanitation.

5.1.1.1 Mixed Liquor Suspended Solids

The TSS measured within reactor model one is depicted below in Figure 5.1. Note that the jump in biomass concentration is primarily due to the change of sampling method. The sampling syringe attached to a tube was initially near the middle of the reactor where diluted flocs were being extracted rather than the biomass itself. Hence, the syringe and tubing were lowered to obtain a more accurate sample of the reactor biomass content.

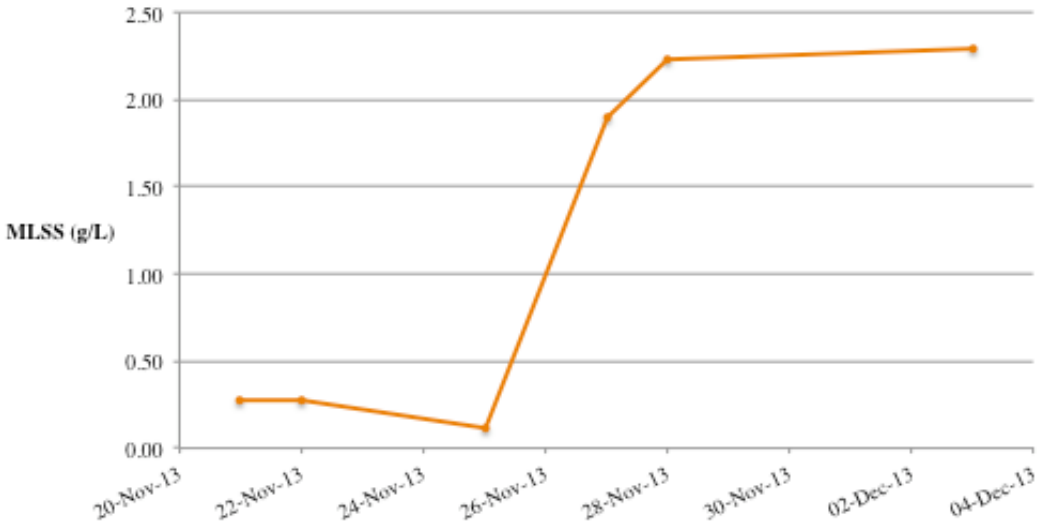


Figure 5.1 **TSS in reactor model one.**

5.1.1.2 Effluent Suspended Solids

The TSS discharged with the effluent from reactor model one is illustrated below in Figure 5.2.

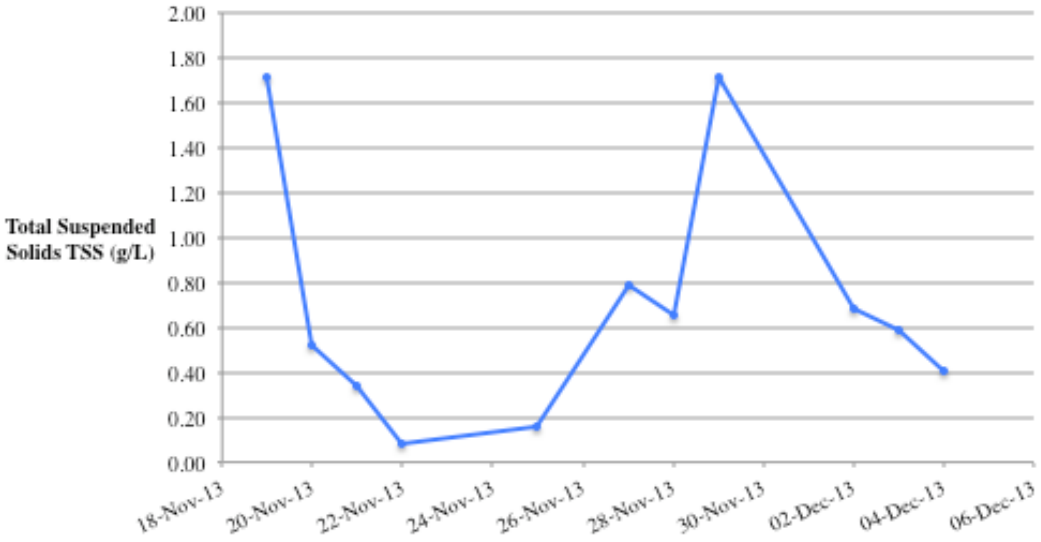


Figure 5.2. **TSS discharged with effluent from reactor model one.**

5.1.1.3 Chemical Oxygen Demand

A comparison between the influent COD and effluent COD in reactor model one is depicted in Figure 5.3.

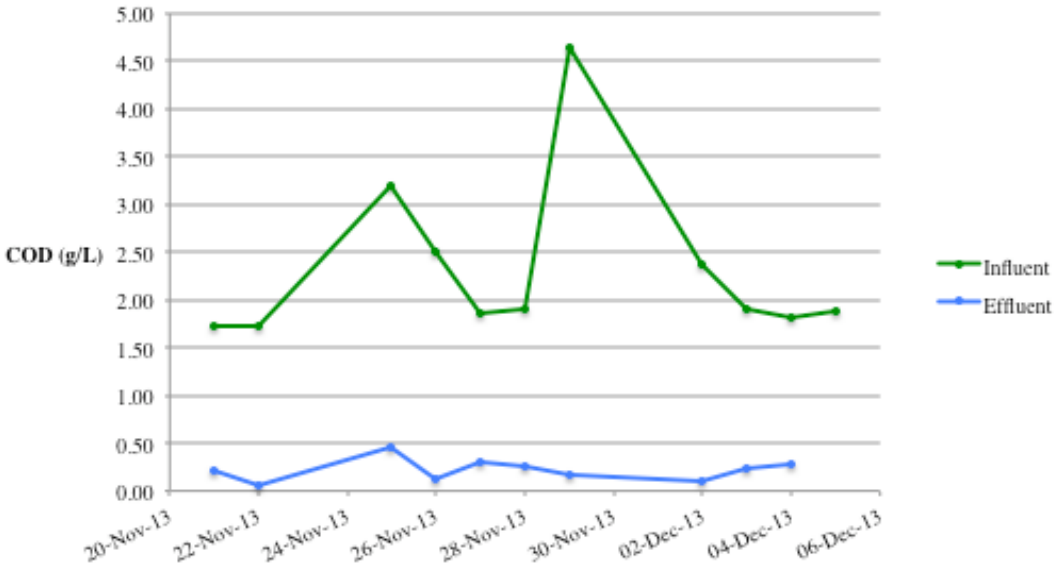


Figure 5.3 **COD influent vs. effluent in reactor model one.**

5.1.1.4 pH

The pH level in reactor model one is presented below in Figure 5.4.

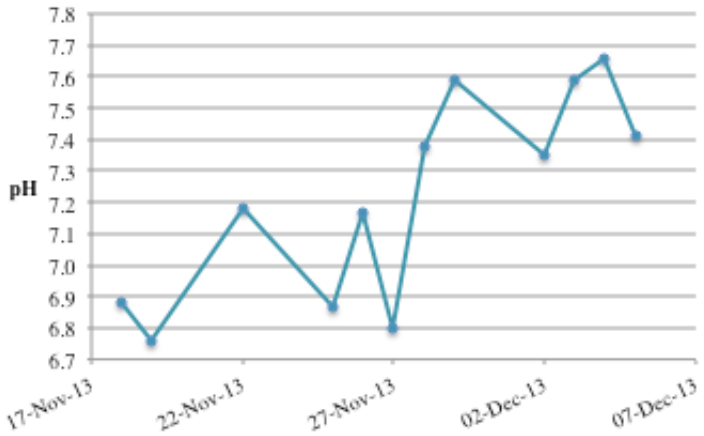


Figure 5.4 **pH in reactor model one.**

5.1.2 Reactor Model Two

Reactor model two, as mentioned previously, was another attempt at rearranging the traditional SBR configuration and was, in fact, carried out by another participating group within the same company. In this trial, two parallel SBR reactors, Train One and Train Two, were setup to assess the reliability of the design and to possibly run different operational features simultaneously in the future. The preliminary results of this investigation are presented in the following section.

5.1.2.1 Train One

5.1.2.1.1 Sludge Volume Index

The SVI for reactor model two train one is depicted below in Figure 5.5. SVI 5, SVI 10, SVI 15, SVI 20 and SVI 30 correspond to the measured SVI in the graduated cylinder after 5, 10, 15, 20 and 30 minutes, respectively.

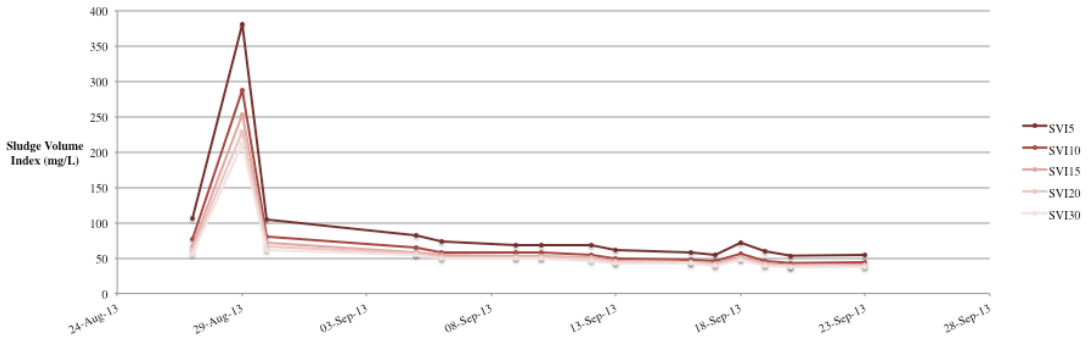


Figure 5.5. **SVI in reactor model two, train one.**

5.1.2.1.2 Mixed Liquor Suspended Solids

The TSS of reactor model two train one is illustrated below in Figure 5.6.

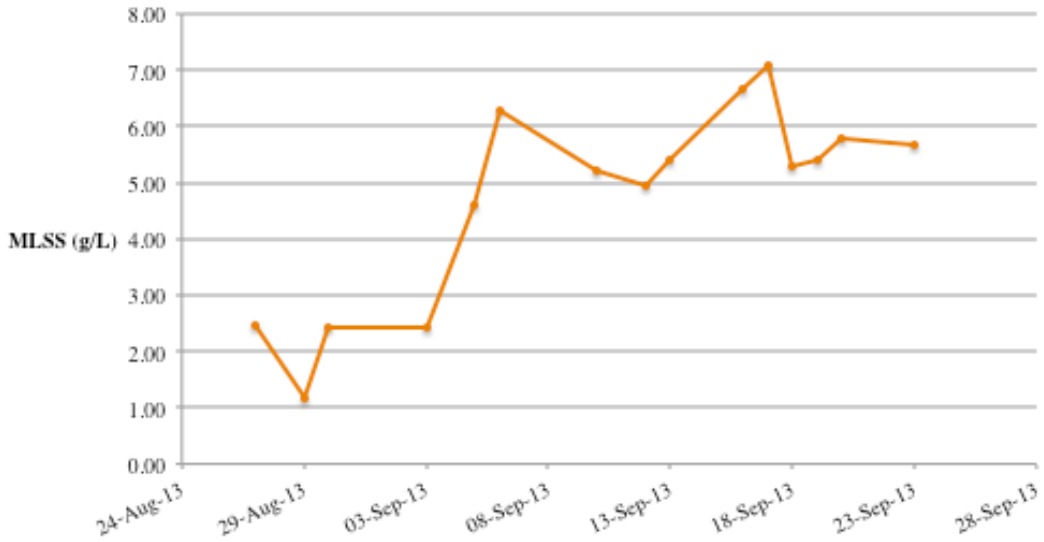


Figure 5.6 **TSS in reactor model two, train one.**

5.1.2.1.3 pH

The pH level in reactor model two train one is depicted below in Figure 5.7.

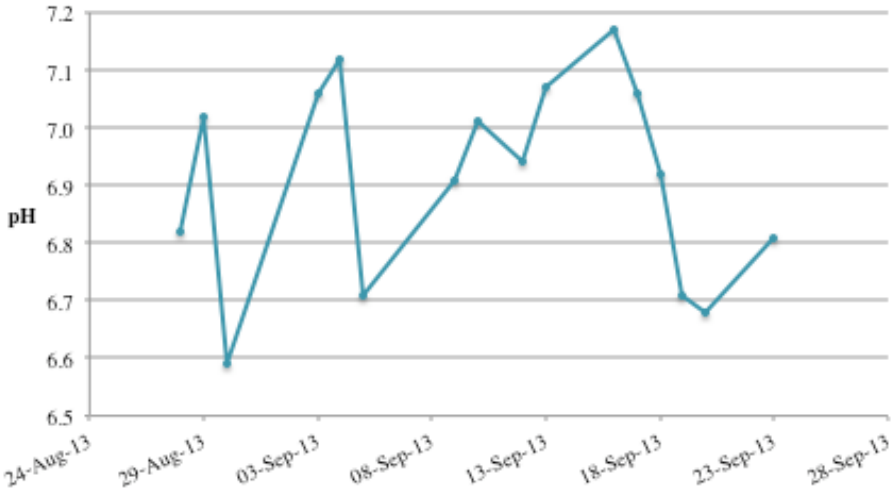


Figure 5.7. **pH in reactor model two, train two.**

5.1.2.2 Train Two

5.1.2.2.1 Sludge Volume Index

The SVI for reactor model two train two is depicted below in Figure 5.8. SVI 5, SVI 10, SVI 15, SVI 20 and SVI 30 correspond to the measured SVI in the graduated cylinder after 5, 10, 15, 20 and 30 minutes, respectively.

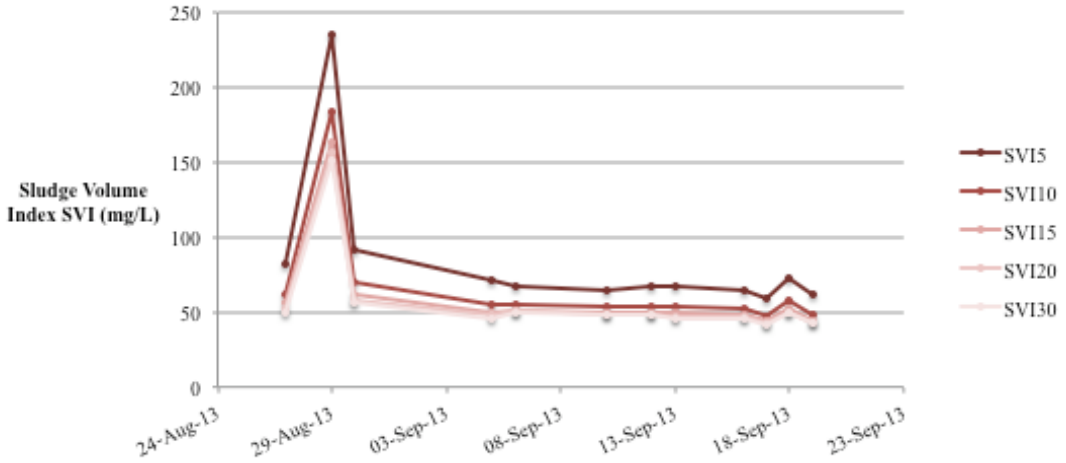


Figure 5.8 **SVI in reactor model two, train two.**

5.1.2.2.2 Mixed Liquor Suspended Solids

The TSS of reactor model two train two is illustrated below in Figure 5.9.

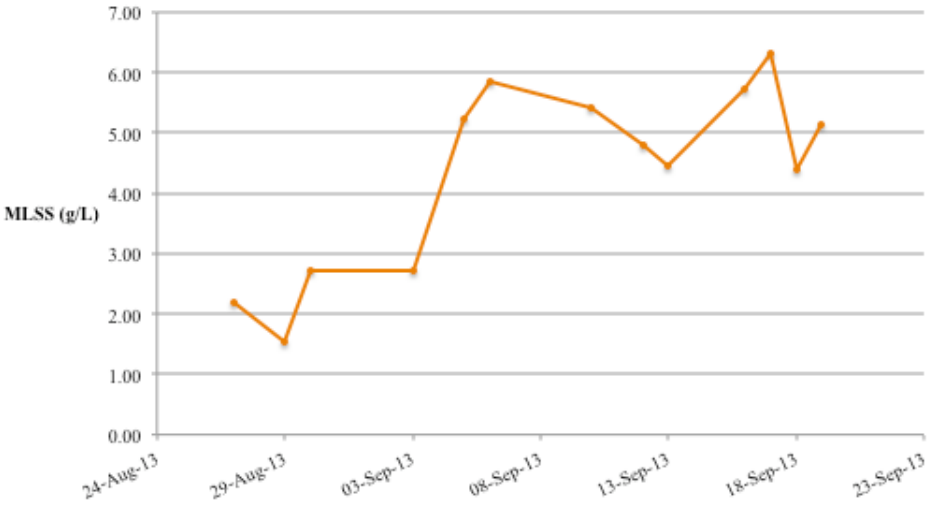


Figure 5.9 **TSS in reactor model two, train two.**

5.1.2.2.3 pH

The pH level in reactor model two train two is depicted below in Figure 5.10.

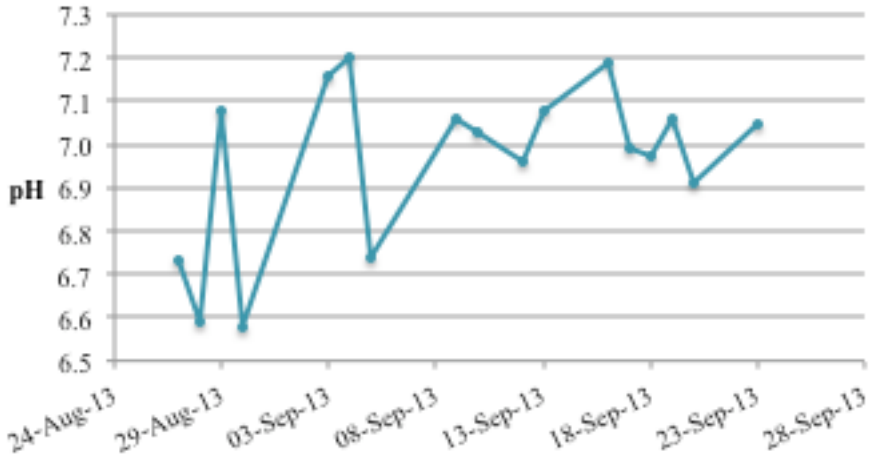


Figure 5.10 **pH in reactor model two, train two.**

5.2 Chalmers Laboratory-Scale Experiment

The results of the lab work carried out for the investigation of the effect various nitrogen loads impart on aerobic granulation is presented in the following section. The findings shall be discussed in the subsequent chapter.

5.2.1 Sludge Height

The sludge height of the three reactors is depicted below in Figure 5.11. The beige text outlines the decrease in settling time during the start-up period of the reactors.

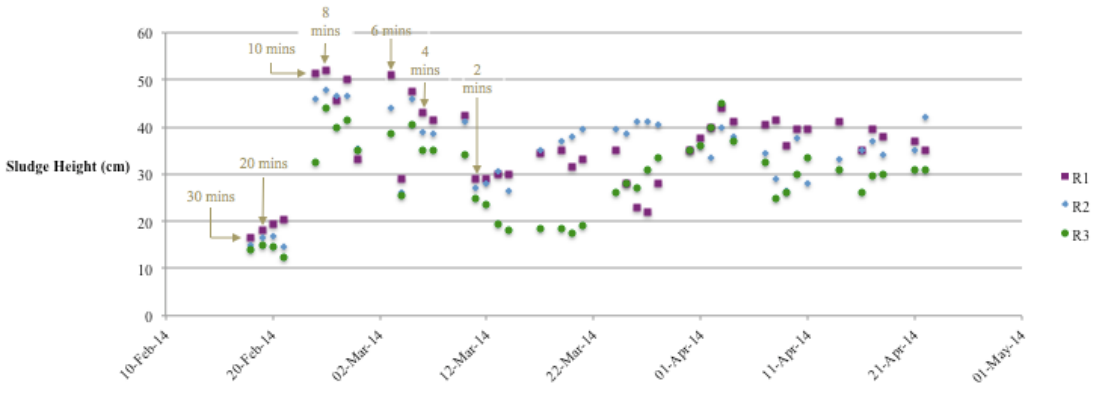


Figure 5.11 **Sludge height in R1, R2 and R3.**

5.2.2 Settling Time

Figure 5.12 depicts the rate of decrease in settling time during the start-up period of the reactors. From 12th March 2014 onwards, the settling time remained at two minutes.

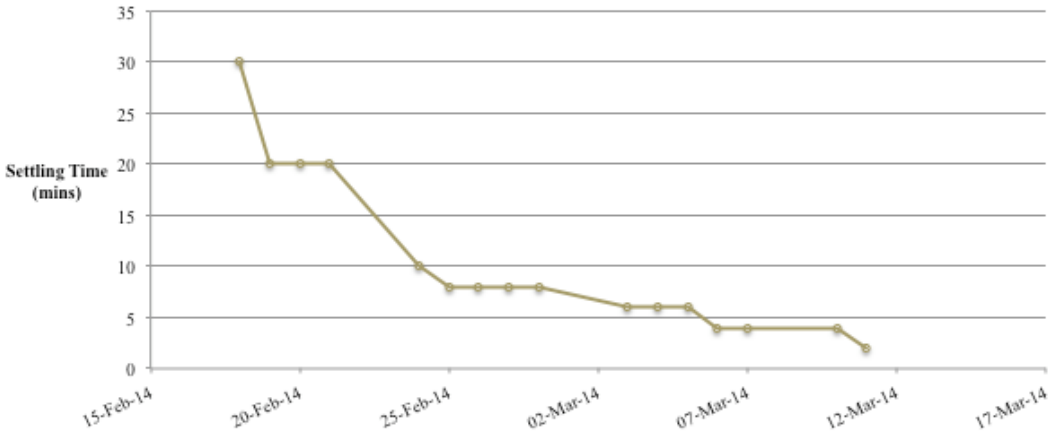


Figure 5.12 **Decrease in settling time during start-up period in R1, R2 and R3.**

5.2.3 Sludge Volume Index

The SVI of all three reactors is illustrated below in Figure 5.13. Note that the two evident peaks on the 12th March and 28th April 2014 indicate plausible anomalies, possibly from errors in measurement method.

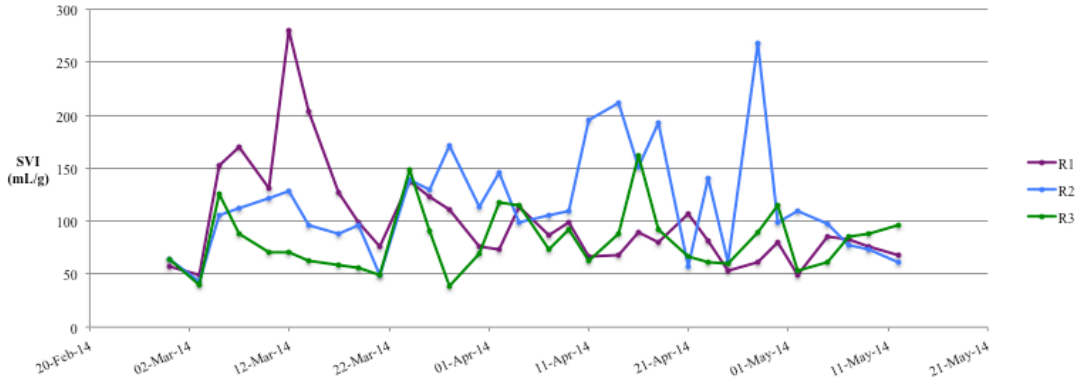


Figure 5.13. **Sludge volume index.**

5.2.4 Mixed Liquor Suspended Solids

The MLSS ("TSS reactor") and MLVSS ("VSS reactor") of R1, R2 and R3 are demonstrated below in Figure 5.14.

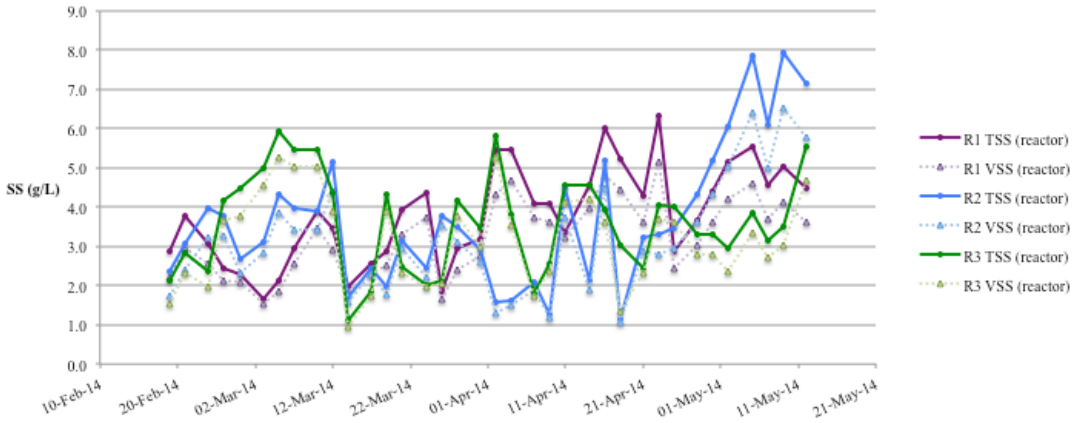


Figure 5.14. **TSS and VSS of reactor mixed liquor.**

5.2.5 Settling Velocity

The settling velocity of R1, R2 and R3 are illustrated below in Figures 5.15, 5.16 and 5.17, respectively. The tests were carried out on 13th May 2014 using samples of granules extracted on the same day.

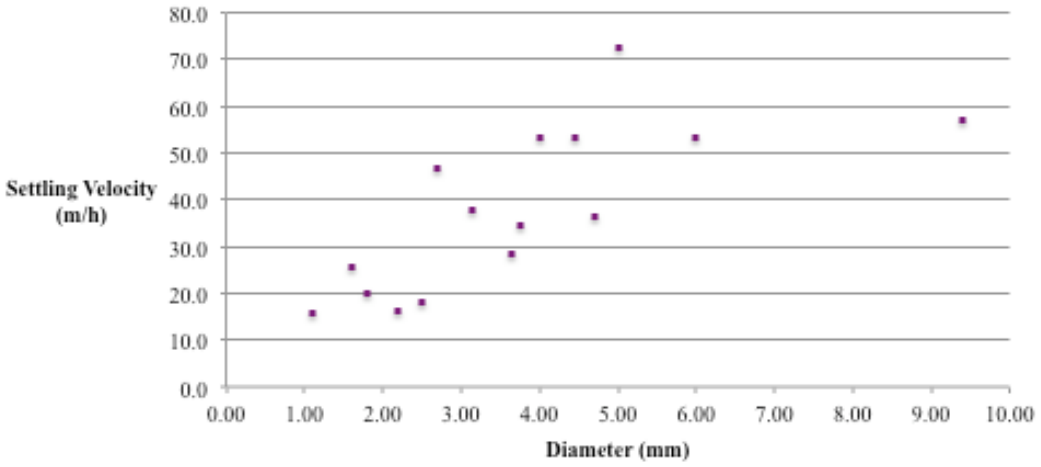


Figure 5.15. **Settling velocity of R1 granules.**

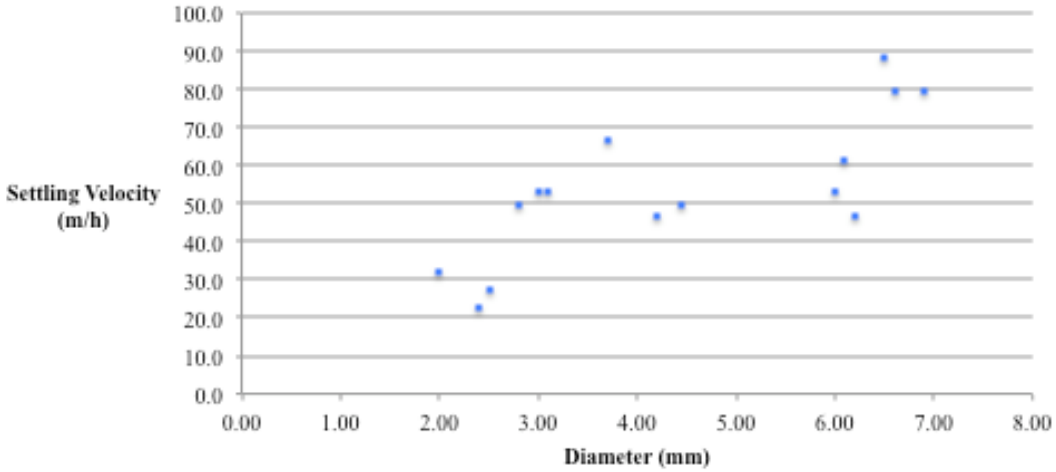


Figure 5.16. **Settling velocity of R2 granules.**

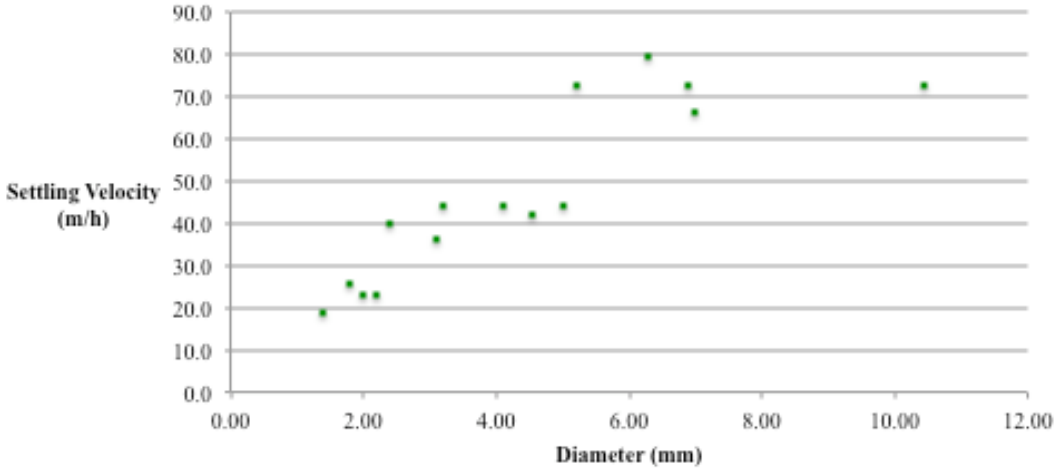


Figure 5.17. **Settling velocity of R3 granules.**

5.2.6 Relative Hydrophobicity

The averaged results of the relative hydrophobicity tests are summarised below in Table 5.1.

Table 5.1. **Average relative hydrophobicity for R1, R2 and R3.**

	Average Relative Hydrophobicity (%)
R1	6.6
R2	14.9
R3	43.3

5.2.7 Effluent Suspended Solids

The TSS and VSS discharged with the effluent from three reactors are illustrated below in Figure 5.18. Note that the peak on the 2nd April 2014 for R1 may be a plausible anomaly due to measurement error.

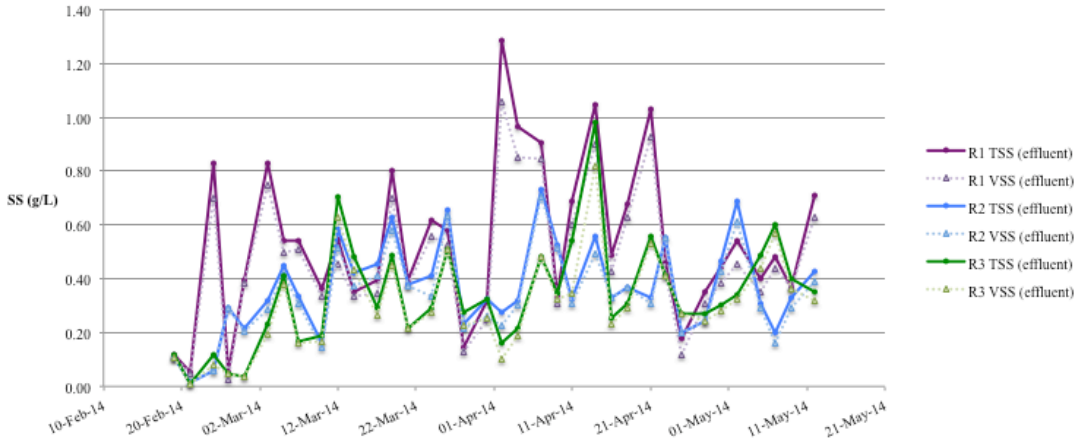


Figure 5.18. **TSS and VSS discharge with effluent.**

5.2.8 Total Organic Carbon

The TOC discharged with the effluent from the three reactors is illustrated below in Figure 5.19. The comparison between influent TOC levels and effluent TOC levels for R1, R2 and R3 are depicted in Figures 5.20, 5.21 and 5.22, respectively. The TOC removal established within all three reactors is then presented in the subsequent sub-section 5.2.8.1.

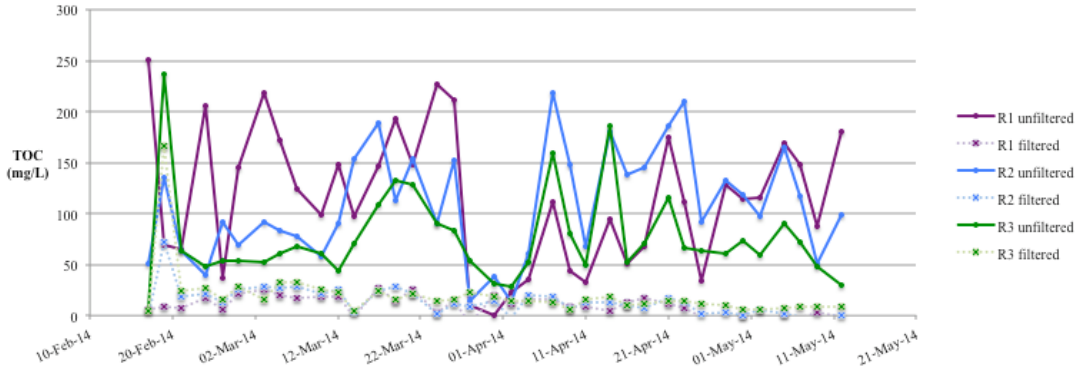


Figure 5.19. **TOC in effluent of R1, R2 and R3.**

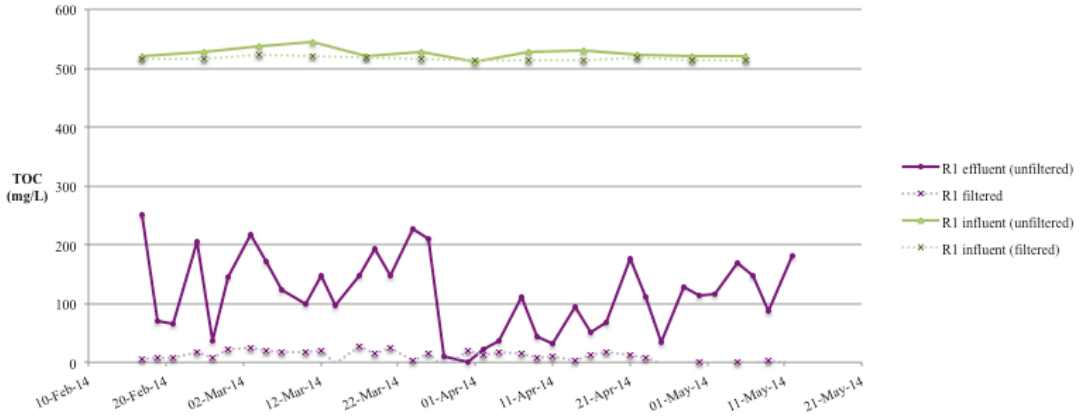


Figure 5.20. **Influent TOC vs. effluent TOC for R1.**

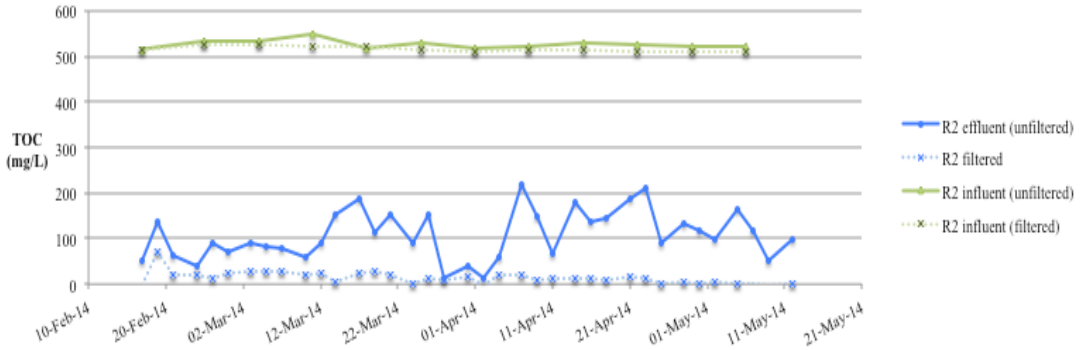


Figure 5.21. **Influent TOC vs. effluent TOC for R2.**

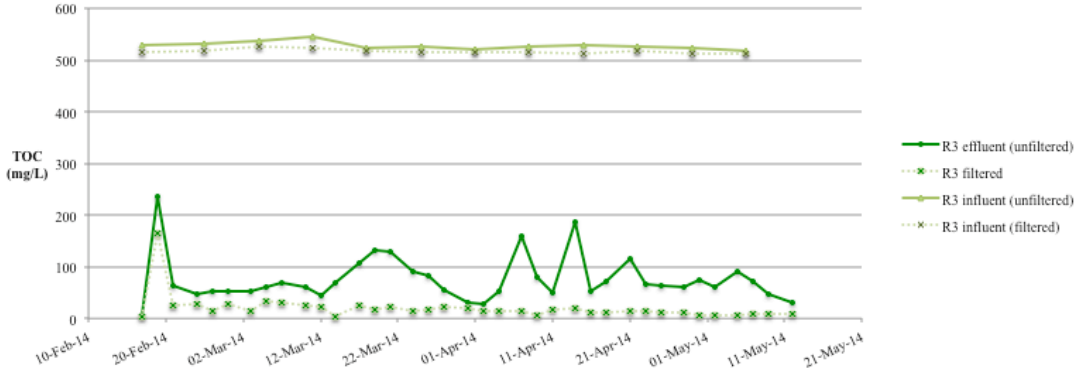


Figure 5.22. **Influent TOC vs. effluent TOC for R3.**

5.2.8.1 Total Organic Carbon Removal

The amount of TOC removed from the three reactors and their corresponding removal efficiency are presented in Table 5.2.

Table 5.2. **TOC removal amount and removal efficiency.**

		TOC Removal Amount (mg/L)	TOC Removal Efficiency (%)
R1	Unfiltered TOC	427.1	81.0
	Filtered TOC	510.9	98.9
R2	Unfiltered TOC	396.7	75.2
	Filtered TOC	508.9	98.5
R3	Unfiltered TOC	450.5	85.2
	Filtered TOC	505.6	97.9

5.2.9 Total Carbon

The TC discharged with the effluent from all three reactors is depicted below in Figure 5.23.

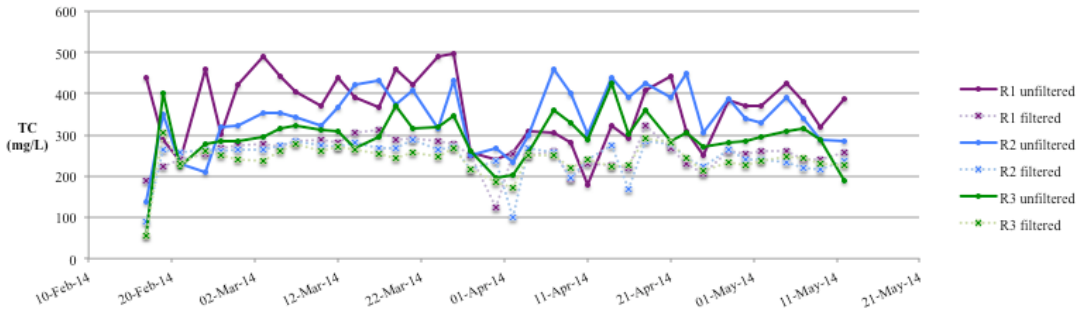


Figure 5.23. **TC discharge with effluent from R1, R2 and R3.**

5.2.10 Total Inorganic Carbon

The IC discharged with the effluent from all three reactors is depicted below in Figure 5.24.

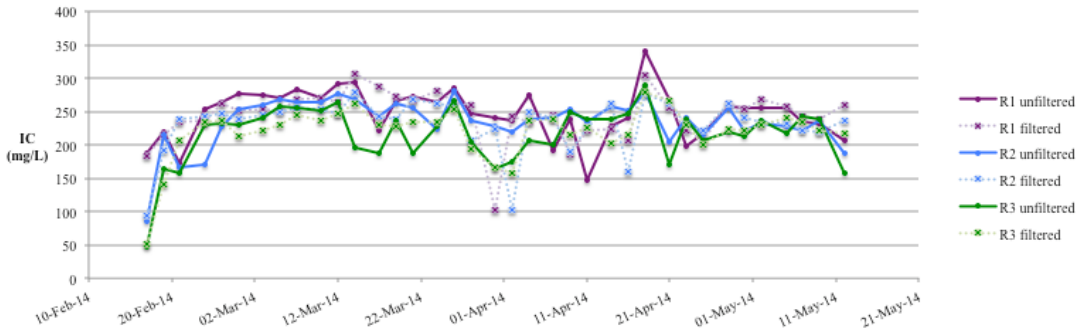


Figure 5.24. **IC discharged with effluent from R1, R2 and R3.**

5.2.11 Total Nitrogen

The TN discharged with the effluent from all three reactors is depicted below in Figure 5.25. The comparison between the influent and effluent TN levels in R1, R2 and R3 are presented in Figures 5.26, 5.27 and 5.28, respectively.

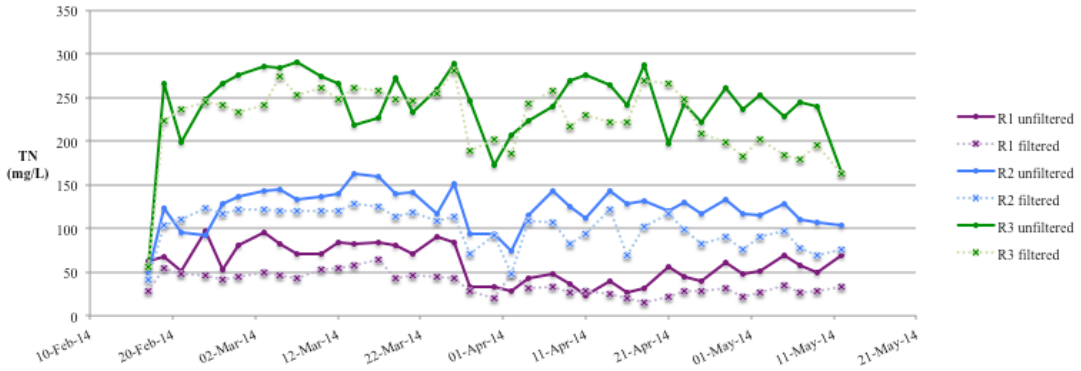


Figure 5.25. **TN discharged with effluent from R1, R2 and R3.**

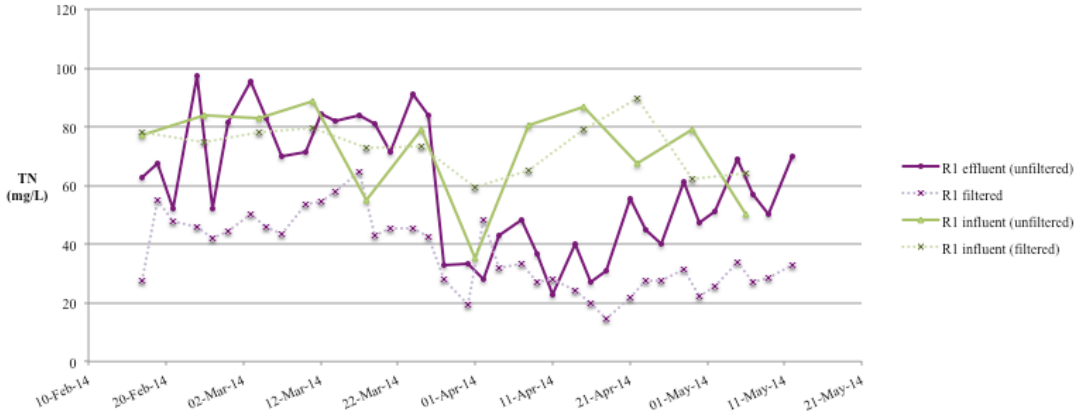


Figure 5.26. **Influent TN vs. effluent TN for R1.**

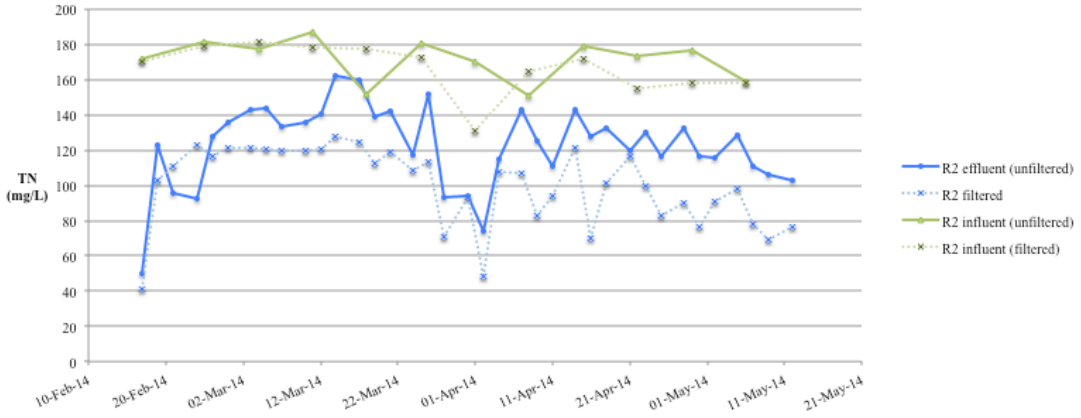


Figure 5.27. **Influent TN vs. effluent TN for R2.**

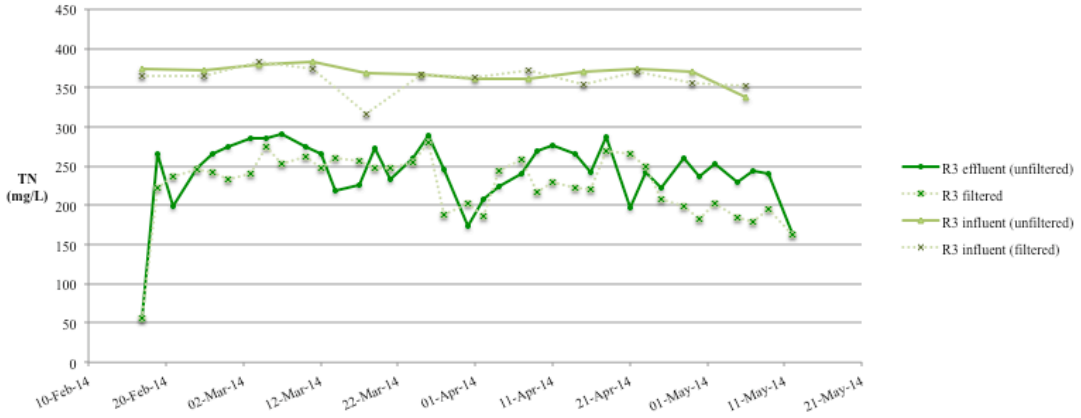


***Figure 5.28*. Influent TN vs. effluent TN for R3.**

5.2.11.1 Total Nitrogen Removal

The amount of TN removed from the three reactors and their corresponding removal efficiency are presented in Table 5.3.

***Table 5.3.* TN removal amount and removal efficiency.**

		TN Removal Amount (mg/L)	TN Removal Efficiency (%)
R1	Unfiltered TOC	28.8	38.1
	Filtered TOC	47.4	63.7
R2	Unfiltered TOC	49.6	28.9
	Filtered TOC	77.9	45.9
R3	Unfiltered TOC	128.4	34.8
	Filtered TOC	144.6	40.0

5.2.12 Ammonium Nitrogen

The NH_4-N discharged with the effluent from all three reactors is illustrated below in Figure 5.29.

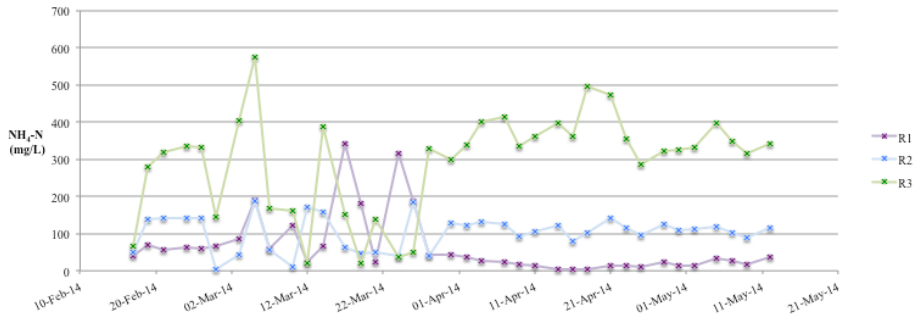


Figure 5.29. **NH_4-N discharged with effluent from R1, R2 and R3.**

5.2.13 Nitrite Nitrogen

The NO_2-N discharged with the effluent from all three reactors is illustrated below in Figure 5.30.

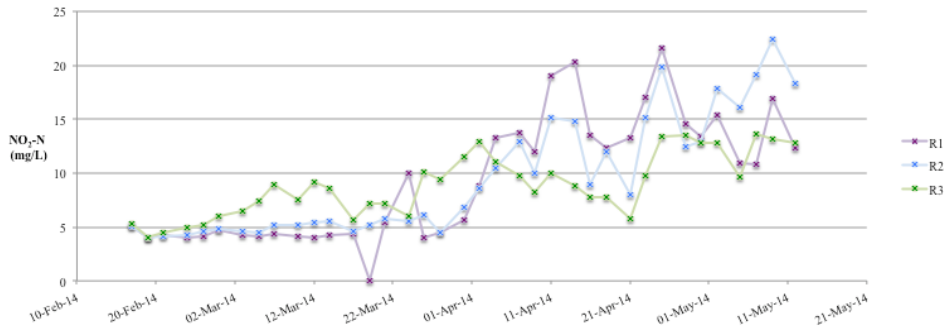


Figure 5.30. **NO_2-N discharged with effluent from R1, R2 and R3.**

5.2.14 Nitrate Nitrogen

The NO_3-N discharged with the effluent from all three reactors is illustrated below in Figure 5.31.

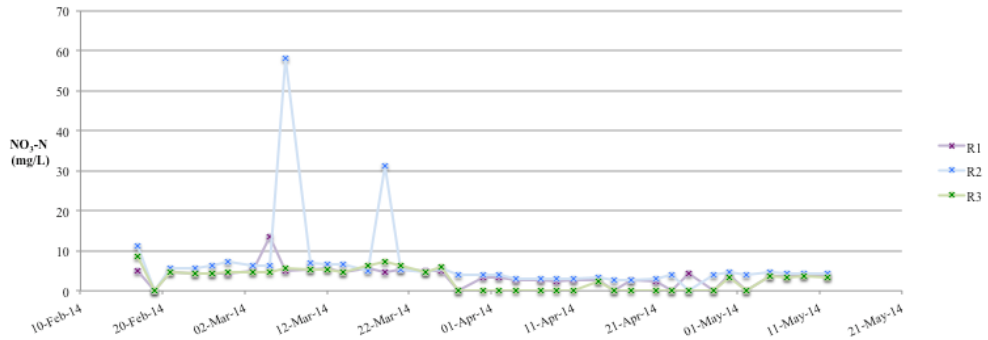


Figure 5.31. **NO_3-N discharged with effluent from R1, R2 and R3.**

5.2.15 pH

The pH logged during week six and the first day of week six is illustrated below in Figures 5.33 and 5.34, respectively. Week six demonstrated the period where granules were fully formed and functioning, hence, held the most valued representation for this study. The following logged weeks did not stray largely from the depicted pattern in the figures below. Refer to Appendix E for the pH log on selected dates that demonstrate an overall summary of pH fluctuations.

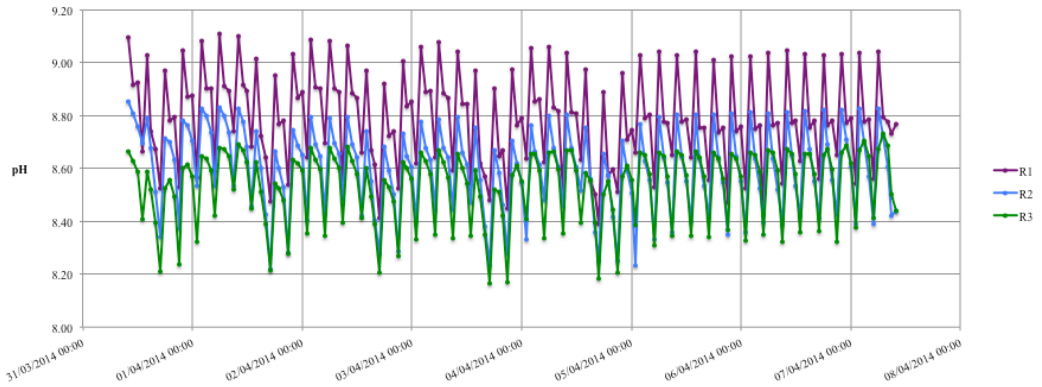


Figure 5.32 **pH log of Week 6.**

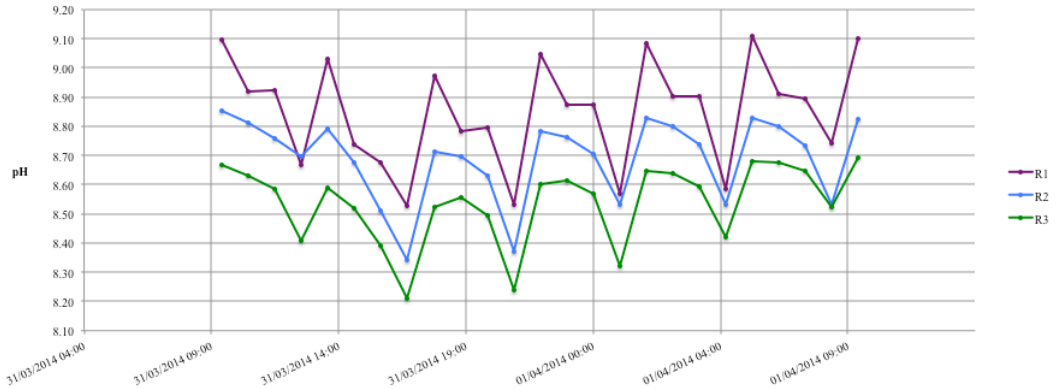


Figure 5.33 **pH log of first day in Week 6.**

5.2.16 AOB, NOB and Heterotrophic Activity

The AOB, NOB and heterotrophic activity tests using original and acidified feed as well as the corresponding standard deviations (σ) are presented below in Tables 5.4, 5.5 and 5.6, respectively. The NOB, AOB and heterotrophic activity were calculated using Equations 4.4, 4.5 and 4.6, respectively. An example of calculating the NOB, AOB and heterotrophic activity for R1 in the original feed is demonstrated below; where the OUR values were obtained from the DO gradients of the DO vs. Time plots.

Table 5.4. **AOB, NOB and heterotrophic activity in original feed.**

Original Feed

	NOB (%)	**AOB** (%)	**Heterotrophs (%)**
R1	14.6	28.0	57.4
R2	4.7	18.2	77.0
R3	15.3	13.1	76.8

Table 5.5. **AOB, NOB and heterotrophic activity in acidified feed.**

Acidified Feed (with HCl)

	NOB (%)	**AOB** (%)	**Heterotrophs (%)**
R1	17.2	23.6	59.2
R2	5.1	25.2	69.8
R3	4.0	13.3	82.7

Table 5.6. **Standard deviations for R1, R2 and R3 in OUR tests .**

Original Feed

R1	NOB	$\sigma = 6.52$
	AOB	$\sigma = 2.10$
	Heterotrophs	$\sigma = 5.23$
R2	NOB	$\sigma = 3.00$
	AOB	$\sigma = 2.41$
	Heterotrophs	$\sigma = 11.67$
R3	NOB	$\sigma = 10.20$
	AOB	$\sigma = 3.09$
	Heterotrophs	$\sigma = 1.26$

(continued)

(continued)

		Acidified Feed
R1	NOB	σ = 4.08
	AOB	σ = 0.58
	Heterotrophs	σ = 4.65
R2	NOB	σ = 4.07
	AOB	σ = 1.45
	Heterotrophs	σ = 5.52
R3	NOB	σ = 0.27
	AOB	σ = 1.34
	Heterotrophs	σ = 1.07

Example for R1 (first triplicate trial) AOB, NOB and heterotrophic activity in original feed:

R1 OUR (original feed) = 0.0053

R1 OUR (original feed with $NaClO_3$) = 0.0049

R1 OUR (original feed with ATU) = 0.0033

NOB activity (using Equation 4.4):

$$\boldsymbol{NOB}\ (\%) = \frac{\boldsymbol{OUR_i - OUR_{NaClO_3}}}{\boldsymbol{OUR_i}} \times \mathbf{100}$$

NOB = (0.0053 – 0.0049) / 0.0053 x 100 = 7.5 %

AOB activity (using Equation 4.5):

$$\boldsymbol{AOB}\ (\%) = \frac{(\boldsymbol{OUR_{NaClO_3} - OUR_{ATU}})}{\boldsymbol{OUR_i}} \times \mathbf{100}$$

AOB = [(0.0049 – 0.0033)] / 0.0053 x 100 = 30.2 %

$$\boldsymbol{Heterotrophs}\ (\%) = \frac{\boldsymbol{OUR_{ATU}}}{\boldsymbol{OUR_i}} \times \mathbf{100}$$

Heterotrophs = (0.0033 / 0.0053) x 100 = 62.3 %

These values were then averaged with their corresponding second and third triplicate trial values to establish the final AOB, NOB and heterotrophic activity, as presented in Tables 5.4 and 5.5. Figures 5.34 and 5.35 depict the diagrammatic visuals of Tables 5.1 and 5.2, respectively. The purple colour represents R1, the blue colour represents R2 and the green colour represents R3, with the error bars indicating the standard deviation.

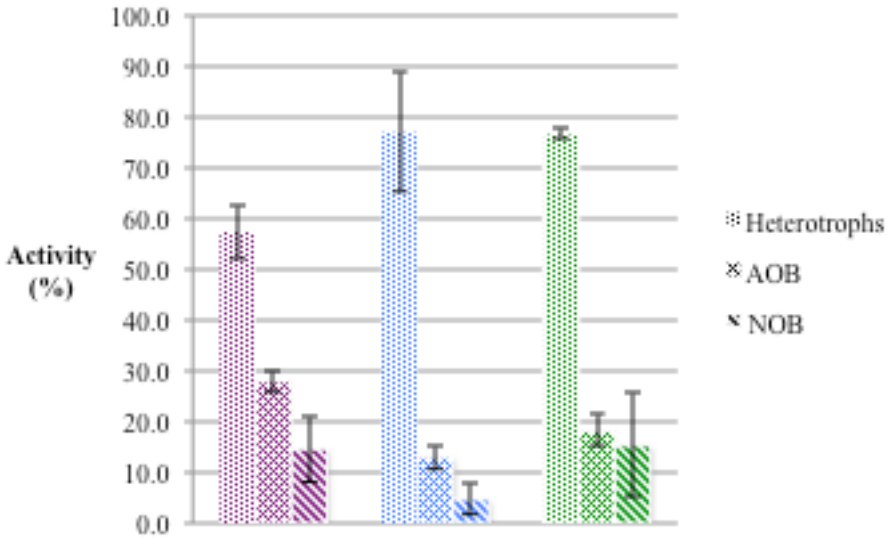


Figure 5.34. **AOB, NOB and heterotrophic activity in original feed.**

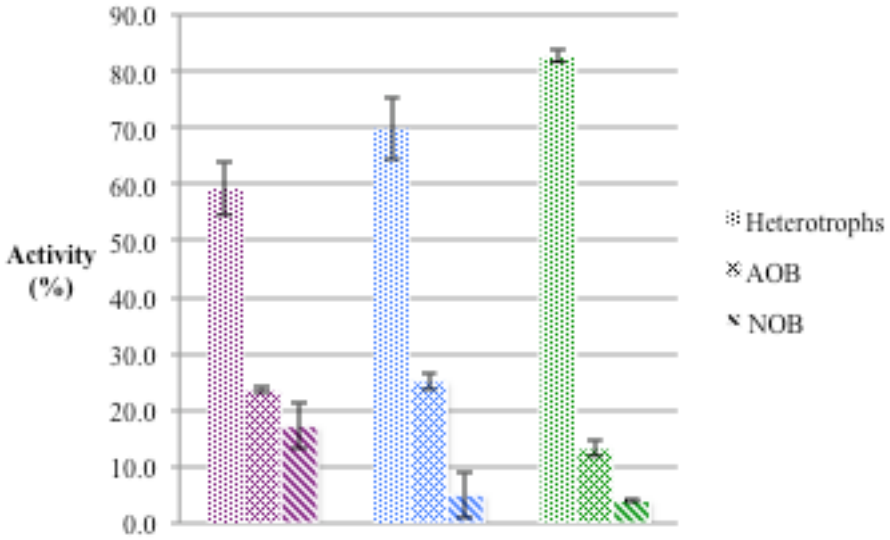


Figure 5.35. **AOB, NOB and heterotrophic activity in acidified feed.**

6 Discussion

The following chapter presents a discussion about the findings attained from the experimental models dabbled with at the start of the thesis and the subsequent study carried out on investigating the effects various nitrogen loads impart onto aerobic granulation.

6.1 The Aerobic Granulation Industry

At the onset of this thesis I was granted a brief experience with a company working in the field of wastewater treatment. The focus of my endeavour was to follow up on current innovative plans in competing with industrial-scale treatment plants implementing aerobic granulation technology. This particular company assigned two teams in carrying out the task of investigating the key parameters of aerobic granular sludge formation on two distinct design models. The first design model presented in this thesis, reactor model one, corresponds to the model I personally undertook studies upon. The second design model, reactor model two, corresponds to the model investigated by an adjacent team. I felt it was useful to include the work of the adjacent team as it portrayed a creative concept, which could be compared and contrasted to reactor model one. A valuable note to consider is that both reactors were run at only a length of two and a half weeks or so. A lagging period at the start where the fabrication of the models were delayed due to late delivery of materials and lack of available personnel to supervise the work constrained the degree of research. Nevertheless, the preliminary models provide an insight into the creative concepts being examined for potential components in future WWTPs.

As mentioned previously, the Nereda technology is the first of its kind in implementing full-scale reactors to biologically treat wastewater using the aerobic granular sludge technique at WWTPs. The Nereda concept is still at a novice stage in its implementation as the field of aerobic sludge granulation requires further research into fully understanding its processes and mechanisms. de Bruin & van Loosdrecht, 2010 convey the overview of research findngs carried out on the five pilot plants in the Netherlands, refer to Appendix F. Since then, the Nereda technology has expanded to South Africa and fututre implementations in various European countries are prominent.

6.1.1 Reactor Model One

As noted before, reactor model one was a lab-scale attempt at rearranging the traditional configuration of an SBR to establish whether aerobic granulation can be manifested within a novel reactor design. The model demonstrates two components to the aerobic granulation process, a main SBR body and a minor selector tank. The selector tank is situated closer to the top of the reactor, as depicted in Figure 4.1. Its main purpose and function was to foster anoxic conditions for optimal denitrification as well as recycle the reactor contents. The selector's purpose seemed defeated as at the start of the operation sludge began to accumulate and layer rather than pose as a clear anoxic zone. Figure 5.1 depicts the amount of biomass within the reactor was at

an undesirable level of 2.5 mg/L; where an amount of 5mg.L was desired. The sudden jump in MLSS in Figure 5.1 was caused by adjusting the level of the measuring syringe to a deeper, more appropriate sludge-filled region. The reactor was constantly aerated expect during the settling and decanting phase. It was found difficult to maintain biomass within the reactor as the biomass was not given enough time to aggregate during an early stage and so a large portion of sludge was washed out during the decanting phase. Figure 5.2 depicts the biomass concentration washed out with the effluent and shows a large portion of the reactor biomass concentration was released, especially in the last week where almost 70% of the biomass was discharged. Had the reactor been running for a longer period, it would have been recommended to pour in more seed sludge and investigate its concentration levels further. Most likely the cycle operation would need to be adjusted to allow a longer settling phase at the start to encourage aggregation. Knowing that conventional sludge flocs settle a great deal slower than aerobic granules, incorporating a longer settling period at the start of the operation and gradually decreasing the settling phase over the first month would encourage the biological processes to occur. This, in fact, was applied to the Chalmers experiment and proved successful; refer to Figure 5.12 for the illustration of the decrease in settling time applied to the Chalmers experiment during the start-up period.

The failure of the selector concept may primarily stem from permitting too short a setting phase at the start-up period. A possible alternative for incorporating a selector tank could be to shut off connection, by means of a valve, to the selector at the start of the run and once flocs begin to nourish enable connection with the selector. The effect on granule formation could be investigated using such a technique compared to the formation of granules in a conventional SBR. However, this may be deemed rather costly regarding pilot- and full-scale applications An additional disadvantage of the design model includes feeding the influent from the selector. Feeding directly into "anoxic" conditions and then contacting the biomass within the main SBR body could alter the chemical and biochemical processes that take place.

The sludge flocs in reactor model one did begin to demonstrate a fluffy consistency after a week of operation, which indicated signs of slight aggregation; meaning the flocs were consuming the nutrients supplied by the feed that of which consisted of diluted cola and a blend of chemicals replicating industrial wastewater. Figure 5.3 demonstrates the consumption of nutrients as the COD concentration in the influent far surpassed the COD concentration in the effluent. COD is a food source for the biomass and its depleted concentration within the effluent signifies the biomass consuming the nutrients for its development into fluffy flocs. Although the reactor established an environment for slight biomass activity, the scope of its operation was deemed unsuccessful. Throughout the run of its operation the reactor never maintained a stable condition, the pH level fluctuated at relatively acceptable levels, however, the influent and effluent flowrates were constantly unstable. Exhibiting an exchange ratio of 8% rather than the expected 50% presented detrimental effects on the amount of substrate the biomass was exposed to as well as the amount of nutrients available to nourish the biomass.

6.1.2 Reactor Model Two

In comparison to reactor model one, reactor model two consists of one SBR column, which incorporates an effluent discharge contraption that is unique to the conventional SBR setup. The coiled tubing bound to the float at the surface-liquid level was a successful method in discharging the utmost treated effluent. The upmost region of the reactor liquid supplied the cleanest effluent as it was situated at the furthest point away from the biomass. Figures 5.5 and 5.8 depicting the SVI of the MLSS in train one and two, respectively, presented a reasonable drop between the five to ten minute intervals of measurement within the timespan suggesting appropriate granule forming qualities. With time, the SVI five to ten minute intervals reduced. This demonstrated that the sludge began to increase in density as it increased in settling velocity. Figures 5.6 and 5.9 illustrate that the amount of MLSS within trains one and two, respectively, gradually increased throughout the operation; further depicting growth in biomass within the reactor. The largest comparison to reactor model one was the cycle operation, where an aeration period of six hours and fifty-five minutes was implemented. The requirement of an anaerobic phase is not necessary, however, an excess of an aeration phase tampers with the biological processes within the reactor contents; it supplies a surfeit of oxygen to the microorganisms. The nitrification and denitrification populations would be affected by such an extent of oxygen being supplied to them; hindering the rate of denitrification as it requires organic carbon and lack of oxygen to carry out its task. The results from train one and train two are largely comparable, having only slight deviations in pH levels. A greater amount of investigation is required to assess whether or not reactor model two is adequate to expand to a pilot-scale prototype for wastewater treatment. An understanding of its lab-scale function in the long-term is necessary to deduce its adequacy for pilot-scale operation.

6.2 Chalmers Laboratory-Scale Experiment

The study of investigating the effects various nitrogen loads have on aerobic granule formation in a SBR shifts interest from the exploratory design of SBRs to the appreciation of granule structure and composition yielded. The Chalmers reactor vastly differs in design and operation of reactor models one and two, hence trifling comparisons can be made between the two. The Chalmers reactor integrates an anaerobic phase spanning fifty-five minutes following the feeding phase. The feeding phase itself is anaerobic. The anaerobic phase was implemented to encourage the microorganisms within the biomass to acclimatise to the surrounding environment. The greatest concentration of nitrogen fed was to R3, with the intermediate amount to R2 and the least to R1; Figure 5.25 clarifies these applications. The same amount of COD, 5 mg/L, was fed to all reactors. The first three weeks of the reactor operation experienced a gradual decrease in settling time. This was proposed to encourage the sludge flocs to aggregate to an adequate degree in order to avoid being washed out too soon, as demonstrated in reactor model one. It is evident from Figure 5.11 that a healthy jump in sludge height followed by a slight decrease and gradual maintenance in height complimented the rate in change of settling time. An initial settling time of 30 minutes was established, the next day a time of 20 minutes was implemented, three days after that a time of ten minutes was applied, the following day eight minutes, four days after that six minutes, three days after that four minutes and finally three days after that the desired settling time of two minutes was applied. Although this

application prompts the granulation process, R1 remained excessively flocculated for a lengthy period of time at the start. Figure 5.14 shows that R1 contained the least amount of biomass where it most likely represented the flocculated state of the reactor contents. Appendix D illustrates the macro-scale structure of the granules throughout the experiment at selected dates and correlates to the results obtained from the reactors. Figure 5.14 also shows that, during the start-up period, R3 corresponded to having the highest biomass concentration and R2 owning the intermediate amount. This conveys that R3 cultivated the most efficient granules at the start and R1 cultivating the least efficient. During the intermediate period of the experiment the biomass concentration fluctuated a great deal between all reactors. This may be due to the diverse microorganism populations reacting to the significantly high pH levels propagated. An average pH of 9.1, 8.8 and 8.6 in R1, R2 and R3, respectively, demonstrate a cause for concern. A suggested pH range between 6.5 and 7.5 is recommended for optimal growth of microorganisms. Figure 5.32 illustrates the pH logged during week six and the high pH levels are evident. The pH in the reactors peaks during the aeration period when all the biological processes are underway. From Figure 5.32 and the pH logs presented in Appendix E, a noticeable wave pattern is illustrated. This wave pattern is believed to correlate to the rise and fall in temperature within a day. The pH level is highest during the afternoon period where most exposure to the day's warmth has passed. The lowest pH level corresponds to the time period before the sun has risen. Temperature has played a role in fluctuating the pH levels between each cycle, however, only to a small extent. The fact that hourly temperature changes imparted an effect on the pH within the reactors shows that the plastic material used to construct the SBR model permitted relative temperature exchange. In a full-scale model, reinforced concrete is the main construction material of the aerobic granulation tank; hence, it is useful to acknowledge the limitations a lab-scale SBR carries in relation to the developed pilot- or full-scale application. It was debated whether or not to dose hydrochloric acid (HCl) in order to reduce the pH, however, it was decided to instead observe whether any changes would take place in time. Slight reductions were noticed, nonetheless, not to an extent that would propagate optimal conditions.

The sludge volume index, excluding the anomalies of a SVI greater than 200 mL/g, demonstrated a relative indication of the sludge settling characteristics of the granules within each reactor. Figure 5.13 shows that R2 granules dominated for an extended period of time as having the greatest settling properties. However, at the end of the experiment it is evident that R3 granules claimed to own the best settling properties. The erratic behaviour of the granules, once again, is strongly believed to be in response to the extremely high pH levels established within the reactors; the root to which remains rather unclear. The settling velocity test, illustrated in Figures 5.15, 5.16 and 5.17, confirms that R3 granules maintain excellent settling properties and the highest settling velocity were achieved by R2 granules. This may indicate that R2 granules are slightly denser than R3 granules. The relative hydrophobicity test, depicted by Table 5.1, demonstrated R3 granules to be the most hydrophobic (43.3%), R2 the intermediate amount (14.9%) and R1 the least (6.6%). This shows that R3 granules held the greatest ability to aggregate as they repelled away from water and attracted to microorganisms present in the mixed liquor. The combination of having a high relative hydrophobicity and fast settling characteristics suggests that a healthy

amount of EPS was produced in R2 and R3; engaging in a protective environment that maintains the structural integrity of the granules.

The consumption of total organic carbon in all three reactors signifies the utilisation of energy that supports the growth of biomass. Figures 5.20, 5.21 and 5.22 depict the diminished levels of TOC in the effluent compared with the influent in R1, R2 and R3, respectively. The TOC comparison figures prove successful granulation took place within all reactors and Appendix D further illustrates this. It was observed that R3 granules removed the greatest amount of TOC (450.5 mg/L) and demonstrated the highest removal efficiency (85.2%) regarding the unfiltered proportion of the TOC. With regards to the filtered proportion of the TOC, R1 proved to removal the greatest amount (510.9 mg/L) with the high efficiency (98.9%). The TOC proportion of greater interest depends on whether or not there is a requirement for post-treatment after the SBR biological treatment in a full-scale application. The unfiltered TOC case represents the removal amount and efficiency required for an application without post-treatment and the filtered TOC case represents the removal and efficiency required for an application with post-treatment. The total nitrogen trendlines for R1 and R2 depicted in Figures 5.26 and 5.28 respectively, display a peculiar nonlinear trend at the start. The measurements taken up till the 28th April 2014 should be discarded as they correspond to measurement errors made by the ion chromatography machine. Applying that case, it is more apparent that an appropriate trend is observed with the TN in the influent remaining greater than the TN in the effluent, indicating consumption of the nitrogen that is a staple nutrient for granule growth and sustenance. R1 does not depict this on the 6th May 2014, however, this can also be assumed as a measurement error in the ion chromatography machine. From Table 5.3, it is evident that R3 proves to remove the greatest amount of filtered and unfiltered TN with a removal amount of 144.6 mg/L and 128.4 mg/L, respectively. One month into the operation of the reactors, lack of signs indicating an increase in nitrite concentrations placed uncertainty in whether the granules were nitrifying. Appreciating that the results attained from the ion chromatography machine during this period were unavailing, oxygen uptake rate tests were carried out to establish the AOB, NOB and heterotrophic activity within the granules. This intended to decipher whether or not nitrification was occurring. Nitrification is a crucial part of the nitrogen cycle that subsequently enables denitrification to take place within the granules in order to remove nitrogen present in the wastewater. Too high a level of nitrogen discharged into the environment leads to eutrophication of water sources. The OUR tests carried out tested granules in the original feed as well as in acidified feed. The acidified feed was examined for the purpose of assessing the affect in AOB, NOB and heterotrophic activity given the pH of the reactor was lowered to a more optimal condition. To great avail, as depicted in Figures 5.34 and 5.35, the OUR tests demonstrated that nitrification was taking place within the reactors with both NOB and AOB populations found present within the granules. The higher activity in AOBs indicated that most of the nitrification was in the form where ammonium is converted into nitrite. Less NOB activity meant that not as many granules transformed nitrite into nitrate. This did not hinder the extent to which denitrification was taking place, as the heterotrophic activity was significantly high. The difference between the original and acidified feed tests was marginal. However, the acidified feed promoted further heterotrophic activity in R1 and R3, further AOB activity in R2 and further NOB activity in R1 and R2.

7 Conclusions

To conclude, R3 composed the most desirable granule structure that of which demonstrated excellent settling characteristics and emphasised dense and hydrophobic qualities. It proved to remove the greatest amount of unfiltered TOC as well as unfiltered and filtered TN and is deemed most effective in terms of treatment capacity aiming to apply its configuration to a full-scale reactor without post-treatment. R1 treated the greatest amount of filtered TOC and governed the highest removal efficiency for TOC and TN. The decision factor in assessing which reactor configuration provided the ultimate outcome depends on the intended use of the treated wastewater. If it is intended to use reclaimed wastewater for treatment to provide potable water then R1 demonstrates a more qualified model. If it is intended to discharge the treated wastewater back to the environment then R3 presents the most compelling configuration.

The chief investment that is recommended to advance the Chalmers experiment is to dose HCl into the feed in order to reduce the pH levels and investigate the operation of the SBR at optimal conditions. Regarding the field of aerobic sludge granulation, developing further understanding of the mechanisms involved in the granulation process as well as grasping the role and production of EPS within granules are recommended.

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Appendix A

Trends in Drinking-Water Sources and Sanitation Facilities (WHO, 2014)

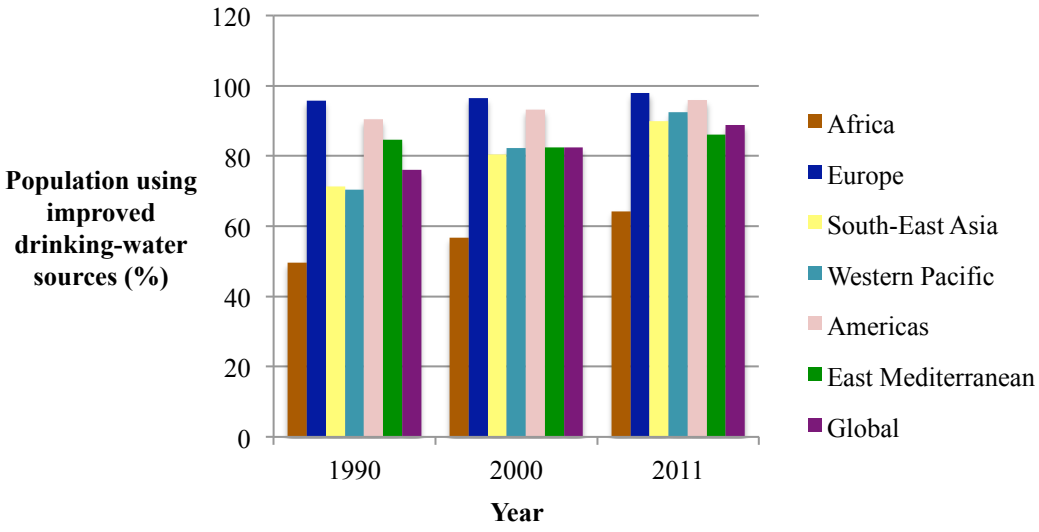

Figure A 1. **Improved drinking-water sources.**

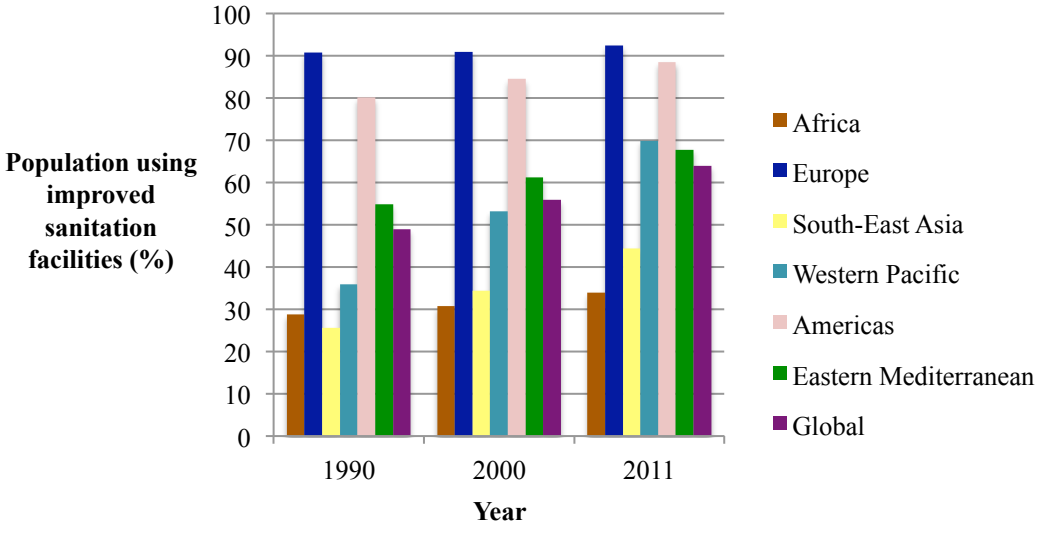

Figure A 2. **Improved sanitation facilities.**

Appendix B

Reactor Model One – Dissolved Oxygen, Oxygen Uptake Rate and Temperature

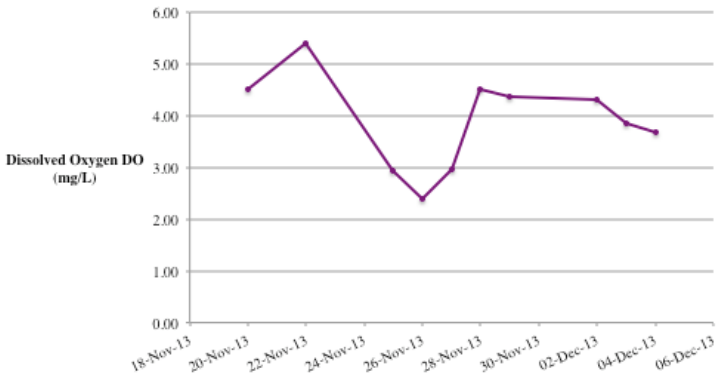

Figure B 1. **DO in reactor model one.**

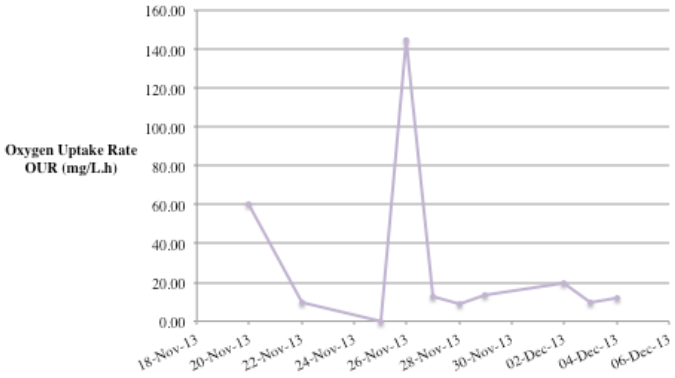

Figure B 2. **OUR in reactor model one.**

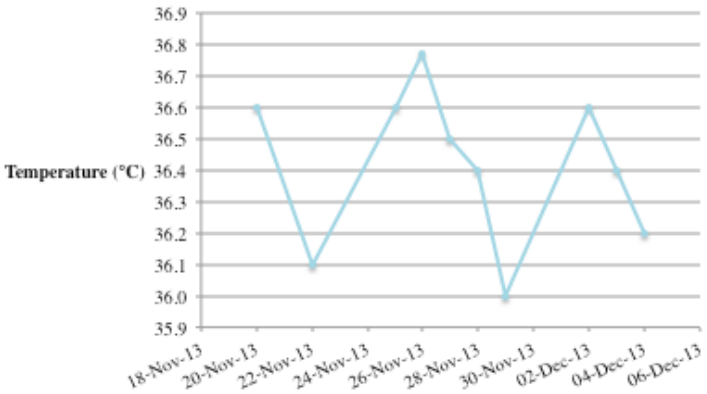

Figure B 3. **Temperature in reactor model one.**

Appendix C

Reactor Model Two, Train One and Two – Dissolved Oxygen, Oxygen Uptake Rate and Temperature

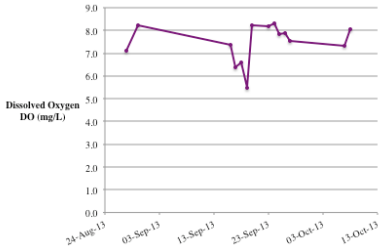

Figure C 1. **DO in reactor model two, train one.**

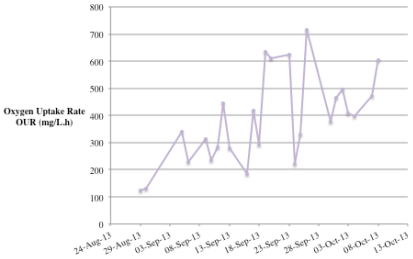

Figure C 2. **OUR in reactor model two, train one.**

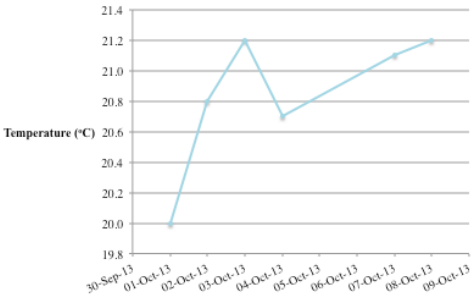

Figure C 3. **Temperature in reactor two, train one.**

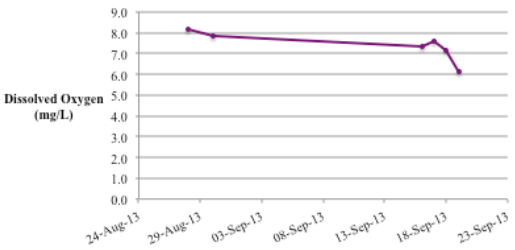

Figure C 4. **DO in reactor model two, train two.**

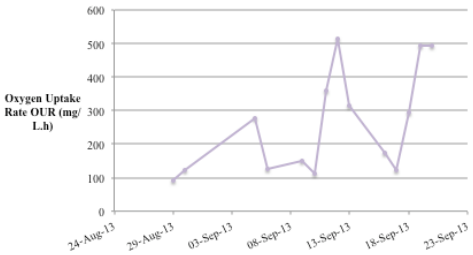

Figure C 5. **OUR in reactor model two, train two.**

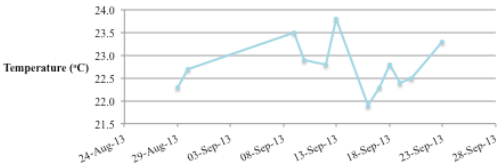

Figure C 6. **Temperature in reactor model two, train two.**

Appendix D

Macro-scale Biomass Photo Diary

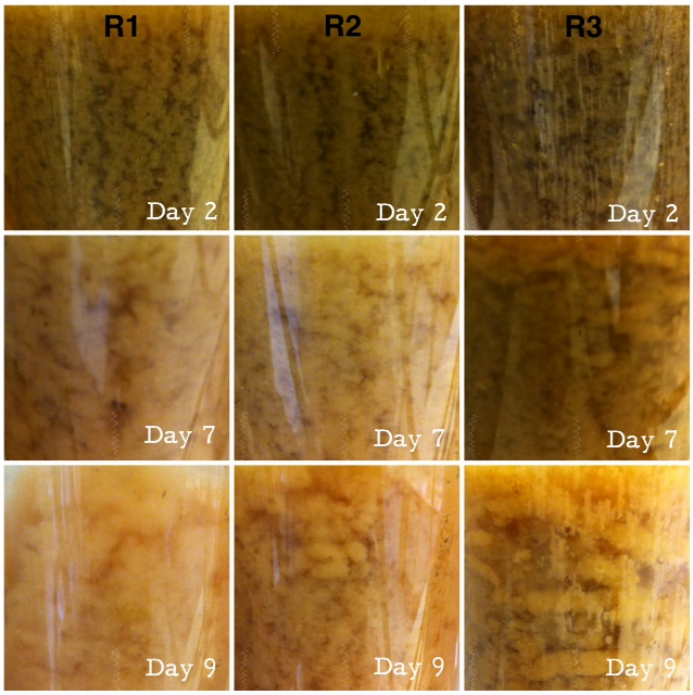

Figure D 1. **Macro-scale aerobic biomass photos on days 2, 7 and 9.**

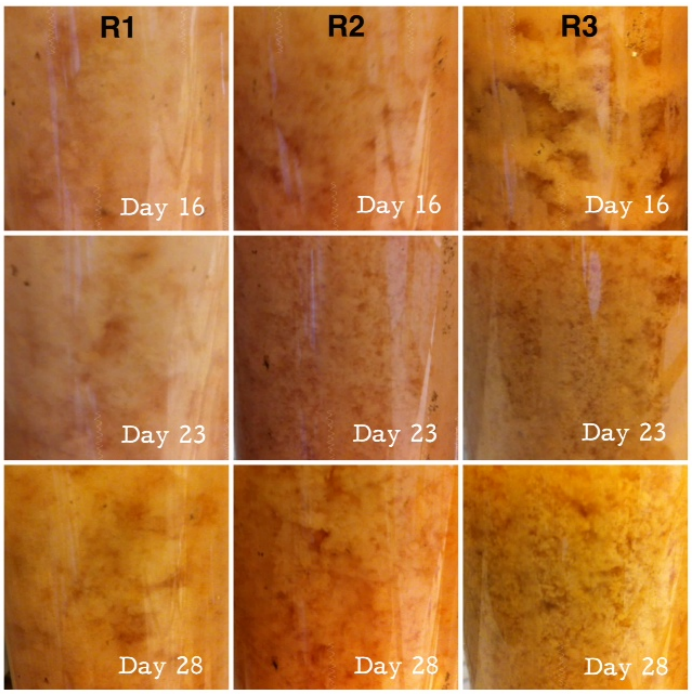

Figure D 2**. Macro-scale aerobic biomass photos on days 16, 23 and 28.**

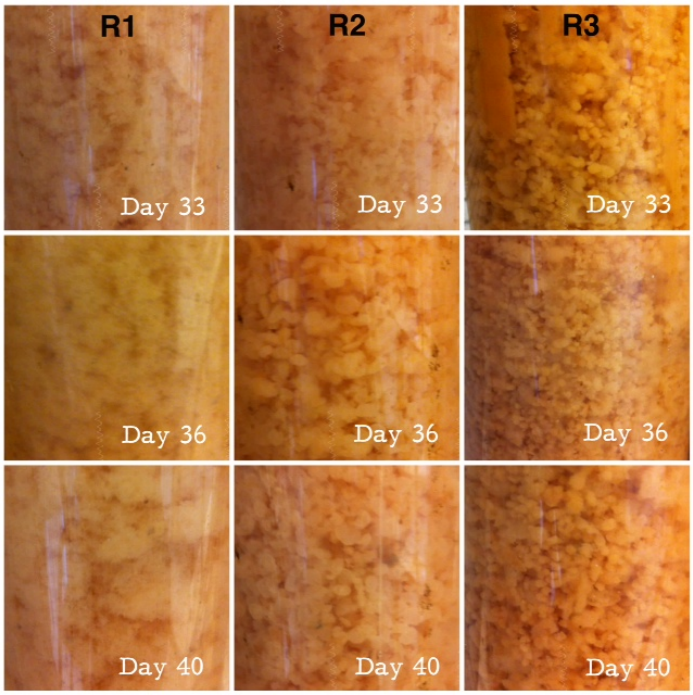

Figure D 3. **Macro-scale aerobic biomass photos on days 33, 36 and 40.**

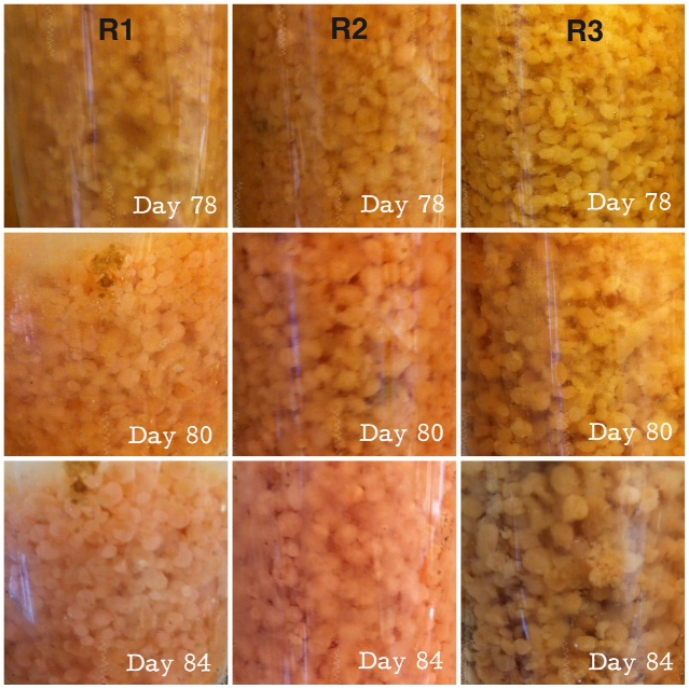

Figure D 4. **Macro-scale aerobic biomass photos on days 78, 80 and 84.**

Appendix E

pH Logs

(blue line = R1, red line = R2 and green line = R3)

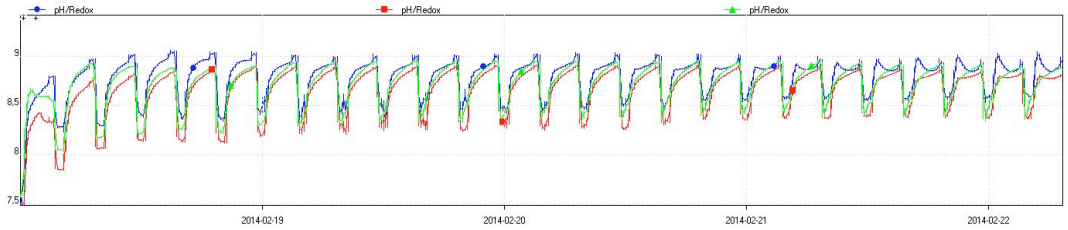

***Figure E 1*. pH log between 18-02-2014 and 22-02-2014 for R1, R2 and R3.**

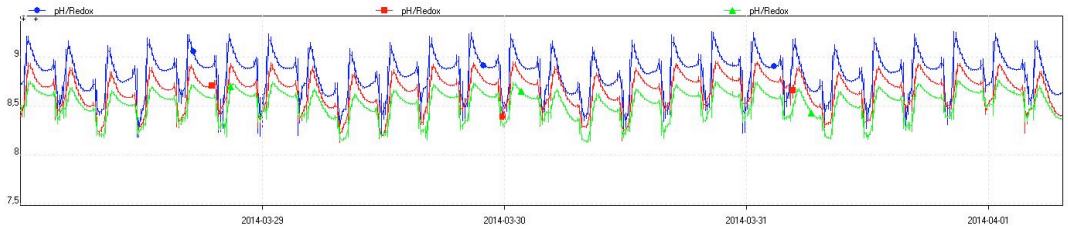

***Figure E 2*. pH log between 28-03-2014 and 01-04-2014 for R1, R2 and R3.**

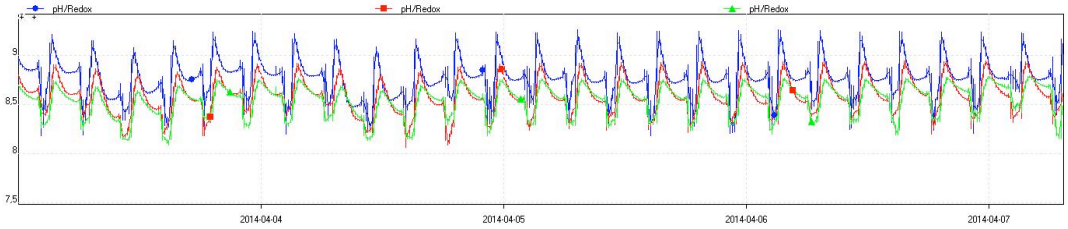

***Figure E 3*. pH log between 03-04-2014 and 07-04-2014 for R1, R2 and R3.**

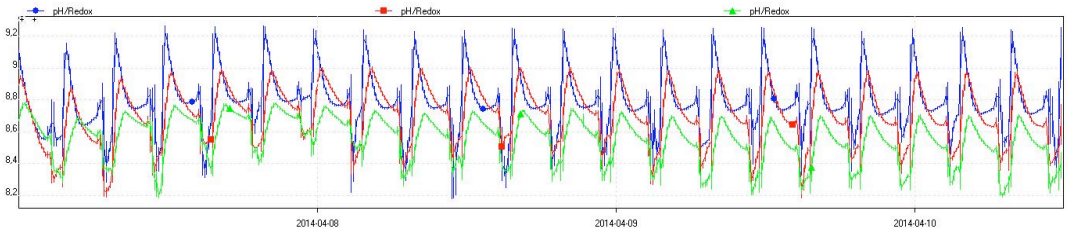

***Figure E 4*. pH log between 07-04-2014 and 10-04-2014 for R1, R2 and R3.**

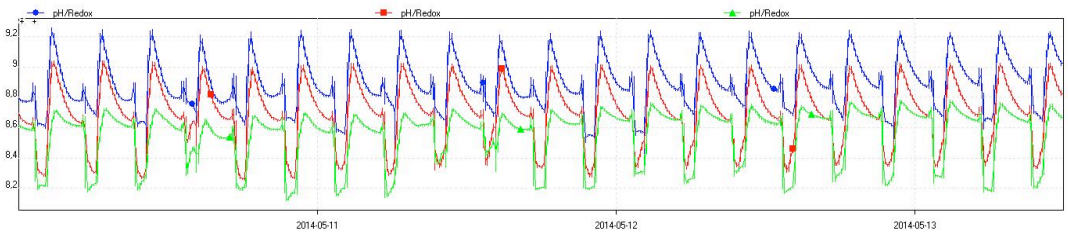

***Figure E 5*. pH log between 10-05-2014 and 13-05-2014 for R1, R2 and R3.**

Appendix F

Overview of Nereda Pilot Research

WWTP	Ede	Aalsmeer	Epe	Hoensbroek	Dinxperlo
Aspect / Period	**2003 – 2005**	**2006**	**2006 – date**	**2007**	**2008 - 2009**
Granulation	Yes	yes	yes	inoculation with anaerobic granules	inoculation with aerobic granules
Substrate	pretreated influent	raw influent	raw influent	raw influent	raw influent
Selection pressure during granulation	High	low	high	n.a.	n.a.
Supplementary acetate dosing during granulation	no	yes	no	no	No
Reactor type	Bubble column and air-lift reactor	bubble column	bubble column	bubble column	bubble column
Process control nitrogen removal	O_2	O_2	O_2 / NH_4 / NO_3	O_2	O_2 / NH_4 / NO_3
Cycle time	Fixed	fixed	fixed / dynamic	fixed	fixed / dynamic
Duration cycle phases	fixed	fixed	dynamic	fixed	Dynamic
Supplementary simultaneous chemical P-removal	no	no	no	no	yes

Figure F 1. **Overview of Nereda pilot research, (cited in de Bruin & van Loosdrecht, 2010).**