INVESTIGATION OF INFLUENT COD/TAN RATIO AND LOADING RATE EFFECTS ON CARBON AND NITROGEN REMOVAL VIA AEROBIC GRANULES

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ABSTRACT

INVESTIGATION OF INFLUENT COD/TAN RATIO AND LOADING RATE EFFECTS ON CARBON AND NITROGEN REMOVAL VIA AEROBIC GRANULES

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Aerobic granulation serves as a promising treatment technology over the conventional systems due to its advantages such as high biomass retention capacity, high settleability, ease of separation from the effluent, toxicity resistance, capacity to handle high organic loading rates and suitability for high chemical oxygen demand (COD) wastewaters.

The scope of the thesis is to investigate the influent COD/total ammonifiable nitrogen (TAN = NH_4-N + NH_3-N) ratio and loading rate effects for the removal of carbon and nitrogen from wastewaters by aerobic granular SBR operation.

Firstly, the effects of influent COD/TAN ratios (1 to 30) were investigated. The optimum influent COD/TAN ratio leading to both the highest COD (75%) and TAN (90%) removal efficiencies and maintenance of granular stability under the studied conditions was determined as 7.5. It was speculated that the influent COD/TAN ratio affected the aerobic granular composition via enrichment of heterotrophic bacteria at higher ratios (10-20-30) and nitrifiers at lower ratios (5-3.5-2-1).

Secondly, the effects of increasing organic loading rates from 0.75-12 g COD/Lday, and nitrogen loading rates from 0.1-1.6 g TAN/Lday were investigated at a constant influent COD/TAN ratio of 7.5. The optimum loading rates were determined as 1.5 g COD/Lday and 0.2 g TAN/Lday, regarding the provision of the highest COD (85±1%), TAN (87±2%) and total nitrogen (TN) (49±17%) removal efficiencies, as well as formation of granules with high stability.

The utilization of aerobic granular systems for sugar beet processing wastewater treatment, and the effect of this wastewater on aerobic granules were also investigated. It was found that the raw sugar beet processing wastewater with high solids content (2255±250 mg/L total suspended solids, i.e TSS) could be treated successfully via aerobic granular sludge with removal efficiencies of 65±5% total chemical oxygen demand (tCOD), 87±1% soluble chemical oxygen demand (sCOD), 58±10% TSS and 61±4%TAN.

Results of this study are expected to be used for the determination of the suitable influent COD/TAN ratio and the loading rates that provide high carbon and nitrogen treatment efficiencies and maintenance of granular stability in the upcoming studies employing aerobic granular systems.

Key Words: simultaneous nitrification and denitrification, granulation, industrial wastewater.

ÖZ

GİRİŞ KOİ/TAN ORANI VE YÜKLEME HIZININ AEROBİK GRANÜLLERLE KARBON VE AZOT GİDERİMİNE ETKİLERİNİN ARAŞTIRILMASI

KOCATÜRK, İREM
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Aerobik granülasyon; yüksek biyokütle tutma kapasitesi, yüksek çökelebilirlik, çıkış suyundan ayrılma kolaylığı, toksisite direnci, yüksek organik yükleme hızlarının üstesinden gelebilme kapasitesi ve yüksek kimyasal oksijen ihtiyacı (KOİ) içeren atıksulara uygunluk gibi avantajları sayesinde, konvansiyonel sistemlere kıyasla gelecek vaat eden bir arıtım teknolojisi sunmaktadır.

Bu tezin kapsamı giriş KOİ/toplam amonyak azotu (TAN = NH_4-N + NH_3-N) oranı ve yükleme hızının aerobik granüler AKRlerle atıksulardan karbon ve azot giderimine etkilerinin araştırılmasıdır.

Öncelikle, (1-30 arası) giriş KOİ/TAN oranlarının etkisi araştırılmıştır. Hem KOİ (%75) hem de TAN (%90) için en yüksek giderim verimlerinin sağlandığı ve çalışılan koşullar altında granüler stabilitenin korunduğu optimum giriş KOİ/TAN oranı 7,5 olarak saptanmıştır. Giriş KOİ/TAN oranının aerobik granüllerin içeriğini (10-20-30 gibi) yüksek oranlarda heterotrof bakterilerce, (5-3,5-2-1 gibi) düşük oranlarda ise nitrifikasyon bakterilerince zenginleştirerek etkilediği spekülе edilmektedir.

İkinci olarak, giriş KOİ/TAN oranı 7,5'ta sabitlenerek, artan organik yükleme hızlarının (0,75'ten 12 g KOİ/Lgün'e) ve azot yükleme hızlarının (0,1'den 1,6 g

TAN/Lgün‘e) etkileri araştırılmıştır. En yüksek KOİ (%85±1), TAN (%87±2) ve toplam azot (%49±17) giderim verimlerinin sağlanması ve yüksek stabilitede granüllerin oluşumu açısından optimum yükleme hızları 1,5 g KOİ/Lgün ve 0,2 g TAN/Lgün olarak saptanmıştır.

Aerobik granüler sistemlerin şeker pancarı işleme atıksuyu arıtımında kullanımı ve bu atıksuyun aerobik granüllere etkisi de araştırılmıştır. Yüksek katı içeriğine (2255±250 mg/L askıda katı madde; AKM) sahip olan ham şeker pancarı işleme atıksuyunun aerobik granüler çamur ile %65±5 toplam kimyasal oksijen ihtiyacı (tKOİ), %87±1 çözünebilir kimyasal oksijen ihtiyacı (çKOİ), %58±10 AKM ve %61±4 TAN giderim verimlerinde başarılı olarak arıtılabildiği saptanmıştır.

Bu çalışmadan elde edilen sonuçların, aerobik granüler sistemlerle yapılan bundan sonraki çalışmalarda yüksek karbon ve azot arıtımı eldesi ve granüler stabilitenin korunması için uygun giriş KOİ/TAN oranı ve yükleme hızlarının belirlenmesinde kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: simultane nitrifikasyon ve denitrifikasyon, granülasyon, endüstriyel atıksu.

To my grandparents,

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LIST OF ABBREVIATIONS

ANAMMOX Anaerobic ammonia oxidation

AOB Ammonia-oxidizing bacteria

BOD Biological oxygen demand

CAS Conventional activated sludge

CMTR Completely mixed tank reactor

COD Chemical oxygen demand

DGGE Denaturing gradient gel electrophoresis

DN Denitrification

DNPAO Denitrifying phosphate-accumulating organism

DO Dissolved oxygen

EBPR Enhanced biological phosphorus removal

EPS Extracellular polymeric substance

FA Free-ammonia (NH3-N)

H/D Height to diameter ratio

HAc Acetic acid

HRT Hydraulic retention time

MBR Membrane bioreactor

MBS Membrane bioreactor sludge

METU Middle East Technical University

NLR Nitrogen loading rate

NNOP National Nereda Onderzoeks Programma

NOB Nitrite-oxidizing bacteria

OLR Organic loading rate

PAO Phosphate-accumulating organism

PN Protein

POME Palm oil mill effluent

PS Polysaccharide

SBAR Sequencing batch airlift reactor

SBR Sequencing batch reactor

sCOD Soluble chemical oxygen demand

SNDN Simultaneous nitrification-denitrification

SOUR Specific oxygen uptake rate

SRB Sulfate reducing bacteria

SRT Solid retention time

SS Suspended solids (synonm for TSS)

SVI Sludge volume index

TAN Total ammonifiable nitrogen (NH4-N + NH3-N)

tCOD Total chemical oxygen demand

TKN Total Kjeldahl nitrogen

TN Total nitrogen

TOC Total organic carbon

TON Total oxidized nitrogen

TP Total phosphorus

TSS Total suspended solids

UAFB Upflow anaerobic fixed bed

UASB Upflow anaerobic sludge blanket

VER Volumetric Exchange ratio

VSS Volatile suspended solids

CHAPTER 1

INTRODUCTION

The biogranulation process may be defined as a special biofilm composed of different types of microorganisms, forming compact, dense and spherical aggregates via biological physical and chemical processes (Liu and Tay, 2004). Biogranulation may be sorted as aerobic and anaerobic granulation. Due to the intercellular repulsive electrostatic forces and hydration interactions, the microorganisms do not tend to gather on their own (Watnick and Kolter, 2000; Liu and Tay, 2004). Biogranulation, which is thought to be a result of microbial evolution, serves as a defense mechanism providing self-immobilization of microorganisms under environmental stress (Liu et al., 2004a; Liu and Tay, 2004). Instead of random aggregation, the granules are formed by the contribution of microorganisms which establish a symbiotic relationship to cater to each others' needs in order to survive under stressful conditions (Watnick and Kolter, 2000). Biogranules are distinguished from flocs by their dense and compact structures, 0.14-5 mm average diameter, and fast settling velocity of 20-94 m/hour (Schmidt and Ahring, 1996; Morgenroth et al, 1997; Liu and Tay, 2004; Hu et al., 2005a).

The first granular sludge consisted of anaerobic granules developed by Lettinga et al. (1980) in upflow anaerobic sludge bed (UASB) reactors. Compared to activated sludge flocs, granular sludge is advantageous in terms of its compact, dense and robust structure, high biomass retention (30-80 g/L), toxicity resistance, ability to tolerate high organic loadings, high settleability, ease of separation from the effluent and suitability for high COD, high strength wastewaters (Speece,1996; Liu and Tay, 2004; Adav et al., 2008a).

Aerobic granules were first cultivated in a continuous aerobic upflow sludge blanket reactor by Mishima and Nakamura (1991). Since then, numerous aerobic granulation studies have been conducted (Morgenroth et al., 1997; Beun et al., 1999; Peng et al., 1999; Etterer and Wilderer, 2001; Tay et al., 2001a; Liu and Tay, 2002; Beun et al., 2002; Adav et al., 2008a). Anaerobic granulation technology, which is unsuitable for N and P removal, requires at least 2-4 months (Lettinga et al., 1980; Liu and Tay, 2004) cultivation period as well as high operation temperatures. Aerobic granulation technology overcomes these obstacles by its fast cultivation (2-4 weeks depending on the wastewater type) and ability to remove nitrogen and phosphorus as well as carbon (Adav et al., 2008a; Gao et al., 2011). However, in long-term operations, granular stability may be distrupted causing deteriorated treatment efficiency due to the limited diffusion of oxygen and nutrients to the inner parts of the granular structure (Morgenroth et al., 1997; Tay et al., 2002b; Liu and Tay, 2004). In order to overcome the stability interferences and improve the treatment efficiency of aerobic granules, the research is in development. Furthermore, investigations into the use of aerobic granulation technology for the treatment of different industrial wastewaters are widely conducted.

Formation and characteristics of aerobic granules including stability and nutrient removal performance are determined by the operational conditions of the reactor to great extent (Liu and Tay, 2004; Gao et al., 2011). The influent loading rates and carbon to nitrogen (COD/N) ratio are two major operational parameters in aerobic granular reactors.

1.1 Scope and Objective of the Thesis

This thesis, which is based on carbon and nitrogen removal from wastewaters by aerobic granular SBR operation, aims

- to determine the optimum influent COD/N ratio and
- to determine the maximum influent organic and nitrogen loading rates

providing both high nitrogen and COD removal efficiencies while maintaining the structural stability of the granular sludge.

- to investigate the treatability of sugar beet wastewater rich in solid content by aerobic granular sludge for the first time. To this purpose, an aerobic granular SBR was operated with raw sugar beet processing wastewater and the effluent of an anaerobic digester treating sugar beet processing wastewater.

CHAPTER 2

LITERATURE REVIEW

2.1 Biogranulation Process

Studies have revealed that the aerobic biogranulation process initiated by activated seed sludge continues with the conversion of compact aggregates into granular sludge that eventually forms mature granules (Liu and Tay, 2004). Tay et al. (2001a) studied aerobic granulation from conventional activated sludge in SBRs. The results indicated that the granulation was a gradual process including compact aggregation (Week 1), appearance of clear spherical granules (Week 2), and observation of mature aerobic granules (Week 3) with regular round shapes. It was also seen that there was no carrier support requirement for granulation. A remarkable increase in reactor volatile suspended solids (VSS) content was reported during the development of mature aerobic granules from activated seed sludge (Sun et al., 2006). Hailei et al. (2006) observed 5 phases of granulation in their study employing aerobic granulation by papermaking wastewater. These were namely, microbe multiplication phase, appearance of 0.3-0.8 mm flocs, floc cohesion, maturation of highly settlable flocs of 3-6.5 mm, and aerobic granule phase. The aerobic granules were formed by compaction of flocs and the process was completed by detachment of filaments and other isolated bacteria on floc surfaces by shear force.

Numerous hypothetical proposals and mathematical models have been developed to explain the complex mechanism of the aerobic granulation process (Liu and Tay, 2002; Adav et al., 2008a), despite their inadequacy to explain the whole mechanism. The complexity of the process arises from the contribution of a wide range of microbial species as well as the diversity of degraded substrates. The on-going research about the aerobic granulation mechanism lacks knowledge especially about the microbial structures and process dynamics (Gao et al., 2011).

Cell surface hydrophobicity is a significant factor to start aerobic granulation in terms of favoring intercellular aggregation via hydrophobic binding (Liu and Tay, 2004). According to thermodynamics, high cell surface hydrophobicity may enhance the intercellular interactions by lowering the surface Gibbs free energy. Hence the microorganisms accumulate together and move away from the liquid (hydrophilic) phase (Liu and Tay, 2004). When the microbes are highly hydrophobic, formation of more compact granules can be achieved due to the forceful attractions among the microorganisms. The aggregated unity of numerous cells can be stabilized by the extracellular polymeric substances (EPS) polysaccharides, which have adhesive and cohesive effects. The polysaccharides, which promote aerobic granulation, are reported to be more abundant in aerobic granules with respect to flocculent sludge (Liu and Tay, 2004; Tay et al., 2001c). The polysaccharide secretion of granular bacteria is favored by selection pressure such as short settling time in SBR operations (Liu and Tay, 2004; Qin et al., 2004a). The effects of settling time are discussed in Section 2.2.3.1.

2.2 Factors Affecting Aerobic Granulation

The factors affecting granulation can be listed as substrate type and composition, organic loading rate (OLR), hydraulic selection pressure, reactor configuration, sludge retention time (SRT), superficial upflow air velocity, starvation, presence of divalent metal ions, intermittent feeding strategy, dissolved oxygen concentration, pH, temperature, seed sludge characteristics (microbial activity, concentration, settleability), possible inhibition, influent carbon to nitrogen ratio, EPS, and non-aerated periods in SBR cycle.

2.2.1 Substrate type and composition

Substrate type is known to affect the aerobic granular composition, formation, species diversity, stability, storage and structure (Schmidt and Ahring, 1996; Tay et al., 2001a; Tay et al., 2002c; Liu and Tay, 2004; Sun et al., 2006). As explained in the following sections (Section 2.2.1.1. and Section 2.2.1.2.), the studies employed synthetic wastewater with various carbon sources or real wastewater. It was denoted that the aerobic granulation is independent of the type of carbon source, because successful aerobic granulation via numerous substrates was reported in the literature. Furthermore, due to the high growth rates of aerobic microorganisms the substrate energy content does not affect the aerobic granule cultivation (Liu and Tay, 2004).

2.2.1.1 Synthetic wastewater

Aerobic biogranule development is possible via carbon sources such as ethanol, acetate, glucose, phenol, starch, molasses, peptone, fecula, sucrose, phtalic acid (Tay et al., 2001a; Liu and Tay, 2004; Hu et al., 2005a; Sun et al., 2006; Zeng et al., 2007; Adav et al, 2008a; Luo et al., 2014). The substrate carbon type is a significant factor in terms of granular composition, morphology, stability and species selection and diversity (Tay et al., 2001a; Tay et al., 2002c; Moy et al., 2002; Hu et al., 2005a; Sun et al., 2006; Adav et al, 2008a). Utilization of glucose as carbon source for aerobic granulation has been observed to promote filamentous growth (Tay et al., 2001a; Tay et al., 2002c; Moy et al.,2002; Liu and Tay, 2004). In addition, Chudoba (1985) claimed that the carbohydrate containing glucose and maltose was observed to favor filaments.

Moy et al. (2002) showed that the type of substrate affected the OLR resistance and structure of the aerobic granules by the selection and cultivation of different microbial communities. Two SBRs were operated with glucose or acetate in order to develop aerobic granules at various OLRs (6-15 g COD/Lday). Although the granulation occured within 3 weeks for both of the reactors, the acetate-fed reactor faced granular disintegration at the OLR of 9 g COD/Lday, while the glucose-fed reactor could resist up to 15 g COD/Lday. However, the granular structure of the glucose-fed reactor was fluffy, loose and filamentous, which later became irregular shaped, larger, smooth, compact granules with folds and crevices. The acetate-fed reactor, on the other hand, had dense compact and regular shaped granules dominated by rod-like species without any filamentous growth.

Similar results were reported by Tay et al. (2001a), who developed aerobic granules from filamentous activated sludge using acetate or glucose. The acetate-fed reactor showed gradual disappearance of filamentous microorganisms and formation of compact granules dominated by rod-like bacteria. The glucose-fed reactor was dominant in filaments and had granules with a fluffy outer surface.

The effect of carbon source on selection of microbial species contributing to aerobic granulation was implied by Tay et al. (2002c), who developed aerobic granules with both acetate and glucose. Although the granules had similar shape, settleability, hydrophobicity, strength, biological activity and COD removal efficiency; their microbial diversity, size and storage stability were different. The observations showed that glucose-fed granules were larger (2.4 mm) and showed filamentous growth while the acetate-fed ones were smaller (1.1 mm) and dominated by rod-like species. Storage stability of glucose-fed granules were higher since they displayed less specific oxygen utilization rate (SOUR) reduction, indicating less microbial activity decrease than that of the acetate-fed granules.

Sun et al. (2006) implied that slowly biodegradable carbon sources (such as peptone) supported the enrichment of slow-growing microorganisms, providing smaller aerobic granules with high stability and settleability. Granulation was achieved in 4 SBRs fed with acetate, glucose, peptone and fecula, respectively. The most stable granules with high settleability were obtained in the peptone-fed reactor, although their sizes were small.

2.2.1.2 Real wastewater

The real wastewaters used in aerobic granulation and/or treatment studies have included domestic wastewater (de Bruin et al., 2004; de Kreuk and van Loosdrecht, 2006; Liu and Tay, 2007a), urban wastewater (Liu et al., 2010a), municipal wastewater (di Iaconi et al., 2007), landfill leachate (di Iaconi et al., 2007; Wei et al., 2012), malting wastewater (Schwarzenbeck et al., 2004a, 2004b), brewery wastewater (Wang et al., 2007a), soybean processing wastewater (Su and Yu, 2005), dairy wastewater (Arrojo et al., 2004; Schwarzenbeck et al., 2005), abattoir wastewater (Yılmaz et al., 2008; Lemaire et al., 2008), swine slurry (Figueroa et al., 2011), textile wastewater (Muda et al., 2010; Lotito et al., 2012; Lotito et al., 2014), palm oil production wastewater (Figueroa et al., 2009; Abdullah et al., 2011), rubber wastewater (Rosman et al., 2014) and livestock wastewater (Kishida et al., 2009).

The studies with real wastewater are explained in Sections 2.3.1.1.2 and 2.3.1.1.3 where the applications of aerobic granular sludge technology are listed. The accomplishment of aerobic granulation via various real wastewaters reveal, once again, the independency of the process from the carbon type.

2.2.2 Organic loading rate (OLR)

Appropriate selection of OLR can favor granulation, since aggregation or granulation of the sludge is provided by adjusting the OLR between famine and feast status (Gao et al., 2011). The studies investigating the OLR effect on aerobic granules were in the range of 0.13-21.3 g COD/Lday OLR (Moy et al., 2002; Tay et al., 2004a,b; Zheng et al., 2006; Li et al., 2008a; Kim et al., 2008; Chen et al., 2008; Thanh et al., 2009; Adav et al., 2009a,2010; Peyong et al., 2012; Lotito et al., 2012; Abdullah et al., 2013). Even 30 g COD/Lday OLR was studied (Thanh et al., 2009), however the overgrown granules due to the high substrate concentration created clogging problems at lab scale.

Independence of aerobic granule formation from the applied OLR was demonstrated by numerous studies that achieved aerobic granulation in a wide OLR ranging from 0.6 to 15 g COD/Lday (Peyong et al., 2012; Liu and Tay, 2004). Cydzik and Baryla (2011), who seeded an SBR with aerobic granules, reported that the formation of new aerobic granules was possible at OLR values even as low as 0.45 g COD/Lday. However, the OLR has major influence on the granulation rapidity as well as granular characteristics such as physical properties and species diversity (Liu and Tay, 2004; Gao et al., 2011).

The granule sizes tend to increase with increasing OLRs (Moy et al., 2002; Li et al., 2008a; Tay et al., 2004a,b; Liu and Tay, 2004; Chen et al., 2008). Increasing OLR provided faster granulation while yielding large and fast-growing granules (Liu and Tay, 2004; Li et al., 2008a). For instance, Li et al. (2008a) cultivated tightly packed small (2 mm) granules in 50 days at the OLR of 1.5 g COD/Lday, while the OLR increase to 4.5 g COD/Lday provided fast formation of larger (4-5 mm) granules with in a shorter period of 12 days. On the other hand, formation of loose filamentous granules with decreased hydrophobicity and lower specific gravity (Zheng et al., 2006; Liu and Tay, 2004) were reported at high OLR values. Hence the deterioration of granular strength and robustness may create granular stability problems (Liu and Liu, 2006), leading to partial detachment (Lotito et al., 2012) and/or disintegration of granular biomass (Zheng et al., 2006) at high OLR values (Liu and Tay, 2004; Gao et al., 2011).

Granular disintegration can be observed at OLRs high as 21.3 g COD/Lday (Adav et al., 2009a, 2010) and at moderate values of 12 g COD/Lday (Chen et al., 2008), 8 g COD/Lday (Tay et al., 2004a,b), 9-15 g COD/Lday (Moy et al., 2002), 6 g COD/Lday (Zheng et al., 2006) and 5 g COD/Lday (Thanh et al., 2009). Lotito et al. (2012) reported partial detachment of granular biomass due to fast but unstable bacterial growth at an OLR of 3.4 g COD/Lday. Thanh et al. (2009) claimed that stable dense aerobic granules could be formed between 2.5-15 g COD/Lday, despite the disintegration and the following recovery at the OLR of 5 g COD/Lday. Adav et al. (2009a) claimed that the structural integrity of granules and high COD removal efficiency (94-96%) had been maintained at 19.5 g COD/Lday. However, moving on to the OLR of 21.3 g COD/Lday, foaming and granular disintegration were reported. In contrast to common declaration where granular disintegration at high OLRs was accompanied by filamentous growth (Zheng et al., 2006; Liu and Tay,2004), overgrown filaments were not observed by Adav et al. (2009a). Granular disintegration at very low OLR values was reported by Peyong et al. (2012) who observed granular disintegration with OLR decrease from 1.2 to 0.13 g COD/Lday most probably due to the limited substrate amount. The granules recovered when the OLR was increased to 0.6 g COD/Lday. Generally, granular disintegration is attributed to limited mass transfer and formation of anaerobic zones in the granule with larger sizes under high OLRs (Zheng et al., 2006), the decreased aggregation tendency of cells (Adav et al. 2010), or the reduced cell hydrophobicity (Thanh et al., 2009).

The effect of OLR on microbial diversity of the aerobic granules was claimed by Li et al. (2008a), who reported less diverse populations with the OLR increase from 1.5 to 4.5 g COD/Lday. Gradual adjustments in OLR may shift the dominant species, contributing to granulation via creating microbial selection pressure (Chen et al., 2008). A population shift created by the gradual OLR increase from 6 to 12 g COD/Lday was reported to favor the formation of larger, denser, more settlable but less-bioactive granules (Chen et al., 2008). Similar observations were reported by Abdullah et al. (2013), who reported microbial evolution with OLR increase from 1.5 to 3.5 g COD/Lday. It can be concluded that each OLR promotes the enrichment of different species, indicating microbial adaptation. Therefore, the species diversity may be changed and the dominant species may be shifted by OLR adjustment.

2.2.3 Hydraulic selection pressure

The major driving force behind the aerobic granulation process is the hydraulic selection pressure, which aims to select the functional bacteria for granulation, and discard the rest (Liu and Tay, 2004; Tay et al., 2001a). The hydraulic selection pressure is exterted by 3 major components; namely, settling time, hydraulic retention time (HRT) and volumetric exchange ratio (VER) of the reactor (Liu et al., 2005a). By adjusting these 3 parameters, bioflocs and biogranules faster than some certain settling velocity are held and enriched, while the light slow settling particles are discharged from the aerobic granular SBR (Liu et al, 2005a; Li et al., 2008b). The microbial species that are held and cultivated in the system are determined by hydraulic selection pressure to a certain extent (Liu and Tay, 2004).

2.2.3.1 Settling time

The main conductor of the hydraulic selection pressure is the settling time, which is the period where the reactor content is left to settle, just before the effluent discharge, at the end of the SBR cycle (Liu and Tay, 2004; Tay et al., 2001a). Since the hydraulic selection pressure aims to hold fast-settling particles and discard the poorly-settlable portion, the sludge discharge rate from the SBR increases with shortening settling time (Qin et al., 2004b). At short settling times, only the fast-settling bacteria are able to reach the reactor bottom; while the slow-settling biomass does not settle deep enough before the discharge port is opened. Hence the fast-settling biomass (contributing to granulation) is held and enriched in the reactor, while the slow-settling and non-flocculating portion is washed out. In a way, the selection pressure influences the type of microorganisms that are retained in the system. Consequently, determination of the optimum settling time is significant in terms of creating selection pressure for the management of granule development and properties (Liu and Tay, 2004).

At long settling times, the flocs with poor settleability may not be eliminated from the reactor to a sufficient extent. The functional biomass that contributes to the granule formation can be surpassed by these suspended flocs, which adversely affects the granulation process (Liu et al., 2005a). The supporting effect of short settling times on aerobic granulation was expressed by various research (Qin et al., 2004b,c; Adav et al., 2009b; Beun et al., 2000; Beun et al.,2002; Liu et al.,2005a;

McSwain et al., 2004a). Short settling times (1-2 min) (at max 5 min) enhance the cultivation of highly settlable, hydrophobic aerobic granules with high EPS production (Adav et al.,2009b; Qin et al., 2004b,c; Tay et al., 2001a). These studies are in compliance with the claim that high selection pressure and shear force provided higher EPS production (McSwain et al, 2004a). Application of short settling times in order to select and enrich the granules via settling velocity difference was also conducted by Beun et al. (2000; 2002).

Improved granular characteristics such as settleability, size, stability, EPS secretion and hydrophobicity at short settling times were reported by Qin et al. (2004b,c), Adav et al. (2009b) and McSwain et al. (2004a). It can be concluded that short settling times (≤5 min) may be appropriate to achieve stable aerobic granulation by SBR operation.

Qin et al. (2004b,c), who operated 4 aerobic granular SBRs with settling times of 5-10-15-20 min, claimed that short settling times (<15 min) enhanced the formation and characteristics of aerobic granules. Although there was aerobic granulation in the SBRs operated at less than 15-min settling time, only the SBR with 5-min settling time was dominated by aerobic granular sludge. Granulation failed in the SBR with 20-min settling time where the sludge was only flocculated. Formation of more hydrophobic and settlable aerobic granules were reported with decreasing settling time. This which was attributed to the stimulation of the hydraulic selection pressure, which accelerated the microbial metabolism, and enhanced the secretion of extracellular polysaccharides (PS). Similar findings were reported by Adav et al. (2009b), who studied 10, 7 and 5 min settling times. It was stated that settling times short as 5 minutes may promote the formation of more settlable and larger aerobic granules by selecting functional microbial strains and eliminating the non-flocculating ones. The study also implied the secretion of excess EPS due to the enrichment of functional strains, in the absence of competitors, which had already been discarded. According to McSwain et al. (2004a), short settling times (such as 2 min) provided aerobic granules with higher EPS protein content and a less diverse but more stable population.

2.2.3.2 Hydraulic retention time (HRT)

As stated before, the hydraulic selection pressure aims to discharge the poor settling dispersed sludge, while keeping the denser and well settling portion in the reactor. HRT together with cycle duration and volumetric exchange ratio, determines the discharge frequency of the SBR (Liu and Tay, 2004). In a column type SBR, HRT can be defined as the cycle time divided by the % volumetric exchange ratio. At a constant volumetric exchange ratio, shorter cycle time results in shorter HRT. Short cycle times and thus short HRT values may enhance granulation by obstructing the growth of poor-settling sludge via frequent discharges. The cycle time and the resulting HRT should be short enough to favor microbial growth, stimulate EPS secretion and increase cell hydrophobicity; thus granulation can be promoted (Tay et al. 2002d; Liu and Tay, 2004).

However, at too short cycle times (≤ 1.5 h) and HRT values (≤ 3 h), the hydraulic selection pressure becomes too strong and over-frequent discharge of biosolids leads to granular disintegration (or prevent the formation of new ones), sludge loss and thus reactor failure (Pan et al., 2004; Liu and Tay, 2008a; Liu and Tay, 2004). In other words, the biomass would be exposed to hydraulic washout before finding time to grow (Liu and Tay, 2004). On the other hand, too long cycle times (≥12 h) and HRT values (≥24 h) are not effective in creating hydraulic selection pressure since they allow the growth of poor-settling suspended biomass (Liu and Tay, 2004). HRT should be long enough for bacterial acclimation and growth and short enough to prevent suspended growth. Kishida et al. (2009) reported that the gradual increase in HRT from 0.75 to 7.5 days caused deterioration of aerobic granular structure and decrease in N and P removal efficiencies due to the decreased hydraulic selection pressure.

According to Liu and Tay (2008a), 4-8 h cycle times (corresponding to 8-16 h HRTs) were suitable for the formation of stable and robust granules. When the cycle time and HRT were decreased to 1.5 h and 3 h, respectively, the aerobic granules gained a floccy appearance by 2 months of operation. Pan et al. (2004) stated that HRT should be between 2-12 hours (corresponding to 1-6 h cycle times) in order to provide sufficient hydaulic selection pressure to develop and maintain compact aerobic granules with high stability, settleability, hydrophobicity, COD removal efficiency, structural strength and high PS/PN ratios. The study implied that very short HRTs like 1 h created too strong hydraulic pressure that resulted in biomass washout. The hydraulic selection pressure was insufficient at long HRTs like 24 h, thus the aerobic granules were disintegrated and reactor content was dominated by flocs. Rosman et al. (2014) operated an aerobic granular SBR at the HRTs of 6, 12 and 24 h (with corresponding 3, 6 and 24 h cycle times). The granular characteristics improved as the HRT decreased from 24 to 6 hours. The largest (2 mm) and most settlable granules with highest nurtient removal performances (98.4% COD, 92.7% NH_4^+-N, 89.5% TN) were observed at the operation with 6 h of HRT. Accumulation of Mg^{2+} and Ca^{2+} were also reported for operation at an HRT of 6h.

The suitable HRT and cycle time range may vary according to the microbial species contributing the granulation. Tay et al. (2002d) reported the successful cultivation of slow-growing nitrifying granules at 6-12 h cycle times and 12-24 h HRT values. The study revealed that a cycle time of 3 h (and HRT of 6 h) was too short, while a cycle time of 24 h (and HRT of 48 h) was too long for the formation of nitrifying granules.

Although the proper selection of cycle time and HRT are significant for aerobic granular SBR operation, settling time stands to be a more critical parameter. In other words, even the proper selection of cycle time and HRT may not prevent aerobic granulation failure at settling times above 15 minutes (Qin et. al, 2004 b,c; Liu et al. 2005a).

2.2.3.3 Volumetric exchange ratio (VER)

Volumetric exchange ratio (VER) is defined as the percent ratio of the discharged effluent volume to the total working volume of the SBR (Liu and Tay, 2004). Assuming the diameter is constant, the VER of a column type SBR is proportional to “L” (the height from the discharge level to the water surface) (Figure 2.1; Wang, 2004).

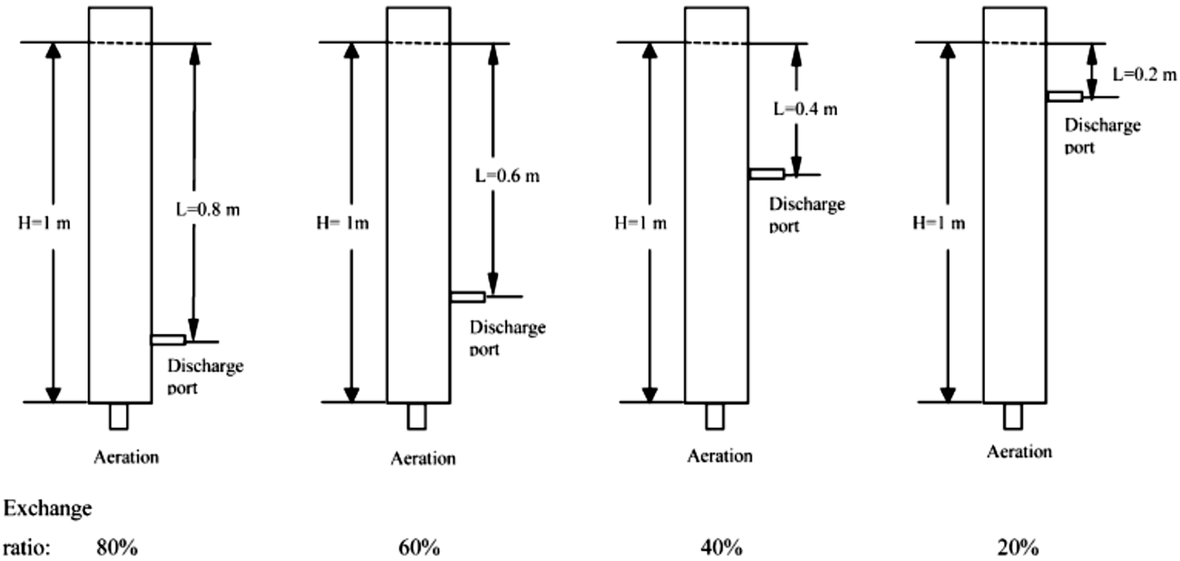

Figure 2.1. Demonstration of volumetric exchange ratio in column-type SBRs (Wang, 2004).

The effect of VER on the formation of aerobic granules was stressed by Zhu and Wilderer (2003), who achieved aerobic granulation in an SBR with 75% volumetric exchange ratio. Wang (2004) investigated the SBR operation at different VERs between 20%-80%. The experiments were conducted at a constant settling time of 5 minutes. The results showed that at higher exchange ratios (60-80%), the percent of aerobic granules in the total biomass was also greater. The reactor content showed aerobic granular dominancy at the SBRs operated at 60% and 80% VERs. On the other hand, at lower exchange ratios such as 40% and 20%, the SBR contained a mixture of aerobic granules and suspended sludge (Liu et al., 2005a; Wang, 2004).

2.2.4 Reactor configuration

2.2.4.1 Hydraulic behaviour

The reactor configuration affects the reactor's hydraulic behaviour, which influences aggreagated cells. The flow patterns of the liquid and biomass in the reactor are affected via reactor configuration (Liu and Tay, 2004). Aerobic granule formation and/or treatment studies are conducted in column type upflow reactors, most commonly in SBRs (Liu and Tay, 2004; Liu et al., 2005a), with a few exceptions including sequencing batch airlift reactor (SBAR) (Beun et al., 2000) and even batch reactors (Wang et al., 2006a). The SBRs are advantageous over many biological treatment systems due to their flexible operation conditions, small surface area, and compact reactor system (Tchobanoglous et al., 2004). SBR operation consists of 4-5 sequencing periods namely; feeding, aeration, settling, (idle) and discharge periods. The operational parameters and conditions are known to have significant effects on granular sludge development from suspended sludge.

In column type reactors, the air or liquid follows an upflow pattern, providing nearly homogenous circular flow and localized vortex along reactor's axis. The aggregated cells are converted into granular shape by this circular flow. If the height to diameter (H/D) ratio is high in an upflow column reactor, the length of the circular flow trajectory increases. As a result, the microbial aggregates are subjected to stronger hydraulic corrasion (Liu and Tay, 2004). The configuration of upflow column type reactors creates hydraulic attrition due to the upflow pattern, circular flow and resulting vortex along the reactors axis. The microbial aggregates gain a granular shape by this hydraulic attrition; which brings us to the conclusion that granulation is supported by upflow column type reactors (Liu and Tay, 2004; Liu et al., 2005a).

Different from the column type reactors, the completely mixed tank reactors (CMTRs) have a flow pattern that allows multi-directional movement of aggregated cells in accordance to dispersed flow. Hydrodynamic shear force affects the aggregated cells from many directions, creating various upflow trajectories and random collisions. This hydraulic behaviour in CMTRs, explained above, provides the occasional formation of irregular shaped flocs in various sizes, rather than regular shaped granules (Liu and Tay, 2004; Liu and Tay, 2002). Regarding these drawbacks, CMTRs are not suitable for aerobic granulation.

2.2.4.2 H/D ratios used in SBRs

In SBR operation, to prevent being discharged from the reactor, the particles should have a minimum threshold settling velocity to travel the distance to the discharge port during the settling time. This threshold particle settling velocity may be adjusted by the H/D ratio of the SBR as well as by the settling time and VER. Assuming the settling time and VER are fixed, if the reactor H/D ratio increases, the minimum settling velocity for the particles to retain in the reactor also increases. In other words, a particle should be faster to retain in the reactor as the H/D ratio increases. Thus, higher H/D ratio in SBRs (such as 20-30), supports hydraulic selection by selecting more settlable granules via taking the advantage of settling velocity differences (Liu and Tay, 2004; Beun et al., 1999). Beun et al. (2002), who operated an SBAR at the H/D ratio of 16, claimed that high H/D ratios decreased the reactor footprint by providing enhanced oxygen transfer and more efficient use of the reactor volume since the reactor was only occupied by fast-settling particles.

According to Heijnen and van Loosdrecht (1998), the minimum settling velocity for aerobic granulation was defined to be above 10 m/h. Hence, H/D ratios about 20-30 are used in aerobic granulation studies to provide that threshold settling velocity (Liu and Tay, 2007b; Wang et al., 2007b; Li et al., 2006a). Kong et al. (2009) achieved granulation in reactors with H/D ratios between 4-24, corresponding to the settling velocities between 12-2 m/h. However, it was revealed that the studied H/D ratios from 4 to 24 had no considerable impact on aerobic granule formation and properties such as granule volume percentage, settleability, size, density, SOUR, EPS content, microbial species and the shift of species during the start-up period (Kong et al., 2009). Tsuneda et al. (2003a) developed nitrifying granules setting the H/D ratio as 64 (Liu et al., 2005b). Unfortunately, the H/D ratio of 64 is unfeasibly high for real-life applications (Kong et al, 2009). Liu et al. (2005a) concluded that, although the particles with the desired settling velocity can be retained in SBRs by adjusting the H/D ratio, aerobic granulation was not very much dependent on the H/D ratio which was not a selection pressure for aerobic granulation.

2.2.5 Solids retention time (SRT)

The SRT is determined by the amount of sludge that is discharged from the reactor, which is mainly dependent on the hydraulic selection pressure provided by the settling time for aerobic granular SBRs. The VER, temporal parameters for reactor operation (such as cycle duration, discharge time etc.), and sludge characteristics are also effective. Since the granulation process involves the enrichment of fast-settling species that tend to aggregate, and discharging the remaining suspended sludge portion; low SRT values are essential at the commencement of the granulation from flocculent activated sludge (Liu et. al, 2005a; Liu and Liu 2006).

Pan (2003) operated SBRs in order to achieve aerobic granulation. The results showed that, although the aerobic granulation in SBRs was obtained at a wide range of SRT values (1-40 days), the SRT values up to 30 days had negligible effect on aerobic granule development process (Li et al., 2008b; Liu et al., 2005a).

Li et al. (2008b) investigated the effect of SRT in an aerobic granular SBR. In order to provide negligible hydraulic selection pressure, the SBR settling time was adjusted to 30 minutes, which can prevent the selection of bacteria according to their settling velocity. Failure of granulation was reported at the studied SRTs between 3-40 days. Supporting Liu et al. (2005a), to favor heterotrophic aerobic granulation, Li et al. (2008b) recommended that the hydraulic selection pressure should be created according to the minimum particle settling velocity instead of controlling the SRT, which had no significant effect on aerobic granulation.

Liu and Liu (2006) stated that aerobic granular reactors operated with long SRT values tend to experience filamentous growth due to the inverse proportion between SRT and specific microbial growth rate. Slow-growing species such as filaments are supported at long SRT values (>10 days) (Richard, 1989), while lower SRT operations promoted the enrichment of fast-growing microbes such as floc forming bacteria. According to Lin (2003), a small SRT of 10 days provided stable and small granules; whereas the granules cultivated at 70 days of SRT were fluffy and filamentous, and were inevitably washed-out.

Due to the increasing settleability of the reactor content through the continuing stages of the granulation process, a stepwise stabilization is likely to be observed in SRT values (e.g. at 25 days). In their study independent of SRT, Beun et al. (2000) claimed that during the formation and maturation of aerobic granules in the 50 days of operation via SBARs, the SRT first increased from 2 to 30 days, then dropped to 17 days, and stabilized at 9 days (Li et al., 2008b). Generally, the aerobic granular SBRs are not driven by an SRT-based control strategy for the improvement of granulation. Instead, the SRT varies with sludge settleability under the main driving force, i.e. the hydraulic selection pressure in terms of settling time (Liu and Liu, 2006). Although Tchobanoglous et al. (2004) stressed the essence of SRT adjustment in aerobic granular SBRs, SRT, which is influenced by settling characteristics of the sludge and the reactor settling time, is not an obligatory control parameter for aerobic granulation in SBRs.

2.2.6 Hydrodynamic shear force and superficial upflow air velocity

Hydrodynamic shear force, which enhances granulation, is provided by upflow air velocity for aerobic granule development. Hydrodynamic shear force, which is one of the fundamental aspects for the accomplishment of granulation, influences the cultivation and characteristics of aerobic granules (Dulekgurgen et al., 2008). Shear force favors aerobic granulation by applying selective pressure; hence the granule-forming microorganisms retain in the reactor while the ones that can not contribute the granular structure are eliminated. Furthermore, shear force affects the granular density and strength. The EPS secretion is enhanced under high shear force provided by high upflow air velocity (Schmidt and Ahring, 1996).

The minimum upflow air velocity for aerobic granulation in column type SBRs is defined as 1.2 cm/s (Tay et al., 2001a). Tay and Pan (2002) observed successful granulation at superficial air velocity of 2.5 cm/s. The results showed that the superficial air flow velocity should not be below 0.3 m/s, otherwise the aerobic granulation was observed to fail in SBRs. The reason was that the flocculent sludge was unable to form aggregates at low upflow air velocity below 0.3 cm/s. If enough shear force was provided, the surface hydrophobicity of the cells would increase due to the changes in EPS content. Higher EPS and cell surface hydrophobicity were reported under high shear force (Tay and Pan, 2002).

2.2.7 Starvation and intermittent feeding strategy

The tendency of microorganisms to aggregate under starvation conditions was explained as a defense mechanism against starvation (Tay et al., 2001a; Liu and Tay, 2004). Tay et al. (2001a) reported that the microorganisms that are exposed to starvation are capable of modifying their surface characteristics. Starvation influences the aggregation tendency of microorganisms via changing their hydrophobicity. The studies on xenobiotic-degrading (Sanin, 2003; Sanin et al., 2003) and e.coli bacteria to (Chen and Strevett, 2003), revealed that the starvation for N decreased the hydrophobicity, and in turn the aggregation tendency of cells via increasing their PS content. On the other hand carbon starvation increased/had no effect on the hydrophobicity of e.coli (Chen and Strevett, 2003) and xenobiotic-degrading bacteria (Sanin, 2003; Sanin et al., 2003), respectively.

Due to the cyclic SBR operation consisting of feeding, aeration, settling, and discharge periods, the biomass inside the reactor is exposed to various environmental conditions including aerobic substrate starvation (Liu and Tay, 2004; Tay et al., 2001a). The aerobic starvation phase occurs after the substrate is degraded extensively in aerobic period. In starvation phase, the external substrate is not available for the microorganisms (Liu and Tay, 2004). The control of the aerobic starvation phase in SBR operation may fasten the granulation rate while influencing granular stability (Gao et al., 2011; Liu and Tay, 2008a). The starvation period, which is related to the cycle duration, may take up more than 80% of the aeration period (Liu and Tay, 2006).

Starvation favors granulation by promoting microbial aggregation via increasing the cell hydrophobicity (Bossier and Verstraete, 1996; Liu and Tay, 2004, Liu et al., 2007c) and enhancing EPS secretion (Tay et al., 2001a; Liu and Tay, 2008a; Gao et al., 2011). Long starvation periods may provide stronger and denser granules by the selection of microorganisms with higher EPS secretion capability (Liu and Tay, 2004; Gao et al., 2011; Tay et al., 2001a; Li et al., 2006b). However, the treatment efficiency and characteristics of granules (such as settleability and density) may deteriorate under too long or too short starvation periods due to the changes in EPS polysaccharide/protein ratio (Gao et al., 2011; Liu et al., 2007b; Liu and Tay, 2008).

Li et al. (2006b) reported that aerobic starvation phase during SBR start-up initiated aerobic granulation, by decreasing the negative surface charges and increasing the relative hydrophobicity of the granules. Liu and Tay (2008a) operated 3 SBRs at different cycle times of 1.5, 4 and 8 h, respectively, and provided different starvation periods. The study showed that short cycle times such as 2 h resulted in short starvation periods that fasten granulation by increasing the hydraulic selection pressure; however forming fluffy large unstable granules with poor settleability. On the other hand, longer cycle times (4-8 h) provided longer starvation periods that lead to slower granulation while forming more stable granules allowing long-term operation. Wang et al. (2006a) subjected 4 aerobic granular batch reactors to C,N,P, and K starvation, respectively. Although all of the reactors experienced EPS content decrease, deterioration of settleability and structural integrity, and inhibitied microbial activity, the starvation of the studied nutrients did compeletely destroyed the aerobic granular stability.

Intermittent feeding strategy is applied in SBRs in order to provide starvation which enhances granulation. In aerobic SBRs, the microorganisms are subjected, firstly, to high carbon concentrations in the aeration period just after the feeding. Since the aeration period continues even after the substance is consumed, the aerobic bacteria are exposed to long-term starvation (Tay et al., 2001a). The periodic starvation phase during the cyclic SBR operation and cell surface hydrophobicity were stated to be proportionally related (Liu and Tay, 2004; Tay et al., 2001a; Liu et al., 2003c). Intermittent SBR feeding strategy and pulse feeding provided compact and dense aerobic granulation; since starvation increased the cell surface hydrophobicity which favored aerobic granulation by promoting bacretial aggegation and adhesion (Bossier and Verstraete, 1996; Liu and Tay, 2004, McSwain et al., 2004b).

2.2.8 Presence of divalent metal ions

Divalent cations such as Ca^{+2}, Mg^{+2} and Fe^{+2} have supporting effects on granulation rate and granular properties (Adav et al., 2008a). Especially, Ca^{+2} is reported to favor anaerobic (Yu et al., 2001) and aerobic granulation (Jiang et al., 2003; Ren et al., 2008; Liu et al.,2010b); while the positive effects of Mg^{+2} augmentation on aerobic granulation (Li et al., 2009; Liu et al., 2010b) are also reported (Gao et al., 2011). In contrast, the adverse effects of Ca accumulation of granular bioactivity was also indicated (Ren et al., 2008).

Liu et al. (2010b) augmented an aerobic granular SBR with 40 mg/L Ca^{2+} and the other one with 40 mg/L Mg^{2+}. The calcium-augmented reactor showed faster granulation, yielding better settleability, compactness and density; while the magnesium augmentation provided granules with higher polysaccharide and protein content, faster biodegradation capability and higher species diversity dominated by *β-proteobacterium.* The study indicated the significance of Ca^{2+} augmentation on physical characteristics of aerobic granules, whereas improved biological features were provided via Mg^{2+} augmentation. Addition of Ca^{+2} about 100 mg/L, is reported to fasten the aerobic granulation process by enhancing bacterial adhesion via binding the negative charges of the extracellular polysaccharides on the bacterial surface.

Calcium cations augmentation decreased the granulation period from 32 to 16 days. The settleability, strength and polysachharides content of the granules were also improved. Polysaccharides contribute the structural integrity of aerobic granules by forming a strong sticky nondeformable polymeric gel-like matrix (Liu and Tay, 2004; Jiang et al., 2003). Ren et al. (2008) reported that calcium augmentation created calcium precipitates mainly in the form of $CaCO_3$ in aerobic granular nuclei. The calcium-rich granules showed higher rigidity and strength compared to the granules cultivated without calcium. On the other hand, at calcium addition more than 20 mg/L, granules showed decreased SOUR, thus deteriorated bioactivity. Similarly, Li et al. (2009) observed that the amendment of 10 mg/L Mg^{+2} fastened the granulation process while forming larger aerobic granules with higher EPS content.

The supporting effects of divalent cations are explained by various mechanisms enhancing microbial aggregation and thus granulation. The mechanisms include; neutralization of negative charges on microbial surfaces via inorganic ions (including Ca^{+2} and Mg^{+2}) (Morgan et al., 1990); enhancement of microbial aggregation by nucleation around cation precipitates (especially Ca^{+2}); enhancement of granulation by the cation bridges by ionic bonds between aggregates and inorganic ions (including the divalent cations such as Ca^{+2} and Mg^{+2}) (Gao et al., 2011). The granular structure is strengthen by the cross links between EPS and Ca^{+2}, after granulation (Gao et al., 2011; Yu et al., 2001; Ren et al., 2008). Gao et al. (2011) observed the augmentation of Ca^{+2}, resulted in calcium precipitates in granular structure; however precipitation was not observed for Mg^{+2}addition. The promoting effect of divalent cations of calcium and magnesium was stressed by Gao et al. (2011), stating their augmentation is not indispensable for granulation.

2.2.9 Dissolved oxygen, pH, temperature

Dissolved oxygen, pH, temperature are effective operational parameters for aerobic granular sludge technology, although they are not decisive.

Despite its influence on operation of aerobic granular systems, DO concentration is not a decisive parameter in aerobic granule formation. Aerobic granulation was accomplished at a wide range of DO concentrations, low as 0.7-1.0 mg/L, and high as above 2 mg/L (Peng et al., 1999; Tay et al.,2002c; Liu and Tay, 2004; Liu et al.,2005a). Many authors concluded that, compared to the effects of pH and temperature, DO concentration is not a key parameter in aerobic granulation if aerobic condition and shear force are provided via adequate aeration (Liu and Tay, 2004; Liu et al., 2005a; Gao et al., 2011). However, it was also claimed that DO concentration and substrate removal kinetics were more effective over shear force from the aerobic granulation point of view (McSwain and Irwine, 2008). McSwain and Irwine (2008), operated 2 SBRs at same shear force of 1.2 cm/s, applying 2 different DO concentrations (8-8.5 mg/L and less than 5 mg/L DO). Despite the high shear force, granulation failed below 5 mg/L DO. The other part of the study investigated the effect of shear force with 1 SBR operated at gradually decreasing superficial upflow air velocities from 1.2 to 0.4 cm/s. This paper was of debate due to the granule cultivation at low DO levels with the inclusion of anoxic conditions sequencing the aerobic period (Gao et al., 2011; Vlaeminck et al., 2008).

The pH and temperature are not key parameters for aerobic granulation, unlike their significance to anaerobic granulation (Liu and Tay, 2004). However, they are known to influence the treatment performance and microbial characteristics of aerobic granular systems (Bao et al., 2009; Yang et al., 2008; Adav et al., 2008a).

Despite the effect of pH on microbial growth rate and species selection, there is no detailed information about the effect of pH on aerobic granulation (Adav et al., 2008a). The pH influence on granular species, morphology, strength and formation rate was observed by Yang et al. (2008), who proposed that aerobic granulation with desired species, structure and rate may be provided via pH adjustments. The operation at pH 3 yielded fast development of fungi-dominated fluffy loose large (7 mm) aerobic granules; wheras the slower cultivation of bacteria-dominated compact relatively smaller (4.8 mm) granules was observed at pH 8.1. Although the granules were similar in terms of treatment performance, settleability and EPS distribution the structural strength of bacterial granules were higher than that of fungal.

Aerobic granulation studies are carried out commonly at room temperature of 20–25 °C (Adav et al., 2008a). Temperature affects the stability, microbial activity, species, and hence treatment performance of the aerobic granules (de Kreuk et al., 2005a; Bao et al., 2009; Song et al., 2009). Despite the successful granulation study at 10°C (Bao et al.,2009); de Kreuk et al. (2005a) observed the unsuitability of cold climates like 8°C for aerobic granule formation, supporting Adav et al. (2008a). Song et al. (2009), who compared the aerobic granule formation at 25-30-35°C, claimed the optimum temperature for granulation was 30°C in terms of stability, compactness, settleability and bioactivity. The operation at 30°C yielded 97% COD, and 75% total P removal. de Kreuk et al. (2005a) investigated the effect of temperature on aerobic granulation in the range of 8°C - 25°C. The reactor start-up at 8°C resulted in formation of unstable and filamentous granules with low N removal efficiency. According to de Kreuk et al. (2005a) at cold temperatures the decreased activity in the outer layers of granules increased the oxygen penetration depth and decreased the denitrification efficiency of the granules. In contrast, when the reactor start-up was at 20°C, and then the temperature was gradually decreased to 15°C and finally down to 8°C, stable granule cultivation was accomplished. The development of aerobic granules at the thermophilic temperature range was studied by Zitomer et al. (2007), who obtained aerobic granular sludge accompanied by bioflocs at 55°C. Since the temperature increase brings lower solubility for gases including oxygen, the applicability of aerobic granular sludge technology was claimed to be unfeasible and uneconomical at termophilic conditions (Gao et al., 2011). Bao et al. (2009) explored the aerobic granulation and nutrient removal performance in an SBAR operated at a low temperature of 10°C. At stable operation aerobic granules with clear compact surfaces were successfully formed with the average size of 3.4 mm. The COD, NH4-N and PO4-P removal performances reached 95%, 82% and 98%, respectively. It was seen that the reactor operation at low temperature of 10°C did not influence phosphorus removal efficiency; although the nitrification was adversely affected, supported by the observation of intermittent nitrite accumulation. It was stressed that the temperature was not a key factor affecting aerobic granulation.

2.2.10 Seed sludge

Most of the aerobic granulation studies employ active sludge as seed (Liu and Tay, 2004; Adav et al., 2008a), despite a few studies where the anaerobic seed sludge was used (Hu et al., 2005a; Muda et al., 2010). The properties of seed sludge such as bacterial composition, settling velocity, surface characteristics and microbial activity are effective on granulation process in terms of granulation rate and granular characteristics. High hydrophobicity and low surface charge of the seed sludge provide faster granulation by enhancing aggregation of microorganisms, hence improved settleability (Adav et al., 2008a; Liu and Tay, 2004). Since the membrane bioreactor (MBR) sludge is more hydrophobic than the conventional bioreactor sludge (Harper et al., 2006), it may be suitable to develop aerobic granules. Erşan (2013) investigated the effect of seed sludge type on aerobic granulation by comparing the conventional activated sludge (CAS) and membrane bioreactor sludge (MBS). Although aerobic granulation was achieved in both of the sludge types, the results revealed that MBS was more efficient as seed sludge for aerobic granulation. The granulaes developed by MBS were non-filamentous and had smooth structure, high settleability, high biomass retention, toxicity resistance, acclimation capability to new conditions, higher recoverability and ability to maintain structural stability at higher loading rates of 3.93 g COD/Lday and 0.86 g N/Lday. The CAS-developed granules were filamentous and had lower treatment performance (12% TAN and 36% COD) than that of the MBR-developed ones (37% TAN and 70% COD).

2.2.11 Inhibition to aerobic granulation

The substances that may pose inhibitory effects on aerobic granules include free ammonia (Yang et al., 2004a,b). Yang et al. (2004a,b) claimed that free ammonia obstructed aerobic granulation by repressing the biomass metabolism. The inhibition is characterized by drastically decreased cell hydrophobicity, and limited extracellular polysaccharide production which may eventually lead to collapse of the reactor. The maximum free ammonia threshold concentration for successful aerobic granulation was stated to be 23.5 mg/L NH_3-N (Yang et al., 2004a,b). Complete inhibition of nitrification was reported above 10 mg/L free ammonia concentration. It was seen that, with the increase in free ammonia concentration from 2.5 to 39.6 mg/L NH_3-N, cell surface hydrophobicity decreased from 70 to 40%, obstructing microbial aggregation (Gao et al., 2011; Yang et al., 2004a). In addition, the SOUR of heterotrophic bacteria dropped 5 times, while the nitrifiers experienced SOUR decrease by a factor of 2.5. The SOUR decrease indicated the deteriorated biological activity due to the presence of free ammonia (Yang et al., 2004a,b; Liu and Tay, 2004). As stressed by Adav et al. (2008a), the literature lacks research on the inhibitory substances to aerobic granulation as well as the mechanisms involved in ammonium inhibition.

2.2.12 Carbon to nitrogen ratio in feed

Effect of influent COD/N ratios on aerobic granular characteristics in terms of formation, species, morphology, elemental composition and treatment efficiency was studied by Liu et al. (2003b), Yang et al. (2005), Li et al. (2011), Cydzik and Baryla (2011), Wu et al. (2012) and Luo et al. (2014). COD and nitrogen (COD/N, COD/TAN etc.) of the substrate, have direct effect on the composition and characteristics of the aerobic granules (Liu et al., 2003b). It was found that aerobic granulation was possible at COD/N ratios of 1-20 (Yang et al., 2005; Wu et al., 2012; Liu et al., 2003b). The amount of nitrifiers in the aerobic granules increase with decreasing COD/N ratio (Liu et al., 2003b; Wu et al., 2012; Yang et al., 2005; Cydzik-Kwiatkowska and Wojnowska-Baryla, 2011; Luo et al., 2014).

Liu et al. (2003b), who first showed the influence of influent C/N ratio on elemental composition, microbial distribution and characteristics of aerobic granules, operated 4 SBRs with influent COD/N ratios from 20 to 3.3. Decreasing COD/N ratio from 20 to 3.3 yielded smaller granules rich in nitrifiers, while influencing their elemental composition in terms of O, N, Ca and C. It was proved that the nitrification efficiency was adversely affected by organic carbon presence and substrate COD/N ratio influences the distribution of heterotrophic and nitrifying bacteria in biofilms. Wu et al. (2012), who operated 4 SBRs with influent COD/N ratios as 0-1-2 and 4 to explore the microbial population shift in aerobic granular sludge, claimed that nitrifiying populations are enhanced at low COD/N ratios such as 2. The SBRs with COD/N ratios of 1 and 2 yielded granules with clear outer shapes, however there was no granulation in those with COD/N ratios of 4 and 0, which was attributed to either excess or limited EPS production. It was seen that rod shaped bacteria dominated the granules at COD/N ratio of 1, while those at 2 were rich in filaments. The study showed that as the ratio increased from 0 to 4; the denitrification efficiency increased from 72 to 88%. Li et al. (2011) developed nitritation granules in an SBR by gradually decreasing the influent C/N ratio from 8 to 2, which favored ammonia oxidizing bacteria (AOB) rather than the nitrite oxidizing bacteria (NOB) in the aerobic granules. Over 95% ammonia removal efficiency was reported. The nitrite accumulation indicating partial nitrification, was attributed to the difference between the amounts of AOB and NOB in the granular structure. The study of Yang et al. (2005) reveals that the anoxic denitrification performance increases with decreasing

COD/N ratio from 3.3 to 10. The specific N removal rates in the anoxic period of SBR operation with 0.5 mg/L DO were measured for the reactors with COD/N ratio of 10, 5 and 3.3; the results were 0.42-0.85-0.91 mg N/g SS min, respectively. It was seen that the mg TON converted to $N_{2(g)}$ by denitrification was highest for the reactor with COD/N ratio of 3.3, and the lowest for the ratio of 10.

The co-existence of denitrifying and nitrifying populations in aerobic granules allow complete N removal by simultaneous nitrification and denitrification (Liu and Tay, 2004; Yang et al, 2004c; Jang et al., 2003). High N/COD ratios (i.e. low COD/N ratios) are reported to favor denitrifying and nitrifying populations, while the aerobic heterotrophic populations are adversely affected (Liu and Tay, 2004; Liu et al., 2003b). The COD/N ratio decrease from 20 to 3.3 enhanced nitrifiers and denitrifiers, while the heterotrophic populations were not favored in aerobic granules (Yang et al.,2005).

The literature research on COD/N ratio effect on aerobic granulation was generally limited to a carbon/nitrogen range of 1 to 20. The substrate C/N ratio effect on aerobic granulation and biogranular structure has been investigated; whereas the optimum range for both high COD and N removal has not been determined yet. The aim of this thesis includes the determination of the optimum influent COD/TAN ratio and COD, TAN concentration range that provides the maximum TAN and COD removal efficiencies, denitrification and stable aerobic granules. To this purpose, the effect of different influent COD/TAN ratios, in a wider range of 1 – 30, on aerobic granular sludge was investigated in terms of carbon and nitrogen removal efficiency, denitrification - nitrification performances and granular stability.

2.2.13 EPS

Extracellular polymeric substances (EPS), which are defined as sticky substances secreted by microorganisms, contribute to cell adhesion and microbial matrix formation of granules, promoting their stability and structural integrity (Liu et al., 2004a). Microbial cultivation involves EPS secretion as a survival mechanism under stressful conditions to provide stable cell membrane and act as a protective barrier. Microbial EPS production increases due to the changes in surface hydrophobicity as a response to stressful envionmental conditions including high shear force, high selective pressure (i.e. low settling time in SBRs), long-term starvation, nutrient limitation, high pH and temperature (Tay et al., 2001b; Liu et al., 2003d; Qin et al., 2004a,b; Nichols et al., 2004). The high cell surface hydrophobicity resulting from environmental stress is thought to initiate aerobic granulation by favoring microbial adhesion (Liu et al., 2003d; Liu and Tay, 2004; Qin et al., 2004a).

Although the sequence of cell aggregation and EPS secretion is not entirely explained, it may be possible that cell aggregation facilitates EPS secretion by providing the essential conditions (Liu et al., 2004a). Thus, EPS promotes the formation, stability and structural integrity of biogranules by enhancing cell aggregation; via preventing the repulsion between cells by decreasing their negative surface charge density (Tsuneda et al., 2003b), via forming a bridge to physically bind the adjacent cells (Liu et al., 2004a; Shen et al., 1993 ; Schmidt and Ahring, 1994; Tsuneda et al., 2003c), and via forming extensive binding sites around the cells (Liu et al., 2004a; Yu et al., 2001; Sponza, 2002). On the other hand, excess EPS secretion may destroy the granular structure and/or favor the repulsive forces among bacteria (Schmidt and Ahring, 1996).

The decreased EPS production with increasing depth in heterotrophic biofilms (Zhang and Bishop, 2001) was attributed to the lower microbial activity caused by lower nutrient availability in the deeper parts of the biofilms (Liu et al., 2004a). EPS can be used as secondary substrate at deeper sections of biofilms and biogranules in case of substrate deficiency. Biogranular strength and stability are also affected by spatial EPS distribution, since the EPS on the edges of the granules provided higher shear strength, than centrally located EPS (Batstone and Keller, 2001).

Biogranular EPS contains protein (PN), polysaccharides (PS), nucleic acids, humic-like substances, lipids, and heteropolymers such as glycoproteins at varying quantities (Nielsen et al., 1997). The amount and contents of EPS are influenced by type, growth phase and physiological state of microbial species, including the shift of microbial species during granulation (Etchebehere et al., 2003; Yi et al., 2003), as well as operational conditions including oxygen and nutrient limitations, ionic strength, temperature, shear force, and many other factors (Liu et al., 2004a). Granules cultivated on protein-rich substrate were reported to secrete protein-rich

EPS, whereas EPS with high PS content is produced by granules fed by other types of substrates (Batstone and Keller, 2001).

The ratio of carbohydrate to protein, i.e. PS/PN, in biogranular EPS is of debate (by researchers). Predominant component of EPS was reported to be carbohydrates (Fang et al., 2002), while Fukuzaki et al. (1995) stated the domination of protein in anaerobic granules. The PN/PS ratio, which was reported to be between 2-16 by weight, is significant for aerobic granulation since the amount of cellular PS was reported to increase significantly with respect to PN during the formation of aerobic granules (Tay et al., 2001b,c,2002d; Punal et al., 2003). PS is known to form a bioglue and improve the structural strength by forming a polymeric matrix that enhances the cellular interactions (Liu et al., 2004a). Since PS is generally higher than PN, the higher PS/PN ratios provide more compact and stronger aerobic granules with higher specific gravity (Tay et al., 2001b,c,2002d; Liu and Tay, 2002d; Qin et al., 2004a,b). The PS/PN ratio was also reported to increase in SBR operation with increasing environmental stress via high shear force, short settling time, short HRT, and periodical feast-famine regime (Tay et al., 2001b, 2002d; Liu et al., 2003a; Qin et al., 2004a,b). Deterioration of strength and settleability was reported for anaerobic granules and aerobic bioflocs with low PS/PN ratios (Liu et al., 2004a; Batstone and Keller, 2001; Martinez et al., 2004). In contrast, Adav and Lee (2008) claimed that PS might be lower than PN which resulted in a PN/PS ratio of 3.4-6.2 (i.e. PS/PN ratios of 0.16-0.29) for the mature granules.

2.2.14 Non-aerated periods in SBR cycle

Presence of non-aerated periods in SBR cyclic operation was studied by many researchers (Jang et al.,2003; Qin and Liu, 2006; Yilmaz et al., 2008; Adav et al., 2009c; Wan et al., 2009; Su et al., 2012; Ersan and Erguder, 2013). Formation of denser, more stable and compact aerobic granules rich in denitrifiers was reported in the presence of anoxic (and anaerobic) phase of the SBR cycle (Wan et al., 2009; Zhang et al., 2011a; Pijuan et al., 2009; Su et al., 2012). Considering SBR with pre-anoxic operation, in other words aerobic period sequencing the anoxic period, major portion of the organics is consumed during the non-aerated period. Hence, the sequencing aerobic period will be subjected to longer starvation time, which was known to promote cell aggregation (de Kreuk and van Loosdrecht, 2006) (also stated in Section 2.2.7). Furthermore, the filamentous growth arising from the lack of DO and substrate in the granular core, may be prevented in the presence of non-aerated periods in SBR cycle (Adav et al., 2009c); which was attributed to the enrichment of denitrifiers in the granular core. Ersan and Erguder (2013) compared the effect of anoxic/aerobic period sequence in aerobic granular SBR operation. Aerobic granulation was achieved in 2 SBRs operated by aerobic-anoxic cycles and anoxic-aerobic cycles. Although the carbon and nitrogen removal performances were similar for both SBRs, the pre-anoxic SBR yielded larger granules with higher stability while the complete denitrification was seen during anoxic period. On the other side, granular disintegration due to the carbon deficiency and resulting nitrate accumulation were reported in post-anoxic SBR.

2.3 Applications of Aerobic Granules

Aerobic granular systems can adopt to many different types of wastewater at various loading rates (Liu and Tay, 2004). Due to the high settleability, metabolic activity, biomass retention, resistance to toxic and shock loads due to the defence of EPS matrix, the aerobic granulation poses a wide application area for wastewater treatment (Adav et al., 2008a). The aerobic granular systems have been used for the treatment of numerous synthetic, domestic and industrial wastewaters in terms of carbon, nitrogen and phosphorus, organic toxic compounds, dyestuffs, heavy metals and even nuclear wastes (Adav et al., 2008a; Gao et al., 2011).

2.3.1 Laboratory scale studies

2.3.1.1 Removal of organic carbon

2.3.1.1.1 Synthetic wastewater treatment

As also stated in Section 2.2.1.1, aerobic granular technology is capable of removing numerous types of organics including ethanol, acetate, glucose, phenol, starch, molasses, peptone, fecula, sucrose, phtalic acid. The studies employing synthetic wastewater with acetate (Dulekgurgen et al., 2003; de Kreuk et al., 2005b; Kishida et al., 2006; Chen et al., 2008; Adav et al., 2010; Cydzik and Baryla, 2011; Peyong et al., 2012; Bassin et al., 2012), with glucose (Jang et al., 2003; Li et al., 2008a; Kim et al., 2008; Thanh et al., 2009), glucose-acetate (Tay et al., 2001a; Tay et al., 2002c; Moy et al., 2002); ethanol (Qin and Liu, 2006; Wu et al., 2012), sucrose (Zheng et al., 2006), their combinations with propionate (Wan et al., 2009) or with peptone and fecula (Sun et al., 2006) genereally yielded 80-100% COD, 79-100% NH_4^+-N, 71-100% total N, 94-100% total P removal efficiencies. Phthalic acid containing synthetic wastewater was also treated by aerobic granules with a total organic carbon (TOC) removal efficiency of 100% (Zeng et al., 2007). Yet, very low removal efficiencies were also reported by Wu et al. (2012) who obtained 59% COD and 26% NH_4^+-N removal efficiencies, and by Kim et al. (2008) who reported 48-50% NH_4^+-N and 50% total N removal efficiencies. Wu et al. (2012) reported a very low NH_4^+-N removal efficiency of 26%, which was attributed to the incapability of nitrifiers to grow at an high organic substrate concentration (and COD/N ratio as high as 4). Wu et al. (2012) also reported COD removal efficiency as low as 59%, which may be attributed to the decreased performance of heterotrophic bacteria at a low COD/N ratio of 1. Kim et al. (2008) reported TN removal efficiency as low as 50% which was attributed to the low denitrification efficiency at the loading rates as low as 1.76 g COD/Lday OLR and 0.1 g NH_4^+-N/Lday. With increasing loading rates to 2.52-2.84 g COD/Lday OLR and 0.14-0.16 g NH_4^+-N/Lday nitrogen loading rate (NLR), Kim et al. (2008) observed NH_4^+-N removal efficiencies as 48-50%, which were attributed to the limited mass transport to inner parts of the granule since most of the oxygen was consumed for the formation of new aerobic granules.

High strength synthetic organic wastewater treatment is possible by aerobic granular systems due to their dense compact structure with high biomass retention (Liu and Tay, 2004). Moy et al. (2002) used aerobic granules to treat high-strength organic wastewater with stable COD treatment efficiency over 89%. Liu et al. (2003a) indicated the independency of granulation from substrate concentration, stating that the granule development was possible at influent COD concentrations from 500 to 3000 mg/L. Instead, oxygen mass transfer was thought to limit the aerobic granular COD removal performance (Gao et al., 2011). Aerobic granulation technology applications for the treatment of wastewaters with high concentrations and OLRs (> 5–7 g COD/Lday) may be disadvantageous in terms of operational costs for aeration and mass transfer limitations. Instead high-rate anaerobic reactors, like UASB, can be used since they do not require aeration while producing biogas (Gao et al., 2011). Yet, the advantages of coupled nitrogen removal via aerobic granules in the same reactor should be also taken into consideration.

2.3.1.1.2 Treatment of domestic, municipal and urban wastewaters

Low-strength domestic wastewater (Liu and Tay, 2007a) was treated by aerobic granules at 25°C and 35^{0}C. Municipal wastewater and landfill leachate were treated by an aerobic granular sequencing batch biofilter reactor by di Iaconi et al. (2007). Municipal wastewater was treated with over 87% TKN removal efficiency at an OLR of 5.7 g COD/Lday and NLR of 0.8 g TKN/Lday. The maximum OLR was 7 g COD/Lday which yielded effluent COD concentrations lower than 50 mg/L. The landfill leachate was treated at an OLR of 1.1 g COD/Lday and 80% of COD content was removed, corresponding to all biodegradable portion. The landfill leachate with high COD (4298-5992 mg/L) was treated with high removal efficiencies up to 84% COD, 96% NH_4^+-N and 75% TN by Wei et al. (2012). de Kreuk and van Loosdrecht (2006) developed aerobic granules with pre-settled domestic wastewater at an OLR of 1 g COD/Lday and stated that high COD loading was a key factor for the aerobic granulation.

2.3.1.1.3 Industrial wastewater treatment

Despite the capability of aerobic granulation technology for industrial wastewater treatment, the literature lacks information about the performance of such systems since most of the studies employed synthetic wastewater (Adav et al., 2008a; de Bruin et al., 2004; de Villiers and Ibala, 2003; Schwarzenbeck et al., 2004b). As previously mentioned (Section 2.1.1), numerous industrial wastewaters have been employed in aerobic granule formation and/or treatment studies. A summary of these studies are given in Table 2.1.

Table 2.1. Industrial wastewater studies employing aerobic granules.

Wastewater type	**Parameter (mg/L)**	**Ratio**	**Loading rate (g/Lday)**	**[f]Treatment efficiency (%)**	**Ref[e]**
[a]Papermaking ww	tCOD: 2100-3000 TSS: 1050	COD/TN:52-130	COD:[b]4.1	[b]COD>90 [b]TSS: 97-98	1
Dairy products ww	tCOD: 2800 sCOD: 1500 TN : 140 TSS: 300	[b]tCOD/TN:20 [b]sCOD/TN:10.7	tCOD: 4.5-5.9 sCOD:2.4- 2.9 TN:[b]0.23-0.3	tCOD: 90 TN: 80 TP:67 [b]sCOD: 92	2
Abattoir ww with anaerobic treatment	tCOD: 1480 sCOD: 1072 NH4-N: 222 TN: 237 TSS: 205	tCOD/NH4-N: 6.7 sCOD/NH4-N: 4.8	tCOD:2.7 sCOD:1.9 NH4-N:0.4 TN:0.43	tCOD:68 sCOD:85 TN: 86 NH4-N:100	3
Brewery ww	tCOD: 1300-2300 sCOD: 1000-2000 NH4-N:15-28 TN: 30-37	COD/NH4-N:83.7[c] COD/TN:53.7[c] COD/N/P ratio of 100/10/1 via nutrient addition	tCOD:2.6–4.6 sCOD:2-4 NH4-N:0.18-0.32	tCOD: 89 [b]sCOD:91 TN: 89	4
40% domestic 60% industrial ww	sCOD: 1000 NH4-N: 60	sCOD/NH4-N: 16.7	-	sCOD: 80 NH4-N:98 Total inorganic N:50	5
Industrial ww rich in toxic organics and anaerobic acidogenic reactor effluent	[a]COD: 1000 -500 NH4-N: 50 TN: 100 TSS: <10	[a]COD/NH4-N: [c]15 [a]COD/TN:[c]7.5	[a]COD:[b]1.5 NH4-N: [b] 0.1 TN:[b] 0.2	[a]COD:80 TN:40 NH4-N:90	6
Malt industry ww	tCOD: 1700 sCOD: 470 NH4-N: 3 TN: 45 TSS: 950	tCOD/NH4-N: 567 tCOD/TN:37.8 sCOD/NH4-N: 157	tCOD:3.2 TN:[b]0.006-0.085	tCOD: 50 sCOD: 80 pCOD:40-46	7
Soybean processing ww	sCOD: 2110±260 TN: 97± 11 TSS: 31±2	sCOD/TN: [b]22	sCOD:6 NH4 –N:0.29	sCOD: 98-99	8
Abattoir ww	tCOD: 7685±646 sCOD: 5163±470 NH4-N: 50±12 TKN: 1057 TSS: 1742± 116 VSS: 1520±128	tCOD/NH4-N: 154 tCOD/TKN: 7.3 sCOD/NH4-N:103.3	tCOD:2.6 -2.7 NH4–N: [b]0.02 TKN:[b]0.37	tCOD>98 sCOD:98 VSS>97 N> 97 P>98	9
Palm oil production ww	[b]sCOD: 6100	-	sCOD:3-6	sCOD: 88 Turbidity:99	10
Petrochemical industry ww	[a]COD: 282± 20 NH4-N: 42.9	[a]COD/NH4-N: 6-7	[a]COD:2 NH4-N:0.16	[a]COD: 89 NH4-N: 94 TN:67	11
Industrial effluents prepared in lab [d]	tCOD: 350-1750 sCOD: 200-1300 NH4-N: 25-220 TSS: 30-900 VSS:20-700	tCOD/NH4-N: [b]6-10	[a]COD:0.7-5 NH4-N:0.15-0.65	sCOD: 60-97 NH4-N:15-76 VSS: [b]17-60	12
(Synthetic) Textile industry ww	[b]sCOD: 600 [b]NH4-N:38-42 sCOD=tCOD=1270 TSS= 0	[b]sCOD/NH4-N :14.3	sCOD:2.4 NH4-N: [b]0.168	sCOD: 94 NH4-N: 95 Color:62	13

Swine slurry	tCOD: 579-15932 sCOD: 560-13689 NH4-N:95-1823 TSS: 100-5900 VSS:90-4900	COD/TN:5.5-8	sCOD:2.2-7 TN:0.39-1.26	TOC: 87 TN:70 TSS: [b]25-30	14
Synthetic ww and dairy products industry ww	tCOD: 500-1800 sCOD : 300-1500 NH4-N: 30-180 TN:25-200 TSS: 200 -900	[b]tCOD/NH4-N: 11 sCOD/NH4-N:8.6	sCOD:1-7 NH4-N: 0.1-0.7	tCOD: 85-95 TN: 80 TSS:80	15
Anaerobically treated abattoir ww	tCOD: 600–783 sCOD: 265–384 NH4-N: 215–240 TN: 225–277 TSS: 185–217	[b]tCOD/NH4-N: 3 [b]tCOD/TN: 2.76 [b]sCOD/ NH4-N: 1.4	sCOD:2.7 TN:0.43	tCOD:68 sCOD: 85 TN: 86 sN: 93 tP:74 sP: 89	16
Dilute palm oil production ww	sCOD: [b] 625 NH4-N: 45 TN: [b] 31.3 TSS:185–217	[b]sCOD/TN:20 [b]sCOD/NH4-N: 13.8	sCOD:2.5 NH4-N:[b]0.125	sCOD: 91 NH4-N: 98 Color:38	17
[a] Rubber ww	COD: 1820 NH4-N: 59 TN: 250 TSS:270	[b]COD/NH4-N: 31 [b]COD/TN:7.3	[a]COD:0.9-3.6 [b]NH4-N:0.03-0.12	COD: 98.4 NH4-N: 92.7 TN: 89.5	18
[a] Textile industry ww and municipal ww	COD: 249 NH4-N: 95 TKN: 34.2 TSS:87	[b]COD/NH4-N: 2.62	[a]COD: [b]0.74-2	COD: 82.1 NH4-N: 95 TKN: 87.5 TSS: 94.7 Total surfactants: 77.1 Colour:33.9-52.6	19
Livestock ww and synthetic ww	TOC: 120-1100 NH4-N: 65-510 TN: 65-650 TSS: 0-2250 TP:12.5-125 PO4-P:12.5-80	[b]TOC/TN: 1.69-1.85 [b]TOC/NH4-N: 1.85-2.6	TOC: 0.15-0.16 NH4-N: 0.06-0.09 TN: 0.09 TSS: 0-0.32 TP: 0.02 PO4-P: 0.01-0.02	NH4-N: over 99 PO4-P: 98.5	20
[a]Dyeing textile ww	COD: 365-1345 TKN: 9.8-32.2 TSS: 45.6-295.2 Total surfactants: 4.3-35.7	[b]COD/TKN: 37-42	[a]COD:0.4-3.4	COD: 54.1-78.3 TKN: 90.4-28.6 TSS: 91.9-29.6 Total surfactants: 65-80.4 Colour:0-38.2	21

[a] Soluble or total COD not defined.
[b]calculated from the related document.
[c]average values
[d] Dairy products, fish canning, sea products industry, pig farm swine slurry were used.
[e] References: 1) Hailei et al., 2006; 2) Schwarzenbeck et al., 2005; 3) Lemaire et al., 2008; 4) Wang et al., 2007a; 5) Liu et al., 2010a; 6) Liu et al., 2011a; 7) Schwarzenbeck 2004a; 8) Su and Yu, 2005; 9) Cassidy and Belia, 2005; 10) Gobi et al., 2011; 11) Zhang et al., 2011b; 12) Val del Rio et al., 2012; 13) Muda et al., 2010; 14) Figueroa et al., 2011; 15) Arrojo et al., 2004; 16) Yilmaz et al., 2008; 17) Abdullah et al., 2011; 18) Rosman et al., 2014; 19) Lotito et al., 2014; 20) Kishida et al., 2009; 21) Lotito et al., 2012.
[f]Average treatment efficiency.

Schwarzenbeck et al. (2004a) developed aerobic granules to treat malting industry wastewater containing high particulate matter about 0.95 g/L TSS. The SBR operation yielded 50% tCOD and 80% sCOD removal efficiencies. Schwarzenbeck (2005) achieved aerobic granulation in an SBR and treated dairy wastwater. The study stressed the inevitability of the presence of SS in the aerobic granular effluents due to the hydraulic selection that rejects less settlable biomass. When the effluent SS was removed via 15-30 min sedimentation, the efficiencies increased to 90% tCOD, 80% total N and 67% total P. Kishida et al. (2009) treated the livestock wastewater in an SBR seeded with aerobic granules, and obtained over 99% NH4-N and 98.5% PO4-P removal efficiencies. The high SS content of the livestock watewater (2250 mg/L) did not influence the granular structure or settleability. This was attributed to the fact that the livestock wastewater was diluted and mixed with SS-free synthetic wastewater during most of the operational period, before the introduction of the non-diluted livestock wastewater. Thus the average SS concentration was gradually increased from 0 to 2250 mg/L during the operation. The SS loading rate varied between 0 and 0.32 g SS/Lday. Introduction of the non-diluted livestock wastewater to the reactor resulted in decreased granular size and denitrification efficiency. This was attributed to the decreased hydraulic selection pressure due to the HRT increase from 0.75 to 7.5 days in order to provide constant loading rates for total N and total P. Yilmaz et al. (2008), who operated an aerobic granular SBR, achieved simultaneous nitrification and denitrification, and P removal from an abattoir wastewater which was initially subjected to anaerobic pretreatment. Although the removal efficiencies for sCOD, soluble N and soluble P were 85%, 93% and 89%, respectively the high solids content of the effluent decreased the overall efficiency to 68%, 86%, and 74% for total COD, TN, and TP, respectively.

Cassidy and Belia (2005), studied aerobic granular treatment of abattoir wastewater. By gradual settling time decrease, fast granule formation was observed in 4 days. The removal efficiencies were over 98% for COD and P, whereas over 97% N and VSS removals were reported. Lemaire et al. (2008) used aerobic granules to treat nutrient-rich abattoir wastewater which was subjected to anaerobic pre-treatment. The breaking of mature granules due to the particulates, colloids, oily substances in the abattoir wastewater was reported. The complex constituents of the wastewater were observed to increase the microbial diversity of the granular structure, providing the secretion of different types of EPS.

Muda et al. (2010) developed aerobic granules via synthetic textile wastewater. The granule size and settling velocity reached 2.3±1.0 mm and 80±8 m/h, respectively, while the granular strength improved. The operation yielded 94% sCOD and 95% NH_4^+-N treatment efficiencies, with 62% color removal. Lotito et al. (2012) treated dyeing textile wastewater in a sequencing batch biofilter granular reactor (SBBGR) with the removal efficiencies of 54.1-78.3% COD, 28.6-90.4% TKN, 29.6-91.9% TSS, 60-80.4% total surfactants and 0-38.2% color. The SBBGR was also used by Lotito et al. (2014) who treated textile wastewater mixed with municipal sewage with the removal efficiencies of 82.1% COD, 95% NH_4^+-N, 94.7% TSS, 87.5% TKN and 77.1% surfactants, while the color removal varied between 33.9-52.6% for different wavelengths between 426 to 660 nm.

Gobi et al. (2011) achieved 88% sCOD removal in aerobic granular treatment of palm oil mill effluent (POME). About 21% of the remaining COD was removed by adsorption on to waste aerobic granules, whereas 99% turbidity removal was obtained. Abdullah et al. (2011) cultivated stable aerobic granules on POME, achieving 91% COD, 98% NH_4^+-N and 38% color removal efficiencies.

Arrojo et al. (2004) operated aerobic granular SBRs with industrial wastewater produced in a laboratory for analysis of dairy products and obtained 70% N and 85-95% tCOD removal efficiencies. Val del Rio et al. (2012) operated 4 aerobic granular SBRs with industrial effluents prepared in lab, namely dairy products, fish canning, sea products industry and pig farm swine slurry. The reactor performances fluctuated between 60-97% for sCOD, 15-76% for NH_4^+-N and 17-60% for VSS removal efficiencies.

Figueroa et al. (2011) studied the removal of organics and nitrogen from swine slurry at different dilution ratios by an aerobic granular SBR. Aerobic granulation was reported providing 5 mm granules capable of removal efficiencies up to 87% and 70% for organics and N, respectively. Hailei et al. (2006) accomplished aerobic granulation with papermaking wastewater, with variable COD treatment efficiencies up to 90% (Table 2.1). Wang et al. (2007a) used brewery wastewater to form 2-7 mm aerobic granules with high settleability. The removal efficiencies were 89% for both tCOD and NH_4^+ -N. Su and Yu (2005) cultivated aerobic granules in an SBR treating soybean-processing wastewater with 98-99% sCOD removal efficiency. Liu et al. (2011a) developed aerobic granules in an SBR treating industrial wastewater rich in toxic organics and effluent from an anaerobic acidogenic reactor. The cultivated granules showed toxicity resistance due to their rougher surface, in comparison to the synthetically-fed granules. There were 80% COD, 90% NH_4^+ -N and 40% total N removal efficiencies. Zhang et al. (2011b) gradually introduced petrochemical wastewater to an SBAR seeded with synthetically developed aerobic granules. As the percent petrochemical wastewater content of the influent increased, the granular characteristics such as settleability, EPS secretion and carbon and nitrogen removal deteriorated, while the granular size decreased. Addition of sodium propionate as co-substrate provided higher removal efficiencies, namely, 89% COD, 94% NH_4^+-N and 67% total N. Liu et al. (2010a) developed stable aerobic granules in a pilot-scale SBR treating real wastewater, including 40% domestic and 60% industrial influents (also mentioned in Section 2.3.2). The study demonstrated over 80% COD, over 98% NH_4^+-N, and 50% total inorganic N removal efficiencies. Rosman et al. (2014) treated rubber wastewater with an aerobic granular SBR at various HRT values from 6 to 24 hours. The removal efficiencies, which increased with decreasing HRTs, were from 72.8 to 98.4% for COD, 73 to 92.7% for NH_4^+-N and 70 to 89.5% for total N removal.

The industrial wastewater studies (also shown in Table 2.1) provided treatment efficiencies in the range of 60-99% sCOD, 50-95% tCOD, 15-100% NH_4^+-N, 40-over 97% TN, 25-98% TSS. Generally sCOD removal efficiency ranged between 80% and 99%, however lower values such as 60% sCOD removal efficiency were also reported (Val del Rio et al., 2012). Schwarzenbeck et al. (2004a) reported 50% tCOD, 80% sCOD and 40-46% pCOD removal efficiencies for malting wastewater with 950 mg/L influent TSS content indicating that the overall COD removal efficiency was decreased by the particulates in the effluent. It was seen that the influent TSS of 950 mg/L made up 89% of total COD. The findings are important, since this thesis study includes the investigation of the use of sugar beet processing wastewater rich in solids in aerobic granular systems.

The studies listed in Table 2.1 include various types of wastewaters with a wide range of solids content as high as swine slurry with 5900 mg/L TSS and 4900 mg/L VSS (Figueroa et al., 2011), and livestock wastewater with 2250 mg/L TSS (Kishida et al., 2009), or wastewaters with low solids content (<100 mg/L TSS) (Liu et al., 2011a; Su and Yu, 2005; Lotito et al., 2014) and even solid-free industrial effluents prepared in the laboratory (Muda et al., 2010). Total solids removal efficiency between 25% to 98% was reported in the studies listed in Table 2.1. The highest solid removal efficiency was obtained by Hailei et al. (2005) who achieved 98% TSS removal for papermaking wastewater containing 1050 mg/L TSS. Removal efficiencies as high as 92-95% TSS were also reported for textile industry wastewaters (Lotito et al., 2012; 2014), while Cassidy and Belia (2005) provided over 97% VSS removal for abattoir wastewater containing 1520 mg/L influent VSS. Lotito et al. (2012) reported TSS removal efficiency as low as 29.6% which was attributed to the detachment and washout of biomass due to the stability problems at high OLRs (3.4 g COD/Lday).

Since the aerobic granular SBR operation is based on the elimination of slow settling biomass due to the application of hydraulic selection pressure (Section 2.2.3), the presence of solids and biomass in the effluent is inevitable. The effluent solids content may even be higher than that of the influent (Schwarzenbeck et al., 2005; Lemaire et al., 2008; Val del Rio et al., 2012; Yilmaz et al., 2008), even for the solid-free influents like synthetic textile wastewater (Muda et al., 2010), the effluent of which after treatment in granular SBR was reported to contain solids as high as 400 mg/L TSS. The biomass in the effluent may be a result of the granular fragmentation caused by the cell degradation due to the limited diffusion (Arrojo et al. 2004). Therefore, aerobic granular systems are not suitable for the treatment of wastewaters with high solids content, since the effluent is likely to be rich in solids (Speece, 1996; Schwarzenbeck et al., 2005). In order to decrease the effluent solids content, 15-30 min settling of the effluent was suggested by Schwarzenbeck et al. (2005), while Arrojo et al. (2004) increased the TSS removal efficiency from 30% to 80% by shortening the discharge period.

2.3.1.2 Removal of nitrogen and phosphorus

Total nitrogen removal from wastewater requires simultaneous nitrification and denitrification (SNDN). SNDN involves the production of oxidized N forms, namely nitrite and nitrate, by nitrification and denitrification where the nitrite and nitrate are converted to gaseous nitrogen(Adav et al., 2008a).

N removal by aerobic granules was reported by numerous studies investigating the cultivation of nitrifying granules by high ammonia containing wastewater (1000–1400 g N/m^3) (de Kreuk and Van Loosdrecht, 2004; Tsuneda et al., 2006), development of heterotrophic granules with SNDN capability (Beun et al., 2000) and removal of total N as well as carbon (Jang et al., 2003; Mosquera-Corral et al., 2005; Wang et al., 2008a). Jang et al. (2003) observed alternate nitrification and denitrification by heterotrophic nitrifying populations in the granules with 95% COD removal efficiency. Wang et al. (2008a) obtained 85–92% of TOC, nearly 85-100% of NH_4^+-N and 42-78% of TN removal in an aerobic granular sludge membrane bioreactor treating synthetic wastewater.

N removal from real wastewaters such as landfill leachate was reported by Wei et al. (2012) with gradually decreasing removal efficiencies between 75-35% for TN and 96-39% for NH_4^+-N, at increasing influent ammonium concentrations. N removal from industrial effluents were also reported (Table 2.1), while many of the industrial wastewater studies reported SNDN (Table 2.1: Cassidy and Belia, 2005; Wang et al., 2007a; Yilmaz et al., 2008; Figueroa et al., 2008; Kishida et al., 2009; Lotito et al., 2012). Zhang et al. (2011b) provided 94% NH_4^+-N and 67% total N removal for petrochemical wastewater. N removal from textile wastewater was reported by Muda et al. (2010) who achieved 95% NH_4^+-N removal efficiency, and by Lotito et al. (2012) who obtained 29-90% TKN removal from dyeing textile wastewater. In addition, Lotito et al. (2014) achieved 95% NH_4^+-N and 88% TKN removal efficiencies for the mixture of textile and municipal wastewaters. Rosman et al. (2014) obtained 93% NH_4^+-N and 90% total N removal efficiencies by the treatment of rubber wastewater. Figueroa et al. (2008) treated fish canning industry wastewater with aerobic granules, by providing up to 40% ammonia N removal via SNDN at N loading rate of 0.18 kg N/m^3day. Wang et al. (2007a) achieved 89% NH_4^+-N removal efficiency for brewery wastewater. Yilmaz et al. (2008) accomplished 93% soluble N and 86% total N removal efficiencies for abattoir wastewater. Cassidy and Belia (2005) reported complete nitrification and over 97% N removal for abattoir effluent.

In addition to N and organics removal, aerobic granules are reported to achieve biological phosphorus removal (Lin et al., 2003; Carvalho et al., 2007; You et al., 2008; Lemaire et al., 2008). The ability of aerobic granules for simultaneous removal of COD, N and P was shown by many studies (Dulekgurgen et al., 2003; de Kreuk et al., 2005b; Cassidy and Belia, 2005; Schwarzenbeck et al.,2005; Yilmaz et al., 2008; Kishida et al., 2006, 2009; Lemaire et al., 2008; Bassin et al., 2012). Aerobic granules can achieve simultaneous removal of N, P and organics due to the different zones in granular structure, which allows the coexistence of heterotrophic, nitrifying, denitrifying and polyphosphate accumulating populations (Yang et al., 2004b; de Kreuk et al., 2005b; Kishida et al., 2009). The aerobic granular structure has 3 main parts, namely aerobic zone which is responsible for organic matter degradation, micro-oxygen zone where nitrification takes place by nitrifiers, and anoxic (or anaerobic) zone which is responsible for denitrification, P removal, anaerobic degradation and ANAMMOX (anaerobic ammonium oxidation) activity (Gao et al., 2011) (Figure 2.2).

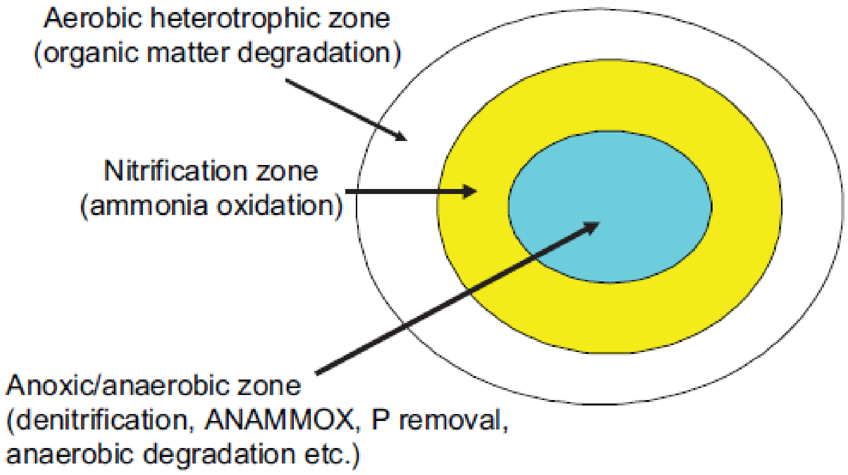

Figure 2.2. Aerobic granular structure (Gao et al., 2011).

P removal is conducted in the inner most part of the granule, namely anoxic/anaerobic zone where there are polyphosphate accumulating organisms (PAOs) (Gao et al., 2011). PAOs are known to remove P from wastewater by accumulating high quantities of polyphosphate in their burden in addition to their essential P uptake (Tchobanoglous et al., 2004). Some of the PAOs, which are able to remove nitrate and P simultaneously, are denitrifying P accumulating organisms (DNPAOs) (Yuan et al., 2007). Aerobic granules were reported to achieve denitrifying P removal, as well as biological essential P uptake (Carvalho et al., 2007; Gao et al., 2011).

Lemaire et al. (2008) conducted simultaneous nitrification, denitrification and phosphorus removal (SNDPR) in an aerobic granular SBR. They observed a PAO, namely *Accumulibacter sp.*, to be dominant in outer layer of the granules, and inner part was dominated via *Competibacter sp*. Lin et al. (2003) observed phosphorus accumulation in aerobic granules fed at influent P/COD ratios between 1/100-10/100 by weight. The increase in substrate P/COD ratio resulted in smaller but more compact and dense granules, and decreased P accumulation. The phosphorus uptake of the system was 1.9-9.3% by weight, which was comparable to the conventional enhanced biological phosphorus removal (EBPR) process (Liu and Tay, 2004). You et al. (2008) achieved 95% COD, and nearly 100% P removal efficiency via P accumulating aerobic granule cultivation. The study stressed the correlation between the elemental composition and influent P/COD ratio.

Simultaneous removal of N, P and organics were reported by many studies as follows. Removal of phosphorus up to 100% was reported by Dulekgurgen et al. (2003), while the COD and total N removal efficiencies were 95% and 71%, respectively. Simultaneous removal of 90% total COD, 80% total N and 67% total P was reported for dairy wastewater by Schwarzenbeck et al. (2005) (Table 2.1). Kishida et al. (2009) observed simultaneous nitrification, denitrification and phosphate uptake under aerobic conditions and reported over 99% NH4-N and 99% PO4-P removal efficiencies for livestock wastewater (Table 2.1). Simultaneous removal of N, P and organics were reported for abattoir wastewater (Table 2.1) (Cassidy and Belia, 2005; Yilmaz et al., 2008). Yilmaz et al. (2008), who studied pre-treated abattoir wastewater, provided 68%, 86%, and 74% removal efficiencies for total COD, total N, and total P, respectively. The removal efficiencies were reported to be 85%, 93% and 89% for sCOD, soluble N and soluble P, respectively. Cassidy and Belia (2005) also accomplished more than 97% N and more than 98% COD and P removal efficiencies for abattoir wastewater.

Since DO gradient exists in aerobic granules, diffusive transport of oxygen is significant for reactions such as nitrification and denitrification to take place (Gao et al., 2011). Low DO concentrations (<2 mg/L) enhance total N removal via promoting denitrification and SNDN, allow simultaneous N, P and COD removal over 90% and reduce the aeration costs (Beun et al., 2001; de Kreuk et al., 2005b; Mosquera-Corral et al., 2005; Vlaeminck et al., 2008; Bassin et al., 2012). Mosquera-Corral et al. (2005) observed increasing N removal efficiency from 8% to 45% with decreasing DO concentrations from 9.1 to 3.6 mg/L (decreasing DO saturation from 100% to 40% at 20^{o}C). Although low DO concentrations such as 3.6 mg/L increased the N removal efficiency by favoring denitrification; stability problems, granular disintegration and washout were reported at the long-term operation (Mosquera-Corral et al., 2005). Vlaeminck et al. (2008), who cultivated aerobic granules with partial nitritation and ANAMMOX activity, reported the highest N removal efficiency over 50% at a DO concentration as low as 1.1 mg/L. de Kreuk et al. (2005b) reported simultaneous removal of 100% COD, 100% NH_4^+-N, 94% total N and 94% total P at DO concentrations as low as 20% (1.8 mg/L DO at 20^{o} C). Bassin et al. (2012), who achieved full nitrification, denitrification and P removal at DO concentrations below 2 mg/L, stressed the supporting effect of low DO concentrations on the growth of denitrifying PAOs, which provided denitrification and P removal.

2.3.1.3 Degradation of toxic substances

Aerobic granules have been reported to degrade toxic substances including phenol (Tay et al., 2004c; Moussavi et al., 2010; Ho et al., 2010; Shams et al., 2008; Jiang et al., 2002, 2004; Tay et al., 2005a,b;), phenolic compounds (Yi et al., 2006; Adav et al., 2007a; Liu et al., 2008), chlorinated phenolic compounds known for serious toxicity risk (Carucci et al., 2009; Wang et al., 2007c); as well as methyl tert-butyl ether (MTBE) (Zhang et al.,2008). Adav et al. (2007a) reported high phenol degradation rates (1.18 g phenol/gVSSday), which were attributed to the partial decrease of the phenol concentrations by mass transfer barrier formed by granular matrix (Liu and Tay, 2004). Both phenol and pyridine degradation was reported by Adav et al. (2007a) who observed the microbial community shift in the granular sludge supporting *Acinetobacto*r sp. dominancy after the addition of pyridine. The use of aerobic granules in phenol degradation may be preffered due to the toxicity resistance of aerobic granules since their densely packed structure decreases the exposure (Liu and Tay, 2004). Aerobic granules may use phenol as a carbon source despite the risk of unstable granulation leading to microbial washout (Allsop et al., 1993; Liu and Tay, 2004).

2.3.1.4 Biosorption of heavy metals, dyestuffs and nuclear wastes

Aerobic granules can achieve biosorption of heavy metals and dyestuffs (Liu et al., 2002, 2003e,f; Xu et al., 2006; Yao et al., 2009; Sun et al., 2008a, 2008b; Xu et al., 2004; Zheng et al., 2005) due to their large surface area, structural strength, high porosity, and excellent settleability (Liu and Tay,2004; Gao et al, 2011). Aerobic granules have 3 times higher maximum adsorption density compared to flocs (Adav et al., 2008a). The biosorption mechanism includes ion exchange, binding to extracellular polymers and chemical precipitation (Gao et al., 2011). Biosorption of Cd^{2+} and Zn^{2+} were reported at the maximum capacities of 566 mg/g and 270 mg/g of aerobic granules, respectively (Liu et al., 2002; 2003e,f). According to Xu et al. (2004), the maximum adsorption capacity of aerobic granules for Cu^{2+} and Zn^{2+} were 246.1 mg/g and 180 mg/g, respectively. Studies including Xu et al. (2006) and Yao et al. (2009) concluded that the optimum pH was found as pH 5-6 for Ni^{2+} and Cr^{3+} biosorption by aerobic granules (Gao et al., 2011). Sun et al. (2008a) studied competitive metal adsorption with Zn^{2+} and Co^{2+} on aerobic granules. It was stated that the maximum adsorption capacities were higher (55.25 mg Co/g; 62.50 mg Zn/g) for single systems, compared to that of binary (54.05 mg Co/g; 56.5 mg Zn/g). The studies employing dyestuffs include removal of cationic dyes, namely rhodamine B (Zheng et al., 2005) and malachite green (Sun et al., 2008b). Furthermore, the capability of aerobic granules to remove nuclear wastes was shown by Nancharaiah et al. (2006), who achieved almost complete biosorption of soluble uranium [U(VI)] of 6–100 mg/L in less than 1 hour.

2.3.2 Pilot-scale and full-scale applications

The application of aerobic granulation technology for large-scale wastewater treatment plants may enhance the treatment efficiency and decrease the reactor volume, due to the high biomass concentration, biodegradation rates, and settleability of the system (Liu and Tay, 2004). Although the aerobic granular sludge tecnology offers a promising alternative for wastewater treatment regarding the feasibility studies in both lab and pilot-scales (de Bruin et al., 2004; de Kreuk et al., 2007), the pilot-scale and full-scale applications are limited (de Kreuk et al., 2007 ; Gao et al., 2011). Literature lacks detailed information about the pilot applications and especially full-scale aerobic granular plants, which are found in Netherlands, Portugal and South Africa (Giesen et al., 2012), China (Ni et al., 2009) and Italy (Gao et al., 2011). The application of aerobic granulation technology on a full-scale basis requires technical experience and economic analysis as well as feasibility studies (Gao et al.,2011).

The scaling-up of aerobic granular systems from lab-scale to pilot-scale and full-scale took place in Netherlands, in 2003, by a research group named National Nereda Onderzoeks Programma (NNOP) who developed Nereda Technology (Giesen et al., 2012). The first full-scale Nereda system was installed in 2005, and had 50-250 m^3/day treatment capacity. In 2009, the research group modified a pilot plant for food industry wastewater treatment, and came up with a plant at a capacity of 1–8 g COD/L with 95% removal efficiency. In 2008, South Africa (Gansbaai, Western Cape) facing water scarcity, employed Nereda with a design capacity of 4000 m^3/d to treat municipal wastewater and high strength septic sewage, aiming water reuse for irrigation. The first full-scale aerobic granular treatment plant was built in Netherlands, Epe in 2012 and employed Nereda Technology. The system had 1500 m^3/d capacity and treated municipal sewage containing 15% of slaughterhouse wastewater. The effluent qualities for nitrogen and phosphorus were below 5mg/L and below 0.3 mg/L, respectively. The aerobic granular wastewater treatment is conducted by Nereda Technology, in Netherlands, Portugal and South Africa, while the system is planned to be used in Australia, Europe and South America in the future (URL 1).

Italy employs a sequencing batch biofilter granular reactor of 3.1 m^3 volume in a sewage treating plant. A pharmaceutical company in Hungary has a pilot plant of 2.7 m^3 that was seeded with aerobic granules developed in on-site bioreactors. China has a pilot plant with 2 column SBRs with working volume of 1 m^3 treating municipal wastewater (Ni et al., 2009; Gao et al.,2011). The reactor showed 90% tCOD and 95% NH_4^+-N removal efficiencies on the average. The cultivated aerobic granules had 0.2-0.8 mm diameter, and 18–40 m/h settling velocity, indicating good settleability. The major species contibuting to granulation were identified to be rod, coccus and filamenous bacteria (Ni et al., 2009). It was seen that granulation, which took 1-2 months in lab-scale SBRs, was achieved in 10-13 months in pilot-scale reactors (Liu et al., 2010a; Ni et al., 2009).

Liu et al. (2010a) achieved aerobic granulation in a pilot-scale SBR on site and treated real (mixed domestic and industrial) wastewater. During the granulation process for 400 days, despite the variations in floc ratio from 5 to 30%, the sludge showed a stable SVI_5 value of 30 mL/g. The denaturing gradient gel electrophoresis (DGGE) results indicated that there was no considerable microbial selection after the granulation was completed. The reactor had stable removal efficiencies; namely more than 80% COD, more than 98% NH_4^+-N and total inorganic N of 50%. Long-term stability of granular sludge was provided by high influent quality fluctuations.

Granular SBRs either with pre-treatment and post-treatment were found to be more advantageous with respect to conventional activated sludge systems in terms of annual costs including land price and footprint (de Bruin et al., 2004; de Kreuk et al., 2007; Gao et al. 2011). Unfortunately, it was revealed that a granular SBR with only pre-treatment was unable to fulfill the municipal wastewater effluent standards due to the microbial washout of less settlable biomass (de Bruin et al., 2004; de Kreuk et al., 2007; Gao et al. 2011).

2.4 Storage Stability and Re-activation of Aerobic Granules

Storage and reactivation of aerobic granules is significant in real-life applications, where the wastewater flow rates and pollution loads pursue fluctuating trends in treatment plants (Liu and Tay, 2004), e.g. due to seasonal variations. Lack of storage stability and re-activation difficulties of aerobic granular sludge, limit the utilisation of aerobic biogranules in pilot and full-scale reactors (Zeng et al., 2007; Gao et al., 2011) as well as their long range transport (Zeng et al., 2007). Nevertheless, it was reported that stability and structure of the granules were preserved for weeks or months under starvation or storage (Gao et al., 2011). Main mechanism behind the loss of stability and structural integrity of aerobic granules is microbial activity decrease caused by hydrolysis, endogenous respiration, and anaerobic degradation due to long periods of storage in a high temperature media, without any substrate

(Ng, 2002; Liu and Tay, 2004; Liu et al., 2004b; Adav et al., 2007b; Gao et al., 2011). Storage conditions (such as temperature, duration and media) as well as the features of aerobic granules (including microbial activity and structural integrity) affect the extent of these mechanisms (Liu et al., 2004b; Gao et al., 2011). Biological activity and storage stability are supported by storage at cold temperatures in carbon containing solutions, for short storage periods (Adav et al., 2007b; Gao et al., 2011).

There are numerous studies about the re-activation and storage of aerobic granules (Zhu and Wilderer, 2003; Zhang et al.,2005; Zeng et al., 2007; Wang et al., 2008b) including the ones investigating the effect of storage solution (Tay et al., 2002c; Ng, 2002) and temperature (Adav et al., 2007b) and the start-up of a pilot-scale reactor with stored aerobic granules (Liu et al., 2005c). Liu et al. (2005c), for the first time, achieved the start-up of a pilot-scale reactor by seeding the aerobic granules subjected to 4 months of storage. Although the initial biomass concentration was 1.03 g/L, the microbial activity of granules recovered in 2 days, and later on 6.0 g/L biomass concentration was achieved in the system. Wang et al. (2008b) stated that the aerobic granules stored for 7 months showed decreased settleability, but maintained their structural integrity and size. It was observed that both the decay and recovery of the nitrifiers were faster than the heterotrophic populations. The recovery took 16 and 11 days for heterotrophs and nitrifiers, respectively. The EPS content of the granules showed a drastical decrease during 1 month storage, however gradual EPS accumulation was observed during the remaining 6 months. The study displayed that the long-term storage stability of aerobic granules was favored by the EPS accumulation (Wang et al., 2008b; Gao et al., 2011; Di Iaconi et al., 2007; de Kreuk and van Loosdrecht, 2004).

2.5 Treatment of Sugar Beet Processing Wastewaters

Sugar is produced from beet instead of cane in Turkey (URL 2; URL 3). The sugar beet processing wastewater is rich in organics, COD, BOD, suspended and dissolved solids, alkalinity, nitrogen, phosporus, pathogens and pesticide residues (Güven et al., 2009; Aliplik Akın, 2010). The pollution loads were reported to be 4000–7000 mg/L BOD_5, COD up to 10000 mg/L, and SS up to 5000 mg/L (Güven et al., 2009; The World Bank Group, 2007; Aliplik Akın, 2010). Due to their high organic content, direct discharge of sugar industry wastewaters to the aquatic environments, may jeopardize the aquatic life by causing oxygen depletion (Hampannavar and Shivayogimath, 2010).

In Turkey, sugar beet processing wastewaters are generally stored in stabilization ponds and lagoons, with possible application of agricultural reuse; or directly discharged to the receiving body (Aliplik Akın, 2010). However, many treatment options such as biological treatment (e.g. sequencing anaerobic and aerobic systems), physico-chemical processes (e.g. electrochemical oxidation) and combinations are available. Table 2.2 lists the studies employing the treatment of sugar beet processing wastewater. Electrochemical oxidation (Güven et al., 2009) and chemical coagulation (Aliplik Akin, 2010) were among the lab-scale processes for the treatment of sugar beet processing wastewater (Table 2.2). According to the literature knowledge, sugar beet processing wastewater is commonly treated by anaerobic systems such as UASB reactors (Hampannavar and Shivayogimath, 2010; Taniksali, 2013; Thaveesri et al., 1995), upflow anaerobic fixed bed (UAFB) (Farhadian, 2007), downflow stationary fixed film reactor (Hamoda and Kennedy, 1986), anaerobic batch reactor (Alkaya and Demirer, 2011) (Table 2.2). The COD removal efficiency was reported to be between 75-96% for anaerobic systems (Table 2.2).

The incapability of anaerobic systems for nitrogen and phosphorus removal, revived the use of aerobic treatment systems such as aerobic biogranulation. Despite the variety of industrial wastewaters used in the formation and/or treatment studies with aerobic granules (Table 2.1), there is no information about the treatability of sugar beet processing wastewaters with aerobic granular systems, which can also be seen from Table 2.2. Therefore, this thesis includes the investigation of the use of aerobic granules for sugar beet processing wastewater treatment.

Table 2.2. Sugar beet processing wastewater treatment studies.

[a]Reactor type	COD removal %	Sugar beet ww COD (mg/L)	Reference
UASB	89	1000-4340	Hampannavar and Shivayogimath (2010)
UAFB	75-93	2000-8000	Farhadian (2007)
UASB	80-96	2000	Tanksali (2013)
UASB	-	7500-12500	Thaveesri et al. (1995)
Anaerobic batch reactor	87	4500	Alkaya and Demirer (2011)
Downflow stationary fixed film reactor	87	5000-15000	Hamoda and Kennedy (1986)
Electrochemical oxidation	86	6300	Güven et al. (2009)
Chemical coagulation	49	2050-2550	Aliplik Akın (2010)

[a]Reactor type: UASB (upflow anaerobic sludge blanket), UAFB (upflow anaerobic fixed bed).

CHAPTER 3

MATERIALS AND METHODS

The thesis study consists of three experimental sets (Set-1, Set-2, Set-3) conducted with aerobic granular SBR operation. The seed sludge, experimental procedure, reactor operations, and analytical methods employed in the thesis study are explained in this chapter.

3.1 Seed Sludge

Aerobic granular sludge cultivated in a previous study (Ersan, 2013) was used as seed sludge in the experiments. In this study, the membrane bioreactor sludge, obtained from the membrane unit of METU-Vacuum Rotation Biomembrane Plant, was used for aerobic granule development via anoxic-aerobic SBR operation. The seed sludge properties of each reactor set conducted within this thesis study are tabulated in Table 3.1.

Table 3.1. The properties of seed sludge used in the SBR operation.

Reactor set	Sludge type	TSS (mg/L)	VSS (mg/L)	SVI_{30} (mL/g)
Set-1	Aerobic granular sludge	6050	4760	36
Set-2	Aerobic granular sludge	4910	4180	37
Set-3	Aerobic granular sludge	10920	6000	18

3.2 Wastewater Composition

In Set-1 and Set-2, the SBRs were operated with synthetic wastewater. In Set-3, an SBR was operated with 2 types of sugar beet processing wastewaters after start-up with synthetic wastewater. The composition of the synthetic wastewater used in the study is given in Table 3.2. The industrial wastewaters used in Set-3 are explained in Section 3.2.3.

Table 3.2. Composition of the synthetic wastewater.

Solution			**Micronutrient stock solution*		
HAc (CH_3COOH)	1.34	mL/L	$FeCl_2.4H_2O$	1.5	g/L
NH_4Cl	765	mg/L	H_3BO_3	0.15	g/L
$NaNO_3$	486	mg/L	$CuCl_2.2H_2O$	0.02	g/L
$NaHCO_3$	1800	mg/L	KI	0.18	g/L
$MgSO_4.7H_2O$	180	mg/L	$MnSO_4.H_2O$	0.1	g/L
$CaCl_2.2H_2O$	160	mg/L	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.044	g/L
$Na_3PO_4.12H_2O$	244	mg/L	$ZnCl_2$	0.057	g/L
Yeast extract	2	mg/L	$CoCl_2.6H_2O$	0.15	g/L
*Micronutrient stock solution**	0.6	mL/L	EDTA	10	g/L

(Smolders et al. 1994; Erguder and Demirer, 2005a; Erguder and Demirer, 2005b; Fang et al., 2009; Shi et al., 2010).

The composition of the synthetic wastewater given in Table 3.2 was determined by the partial modification of the nutrient solutions and concentrations used in the aerobic- anoxic (or microaerobic) granulation studies and research on nitrogen-phosphorous removal (Smolders et al. 1994; Erguder and Demirer, 2005a; Erguder and Demirer, 2005b; Fang et al., 2009; Shi et al., 2010). The carbon source was acetic acid (HAc) for heterothophic bacteria and bicarbonate (HCO_3^-) for autotrophic nitrification bacteria. The nitrogen source for denitrification bacteria was $NaNO_3$. The pH of the synthetic wastewater was adjusted to 7 using 0.1N HCl and NaOH solutions. The synthetic wastewater was frequently prepared, stored at +4 ^{0}C, and introduced to the SBRs by peristaltic pump.

The composition in Table 3.2 stands for 1500 mg/L COD, 200 mg/L TAN (NH_4-N), 80 mg/L NO_3-N and 10 mg/L PO_4-P. To serve the aim of each experimental study, the composition in Table 3.2 was adjusted according to the aim of each reactor set as well as the reactor performances. The adjustments were conducted in terms of HAc, NH_4Cl, $NaNO_3$ and $NaHCO_3$ concentrations and are explained below for each set.

3.2.1 Wastewater composition for Set-1

Set-1 aimed to assay the effect of different influent COD/TAN ratios on treatment efficiencies. The influent COD concentration of the synthetic wastewater was changed, by adjusting the quantity of HAc. For instance, 1.34 mL/L HAc (Table 3.2); which corresponded to 1500 mg/L influent COD, was increased to 1.79 mL/L in order to provide 2000 mg/L influent COD at the influent COD/TAN ratio of 10, and the influent HAc concentration was increased to 5.36 mL/L to provide the influent COD concentration of 6000 mg/L corresponding to the COD/TAN ratio of

30. During the operation period of 101 days, influent COD/TAN ratios from 1 to 30 were studied, while the influent HAc concentrations varied between 0.18-5.36 mL/L. For both of the reactors, R1 and R2, the influent TAN concentration was constant at 200 mg/L, and influent NLR was constant at 0.4 g TAN/Lday, provided by 765 mg/L NH_4Cl addition, through Set-1. Since enough nitrate was produced by nitrification, $NaNO_3$ was halved from 486 mg/L to 243 mg/L by the end of Week 4 (Day 20). Hence the corresponding NO_3-N concentration was also halved from 80 to 40 mg/L NO_3-N. The adjustments in synthetic wastewater composition for Set-1 are explained in Table 3.3. The rest of the synthetic wastewater composition is as tabulated in Table 3.2.

Table 3.3. The adjustments in the synthetic wastewater composition for Set-1.

Set-1										
Parameter	**Start -up**	**R1**				**R2**				
COD/TAN ratio	7.5	7.5	10	20	30	7.5	5	3.5	2	1
HAc (mL/L)	1.34	1.34	1.78	3.56	5.34	1.34	0.89	0.63	0.36	0.18
[a]COD (mg/L)	1500	1500	2000	4000	6000	1500	1000	700	400	200
NH_4Cl (mg/L)	765	765	765	765	765	765	765	765	765	765
[b]TAN (mg/L)	200	200	200	200	200	200	200	200	200	200
[c]$NaNO_3$ (mg/L)	486	486-243	243	243	243	243	243	243	243	243
[d]NO_3-N (mg/L)	80	80-40	40	40	40	40	40	40	40	40
$NaHCO_3$ (mg/L)	1800	1800	1800	1800	1800	1800	1800	1800	1800	1800
[e]OLR	3	3	4	8	12	3	2	1.4	0.8	0.4
[e]NLR	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

[a]Theoretical COD concentration corresponding to the HAc content; i.e. 1.34 mL/L influent HAc corresponds to 1500 mg/L influent COD.
[b]Theoretical TAN concentration corresponding to the NH_4Cl content; i.e. 765 mg/L influent NH_4Cl corresponds to 200 mg/L influent TAN.
[c]Since enough nitrate was produced by nitrification, $NaNO_3$ was halved by the end of Week 4 (Day 20). Hence the corresponding NO_3-N concentration was also halved from 80 to 40 mg/L NO_3-N.
[d]Theoretical NO_3-N concentration corresponding to the $NaNO_3$ content; i.e. 486 mg/L influent $NaNO_3$ corresponds to 80 mg/L influent NO_3-N.
[e]OLR in terms of (g COD/Lday), NLR in terms of (g TAN/Lday).

3.2.2 Wastewater composition for Set-2

The effects of increasing OLR and NLR values on aerobic biogranules were researched in Set-2 where one SBR, namely R3, was operated for 70 days. The influent HAc and NH_4Cl concentrations were gradually increased to obtain the desired loading rates. Throughout the operation, the studied theoretical OLR and NLR values were in the range of 0.75-12 g COD/Lday and 0.1-1.6 g TAN/Lday, respectively. The corresponding influent COD and TAN (in the form of NH_4-N) concentrations were 375-6000 mg/L COD and 50-800 mg/L TAN, respectively. To fit those loading rates and concentrations, the amounts of HAc and NH_4Cl in the synthetic wastewater composition in Table 3.2 was adjusted to be between 0.34-5.36 mL/L HAc and 191.3-3060 mg/L NH_4Cl, respectively.

In Set-1, the synthetic wastewater contained nitrate in the form of $NaNO_3$. However, in Set-2, there was no nitrate in the synthetic wastewater. The reason is that, in Set-1, nitrification was accomplished, with sufficient production of nitrate serving as denitrification source for the upcoming cycle. This eliminated the requirement of adding any extra nitrate as denitrification source, like $NaNO_3$. Regarding the Set-1 experience, the amount of $NaHCO_3$, which was 1800 mg/L in Set-1, was decreased to 551 mg/L in Set-2. Since the carbon source for nitrification was provided via $NaHCO_3$, the amount of influent $NaHCO_3$ was determined aiming to reach a HCO_3/NH_4-N ratio of at least 8 (Yang et al., 2004b). The adjustments in synthetic wastewater for Set-2, apart from the composition in Table 3.2, are given in Table 3.4.

Table 3.4. The adjustments in the synthetic wastewater composition for Set-2.

	Set-2					
Reactor	**Start-up**	**R3**				
OLR (g COD/Lday)	3.0	0.75	1.5	3.0	6.0	12
NLR (g TAN/Lday)	0.4	0.1	0.2	0.4	0.8	1.6
HAc (mL/L)	1.34	0.34	0.67	1.34	2.68	5.36
[a]COD (mg/L)	1500	375	750	1500	3000	6000
NH_4Cl (mg/L)	765	191.3	383	765	1530	3060
[b]TAN (mg/L)	200	50	100	200	400	800
$NaNO_3$ (mg/L)	-	-	-	-	-	-
$NaHCO_3$ (mg/L)	551	551	551	551	551	551
COD/TAN ratio	7.5	7.5	7.5	7.5	7.5	7.5

[a]Theoretical COD concentration corresponding to the HAc content; i.e. 1.34 mL/L influent HAc corresponds to 1500 mg/L influent COD.
[b]Theoretical TAN concentration corresponding to the NH_4Cl content; i.e. 765 mg/L influent NH_4Cl corresponds to 200 mg/L influent TAN.

3.2.3 Wastewater composition for Set-3

For the investigation of the treatability of sugar beet industry wastewaters via aerobic granular sludge and the effects of this wastewater on aerobic granular sludge, Set-3 employed 3 types of wastewaters. After the start-up with synthetic wastewater, one SBR, namely R4, was operated with the effluent of anaerobic digester treating sugar beet processing wastewater (Days 1-32), and sugar beet processing wastewater (Days 33-54), respectively.

3.2.3.1 Synthetic wastewater composition for Set-3

The composition of the synthetic wastewater used in Set-3 during the start-up period of 21 days is given in Table 3.2, with the adjustments in Table 3.5.

Table 3.5. The adjustments in synthetic wastewater composition for Set-3.

Set-3 Start-up period								
[a]OLR	[a]NLR	HAc (mL/L)	[b]COD (mg/L)	NH_4Cl (mg/L)	[c]TAN (mg/L)	$NaNO_3$ (mg/L)	$NaHCO_3$ (mg/L)	COD/TAN ratio
0.75	0.1	1.34	1500	765	200	-	551	7.5

[a] OLR in terms of (g COD/Lday), NLR in terms of (g TAN/Lday).
[b]Theoretical COD concentration corresponding to the HAc content; i.e. 1.34 mL/L influent HAc corresponds to 1500 mg/L influent COD.
[c]Theoretical TAN concentration corresponding to the NH_4Cl content; i.e. 765 mg/L influent NH_4Cl corresponds to 200 mg/L influent TAN.

The synthetic wastewater contained influent COD:TAN concentration ratio of 7.5 (1500 mg/L/200 mg/L). The composition was similar to that of 'Set-2 Start-up period' (Table 3.4, Section 3.2.2), with lower loading rates. In Set-3, in order to acclimate the aerobic granular sludge to the low COD and TAN content as that of the anaerobic digester, the reactor was subjected to 1 feeding per day instead of 4 feedings. The loading rates were ¼ times of the conventional SBR operation. The theoretical OLR and NLR values applied during the start-up period of R4 were 0.75 g sCOD/Lday and 0.1 g TAN/Lday, respectively.

3.2.3.2 Industrial wastewaters in Set-3

The industrial wastewaters were the effluent of the anaerobic digester treating sugar beet processing wastewater (i.e. anaerobically-pretreated effluent) and the raw sugar beet processing wastewater, which were obtained from Ankara Sugar Factory. The effluent of the anaerobic digester will be defined as anaerobically-pretreated effluent for the rest of the document. Except the pH adjustments with dilute NaOH and HCl solutions, the original wastewaters stayed untouched in composition. The wastewaters were introduced to the reactor after 1 hour settlement in order to provide a realistic simulation of primary sedimentation tanks in treatment plants by removing settlable solids. The pH of the wastewaters were initially set to 7 and stored at +4°C during the study. The compositions of the industrial wastewaters are shown in Table 3.6.

Table 3.6. The properties of the industrial wastewaters used in the study (after 1 hour settlement).

Parameter	Value	
	Anaerobically-pretreated effluent	Raw sugar beet processing wastewater
Total COD (tCOD) (mg/L)	245±16	4280±260
Soluble COD (sCOD) (mg/L)	111±16	3055±183
TSS (mg/L)	274±14	2255±250
VSS (mg/L)	167±29	1143±160
NO_3-N (mg/L)	28.5±13.2	9.5±1.5
NO_2-N (mg/L)	21.3±3	3.1±1.5
TAN (mg/L)	37.8±2.8	32.9±4.37
Total phosphorus (TP) (mg/L)	2.2±1	7.8±0.8
Sulfate (mg/L)	4±1	10±2
pH	8.1	6.35±0.5
Alkalinity (mg/L $CaCO_3$)	755±78	1480±108

3.3 Experimental Procedure

Three reactor sets were operated to investigate the following:

Set-1: the effect of different influent COD/TAN ratios on characteristics and treatment performance of aerobic granules,

Set-2: the effect of increasing OLR and NLR values on characteristics and treatment performance of aerobic granules,

Set-3: the treatment of sugar beet industry wastewaters and the effect of this wastewater application on aerobic granular sludge.

3.3.1 Reactor configuration

Set-1 employed 2 identical sequencing batch reactors (SBRs), whereas 1 SBR was used in Set-2 and Set-3. The plexiglass SBRs were cylindrical in shape with 8 cm inner diameter, 60 cm depth and working volume of 2.45 L. The volumetric exchange ratio (VER) was 50%.

3.3.2 Sequencing batch reactor operational conditions

For all the three sets; Set-1, Set-2 and Set-3; the reactors were operated continuously 4 cycles a day, each lasting 6 hours. One cycle consisted of 5 min-feeding, 43 min-anoxic, 309-305 min-aerobic, 1-5 min-settling and 2 min-withdrawal periods. The timing of these periods (the cyclic operation of the SBRs) was managed via automatic time controllers. The HRT was 12.3 hours. Table 3.7 summarizes the cyclic operation of SBR in each set.

Table 3.7. Operational conditions for Set-1, Set-2 and Set-3.

Parameter	**Operational conditions**		
	Set-1	**Set-2**	**Set-3**
Feeding period (min)	5	5	5
Anoxic period (min)	43	43	43
Aerobic period (min)	309-305	309	305
Settling period (min)	1-5	1-5	1-5
Withdrawal period (min)	2	2	2
Cycle time (h)	6	6	6
Volumetric exchange ratio	50%	50%	50%
HRT (h)	12.3	12.3	12.3
Upflow air velocity (cm/s)	1.5	1.5	1.5
OLR (g COD/Lday)	0.4-12	0.75-12	0.07-6.97
[a]NLR (g NH_4-N/Lday)	0.4	0.1-1.6	0.01-0.11
Influent COD concentration (mg/L)	200-6000	375 – 6000	68 – 3845
Influent TAN concentration (mg/L)	200	50 – 800	22 – 56
COD/TAN ratio	1-30	7.5	2.26 – 73.2

[a]For Set-1 and Set-2 TAN = TN thus NLR was given as g TAN/Lday = g TN/Lday.

During aerobic periods, the SBRs were aerated at an airflow rate of 180 L/hour by air pumps. The aeration aimed to provide DO and homogeneous mixing, as well as to enhance granulation by providing hydraulic shear force. The experiments were conducted at 20-26^{o}C laboratory medium. The temperature inside the reactors varied between 16 to 24^{o}C. The reactor pH was monitored and ensured to be kept in the optimum range, which is 7.5–8.6 for nitrification and 7–8 for denitrification (Yoo et al., 1998) using 0.1N HCl and NaOH solutions.

3.3.2.1 Set-1: The effects of influent COD/TAN ratio

Set-1 was conducted to investigate the effects of influent COD/TAN ratios among 1-30 on aerobic granular characteristics, carbon and nitrogen removal efficiency, and nitrification-denitrification performance. The optimum initial COD/TAN ratio providing maximum nitrogen and COD removal efficiencies and formation of stable aerobic granules were explored.

Firstly, 1 SBR was seeded with aerobic granular sludge having 6050±500 mg/L TSS, 4760±450 mg/L VSS and 35.9 mL/g SVI. The SBR was fed with synthetic wastewater (Table 3.2) for 1 week, in order to acclimate the previously developed aerobic granular seed sludge to the studied operational conditions (Table 3.7). Composition of the synthetic wastewater during the start-up period had 1500 mg/L COD and 200 mg/L TAN influent concentrations, corresponding to the COD/TAN ratio of 7.5.

When the stable treatment efficiencies (79±4% for TAN and 74±1% for COD) were obtained, i.e when the standard deviation of 3 consecutive analyses was ≤5% of their average, and sufficient VSS concentration (5743±1165 mg/L) was present, the reactor content was divided equally into 2 identical SBRs, namely, R1 and R2. The initial VSS contents were 3040±350 mg/L and 3150±350 mg/L for R1 and R2, respectively.

Through the 101-day operation period, the reactors R1 and R2, which were identical to the start-up SBR, were operated under the same SBR conditions as in the start-up period (Table 3.7). Both SBRs were fed with synthetic wastewater with a COD/TAN ratio of 7.5 for the initial 10 days (Table 3.3). During the rest of the study, the operation with desired influent COD/TAN ratios was provided by changing the influent COD concentrations via adjusting the HAc quantity in the synthetic wastewater. Both for R1 and R2, the amount of influent TAN was constant at 200 mg/L NH4-N, providing a constant NLR of 0.4 g TAN/Lday. R1 experienced gradually increasing COD/TAN ratios as 7.5, 10, 20 and 30; whereas R2 experienced gradually decreasing COD/TAN ratios as 7.5, 5, 3.5, 2 and 1. The ratio changes were conducted after at least 20 cycles (> 9 HRTs) from the previous ratio change, and in the condition that the reactor reached a stable treatment efficiency. The reactors were operated as explained in Table 3.7.

The operational conditions in terms of the applied loading rates for Set-1 are summarized in Table 3.8. Both R1 and R2, were operated according to the anoxic-aerobic operation of SBR cycles, as explained in Table 3.7. The HRT was 12.3 hours. The reactors were aerated at an upflow air velocity of 1.5 cm/s during aerobic periods.

Table 3.8. Set-1: Operational conditions.

One week of start-up period					
COD (mg/L)	TAN (mg/L)		COD/TAN	OLR (g COD/Lday)	NLR (g TAN/Lday)
1500	200		7.5	3	0.4
[a]R1					
Day	COD (mg/L)	TAN (mg/L)	COD/TAN	OLR (g COD/Lday)	NLR (g TAN/Lday)
1-10	1500	200	7.5	3	0.4
11-67	2000	200	10	4	0.4
68-81	4000	200	20	8	0.4
82-101	6000	200	30	12	0.4
R2					
Day	COD (mg/L)	TAN (mg/L)	COD/TAN	OLR (g COD/Lday)	NLR (g TAN/Lday)
1-10	1500	200	7.5	3	0.4
11-28	1000	200	5	2	0.4
29-59	700	200	3.5	1.4	0.4
60-79	400	200	2	0.8	0.4
80-101	200	200	1	0.4	0.4

[a]Day 46-47: Influent COD/TAN ratio for R1 was 1000/100 for cleaning the accumulated TAN in the reactor content; Day 68-69: Influent COD/TAN ratio for R1 was 4000/100 to avoid shock loading.

3.3.2.2 Set-2: The effects of increasing influent OLR and NLR values

It was aimed in Set-2 to investigate the effects of increasing OLR and NLR values on aerobic granules and their treatment efficiency in terms of carbon and nitrogen removal. The maximum OLR and NLR values allowing a high treatment efficiency without destructing the granular stability were determined.

One SBR, namely R3, was seeded with aerobic granular sludge, at 4180±450 mg/L VSS, 4910±450 mg/L TSS and 37 mL/g SVI initially. Then the reactor was subjected to a start-up protocole for 21 days, aiming to acclimate the aerobic granular sludge to SBR operation at low loading rates. Composition of the synthetic wastewater during the start-up period included 1500 mg/L COD, 200 mg/L TAN influent concentrations, corresponding to the OLR and NLR values of 3 g COD/Lday and 0.4 g TAN/Lday, respectively. When stable treatment efficiency and sufficient solids content (9680 mg/L VSS) were reached, R3 was subjected to 70 days of operation with gradually increasing influent loading rates. The procedure employed the stepwise increase in influent COD and TAN concentrations as shown in Table 3.4, and in turn the stepwise increase in OLR and NLR values as 0.75-1.5-3.0-6.0-12 g COD/Lday and 0.1-0.2-0.4-0.8-1.6 g TAN/Lday, respectively. The COD/TAN ratio was kept constant at 7.5 during the operational period of R3. In addition to the cycle operation in Table 3.7, the operational procedure of R3 is tabulated below in Table 3.9. Regarding the results of Set-1, the influent COD/TAN ratio was kept constant at

7.5 during the 70 days of operation. The influent concentrations were gradually increased from 375 mg/L COD and 50 mg/L TAN to 6000 mg/L COD and 800 mg/L TAN, respectively.

Table 3.9. Set-2: Operational conditions.

Day	Influent COD/TAN concentrations (mg/L/mg/L)	OLR (g COD/Lday)	[a]NLR (g TAN/Lday)	COD/TAN ratio
Start-up (21 Days)	1500/200	3.0	0.4	7.5
1-16	375 / 50	0.75	0.1	7.5
17-29	750 / 100	1.5	0.2	7.5
30-49	1500 / 200	3	0.4	7.5
50-61	3000 / 400	6	0.8	7.5
62-70	6000 / 800	12	1.6	7.5

[a]Since there is no nitrite or nitrate in the influent, NLR indicates both nitrogen (g N/Lday) and TAN loading rates (g TAN/Lday).

As previously stated in Table 3.7, the cyclic SBR operation consisted of anoxic-aerobic sequencing cycles, lasting for 6 hours. The volumetric exchange ratio was 50% and HRT was 12.3 hours. During the aerobic periods, the reactor was aerated at an upflow air velocity of 1.5 cm/s.

3.3.2.3 Set-3: The application of sugar beet industry wastewaters

Set-3 was conducted to investigate the utilization of aerobic granular systems for sugar beet processing wastewater treatment, and the effect of wastewater on aerobic granules. One SBR, namely R4, was operated with the effluent from anaerobic digester treating sugar beet processing wastewater and further with the raw wastewater from sugar beet processing. The operational conditions for R4 are shown below in Table 3.10.

Table 3.10. Set-3: Operational conditions.

Set-3						
Day	WW type	Number of feedings per day	[a]OLR	[a]NLR	Influent sCOD (mg/L)	Influent TAN (mg/L)
Start-up (21 days)	Synthetic	1	0.75	0.1	1500	200
1-6	Anaerobically-pretreated effluent	1	0.07±0.01	0.02±0.004	149±13	32±8
7- 13		2	0.09±0.01	0.02±0.01	93±6	22±0
14-32		4	0.22±0.01	0.07±0.01	112±37	35±4
33-54	Sugar beet processing ww	4	6.11±0.37	0.1±0.01	3055±183	49±5

[a]OLR: Average organic loading rate (g sCOD/Lday), NLR: Average nitrogen loading rate (g TAN/Lday).

R4 was seeded with aerobic granular sludge having 10920±900 mg/L TSS, of 6000±500 mg/L VSS, and 18 mL/g SVI. The reactor was subjected to a start-up period with synthetic wastewater for 21 days (Table 3.5, Section 3.2.3.1). It was aimed to acclimate the aerobic granular sludge to low influent COD and TAN concentrations and loadings, similar to that of anaerobic digester's effluent. To this purpose, during the start-up period (21 days), R4 was fed 1 time each day, instead of conventional operation with industrial wastewaters (employing 4 feedings a day). Therefore the reactor was subjected to low pollution load; i.e. the OLR and NLR values were 0.75 g sCOD/Lday and 0.1 g TAN/Lday, respectively during the start-up period. When stable treatment efficiencies were reached, i.e. when the standard deviation of 3 consecutive analyses were ≤5% of their average, the industrial wastewater application was initiated.

The anaerobically-pretreated effluent was gradually introduced to the SBR during Days 1-32 to facilitate the adaptation. The feeding protocole for anaerobically-pretreated effluent included; 1 feeding per day (Days 1-6), 2 feedings per day (Days 7-13), and finally 4 feedings per day (Days 14-32). The highest loading rate with anaerobically-pretreated effluent was reached on Day 14-32, when 4 feedings were applied per day. Gradual OLR rise from 0.07 to 0.22 g sCOD/Lday aimed to acclimate the granular sludge to anaerobically treated sugar beet processing wastewater. During the application of anaerobically-pretreated effluent, the average OLR and NLR values were 0.18±0.1 g sCOD/Lday and 0.05±0.03 g TAN/Lday, respectively. The corresponding influent concentrations were 115±35 mg/L sCOD and 32±6 mg/L TAN on the average.

During Days 33-54, the raw sugar beet processing wastewater was introduced to R4, pursuing the conventional procedure with 4 feedings a day. The sugar beet processing wastewater (Table 3.6, Section 3.2.3.2) had higher sCOD, tCOD, and solids content compared to that of anaerobically-pretreated effluent. During the operation with sugar beet processing wastewater, the average influent concentrations were 3055±183 mg/L sCOD and 49±5mg/L TAN, while the corresponding loading rates were 6.11±0.37 g sCOD/Lday and 0.1±0.01 g TAN/Lday.

3.4 Analytical Methods

3.4.1 Reactor performance

In order to explore the carbon and nitrogen removal efficiencies and the nitrification-denitrification performance of the reactors, frequent analyses of sCOD, TAN, NO_2-N and NO_3-N were conducted. For validation of the analyses, calibration curves were prepared regarding the methods, devices and compounds. The calibration curves are given in Appendix A. The treatment efficiencies were determined by alternate sampling. The samples were taken from feed (influent), at the end of feeding period (initial sample) and at the end of the anoxic and aerobic periods (the samples can be named as anoxic and aerobic samples, respectively). The removal efficiencies were calculated by percent difference between the initial and aerobic samples for total removal efficiency, the initial and anoxic samples for anoxic period removal efficiency, and between anoxic and aerobic samples for aerobic period removal efficiency determinations. The samples were filtered through 0.45 μm cellulose acetate filterpapers before sCOD, TAN, NO_2-N and NO_3-N analyses. At least duplicate analyses were conducted and average values are given in tables and figures. The pH and DO monitoring was carried out on a daily basis. The methods for the analysis of each parameter are explained as follows. The N removal efficiency of the reactors were determined in terms %TN removal, %DN (% denitrification) and %SNDN (% simultaneous nitrification and denitrification) according to Appendix B.

<u>Soluble chemical oxygen demand (sCOD):</u> After the filtration, the samples were subjected to sCOD analyses via EPA approved digestion method suitable for the ranges (0-150 mg/L COD) and (0-1500 mg/L COD) (Hach Water Analysis Handbook, 2012). The COD solution was manually prepared. The measurements were carried out by spectrophotometric analysis using Aqualytic AL 38 heater and PC Multidirect Spectrophotometer (Program 130-131). The calibration curves are given in Appendix A, Figure A.1 and Figure A.2.

<u>Total ammonifiable nitrogen (NH_4-N+NH_3-N=TAN):</u> Total Ammonifiable Nitrogen, namely TAN, analyses were performed via Standard Methods (4500- NH_3 Steps B and C) after the samples were filtered through 0.45 μm cellulose acetate fitlerpapers (APHA, AWWA, WEF, 2005). The TAN in the samples were completely converted to NH_3 form and then to NH_4 form with distillation via Gerhardt Vapodest distillation

device. Then the distillates were titrated via 0.02 N sulfuric acid (H_2SO_4). The calibration curve is given in Appendix A, Figure A.3.

Nitrate nitrogen (NO_3-N) : Nitrate nitrogen (NO_3-N) analyses were conducted by EPA approved cadmium reduction method (NitraVer®) after the samples were filtered. The method was applicable for the range of 0.3 – 30 mg/L NO_3-N (Hach Water Analysis Handbook, 2012). The measurements were conducted by spectrophotometric analysis using Hach DR2800 Spectrophotometer (Hach program no 355). The calibration curve is given in Appendix A, Figure A.4.

Nitrite nitrogen (NO_2-N): Nitrite nitrogen (NO_2-N) analyses were conducted by EPA approved ferrous sulfate method (NitriVer®) after the samples were filtered. The method was applicable for the range of 2 – 250 mg/L NO_2 (Hach Water Analysis Handbook, 2012). Hach DR2800 Spectrophotometer (Hach program no 373) was utilised to accomplish the measurements. The calibration curve is given in Appendix A, Figure A.5.

pH: pH measurements were performed daily in a frequent manner, i.e. in the middle and end of the anoxic and aerobic periods. The instruments were Cyberscan pH510 pH meter (Eutech instruments,) and HI 1230 pH probe (Hanna Instruments).

Dissolved oxygen (DO): Dissolved oxygen (DO) measurements were performed daily in a frequent manner, i.e. in the middle and end of the anoxic and aerobic periods. The DO meter and DO probe were used (Extech Instruments Model 407510 Heavy Duty DO Meter). The DO concentrations of the SBRs in Set-1, Set-2 and Set-3 throughout the operation are given in Appendix C Figure C.1, Figure C.2 and Figure C.3 respectively.

Alkalinity: Alkalinity of the industrial wastewaters, namely the effluent from anerobic digester treating sugar beet processing wastewater (anaerobically-pretreated effluent) and raw sugar beet processing wastewater, in Set-3 was determined via Standard Methods (APHA, AWWA and WEF, 2005).

3.4.2 Aerobic granular sludge properties

For the evaluation of granular properties weekly analyses of TSS, VSS, EPS, granular size, granular settling velocity and SVI were accomplished. For validation of the analyses, calibration curves were prepared regarding the methods, devices and compounds. The calibration curves are given in Appendix A. The methods for the analysis of each parameter are explained as follows.

Total suspended solids (TSS) and volatile suspended solids (VSS): To determine the biomass concentration in the reactor, TSS and VSS were analyzed weekly by Standard Methods (2540 D, E) (APHA, AWWA and WEF 2005). The sampling was achieved during the aeration period of the reactor, to provide complete mixing of the reactor content. Triplicate analyses were conducted.

Average granule size: Particle sizes of randomly selected granules (at least 25 granules for each sampling) were measured weekly via light microscope and stage micrometer (Leitz Wetzlar, 6.3 x 4 magnification). The average values and standard deviations were calculated for each weekly sampling (Erguder and Demirer, 2005a). The photographs were taken by 8 megapixel mobile phone camera.

Settling velocity: The settling velocities of the granules were measured based on the study of Etterer and Wilderer (2001). One meter high glass cylinders, with 5 cm diameter and 90 cm effective height were filled with water. Randomly selected granules were allowed to free fall, flowing through water in the cylinder. The granules were assumed to reach their terminal settling velocity during the last 50 cm of the cylinder. The time at which granules travel the last 50 cm, was recorded for to calculate the average settling velocity. At least 15 randomly selected granules were analyzed weekly for each sample.

Extraction of extracellular polymeric substances (EPS):

Deficiency of EPS extraction methods for biofilms due to the leakage of intracellular substances was reported by Azeredo et al. (1999), supporting the comparison of biofilm EPS extraction methods by Zhang et al. (1999). Although numerous physical and chemical methods (including centrifugation, boiling in acid or alkali, cation exchange resins (CER)) are used for EPS extraction from biogranules, a standard procedure has not been accepted, which creates interference for comparability of the results of the EPS studies (Liu et al., 2004a). Biogranular EPS studies employ scanning electron microscopy (SEM) and transmission electron microscopy for detailed exploration (Liu et al., 2004a).

In a preliminary study conducted by Ersan (2013), 3 different EPS extraction methods for aerobic granules were compared to determine the most efficient method that provides the highest EPS extraction from total organic matter without cell lysis. According to the results, the best EPS extraction method was selected as Adav and Lee‘s (2008) ultrasound + formamide + NaOH + centrifuge (UFNC) method, among the other 2 methods which were cation exchange resin (CER) method (Durmaz, 2001; Liu and Fang, 2002; Wang et al., 2007d) and ultrasound + centrifuge (UC) method (Juang et al., 2010; Dignac et al., 1998).

Based on the preliminary study of Ersan (2013), the EPS extraction method used in the experiments was selected as UFNC method proposed by Adav and Lee (2008). The EPS extraction procedure which was used in the experiments is explained as follows. The 10 ml sludge samples were homogenously collected in falcon tubes during the aeration phase of SBR operation. Before the extraction process, to remove the soluble interferences from supernatant, the sludge samples were washed 3 times with ultra pure water. Each time, the falcon tubes were filled with ultra pure water, mixed and subjected to centrifugation at 5000 rpm for 1 min, in order to provide better settling and prevention of sludge loss. After centrifugation, the supernatant was removed until 15-20 mL sludge-liquid mixture retains in the falcon tube. The samples were put into glass tubes which were located in an ice bath later on. The ice bath carrying samples was subjected to ultrasound at 120 Watt for 5 mins, in order to accomplish cellular EPS release. Formamide of 0.06 mL was added to each sample, and the samples were subjected to vortex for 15-20 sec before they were put into the refrigerator for 1h at 4^0C. Then 4mL of 1N NaOH solution was added to the samples. After 3 hours, the samples were centrifuged at constant temperature of $+4^0$C, 10000 rpm, for 20 min. The supernatant of the samples were collected by vacuum filtration with 0.2 μm filterpapers (Adav and Lee, 2008). If required, the extracts were maintained at -15^0C, before the protein and polysaccharide concentrations of EPS were determined.

EPS protein content: The protein content of EPS was determined based on the colorimetric method by Lowry et al. (1951). Bovine albumine solution was used as the calibration material for the preparation of the calibration curve in 0-200 μg/0.6 mL absorbance range (Appendix A, Figure A.6). The EPS of the sludge samples were extracted and subjected to 1/10 dilution. Spectrophotometric measurement was performed at 750 nm wavelength.

EPS carbohydrate content: For the determination of carbohydrate content of EPS the colorimetric method proposed by Dubois et al. (1956) was used. Alginate solution was used as the calibration material for the preparation of the calibration curve in 0-80 μg/2 mL absorbance range (Appendix A, Figure A.7). The EPS of the sludge samples were extracted and subjected to 1/10 or 1/20 dilution. Spectrophotometric measurement was performed at 480 nm wavelength.

Sludge volume index (SVI): SVI of the seed sludge and reactor contents were weekly measured according to the Standard Methods (2710 D) (APHA, AWWA and WEF, 2005).

Granulation Percentage: The granulation percentage of the sludge was determined based on the dynamic SVI measurement suggested by Schwarzenbeck et al. (2005). Dynamic SVI is based on the computation of consecutive SVI values of a sludge sample in a 1 L-graduated cylinder, over a settling period of 30 minutes. The dynamic SVI method used by Schwarzenbeck et al. (2005) is a slight modification of the SVI measurement which aims to relate the sludge settling behaviour and structural characteristics. A homogenous sludge sample in a 1 L graduated cylinder was allowed to settle for 30 min. Its volume was recorded every 30 seconds during 10 min, and then every 2 min for the rest 20 min. The sludge volume data was normalized according to the MLSS content of the sample. For instance SVI_5 represents the sludge volume (mL) per gram of MLSS, during the first 5 min of a total settling period of 30 min. Thus SVI_{30} is the sludge volume (mL) per gram of MLSS, at the end of the 30 min settling period.

Based on the observations of Schwarzenbeck et al. (2005), it can be assumed that the flocculent sludge portion reaches its terminal SVI in 30 min, whereas the granular portion becomes almost settled during the first 5 min. Using the 5 min-SVI and 30 min-SVI values, i.e. SVI_5 and SVI_{30}, the granulation percentage of the sludge was computed, according to Equation 3.1 (Thanh et al., 2009; Zhang et al., 2011a). According to Liu et al. (2010a), this equation is not valid when the average granule sizes are below 0.2 mm and when there is no visual observation of granules.

$$\text{Granulation percent (\%)} = 100\text{x } (SVI_{30}/SVI_5) \qquad \text{(Equation 3.1)}$$

CHAPTER 4

RESULTS AND DISCUSSION

The thesis study consists of 3 experimental sets namely, Set-1 (the effects of influent COD/TAN ratio), Set-2 (the effects of increasing OLR and NLR values) and Set-3 (the treatment of sugar beet processing wastewaters). The results of each set are explained and discussed in this chapter.

4.1. Set-1: The effects of influent COD/TAN ratio on aerobic granules and their treatment efficiency

The relative ratios of influent carbon and nitrogen such as COD/N and COD/TAN affect the aerobic granules in terms of formation, elemental composition, microbial distribution, physical characteristics and treatment efficiency (Liu et al., 2003b; Wu et al., 2012; Li et al., 2011; Yang et al., 2005; Cydzik and Baryla, 2011). The influent COD/TAN ratio adjustments may provide microbial selection pressure to enrich either heterotrophic or nitrifying species contributing to granulation (Wu et al., 2012). A wide range of influent COD/N ratios from 1 to 20 allowed aerobic granulation in previous studies (Yang et al., 2005; Wu et al., 2012; Liu et al., 2003b). High COD/N ratios such as 20 were known to enhance the relative amounts and activities of heterotrophs with respect to nitrifiers in aerobic granules, while the enrichment of slow-growing nitrifiers in the granular structure were reported at low COD/N ratios such as 3.3 and below (Liu et al., 2003b; Wu et al., 2012; Yang et al., 2005; Cydzik and Baryla, 2011). Set-1 aimed to determine the optimum influent COD/TAN ratio that allowed the highest possible removal efficiencies in terms of COD and TAN, without destroying structural stability of the granules. Set-1 was conducted to investigate the effects of influent COD/TAN ratios among 1-30, and in turn the influent COD/TAN concentrations from 200/200 (mg/L / mg/L) to 6000/200 (mg/L / mg/L) on the treatment efficiencies and characteristics of aerobic granules. In Set-1 R1 and R2 were operated at gradually increasing influent

COD/TAN ratios (7.5, 10, 20, 30) and gradually decreasing influent COD/TAN ratios (7.5, 5, 3.5, 2, 1), respectively.

4.1.1. Treatment efficiency

Both R1 and R2 were operated for 101 days. During the first 10 days, R1 and R2 were operated at an influent COD/TAN ratio of 7.5 and the corresponding influent concentrations of 1500/200 (mg/L / mg/L). On Day 10, the COD and TAN removal efficiencies of each reactor stabilized and the average of 3 consecutive removal efficiency analyses for R1 and R2 differed less than 3%. Therefore, the influent COD/TAN ratio changes were started by Day 11. The operational period of R1 and R2 for 101 days is explained, in terms of the changes in N removal, organics removal and granular structure.

4.1.1.1. N removal

Removal of nitrogen in Set-1 was calculated based on TAN and TN removal efficiencies. The percentages of anoxic period denitrification (%DN) and the simultaneous nitrification denitrification (%SNDN) were computed according to Equations B.5-B.9 in Appendix B.2. The calculation of SNDN and TAN values were not conducted unless the pH is less than 9.3, in order to prevent the interference from free-ammonia stripping as explained in Appendix B.3.

4.1.1.1.1. N removal efficiency in R1

Figure 4.1 shows the effluent TAN concentrations of aerobic and anoxic periods of R1, the percent TAN removal efficiencies obtained during anoxic periods, aerobic periods and the overall cycle of concern (i.e. cyclic removal efficiency), and the theoretical influent OLR and NLR values throughout the operational period. During the whole operation, TAN treatment was mainly observed in the aerobic periods, as expected. The reason is that TAN treatment includes the oxidation of TAN to TON, which is possible by nitrification. Nitrification, which requires oxygen and thus aeration of the reactor, can only take part in the aerobic period (Ni et al., 2008). The TAN losses rarely observed during anoxic periods were attributed to ammonia stripping but not oxidation which was discussed further in detail.

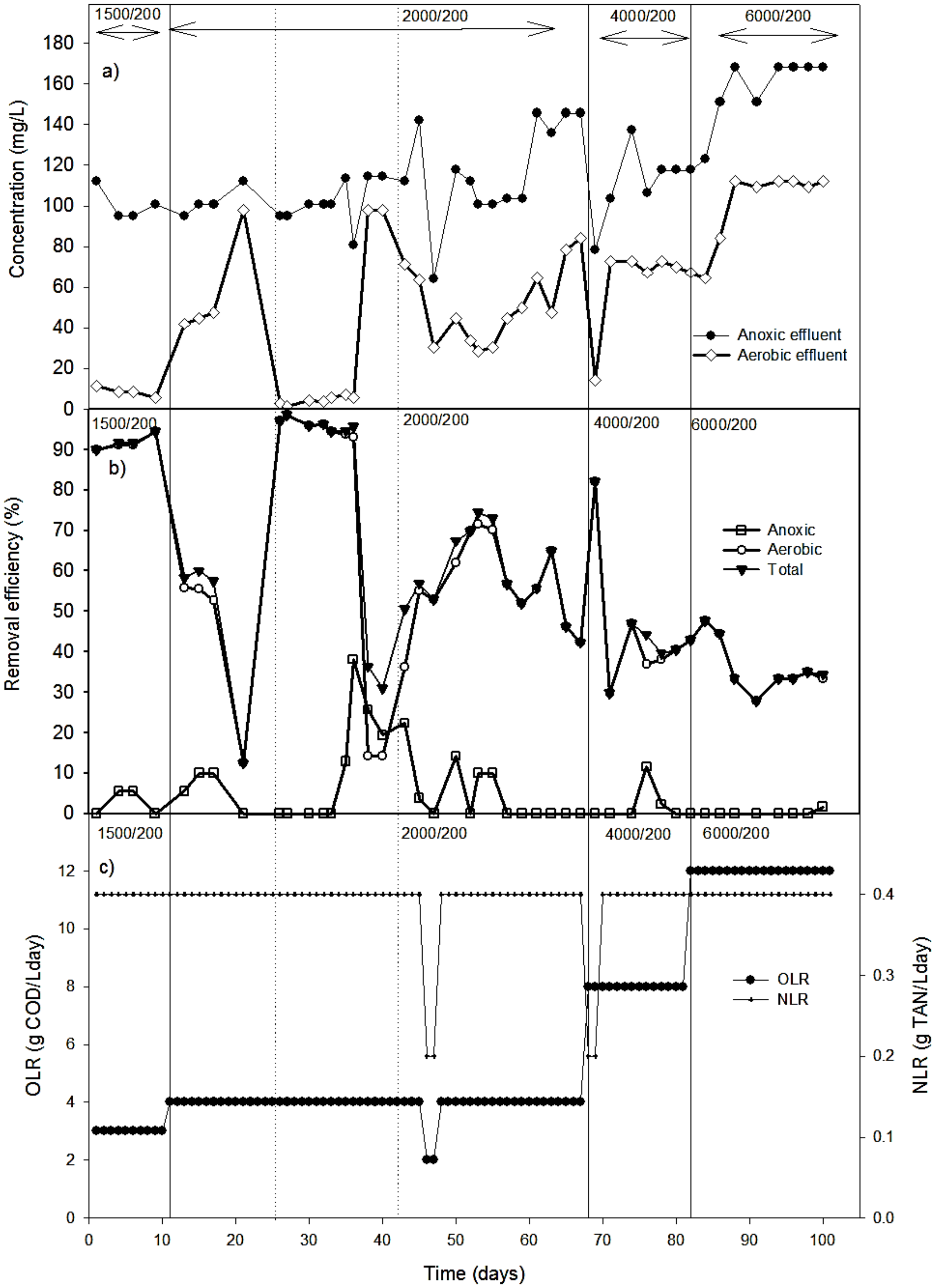

Figure 4.1. TAN graph for R1; a) Effluent TAN concentrations of anoxic and aerobic periods, b) TAN removal efficiency for anoxic period, aerobic period and total cycle, c) OLR and NLR of R1 during the operational period. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations). (Dashed lines represent the washing of the reactor content, while the influent COD/TAN ratio changes are indicated by the solid lines).

During the first 10 days of the operational period, R1 was operated at an influent COD/TAN ratio of 7.5 and showed 92±2% TAN removal efficiency on the average (Days 1-10) (Figure 4.1). During the first 10 days, the average pH values were measured as 9.0±0.1 in anoxic periods and 8.6±0.2 in aerobic periods (Figure 4.2). On Day 11, the influent COD/TAN ratio was increased from 7.5 to 10, the influent concentrations were increased from 1500/200 to 2000/200 (mg/L COD / mg/L TAN). Accordingly the influent OLR was increased from 3 to 4 g COD/Lday while TAN loading rate was kept constant at 0.4 g TAN/Lday (Figure 4.1c). These conditions were applied during Days 11-67. During Days 11-67, TAN removal efficiency of R1 showed a fluctuating trend with frequent decreases (Figure 4.1). The cyclic average TAN removal efficiency of R1 at the influent COD/TAN ratio of 10 was calculated as 65±23% (Days 11-67).

The optimum pH range for nitrification-denitrification is defined to be 7-8.6 (Tchobanoglous et al., 2004). During the first ten days of the influent COD/TAN ratio of 10 application, i.e. Days 11-20, the pH values ranged between 6.8-8.9 for anoxic and 8.5-8.8 for aerobic periods (Figure 4.2). However, starting from Day 21, the pH values increased up to 9.2 in anoxic and 9.7 in aerobic periods. The influences and reasons of this undesired pH increase are discussed below.

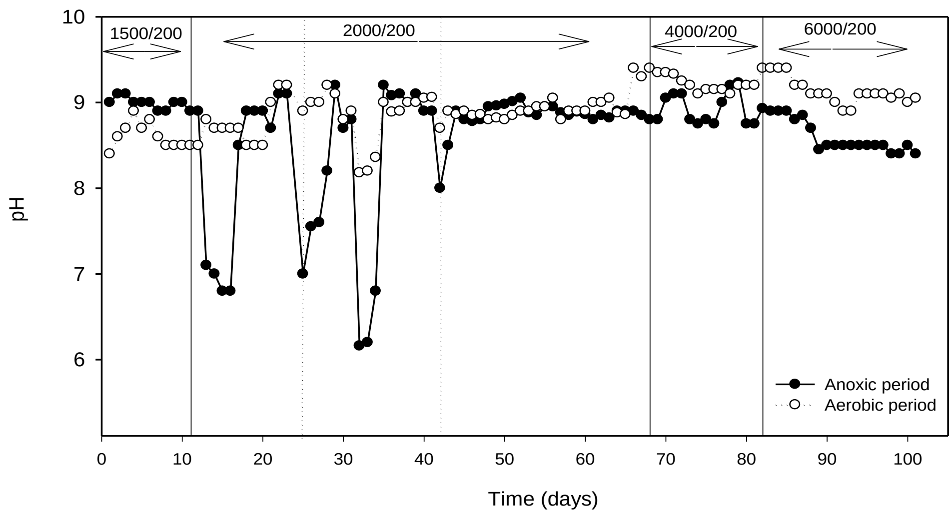

Figure 4.2. pH values of R1 during the operational period. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations). (Dashed lines represent the washing of the reactor content, while the influent COD/TAN ratio changes are indicated by the solid lines).

Above pH 9.3 (at 25^0C), the TAN in the medium becomes dominant in free-ammonia (FA) nitrogen (NH_3-N) instead of the ammonium nitrogen (NH_4-N) (Appendix B.3). FA concentrations of 10-150 mg/L, corresponding to NH_3-N concentration of 8.2-123 mg/L, is known to be inhibitory for nitrifiers (Anthonisen et al., 1976). Yang et al. (2004b) observed the inhibition of aerobic granulation above 23.5 mg/L FA. Serious decrease in the aerobic granular metabolic activity was observed at 39.6 mg/L FA concentration (Peyong et al., 2012). Increasing the FA concentration from 2.5 to 39.6 mg/L NH_3-N decreased the respirometric activity of nitrifiers and heterotrophic bacteria 2.5 and 5 times, respectively (Yang et al., 2004b). In order to assess the suspected FA toxicity, the maximum available FA nitrogen concentrations were calculated regarding the temperature, pH and TAN concentrations (Anthoniessen et al., 1976) (See Appendix B.3, Eqn B.10-11). The calculated FA concentrations were shown in Figure 4.3 and the ranges are listed in Table 4.1.

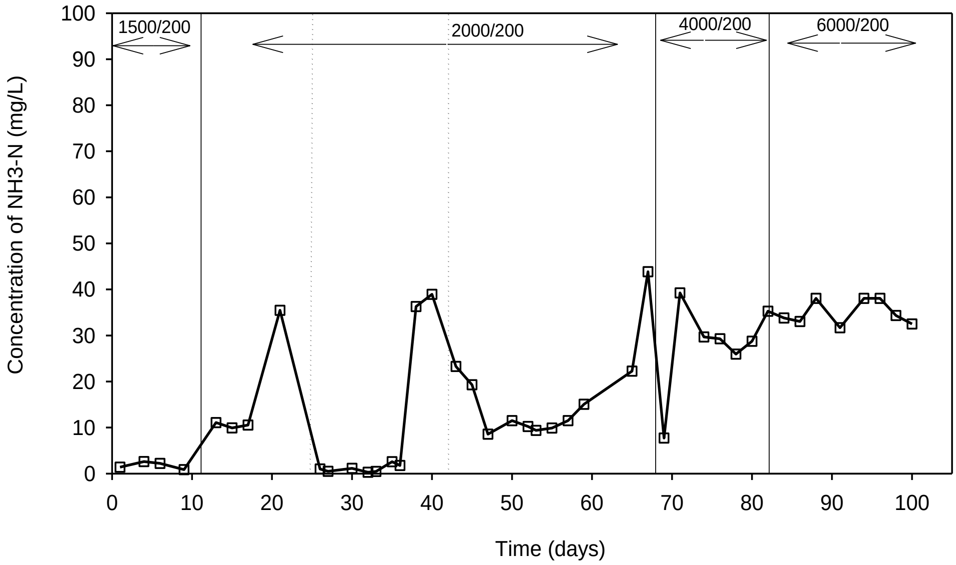

Figure 4.3. The FA concentrations calculated based on the TAN concentrations, pH and temperature during the operational period of R1. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations). (Dashed lines represent the washing of the reactor content, while the influent COD/TAN ratio changes are indicated by the solid lines).

On Day 21, when TAN removal efficiency decreased down to 13% (Figure 4.1), the FA concentration was calculated to be 35.5 mg/L NH_3-N which was inhibitory (Yang et al., 2004b) (Figure 4.3). Thus the gradual drop in TAN removal efficiency from

58% (Day 13) to 13% (Day 21) (Figure 4.1) was thought to be related to the inhibition of nitrifier activity due to the FA toxicity.

Table 4.1. The FA nitrogen concentrations calculated with respect to the observed pH and temperature for R1 with increasing influent COD/TAN ratios.

Day	Influent COD/TAN ratio	Anoxic pH		Aerobic Ph		Theoretical NH_3-N (mg/L)		[a]T^0C
		Ave	Max	Ave	Max	Ave	Max	
1-10	7.5	9±0.1	9.1	8.6±0.2	8.9	1.8±0.8	2.6	24.5
11-67	10	8.5±0.8	9.2	8.9±0.2	9.4	13.9±13.1	43.8	24.5
68-81	20	8.9±0.2	9.2	9.2±0.1	9.4	26.8±10.4	39.2	23
82-101	30	8.6±0.2	8.9	9.1±0.2	9.4	35±2.5	38.1	20

[a]Observed temperature values used in the FA calculations.

Paralel to the deterioration of TAN removal efficiency, as further discussed in Section 4.1.2.1, the granular structure was adversely affected and the biomass content of the reactor decreased from 3410 mg/L VSS on Day 11 to 2580 mg/L VSS on Day 17. It was likely that the FA toxicity started by Day 17, when the FA concentration was 10.5 mg/L (Figure 4.4). Thus the nitrifiers were likely to be washed-out due to the inhibited nitrification as a result of FA toxicity.

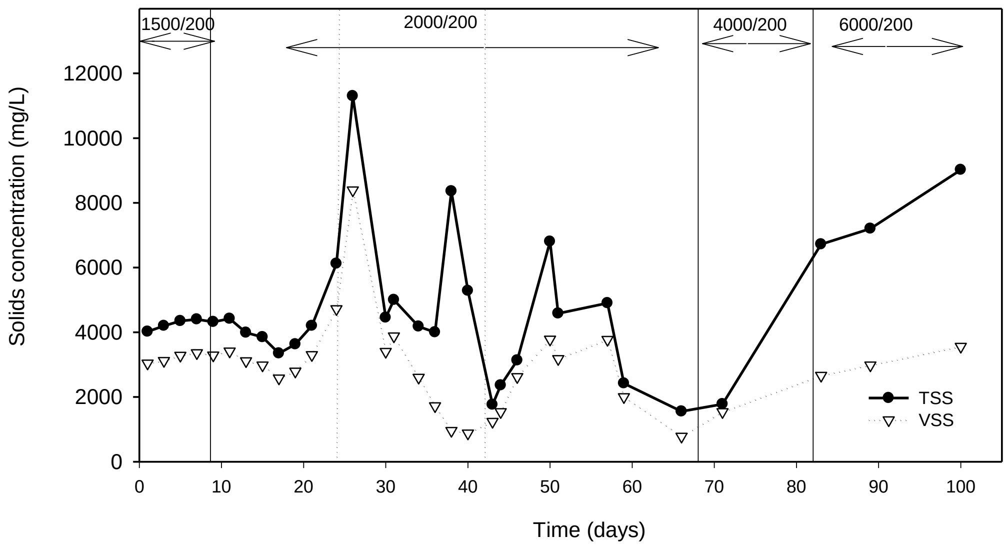

Figure 4.4. Concentration of solids in terms of TSS and VSS in R1 throughout the operation. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations). (Dashed lines represent the washing of the reactor content, while the influent COD/TAN ratio changes are indicated by the solid lines).

In order to prevent the inhibition of bacteria due to long term ammonia toxicity exposure, the reactor content was washed with tap water on Day 25. In addition, to decrease the alkalinity and in turn minimize the conversion of NH_4 to NH_3, the influent bicarbonate was decreased from 1800 to 1200 mg/L $NaHCO_3$. Following the sludge wash, the pH was observed to decrease (Figure 4.2), and the maximum TAN removal of the operation (above 98%) was observed on Day 27 (Figure 4.1). Starting from Day 35, the undesired pH increase (>9) was noted during the anoxic and aerobic periods (Figure 4.2). In order to adjust the pH to the optimum ranges for nitrification-denitrification, dilute HCl solution was added to the reactor whenever the aerobic period's pH reached the values of 9.7. Although the TAN removal efficiency of the system was as high as 96% on Day 36 (Figure 4.1), the delayed effect of the undesired pH increase was observed on Day 38 when the cyclic TAN removal efficiency dropped to 36% (Figure 4.1).

The pH increase was uncontrollable and caused unsuitably high pH values (above 8.6) for nitrification and denitrification. When the pH exceeded 9.3, which is the pKa value of the ammonium-ammonia (Appendix B.3), the TAN in the soluble ammonium form shifted to ammonia which is known to be inhibitory for nitrifiers. This caused inhibition of nitrifier activity, i.e. inhibited the nitrification and TAN removal. Since the TAN cannot be removed via nitrification in the system, the untreated TAN got accumulated. Due to the high pH>9.3, the accumulated TAN shifted to the toxic ammonia above a level. As the TAN treatment via nitrification became deteriorated, some of the nitrifiers might have been washed-out of the reactor due to the ammonia toxicity. The granular structure became disintegrated, and the biomass content of the reactor decreased, as explained in Section 4.1.2.1.

The pH increase could not be prevented and R1 experienced its second highest NH_3-N concentration of 38.9 mg/L while the TAN removal efficiency decreased to 30% (Day 40, Figure 4.1, Figure 4.2, Figure 4.3). During this period (Days 38-41), the cyclic TAN removal efficiency was as low as 31-36% (Figure 4.1) and the granular structure of R1 was seriously deteriorated (as explained in Section 4.1.2.1). In order to cleanse the accumulated ammonia nitrogen and eliminate the undesired pH, R1 content was washed for the second time on Day 42, aiming minimum sludge loss. However the recovery capability of the system worsened after the second wash (on

Day 42) with respect to the first (on Day 25). After the second sludge wash, the pH values decreased to 8-8.5 for anoxic period and to 8.7-8.9 for aerobic period on Days 42-43 (Figure 4.2). The VSS and TSS were measured to be as low as 1240 and 1760 mg/L, respectively, by Day 43 (Figure 4.4). Despite this relatively low biomass content, the cyclic TAN removal efficiency of the system increased to 51-57% on Days 43-45, which was attributed to the removal of accumulated TAN via second sludge wash (Figure 4.1). In order to decrease the loading rates applied to low concentration of microorganisms, and increase the TAN removal efficiency, the COD/TAN concentrations of 1000/100 (mg/L/mg/L) were applied (at constant COD/TAN ratio of 10) to the system for 2 days starting from Day 46 (Figure 4.1c). Halving the organic and nitrogen loading rates at constant COD/TAN ratio improved the treatment efficiency, while the pH decreased below 9.1 (Figure 4.1b, Figure 4.2). To fullfill the research goals, the COD/TAN concentrations were increased back to 2000/200 (mg/L/mg/L) on Day 48, which resulted in undesired pH increase (>9). Although the pH values were in the ranges of 8.9-9.05 in anoxic and 8.8-9 in aerobic periods (Days 48-55, Figure 4.2), the cyclic TAN removal efficiency gradually increased from 67% on Day 50 to 75% on Day 53 (Figure 4.1). This increase in cyclic TAN removal efficiency was attributed to the decrease in accumulated TAN concentration achieved by the second sludge wash (on Day 42) as well as the halved loading rates (on Days 46-47). Therefore, despite the high pH range (Days 48-55), the FA nitrogen concentration did not reach the inhibitory levels (Figure 4.2) and TAN removal efficiency still improved.

As mentioned so far, it was observed that pH increased within the duration time of anoxic periods from 8.7 to 9.2 and aerobic periods from 8.5 to 9.2. The increase in pH during anoxic periods was expected and attributed to denitrification (Appendix B, Eqn B.4). Yet, the increase in pH observed during aerobic periods, where nitrification and in turn pH decrease were expected, was interesting. Despite the presence of nitrification (i.e. TAN oxidation), the increase in pH during the aerobic periods was attributed to the simultaneous denitrification (Appendix B, Eqn B.7) and sulfate reduction (Eqn 4.1) reactions occuring within the aerobic granules. The sulfate reduction; i.e. the reduction of SO_4^{2-} to HS^- (or H_2S and $S^=$ depending on the pH) by the sulfate-reducing bacteria (SRB) (Sawyer et al., 2003), involves the consumption of H^+ ions and production of alkalinity as bicarbonate (HCO_3^-), which increases the pH. The electron acceptor is acetate in our case as shown below (Eqn 4.1).

$$CH_3COO^- + SO_4^{2-} + H^+ \rightarrow CO_2 + HCO_3^- + HS^- + H_2O \quad \text{(Eqn 4.1)}$$

Due to the presence of SRB in aerobic biofilms (Lens et al., 1995), the SRB might participate in aerobic granule formation. Also the seed sludge used in this study was claimed to contain SRBs by Ersan and Erguder (2013). Since the TON reduction in the aerobic period, indicating denitrification simultaneous to the nitrification, was insufficient to explain the pH increase, the presence of SRB activity was likely. In addition, the kinetic studies showed that the amount of TON oxidized in the aerobic period, and in turn the alkalinity production, was not enough to increase the pH over 9 (data not shown). Thus, the aerobic denitrification, itself, was not the main cause of the pH increase.

To eliminate the high pH arising from potential sulfate reduction, on Day 52 the concentration of influent $MgSO_4 \cdot 7H_2O$ in the basal medium, which might have been the source of sulfate, was decreased from 90 to 40 mg/L (SO_4 concentration was decreased from 35.1 to 15.6 mg/L). This was followed by a slight pH decrease, and TAN removal efficiency increased to 75% by Day 53 (Figure 4.1). Unfortunately after 2 days (8 cycles), high pH such as 9.1 was observed again during aerobic period on Day 56. The pH even reached 9.4 in aerobic period on Day 66, and this high pH lead to an increase in NH_3-N concentration to the peak of 43.8 mg/L on Day 67 (Figure 4.3). In the mean time TAN treatment efficiency deteriorated and decreased down to 42% on Day 67 (Figure 4.1). Despite the reduction in the influent sulfate concentration and potential SRB activity, the pH increase continued. Thus it was likely that the SRBs had been already enriched in the aerobic granular sludge. Since there was only slight increase about 5% in SNDN%, the pH increase could not be attributed to aerobic denitrification only (data not shown). Further pH increase, despite the reduction in sulfate concentration and the pH control, remained unexplained.

To summarize, the application of influent COD/TAN ratio of 10 during Days 11-67 adversely affected TAN removal efficiency of R1. The uncontrollable pH increases decreased the TAN removal efficiency which caused untreated TAN to accumulate in the reactor at the beginning of the cycles. Considering the 50% VER and 200 mg/L influent TAN concentration, 100 mg/L initial TAN concentration at the beginning of each cycle was expected in the reactor in the case of 100% TAN removal efficiency. However due to the decreased TAN removal efficiency and remained TAN at the end of the cycles, initial TAN concentrations were higher than the aimed concentration of 100 mg/L. Thus, at high pH, the dominant TAN form shifted to NH_3-N, which created ammonia inhibition above a certain level and caused granular disintegration. It was suspected that the inhibited nitrifiers had been washed-out of the reactor, justifying the VSS decrease down to 780 mg/L on Day 67 (Figure 4.4). The initial TAN concentration was measured as high as 150 mg/L on Day 67, which was close to the maximum achievable TAN concentration of 200 mg/L when there is no TAN removal. Before shifting to the COD/TAN ratio of 20, in order to eliminate the accumulated TAN by nitrification bacteria, the influent TAN concentration was halved to 100 mg/L for 2 days (Days 68-69). In other words, R1 was fed with COD/TAN concetration ratio of 4000/100 instead of COD/TAN of 4000/200 for 2 days so that the accumulated TAN could be consumed by the nitrifiers. This indeed increased the TAN removal efficiency over 80% (Day 69) (Figure 4.1). Therefore, the influent TAN concentration was increased back to 200 mg/L on Day 71. During Days 71-81, the reactor was operated at the influent COD/TAN concentrations of 4000/200 (mg/L/mg/L), corresponding to the influent COD/TAN ratio of 20. In this period the NH_3-N concentration varied between 25-40 mg/L (Figure 4.3). As seen in Figure 4.1, following the ratio change to 20, TAN removal efficiency decreased again, a little regarding the effect of ratio 10, and was recorded as 40±7% on the average during Days 68-81. Since the TAN removal efficiency was stable at 40-45% during Days 76-81 (Figure 4.1), the influent COD/TAN ratio was increased to 30 on Day 82, in order to observe the effect of the higher COD/TAN ratio on granular structure as well as the treatment efficiency.

For the last COD/TAN ratio of 30 (6000/200) studied, about 37±7 % TAN removal efficiency was observed on the average (Days 82-101) (Figure 4.1). The TAN removal efficiency was nearly constant at 33% during Days 92-101 (Figure 4.1). During this period, the pH of anoxic period was in the range of 8.6-8.95, and the aerobic period pH decreased from 9.4 to 9.1±0.2 (Days 82-101) (Figure 4.2). Although the high pH problem was not completely solved, there was a slight decrease in pH values. TAN treatment performance of the system remained almost constant at around 33%. At the influent COD/TAN ratio of 30, due to the increase in the influent carbon concentrations, heterotrophic bacteria growth was thought to be favored and average VSS concentration of R1 increased from 2660 mg/L on Day 83 to 3560 mg/L on Day 100 (Figure 4.4). However, filamentous growth was also observed.

Consequently, as COD/TAN ratio increased from 7.5 to 30, the TAN removal efficiency was adversely affected and showed a gradual decrease from 92% down to 37%. In other words, at the constant influent (feed) TAN concentration of 200 mg/L, the increase in the influent COD concentration from 1500 to 6000 mg/L, and in turn the increase in influent OLR from 3 to 12 g COD/Lday, adversely affected the TAN removal efficiency. The influent COD/TAN ratio of 10, and the corresponding influent concentrations of 2000 mg/L COD and 200 mg/L TAN, were observed as the transitional operational conditions that cause fluctuations and decrease in TAN removal efficiency. The results are in compliance with the literature findings; since nitrifier abundancy in the granules relative to that of the heterotrophic bacteria decreased with increasing COD/TAN ratios (Yang et al., 2005; Liu et al., 2003b; Cydzik and Baryla, 2011). At the influent COD/TAN ratio of 10, both aerobic heterotrophic and nitrification bacteria were thought to be effective. At ratios 20 and 30, the relative amounts of nitrification bacteria in the aerobic granular structure were likely to be further decreased and then stabilized, as also indicated by the low but almost constant TAN removal efficiencies.

Denitrification and nitrification performances as well as TN removal efficiency of R1, which are calculated by the equations in Appendix B (Eqn B.5- B.9), are shown in Table 4.2. During the one week start-up period in one SBR, no SNDN was observed; while the anoxic denitrification was 89±2% (data not shown). Following the sludge division to 2 SBRs, R1 showed high anoxic denitrification efficiency of 92±10%, but very low SNDN performance of 10±2% and moderate TN removal of 48±8% during Days 1-10, at the influent COD/TAN ratio of 7.5 (Table 4.2). The influent COD/TAN ratio was increased to 10 (Days 11-67). The nitrate-nitrogen required for anoxic denitrification is the nitrate produced by nitrification in the previous cycle and the nitrate in the feed (nutrient solution). Since sufficient amount of nitrate (80 mg/L) was produced during the aerobic periods between Days 1-19, the nitrate nitrogen in the nutrient solution was decreased from 160 to 80 mg/L NO_3-N on Day 20. During the application of the influent COD/TAN ratio of 10, %DN decreased to 31±17%, while the %SNDN increased to 25±19% on the average (Table 4.2). The TN removal decreased to 18±10%, which was attributed to the decreased TAN removal (Figure 4.1), as a result of FA toxicity and inhibition of nitrifiers at this period (Days 11-67). Moving on to the influent COD/TAN ratio of 20 (Days 68-81), the average %DN decreased to 26±17%, and %SNDN slightly increased to 31±6%, while the TN removal was 17±4% (Table 4.2). The increase in influent COD/TAN ratio to 30 (Days 82-101) did not change the N removal performance of R1, since the average %DN was 27±14%, %SNDN was 30±4% and the TN removal was nearly stable at 17±6% (Table 4.2).

Table 4.2. Nitrification and denitrification performances and TN removal efficiencies of R1 and R2.

SBR No	COD /TAN ratio	Influent COD/TAN (mg/L/mg/L)	[a]DN (%)	[b]SNDN (%)	[c]TN (%)
R1	7.5	1500/200	92±10	10±2	48±8
	10	2000/200	31±17	25±19	18±10
	20	4000/200	26±17	31±6	17±4
	30	6000/200	27±14	30±4	17±6
R2	7.5	1500/200	89±10	8±2	46±10
	5	1000/200	66±18	42±5	54±5
	3.5	700/200	8±6	43±10	35±10
	2	400/200	12±3	65±9	43±4
	1	200/200	11±4	58±17	26±4

[a] DN(%): the denitrification percent at the anoxic periods
[b]SNDN(%):the denitrification efficiency simultaneous to nitrification during aerobic period
[c]TN(%): total nitrogen removal percent, calculated using TAN and TON values

The drastic decrease in TN removal efficiency from 48±8% at the influent COD/TAN ratio of 7.5 (Days 1-10) to 18±10% at the influent COD/TAN ratio of 10 (Days 11-67) was attributed to the deteriorated nitrifier activity and thus the decreasing TAN removal performance of the reactor (Figure 4.1) (Table 4.2). When the influent COD/TAN ratios were further increased to 20 and 30, the TN removal efficiency was nearly constant at 17% (Days 68-101) (Table 4.2), indicating more or less constant nitrification-denitrification activity. As seen from Table 4.2, the %DN gradually decreased from 92% to 26-27%, and the %SNDN slightly increased from

10% to over 26-31% as the influent COD/TAN ratio of R1 stepwise increased from 7.5 to 30.

Denitrification bacteria are located at the inner side of the granules and are surrounded by nitrifiers and heterotrophic bacteria at the outer granular surface (Gao et al, 2011; Figure 2.2). The oxidation of TAN, via nitrification, is carried out in the outer layers, while the TON reduction via denitrification occurs in the deeper granular structure (de Kreuk et al., 2005b). At high influent COD concentrations, in turn the high influent OLRs and COD/TAN ratios, higher carbon source is present favoring the carbon-consuming heterotrophic bacteria. Thus, at increasing COD/TAN ratios, aerobic heterotrophic bacteria outcompete the nitrifiers for DO and space on the outer granular layer. The enrichment of carbon-consuming heterotrophic bacteria is indicated by the high cyclic COD removal efficiencies of R1 with the increasing influent COD/TAN ratios as explained in Section 4.1.1.2. Higher carbon concentrations support the growth of aerobic heterotrophic biomass and granular size increases. The diffusion of DO and nutrients are prevented on the other hand when granule size exceeds a certain diameter (such as 1.8-2 mm) (de Kreuk et al., 2005b; Su and Yu, 2005). Such mass transfer limitations cause nutrient defficiency for the denitrifiers located in the deeper parts of the granules. As explained in Section 4.1.2, the granule sizes were smaller (1.5-2.5 mm) at the COD/TAN ratios of 10 and above (Days 11-101), compared to the size (3.2 mm) at the COD/TAN ratio of 7.5 (Days 1-10). However the filaments and flocs formed around the granules were likely to obstruct the diffusion of carbon and nitrate into the deeper granule. Thus the decreasing anoxic denitrification from 92% (Days 1-10) to 26-31% (Days 11-101) could be attributed to the mass transfer limitations of nitrate and carbon source in the reactor wastewater content as a result of higher influent COD/TAN ratios and favored filamentous growth around granules (Table 4.2). In addition, as previously mentioned, the decreased relative abundancy of nitrifiers in the granular structure with increasing influent COD/TAN ratios (Yang et al., 2005; Liu et al., 2003b) might have decreased the nitrification efficiency as indicated by the deteriorated TAN removal efficiency from 92% (Days 1-10) down to 37% (Days 82-101) (Figure 4.1). Due to the decreased nitrification, and in turn the decreased nitrate production, the amount of nitrate that remained in the reactor after the settling period for the following cycle was also lower. Hence, in the anoxic period of the following cycle, lower nitrate became available for the denitrification bacteria, which resulted in decreasing %DN. % SNDN was around 25-31% between Days 11-101. The initial increase in %SNDN from 10±2% to 25±19% might be attributed to the better diffusion of TON produced by nitrifiers closely located to the denitrifiers within the granular structure (Figure 2.2).

4.1.1.1.2. N removal efficiency in R2

Figure 4.5 shows the effluent TAN concentrations of aerobic and anoxic periods of R2, the percent TAN removal efficiencies obtained during anoxic periods, aerobic periods and the overall cycle of concern (i.e. cyclic removal efficiency), and the theoretical influent OLR and NLR values throughout the operational period.

During the whole operation, TAN treatment was mainly observed in the aerobic periods, as expected. The anoxic period TAN removal was below 10% during Days 1-33, which can be explained by the experimental errors (Figure 4.5). However higher anoxic period TAN removal efficiencies between 11% to 25% during Days 25-53 (Figure 4.5), might be attributed to the ammonia stripping due to the high pH. The pH values are discussed in the following parts of this section and shown in Figure 4.7. Nevertheless, due to the high TAN oxidation efficiencies, the FA levels were not inhibitory for the nitrification bacteria. The FA concentrations calculated based on the TAN concentrations, pH and temperature during the operational period of R2 are given in Appendix B, Figure B.1.

The average TAN removal efficiency was 92±5% at a COD/TAN ratio of 7.5 during the first 10 days. On Day 11, the influent COD/TAN ratio of R2 was decreased to 5 (1000/200 mg/L of COD/TAN). The COD loading rate was decreased from 3 to 2 g COD/Lday, while TAN loading rate was contant at 0.4 g TAN/Lday (Figure 4.5c). The TAN removal efficiency showed an increase and followed a stable trend about 99% at the COD/TAN ratio of 5 (Days 11-28) (Figure 4.5). The disintegration in the granular structure and formation of smaller granules were observed; whereas the solid content was increasing. The VSS concentration of R2 increased from 3620 mg/L (Day 11) to 5920 mg/L (Day 24) (Figure 4.6). These observations, together with the improved TAN removal, might have indicated the supporting of nitrifiers in R2 under the studied conditions.

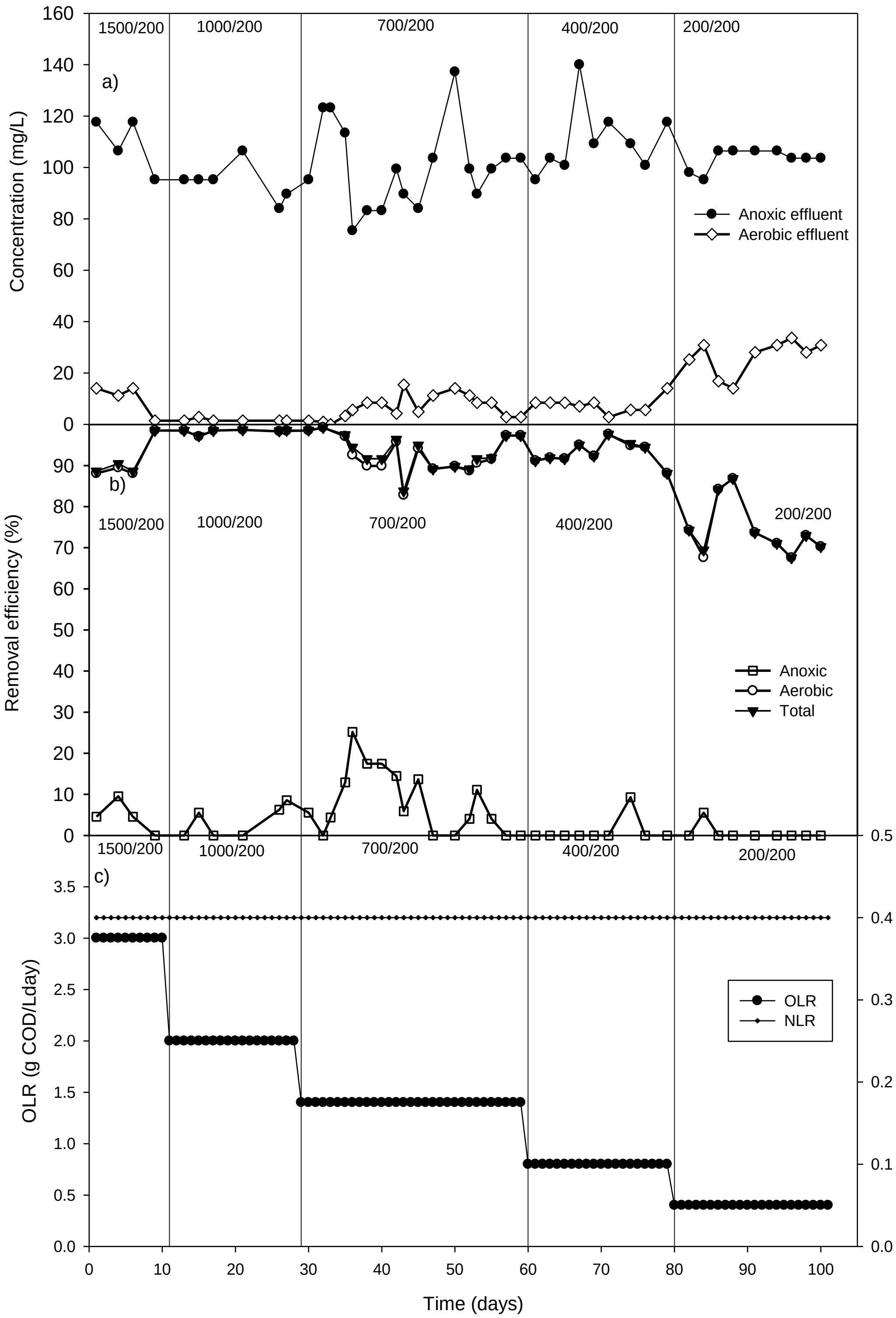

Figure 4.5. TAN graph for R2; a) Effluent TAN concentrations of anoxic and aerobic periods, b) TAN removal efficiency for anoxic period, aerobic period and total cycle, c) OLR and NLR of R2 during the operational period. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations).

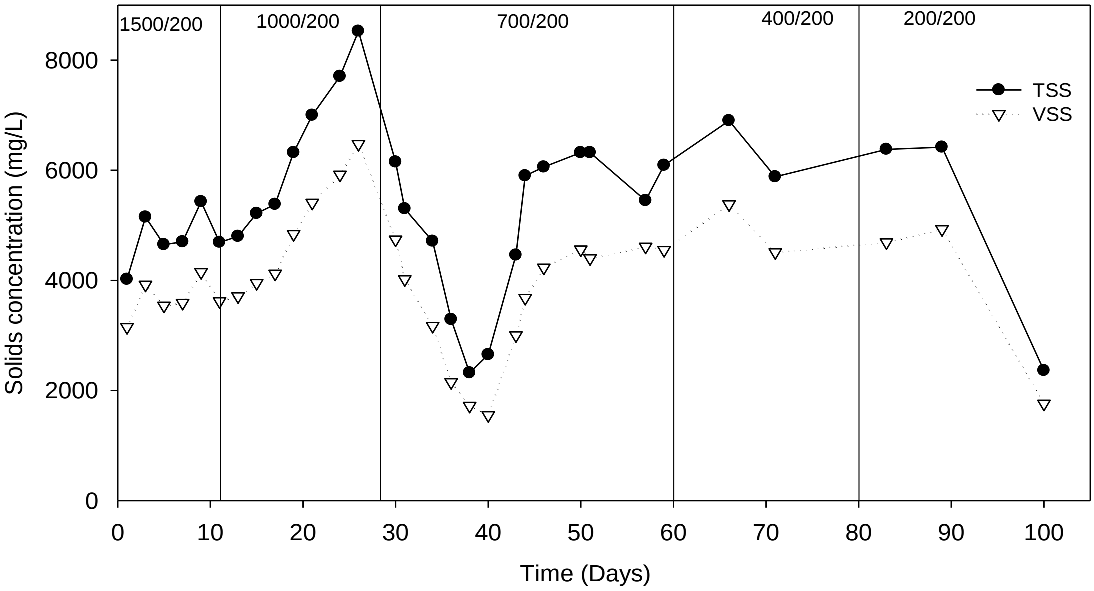

Figure 4.6. Concentration of solids in terms of TSS and VSS in R2 throughout the operation. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations).

The influent COD/TAN ratio was further decreased to 3.5 on Day 29 and applied till Day 59. The COD loading rate was 1.4 g COD/Lday, with the constant TAN loading rate of 0.4 g TAN/Lday by Day 29 as well (Figure 4.5c). During Days 29-36, the cyclic TAN removal efficiency of R2 was as high as 95-100% (Figure 4.5). However, on Days 38-40, the cyclic TAN removal efficiency decreased below 92% (Figure 4.5), which was attributed to the low VSS concentrations (1550-1720 mg/L) on Days 38-40 (Figure 4.6). Regarding Figure 4.6, the gradual decrease in the biomass concentration of the reactor during Days 29-40 (from 4740 to 1550 mg/L VSS and 6150 to 2650 mg/L TSS) are seen. This biomass decrease in the reactor was thought to be due to the decreasing heterotrophic bacteria percentage in granules with the decreasing COD/TAN ratio and influent COD concentration (Yang et al.,2005). When the decreasing TAN removal efficiency from 95-100% (Days 29-36) to below 85% (Days 38-40) are considered (Figure 4.6), it was also likely that a small amount of nitrifiers was also washed-out from the reactor. However the VSS decrease in the reactor was mostly attributed to the wash-out of heterotrophic bacteria since the nitrifiers with low growth rate (0.014-0.064/hr) were thought to be dominant over fast-growing heterotrophic bacteria (1.00-1.44/hr) at low influent COD/TAN ratios (like 1-2) (Wu et al.,2012). To avoid any further VSS decrease and the potential wash-out of slowly-growing nitrifiers, settling time was increased from 2 to 5 minutes during Days 41-47. Longer settling time provided 4500 mg/L VSS and about 97% TAN removal efficiency by the end of the period (Day 59) where COD/TAN ratio was 3.5 (Figure 4.5, Figure 4.6). The average cyclic TAN removal efficiency of R2 during Days 29-59 was 94±4%.

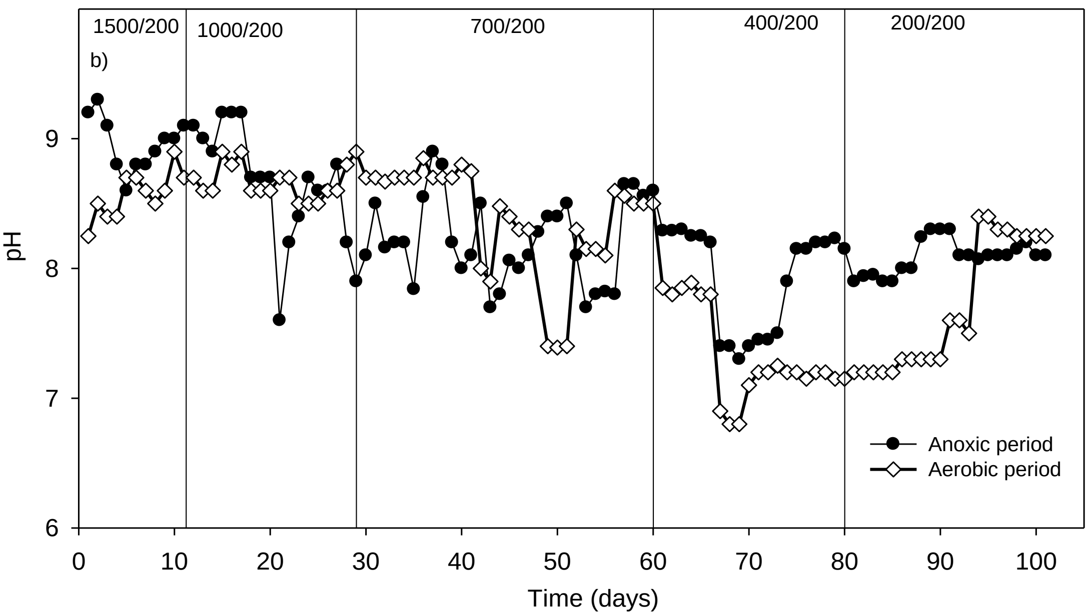

Figure 4.7. pH values for R2 during the operational period. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations).

Shifting to COD/TAN ratio of 2 (400/200 mg/L of COD/TAN) between Days 60-79, the COD loading rate was decreased from 1.4 to 0.8 g COD/Lday, whereas the constant TAN loading rate of 0.4 g TAN/Lday was applied (Figure 4.5c). The average pH values were 7.9±0.4 in anoxic and 7.4±0.4 in aerobic periods, respectively (Days 60-79) (Figure 4.7). The sudden pH drops observed on Days 67-73 was due to manual HCl addition to adjust the pH in the reactor. Yet, R2 had high TAN removal efficiency at the influent COD/TAN ratio of 2. The average cyclic TAN removal efficiency was measured as 93±3% (Days 60-79) (Figure 4.5).

Both TAN and COD removal efficiencies decreased when COD/TAN ratio was decreased to 1 (200/200 mg/L of COD/TAN) between Days 80-101. The corresponding COD and TAN loading rates were 0.4 g COD/Lday and 0.4 g TAN/Lday, respectively (Figure 4.5c). The average TAN removal efficiency was

75±7% (Days 80-101) (Figure 4.5). Bacterial wash-out was observed, with decreasing VSS concentrations down to 1760±155 mg/L on Day 100 (Figure 4.6).

To summarize, TAN removal efficiency was measured to be high and stable in R2 (such as 96%) at COD/TAN ratios of 7.5 and 5 (COD loading rates of 3 and 2 g COD/Lday) (Figure 4.5) (Days 1-28). TAN removal pursued a gradually decreasing trend when the ratio further decreased from 5 to 1. Low COD/TAN ratios (below 5) and loading rates (below 2 g COD/Lday) created unfavorable conditions for heterotrophic bacteria, which might have eventually become washed-out. Decreased abundance of heterotrophic bacteria and/or increased abundance of nitrification bacteria with decreasing influent COD/TAN ratios were also reported by Liu et al. (2003b), Wu et al. (2012), Yang et al. (2005) and Cydzik and Baryla (2011).

Denitrification performances during aerobic and anoxic periods as well as TN removal efficiency of R2 are shown in Table 4.2. At the influent COD/TAN ratio of 7.5 (Days 1-10), the TN removal efficiency of R2 was 46±10%, with 89±10% DN and 8±2% SNDN. During Days 11-28, the influent COD/TAN ratio was decreased to 5. On Day 20, since sufficient nitrate (80 mg/L NO_3-N) was produced, the NO_3-N in the nutrient solution was decreased from 160 to 80 mg/L. The TN removal efficiency increased to 54±5% during Days 11-28, which was attributed to the increased %SNDN to 42±5% (Table 4.2), while the TAN treatment efficiency was as high as over 98% (Figure 4.5). The anoxic DN% was measured as 66±18% on the average during Days 11-28 (Table 4.2). Moving on to the influent COD/TAN ratio 3.5 (Days 29-59), %DN seriously decreased to 8±6% that affected the TN removal efficiency to be as low as 35±10%, while the %SNDN was stable at 43±10% (Table 4.2). When the influent COD/TAN ratio decreased to 2 (Days 60-79), the efficiencies were calculated at 12±3% for DN, 65±9% for SNDN and 43±4% for TN removal (Table 4.2). At the final COD/TAN ratio of 1 (Days 80-101), the %DN was 11±4%, while the %SNDN slightly decreased to 58±17%. The TN removal efficiency was 26±4% for this period (Table 4.2).

The decrease in anoxic denitrification from 89% to 8% with decreasing influent COD/TAN ratio from 7.5 to 3.5 (Days 1- 59) (Table 4.2) may be due to the decreasing relative abundancy of heterotrophic denitrifiers with decreasing influent

COD concentration. After that, the DN slightly increased and was nearly constant at 11-12% at the influent COD/TAN ratios of 2 and 1 (Days 60-101) (Table 4.2). This, as explained in Section 4.1.2, was attributed to the change in the average granule sizes. The average granular sizes decreased from 2.1 to 1.4 mm, with decreasing COD/TAN ratio from 3.5 to 2. The optimum granule size that provided an appropriate anoxic volume was defined to be 1.2-1.8 mm (de Kreuk et al., 2005b). Thus, the slight increase in %DN from 8% to 12% with decreasing influent COD/TAN ratio from 3.5 to 2 (Table 4.2) was related to the decreasing granular size that provided an efficient anoxic volume. Despite the slight increase, DN efficiency during anoxic periods was still low due to low abundance of denitrifiers (compared to the conditions of 7.5 COD/TAN ratio).

The gradual increase in %SNDN from 8% to 65% (Days 1-79) with decreasing influent COD/TAN ratio from 7.5 to 2 (Table 4.2) can be related to the enhanced nitrification due to the increase in relative abundance of nitrifying populations at low influent COD/TAN ratios ($\leq$5) (Yang et al., 2005). Since higher amounts of TON were produced via enhanced nitrification, higher TON was available for the denitrification bacteria in the granule interior, which provided higher %SNDN. The enhanced nitrification during this period (Days 1-79) was indicated by the high TAN removal efficiency (89-99%) (Figure 4.5). In addition, Yang et al. (2005) reported increasing simultaneous denitrification efficiency with decreasing influent COD/TAN ratios from 10 to 3.3. The authors concluded that the abundance of denitrifying populations was related to that of the nitrifying populations (Yang et al., 2005). Thus the enrichment of both nitrifying and denitrifying populations could have provided the increase in SNDN during Days 1-79. The better diffusion of TON produced by nitrifiers close to the denitrifier layer also explains the high denitrification efficiency during aerobic periods (Figure 2.2). During Days 80-101, at the influent COD/TAN ratio of 1, the decreasing %SNDN (and also the decreasing TN removal efficiency) was attributed to the decreasing amount of biomass. Since the biomass was dominant in nitrification bacteria at low COD/TAN ratios, the decreasing biomass concentration to 1760±155 mg/L VSS on Day 100 caused lower %SNDN as well as lower TAN (Figure 4.5) and TN removal efficiencies (Table 4.2, Figure 4.5, Figure 4.6).

4.1.1.2. Organics removal

4.1.1.2.1 COD removal in R1

Figure 4.8 shows the effluent COD concentrations of aerobic and anoxic periods of R1, the percent COD removal efficiencies obtained during anoxic periods, aerobic periods and the overall cycle of concern (i.e. cyclic removal efficiency), and the theoretical influent OLR and NLR values throughout the operational period.

At the influent COD/TAN ratio of 7.5 (Days 1-10), R1 showed moderately stable COD treatment efficiency of 75±7% on cycle basis (Figure 4.8). Moving on to the influent COD/TAN ratio of 10 (Days 11-67), the COD treatment efficiency of R1 increased gradually and reached 91% on Day 15 (Figure 4.8). Between Days 15-45 the COD treatment efficiency fluctuated between 76% and 90%. This was attributed to the granular disintegration and biomass wash-out (Section 4.1.2.1) due to ammonia toxicity (Section 4.1.1.1) during this period. To remove the accumulated TAN due to the inhibited nitrification, the reactor content was washed 2 times on Days 25 and 42, respectively (Figure 4.8). On Days 46-47, the influent COD and TAN concentrations were halved at the constant COD/TAN ratio of 10, in order to recover the TAN removal efficiency. The halved influent COD/TAN concentrations of 1000/100 (mg/L / mg/L), provided increase in COD treatment efficiency. As seen in Figure 4.8, the total cyclic and aerobic period's COD treatment efficiencies exceeded 90% on Day 50. The COD treatment efficiency of R1 remained stable and high (89-93%) until the end of COD/TAN ratio 10 during Days 50-67 (Figure 4.8). Thus, the recovery of the COD treatment efficiency which stayed at high values such as 89-93% during Days 50-67 (Figure 4.8), was attributed to the decreased ammonia toxicity following the second sludge wash on Day 42 and halved loading rates.

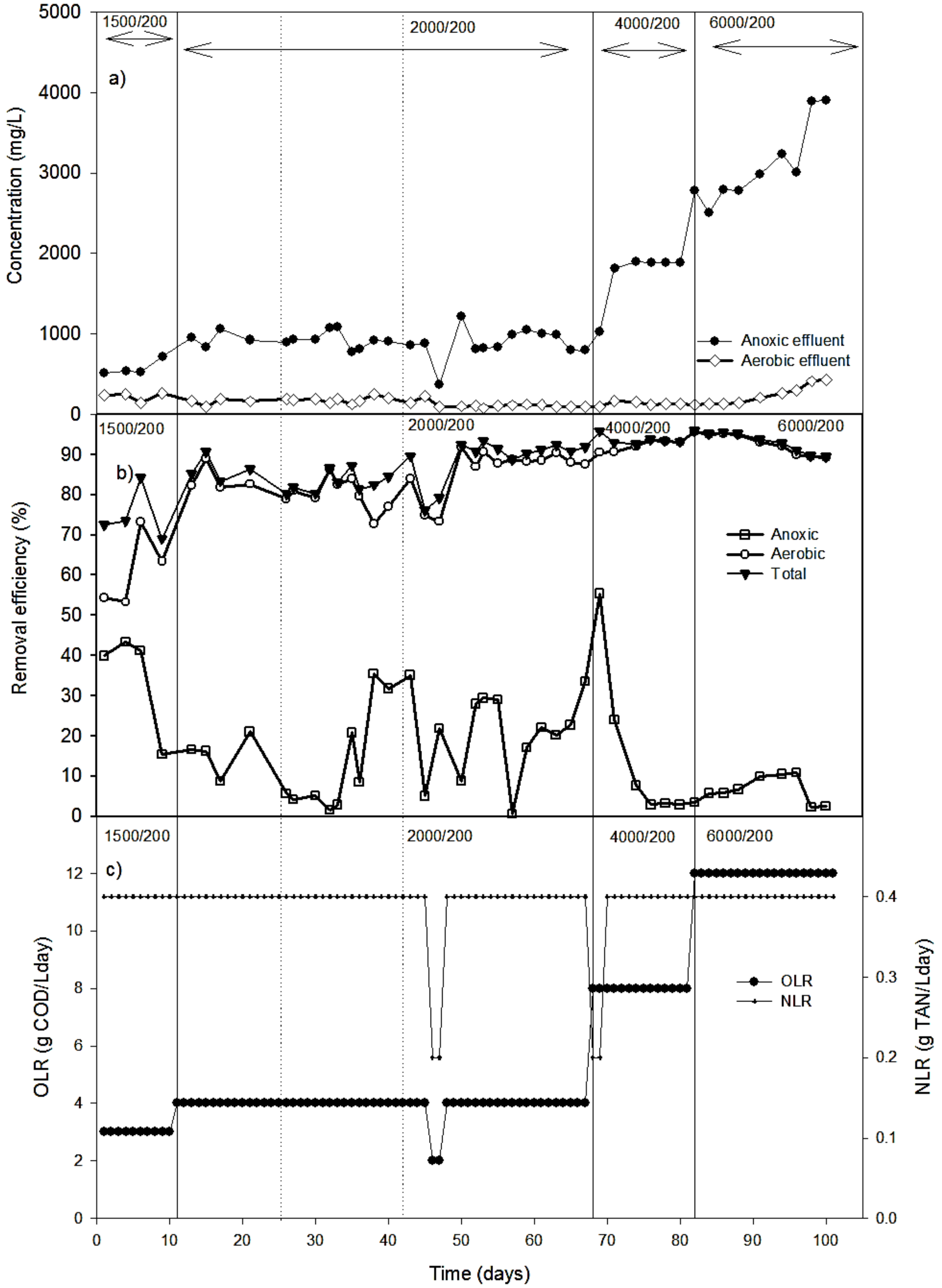

Figure 4.8. COD graph for R1: a) Effluent COD concentrations for anoxic and aerobic periods, b) COD removal efficiency for anoxic period, aerobic period and total cycle, c) OLR and NLR of R1 during the operational period. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations). (Dashed lines represent the washing of the reactor content, while the influent COD/TAN ratio changes are indicated by the solid lines).

When the influent COD/TAN ratio was increased to 20 (Days 71-81), the COD treatment efficiency of R1 was stable and as high as 94±1% on the average. The further increase in influent COD/TAN ratio to 30 (Days 82-101) provided relatively high COD treatment efficiencies between 89-96% (Figure 4.8). The average COD treatment efficiency during Days 82-101 was measured to be 93±3%. It is seen that high COD treatment efficiency of 93-94% was obtained in R1 at the influent COD/TAN ratios of 20-30 (Days 71-101). Based on the observations by Yang et al. (2005), aerobic heterotrophic bacteria were likely to be dominant over the nitrification and denitrification bacteria at the influent COD/TAN ratios of 10, 20 and 30. Since the increasing influent COD concentrations (2000-6000 mg/L), OLR values (4-12 g COD/Lday), and in turn the carbon content supported the growth of aerobic heterotrophic bacteria in the aerobic granular biomass, the aerobic heterotrophs gained resistence to inhibitory effects of ammonia nitrogen to some extent. As a result of this resistence, there was stable and high COD treatment, despite the higher ammonia concentrations calculated towards the end of the operational period (Figure 4.3).

4.1.1.2.2 COD removal in R2

Figure 4.9 shows the effluent COD concentrations of aerobic and anoxic periods of R2, the percent COD removal efficiencies obtained during anoxic periods, aerobic periods and the overall cycle of concern (i.e. cyclic removal efficiency), and the theoretical influent OLR and NLR values throughout the operational period. As seen in Figure 4.9, the majority of COD was removed during the aerobic periods. The anoxic period COD removal efficiency, which was 22±14% on the average, was limited to the available TON which was either remained from the previous cycle and introduced via the influent. The cyclic COD removal efficiency of R2 was observed to follow an oscillating and decreasing trend with decreasing COD/TAN ratio (Figure 4.9), which was opposite of R1 experiencing the increasing influent COD/TAN ratio.

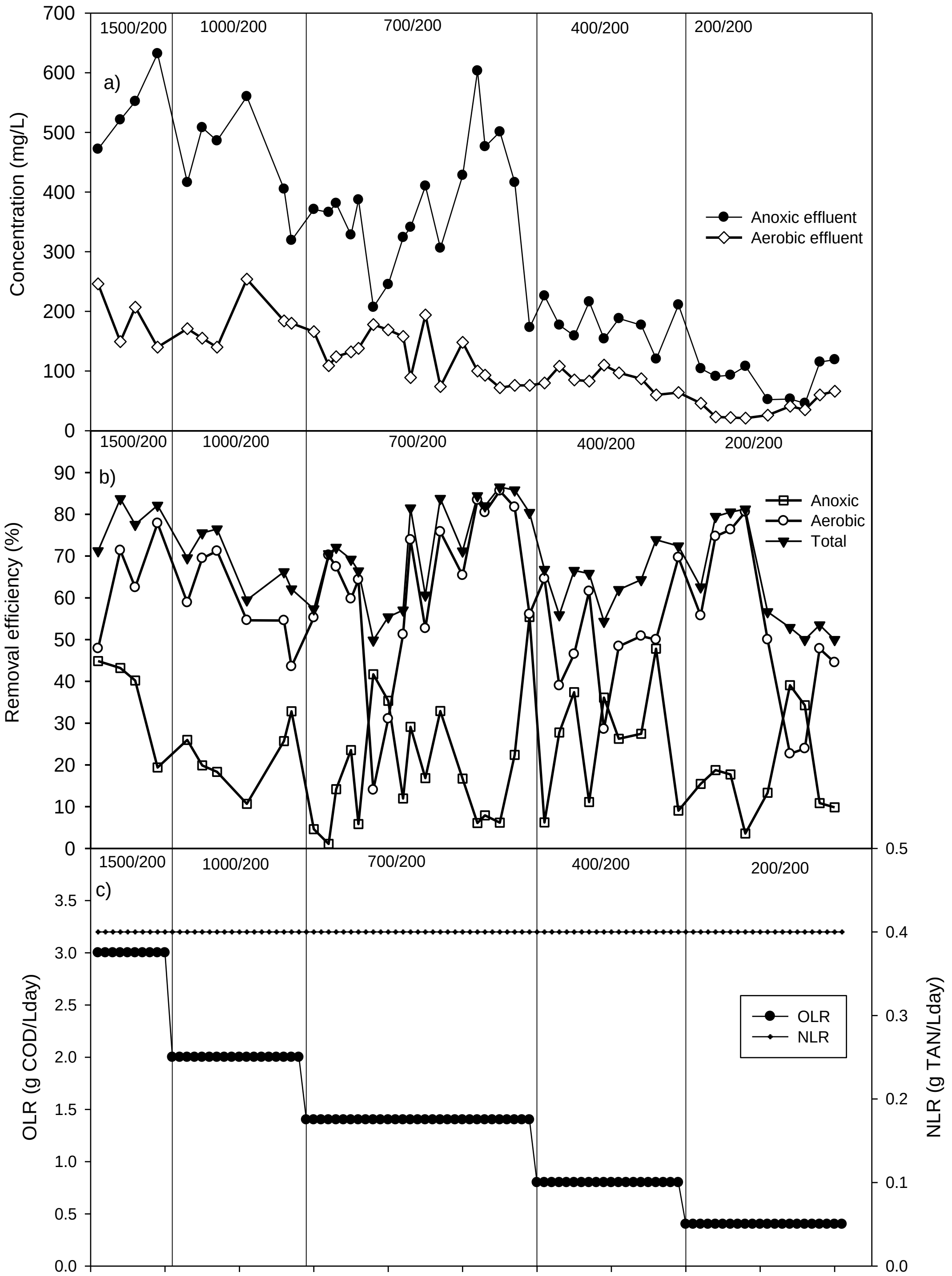

Figure 4.9. COD graph for R2: a) Effluent COD concentrations for anoxic and aerobic periods, b) COD removal efficiency for anoxic period, aerobic period and total cycle, c) OLR and NLR of R2 during the operational period. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations).

During the application of influent COD/TAN ratio of 7.5 (Days 1-10), the average cyclic COD removal efficiency of R2 was measured as 79±6% (Figure 4.9). This value decreased to 68±7% on the average with the shift to the COD/TAN ratio of 5 (Days 11-28), despite the VSS increase from 3620 to 6475 mg/L (Figure 4.6). The decrease in COD removal efficiency of the system could not be related to the ammonia inhibiton because almost 100% TAN removal (oxidation) was obtained during Days 11-28 (Figure 4.5). Therefore, the decreasing COD removal efficiency was explained by the change of dominant species in the system. In other words, the decrease in the influent COD/TAN ratio from 7.5 to 5 and influent COD concentration from 1500 to 1000 mg/L (Days 11-28) supported the growth of slow-growing nitrifiers instead of the aerobic heterotrophic bacteria in the granular structure. Enhanced nitrifier activity was commonly reported at low influent COD/TAN ratios such as 1-3.3 (Yang et al., 2005; Liu et al., 2003b; Cydzik and Baryla, 2011; Wu et al., 2012).

When the influent COD/TAN ratio was decreased to 3.5 (Days 29-59), the COD removal efficiency initially decreased and dropped below 50%, showing oscillations (Figure 4.9). The average COD removal efficiency at the influent COD/TAN ratio of 3.5 was 71±12% (Days 29-59). On the other hand, as previously mentioned in Section 4.1.1.1, the TAN removal efficiency of R2, which was 94±4%, was not adversely affected during this period (Days 29-59). There was also a drastical decrease in reactor biomass content. The average VSS concentration was 3568±1145 mg/L for Days 29-59; however, on Day 40, the VSS concentration decreased down to 1550 mg/L (Figure 4.6). Hence settling time was increased from 2 to 5 mins to maintain microorganisms in the system and prevent biomass loss (Days 41-47). When the VSS content increased from 4610 to 5380 mg/L during Days 55-65 (Figure 4.6), the COD removal efficiency increased and exceeded 86% (Day 55, Figure 4.9). This was attributed to the favoring of fast-growing heterotrophic bacteria with the decrease in settling time and in turn increase in VSS concentration.

Despite the increase in COD treatment efficiency to 86%, with the further decrease in influent COD/TAN ratio to 2 (Days 60-79), the COD treatment efficiency started to fluctuate and was measured as 65±7% on the average. When the influent COD/TAN ratio was further decreased to 1 (Days 80-101), the COD treatment efficiency of R2 decreased to 63±14% (Figure 4.9). During this period (Days 80-101), the reactor biomass content and TAN removal efficiency also decreased. The VSS concentration in the reactor decreased down to 1760 mg/L on Day 100 (Figure 4.6). Although the TAN removal efficiency and the biomass concentration in the reactor diminished during Days 80-101, the decrease in COD removal was more drastic; even down to 50% on Day 100 (Figure 4.9).

As shown in Figure 4.9, the average anoxic COD treatment efficiency of R2 pursued a decreasing trend from 45% to 1% during the first 31 days (Figure 4.9). This was attributed to the deteriorated denitrification and in turn the decreased COD consumption (Table 4.2). After Day 31, the COD treatment efficiency in the anoxic period fluctuated between 1-55% (Days 31-101). This was attributed to the decrease in the relative amounts of denitrification bacteria in the granular structure due to the unfavorable conditions created by the decreasing influent COD/TAN ratio, and in turn the decreasing OLR from 3 to 0.4 g COD/Lday (Figure 4.5c). The unstable treatment efficiency might have been due to the decreased amount of denitrification bacteria that became more sensitive to changes in the operational conditions.

As a result, it can be concluded that the decreasing influent COD/TAN ratio from 7.5 to 1 adversely affected the COD treatment performance of R2, which showed a decreasing trend during the operation. This was attributed to the decrease in the relative amounts of both aerobic and anoxic heterotrophic bacteria in the granular structure as a result of the decreasing influent COD concentration from 1500 to 200 mg/L, and in turn the decreasing influent OLR from 3 to 0.4 g COD/Lday. In other words, low COD/TAN ratios (≤5) were likely to promote the dominancy of nitrification bacteria in the granular structure.

4.1.2. Granular structure

During the operational period of 101 days, the effects of different influent COD/TAN ratios and concentrations on the granular structure of R1 and R2 were investigated. Generally, R1 showed fast-growing, large but loose and fluffy granules with increasing influent COD/TAN ratio from 7.5 to 30; while R2 had slow-growing granules with smaller sizes as the influent COD/TAN ratio decreased from 7.5 to 1.

Aerobic granules are typically yellow-brown in colour (Zheng et al., 2006). Figure 4.10 shows the granular seed sludge, which consisted of spherical, rigid, compact, brown granules with the average size of 3.4 ±1 mm and small amount of flocs.

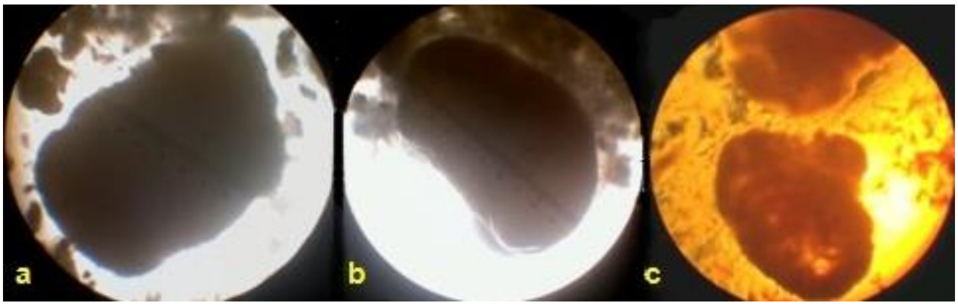

Figure 4.10. Seed sludge used in Set-1; a,b) During seeding; c) After the start-up period of 1 week. (At the influent COD/TAN ratio of 7.5; corresponding to the influent concentrations of 1500 mg/L COD and 200 mg/L TAN).

Filamentous growth was not detected in the seed sludge (Figure 4.10). The average particle sizes of the granules of R1 and R2 were initially 3.7±0.6 mm at the end of the start-up period. The structure of aerobic granular seed sludge showed significant changes at the constant influent TAN concentration of 200 mg/L, and increasing influent COD/TAN ratio from 7.5 to 30 (as in R1) or decreasing influent COD/TAN ratio from 7.5 to 1 (as in R2).

4.1.2.1. Granular structure in R1

The microscopic photographs of R1 during the 101-day operation with increasing influent COD/TAN ratios from 7.5 to 30 are shown in Figure 4.11, while the changes in granular structure of R1 are given in Table 4.3. During the application of the influent COD/TAN ratio of 7.5 (Days 1-10), R1 had compact, spherical, brown granules ressembling the seed sludge, just like R2 (Figure 4.11a, Figure 4.12a). When the COD/TAN ratio was increased to 10 (Days 11-67), the reactor content became light-brown/white and fluffy, had floccy appearence and the granular structure was distrupted (Figure 4.11b,c), which brought granular disintegration and partial biomass wash-out. Biomass wash-out was also indicated by the decrease in VSS down to 880 mg/L on Day 40 (Figure 4.4). The decrease in average particle size to 2.5±0.1 mm (Table 4.3) during Days 11-67 was attributed to the granular disintegration caused by the ammonia toxicity and stability problems due to the high pH in the system (as discussed in Section 4.1.1.1). Toxic effects of ammonia might have caused further wash-out of the nitrifiers from the system, while the TAN removal efficiency was adversely affected (Figure 4.1, Figure 4.4).

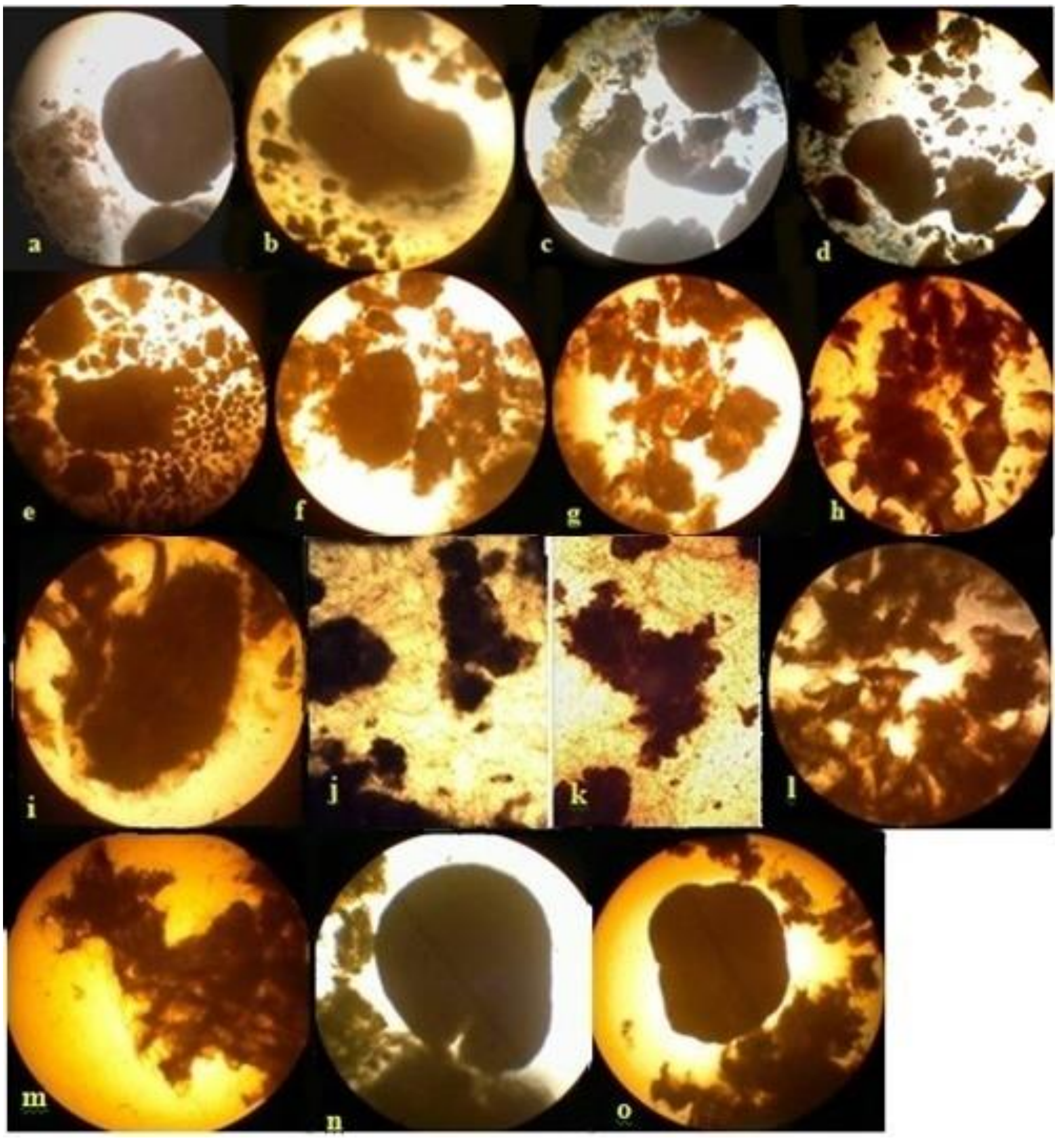

Figure 4.11. Microscopic photographs of R1 during the operational period of 101 days (6.3 x 4 magnification), a) Day 10 (COD/TAN ratio 7.5); b,c) Day 29 (COD/TAN ratio 10); f,g) Day 80 (COD/TAN ratio 20); h,i,j) Day 83 (COD/TAN ratio 30); k) Day 86 (COD/TAN ratio 30); l,m) Day 92 (COD/TAN ratio 30); n,o) Day 101 (COD/TAN ratio 30).

As previously mentioned, in order to remove the accumulated TAN in the reactor, the reactor content was washed with tap water 2 times. Following the second wash of the reactor content on Day 42, despite the recovery in COD and TAN treatment performances, the reactor content gained floccy appearence. The granules maintained their light-brown/white and fluffy structure. Therefore, it can be claimed that the changes in the granular structure of R1 during Days 11- 67, may not be only due to the increase in influent COD/TAN ratio to 10, but also due to the uncontrollable pH increase.

Table 4.3. Granular characteristics of R1 and R2.

R1						
Days	Influent COD/TAN ratio	Average diameter (mm)	SVI_5 (mL/g)	SVI_{30} (mL/g)	[a]Granulation percent	Settling velocity (m/h)
1-10	7.5	3.2±0.7	55±1	43±2	78%	74±13
11-67	10	2.5±0.1	109±18	58±10	53%	63±13
68-81	20	1.5±1.5	61±60	30±23	49%	63±21
82-101	30	2.4±1.8	37±25	33±27	NA	89±14
R2						
Days	Influent COD/TAN ratio	Average diameter (mm)	SVI_5 (mL/g)	SVI_{30} (mL/g)	[a]Granulation percent	Settling velocity (m/h)
1-10	7.5	3±1	54±2	43±2	80%	74±13
11-28	5	2.3±0.1	55±3	45±5	82%	61±1
29-59	3.5	2.1±0.3	58±4	42±14	72%	54±9
60-79	2	1.4±0.9	49 ±3	30±1	61%	45±3
80-101	1	0.5±0.1	70 ±33	62±44	89%	39±5

[a]The granulation percents are calculated by the relative ratios of the 30 and 5 min SVI values; i.e. 100x (SVI_{30}/SVI_5).
NA: Not applicable for the granulation percent analysis.

When the COD/TAN ratio was increased to 20 (Days 68-81), white fluffy flocs were enriched in R1 which contained light-brown coloured small granules with the average diameter of 1.5±1.5 mm (Table 4.3, Figure 4.11f,g). Although the filamentous microorganisms were not observed at the influent COD/TAN ratios of 7.5-20, the filaments were reported in R1 when the influent COD/TAN ratio was increased to 30 on Day 83 (Figure 4.11h, i, j). The operation at the influent COD/TAN ratio of 30 (Days 82-101) yielded larger granules of 2.4±1.8 mm in R1 (Table 4.3), while the reactor showed foaming with high amount of filamentous growth (Figure 4.11k). Deteriorated granular robustness/stability and enrichment of filamentous growth were reported at OLR values as high as 6 g COD/Lday (Zheng et al., 2006). In this study, similar observations were reported at a higher OLR value of 12 g COD/Lday, corresponding to the influent COD/TAN ratio of 30 and influent COD concentration of 6000 mg/L. With the increase in the influent COD/TAN ratio to 30, the increase in average particle size to 2.4±1.8 mm was attributed to the increased influent COD concentrations, and in turn increased carbon content that supported the growth of heterotrophic bacteria. On Day 87, the reactor content gained the appearence of milk turning sour and light-brown/white particles/aggregates and flocs (Figure 4.11l, m) were observed. Since the aggregates ressembled the microbial groups just at the start of a granulation process, the settling time of SBR was decreased from 5 to 3 mins in order to increase the hydraulic selection pressure and facilitate the granulation. On Day 92, the average particle size in the reactor increased to 3.9±0.9 mm (data not shown). The white/light-brown flocs were partially converted to rigid granules with the decrease in the SBR settling time (Figure 4.11n, o). The recovery in the granular structure during Days 82-101 is also implied by the SVI values (Table 4.3). The average particle size was measured as 2.4±1.8 mm during Days 82-101, at the influent COD/TAN ratio of 30. The formation of light-brown spherical granules was also detected at the end of the operational period (Day 101), while the filamentous growth was eliminated to some extent (Figure 4.11n,o). However, the reactor content still had floccy and filamentous appearence. Filamentous growth supports the aerobic granular stability only if present at moderate levels (Liu and Liu, 2006). Yet, the presence of excess filamentous growth negatively influences the stability of granules. Thus, the presence of high amounts of filaments and flocs around the granules were likely to be responsible for the deteriorated structural integrity of the granules.

The granulation percents of R1 (Table 4.3) gradually decreased from 78% to 49% as the influent COD/TAN ratio increased from 7.5 to 20. This decrease was expected as a result of the granular disintegration and biomass wash-out arising from the distrupted system stability by the ammonia toxicity. The granules were surrounded by high amounts of flocs and filamentous microorganisms, especially at the influent COD/TAN ratio of 30. This led to a fluffy and sticky reactor content containing granules surrounded by flocs and filaments. Therefore, the granulation percent analyses could not be performed for R1 during Days 82-101 (COD/TAN ratio of 30). Because, as mentioned in Section 3.4.2, for the granulation percent analysis to be valid the granules should be visually observed and the average particle size should be ≥0.2 mm (Liu et al., 2010b).

It should be noted that, in spite of the floccy and filamentous sticky reactor content, and inability to perform granulation percent analysis, formation of new granules was visually reported at the COD/TAN ratio of 20 and even 30. This study, therefore, indicated that granulation is possible even at FA concentrations of 26.8-35 mg/L, above the value given as threshold level unsuitable for granule formation (Yang et al., 2004b).

The SVI of the aerobic granules is generally below 80 mL/g, and even as low as 20 mL/g (Gao et al., 2011). The SVI_{30} values for R1, which were between 30-58 mL/g, are in this typical range given in the literature. The SVI_5 and SVI_{30} values of R1 indicated the well-settling characteristics of the granules (Table 4.3). The granular disintegration observed at the COD/TAN ratio of 10 (Days 11-67) in the reactor was implied by the rise of SVI_5 value from 55 to 109 mL/g. Yet, the granular system recovered itself which was also indicated by the decrease in SVI values even at higher influent COD/TAN ratios (Table 4.3). Despite the floccy and filamentous growth around the granules, especially at the influent COD/TAN ratio of 30, the system displayed well settling characteristics, most probably due to the sticky nature of the reactor content.

The aerobic granules can have settling velocities between 18-90 m/h and even as high as 130 m/h was reported due to their high biomass retention capacity (Gao et al., 2011). As seen in Table 4.3, the settling velocities of the granules in R1 varied between 63-89 m/h, which are in the typical range. Generally, the decreasing particle settling velocity from 74 to 63 m/h (Table 4.3) with increasing influent COD/TAN ratio from 7.5 to 20 (Days 1-81) can be attributed to the formation of smaller and less dense granules as a result of granular disintegration. This is also indicated by the decreasing granular size from 3.2 to 1.5 mm with the increasing influent COD/TAN ratio from 7.5 to 20 (Days 1-81) (Table 4.3). With the COD/TAN ratio increase to 30, the average settling velocity of the individual granules increased to 89±14 m/h due to the increased average sizes (Days 82-101).

The EPS concentrations of the aerobic granular sludge in R1 are shown in Table 4.4. As the COD/TAN ratio increased from 7.5 to 30, the EPS polysaccharide (PS) content of R1 decreased about 21% (from 122 to 96 mg PS per g VSS), while the protein content in EPS (PN) increased by 22% (from 192 to 235 mg PN per g VSS). Therefore, the increasing influent COD/TAN ratio in R1 led to decreasing PS and increasing PN concentrations parallel to the granular disintegration (i.e. loss of granular stability, rigidity and structural integrity with floccy appearence), as the granules were surrounded with high filamentous growth.

Table 4.4. EPS values of R1 and R2 through the operational period.

R1				
Days	Influent COD/TAN ratio	EPS (mg/g VSS)		
		PN	PS	PS/PN
1-10	7.5	192±0.1	122±0.02	0.64±0.06
11-67	10	168±0.01	106±0.1	0.63±0.1
68-81	20	212±0.9	99±0.01	0.47±0.05
82-101	30	235±0.1	96±0.01	0.41±0.05
R2				
Days	Influent COD/TAN ratio	EPS (mg/g VSS)		
		PN	PS	PS/PN
1-10	7.5	153±0.1	96±0.5	0.63±0.06
11-28	5	134±0.1	84±0.2	0.63±0.02
29-59	3.5	137±0.1	87±0.1	0.64±0.03
60-79	2	130±0.1	79±0.1	0.61±0.05
80-101	1	142±0.1	74±0.5	0.52±0.05

As explained in Section 2.2.13, EPS secretion supports the formation of stable granules with structural integrity via facilitating cell aggregation. Thus, the concentrations of both PS and PN are expected to increase during the granulation process (Adav et al., 2008b). The increase in PS content of EPS contributes to microbial aggregation via bridging the cells (Tay et al., 2001c). PS acts as a bioglue to the granular structure and contributes to the strength, stability and structural integrity of the granules (Liu et al., 2004a; Adav et al., 2008b). Poor PS synthesis is known to have a negative effect on microbial aggregation (Wu et al., 2012). On the other hand, PN, which influences the density and stability of granules (Zhu et al., 2012), is known to increase with stimulated EPS production at stressful conditions (Nichols et al., 2004; Qin et al., 2004a; Yu et al., 2009). The granulation is supported by the increase in PN content of EPS, which facilitates the formation of cross-linked network between adjacent cells via attraction of organic and inorganic materials (Liu and Tay, 2004). Increasing PN concentration was claimed to support aerobic granulation via affecting the relative hydrophobicity of the granules (Zhang et al., 2007). Yet, excess EPS production is known to deteriorate the granular stability and/or increase the repulsive forces among bacteria (Schmidt and Ahring, 1996).

Therefore, due to the role of PS as a backbone for the granular structure, the decreasing PS concentrations in R1 at the influent COD/TAN ratios ≥10 were attributed to the destruction of structural stability and in turn granular disintegration.

The PS/PN ratio of the aerobic granular EPS in R1 decreased from 0.63 to 0.41 (Table 4.4), parallel to the deteriorating granular structure with increasing influent COD/TAN ratios from 10 to 30 (Days 11-101). Since PS supported the structural integrity of the granules, higher PS/PN ratios provided more compact and stronger aerobic granules with higher specific gravity (Tay et al., 2001b,c,2002d; Liu and Tay, 2002d; Qin et al., 2004a,b). Low PS concentrations and in turn low PS/PN ratios were reported to cause granular disintegration (PS/PN <2) (Tay et al., 2004b), and granule development failure (PS/PN: 0.5) (Wu et al.,2012). The minimum PS/PN ratio required for aerobic granulation was generally above 2 (Tay et al., 2004b; Wu et al., 2012) and even higher PS/PN ratios such as 3.4-6.2 were reported for aerobic granules (Adav et al., 2008b). On the other hand, stable aerobic granules were reported at a PS/PN ratio as low as 0.53 (Luo et al., 2014), and it was speculated that the suitable PS/PN ratio for aerobic granulation was 0.6 (Li et al., 2008c). In R1, the granular disintegration with high amount of filamentous growth was observed below PS/PN ratio of 0.6 (observed at influent COD/TAN≥10) (Table 4.4), which supports the claims of Li et al. (2008c). The wastewater content is known to affect the EPS PS/PN ratios. Wastewaters with high C/N content (such as C/N:40) increases PS/PN of EPS; while the low C/N wastewaters (such as C/N:5) result in low PS/PN ratios for the activated sludge (Durmaz, 2001). According to Wu et al. (2012), the increasing influent COD/N ratios (from 0 to 4) facilitated microbial attachment via enhancing PS production of aerobic granules. However, in this study, both high and low COD/TAN influent content brought low PS/PN ratios in terms of EPS content of the aerobic granules. This was expected for R2 (Section 4.1.2.2), not for R1. The deterioration of the granular structure in R1 with increasing influent COD/TAN ratio resulted in decreasing PS concentrations and in turn lower PS/PN ratios.

4.1.2.2 Granular structure in R2

The microscopic photographs of R2 during the 101-day operation with decreasing influent COD/TAN ratios from 7.5 to 1 are shown in Figure 4.12, while the changes in granular structure of R2 are given in Table 4.3.

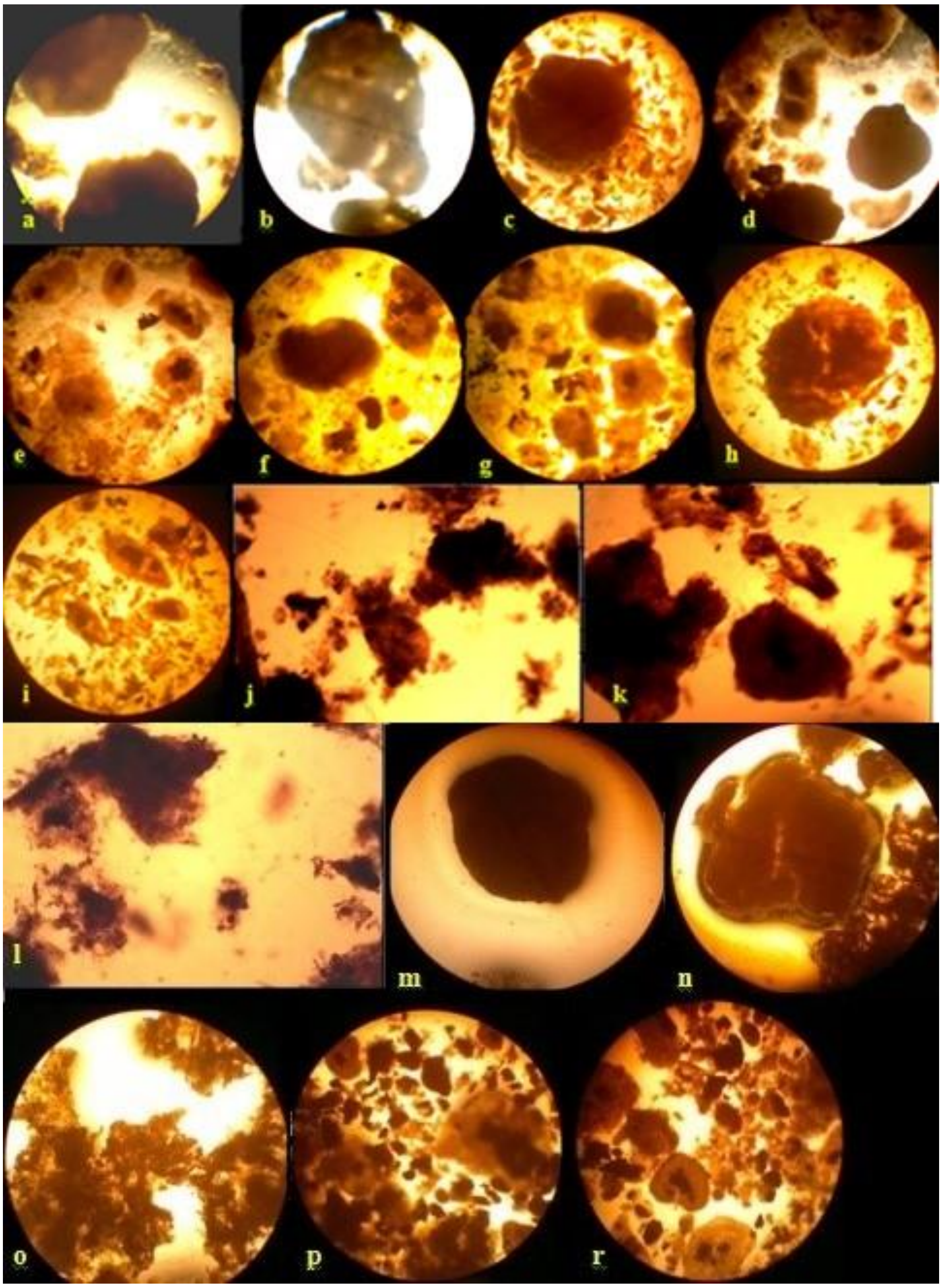

Figure 4.12: Microscopic photographs of R2 during the operational period for 101 days (6.3 x 4 magnification), a,b) Day 10 (COD/TAN ratio 7.5); c)Day 28 (COD/TAN ratio 5); d,e) Day 59 (COD/TAN ratio 3.5) f,g) Day 70 (COD/TAN ratio 2); h,i) Day 79 (COD/TAN ratio 2); j,k,l) Day 83 (COD/TAN ratio 1); m,n,o) Day 92 (COD/TAN ratio 1); p,r) Day 101 (COD/TAN ratio 1).

The average particle sizes decreased from 3±1 to 0.5±0.1 mm with decreasing influent COD/TAN ratios from 7.5 to 1, which was attributed to the decreasing relative abundance of heterotrophic bacteria in the granules, while the slow-growing nitrification bacteria became dominant in the granular structure (Yang et al., 2005). Thus some of the properties of nitrifiers, such as slow-growing biomass and smaller size were represented via the aerobic granules.

During the operation at the influent COD/TAN ratio of 7.5 (Days 1-10), compact rigid spherical and brown granules were observed in R2 (Figure 4.12a,b), similar to R1. At the COD/TAN ratio of 7.5, the average granule size was measured as 3±1 mm in R2 (Table 4.3). When the influent COD/TAN ratio was decreased to 5 (Days 11-28), granular fragmentation was observed in R2 and the average granule size decreased to 2.3±0.1 mm (Table 4.3). The granules were small and orange in colour, ressembling pebble-stones (Figure 4.12c). The colour difference between the reactors (R1 had light-brown/white content, while R2 yielded orange/brown granules) may indicate the enrichment of different species contributing to granulation and the different chemical composition of the granules (Gao et al., 2011). Different microbial species were enhanced in each reactor with respect to the different influent COD/TAN ratios and in turn different influent COD concentrations and OLR values.

The granular fragmentation in R2 at the COD/TAN ratio of 5 (Days 11-28) was attributed to the carbon defficiency due to the decreased influent carbon concentration. To explain, the influent COD/TAN ratio was decreased from 7.5 to 5 via decreasing the influent COD concentration from 1500 mg/L to 1000 mg/L (at a constant influent TAN concentration of 200 mg/L). Due to the decreasing influent COD concentration, and in turn the decreased concentration gradient between the outer and inner granular layers, diffusive transport of COD to the inner granule might have been obstructed. It may be the fact that the whole COD was consumed via aerobic heterotrophs on the outer granular layers during the aerobic period. As a result of the limited diffusion of COD through the inner parts of the granule, the denitrification bacteria in the deeper granular structure were thought to be subjected to carbon defficiency (de Kreuk et al., 2005b; Gao et al., 2011). Degradation of denitrification bacteria after starvation period is known to cause fragmentation, by forming empty regions in granular structure. As the inner side heterotrophic denitrifiers were eliminated from the system, the granule size showed an obvious decrease and smaller but dense granules were formed (Table 4.3). The decrease in granular size was not only attributed to the denitrifiers' elimination, but also at decreasing influent COD concentrations the aerobic heterotrophic bacteria were thought to decrease in quantity. The ease of COD and TON to reach the inner parts of the granules was likely to increase by the decreasing granule sizes. Denitrification favoring conditions were then established, when carbon and oxidized forms of nitrogen achieved to reach the inner side of the granules. Simultaneous denitrification was achieved at smaller COD/TAN ratios (Table 4.2).

During the application of the influent COD/TAN ratio of 3.5 (Days 29-59), the average granule size was measured as 2.1±0.3 mm (Figure 4.12d,e). When the COD/TAN ratio was further decreased to 2 during Days 60-79, the average granule size decreased to 1.4±0.9 mm (Figure 4.12f,g,h,i). The final COD/TAN ratio of 1 (Days 80-101) provided smaller granules at an average size of 0.5±0.1 mm (Table 4.3, Figure 4.12m,n,p,r). As Table 4.3 implies, gradually decreasing influent COD/TAN ratio from 3.5 to 1 (Days 29-101), provided the development of compact dense orange granules with decreasing sizes.

The average particle settling velocities of R2 ranged between 39-74 m/h (Table 4.3), which is in the typical range (between 18-130 m/h) of aerobic granular settling velocity (Gao et al., 2011). During 101 days, the average settling velocity of the particles gradually decreased from 74±13 to 39±5 m/h with decreasing COD/TAN ratios from 7.5 to 1, as a result of fragmentation and decreasing size, as expected (Table 4.3). The settling velocity decrease does not imply poor settleability in R2; as long as the SVI values pursue a decreasing trend. The SVI_{30} values for R2, which are between 30-62 mL/g (Table 4.3) are in the typical range for aerobic granular SVI which is between 20-80 mL/g (Gao et al., 2011). Despite the decreasing granule size with decreasing COD/TAN ratios, SVI values were nearly constant until the COD/TAN ratio was 1. The stable SVI values indicated that the high settleability of the sludge was dominant in small but compact and dense granules rather than flocs as also justified by visual observations. At the COD/TAN ratio of 1 (Days 80-101), the SVI values were observed to increase with decreasing VSS concentrations from 4930 mg/L (Day 89) to 1760 mg/L VSS (Day 100) (Figure 4.6). The increase in SVI values at the COD/TAN ratio of 1 was attributed to the relatively low granular sized sludge bed and decreased settling velocities (Table 4.3).

The granule percentages of R2 are nearly stable at 80-82% during the influent COD/TAN ratios of 7.5 and 5 (Days 1-28) (Table 4.3). The granulation percent as high as 82% may indicate the formation of compact and dense granules as a result of the fragmentation at the influent COD/TAN ratio of 5 (Days 11-28) (Table 4.3). When the influent COD/TAN ratio was decreased to 3.5 and then to 2, the granulation percent decreased to 72% and then to 61%, respectively (Table 4.3). This was expected as a result of the decreasing particle size (Table 4.3) (Days 29-79), which support the enrichment of slow-growing nitrifiers with smaller sizes in the aerobic granular structure of R2. When the influent COD/TAN ratio was further decreased to 1 (Days 80-101), the granulation percent was calculated to be 89% (Table 4.3). As previously mentioned, the biomass concentration in the reactor decreased from 4930 mg/L (Day 89) to 1760 mg/L VSS (Day 100) (Figure 4.6). This microbial wash-out was attributed to the dominancy of slow-growing nitrifiers which required longer SRTs to retain in the reactor. Despite the microbial wash-out, the remaining portion of granular biomass in R2 had very small but dense and compact structures with good settleability as the SVI values indicated (Table 4.3).

The EPS concentrations of the aerobic granular sludge in R2 are shown in Table 4.4. As the influent COD/TAN ratio decreases from 7.5 to 1, the EPS values of R2 showed 23% decrease with nearly constant protein content (Table 4.4). The PS of R2 decreased about 23% (from 96 to 74 g PS per mg VSS), while there was 15% decrease in PN (from 153 to 130 g PN per mg VSS) among the COD/TAN ratios 7.5 to 2 (Days 0-79). When the COD/TAN ratio was further decreased to 1 (Days 80-101), the PN value increased from 130 to 142 g PN per mg VSS, indicating 9% increase. Generally during the operational period, the PN concentrations were measured to be higher than PS, which is expected for aerobic granular systems operated at low settling times (<10 min) (Zhu et al., 2012).

Therefore, it can be stated that the decreasing influent COD/TAN ratio in R2 led to the decreasing PS concentrations, while the PN slightly decreased. Due to the contribution of PS to structural integrity, the decreasing PS concentrations were attributed to the granular fragmentation (i.e. division of granules into small pieces without any floccy appearence or granular stability destruction) (Adav et al, 2008b; Zhu et al, 2012). Decreasing PS/PN ratios were reported for decreasing influent

COD/N ratios such as 4 and below (Wu et al., 2012; Luo et al., 2014). Since the EPS secretion increases in terms of both PS and PN during the granulation process, the opposite can be expected during granular fragmentation; showing that the slightly decreasing PN concentrations were not surprising.

The PS/PN ratio was nearly stable around 0.62 g PS/PN, showing 2-3% fluctuations as the COD/TAN ratio decreased from 7.5 to 2 (Days 0-79). When the COD/TAN ratio decreased to 1, the PS/PN ratio decreased about 18%, from 0.61 to 0.52 g PS/PN (Table 4.4). The decreasing PS/PN ratio was related to the enrichment of nitrifiers. The autotrophic nitrifiers are known to have low growth rates and poor EPS production, which obstructed their biofilm adhesion (Wang et al., 2007b). At the COD/TAN ratio of 1, there was microbial wash-out in R2 which was attributed to the dominancy of slow-growing nitrifiers that required longer SRTs to retain in the reactor. It was likely that the PS/PN ratios below 0.6 were unsuitable for aerobic granulation, which was valid for both R1 and R2.

4.2. Set-2: The effects of increasing OLR and NLR values on aerobic granules and their treatment efficiency

Due to the importance of OLR selection in aerobic granular stability, Set-2 was conducted to investigate the effects of gradually increasing organic and nitrogen loading rates, i.e., OLR and NLR values, on the treatment performance and characteristics of aerobic granules. One SBR, namely R3, was operated for 70 days at stepwise increasing OLR values as 0.75-1.5-3.0-6.0-12 g COD/Lday, and the corresponding NLR values of 0.1-0.2-0.4-0.8-1.6 g TAN/Lday. During the operation the influent COD/TAN ratio was kept constant at 7.5, based on the results obtained from Set-1. This section explains the treatment performance and granular characteristics of R3.

4.2.1. Treatment Efficiency

After the seeding of the aerobic granular sludge, the start-up period for 21 days yielded 91±2% COD and 51±23% TAN removal efficiencies on the average, while the average VSS concentration was 8086±2992 mg/L in the reactor (Table 4.5). The average TAN and COD removal efficiencies of R3 are tabulated in Table 4.5.

Table 4.5. TAN and COD removal efficiencies of R3 through the operation (influent COD/TAN=7.5).

Start-up period of R3					
Period	[a]OLR	[b]NLR	COD removal (%)	[c]TAN removal (%)	[d]VSS (mg/L)
Start-up (21 Days)	3	0.4	91±2	51±23	8086±2992
Operational period of R3					
Days	[a]OLR	[b]NLR	COD removal (%)	[c]TAN removal (%)	[d]VSS (mg/L)
1-16	0.75	0.1	86±2	65±9	5960 ±725
17-29	1.5	0.2	85±1	87±2	6865±488
30-49	3	0.4	88±1	61±3	6689±888
50-61	6	0.8	96±1	45±1	6553±392
62-70	12	1.6	81±2	51±1	6208±308

[a] OLR: Theoretical influent organic loading rate, g COD/Lday
[b] NLR: Theoretical influent nitrogen loading rate (in terms of TAN), g TAN/Lday
[c] TAN: Total ammonifiable nitrogen (NH_4-N + NH_3-N)
[d] VSS: Average VSS concentration in the reactor.

4.2.1.1. N removal efficiency in R3

Similar to Set-1, the N removal efficiencies in Set-2 were calculated regarding the Equations B.5-B.9 (Appendix B.2). Figure 4.13 shows the theoretical influent TAN concentrations and NLR values of R3, as well as the percent TAN removal efficiencies of anoxic period, aerobic period and the overall cycle of concern (i.e. cyclic removal efficiency). During the whole operation, TAN treatment was observed only in the aerobic periods, as expected. Average cyclic TAN removal efficiencies of R3 are tabulated in Table 4.5.

The operation for 70 days with the increasing NLR from 0.1 to 1.6 g TAN/Lday, and the OLR from 0.75 to 12 g COD/Lday, resulted in decreasing TAN treatment efficiency in R3 (Figure 4.13, Table 4.5). Application of the organic and nitrogen loading rates of 0.75 g COD/Lday OLR and 0.1 g TAN/Lday NLR (Days 1-16) yielded 65±9% TAN removal efficiency (Table 4.5, Figure 4.13). This was obviously higher from those achieved during start-up period where 51±23% TAN removal efficiency was observed (Table 4.5). This increase was attributed to the decrease in the influent NLR from 0.4 to 0.1 g TAN/Lday, and influent TAN concentration from 200 mg/L to 50 mg/L regarding the R1 experience. Since each OLR provides the dominancy of different bacterial species (Chen et al., 2008), it can be commented that the heterotrophic bacteria dominant in start-up period at higher loading rates were decreased and/or eliminated in number and the nitrifiers within the granules might have been supported. The lower influent COD and OLR values might have also resulted in much higher DO left for the nitrifiers.

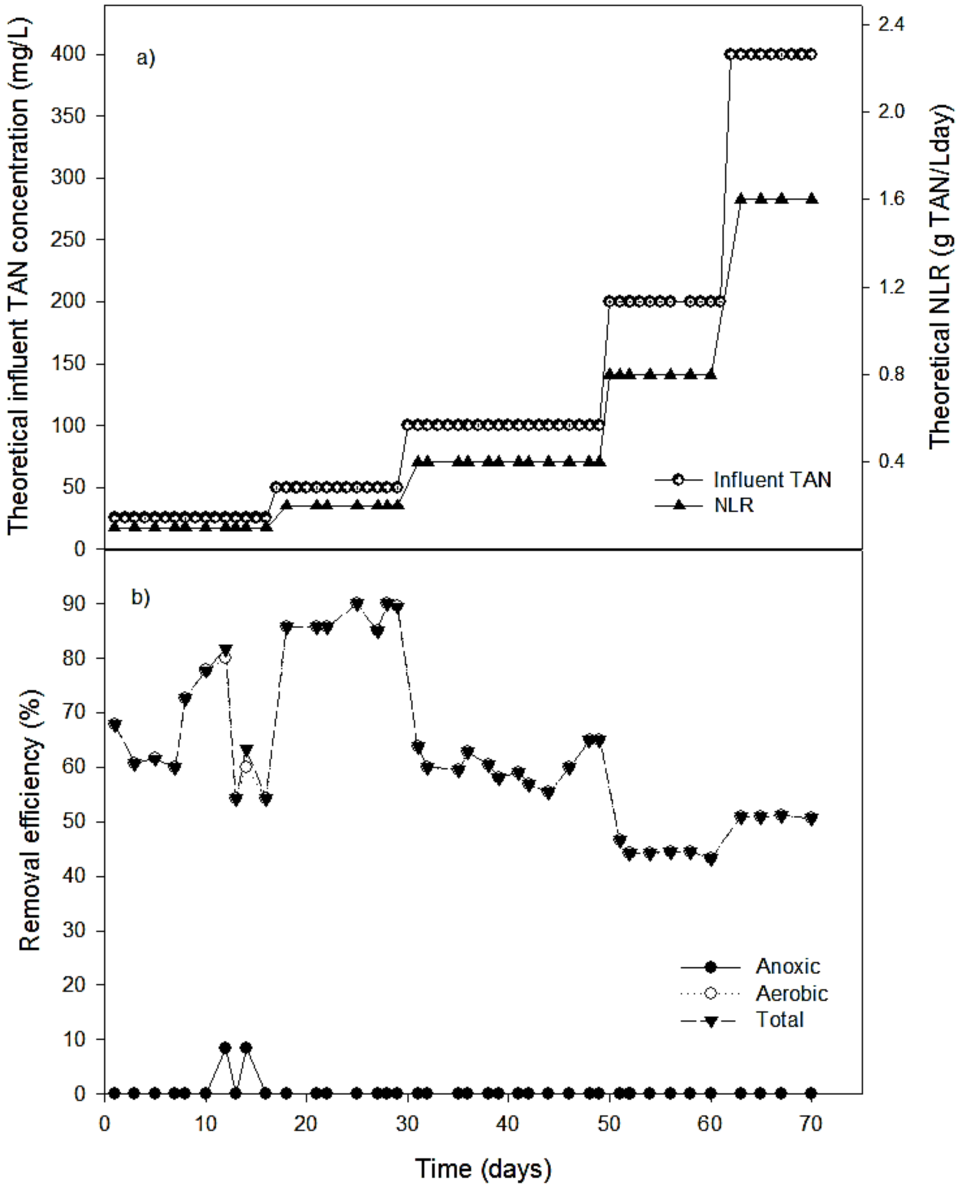

Figure 4.13. TAN graph for R3: a)Theoretical influent NLR (g COD/Lday) and TAN concentration (mg/L); b) TAN removal efficiencies for anoxic period, aerobic period and the overall cycle.

During Days 17-29, the loading rates of 1.5 g COD/Lday OLR and 0.2 g TAN/Lday NLR were applied, and granular fragmentation was observed as explained in Section 4.2.2. Despite the granular fragmentation in the reactor, the average TAN treatment efficiency increased to 87±2% (Days 17-29) (Table 4.5). The maximum TAN removal efficiency of 90% was observed on Day 25, Day 28 and Day 29 at these loading rates (Figure 4.13). The average biomass concentration in the reactor, which was 5960±725 mg/L during Days 1-16, increased to 6865±488 mg/L during Days 17-29 (Table 4.5). When the increased biomass concentration and high TAN removal efficiency during Days 17-29 are considered (Figure 4.13, Table 4.5), it is likely that the nitrifiers were enriched in the granules. The enrichment in nitrifiers also supported the denitrification during aerobic periods as discussed in the following parts. The granular fragmentation observed in this period might have been attributed to the enrichment of nitrifiers and then denitrifiers to achieve efficient granule sizes (1.2-1.8 mm), which improves the mass transport (Tay et al., 2002b; de Kreuk et al., 2006). Rosman et al. (2014) reported increased TAN removal efficiency from 73% to 91% with increasing OLR from 0.9 to 3.6 g COD/Lday, which might be related to the increased denitrification and denitrifier population.

When the loading rates of 3 g COD/Lday and 0.4 g TAN/Lday were studied (Days 30-49), significant decrease in TAN removal efficiency around 60% was reported (Figure 4.13, Table 4.5). About 61±3% TAN was removed on the average (Table 4.5). The deterioration of TAN removal efficiency around 60% and high COD removal efficiency (88±1% on the average) observed during Days 30-49 with the increasing OLR of 3 g COD/Lday was attributed to the DO defficiency. Since the competition between slow-growing autotrophic nitrifiers and fast-growing heterotrophs for DO and inhabitation area commonly results in the dominance of heterotrophs in the outer granular layer (de Kreuk et al.,2005b), the outcompeted nitrifiers may have been subjected to DO defficiency (Liu et al., 2008; Lotito et al., 2012). The tendency of heterotrophic bacteria to outcompete the nitrifiers in the competition for DO and space results in the enhancement of heterotrophs at high OLRs, since most of the DO is consumed by the heterotrophic bacteria (Kim et al., 2008). It can be stated that the increasing influent COD concentrations at the higher OLR of 3 g COD/Lday, increased the relative abundance of heterotrophic bacteria, which are carbon consumers, while the amount of autotrophs (i.e. nitrifiers) decreased in the aerobic granular structure (Ni et al., 2008) as implied by the decreasing TAN removal efficiency during Days 30-49. Similarly the studies such as Lotito et al. (2012) and Kim et al. (2008) reported decreasing N removal despite high COD treatment efficiencies, which was explained by the decreasing abundancy of nitrifiers over aerobic heterotrophic bacteria.

When the loading rates were increased to 6 g COD/Lday and 0.8 g TAN/Lday on Day 50, the TAN treatment efficiency was observed to decrease and follow a stable trend around 45±1% (Days 50-61) (Figure 4.13, Table 4.5). Finally, during the application of 12 g COD/Lday OLR and 1.6 g TAN/Lday NLR, the average TAN removal efficiency, which was still low, slightly increased to 51±1% (Days 62-70) (Table 4.5). When the low TAN removal efficiency (51%) is considered with the dropping COD removal efficiency (to 81%) and VSS decrease in the reactor (to 5990 mg/L on Day 65), it is likely that some portion of biomass was slightly washed-out of the reactor due to the granular stability problems with OLR increase to 12 g COD/Lday as discussed further in Section 4.2.2 (Figure 4.13, Figure 4.14). Granular disintegration and wash-out at high OLRs implied by the biomass decrease in the reactor is a common observation for this topic (Tay et al.,2004a, b; Adav et al., 2009; Lotito et al., 2012).

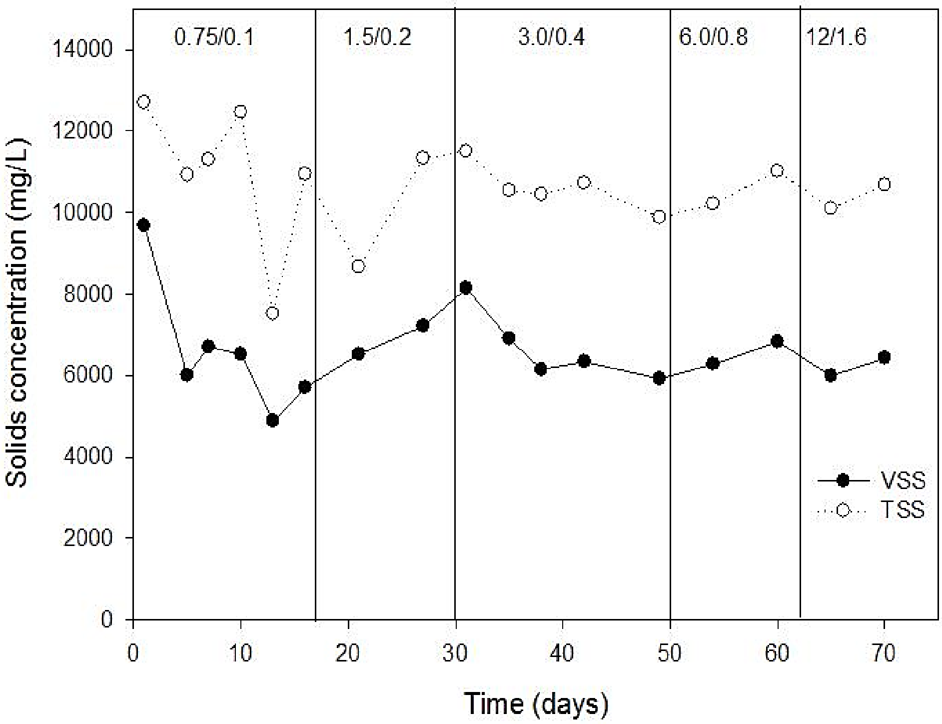

Figure 4.14. The concentration of VSS and TSS in R3. (0.75/0.1 notation refers to the OLR of 0.75 g COD/Lday and the NLR of 0.1 g TAN/Lday)

The nitrification and denitrification efficiencies are significant in terms of total N removal. As a result of the limited DO diffusion, the coexistence of anaerobic, anoxic and aerobic regions in the granular structure allows the total N removal via SNDN (de Kreuk et al., 2005b; Gao et al., 2011). Table 4.6 shows the denitrification and nitrification performances as well as TN removal efficiency of R3. During the start-up period of 21 days, there was 10±11% DN, 48±15% SNDN in the reactor, while the TN removal efficiency was 31±15% (Table 4.6). Moving on to the operational period, the application of the loading rates of 0.75 g COD/Lday OLR and 0.1 g TAN/Lday NLR (Days 1-16) yielded 6±10% DN, 34±16% SNDN and 25±10% TN removal efficiency (Table 4.6). When the loading rates were increased to 1.5 g COD/Lday and 0.2 g TAN/Lday (Days 17-29), TN removal increased to 49±17%, while the average %DN and %SNDN values increased to 23±12% and 58±18%, respectively (Table 4.6). The increase in %DN from 6% to 23% (Days 1-29) may be related to the enrichment of heterotrophic denitrifiers with increasing influent COD concentration and in turn the OLR. The increase in %SNDN from 34% to 58% during Days 1-29, could be explained by the enrichment of nitrifiers and in turn the nitrification and production of TON, which provides substrate for the aerobic period denitrification (Table 4.6). As previously mentiioned, the enrichment of nitrifiers was likely when the increases in average TAN removal efficiency (to 87±2%) and the average reactor VSS content (to 6865±488 mg/L) during this period (Days 17-29) are considered (Table 4.5). As shown in Figure 4.14, the VSS concentration in R3 pursued an increasing trend (from 5700 mg/L on Day 16 to 8140 mg/L on Day 31) during the application of the loading rates of 1.5 g COD/Lday OLR and 0.2 g TAN/Lday NLR.

Moving on to the loading rates of 3 g COD/Lday and 0.4 g TAN/Lday (Days 30-49), denitrification efficiency in anoxic periods (%DN) increased to 31±17% (Table 4.6), which was related to the increasing abundancy of anoxic heterotrophic denitrifiers in the granular structure as a result of the increasing carbon source. The %SNDN and TN removal efficiencies decreased to 41±21% and 27±14%, respectively (Table 4.6). The low TAN treatment efficiency (61±3%) during this period (Days 30-49), indicated the decreased nitrification and in turn decreased TON production. Since TON was produced during aerobic periods, the decreasing %SNDN to 41±21% parallel to the decreased nitrification was likely (Table 4.6).

Table 4.6. Nitrification and denitrification performances and TN removal efficiencies of R3.

Start-up protocol of R3					
Period	aOLR	bNLR	cDN (%)	dSNDN (%)	eTN removal (%)
Start-up (21 Days)	3	0.4	10±11	48±15	31±15
Operational period of R3					
Days	aOLR	bNLR	cDN (%)	dSNDN (%)	eTN removal (%)
1-16	0.75	0.1	6±10	34±16	25±10
17-29	1.5	0.2	23±12	58±18	49±17
30-49	3	0.4	31±17	41±21	27±14
50-61	6	0.8	43±16	83±10	41±11
62-70	12	1.6	39±16	97±10	45±11

a OLR: Theoretical influent organic loading rate, g COD/Lday
b NLR: Theoretical influent nitrogen loading rate (in terms of TAN), g TAN/Lday
c DN(%): the denitrification percent at the anoxic periods
dSNDN(%):the denitrification efficiency simultaneous to nitrification during aerobic periods
eTN(%): total nitrogen removal percent, calculated using TAN and TON values

When the loading rates were increased to 6 g COD/Lday and 0.8 g TAN/Lday (Days 50-61), %DN and %SNDN increased to 43±16% and 83±10%, respectively, while the TN removal efficiency also improved as 41±11% (Table 4.6). Considering the decreasing TAN removal (45±1%) and increasing COD removal (96±1%) efficiencies during this period (Table 4.5), it can be stated that denitrification bacteria in the deeper granular layer were favoured as a result of increasing carbon content. The improvement of SNDN was attributed to the increase in the average granule size (to 2.2 mm as explained in Section 4.2.2-Table 4.7), and limited DO diffusion which allowed the enrichment of denitrifiers in the anoxic zone inside the granule (de Kreuk et al., 2005b). Similarly Lotito et al. (2012) reported gradually increased SNDN of granular sludge with stepwise OLR increase from 0.4 to 3.4 g COD/Lday, which was attributed to the co-existence of aerobic and anoxic zones in the granular structure. At the final loading rates of 12 g COD/Lday OLR and 1.6 g TAN/Lday NLR (Days 62-70), the %SNDN and TN removal efficiencies increased to 97±10% and 45±11%, respectively, with the slight decrease in DN to 39±16% (Table 4.6). The further decrease in %SNDN and TN removal efficiency was attributed to the slight decrease in TAN removal efficiency (to 51±1%) (Table 4.5). This result indicated that even at these highest loading rates studied, N removal performance of the reactor did not collapse. However it may be claimed that if the loading rates had been further increased, serious deterioration in reactor performance as well as granular structure would have occurred. The granular stability problems observed at the highest loading rates (12 g COD/Lday and 1.6 g TAN/Lday), as explained in Section 4.2.2, also support that claim. Yet, it can be concluded that, as the NLR and OLR increased gradually to 1.6 g TAN/Lday and 12 g COD/Lday, respectively, the denitrification efficiencies during both anoxic and aerobic periods increased. Denitrification during the aerobic periods (%SNDN) was likely to be improved with the increase in nitrification efficiency.

4.2.1.2. Organics removal efficiency in R3

Figure 4.15 shows the theoretical influent COD concentrations and OLR values of R3, as well as the percent COD removal efficiencies of anoxic period, aerobic period and the overall cycle of concern (i.e. cyclic removal efficiency). During the start-up period (21 Days), the application of the loading rates of 3 g COD/Lday OLR and 0.4 g TAN/Lday NLR yielded 91±2% cyclic COD removal efficiency on the average (Table 4.5). Generally the total (cyclic) COD removal efficiency of R3 was more or less stable (around 88±5%) with slight increases during Days 1-61, at the OLRs of 0.75-6 g COD/Lday and the NLRs of 0.1-0.8 g TAN/Lday (Figure 4.15). Only at the final loading rates of 12 g COD/Lday and 1.6 g TAN/Lday (Days 62-70), the cyclic COD removal efficiency of R3 decreased. Despite the fluctuations, COD removal efficiency during anoxic periods followed a decreasing trend with increasing OLR and NLR values during the operation (Days 1-70) (Figure 4.15). In contrast to that of the anoxic period, the COD removal efficiency in the aerobic period showed an increasing trend with fluctuations during Days 1-61, with increasing loading rates from 0.75 to 6 g COD/Lday OLR and 0.1 to 0.8 g TAN/Lday NLR. However application of the highest loading rates of 12 g COD/Lday and 1.6 g TAN/Lday (Days 62-70) resulted in decreasing COD removal efficiency in aerobic periods (Figure 4.15). It was also seen that when the anoxic period COD treatment was high, the following aerobic period COD treatment was low, and vice versa. To explain, the COD present at the beginning of the cycle was consumed at the anoxic period, simultaneous to TON limited denitrification reaction. The unconsumed COD, then, shifted to the aerobic period.

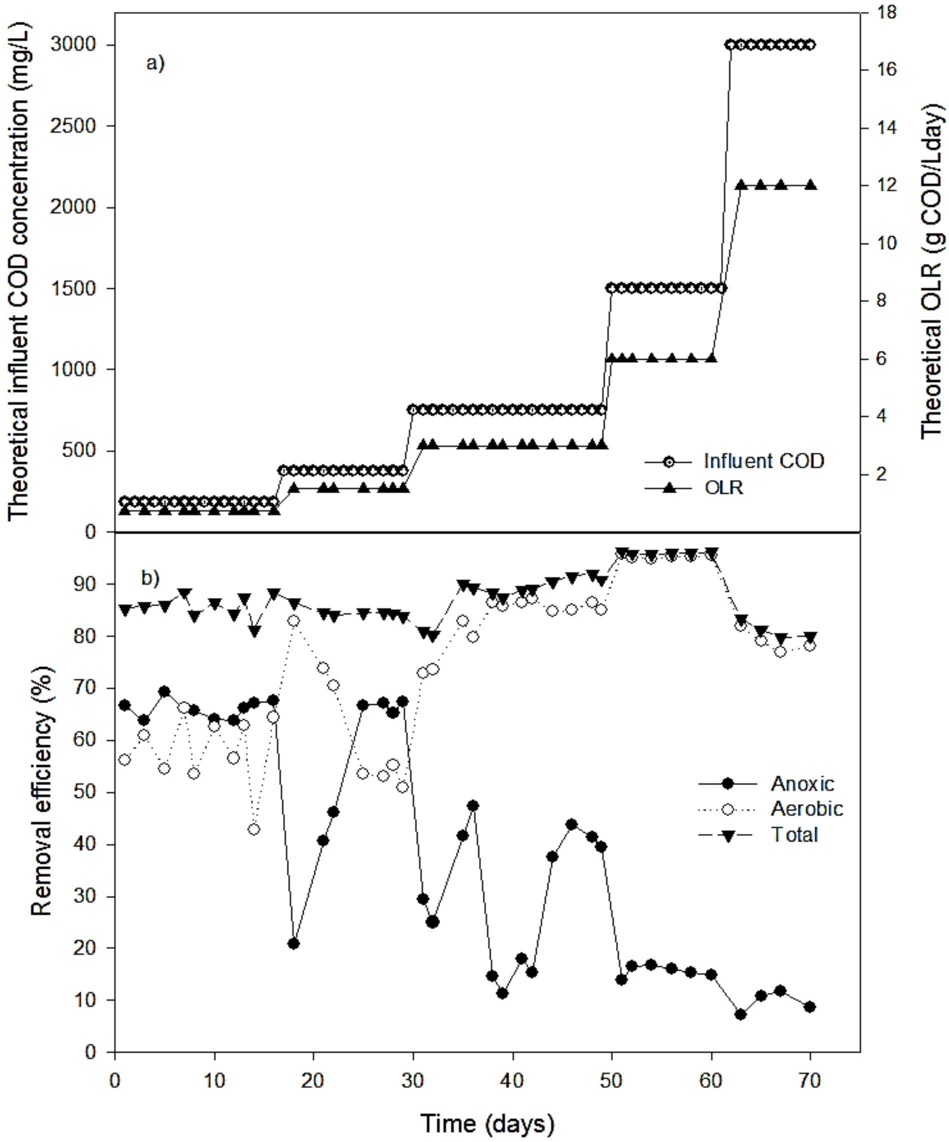

Figure 4.15 COD graph for R3: a)Theoretical influent OLR (g COD/Lday) and COD concentration (mg/L); b) COD removal efficiencies for anoxic period, aerobic period and the overall cycle.

As seen in Table 4.5, after the start-up, with the application of 0.75 g COD/Lday OLR and 0.1 g TAN/Lday NLR (Days 1-16), the average cyclic COD removal efficiency was measured as 86±2%. The slight decrease in COD treatment efficiency was attibuted to the decrease in OLR from 3 g COD/Lday in start-up period to 0.75 g COD/Lday during Days 1-16. This might be due to the fact that OLR decrease results in lower F/M ratios, which causes limited diffusion leading to substrate scarcity at the inner granular structure. Thus, the microbial activities in the deeper granule (i.e.

heterotrophic denitrifiers) are adversely affected, causing some portion of the granules to disintegrate (Peyong et al., 2012). The average VSS content of the reactor, which was 8086±2992 mg/L in start-up period at the OLR of 3 g COD/Lday, decreased to 5960±725 mg/L when the operational period started at the OLR of 0.75 g COD/Lday (Days 1-16, Table 4.5). Peyong et al. (2012) observed formation of smaller granules and then granular disintegration and wash-out of some portion with the decrease in OLR from 1.2 to 0.13 g COD/Lday. Yet, they still observed 100% COD removal efficiency, showing that the COD removal efficiency of aerobic granules may remain intact despite the OLR decrease and granular disintegration. When the following loading rates of 1.5g COD/Lday OLR and 0.2 g TAN/Lday NLR were studied, despite the granular fragmentation (which was observed via the formation of smaller granules in the reactor without drastical biomass decrease) in the reactor stable cyclic COD removal efficiency around 85±1% was observed (Days 17-29) (Figure 4.15, Table 4.5). During Days 30-49, the OLR and NLR values were further increased to 3 g COD/Lday and 0.4 g TAN/Lday, respectively. The increase in OLR provided slightly higher cyclic COD treatment efficiency of 88±4% (Figure 4.15, Table 4.5). It may be concluded that the granular fragmentation, which was observed via formation of smaller granules at the OLRs of 1.5-3 g COD/Lday (Days 17-49), did not affect the COD removal efficiency. During the application of 6 g COD/Lday OLR and 0.8 g TAN/Lday NLR (Days 50-61), the average cyclic COD treatment efficiency was 96±1%, which was the maximum value obtained so far in this study (Figure 4.15, Table 4.5). The increase in COD removal efficiency with increasing OLR from 0.75 to 6 g COD/Lday (Days 1-61), and influent COD concentrations, was attributed to the enhancement of diffusion, as the higher substrate concentrations at bulk solution are known to favor diffusion (Moy et al., 2002). In addition, the increased influent COD concentrations and in turn the higher OLR values, provide more carbon source for the heterotrophic bacteria. The average COD removal efficiency increased drastically from 88% at the OLR of 3 g COD/Lday (Days 30-49) to 96% at the OLR of 6 g COD/Lday (Days 50-61) (Table 4.5), which was attributed to the biomass enrichment as the reactor VSS increase (from 5920 mg/L on Day 49 to 6830 mg/L on Day 60) implies (Figure 4.15, Figure 4.14). Similarly, Adav et al. (2009a) reported biomass enrichment and high COD removal efficiency of 95-96% with increasing OLR from 9 to 19.5 g COD/Lday.

The drastic decrease in average COD treatment efficiency from 96±1% (Days 50-61) to 81±2% (Days 62-70) with increasing OLR from 6 to 12 g COD/Lday (Table 4.5, Figure 4.15), was attributed to the distrupted granular stability under high loading rates (Liu and Tay, 2004). Similar observations about decreasing COD removal efficiency due to the granular stability problems at high OLRs were reported by Tay et al. (2004a) at 8 g COD/Lday, by Adav et al. (2009a) at 21.3 g COD/Lday and by Lotito et al. (2012) at 3.4 g COD/Lday. The COD removal efficiency decrease was attributed to the granular stability deterioration caused by the the loss of structural strength and robustness as a result of the fast growth of loose and less compact biomass at higher OLRs (Liu and Liu, 2006; Li et al 2008; Tay et al., 2004a,b; Adav et al., 2009a, 2010; Lotito et al., 2012). Therefore, the granular structure may be subjected to partial detachment and even disintegration, resulting in wash-out (Tay et al., 2004a,b; Adav et al., 2009a; Lotito et al., 2012). Similarly granular disintegration was also reported by Chen et al. (2008) with OLR increase to 12 g COD/Lday, followed by microbial wash-out which was attributed to high substrate concentration inhibition. The wash-out was related to the microbial reselection required for the microbial species shift in order to adapt the increasing OLR (Chen et al., 2008). In our study, the reactor VSS content decreased from 6830 mg/L (Day 60) to 5990 mg/L (Day 65) (Figure 4.14) with the OLR increase from 6 to 12 g COD/Lday. This may indicate granular disintegration and wash-out, supporting the claims of Tay et al. (2004a,b), Adav et al. (2009a), Lotito et al. (2012) and Chen et al. (2008). The granular disintegration was also confirmed by the particle size and settling velocity analyses which are given in the following Section 4.2.2.

4.2.2. Granular structure in R3

The microscopic photographs of the aerobic granular sludge in R3 sampled throughout the operational period are shown in Figure 4.16. Seed sludge used for the start-up had light brown, large, spherical aerobic granules with the average size of 3.4±1 mm slightly surrounded by flocs (Figure 4.16 a,b). The granules had rigid and compact structure, without any filamentous growth. At the end of the start-up period for 21 days (at an OLR of 3 g COD/Lday and an NLR of 0.4 g TAN/Lday), the average granule size increased to 4.1±1 mm. Microscopic analysis revealed the slight presence of filamentous growth after the start-up. The properties of the granular sludge during the operation are shown in Table 4.7.

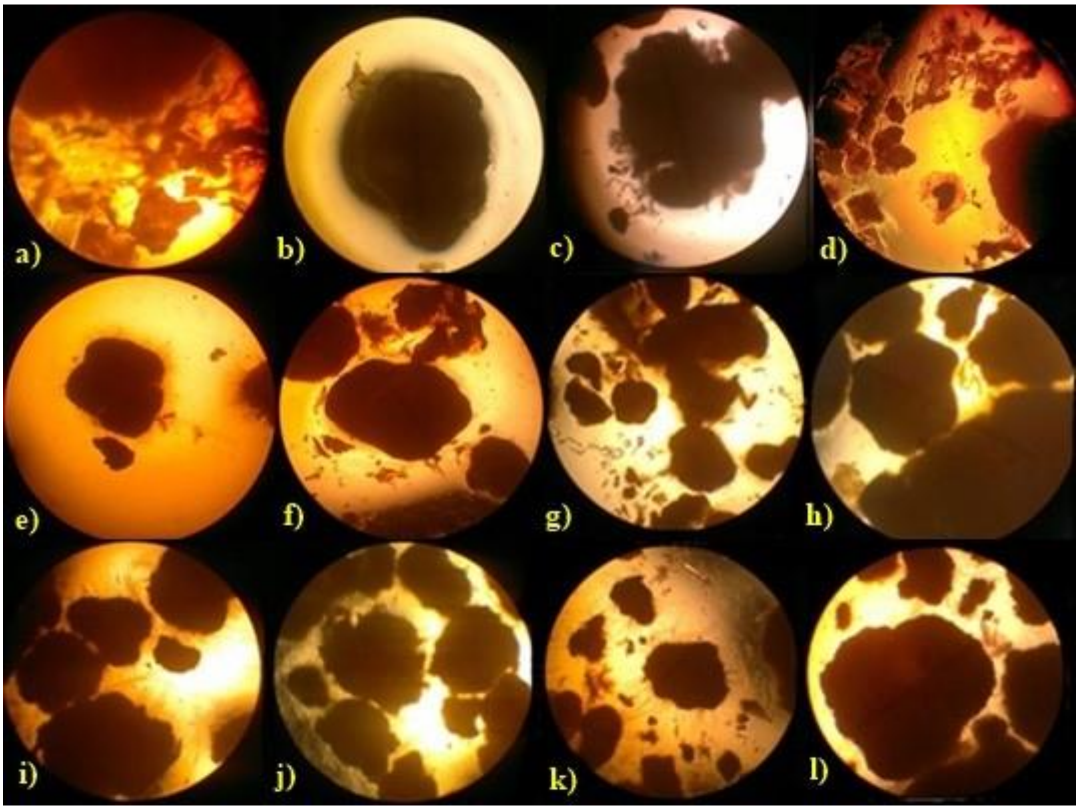

Figure 4.16. Microscopic photographs of aerobic granular sludge during the operational period of R3 (6.3 x 4 magnification). a,b)Seed sludge before the start-up period; c) Day 16; d) Day 30; e) Day 39; f,g) Day 48; h) Day 62; i,j) Day 69; k,l) Day 70.

Table 4.7 Properties of granular sludge in R3 during the operation.

Day	[a]OLR/ NLR	Average diameter (mm)	SVI_5 (mL/g)	SVI_{30} (mL/g)	[b]Granulation percent (%)	Settling velocity (m/h)
1-16	0.75/0.1	3.7±0.7	18±1	17±1	95	91±19
17-29	1.5/0.2	2.2±1.3	18±1	15±1	83	68±14
30-49	3/0.4	1.8±0.4	21±2	19±1	91	60±2
50-61	6/0.8	2.2±0.7	23±1	18±1	78	62±1
62-70	12/1.6	2.7±0.8	22±1	18±1	82	66±5

[a]OLR/NLR: in terms of g COD/Lday / g TAN/Lday
[b]Granulation percent: 100x (SVI_{30} / SVI_5)

During the application of the loading rates of 0.75 g COD/Lday OLR and 0.1 g TAN/Lday NLR (Days 1-16), the average granule size was smaller such as 3.7±0.7 mm, and the average settling velocity was 91±19 m/h (Table 4.7). The granules were compact, rigid, spherical and brown, with some filamentous growth (Figure 4.16c). Since the loading rates (0.75 g COD/Lday OLR and 0.1 g TAN/Lday NLR) were four times smaller than that of the start-up period (3 g COD/Lday OLR and 0.4 g TAN/Lday NLR), granular size decrease was expected as a result of the decreasing influent carbon concentrations and in turn potential decrease in heterotrophic growth. Moving on to the loading rates of 1.5 g COD/Lday and 0.2 g TAN/Lday (Days 17-29), granular fragmentation was observed for granules with average sizes of 2.2±1.3 mm were reported (Table 4.7). In addition, the average particle settling velocity decreased to 68±14 m/h (Table 4.7). The particles were brown-orange in colour and ressembled pebble-stones. The granules lost their sphericity, and filamentous growth was detected (Days 17-29) (Figure 4.16d). Regarding the VSS increase from 5700 mg/L (Day 16) to 8140 mg/L (Day 31) (Figure 4.14), and high N removal performance (49% TN, 87% TAN, 58% SNDN, 23% DN, Table 4.5-4.6) during Days 17-29, the aerobic granular sludge was likely to be enriched in nitrifiers.

During the application of the loading rates of 3 g COD/Lday and 0.4 g TAN/Lday (Days 30-49), the average granular size decreased to 1.8±0.4 mm, and the average particle settling velocity decreased to 60±2 m/h (Table 4.7). The granules were not completely spherical but showed little sphericity and ressembled pebble-stones (Figure 4.16e,f,g). The reactor content was light-brown in colour and filamentous microorganisms were observed. At the loading rates of 6 g COD/Lday and 0.8 g TAN/Lday (Days 50-61), the reactor content was nearly white, light brown-cream in colour, and consisted of spherical rigid granules with filaments (Figure 4.16h). The average granule size increased to 2.2±0.7 mm, and a slight increase in average settling velocity to 62±1 m/h was reported (Table 4.7, Days 50-61). The particle sizes were uniformly distributed with a small standard deviation. At the final loading rates of 12 g COD/Lday and 1.6 g TAN/Lday, the average granule size increased to 2.74±0.8 mm, while the average particle settling velocity increased to 66±5 m/h (Days 62-70, Table 4.7). The reactor content was white-light brown in colour with large and loose granules accompanied by a high amount of filamentous growth (Figure 4.16i,j,k,l).

Although OLR increase provides larger granule sizes in common (Liu and Tay, 2004), the increase in loading rates up to 1.5-3 g COD/Lday in this study, brought decrease in granular sizes via fragmentation. The fragmentation at OLRs 1.5-3 g COD/Lday (Days 17-49) caused the formation of smaller granules (such as granules with the average sizes of 1.8-2.2 mm, much smaller than those of the seed granules). This is also supported by the fact that high settling velocities such as 91±19 m/h during Days 1-16, dropped to 60-68 m/h at the OLRs of 1.5-3 g COD/Lday (Days 17-49, Table 4.7). Since the depth of the granular channels were determined to be 900 µm, i.e.0.9 mm, the granule diameter should not exceed 1.8 mm in order to prevent limited DO diffusion, and the resulting cell death (Tay et al., 2002b). However the average granule sizes ranged between 2.7-3.2 mm at the OLRs of 0.75-1.5 g COD/Lday (Days 1-29) (Table 4.7), which were obviously larger. It may be commented that the granules were fragmented during Days 17-49 in order to reach more efficient sizes in terms of mass transfer; in other words, to facilitate the transport of DO and nutrients. Despite the granular fragmentation, there was biomass growth in the reactor during the application of the loading rates of 1.5 g COD/Lday OLR and 0.2 g TAN/Lday NLR (Days 17-29). The VSS concentration in the reactor, which was 5700 mg/L on Day 16, increased to 8140 mg/L on Day 31 (Figure 4.14). The granular fragmentation, biomass enrichment in the reactor, high TAN removal efficiency and improved %SNDN during Days 17-29 (Figure 4.13, Table 4.6-4.7), indicate the enhancement of nitrification and denitrification bacteria. It should be noted that the COD was already unlimited for denitrification to occur. If the granular fragmentation was resulting from deterioration of granular structure, there would be wash-out in the reactor, and/or deteriorating treatment performance would have been observed. However, during Days 17-29, the average cyclic treatment efficiencies were high (85±1% COD and 87±2% TAN) and the average VSS increased to 6865±488 mg/L (Table 4.5, Figure 4.13, Figure 4.14, Figure 4.15). Thus, it was likely that the granular structure was not deteriorated but instead the granules were fragmented to ease the mass transport.

For OLRs larger than 3 g COD/Lday, the average sizes followed an increasing trend. The increase in granule size between Days 50-70, corresponding to the loading rates of 6-12 g COD/Lday and 0.8-1.6 g TAN/Lday, was mostly related to the increased loading rates. Increase in granular size with OLR increase was commonly reported in literature (Moy et al. 2002; Li et al., 2008a; Tay et al., 2001a, 2001b; Liu and Tay, 2004; Chen et al. 2008). This was due to the increased substrate availability for the biomass growth. In other words, the increased influent COD concentrations provided higher OLRs and in turn higher carbon source which favors the growth of carbon-consuming heterotrophic bacteria (Lotito et al., 2012). However, as the growth of heterotrophic bacteria are fastened, formation of larger but less compact granules with deteriorated structural strength and robustness causes granular stability problems at high OLRs which may result in decreased reactor performance, granular disintegration and wash-out (Liu and Liu, 2006; Liu and Tay, 2004; Gao et al., 2011; Zheng et al., 2006; Lotito et al., 2012). Decrease in reactor VSS content from 6830 mg/L (Day 60) to 5990 mg/L (Day 65) (Figure 4.14) with the OLR increase from 6 to 12 g COD/Lday may be due to the granular disintegration and wash-out, as previously mentioned while explaining COD treatment efficiency (Section 4.2.1.2). It may be claimed that the OLR increase to 12 g COD/Lday during Days 62-70 created granular stability problems and wash-out due to the formation of large (2.7±0.8 mm) but loose granules as implied by the drastic decrease in COD removal efficiency from 96±1% to 81±2% during this period (Days 62-70, Table 4.5). Since the average granule size (2.7±0.8 mm) was larger than the optimum granular sizes of 1.2-1.8 mm (de Kreuk et al., 2006; Tay et al., 2002b), the granular sludge was likely to be disintegrated due to the prevention of nutrients and DO diffusion. On the other hand, although the granular size increase was thought to be responsible for the DO limitations that caused granular disintegration at high OLRs (Moy et al., 2002; Adav et al., 2009a), the disintegration of even smaller granules (0.95-1.32 mm) were reported at the OLRs of 8-12 g COD/Lday (Tay et al., 2004 a,b; Chen et al., 2008). Thus, although large granule sizes may adversely affect mass transport into the granules' depths and cause disintegration, there are other factors (such as microbial composition) influencing the granular stability and the tendency of disintegration.

The sludge settleability, which is indicated via SVI, was nearly stable during the 70 days of operational period. The SVI_{30} values ranged between 15-19 mL/g (Table 4.7), indicating the good settleability of the sludge since the values are below the typical range of 20-80 mL/g given for granules (Gao et al., 2011). The granulation percent of the sludge decreased from 95% to 83% with OLR increase from 0.75 to 1.5 g COD/Lday (Table 4.7), which was attributed to the granular fragmentation during Days 17-29. It was likely that the slow-growing nitrifiers with smaller sizes were enriched in the reactor at the NLR of 0.2 g TAN/Lday and the OLR of 1.5 g COD/Lday (Days 17-29). When the OLR was increased to 3 g COD/Lday, the granulation percent increased to 91%, despite the decrease in granular size and average settling velocity during this period (Days 30-49) (Table 4.7). At the OLR of 6 g COD/Lday (Days 50-61) the granulation percent decreased to 78%. When the increase in VSS concentration from 5920 mg/L on Day 49 to 6830 mg/L on Day 60 is considered (Figure 4.14), with the increasing average granular size to 2.2 mm at the OLR of 6 g COD/Lday (Table 4.7), the decrease in granulation percent may be attributed to the fast enrichment of loose biomass, such as heterotrophic bacteria. Fast growth of loose and unstable biomass was reported at the OLRs of 3.4-6 g COD/Lday and was attributed to the enhancement of carbon-consuming heterotrophs that were likely to be disintegrated and washed-out (Zheng et al., 2006; Lotito et al., 2012). At the OLR of 12 g COD/Lday (Days 62-70), the granulation percent slightly increased to 82%. Despite the granular stability problems in terms of structure, partial disintegration and suspected wash-out in the reactor, formation of larger granules is likely, as also indicated by the increases in granular sizes to 2.7 mm and settling velocities to 66±5 m/h at the OLR of 12 g COD/Lday (Days 62-70) (Table 4.7).

The EPS concentrations of R3 through the operational period are demonstrated in Table 4.8.

Table 4.8. EPS values of R3 through the operational period.

R3					
Days	[a]OLR	[a]NLR	EPS (mg/g VSS)		
			PN	PS	PS/PN
1-16	0.75	0.1	111±1	68±0.1	0.61
17-29	1.5	0.2	78±1	48±0.4	0.62
30-49	3	0.4	103±1	65±0.8	0.63
50-61	6	0.8	117±0.1	78±0.9	0.67
62-70	12	1.6	124±0.9	88±0.1	0.71

[a]OLR: in terms of g COD/Lday, NLR: in terms of g TAN/Lday.

The EPS values decreased for both PS and PN during fragmentation at the OLR of 1.5 g COD/Lday (Days 17-29) (Table 4.8). Moving on to the OLR of 3 g COD/Lday and above, the EPS values pursued an increasing trend in terms of both PS and PN until the end of operation (Days 30-70) (Table 4.8). The decreasing PS and PN concentrations at the OLR of 1.5 g COD/Lday were attributed to the granular fragmentation (i.e. division of granules into small pieces without any floccy appearence or granular stability destruction) (Adav et al, 2008b; Zhu et al, 2012). Similar observation was present in Set-1, R2, which experienced decreasing PS and

PN concentrations with granular fragmentation at the influent COD/TAN ratio of 5 (Section 4.1.2.2). The EPS production of the aerobic granules is known to be stimulated under stressful environmental conditions, including the operations with increasing OLR (Liu et al., 2004a; Zhu et al., 2012). During the granulation process increase in both PS and PN was common (Adav et al., 2008b). Therefore the increasing EPS concentrations with OLR increase from 3 to 12 g COD/Lday was expected. The slight but gradual increase in PS and PN concentrations might have been related to the application of stepwise increasing OLR and NLR values.

The PS/PN ratios of the granular sludge showed a slight but gradual increase from 0.61 to 0.71 with increasing OLR from 0.75 to 12 g COD/Lday during the operational period (Table 4.8). During the operation, the PS/PN ratio values were in the suitable range (≥0.6) for aerobic granulation defined by Li et al. (2008c). A nearly constant PS/PN ratio may indicate the balanced increase in both PS and PN concentrations, which was common during the development of new granules. This nearly constant PS/PN ratio could have been due to the stable influent COD/TAN ratio of 7.5 during the operation. Regarding the Set-1 experience, during the presence of granular stability problems which are commonly followed by deterioration of granular structure, disintegration, wash-out, decreasing PS and increasing PN concentrations, and a PS/PN ratio below 0.6 had been expected. As discussed previously, the granular stability problems related to high OLR were mentioned for R3 at the OLR of 12 g COD/Lday. However, the concentrations of both PS and PN increased and the PS/PN ratio was as high as 0.71, which indicated that the system still maintained its stability in terms of treatment efficiency despite the decrease in both COD and TAN removal efficiencies.

4.3. Set-3: Treatment of sugar beet processing wastewaters by aerobic granules

Sugar beet processing wastewaters may contain organics up to 10000 mg/L and SS up to 5000 mg/L, as well as N, P, alkalinity and pesticide residues (Güven et al., 2009; The World Bank Group, 2007; Aliplik Akın, 2010). Although the sugar beet processing wastewaters are generally subjected to anaerobic treatment (Table 2.2, Section 2.5), anaerobic systems are not suitable for N and P removal, which revived the use of aerobic treatment systems such as aerobic biogranular reactors. Aerobic granulation and/or treatment with industrial wastewaters are known to be studied for the past decade. Effluents of many different industries were used in aerobic granular systems (Table 2.1). However, to our knowledge, the treatability of sugar beet processing wastewaters with aerobic granulation technology has not been studied so far. Therefore, Set-3 was conducted to investigate the treatability of sugar beet processing wastewaters via aerobic granules, and its effects on aerobic granular characteristics. One SBR, namely R4, was operated for 54 days with 2 types of sugar beet processing wastewaters. Part I involved the operation with the effluent of anaerobic digester treating sugar beet processing wastewater (i.e. anaerobically-pretreated effluent) for 32 days (Days 1-32). Raw sugar beet processing wastewater was further employed in Part II during Days 33-54. This section involves the explanation and discussion of the experimental results including the treatment performance and granular characteristics of R4.

4.3.1. Treatment Efficiency

In Set-3, the treatment efficiency of R4 through the operational period of 54 days was investigated in terms of N and organics removal. TN calculations were based on the inorganic soluble N, i.e. the TAN and TON values.

Before the operational period of 54 days, the start-up period with synthetic wastewater application for 21 days yielded 92±1% sCOD, 85±22% TAN, 43±13% TN removal efficiencies on the average (Table 4.9).

Table 4.9. Reactor performance of R4 during the start-up period.

Start-up protocol of R4								
Ww type	Period	[a]Ave. OLR	[b]Ave. NLR	sCOD removal (%)	TAN removal (%)	DN (%)	SNDN (%)	[c]TN removal (%)
Synthetic ww	21 Days	0.75	0.1	92±1	85±22	38±11	50±25	43±13

[a] Ave. OLR: Average organic loading rate, g sCOD/L.day
[b] Ave. NLR: Average nitrogen loading rate (in terms of TAN), g TAN/Lday
[c]TN removal was calculated using TAN and TON values

4.3.1.1. N removal efficiency in R4

The N removal performance of R4 in terms of TAN and TN removal efficiencies as well as anoxic period denitrification (%DN) and the simultaneous nitrification denitrification (%SNDN) percentages are explained in this section. Table 4.10 shows the TAN removal efficiencies of R4 during the operation.

Table 4.10. Cyclic TAN removal efficiencies and the solids concentration of R4 through the operational period.

Wastewater type	Day	Number of feeding per day	[a] Ave. NLR (gTAN/Lday)	Influent TAN (mg/L)	TAN removal (%)	[b]Ave. TSS (mg/L)	[c]Ave. VSS (mg/L)
Anaerobically-pretreated effluent	1-6	1	0.02±0.004	32±8	91±3	4417±203	2870±184
	7-13	2	0.02±0.01	22±0	94±0	7260±764	3560±1301
	14-32	4	0.07±0.01	35±4	89±3	5798±993	2851±413
Sugar beet processing wastewater	33-54	4	0.1±0.01	49±5	61±4	5886±593	3473±259

[a] Ave. NLR: Average nitrogen loading rate, g TAN/Lday
[b] Ave. TSS: Average TSS concentration in the reactor, mg/L
[c] Ave. VSS: Average VSS concentration in the reactor, mg/L
[d]The average influent sCOD/TAN ratios in Part I were calculated as 4.94±1.2 for Days 1-6, 4.15±0.2 for Days 7-13 and 3.15±0.8 for Days 14-32. The overall values for the average influent sCOD/TAN ratios were computed as 3.62±1.1 for Part I, and 63.4±7 for Part II.

Figure 4.17b shows the anoxic period, aerobic period and cyclic TAN treatment efficiencies of the reactor during the operation period of 54 days. TAN treatment was observed only during the aerobic periods as expected. The average influent TAN concentration was 32±6 mg/L, and the average NLR was 0.05±0.03 g TAN/Lday during Part I (Days 1-32, Table 4.10). When SBR was fed 1 time-per day, the TAN removal efficiency was 91±3% (Days 1-6; Table 4.10). The application of 2 feedings per day made TAN removal efficiency increase slightly to 94% (Days 7-14, Table 4.10). Moving on to 4 feedings per day, TAN removal slightly decreased to 89±4% (Days 14-32, Table 4.10). It can be stated that the increase in reactor feeding from 1 to 4 times per day, which resulted in gradual increase in NLRs from 0.02 to 0.07 g TAN/Lday, did not have a serious effect on TAN treatability due to the low concentrations of wastewater composition (Table 4.10, Figure 4.17a). The average cyclic TAN removal efficiency was 90±3% with a maximum of 99% in Part I (Days 1-32, Figure 4.17b).

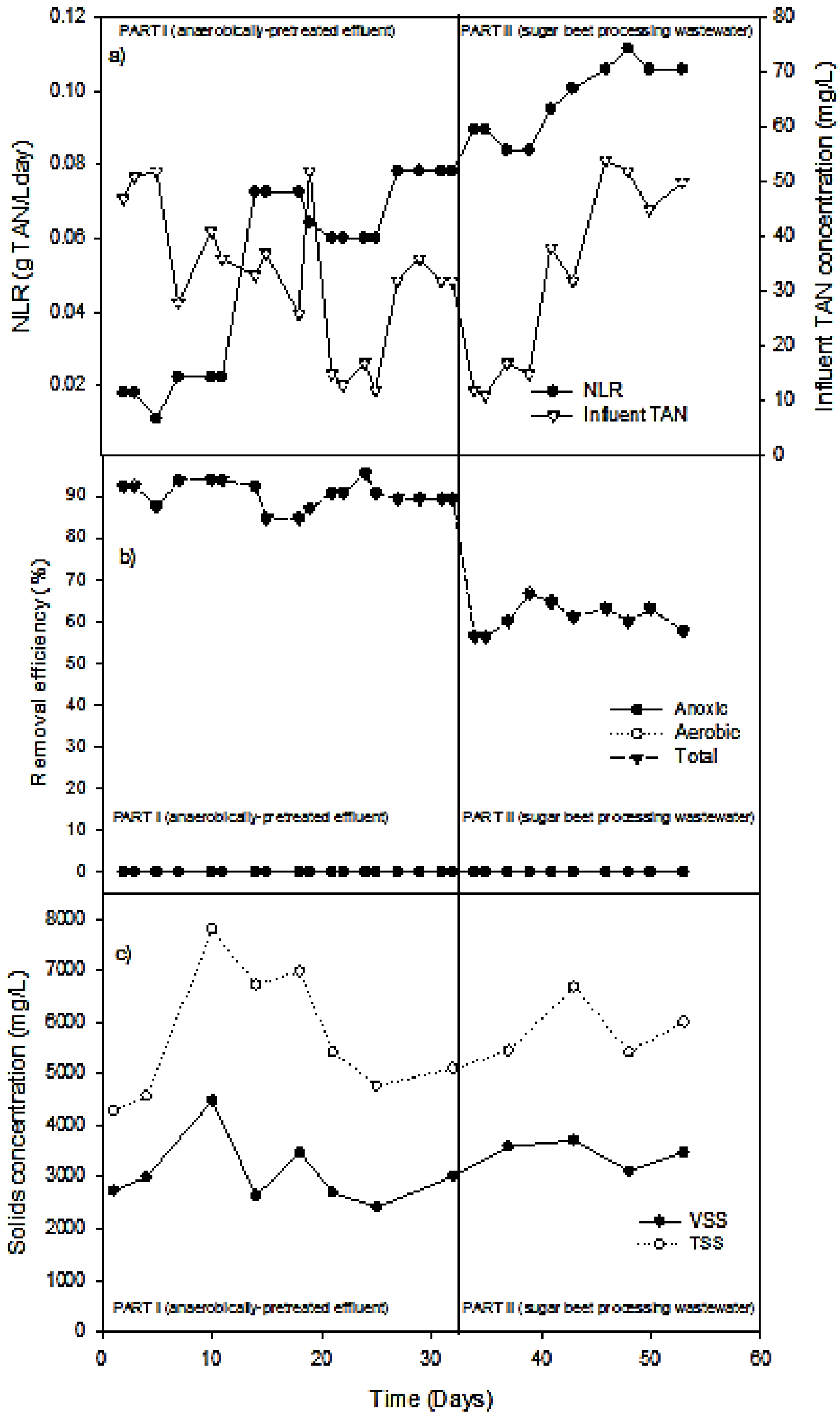

Figure 4.17. TAN and VSS graph for R4 a) The influent NLR (g TAN/Lday) and influent TAN concentrations (mg/L); b) TAN removal efficiency for anoxic period, aerobic period and total cycle; c) reactor TSS and VSS concentrations for R4.

Raw sugar beet processing wastewater, containing 3055±183 mg/L sCOD and 33±4 mg/L TAN was introduced to the SBR during Part II at the loading rates of 6.11±0.4 g sCOD/Lday and 0.1±0.01 gTAN/Lday during Days 33–54 (Table 4.10). The average influent TAN concentration was 49±5 mg/L, and the average NLR was 0.1±0.01 during Part II (Table 4.10). The reactor had 2851±413 mg/L VSS and 5798±993 mg/L TSS on the average during Days 14-32, in Part I (Table 4.10). Moving on to Part II, the application of raw process wastewater with increased pollution load in terms of both COD and TAN, provided biomass increase in the reactor, since there were 3473±259 mg/L VSS and 5886±593 mg/L TSS on the average during Days 33-54 (Figure 4.17c). The average cyclic TAN treatment efficiency obtained during Part II was 61±4% (Table 4.10), with a maximum of 67% (Figure 4.17b). The average influent sCOD/TAN ratio of raw sugar beet wastewater which was 63.4±7 (Part II, Days 33-54), was about 20 times higher than that of the anaerobically-pretreated effluent which was 3.62±1.1 (Part I, Days 1-32). Compared to the difference in COD contents of raw sugar beet processing wastewater and anaerobically-pretreated effluent, both wastewater types had close influent TAN concentrations (Section 3.2.3.2, Table 3.6). Thus, the significant difference between the COD/TAN ratios of the wastewaters was caused by the higher carbon content of the raw processing wastewater. The decrease in TAN removal efficiency from 90±3% to 61±4% with the shift from Part I (anaerobically-pretreated effluent, Days 1-32) to Part II (sugar beet processing wastewater, Days 33-54), might be related to the lower abundance of nitrifiers and in turn lower nitrification with increasing influent sCOD/TAN ratio from 3.62 to 63.4. At high carbon over nitrogen (C/N, COD/TAN, COD/N) ratios such as above 10, enhancement of heterotrophic bacteria and decreased amount of nitrifiers in the granular structure were reported (Liu et al., 2003b; Yang et al., 2005). In addition, Set-1, where the effect of influent COD/TAN ratio on aerobic granules was investigated, also showed that the COD/TAN ratio increase from 7.5 to 30 decreased the TAN treatment efficiency from 90% to 30%.

Table 4.11 states the average anoxic period denitrification (DN%) and aerobic simultaneous nitrification-denitrification (SNDN%) efficiencies, and the percentage of the total nitrogen removal (loss) per cycle (TN%) achieved during the experimental period.

Table 4.11. Denitrification (DN) and simultaneous nitrification-denitrification (SNDN) efficiencies for the industrial wastewaters applied in Set-3.

Wastewater type	Day	Number of feeding per day	Average NLR (gTAN/Lday)	Influent TAN (mg/L)	TAN removal (%)	[a]DN (%)	[b]SNDN (%)	[c]TN removal (%)
Anaerobically-pretreated effluent	1-6	1	0.02±0.004	32±8	91±3	N.D.[e]	N.D.[e]	N.D.[e]
	7- 13	2	0.02±0.010	22±0	94±0	N.D.[e]	23±9	N.D.[e]
	14-32	4	0.07±0.010	35±4	89±3	42±12	48±25	58±16
Sugar beet processing wastewater	33-54	4	0.10±0.01	49±5	61±4	34±29	64±13	57±17

[a] Denitrification achieved during anoxic period
[b] Simultaneous nitrification-denitrification achieved during aerobic period
[c] TN (Total Nitrogen)= TAN + [d]TON
[d] TON (Total oxidized nitrogen) = NO_2-N + NO_3-N
[e] N.D.=Not determined

As previously mentioned, the average NLR and influent TAN concentration for Part I (Days 1-32) were calculated as 0.05±0.03 g TAN/Lday and 32±6 mg/L TAN, respectively (Table 4.10). When SBR was fed 1 time-per day (Days 1-6), the DN, SNDN and TN efficiencies could not be calculated due to the experimental errors observed in the nitrate determination experiments (Table 4.11). During Days 7-13, where SBR was fed 2 times per day, the %DN and TN removal values could not be calculated due to the experimental errors, while the %SNDN was recorded as 23±9%. Despite the experimental errors, it might be possible that the %DN was negligibly small due to the limited substrate availability as a result of the influent COD concentration, since the sCOD removal efficiency also decreased to 78±9% during Days 7-13 (Section 4.3.1.2, Table 4.12). Starting from Day 14, where the SBR was fed 4 times a day, the %DN, %SNDN and TN removal efficiency were 42±12%, 48±25% and 58±16 (Days 14-32), respectively (Table 4.11).

The N removal performance of the reactor improved in Part II; there was 34±29% DN, 64±13% SNDN and 57±17% TN removal (Days 33-54, Table 4.11). The higher denitrification, TN removal and especially simultaneous nitrification-denitrification efficiencies obtained in Part II were attributed to the higher COD/TAN ratio and COD, TAN concentrations of sugar beet processing wastewater as previously mentioned (Section 3.2.3.2, Table 3.6). In addition, it is likely that the higher influent COD concentration in Part II provided higher concentration gradients, enhancing the diffusive transport, hence facilitating the microbial carbon uptake. The improved diffusion might have enhanced DN and SNDN efficiencies by increasing the ease of COD to reach the inner parts of the granule where the denitrification bacteria are found (Gao et al., 2011; de Kreuk et al., 2005b).

4.3.1.2. Organics and solids removal in R4

Table 4.12 summarizes the cyclic sCOD removal efficiencies and the solid content of R4 during the operation.

Table 4.12 Cyclic sCOD removal efficiencies and the solids concentration of R4 through the operational period.

Wastewater type	Day	Number of feeding per day	[a]Ave. OLR	Influent sCOD (mg/L)	sCOD removal (%)	[b]Ave. TSS (mg/L)	[c]Ave. VSS (mg/L)
Anaerobically-pretreated effluent	1-6	1	0.07±0.01	149±13	92±1	4417±203	2870±184
	7-13	2	0.09±0.01	93±6	78±9	7260±764	3560±1301
	14-32	4	0.22±0.01	112±37	72±28	5798±993	2851±413
Sugar beet processing wastewater	33-54	4	6.11±0.37	3055±183	87±1	5886±593	3473±259

[a] Ave. OLR: Average organic loading rate, g sCOD/Lday
[b] Ave. TSS: Average TSS concentration in the reactor, mg/L
[c] Ave. VSS: Average VSS concentration in the reactor, mg/L
[d]The average influent sCOD/TAN ratios in Part I were calculated as 4.94±1.2 for Days 1-6, 4.15±0.2 for Days 7-13 and 3.15±0.8 for Days 14-32. The overall values for the average influent sCOD/TAN ratios were computed as 3.62±1.1 for Part I, and 63.4±7 for Part II.

Figure 4.18 shows the TSS and VSS concentrations of the reactor and sCOD removal efficiency during the operation. In Part I, the operation with the anaerobically-pretreated effluent (Part I, Days 1-32) yielded decreasing and increasing trend in sCOD removal efficiency (Figure 4.18b). The average influent sCOD concentration was 115±35 mg/L and the average OLR was 0.18±0.1 g sCOD/Lday in Part I. When SBR was fed 1 time per day, the cyclic sCOD removal efficiency was 92±1% (Days 1-6). Moving on to 2 feedings per day, the cyclic sCOD removal efficiency dropped gradually down to 78±9% (Days 7-13). Starting from Day 14, SBR was fed 4 times a day; however the sCOD removal efficiency decreased down to 27% on Day 15.

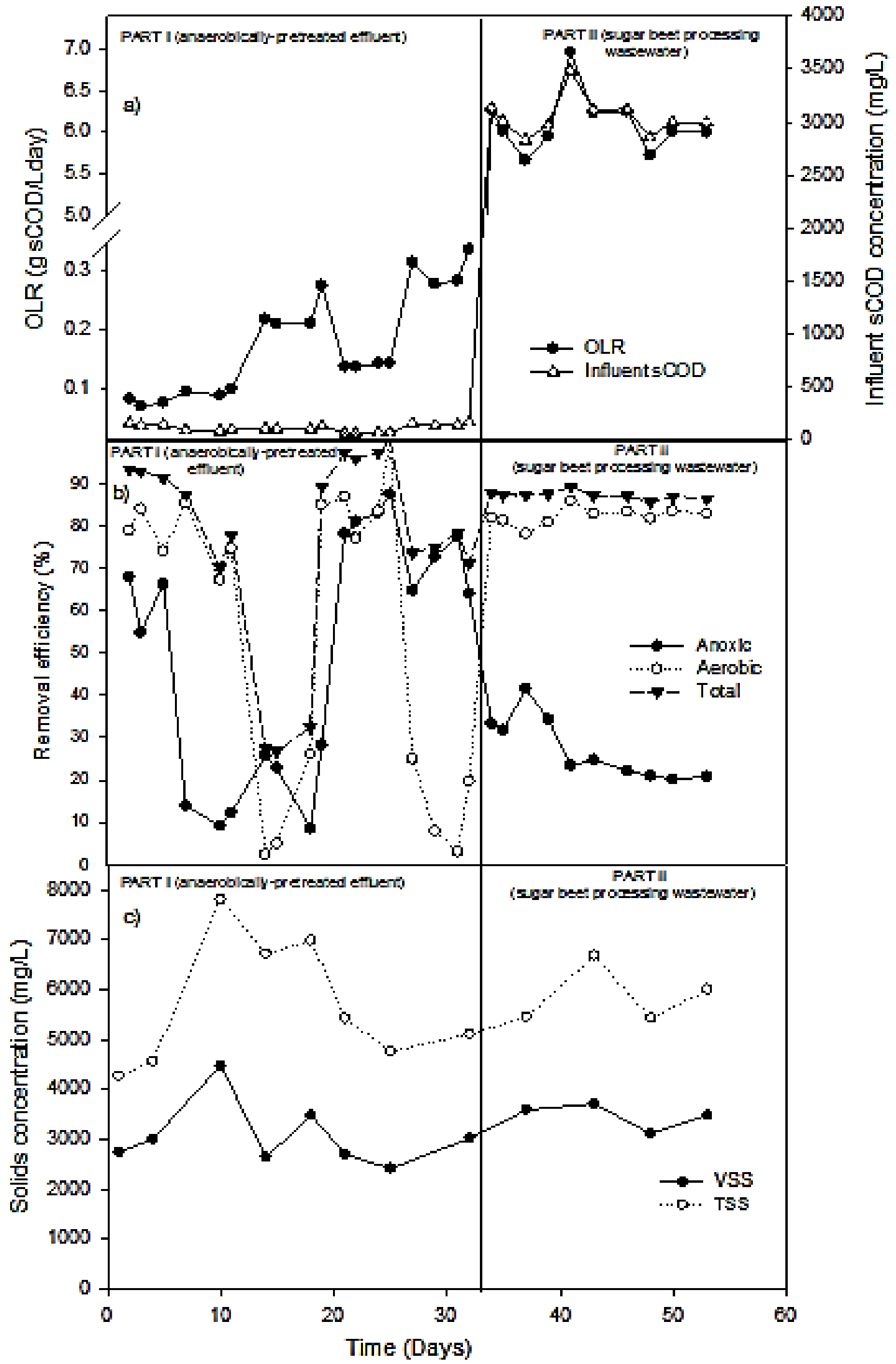

Figure 4.18. COD and VSS graph for R4 a) The influent OLR (g TAN/Lday) and influent COD concentrations (mg/L); b) COD removal efficiency for anoxic period, aerobic period and total cycle; c) reactor TSS and VSS concentrations for R4.

This decrease was attributed to the delayed effect of low influent COD concentrations and in turn substrate limitation. According to Li and Liu (2005), the substrate removal rates are likely to decrease below 100 mg/L COD due to the substrate limited microbial metabolism. Since the influent sCOD concentrations were low, even below 100 mg/L during Days 7-13 (Figure 4.18a), deteriorated sCOD treatment efficiency could be related to the substrate limited metabolism of the aerobic granules. It can be stated that the heterotrophic denitrifiers could not have achieved sufficient carbon uptake and decreased in number and were even partially washed-out, as the VSS decrease in the reactor also implies (Figure 4.18c). As seen in Figure 4.18c, the VSS concentration increased from 2740 mg/L to 4480 mg/L during Days 1-10. Yet, the bacteria were exposed to wash out during Days 11-15, indicated by the drastic VSS decrease down to 2640 mg/L (Day 14, Figure 4.18c). This claim was supported by the deteriorating sCOD removal efficiency from 70 to 27% during Days 10-15 (Figure 4.18b). The VSS decrease during Days 11-15 might have been the result of the decreasing abundancy of aerobic heterotrophic bacteria.

The cyclic sCOD treatment efficiency recovered and reached 89% on Day 19 (Figure 4.18b). This increase was due to the enhanced microorganism growth provided by the OLR increase during Days 14-32 (Table 4.12, Figure 4.18a). Since the number of feedings was increased to 4 starting from Day 14, the OLR was increased to 0.22±0.01 g sCOD/Lday (Table 4.12, Figure 4.18a). The increasing OLR promoted the microbial growth, which was seen by the VSS increase to 3480 mg/L by Day 18 (Figure 4.18c). The maximum cyclic sCOD removal efficiency was obtained as 100% on Day 25 (Figure 4.18b). Despite the further increase in OLR, the cyclic sCOD removal efficiency gradually decreased following Day 25 and dropped down to 71% by Day 32, which remained unclear (Figure 4.18b). This decrease in sCOD removal efficiency to 71% during Days 26-32 might be due to the low influent sCOD/TAN ratio of the anaerobically-pretreated effluent. The influent sCOD/TAN ratio ranged between 3.15 to 4.94 in Part I (Days 1-32) and was 3.62±1.1 on the average (Table 4.12[d]). The similar effect was also observed in Set-1. Operation of R2 (in Set-1) with an influent COD/TAN ratio of 3.5 yielded 94±4% TAN but much lower, i.e. 71±12%, sCOD cyclic treatment efficiencies on the average.

The application of anaerobically-pretreated effluent during Part I (Days 1-32) yielded 76±24% average sCOD treatment efficiency, which was generally lower than that of the conventional aerobic granular systems (Section 2.3.1.1.3, Table 2.1). Since the studies with influent sCOD/TAN ratios ≥13.8 showed sCODremoval efficiencies

≥80%, the lower sCOD removal efficiency in our system may be related to the low average influent sCOD/TAN ratio of 3.62±1.1 during Part I (Table 4.12[d]). Yet, few exceptions reported slightly higher sCOD removal efficiencies such as 85% at lower sCOD/TAN ratios such as 1.4 and 4.8 (Section 2.3.1.1.3; Table 2.1; Yilmaz et al., 2008; Lemaire et al., 2008). The higher sCOD removal efficiencies of these studies were attributed to their influent sCOD concentrations between 265 to 1072 mg/L (Section 2.3.1.1.3, Table 2.1), which were higher than that of the Part I (115±35 mg/L) in our study. The anaerobically-pretreated effluent had a tCOD concentration of 245±16 mg/L, and tCOD treatment efficiency was 71±30% for this period.

In Part II, the operation with sugar beet processing wastewater (Days 33-54) yielded 87±1% average sCOD removal efficiency on cycle basis (Table 4.12, Figure 4.18b). The cyclic sCOD treatment efficiency of R4 pursued a more stable trend than Part I (Figure 4.18b). The influent tCOD and sCOD concentrations were 4280±260 and 3055±183 mg/L on the average during Days 33-54, respectively (Table 4.12). The tCOD removal efficiency was highly stabilized at the value of 65±5%. The tCOD removal efficiencies in Part I (71±30%) and in Part II (65±5%) were comparable to that of Schwarzenbeck et al. (2004a), Yilmaz et al. (2008) and Lemaire et al. (2008), who reported tCOD treatment efficiencies in the range of 50-68% (Section 2.3.1.1.3, Table 2.1). Although Yilmaz et al. (2008) obtained high sCOD removal efficiency of 85% with abattoir wastewater, the reactor effluent had high TSS content which resulted in lower tCOD removal efficiency of 68%. Low tCOD removal efficiency of 68% was attributed to the small HRT (13.3 h) of the system. Both tCOD removal and HRT were comparable to that of our study where 65% tCOD removal in sugar beet processing wastewater was accomplished at 12.3 h of HRT. Higher tCOD removal efficiency was reported by Schwarzenbeck et al. (2005), who increased the tCOD removal efficiency of dairy wastewater from 70% to over 90% by settling the effluent for 15 minutes.

The stable treatment efficiencies achieved for sCOD (87%) and tCOD (65%) in Part II, were attributed to the high influent tCOD concentration of sugar beet processing wastewater (4280±260 mg/L) (Section 3.2.3.2, Table 3.6) providing enough carbon source for bacterial growth. Increase in loading rates and influent substrate concentrations were known to promote the nutrient mass transfer (Liu and Tay, 2004). Thus, it is likely that the drastic increase in the influent wastewater sCOD and tCOD concentrations from Part I to Part II enhanced the carbon uptake of the microorganisms. Since the raw processing wastewater had higher influent COD concentration, the concentration gradient for diffusion also increased, leading to higher COD treatment efficiency and enhanced biomass growth during Part II. This was also verified by the increase in the reactor’s biomass content. The solid content of the reactor reached 3710 mg/L VSS and 6680 mg/L TSS on Day 43 (Figure 4.18c). The average VSS and TSS concentrations were measured as 3473±259 mg/L and 5886±593 mg/L during Days 33-54, respectively (Part II, Table 4.12).

Table 4.13 states the solid removal efficiencies for Part I and Part II. In Part I (Days 1-32), the operation with anaerobically-pretreated effluent containing low solids (274 mg/L TSS and 167 mg/L VSS), yielded 29±4% TSS and 11±2% VSS removal efficiencies. Moving on to Part II (Days 33-54), the solid removal efficiencies increased to 58±10% for TSS and 25±6% for VSS, for the sugar beet processing wastewater with much higher solids content (2255 mg/L TSS and 1143 mg/L VSS).

Table 4.13 Removal efficiency of solids in R4 through the operational period.

Wastewater type	Influent (mg/L)		Effluent (mg/L)		Solids removal (%)	
	TSS	VSS	TSS	VSS	TSS	VSS
Anaerobically-pretreated effluent	274±14	167±29	195±20	149±20	29±4	11±2
Sugar beet processing ww	2255±250	1143±160	945±35	860±50	58±10	25±6

Total solids removal efficiency values ranging from 25% to 98% were reported in the studies with aerobic granular systems treating various types of industrial effluents (Section 2.3.1.1.3, Table 2.1). Similar to 29% TSS removal from anaerobically-pretreated effluent containing 274 mg/L TSS in Part I, Lotito et al. (2012) obtained 29.6% TSS removal for textile wastewater with 284 mg/L TSS content. The 58% TSS removal efficiency obtained in Part II, is comparable to that of Figueroa et al. (2011), who treated swine slurry containing high TSS (6000 mg/L) with 25-30% TSS removal efficiency. On the other hand, the TSS removal efficiencies for both Part I and Part II are lower than that of Arrojo et al. (2004), Hailei et al. (2005) and Lotito et al. (2014), who provided TSS removal efficiencies as high as 80-98%, which may be due to the low influent TSS concentrations (such as 87-1050 mg/L

TSS) compared to our study. Also the higher TSS removal efficiency of Hailei et al. (2005) could be attributed to their HRT of 15 h which was higher than that of our HRT of 12 h. However the HRT of Arrojo et al. (2004) and Lotito et al. (2014), which were 6 and 11 h, respectively were lower than that of our study, thus their TSS removal efficiencies higher than that of our study were not a result of the HRT values. The VSS removal efficiencies (11% for Part I and 25% for Part II) were lower than that of Cassidy and Belia (2005) who provided over 97% VSS removal for abattoir wastewater containing 1520 mg/L influent VSS.

The solids removal efficiencies in this study (29-58% TSS removal) may not seem sufficient; however the aerobic granular systems are known to produce effluents rich in solids and biomass, depending on the settleability of sludge (Schwarzenbeck et al., 2005). The aerobic granular SBR operation is based on the elimination of slow-settling biomass by the application of hydraulic selection pressure via short settling times (Yilmaz et al., 2008; Val del Rio et al., 2012). Therefore, the effluent solids content of aerobic granular systems may be higher than that of the influent (Section 2.3.1.1.3; Table 2.1; Schwarzenbeck et al., 2005; Lemaire et al., 2008; Val del Rio et al., 2012; Muda et al., 2010; Yilmaz et al., 2008). The biomass in the effluent may be a result of the partial detachment, disintegration and washout of the granules as a result of limited diffusion, cell degradation (Arrojo et al., 2004) or stability problems (Lotito et al., 2012).

It was reported that the solid-rich effluent caused lower overall treatment efficiencies, especially in terms of tCOD (Schwarzenbeck et al., 2005; Yilmaz et al., 2008). Although decreasing the SBR discharge period was reported to increase the TSS removal efficiency to 80% (Arrojo et al., 2004), it seems that the application of post-treatment (such as 15-30 min of settling) stands obligatory to decrease the effluent solids content of aerobic granular systems (Schwarzenbeck et al., 2005; Yilmaz et al., 2008).

4.3.2. Granular structure in R4

Table 4.14 shows the properties of the granular sludge during the operation, while Figure 4.19 shows the average granule size during the operation.

Table 4.14. Properties of the granular sludge during industrial wastewater application.

[a]Day	Average diameter (mm)	SVI_{30} (mL/g)	Granulation percent (%)	Settling velocity (m/h)	Observation:
1-32	2.86±0.7	37±0.4	93	74±12	Rigid, non-spherical, small, grizzled, dark brown granules; little filaments.
33-54	2.59±0.4	25±1.4	71	68±6	Large, spherical, brown granules; explicit filamentous growth.

[a]Days 1-32: Part I where anaerobically-pretreated effluent was applied, Days 33-54: Part II where sugar beet processing wastewater was applied.

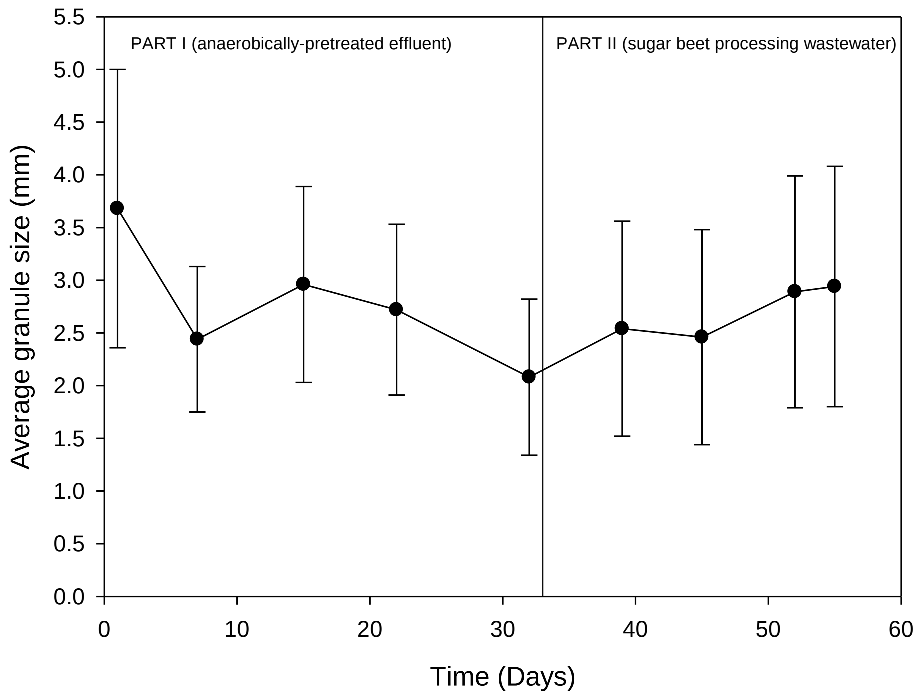

Figure 4.19. Average particle sizes measured during the operation of R4.

Seed sludge containing rigid, spherical, compact and light brown-yellow granules with the average size of 4.14±1.02 mm, showed no filamentous growth. Due to the 21 days of start-up period with the operation at a low OLR of 0.75 g sCOD/Lday, the average granule size was smaller such as 3.68±1.32 mm at the beginning of the operational period (Day 1, Figure 4.19, Figure 4.20a, Figure 4.20b).

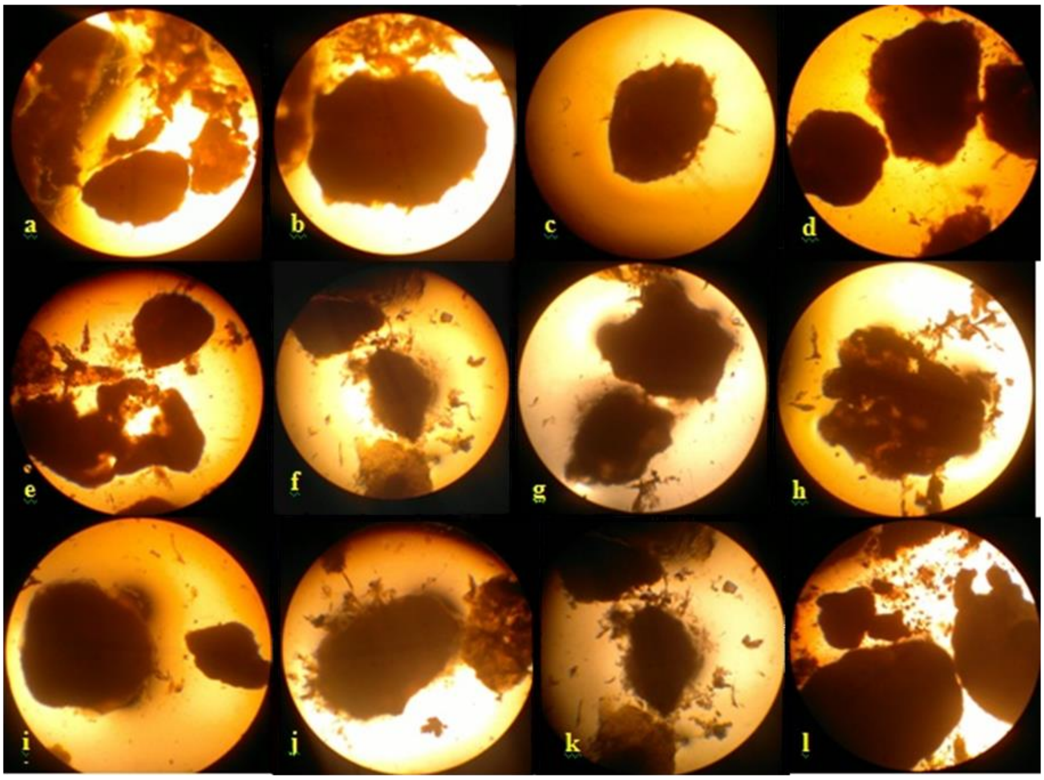

Figure 4.20. Microscopic photographs of aerobic granular sludge during the operational period (6.3 x 4 magnification): Part I: Application of the anaerobically-pretreated effluent: a,b)Day 1; c) Day 15; d,e) Day 22; f,g) Day 31. Part II: Application of the sugar beet processing wastewater: h,i) Day 45; j,k) Day 52; l) Day 54.

The average granule size decreased during the operation with the application of anaerobically-pretreated effluent. Small, grizzled, dark-brown granules about 2.96±0.93 mm were observed on Day 15 (Figure 4.20c, Figure 4.19). The average granule size decreased to 2.72± 0.81 mm on Day 22 (Figure 4.20d, Figure 4.20e, Figure 4.19). At the end of Part I (anaerobically-pretreated effluent application), the average granule size was recorded as 2.08±0.74 mm (Day 32, Figure 4.19). In Part I (Days 1-32) granules had little filamentous growth and were observed to lose their sphericity at the end of the anaerobically-pretreated effluent application (Figure 4.20f, 4.20g). Nevertheless, the decrease in the granular size did not cause operational difficulties; the optimum granular size providing practical and economical SBR operation including large scale ones was determined to be 1-3 mm (Toh et al., 2003). The granular features such as settleability, density and intensity were reported to be better for the granules smaller than 4 mm (Toh et al., 2003).

Moving on to Part II, the application of sugar beet processing wastewater with high pollution load enlarged the granule size to 2.46±1.02 mm by Day 45 (Figure 4.20h, 4.20i; Figure 4.19) and later to 2.89±1.1 mm by Day 52 (Figure 4.20j, 4.20k; Figure 4.19). This was attributed to the increase in OLR from 0.18±0.10 g sCOD/Lday (Part I) to 6.11±0.37 g sCOD/Lday (Part II), due to the increase in influent COD concentrations. Filamentous growth was slightly present. At the end of the whole operation (Day 52), the reactor was observed to contain large spherical dark brown-black granules which ressembled pebble-stones and some smaller granules that were lighter in colour. The final reactor content had 2.94±1.14 mm granules on the average with explicit filamentous growth (Figure 4.20j-k-l, Figure 4.19).

Sludge settleability is indicated by high settling velocity and low SVI values. The granules had settling velocities (68-74 m/h, Table 4.14) comparable to the literature data, reported for the granules as 18-90 m/h (and up to 130 m/h) (Gao et al., 2011). It can be seen in Table 4.14 that the SVI values (25-37 mL/g for SVI_{30} and 35-40 ml/g for SVI_5) are also comparable to the literature, where the aerobic granules are reported to have 20-80 mL/g SVI, with a typical of 50 mL/g (Liu and Tay, 2004; Gao et al., 2011).

As the system shifted from anaerobically-pretreated effluent to sugar beet processing wastewater, the influent COD/TAN ratio, influent sCOD concentration and the OLR increased. Although the SVI_{30} decreased from 37±0.4 mL/g to 25±1.4 mL/g with the shift from Part I (Days 1-32) to Part II (Days 33-54), the settling velocity of individual granules dropped from 74±12 m/h to 68±6 m/h. Furthermore the granulation percents, which were computed by [100 x (SVI_{30}/ SVI_5)], decreased from 93% in Part I (Days 1-32) to 71% in Part II (Days 33-54). This might be attributed to the deteriorated sludge settleability, due to the enriched filaments and poor settleable microorganisms and/or development of flocculent biomass in the sludge. Filamentous microorganism growth is expected with increasing OLR values (Zheng et al., 2006). Similarly, in the study of Morgenroth et al. (1997), the settling velocities of aerobic granules dropped from 38.8 to 33.1 m/h, as the granular structure became more filamentous. Nevertheless, it can be stated that the aerobic granules at both Part I and Part II had good settleability, for the values of both SVI and settling velocity were comparable to those of the granules given in the literature, as previously mentioned.

Table 4.15 shows the EPS concentrations of the granular sludge in R4 measured during the operational period. From Part I to Part II, the PN content in granular EPS increased from 186±60 mg/gVSS to 250±25 mg/gVSS, while the PS content increased from 183±45 mg/gVSS to 211±26 mg/gVSS (Table 4.15).

Table 4.15. EPS content of the granules in R4 through the operational period.

Day	WW type	EPS (mg/g VSS)		
		PN	PS	PS/PN
1-32	Anaerobically-pretreated effluent	186±60	183±45	0.98
33-54	Sugar beet processing ww	250±25	211±26	0.84

During the granulation process increase in both PS and PN components of EPS was expected (Adav et al., 2008b). The changes in PS and PN contents of the granules might have been mainly due to the increase in influent sCOD/TAN ratios with the shift from anaerobically-pretreated effluent (sCOD/TAN ratio: 3.62±1.1) to raw sugar beet processing wastewater (sCOD/TAN ratio: 63.4±7). The PS component of EPS was likely to increase via increasing influent COD/N ratio, and provide higher bacterial attachement to solid surfaces (Wu et al., 2012). Generally the PN concentrations were slightly higher than that of the PS, which was claimed to be an essential feature for aerobic granules (McSwain et al., 2005). Increasing PN content influences the relative hydrophobicity of the granules and this may support aerobic granulation (Zhang et al., 2007). The increase in both PS and PN concentrations were attributed to the formation of new aerobic granules. Although both PS and PN concentrations increased, the PN increase was higher than that of the PS, hence the PS/PN ratio decreased. The PS/PN ratios were calculated as 0.98 and 0.84 for Part I (Days 1-32) and Part II (Days 33-54), respectively. The PS/PN values were higher than 0.6, which was the threshold value below which the stability problems were observed in this study.

Therefore, moving on from Part I to Part II, considering the increasing PS and PN concentrations and decreasing PS/PN ratio, formation of new granules was likely while less dense flocculent biomass might have been also developed in the reactor. This may explain the lower granular settling velocities obtained in Part II despite of the lower SVI values. Considering the decrease in granulation percent from 93% to 71% with the shift from Part I to Part II, the increase in EPS contents (both in PN and PS) may be attributed to the enrichment of flocculent and filamentous biomass in the reactor. Xuan et al. (2012) also reported that the flocculent sludge secreted higher EPS compared to the granular sludge under similar environmental stress.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The summary of the results and outcomes of this thesis study are given below;

In Set-1 the increasing influent COD/TAN ratios from 7.5 to 30, and in turn the increasing OLR from 3 to 12 g COD/Lday, provided

- Large (1.5-2.5 mm), loose and light-brown/white granules containing large amount of flocs and filaments,
- High COD treatment ability (over 90%), but gradually decreasing TAN removal efficiency (from 92% to 33%).
- The relative abundancy of the heterotrophic bacteria in the aerobic granular sludge was thought to increase, while the relative abundancy of the nitrification bacteria decreased with the increasing influent COD/TAN ratios.
- The aerobic granules dominant in heterotrophic bacteria showed fast-growth but were tend to have stability problems since the changes in the granular structure were observed faster.
- Especially at high influent COD/TAN ratios such as 10-30, and in turn high OLRs such as 4-12 g COD/Lday, granular structure may be deteriorated resulting in white floccy sludge due to the inhibition of nitrifiers, unless the ammonia toxicity is avoided by pH maintenance.
- Nevertheless, the improvement of reactor operation (such as adjustment of the SBR settling time) may provide compact, rigid granules with high COD removal efficiency (over 90%); even at high influent COD/TAN ratios such as 30.
- At high influent COD/TAN ratios aerobic granules are speculated to be dominant in heterotrophic bacteria. Aerobic heterotrophic bacteria dominancy in granules bring the ease of adaptation to operational changes. Unfortunately, this may bring the ease of structural distruption and granular

disintegration. Consequently, stability of structure and treatment efficiency may be challenging to achieve, if the aerobic heterotrophs are dominant.

In Set-1 the decreasing influent COD/TAN ratios from 7.5 to 1, and in turn the increasing OLR from 3 to 0.4 g COD/Lday, provided

- Small (0.5-2.3 mm), orange-brown granules ressembling pebble-stones.
- High TAN treatment ability (up to 99%) but gradually decreasing COD treatment efficiency (from 79 to 63%).
- The relative abundancy of the nitrification bacteria in the aerobic granular sludge was thought to increase, while the relative abundancy of the heterotrophic bacteria decreased with decreasing influent COD/TAN ratios, (in contrast to that of the increasing COD/TAN ratios)
- At low influent COD/TAN ratios (1-5) the granular structure becomes dominant in terms of nitrification bacteria, thus granular size and growth rate were controlled by slow-growing nitrifiers. The changes in granular structure were observed slower thus more stable granules were provided; however the system was more sensitive to the changes in operational conditions.

In Set-2, the operation with increasing influent OLR and NLR values from 0.75 to 12 g COD/Lday and 0.1 to 1.6 g TAN/Lday, respectively, at a constant influent COD/TAN ratio of 7.5, resulted in

- High COD treatment efficiency (85-96%) at the OLR values between 0.75 to 6 g COD/Lday and NLR values between 0.1 to 0.8 g TAN/Lday. When the OLR was further increased to 12 g COD/Lday, and the NLR to 1.6 g TAN/Lday, the COD removal efficiency decreased to 81% and high amounts of filamentous growth was present.
- Decreasing TAN removal efficiency trend (from 87% to 45-51%) at the NLR values from 0.1 to 1.6 g TAN/Lday, and the corresponding OLRs from 0.75 to 12 g COD/Lday (decreasing TAN removal efficiency was noted especially above 3 g COD/Lday OLR and 0.4 g TAN/Lday NLR).
- The optimum OLR and NLR values were determined to be 1.5 g COD/Lday and 0.2 g TAN/Lday, respectively, regarding the provision of the highest COD (85%), TAN (87%) and TN (55%) removal efficiencies as well as the

formation of granules with high stability. Both high COD and TAN removal efficiencies were attributed to the formation of efficient granule sizes with suitable anoxic and aerobic volumes at the optimum loading rates. Thus appropriate amounts of aerobic/anoxic heterotrophic and nitrification bacteria were provided to obtain high removal efficiencies.

In Set-3**;**

- It was seen that the aerobic granular SBRs were applicable for the treatment of both sugar beet processing wastewater and anaerobically digested processing wastewater.
- The operation with the effluent of anaerobic digester treating sugar beet processing wastewater achieved average removal efficiencies of 71±30% tCOD, 90±3% TAN, 76±24% sCOD and 29±4% TSS.
- Sugar beet processing wastewater with high solids content (2255 ± 250 mg/L TSS) was treated with 65±5% tCOD, 61±4%TAN, 87±1% sCOD and 58±10% TSS removal efficiencies.
- It was also revealed that the application of raw sugar beet processing wastewater slightly changed the aerobic granular sludge properties such as size, structure, colour, settleability and EPS content, without any drastic and negative effect on treatment performance.

5.2 Conclusion

Considering the whole data of Set-1, it is obvious that the influent COD/TAN ratio of 7.5 provided both high COD (75%) and TAN (90%) removal efficiencies; and maintenance of granular stability. The operation at higher ratios (10, 20 and 30) is suitable when the COD removal from wastewater is mainly aimed, while the operation at lower ratios (5, 3.5, 2 and 1) is suitable when the TAN removal is mainly aimed.

Set-1 revealed that the bacterial population dominancy among nitrifiers and heterotrophic denitrifiers can be adjusted by the variation of influent COD/TAN ratios. In other words, the influent COD/TAN ratio affects the aerobic granular sludge composition in terms of bacterial distribution among nitrifiers and aerobic heterotrophic denitrifiers.

Both Set-1 and Set- 2 revealed that the COD/TAN ratio and OLR and NLR values of the influent wastewater affected the relative abundancy of the microbial populations contributing to the aerobic granular structure. Thus it can be concluded that the influent COD/TAN ratio, OLR and NLR determined the COD and TN removal efficiencies. In order to obtain high TN removal efficiency, the influent wastewater should have appropriate COD/TAN ratio, OLR and NLR values which will promote both nitrifying and denitrifying species.

In Set-3, high sCOD treatment efficiency (87±1%) and relatively low tCOD treatment efficiency (71±30%) were obtained for sugar beet processing wastewater. TAN removal efficiencies for anaerobically-pretreated effluent and raw sugar beet processing wastewater (both containing 33-38 mg/L TAN) were 90±3% and 61±4%, respectively. Lower TAN treatment efficiency for sugar beet processing wastewater was attributed to its higher sCOD/TAN ratio of 63.4±7 (Table 4.10), which was likely to decrease the amount of nitrification bacteria in the granular structure.

The change in the wastewater type from anaerobically digested sugar beet processing wastewater to the raw processing wastewater slightly changed the structure and PN / PS content of the granules. Despite the increase in filamentous growth around the granules, the stable treatment efficiency and settling quality of the granules remained intact.

5.3 Recommendations

- The operation with the high COD/TAN ratio wastewaters are speculated to cause decreasing relative abundancy of nitrifiers in the aerobic granular structure. During the treatment of such wastewaters, the influent TAN concentration and the reactor pH values should be low enough to avoid ammonia toxicity.
- The operation with the low COD/TAN ratio wastewaters are speculated to cause increasing relative abundancy of nitrifiers in the aerobic granular structure. During the treatment of such wastewaters, the parameters (such as

settling time and VER) exerting the hydraulic selection pressure should be selected regarding the slow-growing nitrification bacteria.

- It is recommended that a sedimentation tank should be installed following the granular system in the large scale treatment plants for the accomplishment of higher solids removal from wastewaters of high solid contents such as sugar beet processing wastewater.

In order to improve the TN removal efficiency, a balanced distribution of heterotrophic bacteria and nitrifiers in the aerobic granular structure is required to promote SNDN. To this purpose,

- The influent COD/TAN ratios, OLR and NLR values that support the growth of both denitrification and nitrification bacteria should be chosen.
- DO concentrations should be low (<3 mg/L) enough to provide the dominancy of heterotrophic denitrification bacteria over the aerobic heterotrophic bacteria.
- When the granulation percent of the sludge exceeds 90%, the anoxic period can be excluded from the SBR cycle and the operation of the SBR could be operated at low DO concentrations (<3 mg/L).

REFERENCES

Abdullah N., Ujang Z., Yahya A., 2011. Aerobic granular sludge formation for high strength agro-based wastewater treatment. Bioresource Technology., 102, pp. 6778–6781.

Adav SS, Lee DJ, Ren NQ. 2007a. Biodegradation of pyridine using aerobic granules in the presence of phenol. Water Res, 41, 2903–2910.

Adav SS, Lee DJ, Tay JH. 2007b. Activity and structure of stored aerobic granules. Environ Technol, 28, 1227–1235.

Adav SS, Lee DJ., 2008. Extraction of extracellular polymeric substances from aerobic granule with compact interior structure, J Hazard. Mat., 154, 1120-1126.

Adav, S. S., Lee, D. J., & Lai, J. Y. 2009a. Functional consortium from aerobic granules under high organic loading rates. Bioresource technology, 100(14), 3465-3470.

Adav, S. S., Lee, D. J., & Lai, J. Y. 2009b. Aerobic granulation in sequencing batch reactors at different settling times. Bioresource technology, 100(21), 5359-5361.

Adav, S. S., Lee, D. J., & Lai, J. Y. 2010. Potential cause of aerobic granular sludge breakdown at high organic loading rates. Applied microbiology and biotechnology, 85(5), 1601-1610.

Adav, S.S., Lee, D.J., Lai, J.Y., 2009c. Biological nitrification-denitrification with alternating oxic and anoxic operations using aerobic granules, Appl Microbiol Biotechnol, 84, 1181-1189.

Adav, S.S., Lee, D.J., Show, K.Y., Tay, J.H., 2008a. Aerobic granular sludge: Recent advances, Biotechnology Advances 26, 411–423.

Adav, S.S., Lee, D.J., Tay, J.H. 2008b. Extracellular polymeric substances and structural stability of aerobic granule. Water Research, 42, 1644 – 1650.

Aliplik Akın, B. 2010. Investigation of treatment of the sugar industrial wastewatersby chemical coagulation method and determination of optimum operating conditions (Master's thesis). Retrieved from: acikarsiv.ankara.edu.tr/browse/24395/Banu%20ALPLİK%20AKIN.pdf

Alkaya, E., & Demirer, G. N. 2011. Anaerobic mesophilic co-digestion of sugar-beet processing wastewater and beet-pulp in batch reactors. Renewable Energy, 36(3), 971-975.

Allsop PJ, Chisti Y, Moo-Young M, Sullivan GR. 1993. Dynamics of phenol degradation by Pseudomonas putida. Biotechnol Bioeng ;41:572– 80.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Stinath, E. G., 1976. Inhibition of nitrification by ammonia and nitrous-acid. J. Water Pollut. Control Fed., 48 (5), 835–852.

APHA, AWWA and WEF. 2005. Standard Methods for the Examination of Water and Wastewater. 3rd Edition, Washington, DC.

Arrojo B., Mosquera-Corral A., Garrido J.M., Mendez R., 2004. Aerobic granulation with industrial wastewater in sequencing batch reactors. Water Res. 38, 3389–3399.

Azeredo, J., Lazarova, V., & Oliveira, R. 1999. Methods to extract the exopolymeric matrix from biofilms: a comparative study. Water Science and Technology, 39(7), 243-250.

Bao, R., Yu, S., Shi, W., Zhang, X., & Wang, Y. 2009. Aerobic granules formation and nutrients removal characteristics in sequencing batch airlift reactor (SBAR) at low temperature. Journal of hazardous materials, 168(2), 1334-1340.

Bassin, J. P., Kleerebezem, R., Dezotti, M., & Van Loosdrecht, M. C. M. 2012. Simultaneous nitrogen and phosphate removal in aerobic granular sludge reactors operated at different temperatures. water research, 46(12), 3805-3816.

Batstone DJ, Keller J. 2001. Variation of bulk properties of anaerobic granules with wastewater type. Water Res 35:1723– 1729.

Beun J.J., van Loosdrecht M.C.M. , Heijnen J.J. 2000. Aerobic granulation. Water Sci. Technol., 41, pp. 41–48.

Beun J.J., van Loosdrecht M.C.M., Heijnen J.J., 2002. Aerobic granulation in a sequencing batch airlift reactor. Wat. Res., 36, 702-712.

Beun JJ, Heijnen JJ, van Loosdrecht MCM. 2001. N-removal in a granular sludge sequencing batch airlift reactor. Biotechnol Bioeng;75:82–92.

Beun, J.J., Hendriks, A., van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J. 1999. Aerobic granulation in a sequencing batch reactor. Water Research, 33, 2283– 2290.

Bossier P, Verstraete W. 1996. Triggers for microbial aggregation in activated sludge. Appl Microbiol Biotechnol; 45:1–6.

Carucci A, Milia S, De Gioannis G, Piredda M. 2009. Acetate-fed aerobic granular sludge for the degradation of 4-chlorophenol. J Hazard Mater, 166, 483–490.

Carvalho, G., Lemos, P. C., Oehmen, A., & Reis, M. A. 2007. Denitrifying phosphorus removal: linking the process performance with the microbial community structure. Water Research, 41(19), 4383-4396.

Cassidy, DP, Belia E, 2005. Nitrogen and phosphorus removal from an abattoir wastewater in SBR with aerobic granular sludge. Water Research 39 (19), 4817-4823.

Chen G, Strevett KA. 2003. Impact of carbon and nitrogen conditions on E. coli surface thermodynamics. Colloids Surf B: Biointerfaces;28:135–46.

Chen, Y., Jiang, W., Liang, D. T., & Tay, J. H. 2008. Biodegradation and kinetics of aerobic granules under high organic loading rates in sequencing batch reactor. Applied microbiology and biotechnology, 79(2), 301-308.

Chudoba, J., 1985. Control of activated sludge filamentous bulking VI Formulation of basic principle. Water Res, 19, 1017– 1022.

Cydzik-Kwiatkowska A., Wojnowska-Baryla I., 2011. Nitrifying granules cultivation in a sequencing batch reactor at a low organics-to-total nitrogen ratio in wastewater, Folia Microbiologica, 56(3), 201 – 208.

de Bruin LMM, Kreuk MK, de Roest HFR, van der Uijterlinde C, van Loosdrecht MCM. 2004. Aerobic granular sludge technology: alternative for activated sludge? Water Sci Technol;49:1–7.

de Kreuk M.K., van Loosdrecht, M.C.M., 2006. Formation of Aerobic Granules with Domestic Sewage, Journal of Environmental Engineering 132(6) 694-697.

de Kreuk MK, Kishida N, van Loosdrecht MCM. 2007. Aerobic granular sludge – state of the art. Water Sci Technol, 155, 79–81.

de Kreuk MK, Picioreanu C, Hosseini M, Xavier JB, van Loosdrecht MCM. 2006. Kinetic model of granular sludge SBR: influences on nutrient removal. Biotechnol Bioeng, 97, 801–815.

de Kreuk MK, Pronk M, van Loosdrecht MCM. 2005a. Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures. Water Res, 39, 4476–4484.

de Kreuk MK, van Loosdrecht MCM. 2004. Selection of slow growing organisms as a means for improving aerobic granular sludge stability. Water Sci Technol 2004;49:9–17.

de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M.. 2005b. Simultaneous COD, Nitrogen, Phosphate Removal By Aerobic Granular Sludge, Biotechnology and Bioengineering, 90(6), 761-769.

de Villiers GH, Ibala HM. 2003. Aerobic granulation in a sequencing batch reactor with a petrochemical effluent as substrate. In: IWA, editor. Water, a key to sustainable development in Africa. IWA: Cape Town.

di Iaconi C, Ramadori R, Lopez A, Passino R. 2007. Aerobic Granular sludge systems: The new generation of wastewater treatment technologies. Ind Eng Chem Res, 46, 6661–6665.

Dignac, M.F., Urbain, V., Rybacki, D., Bruchet, A., Snidaro, D., Scribe, P., 1998. Chemical description of extracellular polymers: Implication on activated sludge floc structure. Water Sci and Technol, 38(8-9), 45-53.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350-356.

Dulekgurgen E, Ovez S, Artan N. 2003. Enhanced biological phosphate removal by granular sludge in a sequencing batchreactor. Biotechnol Lett, 25, 687–693.

Dulekgurgen E, Yilmaz M, Wilderer PA. 2008. Shape and surface topology of anaerobic/aerobic granules influenced by shearing conditions.4th IWA Specialized Conference on Sequencing Batch Reactor Technology, Roma, Italy, 7–10 April 2008, 311–320.

Durmaz, B., 2001. Effect of carbon to nitrogen ratio on the composition of microbial extracellular polymers in activated sludge. Master's Thesis, Middle East Technical University.

Environmental Health and Safety Guidelines for Sugar Manufacturing. 2007. World Bank Group Environmental Health and Safety Guidelines, The World Bank Group,Washington, DC.

Erguder T.H., Demirer G.N. 2005a. Investigation of granulation of a mixture of suspended anaerobic and aerobic cultures under alternating anaerobic / microaerobic / aerobic conditions, Proc. Biochem., 40 (12), 3732-3741.

Erguder T.H., Demirer G.N. 2005b. Granulation of a mixture of suspended anaerobic and aerobic cultures under alternating anaerobic / microaerobic / aerobic conditions: A preliminary study. J. Chem. Technol. Biotechnol., 80 (7), 837-842.

Ersan, Y. Ç., & Erguder, T. H. 2013. The effects of aerobic/anoxic period sequence on aerobic granulation and COD/N treatment efficiency. Bioresource technology, 148, 149-156.

Ersan, Y.Ç, 2013. The effects of seed sludge type and anoxic/aerobic period sequence on aerobic granulation and COD, N treatment performance. Master's Thesis, Middle East Technical University.

Etchebehere C, Cabezas A, Dabert P, Muxi L. 2003. Evolution of the bacterial community during granules formation in denitrifying reactors followed by molecular, culture-independent techniques. Water Sci Technol 48:75–79.

Etterer, T., Wilderer, P.A., 2001, Generation and properties of aerobic granular sludge. Water Sci Technol 43, 19– 26.

Fang F, Ni BJ, Li XY, Sheng GP, Yu HQ. 2009. Kinetic analysis on the two-step processes of AOB and NOB in aerobic nitrifying granules. Appl Microbial Biotechnol., 83: 1159-1169.

Fang HHP, Liu H, Zhang T. 2002. Characterization of a hydrogen producing granular sludge. Biotechnol Bioeng 78:44–52.

Farhadian, M., Borghei, M., & Umrania, V. V. 2007. Treatment of beet sugar wastewater by UAFB bioprocess. Bioresource technology, 98(16), 3080-3083.

Figueroa M, Mosquera Corral A, Campos JL, Méndez R. 2008. Treatment of saline wastewater in SBR aerobic granular reactors. Water Sci Technol, 58, 479–485.

Figueroa M, Val del Rio A., Campos JL., Mosquera-Corral A., Mendez R., 2011. Treatment of high loaded swine slurry in an aerobic granular reactor. Wat Sci Tech., 63(9), 1808-1814.

Fukuzaki S, Nishio N, Nagai S. 1995. High rate performance and characterisation of granular methanogenic sludges in upflow anaerobic sludge blanket reactors fed with various defined substrates. J Ferment Bioeng 79:354–359.

Gao D, Liu L, Liang H., Wu WM., 2011. Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment, Critical Reviews in Biotech., 31 (2), 137-152.

Giesen, A., Niermans, R., van Loosdrecht, M.C.M., 2012. Aerobic granular biomass: the new standard for domestic and industrial wastewater treatment?, Water21 April: 28-30.

Gilda C, Paulo C, Adrian O, Maria AM. 2007. Denitrifying phosphorus removal: Linking the process performance with the microbial community structure. Water Res, 41, 4383–4396.

Gobi K, Mashitah MD, Vadivelu VM. 2011. Development and utilization of aerobic granules for the palm oil mill (POM). Chemical Engineering Journal 174, 213– 220.

Güven, G., Perendeci, A., & Tanyolaç, A. 2009. Electrochemical treatment of simulated beet sugar factory wastewater. Chemical Engineering Journal, 151(1), 149-159.

Hach Water Analysis Handbook. 2012. Hach Company, Colorado, U.S.A.

Hailei W, Guangli Y, Guosheng L, Feng P. 2006. A new way to cultivate aerobic granules in the process of papermaking wastewater treatment. Biochemical Engineering Journal 28, 99–103.

Hamoda, M. F., & Kennedy, K. J. 1986. Anaerobic treatment of beet waste in a stationary fixed-film reactor. Agricultural wastes, 17(3), 175-187.

Hampannavar, U. S., & Shivayogimath, C. B. 2010. Anaerobic treatment of sugar industry wastewater by Upflow anaerobic sludge blanket reactor at ambient temperature. International Journal of Environmental Sciences, 1(4), 631-639.

Harper WF, Bernhardt M., Newfield C., 2006. Membrane bioreactor biomass characteristics and microbial yield at very low mean cell residence time. Water SA, 32(2), 193-198.

Heijnen JJ, van Loosdrecht MCM. 1998. Method for acquiring grain-shaped growth of a microorganism in a reactor. European patent EP0826639.

Ho KL, Chen YY, Lin B, Lee DJ. 2010. Degrading high-strength phenol using aerobic granular sludge. Appl Microbiol Biotechnol, 85, 2009–2015.

Hu L., Wang J., Wen X., Qian Y. 2005a. The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules, Process Biochem., 40(1), 5–11.

Hvitved-Jacobsen, T., Vollertsen, J., & Nielsen, A. H. 2013. Sewer processes: microbial and chemical process engineering of sewer networks. CRC press.

Jang A, Yoon YH, Kim IS, Kim KS, Bishop PL. 2003. Characterization and evaluation of aerobic granules in sequencing batch reactor. J Biotechnol;105:71–82.

Jiang HL, Tay JH, Liu Y, Tay STL. 2003. Ca2+ augmentation for enhancement of aerobically grown microbial granules in sludge blanket reactors. Biotechnol Lett;25:95.

Jiang HL, Tay JH, Tay STL. 2002. Aggregation of immobilized activated sludge cells into aerobically grown microbial granules for the aerobic biodegradation of phenol. Lett Appl Microbiol;35:439– 45.

Jiang HL, Tay JH, Tay STL. 2004. Changes in structure, activity and metabolism of aerobic granules as a microbial response to high phenol loading. Appl Microbiol Biotechnol;63:602– 8.

Juang, Y.C., Adav, S.S., Lee, D.J., Tay, J.H., 2010. Stable aerobic granules for continuous flow reactors: Precipitating calcium and iron salts in granular interiors, Bioresource Technology, 101(21), 8051 – 8057.

Kim, I. S., Kim, S. M., & Jang, A. 2008. Characterization of aerobic granules by microbial density at different COD loading rates. Bioresource technology, 99(1), 18-25.

Kishida, K., Tsuneda, S., Kim, J.H., Sudo, R., 2009. Simultaneous nitrogen and phosphorus removal from high-strength industrial wastewater using aerobic granular sludge. J. Environ. Eng. 135, 153–158.

Kishida, N. Kim, J., Tsuneda, S., Sudo, R. 2006. Anaerobic/oxic/anoxic granular sludge process as an effective nutrient removal process utilizing denitrifying polyphosphate-accumulating organisms. Water Res 40:2303–2310.

Kong, Y., Liu, Y.Q., Tay, J.H., Wong, F.S., Zhu, J., 2009. Aerobic granulation in sequencing batch reactors with different reactor height/diameter ratios, Enzyme Microb. Tecnol., 45(5) 379 – 383.

Lemaire R, Webb RI., Yuan Z, 2008. Micro-scale observations of the structure of aerobic microbial granules used for the treatment of nutrient-rich industrial wastewater. ISME J, 2, 528–541.

Lens P.N., Depoorter MPD, Cronenberg CC, Verstraete WH., 1995. Sulfate reducing and methane producing bacteria in aerobic wastewater treatment systems. Water Res., 29:871–80.

Lettinga G., van Velsen A.F.M., Hobma S.W., De Zeeuw W., Klapwijk A., 1980. Use of upflow sludge blanket (USB) concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnol. Bioeng., 22, 699-734.

Li ZH, Kuba T, Kusuda T. 2006b. The influence of starvation phase on the properties and the development of aerobic granules. Enzyme Microb Technol, 38, 670–674.

Li, A. J., Yang, S. F., Li, X. Y., & Gu, J. D. 2008a. Microbial population dynamics during aerobic sludge granulation at different organic loading rates. Water Research, 42(13), 3552-3560.

Li, L.Y., Peng, Y.Z., Wang, S.Y., Wu, L., Ma, Y., Takigawa, A., Li D., 2011, Formation and characteristics of nitritation granules cultivated in sequencing batch reactor by stepwise increase of N/C ratio. Water Science and Technology, 64(7), 1479 – 1487.

Li, X. F., Li, Y. J., Liu, H., Hua, Z. Z., Du, G. C., & Chen, J. 2008c. Correlation between extracellular polymeric substances and aerobic biogranulation in membrane bioreactor. Separation and Purification Technology, 59(1), 26-33.

Li, X. M., Liu, Q. Q., Yang, Q., Guo, L., Zeng, G. M., Hu, J. M., & Zheng, W. 2009. Enhanced aerobic sludge granulation in sequencing batch reactor by Mg^{2+} augmentation. Bioresource technology, 100(1), 64-67.

Li, Y., & Liu, Y. 2005. Diffusion of substrate and oxygen in aerobic granule. Biochemical engineering journal, 27(1), 45-52.

Li, Y., Liu, Y., Xu, H., 2008b. Is sludge retention time a decisive factor for aerobic granulation in SBR?, Bioresource tech. 99, 7672 – 7677.

Li, Z. H., Kuba, T., & Kusuda, T. 2006a. Selective force and mature phase affect the stability of aerobic granule: An experimental study by applying different removal methods of sludge. Enzyme and microbial technology, 39(5), 976-981.

Lin YM, Liu Y, Tay JH. 2003. Development and characteristics of phosphorous-accumulating granules in sequencing batch reactor. Appl Microbiol Biotechnol ;62:430–5.

Lin,Y.M. 2003. Development of P-accumulating microbial granules in SBR. Interim PhD report, Nanyang Technological University, Singapore.

Liu L, Sheng GP, Li WW, Tong ZH, Zeng RJ, Liu JX, Xie J, Peng SC, Yu HQ. 2011a. Cultivation of aerobic granular sludge with a mixed wastewater rich in toxic organics. Biochemical Engineering Journal 57, 7-12.

Liu Y, Liu QS, Qin L, Tay JH. 2004b. Comments on “effect of extended idle conditions on structure and activity of granular activated sludge” by Zhu and Wilderer. Water Res, 38, 3465–3466.

Liu Y, Wang F, Xia SQ, Zhao JF. 2008. Study of 4-t-octylphenol degradation and microbial community in granular sludge. J Environ Sci, 20, 167–171.

Liu Y, Xu HL, Yang SF, Tay JH. 2003f. A general model for biosorption of Cd^{2+}, Cu^{2+} and Zn^{2+} by aerobic granules. J Biotechnol;102:233 –9.

Liu Y, Yang SF, Liu QS, Tay JH. 2003c. The role of cell hydrophobicity in the formation of aerobic granules. Curr Microbiol;46:270– 4.

Liu Y, Yang SF, Tan SF, Lin YM, Tay JH. 2002. Aerobic granules: a novel zinc biosorbent. Lett Appl Microbiol; 35:548–51.

Liu Y, Yang SF, Xu H, Woon KH, Lin YM, Tay JH. 2003e. Biosorption kinetics of cadmium (II) on aerobic granular sludge. Process Biochem;38:995– 9.

Liu Y., Tay J.H., 2002. The essential role of hydrodynamic shear force on the formation of biofilm and granular sludge., Wat. Res., 36, 1653-1665.

Liu Y., Tay J.H., 2004. State of art of biogranulation technology for wastewater treatment. Biotech. Adv., 22, 533-563.

Liu Y., Xu H.L., Yang S.F., Tay J.H., 2003d. Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor. Wat. Res., Vol 37. 661-673.

Liu Y., Yang S.F., Tay J.H. 2003b. Elemental compositions and characteristics of aerobic granules cultivated at different substrate N/C ratios, Appl Microbiol Biotechnol 61:556–561.

Liu YQ, Moy B, Kong YH, Tay JH. 2010a. Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment. Enzyme Microbial Tech., 46, 520–525.

Liu YQ, Moy BYP, Tay JH. 2007b. COD removal and nitrification of low-strength domestic wastewater in aerobic granular sludge sequencing batch reactors. Enzyme Microb Technol, 42, 23–28.

Liu YQ, Tay JH. 2006. Variable aeration in sequencing batch reactor with aerobic granular sludge. J Biotechnol. 124, 338–46.

Liu YQ, Tay JH. 2007a. Characteristics and stability of aerobic granules cultivated with different starvation time. Appl Microbiol Biotechnol, 75, 205–210.

Liu YQ, Tay JH. 2008a. Influence of starvation time on formation and stability of aerobic granules in sequencing batch reactors. Bioresour Technol, 99, 980–985.

Liu, H., Fang, H.H.P. 2002. Extraction of extracellular polymeric substances (EPS) of sludges. J Biotechnol 95:249–256.

Liu, L., Gao, D. W., Zhang, M., Fu, Y. 2010b. Comparison of Ca^{2+} and Mg^{2+} enhancing aerobic granulation in SBR. Journal of hazardous materials, 181(1), 382-387.

Liu, Q. S., Liu, Y., Tay, S. T. L., Show, K. Y., Ivanov, V., Benjamin, M., & Tay, J. H. 2005c. Startup of pilot-scale aerobic granular sludge reactor by stored granules. Environmental technology, 26(12), 1363-1370.

Liu, Q. S., Tay, J. H., & Liu, Y. 2003a. Substrate concentration-independent aerobic granulation in sequential aerobic sludge blanket reactor. Environmental technology, 24(10), 1235-1242.

Liu, Y. Q., & Tay, J. H. 2007b. Influence of cycle time on kinetic behaviors of steady-state aerobic granules in sequencing batch reactors. Enzyme and microbial technology, 41(4), 516-522.

Liu, Y., & Liu, Q. S. 2006. Causes and control of filamentous growth in aerobic granular sludge sequencing batch reactors. Biotechnology Advances, 24(1), 115-127.

Liu, Y., Wang, Z. W., & Tay, J. H. 2005b. A unified theory for upscaling aerobic granular sludge sequencing batch reactors. Biotechnology advances, 23(5), 335-344.

Liu, Y., Wang, Z. W., Qin, L., Liu, Y. Q., & Tay, J. H. 2005a. Selection pressure-driven aerobic granulation in a sequencing batch reactor. Applied microbiology and biotechnology, 67(1), 26-32.

Liu, Y.Q., Liu Y., Tay J.H., 2004a. The effects of extracellular polymeric substances on the formation and stability of biogranules. Appl Microbiol Biotechnol 65: 143–148.

Liu, Y.Q., Wu, W.W., Tay, J.H., Wang, J.L., 2007c. Starvation is not a prerequisite for the formation of aerobic granules, Appl Microbiol Biotechnol, 76(1), 211-216.

Lotito, A. M., De Sanctis, M., Di Iaconi, C., & Bergna, G. 2014. Textile wastewater treatment: Aerobic granular sludge vs activated sludge systems. Water research, 54, 337-346.

Lotito, A. M., Fratino, U., Mancini, A., Bergna, G., & Di Iaconi, C. 2012. Effective aerobic granular sludge treatment of a real dyeing textile wastewater. International Biodeterioration & Biodegradation, 69, 62-68.

Lowry OH, Rosebrough NJ, Farn a, Randall R. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, 265-275.

Luo, J., Hao, T., Wei, L., Mackey, H. R., Lin, Z., & Chen, G. H. 2014. Impact of Influent COD/N Ratio on Disintegration of Aerobic Granular Sludge. Water Research.

Martinez FO, Lema J, Mendez R, Cuervo-Lopez F, Gomez J. 2004. Role of exopolymeric protein on the settleability of nitrifying sludges. Bioresour Technol 94:43–48.

McSwain BS, Irvine RL, Wilderer PA. 2004b. The effect of intermittent feeding on aerobic granule structure. Water Sci Technol;49:19–25.

McSwain BS, Irvine RL. 2008. Dissolved oxygen as a key parameter to aerobic granule formation. Water Sci Technol, 58, 781–787.

McSwain BS, Irvine, RL, Hausner M. 2005. Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. Appl Environ Microbiol, 71, 1051–1057.

McSwain, B., Irvine, R., & Wilderer, P. 2004a. The influence of settling time on the formation of aerobic granules. Water Science & Technology, 50(10), 195-202.

Mishima, K, Nakamura M. 1991. Self-immobilization of aerobic activated sludge–a pilot study of the aerobic upflow sludge blanket process in municipal sewage treatment. Water Sci Technol,1;23: 981– 990.

Morgan JM, Forster CF, Evison L. 1990. A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. Water Res, 24, 743–750.

Morgenroth E., Sherden T., van Loosdrecht M.C.M., Heijnen J.J., Wilderer P.A., 1997. Aerobic granular sludge in a sequencing batch reactor. Wat. Res., 31(12), 3191-3194.

Mosquera-Corral A, de Kreuk MK, Heijnen JJ, van Loosdrecht MCM. 2005. Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor. Water Res;39: 2676–86.

Moussavi G, Barikbin B, Mahmoudi M. 2010. The removal of high concentrations of phenol from saline wastewater using aerobic granular SBR. Chem Eng J, 158, 498–504.

Moy, B. P., Tay, J. H., Toh, S. K., Liu, Y., & Tay, S. L. 2002. High organic loading influences the physical characteristics of aerobic sludge granules. Letters in Applied Microbiology, 34(6), 407-412.

Muda K, Aris A, Salim MR, Ibrahim Z, Yahya A, van Loosdrecht MCM , Ahmad A, Nawahwi MZ., 2010. Development of granular sludge for textile wastewater treatment. Wat Res., 44, 4341-4350.

Nancharaiah YV, Joshi HM, Mohan TVK, Venugopalan VP, Narasimhan SV. 2006. Aerobic granular biomass: a novel biomaterial for efficient uranium removal. Curr Sci;91:503–9.

Ng PH. 2002. Storage stability of aerobic granules cultivated in aerobic granular sludge blanket reactor. Final Year Report of Bachelor of Engineering, Nanyang Technological University, Singapore.

Ni, B. J., Yu, H. Q., & Sun, Y. J. 2008. Modeling simultaneous autotrophic and heterotrophic growth in aerobic granules. Water research, 42(6), 1583-1594.

Ni, B.J., Xie, W.M., Liu, S.G., Yu, H.Q., Wang, Y.Z, Wang, G., Dai, X.L., 2009. Granulation of activated sludge in a pilot scale sequencing batch reactor for the treatment of low-strength municipal wastewater, Water Research, 43, 751-761.

Nichols CAM, Garon S, Bowman JP, Raguenes G, Guezennec Jv. 2004. Production of exopolysaccharides by Antarctic marinevbacterial isolates. J Appl Microbiol 96:1057–1066.

Nielsen PH, Jahn A, Palmgren R. 1997. Conceptual model for production and composition of exopolymers in biofilms. Water Sci Technol 36:11–19.

Pan, S., 2003. Inoculation of microbial granular sludge under aerobic conditions. Ph.D. Thesis, Nanyang Technological University, Singapore.

Pan, S., Tay, J. H., He, Y. X., & Tay, S. L. 2004. The effect of hydraulic retention time on the stability of aerobically grown microbial granules. Letters in applied microbiology, 38(2), 158-163.

Peng D, Bernet N, Delgenes JP, Moletta R. 1999. Aerobic granular sludge—a case report. Water Res 33: 890–893.

Peyong, Y. N., Zhou, Y., Abdullah, A. Z., & Vadivelu, V. 2012. The effect of organic loading rates and nitrogenous compounds on the aerobic granules developed using low strength wastewater. Biochemical Engineering Journal, 67, 52-59.

Pijuan, M., Werner, U., Yuan, Z., 2009. Effect of long term anaerobic and intermittent anaerobic/aerobic starvation on aerobic granules. Water Research, 43, 3622 – 3632.

Punal A, Brauchi S, Rdyes JG, Chamy R. 2003. Dynamics of extracellular polymeric substances in UASB and EGSB reactors treating medium and low concentrated wastewaters. Water Sci Technol 48:41–49.

Qin L, Liu Y, Tay JH. 2004c. Effect of settling time on aerobic granulation in sequencing batch reactor. Biochem Eng J 21:47– 52.

Qin L., Tay J.H., Liu Y., 2004b. Selection pressure is a driving force of aerobic granulation in sequencing batch reactors. Proc. Biochem. Vol. 39, 579-584.

Qin, L, Liu Y., 2006. Aerobic granulation for organic carbon and nitrogen removal in alternating aerobic–anaerobic sequencing batch reactor, Chemosphere 63, 926–933.

Qin, L., Liu, Q.S., Yang, S.F., Tay, J.H., Liu, Y. 2004a. Stressful conditions-induced production of extracellular polysaccharides in aerobic granulation process. Civil Eng Res, 17, 49–51.

Ren, T. T., Liu, L., Sheng, G. P., Liu, X. W., Yu, H. Q., Zhang, M. C., & Zhu, J. R. 2008. Calcium spatial distribution in aerobic granules and its effects on granule structure, strength and bioactivity. Water research, 42(13), 3343-3352.

Richard, M.G. 1989. Activated sludge microbiology, Water Pollut Control Fed, Alexandria, VA.

Rosman, N. H., Nor Anuar, A., Chelliapan, S., Md Din, M. F., & Ujang, Z. 2014. Characteristics and performance of aerobic granular sludge treating rubber wastewater at different hydraulic retention time. Bioresource technology, 161, 155-161.

Sanin SL, Sanin FD, Bryers JD. 2003. Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. Process Biochem;38:909–14.

Sanin SL. 2003. Effect of starvation on resuscitation and the surface characteristics of bacteria. J Environ Sci Health A;38:1517–28.

Sawyer, C.N., Mc Carty, P.L., Parkin, G.F., 2003. Chemistry for Environmental Engineering and Science, 5th Ed, Mc Graw-Hill, 670-672.

Schmidt I, Sliekers O, Schmid M, Bock E, Fuerst J, Kuenen JG, Jetten MSM, Strous M., 2003. New concepts of microbial treatment processes for the nitrogen removal in wastewater, FEMS Microbiol Rev., 27(4), 481-492.

Schmidt JE, Ahring BK.1994. Extracellular polymers in granular sludge from different upflow anaerobic sludge blanket (UASB) reactors. Appl Microbiol Biotechnol 42:457–462.

Schmidt, J.E. ve Ahring, B.K., 1996. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. Biotechnol. Bioeng., Vol.49, 229-246.

Schwarzenbeck N, Erley R, Mc Swain BS, Wilderer PA, Irvine RL, 2004a. Treatment of malting wastewater in a granular sludge sequencing batch. Acta hydrochim. hydrobiol., 32, 16–24.

Schwarzenbeck N, Erley R, Wilderer PA. 2004b. Aerobic granular sludge in an SBR-system treating wastewater rich in particulate matter. Water Sci Technol;49:21–46.

Schwarzenbeck, N., Borges, J.M., Wilderer, P.A., 2005. Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. Appl. Microbiol. Biotechnol., 66, 711–718.

Shams QU, Suhail S, Izharul HF, Ahmad A. 2008. Biodegradation of phenols and p-cresol by sequential batch reactor. International Conference on Environmental

Research and Technology, Cleaner tech control treatment & remediation technique, 906–910.

Shen CF, Kosaric N, Blaszczyk R.1993.The effect of selected heavy metals (Ni, Co and Fe) on anaerobic granules and their extracellular polymeric substance (EPS). Water Res 27:25–33.

Shi XY, Sheng GP, Li XY, Yu HQ. 2010. Operation of a sequencing batch reactor for cultivating autotrophic nitrifying granules. Bioresorce Tech., 101: 2960-2964.

Smolders GJF, van der Meij J, van Loosdrecht. 1994. Model of the anaerobic metabolism of the biological phosphorus removal process: stocihiometry and pH influence. Biotechnology and Bioenrgineering 43: 6, 461-470.

Song ZW, Ren NQ, Zhang K, Tong LY. 2009. Influence of temperature on the characteristics of aerobic granulation in sequencing batch airlift reactors. J Environ Sci, 21, 273–278.

Speece R.E., 1996. Anaerobic Biotechnology for Industrial Wastewater, Archae Press, U.S.A.

Sponza DT. 2002. Extracellular polymer substances and physicochemical properties of flocs in steady- and unsteady-state activated sludge systems. Process Biochem 37:983–998.

Su KZ, Yu HQ, 2005. Formation and characterization of aerobic granules in a sequencing batch reactor treating soybean-processing wastewater. Environ. Sci. Technol., 39, 2818-2827.

Su, B., Cui, X., Zhu, J., 2012. Optimal cultivation and characteristics of aerobic granules with typical domestic sewage in an alternating anaerobic/aerobic sequencing batch reactor, Bioresource Technology 110, 125 – 129.

Sun XF, Wang SG, Liu XW, Gong WX, Bao N, Gao BY, Zhang HY. 2008b. Biosorption of Malachite Green from aqueous solutions onto aerobic granules: Kinetic and equilibrium studies. Bioresour Technol, 99, 3475–3483.

Sun XF, Wang SG, Liu XW, Gong WX, Bao N, Gao BY. 2008a. Competitive biosorption of zinc(II) and cobalt(II) in single- and binary-metal systems by aerobic granules. J Colloid Interface Sci, 324, 1–8.

Sun, F.Y, Yang, C.Y., Li, J.Y., Yang, Y.J., 2006. Influence of different substrates on the formation and characteristics of aerobic granules in sequencing batch reactors. Journal of Environmental Sciences, 18:5, 864 – 871.

Tanksali, A. S. 2013. Treatment Of Sugar Industry Wastewater By Upflow Anaerobic Sludge Blanket Reactor. In IPACT-2013. Proceedings of the National Conference on Industrial Pollution and Control Technology, Annamalai Nagar, Tamil Nadu, India, 14-15 March 2013. (Vol. 5, No. 3, pp. 1246-1253). Sphinx Knowledge House.

Tay JH, Jiang HL, Tay STL. 2004c. High-rate biodegradation of phenol by aerobically grown microbial granules. J Environ Eng, 130, 1415– 1423.

Tay JH, Liu QS, Liu Y. 2001b. The effects of shear force on the formation, structure and metabolism of aerobic granules. Appl Microbiol Biotechnol 57:227–233.

Tay JH, Liu QS, Liu Y. 2002c. Characteristics of aerobic granules grown on glucose and acetate in sequential aerobic sludge blanket reactors. Environ Technol;23:931–6.

Tay JH, Pan S. 2002. The effect of organic loading rate on the aerobic granulation: The development of shear force theory. Water Sci Technol, 47, 235–240.

Tay JH, Yang SF, Liu Y. 2002d. Hydraulic selection pressure-induced nitrifying granulation in sequencing batch reactors. Appl Microbiol Biotechnol;59:332– 7.

Tay STL, Moy BYP, Jiang HL, Tay JH. 2005a. Rapid cultivation of stable aerobic phenol-degrading granules using acetate-fed granules as microbial seed. J Biotechnol;115:387–95.

Tay STL, Moy BYP, Maszenan AM, Tay JH. 2005b. Comparing activated sludge and aerobic granules as microbial inocula for phenol biodegradation. Appl Microbiol Biotechnol;67:708–13.

Tay, J., Pan, S., He, Y., and Tay, S. 2004a. Effect of Organic Loading Rate on Aerobic Granulation. I: Reactor Performance. J. Environ. Eng., 130(10), 1094–1101.

Tay, J., Pan, S., He, Y., and Tay, S. 2004b. Effect of Organic Loading Rate on Aerobic Granulation. II: Characteristics of Aerobic Granules. J. Environ. Eng., 130(10), 1102–1109.

Tay, J.H., Ivanov, V., Pan, S., Tay, S.T.L., 2002b. Specific layers in aerobically grown microbial granules. Lett. Appl. Microbiol., Vol.34, 254-257.

Tay, J.H., Liu, Q.S., Liu, Y., 2001a. Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor. J Appl Microbiol, 91:168– 75.

Tay, J.H., Liu, Q.S., Liu, Y., 2001c. The role of cellular polysaccharides in the formation and stability of aerobic granules. Lett Appl Microbiol 33:222–226.

Tchobanoglous G, Burton FL, Stensel HD. 2004. Wastewater Engineering, Treatment and Reuse. McGrawHill Company, Inc., 4th Ed. New York.

Thanh, B. X., Visvanathan, C., & Aim, R. B. 2009. Characterization of aerobic granular sludge at various organic loading rates. Process Biochemistry, 44(2), 242-245.

Thaveesri, J., Liessens, B., & Verstraete, W. 1995. Granular sludge growth under different reactor liquid surface tensions in lab-scale upflow anaerobic sludge blanket reactors treating wastewater from sugar-beet processing. Applied microbiology and biotechnology, 43(6), 1122-1127.

Toh SK, Tay JH, Moy BYP, Tay STL. 2003. Size-effect on the physical characteristics of the aerobic granule in a SBR. Appl Microbiol Biotechnol, 60, 687–695.

Tsuneda S, Aikawa H, Hayashi H, Yuasa A, Hirata A. 2003c. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol Lett 223:287– 292.

Tsuneda S, Jung J, Hayashi H, Aikawa H, Hirata A, Sasaki H. 2003b. Influence of extracellular polymers on electrokinetic properties of heterotrophic bacterial cells examined by soft particle electrophoresis theory. Colloids Surf B 29:181–188.

Tsuneda S, Ogiwara M, Ejiri Y, Hirata A. 2006. High-rate nitrification using aerobic granular sludge. Water Sci Technol;53:147–54.

Tsuneda, S., Nagano, T., Hoshino, T., Ejiri, Y., Noda, N., & Hirata, A. 2003a. Characterization of nitrifying granules produced in an aerobic upflow fluidized bed reactor. Water Research, 37(20), 4965-4973.

URL 1: http://www.royalhaskoningdhv.com/en-gb/news-room/news/20131205-Nereda-technology-energy-savings/1589

URL 2: http://www.turkseker.gov.tr/hakkimizda.aspx

URL 3: http://www.sekerkurumu.gov.tr/sss.aspx

Val del Rio A., Figueroa M, Arrojo B, Mosquera-Corral A, Campos JL, García-Torriello G, Mendez R. 2012. Aerobic granular SBR systems applied to the treatment of industrial effluents. Journal of Environmental Management 95, S88-S92.

Viaeminck SE, Cloetens LFF, Carballa M, Boon N, Verstraete W. 2008. Granular biomass capable of partial nitritation and anammox. Water Sci Technol, 58, 1113–1120.

Wan, J., Bessiere, Y., Sperandio, M., 2009. Alternating anoxic feast/aerobic famine condition for improving granular sludge formation in sequencing batch airlift reactor at reduced aeration rate, Water Research, 43, 5097 – 5108.

Wang JF, Wang X, Zhao ZG, Li JW. 2008a. Organics and nitrogen removal and sludge stability in aerobic granular sludge membrane bioreactor. Appl Microbiol Biotechnol, 79, 679–685.

Wang SG, Liu XW, Gong WX, Gao BY, Zhang DH, Yu HQ., 2007a. Aerobic granulation with brewery wastewater in a sequencing batch reactor. Bioresource Technol. 98, 2142–2147.

Wang SG, Liu XW, Zhang HY, Gong WX, Sun XF. 2007c. Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor. Chemosphere, 69, 769–775.

Wang XH, Zhang HM, Yang FL, Wang YF, Gao MM. 2008b. Long-term storage and subsequent reactivation of aerobic granules. Bioresour Technol, 99, 8304–8309.

Wang ZW. 2004. PhD thesis, Nanyang Technological University, Singapore.

Wang, X. H., Zhang, H. M., Yang, F. L., Xia, L. P., & Gao, M. M. 2007b. Improved stability and performance of aerobic granules under stepwise increased selection pressure. Enzyme and microbial technology, 41(3), 205-211.

Wang, Z.W., Li, Y., Zhou, J.Q., Liu, Y., 2006a. The influence of short-term starvation on aerobic granules, Process Biochemistry, 41(12), 2373-2378.

Wang, Z.W., Liu, Y., Tay, J.H., 2007d. Biodegradibility of extracellular polymeric substances produced by aerobic granules, Applied Microbiol Biotechnol, 74 (2), 462-466.

Watnick P., Kolter R., 2000. Biofilms, city of microbes. J. Bacteriol., 182, 2675-2679.

Wei, Y., Ji, M., Li, R., & Qin, F. 2012. Organic and nitrogen removal from landfill leachate in aerobic granular sludge sequencing batch reactors. Waste management, 32(3), 448-455.

Wu, L., Peng, C.Y., Peng, Y.Z., Li, L.Y., Wang, S.Y., Ma, Y., 2012. Effect of wastewater COD/N ratio on aerobic nitrifying sludge granulation and microbial population shift, Journal of Environmental Sciences, 24(2), 234–241.

Xu H, Liu Y, Tay JH. 2006. Effect of pH on nickel biosorption by aerobic granular sludge. Bioresour Technol, 97, 359–363.

Xu H, Tay JH, Foo SK, Yang SF, Liu Y. 2004. Removal of dissolved copper(II) and zinc(II) by aerobic granular sludge. Water Sci Technol;50:155–60.

Xuan, W., Bin, Z., Zhiqiang, S., Zhigang, Q., Zhaoli, C., Min, J., Junwen L., Jingfeng, W. 2010. The EPS characteristics of sludge in an aerobic granule membrane bioreactor. Bioresource technology, 101(21), 8046-8050.

Yang SF, Liu QS, Tay JH, Liu Y. 2004a. Growth kinetics of aerobic granules developed in sequencing batch reactors. Lett Appl Microbiol;38:106– 12.

Yang SF, Tay JH, Liu Y. 2004b. Inhibition of free ammonia to the formation of aerobic granules. Biochem Eng J;17:41– 8.

Yang, S. F., Tay, J. H., & Liu, Y. 2005. Effect of substrate nitrogen/chemical oxygen demand ratio on the formation of aerobic granules. Journal of environmental engineering, 131(1), 86-92.

Yang, S.F., Li, X.Y., Yu, H.Q., 2008. Formation and characterization of fungal and bacterial granules under different alkalinity and pH conditions, Process Biochemistry, 43, 8 – 14.

Yang, S.F., Tay JH, Liu Y,. 2004c. Respirometric activities of heterotrophic and nitrifying populations in aerobic granules developed at different substrate N/COD ratios. Curr Microbiol 49, 42–46.

Yao L, Ye ZF, Tong MP, Lai P, Ni JR. 2009. Removal of Cr3+ from aqueous solution by biosorption with aerobic granules. J Hazard Mater, 165, 250–255.

Yi S, Tay JH, Maszenan AM, Tay STL. 2003. A cultureindependent approach for studying microbial diversity in aerobic granules. Water Sci Technol 47:283–290.

Yi S, Zhuang WQ, Wu B, Tay ST, Tay JH. 2006. Biodegradation of p-nitrophenol by aerobic granules in a sequencing batch reactor. Environ Sci Technol, 40, 396–401.

Yilmaz G, Lemaire R, Keller J, Yua Z., 2008. Simultaneous nitrification, denitrification and phosphorus removal from nutrient-rich industrial wastewater using granular sludge. Biotehnol Bioeng., 100(3), 529-541.

Yoo H, Ahn KH, Lee HJ, Lee, KH, Kwak YJ, Song KG. 1998. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently-aerated reactor. Wat. Res. Vol. 33, 145-154.

You Y, Peng Y, Yuan ZG, Li XY, Peng YZ. 2008. Cultivation and characteristic of aerobic granular sludge enriched by phosphorus accumulating organisms. Environ Sci, 29, 2242–2248 (in Chinese).

Yu, G.H., Juang, Y.C., Lee, D.J., He, P.J., Shao, L.M.. 2009. Enhanced aerobic granulation with extracellular polymeric substances (EPS)-free pellets, Bioresource Technology (100) 4611–4615.

Yu, H. Q., Tay, J. H., & Fang, H. H. 2001. The roles of calcium in sludge granulation during UASB reactor start-up. Water Research, 35(4), 1052-1060.

Yuan, L., Han, W., Wang, L., Yang, Y., & Wang, Z. 2007. Simultaneous denitrifying phosphorus accumulation in a sequencing batch reactor. Frontiers of Environmental Science & Engineering in China, 1(1), 23-27.

Zeng P, Zhuang WQ, Tay STL, Tay JH. 2007. The influence of storage on the morphology and physiology of phthalic acid-degrading aerobic granules. Chemosphere, 69, 1751–1757.

Zhang H, He Y, Jiang T, Yang F. 2011b. Research on characteristics of aerobic granules treating petrochemical wastewater by acclimation and co-metabolism methods. Desalination 279, 69–74.

Zhang LL, Chen JM, Fang F. 2008. Biodegradation of methyl t-butyl ether by aerobic granules under a cosubstrate condition. Appl Microbiol Biotechnol, 78, 543–550.

Zhang LL, Zhang B, Huang YF, Cai WM. 2005. Re-activation characteristics of preserved aerobic granular sludge. J Environ Sci,17, 655–658.

Zhang XQ, Bishop PL, Kinkle BK. 1999. Comparison of extraction methods for quantifying extracellular polymers in biofilms. Water Sci Technol 39:211-218.

Zhang XQ, Bishop PL. 2001. Spatial distribution of extracellular polymeric substances in biofilms. J Environ Eng 127:850–856.

Zhang, H., Dong, F, Jiang, T., Wei, Y., Wang, T., Yang, F., 2011a. Aerobic granulation with low strength wastewater at low aeration rate in A/O/A SBR reactor, Enzyme and Microbial Technology, 49, 215 – 222.

Zhang, L., Feng, X., Zhu, N., Chen, J., 2007. Role of extracellular protein in the formation and stability of aerobic granules, Enzyme and Microbial Technology, 41, 551 – 557.

Zheng YM, Yu HQ, Sheng GP. 2005. Physical and chemical characteristics of granular activated sludge from a sequencing batch airlift reactor. Process Biochem;40:645–50.

Zheng, Y. M., Yu, H. Q., Liu, S. J., & Liu, X. Z. 2006. Formation and instability of aerobic granules under high organic loading conditions. Chemosphere, 63(10), 1791-1800.

Zhu J, Wilderer PA. 2003. Effect of extended idle conditions on structure and activity of granular activated sludge. Water Res 37:2013–2018.

Zhu, L., Lv, M.L., Dai, X., Yu, Y.W., Qi, H.Y., Xu, X.Y., 2012. Role and significance of extracellular polymeric substances on the property of aerobic granule, Bioresource Technology 107 46–54.

Zitomer DH, Duran M, Albert R, Guven E. 2007. Thermophilic aerobic granular biomass for enhanced settleability. Water Res, 41, 819–825.

APPENDIX A

CALIBRATION AND ABSORBANCE CURVES

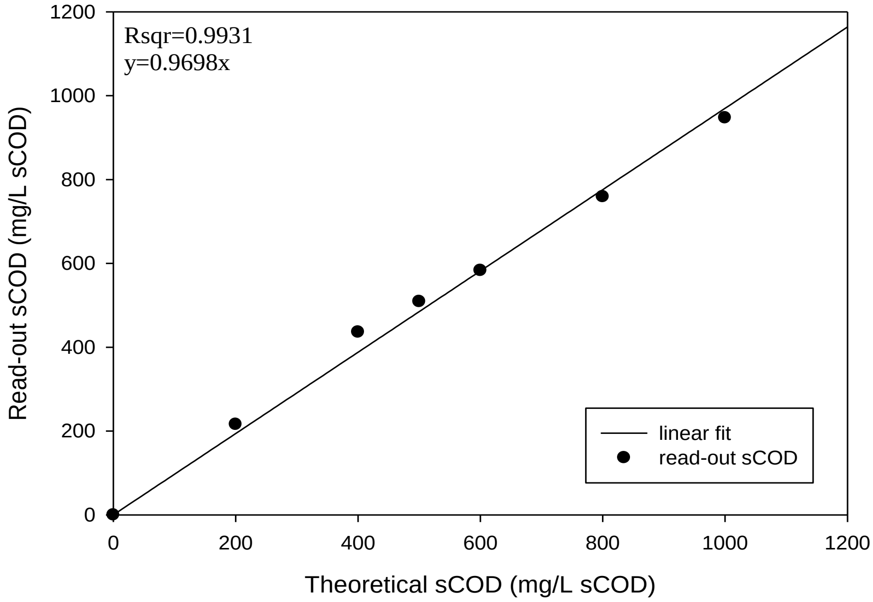

Figure A. 1 Calibration curve for sCOD range of 0-1500 mg/L.

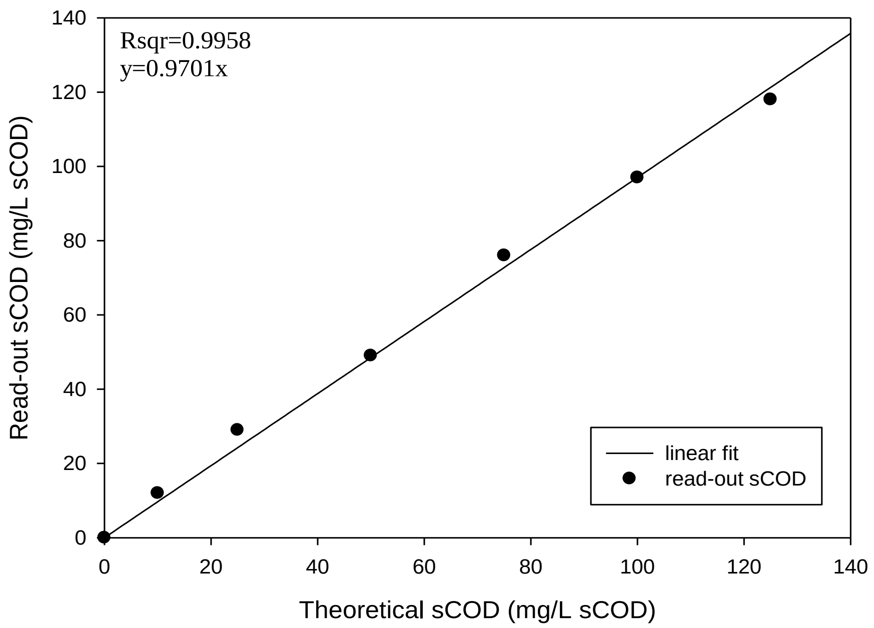

Figure A. 2 Calibration curve for sCOD range of 0-150 mg/L.

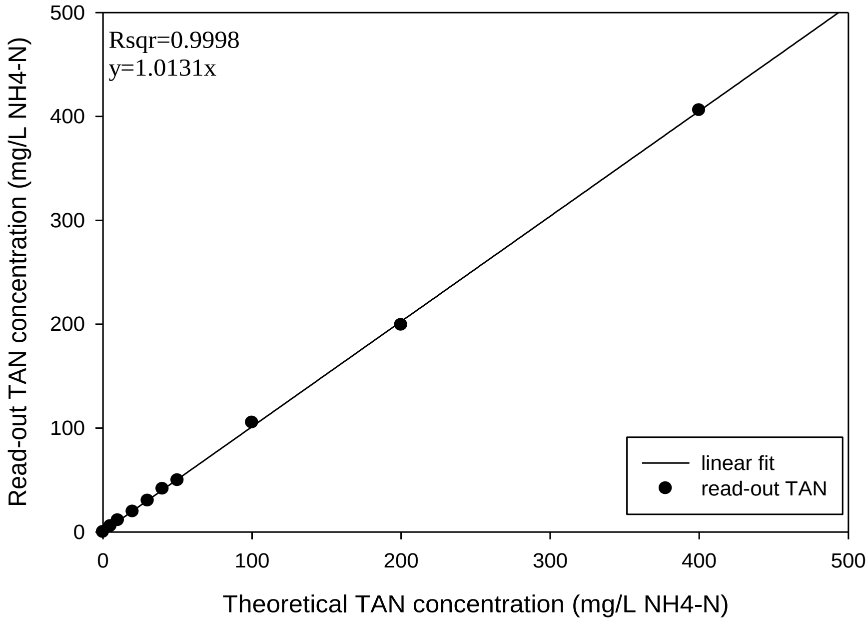

Figure A. 3 Calibration curve for TAN range of 10-400 mg/L.

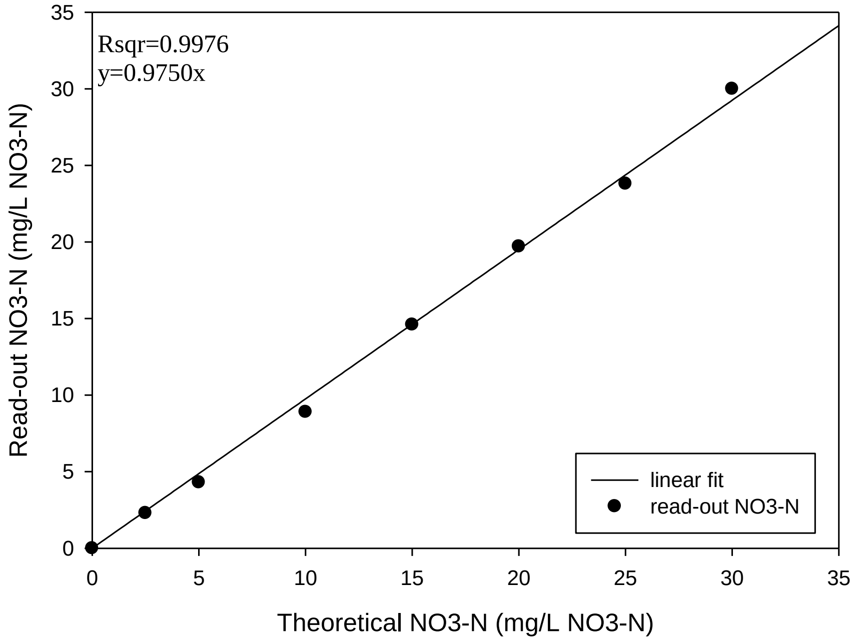

Figure A. 4 Calibration curve for for NO3-N range of 0-30 mg/L.

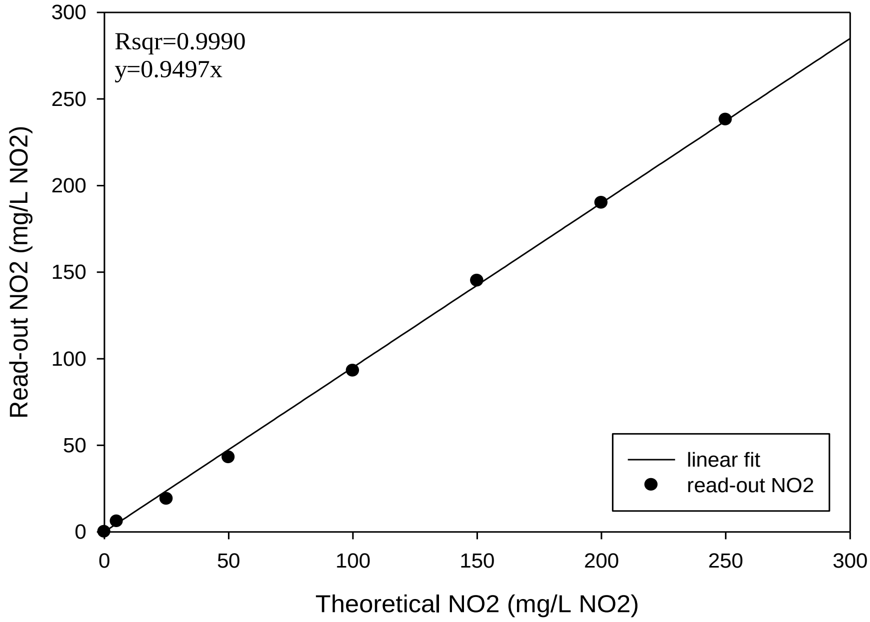

Figure A. 5 Calibration curve for NO2 range of 0-250 mg/L.

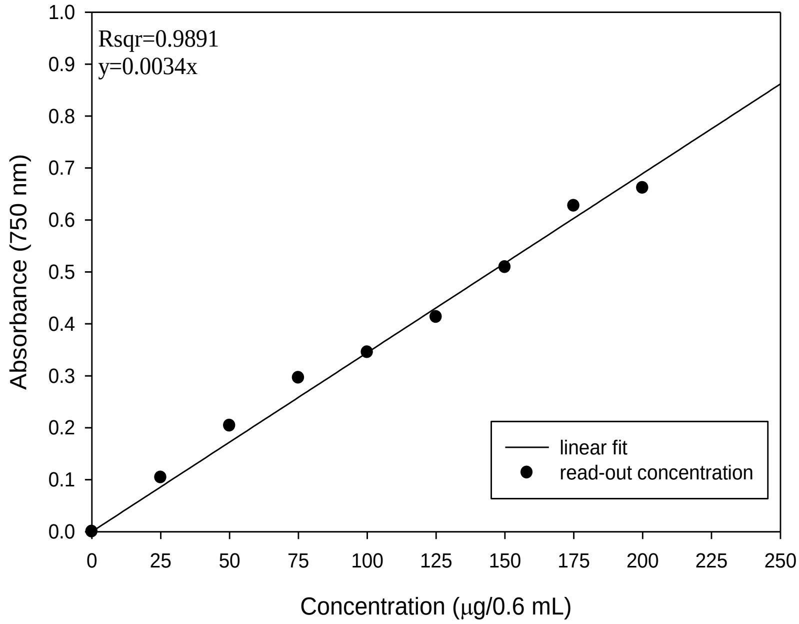

Figure A. 6 Absorbance curve for protein range of 0-200 μg/0.6mL.

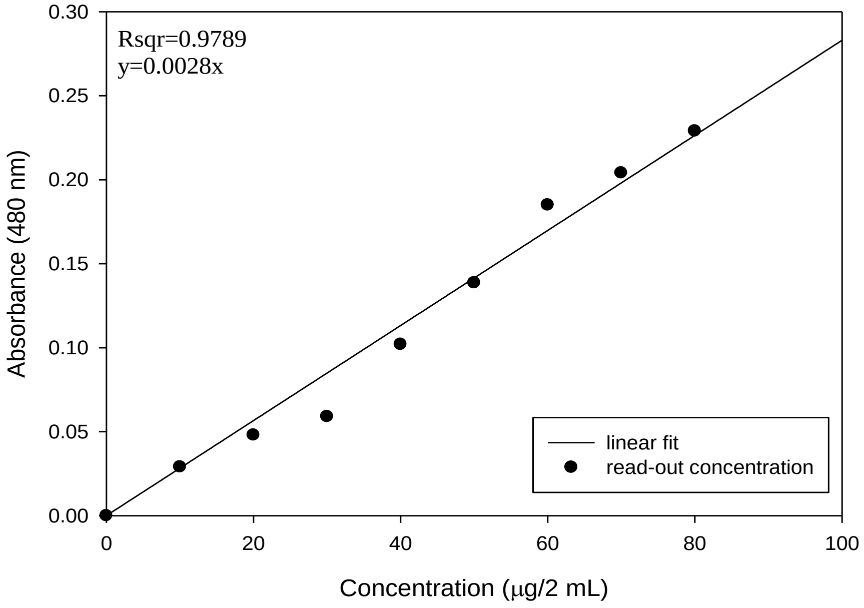

Figure A. 7 Absorbance curve for polysaccharide range of 0-80 μg/2mL.

APPENDIX B

REACTIONS INVOLVED IN THE BIOLOGICAL TOTAL N REMOVAL BY AEROBIC GRANULES

B.1 Nitrification and denitrification reactions

Aerobic granular SBRs can achieve simultaneous nitrification and denitrification due to the granular structure with aerobic outer layers and anoxic inner parts (de Kreuk et al., 2005b). Total N removal involves 2 sequencing biological mechanisms, namely nitrification and denitrification. Nitrification is accomplished in the aerobic media by 2 different types of bacteria, namely ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Nitrification involves the 2 sequencing reactions: the conversion of TAN ((NH_3 + NH_4^+) to nitrite (NO_2^-) via AOBs (Eqn B.1), and then the conversion of nitrite to nitrate (NO_3^-) via NOBs (Eqn B.2). The overall nitrification reaction (Eqn B.3), decreases the pH of the medium as a result of the H^+ ions production. Almost all of the nitrification bacteria are chemo-autotrophs which consume inorganic carbon to cater for their carbon requirement (Tchobanoglous et al., 2004).

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O \qquad \text{(Eqn B.1)}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad \text{(Eqn B.2)}$$

$$NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O \qquad \text{(Eqn B.3)}$$

Denitrification (Eqn B.4) takes place by the catalysis of each specific N compound with a specific enzyme in anoxic or reducing media. Denitrification is conducted by chemo-organotrophic, litotrophic and phototrophic bacteria as well as certain types of fungi (Schmidt et al., 2003). Although the gaseous nitrogen ($N_{2(g)}$) is the ultimate product of denitrification, gaseous $NO_{(g)}$ and $N_2O_{(g)}$ may be observed as the ultimate denitrification products in some cases (such as at high oxygen concentrations) where the NO- and N_2O-reducing enzymes are not completely active (Schmidt et al., 2003). N compounds act as electron acceptors in denitrification reaction, while the electron source is generally organic carbon (like methanol, acetate, ethanol and lactate), even sulfur or hydrogen gas can be used as carbon source by certain types of bacteria. In the absence of any additional carbon source endogenous denitrification can be observed (Tchobanoglous et al., 2004). Denitrification increases the pH of the medium as a result of the alkalinity production, i.e donation of the OH^- ions to the medium.

$$1.25\ CH_3COOH + 2NO_3 \rightarrow N_2 + 1.5H_2O + 2OH^- \qquad \text{(Eqn B.4)}$$

B.2 The calculations for the determination of N removal efficiency

The N removal efficiency of the SBRs were determined in terms of %TAN removal, %TN removal, %DN and %SNDN based on the below equations.

$$\%TAN = 100 \times (TAN_{initial} - TAN_{aerobic}) / (TAN_{initial}) \qquad \text{(Eqn B.5)}$$

$$\%DN = 100 \times (TON_{initial} - TON_{anoxic}) / (TON_{initial}) \qquad \text{(Eqn B.6)}$$

$$\%SNDN = 100 \times [\ 1- (TON_{aerobic} / (\Delta TAN_{aerobic} + TON_{anoxic}))] \qquad \text{(Eqn B.7)}$$

$$\Delta TAN_{aerobic} = TAN_{anoxic} - TAN_{aerobic} \qquad \text{(Eqn B.8)}$$

$$\%TN = 100 \times (TN_{initial} - TN_{aerobic}) / (TN_{initial}) \qquad \text{(Eqn B.9)}$$

where;

%TAN= TAN removal efficiency in the complete cycle

%DN = the denitrification percent at the anoxic period

%SNDN = the denitrification efficiency simultaneously performed during aerobic period

TAN (Total ammonifiable nitrogen) = NH_4-N + NH_3-N

TON (Total oxidized nitrogen) = NO_2-N + NO_3-N

TN (Total Nitrogen)= TAN + TON

$\Delta TAN_{aerobic\ =}$ TAN consumed during aerobic period (i.e. converted to TON)

TN% = the total nitrogen removal (loss) per cycle

Aerobic: Aerobic period

Anoxic: Anoxic period

$TN_{\ initial}$: TN calculated just after the feeding period; in other words summation of TAN, NO_2-N and NO_3-N concentrations measured just after the feeding period.

The subnotations of initial, aerobic and anoxic correspond to the samples which were taken at the cycle initiation just after the feeding period, at end of the anoxic period and at the end of the aerobic period, respectively.

TAN removal efficiency (Eqn B.5) stands for the amount of TAN that has been removed from the system via conversion to TON, i.e. via nitrification. The amount of TAN converted to TON may be further be converted to $N_{2(g)}$ in the presence of denitrification simultaneous to nitrification, i.e. SNDN, in the aerobic period.

The denitrification efficiency (%DN) (Eqn B.6) represents the amount of TON that is converted to $N_{2(g)}$ via denitrification during the anoxic period.

The denitrification efficiency simultaneous to nitrification performed during aerobic period (%SNDN) (Eqn B.7), represents the amount of TON that is converted to $N_{2(g)}$ and lost from the system via denitrification in the aerobic period. This TON value involves both the TON initially present at the beginning of aerobic cycle, and the amount of TAN that is converted to TON via nitrification in aerobic cycle. In other words %SNDN denotes the total N removal during aerobic period, since the TON produced by nitrification in aerobic period is converted to $N_{2(g)}$ by SNDN.

The N removal efficiency in the whole cycle is shown by TN removal efficiency (%TN) (Eqn B.9). TN removal is the sum of TON converted to $N_{2(g)}$ via denitrification during the anoxic period (i.e. %DN) and the TON converted to $N_{2(g)}$ via denitrification simultaneous to nitrification during the aerobic period.

B.3 Free-ammonia (FA) stripping and the calculations for the determination of FA

As previously mentioned, the total ammonifiable nitrogen (TAN) involves the nitrogen in both ammonium and ammonia forms (TAN = NH_4-N + NH_3-N), which are converted to each other depending on the pH and temperature conditions. The pKa for ammonium and ammonia, i.e. NH_4-N and NH_3-N, is 9.3 at 25^0C (Hvitved-Jacobsen et al., 2013). Thus above pH 9.3, NH_4-N portion of TAN shifts to free-ammonia (NH_3-N) form and the TAN becomes dominant of free-ammonia (FA) as the pH rises. Therefore, above pH> 9.3, it is possible to observe ammonia-stripping. FA stripping might occur mostly during the aerobic period of SBR operation, where the aeration is applied. When pH exceeds 9.3, the N loss due to FA stripping could not be distinguished from the N loss caused by SNDN. In order to prevent inaccuracy due to FA stripping interference, the SNDN and TAN removal efficiency measurements were only computed when pH< 9.3, for it is assured that FA stripping, if any, would be minimal.

FA concentration of 10-150 mg/L, corresponding to the NH_3-N concentration of 8.2-123 mg/L, is known to be inhibitory for nitrifiers (Anthonisen et al., 1976). Also the FA concentrations of 2.5-39.6 mg/L NH_3-N decreased the respirometric activity of nitrifiers 2.5 times (Yang et al., 2004b). In order to determine the possible TAN removal inhibition due to the FA toxicity, the FA concentrations in the reactors were determined in terms of NH_3-N, according to the pH, temperature and TAN concentrations in the media (Eqm B.10, Eqn B.11) (Anthonisen et al., 1976).

$$K_b / K_w = \exp (6344 / 273 + {}^0C) \qquad \text{(Eqn B.10)}$$

$$NH_3\text{-N (mg/L)} = [\text{TAN (mg N/L)} \times 10^{pH}] / [K_b / K_w + 10^{pH}] \qquad \text{(Eqn B.11)}$$

The calculations aimed to determine the maximum FA concentration possible at the highest TAN concentration present at the maximum pH. The FA concentrations were calculated for all of the reactors, namely R1, R2, R3 and R4, in this study; however only the FA levels for R1 were found to be inhibitory (Chapter 4, Table 4.1, Figure 4.2). For R3 and R4, according to the maximum possible FA concentrations, the ammonia stripping was seen to be negligible and the TAN removal, which was observed only during the aerobic period, was only due to the TAN oxidation. Only for R2 there was 11-25% TAN removal in anoxic period (Days 25-53) which might have been due to the ammonia stripping at high pH (as also mentioned in Section 4.1.1.1.2). Despite the high pH, the FA levels in R2 did not reach the inhibitory levels for the nitrification bacteria (Figure B.1), since the TAN consumption for the oxidation reaction (nitrification) was high.

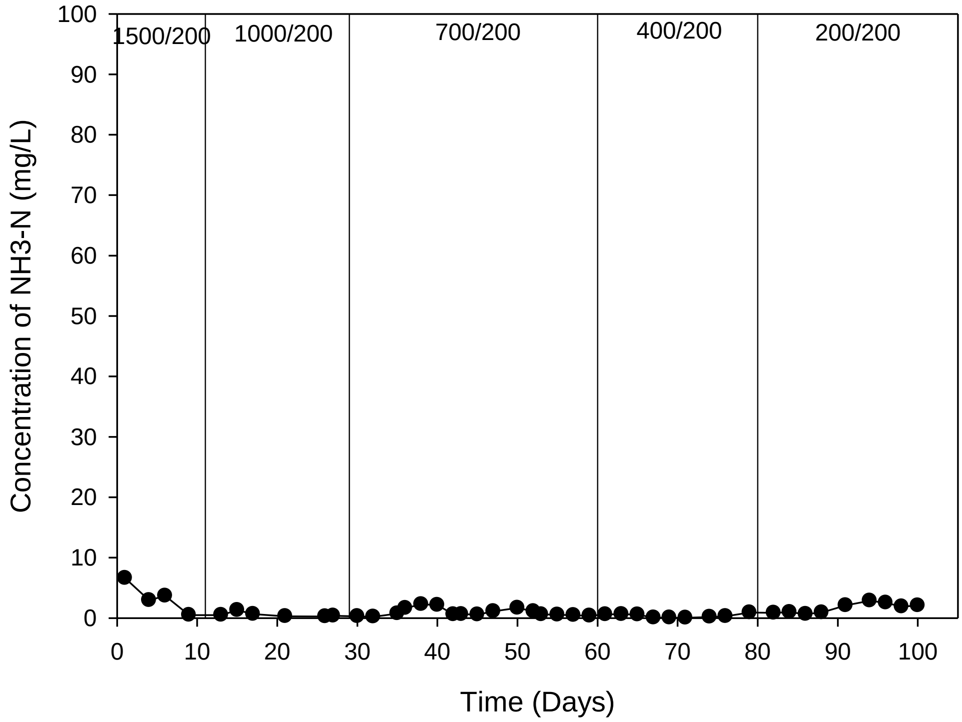

Figure B.1. The FA concentrations calculated based on the TAN concentrations, pH and temperature during the operational period of R2. (1500/200 refers to 1500 mg/L influent COD and 200 mg/L influent TAN concentrations).

APPENDIX C

MEASUREMENT OF DO CONCENTRATIONS

During the operation of the SBRs, anoxic conditions were provided in the anoxic periods since the average DO concentrations of all the reactors were in the range of 0.1-0.4 mg/L in the anoxic period. The average DO concentrations of the reactors during the aerobic period were in the range of 5.8-6.6 mg/L in order to provide aerobic conditions. The DO concentrations of the reactors in each set are given as follows.

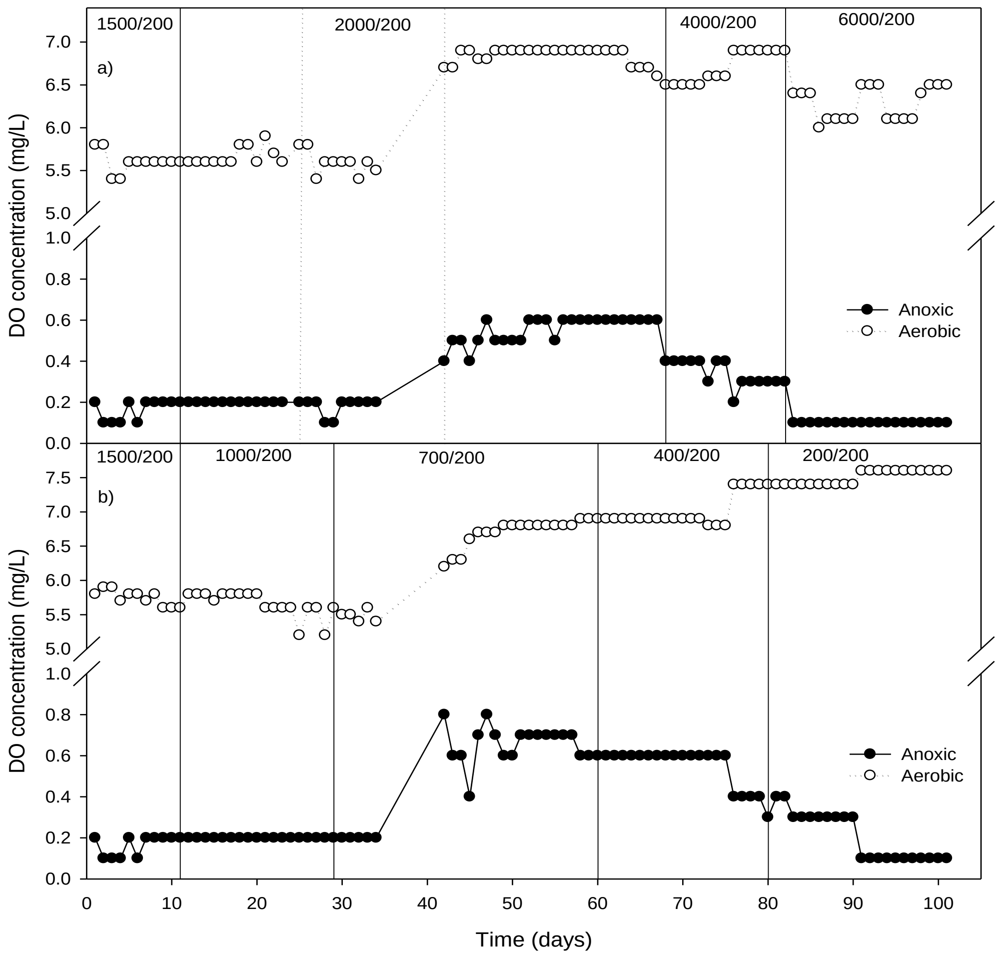

Figure C.1 Anoxic period and aerobic period DO concentrations for a) R1; and b) R2 throughout the operational period of Set-1.

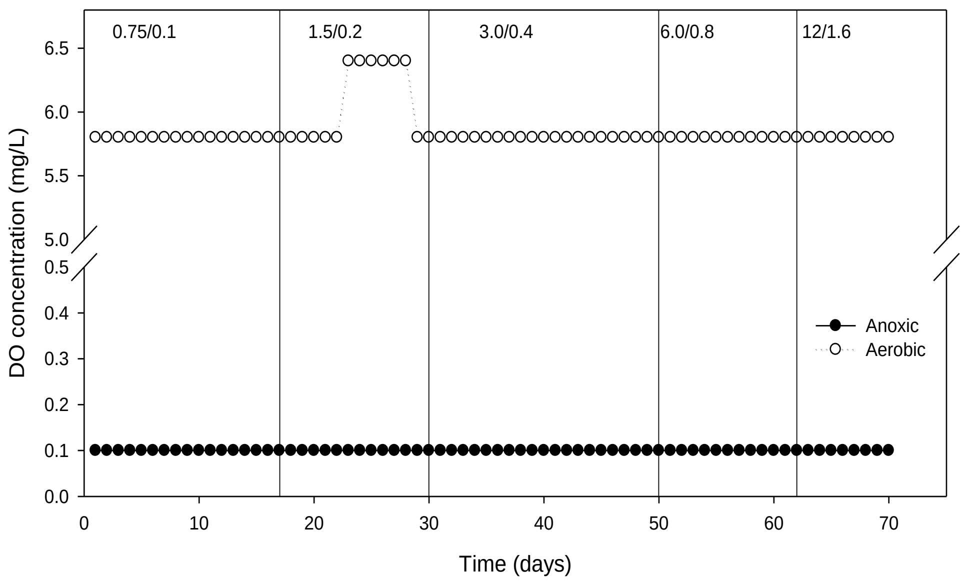

Figure C.2 Anoxic period and aerobic period DO concentrations for R3 throughout the operational period of Set-2.

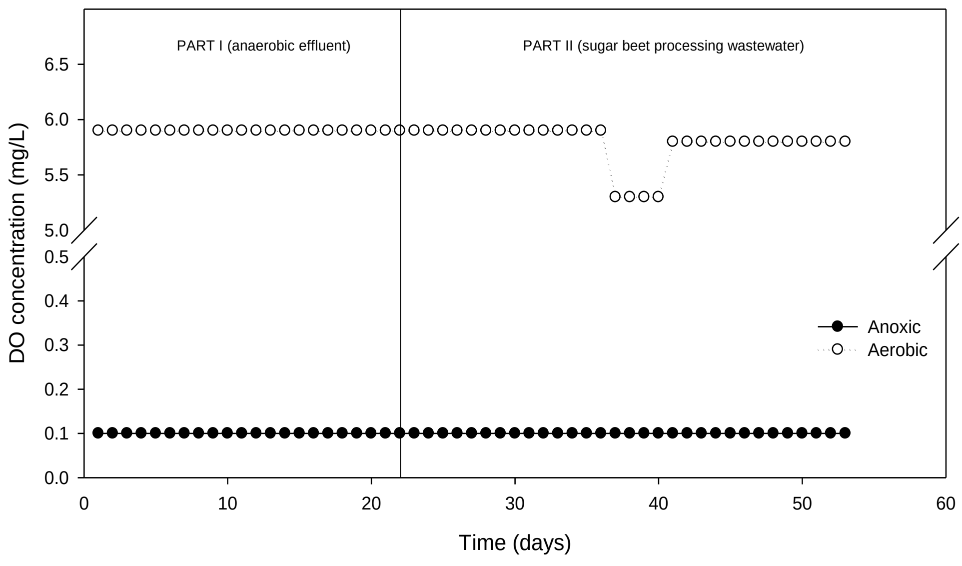

Figure C.3 Anoxic period and aerobic period DO concentrations for R4 throughout the operational period of Set-3.