Aerobic Granular Biomass using Municipal Primary Effluent: A Comparative Monitoring Study

by

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Abstract

In the past two decades, aerobic granular biomass (AGB), has proven to be a viable candidate as a biological wastewater treatment solution, and has been implemented worldwide in the Nereda® process; yet, a lack of research and application of AGB systems in North America, could hinder its application in Canada. As such, two pilot-scale, sequencing batch reactors were operated to cultivate AGB using low-strength, Canadian municipal wastewater. The study was separated into two phases, to fulfil the primary objective: (a) to see the influence of additional volatile fatty acids (VFA) on granulation; and subsequently, a secondary objective: (b) to find out if the granulation start-up time can be decreased by increasing the rate of selection pressure application.

During the first phase, Reactor 2, on average, received 42% more VFA than the other, which acquired VFA solely from the primary effluent feed. Granules were observed in both reactors, however, neither system produced the smooth, dense granules expected with AGB. The granules had outgrowth on the peripheral, and the system that received additional VFA had more outgrowth and higher shape irregularity. It is understood high amounts of VFA breakthrough into the aerobic phase of the cycle led to granular instability, which was more intensely noted in the system that had higher inlet VFA concentrations (both systems ended up using similar amounts of VFA during the anaerobic phase). Preserving high biomass concentrations proved to be challenging in maintaining stability during start-up. Nutrient removal was observed consistently in both systems, but, due to high dissolved oxygen concentrations, denitrification was not achieved significantly in either system. The lack of denitrification caused limitations in orthophosphate removal with VFA competition occurring between denitrifiers and polyphosphate accumulating organisms in the subsequent anaerobic phase. Overall, it was concluded that additional VFA is not a necessity for granulation using local wastewater, during the observed season. Phase 2 was not successful in achieving granulation. Colder temperatures, lower strength wastewater and a short schedule were all factors that attributed. Valuable lessons in granular start-up were attained from Phase 2, despite the initial objective not being fulfilled.

Lay Summary

The necessity of higher levels of wastewater treatment in Canada is increasing. Currently, much of the wastewater produced in the Metro Vancouver area undergoes one level of treatment (solids separation) before being disposed into the aquatic environment. A second level of treatment, in which microorganisms perform the biological removal of contaminants will be required with new federal regulations, and thus, comes the need to investigate novel and effective technologies.

One such technology, called aerobic granular biomass, can perform secondary treatment with reduced space and energy requirements. The technology has been implemented in Europe, Africa, Australia, Asia, and South America, and this study focused on its application in the local area. A pilot-scale study was conducted that compared different conditions and wastewater characteristics using this treatment technique. The results obtained from the study can be used in further studies and applications, and provide evidence on the feasibility in the local area.

Preface

This dissertation is original, unpublished, independent work by the author, Sylvia Woolley

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Nomenclature

°C	Degrees Celsius
"	Inches
%	Percent
μm	Micrometre
AGB	Aerobic granular biomass
AIWWTP	Annacis Island wastewater treatment plant
AND	Alternating nitrification and denitrification
ARC	Annacis Research Centre
BC	British Columbia
BNR	Biological nutrient removal
С	Carbon
Ca ²⁺	Calcium Ion
CaCO ₃	Calcium Carbonate
cm	Centimetre
COD	Chemical oxygen demand
CO ₂	Carbon Dioxide
d	Day
D10	10 th percentile of particle size distribution
D50	50 th percentile of particle size distribution
D90	90 th percentile of particle size distribution
DO	Dissolved oxygen
EPS	Exopolysaccharides
g	Gram
GAO	Glycogen accumulating organism
GC	Gas chromatography
GG	Poly(gulronic acid)
h	Hour
H ₂ SO ₄	Sulfuric acid

HDPE	High-density polyethylene
КНР	Potassium hydrogen phthalate
L	Litre
MDL	Method detection limit
mg	Milligram
MG	Heteropolymeric acid
min	Minute
mL	Millilitre
MLVSS	Mixed liquor volatile suspended solids
mm	Millimetre
MM	Poly(mannuronic acid)
N_2	Dinitrogen gas
N/A	Not applicable
NH ₃	Ammonia
NO ₂ -	Nitrite
NO ₃ -	Nitrate
NPT	National pipe thread
Ortho-PO ₄ ³⁻	Orthophosphate
PAO	Polyphosphate accumulating organism
рН	Power of hydrogen
РНА	Polyhydroxy-alkonates
РНВ	Poly- β -hydroxybutyrate
PP	Polypropylene
PS	Polysytrene
PVC	Polyvinyl chloride
rbCOD	Readily biodegradable chemical oxygen demand
SND	Simultaneous nitrification and denitrification
SBR	Sequencing batch reactor
SRT	Solids retention time
SVI	Sludge volume index
Т	Temperature

TF/SC	Trickling filter solids contact
TSS	Total suspended solids
UBC	University of British Columbia
V	Volt or Volume
Vcrit	Critical settling velocity
VER	Volume exchange ratio
VFA	Volatile fatty acid
VSS	Volatile suspended solids
WAS	Waste activated sludge
WSER	Wastewater Systems Effluent Regulations
WWTP	Wastewater treatment plant

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1 Introduction

In recent years, it has become evident that the current wastewater treatment capabilities in Canada are not adequate to carry the country into a sustainable future. In 2012, only 47% of wastewater treated in the Metro Vancouver region underwent secondary biological treatment (Metro Vancouver, 2014). The remaining 53% was solely treated with the removal of solids by primary clarification. The federal government's Wastewater Systems Effluent Regulations (WSER) were introduced in June 2012, and with the regulations, all wastewater treatment plants (WWTP) in Canada must undergo at least secondary treatment, regardless of where the effluent is being disposed. Many WWTPs in British Columbia, and the Metro Vancouver region were given transitional authorization to upgrade to secondary treatment, within the next fifteen years.

Secondary treatment will, therefore, become a baseline for treatment in the near future, and vast advancements in current infrastructure will be required for such improvements. Although many secondary treatment options are available and have been proven practical for the North America market, there exists an opportunity to seek new technologies that are less resource intensive. To break into the North American market, these novel technologies must be proven to be effective in similar environments elsewhere, to minimize risk of a failure and financial loss. Therefore, a balance between innovative technologies and status-quo must be achieved when seeking treatment solutions.

In recent years, Aerobic Granular Biomass (AGB), has been demonstrated as a potential candidate for efficient secondary treatment of wastewater. With AGB systems, sequencing batch reactors are used to sustain biomass in granular form, which results in a smaller plant footprint and reduced energy requirements (de Bruin et al., 2004). The technology became prominent at the beginning of the millennium and has been commercialized, with one such technology provider being Royal HaskoningDHV with their Nereda[®] process (Giesen et al., 2013). The first, municipal full-scale plant was commissioned in 2011, and since 2012, AGB has been implemented in more than a dozen municipal wastewater treatment facilities, spanning five continents. North America, however, is not included in this list, and as such, investigative work in AGB technologies in local wastewater is required to determine the feasibility of implementation in a Canadian setting.

2 Literature Review

2.1 AGB as a Secondary Treatment Technology

AGB systems biologically remove carbon, nitrogen and phosphorus, categorizing the technology as secondary treatment, with biological nutrient removal (BNR). AGB differs from other conventional processes in the morphology, and structure of the biomass growing within the systems. Granules are large (millimeter scale), dense aggregates, with a smooth peripheral, all of which lead to fast settling biomass. In conventional activated sludge processes, biomass is described as floccular. In contrast, floccular biomass is considerably less dense and takes up higher surface area per volume, and therefore, cannot settle as quickly as granular biomass. AGB can be operated at higher biomass concentrations due to the dense and compact morphology, which can relate to a higher volumetric capacity for AGB systems. Concentrations in large scale developments are noted to be in the range of 10-15 g/L, in comparison to the range of 2-5 g/L for flocculent systems (Keller & Giesen, 2010).

Two requirements have been identified as indicators of granular biomass: (1) the average particle size (D_p) is greater than 0.2 mm, and, (2) the sludge volume index (SVI) after five minutes is equivalent to that after thirty minutes (i.e. SVI5=SVI30) (Keller & Giesen, 2010). SVI is the ratio of the volume of biomass that settles in a specified time-period, in relation to the concentration of biomass, and has units of volume over mass (Standard Methods 2710 D). As such, when the five-minute term and thirty-minute term are equivalent, the biomass is considered fast settling.

$$SVI = \frac{\text{settled sludge volume } \left(\frac{mL}{L}\right) * 1000}{\text{suspended solids } \left(\frac{mg}{L}\right)}$$

The sequencing batch reactor (SBR) design has proven to be an effective way of cultivating stable granular systems at both the lab and full scale. In laboratory and pilot scale, the batch cycle is generally comprised of four phases: (1) a feasting anaerobic-fill phase, (2) a famine aerobic phase, (3) a settling phase, and (4) a decant phase. The batch system allows for some biological control by preferentially growing microorganisms that lead to granular stability, and will be further explained in Section 2.2.1 and 2.3.1. Additionally, the SBR system allows for a selective pressure to be placed on the biomass, by manipulating the time allowed for the biomass to settle before decanting the effluent from the reactor (further discussed in Section 2.2.2).

2.2 Granular Morphology and Formation Mechanisms

2.2.1 Selection of Slow-Growing Organisms and the Effect of Readily Biodegradable Carbon The formation mechanisms of granular sludge have been studied, and are still under investigation, as the parameters affecting stability have yet to be fully understood. The composition of wastewater being fed to the system, is said to have an important role in the granulation process. Readily biodegradable chemical oxygen demand (rbCOD), in the form of volatile fatty acids (VFA) are preferred as they can stimulate the growth of slow-growing microorganisms, whose vital role in granulation is further discussed in Section 2.3.1. In particular, short-chain VFAs, such as acetate and propionate are favoured over longer chain VFAs (butyric, and valeric), as they can be readily taken up, stored, and subsequently oxidized to provide slow organism growth.

Additionally, high substrate concentration gradients have been noted as a selector for slowgrowing organisms, and can be achieved by feeding in plug-flow from the bottom of the sludge bed (Winkler, 2012). In perfect plug flow conditions, the wastewater does not get diluted with the bulk reactor volume, before passing through the biomass, which sits at the bottom of the reactor (Figure 1). This configuration allows for the highest concentration gradient, and has been implemented in many AGB systems, both in research and commercial settings. In a completely mixed fill scenario, the incoming wastewater would be mixed with the bulk fluid (the remainder of the previous cycle), which would lead to immediate dilution of substrate concentrations prior to any biological reactions occurring. As such, lower substrate to biomass concentration gradients are seen; however, this configuration is also known to be successful with forming AGB and reduces the risk of short-circuiting, by increasing the contact time, and thus mass transfer, of the substrate to the biomass (Rocktäschel et al., 2013).



By the end of the anaerobic feast phase:

Figure 1 - Schematic showing differences in feeding regime for a plug-flow, and completely mixed system

In much of the literature, synthetic wastewater is used to better see the direct influence of wastewater characteristics on AGB, without the irregularities that come with real wastewater. Forming aerobic granules on domestic wastewater is deemed more challenging, and wastewater variability in strength must be considered for larger scale developments. In systems that are fed with lower-strength wastewater, it has been found that shorter cycle times are preferential to granular formation, to allow for biomass to uptake more feed in a shorter time (de Kreuk and van Loosdrecht, 2006).

The VER is another parameter that can be manipulated to achieve higher concentrations of wastewater in an SBR. With a higher VER, higher concentrations of wastewater are being treated, as there is less dilution occurring during the feed (due to the higher effluent volumes).

2.2.2 Hydraulic Selection Pressure

A high, selective pressure has been recognized as an important parameter in granular start-up (Qin et al., 2004). Pressure can be induced by decreasing the settling time of the system, and washing away biomass that is not able to settle in the allotted time. The biomass being washed out is at the top of the sludge bed, and is comprised of the less dense, flocculent and smaller biomass, which

are not preferred in an AGB system. As such, the stress asserted on the system in selecting for granular biomass, which settle faster. A high-selection pressure, if applied in the wrong way, can result in complete washout of biomass. (Beun et al., 1999; Lochmatter & Holliger, 2014).

An important parameter in selective pressure for AGB systems is the critical settling velocity (V_{CRIT}) that the biomass must attain in order to avoid washout. Lab-scale AGB systems are reported to have V_{CRIT}'s in the range of 10-50 m/h (van Haandel & van der Lubbe, 2012). Settling velocities of this magnitude, however, are not always reflected in literature findings for AGB systems using real wastewater. In a study conducted using low-strength wastewater that had been supplemented with carbon that had undergone primary fermentation, stable granules were observed only when V_{CRIT} was above 5.3 m/h, and the system was not able to sustain above 7.5 m/h (Sturm et al., 2015). A study using synthetic wastewater suggested that successful aerobic granulation occurred when the V_{CRIT} was above 4 m/h (Wang et al., 2006). Literature, however, lacks a fundamental method at which selection pressure should be induced to achieve stable granulation. As such, biomass concentration is an important tool in determining the washout behavior of a system. Mixed liquor volatile suspended solids (MLVSS), measured with the volatile suspended solids (VSS) test, is used to estimate the biomass concentration. VSS represents the volatile component of the total suspended solids (TSS) concentration (which means that this fraction is not dissolved, and will combust readily at 550°C).

In lab and pilot scale, SBRs often have high height to diameter ratios (typically 10:1), and a volume exchange ratio (VER) of around 50% (McSwain et al., 2004). It should be noted that in practice, full-scale systems are unable to keep high height to diameter ratios, however still granulate proficiently. The VER is the ratio between the volume of the effluent to the working volume of the reactor, which physically relates how far biomass must travel to avoid washout (Figure 2).

$$VER = \left(1 - \left(\frac{L_D}{L_L}\right)\right) * 100$$

The high ratio of height to diameter ensures that a circular flow trajectory for the microbial aggregates, which is believed to support the formation of granular sludge, as per the role of hydrodynamic shear force (Section 2.2.1).



Figure 2 – The VER is the ratio between how much volume is removed at decant, related to the total reactor volume.

2.2.3 Hydrodynamic Shear Force

Granular sludge has been described as a self-immobilization community of bacteria, and hydrodynamic shear force has been identified as a possible influence on the structure and function of granules (Liu and Tay, 2002). The biofilm production theory has been used to hypothesize how granules emerge in biomass. After initial attractive forces within bacterial communities have been established, bacteria communities mature and produce large amounts of extracellular polymer, such as exopolysaccharides (EPS). EPS adds to biofilm mass, and it has been observed that production of EPS is enhanced by high superficial air upflow velocity, or shear force. As such, it has been proposed that the amount of shear force applied to a system can control the shape and size of granules aggregates. Bubble column reactors have proven to perform well for AGB systems to achieve the preferred upflow air velocity.

2.2.4 Exopolysaccharide Content within AGB

Physico-chemical differences between the EPS of flocular and granular biomass have been identified. Granular EPS exists as a strong-gel across normal operating pH conditions, lending to an increase in adhesiveness of the EPS and the ability to form aggregates (Seviour et al., 2009). Flocculent biomass has been observed to have a weak solution-gel transition at lower pH, and with the low gel forming capability, flocs are unable to support the same microbial density.

The copolymer alginate has been identified as a reason for the gel-forming capabilities of granular biomass. Alginate is naturally produced by bacteria by linking together monomers β -D-mannuronic acid and α -L-guluronic acid (Rehm & Valla, 1997). Alginates, extracted from brown seaweed, are manipulated for industrial purposes including in the food industry as thickening and gelling agent, and as a method to immobilize cells in pharmaceuticals. The two monomers can be arranged in three different blocks: poly(mannuronic acid) (MM), poly(gulronic acid) (GG) or heteropolymeric (MG), and depending on the ratio of each block, the polymer may have different physical and chemical properties. The GG block is considered hydrophobic as it is able to cross-link with divalent cations (e.g. Ca²⁺), which contributes to the gel forming capabilities. It has been found that AGB biomass contains 69% of the GG block, significantly higher than what has been found in floccular sludge (Lin et al., 2013).

2.3 Biological Composition of Granules

It is thought that three zones, aerobic, anoxic and anaerobic zones, are formed within granules, and are dictated by the granular size, porosity and bulk dissolved oxygen (DO) concentration. The zones allow for the segregation of microorganism type, based on their oxygen preference (Winkler, 2012) (Figure 3). On the exterior portions of the granule, aerobic microorganisms, requiring oxygen as an electron acceptor, thrive. With oxygen penetration being limited on the interior of the granule, microorganisms that can use (or require) alternative electron acceptors (i.e. nitrate), can be active even when oxygen is being supplied to the system.



Figure 3 - Proposed structure of the aerobic granule and microorganisms found at each level (adapted from Winkler, 2012)

2.3.1 Carbon and Phosphorus Removal in AGB

The importance of slow-growing microorganisms, stimulated by VFA during the anaerobic growth phase, on granulation can be further explained using this granule model. Heterotrophic microorganisms that aerobically consume soluble and particulate carbon substrates are not desired in the outer zone when oxygen is being supplied to the system, as they promote floccular outgrowth on the granule. As such, to remove organic carbon from wastewater, a feeding period that

promotes the removal of the rbCOD by slow-growing glycogen accumulating organisms (GAOs) or polyphosphate accumulating organisms (PAOs) is preferred.

It has been proposed, for stable granulation, a prolonged anaerobic feeding period should be implemented to achieve this preferential removal (de Kreuk & van Loosdrecht, 2004). In a slowgrowing system and under anaerobic conditions, PAOs and GAOs convert short-chain VFAs into storage polymers called polyhydroxy-alkonates (PHA) (mainly in the form of poly- β -hydroxybutyrate (PHB)) (Figure 4). During this uptake, GAOs source energy from stored glycogen, while PAOs derive energy from glycogen, in addition to stored polyphosphate (while releasing orthophosphate (ortho-PO4³⁻) into the bulk liquid phase). The removal of this COD during the anaerobic phase is known as the 'feast' phase.

VFA that is not converted by PAOs or GAOs into storage polymers within the cell during the feast phase will most likely lead to granular instability. This is due to rapid carbon utilization and cell growth by heterotrophic organisms under aerobic conditions, which outcompete slow-growing organisms (Pronk et al., 2015a). The VFA loading is therefore an important parameter in granular stability, and can be manipulated to optimize AGB systems.

When given oxygen and no readily biodegradable carbon source (the 'famine' phase), the PAOs and GAOs can use the internally stored PHB as a carbon source for new cell mass. The release of energy, in the oxidation reaction, allows for GAOs and PAOs to restock on glycogen. PAOs will additionally recover polyphosphate, thereby removing ortho-PO4³⁻ from the water. Systems that have a greater 'feast-famine' regime, and do not continue feeding during times of aeration, have been observed to favour smoother and denser granules (McSwain et al, 2004).

Between the two slow-growing microorganisms, PAOs are favoured, because in granular systems, they have been found to contribute to denser particles (due to stored polyphosphate at the end of the aerobic phase, and higher ash content likely due to enhanced chemical precipitation with higher ortho-PO₄³⁻ release) and additionally, they contribute to nutrient removal through their net uptake of ortho-PO₄³⁻ from wastewater (Winkler, 2012). It has been statistically proven that slight alkaline conditions (pH>7.3) and lower mesophilic conditions (T<20 °C) favour PAO growth over GAO growth (Weissbrodt et al., 2013).



Figure 4 - Depiction of PAO (top) and GAO (bottom) metabolism in wastewater treatment (adapted from van Haandel & van der Lubbe, 2012)

2.3.2 Nitrogen Removal in AGB

In an ideal AGB system, the outermost zone of the granule would be dominated by autotrophic nitrifying bacteria, that are capable of converting ammonia (NH₃) into nitrite (NO₂⁻) and consequently, nitrate (NO₃⁻), with oxygen as the electron acceptor. To ensure nitrification, the solids retention time (SRT), within the reactor, must be great enough for nitrifying microorganisms to grow.

Heterotrophic growth, however, is not limited to the anaerobic phase of the reactor. During the aerobic phase, if sufficient anoxic zones exist in the granule (due to limited oxygen penetration which is a result of an optimum bulk DO concentration), denitrifying heterotrophic bacteria can use the produced NO₃⁻ as an electron acceptor; thus, nitrogen can be fully removed from the system in the form of dinitrogen gas (N₂). In order to achieve denitrification during the aerobic phase, the diameter of the granules (relating to DO penetration depth), and amount of DO in the bulk liquid are important parameters, and can be optimized (de Kreuk et al., 2005a). As such, proper DO control during the aerobic phase in the AGB SBR allows for simultaneous nitrification and denitrification (SND). To achieve the best nitrogen removal, one study found that 10% of oxygen saturation during the aerobic phase led to the optimal removal, while another recommended 20% for highest N-removal (Mosquera-Corral et al., 2005); (de Kreuk et al., 2005a). Maintaining AGB systems at lower oxygen concentrations is beneficial from an operational perspective with a reduction in energy consumption, but, low DO may impact granular stability. The organisms capable of denitrification can include PAOs, meaning simultaneous removal of nitrogen and phosphorus, or denitrifying dephosphation, can also be accomplished (Bassin et al., 2012).

Alternatively, the possibility of achieving optimal nitrogen removal by having alternating nitrification and denitrification (AND) during the aerobic phase has been explored (Lochmatter et al., 2013). Instead of controlling DO at a set-point in the reactor, intermittent aeration, or changing from periods of high and low DO, can be implemented to promote nitrification and denitrification from happening at different times. From a nitrogen perspective, AND has shown increased removal efficiencies; however, a drawback can include the re-release of phosphate. In the intermittent feeding strategy, mixing of the reactor does not occur during the periods without air. It was reported that this strategy did not impact granular stability.

High oxygen concentrations during the aerobic phase can lead to the accumulation of NO_3^- and NO_2^- into the anaerobic feeding phase. The presence of NO_3^- , an electron acceptor for facultative anaerobic denitrifiers, in high concentrations can lead to a competition between denitrifiers and GAOs/PAOs for rbCOD during the consequential, feeding period, and thus, compromise ortho- PO_4^{3-} removal (Lochmatter & Hollinger, 2014). Another potential consequence of denitrification during the anaerobic feeding period is the production of dinitrogen gas causing settled sludge to rise in the reactor. If the feeding regime is bottom fed, the "rising sludge" would completely diminish the ability of the reactor to behave as plug-flow.

2.3.3 Temperature

As the technology is performing BNR and relies on bio-chemical reactions to take place, temperature can become an important parameter in the design and implementation of AGB systems. As such, studies have been conducted to access the effect on performance that temperature can have on different aspects of granular formation, stability and performance. In temperate climates, where water temperature varies seasonally, understanding the influence of temperature on granular systems is important.

The effect of temperature on granular formation has been observed in a study that started-up AGB systems in temperatures ranging from 8°C- 20°C (de Kreuk et al., 2005b). At lower temperatures, outgrowth of filamentous organisms was observed, leading to unstable granules and washout of biomass. At lower temperatures, the uptake of VFAs is less due to slower biological activity, which can lead to readably degradable carbon being available during the aerobic phase, which diminishes the feast-famine effect known to promote granular stability. Additionally, at lower temperatures (up to 4 °C), water becomes denser, making it more difficult for particles to settle. As such, granule formation is not favoured at low temperatures, and stability can be achieved when water temperatures are at 20°C. Operating the reactor at lower temperatures, after granular stability has been achieved, was not seen to influence the system. Consequently, it can be concluded that lower temperatures are least desirable during reactor start-up.

Temperature changes can also impact the removal performance of nutrients in AGB systems. In particular, the GAO/PAO ratio can be impacted with higher temperatures (30°C) favouring the growth of GAOs, leading to decreased phosphorus removal, in comparison to lower temperatures

(20°C) (Bassin et al., 2012). Nitrogen removal capacity is also temperature dependent, as nitrification is known to correlate with temperature, as per the Arrhenius equation.

The V_{CRIT} can be influenced by temperature from both biological and physical forces. One study analyzed the settleability of activated sludge in a feast-famine SBR at different temperatures, and related it to the types of storage polymers present in the biomass. Less PHB was seen to form at higher temperatures (above 30°C), which also correlated with high SVI. The poor sludge settleability at higher temperatures was attributed to the formation of long zoogloeal structures, which led to smaller flocs (Krishna & van Loosdrecht, 1999).

2.4 North American Context

Due to higher water consumption use per capita, North America is known to have lower strength wastewater than other developed parts of the world. Data compiled from available sources show that average ranges of wastewater characteristics for Metro Vancouver are lower than that of Europe, and a few Nereda® installations (Table 1). Although this data does not represent the complete dataset, the shortage of available information on AGB implementation in areas with lower strength wastewater, creates a knowledge gap in the field. As presented in Section 2.2.1, the characteristics, including strength of wastewater, can influence the granulation process, and thus, fuels the need fulfill more research in this area using local conditions.

Table 1 – Raw wastewater influent characteristics for Metro Vancouver, Europe and available Nereda® Installations. *data based off 2014 yearly averages for five treatment facilities in Metro Vancouver (Metro Vancouver, 2014) **data based off averages for six European countries (Pons et al., 2004)

***data based from three Nereda® treatment facilities with published data (Naicker et al., 2015); (Pronk et al., 2015); (Niermans et al., 2014)

Metro

	Metro	Europe**	Nereda®
	Vancouver*		Installations***
COD (mg/L)	300-550	450-650	500-1300
TSS (mg/L)	150-250	230-425	230-450
Total Kjeldahl Nitrogen (TKN) (mg/L)	30-40	35-55	50-115
Total Phosphorus (TP) (mg/L)	3-5	5.5-9	6.7-19

3 Thesis Objectives

With minimal research on using low-strength municipal wastewater to cultivate AGB, the motivation behind this work was to attain better understanding of the influence of the feed characteristics in granular formation and stability at the pilot scale. It was proposed to set-up and control two, AGB SBRs in parallel, using primary effluent from the Annacis Island Wastewater Treatment Plant as feedstock.

The research was conducted to fulfill the primary objective:

 To determine the influence of rbCOD as VFA on the formation and stability of granulation for establishing if local, low strength wastewater is sufficient for granulation. One reactor received VFA exclusively from what already existed in the primary effluent (produced via wastewater fermentation in the collection system). The second reactor received supplementary VFA via sodium acetate (acetic acid) addition during the anaerobic feeding phase. The dosing concentration was not greater than what could be available already at wastewater treatment facility that uses primary sludge fermentation to generate volatile fatty acids for biological phosphorus removal. A plug-flow type feeding strategy was used during this phase, as consistent with many studies in literature.

The goal from analysis of the results was to be able to provide more insight into the feasibility of implementing granular systems using typical Canadian primary effluent, and if the addition of readily degradable carbon adds or deters from the formation of stable AGB.

A secondary objective was added to see the potential of reducing start-up time for future projects:

2. To investigate the challenges associated with the start-up of a fully functioning system. Selection pressure, known to play a vital role in granular morphology, was altered in the two reactors by changing the rate of ramp-up of the V_{CRIT}. Both reactors started with the same initial conditions (i.e., same seed sludge, substrate loading, and V_{CRIT}); however, one reactor had the V_{CRIT} changed at a faster rate. If faster rates are proven to be effective, the study could act as evidence that shorter start-up times are possible.

4 Materials and Methods

4.1 Set-up of Sequencing Batch Reactors

Two identical SBRs, operating in parallel, were set-up at the Annacis Research Centre (ARC) in Delta, BC. The ARC, operated by Metro Vancouver, is equipped with a research hall, designed to accommodate wastewater related research by supplying primary effluent, and other streams, from the Annacis Island Wastewater Treatment Plant (AIWWTP) to the facility continuously.

The cylindrical reactors were constructed with clear PVC, had a height of 109.2 cm (43"), an outer diameter of 11.5 cm (4.5"), and inner diameter of 9.72 cm (3.83"). The working volume for each reactor could be maximized at 7.7 L. Three $\frac{1}{2}$ " NPT sampling ports were utilized during the experiments at a height of 19.2 cm (7.55"), 76.9 cm (30.29"), and 86.5 cm (34.07"). To decant the reactors, a $\frac{1}{2}$ " NPT port at 48.1 cm (18.93") was equipped with a solenoid valve. With this configuration, at full capacity, the VER would be at 50%; however, the VER could be reduced easily by decreasing the volumetric flowrate of the feed, and consequently the working volume of the reactors. An outlet port at 96.2 cm (37.86") was designed to prevent overflow if the solenoid valve failed, by removing liquid in attached tubing. Blind flange end caps, with three $\frac{1}{4}$ " NPT ports sealed with a Buna-N gasket, acted as the bottom of the reactors. The first port allowed for the placement of an inlet liquid feed diffuser to promote plug flow feeding. The second port was commissioned to allow for aeration of the reactor. One air-stone diffuser was installed in this port for each reactor. The top reactor flange was left open, to allow for gases to vent off into the surrounding environment.

The set-up allowed for cycling between an anaerobic fill phase, an aerobic phase, a settling phase, and a decant phase (Figure 5). Each phase was triggered with use of a timed controller (ChronTrol XT). For the anaerobic fill phase, a peristaltic pump was activated for each reactor allowing liquid to enter the reactor. To start the aerobic phase, the peristaltic pump was immobilized, and an air pump (HiBlow HP-80) was activated, allowing oxygen to be distributed in an up-flow direction into the reactor. Air flow rate was manually controlled with a rotameter. Aeration was ceased for the settling phase, to allow for biomass to fall under the gravitational force. Finally, to activate the decant phase, power was sent to the solenoid valve. The treated effluent flowed into a recovery clarifier, designed to preserve biomass that may be washed out with the effluent.



Figure 5 – Reactor set-up schematic. Note that the set-up is identical for the second reactor

4.2 Operation of Sequencing Batch Reactors

4.2.1 Reactor Seed

The reactors were seeded with sludge from the AIWWTP, which operates a trickling filter solids contact (TF/SC) process, followed by secondary clarification. The TF/SC process was not intentionally designed for nutrient removal. Biomass was collected for the AGB reactors on February 1, 2016 and October 5, 2016, before each research phase. On February 1, thickened sludge from the secondary clarifiers was retained and initial reactor concentrations were over 5000 mg/L. On October 5, biomass was sourced from the Waste Activated Sludge (WAS) return line and underwent gravitational thickening at the ARC, so that the initial suspended solids concentration was approximately 4000 mg/L.

4.2.2 Cycle Timing and Phase Conditions

Based on previous start-up studies (Rocktäschel et al., 2013; de Kreuk et al., 2005a; Lochmatter et al., 2013), the cycle time distribution was determined for each phase in the cycle (Table 2). A four-hour cycle was chosen and repeated six times per day, to maintain a consistent daily schedule. The cycle timing was altered frequently to allow for the application of selection pressure on the biomass, as is required when starting up AGB with a floccular seed. The selection pressure was induced by increasing the V_{CRIT} and subsequently encouraging the washout of slow settling floccular biomass and accumulating denser aggregates. As such, the amount of time for settling decreased in both reactors as the study progressed. The time reduced in this stage was added to the aerobic period, to ensure each cycle remained at four hours. The amount of time allocated for the anaerobic fill and decant phase remained constant throughout the duration of the study.

	Duration (mins)
Anaerobic Fill	60
Aerobic	135-172.25
Settling	2.75-30
	$(V_{CRIT} = 0.5-7 \text{ m/h})$
Decant	5
Total	240

Table 2 - Time ranges allocated for each phase during the duration of the experiment

Anaerobic Feed Phase

The feed for each reactor was composed of 92% (by volume) primary effluent, pumped directly from the AIWWTP, and 8% sodium bicarbonate, in the water make-up solution. Concentrations ranged from 1000-3000 mgCaCO₃/L in the make-up, meaning that the natural wastewater was supplemented with an additional 80-240 mgCaCO₃/L of alkalinity. The additional alkalinity was added to ensure nitrification (which consumes alkalinity), would not be hindered in the naturally soft waters of the local region. Initially, the flowrate into the reactors was at 65 mL/min, allowing for a VER of 50%, with a working volume of 7.7 L. The VER was changed on Day 96 to 40%, requiring 42 mL/min, with a working volume of 6.2 L.

To fulfil the first objective, additional VFA in the form of sodium acetate was added to the makeup solution entering the second reactor. A concentration of approximately 23 mg acetic acid/L was added to the feed (by dissolving 6.4 g of sodium acetate into the 16 L make-up solution that was supplied as 8% of the feed). The amount of additional VFA that was inputted into the system is representative of the amount of VFA that could be available if primary sludge fermentation existed at the treatment plant (Tchobanoglous et al., 2003).

Aerobic Phase

To allow for both carbon and nutrient removal in the AGB system, the optimum DO range had to be kept around 2 mg/L, with concentrations staying below 5 mg/L (to encourage simultaneous denitrification within the granules as presented in Section 2.3.2). During the Phase 1 portion of the study (May through August), the flow rate of air entering the reactor remained consistent through each aeration cycle, and the flowrate was manually manipulated using the rotameter from acquired data (1-2 L/min). Without the use of DO controllers, timers were used to pulse-aerate the system, and to encourage the DO to stay within the prescribed conditions. Air was diffused into the system for 1-2 minutes before being switched off for 0.5-1 minute (the timing scheme was adapted based on experience, and limitations of the timers). This strategy, was insufficient at satisfying the DO requirements. Breakthrough of readily degradable carbon into the aerobic phase caused the DO to remain low (under 1 mg/L) for up to 30 mins into the aerobic phase. If the air flow rates were increased to allow faster break-up of these constituents, the reactors would reach DO saturation earlier, and discourage denitrification. Additionally, the timers did not allow for

the air to be off for less than thirty seconds, resulting in biomass settling and poor mixing, especially as the study progressed and the biomass became faster at settling. Moving forward with Phase 2 of the study (October-December), the aeration strategy was optimized for enhanced DO control and mixing. A timer was introduced that could be programmed to switch between two rotameters after a fixed time of being actuated. The timer was programmed to allow for higher air flow rates for the first segment of aeration (between 7-12 minutes), followed by a lower flow rate for the remainder of the aeration cycle. Consequently, the biomass remained fully mixed during the duration of the aeration cycle, and the DO stayed at the optimum range for a larger portion of the cycle.

4.2.3 Biomass Wasting

There was no detailed SRT control strategy implemented for the system. As wasting can occur naturally with AGB start-up (through manipulation of the selection pressure), it was uncertain at the start of the study, if the SRT would have to be further reduced through intentional wasting of the sludge bed. Alternatively, the recovery clarifiers provided the option to supplement the systems with additional biomass, if the SRT dropped significantly. All the changes made to the systems that had an effect on the SRT are detailed in Section 5.2.

4.2.4 Maintenance

Routine maintenance was required to keep the reactors performing adequately. The reactors were drained and cleaned with diluted bleach once a week. Shear forces induced by a high air flow rate, in addition to a scrubbing brush, were used to remove any biofilm growth on the reactor surface. With high surface area to volume ratios, due to high height to diameter ratios, biofilm growth has been described as an issue in another pilot scale demonstration, and a similar weekly cleaning approach was used to control biofilm growth in this system (Jezek et al., 2015).

The feed lines into the reactor were cleaned monthly with bleach solution, to remove biomass growth, which could potentially alter the concentration of analytes in the wastewater before entering the reactor.

4.3 Analytical Methods

4.3.1 Sampling: Type and Frequency

The reactor systems were sampled and maintained three days per week during the duration of the study. The 9:00 am cycle was monitored each visit for consistency, considering that wastewater experiences diurnal trends. Two different sampling strategies, Daily Monitoring and Cycle Monitoring were implemented, which allowed for a holistic data set to be collected (Table 3). Daily Monitoring consisted of measuring the influent and effluent concentrations of the reactors for many analytes, as well as doing physical tests on the biomass during the aerobic phase. These monitoring days were a means to observe how each reactor was adapting over time to new conditions. By contrast, for Cycle Monitoring, samples were taken regularly throughout the four-hour cycle, and less physical parameters were measured. The purpose of conducting cycle profiles was to be able to receive insight into the bio-chemical reactions taking place in the system. On average, a Cycle Monitoring was implemented every third or fourth visit.

	Primary	effluent	Reactors	30 mins	Reactors	60 mins	Reactors	70 mins	Reactors	80 mins	Reactors	90 mins	Reactors	120 mins	Reactors	150 mins	Reactors	180 mins	Reactors	210	End of	Phase 2	71JJJ	EIIIueilt	Make-Up	Solution
	D	C	D	C	D	C	D	С	D	C	D	С	D	С	D	C	D	С	D	C	D	С	D	С	D	С
Alkalinity	x	x																					x	x	x	х
Ammonia	x	x				x		х		х		х		х		x		х		x			x	x		
COD	Х																						Х			
DO			х	х	х	х	х	Х	х	Х	х	Х	Х	Х	Х	х	Х	Х	х	Х	Х	Х				
Image													Х													
Nitrate	Х	Х				Х		Х		Х		Х		Х		Х		Х		Х			Х	Х		
Nitrite	Х	Х				Х		Х		Х		Х		Х		Х		Х		Х			Х	Х		
Ortho-PO ₄ ³⁻	Х	Х				Х		Х		Х		Х		Х		Х		Х		Х			Х	Х		
PSD													Х													
pН	Х	Х			Х	Х		Х		Х		Х		Х		Х		Х		Х			Х	Х		
PHB						Х																Х				
SVI													Х													
TSS/VSS	х												Х										х			
VFA	х	х				х		х		х		х				х								х	х	Х

Table 3 - Analytes measured on each sampling day. D - Daily Monitoring; C - Cycle Monitoring

4.3.2 Summary of Analytical Methods

For each analyte, a method for analysis was chosen based on convention and resources that were available for use at the ARC and the UBC Environmental Lab (Table 4).

Table 4 – Summary of Analysis Techniques chosen for each measured analyte.

Analyte	Method	Instrument	Preparation & Preservation	Container
Alkalinity	Standard Method 2320 B (Titration)	Mantech TitraSip™ System	Immediate Analysis	300 mL HDPE
Ammonia (NH ₃ -N)	Standard Method 4500-NH ₃ H (Flow Injection)	Lachat QuikChem® 8000 Series	0.45 μm filtration on nitrocellulose, pH<2 with H ₂ SO ₄ , refrigerated at 4 °C	PS Culture Tube
Chemical Oxygen Demand (COD)	Standard Method 5220 D (Closed Reflux, Colorimetric)	Hach DR2800 Spectrophotometer Bioscience, Inc. COD Reactor Block	Immediate Analysis; for soluble COD: 0.45 μm filtration on nitrocellulose	10 mL Glass Vial with PP cap
Dissolved Oxygen (DO)	Standard Method 4500-O G (Membrane Electrode)	OMEGA® DOH-SD1	Immediate Analysis	N/A In-Situ
Nitrate (NO ₃ -N)	Standard Method 4500-NO ₃ ⁻ I Cadmium Reduction Flow Injection	Lachat QuikChem® 8000 Series	0.45 μm filtration on nitrocellulose, preserved with phenylmercuric acetate solution, refrigerated at 4 °C	PS Culture Tube
Nitrite (NO ₂ -N)	Standard Method 4500-NO ₃ ⁻ I Cadmium Reduction Flow Injection	Lachat QuikChem® 8000 Series	0.45 µm filtration on nitrocellulose, preserved with phenylmercuric acetate solution, refrigerated at 4 °C	PS Culture Tube
Ortho Phosphate (ortho-PO ₄)	Standard Method 4500-P G (Flow Injection)	Lachat QuikChem® 8000 Series	0.45 μm filtration on nitrocellulose, preserved with phenylmercuric acetate solution, refrigerated at 4 °C	PS Culture Tube
Particle Size Distribution	Standard Method 2560 D Light Scattering	Mastersizer 2000	Refrigerated at 4 °C	50 mL PP Centrifuge Vial
рН	Standard Method 4500-H ⁺ B (Electrometric)	Mantech TitraSip™ System	Immediate Analysis	300 mL HDPE

Analyte	Method	Instrument	Preparation & Preservation	Container
Polyhydroxy- butyrate (PHB)	Adapted from Comeau et al, 1988 (Gas Chromatography)	HP5580 Series 2	Water removed from biomass, frozen at - 85 °C, freeze-dried	GC Vial
Sludge Volume Index (SVI)	Standard Method 2710 D	n/a	Immediate Analysis	1 L graduated cylinder
Total and Volatile Suspended Solids (TSS/VSS)	Standard Methods 2540 D and 2540 E	VWR Signature [™] Oven Thermolyne [™] Muffle Furnace Mettler Toledo X Analytical Balance	Immediate Analysis	300 mL HDPE
Volatile Fatty Acids (VFA)	Standard Method 5560 D (Gas Chromatography)	HP5580 Series 2	0.45 μm filtration on nitrocellulose, pH<2 with H ₂ SO ₄ , refrigerated at 4 °C	GC Vial

4.3.3 Adaptation of Polyhydroxybutyrate Method

Although an important parameter in wastewater treatment, a method for determining PHB is not as established as other wastewater parameters. The gas-chromatography method used in the study was adapted from a paper published at UBC in the late 1980s (Comeau et al., 1988). Modifications were made in the derivatization steps, as well as the column set-up. The re-extraction step was modified such that the acidified methanol layer was removed from the tube, instead of transferring the chloroform phase into a new tube. Additionally, hydrogen was used as the mobile phase in the gas chromatography column instead of helium, due to the current lab set-up.

4.4 Quality Control and Assurance

Measures were implemented, where appropriate, to ensure the quality of the analytical methods. Preservation strategies were taken when samples could not be analyzed immediately (Table 4).

For every sample taken, triplicates were analyzed for TSS/VSS and COD. The average and standard deviation were calculated for each sample, which is represented in the results. For COD analysis, a calibration curve, consisting of KHP standards, was created for every new batch. A line of best fit relating the absorbance value to the COD concentration was created for standards of 0, 100, 200, 300 and 400 mg/L.

Calibrations were performed for each analyte using the Lachat Quickchem 8000. For ortho-PO₄³⁻ analysis, seven standards were used at 0.25, 0.5, 1, 2.5, 5, 10 and 25 mg/L. A seven-point calibration curve was also used for NH₃-N at 0.5, 1, 2, 5, 10, 20 and 50 mg/L. For NO₃-N and NO₂-N, a five-point calibration curve was made using 0.5, 1, 5, 10 and 25 mg/L standards. The method detection limit (MDL) for each analyte were found from previous study and have been determined as 0.04 mg/L for ortho-PO₄³⁻, 0.02 mg/L for NH₃-N, and 0.03 mg/L for NO₃-N and NO₂-N (Leong, 2014).

The volatile fatty acid analysis also required a calibration curve, relating the peak area on the chromatogram to the concentration of each volatile fatty acid. A 1000 mg/L stock solution of acetic, proprionic, n-butyic, iso-butyric, n-valeric, and iso-valeric acid was diluted to create six standards: 2, 10, 20, 50, 100 and 200 mg/L. The standards were analyzed at the beginning and end of the run as a check that the instrument was running appropriately.

Similarly, a calibration curve was created for PHB analysis. 3-hydroxybutyric acid, sodium salt was used to create four standards of 3-hydroxybutyric acid at 10 mg/L, 50 mg/L, 100 mg/L, and 300 mg/L. Benzoic acid was used in all samples and calibration standards as an internal standard to represent the effectiveness and reliability of the sample preparation procedure.

The pH probes used for both pH and alkalinity samples was calibrated every sampling day using pH 4, 7, and 10 buffer solutions. The OMEGA® DOH-SD1 units were also calibrated with openair calibration prior to each use.

Replicates were conducted for the remainder of the analytes, when deemed appropriate. No replicates were taken for PHB samples as to not interfere greatly with reactor biomass concentrations. As such, these data act as solely as insight into the reactor performance and no statistical significance can be made.

When determining 95% confidence for the population means in the data analysis, a normal distribution was assumed.
5 Results and Discussion

5.1 Overview of Phases

To fulfil the objectives in Section 3, the reactor's operation time was split into two phases, with each phase beginning with floccular seed sludge. The first phase ran for 212 days from February 2, 2016 to August 31, 2016, and was meant to compare the addition of rbCOD, in the form of acetate, on the granulation process. The second phase, which ran from October 6th, 2016 to December 12th, 2016, was meant to test the limits of applying selection pressure for starting up granular reactors.

5.2 Phase 1: Comparing external VFA addition

5.2.1 Feed Characteristics

The strategy for the first phase was to impose the same conditions on both reactors, except for the VFA concentrations in the feed, as described in Materials and Methods (Section 4). This strategy worked initially, but as the biomass in both reactors began to differ with time, the changes applied to the systems were tailored to the requirements of each system. The concentrations of analytes measured throughout the duration of the study in the primary effluent, and the diluted reactor inlet have been averaged (Table 5). Recall, that the reactor inlet concentration is the combination of primary effluent (92%) and the alkalinity and VFA (for Reactor 2) make-up solution (8%). The amount of additional alkalinity was based on the nitrification potential of the biomass, and the concentration was adapted on a weekly basis for both reactors, which explains the difference in influent calcium carbonate concentrations between Reactor 1 and 2. Note that samples were taken within the first ten minutes of the reactor cycle, and are meant to represent the sixty-minute feeding period. Reactor 2, on average, received 26.0 mg/L (or 41.9%) more VFA in the inlet feed, which corresponds to 10.6% more COD. The supplemental VFA was chosen to simulate the additional VFA loading provided by a primary sludge fermenter.

The measured inlet feed in this study is typical of the regions primary effluent characteristics, and thus, as expected, the delivery method of wastewater from the AIWWTP did not appear to influence concentrations and are representative of real conditions (e.g. Metro Vancouver, 2014).

		Primary Effluent	Reactor 1 Inlet	Reactor 2 Inlet	
	Ν	Average ± 95% CI	Average ± 95% CI	Average ± 95% CI	
COD (mg COD/L)	61	284 ± 20	263 ± 19	291 ± 19	
VFA (mg COD/L)	77	67 ± 6	62 ± 6	88 ± 6	
NH ₃ -N (mg/L)	83	31.1 ± 1.2	28.6±1.1		
Ortho- PO_4^{3} -P (mg/L)	81	3.1 ± 0.2	2.8 ± 0.2		
TSS (mg/L)	60	80 ± 20	74 ± 18		
VSS (mg/L)	60	74 ± 17	68 ± 16		
Alkalinity	81	166 ± 4	298 ± 8 282 ± 8		
(mgCaCO ₃ /L)					
pН	76	6.8 ± 0.0	N/A		

Table 5 – Primary effluent and diluted reactor inlet concentrations for Phase 1

5.2.2 Overview of Phase 1 Timeline

The following section gives a visual and descriptive journey of the biomass morphology and reactor performance over the course of Phase 1. To help effectively describe the changes in the systems, Phase 1 has been broken down by time into four sections: Day 1-55, Day 56-129, Day 130-178, and Day 179-212. In each section, an explanation of the changes made to each reactor will be given, in addition to progression photos of the biomass, and how analyte effluent concentrations differed with the evolution of the systems.

Figure 6 and Figure 8 show how selection pressure was applied throughout Phase 1 to Reactor 1 and Reactor 2 respectively, and how the MLVSS concentrations differed after the V_{CRIT} was changed. Biomass from the recovery clarifiers was also added when increases in the V_{CRIT} led to excess biomass washout at various times throughout the study. The additions are noted on the figures.

Figure 7 and Figure 9 show the progression in particle size over the course of the study. The D10, D50 and D90 were monitored, and provide evidence on how the floccular biomass morphs slowly into larger aggregates, and granulation. The ratio of SVI₅/SVI₃₀ is also depicted, showing the settling characteristics of the biomass throughout the study. Figure 6 through Figure 9 will be referred to in the following sections, to aid in the description of the reactor and biomass morphology changes.



Figure 6 – Overview of application of selection pressure on the biomass concentration for Reactor 1 during Phase 1



Figure 7 – The changes in D10, D50, D90 and SVI5 to SVI30 ratio for Reactor 1 during Phase 1



Figure 8 - Overview of application of selection pressure on the biomass concentration for Reactor 2 during Phase 1



Figure 9 - The changes in D10, D50, D90 and SVIs to SVI30 ratio for Reactor 2 during Phase 1

5.2.3 Section 1: Day 1 to Day 55

5.2.3.1 Biomass Morphology Changes

To support granulation, the initial aim was to apply selection pressure, by increasing the V_{CRIT}, while keeping the biomass concentration sufficient to support the system (Figure 6 and Figure 8). Both reactors were seeded with high concentration biomass (MLVSS was 8600 ± 526 mg/L and 11993 ± 714 mg/L for Reactor 1 and 2, respectively), but quickly normalized to lower concentrations of 1566 ± 175 mg/L and 1943 ± 163 mg/L, for Reactor 1 and Reactor 2, respectively by Day 12. The systems were unable to support very high concentrations of floccular sludge, resulting in significant washout within the first few cycles of operation. It should be noted that Reactor 2 was unintentionally seeded with a higher biomass concentration, which is likely the reason why higher concentrations were initially observed; however, by Day 55, the biomass concentrations in both reactors were consistent with one another. The V_{CRIT} was increased slowly from 0.5-1.9 m/h within the first 53 days in both systems, and biomass levels continued to fluctuate, however, both systems had reached stability by the end of the period.

This slow ramp-up allowed for biomass acclimation, however, did not encourage the aggregation of flocs, until after Day 50 in both reactors (Figure 7, Figure 9, and Figure 10). As time progressed, there appeared to be larger particles; however, the overall size of the bigger particles did not increase substantially, to indicate granulation. The slow settling capability of the biomass was also evident in the SVI5/SVI30 ratios, which were 2.2 ± 0.1 , and 2.0 ± 0.3 for each reactor (Table 6). The average median particle size for the biomass during this time were $106.5 \pm 9.7 \mu m$ and $120.1 \pm 21.8 \mu m$, for Reactor 1 and Reactor 2, respectively. Recall that by definition, the average particle size in granular biomass should be greater than 200 μm .

Table 6 – Average SVIs/SVI30, average median particle size, and average SRT from Day 1- Day 55 during Phase 1

	n R1	n R2	Value	Value
SVI5/SVI30	14	14	2.2 ± 0.1	2.0 ± 0.3
D50	13	13	106.5 μm ± 9.7 μm	120.1 μm ± 21.8 μm
SRT (days)	15	15	14.1 ± 12.2	22.7 ±4.5

Reactor 1



Seed Sludge

Day 22

Day 48

Figure 10 - Morphology changes in biomass for Reactor 1 and Reactor 2 during the first 55 days of operation of Phase 1

5.2.3.2 Reactor Performance

As the reactor stabilized from the high concentration sludge during the first few weeks, reactor performance was variable. COD removal was similar for both systems (Figure 11a). Removal rates before Day 29 are not shown as during this time, significant washout in the system led to a high frequency of negative removal rates. Results shown are representative of after the biomass had equilibrated within the systems. Reactor 1 saw a slightly lower average effluent concentration, but the percent removal was the same, since Reactor 2 started off with higher COD loading (Table 7).

The samples used for effluent concentrations for COD were centrifuged, as to not include the biomass that has been washed out with the cycle in the COD measurement. As such, the values are used to represent a "soluble" COD measurement. Effluent COD concentrations were typical of AGB COD effluent concentrations (Robertson et al., 2015; Pronk et al., 2015).

Reactor 1 saw nitrification by Day 40 (Figure 11b). Most of the nitrification that was occurring in Reactor 1 appeared to stop at nitrite, as no nitrate accumulation was observed (Table 7). It is possible, but, unlikely that simultaneous denitrification occurred in Reactor 1 during this period. DO concentrations measured in the system towards the end of the period were at saturation, and the particle size of the granules would unlikely support the oxygen gradient required to achieve SND. The importance of average granule size on nitrogen removal has been demonstrated in literature (de Kreuk et al., 2005a).

Reactor 2 did not show evidence of nitrification, as NO_x was not detected in the effluent. Although some NH₃ was removed (on average $18\% \pm 7\%$), the loss could be a combination of reactor dilution and biological uptake of NH₃ for cell growth.

Ortho- PO_4^{3-} removal occurred by Day 10 in both Reactors, meaning the SBR configuration was successful in selecting for slow-growing PAOs during the anaerobic feed (Figure 11c). When nitrification occurred in Reactor 1, ortho- PO_4^{3-} removal was negatively impacted with removal rates decreasing from above 90% to an average of 52%, by the end of the period. It is possible that nitrite carryover into the anaerobic feed phase caused the proliferation of denitrifying organisms, and subsequently competition for VFA with the slow-growing PAOs. As such, although no denitrification occurred during the aerobic phase in Reactor 1, it is likely that the carry over nitrite/nitrate caused some denitrification to occur during the anaerobic feast phase. Reactor 2, which did not see any nitrite/nitrate accumulation, maintained high (>90%) ortho- PO_4^{3-} removal from Day 40, till the end of the period.

	n R1	n R2	Effluent Concentration R1 (mg/L)	Effluent Concentration R2 (mg/L)	Percent Removal R1	Percent Removal R2
COD	14	14	53 ± 15	62 ± 25	77% ±8%	77% ± 6%
PO ₄ ³⁻ -P	17	17	0.4 ± 0.2	0.4 ± 0.3	80% ± 10%	80% ± 19%
NH ₃ -N	20	20	14.2 ± 4.4	19.6 ±1.5	40% ± 17%	18% ±7%
NO ₂ ⁻ -N	18	18	5.1 ± 2.9	0.2 ± 0.1	N/	'A
NO ₃ -N	20	21	0.7 ± 0.4	<0.03	N/	'A

Table 7 – Average effluent analyte concentrations and removal percentages for Day 1-Day 55 during Phase 1



Figure 11 – Percent removal progression for (a) COD (b) NH₃ and, (c) PO₄³⁻ from Day 1-Day 55

5.2.4 Section 2: Day 56 – Day 129

5.2.4.1 Biomass Morphology Changes

After the first 55 days of operation, a more aggressive ramping of the V_{CRIT} was conducted to encourage granulation of the biomass. On Day 56, the V_{CRIT} was increased by 1 m/h from 1.9 m/h to 2.9 m/h in both Reactors (Figure 6 and Figure 8). This was the largest increase in the V_{CRIT} for the system, at the time, and resulted in stable biomass concentrations. The systems were pushed further on Day 60 with the V_{CRIT} set to 3.7 m/h. The systems were not able to maintain the biomass with the increase in the V_{CRIT}, and significant solids washout occurred in both systems, with Reactor 1 falling to 857 ± 106 mg/L, and Reactor 2 falling to 1152 ± 125 mg/L by Day 64.

Despite having less biomass, the particles in Reactor 1 (following the change) appeared to be more granular, with a dense core and smoother edges (Figure 12). The biomass in Reactor 2 did aggregate into larger particles; however, the particles appeared to have outgrowth on the surface, resulting in irregular shapes. The V_{CRIT} of Reactor 1 was then increased to 4.2 m/h on Day 72 to see if the granule-like particles could thrive with a higher selection pressure. This change ended up being detrimental to the system and on Day 75, after 15 days at the higher velocities, the MLVSS concentrations were 412 ± 75 mg/L for Reactor 1 and 385 ± 127 mg/L for Reactor 2.

To ensure the survival of both systems, the V_{CRIT} was reduced to 2.9 m/h (the last successful V_{CRIT} before significant washout occurred) and re-seeded with biomass recovered in the clarifiers, as denoted in Figure 6 and Figure 8. After 17 days with the V_{CRIT} at 2.9 m/h, the biomass concentrations had begun to recover, but, the V_{CRIT} was further reduced on Day 96 to 2 m/h, to ensure a sufficient biomass concentration before ramp-up began again.

From Day 96 to Day 129, the V_{CRIT} was increased at small increments from 2 m/h to 4 m/h. The slower progression of ramp-up rates appeared to allow for the biomass to be maintained in the system at biomass concentrations around 1000 mg/L. During the time following the re-seed, the larger biomass particles were common in both systems. In Reactor 1, the particles appeared to be more compact, denser, but with some outgrowth on the peripheral (Figure 12). Reactor 2 particles were larger, however, less dense, and had more outgrowth. The D50 of Reactor 1 increased with V_{CRIT} and went from 153 to 178 µm during the ramp-up (Figure 7).

Reactor 2, did not experience the same upward trend in particle size with the V_{CRIT} and the D50 declined from 256 to 184 μ m by Day 129 (Figure 9). During this time, Reactor 2 did see an increase in the D90, followed by a significant decrease. It is likely that the irregular and unstable shapes of the particles in Reactor 2 led to fluctuating results in the particle distribution. This variability is also observed with the large confidence intervals on the D50 for Reactor 2 (Table 8).

Table 8 - Average SVI₅/SVI₃₀, average median particle size, and average SRT from Day 56-Day 129 during Phase 1

	n R1	n R2	Value R1	Value R2
SVI5/SVI30	19	18	2.1 ± 0.1	2.4 ± 0.3
D50	23	23	167.6 μm ± 7.4 μm	207.0 μm ± 21.2 μm
SRT (days)	21	20	4.5 ± 2.6	5.5 ±1.7

5.2.4.2 Reactor Performance

The COD removal rate did not experience much change from the first 55 days (Figure 13a). Reactor 1 performed slightly better with a removal rate on average 5% higher (Table 9). It did not appear that biomass loss due to washout at Day 75 influenced COD removal; however, it should be noted that during the time a biofilm layer was prominent on the reactor walls. Therefore, it is likely that excess COD that could not be consumed by the biomass during the anaerobic phase, prompted the growth of a biofilm during the aerobic phase; this is not representative of the biological capacity of the granular biomass.

NH₃ removal stayed consistently high during this period (except for after the washout event), likely due to seasonal changes bringing warmer temperatures, however, complete nitrification was not observed, and effluent NH₃ concentrations were averaged at 5.1 ± 3.0 mg/L. A stark dip in the removal rate is apparent in Figure 13b on Day 75, likely from the decrease in SRT because of significant washout of the biomass. By Day 96, nitrification had picked up again in the system and remained consistently high (above 90% removal). In comparison with the first 55 days which saw higher concentrations of nitrite, the NO_x divide appeared to mostly in the form of nitrate during the period (Table 9). Again, no evidence of denitrification occurring in the aerobic phase was evident in Reactor 1, with the pattern in NO_x effluent concentrations following closely with the NH₃ removal. The DO levels nearing the end of the cycle during this time were still close to saturation, therefore, not promoting SND.





Day 64

Day 88

Day 114

Figure 12 - Morphology changes in biomass for Reactor 1 and Reactor 2 from Day 55 to Day 129 during Phase 1.

Reactor 2 finally showed evidence of nitrification on Day 106. It is difficult to deduce the primary reason for the delay in nitrification in Reactor 2, as the SRT of both systems were unstable due to variability in washout. It can be speculated that when aeration would begin, the VFAs that were not consumed during the anaerobic feast phase would be consumed by heterotrophic organisms, which outcompete nitrifiers for oxygen. The breakthrough of VFA was confirmed with the cycle profile results (showcased in Section 5.2.7), and are consistent with the outgrowth seen in the microscope photos. As such, the increase in VFA appeared to lead to limitations in nitrification in reactor start-up, since the biomass was unable to completely follow the feast-famine regime.

Both Reactor 1 and Reactor 2 experienced a decline in ortho-PO₄³⁻ removal rates following the washout event (Figure 13c). Reactor 1 appeared to show improvements in the removal rate after the washout event from Day 82-96. However, this increase in removal rate coincides with the absence of nitrification, and the removal rate of ortho-PO₄³⁻ became unstable again when nitrification had picked back up, and nitrates were found at higher concentrations in the effluent. The rising of the sludge bed during the anaerobic feed ("rising sludge") was first noticed in Reactor 1 on Day 58. This phenomenon provides additional evidence that competition could have existed between PAOs and denitrifiers, leading to less VFA uptake by the slow-growing organisms.

After the dip in the ortho- PO_4^{3-} removal following the washout event, Reactor 2 experienced consistently high ortho- PO_4^{3-} removal rates (higher than 95% from Days 101-129). This stable removal rate did not seem to be impacted by nitrification, which started on Day 106, unlike the negative effect nitrification had on ortho- PO_4^{3-} removal in Reactor 1. During this time, NO_x appeared only in the form of nitrite (Table 9) and no rising sludge was observed.

	n R1	n R2	Effluent Concentration R1 (mg/L)	Effluent Concentration R2 (mg/L)	Percent Removal R1	Percent Removal R2
COD	20	20	42 ± 8	50 ± 8	82% ±4%	79% ±4%
PO4 ³⁻ -P	30	30	0.8 ± 0.3	0.3 ± 0.1	73% ±9%	92% ±4%
NH ₃ -N	29	30	5.1 ± 3.0	21.4 ± 2.5	82% ±10%	32% ±8%
NO ₂ -N	30	30	2.0 ± 0.8	2.5 ± 1.4	N/	A
NO ₃ -N	30	30	14.9 ± 2.7	0.1 ± 0.1	N/	A

Table 9 - Average effluent analyte concentrations and removal percentages for Day 56- Day 129 during Phase 1



Figure 13 - Percent removal progression for (a) COD (b) NH3 and, (c) PO43- from Day 56- Day 129 during Phase 1

5.2.5 Section 3: Day 130 – Day 178

5.2.5.1 Biomass Morphology Changes

During this period, as the biomass became increasingly different in each system both physically, and bio-chemically, it was deemed appropriate to apply different selection pressure strategies for each reactor. The differences made in each system were based on performance and are described in detail below.

After 17 days at a V_{CRIT} of 4 m/h, and with a strong, consistent biomass concentration (average of 1052 ± 118 mg/L), the V_{CRIT} of Reactor 1 was changed from 4 to 6 m/h on Day 147. The change was made after reviewing a published study, which showed that increasing the V_{CRIT} from 2 to 6 m/h was effective, and did not have detrimental effects on the biomass concentration (Jezek et al., 2015). The study used North American low-strength wastewater, which was supplemented with fermented, primary-sludge supernatant. The study also found that 15 m/h appeared to be too aggressive for the system, and that a V_{CRIT} of 7.5 m/h was sufficient to promote stable granulation. As such, the method of increasing the ramp-up rate with larger changes in the V_{CRIT} was considered and applied to the system. The system lost significant biomass, but was maintained at an average of 796 \pm 102 mg/L from Days 157 to 178 (Figure 6). On Day 157, it was observed that the biomass in Reactor 1 had a high number of granules present (Figure 14).

The average median particle size for Reactor 1 during the period was $284.9 \pm 49.8 \mu m$, and the particle size saw a steep increase after the change in the V_{CRIT}. The D50 climbed from 180.7 μm on Day 144, to 421.3 μm on Day 161, and back down to 318.8 μm by the end of the period (Figure 7). The loss in biomass, around Day 157, may have led to the decrease in particle size experienced in Reactor 1. On Day 164, the SVI₅/SVI₃₀ was 1.2, the lowest it had ever been recorded, however, not at the prescribed 1.0, as defined of granular biomass. The biomass during this period was consistent to what had previously been experienced with dense granule particles, with some outgrowth on the edges (Figure 15).

For Reactor 2, a noticeable drop in the MLVSS, after the V_{CRIT} was changed to 4 m/h, prompted a decrease in the V_{CRIT} to 3.9 m/h on Day 142 (the last V_{CRIT} before significant washout occurred), and biomass was supplemented to the system from the clarifier. The V_{CRIT} stayed at 3.9 m/h until Day 163, when it was increased back to 4 m/h when biomass concentrations had appeared to have recovered. Reactor 2 endured the increase back to 4 m/h and was stable at 1037 \pm 80 mg/L by Day 178.

The biomass during the period had evolved to be denser than what had previously been observed; however, the shapes of particles were still irregular, and not spherical as typical of AGB (Figure 15). The average median particle size was $182.5 \pm 19.4 \mu m$, which was 36% lower than the average median particle size of Reactor 1. The disparity is consistent with the differences in V_{CRIT}, which was also around 35% lower in Reactor 2 during the period (the closeness in values is likely more a coincidence then direct correlation). The average SVI₅/SVI₃₀ ratio was, on average, similar in the reactors, despite the differences in biomass characteristics (Table 10).

Table 10 - Average SVI5/SVI30, average median particle size, and average SRT from Day 130-Day 178 during Phase 1

	n R1	n R2	Value R1	Value R2
SVI5/SVI30	15	15	1.8 ± 0.8	2.0 ± 0.2
D50	15	15	284.9 μm ± 49.8 μm	182.5 μm ± 19.4 μm
SRT (days)	13	14	4.9 ± 2.8	3.6 ± 1.1



Figure 14 - Granules observed in Reactor 1 on Day 157.





Day 149

Day 159

Day 173

Figure 15- Morphology changes in biomass for Reactor 1 and Reactor 2 from Day 130 to Day 178 during Phase 1

5.2.5.2 Reactor Performance

The COD removal rates for both Reactor 1 and Reactor 2 increased, on average, 5-6% from the previous period. Reactor 1 again, experienced slightly higher removal rates. A small dip in the Reactor 2 removal rate can be seen after the biomass washout event at Day 142; however, the system recovered with the introduction of additional biomass to the system (Figure 16a).

The NH₃ removal in Reactor 1 was extremely consistent until Day 157, when the removal rate dropped down, eventually reaching 50% (Figure 16b). The drop in NH₃ removal is consistent with the decrease in MLVSS, as reported above. The decrease in SRT (dropped below 1 day) with increased washout, likely resulted in the loss of nitrification potential of the system. As the system stabilized, nitrification rates recovered, and were above 90% by the end of the period. Consistent with what was experienced in the previous period, nitrification in Reactor 1 led to the accumulation of NO₃⁻ in the system by the end of the aerobic phase and rising sludge was observed in the subsequent anaerobic phase. Reactor 2 maintained nitrification throughout the period; however, removal rates showed more variability, likely due to the variability in biomass concentrations.

Reactor 1 experienced a decrease in the average ortho-PO4³⁻ removal rate for the period. Again, competition for VFA during the anaerobic phase from denitrifiers likely caused unstable and low removal rates (Figure 16c) from Day 130-Day 157. Stable removal, averaged at 29%, was observed in Reactor 1 from Days 157 to Day 178.

The ortho-PO₄³⁻ removal rate for Reactor 2 averaged above 99% for the entire period despite significant nitrification occurring (Table 11). The strong, existing community of slow-growing organisms combined with the extra rbCOD in the feed of Reactor 2 may have led to the increased PAO stability, in comparison to Reactor 1. This point is broadened more in Section 5.2.7, which looks at average analyte profiles during this period. Small amounts of rising sludge during the anaerobic feed phase were first observed in Reactor 2 on Day 175, meaning that denitrification was occurring with the breakthrough of nitrites/nitrates into the subsequent cycle.

	n R1	n R2	Effluent Concentration R1 (mg/L)	Effluent Concentration R2 (mg/L)	Percent Removal R1	Percent Removal R2
COD	14	14	39 ± 7	53 ±9	88% ±2%	84% ±3%
PO4 ³⁻ -P	19	19	1.9 ± 0.4	< 0.04	37% ±13%	>99%
NH ₃ -N	19	19	3.2 ± 2.4	7.5 ± 2.1	89% ±8%	74% ±7%
NO ₂ ⁻ -N	19	19	1.2 ± 0.6	7.7 ± 1.5	N	/A
NO ₃ ⁻ -N	19	19	16.4 ± 3.0	2.8 ± 1.5	N	/A

Table 11 - Average effluent analyte concentrations and removal percentages for Day 130-Day 178 during Phase 1



Figure 16 – Percent removal progression for (a) COD (b) NH₃ and, (c) PO₄³⁻ from Day 130 – Day 178 during Phase 1

5.2.6 Section 4: Day 179 – Day 212

5.2.6.1 Biomass Morphology Changes

As Reactor 1 had persisted after the previous change to 6 m/h, the V_{CRIT} was adjusted on Day 179 to 7 m/h to see if the system could reach a more complete and stable granular state. The modification was seriously affected in the system and the MLVSS in Reactor 1 dropped from 850 \pm 87 mg/L to 450 \pm 99 mg/L, by Day 183.

It was decided, at the time, to not re-seed or revert to a lower V_{CRIT} to help recover biomass concentrations. The study was reaching completion, and there would be value in witnessing how the system would respond with a significant change in biomass concentration. Initially, the biomass stayed granular in shape; however, with low biomass concentrations not being able to support the feast phase, the large particles started to accumulate more filamentous outgrowth (Figure 18). The outgrowth differed greatly from the surface outgrowth described in the previous periods; by Day 197, the remains of the granules were overcome with a large amount of filamentous outgrowth, causing the particles to increase their surface area to volume ratio. The particles did become increasingly bigger and the D50 averaged the highest of any of the other time periods at 330.4 \pm 36.5 μ m, but, the loss of structural compactness led to the inability to settle efficiently, and the SVI₅/SVI₃₀ ratio increased with time (Figure 7 & Table 12). Overall, after the change in V_{CRIT}, the larger granules appeared to be the only biomass that could survive and the D10 reached 432 µm on Day 198; however, with the loss of significant biomass concentration, it is likely that the feast-famine condition and slow-growing organisms were lost, and with it, the ability to grow in a compact nature.

Since Reactor 2 had shown to be more sensitive to increases in the V_{CRIT}, the next change was made from 4 m/h to 5 m/h on Day 179 (Reactor 1 had been pushed previously pushed from 4 m/h to 6 m/h). The MLVSS after the adjustment did not seem to be impacted, and averaged 959 \pm 226 mg/L at 5 m/h (Figure 8). At this time, a significant amount of larger, white, fluffy granules were observed in the system (Figure 17). The D50 peaked on Day 190 with a value of 361 μ m (Figure 9).

On Day 191, the system was pushed from 5 m/h to 6 m/h. The system responded with a loss of MLVSS, with a final average concentration of 808 ± 128 mg/L (Day 197-212). The change also appeared to lead to a decrease in the particle size, and the loss of the white granules. Outgrowth on the biomass was still observed during this period; however, the particles appeared to be more spherical in shape (Figure 18).

Both systems were terminated on Day 212, marking the completion of Phase 1.

Table 12 - Average SVI₅/SVI₃₀, average median particle size, and average SRT from Day 179-Day 212 during Phase 1

	n R1	n R2	Value R1	Value R2
SVI5/SVI30	9	10	1.6 ± 0.2	1.6 ± 0.1
D50	9	8	330.4 μm ± 36.5 μm	314.4 μm ± 33.0 μm
SRT (days)	9	10	2.9 ± 3.0	4.7 ± 1.0



Figure 17 - Appearance of "white and fluffy" granules in Reactor 2. Photo from Day 190.

Reactor 1



Day 185

Day 197

Day 206

Figure 18 - Morphology changes in biomass for Reactor 1 and Reactor 2 from Day 179 to Day 212 during Phase 1

5.2.6.2 Reactor Performance

There was a decrease in the average COD removal for Reactor 1, and an increase in the removal for Reactor 2 (Table 13). Overall, the COD removal rate did not vary significantly, and was consistent to what has been observed throughout the duration of the study (Figure 19a). As postulated above, the loss of feast-famine resulted in less growth during the anaerobic feed phase, and more carbon utilization during the aerobic phase, which caused the filamentous outgrowth.

The NH₃ removal was significantly impacted in Reactor 1 following the increase to 7 m/h, with removal rates going as low as 13.5% (Figure 19b). The ammonia removal rate recovered after Day 206, as biomass accumulated in the system; however, the system was no longer granular. Due to the increased biomass washout, the SRT of Reactor 1 decreased to less than 2 days by Day 185, the lowest it had been since the first washout event at Day 75. The SRT began to increase by Day 199, which coincides with the increase in NH₃ removal.

Reactor 2 saw consistent NH₃ removal rates from Day 179-Day 190, when the system was at 5 m/h. During this time, low NO_x concentrations (<0.5 mg/L), were observed, indicating that SND likely occurred. Since there was no change in the air flow rates during aeration, it is likely that the granules formed at the new V_{CRIT} could provide a sufficient oxygen gradient within the granule, as is required for SND. With the push to 6 m/h, the system no longer experienced complete NO_x removal, and thus, likely lost the conditions that promoted the formation of the large, dense granules (Figure 19b). The NH₃ removal rate also decreased after the change in the V_{CRIT}; however, it appeared to be increasing by the time the system was shut down. Rising sludge was observed during this period in Reactor 2, except during the time of SND, when the system was at 5 m/h.

Ortho- PO_4^{3-} removal remained low and unstable in Reactor 1 for the remainder of the study. As suggested, it is most likely the system was no longer able to support the feast-famine regime with such a low biomass concentration. Reactor 2 ortho- PO_4^{3-} removal rates remained consistently high, but, faltered briefly after the washout event on Day 197. (Table 13 and Figure 19c).

	n R1	n R2	Effluent Concentration R1 (mg/L)	Effluent Concentration R2 (mg/L)	Percent Removal R1	Percent Removal R2
COD	9	10	45 ± 10	43 ± 10	86% ±3%	87% ±3%
PO4 ³⁻ -P	11	12	1.7 ± 0.5	0.3 ± 0.4	41% ±17%	90% ±14%
NH ₃ -N	11	12	14.1 ± 4.9	5.2 ± 3.3	45% ±19%	81% ±12%
NO ₂ ⁻ -N	11	12	2.6 ± 1.4	2.9 ± 1.5	N	/A
NO ₃ ⁻ -N	11	12	7.1 ± 4.6	6.7 ± 3.8	N	/A

Table 13 - Average effluent concentrations and removal percentages for Day 179 - Day 212 during Phase 1



Figure 19 – Percent removal progression for (a) COD (b) NH3 and, (c) PO43- from Day 179- Day 212 during Phase 1

5.2.7 Cycle Profiles for Granular Behavior in Phase 1

Based on the findings in the previous analysis, Reactor 1 performed consistently with evidence of a notable granulation biomass fraction during the second portion of Section 2 and the beginning of Section 3. This period corresponds to when the V_{CRIT} was slowly pushed from 2.3 m/h to 4 m/h, and the first week after it was pushed to 6 m/h; it is characterized by stable MLVSS concentrations, high NH₃ removal, and adequate ortho-PO₄³⁻ removal. The seven cycle profiles spanning this period were averaged, to illustrate the average profile for Reactor 1. The cycle profiles included were from Day 99, Day 108, Day 117, Day 124, Day 134, Day 148, and Day 159.

Reactor 2 showed stability, with granular biomass during Section 3 and the six cycle profiles spanning that period were averaged. This time portion corresponds to when the V_{CRIT} was kept between 3.9 m/h to 4 m/h, and is characterized by complete ortho-PO₄³⁻ removal, and some NH₃ removal. The cycle profiles were performed on Day 124, Day 134, Day 148, Day 159, Day 166 and Day 178. Reactor 2 also performed well at 5 m/h (Day 179-Day190); however, no cycle profile was conducted during this period.

Figure 20 through Figure 25 show the averaged, normalized analyte concentrations during the cycle for VFA, ortho-PO4³⁻, DO, NH₃, NO₂⁻ and NO₃⁻, and pH, respectively. Note that initial concentrations marked at time zero are normalized to the primary effluent, for most analytes. This includes the VFA for Reactor 2, which is above 1 at time zero, due to the addition of supplemental acetate. The NO_x profiles were normalized to the effluent concentration, because the compounds are products of nitrification and are not found in primary effluent. Finally, the pH profile was not normalized, and the measured values are shown for easier interpretation.

The sixty-minute mark samples were taken from the reactors after aeration began and represent the mixed influent wastewater (40%) with the remaining contents from the previous cycle (60%). Depending on the analyte, the mixing may cause dilution of the analyte (VFA, NH₃, and ortho- PO_4^{3-}) or may inflate the feed concentration (if NO_x species remained at the end of the previous aeration cycle). The effluent concentrations from the previous cycle were not measured, and thus mass balances on each analyte were not undertaken for the study.

5.2.7.1 VFA Cycle Profile

The averaged VFA cycle profile shows that breakthrough of VFA, from the anaerobic to aerobic phase, was prevalent in both reactors, consistently, during the averaged time (Figure 20). At the sixty-minute transition into the aerobic phase, average normalized concentrations were 0.23 ± 0.09 $(11.3 \pm 4.3 \text{ mgVFA/L})$, and $0.42 \pm 0.06 (20.8 \pm 2.9 \text{ mgVFA/L})$ for Reactor 1 and 2, respectively. With dilution alone, the normalized concentrations should be 40% of what was in the inlet or 0.40 and 0.56 normalized concentration, for each reactor (Reactor 2 had on average had 1.40 times more VFA in the inlet). By subtracting the sixty-minute measured concentrations from the diluted concentrations, it was found that Reactor 1 was using more VFA than Reactor 2, during the anaerobic phase, despite receiving lower concentrations. In particular, Reactor 1 removed 0.17 normalized concentration (8.7 mgVFA/L), while Reactor 2 only removed 0.14 (7.0 mgVFA/L). As such, the extra VFA provided to Reactor 2, did equate to increased VFA uptake during the anaerobic phase. Interestingly, although Reactor 1 was found to use more VFA during the anaerobic phase, evidence from PHB and ortho-PO4³⁻ data suggest that Reactor 2 was better at promoting the growth of PAOs/GAOs. These results will be further discussed in Section 5.2.8. It should be noted that during the averaged time, there were many changes in the reactor biomass, and the averaged data only depicts overarching trends, rather than time specific performance.

The VFA that did not get used in the anaerobic phase, were consumed within the first ten minutes of the aerobic phase. As described, the breakthrough allowed for heterotrophs to thrive, when air was introduced into the system, leading to an unstable feast/famine. These microorganisms most likely led to the outgrowth observed on the granular surface. To prevent feast/famine instability, maintaining more control on the biomass concentration (i.e. prevent significant washout events).

Additionally, it is possible that the plug flow type configuration may have led to VFA breakthrough. Throughout the study, short-circuiting of biomass, due an uneven distribution of the influent into the reactor, was a concern. The feed distribution mechanism was altered several times after noticing that wastewater was coming up quickly along the reactor walls, and bypassing the settled biomass. Often, holes in the distribution system would become plugged with solid materials, causing an uneven flow and short-circuiting. This would lead to VFA availability during aeration, when the reactor contents were mixed. Although a challenge for this system, short-circuiting and uneven distribution could be combatted with an improved influent mechanism.

As described previously, rising sludge in the anaerobic phase due to carry over of NO_x also altered the feeding regime, and potentially led to VFA carry-over, in both reactors. With much of the biomass at the top of the liquid bed due to the release of N_2 , the influent was not in direct contact with the biomass during the anaerobic phase. As such, there was potential for VFA to be unused due to the unmixed feed. Although high substrate concentrations, which are supported by a plug flow regime, are known to be an influential in granular development, the plug flow configuration brought about additional challenges that negatively impacted start-up. If the feed were to be completely mixed in an anaerobic setting, the substrate gradient may not be as high; however, the risk of VFA carry-over into the aerobic phase would be diminished.

5.2.7.2 Ortho-PO₄³⁻ Cycle Profile

As expected, a spike in ortho-PO₄³⁻ concentration was observed in both reactors following the anaerobic phase (Figure 21). The average peak was recorded seventy minutes into the cycle, when theoretically, the peak should have been observed at the sixty-minute mark, or as soon as aeration began. Two explanations exist for this discrepancy. First, it is possible that the system was not completely mixed when the sample was taken, and the sample appeared to be more dilute because of mixing with the previous cycle water. At the time of sampling, this problem was considered, and it was thought that adequate time was given for mixing to occur.

Both reactors ortho-PO4³⁻ concentration peaked at a normalized concentration of 1.4 (1.40 ± 0.51 and 1.40 ± 0.49 for Reactor 1, and Reactor 2, respectively) times the inlet concentration, which again, does not adequately represent the true normalized peak, due to dilution of the wastewater upon mixing. Reactor 2 would be releasing more ortho-PO4³⁻ in the anaerobic phase when compared to Reactor 1 (1.0 mgPO4^{3-} -P/L and 1.3 mgPO4^{3-} -P/L, for Reactor 1 and Reactor 2), if the effluent concentrations were similar to that of the previous cycle. Since Reactor 2, on average, removed all ortho-PO4³⁻, while Reactor 1 did not, the mixed reactor concentration in Reactor 1 would be a combination of the remaining ortho-PO4³⁻ and whatever was produced during the anaerobic phase, which is why the two reactors appear to peak at the same value. As such, Reactor 2 likely had more PAO activity than Reactor 1.



Figure 20 – Average VFA profile for Reactor 1 and Reactor 2 during periods of stable performance in Phase 1 and normalized to the primary effluent concentration. Note that Reactor 2 on average received 1.4 times more VFA during the period.



Figure 21 - Average ortho- PO_4^{3-} profile for Reactor 1 and Reactor 2 during periods of stable performance in Phase 1 and normalized to the primary effluent concentration

Assuming mixing was complete before sampling, another explanation for the delayed peak is that breakthrough VFA into the aerobic phase caused lower DO concentrations, and PAOs were still active at converting VFA into PHB in the aerobic phase, within the granule. A lag was seen in the DO concentrations after aeration began, and the average concentrations remained below 1 m/L in Reactor 1 for the first six minutes, and the first twelve minutes for Reactor 2 (Figure 22). Note that in Figure 22, a moving average trend-line was applied to the averaged data to better depict the trend in DO. The measurement technique used for DO was impacted by the intermittent aeration applied to the system (biomass would settle on DO membrane surface during times of no aeration causing deflated DO readings), which resulted in noisy data. It is possible that either effect or combination of the two, resulted in the ortho-PO4³⁻ peak at seventy-minutes.



Figure 22 - Average DO concentration profile for Reactor 1 and Reactor 2 during periods of stable performance in Phase 1

As DO became more available after the remaining VFA had been consumed during the first ten minutes of aeration, the PAOs were able to use internal carbon and uptake ortho- PO_4^{3-} from the wastewater. A linear uptake was observed in Reactor 1 until 180 minutes into the cycle, and Reactor 2 saw a higher rate (steeper slope) of uptake which lasted for the first 120 minutes. The uptake rate then declined in both reactors. Reactor 2 was able to slowly uptake the reamining ortho- PO_4^{3-} by 180 minutes into the cycle. The rapid uptake of ortho- PO_4^{3-} from the PAO biomass in Reactor 2, meant a prolonged period of aeration without substrate, which can lead to endogenous decay of the biomass. An indication of PAO aerobic starvation would be the re-release of ortho- PO_4^{3-} as the cell undergoes maintenance (Lopez et al., 2006). Since this was never observed in either reactor, the prolonged famine phase did not seem to impact the biomass.

The Reactor 1 effluent contained on average 29% of the ortho- PO_4^{3-} in the influent. The slope after 180 minuntes flattened, indicating that the internally stored carbon had been depleted, and polyphoshate production had ceased. The lack of adequate slow-growing organisms during the anaerobic feast phase, likely led to this occurance. Overall, Reactor 2 was better at biological phosphorus removal, despite having used less VFA during the anaerobic phase. All of these results are supported by the PHB findings presented in Section 5.2.8.

5.2.7.3 NH₃ and NO_x Cycle Profiles

The profile for normalized NH₃ concentrations is shown with NO_x generation (Figure 23). The calculated normalized NH₃ concentration, incorporating dilution, after the sixty-minute anaerobic feed, was expected to be just below 0.40, assuming no nitrification and some biological uptake of NH₃ during the feed. The measured normalized concentration at 60 minutes was 0.33 ± 0.06 (11.3 \pm 1.8 mgNH₃-N/L), and 0.57 ± 0.07 (19.4 \pm 3.9 mgNH₃-N/L), for Reactor 1 and Reactor 2, respectively. Since Reactor 1 experienced complete NH₃ removal during this period, the measured concentration is in the expected range at sixty minutes. The higher normalized concentration observed in Reactor 2 is the result of carry-over NH₃ from the previous cycle, which reduces effect of the dilution.

The removal rates of NH₃ appear to be similar for both reactors as the linear trends have similar slopes; however, only Reactor 1 saw complete removal by the end of the cycle, as it started with a

lower initial amount of NH₃. NO_x production appears to be symmetrical with NH₃ degradation, and qualitatively, it does not appear that simultaneous nitrification/denitrification occurred during the aerobic phase in either reactor during the aerobic phase. Additionally, as discussed, the DO concentrations in the reactor, during the aerobic phase, were likely too high to allow for SND (Figure 22). With the lack of larger dense granules, lower DO concentrations would likely be required to allow for a significant oxygen gradient within the particles.

If no carry-over of NO_x from the previous cycle occurred in the system, at the sixty-minute mark the expected NO₂⁻ and NO₃⁻ concentrations should be zero. Instead, Reactor 1 saw NO₃⁻ and NO₂⁻ concentrations to be 0.55 ± 0.07 ($11.0 \pm 2.0 \text{ mgNO}_3^{-}\text{-N/L}$), and 0.02 ± 0.02 ($0.4 \pm 0.3 \text{ mgNO}_2^{-}$ N/L), respectively (Figure 24). As such, around 55% of the NO_x observed in the effluent appeared to come from carry-over, and mixing with the effluent from the previous cycle. The remaining 45% came from nitrification within the aerobic phase. There appeared to be little to no NO₂⁻ accumulation throughout the cycle, which likely means that NO₂⁻ was further oxidized as soon as it was produced. Interestingly, using a simplified mass balance, it would not appear that NO₃⁻ got consumed during the anaerobic phase, as hypothesized with the rising sludge. Assuming a normalized effluent concentration from the previous cycle being 1, the reactor should expect 60% NO_x at the sixty-minute mark, and 55% is what was observed in Reactor 1, thus contradicting the idea that significant denitrification occurred during the feed. However, without data from the previous cycle, no comments can be made with certainty regarding this simplified mass balance of NO_x.

Reactor 2 saw about 12% of NO₂⁻ found in the effluent coming from the previous cycle, and no carry-over from NO₃⁻. NO₃⁻ appeared in the system by the 120-minute mark, after significant NO₂⁻ (0.20 ± 0.1 normalized concentration or $2.4 \pm 1.0 \text{ mgNO}_2$ ⁻-N/L) had accumulated in the system. The concentration of both analytes rose in the system until the end of the cycle at which roughly 80% of the NO_x was in the form of NO₂⁻, and 20% was in the form of NO₃⁻. Since no carry-over of NO₃⁻ was observed at the sixty-minute mark, and assuming effluent concentrations were similar to the previous cycle, this result promotes the idea that denitrification was occurring during the anaerobic phase.



Figure 23 – Average NH3 and NOx profile for Reactor 1 and Reactor 2 during periods of stable performance in Phase 1



Figure 24 - Average NO₂ and NO₃ profile for Reactor 1 and Reactor 2 during periods of stable performance in Phase 1

5.2.7.4 pH Cycle Profile

The pH of the primary effluent was consistent with local wastewater and, on average, the pH was measured just below neutral (Figure 25). By the thirty-minute mark, an increase in pH was observed in both reactors, which can be attributed to the residual subnatant from the previous cycle and the alkalinity that was added to the feed. The pH in both reactors follow a similar trend, with the pH in Reactor 1 being initially lower when the aerobic feed began, likely due to the faster consumption of alkalinity in nitrification. Reactor 2 stayed at a consistent pH for most of the aeration cycle. Both reactors experienced increases in pH by the end of the cycle, most likely due to excess oxygen stripping CO₂ from the wastewater, reducing the effect of the carbonic acid on the pH.



Figure 25 – Average pH profile for Reactor 1 and Reactor 2 during a periods of stable performance in Phase 1

5.2.8 Polyhydroxybutyrate in Biomass

Biomass samples were collected at the beginning and end of the aeration phase during each cycle profile sampling day, and analyzed for PHB. PHB was found in higher concentrations in Reactor 2 for all samples taken at the transition between the anaerobic and aerobic phase (Figure 26). By the end of the aerobic phase, PHB concentrations were more similar in the reactors, and in some cases Reactor 2 had lower final values, indicating more GAO/PAO activity. The lighter coloured bars are representative of the difference between the beginning and end of the aerobic phase, and on average, PHB consumption was 3.3 times greater for Reactor 2 (Table 14).



Figure 26 - PHB at the beginning and end of the aerobic phase for Reactor 1 and 2 throughout Phase

Table 14 - Average removal of PHB during the aerobic phase for Reactor 1 and 2

	Average PHB Removed in the Aerobic Phase		
	(ng/mg biomass)		
Reactor 1	1475 ± 460		
Reactor 2	4910 ± 1275		

With higher concentrations of VFA in the influent of Reactor 2, more PHB activity was expected; however, as discovered, since Reactor 2 did not use the supplemental VFA in the anaerobic phase (as discovered in in Section 5.2.7), this finding cannot be directly attributed to the higher VFA load. Considering the biomass concentration, the amount of PHB produced per mass of VFA utilized in the anaerobic phase was found to be higher in Reactor 2, than Reactor 1 (Figure 27). This provides circumstantial evidence that higher competition existed in the anaerobic phase in
Reactor 1 for the VFA, and slow-growing organisms were, as a result, not as active in producing PHB. As such, the increase in PHB observed in Reactor 2 may not been directly related to the higher concentrations of VFA in the feed, but instead, related to the types microorganisms active in the system. As noted, Reactor 1 had high levels of nitrate carrying over into the anaerobic phase, which most likely led to the proliferation of denitrifiers that used the VFA directly, instead of converting it to storage polymers during the anaerobic feed.



Figure 27 – Amount of PHB produced for the amount of VFA used during the anaerobic feed in Phase 1

To supplement this argument, nitrates first appeared in Reactor 2 effluent on Day 106, and were present until the end of Phase 1 (Figure 13b). This timing also corresponds to a significant decrease in PHB production on all cycle profiles in Reactor 2 from Day 108 onwards (Figure 26). Therefore, for this system, it would appear that denitrification, during the anaerobic phase, had a stronger influence in determining the production of PHB during the anaerobic phase, than the amount of VFA in the influent. Adding VFA to a system that cannot adequately utilize it during the anaerobic phase led to break-through and subsequent aerobic heterotrophic outgrowth on the granule surface; this ultimately led to granular instability, by reducing the granule's ability to settle efficiently, and led to faster washout of biomass. The decrease in settling efficiency was a result of larger aspect ratios of the granules, due to filamentous outgrowth and the loss of biomass, led to increased oxygen and substrate penetration of the granules and consequently, further instability.

Since a benefit of selecting slow-growing organisms is the removal of ortho-PO₄³⁻ from wastewater, it is important to consider if the PHB being produced is from PAOs or GAOs. An indirect method of determining this is to see how much PHB has been removed in the aerobic

phase compared to the corresponding amount of ortho-PO4³⁻ removal, during the same period. A close to linear relationship exists for Reactor 1, and with higher ortho-PO4³⁻ removal, higher utilization of PHB exists, suggesting that PAOs and PHB are related (Figure 28). With Reactor 2, higher ortho-PO4³⁻ removal is seen throughout, however, much more PHB removal is seen at lower ortho-PO4³⁻ removal concentrations than in Reactor 1. As such, it can be hypothesized that GAOs were more active in Reactor 2, and that the PAO/GAO ratio varies, as experienced with the PHB concentrations.

While slow-growing organisms influence the morphology of granules, from a purely nutrient removal perspective, natural VFA levels in the local wastewater, and thus primary effluent, proved to be sufficient in removing ortho-PO4³⁻ when a strong feast-famine regime existed. The three first data points for Reactor 1 on Figure 28, that show ortho-PO4³⁻ removal below 30%, correspond to Days 148, 159 and 178, after the V_{CRIT} had been changed to 6 m/h, and are a result of low biomass concentrations (less than 900 mg/L). As previously hypothesized in Section 5.2.6, these low biomass concentrations caused the feast-famine regime, and subsequently granulation and ortho-PO4³⁻ removal to stop. As such, the importance of maintaining a high biomass concentration, while applying selection pressure via altering the V_{CRIT}, cannot be understated.



Figure 28 – The influence of PHB removal on ortho- PO_4^{3-} removal during Phase 1

5.2.9 Summary of Outcomes for Phase 1

Overall, there were many differences in the system that did not receive supplemental rbCOD, Reactor 1, to the system that was given more VFA, Reactor 2. Neither system reached a stable granule-dominated state, due to constant changes in the V_{CRIT} and the changing feast-famine regime that was influenced in part by varying primary effluent characteristics; however, large amounts of granular biomass was observed in both systems at different times. The constant changes led to system instability and made the progression of biomass morphology challenging to follow and analyze, however, overarching trends have emerged from the data. The differences observed can be categorized as being physical and relating to the morphology, or biochemical and relating to the reactor performance.

5.2.9.1 Morphology

- The most granular biomass (by appearance) observed during the study was in Reactor 1 from Days 157-170, after the V_{CRIT} was set to 6 m/h. During this time, the SVI₅/SVI₃₀ went as low as 1.2. The appearance of more granular biomass did not lead to better performance, as lower biomass concentrations (due to increases in the V_{CRIT}), would ultimately lead to system instability and the inability of the system to perform in a feast/famine regime.
- Throughout the study, granules in both reactors were seen to have outgrowth around the peripheral, and varied in size and shape at any given time. Reactor 1 saw overall smoother and denser particles, which has been attributed to less VFA breakthrough into the aerobic phase. Consequently, Reactor 2 granules were often more irregular in shape due to large amounts of filamentous outgrowth because of breakthrough of VFA into the aerobic phase. As such, the idea that supplemental VFA would lead to enhanced granular formation in low strength wastewater was not observed. More importantly, ensuring system configuration and influent conditions to promote full VFA take-up during the anaerobic phase, proved to be more influential on granular stability. Overall, not observing smooth, dense granules is not surprising, given that literature has noted that granules grown with domestic wastewater are "more heterogeneous than granules found with synthetic influent." (de Kreuk & van Loosdrecht, 2006); however, there was potential for the system to reach a more stable granular state, if VFA breakthrough had been controlled.

- Applying selection pressure, by modifying the V_{CRIT}, proved to have an observable effect on the size of granules found in the reactors, and led to challenges with maintaining sufficient biomass concentrations. During the first 45 days of the study, when the V_{CRIT} was set to or below 1 m/h, no significant change was seen to the particle size. Larger particles were observed after increasing the V_{CRIT}, and the largest particles that were observed occurred in Reactor 1, after the V_{CRIT} was set to 7 m/h. Large particle size, however, did not always relate to better granule behavior, as this was witnessed with the last stage of Reactor 1, where low biomass concentrations led to the inability of the system to perform in a feast-famine regime.
- The low biomass concentration in the reactors, as a result of large washout events following increases in the V_{CRIT}, was not desirable and likely led to the instability of the granules. Low biomass concentrations meant higher organic loading rates, which although can be beneficial for promoting granulation, led to increased breakthrough of VFA, which ultimately caused instability. Maintaining higher biomass concentrations, reducing cycle time, or altering the feed configuration are ways to promote granular stability by ensuring minimal breakthrough. Based on observations, it is unlikely that granules supported on this wastewater could achieve the V_{CRIT} prescribed in literature of 10-50 m/h.

5.2.9.2 Performance

- Both reactors performed consistently in overall COD removal throughout Phase 1. The total removal for the entire period was 83% ± 3%, and 81% ± 2%, for Reactor 1 and Reactor 2, respectively. A biofilm on the reactor walls would develop over time during routine operation, and would be more prevalent at times following large washout events. As such, the stability in overall COD removal throughout the study, even at times with low MLVSS, may be partially attributed to the growth of a biofilm during the aerobic phase of the cycle.
- Removal of NH₃ was consistent in both reactors during the period of enhanced granular biomass, however, Reactor 1 showed more stability throughout the study. While Reactor 1 started nitrifying at Day 40, Reactor 2 did not experience significant nitrification until Day 106. The SRT for both systems varied significantly during the system due to changes in

the amount of biomass washout at different V_{CRITS}. As such, the variability in SRT may have also led to the delay experienced in Reactor 2, while Reactor 1 may have not been impacted. Additionally, more VFA carry-over into the aerobic phase for Reactor 2, may have caused more competition for oxygen for nitrifiers which delayed their proliferation.

- SND did not appear to be significant in either reactor during the study. As observed in literature, SND can be inhibited if DO concentrations are too high and/or the granule size is too small to create an oxygen gradient (Kreuk, 2005). The lack of DO control in the system during the analysis likely led to an environment which could not encourage SND. Reactor 2 biomass appeared to perform SND for three monitoring days, which corresponded to when the V_{CRIT} was at 5 m/h. Large granules were observed during this time, and it is possible that the biomass morphology had altered enough to support the required oxygen gradient for denitrification.
- Soluble ortho-PO4³⁻ removal occurred quite readily in both reactors following start-up, but, throughout the study, Reactor 2 performed consistently better. It is believed that the extra VFA in the feed was not the sole reason for more consistent ortho-PO4³⁻ uptake, as it was found that VFA uptake during the anaerobic phase was, on average, higher in Reactor 1. It has been postulated that the increased nitrification in Reactor 1 led to carry-over of highlevels of nitrite and nitrate, which in turn prompted competition for VFA in the anaerobic phase between slow-growing PAOs and denitrifiers. Reactor 2 had higher PHB activity throughout the study; however, a decrease in activity was noted after Day 108, which corresponded to when the reactor experienced nitrification, providing more evidence that competition existed. It was found that the low strength wastewater during the study contained sufficient VFA to promote granulation, as well as biological phosphorus removal, and that supplemental VFA would not be required. It should be noted, however, that the study was conducted during the driest and warmest seasons of the local area, and that wet-weather concentrations were not fully represented in this study.

5.3 Phase 2: Testing the limits of Selection Pressure

5.3.1 Feed Characteristics

For the second phase, both reactors received the same primary effluent loading, and without supplemental VFA addition, but differed in the changes made to the V_{CRIT} . The concentrations of analytes measured throughout the duration of the study in the primary effluent, and reactor inlets have been averaged (Table 15). Again, for the reactor inlet concentrations, the primary effluent was diluted, as the make-up made up 8% of the total flow. The same sampling strategy was undertaken and a sample from the first ten minutes were used to represent the entire feeding period.

The measured inlet feed in this study is typical of the regions wastewater characteristics, and thus, as expected, the delivery method of wastewater from the AIWWTP did not appear to influence concentrations and are representative of real conditions (Metro Vancouver, 2014). In comparison to Phase 1, the wastewater during Phase 2 was of lower strength, likely due to the increased precipitation in the wastewater catchment area. The VFA, NH₃, and ortho-PO₄³⁻ concentrations were all at least 30% less, than the average concentrations in Phase 1, while TSS and VSS saw a 20% difference, and the overall COD inlet concentration was only 2% less. The reason for the little change in COD loading could be that wet-weather flows allowed for settled solids to be pulled from the bottom of piping infrastructure, in addition to poorer performance of primary clarifiers with increased volumes.

		Primary Effluent	Reactor Inlet	
	Ν	Average ± 95% CI	Average ± 95% CI	
COD (mg COD/L)	14	277 ± 65	255 ± 60	
VFA (mg COD/L)	21	46 ±16	42 ± 14	
NH ₃ -N (mg/L)	23	20.9 ± 1.7	19.2 ± 1.6	
Ortho-PO ₄ ³⁻ -P (mg/L)	23	2.1 ± 0.2	1.9 ± 0.2	
TSS (mg/L)	17	63 ±10	58 ±9	
VSS (mg/L)	17	60 ± 8	55 ±7	
Alkalinity (mg CaCO ₃ /L)	20	154 ± 9	253 ±17	
pH	20	6.6 ± 0.1	N/A	

Table 15 - Feed and Reactor inlet concentrations for Phase 2

5.3.2 Overview of Phase 2 Timeline

The ramp-up strategy to help stimulate granulation was based off a working schedule of 80 days, beginning in October through to late December (Figure 29). Both systems were selected to begin at 1 m/h, based off of experiences in Phase 1. Recall, that no significant changes occurred in the system until the V_{CRIT} was above 1.5 m/h in the first phase, which provided justification for the initial set point. The final V_{CRIT} was selected to be 5 m/h, which was found to promote granulation during Phase 1. For this scheme, Reactor 1 would reach 5 m/h on Day 70, with 1 m/h increments every 16-19 days. Reactor 2 would reach 5 m/h after 40 days, with 1 m/h increments occurring every 8-10 days. The idea was to not supplement either system with biomass from the recovery clarifier if a washout event occurred, in order to see how the system would truly respond to the ramp-up regime.



Figure 29 – Proposed ramp-up strategy for Phase 2 to discover if a faster ramp-up strategy could be implemented to promote granulation

Due to several challenges, the selected scheme could not be applied to the reactors, and Figure 30 and Figure 32 show the real changes to the selection pressure for Reactor 1 and Reactor 2 respectively, and how the MLVSS concentrations differed after the V_{CRIT} was changed. Note that from Day 28 to 35, both systems were shut off, and the reactor contents stored at 4°C. During this time, the pump delivering primary effluent to the ARC failed and as such, without a reliable feed source, the study was paused.

Figure 31 and Figure 33 show the progression in particle size over the course of the study. The D10, D50 and D90 were monitored. The ratio of SVI₅/SVI₃₀ is also depicted, showing the settling characteristics of the biomass throughout the study. Figure 6 through Figure 9 will be referred to in the following sections to aid in the description of the reactor and biomass morphology changes.



Figure 30- Overview of application of selection pressure on the biomass concentration for Reactor 1 during Phase 2



Figure 31 - The changes in D10, D50, D90 and SVI5 to SVI30 ratio for Reactor 1 during Phase 2



Figure 32 - Overview of application of selection pressure on the biomass concentration for Reactor 2 during Phase 2



Figure 33 - The changes in D10, D50, D90 and SVI₅ to SVI₃₀ ratio for Reactor 2 during Phase 2

5.3.3 Day 1-27

5.3.3.1 Biomass Morphology Changes

Both reactors were seeded with high concentration biomass (~4000 mg/L) from the WAS line, but quickly normalized to lower concentrations of 1665 ± 149 mg/L and 1607 ± 371 mg/L, for Reactor 1 and Reactor 2, respectively at the V_{CRIT} of 1 m/h (Figure 30 and Figure 32). This initial loss was also experienced during Phase 1, which equilibrated to similar biomass concentrations.

As prescribed, on Day 10, the V_{CRIT} for Reactor 2 was increased to 2 m/h, and the biomass was reduced, but stabilized at 1200 ± 54 mg/L. The push to 3 m/h on Day 20, however, led to a severe washout and on Day 27, the biomass concentration was 417 ± 38 mg/L. Reactor 1 was pushed to 2 m/h on Day 17, and similarly to Reactor 2, the biomass reduced to 1137 ± 54 mg/L by Day 27.

No noteworthy changes were observed in the biomass morphology during this period. In both reactors, the settling efficiency decreased with the SVI₅/SVI₃₀ getting larger after the V_{CRIT} was pushed to 2 m/h (Figure 31 and Figure 33). The SVI₅/SVI₃₀ appeared to drop after the push to 3 m/h; however, it was still above 2 by the end of the period. The average median particle size for the biomass during this time were smaller, relative to what was experienced in Phase one and averaged at 92.9 \pm 12.9 µm and 91.4 \pm 12.9 µm, for Reactor 1 and Reactor 2 respectively. Images taken from the first 27 days did not show any granular behavior in the biomass (Figure 34).

As mentioned, both systems were shut down on Day 27, because of a malfunction in the primary effluent delivery system.

	n R1	n R2	Value R1	Value R2
SVI5/SVI30	7	7	2.0 ± 0.4	2.3 ± 0.5
D50	9	9	92.9 ± 12.9	91.4 ± 12.9
SRT (days)	7	7	21.9 ± 13.4	9.2 ± 9.9

Table 16 - Average SVI₅/SVI₃₀ and average median particle size from Day 1- Day 27 during Phase 2





Seed Sludge

Day 12

Day 21

Figure 34 - Morphology changes in biomass for Reactor 1 and Reactor 2 from Day 1 to Day 27 during Phase 2. Note that a change in scale for the Day 21 photographs. The magnification of the microscope was doubled for these images

5.3.3.2 Reactor Performance

The average performance during the first 27 days of Phase 2 have been summarized (Table 17). As with Phase 1, COD removal rates remained high and constant throughout the period (Figure 35a). The removal rates were $85\% \pm 3\%$ and $86\% \pm 4\%$, for Reactor 1 and Reactor 2, respectively.

There was no delay in nitrification in either reactors with NH₃ removal starting by Day 13 in both reactors (Figure 35b). The removal rate was variable in both systems; however, Reactor 2 did not experience lower removal after the significant drop in biomass concentrations, despite the reduction in SRT. The NO_x concentration in the effluent matched very closely with NH₃ removal, and because of the lack of granular biomass in the system, it is unlikely that SND occurred during this period.

Ortho-PO4³⁻ removal started immediately in both reactors with removal rates above 95% for the first 13 days in both reactors (Figure 35c). Reactor 1 remained high until Day 22, in which ortho-PO4³⁻ ceased completely until Day 27. The reason for the absence of ortho-PO4³⁻ removal in Reactor 1 during this time is unclear, since the biomass concentration was adequate, and no significant changes in VFA concentrations in the feed were experienced. It is possible, that like with Phase 1, the presence of NO_x in the feed caused competition between denitrifiers and PAOs during the anaerobic feed; however, it is unlikely that this competition would result in no PAO activity. Another explanation is that GAOs dominated during this period; however, literature would suggest that this is unlikely. Between 10-20°C, and with acetic to propionic acid ratio being between 75-25%, PAOs are likely to dominate (Lopez-Vasquez et al., 2009). A third reason could be that an unintentional change to the reactor configuration (i.e. severe short-circuiting), resulted in the loss of slow-growing organisms. Unfortunately, no cycle profile was captured to confirm if any fluctuations occurred in the ortho-PO4³⁻ concentrations during the aerobic phase.

Reactor 2 experienced some variation in ortho- PO_4^{3-} concentrations, and after the V_{CRIT} was pushed to 3 m/h on Day 20, the ortho- PO_4^{3-} removal rate decreased, likely due to the drop in biomass concentration (Figure 35c).

	n R1	n R2	Effluent Concentration R1 (mg/L)	Effluent Concentration R2 (mg/L)	Percent Removal R1	Percent Removal R2	
COD	7	7	45 ± 14	40 ± 3	85% ±3%	86% ±4%	
PO4 ³⁻ -P	9	9	0.7 ± 0.9	0.5 ± 0.5	70% ± 36%	76% ±23%	
NH ₃ -N	10	9	15.0 ± 5.9	14.2 ± 6.5	38% ±21%	41% ±23%	
NO ₂ ⁻ -N	5	5	7.0 ± 2.6	6.6 ±1.7	N/A		
NO ₃ ⁻ -N	10	10	2.1 ± 2.4	3.6 ± 5.3	N	/A	

Table 17 - Average effluent analyte concentrations and removal percentages for Day 1-Day 27 during Phase 2



Figure 35 - Percent removal progression for (a) COD (b) NH₃ and, (c) PO4³⁻ from Day 1- Day 27 during Phase 2

5.3.4 Day 39-Day 69

5.3.4.1 Biomass Morphology Changes

During the hiatus, it was realized that the planned ramp-up schedule could not be completed due to time and resource limitations. To account for the changes in biomass properties, as a result of endogenous decay during the break, the V_{CRIT} for both reactors was set to 1.5 m/h on Day 35 to allow for acclimatization (Figure 30 and Figure 32). Reactor 2 had very low biomass concentrations before the system was shut down, so this lower V_{CRIT} would be an opportunity for biomass regrowth. Since the original plan could no longer be followed, a more adaptive strategy was considered, with changes made to each system, based on performance.

After 4 days, Reactor 1 was changed back to 2 m/h, and was increased to 2.3 m/h on Day 48 (Figure 30). The biomass concentration remained strong before the change (1314 ± 190) , and the reactor was further pushed to 2.5 m/h on Day 52. Despite the small increase, the biomass could not support the change and the concentration dropped to 490 ± 66 mg/L. This result came as a surprise, since during Phase 1, no severe washout was observed until the V_{CRIT} had reached 4 m/h (Figure 6 and Figure 8). The V_{CRIT} was reduced to 1 m/h, and remained at that level for the rest of the period, while the biomass concentration increased slowly with time. The loss of biomass from 2.3 to 2.5 m/h can be attributed to the lower temperatures impacting settling efficiencies. At the time, the wastewater temperature was 15.2°C. During Phase 1, the ramp-up that led to granulation took place when the wastewater temperatures would impact the buoyant force acting on the particle, and therefore, the ability for it to settle. In particular, the terminal velocity of a particle, of diameter 300 μ m, and density 1010 kg/m³ would be 2.1 m/h at 20°C, and 1.7 m/h at 15°C (Appendix A). This discrepancy could be enough to impact granular start-up.

Reactor 2 was left to recover at 1.5 m/h until Day 55, at which point the biomass concentration had reached 908 \pm 78 mg/L. The V_{CRIT} was increased to 2 m/h, and remained there until the end of Phase 2 on Day 69. The biomass concentration slowly dropped, as did the reactor temperature, which reached 11.3°C by Day 62

Overall, this period saw inefficient settling, and the SVI₅/SVI₃₀ remained unsteady and above 2 in each reactor (Figure 31 and Figure 33). The average median particle did increase from the first period, although still remained low, relative to what would be expected for a granular system (Table 18).

The microscopic images during this time show that the biomass in both reactors was getting denser over time, signaling that the formation mechanisms of granulation may be active (Figure 36). The size of the particles was small, relative to what was experienced in Phase 1.

n R1 n R2 Value Value SVI₅/SVI₃₀ 10 10 2.2 ± 0.3 2.3 ± 0.4 **D50** 10 10 133.5 ± 11.2 147.1 ± 18.4 SRT (days) 9 10 10.4 ± 8.3 5.1 ± 1.7

Table 18 - Average SVI₅/SVI₃₀ and average median particle size from Day 39- Day 69 during Phase 2





Day 41

Day 55

Day 69

Figure 36 - Morphology changes in biomass for Reactor 1 and Reactor 2 from Day 39 to Day 69 during Phase 2. Note the photos are double the magnification of what was used for Phase 1.

5.3.4.2 Reactor Performance

The reactor performance for the last thirty days of operation has been summarized (Table 19). No significant fluctuations were observed in COD removal for either reactor (Figure 37a).

Reactor 1 saw strong nitrification (>70% removal) until Day 57, at which the nitrification rate remained variable for the rest of the study (Figure 37b). As seen in the past, the variability was derived from the flux in SRT following biomass washout. Nitrification in Reactor 2 did not return until Day 43, eight days after the system was brought back online, allowing for enough biomass to accumulate in the system. The removal rate of NH₃ stayed strong for the rest of the study, with the exception of a falter on Day 63, (which cannot be attributed to changes in biomass concentration and SRT). During the last week of operation, the local weather shifted, which brought a major snow storm to the area on Day 62 (December 5, 2016). The change in local weather, meant the reactors experienced colder temperatures, which likely caused the interruption in nitrification, which is known to be temperature sensitive. Again, NO_x levels followed NH₃ removal quite closely, and the size of particles at the time would likely not promote SND.

Ortho-PO₄³⁻ removal was variable in both reactor systems throughout the remainder of the study (Figure 37c). The VFA concentrations in the primary effluent during this period ranged from 12.2 mgCOD/L to 49.8 mgCOD/L, with an average of 32.5 ± 7.2 mgCOD/L. The average concentration in the primary effluent for Phase 2 was 46 ± 16.0 mgCOD/L, hence this period saw the lowest VFA concentrations in the inlet throughout the study, and along with the colder temperature, likely led to the variability in ortho-PO₄³⁻ experienced in the system.

	n R1	n R2	Effluent Concentration R1 (mg/L)	Effluent Concentration R2 (mg/L)	Percent Removal R1	Percent Removal R2	
COD	7	7	41 ±9	36 ± 6	82% ±5%	84% ±3%	
PO4 ³⁻ -P	13	13	0.6 ± 0.2	0.6 ± 0.3	69% ±13%	69% ±15%	
NH3-N	12	13	3.9 ± 2.9	7.0 ± 3.3	81% ±14%	63% ±18%	
NO ₂ ⁻ -N	13	13	7.5 ± 2.8	8.1 ± 2.5	N/A		
NO ₃ ⁻ -N	12	12	3.6 ± 1.5	1.4 ± 0.8	N/A		

Table 19 - Average effluent analyte concentrations and removal percentages for Day 39-Day 69 during Phase 2



Figure 37 - Percent removal progression for (a) COD (b) NH₃ and, (c) PO4³⁻ from Day 39-Day 69 during Phase 2

5.3.5 Summary of Outcomes for Phase 2

Overall, fulfilling the objectives of Phase 2 proved to be challenging; however, valuable lessons associated with granular start-up were reinforced from the findings. Neither system cultivated granules during the 59 days of operation, due to a shift in environmental factors, which overall impacted the goals of this study. The outcomes have been summarized below.

- Before the system was put on hold, the planned ramp-up schedule was being followed. Reactor 2 was not able to survive the push to 3 m/h on Day 20, providing evidence that a fast ramp-up regime could not lead to faster granulation. It is unclear, however, if this result would be true in all conditions, or if seasonal rains, and colder temperatures were the main reason for poor settling characteristics. Completing this study in the summer, when VFA concentrations are higher, and higher temperatures promote enhanced settling, may have led to alternative results.
- The importance of temperature on the start-up process was a notable outcome of Phase 2. In literature, this phenomenon has been studied, and start-up is not recommended at temperatures below 15°C (de Kreuk et al., 2005b). Temperature is a crucial parameter in governing biological capabilities of the system, and with lower temperatures.
- Seasonal deviations on wastewater strength can lead to varied reactor performance. Phase
 1 found that supplemental VFA would not be required in the granular system to promote
 granulation or achieve biological phosphorus removal; however, results from Days 39-69,
 indicate variable VFA loading may have contributed to variable ortho-PO4³⁻ removal. In
 future studies, it would be valuable to see how a stable granular biomass would be impacted
 by the seasonal VFA loading, and if, supplemental VFA would be required to achieve yearround nutrient removal.

5.4 Conclusions

With the completion of two research phases, and subsequent analysis, the outcomes from the objectives sought out in Section 3, have been summarized. From the primary objective, it was discovered that the addition of VFA, to supplement the primary effluent, was not effective, as the system was not able to uptake the extra VFA during the anaerobic feed phase. Challenges with maintaining a proper plug-flow configuration, in addition to too low biomass concentrations, ultimately led to VFA breakthrough into the aerobic phase. Granular formation was hindered with unstable feast/famine regimes that resulted from the presence of VFA during the aerobic phase. During the summer months, when the study occurred, natural wastewater concentrations proved to be sufficient at promoting granulation and nutrient removal, and therefore it was recommended that additional VFA would not be a necessity when starting up during this time.

The subsequent, secondary objective was partially fulfilled and environmental factors, ultimately led to unsuitable start-up conditions. Granulation was not observed in either system, and ramping up the rate at which V_{CRIT} was increased by, led to faster washout of biomass, and system instability.

5.5 Recommendations

With the completion of the study, a few recommendations for new research can be made:

- Since Phase 1 was conducted in the summer months, when VFA concentrations were the highest, the need to see the impact of this study using winter conditions may be deemed appropriate. Phase 2 results showed that winter start-up was not feasible under the study conditions, so, starting-up in the summer, and continuing the study through the subsequent winter, could be an effective way of showing the possibility of AGB during all local seasons. The need to add VFA during periods of wet-weather, and insulation to keep water temperatures at a sufficient temperature should be considered.
- The plug-flow feeding regime used in this study proved to be challenging, and it is believed that short-circuiting may have led to the diminishment of the feast/famine regime. A completely-mixed feeding regime has been found to be effective for granulation in literature, and thus, it may be beneficial to see the possibility of promoting granulation

using this feeding strategy in local low-strength wastewater conditions. Additionally, if using a plug-flow configuration, it is recommended to put emphasis on its design to ensure that short-circuiting would be minimal. This includes an even distribution through the entirety of the reactor bottom surface, and sizing holes to ensure solids material does not regularly plug the dispensing unit.

- Using the local conditions, it is recommended to keep reactor MLVSS concentrations sufficient, around 1500 mg/L, or above 1000 mg/L, or, high enough to ensure a stable feast/famine regime. Results from the current study suggest that the V_{CRIT} does not need to be pushed above 5 m/h to have sufficient granulation. Setting the V_{CRIT} higher poses a higher risk of significant biomass washout. When increasing the V_{CRIT} of a system, it is important to ensure that biomass concentrations have reached stability before more changes are made.
- In order to better promote total nitrogen removal, high emphasis should be put on DO control. At start-up, when particle sizes are still representative of floccular biomass, the denitrification potential is low. As such, to achieve denitrification during start-up, low DO concentrations (around 10-20%) would be required to promote SND. Another possibility would be to add an anoxic phase during the famine phase to help promote denitrification. Negative consequences could result from this addition (i.e. re-release of ortho-PO4³⁻), and therefore, it may be required to always finish the cycle with aeration, before settling and decant. When starting an AGB system, much emphasis should be put on the strategy for DO control and manipulation, especially when nutrient removal is the motivation.
- When conducting AGB research, having the resources to perform genetic sequencing and microorganism identification could be beneficial. These techniques can help identify how changing parameters can alter the bacterial communities, and help explain, with better certainty, why one system behaves differently from another.

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Appendix A

Stoke's Law Calculation

Constants and Inputs

Gravitational constant (g)= $9.81 \frac{m}{s^2}$ Radius of particle (r) = $1.5 \times 10^{-4} m$ Density of the particle (ρ_p) = $1010 \frac{kg}{m^3}$ Density of water at $15^\circ C$ (ρ_{w-15}) = $999.1 \frac{kg}{m^3}$ Density of water at $20^\circ C$ (ρ_{w-20}) = $998.2 \frac{kg}{m^3}$ Dynamic viscosity of water at $15^\circ C$ (μ_{w-15}) = $1.138 \times 10^{-3} \frac{kg}{sm}$ Dynamic viscosity of water at $20^\circ C$ (μ_{w-20}) = $1.002 \times 10^{-3} \frac{kg}{sm}$ Velocity of particle (v) Gravitational forces acting on particle: $F_g = mg = V\Delta\rho g = \frac{4}{3}\pi r^3 (\rho_p - \rho_w)g$ Drag force acting on particle (Stoke's Law): $F_D = 6\pi r\mu v$

Terminal velocity when occurs when,

$$F_g = F_D$$

$$\frac{4}{3}\pi r^3 (\rho_p - \rho_w)g = 6\pi r\mu v$$

$$v = \frac{2}{9} \frac{(\rho_p - \rho_w)r^2g}{\mu}$$

At 15° C,

$$v = \frac{2}{9} \frac{\left(1010\frac{kg}{m^3} - 999.1\frac{kg}{m^3}\right)(1.5 \times 10^{-4} \, m)^2 * 9.81\frac{m}{s^2}}{1.138 \times 10^{-3}\frac{kg}{sm}} * \frac{3600 \, s}{h} = 1.69\frac{m}{h}$$

At 20° C,

$$v = \frac{2}{9} \frac{\left(1010\frac{kg}{m^3} - 998.2\frac{kg}{m^3}\right)(1.5 \times 10^{-4} m)^2 * 9.81\frac{m}{s^2}}{1.002 \times 10^{-3}\frac{kg}{sm}} * \frac{3600 s}{h} = 2.08\frac{m}{h}$$