Construction and Start-up of

Continuous Flow Aerobic Granular Sludge Reactors with Side Stream Selective Wasting

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Civil Engineering

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Winnipeg

Abstract

This study upscales a previously examined continuous plug flow conventional activated sludge (CAS) process with side stream selective wasting to gather comprehensive data for process optimization and uncover potential issues that may not be apparent at a smaller scale. The proposed side stream selective wasting mechanism was uniquely designed to facilitate granulation within a continuous plug flow system. It requires a small footprint and can be seamlessly integrated into existing continuous flow systems without introducing the adverse effects of aggressive shear stress, which is a common issue with hydrocyclones.

The study was conducted in three stages: the first stage focused on testing split feeding, the second stage evaluated combined feeding, and the third stage assessed the implementation of side stream selective wasting. Although mechanical challenges occurred, the system achieved general biological nutrient removal. However, maintaining a consistent hydraulic retention time (HRT) and solids retention time (SRT) within the given process installation emerged as the most significant challenge throughout the study.

This research contributes valuable insights into the optimization of medium size CAS pilot processes and highlights the potential of side stream selective wasting as a viable solution for granulation. The findings underscore the importance of careful process design and system integration in overcoming operational challenges, ultimately paving the way for more efficient and scalable wastewater treatment solutions.

Acknowledgements

I extend my heartfelt thanks to all individuals who contributed to the completion of this pilot study. Special thanks to Professors Dr J. Oleszkiewicz, Dr. Q. Yuan, Dr. T. Devlin, Dr. K.Sears, and Dr. R. Sparling for their invaluable guidance, expertise, and unwavering support throughout the entire process. I am deeply grateful to Dr. Wei for his technical assistance and patience in helping me navigate the complexities of the lab work. My gratitude also extends to Jennifer Garica for her collaborative spirit and valuable assistance during the experimental setup and data collection phases. I am particularly indebted to Michelle Paetkau, Jong Hwang, Mark Jacobson, Jorge Martins, and Ian Lucas for their support and coordination at the plant site, which was instrumental in ensuring the smooth operation of the pilot study. I would also like to thank Kevin Magura, Keith Prevost, Shane McBey, and Henry Koch for their hard work, attention to detail, and dedication in aiding the pilot setup, with Henry Koch's specific contribution to the clarifier interior structure being particularly noteworthy. This project would not be possible without the collective expertise, patience, and dedication of everyone involved. Thank you all for your invaluable contributions.

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Introduction

Current wastewater treatment infrastructure often exhibit inadequate nutrient removal capabilities. Rapid urbanization—marked by population growth and industrial/commercial expansion—demands increased treatment capacity. Concurrently, climate change exacerbates wet weather flows, further straining aging systems. Stringent discharge regulations highlight the urgent need for infrastructure upgrades.

The CAS process enables biological nutrient removal (BNR), where treated water is separated from biological sludge in the secondary clarifier before discharge as final effluent. However, solids-liquid separation frequently becomes a bottleneck in this system. Clarifier efficiency directly impacts mixed liquor concentration—a critical factor for nutrient removal performance. To boost treatment capacity, optimizing mixed liquor concentration and improving sludge settleability are essential.

Aerobic granular sludge (AGS) presents a promising solution to this challenge. The AGS process has been rigorously tested in sequencing batch reactors (SBRs) for over two decades, with commercial solutions like AquaNereda® available for both greenfield projects and select brownfield retrofits. However, in densely developed urban areas, space constraints and integration complexities with existing infrastructure often hinder the adoption of these proven technologies.

Other selective wasting sidestream systems, such as the inDENSE® hydrocyclone, effectively enhance biological sludge settleability and mitigate bulking. While hydrocyclones have been tested for aerobic granular sludge (AGS) processes, they remain less studied than the Nereda® process. Although the hydrocyclone’s shear stress aids in removing light fractions, excessive shear can compromise floc or granular integrity beyond a critical threshold.

This thesis project conducted a medium-scale pilot study to test the CAS process in a continuous plug-flow reactor with side-stream selective wasting. The primary objectives of the project is to (1) upscaling a previously studied process and (2) collecting comprehensive operational data for optimization. Key responsibilities involved designing and constructing a system capable of treating 21 m^3/day of wastewater, as well as managing startup procedures and operational monitoring. The study aimed to evaluate large-scale performance, providing critical insights for improving real-world wastewater treatment applications.

AGS aggregates and CAS flocs exhibit distinct physical and biological characteristics that significantly impact treatment efficiency optimization. While small-scale pilot studies have successfully demonstrated AGS feasibility in continuous plug-flow configurations, scaling up remains critical to uncover latent operational challenges. Prior to full-scale implementation, key challenges must be addressed including mixing efficiency, aeration performance, system control strategies, shear stress management, and hydraulic profile optimization. This pilot study serves as a pivotal bridge between conceptual research and full-scale deployment. By proactively identifying and resolving scaling-related issues, the technology's robustness and reliability for real-world applications can be validated, thereby mitigating deployment risks.

The scope of this thesis includes system construction, startup procedures, and achieving initial biological nutrient removal. Due to space and height restrictions, the ten reactors used in the study were arranged in two rows of five during construction—one row aligned with the sloped ground and the other along the sloped floor. All reactors were placed at nearly the same elevation, the two-row configuration and similar elevation placement introduced hydraulic profile challenges. The non-standard secondary clarifier struggled to maintain effective liquid/solid separation, while manual monitoring and maintenance further complicated hydraulic and solids management issues. As a result, maintaining steady HRT and SRT was difficult.

The thesis is structured into three chapters, each addressing distinct research components. Chapter 1 provides foundational background on aerobic granular sludge, including factors influencing granulation, applications, and process configurations. Chapter 2 details system design, construction methodology, and the proposed side-stream selective wasting mechanism. Chapter 3 presents study findings, analyzing nutrient removal performance, selective wasting efficiency, and solids management challenges. It also documents key operational issues and their resolutions, offering a comprehensive assessment of research outcomes. The conclusion synthesizes findings and validates the study's significance.

Chapter 1: Literature Review

Introduction

The escalating global urban population, deteriorating infrastructure networks, increasingly stringent environmental regulations, proliferation of emerging contaminants, and compounded economic-energy crises collectively necessitate the development of innovative wastewater treatment systems that are simultaneously operationally robust, space-efficient, cost-effective, and amenable to technological upgrades. The AGS process presents a compelling technological paradigm capable of achieving simultaneous carbon, nitrogen, and phosphorus removal while facilitating resource recovery—demonstrating a substantial 75% reduction in physical footprint compared to CAS systems (de Bruin et al., 2004).

AGS represents a specialized biofilm structure characterized by self-aggregation of microbial communities in the absence of artificial carriers (de Kreuk et al., 2005). This phenomenon was initially documented in the Aerobic Upflow Sludge Blanket (AUSB) reactor system, a technology originally developed for municipal wastewater treatment (Mishima & Nakamura, 1991). Over the past three decades, AGS has emerged as a focal point of wastewater research, owing to its distinctive physicochemical properties and demonstrated efficacy in simultaneous nutrient removal (Gao et al., 2011; Giesen et al., 2016; Sharma et al., 2019).

CAS processes require secondary clarifiers for liquid-solid separation. However, the slow settling velocity of flocculent biomass restricts clarifier hydraulic capacity, thereby reducing overall system efficiency. This settling limitation directly affects biomass retention, as clarified effluent inevitably carries suspended solids. In comparison to CAS systems, AGS demonstrates distinct operational advantages, most notably enhanced settling characteristics and reduced sludge production. AGS granules, which exhibit a spherical shape and range in size from 0.2 mm to 10 mm, settle up to 17 times faster than conventional flocs (Li et al., 2019; Long et al., 2019; Nguyen Quoc et al., 2021; Winkler & van Loosdrecht, 2022). This superior settling velocity enables efficient liquid-solid separation within a single reactor, eliminating the need for a secondary clarifier, while the same system configuration can sustain higher biomass concentrations (Beun et al., 2002; Ekholm et al., 2022). The distinct operational conditions of AGS further promote the proliferation of slow-growing microorganisms, including ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and phosphate-accumulating organisms (PAO), while suppressing fast-growing heterotrophs (M. K. de Kreuk et al., 2010). This selective microbial enrichment results in substantially lower sludge yields, enhancing sludge settleability.

AGS exhibits a dense, layered structure that allows different functional microorganisms to reside at various depths within the granules (Winkler, Kleerebezem, Strous, et al., 2013). However, this structural complexity presents significant challenges for both granulation processes and research methodologies. Most studies on AGS have employed SBRs because the sequential timing of SBR operations can simulate various operating conditions - including anaerobic feeding, feast and famine cycles, and selective wasting - all within a single reactor configuration (Beun et al., 1999). Conventional continuous plug flow shallow depth reactors cannot simultaneously provide all the necessary competitive conditions, hindering the implementation of AGS in continuous flow reactors and existing plant retrofits. Devlin demonstrated that a side stream selective wasting regime is a promising approach for achieving the desired operating conditions (Devlin & Oleszkiewicz, 2018). More recently, innovative approaches utilizing partial return activated sludge (RAS) with side stream hydrocyclone separation have been shown to decouple the SRT of granules and flocs, thereby favoring slow-growing microorganisms (Regmi et al.,2022). These technological advancements are particularly encouraging as they provide critical insights into the potential configuration of continuous flow reactor-based AGS (CFR AGS) systems.

The conventional wastewater treatment plant (WWTP) was designed primarily to recover water rather than resources extraction. However, in the modern water industry, treated wastewater is increasingly recognized as a valuable source of both high-quality water and recoverable resources. The research community has made significant strides in advancing water and resource recovery technologies, with the Wastewater Resource Recovery Facility (WRRF) emerging as a strategic framework for fostering a sustainable circular economy. As highlighted by Amorim de Carvalho's review, while resource recovery is highly desirable, greater emphasis is required to effectively implement these technologies, particularly within the aerobic granular sludge process (Amorim de Carvalho et al., 2021). Supporting this, Bahgat's comprehensive study—which included a detailed mass balance analysis—demonstrated the feasibility of recovering combined extracellular polymeric substances (EPS), methane, phosphorus, and nitrogen (Bahgat et al., 2023).

Physical characteristics

Granules morphology and size

Aerobic granules are compact, dense particles with spherical or elliptical shapes. Their visual appearance differs significantly from conventional activated sludge. The granulation process, influenced by conditions such as substrate, organic loading rate (OLR), and operating parameters, shapes the granules and enhances the performance of the AGS process. Aerobic granules are typically yellow to brown in colour.

Granule size increases during acclimation until maturity under the given operating conditions, with size variation highly dependent on operating parameters. High OLR promotes faster granulation and produces larger granules, while low OLR results in smaller granules and slower granulation (Tay et al., 2004). For granules smaller than 4 mm, Toh observed that settling velocity and density positively correlate with granule size, while granule strength, surface hydrophobicity, and Sludge Volume Index (SVI) are negatively correlated (Toh et al., 2003). However, larger granules may experience mass transfer limitations, particularly at greater depths. For example, the oxygen penetration depth is approximately 100 µm for a 0.5 mm granule (L. Liu et al., 2010), while acetate and glucose can penetrate up to 1750 µm and 1250 µm, respectively (Chou et al., 2011). Mass transfer limitations can lead to granule core decay and disintegration in large granules (M. K. de Kreuk et al., 2007; Winkler et al., 2015). Nguyen Quoc's research on nitrogen and phosphorus removal revealed that smaller granules (331 µm) exhibit 4.72 times higher specific ammonia oxidizing rates by weight than larger granules (2225 µm), though their rates are 1.43 times lower by surface area. Selecting smaller granules (212 µm to1,000 µm) can significantly reduce aeration energy costs, as they require less dissolved oxygen (DO) , less than 3 mg/L(Nguyen Quoc, Armenta, et al., 2021; Nguyen Quoc, Wei, et al., 2021).

Specific gravity

Shi and Zheng reported that seed sludge has a wet specific gravity of 1.005 g/cm^3, while mature granules range from 1.036 g/ cm^{-3} to 1.1 g/ cm^{-3}. High wet specific gravity contributes to fast settling granules, which is advantageous for liquid-solid separation (Shi et al., 2009; Zheng et al., 2005).

Porosity

The porous structure of granules facilitates nutrient and oxygen transfer to the interior, with granule porosity typically ranging from 0.78 to 0.92 (Xiao et al., 2008), which is slightly lower than the 0.95 porosity of conventional flocs (Gao et al., 2011). However, dead cells or excess EPS can clog pores, leading to mass transfer limitations and granule disintegration (Corsino, Capodici, Torregrossa, et al., 2016).

Settling velocity and SVI

Fast settling is a key advantage of aerobic granules, as demonstrated by Zheng's study, where granules settled 18 times faster than seed sludge (Shi et al., 2009; Zheng et al., 2005). Larger and denser granules exhibit superior settling properties and lower SVI than conventional flocs (Su et al.,2012).

Surface chemistry

Higher cell hydrophobicity reduces Gibbs's energy, favouring cell-to-cell aggregation and granulation (Y. Liu et al., 2004; Zita & Hermansson, 1997). Tay found that acetate-fed and glucose-fed granules had hydrophobicity levels of 73% and 68%, respectively, compared to seed sludge at 39% (J. H. Tay et al., 2002). Qin's research further demonstrated a negative correlation between cell hydrophobicity and settling time, indicating that shorter settling times selectively retain fast-settling particles with high hydrophobicity, thereby enhancing granulation (Qin et al., 2004).

Biological characteristics

Extracellular polymeric substances

EPS are secreted by microorganisms and released through cell lysis and hydrolysis. The composition of EPS plays a crucial role in determining biofilms' strength, elasticity, and sorption capacity. The primary components of EPS include carbohydrates, such as polysaccharides (PS) , proteins (PN) , lipids, and nucleic acids (Christensen, 1989; Lazarova, 1995). Zhang and Bishop reported that EPS is biodegradable and can serve as an energy source, though its utilization rate is higher in suspended cultures compared to biofilms (X. Zhang & Bishop, 2003). Wang et al. further categorized EPS into biodegradable and non-biodegradable components, noting that up to 50% of EPS PS and 30% of EPS PN are biodegradable. However, the biodegradable EPS utilization rate is significantly lower than that of acetate utilization rate, being five times less, but 50 times faster than non biodegradable EPS. They suggested that biodegradable EPS is unlikely to contribute to the structural integrity of granules but may serve as an energy reserve during extended starvation periods. Sutherland and Wang demonstrated that insoluble β-linked EPS in the outer shell of granules is non-biodegradable even after long-term starvation, likely functioning as a structural barrier or backbone. (Sutherland, 2001; Z. Wang et al., 2005, 2007).

Adav compared eight EPS extraction methods and concluded that the PN/PS ratio increases from 0.9 in C to 3.4 to 6.2 in granules (Adav & Lee, 2008). Zhu et al. reported that suitable operating conditions, such as settling and surface gas velocities, promote EPS secretion and granulation. The granules' minimal settling velocity, hydrophobicity, and surface charge are positively correlated with PN content, while the SVI is negatively correlated. Zhu et al. also noted that loosely bound EPS and tightly bound EPS for granules were 33.6 ±9.7 mg/gVSS (Volatile suspended solids) and 96.8 ±11.9 mg/gVSS, respectively, compared to 8.5 ±1.5 mg/gVSS and 43.1 ±2.7 mg/gVSS for conventional activated sludge. There was no significant difference in PS content between granules and conventional activated sludge. They concluded that proteins with high molecular weight likely assist in granulation and enhance structural stability (Zhu et al.,2012,2015) (L. Zhu et al., 2012, 2015).

Microbial composition

Aerobic granules exhibit a unique biofilm structure characterized by a layered structure, which emerges as an adaptive response to particular operational parameters. This stratified configuration establishes heterogeneous microenvironments that support the proliferation of diverse microbial communities at different depths within the granule matrix. The microbial population within aerobic granules is shaped by multiple factors including inoculum properties, substrate characteristics, and operational conditions. Notably, upon completion of granulation, the microbial consortium undergoes substantial compositional divergence from its original inoculum (Lv et al., 2014; Winkler, Kleerebezem, de Bruin, et al., 2013).

The layered structure of aerobic granules allows partial penetration of substrate and oxygen through interstitial pores (Zheng & Yu, 2007) , establishing pronounced gradients of both (Y. Li et al., 2008). These gradients drive spatial microbial stratification: the oxygen-rich outer layer predominantly hosts ordinary heterotrophic organisms (OHO), ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), polyphosphate-accumulating organisms (PAO), and glycogen-accumulating organisms (GAO), while the oxygen-limited inner zone may support denitrifying counterparts including denitrifying OHO (DOHO), denitrifying PAO (DPAO), and denitrifying GAO (DGAO) (S. T. L. Tay et al., 2002). This redox-driven compartmentalization facilitates simultaneous carbon oxidation, nitrification, denitrification, and phosphorus removal within a single granule.

The diverse microbial architecture and nutrient gradients within aerobic granules enable efficient nutrient removal across both fully aerobic and alternating anaerobic/aerobic treatment processes. The complex microbial consortium facilitates self-regulation through the recycling of intermediate oxidation and reduction products. Moreover, the granulation process selects for slow-growing microorganisms, resulting in significantly reduced sludge production compared to CAS.

Granulation Mechanism

Despite extensive research, the granulation mechanism remains incompletely understood. Liu and Tay proposed a four step model: (1) microbial/non-microbial contact via physical movement, (2) aggregation driven by physical and microbial attractive forces, (3) maturation of attached microbes through microbial interactions, and (4) hydrodynamic shaping of the 3-D structure. Steps 1 and 2 critically depend on microbial aggregation propensity, which is governed by cellular profiles and physicochemical properties (Y. Liu & Tay, 2002). Operational conditions, such as a high PN/PS ratio, stimulate EPS secretion. Cell hydrophobicity, positively correlated with the PN/PS ratio, enhances aggregation and granulation efficiency (Y. Liu et al., 2003, 2004; Guo et al., 2011; L. Zhang et al., 2007). Wilén et al. hypothesizes that granulation is not a single action but a combination of multiple factor granulation results from multifactorial interactions rather than a single process (Wilén et al., 2018). Similarly, van Dijk et al. identified six mechanisms contributing to granulation: microbial selection, selective wasting, optimized substrate transport into the biofilm, selective feeding, substrate type (modulation), and breakage management , demonstrating their compensatory flexibility (van Dijk et al., 2022).

Factors affecting granulation and granular stability

Influent feeding regime

The SBR system employs two predominant feeding strategies for AGS cultivation: fast aerobic feeding and slow anaerobic plug flow feeding. Early studies predominantly utilized fast aerobic feeding with continuous aeration, which often resulted in granulation but was frequently accompanied by surface overgrowth of loose filamentous biomass (Morgenroth, 1997; Moy et al., 2002). In contrast, slow anaerobic feeding from settled sludge has been demonstrated to enhance anaerobic polymer storage, offering an alternative approach to granulation control. (M. K. de Kreuk & van Loosdrecht, 2004).

In fast aerobic feeding, high nutrient levels often lead to diffusion limitations. The outer layer of the granule consumes nutrients for growth or stores them as polymers, while the inner core receives limited or no nutrients. Readily available nutrients under aerobic conditions are known to favour filamentous growth, which explains the frequent reports of filamentous overgrowth on granule surfaces in early studies.

In slow plug flow anaerobic feeding, PAOs and GAOs consume substrates and store them as polyhydroxyalkanoates (PHAs) for slower growth during the subsequent aerobic step (M. K. de Kreuk & van Loosdrecht, 2004). van Dijk's modelling on granulation suggested that fast settling granules accumulate at the bottom of the sludge layer, where they receive the most granule forming nutrients and are less likely to be washed out (van Dijk et al., 2022).

Organic loading rate

OLR regulates granule morphology, structure, and granulation rate. High OLR was considered a key inducer of granulation in early studies of fully aerobic reactions. High OLR appeared to produce larger granules in shorter times, while low OLR often resulted in smaller granules or required longer granulation periods (A. Li et al., 2008, 2011). Wang achieved granulation at a low OLR of 1 - 2 kg COD m^{-3} d^{-1} , though the granules were less compact compared to those from other studies. Tay, however, could not achieve granulation at a low OLR of 2 kg COD m^{-3} d^{-1} (J.-H. Tay et al., 2004;S.-G. Wang et al., 2009). Tay's failure was attributed to aggressive settling times of two minutes, which caused significant biomass washout and an unmanageable F/M ratio, preventing granulation. Granulation under high OLR using glucose was also tested, and granules could sustain an OLR of 15 kg COD m^{-3} d^{-1}. However, the granules were irregular with folds (Moy et al., 2002).

Contrary to earlier findings, granulation under low OLR was achieved with anaerobic feeding. Devlin successfully granulated at low OLR using an anaerobic feeding regime (Devlin et al., 2017). OLR is critical in fully aerobic reaction configurations but has less impact on granulation when operating conditions like anaerobic feeding are used to suppress fast growing organisms.

Feast and famine ratio

Particulate or slow biodegradable substrates tend to favour filamentous growth, leading to substrate gradients on the granules' surfaces (M. K. de Kreuk et al., 2010). These gradients can result in structural deterioration of the granules. Anaerobic feeding promotes the storage of substrates as PHA, although not all substrate is converted (Wagner et al., 2015). The feast and famine regime creates a temporal substrate gradient and extends endogenous decay, which helps counteract the overgrowth of filamentous organisms (Chiesa et al., 1985).

Research by Liu and Tay demonstrated granulation in an SBR AGS with a 38% feast in a sodium acetate fed fully aerobic reactor. However, filamentous organisms appeared on the granules' surfaces by the fourth month (Y.-Q. Liu & Tay, 2008). Corsino's study with brewery wastewater indicated that extended famine conditions favour polymer storage microorganisms, enhancing granule stability (Corsino et al., 2017). Sun's investigation with a multiple chamber CFR AGS suggested that a minimum of 50% feast is required, with 33% feast yielding the best results (Sun et al., 2021). This implies that OHO decays faster than substrate storing, slow growing microorganisms under aerobic conditions (Advances in Wastewater Treatment, 2019), leading to the selection of the latter.

Famine conditions enhance cellular hydrophobicity and promote microbial aggregation, as demonstrated by Tay's observations of microbial cell densification following periodic starvation (Y. Liu et al., 2004; J.-H. Tay et al., 2001).

Settling velocity

Settling velocity is a critical factor in the SBR AGS study. Liu et al. observed that aggressive two minute settling at high OLR resulted in granule formation within 24 hours (Y.-Q. Liu et al., 2016). In a full scale SBR AGS plant, a 30 minute settling time achieved a hybrid culture with 80% granules greater than 0.2 mm (Pronk, de Kreuk, et al., 2015). Settling velocity effectively decouples the SRT of granules or granule ready particles from flocs or fragments. Granules have an SRT close to the system's SRT, while flocs have a much shorter SRT, often nearing the HRT. Consequently, granules are retained in the reactor, while flocs are wasted during effluent discharge.

Modelling by van Dijk revealed that settling velocity effectively removes light fractions during the start-up phase. However, it is less effective in maintaining the optimal mature granule size distribution. Over time, large granules develop, which can encounter diffusion limitations, potentially leading to disintegration(McSwain et al., 2004; van Dijk et al., 2020).

Selective Wasting

Settling velocity serves as a key parameter for selective wasting in SBR AGS, where higher velocities preferentially remove light biomass fractions. However, excessively aggressive initial settling velocities or short settling durations may induce premature biomass washout, potentially compromising microbial diversity (Weissbrodt et al., 2012). In the context of CFR AGS systems, Ford demonstrated that hydrocyclone-based selective wasting provides granule densification without affecting critical microbial populations, including AOB, NOB, OHO, PAO, and DPAO (Ford, 2018).

Conventional settling strategies primarily target light fractions without distinguishing between microbial functional groups or granule sizes. Winkler's comparative study of PAOs and

GAOs revealed differential settling behavior, with GAOs preferentially accumulating at the top of the sludge layer while PAOs settled at the bottom, enabling selective GAO removal to achieve complete phosphorus removal (Winkler et al., 2011). Although granule growth enhances settleability, excessively large granules may develop diffusion limitations, potentially causing surface filamentous overgrowth or core deterioration. Selective removal of these large granules from the sludge bed can effectively.

Hydrodynamic shear

The majority of SBR AGS research employs cylindrical column reactors, where aeration and mixing are induced by airflow, quantified as upflow superficial velocity. Early investigations predominantly operated under fully aerobic conditions, which favored rapid-growing OHO over slower-growing microbial groups due to the abundant availability of organic substrates. This environment frequently resulted in pronounced heterotrophic proliferation on granule surfaces. However, hydrodynamic shear forces exerted by aeration effectively suppressed filamentous overgrowth, contributing to the development of smoother and denser granules.

In contrast, Devlin's study demonstrated granule formation under low hydrodynamic shear (minimal upflow superficial velocity) with anaerobic feeding at low organic loading rates (OLR). These findings suggest that hydrodynamic shear is not an absolute prerequisite for granulation but rather functions as a regulatory mechanism to manage high OLR conditions (Devlin et al., 2017; J-h et al., 2001; Y. Liu & Tay, 2002; Wu et al., 2020).

Quorum sensing

Quorum sensing (QS) constitutes a fundamental bacterial communication mechanism mediated by diffusible signaling molecules including acyl-homoserine lactones (AHLs) and autoinducing peptides (AIPs) (Vadakkan et al., 2018). These autoinducers accumulate proportionally with cell density and elicit population-wide coordinated responses upon reaching critical threshold concentrations, enabling synchronized regulation of gene expression and collective behaviors (Galloway et al., 2011).

1) QS has been demonstrated to play a pivotal role in granule formation through its regulatory effects on EPS production, as evidenced by Liu et al.'s findings that QS mechanisms participate in both EPS secretion and the upregulation of quorum sensing-related enzyme genes, particularly under low-temperature conditions (W. Liu et al., 2022). This observation suggests that QS-mediated processes facilitate the selective enrichment of low-temperature-adapted microbial populations while simultaneously promoting EPS biosynthesis - a critical determinant for granule structural stability and microbial aggregation.
2) QS significantly enhances microbial activities essential for granulation through two distinct pathways: (1) functional activation, as demonstrated by Wang et al.'s findings that exogenous AHL molecules (C6-HSL, C8-HSL, and 3-oxo-C10-HSL) stimulate nitrification, biomass accumulation, and self-aggregation in Acinetobacter sp. - a critical nitrifying bacterium involved in granule formation (X. Wang et al., 2018) ; and (2) structural reinforcement, with Yang et al. documenting that AHLs promote EPS production and biofilm development in Cylindrotheca sp., thereby contributing to both granule matrix stability and microbial community organization (Yang et al., 2016). These studies collectively illustrate QS's dual role in enhancing both the metabolic activities and structural integrity of aerobic granules.
3) QS significantly enhances enzymatic activities critical for nutrient cycling in granular systems, as demonstrated by Zhang et al.'s findings that functional AHL molecules can elevate starch hydrolase activity by 4-6-fold, thereby substantially improving substrate degradation efficiency within microbial aggregates (Z. Zhang et al., 2023).

Despite the existing evidence, our understanding of QS in complex, real-world wastewater systems remains incomplete. Current research predominantly focuses on individual signalling molecules, while the intricate interactions among multiple QS molecules in these systems are still not well characterized. This knowledge gap underscores the necessity for further investigations to elucidate QS's regulatory mechanisms in diverse microbial communities and its adaptive responses to varying environmental conditions.

AGS applications

Municipal applications

Municipal wastewater characteristics exhibit significant regional variation, influenced by both geographic conditions and infrastructure design. Warmer regions with flat terrain typically produce septic wastewater due to prolonged retention in sewer systems, whereas hilly, colder climates generate fresher, less degraded influent as a result of rapid drainage and lower temperatures (Oleszkiewicz, et al., 2015). Infrastructure age further compounds these variations: older combined sewer systems experience substantial dilution from spring meltwater, storm events, and infiltration, leading to weaker wastewater flows during wet weather conditions. In contrast, modern separate sewer systems effectively minimize such dilution, maintaining consistently stronger wastewater characteristics throughout the year. This systematic variability underscores the importance of considering regional and infrastructural factors when developing wastewater treatment strategies.

Wastewater consists of soluble and particulate (solid) fractions, each containing biodegradable and non-biodegradable components. During anaerobic phases, microorganisms metabolize and store soluble biodegradable nutrients (e.g., volatile fatty acids). However, particulate biodegradable matter must undergo hydrolysis prior to microbial uptake.

Unhydrolyzed solids may persist into subsequent aerobic phases or treatment cycles, adsorbing to granule surfaces and gradually releasing nutrients under aerobic conditions. This sustained nutrient supply promotes the proliferation of fast-growing OHOs, whose filamentous overgrowth on granule surfaces exacerbates diffusion limitations—a key contributor to granule disintegration in early fully aerobic AGS systems. Therefore, effective biodegradable solids management is crucial for granule stability. Advances in AGS research, such as the implementation of anaerobic feeding and feast-famine regimes, have demonstrated improved operational stability by favoring substrate-accumulating microorganisms (e.g., PAOs) over OHOs. These strategies facilitate anaerobic hydrolysis of biodegradable solids, reducing particulate carryover into aerobic phases and thereby mitigating filamentous overgrowth.

Most AGS systems are designed and operated as SBRs. The SBR cycle consists of four key phases: anaerobic feeding, aerobic reaction, settling, and effluent withdrawal. During the settling phase, dense mature granules settle to the reactor bottom, while lighter biomass fractions (e.g., flocs) are removed with the effluent. However, inert solids (e.g., grit, sand), which exhibit faster settling velocities than granules, may accumulate at the reactor base. This accumulation can disrupt biomass dynamics and reactor hydraulics, potentially compromising system performance.

Effective solids management is crucial for reactor performance: poorly controlled biodegradable solids can destabilize granules by promoting filamentous overgrowth during aerobic phases, while non-biodegradable solids may cause piping clogging or reduce reactor efficiency. To mitigate these issues, screening, grit removal, and primary clarification are essential pretreatment steps, particularly in systems treating wastewater with high solids loads.

Additionally, primary sludge fermentation can generate volatile fatty acids (VFAs) to supplement carbon-deficient influent when primary treatment reduces nutrient availability.

Nutrient leakage into aerobic phases poses a significant risk to granule stability, as residual substrates promote the proliferation of fast-growing heterotrophs that degrade granular integrity. Evolving wastewater characteristics, driven by water conservation practices, climate change-induced wet weather flows, or stormwater surges, further compound this challenge. To address this, SBR AGS systems must incorporate:

1) Adequate anaerobic retention time to maximize substrate uptake by storage organisms (e.g., PAOs).
2) Dynamic DO control to adapt feast and famine ratios to variable loads.
3) Operational flexibility (e.g., adjustable cycle times, redundant configurations) to withstand hydraulic or organic shocks.

The volume exchange ratio (VER) dictates the proportion of light biomass wasted per cycle. During granulation, aggressive wasting (characterized by high VER and short settling times) accelerates granular dominance but risks reducing microbial diversity. Post granulation, VER adjustments enable fine-tuning of biomass retention, though continuous monitoring is required. Granule size distribution also demands attention: oversized granules (>2 mm) are prone to diffusion limitations and disintegration, while stratification within the settled sludge blanket concentrates lighter GAOs at the top and denser PAOs at the bottom. To enhance system resilience, targeted wasting is recommended:

1) Bottom wasting removes oversized granules to prevent fragmentation.
2) Top wasting eliminates GAO rich biomass to favor PAOs, improving settleability.

3) Flexible sludge blanket extraction balances these approaches, adapting to real time operational needs.

Industrial applications

AGS systems—characterized by rapid settling, high biomass retention, and robust pollutant removal—represent a promising alternative to CAS processes. Growing research explores AGS adoption across diverse industries, including wastewater containing toxic or inhibitory compounds. However, industrial effluents often contain recalcitrant or inhibitory substances that challenge AGS stability and performance. Below, industrial case studies are reviewed in ascending order of complexity.

Brewery wastewater is characterized by high COD, with nitrogen derived primarily from malt and cleaning agents and phosphorus from detergents (Brewers of Europe, 2002; Enitan et al., 2015; Gebeyehu et al., 2018; Jaiyeola & Bwapwa, 2016; Natural Resources Canada & Brewers Association of Canada, 2011). Wang et al. achieve granulation within nine weeks, reporting 88% COD and ammonium (NH_4^+) removal (S.-G. Wang et al., 2007). Similarly, Corsino et al. cultivate granules but observe structural deterioration under short cycle operation due to particulate leakage into aerobic phases, which triggers filamentous overgrowth. In contrast, extended cycles with balanced feast and famine conditions yield compact, stable granules (Corsino et al., 2017; Nancharaiah & Kiran Kumar Reddy, 2018; Weber et al., 2007)

Dairy effluent exhibits high COD and solids, with TN and TP constituting 3% – 6% and 0.6% - 0.7% of BOD_5, respectively (Kolev Slavov, 2017). Arrojo et al. demonstrate successful granulation both with and without anoxic phases, achieving 70% nitrogen removal. The authors note that low effluent solids correlate with reduced COD to volatile suspended solids (COD/VSS) ratios or shortened withdrawal times (Arrojo et al., 2004). Schwarzenbeck et al. observed elevated effluent solids across varying OLRs. Compared to synthetic wastewater, raw dairy effluent generates a less pronounced feast and famine dynamic, likely due to high particulate content and inadequate famine phases, exacerbating solids washout (Schwarzenbeck et al., 2005).

Fish canning wastewater is characterized by high organic content (10,000 - 50,000 mg/L COD), elevated nitrogen (0.3 - 69.7 mg/L total nitrogen) derived from proteins, blood, and slime, and significant salinity (Chowdhury et al., 2010; Mendez et al., 1995; NovaTec Consultants Inc & EVS Environment Consultants, 1994). Corsino et al. demonstrated that saline adapted aerobic granules maintain organic carbon removal at salinities up to 75 g NaCl/L. However, nitritation was inhibited at 30 g NaCl/L, evidenced by effluent nitrite concentrations of 50 -70 mg/L and negligible ammonium/nitrate levels. Simultaneous nitrification denitrification (SND) persisted at ⩽50 g NaCl/L. Another factor was the observed high free ammonium further suppressed nitrification (Corsino, Capodici, Morici, et al., 2016; S.-F. Yang et al., 2004).

Petrochemical wastewater or produced water was estimated to be three times the oil production in 2011. Petrochemical wastewater composition varies significantly from one process to another. It contains hydrocarbon, metal, salt, and production chemical components (Fakhru'l-Razi et al., 2009). Zhang gradually acclimated aerobic granules from synthetic wastewater to 100% petrochemical wastewater in the course of 192 days. However, full-strength petrochemical wastewater reduced system performance and granule stability. Co-metabolism supplementation improved treatment performance, increasing COD and ammonium removal from 65% and 75% to 89% and 94%, respectively (H. Zhang et al., 2011). Similarly, Campo's study on slop wastewater demonstrated that gradual acclimation with synthetic and slop mixtures enhanced total petroleum hydrocarbon (TPH) removal (Campo & Di Bella, 2019).

Pharmaceutical and personal care products (PPCPs) originate from various aspects of modern life and ultimately enter wastewater systems, where their bioactive compounds may affect organisms through similar pathways as in humans (Fent et al., 2006). CAS, not designed to address PPCPs, often struggle with their inhibitory effects on biological treatment. In WRRF, PPCP removal efficiency ranges from 12% to 100%, making effluent a primary environmental source (Luo et al., 2014). However, AGS shows promise: Amorim's study on fluoroquinolone (FQ)-containing synthetic wastewater revealed that shock doses caused reversible performance impacts, whereas continuous low-concentration doses did not impair organic removal or nitrification. Prolonged FQ exposure, however, induced granule disintegration, reducing bed volume and denitrification capacity—particularly at high DO levels (~100% air saturation)—leading to elevated effluent nitrate levels. Polyphosphate-accumulating organisms (PAO) were also inhibited during continuous dosing. Remarkably, AGS performance recovered after FQ removal, with adsorption/desorption dynamics observed in mixed liquor and effluent (Amorim et al., 2014). The author hypothesized that the EPS matrix provided some degree of protection against FQs (Ding et al., 2015).

AGS configurations

Since the first AGS was reported two decades ago, the SBR has been the preferred method for most AGS studies and operations. Most full-scale AGS applications, branded under Nereda®, utilize SBRs. The S::Select® and inDENSE® systems, based on hydrocyclones, were initially designed to address floating or bulking sludge issues. A side-stream hydrocyclone has been shown to produce hybrid floc-granular sludge, gaining interest for continuous-flow reactors. However, S::Select® has only a few installations, and performance data remain limited (*S*-

Select® References | Granular Sludge in the Wastewater Treatment, 2023). This section focuses on the Nereda® SBR and inDENSE® systems.

Sequencing Batch Reactors

The development of the Nereda® SBR AGS has advanced significantly since its inception in 1993, with the first full-scale system commissioned in 2011 in Epe, The Netherlands, following a successful four-year pilot trial (Krishna, 2013). This innovative system offers four distinct configurations tailored to different applications: the first and second configurations are designed for greenfield or parallel expansion projects, with the former requiring a minimum of three parallel reactors to ensure continuous feeding and the latter incorporating an equalization basin and at least two parallel reactors to buffer flow during reaction periods. For brownfield or retrofitting applications, the third and fourth configurations are recommended; the third operates in parallel with a conventional CAS system, treating a portion of the flow and directing surplus granules to the CAS to enhance its performance, while the fourth converts existing SBR or CAS systems into Nereda® SBR AGS (Otten et al., 2018). Approximately half of current installations are greenfield projects, with the remainder comprising retrofit or brownfield applications (Hamza et al., 2022). As of 2023, Nereda® has over 100 installations worldwide, demonstrating its widespread adoption and success (*Projects | Royal HaskoningDHV*, 2023).

The Nereda® SBR AGS operates as a constant level SBR with three distinct phases: simultaneous fill and withdraw, reaction, and settle. During the anaerobic feeding phase, flow enters the reactor from the bottom, passes through the settled sludge blanket, and exits as treated effluent from the top, with readily available nutrients hydrolyzed and stored as PHA. The aerated reaction phase is meticulously controlled to create short feast and long famine conditions, which are crucial for selecting slow-growing substrate-storing microorganisms. In the settling phase, a critical settling velocity is established to select dense and compact granules, ensuring the system's optimal performance and efficiency.

Continuous Flow Reactors

The inDENSE® hydrocyclone, introduced in 2016, is a gravimetric sludge selection system specifically designed to address operational challenges such as floating or bulking sludge in CAS processes (World Water Works, 2016b). Functioning as a modular side stream hydrocyclone installed parallel to the RAS line, this innovative technology enhances the treatment process through improved sludge management by selectively retaining denser biomass while removing lighter, problematic solids. The system's gravimetric separation mechanism optimizes sludge quality, thereby contributing to more stable and efficient CAS process performance.

The inDENSE® hydrocyclone separates sludge into two distinct flows: a denser stream and a lighter stream. The denser flow is reintroduced into the mainstream RAS system, while the lighter flow is directed to the sludge handling module. This selective separation ensures the retention of viable microorganisms in the biological treatment process while managing excess biomass, thereby maintaining optimal sludge conditions for efficient wastewater treatment.

The hydrocyclone can be operated continuously or on a scheduled basis, offering flexibility in its application (World Water Works, 2016a). Guo's computational fluid dynamics (CFD) modeling revealed that tangential velocity plays a crucial role in the swirling flow within the hydrocyclone, significantly affecting separation efficiency. High tangential feed flow velocity and large granule size enhance separation efficiency but reduce particulate residence time. This reduction is linked to higher turbulence and hydraulic shear, which can promote granulation by creating dense and compact granules. However, excessive hydraulic shear or tangential velocity may lead to premature granule disintegration, highlighting the need for balanced system operation (D. Guo et al., 2022). Despite successful testing in the United States, France, and likely Poland, there are no known full-scale applications of the inDENSE® hydrocyclone as of the current knowledge cutoff in 2023. Similarly, the S::Select® system, another gravimetric sludge selection system, has approximately 14 installations, but limited information is available about its performance (Hamza et al., 2022).

Studies conducted by Avila and Regmi demonstrate the potential of the inDENSE® system in enhancing granulation and clarifier performance. At the Robert W. Hite Treatment Facility in Colorado, Avila's study showed that the inDENSE® system achieved good SVI and partial granulation, with a granulation rate of 40% in the test train compared to 16% in the control train. The test train clarifier performed comparably to the control train clarifier at a 32% higher solids loading rate, indicating improved efficiency (Avila et al., 2021). Regmi's study at the James R. Dolorio Water Reclamation Facility further highlighted the benefits of the inDENSE® system. By selectively retaining dense biomass, the system stabilized and improved phosphorus removal to meet permit levels within three months, showcasing its adaptability to regulatory requirements (Regmi et al., 2022).

The Dijion WRRF Test train demonstrated improved effluent TSS but did not show significant improvement in NH_4^+, NO_x, and PO_4^{3+}. The hydrocyclone's gravimetric selection maintained a hybrid floc granular sludge, with approximately 40% of the biomass by weight in the Test train exceeding 200 μm. However, the induced hydraulic shear stress appeared to limit the development of larger granules. The presence of biomass larger than 200 μm, combined with a good form factor (floc granular ratio), improved settling velocity, thereby enhancing SVI or dSVI. During the first winter, in the transition period, the Test and Control train SVIs were 82 ±

17 mL/g and 149± 45 mL/g, respectively. The Test train's dSVI further reduced and stabilized around 50± 6.8 mL/g for one year. This resulted in a reduced SVI baseline and the elimination of seasonal variation.

The hydrocyclone underflow retained dense biomass, while the overflow removed light flocs, enabling selective wasting of light flocs and uncoupling the SRT of granular and flocculent sludge. In the Test and Control trains, the SRTs were 70% and 270% of the overall SRT, respectively, favoring AOB over NOB (Roche et al., 2022). Partin's six-month study at the James River Wastewater Treatment Plant (Virginia) further demonstrated the hydrocyclone's effectiveness in reducing SVI values. Testing various hydrocyclone nozzle sizes, Partin observed that the system reduced plant SVI_5 and SVI_{30} to 200 mL/g and 120 mL/g, respectively. However, SVI levels rebounded after the hydrocyclone was taken offline, highlighting its continued necessity for sustained performance improvements (Partin, 2019).

While most studies suggest that hydrocyclone densification can be observed within weeks and leads to significant SVI improvement, limited information is available regarding the hydrocyclones used in Roche’s and Partin’s studies. Two hypotheses have been proposed: first, that Partin’s case may require a longer transition period, and second, that there might have been a redesign of the hydrocyclone during the study. Nevertheless, it is worth verifying the "no hydrocyclone" mode. For instance, would the SVI in Roche’s study rebound when the hydrocyclone is offline? Addressing this question could fill a critical gap in the existing research and provide valuable insights for planning and operator confidence.

The hydrocyclone’s ability to retain and return dense biomass to the mainstream is particularly advantageous, as densification can be maintained even during cold months, reducing the risk of sludge bulking in winter. However, fast settling dense biomass and higher biomass levels may challenge current clarifier design criteria and operational procedures. Conducting a clarifier audit could help assess performance under these new operating conditions and provide valuable findings for future greenfield CFR ASG projects incorporating side stream hydrocyclones for selective wasting.

Lab Scale Reactor Design

Selection pressures, such as settling velocity and feast and famine conditions, are crucial for the success of AGS in SBR. The SBR's batch-process nature enables precise management of feast-famine cycles and selective wasting, whereas CFR systems operate continuously, presenting greater challenges in maintaining optimal feast-famine conditions for granular sludge development.

Most lab scale CFR AGS designs incorporate an internal or external settling or separation module into the system, with three-phase separators being a common choice for internal separation. Zhou et al. demonstrated granulation in about 20 days using a three-phase separator assisted continuous flow airlift fluidized bed reactor (CAFB). However, routine removal of homogeneous sludge as waste activated sludge (WAS) maintained a low SRT of 8–10 days, combined with low bulk substrate concentrations, which led to filamentous overgrowth and granule disintegration (Y. Liu & Liu, 2006; Y. Yang et al., 2014; D. Zhou et al., 2013). Alternatively, Zou investigated a fully aerated CFR system utilizing a two zone sedimentation tank featuring a variable-depth baffle to adjust settling velocity thresholds. `By adding external nuclei, Zou achieved granulation and maintained steady operation for 100 days (Zou et al., 2018). Liu et al. employed a similar external two zone sedimentation tank in their anaerobic-anoxic-oxic CFR system, separating granules based on density: faster-settling granules (ST-1, density >1.011 kg/m^3) were retained in the first zone and returned as RAS, while slower-settling granules (ST-2, density <1.011 kg/m^3) were retained in the second zone and periodically wasted as WAS. Microbial analysis revealed that ST-1 granules were dominated by AOBs and NOBs, whereas ST-2 granules were dominated by PAOs, GAOs, and other OHOs. This selective retention and wasting strategy provided a longer SRT for chemoautotrophic bacteria in ST-1 granules while facilitating phosphorus removal through ST-2 wasting (W. Liu et al., 2020).

Baffles and sequentially arranged reactors or zones are essential for creating a spatial feast and famine regime. Li et al. developed a fully aerated Reverse Flow Baffled Reactor (RFBR) that operated with alternating forward and backward flow, featuring two identical mixing/settling modules at each end. This design eliminated the need for a RAS pump through periodic flow reversal, while the zones between adjacent baffles prevented short-circuiting and facilitated extended aeration. In the absence of a clarifier, lighter biomass washout occurred during Phase I. Granulation was achieved by day 21, with the system maintaining a low SVI of 33 mL/g until day 135. Along the flow direction, BOD_5 and NH_4 concentrations, as well as the F/M ratio, gradually decreased while mixed liquor suspended solids concentration increased. The system achieved BOD_5 and NH_4 removal efficiencies of 90 - 94% and 97 - 100%, respectively (J. Li et al., 2015).

Corsino et al. designed a granular continuous-flow membrane bioreactor with distinct high- and low-load zones to promote feast-famine conditions. However, the prolonged feast step created nutrient overabundance, triggering excessive filamentous microorganism growth on granule surfaces. This biofilm overgrowth caused oxygen and nutrient diffusion limitations within granules, resulting in decreased granule density, increased water content, and significantly reduced granulation rates. The structural integrity of mature granules was compromised, leading to disintegration. To address the extended feast period, intermittent feeding was implemented.

The lack of selective wasting combined with long SRT caused bacteria to hydrolyze EPS, increasing mixed liquor viscosity and impairing oxygen transfer efficiency (Corsino, Campo, Di Bella, et al., 2016).

Resource Recovery

The growing global population, particularly in metropolitan regions, necessitates improved planning for resource utilization to address escalating pressures such as resource scarcity and climate change. Reusing recovered materials represents a cornerstone of sustainable development within the circular economy framework. In wastewater treatment systems, WRRFs exemplify this principle by recovering multiple resources: treated wastewater for reuse, thermal energy from water sources, biogas (upgraded to biomethane for heat and power generation), phosphorus in the form of $NH_4MgPO_4 \cdot 6H_2O$ (struvite) for fertilizer applications, and alginate-like exopolymers (ALE) extracted from EPS.

Treated wastewater (or reclaimed water) represents a consistently available resource with minimal temperature fluctuations, making it well-suited for general cooling water and irrigation applications. When subjected to advanced treatment processes, this water can meet non-potable process water quality standards, thereby enabling industrial or agricultural reuse. However, the implementation of such reuse strategies necessitates city- or region-wide strategic planning. By co-locating high water-consumption industries or businesses with non-potable water demands, infrastructure efficiency can be optimized while creating economic incentives for reuse. Advanced treatment technologies offer additional flexibility to refine water quality as required. This integrated approach not only reduces discharge into natural water bodies but also decreases dependence on fresh, potable water sources.

The stable thermal properties of treated wastewater present additional opportunities for energy recovery. Water-source heat pumps—a mature heating, ventilation, and air conditioning (HVAC) technology—leverage this resource year-round to significantly reduce energy consumption for heating and cooling. In cooling mode, the system extracts thermal energy from indoor spaces and transfers it to the wastewater stream. Conversely, in heating mode, it absorbs thermal energy from the wastewater and delivers it indoors or preheats sub-zero ambient air before furnace intake. Unlike geothermal heat pumps, water-source systems eliminate the need for ground loops, thereby reducing installation complexity and cost.

Biogas, primarily composed of methane, is produced through anaerobic digestion or co-digestion of WAS, organic waste from the meat industry (e.g., manure), and municipal or industrial organic waste. This biogas can fuel fuel cells or combined heat and power (CHP) systems, thereby offsetting energy demands at WRRFs or enabling decentralized energy generation for remote agricultural operations. Harnessing biogas from organic waste exemplifies an effective circular economy practice.

Aerobic granules represent dense, quasi-spherical aggregates characterized by a cation-rich core and an outer layer of structural EPS. Similar to WAS, wasted AGS demonstrates 44% anaerobic biodegradability and achieves 32% solids reduction under mesophilic conditions (Val Del Río et al., 2014). As reported by Bernat et al., WAS exhibits a biogas potential of 536-781 dm^3/kg (total solids), with methane comprising 60.8-62.4% of the gas. In contrast, AGS yields a lower biogas potential of 320-410 dm^3/kg TS, with methane accounting for 56.7-59.5%. Comparative studies indicate that WAS produces 60-80% more biogas than AGS, a disparity primarily attributed to the recalcitrant lignin content in AGS, which constitutes 54% of its fibrous material (Bernat et al., 2017).

Further analysis by H. Guo et al. demonstrates that during anaerobic digestion, AGS exhibits slower degradation rates for structural EPS (26%), polysaccharides (39%), and proteins (24%) compared to WAS (41%, 62%, and 33%, respectively) (H. Guo et al., 2020). To enhance AGS biogas recovery, Cydzik-Kwiatkowska et al. investigated ultrasound pretreatment, finding that pretreated AGS achieved higher biogas yields at reduced digestion times (Cydzik-Kwiatkowska et al., 2022). These studies confirm that aerobic granules are anaerobically degradable but underperform WAS in biogas production. While biogas recovery from AGS offers environmental benefits, further research is required to optimize practical applications.

Under alternating anaerobic/aerobic conditions, PAOs uptake and store excess phosphate as intracellular polyphosphate. In PAO-rich sludge, phosphorus can constitute up to 20% of cellular dry weight (Z. Yuan et al., 2012). Recovering phosphorus from PAO-rich sludge involves a two step process: phosphate stripping followed by chemical precipitation. Anaerobic digestion or storage of wasted sludge with primary sludge fermentate releases phosphate into solution. The phosphate-rich dewatering liquor is then treated with soluble magnesium salts under controlled alkaline conditions to precipitate struvite ($NH_4MgPO_4 \cdot 6H_2O$). The resulting struvite pellets are separated, dried, and packaged as the commercial fertilizer Crystal Green® (Gysin et al., 2018). A similar strategy can be applied to high nutrient mainstream wastewater by supplementing external carbon sources.

EPS have demonstrated efficacy as biopolymers for raw water treatment, including coagulation and the removal of COD, turbidity, colour, and heavy metals. For instance, Li et al. reported that EPS achieved superior COD (61.2%) and turbidity (95.6%) removal compared to synthetic coagulants when treating low temperature drinking water (Z. Li et al., 2009). In aqueous solutions, EPS exhibits variable dye removal efficiency (20 - 99.99%) depending on operational parameters (Buthelezi, 2008). Heavy metals, which are toxic, non biodegradable, and prone to bioaccumulation in food chains, pose significant environmental risks. Biosorption using microbial biomass offers a cost effective alternative to physicochemical methods for detoxifying industrial effluents (Kratochvil & Volesky, 1998). While activated sludge has been widely studied as a biosorbent, AGS — with its high toxicity tolerance, rapid settling properties, and diverse microbial consortia — emerges as a promising candidate. The functional groups produced by AGS microbial communities enhance metal binding capacity (L. Wang et al., 2018). Although biosorbents like AGS reduce reliance on chemical agents, challenges remain in regenerating sludge and recovering metals post adsorption, necessitating further research.

Natural alginate is commercially derived from the cell walls of brown seaweed, which is often associated with seasonal production variations and high wastewater generation during extraction (Zahra et al., 2022). Its gel forming capability with Ca^{2+} ions makes it a preferred material for gelling and thickening applications (Amorim de Carvalho et al., 2021). As noted by Leeuwen et al. (2018), earlier studies by McHugh (1987) and Lee and Mooney (2012) have demonstrated alginate's suitability for biomedical and tissue engineering applications. The polyanionic gel forming alginate like exopolysaccharides (ALE), one of the primary exopolysaccharides produced by aerobic granules, significantly influence granular hydrophobicity, compactness, and structural integrity (Lin et al., 2010). Bio based ALE (Bio-ALE) shows promise for horticultural, paper, medical, and construction industries, with applications ranging from fibres (e.g., absorbent tissues) to gels (e.g., adhesives), foams (e.g., fire resistant boards), and liquids (e.g., thickeners). The first Bio-ALE recovery system was installed in Zutphen and Epe in 2019 in the Netherlands; projections suggest that global ALE production could reach 85,000 tonnes by 2030, accounting for over 50% of the value recovered in WRRFs in the Netherlands (Leeuwen et al., 2018).

Kehrein et al. modeled three on site COD recovery scenarios for EPS production(Kehrein et al., 2020): (1) EPS extraction alone yields 3.3 tonnes of EPS and 7.1 MWh of electricity daily; (2) anaerobic digestion followed by EPS extraction produces 3.4 tonnes of EPS, 8.4 MWh of electricity, and 5.8 MWh of heat daily; and (3) anaerobic digestion combined with EPS extraction and chemical enhanced primary treatment generates 1.6 tonnes of EPS, 22.5 MWh of electricity, and 15.6 MWh of heat daily.

The results indicate that COD derived energy can be recovered as electricity, heat, and value added EPS; however, optimizing these outputs requires a comprehensive master plan. The model did not account for thermal energy recovery from treated wastewater or phosphate recovery. Integrating thermal energy utilization could enhance EPS production efficiency, while phosphate recovery might further improve WRRF profitability.

Design Considerations

After more than two decades of research and development, three commercially viable AGS systems are now operational: Nereda® employs an SBR configuration, while inDENSE® and S::Select® utilize gravimetric separation-enhanced continuous flow designs. Among these, Nereda® typically achieves the highest proportion of granules (>80% granular biomass), whereas inDENSE® and S::Select®—originally developed to improve biomass settleability—retain a lower granular fraction (40-60%). Nonetheless, all three systems deliver robust nutrient removal, operational simplicity, and reduced operating costs compared to conventional activated sludge. However, full-scale AGS systems remain prone to prolonged granulation periods (2-6 months) and granule disintegration during long-term operation.

The initial granulation phase is time intensive. Studies propose strategies to accelerate this process, such as seeding reactors with multivalent cations (e.g., Ca^{2+}, Mg^{2+}) (Kończak et al., 2014; Z. Li et al., 2021) crushed/dried sludge (J. Liu, 2020; Ogura, 2020), or granular activated carbon (GAC) (Liang et al., 2019; Tao, 2017; J. Zhou, 2015) as nucleation media. Other research highlights that crushed or intact mature granules can reduce start up times by up to 50%, serving as a rapid inoculation strategy (F. Liu, 2014; S. T.-L. Tay et al., 2005; Verawaty et al., 2012). However, these materials are often unavailable to new facilities during initial commissioning.

Recent advances demonstrate that cold stored granules (4°C) from operational AGS systems can be reactivated after long term storage, with nutrient removal performance restored within 1 - 2 weeks (Campo et al., 2021; D. Gao et al., 2012; W. Gao et al., 2021; X. Wang et al., 2008). This suggests that surplus biomass could be preserved and transported for new plant start ups. However, post storage granule efficacy depends on system compatibility: cold stored granules perform optimally in their original configuration, exhibit reduced efficiency in similar systems, and require extended acclimation (2 - 4 weeks) in dissimilar wastewater or reactor designs. While promising, these findings derive from lab scale trials and necessitate pilot scale validation before full scale adoption.

Early studies have documented granule disintegration under diverse operational conditions. In fully aerobic systems, high OLRs promote filamentous microorganism overgrowth on granule surfaces (Y. Liu & Liu, 2006; Y. Liu & Tay, 2004) and degrade the anaerobic core (Long et al., 2015; Zheng et al., 2006). Similarly, an initially high F/M ratio accelerates granule formation but generates oversized granules prone to diffusion limitations and subsequent disintegration (A. Li et al., 2011). Wastewater composition also plays a critical role: slowly biodegradable compounds or colloidal substances act as slow release nutrient sources, which sustain filamentous growth and destabilize granules (Moy et al., 2002; Nor-Anuar et al., 2012; J.-H. Tay et al., 2001).

Under anaerobic feeding conditions, PAOs and GAOs metabolize these slow release substrates, converting them into PHA for later energy use. Additionally, such systems' feast and famine regime suppresses filamentous overgrowth by eliminating continuous nutrient availability. However, optimizing the feast and famine ratio requires case specific evaluation to balance granule stability and nutrient removal efficiency.

SBR AGS reactors inoculated with activated sludge, elevated effluent solids—often loose or lightweight biomass—during granulation due to progressively shortened settling times. Depending on operational parameters, effluent may still contain fragmented or undersized granules after granulation. While limited data exist on full scale AGS effluent solids composition, recovering and repurposing these granular fractions as future inoculum presents a circular economy opportunity. As noted earlier, greenfield AGS plants face prolonged start up phases due to the absence of seed granules. Harvesting residual granules from effluent improves final effluent quality and generates a cost effective inoculum source. This approach transforms a perceived waste stream into a resource, aligning with sustainable wastewater management practices.

In CAS systems, nitrification, nitritation, and phosphate uptake occur in the aerobic zone, with reactors typically maintaining a fixed DO level. AGS, however, exhibits a spherical layered structure: the outer oxygen rich layer hosts OHOs, AOBs, NOBs, PAOs, and GAOs, while deeper anoxic / anaerobic layers harbor DHOs, DPAOs, and DGAOs. As granules grow in size, the oxygenated outer layer thickens, necessitating dynamic DO adjustments to balance nitrification / nitritation in aerobic zones with denitrification in anoxic regions. Ammonia based aeration control — a strategy linking DO or airflow to real time ammonia concentrations (Paper & Higgins, 2021) — has emerged as a promising tool for optimizing this balance while reducing energy costs. For instance, Miyake et al. (2023) achieved 40% simultaneous nitrification denitrification (SND) and 50% total nitrogen (TN) removal by maintaining DO at 0.5 mg/L through ammonia driven aeration (Miyake et al., 2023). This underscores the potential of adaptive DO control to harmonize competing microbial processes within granules.

Gravimetric separation enhanced hydrocyclone offer a novel method for segregating dense granules from lighter flocs. Trials report improved settling properties in hybrid floc granule systems, even under winter conditions, with final effluent total suspended solids (TSS), total phosphorus (TP), and TN reductions attributed to WAS removal via hydrocyclone overflow. However, soluble nutrient removal showed negligible improvement, and while granule like aggregates were observed in hydrocyclone underflow, their maturation status remains unclear (Avila et al., 2021; Gemza & Kuśnierz, 2022; Roche et al., 2022). True granulation requires prolonged microbial structural evolution, where simultaneous nitrification denitrification depends on stratified DO gradients—a feature absent in Avila and Gemza’s trials, which reported no denitrification despite enhanced settling. Thus, hydrocyclones may primarily address settleability rather than comprehensive nutrient removal. Further research is needed to evaluate their role in fostering functional granule development.

Chapter 2: Methodology

Process Description

The CAS process features a large footprint, high energy demand, and high sludge yield. However, the poor settleability of flocs can lead to challenges in the solids handling process. In contrast, self immobilized aerobic granular sludge requires a smaller footprint, lower energy consumption, and produces less sludge. Compared to conventional activated sludge, the granules' improved settleability enables higher mixed liquor suspended solids (MLSS), resulting in greater nutrient treatment capacity.

Anaerobic feeding involves a deep anaerobic feast followed by a prolonged aerobic famine. During the anaerobic phase, biomass converts and stores substrate as PHA internally, leaving minimal substrate for external or suspended filamentous microorganisms in the aerobic phase. This selective feeding regime eliminates filamentous microorganisms. Linear settling velocity distinguishes between lighter flocs and denser granules. Selective wasting applies gradual selective pressures based on linear settling velocity, eliminating lighter flocs.

The pilot study utilized a combination of feast and famine cycles and side stream selective wasting to apply selective pressure. The anaerobic and aerobic steps were designed to eliminate filamentous microorganisms and light flocs while promoting granulation. The process involved a series of anaerobic reactors followed by aerobic reactors, with side stream selective wasting connected to and from the middle of the aerobic zone. In the selective wasting tank, mixed liquor was allowed to settle before denser sludge and lighter flocs were returned to the mainstream and decanted as waste. Settling time was gradually reduced to increase linear settling velocity.

Three stages of the study

The study involved three stages. In Stage 1, three anaerobic reactors evenly received the primary effluent. Initially, the system included three anaerobic and seven aerobic reactors, but the last two were deactivated to reduce the HRT. Figure 1 (Stage 1 Schematic Flow Diagram) shows the configuration for Stage 1 (June–December 2023). In Stage 2 (December 2023–April 2024), primary effluent was directed to the first anaerobic reactor to create a true anaerobic feast, as illustrated in Figure 2 (Stage 2 Schematic Flow Diagram). In Stage 3 (April–July 2024), side-stream selective wasting was introduced, with the configuration depicted in Figure 3 (Stage 3 Schematic Flow Diagram). Additionally, internal recycling was investigated to evaluate its impact on denitrification and selective wasting.

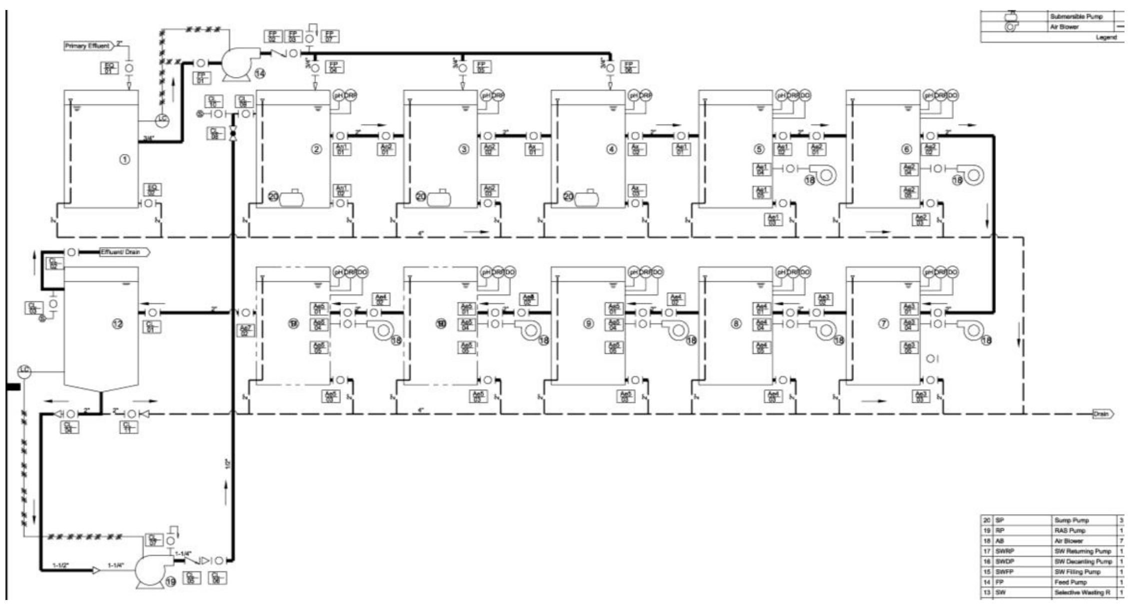

Figure 1 Stage 1 Schematic Flow Diagram

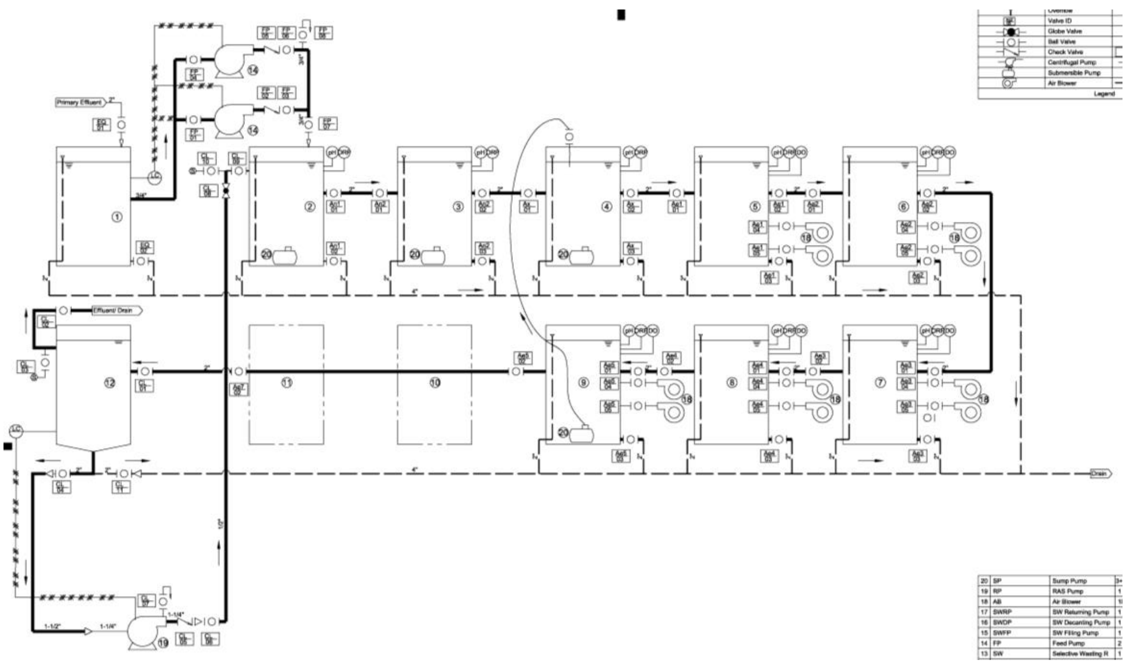

Figure 2 Stage 2 Schematic Flow Diagram

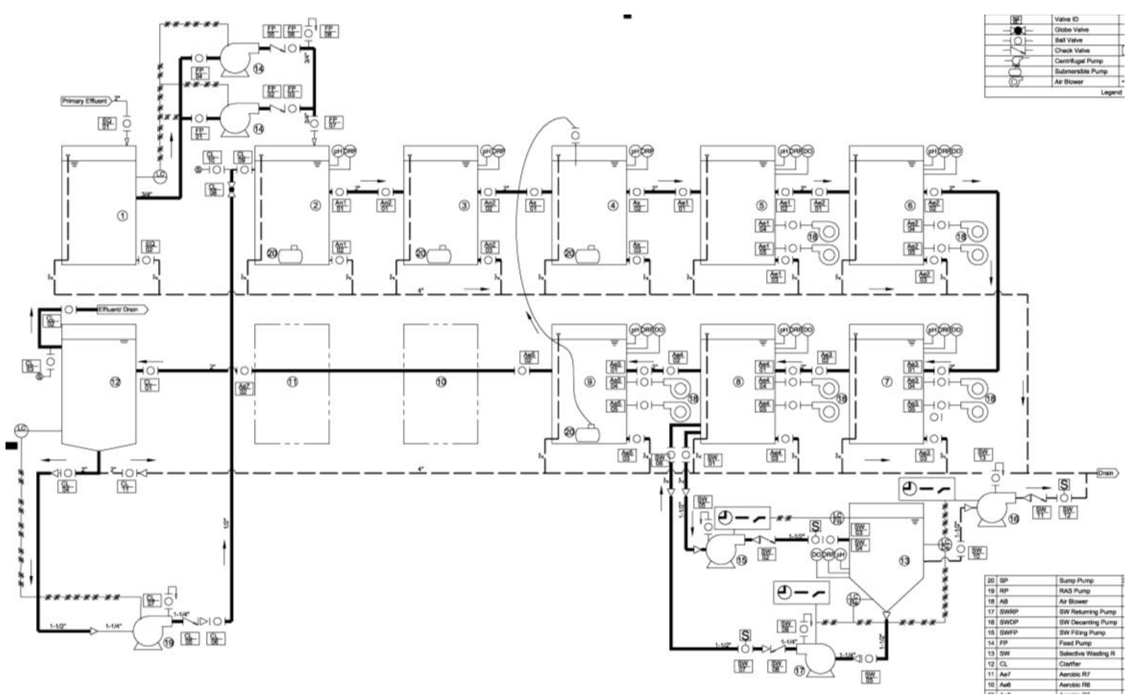

Figure 3 Stage 3 Schematic Flow Diagram

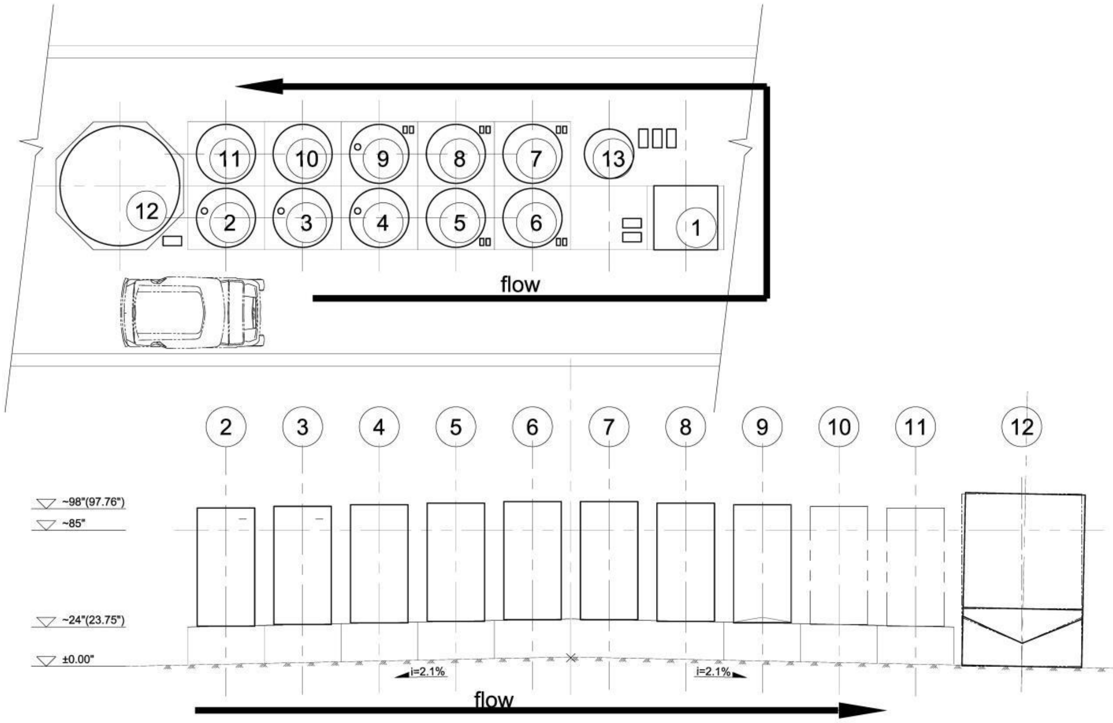

Figure 4 Pilot Layout

Note. The upper diagram illustrates a top-down perspective of the pilot reactor layout, while the lower diagram provides an expanded, detailed cross-sectional view of the reactor design.

Reactor configurations

Figure 4 illustrates the reactor layout. The system featured ten reactors arranged in two rows of five, labeled R2 through R11. Each reactor had a diameter (D) of 35 inches (0.89 m) and a height (H) of 74 inches (1.88 m). The primary effluent tank measured 48 inches (1.22 m) in width (W), 40 inches (1.02 m) in length (L), and 42 inches (1.07 m) in height (H). All units were elevated on cinder blocks and pallets to align with the clarifier's overflow level. Reactors were interconnected using 2-inch (0.05 m) soft PVC tubing. The clarifier had a diameter (D) of 75 inches (1.91 m) and a height (H) of 100 inches (2.54 m). Settled sludge from the clarifier was partially recirculated as RAS.

Submersible sump pumps with reduced flow rates were installed in the anaerobic reactors for mixing purposes. Both fine and coarse bubble diffusers were tested for aeration in the aerobic reactors, with occasional use of an additional submersible sump pump for enhanced mixing. The conical-bottom selective wasting tank, measuring 33 inches (0.84 m) in diameter (D) and 74 inches (1.88 m) in height (H), was operated by a programmable logic control module that managed the filling, settling, decanting, and returning processes. Process and air pumps were positioned beneath the reactors, while online analyzers with suspended sensors were integrated into the reactors for continuous monitoring.

Selective Wasting Controller

The controller allows manual input of the number of selective wasting cycles and the duration of the settling time. At the set time, the fill pump activates, opening the normally closed solenoid filling ball valve to transfer mixed liquor from R8 into the selective wasting tank. When the tank reaches the high level, the fill pump deactivates, and the system returns to its default state, awaiting the next cycle. An internal countdown timer initiates the settling period. Upon completion, the decant pump activates, opening the normally closed solenoid decanting ball valve to allow the lighter fraction to decant into the drain. When the middle level switch is triggered, the decant pump deactivates, and the system resets for the next cycle. The middle and low level switches then activate the return pump, opening the normally closed solenoid returning ball valve to transfer the settled, denser sludge back to R8. Once the low level switch is triggered, the return pump deactivates, and the system resets, preparing for the next cycle.

Analysis Methodology

Triplicate samples were collected weekly on Monday, Wednesday, and Friday. During long weekends, sampling was adjusted to Tuesdays and Fridays. Collected samples were stored in 1,000-mL containers within coolers to maintain integrity until laboratory analysis. The settleable solids volume index (SVI) test was conducted onsite immediately after sample collection.

Influent (primary effluent), mixed liquor, and effluent (clarifier final effluent) samples were analyzed for chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD). Additionally, the same samples were analyzed for total ammonia nitrogen (TAN), nitrite, nitrate, and ortho-phosphate (OP) using the Lachat QuikChem 8500 system (Hach, CA). Mixed liquor, final effluent, and RAS were tested for total suspended solids (TSS) and volatile suspended solids (VSS) according to Standard Methods (Water Environment Federation, 2012). All analyses were performed in duplicate to ensure accuracy.

Chapter 3: Data and Discussion

Nutrient Removal Performance

The study aimed to achieve granulation by introducing a side-stream selective wasting process into a CAS system. The investigation comprised three distinct stages: Stage 1 implemented split feeding; Stage 2 employed combined feeding; and Stage 3 evaluated selective wasting.

Wastewater composition

The primary effluent composition throughout the study period is summarized in Figure 5 Primary Effluent Composition. The primary effluent sCOD averaged approximately 200 mg/L; however, due to wet weather conditions, it dropped to around 100 mg/L between April and June 2024. Data collection was paused from July to August 2024 due to system troubleshooting following selective wasting trials in Stage 3. The average values of the primary effluent characteristics are provided in *Figure 5 Primary Effluent Composition*

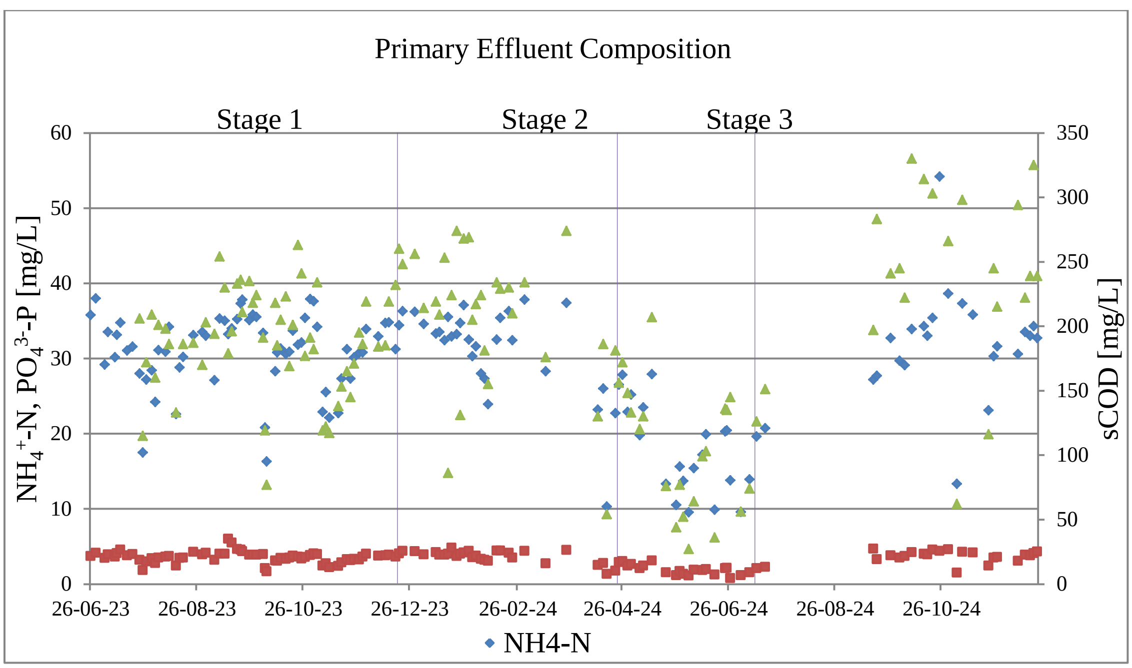

Figure 5 Primary Effluent Composition

Table 1 Average values of Primary Effluent Composition

		JUN 23/ DEC 23	DEC 23/ APR 24	APR 24/ JUL 24	SEP 24/ DEC 24
Average Value		Stage 1	Stage 2	Stage 3	Stage [4]
sCOD	[mg/L]	188	208	104	235
NH_4^+-N	[mg/L]	31	31	18	31
sCOD/NH_4^+-N	[n.a.]	6	7	6	8
PO_4^{3-}-P	[mg/L]	3.6	3.7	1.9	3.6
sCOD/PO_4^{3-}-N	[n.a.]	52	56	55	65

Figure 5 and Table 1 demonstrate the variability in primary effluent composition throughout the study period. The significant reduction in sCOD levels during wet weather conditions highlights the influence of external factors on system performance. In Stage 3, the implementation of selective wasting presented operational challenges, as indicated by the subsequent troubleshooting period. The temporary suspension of data collection during this phase underscores the inherent complexities of integrating selective wasting into CAS systems.

Table 2 Summary of NH4+-N and PO43--P Removal

	JUN 23/ DEC 23	DEC 23/ APR 24	APR 24/ JUL 24	SEP 24/ DEC 24
	Stage 1	Stage 2	Stage 3	Stage [4]
>90% NH_4^+-N Removal	56%	86%	90%	75%
>90% PO_4^{3-}-P Removal	70%	79%	19%	50%

Nutrient removal performance

The pilot system encountered multiple operational challenges, including power outages, mechanical failures, hydraulic limitations, online analyzer malfunctions, weak wastewater strength, and solids management difficulties. Despite these adversities, the system achieved biological nutrient removal. Nitrogen and phosphorus removal performance is summarized in Table 2, which shows the percentage of sampling days in each stage where NH_4^+-N and PO_4^{3-}-P removal exceeded 90%. In Stage 3, phosphorus removal efficiency significantly declined to 19% of operational days. Internal recycling-related dilution and carbon competition persisted in Stages 2 and 3. Compared to Stage 2, the reduced phosphate removal in Stage 3 primarily resulted from biomass loss during selective wasting trials, leading to PAO depletion.

During Stage 1, split feeding was tested by equally distributing primary effluent to anaerobic reactors R2, R3, and R4, followed by seven aerobic reactors (R5-R11) maintaining long aerobic famine conditions. R11 and R10 were sequentially removed to achieve shorter HRT, and internal recycling from R9 to R4 was evaluated. From July to September 2023, seven aerobic reactors provided a feast-famine ratio of 0.75; from September to October 2023, six reactors achieved a ratio of 1; and from October to December 2023, five reactors maintained a ratio of 1. These ratios exceeded literature values. The higher ratio shortened famine duration, reducing filamentous bacteria control as aerobic reactors were removed.

Primary effluent was directed into anaerobic reactors R2-R4, where sCOD concentration increased slightly from 30 to 50 mg/L through dilution and accumulation. Under these conditions, PAOs exhibited first-order kinetic carbon uptake at a slower rate. While PHA storage/consumption rates and aerobic OUR were not measured, aerobic phosphate uptake rates approximated feast-famine cycles. The observed shorter, weaker aerobic famine conditions may have contributed to floc destabilization.

The observed shorter and weaker aerobic famine condition might have contributed to loose or destabilized flocs.

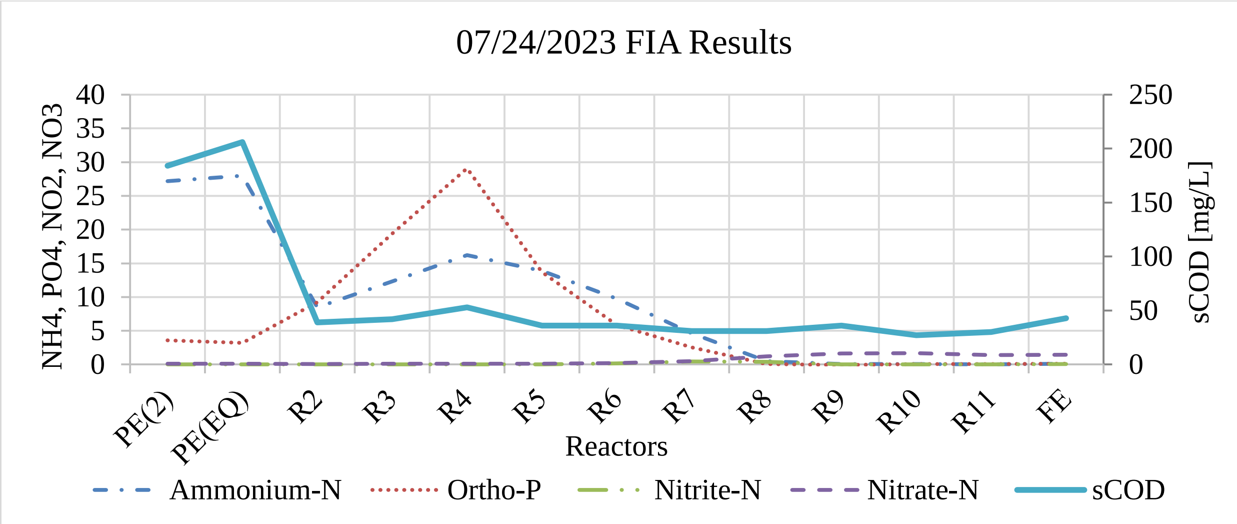

Figure 6 07/24/2023 FIA Results

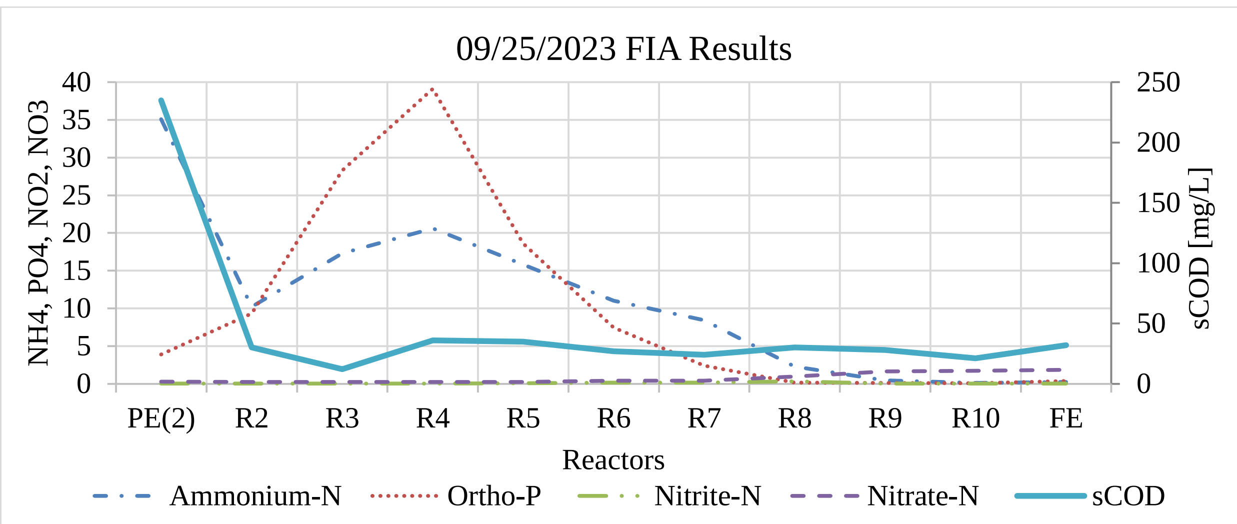

Figure 7 09/25/2023 FIA Results

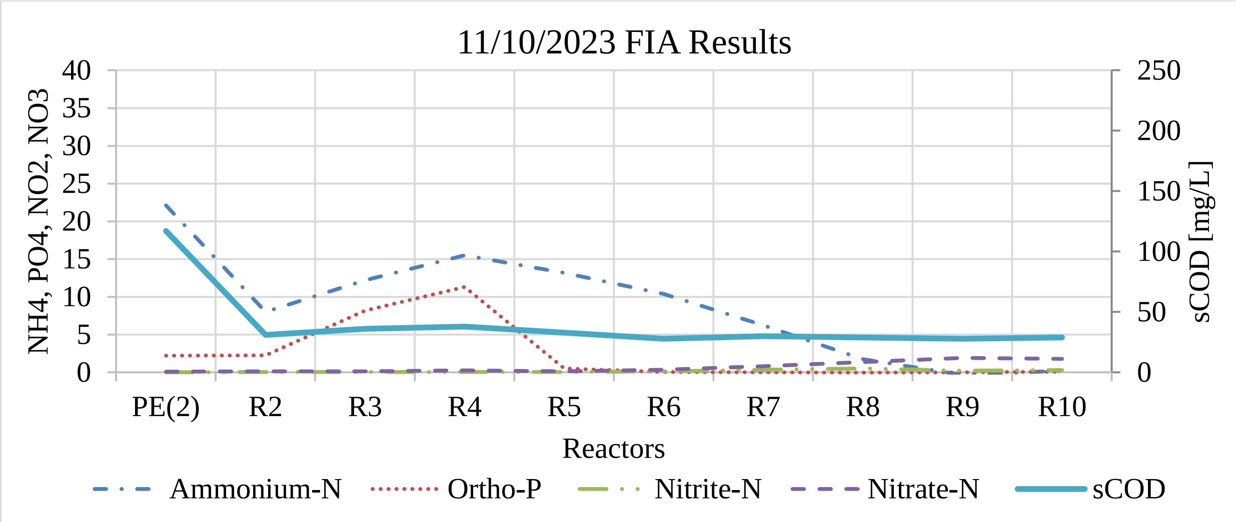

Figure 8 11/10/2023 FIA Results

Figures 6, 7, and 8 illustrate the nutrient removal performance during Stage 1, with seven, six, and five aerobic reactor configurations operated for two months, one month, and one month, respectively. Phosphate, ammonium, and sCOD removal were generally effective throughout the testing period. The duration may have been insufficient to fully assess the impact of removing R11 or R10; however, the feast-famine ratio increased from 0.75 to 1, creating conditions favorable for OHO or filamentous bacteria. Weakened aerobic famine conditions, compounded by fluctuations in F/M ratio, HRT, SRT, DO, DO, and mixing, subsequently posed operational challenges.

In Stage 2, primary effluent was introduced into anaerobic reactor R2, followed by sequential processing in one additional anaerobic reactor, one anoxic reactor, and five aerobic reactors (R5-R9) using a combined feeding strategy. Internal recycling redirected approximately 70% of the flow rate (Q) from R9 to R4. Figure 9 demonstrates the nutrient removal performance under this configuration. The combined feeding aimed to enhance anaerobic feast conditions and amplify the phosphate release peak in R4. However, the 0.7Q internal recycling diluted nutrient concentrations, inducing anoxic conditions in R4 and relocating the phosphate release peak to R3.

Despite carbon competition between PAOs and denitrifiers in R4, phosphate removal efficiency remained unaffected.

Ammonium serves as a nitrogen source for bacterial protein synthesis, with heterotrophic bacteria incorporating ammonium into biomass during denitrification. The observed ammonium reduction in anoxic reactor R4 likely resulted from biomass synthesis and recycling-induced dilution. Nitrification persisted through R9 but at a significantly slower rate than in Stage 1. The reduced nitrification rate, accounting for internal recycling, may stem from decreased effective HRT. AOB exhibited diminished ammonium processing efficiency under these HRT constraints.

The combined feeding achieved an initial sCOD concentration of 50 mg/L in R2, though 0.7Q internal recycling further diluted nutrients in R4. PAOs displayed first-order kinetic carbon uptake at a reduced rate.

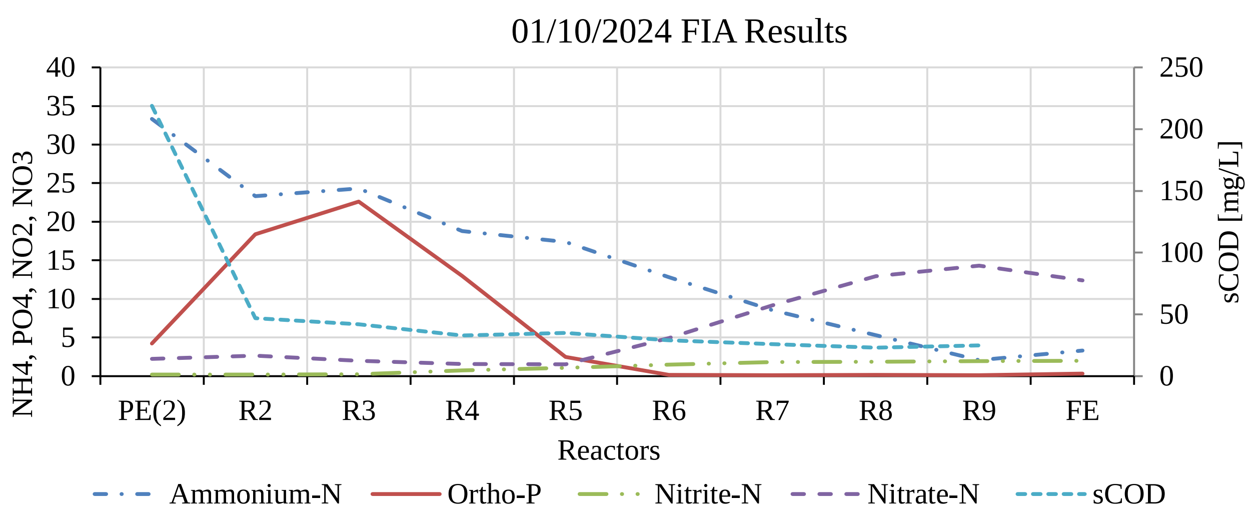

Figure 9 01/10/2024 FIA Results

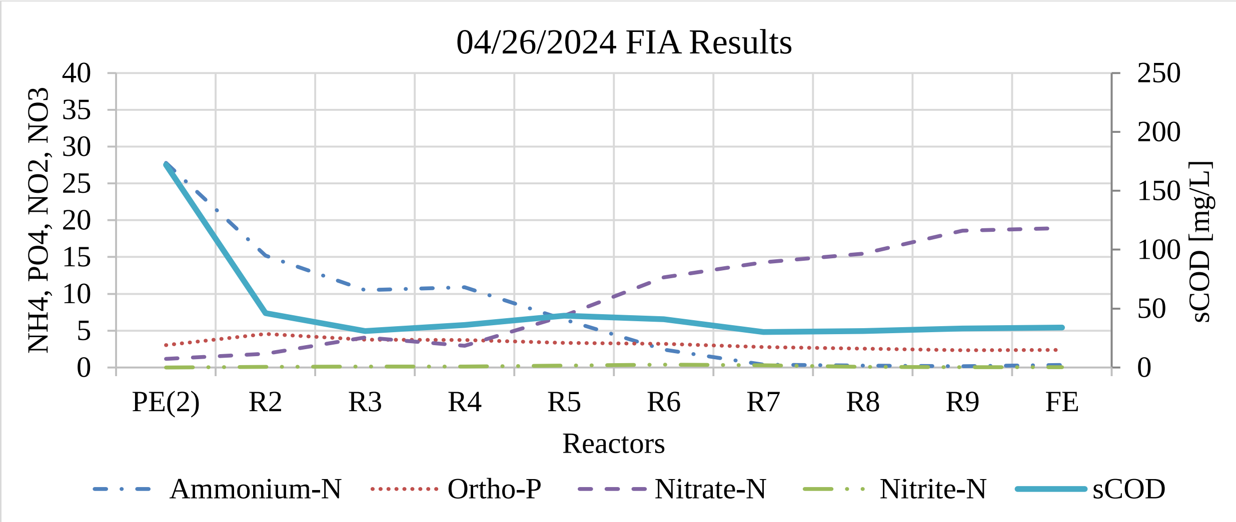

Figure 10 04/25/2024 FIA Results

During Stage 3, selective wasting was implemented through programmed and controlled cycles, with mixed liquor transferred to and from R8 for the process. Figure 10 demonstrates the nutrient removal performance during this stage. The settling process in selective wasting typically required longer durations than those observed in a benchtop settleometer. Encouraged by initial good separation, operators progressively increased the number of wasting cycles per day from one to seven while maintaining the set settling duration. In May, persistent scum formation occurred in the clarifier, which was later attributed to selective wasting-induced reduced settling efficiency and impaired denitrification. The process preferentially decanted high-solids mixed liquor rather than the lighter fraction, leading to a rapid decline in mixed liquor total solids concentration from 2,900 mg/L to below 1,900 mg/L within one month.

The significant solids loss adversely affected system performance, particularly phosphate removal. The absence of phosphate release during anaerobic conditions resulted from PAOs depletion and a low sCOD/P ratio. This allowed residual sCOD to migrate into aerobic reactors. On April 26, the RAS, DO concentration measured approximately 1.8-2.0 mg/L, creating small aerobic zones in R2 that may have supported unintended nitrification.

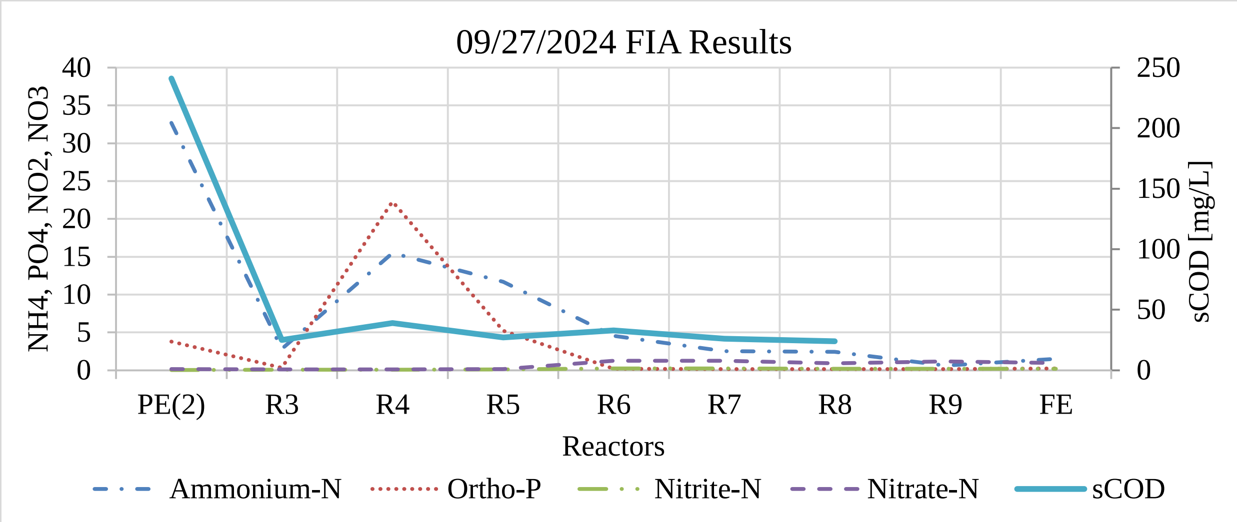

Figure 11 09/27/2024 FIA Results

A SBR AGS system employs one hour of anaerobic feeding followed by an aerobic reaction phase. To evaluate the effects of reduced anaerobic retention time, reactor R2 was decommissioned after Stage 3. The RAS flow was redirected to R3, while primary effluent and internal recycling were introduced into R4. Figure 11 presents the nutrient removal performance post-R2 removal, demonstrating highly effective ammonium and phosphate removal. However, the observed nitrification rate reduction may correlate with decreased effective HRT.

Despite generally satisfactory nutrient removal throughout the study period, operational challenges emerged. These issues required extensive process reconfiguration and numerous incremental adjustments to accommodate major modifications. The operational difficulties stemmed not from isolated problems but from complex interactions between multiple factors. While conventional continuous plug-flow activated sludge processes typically achieve granulation through feast-famine cycles and selective wasting, the cumulative effect of these minor issues ultimately prevented granulation in this system.

Possible reasons for poor performance

Unstable HRT and SRT

The pilot system initially consisted of ten reactors: three anaerobic reactors (R2, R3, R4) and seven aerobic reactors (R5-R11). The HRT of this configuration exceeded that of the full-scale West End Water Pollution Control Centre (WEWPCC). To align the pilot's HRT with the full-scale facility, operators sequentially removed reactors R11 and R10, thereby modifying the system configuration.

HRT and SRT values across different configurations are summarized in Tables 3 to 6. In Stages 1 and 2, HRT variations were noticeable but manageable, averaging 16% difference. However, in Stages 3 and 4, these variations increased to 24% and 33%, representing 50% and 100% increases, respectively. SRT variation was more significant, with a minimum of 72% recorded in Stage 3. These findings suggest that SRT variability had a greater impact on nutrient removal performance than HRT. The substantial increase in SRT variation in later stages highlights the challenges of managing retention times in dynamic pilot systems.

The data presented in Tables 3 to 6 and Figures 6 and 7 offer valuable insights into the system's operational dynamics. They underscore the necessity for robust control strategies to mitigate the effects of SRT variability and improve overall performance. Future research could investigate advanced control mechanisms or adaptive management approaches to effectively address these challenge.

Table 3 HRT Standard Deviation and Coefficient of Variation in Stage 1

		JUN 23/ SEP 23	SEP 23/ OCT 23	OCT 23/ DEC 23
Number of Reactors	[n.a.]	10	9	8
Average	[hrs]	13	12	9
HRT'/HRT	[n.a.]	1.3	1.4	1.1
STDEV.S	[n.a.]	2	2	2
CV	[%]	16%	17%	24%

Table 4 SRT Standard Deviation and Coefficient of Variation in Stage 1

		JUN 23/ SEP 23	SEP 23/ OCT 23	OCT 23/ DEC 23
Number of Reactors	[n.a.]	10	9	8
Average	[days]	38	32	17
STDEV.S	[n.a.]	44	25	14
CV	[%]	115%	79%	80%

Table 5 HRT Standard Deviation and Coefficient of Variation

		JUN 23/ DEC 23	DEC 23/ APR 24	APR 24/ JUL 24	SEP 24/ DEC 24
		Stage 1	Stage 2	Stage 3	Stage 4
Number of Reactors	[n.a.]	10~8	8	8	7
Average	[hrs]	13	12	9	13
HRT'/HRT	[n.a.]	1	1	1	2
STDEV.S	[n.a.]	2	2	2	4
CV	[%]	16%	17%	24%	33%

Table 6 SRT Standard Deviation and Coefficient of Variation

		JUN 23/ DEC 23	DEC 23/ APR 24	APR 24/ JUL 24	SEP 24/ DEC 24
		Stage 1	Stage 2	Stage 3	Stage 4
Number of Reactors	[n.a.]	10~8	8	8	7
Average	[days]	27	12	15	28
STDEV.S	[n.a.]	31	8	13	34
CV	[%]	115%	72%	87%	122%

The primary effluent flowed through the reactors and clarifier by gravity, without devices to control individual reactor volumes, leading to dynamic shifts in reactor volumes based on hydraulic conditions. Operators adjusted the primary effluent flow rate by throttling a ball valve to stabilize HRT. FOG in the primary effluent increased tube friction loss, while fine grit frequently clogged the throttled valve. In rare instances, complete ball valve clogging halted primary effluent flow. Although regular cleaning and adjustments mitigated these issues, flow fluctuations persisted. To prevent potential clogs, operators occasionally set the initial flow rate 30% above calculated values, inadvertently reducing HRT and increasing hydraulic pressure, which elevated final effluent solids concentrations. Flow rates typically stabilized at target levels within hours.

Operators manually wasted WAS from the RAS line to maintain SRT. WAS and final effluent solids constituted primary waste streams, while scum—observed on 27% of sampling days (43/159)—was occasionally disposed of as waste. SRT calculations incorporated WAS, effluent solids, and scum. However, aerobic reactor foaming contributed to unquantifiable biomass losses. Prolonged, undetected foaming events (lasting hours to days) caused catastrophic biomass loss necessitating reseeding. These unaccounted foaming losses distorted SRT calculations, yielding overestimated values.

The study reveals the inherent difficulties in maintaining stable HRT and SRT within a gravity-driven, manually adjusted system. Reactor volumes fluctuated by 1-2% during the study. Hydrostatic pressure, FOG, and grit collectively challenged flow management, highlighting the need for enhanced HRT monitoring and control strategies. Manual WAS wasting and unquantifiable foam-related biomass losses further complicated SRT management. These operational challenges underscore the potential benefits of advanced automation and real-time biomass tracking methodologies.

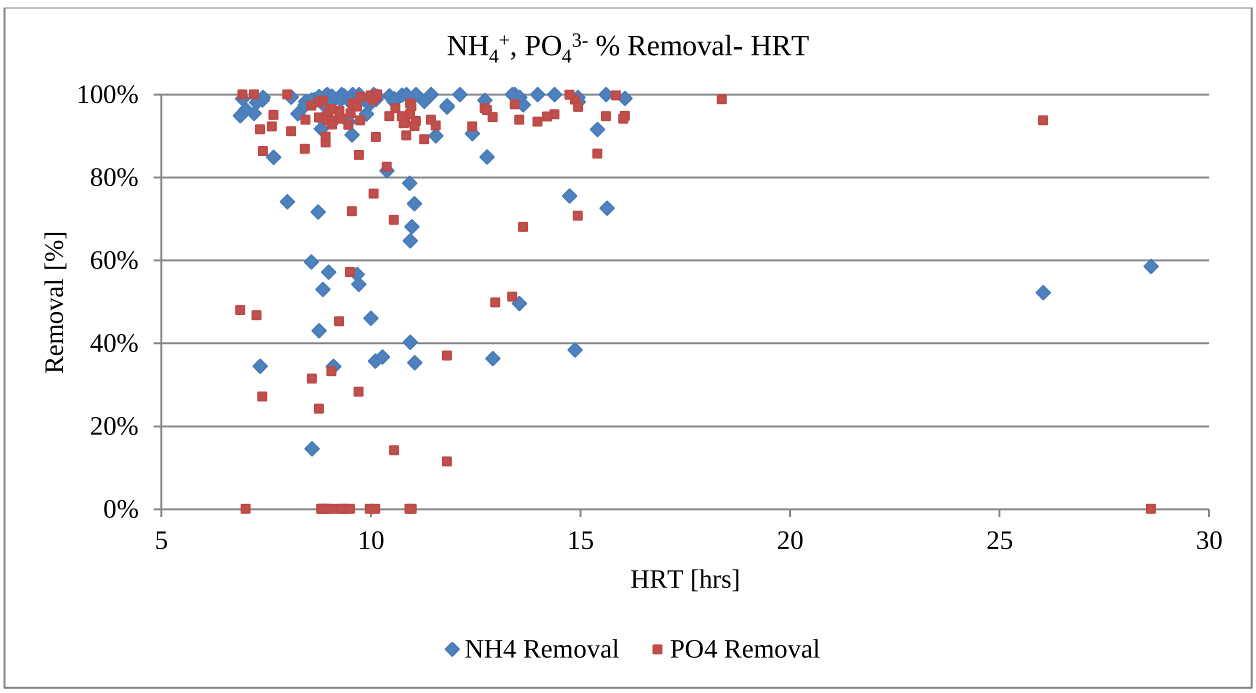

Figure 12 NH4-N and PO4-P removal vs HRT

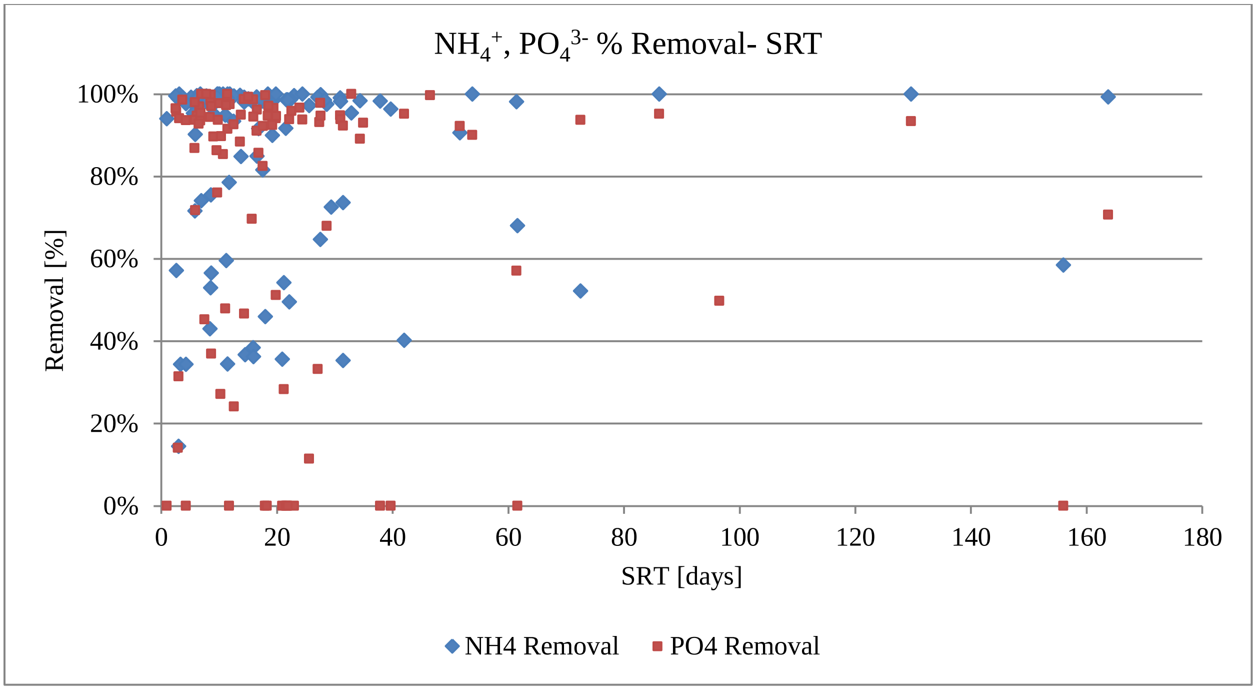

Figure 13 NH4-N and PO4-P removal vs SRT

HRT does not directly impact BNR performance. However, short HRT frequently induces biomass washout, thereby compromising BNR efficiency. Conversely, prolonged HRT—resulting from reduced feeding flow—leads to a F/M ratio, favoring heterotrophic bacteria over slow-growing nitrifiers due to competitive exclusion for nutrients and oxygen. This dynamic further diminishes nitrification efficiency. The most detrimental scenario involves fluctuating HRT, which disrupts microbial community stability. Figure 12 visually demonstrates these HRT-nutrient removal relationships.

Nitrifying bacteria exhibit significantly slower growth rates than heterotrophs. Short SRT enables heterotrophic dominance, expelling nitrifiers and degrading nitrification. Extended SRT exacerbates low F/M conditions and endogenous respiration. Filamentous bacteria, with their high surface-area-to-volume ratio, outcompete floc-forming bacteria under nutrient-limited conditions, while endogenous respiration further weakens floc formers. Stagnant zones from uneven mixing/aeration create low DO microenvironments that promote filamentous proliferation. Systemic dominance by filaments under low F/M or low DO conditions can induce bulking and foaming.

Phosphate removal performance remained generally stable across HRT variations, except during significant biomass loss events. PAOs require marginally shorter minimum SRT than nitrifiers, rendering them less susceptible to SRT fluctuations. Systems meeting nitrification SRT criteria inherently support PAO populations. However, prolonged SRT triggers endogenous respiration, elevating effluent phosphate concentrations. As with nitrifiers, stable SRT is essential for consistent phosphate removal. Figure 13 illustrates the SRT-nutrient removal correlation.

Stable HRT and SRT are paramount for optimal wastewater treatment system operation. Implementing daily HRT/SRT controls—adjusted for varying F/M ratios—provides real-time diagnostic data crucial for system optimization. This approach ensures operational consistency, underscoring the necessity for continuous monitoring and adaptive management in modern treatment facilities.

F/M ratio

The F/M ratio, a key parameter reflecting substrate availability, averaged 0.42 kg sCOD/kg MLVSS during the study (200 mg/L primary effluent sCOD), dropping to 0.24 kg sCOD/kg MLVSS during 2024 spring snowmelt events (50 mg/L sCOD). These values fall within the mid-to-low range for CAS systems, as detailed in Table 7.

Table 7 F/M ratio

	JUN 23/ SEP 23	SEP 23/ OCT 23	OCT 23/ DEC 23	DEC 23/ APR 24	APR 24/ JUL 24	SEP 24/ DEC 24
	Stage 1	Stage 1	Stage 1	Stage 2	Stage 3	
Number of Reactors	10(3+7)	9(3+6)	8(3+5)	8(3+5)	8(3+5)	7(2+5)
Average	0.24	0.25	0.42	0.48	0.23	0.41
STDEV.S	0.10	0.09	0.14	0.16	0.11	0.26
CV	43%	37%	35%	33%	47%	64%

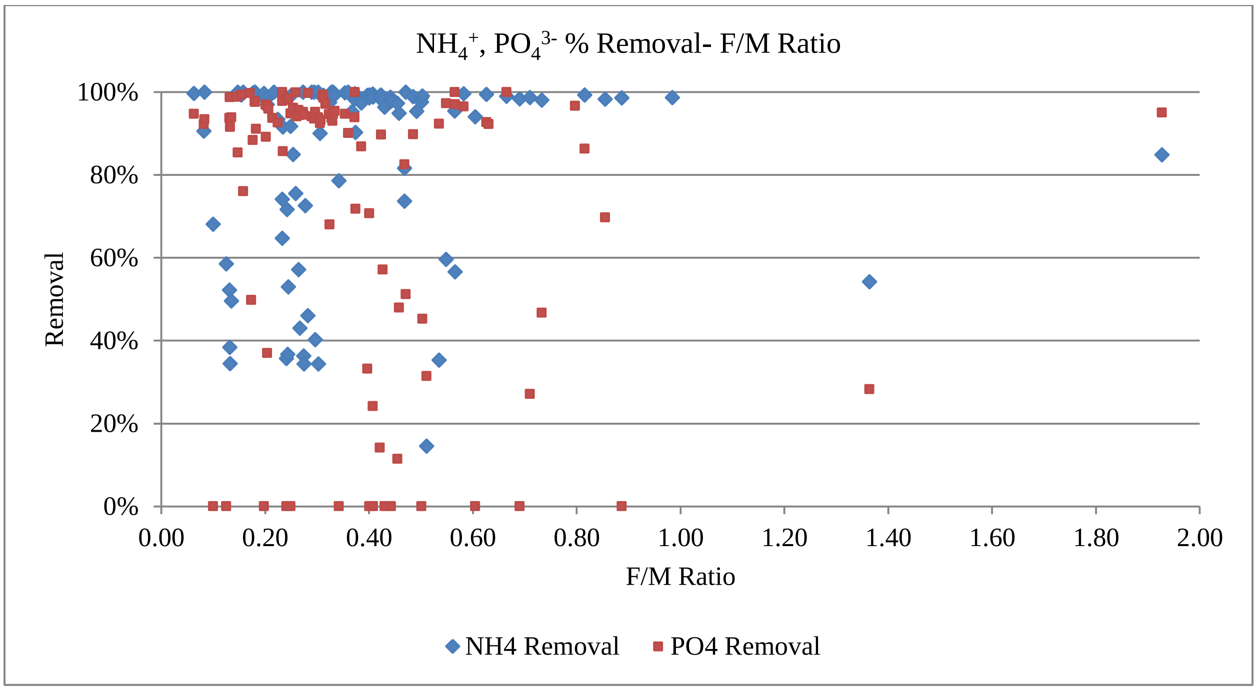

Figure 14 NH4 and PO4-P removal vs overall F/M

A low and fluctuating F/M ratio destabilizes the system, promoting shifts in microbial populations toward bubble-trapping filamentous bacteria, which can cause bulking and foaming. Unstable F/M conditions also trigger EPS production, stabilizing gas bubbles, trapping air, slowing separation, and increasing viscosity. Poor aeration exacerbates foam persistence, complicating biomass loss quantification. Foam accumulation led to spit or overflow issues, making accurate biomass measurement challenging. FOG fouling observed in the tubing during fall 2024 acted as a foam stabilizer, intensifying the foaming problem.

In mid-June 2024, operators added sodium acetate to the anaerobic reactor to elevate system sCOD (and F/M ratio) and enhance phosphate uptake. The sCOD profiles showed no significant spikes in anaerobic or aerobic reactors, indicating rapid microbial utilization of the added sodium acetate. The F/M ratio fluctuated by approximately 47%, obscuring the impact of sodium acetate addition. Significant biomass loss later occurred due to excessive foaming in R5. Complications from F/M ratio fluctuations, mixing, and aeration rendered foaming control ineffective, leading to sodium acetate addition cessation in July.

Maintaining a steady SRT mitigates F/M fluctuations. For weak primary effluent, increasing WAS volume (while ensuring MLSS remains above critical levels) stabilizes the F/M ratio. For strong primary effluent, reducing WAS volume (while keeping MLSS within secondary clarifier limits) maintains SRT stability. External carbon sources (e.g., sodium acetate or primary sludge fermentate) should be considered when primary effluent carbon levels inadequately support basic BNR requirements. These strategies optimize F/M and SRT management, ensuring microbial population stability and preventing operational issues like foaming and bulking.

Aeration and mixing

Aeration and mixing configurations were evaluated to optimize performance. The submersible sump pump mixed anaerobic reactors at reduced flow rates, balancing turbulence with minimal surface aeration. Portable DO probe measurements confirmed DO levels remained below 0.18 mg/L. Five aerobic reactor configurations were tested: fine bubble diffusers, coarse bubble diffusers, hybrid fine/coarse bubbles, fine bubbles with submersible sump pump, and coarse bubbles with submersible sump pump.

(1)Fine bubble diffusers achieve high oxygen transfer efficiency but struggle to mix deep or large tanks effectively. Each aerobic reactor used two evenly spaced fine bubble diffusers to enhance coverage and aeration uniformity. Despite this, DO gradients persisted along the reactor's depth and cross section. Sludge trapped in dead zones likely harboured filamentous bacteria, which could later dominate under favourable conditions. Weak mixing also caused surface scum and foam, highlighting the limitations of fine bubble diffusers in achieving complete mixing.

(2)Coarse bubble diffusers generate larger bubbles, requiring higher airflow than fine bubbles to achieve equivalent DO levels. These bubbles provide vigorous vertical mixing, reducing dead zones and breaking up surface scum. Compared to fine bubble systems, reactors with coarse diffusers showed less pronounced DO gradients across depth and cross sections. However, upward facing diffuser placement left DO critically low at the bottom of the reactor.

(3)A fine coarse bubble diffuser hybrid achieved high oxygen transfer and adequate mixing on the coarse bubble side but failed to eliminate light fraction accumulation in poorly mixed zones. This suggests that while the hybrid approach improved certain aspects of reactor performance, it did not fully address the challenges associated with mixing and aeration in all areas of the reactor.

(4)Submersible sump pumps were installed to enhance mixing and reduce dead zones in reactors. While the discharge stream became visible in some reactors, it was not consistently observed across all units. Repositioning the pumps restored visibility in affected units, demonstrating the importance of proper pump placement. However, the interaction between the original aeration system and the added pump created separate mixing paths, and misalignment between these paths caused interference, resulting in low DO zones and poor aeration.

(5)After evaluating all configurations, the study concluded that two coarse bubble diffusers emerged as the choice for the pilot. This configuration provided vigorous mixing, reduced dead zones, and maintained relatively uniform DO levels across the reactor.

The operation of a pilot reactor with a depth of 60 inches (1.52 m) presents unique operational challenges compared to full-scale aeration basins, which typically range from three to eight meters in depth. The shallower depth of the pilot reactor results in significantly shorter bubble rise times and distances, leading to diminished oxygen transfer efficiency and the establishment of pronounced oxygen gradients within the reactor. These gradients can promote excessive EPS production in high DO zones while simultaneously encouraging filamentous bacterial proliferation in low DO zones (Huang et al., 2024; N. Zhu et al., 2015). The reduced bubble rise time also intensifies surface turbulence, which can entrap hydrophobic compounds and stabilize foam formations, thereby further complicating the treatment process.

The transition from anaerobic to aerobic conditions exacerbates operational challenges by triggering EPS secretion. In reactor R5, localized high DO zones intensified this secretion, leading to scum formation, foaming, and filamentous bacterial proliferation in low-oxygen zones. When combined with low F/M ratios and prolonged HRT, these conditions resulted in catastrophic biomass loss, significantly compromising treatment efficiency.

A low-speed mechanical mixer with a right-handed impeller mitigates these issues by eliminating dead zones through gentle upward mixing. When paired with DO-controlled fine-bubble aeration, this configuration ensures an upward aeration path that avoids flow interference. The reduced mixing speed minimizes surface turbulence, preventing hydrophobic compound entrapment and foam stabilization. This approach enables operators to maintain consistent SRT and HRT while optimizing F/M ratios.

DO sensor fouling presents another critical challenge. Standard sensors' rough, semipermeable polymer membranes are prone to microbial colonization, causing biofilm-associated fouling that traps mineral deposits and particulates. Pilot reactors initially relied on DO-controlled aeration, but fouling rates exceeded daily cleaning capacity. Operators subsequently implemented high-output aeration in the first two aerobic reactors and low-output aeration in downstream units. However, manual DO measurements provided insufficient reference points due to poor mixing and aeration, while fluctuating HRT and SRT compounded operational uncertainties. Delayed aeration adjustments likely contributed to numerous aeration-related malfunctions.

A self-cleaning DO sensor system is proposed as a superior alternative to conventional sensors. This system would more effectively address fouling issues while strategic sensor placement and improved reactor mixing could reduce maintenance demands and ensure reliable real-time DO monitoring.

Optimal wastewater treatment performance requires comprehensive optimization of reactor depth, mixing dynamics, aeration strategies, and sensor maintenance protocols. Addressing these factors can mitigate shallow-reactor depth challenges and enhance overall process stability.

Split feeding vs Combined feeding

During the startup phase (June 26 - December 18, 2023), anaerobic reactors R2, R3, and R4 received equal volumes of primary effluent. On December 20, 2023, the pilot plant transitioned to combined feeding in reactor R2. Operators conducted internal recycling tests (November 27, 2023 - July 8, 2024) to evaluate denitrification impacts and implemented selective wasting trials (April 24 - July 10, 2024). A subsequent operational test (July 11 -

December 20, 2024) assessed the effects of shortened anaerobic phases following reactor R2's removal, as illustrated in Figure 15. Despite these operational challenges, the CAS configuration achieved biological nutrient removal.

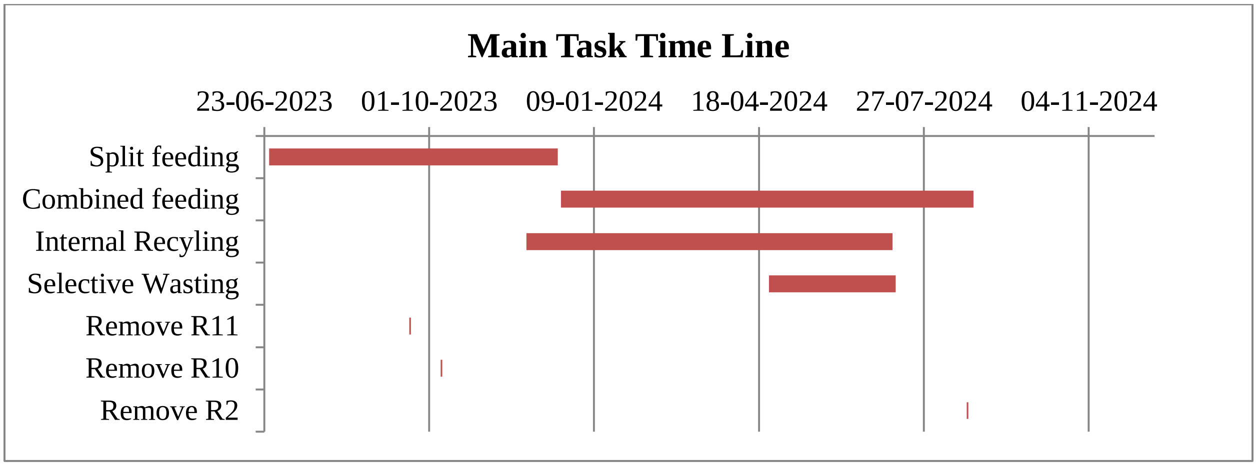

Figure 15 Main Tasks Time Line

The study aimed to cultivate aerobic granular sludge in a continuous plug-flow reactor using feast-and-famine conditions and side-stream selective wasting as selective pressures. The operational cycle comprised a robust anaerobic feast (feeding phase) followed by an extended aerobic famine. During the split-feeding period (November 27 to December 18, 2023), nutrient concentrations gradually increased across the three anaerobic reactors, peaking in R4 to maximize anaerobic phosphate release. Concurrent internal recycling tests introduced nitrate (NOx) into the reactor train, creating competition between PAOs and denitrifiers. Despite this competition, phosphate removal efficiency remained stable. In the combined-feeding phase (December 20, 2023 to July 8, 2024), operators centralized feeding in R2 (the primary anaerobic reactor) to intensify the anaerobic feast. Phosphate release peaked in R3, demonstrating proportionality to nutrient concentration, while internal recycling maintained PAO-denitrifier competition in R4. Although phosphate uptake typically occurs via internally stored PHA in aerobic zones, certain PAOs were observed to oxidize stored PHA using nitrate/nitrite as electron acceptors under anoxic conditions, enabling denitrifying phosphate uptake. This phenomenon correlated with strong nitrification activity, suggesting that RA S introduced anoxic conditions to R2. This finding aligns with intermittent peak phosphate release in R2, where PAO carbon competition likely limited further phosphate release into R4. Anoxic RAS delivered nitrite/nitrate to R2, creating localized anoxic zones that facilitated rapid phosphate absorption via DPAOs. During periods of poor nitrification, phosphate release shifted to R3 as RAS-derived anoxic conditions became insufficient to drive denitrifying uptake, prompting DPAO-mediated uptake in R4 where internal recycling established favorable anoxic conditions.

Internal recycling introduced nitrite/nitrate to the reactor train, driving denitrification. The resulting localized anoxic conditions triggered denitrifying phosphate uptake, likely explaining the weak phosphate release. Despite this, PAOs effectively stored carbon as PHA during anaerobic feeding, maintaining robust phosphate removal overall.

A short feast phase followed by a prolonged famine phase is ideal for promoting microbial selection. However, poor mixing, uneven aeration, and manual DO adjustments reduced the responsiveness and precision of DO control. Despite these challenges, the system generally achieved biological nutrient removal. At reactor R9, most carbon, ammonium, and phosphate were depleted. Operators removed aerobic reactors R11 and R10 on September 25, 2023, and October 10, 2023, shortening the HRT by two hours to an average of 10 hours. Phosphate uptake rate in aerobic reactors differentiated feast and famine conditions—a simplified approach. The feast and famine ratio ranged between 0.75~1 in stage 1 and about 1 in stage 2. Removing two reactors shortened the HRT and increased the feast and famine ratio. Retesting the combined feeding and ten-reactor configuration could validate these findings and clarify the relationship between operational adjustments and nutrient removal efficiency, given improvements in reactor mixing, aeration control, and hydraulic management.

Hydraulic Challenges

The pilot system experienced persistent hydraulic instability, characterized by tube fouling, reduced flow rates, clogged ball valves, and fluctuating HRT/SRT due to variable tank volumes and inconsistent feed flows. Operators installed the reactors on concrete cinder blocks and wooden pallets, with connection tubes initially dimensioned for the design flow rate. However, accumulation of sludge, bubbles, biofilm, and FOG significantly decreased flow rates, prolonging HRT by two hours beyond theoretical calculations. Both primary effluent and RAS pumps operated at excessive capacities, requiring throttling—an inefficient control strategy. Throttling ball valves to stabilize flow exacerbated clogging as fine grit and grease accumulated at restricted openings. Space constraints limited reactor freeboard to 8-11 inches (0.2-0.3 m), precluding higher flow rates. These compounding factors rendered flow regulation and HRT maintenance particularly challenging.

The ten cylindrical reactors configured in series frequently encountered flow disruptions from inter-reactor tubing. Employing oversized rigid piping between reactors could reduce fouling incidence, while variable-frequency drives for primary and RAS pumps would improve flow consistency. A full-scale channel-shaped continuous plug-flow reactor design might entirely eliminate tubing-related complications, thereby simplifying operational procedures.

Selective Wasting

A side-stream selective wasting system was designed to establish a linear settling velocity gradient, enabling differentiation between lighter and denser biomass fractions. The lighter fractions primarily comprised filamentous bacteria, loose floc, and scum. The system integrated with a CAS process by connecting to and returning flows from the midpoint of the aerobic zone. Figure 10 (*Selective Wasting Schematic Flow Diagram*) illustrates the process configuration, highlighting the separation mechanism and flow pathways.

Operationally, the selective wasting module functioned as described in the Methodology section. A programmable logic controller automated the complete cycle: transferring mixed liquor from R8, permitting settling, decanting lighter fractions as waste, and returning denser biomass to R8. Initial performance demonstrated effectiveness in selectively decanting lighter fractions (e.g., filamentous bacteria, scum) while retaining denser biomass in R8, evidenced by improved SVI_{30} values indicating enhanced settleability.

However, selective wasting performance deteriorated rapidly due to systemic issues. The primary operational challenge stemmed from settling times in the selective wasting tank exceeding those observed in SVI_{30} tests conducted under identical conditions using a graduated settleometer. Extended settling trials proved inadequate for proper separation of light and dense biomass fractions. Prolonged settling additionally induced denitrification in settled sludge, causing refloatation and subsequent unintended wasting of high-solids floating sludge during decanting. The programmable controller’s implementation of multiple daily wasting cycles exacerbated biomass loss, culminating in catastrophic depletion. Potential factors contributing to settling efficiency degradation and corresponding mitigation strategies are subsequently examined.

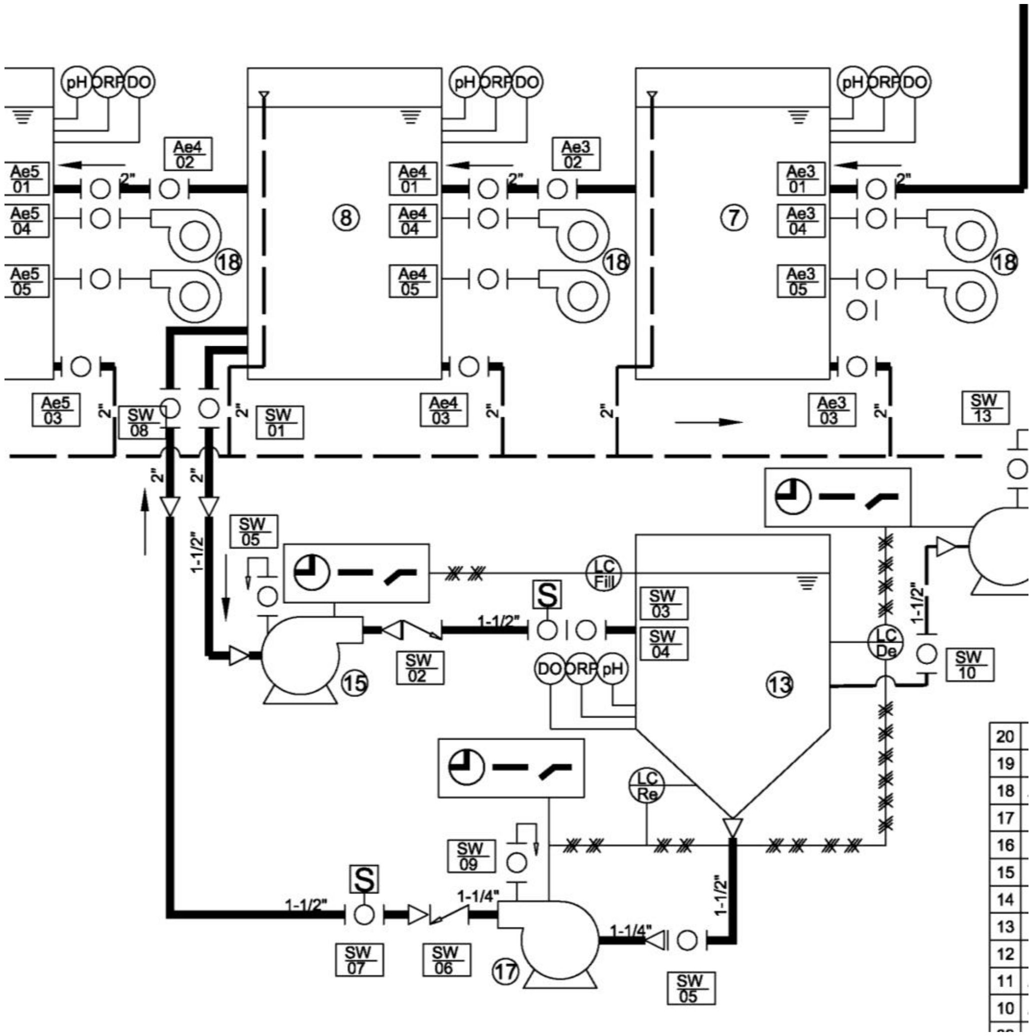

Figure 16 Selective Waste Schematic Flow Diagram

1) The initial filling flow rate was observed to be excessively high, resulting in strong turbulence within the selective wasting tank. This turbulent condition was linked to prolonged settling times. Consequently, operational adjustments were made to reduce the filling flow rate, which facilitated a more consistent and smoother transfer of mixed liquor from reactor R8.

2) A pressure-assisted, normally closed solenoid valve was employed during the filling step. This valve necessitated 20 psi to open, requiring an upstream pump to provide the operating pressure. Substitution with a zero-pressure-drop solenoid valve would obviate the need for the pump, enabling direct gravity-fed transfer of mixed liquor into the selective wasting tank.

3) The absence of an energy-dissipating device at the inlet of the selective wasting tank was identified as a contributing factor to hydraulic inefficiencies. Installation of baffles at this location was proposed as a remedial measure, which would serve to dissipate excess hydraulic energy and attenuate turbulence within the tank.
4) Activated sludge flocs exhibit high susceptibility to shear stress. At reduced filling flow rates, the estimated velocity gradient at the fill pump (2,000 - 4,900 s^{-1}) exceeded typical rapid mixing thresholds (>1,000 s^{-1}) in wastewater treatment, corresponding to shear stresses of 9.5 - 16.4 Pa (Mikkelsen, 2001; Mikkelsen & Keiding, 2002; Seka & Verstraete, 2003; Wilén et al., 2003; Y. Yuan & Farnood, 2010). Elevated shear stress disperses floc-associated substances into the aqueous phase. Guo's study demonstrated floc regrowth at 469 s^{-1} (2 minutes) but not under higher shear stress, indicating a critical recovery threshold (X. Guo et al., 2019). The excessive shear stress generated by the filling pump likely destabilized the sludge flocs, accounting for the observed discrepancy between favorable settling in laboratory settleometers and suboptimal performance in the selective wasting tank under field conditions.
5) A higher H/D ratio in a batch settling tank increases the vertical distance for particle settling, allowing particles to travel longer distances and times before reaching the bottom. This enhanced settling distance facilitates improved separation of light and dense fractions at a specific critical settling velocity. In contrast, a lower H/D ratio results in shorter settling distances and times, preventing effective differentiation between particle fractions. The effective reactor H/D ratio ($\approx$1) was significantly lower than that of the graduated settleometer (H/D = 5.5), which accounted for the superior settling performance observed in the settleometer.
6) Denitrification was observed in settled sludge within the selective wasting tank, a phenomenon originating from mainstream process performance rather than tank design. Comparable

denitrification occurred in the clarifier, where nitrogen gas release from settled sludge resulted in floating scum formation. The implications of this process for solids management will be thoroughly examined in subsequent analysis.

7) Operator inexperience with the selective wasting module resulted in premature biomass loss. Initial positive outcomes prompted operators to increase cycle frequency, reduce settling times, or implement both modifications concurrently. These operational adjustments caused system destabilization, manifesting as a rapid decrease in phosphate removal efficiency and compromised nitrification performance.

Selective wasting was designed to remove light biomass fractions from the system. Despite the operational drawbacks outlined in points (1), (2), and (3), the system demonstrated initial effectiveness. As indicated in (4), fill pump-induced shear stress may have contributed to poor settling in the selective tank. The high H/D ratio discussed in (5) - derived from granular sludge cultivation in SBRs - underscored the limitations of the installed selective wasting tank's low H/D ratio ($\approx$1), which restricted its ability to apply sufficient selective pressure. Testing a higher H/D ratio could potentially enhance separation efficiency.

Denitrification, as noted in (6), likely served as the primary driver of biomass loss in the selective wasting tank. Addressing this issue necessitates integrating denitrification control with comprehensive mainstream process adjustments. The controller program (7) governed settling times and daily cycles. Gradual reduction of settling times is recommended to ensure smooth transition in settling velocity, thereby preventing premature biomass loss and system destabilization. Aggressive adjustments, however, risked rapid biomass depletion, identified as the secondary cause of losses in the tank.

Compared to the side stream selective wasting module tested in this study, the hydrocyclone presents an alternative sludge intensification method. Under pressure, sludge enters the hydrocyclone's tangential feed inlet, generating a vortex that forces heavier fractions outward and downward to the conical bottom (returned as sludge) while lifting lighter fractions upward (decanted as waste). Compact, modular, and designed as a plug-and-run unit, the hydrocyclone - originally developed to enhance sludge settleability during cold weather operations - has been widely applied for separating particles of differing densities. However, limited studies beyond the Dijon case have explored its potential for granular sludge cultivation. Further investigation into operating parameters (e.g., pressure, feed velocity), particularly regarding shear stress/floc (granular) stability comparisons between selective wasting and hydrocyclone systems, could provide valuable insights for decision-making.

Solids Management

Fluctuating HRT disrupts nitrification consistency, placing stress on slow-growing, environmentally sensitive autotrophic nitrifying bacteria. AOB and PAOs, both requiring stable, prolonged conditions, experience population declines under variable SRT. These microorganisms cannot recover as rapidly as fast-growing heterotrophs, such as filamentous bacteria, thereby exacerbating poor nutrient removal.

Persistent foaming in aerobic reactors, combined with scum accumulation in the clarifier, contributed to recurring biomass loss during the study. This biomass loss destabilized system operations, particularly under wet weather conditions that diluted primary effluent strength. Mechanical shortcomings, including poor mixing, inconsistent aeration, and suboptimal hydraulic profiles, further intensified system-wide challenges and operational complexity.

Maintaining a steady SRT is critical for sustaining BNR performance in CAS processes. The system's ability to maintain steady SRT is influenced by several factors, categorized as external and operational. External factors primarily relate to wastewater characteristics, which may exhibit seasonal variations and are affected by wet weather conditions, depending on the wastewater collection system. Operational factors include HRT, WAS rate, and DO levels, all of which play significant roles in achieving stable SRT and optimizing BNR performance.

HRT

The HRT and SRT are two critical parameters for BNR efficiency. HRT determines the duration microorganisms have to process incoming nutrients, influencing biomass growth and overall system performance. Maintaining a steady SRT is essential, as it requires continuous adjustments based on biomass growth to ensure optimal microbial activity and nutrient removal.

In the pilot study, primary effluent was pumped to Reactor 2 (R2) using a submersible sump pump and conveyed through the system by gravity. The absence of dedicated volume control devices resulted in fluctuating reactor volumes. The sump pump's flow rate frequently exceeded system requirements, necessitating manual ball valve adjustments. Over time, operational challenges including tube fouling and valve clogging caused additional flow rate fluctuations, further complicating HRT management. The variability in HRT calculation parameters made precise control difficult. Elevated HRT was associated with higher F/M ratios, potentially promoting excessive EPS secretion in aerobic reactors. Conversely, reduced HRT was linked to lower F/M ratios, which may have fostered filamentous bacterial growth and system destabilization.

The reactor configuration significantly contributed to the observed poor hydraulic profile. Ten reactors were arranged in two rows of five each. Reactors R2 through R6 were positioned against the ground slope (from low to high elevation), while Reactors R7 through R11 were aligned along the slope. This arrangement exacerbated hydraulic imbalances, further compromising system performance and operational stability.

Table 8 Revised Hydraulic Profile at Current Reactor Layout

Reactor	Ground Elevation	Block Height	Base Elevation	Water Level	Hydraulic Level	Δ Head	Reactor Volume	Retention Time
	[in]	[in]	[in]	[in]	[in]	[in]	[L]	[hr]
R2	±0.00	23.75	23.75	62.00	85.75	1.04	977.50	1.09
R3	0.96	23.75	24.71	60.00	84.71	1.04	945.97	1.05
R4	1.92	23.75	25.67	58.00	83.67	1.04	914.44	1.02
R5	2.88	23.75	26.63	56.00	82.63	1.04	882.91	0.98
R6	3.84	23.75	27.59	54.00	81.59	1.50	851.37	0.95
R7	3.84	23.75	27.59	52.50	80.09	0.96	827.73	0.92
R8	2.88	23.75	26.63	52.50	79.13	0.96	827.73	0.92
R9	1.92	23.75	25.67	52.50	78.17	0.96	827.73	0.92
R10	0.96	23.75	24.71	52.50	77.21	0.96	827.73	0.92
R11	±0.00	23.75	23.75	52.50	76.25	1.25	827.73	0.92
			Clarifier inlet elevation≈		75.00			Total=9.68

The secondary cause of the poor hydraulic profile was the design of the connection tubes between reactors. The reactors' inlet and outlet were positioned at the lower part of the reactor, necessitating gravity flow to overcome the hydrostatic head of the subsequent reactor. When this head could not be overcome, the water level in the preceding reactor would rise to compensate for the resistance, resulting in uneven flow distribution and further destabilizing the system's hydraulic performance. To address this issue, the following mitigation measures are proposed:

1) Fixed Reactor Volume: An overflow-style outlet can be installed at the water level to ensure consistent reactor volume. This configuration will force water to exit at a predetermined level, thereby maintaining stability within the system.
2) Improved Hydraulic Profile: The clarifier inlet is positioned at an elevation of 70 inches. By configuring outlets at a predetermined elevation, a positive head can be maintained between each reactor and the subsequent one, thereby ensuring consistent water flow throughout the system.
3) Use VFD Pump: A Variable Frequency Drive (VFD)-fed pump can be employed to regulate flow rate and maintain a consistent HRT.

R2 through R6: These reactors, positioned against the ground slope, would benefit from an increased drop between reactors to promote efficient water flow. R7 through R11: In contrast, these reactors, aligned along the slope, may necessitate a reduced drop to avoid overloading the clarifier and maintain optimal performance. A comprehensive summary of these configurations is presented in Table 8.

Low F/M and Unstable F/M ratio

At a low F/M ratio, insufficient food availability for microorganisms may induce endogenous respiration, thereby reducing biomass concentration and weakening or altering the microbial community structure. This alteration can impede BNR efficiency, potentially resulting in sludge bulking, poor settling, and high final effluent solids. Under such conditions, filamentous bacteria thrive and outcompete nitrifiers and PAOs for oxygen or residual food in the aerobic tank. Similarly, an unstable F/M ratio may hinder microbial adaptation to changing conditions, further compromising BNR efficiency. In the pilot study, both low and unstable F/M ratios repeatedly caused foaming in the first aerobic reactor. The unquantifiable biomass loss via foam from the aerobic tank significantly complicated SRT calculations. To address these issues, the following mitigation measures are proposed:

1) Increase WAS wasting: More WAS wasting reduces the biomass inventory, thus increasing or restoring the F/M ratio to a higher level.
2) Adjust and maintain DO: The Controlled aeration will regulate DO levels to sustain optimal conditions for nitrifiers and PAOs while simultaneously suppressing filamentous bacteria.
3) Avoid Shock Load: A sudden increase in food input may further disrupt the F/M ratio, potentially exacerbating operational challenges in the biological treatment process.
4) Frequent Monitoring: Continuous monitoring of WAS rate, F/M ratio, DO level, MLSS, and BNR efficiency enables real-time process assessment, thereby facilitating more effective operational guidance.

Mixing and aeration

Low F/M ratio promotes the proliferation of filamentous bacteria. The transition from anaerobic to aerobic phases induces EPS production. Observed uneven mixing or aeration creates DO gradients, where filamentous bacteria dominate low-DO zones while excessive EPS secretion occurs in high-DO zones. During the study, elevating DO concentration or airflow rate was routinely employed to mitigate foaming under low F/M conditions. However, over-aeration in certain areas may have exacerbated EPS secretion. Quantifying EPS production in the primary aerobic reactor could provide comprehensive process understanding.

Repeated excessive foaming in aerobic zones caused unquantifiable biomass loss, significantly complicating SRT calculations. To address this issue, the following mitigation measures are proposed:

1) An overhead blade mixer ensures comprehensive vertical and lateral mixing, thereby minimizing stagnant zones induced by sump pump streams or coarse bubble diffuser flows.
2) The gearbox substantially decreases mixing velocity, enabling optimal mixing conditions by minimizing undesirable surface turbulence and shear stress.
3) DO-controlled fine bubble aeration enables efficient oxygen transfer precisely when required, thereby preventing both under-aeration and over-aeration scenarios.

Slow mixing and on-demand oxygen transfer effectively minimize oxygen gradients, particularly under low F/M conditions. Eliminating low DO or under-aerated zones promotes nitrifying bacteria and PAOs while suppressing filamentous bacteria proliferation, especially in the primary aerobic reactor. Conversely, eliminating high DO or over-aerated zones mitigates sudden EPS secretion. Aerobic reactor foaming can be more effectively controlled by managing filamentous bacteria growth and EPS secretion, thereby reducing uncontrolled biomass loss and enhancing system stability.

Clarifier

Primary effluent (Q $\approx$15 L/min) and RAS (Q$\approx$12 L/min) entered the clarifier at a combined flow rate of 27 L/min, with an MLSS concentration of 2,000 mg/L. The calculated clarifier surface and solids loading rates were 13.6 m^3/m^2/day and 27.3 kg TSS/m^2/day, respectively, while the HRT averaged 2.8 hours. Both loading rates operated below recommended thresholds for optimal clarifier performance.

Under low surface overflow rates (SOR), the clarifier's performance was significantly compromised by uneven influent distribution, leading to short circuiting, surface scum accumulation, and temperature gradients. Short circuiting caused solids to settle near the center inlet baffle, reducing effective detention time and risking stagnant sludge blanket formation. As surface scum thickened over time, localized anaerobic conditions developed. Slow-moving water induced density stratification through temperature gradients, impairing sludge settling and compaction. The low HRT further limited settling duration, exacerbating solids management issues. The absence of a reaction wall allowed sludge to bypass the thickening zone entirely, resulting in dilute RAS effluent. Combined with suboptimal SOR, this bypass critically reduced RAS concentrations, undermining the system's capacity to maintain a robust biomass inventory for efficient treatment.

At low solids loading rates, prolonged sludge retention exceeded optimal durations, potentially inducing denitrification where denitrifying bacteria reduce nitrate to nitrogen gas. The resulting gas bubbles decreased sludge density, impairing settling and compaction efficiency while lifting sludge flocs to form surface scum or elevate effluent solids concentrations. Extended anaerobic retention times may also promote endogenous respiration, weakening floc structure and reducing settling velocity.

Non-standard clarifier design features, particularly the absence of a reaction wall and notched weir coupled with an elevated H/D ratio of 0.77 (exceeding the recommended 0.1-0.3), compounded operational challenges. The reaction wall's absence eliminated hydraulic buffering, energy dissipation, and sludge blanket stabilization, decreasing effective settling volume and causing poor solids capture, effluent surges, and upward sludge blanket displacement that formed surface scum. The scraper assembly's rotational motion likely created rotational dead zones, allowing sludge and scum accumulation. The simplified 6" circular overflow (instead of a notched weir) caused localized flow paths, short circuiting, and uneven effluent withdrawal, trapping floating flocs in accumulating scum. These design factors collectively contributed to stagnant sludge conditions, triggering denitrification and floating floc formation. To address these issues, the following mitigation measures are proposed:

1) MLSS Control: Prevent excessive MLSS concentrations from entering the clarifier to maintain optimal operational conditions.
2) WAS Rate Adjustment: Regulate WAS rates based on the F/M ratio and SVI to ensure process stability.
3) Daily SVI Monitoring: Assess sludge settleability daily and adjust the solids loading rate accordingly to enhance clarifier performance.
4) RAS Optimization: Balance RAS rates to achieve equilibrium between biomass retention and clarifier loading.
5) Performance Modeling: Utilize secondary clarifier performance models (e.g., State Point Analysis) to predict and optimize operational parameters.
6) Clarifier Modification 1: Install a reaction wall to dissipate hydraulic energy and promote uniform flow distribution within the clarifier.
7) Clarifier Modification 2: Equip a notched weir along the interior wetted perimeter to facilitate efficient scum removal.
8) Automated WAS Wasting: Implement automated WAS wasting systems controlled by process SRT and MLSS concentrations.
9) Sludge Level Sensors: Deploy sensors to monitor sludge depth in real-time, enabling proactive adjustments.
10) Flow Meter Installation: Track influent and effluent flow rates to maintain hydraulic balance and detect anomalies.

11) pH/DO Probes: Install probes to monitor pH and DO levels, ensuring optimal biological process conditions.

12) Redundant Design: Incorporate redundancy (e.g., multiple clarifiers) to accommodate peak loads and improve system resilience.

13) Machine Learning Integration: Leverage artificial intelligence (AI) for real-time process diagnostics and adaptive control strategies.

Split Feeding, or Combined Feeding

At the study's outset, split feeding to the three anaerobic reactors directed all carbon to PAOs, leaving minimal residual carbon in the aerobic zone. Later tested combined feeding intensified the anaerobic feast phase, further depleting aerobic carbon availability. Both strategies prioritized anaerobic feast conditions. The initial process configuration included seven aerobic reactors, establishing a robust aerobic famine phase (feast and famine ratio of 0.75~1) that effectively suppressed fast-growing filamentous bacteria. Later configurations removed aerobic reactors R10 and R11, weakening the famine phase (feast and famine ratios of 0.25~0.755). This shift increased system susceptibility to filamentous bacterial growth. Compounding these issues, fluctuating HRT and SRT, low and fluctuating F/M ratios, poor reactor mixing/aeration, and mechanical failures destabilized the microbial community. Biomass loss and structural weakening created an environment favoring filamentous bacteria, further degrading floc integrity.

To address the issue, the following mitigation measures are proposed: Restore R10 and R11 under the current reactor layout, which with an improved hydraulic profile would stabilize HRT closer to the WEWPCC's HRT. Longer aerobic famine or a smaller feast/famine ratio can effectively eliminate filamentous bacteria.

Conclusion

The pilot successfully achieved BNR across three operational stages. In Stage 1, the system achieved 90% ammonium removal 56% of the time and 90% phosphate removal 70% of the time. Performance improved in Stage 2, attaining 90% ammonium removal 86% of the time and 90% phosphate removal 79% of the time. Stage 3 demonstrated 90% ammonium removal 90% of the time, but phosphate removal efficiency declined significantly to 19% of the time, likely due to premature biomass loss from selective wasting testing.

Regarding feeding strategies, combined feeding created a marginally better aerobic environment for phosphate uptake compared to split feeding. However, competition with denitrification through internal recycling limited the effectiveness of combined feeding. Process constraints resulted in weak feast and famine stress, particularly after removing the last two aerobic reactors. The initial longer aerobic step had produced relatively stronger feast-and-famine conditions. Selective wasting was employed to retain fast-settling, denser biomass, but its efficacy was reduced by pump shear stress, mainstream denitrification, and premature biomass wasting.

Rigorous testing of process configurations and operational scenarios was conducted to enhance performance. Despite meticulous efforts, operators observed significant fluctuations in MLSS, HRT, SRT, and F/M ratio. These fluctuations correlated with poor settling, floating flocs, scum accumulation, and eventual biomass loss via scum, preventing achievement of long-term steady-state conditions.

While the pilot aimed to scale up previously validated small-scale concept research, successful implementation required more than conceptual replication - it demanded rigorous integration of hydraulic, mechanical, and microbial factors. The pilot project served as a critical bridge between original concept design and potential full-scale operations, revealing unexpected challenges not apparent in smaller-scale studies. Despite these challenges, the pilot successfully demonstrated BNR capabilities across all three operational stages.

Thoughts on improvement or future project

The pilot project encountered several operational challenges, including difficulties in hydraulic and process flow management, biomass management, aeration (including mixing), solids separation, and selective wasting. These issues led to instability in key operating parameters such as HRT, SRT, F/M ratio, DO, MLSS, and BNR performance. To address these challenges, an improved process design is essential, providing more reliable, practical, repeatable, and simplified operating conditions. Additionally, operating the process until steady state is crucial to fully evaluate the impact of operational parameters.

Hydrocyclones were found to limit floc and granule size due to shear stress, though their granulation effects and long-term BNR performance remain poorly understood. In contrast, side-stream selective wasting emerges as a more favorable option for granulation in continuous flow processes, being less invasive and subjecting flocs or granules to reduced shear stress—thereby better maintaining granulation integrity.

The pilot received weak primary effluent from the WEWPCC, limiting aerobic feast intensity and feast-and-famine strength. Future process designs could enhance denitrification by combining or pulse feeding without internal recycling, achieving more pronounced short aerobic feasts and stronger feast-and-famine stress. However, the absence of denitrification may initially challenge secondary clarifier performance, where modeling could prove instrumental in establishing or calibrating design parameters.

Initially, the feast-and-famine ratio was estimated using aerobic phosphate uptake rates; however, measuring PHA and oxygen uptake rate would offer more comprehensive insights into these conditions' intensity and duration. Reactor number constraints limited feast-and-famine resolution, suggesting a new reactor design with higher resolution could refine process parameter optimization.

Microbial community analysis has become a powerful tool in wastewater treatment research. Incorporating near real-time monitoring of microbial communities under varying operational conditions could significantly enhance process understanding and optimization, providing valuable insights into microbial dynamics and their responses to operational parameters—ultimately aiding in developing more efficient and adaptive treatment processes.

Reference

Adav, S. S., & Lee, D.-J. (2008). Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. *Journal of Hazardous Materials*, *154*(1), 1120–1126. https://doi.org/10.1016/j.jhazmat.2007.11.058

Amorim, C. L., Maia, A. S., Mesquita, R. B. R., Rangel, A. O. S. S., van Loosdrecht, M. C. M., Tiritan, M. E., & Castro, P. M. L. (2014). Performance of aerobic granular sludge in a sequencing batch bioreactor exposed to ofloxacin, norfloxacin and ciprofloxacin. *Water Research*, *50*, 101–113. https://doi.org/10.1016/j.watres.2013.10.043

Amorim de Carvalho, C. de, Ferreira dos Santos, A., Tavares Ferreira, T. J., Sousa Aguiar Lira, V. N., Mendes Barros, A. R., & Bezerra dos Santos, A. (2021). Resource recovery in aerobic granular sludge systems: Is it feasible or still a long way to go? *Chemosphere*, *274*, 129881. https://doi.org/10.1016/j.chemosphere.2021.129881

Arrojo, B., Mosquera-Corral, A., Garrido, J. M., & Méndez, R. (2004). Aerobic granulation with industrial wastewater in sequencing batch reactors. *Water Research*, *38*(14–15), 3389–3399. Scopus. https://doi.org/10.1016/j.watres.2004.05.002

Avila, I., Freedman, D., Johnston, J., Wisdom, B., & McQuarrie, J. (2021). Inducing granulation within a full-scale activated sludge system to improve settling. *Water Science and Technology*, *84*(2), 302–313. https://doi.org/10.2166/wst.2021.006

Bahgat, N. T., Wilfert, P., Korving, L., & van Loosdrecht, M. (2023). Integrated resource recovery from aerobic granular sludge plants. *Water Research*, *234*, 119819. https://doi.org/10.1016/j.watres.2023.119819

Brewers of Europe. (2002). *Guidance note for establishing BAT in the brewing industry*. https://docplayer.net/27092569-The-brewers-of-europe.html

Buthelezi, S. (2008). *APPLICATION OF BACTERIAL BIOFLOCCULANTS FOR WASTEWATER AND RIVER WATER TREATMENT*. https://d1wqtxts1xzle7.cloudfront.net/73311343/Buthelezi_S_2008-libre.pdf?1634836328=&response-content-disposition=inline%3B+filename%3DApplication_of_bacterial_bioflocculants.pdf&Expires=1704684859&Signature=Fvf1PxHMgF-8WxBegU7FglX4oz9y93btg-Q6Obao0D25K-YFNMb22mJ4sF2lm8t7OBev25sKi0LZfjYjBi9FWe07hCzuZmkHLWklgB68JqWbCzqvZOlo62vDvo-wvd7jkXex9R9iMiT4hc9OrLAhtHXXerALnBDjZUnJkc4JqKeQxdrdj5Ob1piIyUcnK~ETLsoppIEVtm0oAD7U2M3ZobNQvwDdJEpq54yGzvDF8avf5S7VMT68fgtb35ub7Q7rzhn5j5osdQibVDu5EVLj1IMhFXNkXHsjFcGxLpYLPztDGENS2T9dB7gLh4SfFfDH5RlctqDwVyWOeE59SvT8kQ__&Key-Pair-Id=APKAJLOHF5GGSLRBV4ZA

Campo, R., & Di Bella, G. (2019). Petrochemical slop wastewater treatment by means of aerobic granular sludge: Effect of granulation process on bio-adsorption and hydrocarbons removal. *Chemical Engineering Journal*, *378*, 122083. https://doi.org/10.1016/j.cej.2019.122083

Campo, R., Vassallo, A., Rabbeni, G., Arancio, W., Gallo, G., & Di Bella, G. (2021). Reactivation of aerobic granular sludge for the treatment of industrial shipboard slop wastewater: Effects of long-term storage on granules structure, biofilm activity and microbial community. *Journal of Water Process Engineering*, *42*. Scopus. https://doi.org/10.1016/j.jwpe.2021.102101

Chiesa, S. C., Irvine, R. L., & Manning, J. F. (1985). Feast/famine growth environments and activated sludge population selection. *Biotechnology and Bioengineering*, *27*(5), 562–568. https://doi.org/10.1002/bit.260270503

Chou, H.-H., Huang, J.-S., Tsao, C.-W., & Lu, Y.-C. (2011). Comparative influential effects of mass transfer resistance in acetate-fed and glucose-fed sequential aerobic sludge blanket reactors. *Chemical Engineering Journal*, *174*(1), 182–189. https://doi.org/10.1016/j.cej.2011.08.072

Christensen, B. E. (1989). The role of extracellular polysaccharides in biofilms. *Journal of Biotechnology*, *10*(3–4), 181–202. https://doi.org/10.1016/0168-1656(89)90064-3

Corsino, S. F., Campo, R., Di Bella, G., Torregrossa, M., & Viviani, G. (2016). Study of aerobic granular sludge stability in a continuous-flow membrane bioreactor. *Bioresource Technology*, *200*, 1055–1059. https://doi.org/10.1016/j.biortech.2015.10.065

Corsino, S. F., Capodici, M., Morici, C., Torregrossa, M., & Viviani, G. (2016). Simultaneous nitritation–denitritation for the treatment of high-strength nitrogen in hypersaline wastewater by aerobic granular sludge. *Water Research*, *88*, 329–336. https://doi.org/10.1016/j.watres.2015.10.041

Corsino, S. F., Capodici, M., Torregrossa, M., & Viviani, G. (2016). Fate of aerobic granular sludge in the long-term: The role of EPSs on the clogging of granular sludge porosity. *Journal of Environmental Management*, *183*, 541–550. https://doi.org/10.1016/j.jenvman.2016.09.004

Corsino, S. F., di Biase, A., Devlin, T. R., Munz, G., Torregrossa, M., & Oleszkiewicz, J. A. (2017). Effect of extended famine conditions on aerobic granular sludge stability in the treatment of brewery wastewater. *Bioresource Technology*, *226*, 150–157. https://doi.org/10.1016/j.biortech.2016.12.026

Cydzik-Kwiatkowska, A., Bernat, K., Zielińska, M., Gusiatin, M. Z., Wojnowska-Baryła, I., & Kulikowska, D. (2022). Valorization of full-scale waste aerobic granular sludge for biogas production and the characteristics of the digestate. *Chemosphere*, *303*, 135167. https://doi.org/10.1016/j.chemosphere.2022.135167

de Bruin, L. M. M., de Kreuk, M. K., van der Roest, H. F. R., Uijterlinde, C., & van Loosdrecht, M. C. M. (2004). Aerobic granular sludge technology: An alternative to activated sludge? *Water Science and Technology*, *49*(11–12), 1–7. Scopus. https://doi.org/10.2166/wst.2004.0790

de Kreuk, M. K., Kishida, N., Tsuneda, S., & van Loosdrecht, M. C. M. (2010). Behavior of polymeric substrates in an aerobic granular sludge system. *Water Research*, *44*(20), 5929–5938. https://doi.org/10.1016/j.watres.2010.07.033

de Kreuk, M. k., Picioreanu, C., Hosseini, M., Xavier, J. b., & van Loosdrecht, M. c. m. (2007). Kinetic model of a granular sludge SBR: Influences on nutrient removal. *Biotechnology and Bioengineering*, *97*(4), 801–815. https://doi.org/10.1002/bit.21196

de Kreuk, M. K., & van Loosdrecht, M. C. M. (2004). Selection of slow growing organisms as a means for improving aerobic granular sludge stability. *Water Science and Technology*, *49*(11–12), 9–17. Scopus. https://doi.org/10.2166/wst.2004.0792

Devlin, T. R., di Biase, A., Kowalski, M., & Oleszkiewicz, J. A. (2017). Granulation of activated sludge under low hydrodynamic shear and different wastewater characteristics. *Bioresource Technology*, *224*, 229–235. https://doi.org/10.1016/j.biortech.2016.11.005

Devlin, T. R., & Oleszkiewicz, J. A. (2018). Cultivation of aerobic granular sludge in continuous flow under various selective pressure. *Bioresource Technology*, *253*, 281–287. https://doi.org/10.1016/j.biortech.2018.01.056

Ding, Z., Bourven, I., Guibaud, G., Van Hullebusch, E. D., Panico, A., Pirozzi, F., & Esposito, G. (2015). Role of extracellular polymeric substances (EPS) production in bioaggregation: Application to wastewater treatment. *Applied Microbiology and Biotechnology*, *99*(23), 9883–9905. https://doi.org/10.1007/s00253-015-6964-8

Ekholm, J., Persson, F., de Blois, M., Modin, O., Pronk, M., van Loosdrecht, M. C. M., Suarez, C., Gustavsson, D. J. I., & Wilén, B.-M. (2022). Full-scale aerobic granular sludge for municipal wastewater treatment – granule formation, microbial succession, and process performance. *Environmental Science: Water Research & Technology*, *8*(12), 3138–3154. https://doi.org/10.1039/D2EW00653G

Enitan, A. M., Adeyemo, J., Kumari, S. K., Swalaha, F. M., & Bux, F. (2015). Characterization of brewery wastewater composition. *International Journal of Environmental and Ecological Engineering*, *9*(9), 1073–1076. https://doi.org/10.51415/10321/2992

Fakhru'l-Razi, A., Pendashteh, A., Abdullah, L. C., Biak, D. R. A., Madaeni, S. S., & Abidin, Z. Z. (2009). Review of technologies for oil and gas produced water treatment. *Journal of Hazardous Materials*, *170*(2), 530–551. https://doi.org/10.1016/j.jhazmat.2009.05.044

Fent, K., Weston, A. A., & Caminada, D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology*, *76*(2), 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009

Ford, A. C. (2018). *The Evaluation of Enhancing Biological Phosphorus Removal and Improving Settleability Using Mainstream Hydrocyclones for External Selection*. https://doi.org/10.25777/EHXZ-3V28

Galloway, W. R. J. D., Hodgkinson, J. T., Bowden, S. D., Welch, M., & Spring, D. R. (2011). Quorum Sensing in Gram-Negative Bacteria: Small-Molecule Modulation of AHL and AI-2 Quorum Sensing Pathways. *Chemical Reviews*, *111*(1), 28–67. https://doi.org/10.1021/cr100109t

Gao, D., Liu, L., Liang, H., & Wu, W.-M. (2011). Aerobic granular sludge: Characterization, mechanism of granulation and application to wastewater treatment. *Critical Reviews in Biotechnology*, *31*(2), 137–152. https://doi.org/10.3109/07388551.2010.497961

Gao, D., Yuan, X., & Liang, H. (2012). Reactivation performance of aerobic granules under different storage strategies. *Water Research*, *46*(10), 3315–3322. Scopus. https://doi.org/10.1016/j.watres.2012.03.045

Gao, W., Hu, Y., Jiao, X., Gao, M., & Wang, X. (2021). Recovery of structure and activity of disintegrated aerobic granular sludge after long-term storage: Effect of exogenous N-acyl-homoserine lactones. *Chemosphere*, *281*, 130894. https://doi.org/10.1016/j.chemosphere.2021.130894

Gebeyehu, A., Shebeshe, N., Kloos, H., & Belay, S. (2018). Suitability of nutrients removal from brewery wastewater using a hydroponic technology with Typha latifolia. *BMC Biotechnology*, *18*(1), 74. https://doi.org/10.1186/s12896-018-0484-4

Gemza, N., & Kuśnierz, M. (2022). Gravimetric selection of activated sludge for settling properties improvement and granular sludge formation – full scale case study. *Water Practice and Technology*, *17*(5), 1169–1176. https://doi.org/10.2166/wpt.2022.044

Guo, D., Jiang, X., Guo, M., Zeng, M., Wu, N., Hao, L., & Wang, C. (2022). Role of hydrocyclone separator on the formation and separation of aerobic granular sludge: Evaluating granulation efficiency and simulating hydrodynamic behavior. *Separation and Purification Technology*, *283*, 120231. https://doi.org/10.1016/j.seppur.2021.120231

Guo, H., Felz, S., Lin, Y., van Lier, J. B., & de Kreuk, M. (2020). Structural extracellular polymeric substances determine the difference in digestibility between waste activated sludge and aerobic granules. *Water Research*, *181*, 115924. https://doi.org/10.1016/j.watres.2020.115924

Guo, X., Zhang, C., & Liu, J. (2019). Optimal hydraulic shear strength and mechanism of activated sludge floc re-growth after breakage. *Colloids and Surfaces B: Biointerfaces*, *176*, 202–211. https://doi.org/10.1016/j.colsurfb.2019.01.001

Gysin, A., Lycke, D., & Wirtel, S. (2018). The Pearl® and WASSTRIP® processes (Canada). In C. Schaum (Ed.), *Phosphorus: Polluter and Resource of the Future – Removal and Recovery from Wastewater* (pp. 359–365). International Water Association. https://doi.org/10.2166/9781780408361_359

Hamza, R. A., Rabii, A., Ezzahraoui, F., & Morgan, G. (2022). *A review of the state of development of aerobic granular sludge technology over the last 20 years: Full-scale applications and resource recovery | Elsevier Enhanced Reader*. https://doi.org/10.1016/j.cscee.2021.100173

Huang, B., Huang, J., Wang, X., Chen, Y., Ni, M., Ding, Y., Huang, Y., Li, L., & Pan, Y. (2024). Enhancing phosphorus uptake in biofilms by dissolved oxygen: Mechanistic insights from cellular and EPS perspectives. *Journal of Water Process Engineering*, *65*, 105860. https://doi.org/10.1016/j.jwpe.2024.105860

Jaiyeola, A. T., & Bwapwa, J. K. (2016). Treatment technology for brewery wastewater in a water-scarce country: A review. *South African Journal of Science*, *112*(3/4), Article 3/4. https://doi.org/10.17159/sajs.2016/20150069

J-h, T., Liu, Q.-S. Q., & Liu, Y. Y. (2001). The effects of shear force on the formation, structure and metabolism of aerobic granules. *Applied Microbiology and Biotechnology*, *57*(1–2), 227–233. https://doi.org/10.1007/s002530100766

Kehrein, P., Van Loosdrecht, M., Osseweijer, P., & Posada, J. (2020). Exploring resource recovery potentials for the aerobic granular sludge process by mass and energy balances – energy, biopolymer and phosphorous recovery from municipal wastewater. *Environmental Science: Water Research & Technology*, *6*(8), 2164–2179. https://doi.org/10.1039/D0EW00310G

Kolev Slavov, A. (2017). Dairy Wastewaters – General Characteristics and Treatment Possibilities – A Review. *Food Technology and Biotechnology*, *55*(1). https://doi.org/10.17113/ftb.55.01.17.4520

Kończak, B., Karcz, J., & Miksch, K. (2014). Influence of Calcium, Magnesium, and Iron Ions on Aerobic Granulation. *Applied Biochemistry and Biotechnology*, *174*(8), 2910–2918. https://doi.org/10.1007/s12010-014-1236-0

Kratochvil, D., & Volesky, B. (1998). Advances in the biosorption of heavy metals. *Trends in Biotechnology*, *16*(7), 291–300. https://doi.org/10.1016/S0167-7799(98)01218-9

Krishna. (2013). *Epe Sewage Treatment Plant (STP)—Water Technology*. Www.Water-Technology.Net. https://www.water-technology.net/projects/epe-sewage-treatment-plant-netherlands/

Lazarova, V. (1995). Biofilm characterization and activity analysis in water and wastewater treatment. *Water Research*, *29*(10), 2227–2245. https://doi.org/10.1016/0043-1354(95)00054-O

Leeuwen, K. van, Link to external site, this link will open in a new window, de Vries, E., Koop, S., & Roest, K. (2018). The Energy & Raw Materials Factory: Role and Potential Contribution to the Circular Economy of the Netherlands. *Environmental Management*, *61*(5), 786–795. https://doi.org/10.1007/s00267-018-0995-8

Li, A., Li, X., & Yu, H. (2011). Effect of the food-to-microorganism (F/M) ratio on the formation and size of aerobic sludge granules. *Process Biochemistry*, *46*(12), 2269–2276. https://doi.org/10.1016/j.procbio.2011.09.007

Li, A., Yang, S., Li, X., & Gu, J. (2008). Microbial population dynamics during aerobic sludge granulation at different organic loading rates. *Water Research*, *42*(13), 3552–3560. https://doi.org/10.1016/j.watres.2008.05.005

Li, J., Cai, A., Ding, L., Sellamuthu, B., & Perreault, J. (2015). Aerobic sludge granulation in a Reverse Flow Baffled Reactor (RFBR) operated in continuous-flow mode for wastewater treatment. *Separation and Purification Technology*, *149*, 437–444. https://doi.org/10.1016/j.seppur.2015.04.045

Li, Y., Liu, Y., Shen, L., & Chen, F. (2008). DO diffusion profile in aerobic granule and its microbiological implications. *Enzyme and Microbial Technology*, *43*(4–5), 349–354. https://doi.org/10.1016/j.enzmictec.2008.04.005

Li, Z., Li, H., Zhao, L., Liu, X., & Wan, C. (2021). Understanding the role of cations and hydrogen bonds on the stability of aerobic granules from the perspective of the aggregation and adhesion behavior of extracellular polymeric substances. *Science of The Total Environment*, *795*, 148659. https://doi.org/10.1016/j.scitotenv.2021.148659

Li, Z., Zhong, S., Lei, H. -y., Chen, R. -w., Yu, Q., & Li, H.-L. (2009). Production of a novel bioflocculant by Bacillus licheniformis X14 and its application to low temperature drinking water treatment. *Bioresource Technology*, *100*(14), 3650–3656. Scopus. https://doi.org/10.1016/j.biortech.2009.02.029

Li, Z.-H., Zhu, Y.-M., Zhang, Y.-L., Zhang, Y.-R., He, C.-B., & Yang, C.-J. (2019). Characterization of aerobic granular sludge of different sizes for nitrogen and phosphorus removal. *Environmental Technology*, *40*(27), 3622–3631. https://doi.org/10.1080/09593330.2018.1483971

Liang, Z., Tu, Q., Su, X., Yang, X., Chen, J., Chen, Y., Li, H., Liu, C., He, Q., & Link to external site, this link will open in a new tab. (2019). Formation, extracellular polymeric substances, and structural stability of aerobic granules enhanced by granular activated carbon. *Environmental Science and Pollution Research*, *26*(6), 6123–6132. https://doi.org/10.1007/s11356-018-04101-1

Lin, Y., de Kreuk, M., van Loosdrecht, M. C. M., & Adin, A. (2010). Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant. *Water Research*, *44*(11), 3355–3364. https://doi.org/10.1016/j.watres.2010.03.019

Liu, F. (2014). Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor. *Bioresource Technology*.

Liu, J. (2020). *Improving aerobic sludge granulation in sequential batch reactor by natural drying: Effluent sludge recovery and feeding back into reactor*.

Liu, L., Li, W.-W., Sheng, G.-P., Liu, Z.-F., Zeng, R. J., Liu, J.-X., Yu, H.-Q., & Lee, D.-J. (2010). Microscale Hydrodynamic Analysis of Aerobic Granules in the Mass Transfer Process. *Environmental Science & Technology*, *44*(19), 7555–7560. https://doi.org/10.1021/es1021608

Liu, W., Wang, C., Chen, J., Jiang, K., Zhang, L., Wang, J., Wu, P., Ji, X., Shen, Y., Yang, D., & Shen, Y. (2022). Cold temperature drives the re-granulation of disintegrated partial nitritation granules in a continuous-flow reactor. *Journal of Cleaner Production*, *378*, 134530. https://doi.org/10.1016/j.jclepro.2022.134530

Liu, W., Wu, Y., Zhang, S., Gao, Y., Jiang, Y., Horn, H., & Li, J. (2020). Successful granulation and microbial differentiation of activated sludge in anaerobic/anoxic/aerobic (A2O) reactor with two-zone sedimentation tank treating municipal sewage. *Water Research*, *178*, 115825. https://doi.org/10.1016/j.watres.2020.115825

Liu, Y., & Liu, Q.-S. (2006). Causes and control of filamentous growth in aerobic granular sludge sequencing batch reactors. *Biotechnology Advances*, *24*(1), 115–127. https://doi.org/10.1016/j.biotechadv.2005.08.001

Liu, Y., & Tay, J.-H. (2002). The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. *Water Research*, *36*(7), 1653–1665. https://doi.org/10.1016/S0043-1354(01)00379-7

Liu, Y., & Tay, J.-H. (2004). State of the art of biogranulation technology for wastewater treatment. *Biotechnology Advances*, *22*(7), 533–563. https://doi.org/10.1016/j.biotechadv.2004.05.001

Liu, Y., Yang, S.-F., Liu, Q.-S., & Tay, J.-H. (2003). The Role of Cell Hydrophobicity in the Formation of Aerobic Granules. *Current Microbiology*, *46*(4), 0270–0274. https://doi.org/10.1007/s00284-002-3878-3

Liu, Y., Yang, S.-F., Tay, J.-H., Liu, Q.-S., Qin, L., & Li, Y. (2004). Cell hydrophobicity is a triggering force of biogranulation. *Enzyme and Microbial Technology*, *34*(5), 371–379. https://doi.org/10.1016/j.enzmictec.2003.12.009

Liu, Y.-Q., & Tay, J.-H. (2008). Influence of starvation time on formation and stability of aerobic granules in sequencing batch reactors. *Bioresource Technology*, *99*(5), 980–985. https://doi.org/10.1016/j.biortech.2007.03.011

Long, B., Xuan, X., Yang, C., Zhang, L., Cheng, Y., & Wang, J. (2019). Stability of aerobic granular sludge in a pilot scale sequencing batch reactor enhanced by granular particle size control. *Chemosphere*, *225*, 460–469. https://doi.org/10.1016/j.chemosphere.2019.03.048

Long, B., Yang, C., Pu, W., Yang, J., Liu, F., Zhang, L., Zhang, J., & Cheng, K. (2015). Tolerance to organic loading rate by aerobic granular sludge in a cyclic aerobic granular reactor. *Bioresource Technology*, *182*, 314–322. https://doi.org/10.1016/j.biortech.2015.02.029

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of The Total Environment*, *473–474*, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065

Lv, Y., Wan, C., Lee, D.-J., Liu, X., & Tay, J.-H. (2014). Microbial communities of aerobic granules: Granulation mechanisms. *Bioresource Technology*, *169*, 344–351. https://doi.org/10.1016/j.biortech.2014.07.005

McSwain, B. S., Irvine, R. L., & Wilderer, P. A. (2004). The influence of settling time on the formation of aerobic granules. *Water Science and Technology*, *50*(10), 195–202. https://doi.org/10.2166/wst.2004.0643

Mikkelsen, L. H. (2001). The shear sensitivity of activated sludge: Relations to filterability, rheology and surface chemistry. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *182*(1), 1–14. https://doi.org/10.1016/S0927-7757(00)00772-X

Mikkelsen, L. H., & Keiding, K. (2002). The shear sensitivity of activated sludge: An evaluation of the possibility for a standardised floc strength test. *Water Research*, *36*(12), 2931–2940. https://doi.org/10.1016/S0043-1354(01)00518-8

Miyake, M., Hasebe, Y., Furusawa, K., Shiomi, H., Inoue, D., & Ike, M. (2023). Enhancement of nutrient removal in an activated sludge process using aerobic granular sludge augmentation strategy with ammonium-based aeration control. *Chemosphere*, *340*, 139826. https://doi.org/10.1016/j.chemosphere.2023.139826

Morgenroth, E. (1997). *AEROBIC GRANULAR SLUDGE IN A SEQUENCING BATCH REACTOR*. 4.

Moy, B. Y.-P., Tay, J.-H., Toh, S.-K., Liu, Y., & Tay, S. T.-L. (2002). High organic loading influences the physical characteristics of aerobic sludge granules. *Letters in Applied Microbiology*, *34*(6), 407–412. https://doi.org/10.1046/j.1472-765X.2002.01108.x

Nancharaiah, Y. V., & Kiran Kumar Reddy, G. (2018). Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications. *Bioresource Technology*, *247*, 1128–1143. https://doi.org/10.1016/j.biortech.2017.09.131

Natural Resources Canada & Brewers Association of Canada. (2011). *Guide to energy efficiency opportunities in the Canadian Brewing Industry*. https://natural-resources.canada.ca/sites/nrcan/files/oee/files/pdf/publications/industry/Brewers_Guide_access_e.pdf

Nguyen Quoc, B., Armenta, M., Carter, J. A., Bucher, R., Sukapanpotharam, P., Bryson, S. J., Stahl, D. A., Stensel, H. D., & Winkler, M.-K. H. (2021). An investigation into the optimal granular sludge size for simultaneous nitrogen and phosphate removal. *Water Research*, *198*, 117119. https://doi.org/10.1016/j.watres.2021.117119

Nguyen Quoc, B., Wei, S., Armenta, M., Bucher, R., Sukapanpotharam, P., Stahl, D. A., Stensel, H. D., & Winkler, M.-K. H. (2021). Aerobic granular sludge: Impact of size distribution on nitrification capacity. *Water Research*, *188*, 116445. https://doi.org/10.1016/j.watres.2020.116445

Nor-Anuar, A., Ujang, Z., Van Loosdrecht, M. C. M., De Kreuk, M. K., & Olsson, G. (2012). Strength characteristics of aerobic granular sludge. *Water Science and Technology*, *65*(2), 309–316. Scopus. https://doi.org/10.2166/wst.2012.837

Ogura, S. (2020). Dried aerobic granules for fast startup of aerobic granular sludge reactors_ Reactivation and performance. *Journal of Water Process Engineering*.

Oleszkiewicz, J., Kruk, D., Devlin, T., Lashkarizadeh, M., & Yuan, Q. (2015). *Options for Improved Nutrient Removal and Recovery From Municipal Wastewater in the Canadian*

Context. https://cwn-rce.ca/wp-content/uploads/2015/12/Oleszkiewicz-KI-Report-March-2015.pdf

Otten, A., Zilverentant, A., de Bruin, B., & Zandbergen, E. (2018). *CAPABILITY STATEMENT Worldwide Nereda Variants and Applications*. https://www.ringsendwwtpupgrade.ie/planning-sites/ringsend-planning/docs/environmental-documents/volume-2b/V2B%204B%20RGD%20EIAR%20Nereda%20Capability.pdf

Paper, W., & Higgins, P. (2021). *Ammonium-Based Aeration Control in Wastewater*.

Partin, A. (2019). *Hydrocyclone Implementation at Two Wastewater Treatment Facilities to Promote Overall Settling m Improvement* [Virginia Polytechnic Institute and State University]. https://vtechworks.lib.vt.edu/bitstream/handle/10919/95488/Partin_AK_T_2019.pdf?sequence=1

Pronk, M., de Kreuk, M. K., de Bruin, B., Kamminga, P., Kleerebezem, R., & van Loosdrecht, M. C. M. (2015). Full scale performance of the aerobic granular sludge process for sewage treatment. *Water Research*, *84*, 207–217. https://doi.org/10.1016/j.watres.2015.07.011

Qin, L., Tay, J.-H., & Liu, Y. (2004). Selection pressure is a driving force of aerobic granulation in sequencing batch reactors. *Process Biochemistry*, *39*(5), 579–584. https://doi.org/10.1016/S0032-9592(03)00125-0

Regmi, P., Sturm, B., Hiripitiyage, D., Keller, N., Murthy, S., & Jimenez, J. (2022). Combining continuous flow aerobic granulation using an external selector and carbon-efficient nutrient removal with AvN control in a full-scale simultaneous nitrification-denitrification process. *Water Research*, *210*, 117991. https://doi.org/10.1016/j.watres.2021.117991

Roche, C., Donnaz, S., Murthy, S., & Wett, B. (2022). Biological process architecture in continuous-flow activated sludge by gravimetry: Controlling densified biomass form and function in a hybrid granule–floc process at Dijon WRRF, France. *Water Environment Research*, *94*(1), e1664. https://doi.org/10.1002/wer.1664

Schwarzenbeck, N., Borges, J. M., & Wilderer, P. A. (2005). Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. *Applied Microbiology and Biotechnology*, *66*(6), 711–718. https://doi.org/10.1007/s00253-004-1748-6

Seka, M. A., & Verstraete, W. (2003). Test for assessing shear sensitivity of activated sludge flocs: A feasibility study. *Water Research*, *37*(14), 3327–3334. https://doi.org/10.1016/S0043-1354(02)00558-4

S-Select® References | Granular sludge in the wastewater treatment. (2023, April). https://www.essde.com/en/sselect/references_118

Sun, Y., Angelotti, B., Brooks, M., & Wang, Z.-W. (2021). Feast/famine ratio determined continuous flow aerobic granulation. *Science of The Total Environment*, *750*, 141467. https://doi.org/10.1016/j.scitotenv.2020.141467

Sutherland, I. W. (2001). Biofilm exopolysaccharides: A strong and sticky framework. *Microbiology*, *147*(1), 3–9. https://doi.org/10.1099/00221287-147-1-3

Tao, J. (2017). Effect of granular activated carbon on the aerobic granulation of sludge and its mechanism. *Bioresource Technology*.

Tay, J. H., Liu, Q. S., & Liu, Y. (2002). Characteristics of Aerobic Granules Grown on Glucose and Acetate in Sequential Aerobic Sludge Blanket Reactors. *Environmental Technology*, *23*(8), 931–936. https://doi.org/10.1080/09593332308618363

Tay, J.-H., Liu, Q.-S., & Liu, Y. (2001). Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor. *Journal of Applied Microbiology*, *91*(1), 168–175. https://doi.org/10.1046/j.1365-2672.2001.01374.x

Tay, S. T.-L., Ivanov, V., Yi, S., Zhuang, W.-Q., & Tay, J.-H. (2002). Presence of Anaerobic Bacteroides in Aerobically Grown Microbial Granules. *Microbial Ecology*, *44*(3), 278–285.

Tay, S. T.-L., Moy, B. Y.-P., Jiang, H.-L., & Tay, J.-H. (2005). Rapid cultivation of stable aerobic phenol-degrading granules using acetate-fed granules as microbial seed. *Journal of Biotechnology*.

Toh, S., Tay, J., Moy, B., Ivanov, V., & Tay, S. (2003). Size-effect on the physical characteristics of the aerobic granule in a SBR. *Applied Microbiology and Biotechnology*, *60*(6), 687–695. https://doi.org/10.1007/s00253-002-1145-y

Vadakkan, K., Choudhury, A. A., Gunasekaran, R., Hemapriya, J., & Vijayanand, S. (2018). Quorum sensing intervened bacterial signaling: Pursuit of its cognizance and repression. *Journal of Genetic Engineering & Biotechnology*, *16*(2), 239–252. https://doi.org/10.1016/j.jgeb.2018.07.001

Val Del Río, Á., Palmeiro-Sanchez, T., Figueroa, M., Mosquera-Corral, A., Campos, J. L., & Méndez, R. (2014). Anaerobic digestion of aerobic granular biomass: Effects of thermal pre-treatment and addition of primary sludge. *Journal of Chemical Technology & Biotechnology*, *89*(5), 690–697. https://doi.org/10.1002/jctb.4171 van Dijk, E. J. H., Haaksman, V. A., van Loosdrecht, M. C. M., & Pronk, M. (2022). On the mechanisms for aerobic granulation—Model based evaluation. *Water Research*, *216*, 118365. https://doi.org/10.1016/j.watres.2022.118365

van Dijk, E. J. H., Pronk, M., & van Loosdrecht, M. C. M. (2020). A settling model for full-scale aerobic granular sludge. *Water Research*, *186*, 116135. https://doi.org/10.1016/j.watres.2020.116135

Verawaty, M., Pijuan, M., Yuan, Z., & Bond, P. L. (2012). Determining the mechanisms for aerobic granulation from mixed seed of floccular and crushed granules in activated sludge wastewater treatment. *Water Research*, *46*(3), 761–771. https://doi.org/10.1016/j.watres.2011.11.054

Wagner, J., Weissbrodt, D. G., Manguin, V., Ribeiro Da Costa, R. H., Morgenroth, E., & Derlon, N. (2015). Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors. *Water Research*, *85*, 158–166. https://doi.org/10.1016/j.watres.2015.08.030

Wang, L., Liu, X., Lee, D.-J., Tay, J.-H., Zhang, Y., Wan, C.-L., & Chen, X.-F. (2018). Recent advances on biosorption by aerobic granular sludge. *Journal of Hazardous Materials*, *357*, 253–270. https://doi.org/10.1016/j.jhazmat.2018.06.010

Wang, S.-G., Gai, L.-H., Zhao, L.-J., Fan, M.-H., Gong, W.-X., Gao, B.-Y., & Ma, Y. (2009). Aerobic granules for low-strength wastewater treatment: Formation, structure, and microbial community. *Journal of Chemical Technology & Biotechnology*, *84*(7), 1015–1020. https://doi.org/10.1002/jctb.2127

Wang, S.-G., Liu, X.-W., Gong, W.-X., Gao, B.-Y., Zhang, D.-H., & Yu, H.-Q. (2007). Aerobic granulation with brewery wastewater in a sequencing batch reactor. *Bioresource Technology*, *98*(11), 2142–2147. https://doi.org/10.1016/j.biortech.2006.08.018

Wang, X., Wang, W., Li, Y., Zhang, J., Zhang, Y., & Li, J. (2018). Biofilm activity, ammonia removal and cell growth of the heterotrophic nitrifier, *Acinetobacter* sp., facilitated by exogenous *N* -acyl-homoserine lactones. *RSC Advances*, *8*(54), 30783–30793. https://doi.org/10.1039/C8RA05545A

Wang, X., Zhang, H., Yang, F., Wang, Y., & Gao, M. (2008). Long-term storage and subsequent reactivation of aerobic granules. *Bioresource Technology*, *99*(17), 8304–8309. Scopus. https://doi.org/10.1016/j.biortech.2008.03.024

Wang, Z., Liu, Y., & Tay, J. (2005). Distribution of EPS and cell surface hydrophobicity in aerobic granules. *Applied Microbiology and Biotechnology*, *69*(4), 469–473. https://doi.org/10.1007/s00253-005-1991-5

Wang, Z., Liu, Y., & Tay, J. (2007). Biodegradability of extracellular polymeric substances produced by aerobic granules. *Applied Microbiology and Biotechnology*, *74*(2), 462–466. https://doi.org/10.1007/s00253-006-0686-x

Weber, S. D., Ludwig, W., Schleifer, K.-H., & Fried, J. (2007). *Microbial Composition and Structure of Aerobic Granular Sewage Biofilms*. https://doi.org/10.1128/AEM.01002-07

Weissbrodt, D., Lochmatter, S., Ebrahimi, S., Rossi, P., Maillard, J., & Holliger, C. (2012). Bacterial Selection during the Formation of Early-Stage Aerobic Granules in Wastewater Treatment Systems Operated Under Wash-Out Dynamics. *Frontiers in Microbiology*, *3*. https://www.frontiersin.org/articles/10.3389/fmicb.2012.00332

Wilén, B.-M., Jin, B., & Lant, P. (2003). Impacts of structural characteristics on activated sludge floc stability. *Water Research*, *37*(15), 3632–3645. https://doi.org/10.1016/S0043-1354(03)00291-4

Wilén, B.-M., Liébana, R., Persson, F., Modin, O., & Hermansson, M. (2018). The mechanisms of granulation of activated sludge in wastewater treatment, its optimization, and impact on effluent quality. *Applied Microbiology and Biotechnology*, *102*(12), 5005–5020. https://doi.org/10.1007/s00253-018-8990-9

Winkler, M.-K. H., Bassin, J. P., Kleerebezem, R., de Bruin, L. M. M., van den Brand, T. P. H., & van Loosdrecht, M. C. M. (2011). Selective sludge removal in a segregated aerobic granular biomass system as a strategy to control PAO–GAO competition at high temperatures. *Water Research*, *45*(11), 3291–3299. https://doi.org/10.1016/j.watres.2011.03.024

Winkler, M.-K. H., Ettwig, K. F., Vannecke, T. P. W., Stultiens, K., Bogdan, A., Kartal, B., & Volcke, E. I. P. (2015). Modelling simultaneous anaerobic methane and ammonium removal in a granular sludge reactor. *Water Research*, *73*, 323–331. https://doi.org/10.1016/j.watres.2015.01.039

Winkler, M.-K. H., Kleerebezem, R., de Bruin, L. M. M., Verheijen, P. J. T., Abbas, B., Habermacher, J., & van Loosdrecht, M. C. M. (2013). Microbial diversity differences within aerobic granular sludge and activated sludge flocs. *Applied Microbiology and Biotechnology*, *97*(16), 7447–7458. https://doi.org/10.1007/s00253-012-4472-7

Winkler, M.-K. H., Kleerebezem, R., Strous, M., Chandran, K., & van Loosdrecht, M. C. M. (2013). Factors influencing the density of aerobic granular sludge. *Applied Microbiology and Biotechnology*, *97*(16), 7459–7468. https://doi.org/10.1007/s00253-012-4459-4

Winkler, M.-K. H., & van Loosdrecht, M. C. M. (2022). Intensifying existing urban wastewater. *Science*, *375*(6579), 377–378. https://doi.org/10.1126/science.abm3900

World Water Works. (2016a). *inDENSE^{TM} Gravimetric Selection Technology*. https://worldwaterworks.directus.app/assets/288b395e-c05f-4984-bcb7-796bab0fda2e

World Water Works. (2016b). *World Water Works Unveils New inDENSE^{TM} Technology*. https://www.worldwaterworks.com/resources/press-room/world-water-works-unveils-new-indense-technology

Wu, J., de los Reyes, F. L., & Ducoste, J. J. (2020). Modeling cell aggregate morphology during aerobic granulation in activated sludge processes reveals the combined effect of substrate and shear. *Water Research*, *170*, 115384. https://doi.org/10.1016/j.watres.2019.115384

Yang, C., Fang, S., Chen, D., Wang, J., Liu, F., & Xia, C. (2016). The possible role of bacterial signal molecules N-acyl homoserine lactones in the formation of diatom-biofilm (Cylindrotheca sp.). *Marine Pollution Bulletin*, *107*(1), 118–124. https://doi.org/10.1016/j.marpolbul.2016.04.010

Yang, S.-F., Tay, J.-H., & Liu, Y. (2004). Inhibition of free ammonia to the formation of aerobic granules. *Biochemical Engineering Journal*, *17*(1), 41–48. https://doi.org/10.1016/S1369-703X(03)00122-0

Yang, Y., Zhou, D., Xu, Z., Li, A., Gao, H., & Hou, D. (2014). Enhanced aerobic granulation, stabilization, and nitrification in a continuous-flow bioreactor by inoculating biofilms. *Applied Microbiology and Biotechnology*, *98*(12), 5737–5745. https://doi.org/10.1007/s00253-014-5637-3

Yuan, Y., & Farnood, R. R. (2010). Strength and breakage of activated sludge flocs. *Powder Technology*, *199*(2), 111–119. https://doi.org/10.1016/j.powtec.2009.11.021

Yuan, Z., Pratt, S., & Batstone, D. J. (2012). Phosphorus recovery from wastewater through microbial processes. *Current Opinion in Biotechnology*, *23*(6), 878–883. https://doi.org/10.1016/j.copbio.2012.08.001

Zahra, S. A., Abdullah, N., Iwamoto, K., Yuzir, A., & Mohamad, S. E. (2022). Alginate-like exopolysaccharides in aerobic granular sludge: A review. *Materials Today: Proceedings*, *65*, 3046–3053. https://doi.org/10.1016/j.matpr.2022.04.032

Zhang, H., He, Y., Jiang, T., & Yang, F. (2011). Research on characteristics of aerobic granules treating petrochemical wastewater by acclimation and co-metabolism methods. *Desalination*, *279*(1), 69–74. https://doi.org/10.1016/j.desal.2011.05.060

Zhang, X., & Bishop, P. L. (2003). Biodegradability of biofilm extracellular polymeric substances. *Chemosphere*, *50*(1), 63–69. https://doi.org/10.1016/S0045-6535(02)00319-3

Zhang, Z., Wang, L., Ji, Y., Cao, R., Zhou, J., Li, M., Zhu, L., & Xu, X. (2023). Understanding the N-acylated homoserine lactones(AHLs)-based quorum sensing for the stability of aerobic granular sludge in the aspect of substrate hydrolysis enhancement. *Science of The Total Environment*, *858*, 159581. https://doi.org/10.1016/j.scitotenv.2022.159581

Zheng, Y.-M., & Yu, H.-Q. (2007). Determination of the pore size distribution and porosity of aerobic granules using size-exclusion chromatography. *Water Research*, *41*(1), 39–46. https://doi.org/10.1016/j.watres.2006.09.015

Zheng, Y.-M., Yu, H.-Q., Liu, S.-J., & Liu, X.-Z. (2006). Formation and instability of aerobic granules under high organic loading conditions. *Chemosphere*, *63*(10), 1791–1800. https://doi.org/10.1016/j.chemosphere.2005.08.055

Zhou, D., Dong, S., Gao, L., Liu, M., & Niu, S. (2013). Distribution characteristics of extracellular polymeric substances and cells of aerobic granules cultivated in a continuous-flow airlift reactor. *Journal of Chemical Technology & Biotechnology*, *88*(5), 942–947. https://doi.org/10.1002/jctb.3927

Zhou, J. (2015). Granular activated carbon as nucleating agent for aerobic sludge granulation: Effect of GAC size on velocity field differences (GAC versus flocs) and aggregation behavior. *Bioresource Technology*.

Zhu, L., Lv, M., Dai, X., Yu, Y., Qi, H., & Xu, X. (2012). Role and significance of extracellular polymeric substances on the property of aerobic granule. *Bioresource Technology*, *107*, 46–54. https://doi.org/10.1016/j.biortech.2011.12.008

Zhu, L., Zhou, J., Lv, M., Yu, H., Zhao, H., & Xu, X. (2015). Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE. *Chemosphere*, *121*, 26–32. https://doi.org/10.1016/j.chemosphere.2014.10.053

Zhu, N., Liu, L., Xu, Q., Chen, G., & Wang, G. (2015). Resources availability mediated EPS production regulate microbial cluster formation in activated sludge system. *Chemical Engineering Journal*, *279*, 129–135. https://doi.org/10.1016/j.cej.2015.05.017

Zita, A., & Hermansson, M. (1997). *Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs*. https://doi.org/10.1128/aem.63.3.1168-1170.1997

Zou, J., Tao, Y., Li, J., Wu, S., & Ni, Y. (2018). Cultivating aerobic granular sludge in a developed continuous-flow reactor with two-zone sedimentation tank treating real and low-strength wastewater. *Bioresource Technology*, *247*, 776–783. https://doi.org/10.1016/j.biortech.2017.09.088