DEVELOPMENT OF AEROBIC GRANULAR SLUDGE FOR THE REMOVAL OF ANTIBIOTIC SULFAMETHOXAZOLE FROM MUNICIPAL WASTEWATER

By

ABBASS JAFARI KANG

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Civil Engineering

University of Manitoba

Winnipeg Manitoba R3T 5V6

Canada

Abstract

Aerobic granular sludge (AGS) was developed in anoxic/anaerobic/oxic sequencing batch reactors (SBR) for the removal of antibiotic sulfamethoxazole from municipal wastewater. First, the stability and biological nutrient removal (BNR) efficiency of aerobic granules at low organic loads (when applied to municipal wastewater) was studied. For this aim, granules obtained with high-strength (COD = 1400 mg/L) wastewater were fed with medium (COD = 700 mg/L) and then low-strength (COD = 400 mg/L) municipal wastewater. The granules rapidly acclimated to the medium-strength wastewater. However, feeding with low-strength wastewater reduced the food/microorganisms (F/M) ratio from 0.4 to 0.2 gCOD/gVSS d and granules disintegration occurred. Re-granulation was obtained after poor settling biomass was washed out and the F/M ratio reached 0.4 gCOD/gVSS d.

Second, the BNR treatment performance of the granules was evaluated under two operation strategies: i) continuous anaerobic feeding and ii) anaerobic contact. Results indicated that, anaerobic contact increased anaerobic COD utilization from 17-24% to 45-53% with improving mass transfer and consequently, improved phosphorus removal from 63 to 93%. The anaerobic contact also improved the effluent quality from 87 to 46 mg/L VSS with prevention of flocs formation. With the application of anaerobic contact retention time (SRT) increased from 15 to 32 days.

Third, the removal of SMX in aerobic granular sludge was compared with conventional activated sludge in anoxic/anaerobic/oxic SBRs under the same operation conditions. For three months, 2µg/L SMX was spiked into the reactors' feeds (synthetic municipal wastewater). The presence of SMX had no significant impact on treatment performance of the suspended and granular biomass in terms of removing organics and nutrients. Overall, with 12 h of hydraulic retention time (HRT), mean removal efficiencies of 84±8% and 73±10% were obtained for the granular and suspended biomass, respectively. Results of SMX elimination during eight hours of a cycle consisting of anaerobic/anoxic and aerobic phases showed that SMX removal was obtained only under aerobic condition while mixing under anoxic/anaerobic condition did not remove SMX. This confirms the insignificance of SMX removal via anoxic/anaerobic transformation and/or sorption onto activated sludge. The pseudo-first order SMX biodegradation constant rate constants (k_{bio}) in the granular and suspended biomass at steady-state operating condition, were 2.25±0.30 and 1.34±0.39 L/gVSS.d, respectively. The mean value of k_{bio} obtained with granular sludge was significantly greater than that with the suspended biomass. These results, suggest that aerobic granular sludge, which has advantages such as high biomass retention and high biomass concentration, could be used as an efficient process for the removal of such organic micropollutants.

Acknowledgement

I would like to acknowledge my advisor Dr. Qiuyan Yuan for giving me the opportunity to pursue my graduate studies at the University of Manitoba and also for her precious advice and guidance during my master's program. I would also like to thank my advisory committee, Dr. Giulio Munz and Dr. Charles Wong for their valuable comments and time to look into my thesis. I wish to extend my appreciation to Dr. Zhiyong Huang and Alistair Brown for their contributions.

Sincere thanks goes to Dr. Victor Wei who helped me during the experimental part of this study. I would like to acknowledge my dear friend Cornelia Badila, for being a great research partner and a wonderful friend. I would like to thank my colleagues Cici, Fernanda, Mahrooz, Pouria, Juno, Alice, Simon, Tanner, Mufizol, Kelsey, Dan, Alireza and Parvin who were bringing me memorable moments aside from research and outside of the Lab. Also, I would like to thank my wonderful friends Ramin, Alireza, Farimehr, Elham, Zahra, Shahram, Saeed, Sina and Amin for their support and being there for me.

Finally, of course, I would like to thank my parents and to my brothers Mehdi, Sadegh, Saeed and Masood who are now far from me but they have always been a great encouragement and support. My undergraduate and graduate studies would not have been accomplished without them.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

A2O	anoxic/anaerobic/oxic process
A/O	anaerobic/oxic process
AGS	Aerobic granular sludge
AMO	Ammonia monooxygenase
AOB	Ammonia oxidizing bacteria
AOP	Advanced oxidation process
BOD	Biochemical oxygen demand
BNR	Biological nutrient removal
COD	Chemical oxygen demand
d_{mean}	Mean diameter
DO	Dissolved oxygen
EDC	Endocrine disrupting compound
EPS	Extracellular polymeric substances
ETSS	Effluent total suspended solids
F/M	Food to microorganism ratio
GAO	Glycogen accumulating organisms
HRT	hydraulic retention time
K_{bio}	Biodegradation constant rate constants
K_d	Sorption coefficient
K_{ow}	Octanol-water distribution coefficient
K_a	Dissociation constant
MBR	Membrane bioreactor

MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NOB	Nitrite oxidizing bacteria
OHO	Ordinary heterotrophic organisms
P/C	Protein to carbohydrate ratio
PAO	Poly-phosphate accumulating organisms
PCoA	Principal coordinates analysis
LEfSe	Linear discriminant analysis effect size
rbCOD	Readily biodegradable chemical oxygen demand
SBR	Sequencing batch reactor
sCOD	Soluble chemical oxygen demand
SMX	Sulfamethoxazole
SRT	Solids retention time
SVI_5	Sludge volume index after 5 min
TN	Total nitrogen
TP	Total phosphorous
TSS	Total suspended solids
VER	Volumetric exchange ratios
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

Chapter 1: Introduction and Objectives

1.1. INTRODUCTION

Uncontrolled release of endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) in the aquatic environment has been a global health concern (Rahman et al., 2009). Lately, antibiotics has become a serious issue due to: i) the increasing use in human and veterinary medicine, ii) potential adverse effects on aquatic animals and plants, and iii) potential impacts on the micro-ecosystem (e.g., formation of antibiotic resistance) (Ding & He, 2010; Kumar et al., 2012; Oberle et al., 2012). Municipal wastewater treatment plants are known as a main source from which these micropollutants are discharged into the environment (Michael et al., 2013). Sulfamethoxazole (SMX) is one of the most commonly used antibiotics, and was reported as one of the most detected (100%) organic micropollutants monitored in 139 streams in the U.S. (Kolpin et al., 2002).

Current wastewater treatment facilities, which are designed for biological removal of organics (COD) and nutrients (nitrogen and phosphorus), are not capable of effective removal of all micropollutants. Application of advanced treatment technologies, such as adsorption onto activated carbon and degradation with advanced oxidation processes (AOPs) before effluent discharge, has been suggested for the effective removal of poorly biodegradable micropollutants. However, this will increase capital and operational costs of the treatment (Michael et al., 2013).

Different values have been reported for the removal efficiency of SMX in full-scale wastewater treatment plants (WWTP) using activated sludge processes. Batt et al. (2007) compared removal of antibiotics in four WWTPs in Erie County, New York. First, the Amherst WWTP with two-stage activated sludge (stage 1: BOD removal with HRT= 1h and SRT- 6d; stage 2: nitrification with HRT= 2h and SRT=49d) receiving 2.8 μg/L SMX achieved 57 and 17

% removal via the first and second stages, respectively. Second, the East Aurora WWTP with an extended aeration process (HRT= 28-31h and SRT= 17d and ferrous chloride addition) removed 72% SMX from wastewater containing 0.88 μg/L SMX. Third, the Holland WWTP with RBC (rotating biological contactor) treatment system (HRT= 4h) obtained 36% SMX removal (influent SMX = 0.75 μg/L). Fourth, the Lackawana WWTP with pure oxygen activated sludge (HRT= 1h and SRT= 15d) receiving 0.72 μg/L SMX also achieved 36 % removal efficiency. Gao et al. (2012) reported 89% removal of SMX (1.57 μg/L in raw influent) in a full-scale conventional treatment plant (East Lansing WWTP, Michigan, US) containing preliminary treatment (aerated grit removal, equalization basin), primary treatment (primary clarifier), secondary treatment (aeration tank followed by secondary clarifier) and tertiary treatment (disinfection, rapid gravity sand filters and post-filtration aeration). The secondary biological treatment process removed 63% of the receiving SMX. Another full-scale plant (Kloten-Opfikon WWTP, Zurich, Switzerland) receiving 1.7 μg/L SMX obtained an overall removal of 62% using primary treatment (screens, aerated grit-removal and primary clarifier), secondary treatment (activated sludge system capable of nitrification and denitrification) and tertiary treatment (sand filter). The secondary treatment obtained 59% SMX removal. Rosal et al. (2010) reported 17.3 % SMX removal (with 279 ng/L SMX in the influent) in a full-scale WWTP (Alcala´ de Henares, Madrid, Spain) operated for BNR (A2O with anoxic, anaerobic, and oxic zones).

The effectiveness of biological wastewater treatment for degrading these compounds is mostly determined by operational conditions. The A2O (anoxic/anaerobic/oxic) process, used for nitrification, denitrification, BOD and phosphorus removal, and the A/O (anaerobic/oxic) process used for nitrification, phosphorus and BOD removal, both had higher efficiencies in elimination of antibiotics compared to conventional activated sludge (oxic) process which uses only BOD removal (Ghosh et al., 2009). Increasing solids retention time (SRT) was found to improve the removal of micro-pollutants by enhancing the microbial diversity and hence enzymatic activity of activated sludge (Joss et al., 2006; Kreuzinger et al., 2004; Polesel et al., 2016). Enrichment of nitrifying bacteria by increasing SRT was reported to enhance biotransformation of pharmaceuticals in activated sludge (Suarez et al., 2010). Using a membrane bioreactor (MBR) was found to be effective for the removal of antibiotics (erythromycin, sulfamethoxazole, ofloxacin, and trimethoprim) when run with high SRTs (Radjenovic et al., 2009). According to Yu et al. (2009), extended sludge age process with providing high SRT (higher than 200 days) and biomass concentration (16 gTSS/L) was reported to be effective for the removal of pharmaceuticals. Removal efficacies of 91 and 86% were obtained for SMX when initial concentrations of 1 and 5 μg/L were applied, respectively. Immobilized cell bio-carrier process which also provides high solid retention time was applied for the removal of antibiotics (Yu et al., 2011). This type of treatment was found to obtain above 40% removal of sulfamethoxazole by degradation and less than 40% removal by bio-sorption.

Aerobic granular sludge (AGS) is a promising treatment technology for simultaneous removal of organics and nutrients from wastewater. AGS is basically a type of biofilm formed via self-immobilization of activated sludge without any carrier. Granulation process is controlled by different factors such as: i) hydrodynamic force which provides physical movement to initiate bacterium to bacterium contact (Liu & Tay, 2002); ii) hydraulic selection pressure by which suspended flocs and sludge with poor settling capability are washed out (Wang et al., 2004); iii) production of extracellular polymeric substances which enhance polymeric interaction and promote aerobic granulation and is associated with the shear force (Tay et al., 2001; Wang et al., 2006); and iv) organic loading rate (OLR) which is a crucial factor in fast formation of stable granules and determines granules size (Tay et al., 2004).

AGS technology has attracted a lot of attention because of the unique characteristics of granules which provide: i) high biomass concentration without settling problems; ii) high biomass retention by fixing slow-growing organisms inside the granular structure; and iii) strong microbial structure by favouring the growth of organisms with different functionality inside one small unit. Additionally, due to the production of extra-cellular polymeric substances, bacteria inside biofilms are more resistant to antibiotics compared to freely floating bacteria (Schmidt et al., 2012). Despite the advantages of aerobic granules, there are limited studies on their application for degradation of such micropollutants. Balest et al. (2008) investigated the effectiveness of an aerobic granular bio-filter with SRT over 6 months and biomass concentration greater than 40 g/L for removing EDCs (estrogens, bisphenol A, and 4-tert-octylphenol) from sewage. The process achieved higher removal efficiencies (60-90 %) for all tested EDCs compared to conventional activated sludge process (41-72%). Using nitrifying granules, Wang and Ivanov (2009) obtained complete degradation of estrogens (17β-estradiol, estriol, and 17a-ethynylestradiol) with initial concentrations of 100 μg/L over 60 days.

1.2. OBJECTIVES AND HYPOTHESES

This research was conducted in three experimental phases with the following objectives:

- Phase I was aimed to study the long-term stability as well as organics and nutrient removal performance of aerobic granules cultivated with high-strength wastewater when applied for treatment of low-strength municipal wastewater.
- The purpose of phase II was to study the effect of anaerobic contact on treatment performance (effluent quality and nutrient removal efficiency) as well as properties of

granular sludge including solid residence time, settling properties, granules size, and EPS content the aerobic granules.

- Phase III was aimed to evaluate the effectiveness of aerobic granules for the removal of antibiotic sulfamethoxazole compared to conventional activated sludge. Additionally, treatment performance and microbial community structure of the two types of biomass exposed to SMX were investigated.

Hypotheses of this research are as follows:

- The research hypothesis of phase I was that the granular sludge could acclimate to the change in wastewater strength.
- In phase II, increasing contact during anaerobic phase was hypothesised to increase mass transfer and enhance anaerobic COD uptake.
- The research hypothesis in phase III was that compared to conventional activated sludge, aerobic granules could be more effective in SMX removal by providing higher SRT.

1.3. ORGANIZATION OF THIS THESIS

This document was prepared with three stand-alone manuscripts presented in chapter two, three and four. Each chapter contains its own introduction, materials and methods, results and discussion, and conclusion. Chapter two discusses the development of aerobic granular sludge with high-strength wastewater and its application for nutrient removal from low-strength wastewater. Chapter three, investigate the improvement of treatment performance of aerobic granules by increasing anaerobic COD utilization. The forth chapter studies the application of aerobic granules for the removal of antibiotic sulfamethoxazole in comparison with conventional activated sludge.

Chapter 2: Long-term stability and nutrient removal efficiency of aerobic granules at low organic loads

Abstract

The feasibility of application of aerobic granular sludge cultivated with high organic loads for biological nutrient removal (BNR) from low-strength wastewater was studied. Granules obtained with high-strength (COD = 1400 mg/L) wastewater were fed with medium (COD = 700 mg/L) and then low-strength (COD = 400 mg/L) wastewater. The granules rapidly acclimated to the medium-strength wastewater. However, feeding with low-strength wastewater reduced the F/M ratio from 0.4 to 0.2 gCOD/gVSS d. Consequently, granules' disintegration occurred and biomass settling was deteriorated. Re-granulation was obtained after the poor settling biomass (flocs) was washed out and the F/M ratio reached 0.4 gCOD/gVSS d. Disintegration of granules coincided with the decrease in extracellular polymeric substances (EPS) content and protein-to-carbohydrate ratio and re-granulation was assisted with the increase in EPS and protein-to-carbohydrate ratio. This confirms the suggested theory that granules' stability is determined by EPS content and EPS matrix. The results indicated that cultivation of aerobic granules with high organic loads and its implication for BNR treatment of low-strength wastewater while balancing the F/M ratio can be an alternative to reduce start-up period.

***Keywords**:* Aerobic granules, Long-term stability, Low organic loads, Extracellular polymeric substances, F/M ratio

2.1. INTRODUCTION

Aerobic granular sludge is a promising technology capable of removing a wide range of organics and nutrients from different types of wastewater ranging from low-strength municipal wastewater (Coma et al., 2012; Lashkarizadeh et al., 2015) to high-strength industrial effluents such as that of palm oil mills (Abdullah et al., 2011), fish canning (Figueroa et al., 2008), piggeries (Kishida et al., 2009), textiles (Muda et al., 2010) breweries (Corsino et al., 2016) and landfill leachates (Ren and Yuan, 2016).

To date, various research works have been conducted on cultivation of aerobic granules in sequencing batch reactors (SBR) and different factors such as reactor design, seed sludge, carbon source, hydrodynamic selective pressure, shear force, organic loading rate (OLR), hydraulic retention time (HRT), production of extracellular polymeric substances (EPS), F/M ratio, COD to nitrogen ratio, feast/famine balance and starvation time were found to control the granulation process. However, different authors reported different requirements for development of aerobic granules. For example, a minimum OLR of 2 kgCOD/m^3 d was first reported by Tay et al. (2004). However, successful granulation by utilizing lower OLRs was reported later (Ni et al., 2009). Although higher OLRs encourage granulation, when high-strength wastewater was used, longer HRT resulting in lower OLR was found be more effective by providing longer starvation time (Corsino et al., 2016). The high shear force with the application of up-flow air velocity higher than 1.2 cm/s was suggested as the requirement for granulation (Liu and Tay, 2002; Tay et al., 2001). Although, a recent study successfully achieved stable granules by applying hydrodynamic shear as low as 0.4 cm/s (Henriet et al., 2016). Devlin et al. (2017) suggested that removal of fast growing organisms such as ordinary heterotrophic organisms (OHO) from granule surfaces was the mechanism through which hydrodynamic shear benefits the granulation.

The granulation was achieved with low-strength wastewater at low hydrodynamic shear; while when medium and high-strength wastewater was used, unstable granules with undesirable surface coating were obtained. Therefore, the effect of different parameters should be considered together.

One of the challenges in the granulation process is biological nutrient removal (BNR) from low-strength wastewater. Successful granulation with the application of short HRT resulting in high OLR has been reported in previous studies (De Kreuk and van Loosdrecht, 2006; Ni et al., 2009). However, nitrification, denitrification, and phosphorus removal were not achieved. Coma et al. (2012) reported that granulation with low-strength wastewater for BNR purposes was unsuitable with long start-up periods. On the contrary, granules capable of nitrogen and phosphorus removal were cultivated with low-strength wastewater in few works (Devlin et al., 2017; Lashkarizadeh et al., 2015). A study by Coma et al. (2012) could enhance the granulation of flocs with the addition of crushed granules and also achieve granules capable of BNR treatment of domestic wastewater. Cultivation of aerobic granules using wastewater with higher organic loads allows the application of a longer HRT with anoxic/anaerobic and aerobic conditions that seem to be easily feasible and also beneficial for reducing start-up time. Although the application of granules cultivated with high organics for BNR treatment of low-strength wastewater could be an option to reduce start-up period, to date, no study in this area has been conducted.

The purpose of this work, was to study the stability as well as organics and nutrient removal performance of aerobic granules cultivated with high-strength wastewater when applied for treatment of medium and low-strength wastewater.

2.2. MATERIALS AND METHODS

2.2.1. Reactors configurations and operation strategies

In this study, granulation of conventional activated sludge for COD, nitrogen and phosphorus removal from high-strength wastewater (COD = 1400 mg/L) was performed in SBRs. After stable granules were achieved, the reactors were fed with medium-strength (COD = 700 mg/L) and low-strength (COD = 400 mg/L) wastewater, respectively, under the same operation conditions.

Three identical reactors named R1, R2 and R3 with 3L working volume (D = 10 cm) and the exchange ratio of 2/3 were used. A schematic diagram of the reactors is shown in Figure 2.1. Fine air bubbles were introduced from the bottom of the reactors with a flow rate of 2 L/min resulting in superficial air velocity of 0.43 cm/s. The SBRs were operated with 8-h cycles including 90 min of anaerobic feeding, 370 min of aeration, 4 min of settling and 15 min of discharge. Reactors were inoculated with conventional activated sludge taken from the South End sewage treatment plant in Winnipeg, MB, Canada. The plant was operated with SRT of 1–2 days for BOD removal from municipal wastewater. During the first stage of the experiment, reactors were operated with high-strength wastewater with COD concentration of 1400 mg/L resulting in OLR of 2.8 kgCOD/m^3 d. After 75 days, medium-strength wastewater with COD of 700 mg/L was used as feed resulting in OLR of 1.4 kgCOD/m^3 d. In the third stage, the reactors were fed with low-strength wastewater representing municipal wastewater characteristics. The operating parameters are presented in Table 2.1.

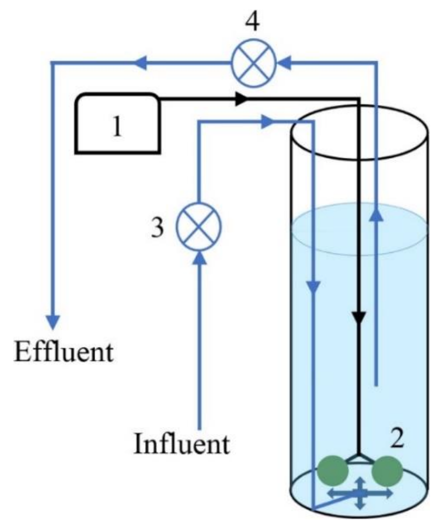

Figure 2.1. The schematic diagram of SBRs (1. Air pump; 2. Fine bubble air diffuser; 3. Feeding pump; 4. Discharge pump).

Table 2.1. Operation parameters and wastewater characteristics.

Parameter/Component	**Stage I**	**Stage II**	**Stage III**
Operating day	1–75	76–166	167–304
Yeast extract (mg/L)	1250	625	357
NH_4Cl (mg/L)	33	55	29
$K_2HPO_4 \cdot 3H_2O$ (mg/L)	9	19	20
$MgSO_4$ (mg/L)	5.8	5.8	5.8
$CaCl_2$ (mg/L)	12	12	12
EDTA (mg/L)	3	3	3
Mineral solution (mL/L)	0.3	0.3	0.3
COD (mg/L)	1400	700	400
TN (mgN/L)	140	80	45
TP (mgP/L)	20	12	8
HRT (h)	12	12	12
OLR (kgCOD/m^3 d)	2.8	1.4	0.8

2.2.2. Wastewater characteristics

Synthetic wastewater was prepared using different amounts of yeast extract, ammonium chloride (NH_4Cl) and dipotassium phosphate ($K_2HPO_4 \cdot 3H_2O$) to obtain different levels of COD, TN and TP as listed in Table 2.1. Other components such as magnesium sulfate, calcium chloride and ethylenediaminetetraacetic acid (EDTA) as well as mineral solution containing 0.15 g/L H_3BO_3, 0.03 g/L $CuSO_4 \cdot 5H_2O$, 0.03 g/L KI, 0.12 g/L $MnCl_2 \cdot 4H_2O$, 0.06 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.12 g/L $ZnSO_4 \cdot 7H_2O$, 0.15 g/L $CoCl_2 \cdot 2H_2O$, and 1.5 g/L $FeCl_3 \cdot 6H_2O$ were also used. In order to achieve complete nitrification, sufficient alkalinity (7.14 g $CaCO_3$/g NH_4^+-N) was provided by adding sodium bicarbonate (12 g $NaHCO_3$/g NH_4^+-N) to the feed during the entire operation period.

2.2.3. Analytical methods

The concentration of suspended solids in the mixed liquor (MLSS) and effluent (ETSS), influent and effluent COD and Sludge Volume Index (SVI) after 5 and 30 min of settling were measured according to standard methods. Concentrations of phosphorous in form of orthophosphate (PO_4^{3-}-P) and nitrogen in forms of ammonium (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N) were measured via a flow injection analyser (Quick Chem 8500, LACHAT Instruments) with a detection limit of 0.2 mg/L. All the measurements for nitrogen and phosphorus were performed using filtered samples, which represented the concentration of soluble nitrogen and phosphorus. In the case of COD, the measurement for filtered samples were presented as sCOD. Granule size distribution was measured using Malvern laser light scattering instrument (Mastersizer 2000 series, Malvern Instruments), which measured particle sizes ranging from 0.02 to 2000 μm. Surface morphology of the granules was observed using a stereomicroscope (Axio Vert.A1, ZEISS).

Extracellular polymeric substance content of the biomass was extracted according to Le-Clech et al. (2006). For EPS extraction, 50 mL of mixed liquor was centrifuged at 5000 rpm for 5 min. The supernatant was removed and the solids were re-suspended with deionized water up to 50 mL. The suspension was placed in a water bath at 80 °C for 10 min. After cooling down to room temperature, the sample was centrifuged at 7000 rpm for 10 min at 4 °C. The supernatant was filtered and protein and carbohydrate concentrations were measured using phenol–sulphuric acid method and Lowry protein assay, respectively. In this study, the summation of protein and carbohydrate of biomass was reported as EPS content.

In this paper, the results for MLSS, ETSS, SVI_5, granules' size and EPS are the average of replicates of the three identical reactors and error bars represent the standard error of the mean (SEM). However, the results of effluent sCOD, nitrogen and phosphorus concentrations are individually reported for each reactor.

2.3. RESULTS AND DISCUSSION

2.3.1. Aerobic granulation with high-strength wastewater

The concentrations of MLSS and SVI_5 are presented in Figure 2.2. At the beginning of the experiment, the reactors were inoculated with more than 6 g/L of conventional activated sludge. However, MLSS decreased to 5.2 ± 0.2 g/L after one day of operation, due to the discharge of poor settling flocs. The decrease in the reactors MLSS continued until day 40 when the biomass concentration of 3.3 ± 0.1 gTSS/L was observed. It should be noted that the biomass reduction increased food to microorganisms (F/M) ratio from around 0.45 to 0.99 ± 0.03 gCOD/gVSS d. It was reported that higher F/M ratios favor production of extracellular polymeric substances and consequently enhance granulation (Li et al., 2011; Tay et al., 2004). From day 40 to 69, biomass accumulation was observed and MLSS reached 6.7 ± 0.4 g/L. With the increase in MLSS, F/M ratio decreased to 0.50 ± 0.03 gCOD/gVSS d at the end of the first stage. The results were in agreement with the suggested F/M ratios to initiate granulation and to maintain stability. Tay et al. (2004) reported that stable granules with 0.33 gCOD/gVSS d of F/M were obtained when initial F/M ratio of 1.35 gCOD/gVSS d was applied at high OLR (4 gCOD/gVSS d). Li et al. (2011), suggested F/M ratio higher than 1.1 gCOD/gVSS d for initializing granulation and F/M ratio lower than 0.3 gCOD/gVSS d for granules stabilization.

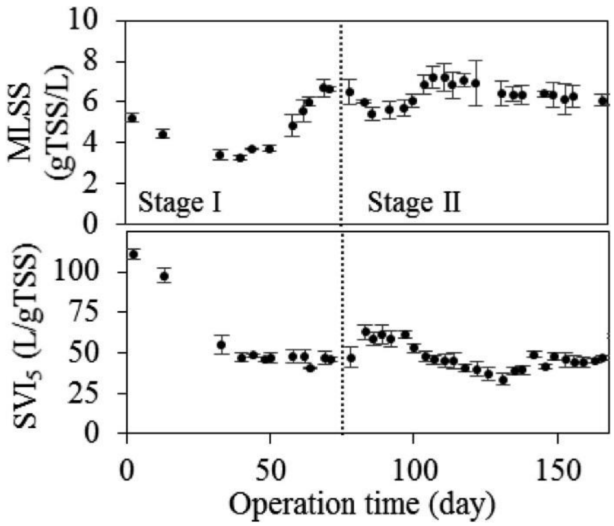

Figure 2.2. Mixed liquor suspended solids (MLSS) concentration and sludge volume index after 5 min (SVI_5) during the first two stages.

Although the application of selective pressure resulted in the residence of biomass aggregates, it caused high suspended solids concentrations in the effluent (ETSS) up to 429 ± 65 mg/L on day 48. However, after the appearance of granules and biomass accumulation, ETSS decreased to 42 ± 5 mg/L at the end of the first stage. The effluent quality confirmed that the applied hydrodynamic shear and HRT was appropriate to avoid accumulation of fast growing organisms such as OHO in the suspension. It was recently reported that OHOs accumulated in the suspension and also appearing on granules' surface caused undesirable surface coating when hydrodynamic shear of 0.41 cm/s was applied for granulation with high-strength with wastewater

(Devlin et al., 2017). Based on the reactors MLSS and ETSS, the average solid retention time of 34 ± 3 days was calculated for the granules in the first stage, suggesting the proliferation of slow growing organisms in the granular sludge.

The sludge volume index after 5 min of settling decreased from 111 ± 3 to 55 ± 6 mL/g after 33 days of operation. Granules with $SVI_5 < 50$ mL/g and mean diameter greater than 200 μm were obtained after 40 days of operation. Results from microscopic analyses indicated that granules were uniform and compact with a smooth surface. The granules were yellowish-brown in color with a thin outer layer of a white substance which could be active cells or EPS. Mature granules with SVI_5 of 46 ± 2 mL/g and a diameter greater than 2 mm were obtained at the end of the first stage of operation.

The results of the first stage confirmed the important role of selective pressure and F/M ratio in granulation of activated sludge. Stable granules were successfully cultivated with high-strength wastewater by applying HRT of 12 h which resulted in OLR of 2.8 kgCOD/m^3 d and about 4.5 h of starvation time. This strengthened the importance of starvation time over OLR, as suggested by Liu and Tay (2007). Compact granules with desirable surface properties could be successfully achieved at low hydrodynamic shear (superficial upflow air velocity of 0.43 cm/s). Successful granulation was also achieved at the dynamic shear as low as 0.42 cm/s as reported by Henriet et al. (2016). The application of high superficial air velocity (higher than 1.2 cm/s) was suggested as a crucial factor in the granulation process (Liu and Tay, 2002; Tay et al., 2001). However, Devlin et al. (2017) also reported that low dynamic shear applying up-flow air velocity of 0.41 cm/s resulted in the undesirable surface coating when medium and high-strength wastewaters were used.

2.3.2. Stability of the granules at medium-strength wastewater

In the second stage of operation, mature granules cultivated with high-strength wastewater were fed medium-strength wastewater that resulted in the reduction of OLR from 2.7 to 1.4 kgCOD/m^3 d. Consequently, F/M ratio decreased from 0.50 ± 0.03 to 0.29 ± 0.03 gCOD/ gVSS.d. As a result of this change, SVI_5 rapidly increased from 46 ± 2 to 63 ± 4 mL/g. Consequently, MLSS decreased from 6.7 ± 0.4 to 5.4 ± 0.3 g/L due to the discharge of biomass with poor settling and F/M rose to 0.33 ± 0.02 gCOD/gVSS d. However, in less than 20 days, stable granules with SVI_5 of 45 ± 3 mL/g and MLSS concentration of 7.2 ± 0.5 g/L was recorded again.

As a result of the discharge of poor settling biomass, ETSS rapidly increased up to 302 ± 37 mg/L on day 93, however, after obtaining well settling biomass, the ETSS gradually decreased to 69 ± 13 mg/L on day 118. High levels of solids up to 424 ± 91 mg/L were found in the effluent again (day 153). At this time, the presence of granules in the effluent caused high ETSS. Biomass accumulation to the level exceeding the capacity of the reactors was the potential reason. A gradual decrease in both MLSS and ETSS was observed until the end of this stage with the concentrations of 6.2 ± 0.2 g/L and 58 ± 12 mg/L for MLSS and ETSS, respectively. The average solid residence time of 34 ± 2 days was calculated for the stable granules at the end of this stage.

The change in OLR also affected the granules' size. An expected gradual decrease in the granules' size was observed during this stage. It has been previously reported that organic loading rate determines granules' size (Tay et al., 2004). More compact granules with a mean diameter of 1.11 ± 0.04 mm were obtained at the end of the second stage. The EPS content (protein + carbohydrate) of the granules at the end of this stage was measured as 150.3 ± 3.0

mg/gVSS with a protein-to-carbohydrate ratio of 25.2 ± 3.2. These values for a suspended activated sludge taken from a lab-scale reactor operating at the same HRT and feed characteristics (but SRT of 10 days) were measured as 115.8 ± 1.1 mg/gVSS and 12.9 ± 0.8, respectively. However, the suspended biomass was a well-settling sludge with SVI_{30} of 33 ± 2 mL/g. The results of EPS measurements confirmed the higher levels of EPS and a higher ratio of protein to carbohydrate in granular sludge when compared to the flocs as reported by other researchers (Wang et al., 2006; Zhang et al., 2007; Lashkarizadeh et al., 2015).

2.3.3. Stability of the granules at low-strength wastewater

In the third stage of operation, stable granules obtained at the end of the second stage were fed with a low-strength wastewater, resulting in OLR of 0.8 kgCOD/m^3 d. The results from monitoring biomass concentration and settling properties of the granules during this stage are presented in Figure 2.3a. With the change in COD load, the F/M ratio decreased from 0.35 ± 0.03 to 0.24 ± 0.02 gCOD/gVSS d. Consequently, the granules disintegrated and the biomass settling was demolished. As can be seen in Figure 2.3a, rapid changes in SVI_5 occurred in the first 2 weeks of this stage. The concentration of MLSS decreased from 6.2 ± 0.2 to 3.7 ± 0.1 g/L in 10 days due to the discharge of poor settling biomass. The MLSS continued to decline for more than 2 months and the lowest concentration was recorded as 2.2 ± 0.1 g/L on day 243. This resulted in a gradual increase in F/M up to 0.45 ± 0.03 gCOD/gVSS d. After this point, re-granulation was observed followed by improvement of the biomass settleability with the decrease in SVI_5. At the end of this stage, the granular sludge achieved SVI_5 and MLSS concentration of 47 ± 3 mL/g and 3.0 ± 0.3, respectively. Also, the F/M ratio was calculated as 0.33 ± 0.03 gCOD/gVSS d. Although after re-granulation, quality of the effluent in terms of suspended solids improved; however, fluctuation in the ETSS concentration was observed. The average concentration of 87 ± 3 mg/L was calculated for the TSS in the effluent for this stage. Based on the concentration of the biomass in the reactor and in the effluent, the average SRT of 16 ± 0.6 days was calculated.

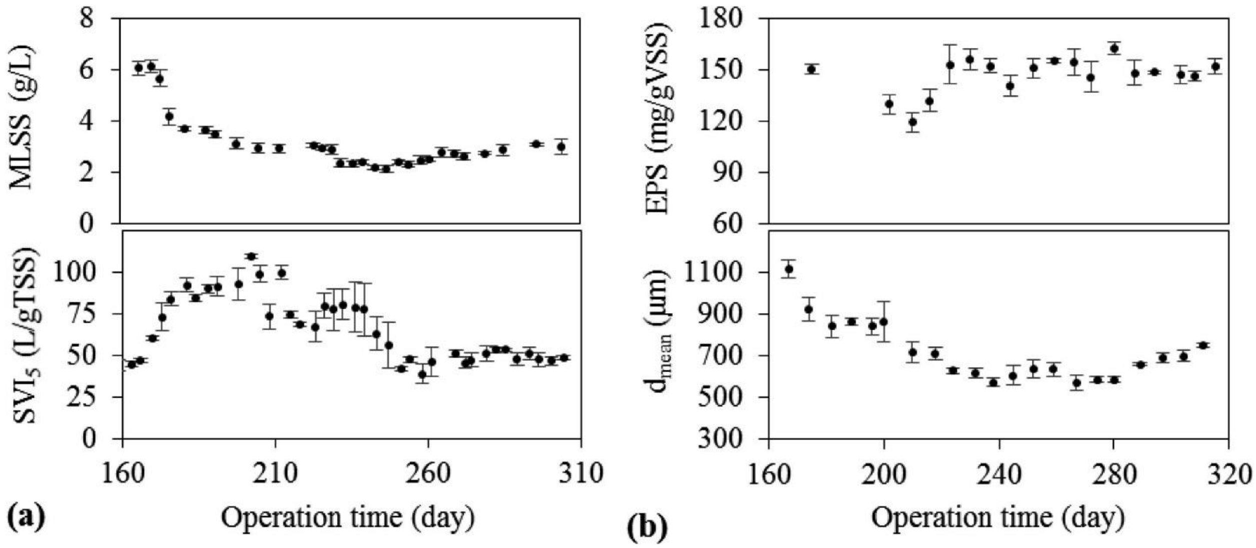

Figure 2.3. (a) MLSS and SVI_5 and (b) EPS and granule sizes under low OLR (stage 3).

The data of EPS and granules' size measurements are presented in Gigure 2.3b. As can be seen, the granules size gradually decreased from 1.11 ± 0.04 to 0.57 ± 0.02 mm on day 238 and no further reduction was observed afterward. The reduction in granules' size caused by disintegration is in agreement with the results of biomass settling which showed an increase in SVI_5. After the start of re-granulation, the mean diameter increased over time, which coincided with the decrease in SVI_5. At the end of this stage the compact granules with mean diameter of 0.75 ± 0.01 mm was obtained.

As shown in Fig. 3b, EPS content (protein + carbohydrate) of the granular biomass decreased from 150.3 ± 3.0 to 119.4 ± 5.7 mg/gVSS from day 175 to 210. The decrease in the EPS coincided with the granules disintegration and deterioration of biomass settling. In addition to EPS content of the biomass, a change in the EPS component was observed during this period, wherein the ratio of protein-to-carbohydrate decreased from 25.2 ± 3.2 to 10.2 ± 0.3. After acclimation of the microorganisms to the new feed, production content increased up to 156.3 ± 6.3 mg/gVSS on day 230 after which re-granulation and improvement of the biomass settling was observed. After this point, a gradual change in the ratio of protein-to-carbohydrate was observed. The ratio increased up to 31.2 ± 1.3 on day 252 when compact granules were achieved. The results showed the role of EPS content especially protein in re-granulation and formation of stable granules. Increase in EPS content as well as the ratio of protein-to-polysaccharide during the formation of granules from flocs and re-granulation was reported in previous studies (Lashkarizadeh et al., 2015; Wang et al., 2006; Zhang et al., 2007). It was also reported that protein content is responsible for stability and settleability of granules (Zhang et al., 2007; Zhu et al., 2012). However, elsewhere, polysaccharides or types of glycosides were found as gel-forming components of EPS and were considered responsible for granules' stability (Seviour et al., 2009).

Although the change in the wastewater strength from medium to low reduced the EPS content; however, after re-granulation, the new granules obtained EPS levels as high the previous stage. This was not observed in the case of granules size and the new granules could never achieve their initial size. This can be explained by the fact that granules size is determined by organic loading rate (Tay et al., 2004) while EPS production is encouraged by shear force and selective pressure (Lin et al., 2008; Tay et al., 2001). EPS production was also reported to be affected by feast/famine balance (Liu and Tay, 2007) and the length of the starvation period (Corsino et al., 2016).

In our work, the organics load decreased with the reduction in COD from 700 to 400 mg/L while the hydraulic retention time (HRT) remained the same. Therefore, fewer organics were available and hence were consumed in a shorter time. Consequently, at lower organic loads, the microorganisms experienced a shorter feast and a longer starvation period. Accordingly, bacteria produced more EPS to utilize as carbon and energy source for endogenous respiration when the presence of substrate was limited (Zhu et al., 2012). Corsino et al. (2016) reported that when lower OLR was applied with increasing HRT, EPS production was enhanced and resulted in more compact and hence smaller granules. They also reported that higher protein-to-carbohydrate ratio was achieved when lower OLR (longer HRT) was used. These results confirmed the role of starvation time in both EPS production and EPS composition of granules.

The results from the third stage indicated that implication of aerobic granules stabilized with medium-strength wastewater for treating low-strength wastewater is feasible. However, a long time is required for the biomass acclimation and re-granulation when the organic loads are reduced but the same MLSS and HRT can be applied. The results confirmed that F/M is a controlling factor in the stability of the granules. Therefore, the granules should be dosed according to COD of the feed to balance the F/M ratio. This could avoid long time required for acclimation and stabilization and also avoid the biomass wash-out and hence high ETSS concentrations can be attained.

2.3.4. Organics and nutrient removal efficiency of the granules

The result of monitoring reactors effluent quality in terms of sCOD, ammonium, nitrite, nitrate, and phosphorus concentration is presented in figure 2.4. Organic and nutrient removal efficiency of the reactors during the different stage are presented in table 2.2. During the first stage, when influent COD was high (1400 mg/L), sCOD in the effluent was not stable and increased up to 120 mg/L. Average COD removals for R1, R2 and R3 were 94.1 ± 0.4, 93.9 ± 0.3 and 94.1 ± 0.3%, respectively. Results of a kinetic test on day 55 showed COD removal between 33 and 55 percent in the 1.5 h anaerobic phase. The remaining COD was utilized very fast in the aerobic zone and it decreased to less than 50 mg/L after 1.5 h.

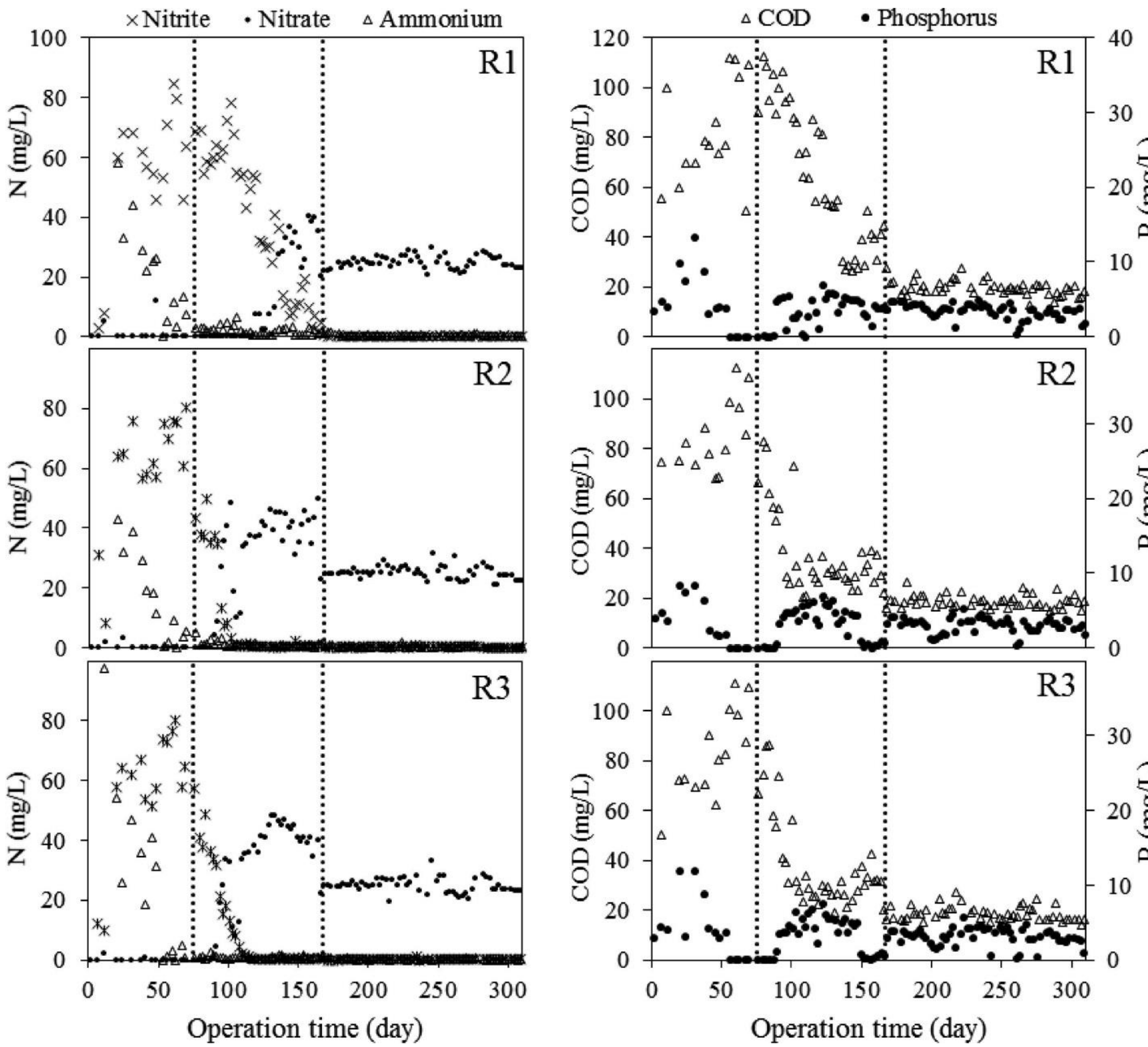

Figure 2.4. Concentrations of sCOD, nitrogen and phosphorus in the effluent during the three stages of operation.

Phosphorus removal higher than 80% was observed in the three reactors in one week after inoculation that improved until day 56; however, no soluble phosphorus was detected in the reactors' effluent thereafter. It was found that the granules are capable of EBPR (enhanced biological phosphorus removal) performed by polyphosphate accumulating organisms (PAOs).

In addition to anaerobic sCOD uptake, 6–11 mgP/gVSS anaerobic phosphorus release in followed by 8–11 mgP/gVSS aerobic uptake was observed in the reactors, confirming the activity of PAOs. In the first stage, phosphorus removal efficiencies of 92.6 ± 3.1, 96.3 ± 1.5 and 92.8 ± 3.0 percent was obtained for R1, R2 and R3, respectively.

Nitrification was not observed until day 20, which was due to the type of activated sludge used as inoculum. The seed sludge was taken from a conventional activated sludge system operated with SRT of 1–2 days for BOD removal. Therefore, nitrification was not achieved until nitrifiers which are relatively slow growth organisms accumulated in the system. After day 53, ammonium removal as high as 99% was observed in the three reactors, confirming the proliferation of ammonia oxidizing bacteria (AOB) in the system. However, after this point, fluctuations in the effluent's ammonium concentration ranging from 0.5 to 13.2 mgN/L as well as effluent nitrite from 43.5 to 84.6 mgN/L were observed. Nitrate was not detected in any of the reactors effluent. This could be due to the absence of nitrite oxidizing bacteria (NOBs) in the system, which was confirmed by the results of the kinetic tests. However, nitrogen removal of 47.0 ± 3.3, 47.8 ± 3.5 and 48.0 ± 2.4 percent was achieved by R1, R2, and R3, respectively.

When the reactors were fed with medium strength wastewater, effluent sCOD decreased to less than 50 mg/L in the three reactors. Average COD removal efficiencies of 93.1 ± 0.5, 95.8 ± 0.1 and 95.9 ± 0.2% was achieved for R1, R2 and R3, respectively. With the reduction in influent ammonium from 140 to 80 mg/L, nitrification was improved and effluent nitrite of the reactors gradually decreased to less than 1 mg/L. Consequently, the concentration of nitrate increased from 35 to 48 mgN/L. Denitrification was also observed in the anaerobic phase of the three reactors. The result of monitoring sCOD showed 52–61 percent anaerobic COD removal which was higher than that of the first stage, confirming the rbCOD uptake by denitrifiers. At this stage, the average nitrogen removal efficiencies for R1, R2, and R3 were 47.0 ± 1.3, 52.5 ± 2.2 and $51.9 \pm 2.1\%$. No soluble phosphorus was detected in the reactors effluent until day 89. After this point, variation in the phosphorus removal of the reactors was observed and concentrations as high as 6.9 mg/L were recorded. This could be due to the transition from the anaerobic phase to anoxic that resulted in nitrate production and caused the competition of PAOs and denitrifiers for rbCOD uptake. Microorganisms that compete with PAOs such as glycogen-accumulating organisms (GAOs) were reported as dominant species in the granules when influent COD ranging from 500 to 800 mg/L was used (Yu et al., 2014). With the acclimation of PAOs to the new condition, phosphorus removal improved in R2 and R3 and effluent concentrations less than 1 mgP/L (soluble phosphorus) was achieved after day 150. However, in the case of R1 stable phosphorus was not achieved and fluctuation between 1.4 and 4.6 mgP/L was observed in the reactor effluent.

In the third stage, when the reactors were fed with low-strength wastewater, the effluent sCOD decreased to below 25 mg/L. In terms of nitrogen, nitrate concentration between 20 and 30 mgN/L was obtained, while ammonium and nitrite were not detected in the reactors' effluent. Nitrogen removal efficiencies of 43.2 ± 0.6, 42.8 ± 0.8, and $45.4 \pm 1.6\%$ were obtained for R1, R2, and R3, respectively. In terms of nitrification and COD removal, the change in the feed and accordingly the granule disintegration did not cause any problems. On the contrary, high phosphorus removal efficiencies (>96%) obtained for R1 and R2 dwindled right after the change in feed. This was caused due to the less available rbCOD which is required for both denitrification and EBPR. The fluctuation between 50 and 95% was observed in phosphorus removal efficiency of the reactors. Fluctuation in the anaerobic COD uptake by denitrifiers and PAOs was also observed. It was found that due to the small size of the reactors, the anaerobic feeding over 1.5 h could not provide mixing or a proper contact between substrate and microorganisms. Liu and Tay (2002) suggested that hydrodynamic turbulence plays an important role in the mass transfer of substrate and hence substrate flux in the biofilm. Rocktäschel et al. (2013) also reported that mixing during the anaerobic phase benefits the nutrient removal with equal distribution of substrate. It should be noted that when it comes to full-scale applications, anaerobic plug-flow feeding at high flow-rates would be effective to produce hydrodynamic turbulence and provide adequate contact.

Table 2.2. Characteristics and performance of granular sludge at different stages of operation.

Parameter	**Stage I**	**Stage II**	**Stage III**
SRT (d)	34 ± 3	34 ± 2	16 ± 1
SVI_5 (mL/g)	46 ± 2	45 ± 3	47 ± 3
MLVSS (g/L)	4.6 ± 0.2	4.4 ± 0.4	2.8 ± 0.3
Effluent VSS (g/L)	38 ± 11	41 ± 10	88 ± 17
Granules’ size (mm)	>2	1.11	0.75
COD removal (%)			
R1	94.1 ± 0.4	93.1 ± 0.5	95.4 ± 0.5
R2	93.9 ± 0.3	95.8 ± 0.1	95.3 ± 0.7
R3	94.1 ± 0.3	95.9 ± 0.2	95.4 ± 0.7
N removal (%)			
R1	47.0 ± 3.3	47.0 ± 1.3	43.2 ± 0.6
R2	47.8 ± 3.5	52.5 ± 2.2	42.8 ± 0.8
R3	48.0 ± 2.4	51.9 ± 2.1	45.4 ± 1.6
P removal (%)			
R1	92.6 ± 3.1	71.3 ± 2.7	62.9 ± 2.6
R2	96.3 ± 1.5	73.9 ± 3.1	64.2 ± 2.5
R3	92.8 ± 3.0	72.2 ± 3.2	63.4 ± 3.2

2.4. CONCLUSION

In this study, stability of aerobic granules at different organic loads was studied. The results demonstrated that: 1) When COD was changed from 1400 to 700 mg/L, granules were rapidly acclimated while, applying COD of 400 mg/L caused disintegration of the granules due to the reduction of F/M ratio from 0.4 to 0.2 gCOD/gVSS d. Re-granulation was obtained when F/M ratio reached 0.4 gCOD/gVSS d after discharge of poor settling biomass. 2) Both Granules' EPS content and protein-to-carbohydrate ratio decreased with the disintegration of the granules and then increased with re-granulation. EPS production play a significant role in granules' stability, therefore, EPS play a significant role in the granules' stability. 3) Lower organic loads resulted in higher EPS content and protein-to-carbohydrate ratio and hence more compact granules were obtained.

Chapter 3. Effect of Anaerobic COD Utilization on the Treatment Performance of Aerobic Granules in Anaerobic/Aerobic SBRs

Abstract

Influence of anaerobic COD consumption on the properties of aerobic granules and their nutrient removal performance was studied in sequencing batch reactors (SBR). Three identical anaerobic/aerobic SBRs were operated under two operation strategies over four months. During the first stage, anaerobic feeding was applied for a 90 min. anaerobic phase, while in the next stage, 15 min. of feeding followed by 75 min. of anaerobic contact was provided with circulating the liquid through settled biomass. Results indicated that, anaerobic contact increased anaerobic COD utilization from 17-24% to 45-53% with improving mass transfer and consequently, improved phosphorus removal from 63 to 93%. The anaerobic contact also improved the effluent quality from 87 to 46 mg/L VSS with prevention of flocs (rapidly-growing organisms) formation. With the application of anaerobic contact, slowly growing organisms dominated the system and solid retention time (SRT) increased from 15 to 32 days. Alerting anaerobic continuous feeding with anaerobic contact also affected granules' properties. More compact granules with less extracellular polymeric substance (EPS) content and lower protein-to-carbohydrate (P/C) ratio were obtained when anaerobic contact was applied.

Keywords: Aerobic granules, Aerobic/anaerobic SBR, Anaerobic COD uptake, Treatment performance, Nutrient removal, Granules' properties.

3.1. INTRODUCTION

Aerobic granular sludge is a type of biofilm formed by self-immobilization of activated sludge in spherical shape when hydrodynamic shear force (resulted from air flow) and hydraulic selective pressure (short settling time) are applied (Devlin et al., 2017). The unique structure of aerobic granules offers different layers with aerobic, anaerobic and anoxic conditions, which provides simultaneous removal of organics, phosphorous, and nitrogen from wastewater (Winkler et al., 2013).

Successful application of aerobic granules for organics removal from municipal wastewater in aerobic SBRs has been reported (De Kreuk and van Loosdrecht, 2006; Ni et al., 2009). However, nitrification, denitrification and phosphorus removal were not achieved due to the high organic loading rate (OLR) applied. Coma et al., (2012) reported that granulation with low-strength wastewater for biological nutrient removal (BNR) is unsuitable and requires long start-up periods the low OLR required to obtain BNR. Results of the second chapter suggested that granulation at high OLR and application of lower OLR can obtain stable granules with BNR.

Stable granules capable of simultaneous nitrogen and phosphorus were obtained with low-strength wastewater in few studies (Devlin et al., 2017; Lashkarizade et al., 2015). However, biological removal of nutrients, especially phosphorus, from low-strength wastewater with granular sludge has been a challenge (Yu et al., 2014; Coma et al., 2012; Henriet et al., 2016). Competition between polyphosphate accumulating organisms (PAO) and glycogen-accumulating organisms (GAO) for carbon uptake during the anaerobic phase was suggested as a possible reason for unsuccessful phosphorus removal in granular systems (Yu et al., 2014). Different strategies were applied to enhance granules' phosphorus removal. Selective biomass removal was found to improve the activity of PAOs (Winkler et al., 2011) and their studied found

Competibacter (GAOs) to be more dominant at the top of the settled sludge while *Accumulibacter* (PAOs) was more dominant at the bottom. However, Henriet et al. (2016) reported that *Candidatus Accumulibacter* (PAO) was more dominant in intermediate density fraction of granules. Therefore, high selective pressure with short settling time has improved phosphorus removal.

Anaerobic COD removal during the feeding period was also beneficial for the development of aerobic granules with the reduction in yield of rapidly growing aerobic microorganisms (Devlin et al., 2017). Rocktäschel et al. (2013) found mixing advantageous to anaerobic COD utilization and hence enhanced phosphorus release and denitrification due to high mass transfer. However, at large scale, anaerobic feeding can be strong enough to provide hydrodynamic turbulence, which benefit mixing. It should be noted that shear force in the anaerobic period can affect the granules' structure. Also, excess shear can be harmful to granules and cause disintegration of the granules. Therefore, a feeding system that provides an equal substrate distribution can provide anaerobic COD uptake and enhance BNR treatment (Rocktäschel et al., 2013), while being cost-effective as compared to anaerobic mixing in large-scale SBRs.

The purpose of this work was to study the effect of anaerobic COD utilization on nutrient removal performance of the aerobic granules. Moreover, the effluent quality as well as properties of granular sludge including solid residence time, settling properties, granules size, and EPS content were monitored under the two operating conditions: (i) continuous anaerobic feeding and (ii) feeding followed by anaerobic contact.

3.2. MATERIALS AND METHODS

3.2.1. Reactor configuration and operation strategies

Three identical reactors named R1, R2, and R3 with 3 L working volume and 10 cm diameter were used. The reactors were fed with 2 L wastewater (resulting in an exchange ratio of 2/3) delivered through a four-way splitter located on the reactors' floor. Fine air bubbles were introduced from the bottom of the reactors through two spherical stone diffusers having 3 cm diameter. Air flow rate of 2 L/min during the aerobic period resulted the superficial air velocity of 0.43 cm/s. Dissolved oxygen (DO) was not controlled during the aeration and DO reached saturation. The sequencing batch reactors with the schematic diagram shown in figure 3.1 were operated in 8 hour cycles including: 90 min of anoxic/anaerobic phase, 370 min of aerobic phase, 4 min of settling and 15 min of discharge.

During the first stage of the experiment, the anaerobic phase was obtained during the feeding over 90 min; while in the second stage, the operation was changed to 15 min feeding followed by 75 min of anaerobic contact. The aerobic phase, settling and discharge time was the same during the two stages. Anaerobic contact was provided by circulation of wastewater with pumping from the top of the reactor to the bottom with 0.1 L/min flow rate, while the biomass was being settled. Solid retention time was not controlled during the whole operation period. Therefore, the only way of wasting biomass was through the effluent solids. The three identical reactors were operated under the same condition in order to ensure reproducibility to the study.

Aerobic granules with mean size of 1.11 ± 0.04 mm and extracellular polymeric substance (EPS) content of 150.3 ± 3.0 mg/gVSS were initially obtained with OLR of 1.4 $kgCOD/m^3d$. The reactors were then operated under the conditions of first stage (resulting in OLR of 0.8

kgCOD/m^3d) for over two months until stable granules were achieved. In this work, data reported for the first stage are for the stable granules.

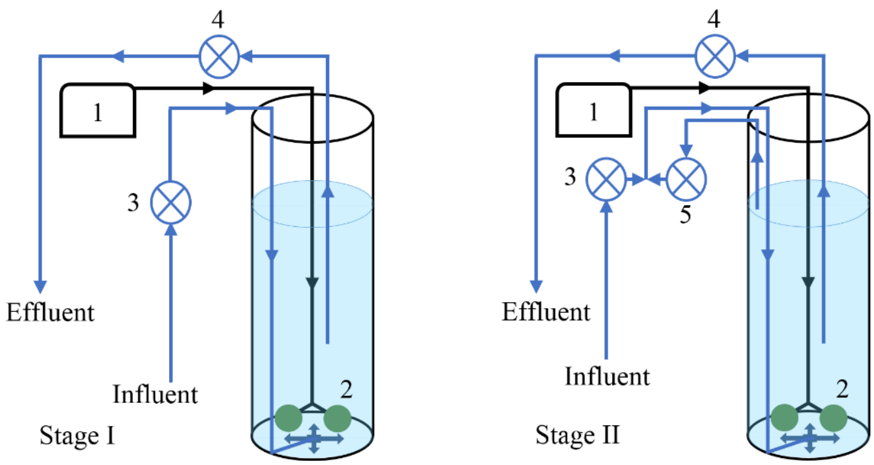

Figure 3.1. The schematic diagram of SBRs for the two stages of operation (1. Air pump; 2. Fine bubble air diffuser; 3. Feeding pump; 4. Discharge pump; 5. Circulating pump)

3.2.2. Wastewater characteristics

Synthetic wastewater simulating the characteristics of typical municipal wastewater with COD, TN and TP concentrations of 400, 45 and 8 mg/L, respectively, was used in this study. Yeast extract (357 mg/L) was used as source of COD (400 mg/L), nitrogen (37 mgN/L) and phosphorus (5.3 mgP/L). In order to obtain characteristics of municipal wastewater, nitrogen and phosphorus concentrations were adjusted using 30m/L NH_4Cl (8 mgN/L) and 20 mg/L $K_2HPO_4.3H_2O$ (2.7 mgP/L). Other ingredients were 5.8 mg/L $MgSO_4$, 12 mg/L $CaCl_2$ and 3 mg/L EDTA. Additionally, 0.3 mL/L of mineral solution containing 0.15 g/L H_3BO_3, 0.03 g/L $CuSO_4.5H_2O$, 0.03 g/L KI, 0.12 g/L $MnCl_2.4H_2O$, 0.06 g/L $Na_2MoO_4.2H_2O$, 0.12 g/L $ZnSO_4.7H_2O$, 0.15 g/L $CoCl_2.2H_2O$, and 1.5g/L $FeCl_3.6H_2O$ was used for the wastewater preparation. In order to achieve complete nitrification, sufficient alkalinity was provided by adding sodium bicarbonate (12g $NaHCO_3$/g NH_4^+-N) to the feed during the whole operation period.

3.2.3. Analytical methods

The concentration of suspended solids in the mixed liquor (MLSS) and effluent (ETSS), influent and effluent COD and Sludge Volume Index (SVI after 5 and 30 minutes settling) were measured according to standard methods for the examination of water and wastewater. Concentrations of phosphorous in form of orthophosphate (PO_4^{3-}-P) and nitrogen in forms of ammonium (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N) were measured via a flow injection analyser (Quick Chem 8500, LACHAT Instruments), with the detection limit of 0.2 mg/L. All the measurement for nitrogen and phosphorus are performed using filtered samples (via 0.45 µm filters), which represented the concentration of soluble nitrogen and phosphorus. In the case of COD, the measurement for filtered samples was presented as sCOD. Granules size distribution was measured using Malvern laser light scattering instrument (Mastersizer 2000 series, Malvern Instruments), with the capacity to measure particle sizes ranging from 0.02 to 2000 µm. Surface morphology of the granules was observed using a stereomicroscope (Axio Vert.A1, ZEISS).

The EPS content of the biomass was extracted and measured according to the method carried out by Le-Clech (2006). Briefly, 50 mL of mixed liquor was centrifuged at 5000 rpm for 5 minutes. The supernatant was removed and the solids were re-suspended with deionized water up to 50 mL. The suspension was placed in a water bath at 80 °C for 10 minutes. After cooling down to room temperature, the sample was centrifuged at 7000 rpm for 10 minutes at 4 °C. The supernatant was filtered and protein and carbohydrate concentrations were measured using phenol–sulphuric acid method and Lowry protein assay, respectively. In this study, the summation of protein and carbohydrate concentrations was reported as EPS.

Mixed liquor samples for MLSS, EPS, granules' size and SVI analyses were grabbed from the middle point of the reactors. Except the samples for EPS which were always taken two hours after the start of cycle (30 min after the start of aerobic phase), other mixed liquor samples were collected at the end of aerobic phase. In this paper, results for sludge properties such as SVI_5, granule sizes and EPS are reported in the form of average of replicates of the three identical reactors and error bars in the graphs represent standard error of the mean. However, the results of MLSS, ETSS, sCOD, nitrogen and phosphorus concentrations are reported for each reactor, individually.

3.3. RESULTS AND DISCUSSION

3.3.1. Biomass quantities and effluent quality

Results of monitoring biomass concentration in the reactors in the form of TSS and VSS during both stages of operation are presented in Figure 3.2. At the beginning of the first stage, R1, R2, and R3 contained 2.0, 1.7 and 2.0 g/L MLVSS, respectively. During this stage, biomass acclimation was observed and the MLVSS increased to 2.6, 2.1 and 2.8 g/L in R1, R2 and R3, respectively. The acclimation was expected in the system because the biomass was only removed by effluent suspended solids. The ratio of VSS/TSS for the biomass was stable in the three reactors during this stage and an average of 0.80 ± 0.01 was calculated for the three reactors.

The concentrations of VSS and TSS in the reactors' effluent over time are shown in Figure 3.2. During the first stage, effluent VSS was found in the range of 30 to 140 mg/L with an average concentration of 87 ± 5 mg/L for the three reactors. With the change in operation from anaerobic feeding to anaerobic contact, an immediate increase was observed in the effluent VSS to 166, 214 and 108 mg/L in R1, R2, and R3, respectively. Consequently, biomass concentration in R1, R2 and R3 was reduced to 2.5, 1.9 and 2.6 g/L VSS. Moreover, after acclimation, the effluent VSS decreased down to 10 mg/L on day 67. However, the effluent VSS was not stable after this day and fluctuated between 10 to 82 mg/L. An average VSS concentration of 46 ± 3 mg/L was calculated for the reactors during this stage, which was 47% less than that of the first stage.

As a result of the decrease in effluent VSS, biomass acclimation was obtained and MLVSS reached 3.0, 3.1 and 3.8 g/L in R1, R2, and R3, respectively, at the end of the second stage. The average ratio of VSS/TSS for the biomass was 0.76 ± 0.01 in all reactors. This biomass acclimation under the condition that the organic load is fixed caused a decrease in F/M ratio. The F/M ratio decreased from 0.42 to 0.33 gCOD/gVSS.d during the first stage and then to 0.25 gCOD/gVSS.d during the second stage of operation.

As can be seen in Figure 3.2, a decrease in the effluent solid resulted in an increase in the MLSS in the reactors and vice versa. Therefore, biomass acclimation was controlled with the effluent quality which was dependent on the settling performance of the biomass. The biomass residence time, which was calculated based on quantities of biomass in the reactors and the effluent, was determined by the effluent quality. The average SRT in the three reactors was 15 ± 1 d during the first stage, while in the second stage, average SRT of 32 ± 3 d was obtained with the decrease in effluent VSS. Results of SRT and effluent quality for the three reactors during the first and second stage of operation are listed in table 3.1.

Results of statistical analysis showed that in the three reactors, SRT and effluent solids was significantly influenced ($p<0.05$) by the change in the operation. Compared to anaerobic feeding strategies, anaerobic contact obtained better effluent quality and resulted in higher biomass residence time in the system. This suggested that in the second stage, the slow-growth organisms could proliferate in the system. Also, less biomass in the effluent suggested that ordinary heterotrophic organisms (OHOs) had less chance to grow in the suspension when potentially no readily biodegradable COD (rbCOD) was present in the aerobic phase. Devlin et al. (2017) also reported that anaerobic uptake of COD (55-77% of total COD removal) reduced the yield of rapidly growing aerobic microorganisms.

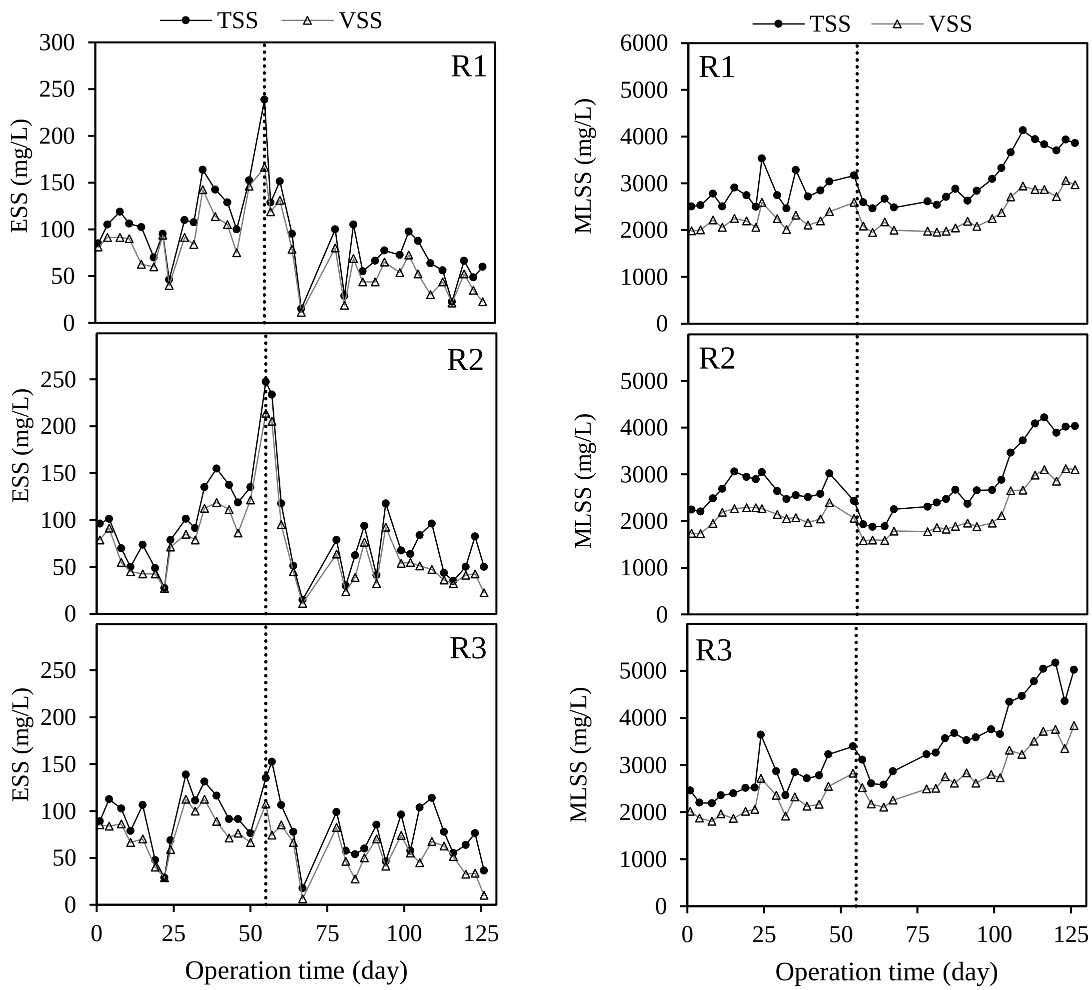

Figure 3.2. Mixed liquor suspended solid and effluent suspended solid concentrations in the reactors

3.3.2. Organics and nutrient removal performance of the granules

The effluent concentrations of COD, nitrogen, and phosphorus removal during the 125 days of operation with low-strength wastewater are presented in figure 3.3. Based on these results, COD, TN and TP removal efficiency of the reactors during the two stages was calculated and presented in Table 1. During the two stages, effluent sCOD ranged from 15 to 25 mg/L with an average removal efficiency of 95% in the three reactors. However, anaerobic COD removal was different in the two stages. COD removal during anaerobic feeding was between 17 to 24%, while contact enhanced COD removal from 45 to 53% in the anaerobic phase. Rocktäschel et al. (2013) also reported that compared to anaerobic feeding, anaerobic mixing achieved higher COD uptakes. However, Devlin et al. (2017) reported 55-77% COD uptake from low-strength wastewater during one hour of anaerobic feeding in four liter reactors without mixing.

During the two stages, nitrate concentration between 20 to 30 mgN/L was obtained, while ammonium and nitrite were not detected in the reactors' effluent. Results presented in Table 3.1 showed that the second stage obtained higher nitrogen removals in the three reactors. This could be due to complete denitrification obtained during anaerobic contact, which is explained later.

The reactors' effluent phosphorus fluctuated between values approximately 1 mgP/L to higher than 4 mgP/L during the first stage. Average phosphorus removal of less than 65% was achieved in the first stage. However, when anaerobic contact was applied, stable phosphorus removal with effluent phosphorus less than 1 mg/L was obtained in one week. The average phosphorus removal in this stage was higher than 90%. Results of statistical analysis showed that anaerobic contact significantly ($p<0.05$) enhanced phosphorus removal in the three reactors.

The results of a cycle test performed in the first and the second stage are presented in figure 3.4. As can be seen in Figure 3.4b, phosphorus release was not achieved during the anaerobic feeding, suggesting that PAOs were not responsible for phosphorus removal. Additionally, incomplete denitrification was observed as 3.8 and 3.4 mgN/L nitrate was measured in R1 and R3, respectively, at the end of the anaerobic phase. The initial nitrate measured was 29.1 and 29.8 mgN in R1 and R3, respectively. Assuming the ratio of 7 gCOD/gNO_3^--N (Lashkarizade et al., 2015), 203.6 and 208.5 gCOD was required for the complete denitrification in R1 and R3, respectively. However the anaerobic COD reduction for R1 and R3 was 164.6 and 154.4 mgCOD, respectively.

As shown in Figure 3.4b, applying anaerobic contact resulted in complete denitrification and anaerobic phosphorus release of 6.4 and 6.2 mgP/gVSS was observed in R2 and R3, respectively. In the aerobic phase, complete phosphorus removal was obtained with the specific uptake rate of 2.1 to 2.5 mgP/VSS.h.

The results indicated that although sufficient carbon source was present in the feed, denitrification and phosphorus release were not achieved with anaerobic feeding. Comparing the two operation strategies, substrate gradient during the anaerobic phase was higher in the second stage. Also, the applied anaerobic contact provided better mass transfer and improved the nutrient removal efficiency of the granules. Rocktäschel et al. (2013) also reported that mechanical mixing during the anaerobic feeding benefits the nutrient removal with equal distribution of substrate. Liu and Tay (2002) suggested the important role of hydrodynamic turbulence in mass transfer of substrate and hence substrate flux in the biofilm. However, the hydrodynamic shear applied with the liquid flow during the anaerobic contact was more than 21 times smaller than that of the aeration.

The results suggest that anaerobic carbon uptake could be obtained without applying a high shear force with mechanical mixing. Although when it comes to full-scale applications, anaerobic feeding at high flow-rates would be effective to produce hydrodynamic turbulence and provide adequate contact. Also, fast anaerobic feeding followed by mixing for large sequential batch reactors may not be feasible due to the cost ineffectiveness. High shear forces produced during the fast feeding could be destructive to the granules. Therefore, design considerations are required for feeding system in SBRs introduce the feed while distributing the substrate and avoid the need for further mixing. The distribution of feed can also reduce the hydraulic turbulence and accordingly prevent the high shear force which could cause granules disintegration.

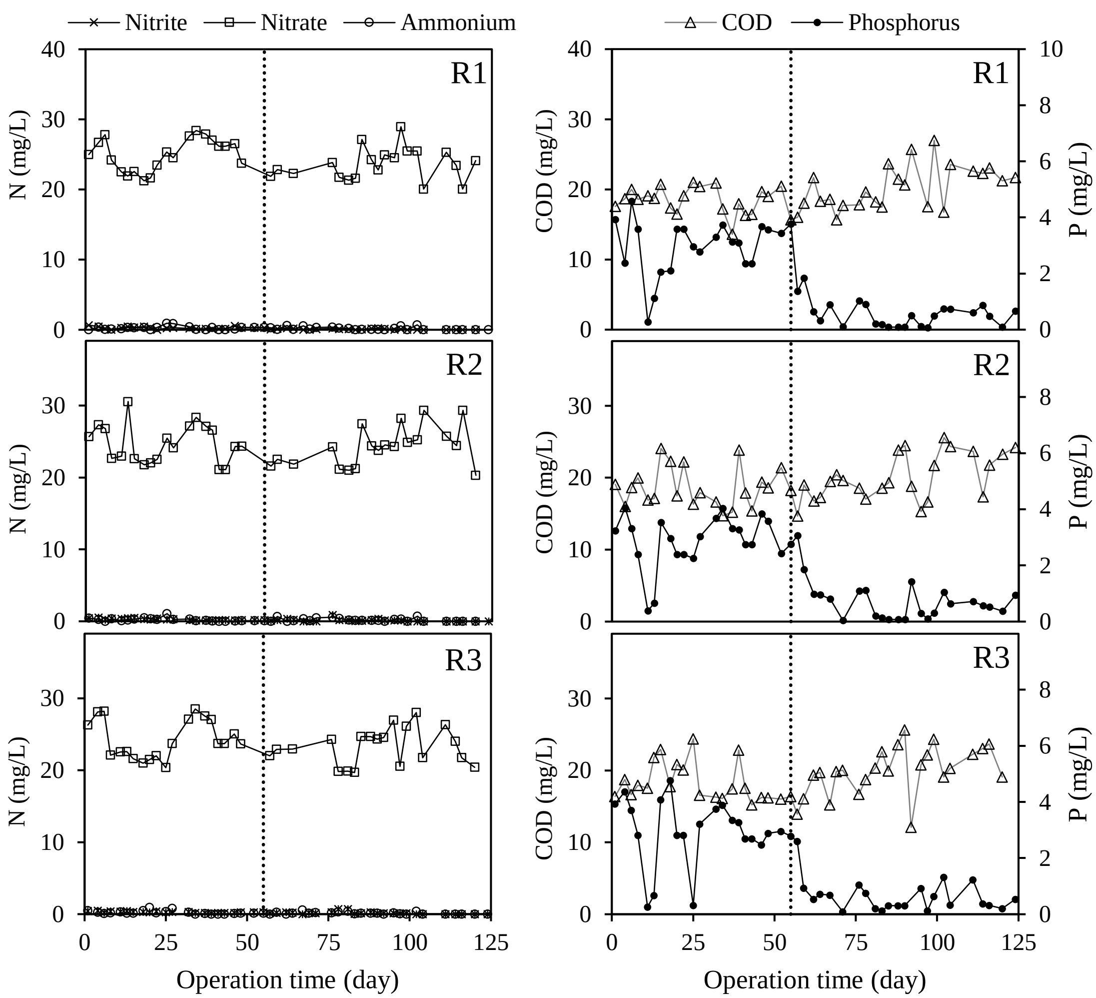

Figure 3.3. Concentrations of sCOD, nitrogen and phosphorus in the effluent

Table 3.1. Overall treatment performance, effluent quality, and SRT of the reactors during both stages

	R1		R2		R3	
	Stage I	*Stage II*	*Stage I*	*Stage II*	*Stage I*	*Stage II*
SRT (d)	14 ± 2	32 ± 5	17 ± 3	30 ± 4	16 ± 2	34 ± 4
Effluent VSS (mg/L)	96 ± 8	47 ± 5	86 ± 11	43 ± 4	78 ± 6	48 ± 5
COD removal (%)	95 ± 1	95 ± 1	95 ± 1	95 ± 1	95 ± 1	95 ± 1
Phosphorus removal (%)	63 ± 3	94 ± 2	64 ± 2	91 ± 2	63 ± 3	93 ± 2
Nitrogen removal (%)	43 ± 1	47 ± 1	44 ± 1	45 ± 1	45 ± 1	48 ± 1

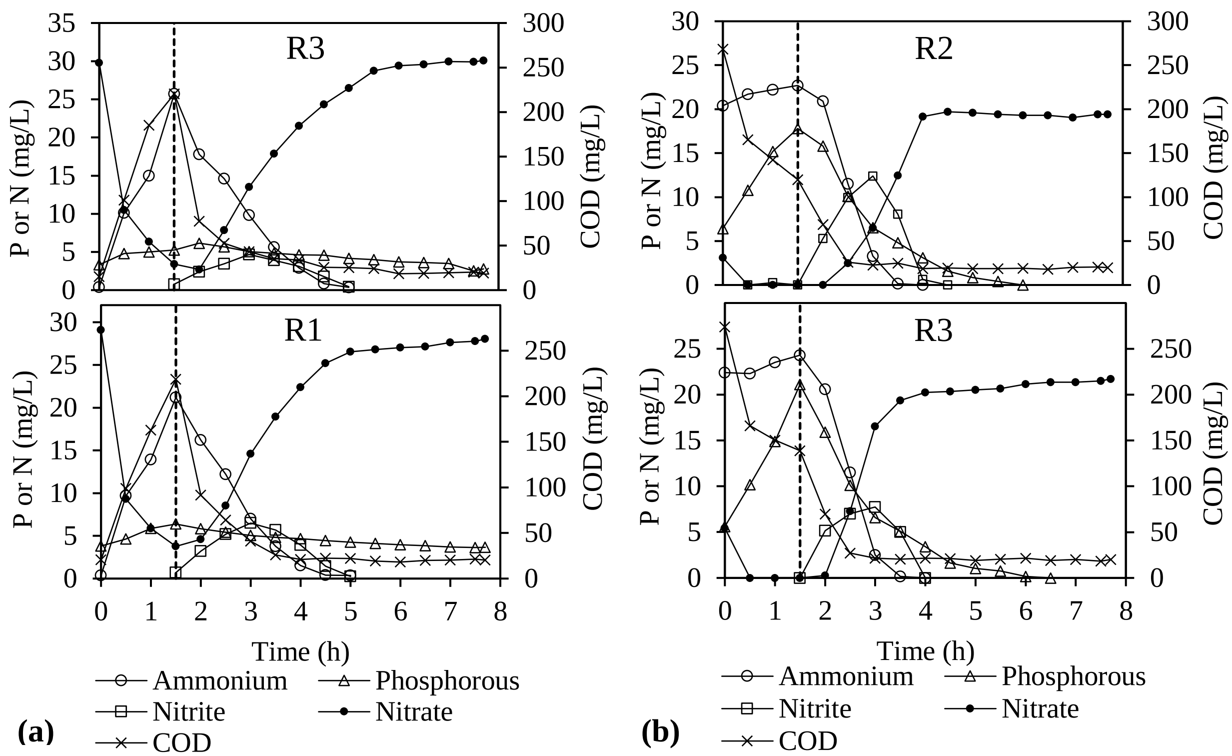

Figure 3.4. COD, nitrogen and phosphorus removal during one cycle when the reactors operated with (a) anaerobic feeding and (b) anaerobic contact

3.3.3. Characteristics of the granules

Characteristics of the granules were monitored during both stages of operation by measuring SVI_5, granules size, EPS content and EPS composition. The results are presented in Figure 3.5. As can be seen, SVI_5 was stable during the first stage, while operating with anaerobic contact that resulted in the change in granules settleability. After the change in operation, SVI_5 increased from 49 ± 1 to 90 ± 7 mL/g on day 68. High SVI_5 (> 80 mL/g) was observed for 20 days. After day 88, the settling improved and stable SVI_5 (≈ 50 mL/g) was obtained after day 103.

As shown in Figure 3.5, EPS content which was stable (≈ 150 mg/gVSS) during the first stage decreased to 113 ± 2 mg/gVSS (day 86) after applying anaerobic contact. After acclimation to the new condition, with the improvement of biomass stability, the EPS content increased and reached 131 ± 1 mg/gVSS at the end of this stage. The EPS content from the first stage could not be achieved in the second stage. The results showed that the protein content of the granules decreased in the second stage while carbohydrate remained the same. This resulted in lower P/C ratio in the second stage. The change in EPS content and EPS matrix (P/C) of the granules could be due to the decrease in the amount of COD in the aerobic phase caused by the anaerobic COD uptake. Less availability of carbon in the aerobic period altered the feast/famine balance. It was reported that EPS production is affected by feast/famine balance (Liu & Tay, 2007; Corsino et al., 2016). Therefore, in the second stage, applying same aeration time with the availability of less carbon could either cause less EPS production during the feast period or more EPS consumption during the starvation period. According to Zhu et al. (2012), under the feast condition, bacteria produced EPS as an energy source to utilize as carbon for endogenous respiration under famine (starvation) condition when the presence of substrate is limited.

It was reported that higher F/M ratios favor production of EPS and consequently enhance granulation (Li et al., 2011). At the end of the second stage, granules stabilized to F/M ratio of

0.33 gCOD/gVSS.d while the biomass increased from 3 to 4 g/L VSS, and the F/M ratio decreased to 0.25 gCOD/VSS.d. Devlin et al. (2017) also observed a decrease in net EPS content when biomass acclimation was obtained in granular SBRs treating low-strength wastewater. Increase in biomass concentration decreased F/M ratio and caused aerobic consumption of EPS.

It should also be mentioned that anaerobic contact could cause hydrodynamic shear and affect granules' properties. Zhang et al. (2011) reported that applying shear during anaerobic and anoxic phase by contact can enhance EPS production and aerobic granulation. However, in this work, shear caused by the applied liquid circulation was very small compared to that of air flow. The shear during the anaerobic phase in the first and second stage was calculated as 0.005 and 0.02 cm/s, respectively, which were considered insignificant and therefore, should not induce any superfluous changes.

As shown in Figure 5, granule size was also affected by the change in operation. During the first stage, a gradual increase in the mean diameter from 0.6 to 0.7 mm was observed. However, in the second stage, the granules' size decreased to less than 0.6 mm, which was later stabilized. As can be seen, the granules' size decreased when the biomass settling was deteriorating (suggesting disintegration of the granules) and it stabilized when the biomass settling was improved (suggesting re-granulation). As previously reported (Tay et al., 2004; Tay et al., 2001; Liu and Tay; 2002), granule size is controlled by organic loading rate and shear force. Although the organic loading rate (OLR) and shear were the same in the two stages, the granules could not achieve their initial size when anaerobic contact was applied. It could be due to the decrease in OLR of the aerobic phase with the increase in removal of organics caused by anaerobic contact.

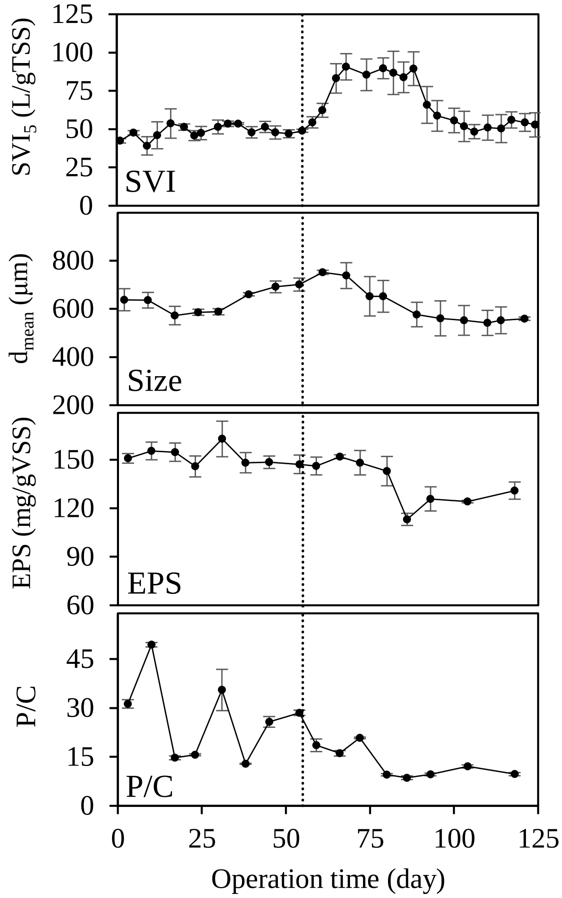

Figure 3.5. Characteristics of granules determined by SVI_5, EPS content and granules' size

(Error bars represent standard deviation)

3.4. CONCLUSION

Two operation strategies were applied to aerobic/anaerobic SBRs: anaerobic feeding and anaerobic contact. Results indicated that the system with anaerobic contact performed better in terms of effluent quality and nutrient removal. Anaerobic contact enhanced mass transfer and increased anaerobic COD removal from 17-24% to 45-53%. Consequently, phosphorus removal was improved from 63 to 93%. Raising mass transfer during anaerobic phase improved the effluent quality (from 87 to 46 mg/L VSS) by reducing the growth of rapidly growing organisms (floccular biomass) and consequently, improved biomass retention time (from 15 to 32 d). The presented results suggest that design considerations should be taken into account to provide sufficient anaerobic contact and hence anaerobic COD utilization in granular SBRs treating low-strength wastewater.

Chapter 4. Removal of Antibiotic Sulfamethoxazole by Anoxic/Anaerobic/Oxic Granular and Suspended Activated Sludge Processes

Abstract

This study investigates the removal of the antibiotic sulfamethoxazole (SMX) in two sets of anoxic/anaerobic/oxic sequencing batch reactors (SBRs) inoculated with suspended and granular activated sludge. For three months, 2μg/L SMX in a synthetic municipal wastewater with COD, TN and TP of 400, 43 and 7 mg/L, respectively, was spiked into the reactor feeds. The presence of SMX had no significant ($p>0.05$) impact on treatment performance of the suspended and granular biomass in terms of removing organics and nutrients. With 12 h of hydraulic retention time (HRT), mean removal efficiencies of 84±8% and 73±10% were obtained for the granular and suspended biomass, respectively. Mixing without aeration (1.5 h anoxic/anaerobic phase) did not remove SMX, confirming the insignificance of SMX removal via anoxic/anaerobic transformation and/or sorption onto activated sludge. The pseudo-first order SMX biodegradation constant rate constants (K_{bio}) in the granular and suspended biomass at steady-state operating condition, were 2.25±0.30 and 1.34±0.39 L/gVSS.d, respectively. The mean value of K_{bio} obtained with granular sludge was significantly greater than that with the suspended biomass. These results, suggest that aerobic granular sludge, which has advantages such as high biomass retention and high biomass concentration, could be used as an efficient process for the removal of such organic micropollutants.

Keywords: Wastewater treatment, Aerobic granular sludge, Organic micropollutants, Antibiotic Sulfamethoxazole, Biodegradation

4.1. INTRODUCTION

Lately, antibiotics has become a serious issue due to: i) the increasing use in human and veterinary medicine, ii) potential adverse effects on aquatic animals and plants, and iii) potential impacts on the micro-ecosystem (e.g., formation of antibiotic resistance) (Ding & He, 2010; Kumar et al., 2012; Oberle et al., 2012). Municipal wastewater treatment plants are known as a main source from which these micropollutants are discharged into the environment (Michael et al., 2013). Sulfamethoxazole (SMX) is one of the most commonly used antibiotics, and was reported as one of the most detected (100%) organic micropollutants monitored in 139 streams in the U.S. (Kolpin et al., 2002).

During biological treatment, micropollutants are eliminated from wastewater mainly via sorption on to biomass, biodegradation, and photolysis. The adsorptive removal of a certain organic compound is determined by sorption coefficient (K_d) of the biomass which is a function of octanol-water distribution coefficient (K_{ow}) as well as the dissociation constant (K_a) of the compound (Joss et al., 2006; Rosal et al., 2010). SMX is resistant to conventional wastewater treatment processes due to the relatively low affinity of the compound to both sorption (caused by the low K_{ow} and polarity of the compound) and biodegradation (Kassotaki et al., 2016). Results from monitoring full-scale wastewater treatment plants also showed the insignificance of adsorptive removal of SMX in activated sludge processes (Batt et al., 2007; Rosal et al., 2010). Gao et al. (2012) mass fraction of SMX in the dewatered sludge to be less than 0.1%. Elseswhere, concentration of 68 μg/kg was reported for SMX in sludge samples (Gobel et al., 2005).

The effectiveness of biological wastewater treatment for degrading these compounds is mostly determined by operational conditions. The A^2O (anoxic/anaerobic/oxic) process, used for nitrification, denitrification, BOD and phosphorus removal, and the A/O (anaerobic/oxic) process used for nitrification, phosphorus and BOD removal, both had higher efficiencies in elimination of antibiotics compared to conventional activated sludge (oxic) process which uses only BOD removal (Ghosh et al., 2009). Enrichment of nitrifying bacteria was reported to enhance biotransformation of pharmaceuticals in activated sludge (Suarez et al., 2010). Kassotaki et al. (2016) reported high degradation capabilities of an enriched culture of Ammonia Oxidizing Bacteria (AOB) for degradation of SMX. They suggested that AOB could co-metabolize the compound by producing ammonia monooxygenase (AMO) enzyme which is capable of oxidation of micropollutants. In activated sludge, SMX could be used as carbon and/or nitrogen source by heterotrophic bacteria which assimilate SMX-C and SMX-N or by autotrophic nitrifying bacteria which oxidize the functional amino group of SMX (Drillia et al., 2005; Muller et al., 2013).

Increasing solids retention time (SRT) was found to improve the removal of micro-pollutants by enhancing the microbial diversity and hence enzymatic activity of activated sludge (Joss et al., 2006; Kreuzinger et al., 2004; Polesel et al., 2016). Using a membrane bioreactor (MBR) was found to be effective for the removal of antibiotics (erythromycin, sulfamethoxazole, ofloxacin, and trimethoprim) when run with high SRTs (Radjenovic et al., 2009). According to Yu et al. (Yu et al., 2009), extended sludge age process with providing high SRT (higher than 200 days) and biomass concentration (16 gTSS/L) was reported to be effective for the removal of pharmaceuticals. Removal efficacies of 91 and 86% were obtained for SMX when initial concentrations of 1 and 5 μg/L were applied, respectively. Immobilized cell bio-carrier process which also provides high solid retention time was applied for the removal of antibiotics (Yu et al., 2011). This type of treatment was found to obtain above 40% removal of sulfamethoxazole by degradation and less than 40% removal by bio-sorption.

Aerobic granular sludge technology which is the formation of biofilm via self-immobilization of activated sludge (without carrier), provides: i) high biomass concentration without settling problems; ii) high biomass retention by fixing slow-growing organisms inside the granular structure; and iii) strong microbial structure by favouring the growth of organisms with different functionality inside one small unit. Additionally, due to the production of extra-cellular polymeric substances, bacteria inside biofilms are more resistant to antibiotics compared to freely floating bacteria (Schmidt et al., 2012). Despite the advantages of aerobic granules, there are limited studies on their application for degradation of such micropollutants. Balest et al. (2008) investigated the effectiveness of an aerobic granular bio-filter with SRT over 6 months and biomass concentration greater than 40 g/L for removing EDCs (estrogens, bisphenol A and 4-tert-octylphenol) from sewage. The process achieved higher removal efficiencies (60-90 %) for all tested EDCs compared to conventional activated sludge process (41-72%). Using nitrifying granules, Wang and Ivanov (2009) obtained complete degradation of estrogens (17β-estradiol, estriol, and 17a-ethynylestradiol) with initial concentrations of 100 μg/L over 60 days.

This study was aimed to evaluate the effectiveness of aerobic granules operated in anoxic/anaerobic/oxic SBRs for the degradation of antibiotic sulfamethoxazole compared to suspended biomass taken from a conventional activated sludge process operated for BNR. Fate of SMX during anoxic/anaerobic and aerobic phases in the presence/absence of organics and nutrients was also studied in the two systems. Additionally, treatment performance and microbial community structure of the two types of biomass exposed to SMX were investigated.

4.2. MATERIALS AND METHODS

4.2.1. Reactor setup

Two sets of SBRs in triplicate were inoculated. All SBRs (Figure 4.1) were operated with 3 L working volume and volumetric exchange ratios (VER) of 2/3. Three identical reactors named S1, S2 and S3 with inner diameter of 15 cm were inoculated with suspended activated sludge. Identical reactors named G1, G2 and G3 with 10 cm inner diameter (to obtain hydrodynamic shear required for stability of granules) were used for granular SBRs. Fine air bubbles were introduced into all reactors through 3 cm diameter spherical stone diffusers with air flow rates of 2 L/min, resulting in saturated oxygen conditions. One stone diffuser was placed near the bottom (2 cm above the bottom) of the suspended activated sludge reactors. However, for the granular reactors, two diffusers placed on the bottom were used to provide air-lift conditions. This aeration produced superficial air velocities of 0.43 cm/s in the granular SBRs. Magnetic stirrers were used to obtain gentle mixing (around 50 rpm) in the suspended reactors, while mechanical mixers were not used in granular SBRs. To provide mixing during the anoxic/anaerobic phase of the granular SBRs, the wastewater was circulated through the settled biomass by pumping the supernatant was pumped from the top of the reactors to the bottom with a flowrate of 0.13 L/min. All reactors were covered with aluminum foil to eliminate phototransformation of SMX.

4.2.2. Operation strategy

The reactors were operated with 8-hour cycles to have hydraulic retention time (HRT) of 12 h based on a VER of 2/3. Each cycle included filling (15 min), anoxic/anaerobic reaction (75 min), aerobic reaction (375 min for granules and 349 min for suspended sludge), settling (4 min for granules and 30 min for suspended sludge), discharge (10 min), and idle stage (1 min). At the end of the aerobic reaction, 100 mL of the mixed liquor was removed from the suspended SBRs, to have a 10 day solid retention time (SRT). In the granular reactors, SRT was not controlled. However, at the end of each cycle, a small portion of the biomass (slow-settling flocs) was removed through the effluent. The SRT of the granular biomass was calculated by mass balance between the mixed liquor suspended solids and the effluent suspended solids.

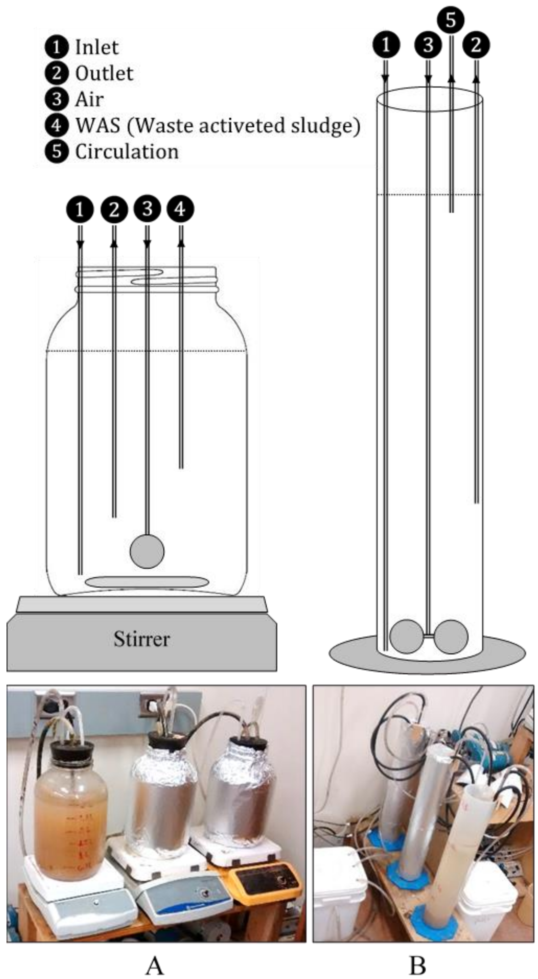

Figure 4.1. (a) Suspended and (b) granular reactors

4.2.3. Granular and suspended sludge inoculation

The suspended SBRs were seeded with conventional activated sludge obtained from a full-scale wastewater treatment plant (West-End Water Pollution Control Center, Winnipeg, MB, Canada). The plant was operated with an SRT of 9 days for biological nutrient removal (BNR) of municipal wastewater. The suspended reactors had been operating for over one year without any change in the feed or operating conditions before the start of the experiment. Also, aerobic granules had been cultivated (from conventional activated sludge) and stabilized for nutrient removal from synthetic municipal wastewater, as previously described (Kang & Yuan, 2017). The granular SBRs had been operating without any change over two months before the start of the experiment.

4.2.4. Synthetic wastewater preparation

A synthetic wastewater (Table 1) representing characteristics of municipal wastewater was used in this study. The feed contained 399.1 ± 20.9 mg/L of COD, 43.0 ± 3.0 mg/L of total nitrogen (TN), and 7.1 ± 0.6 mg/L of total phosphorus (TP). A mineral solution containing 0.15 g/L H_3BO_3, 0.03 g/L $CuSO_4.5H_2O$, 0.03 g/L KI, 0.12 g/L $MnCl_2.4H_2O$, 0.06 g/L $Na_2MoO_4.2H_2O$, 0.12 g/L $ZnSO_4.7H_2O$, 0.15 g/L $CoCl_2.2H_2O$, and 1.5g/L $FeCl_3.6H_2O$ was added to the feed. To achieve complete nitrification conditions, sufficient alkalinity was provided by adding sodium bicarbonate (12g $NaHCO_3$/g NH_4^+-N) to the feed, which resulted in a pH of 7.5 to 7.8.

The feed for G2, G3, S2 and S3 was spiked with 2 μg/L of SMX while S1 and G1 which were control reactors received wastewater without SMX. SMX stock solution was prepared first in methanol (320 mg/L) and then in deionized water (16 μg/L) and added to the feed to obtain nominal concentration of 2 μg/L SMX. The amount of methanol added to the feed was negligible. Reactors Fresh feed was prepared before each cycle as described in Figure 4.2. In two separate buckets, stock solutions of the feed as well as SMX were prepared every 5 days and kept at 4 °C and in the dark. SMX stock solution was prepared with adding 1 mL of 320 ppm

SMX (in methanol) to 20 L of DI water resulting in concentration of 16 μg/L. The two solutions were mixed and diluted with tap water using pumps prior to each cycle.

Table 4.1. Synthetic wastewater ingredients

Component	**Concentration**
Yeast extract (mg/L)	357
NH_4Cl (mg/L)	29
$K_2HPO_4.3H_2O$ (mg/L)	20
$MgSO_4$ (mg/L)	5.8
$CaCl_2$ (mg/L)	12
EDTA (mg/L)	3
Mineral solution (mL/L)	0.3

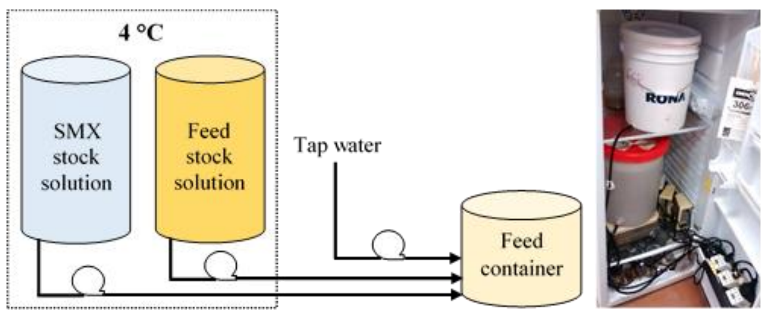

Figure 4.2. Synthetic feed preparation setup

4.2.5. Sampling and analytical methods

The concentration of mixed liquor suspended solids (MLSS), effluent suspended solids (ETSS), and influent and effluent COD were measured according to Standard Methods for the Analysis of Water and Wastewater (APHA et al., 2012). All samples taken for SMX and COD measurement were filtered with 0.45 μm filters. Concentrations of phosphorous (PO_4^{3-}-P) and nitrogen in forms of ammonium (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N) were measured via a flow injection analyser (Quick Chem 8500, LACHAT Instruments) with a detection limit of 0.2 mg/L. Extracellular polymeric substance (EPS) content of the granular biomass was extracted and protein and carbohydrate contents were measured according to Le-Clech et al. (2006).

For SMX measurement, 100 mL of the filtered sample was concentrated by solid phase extraction (SPE) and analyzed by liquid chromatography-tandem mass spectrometry as previously described (Brown & Wong, 2016). In brief, SMX was extracted using hydrophilic-lipophilic balance SPE cartridges, Oasis HLB 3cc, 60mg from Waters Corporation (Milford, MA). 3 ml methanol aliquots were used to elute the SMX, dried the eluents under nitrogen at 42^{0}C, reconstituted in 1 ml of 50:50 methanol: deionized water, and syringe filtered through 0.22 μm white PTFE luer lock inlet syringe filters from Restek (Bellefonte, PA, USA). SMX was separated on an Acquity HSS T3 C18 column (2.1 mm × 50 mm, 1.8 μm dp), directly coupled to a Acquity HSS T3 C18 guard column (2.1 mm × 5 mm) at 42^{0}C at 0.4 mL/min. Chromatographic analysis was performed via ultrahigh-performance liquid chromatography-tandem mass spectrometry using multiple-reaction monitoring in positive electrospray ionization mode. SMX was quantified using internal standards and isotope dilution.

In order to study microbial community of the suspended and granular sludge, biomass samples from the control and SMX spiked reactors were collected over time (day 1, 30, 60 and 90). Community DNA was extracted from the samples using E.Z.N.A™ Soil DNA Kit (Omega Bio-tek, Inc.) according to the protocol provided by the Kit. The DNA samples were sequenced by 16S (V4) for bacteria community composition analysis. The sequencing was conducted by NovoGene Corporation. Variable region 4 of bacterial 16S rRNA genes were amplified and sequenced on an Illumina Hiseq 2000 pyrosequencing machine (Illumina, Inc.), producing 250 bp paired-end (PE) reads. Principal Coordinates Analysis (PCoA) was performed to determine similarities or dissimilarities of sequencing data from different reactors at different operation time. In order to determine the differences between bacteria community composition of samples collected from the reactors over operation time, Linear Discriminant Analysis (LDA) effect size (LEfSe) method was used.

4.2.6. Data analyses

Statistical analysis was performed using IBM SPSS Statistics (Version 22.0). One-way ANOVA was done to determine statistical significances between mean values of different parameters (such as COD, TN, and TP removal efficiencies) between spiked and control reactors. The Student's t-test was used to compare mean values of parameters (such as SMX removal efficiency and biodegradation rate constant) obtained in the granular and suspended biomass. Two-way ANOVA was applied to understand the effects of biomass type (granular/suspended) and operation conditions (aerobic/anaerobic). For all statistical analyses, a significance level (p-value) of 0.05 was used.

4.3. RESULTS AND DISCUSSION

4.3.1. Biomass quantities and treatment performance of the SBRs

Results of monitoring biomass in the reactors as well as the reactors effluent are presented in figure 4.3. At the beginning, the granular reactors contained 2.6 to 2.8 gVSS/L biomass, while the biomass concentration in the suspended reactors was 1.8 to 2.2 gVSS/L (Table 4.2). During the experiment, the suspended biomass concentration was more stable than that of granules. This observation could be because biomass quantity was controlled and sufficient settling time were applied in the suspended reactors; while, biomass quantity was affected by washout of solids through the effluent of the granular reactors, which were operated with much lower settling times. The concentration of effluent solids in the granular reactors was not stable and also much higher than that of the suspended reactors. Mean values of SRT for granular biomass (presented in table 2) were 2.3 to 2.8 greater than the suspended biomass, which confirmed the fact that granulation process increased biomass retention time by fixing slow-growing organisms inside the granules (Devlin et al., 2017). According to the literature, higher SRTs enhance the removal of micropollutants.

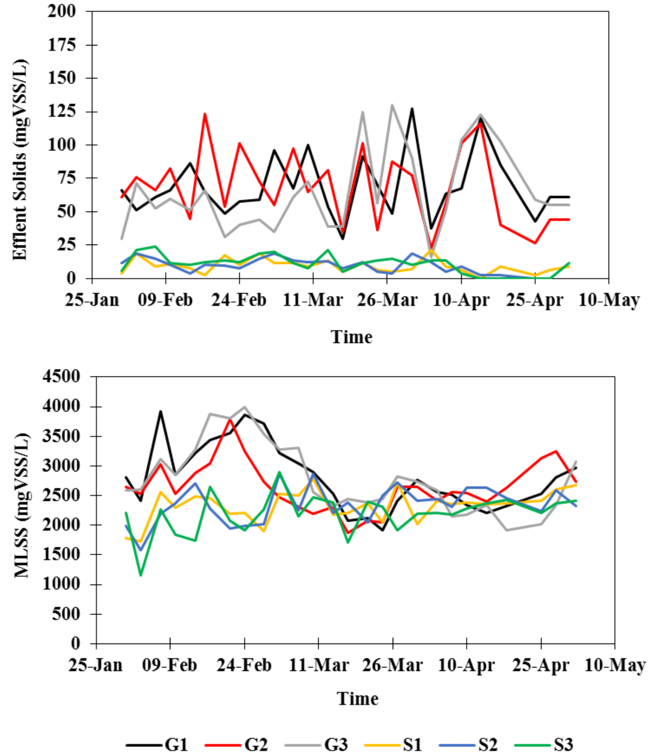

Figure 4.3. Concentrations of mixed liquor suspended solids (MLSS) and effluent suspended solids

No change in the visual appearance of granular and suspended sludge was observed during the operation time. The granules remained compact with mean diameters of 0.6 mm in the three reactors until the end of the experiment. In all reactors, the sludge volume index (SVI) of SVI_5<50 mL/gTSS and SVI_{30}<50 mL/gTSS was maintained by the granular and suspended biomass, respectively. To monitor the stability of the granules, extracellular polymeric substances (EPS) including protein and carbohydrate were measured during the operation period (Table 4.2). As expected, the granular biomass had a higher EPS content compared to that of the suspended activated sludge. EPS content, especially protein content, was reported to influence surface properties of biomass which plays an important role in the adsorption of organic pollutant (Schmidt et al., 2012; Zhu et al., 2012). According to Zhang et al. (2007) compared to suspended biomass, granular sludge is less negatively charged (zeta potential values of −32.4 and −13.3 mV was reported for granular and suspended sludge, respectively). This could reduce the sorption capacity of granules for the removal of polar organic micro-pollutants such as SMX. On the other hand, according to Avella et al. (2010), higher EPS content can be beneficial for the protection of bacteria exposed to antibiotics.

Results of measuring effluent sCOD, nitrogen and phosphorus are presented in Figure 4.4. Overall COD, nitrogen and phosphorus removal efficiencies of the reactors are listed in Table 4.2. During the operation period, all reactors obtained effluent sCOD less than 25 mg/L with as well as complete nitrification. Biological phosphorus removal was also obtained in both the suspended and granular biomass during the experiment, although effluent phosphorus concentration was not stable. Effluent concentrations below the detection limit (less than 0.2 mgP/L) and up to 3 mgP/L were recorded during the operation. In both suspended and granular reactors, in terms of COD, nitrogen and phosphorus removal efficiency, there was no statistically significant difference between the control and SMX spiked reactors. According to Schmidt et al. (2012), elimination of COD and ammonium was not affected by the presence of antibiotics (ciprofloxacin, gentamicin, sulfamethoxazole, trimethoprim, and vancomycin) with total concentration up to 20 and 7.2 mg/L, respectively. No significant change in ammonium removal was reported (Collado et al., 2013) after spiking 50 μg/L SMX. Elsewhere (Ghosh et al., 2009), SMX concentration as high as 0.5 mg/L did not influence nitrification (measured by ammonia dependent oxygen uptake rate); while 20% OUR (oxygen uptake rate) inhibition was reported when 1 mg/L SMX was spiked.

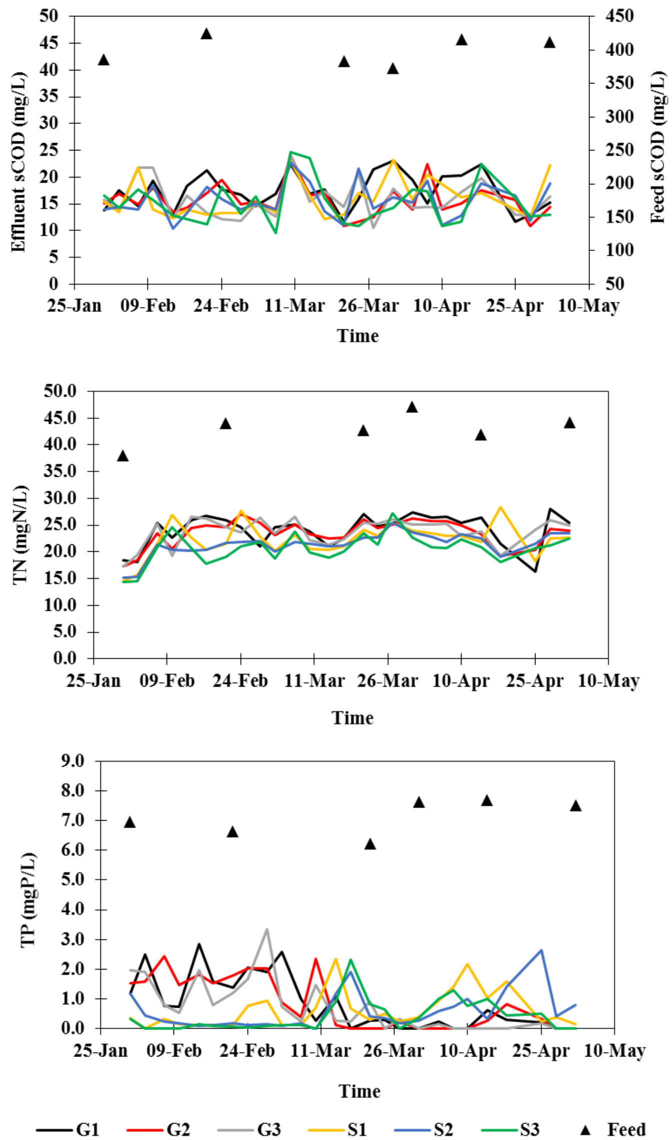

Figure 4.4. Concentrations of sCOD, TN and TP in influent and effluent of the reactors

Table 4.2. Overall treatment performance of the reactors and properties of suspended and granular biomass (error bars represent standard deviation)

Parameter	**G1**	**G2**	**G3**	**S1**	**S2**	**S3**
MLVSS (g/L)	2.8±0.6	2.6±0.4	2.8±0.6	2.3±0.3	2.3±0.3	2.2±0.3
Effluent VSS (g/L)	69±24	68±28	64±31	9±5	10±5	11±7
SRT (d) *	23±8	24±13	28±17	10	10	10
EPS (mg/gVSS)	161±16	153±26	162±14	117±1	117±3	114±1
COD removal (%)	96±1	96±1	96±1	96±1	96±1	96±1
TN removal (%)	43±7	45±5	44±7	47±7	49±4	50±6
TP removal (%)	87±13	87±13	89±13	91±9	91±9	93±8

* SRT in the suspended biomass was controlled via wasting activated sludge and effluent solids were negligible, while in the granular biomass SRT was not controlled and discharge of solids through effluent determined SRT of the system.

4.3.2. Overall removal of SMX in the SBRs

The setup for feed preparation, designed to spike 2 µg/L of SMX into the synthetic wastewater, yielded mean SMX concentrations of 2.19 ± 0.24 µg/L. No SMX (0.5 µg/L instrument limit of quantification) were quantified in influent and effluent samples from the control reactors (G1 and S1). In all spiked reactors, SMX removal was observed from the first cycle of operation after SMX was spiked in the feed (Figure 4.5). However, according to previous studies (Drillia *et al.*, 2005; Muller *et al.*, 2013), SMX removal in SBRs was expected to happen after a couple of days of adaptation of the biomass to biodegrade the compound. Considering the exchange ratio of 2/3 for the SBRs, granular and suspended sludge achieved 73 and 60 % removal, respectively in the first cycle.

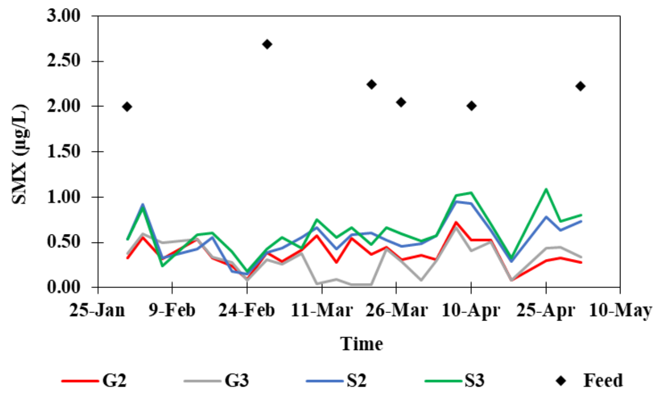

Figure 4.5. Influent and effluent concentrations of sulfamethoxazole

In the first month of operation, decreases in the SMX effluent concentration in all the dosed reactors was observed. On day 22, SMX removal greater than 90% was obtained in all reactors, although the removals were not consistent after this point. Overall, SBRs inoculated with granular sludge contained lesser effluent SMX concentrations than those with suspended sludge. Mean values of 0.38±0.15, 0.31±0.19, 0.55±0.21, and 0.61±0.23 µg/L were obtained for the effluent concentration of SMX in G2, G3, S2, and S3 reactors, respectively. The mean SMX concentrations in the effluent of the granular reactors were significantly lower than those of the suspended reactors. However, there was no significant difference between S2 and S3 and also between G2 and G3 in terms of effluent SMX. Comparing mean removal efficiencies (one-way ANOVA), both reactors with granular sludge obtained statistically greater SMX removal efficiencies (G1=83±7% and G2=86±9%) than the suspended ones (S2=75±11% and S3=72±11%). Granular biomass achieved significantly lower SMX concentrations in effluent (G=34±17 µg/L and S=0.58±22 µg/L) and thus significantly higher SMX removal performance (G=84±8% and S= 73±10%).

Different values have been reported for the removal efficiency of SMX in full-scale WWTPs using activated sludge processes. Batt et al. (2007) compared removal of antibiotics in four

WWTPs in Erie County, New York. First, the Amherst WWTP with two-stage activated sludge (stage 1: BOD removal with HRT= 1h and SRT- 6d; stage 2: nitrification with HRT= 2h and SRT=49d) receiving 2.8 μg/L SMX achieved 75% removal. Second, the East Aurora WWTP with an extended aeration process (HRT= 28-31h and SRT= 17d and ferrous chloride addition) removed 72% SMX from wastewater containing 0.88 μg/L SMX. Third, the Holland WWTP with RBC (rotating biological contactor) treatment system (HRT= 4h) obtained 36% SMX removal (influent SMX = 0.75 μg/L). Fourth, the Lackawana WWTP with pure oxygen activated sludge (HRT= 1h and SRT= 15d) receiving 0.72 μg/L SMX also achieved 36 % removal efficiency. Gao et al. (2012) reported 89% removal of SMX (1.57 μg/L in raw influent) in a full-scale conventional treatment plant (East Lansing WWTP, Michigan, US) containing preliminary treatment (aerated grit removal, equalization basin), primary treatment (primary clarifier), secondary treatment (aeration tank followed by secondary clarifier) and tertiary treatment (disinfection, rapid gravity sand filters and post-filtration aeration). The secondary biological treatment process removed 63% of the receiving SMX. Another full-scale plant (Kloten-Opfikon WWTP, Zurich, Switzerland) receiving 1.7 μg/L SMX obtained an overall removal of 62% using primary treatment (screens, aerated grit-removal and primary clarifier), secondary treatment (activated sludge system capable of nitrification and denitrification) and tertiary treatment (sand filter). The secondary treatment obtained 59% SMX removal. Rosal et al. (2010) reported 17.3 % SMX removal (with 279 ng/L SMX in the influent) in a full-scale WWTP (Alcala´ de Henares, Madrid, Spain) with anoxic, anaerobic, and oxic (A^2O) process.

The greater SMX removal obtained by the granular sludge could result from greater SRT of the granular system (23 to 28 days) compared with that of the suspended biomass (SRT = 10 d). Prolonged SRTs achieved in membrane bioreactors or biofilm reactors could enhance the elimination of pharmaceuticals by expansion of the microbial community or enrichment of slow-growing bacteria which are capable of utilizing or biotransformation of such compounds (Polesel et al., 2016).

Yu et al. (2009) reported high removal efficiencies (65 to 96 % with initial concentration of 0.5 to 10 μg/L) for SMX removal using extended sludge age biological process (SRT>200 d, HRT = 12 h, and MLSS = 16 gTSS/L). However, according to the reported SMX removal efficiency in other studies, high SRTs did not necessarily result in high SMX removal. For example, an activated sludge system with 10 d of SRT 12 h of HRT receiving SMX concentration of 0.25 to 1.3 μg/L obtained 74% removal efficacy (Radjenovic et al., 2009); while Ghosh et al. (2009) and Carballa et al. (2007) reported SMX removal efficiencies of 26% (SRT = 18 d, HRT = 5.5 h and SMX = 0.18 μg/L) and 50% (SRT = 24 d, HRT = 24 h and SMX = 0.6 μg/L), respectively. According to Polesel et al. (2016), variations in the reported removal efficiency of SMX in full-scale WWTPs could be due to the measurement of SMX without considering the deconjugation of SMX conjugates to SMX) during the treatment process.

4.3.3. Fate of SMX in the granular and suspended SBRs

To study the fate of SMX under anoxic/anaerobic and aerobic conditions and the removal of SMX as a source of carbon and nitrogen in the presence of organics and nutrients, sCOD, nitrogen, and SMX were monitored during one cycle (Figure 4.6 and 4.7). During the anoxic/anaerobic phase, the decrease in sCOD, elimination of nitrate left from the previous cycle, and increase in phosphorus concentration confirmed the activity of heterotrophic organisms: polyphosphate accumulating organisms (PAOs) and denitrifying glycogen accumulating organisms (GAOs). In the case of ammonium, an increase in the concentration was observed during the anoxic/anaerobic period. This could be due to the hydrolysis of organic nitrogen present in yeast extract (used for wastewater preparation) into NH_4^+.

In cycle tests performed previously with both granular and suspended systems (under the same conditions), sCOD removal and complete nitrification were obtained in 1 and 3h after aeration, respectively. Therefore, the SMX concentration in the reactors was recorded at time 2.5 and 4.5 h (from the beginning of the cycle) when COD was removed and when ammonium was nitrified, respectively. As can be seen in Figure 4.6, no COD removal was observed after time 2.5h and nitrification was completed (ammonium removal and nitrate production were not observed) at time 4.5h. In the case of SMX, after the start of aeration, a gradual decrease of the concentration over time was observed in both systems. SMX reduction over time was statistically significant. It was also confirmed that in the granular system, SMX concentration was significantly lower than that in the suspended system.

The effect of various processes affecting SMX, such as sorption, volatilization, and biodegradation, can be evaluated by estimating the extent of these processes based on known information from the literature, and from the results obtained in this experiment. The overall specific removal rate of SMX under aerobic conditions was 1.86 ±0.44 and 1.37±0.22 μg/gVSS.d in granular and suspended biomass, respectively, with the former significantly greater than the latter. According to the literature (Joss et al., 2006; Kassotaki et al., 2016; Suarez et al., 2010; Yang et al., 2011), removal of antibiotics in activated sludge system could be obtained through sorption, volatilization, and biodegradation. The amount of compound sorbed onto biomass can be expressed (Joss et al., 2006):

$$C_S = SK_d X_{SS}$$

where C_S is the compound concentration sorbed (μg/L), S is the soluble compound concentration (μg/L), X_{SS} is the concentration of suspended solids (gVSS/L), and K_d is the sorption coefficient of the compound (L/gTSS). K_d could be predicted according to Xia et al. (2005):

$$\log K_d = 0.58 \log K_{OW} + 1.14$$

where K_{OW} is the octanol-water distribution coefficient. Assuming K_{OW} = 0.89 for SMX (Yang et al., 2011), the K_d value is estimated to be 45.3 mL/g. The amount of SMX which can be removed via sorption onto biomass was calculated based on the initial SMX concentration and biomass concentration in the reactor. For the granular and suspended reactors this portion was found to be 11.6±1.2 and 11.1±0.9 % of the initial SMX concentration. Experimental results showed that mixing obtained negligible SMX removals after 90 min; mean removal efficiencies of 1.4±2.0 and 0.7±1.8 percent were obtained in granular and suspended reactors, respectively. To study the removal of SMX via sorption onto biomass, sterilized sludge has been used. Yang et al. (2011) reported 7.2% for the removal of SMX onto sterilized sludge (initial concentration of 100 μg/L). However, elsewhere (Yu et al., 2011), 31% removal was reported for sorption of SMX with in initial concentration of 100μg/L onto sterilized sludge. It should be considered that the sterilization process might change the physical structure as well as surface properties of activated sludge which are responsible for sorption of pollutants.

No significant removal of SMX was observed in any of the reactors during 90 min of anoxic/anaerobic period at the beginning of the cycle. Therefore, the removal of SMX via adsorption onto biomass and biodegradation/biotransformation were not achieved under the anoxic/anaerobic condition in granular and suspended biomass. Ternes et al. (2004) reported that sorption of pharmaceuticals onto activated sludge reached equilibrium very quickly (0.5 h after spike addition). Assuming that the sorption equilibrium was already obtained in the anoxic/anaerobic phase (first 1.5 h of the cycle), sorption could be considered insignificant in the removal of SMX. In some studies on SMX fate in full-scale activated sludge systems (Batt et al., 2007; Gao et al., 2012; Gobel et al., 2005; Rosal et al., 2010), extraction of the compound from secondary sludge indicated that the contribution of sorption in the removal of SMX to be insignificant (<0.1 %).

The kinetics of removal due to volatilization can be expressed (Joss et al., 2006):

$$\frac{dS}{dt} = -Hq_G S$$

where H is the dimensionless Henry gas-water partitioning coefficient (L_{water}/L_{air}) and q_G is the air applied per volume of wastewater per unit of time (L_{air}/L_{water}.d). With a Henry gas-water partitioning coefficient of 2.6×10^{-11} (Suarez et al., 2010), the fraction of SMX stripped via aeration was very low (0.0000006 % which is negligible).

The kinetics of removal due to biodegradation can be described with a pseudo-first order model as follows (Joss et al., 2006):

$$\frac{dS}{dt} = k_{bio} X_{VSS} S$$

where, t is the time (d) and k_{bio} is the biodegradation rate constant (L/gVSS.d). Assuming that SMX sorption onto biomass reached equilibrium during the anoxic/anaerobic period and that SMX removal due to volatilization was insignificant, biodegradation was considered as the only removal pathway in the aerobic phase. To find k_{bio}, ln (C/C_0) was plotted versus time from the data of the cycle test, with C_0 as the concentration of SMX at the beginning of aeration. The degradation rate constant was determined using the trend line slope and the biomass concentration (X_{VSS}). The concentration of biomass was assumed constant during the cycle test. The k_{bio} values for granular and suspended sludge were 2.25±0.30 and 1.34±0.39 L/gVSS.d, with the former significantly greater than the latter. According to Joss et al. (2006), in typical nutrient-removing municipal wastewater treatment, antibiotic sulfamethoxazole is in the class of compounds with k_{bio} < 0.1 L/gVSS.d. These cannot be removed to a significant extent (less than 20%). Suarez et al. (2010) reported SMX with k_{bio} of 0.3 L/gVSS.d persistent to biotransformation in nitrifying activated sludge (22% removal was reported).

Joss et al. (2006) suggested that the biodegradation rate constant is significantly influenced by diversity of the activity of biomass, the fraction of active biomass, and biomass floc size. Diversity of the activity of the biomass is affected by the biomass retention time. Elevated SRTs result in the dominance of slowly growing bacteria and enhance the degradation of micro pollutants by establishment of a more diverse microbial community (Kreuzinger et al., 2004). Polesel et al. (2016) suggested that kinetics of SMX biotransformation was improved when an activated sludge system was operated at SRT greater than 16 days. Elsewhere Clara et al., 2005)reported that WWTPs operating SRTs >10 days (at 10 °C) obtained higher EDC removals. Comparing the granular and suspended activated sludge systems, granular biomass had 23 to 28 days of SRT while SRT of the suspended systems was adjusted to 10 days. This could explain the greater biodegradation rate constant and hence greater removal of SMX by granular sludge.

Comparison of microbial communities of aerobic granules and flocculent sludge fed with the same municipal wastewater revealed that the two systems have different bacteria with the same functionalities (Winkler et al., 2013). This is because during the aerobic granulation of flocculent sludge in anaerobic/anoxic/oxic reactors, the bacterial community of the sludge changes and slow-growing organisms are fixed inside the granules (Devlin et al., 2017). This will provide the system with enrichment of slow-growing bacteria such as nitrifiers or specialist degrader strains which are responsible for specific biotransformation mechanisms, and/or organisms that are capable of utilizing such compounds at low concentrations (Polesel et al., 2016).

In addition to SRT, the food-to-microorganism ratio (F/M) was as an important factor in removal of EDCs because it was reported that higher EDC removals were obtained at lower F/M ratios. F/M ratio of 0.2 to 0.3 $kgBOD_5/kgTSS.d$ was reported to obtain significant removal for most compounds (Kreuzinger et al., 2004). Because granular sludge obtains higher biomass concentrations, it obtains lower F/M ratios (Tay et al., 2004). However, in our study, the F/M ratios for the suspended and granular reactors were calculated 0.11±0.02 and 0.13±0.02 kgBOD/kgTSS.d, which were not significantly different (t-test).

Under aerobic conditions, activated sludge was found capable of utilizing SMX both as a carbon and/or nitrogen source (Drillia et al., 2005). Two groups of bacteria were proposed to be responsible for SMX biodegradation; 1) heterotrophic bacteria which assimilate SMX-C and/or SMX-N and (2) autotrophic nitrifying bacteria oxidizing the functional amino group of SMX (Muller et al., 2013). However, a clear mechanism for SMX biodegradation and biodegradation pathways of this compound in activated sludge has not been established. Herzog et al. (2013) reported *Pseudomonas*, *Brevundimonas*, *Variovorax* and *Microbacterium* as the cultures isolated from activated sludge that are capable of degradation of SMX in the presence/absence of carbon/nitrogen sources.

Based on results of the cycle test (shown in Figure 4.6), during the aerobic phase, elimination of SMX was obtained when COD and ammonium were being removed (time 1.5 to 4.5) and also when COD removal and nitrification were completed (time 4.5 to 7.5h). This confirms the suggested theory by Müller et al. (2013) that with SMX could be utilized as a co-substrate (co-metabolism with organics and ammonium) and also, as sole carbon and nitrogen source. They found that in the presence of readily biodegradable carbon and the absence of nitrogen (NH_4^+), the SMX removal was enhanced. Kassotaki et al. (2016) found ammonium oxidizing bacteria (AOB) capable of degrading SMX without an adaptation period. They also reported that higher ammonium loads, which resulted in higher specific ammonium oxidation rates (SAOR) obtained higher SMX removals. On the contrary, Drillia et al. (2005) reported that SMX removal with activated sludge was not obtained when organic carbon and ammonium were present.

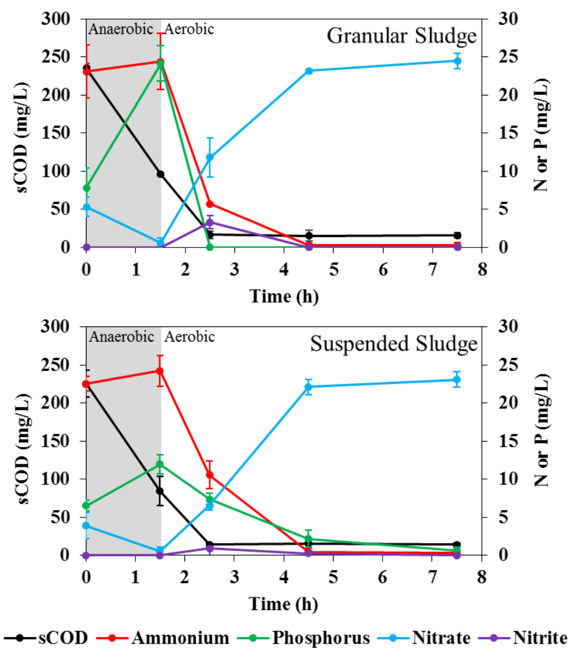

Figure 4.6. Concentrations of sCOD, nitrogen and phosphorus during on cycle (error bars represent standard deviation)

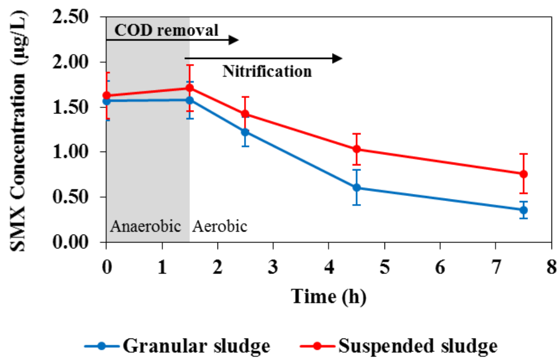

Figure 4.7. Concentration of sulfamethoxazole during one cycle (error bars represent standard deviation)

4.3.4. Bacterial community of the suspended and granular biomass

Results of bacterial community in class level for granular and suspended biomass at different operation times are presented in Figure 4.8. The dominant bacteria (relative abundance>0.02) of both types of sludge for the spiked and control samples in phylum level were *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Planctomycetes* (mostly existing in the suspended biomass). At the class level, *Betaproteobacteria*, *Saprospirae*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, and *Planctomycetia* (only in suspended biomass) were the dominant class (relative abundance>0.02) for all samples.

Results of DNA sequencing (analysed to genus level) for suspended and granular biomass samples at the end of the experiment are presented in figure 4.9. In the granular biomass samples from control reactors, *Zoogloea* > *Dechloromonas* > *Niabella* > *Aeromonas* > *Dokdonella* were the most abundant genus; while *Zoogloea* > *Dechloromonas* > *Staphylococcus* > *Niabella* > *Aeromonas* were found most abundant in spiked samples. In the case of suspended biomass the identified genus were *Gemmata* > *Zoogloea* > *Aeromonas* > *Niabella* > *Shewanella* and *Zoogloea* > *Aeromonas* > *Niabella* > *Shewanella* > *Gemmata* for control and spiked reactors, respectively. The abundance of *Zoogloea* and *Dechloromonas* as dominant denitrifying genera in activated sludge was previously reported (Hagman et al., 2008; Juretschko et al., 2002). *Zoogloea* was reported to be dominant in aerobic granules and play a significant role in aerobic granulation of activated sludge by producing EPS and binding cells together (Amorim et al., 2014; Ebrahimi et al., 2010; Li et al., 2008; Zhao et al., 2013). *Aeromonas* is one of the dominant genera in phosphate-removing sludge (Jeon et al., 2003).

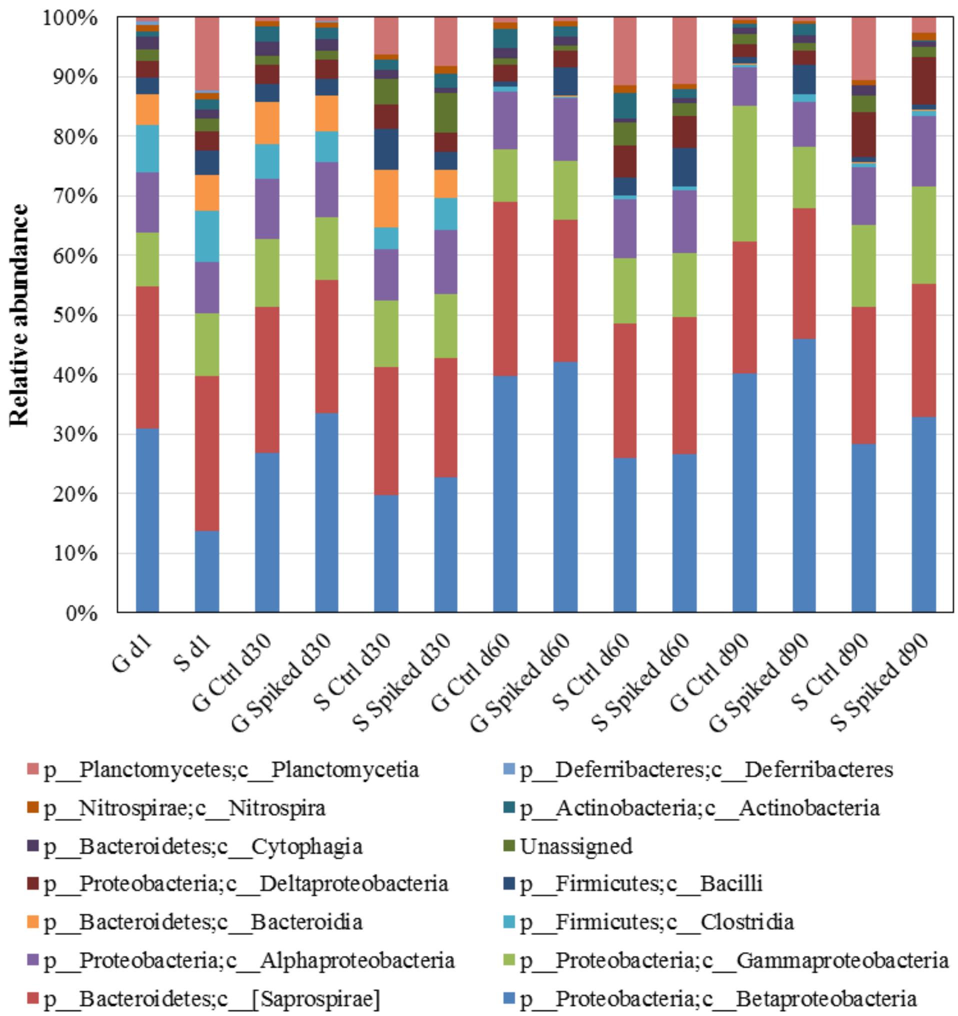

Figure 4.8. Bacterial community composition in Class level for suspended (S) and granular (G) biomass in control (Ctrl) and SMX fed reactors (Spiked) from day one (d1) to 90 (d90)

As discussed before, removal of SMX was mainly carried out by microbial degradation. Studies on microbial degradation of sulfonamide antibiotics using mixed and pure cultures suggested bacteria that are responsible for removal of these compounds in activated sludge. Kassotaki et al. (2016) suggested that AOB could co-metabolize the compound by producing ammonia monooxygenase (AMO) enzyme which is capable of oxidation of micropollutants. Strains of *Microbacterium, Rhodococcus, Tsukumurella, Achromobacter,* and *Ralstonia* isolated from a membrane bioreactor acclimatized to antibiotics sulfamethoxazole, carbamazepine, and diclofenac were reported capable of SMX mineralization (Bouju et al., 2012). Pure culture of *Rhodococcus equi* was found effective for the removal of SMX by producing an enzyme (*arylamine N-acetyltransferase*) capable of degrading aromatic amines (Larcher & Yargeau, 2011). Pure cultures of *Pseudomonas*, *Brevundimonas*, *Variovorax* isolated from an SMX-acclimated activated sludge showed capability of SMX utilization as sole carbon and nitrogen source as well as co-substrate (Herzog et al., 2013). Strains of *Escherichia* and *Acinetobacter* isolated form marine environments were effectively used for biodegradation of sulfonamides sulfapyridine and sulfathiazole (Zhang et al., 2012). According to Yang et al. (2016), *Acinetobacter* and *Pseudomonas* were major bacteria in activated sludge that are involved in removal of sulfonamides (sulfamethoxazole, sulfadimethoxine and sulfamethazine). In our study, ammonia oxidizing bacteria *Nitrosomonas* (relative abundance of 0.5 to 1.3%), *Pseudomonas* (0.2 to 0.3%), *Acinetobacter* (0.1 to 0.2 %) were detected in all biomass samples, which could be responsible for the degradation of SMX. Other genera including *Chryseobacterium* (0.6 to 1.8%), *Dokdonella* (0.7 to 1.3%), *Hydrogenophaga* (0.2 to 0.3%), *Bdellovibrio* (0.1 to 0.2%), and *Steroidobacter* (0.1 to 0.2%) were detected in all biomass samples. According to Yang et al. (2016), these bacteria which are associated with the degradation of aromatic hydrocarbons could be capable of SMX degradation.

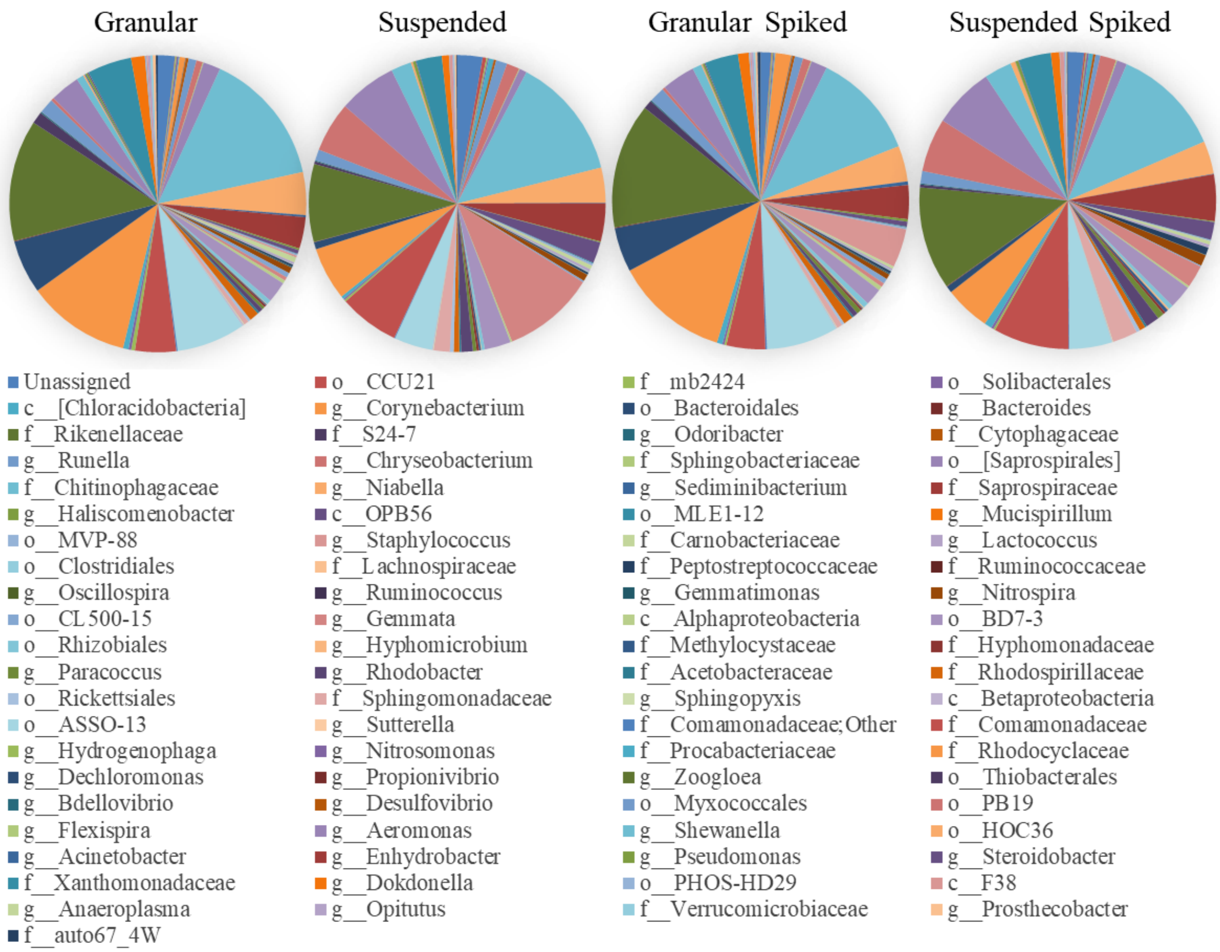

Figure 4.9. Bacterial community composition in genus level at the end of experiment (the last analysed levels are presented as c_, o_, f_ and g_ referring to class, order, family and genus, respectively).

PCoA results (figure 4.10) showed that aerobic granular sludge and conventional activated sludge had different microbial community compositions. In terms of similarity in bacterial communities, the samples can be divided into five groups: a) Samples from suspended reactors on the first day which showed the most similarity) b) Samples of suspended biomass in SMX spiked reactors on day 90 c) Suspended biomass samples from the spiked and control reactors on day 30 and 60 d) Granular biomass samples from spiked and control reactors on day 1 and 30 e) Granular biomass samples from spiked and control reactors on day 60 and 90. PCoA also showed that in both granular and suspended biomass, microbial community composition varied over the operation time. This variation happened even in control reactors which were operated without any change in the feed composition and operation strategy. Kaewpipat and Grady (2002) reported that bacterial communities of activated sludge are highly dynamic. They observed that after 60 day of operation under identical conditions, the microbial communities of two activated sludge system were not identical. In our study, change in microbial communities of suspended and granular sludge due to the exposure to antibiotic sulfamethoxazole could not be distinguished from the change due to the dynamics of microbial communities of activated sludge.

Results of LEfSe analysis for suspended biomass spiked with SMX showed that during the operation time, *Rikenellacea* and *Oscillospira* decreased and *Rhodocyclaceae*, *Zoogloea,* and *Shewanella* increased. In the case of granular biomass increase in *Rhodocyclaceae*, *Zoogloea*, and *Aeromonas* over the operation time was observed. Also *Bacteroidales*, *Ruminococcaceae*, and *Dokdonella* increased in the first month but decreased over time. The increase in abundance of *Rhodocyclaceae*, *Zoogloea*, *Shewanella,* and *Aeromonas* might indicated SMX antibiotic resistance of these bacteria. Hoa et al. (2008) studied sulfonamide-resistant genes in fish pounds receiving piggery waste and hospital and municipal wastewater (*Sul*) and identified *Acinetobacter, Aeromonas, Arthrobacter, Cellulosimicrobium, Escherichia, Shigella, Vitreosciella*, and *Wautersiella* as the major bacteria carrying *Sul* gene. *Zoogloea* was reported to become dominant in activated sludge when exposed to antibiotic erythromycin (Fan & He, 2011). The authors suggested that *Zoogloea* could form biofilm which protects inner microbes from antibiotics. *Shewanella,* and *Aeromonas* were reported as multi-drug (chloramphenicol and tetracycline) resistant bacteria in aquatic farms (Yoo et al., 2003). *Aeromonas* strains were reported to become resistant to quinolone antibiotic as a results of discharging municipal wastewater treatment plant effluent to river (Arga, Spain) water (Goñi-Urriza et al., 2000). Schmidt et al. (2012) also reported *Aeromonas* to be resistant to tetracycline.

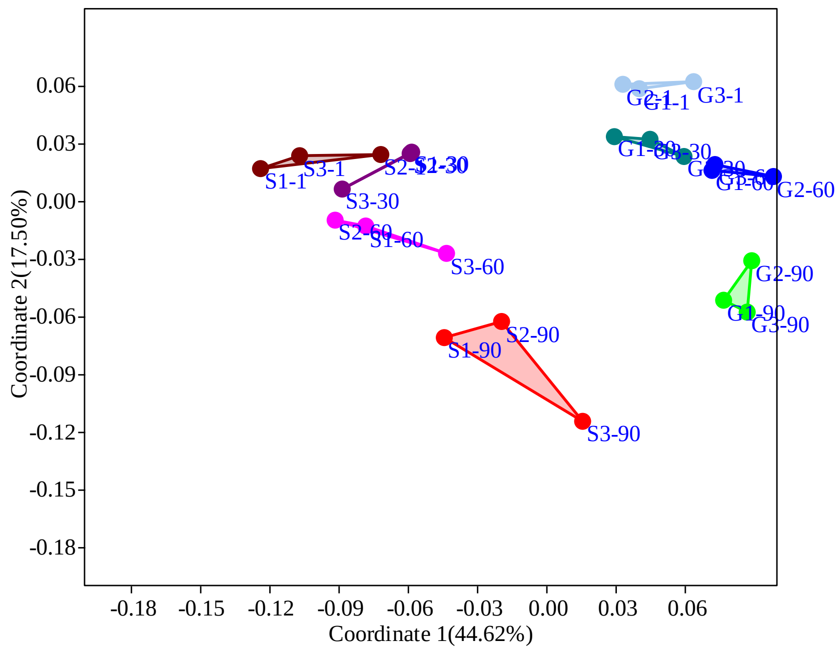

Figure 4.10. Principal Coordinates Analysis (PCoA) for the microbial community composition of granular (G) and suspended (S) biomass in control (1) and spiked (2 and 3) reactors at different operation time (day 1, 30, 60, and 90).

4.4. CONCLUSION

In this study, aerobic granular sludge was compared with conventional activated sludge in terms of removing antibiotic sulfamethoxazole from municipal wastewater. For this aim, anoxic/anaerobic/oxic SBRs inoculated with suspended and granular biomass were operated under the same conditions (HRT = 12h) for over 90 days. Results showed that with initial SMX concentration of 2 μg/L, the granules obtained 84±8% removal efficiency which was significantly higher than that obtained by the suspended sludge (73±10%). It was found that the spiked SMX (2 μg/L) did not have any significant impact on the characteristics of granular and suspended biomass as well as organic and nutrient removal efficiency of the reactors. The results confirmed that aerobic biodegradation was the main pathway for the elimination of SMX while sorption and anoxic/anaerobic treatment were insignificant. The granules achieved biodegradation rate constant (k_{bio}) of 2.25±0.30 L/gVSS.d which was significantly higher than k_{bio}= 1.34±0.39 L/gVSS.d obtained in the suspended biomass. Results of study of DNA sequencing confirmed the microbial community difference between granular and suspended biomass. Over the experimental period, variations in microbial communities of suspended and granular biomass were observed. However, these variations could not be attributed to the exposure to SMX. As the major bacteria responsible for degradation of SMX, *Nitrosomonas Pseudomonas*, and *Acinetobacter* were detected in all biomass samples. Increase in the abundance of *Rhodocyclaceae*, *Zoogloea*, *Shewanella*, and *Aeromonas* suggested these bacteria as possible sulfamethoxazole resistant bacteria.

Chapter 5: Summary and Conclusions

Development and stabilization of aerobic granules

Aerobic granular sludge capable of simultaneous removal of COD, nitrogen and phosphorus from high-strength wastewater (COD = 1400 mg/L) was successfully developed and applied for the treatment of medium (COD = 700 mg/L) and low-strength (COD = 400 mg/L) wastewater. Granules could rapidly acclimate and stabilize at medium-strength wastewater (COD = 700 mg/L). However, when granule's feed changed to low-strength wastewater, F/M ratio reduced from 0.4 to 0.2 gCOD/gVSS d, which caused disintegration of granules. With washout of poor-settling biomass, F/M ratio reached 0.4 gCOD/gVSS d and re-granulation at low COD was obtained. EPS content of the biomass decreased with the disintegration and increased with re-granulation. Lower organic loads obtained more compact granules with higher EPS content.

Enhancement of BNR treatment efficiency of granules

Two operation strategies applied to sequencing batch reactors inoculated with aerobic granular sludge for the BNR treatment of low-strength wastewater: i) continuous anaerobic feeding and ii) anaerobic feeding followed by anaerobic contact. Application of anaerobic contact enhanced mass transfer and increased anaerobic COD removal from 17-24% to 45-53% and consequently, improved phosphorus removal efficiency from 63 to 93%. Anaerobic contact also improved the effluent quality (from 87 to 46 mg/L VSS) by reducing the growth of rapidly growing organisms (floccular biomass) and increased biomass retention time from 15 to 32 days.

Application of granules for SMX removal

Under the same operation conditions (HRT = 12h) aerobic granular sludge and conventional activated sludge were exposed to 2μg/L antibiotic sulfamethoxazole. Aerobic granules showed higher SMX removal efficiency (84%) compared with conventional activated sludge (73%). The spiked SMX did not significantly influence the characteristics of granular and suspended biomass as well as their organic and nutrient removal. Aerobic biodegradation was found as the main SMX removal pathway; while sorption and anoxic/anaerobic treatment were insignificant. Microbial community structure of granular and suspended biomass were different. Over the experimental period, variations in microbial communities of suspended and granular biomass were observed. However, these variations could not be attributed to the exposure to SMX.

Chapter 6: Engineering Significance

Aerobic granular sludge is a small-footprint technology for the effective treatment of organics and nutrients from different types of wastewater. However, due to the significance of high organic loading rate in granulation kinetic, development of aerobic granules for BNR capability with low-strength municipal wastewater requires a long start-up period. This study suggests that cultivation of aerobic granules with high organic loads and its implication for BNR treatment of low-strength wastewater while balancing the F/M ratio can be an alternative to reduce start-up period.

The other issue which could be caused in the application of AGS for BNR treatment of municipal wastewater is inefficiency of mass transfer during anaerobic/anoxic phase. In addition to sufficient carbon source, a good contact during anaerobic/anoxic is required to obtain denitrification and enhance biological phosphorus removal (EBPR) in biofilm systems. Our results suggest that enhancing mass transfer during anaerobic phase can improve BNR performance as well as effluent quality by reducing the chance of growing floc-forming organisms outside of the granular structure.

Anaerobic carbon uptake could be obtained without applying a high shear force with mechanical mixing. Although when it comes to full-scale applications, anaerobic feeding at high flow-rates would be effective to produce hydrodynamic turbulence and provide adequate contact. Also, fast anaerobic feeding followed by mixing for large sequential batch reactors may not be cost-effective. High shear forces produced during the fast feeding could be destructive to the granules. Therefore, design considerations are required for feeding system in SBRs introduce the feed while distributing the substrate and avoid the need for further mixing. The distribution of feed can also reduce the hydraulic turbulence and accordingly prevent the high shear force which could cause granules disintegration.

The effectiveness of biological wastewater treatment for degrading micropollutant such as antibiotics is mostly determined by operational conditions. Increasing solids retention time (SRT) by application of membrane bioreactor (MBR) has been suggested to enhance the microbial diversity and hence improve the biodegradation of such compounds. However, membrane separation has much higher electrical energy demand compare to conventional clarification. Aerobic granular sludge technology offers high biomass retention by fixing slow-growing organisms inside the granular structure and also, high biomass concentration in a small bioreactor without settling problems.

References

Abdullah, N., Ujang, Z., & Yahya, A. (2011). Aerobic granular sludge formation for high strength agro-based wastewater treatment. *Bioresource technology*, **102**(12), 6778-6781.

Amorim, C.L., Maia, A.S., Mesquita, R.B., Rangel, A.O., van Loosdrecht, M.C., Tiritan, M.E., Castro, P.M. 2014. Performance of aerobic granular sludge in a sequencing batch bioreactor exposed to ofloxacin, norfloxacin and ciprofloxacin. *Water research*, **50**, 101-113.

APHA, AWWA, WEF. 2012. Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*.

Avella, A.C., Essendoubi, M., Louvet, J.N., Gorner, T., Sockalingum, G.D., Pons, M.N., Manfait, M., de Donato, P. 2010. Activated sludge behaviour in a batch reactor in the presence of antibiotics: study of extracellular polymeric substances. *Water Science and Technology*, **61**(12), 3147-3155.

Balest, L., Lopez, A., Mascolo, G., Di Iaconi, C. 2008. Removal of endocrine disrupter compounds from municipal wastewater using an aerobic granular biomass reactor. *Biochemical Engineering Journal*, **41**(3), 288-294.

Batt, A.L., Kim, S., Aga, D.S. 2007. Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere*, **68**(3), 428-435.

Bouju, H., Ricken, B., Beffa, T., Corvini, P.F.-X., Kolvenbach, B.A. 2012. Isolation of bacterial strains capable of sulfamethoxazole mineralization from an acclimated membrane bioreactor. *Applied and environmental microbiology*, **78**(1), 277-279.

Brown, A.K., Wong, C.S. 2016. Simultaneous quantification of propranolol and sulfamethoxazole and major human metabolite conjugates 4-hydroxy-propranolol sulfate and sulfamethoxazole-beta-glucuronide in municipal wastewater - A framework for multiple classes of drugs and conjugates. *Journal of Chromatography A*, **1471**, 34-44.

Carballa, M., Omil, F., Lema, J.M. 2007. Calculation methods to perform mass balances of micropollutants in sewage treatment plants. Application to pharmaceutical and personal care products (PPCPs). *Environmental Science & Technology*, **41**(3), 884-890.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H. 2005. The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, **39**(1), 97-106.

Collado, N., Buttiglieri, G., Marti, E., Ferrando-Climent, L., Rodriguez-Mozaz, S., Barcelo, D., Comas, J., Rodriguez-Roda, I. 2013. Effects on activated sludge bacterial community exposed to sulfamethoxazole. *Chemosphere*, **93**(1), 99-106.

Corsino, S. F., di Biase, A., Devlin, T. R., Munz, G., Torregrossa, M., & Oleszkiewicz, J. A. (2016). Effect of Extended Famine Conditions on Aerobic Granular Sludge Stability in the Treatment of Brewery Wastewater. *Bioresource Technology*, **226**, 150-157.

Coma, M., Verawaty, M., Pijuan, M., Yuan, Z., & Bond, P. L. (2012) Enhancing aerobic granulation for biological nutrient removal from domestic wastewater. *Bioresource technology*, **103**(1), 101-108.

De Kreuk, M. K., & van Loosdrecht, M. C. (2006) Formation of aerobic granules with domestic sewage. *Journal of Environmental Engineering*, **132**(6), 694-697.

Devlin, T. R., di Biase, A., Kowalski, M., & Oleszkiewicz, J. A. (2016). Granulation of activated sludge under low hydrodynamic shear and different wastewater characteristics. *Bioresource Technology*, **224**, 229-235.

Ding, C., He, J.Z. 2010. Effect of antibiotics in the environment on microbial populations. *Applied Microbiology and Biotechnology*, **87**(3), 925-941.

Drillia, P., Dokianakis, S.N., Fountoulakis, M.S., Kornaros, M., Stamatelatou, K., Lyberatos, G. 2005. On the occasional biodegradation of pharmaceuticals in the activated sludge process: The example of the antibiotic sulfamethoxazole. *Journal of Hazardous Materials*, **122**(3), 259-265.

Ebrahimi, S., Gabus, S., Rohrbach-Brandt, E., Hosseini, M., Rossi, P., Maillard, J., Holliger, C. 2010. Performance and microbial community composition dynamics of aerobic granular sludge from sequencing batch bubble column reactors operated at 20 C, 30 C, and 35 C. *Applied microbiology and biotechnology*, **87**(4), 1555-1568.

Fan, C., He, J. 2011. Proliferation of antibiotic resistance genes in microbial consortia of sequencing batch reactors (SBRs) upon exposure to trace erythromycin or erythromycin-H 2 O. *Water research*, **45**(10), 3098-3106.

Figueroa, M., Mosquera-Corral, A., Campos, J. L., & Méndez, R. (2008). Treatment of saline wastewater in SBR aerobic granular reactors. Water Science and Technology, **58**(2), 479-485.

Gao, P., Ding, Y.J., Li, H., Xagoraraki, I. 2012. Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. *Chemosphere*, **88**(1), 17-24.

Ghosh, G.C., Okuda, T., Yamashita, N., Tanaka, H. 2009. Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. *Water Science and Technology*, **59**(4), 779-786.

Goñi-Urriza, M., Capdepuy, M., Arpin, C., Raymond, N., Caumette, P., Quentin, C. 2000. Impact of an Urban Effluent on Antibiotic Resistance of Riverine Enterobacteriaceae andAeromonas spp. *Applied and environmental Microbiology*, **66**(1), 125-132.

Hagman, M., Nielsen, J.L., Nielsen, P.H., Jansen, J.l.C. 2008. Mixed carbon sources for nitrate reduction in activated sludge-identification of bacteria and process activity studies. *Water research*, **42**(6), 1539-1546.

Henriet, O., Meunier, C., Henry, P., & Mahillon, J. (2016). Improving phosphorus removal in aerobic granular sludge processes through selective microbial management. *Bioresource technology*, **211**, 298-306.

Herzog, B., Lemmer, H., Horn, H., Müller, E. 2013. Characterization of pure cultures isolated from sulfamethoxazole-acclimated activated sludge with respect to taxonomic identification and sulfamethoxazole biodegradation potential. *BMC microbiology*, **13**(1), 276.

Hoa, P.T.P., Nonaka, L., Viet, P.H., Suzuki, S. 2008. Detection of the sul1, sul2, and sul3 genes in sulfonamide-resistant bacteria from wastewater and shrimp ponds of north Vietnam. *Science of the Total Environment*, **405**(1), 377-384.

Jeon, C.O., Lee, D.S., Park, J.M. 2003. Microbial communities in activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor. *Water Research*, **37**(9), 2195-2205.

Joss, A., Zabczynski, S., Gobel, A., Hoffmann, B., Loffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H. 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. *Water Research*, **40**(8), 1686-1696.

Juretschko, S., Loy, A., Lehner, A., Wagner, M. 2002. The microbial community composition of a nitrifying-denitrifying activated sludge from an industrial sewage treatment plant analyzed by the full-cycle rRNA approach. *Systematic and applied microbiology*, **25**(1), 84-99.

Kaewpipat, K., Grady, C. 2002. Microbial population dynamics in laboratory-scale activated sludge reactors. *Water Science and Technology*, **46**(1-2), 19-27.

Kishida, N., Tsuneda, S., Kim, J. H., & Sudo, R. (2009). Simultaneous nitrogen and phosphorus removal from high-strength industrial wastewater using aerobic granular sludge. *Journal of Environmental Engineering*, **135**(3), 153-158.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environmental Science & Technology*, **36**(6), 1202-1211.

Kassotaki, E., Buttiglieri, G., Ferrando-Climent, L., Rodriguez-Roda, I., Pijuan, M. 2016. Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. *Water Research*, **94**, 111-119.

Kreuzinger, N., Clara, M., Strenn, B., Kroiss, H. 2004. Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. *Water Science and Technology*, **50**(5), 149-156.

Kumar, R.R., Lee, J.T., Cho, J.Y. 2012. Fate, Occurrence, and Toxicity of Veterinary Antibiotics in Environment. *Journal of the Korean Society for Applied Biological Chemistry*, **55**(6), 701-709.

Larcher, S., Yargeau, V. 2011. Biodegradation of sulfamethoxazole by individual and mixed bacteria. *Applied Microbiology and Biotechnology*, **91**(1), 211-218.

Lashkarizadeh, M., Yuan, Q., & Oleszkiewicz, J. A. (2015). Influence of carbon source on nutrient removal performance and physical–chemical characteristics of aerobic granular sludge. *Environmental technology*, **36**(17), 2161-2167.

Le-Clech, P., Chen, V., & Fane, T. A. (2006) Foulin g in membrane bioreactors used in wastewater treatment. *Journal of membrane science*, **284**(1), 17-53.

Li, A. J., Li, X. Y., & Yu, H. Q. (2011). Effect of the food-to-microorganism (F/M) ratio on the formation and size of aerobic sludge granules. *Process Biochemistry*, **46**(12), 2269-2276.

Li, A. J., Yang, S.-f., Li, X.-y., Gu, J.-d. 2008. Microbial population dynamics during aerobic sludge granulation at different organic loading rates. *Water Research*, **42**(13), 3552-3560.

Liu, Y., Tay, J.H. 2002. The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. *Water Research*, **36**(7), 1653-1665.

Liu, Y. Q., & Tay, J. H. (2007). Characteristics and stability of aerobic granules cultivated with different starvation time. *Applied microbiology and biotechnology*, **75**(1), 205-210.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Research*, **47**(3), 957-995.

Muda, K., Aris, A., Salim, M. R., Ibrahim, Z., Yahya, A., van Loosdrecht, M. C., Ahamd, A., & Nawahwi, M. Z. (2010). Development of granular sludge for textile wastewater treatment. *Water research*, **44**(15), 4341-4350.

Muller, E., Schussler, W., Horn, H., Lemmer, H. 2013. Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. *Chemosphere*, **92**(8), 969-978.

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**(3), 751-761.

Oberle, K., Capdeville, M.J., Berthe, T., Budzinski, H., Petit, F. 2012. Evidence for a Complex Relationship between Antibiotics and Antibiotic-Resistant Escherichia Coli: From Medical Center Patients to a Receiving Environment. *Environmental Science & Technology*, **46**(3), 1859-1868.

Polesel, F., Andersen, H.R., Trapp, S., Plosz, B.G. 2016. Removal of Antibiotics in Biological Wastewater Treatment Systems-A Critical Assessment Using the Activated Sludge Modeling Framework for Xenobiotics (ASM-X). *Environmental Science & Technology*, **50**(19), 10316-10334.

Radjenovic, J., Petrovic, M., Barcelo, D. 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water Research*, **43**(3), 831-841.

Rahman, M.F., Yanful, E.K., Jasim, S.Y. 2009. Occurrences of endocrine disrupting compounds and pharmaceuticals in the aquatic environment and their removal from drinking water: Challenges in the context of the developing world. *Desalination*, **248**(1-3), 578-585.

Ren, Y., & Yuan Q. (2016) The Treatment of Landfill Leachate Using Aerobic Granular Sludge. *3rd IWA Specialized International Conference "Ecotechnologies for Wastewater Treatment*" (Ecostp16) 27-30 June 2016, Cambridge, UK.

Rocktäschel, T., Klarmann, C., Helmreich, B., Ochoa, J., Boisson, P., Sørensen, K. H., & Horn, H. (2013). Comparison of two different anaerobic feeding strategies to establish a stable aerobic granulated sludge bed. Water research, 47(17), 6423-6431.

Rosal, R., Rodriguez, A., Perdigon-Melon, J.A., Petre, A., Garcia-Calvo, E., Gomez, M.J., Aguera, A., Fernandez-Alba, A.R. 2010. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Research*, **44**(2), 578-588.

Schmidt, S., Winter, J., Gallert, C. 2012. Long-Term Effects of Antibiotics on the Elimination of Chemical Oxygen Demand, Nitrification, and Viable Bacteria in Laboratory-Scale Wastewater Treatment Plants. *Archives of Environmental Contamination and Toxicology*, **63**(3), 354-364.

Seviour, T., Pijuan, M., Nicholson, T., Keller, J., & Yuan, Z. (2009). Gel-forming exopolysaccharides explain basic differences between structures of aerobic sludge granules and floccular sludges. *Water research*, **43**(18), 4469-4478.

Suarez, S., Lema, J.M., Omil, F. 2010. Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. *Water Research*, **44**(10), 3214-3224.

Tay, J.H., Liu, Q.S., Liu, Y. 2001. The effects of shear force on the formation, structure and metabolism of aerobic granules. *Applied Microbiology and Biotechnology*, **57**(1-2), 227-233.

Tay, J.H., Pan, S., He, Y.X., Tay, S.T.L. 2004. Effect of organic loading rate on aerobic granulation. I: Reactor performance. *Journal of Environmental Engineering-Asce*, **130**(10), 1094-1101.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A. 2004. A rapid method to measure the solid-water distribution coefficient (K-d) for pharmaceuticals and musk fragrances in sewage sludge. *Water Research*, **38**(19), 4075-4084.

Wang, Q., Du, G.C., Chen, J. 2004. Aerobic granular sludge cultivated under the selective pressure as a driving force. *Process Biochemistry*, **39**(5), 557-563.

Wang, X.H., Ivanov, V. 2009. Microbial structure of nitrifying granules and their estrogens degradation properties. *Water Science and Technology*, **59**(9), 1855-1862.

Wang, Z.P., Liu, L.L., Yao, H., Cai, W.M. 2006. Effects of extracellular polymeric substances on aerobic granulation in sequencing batch reactors. *Chemosphere*, **63**(10), 1728-1735.

Winkler, M. K., Bassin, J. P., Kleerebezem, R., De Bruin, L. M. M., Van den Brand, T. P. H., & Van Loosdrecht, M. C. M. 2011. Selective sludge removal in a segregated aerobic granular biomass system as a strategy to control PAO–GAO competition at high temperatures. *Water research*, **45**(11), 3291-3299.

Winkler, M. H., Kleerebezem, R., De Bruin, L. M. M., Verheijen, P. J. T., Abbas, B., Habermacher, J., & Van Loosdrecht, M. C. M. 2013. Microbial diversity differences within aerobic granular sludge and activated sludge flocs. *Applied microbiology and biotechnology*, **97**(16), 7447-7458.

Xia, K., Bhandari, A., Das, K., Pillar, G. 2005. Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids. *Journal of Environmental Quality*, **34**(1), 91-104.

Yang, C.-W., Hsiao, W.-C., Chang, B.-V. 2016. Biodegradation of sulfonamide antibiotics in sludge. *Chemosphere*, **150**, 559-565.

Yang, S.F., Lin, C.F., Lin, A.Y.C., Hong, P.K.A. 2011. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions. *Water Research*, **45**(11), 3389-3397.

Yoo, M.H., Huh, M.-D., Kim, E.-h., Lee, H.-H., Do Jeong, H. 2003. Characterization of chloramphenicol acetyltransferase gene by multiplex polymerase chain reaction in multidrug-resistant strains isolated from aquatic environments. *Aquaculture*, **217**(1), 11-21.

Yu, S., Sun, P., Zheng, W., Chen, L., Zheng, X., Han, J., & Yan, T. (2014). The effect of COD loading on the granule-based enhanced biological phosphorus removal system and the recoverability. *Bioresource technology*, **171**, 80-87.

Yu, T.H., Lin, A.Y.C., Lateef, S.K., Lin, C.F., Yang, P.Y. 2009. Removal of antibiotics and non-steroidal anti-inflammatory drugs by extended sludge age biological process. *Chemosphere*, **77**(2), 175-181.

Yu, T.H., Lin, A.Y.C., Panchangam, S.C., Hong, P.K.A., Yang, P.Y., Lin, C.F. 2011. Biodegradation and bio-sorption of antibiotics and non-steroidal anti-inflammatory drugs using immobilized cell process. *Chemosphere*, **84**(9), 1216-1222.

Zhang, B., Ji, M., Qiu, Z., Liu, H., Wang, J. and Li, J., 2011. Microbial population dynamics during sludge granulation in an anaerobic–aerobic biological phosphorus removal system. *Bioresource technology*, **102**(3), 2474-2480.

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**(5), 551-557.

Zhang, W.-W., Wen, Y.-Y., Niu, Z.-L., Yin, K., Xu, D.-X., Chen, L.-X. 2012. Isolation and characterization of sulfonamide-degrading bacteria Escherichia sp. HS21 and Acinetobacter sp. HS51. *World Journal of Microbiology and Biotechnology*, **28**(2), 447-452.

Zhao, Y., Huang, J., Zhao, H., Yang, H. 2013. Microbial community and N removal of aerobic granular sludge at high COD and N loading rates. *Bioresource technology*, **143**, 439-446.

Zhu, 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.