

REMOVAL OF PFAS FROM SYNTHETIC WASTEWATER USING
AEROBIC GRANULAR SLUDGE

by

Tasnim Sultana Ritu

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DEDICATION

This work is dedicated to my family, who have supported me, encouraged me, and made this accomplishment possible.

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ABSTRACT

The project assesses the performance of the aerobic granular sludge (AGS) to remove poly- and per-fluoroalkyl substances (PFAS) and conventional nutrients like carbon, nitrogen, and phosphorus from synthetic wastewater in a sequencing batch reactor (SBR). AGS is an emerging wastewater treatment biofilm that may be effective in reducing the PFAS concentration in wastewater via sorption. PFAS are a class of man-made chemicals used as surfactants, fire retardants, and coating materials. PFAS compounds are very persistent in the environment and can lead to adverse health outcomes in humans. PFAS can migrate from consumer products and enter the influent of wastewater treatment plant (WWTP). PFAS compounds are poorly removed by conventional wastewater treatment methods making effluent from WWTP a significant source of PFAS in the environment. The project uses two specific PFAS which are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate acid (PFOS). Other objectives of this project are to monitor how PFAS influences the treatment of conventional wastewater constituents and the granules' structure and morphology. Two SBRs were started with floccular sludge from seed granules and continued for 402 days. Some standard laboratory analytical methods for nitrogen, phosphorus, and organic carbon were used to monitor the removal efficiencies of the granules. Solid phase extraction (SPE) and liquid chromatography with mass spectrometry (UPLC with ESI Q-TOF-MS) were used to assess the removal of PFOA and PFOS both from liquid and sludge phases. Maximum removal of 33% for PFOS and 28% for PFOA was achieved by AGS in the test SBR. PFOS/PFOA exposure affected the granule's physical properties, and the properties recovered within approximately 34 days of dosing. PFOS/PFOA contamination produced no significant effect on conventional nutrient removal except nitrification. Thus, the treatment of PFAS by AGS is economical, since AGS can treat several pollutants simultaneously in a single reactor. More research should be done on the disposal of PFAS-contaminated sludge and to increase the treatment efficiency.

CHAPTER ONE

INTRODUCTION

Wastewater is used water containing human wastes, food scraps, oils, and other chemicals. The main sources of wastewater are various industries, residential buildings, human activities, rainfall-runoff, and others. Nature has an amazing ability to cope with small amounts of water waste and pollution, but it would be overwhelming if we don't treat the billions of gallons of wastewater and sewage produced every day before releasing it back into the environment. Effective wastewater treatment has to be ensured to reuse water and sewage which is the most sustainable and long-term solution to the problem of water scarcity. A wastewater treatment plant (WWTP) reduces pollutants in wastewater to a level nature can handle. Generally, treatment plants remove conventional nutrients like carbon (C), nitrogen (N), phosphorus (P), and other organics from wastewater [1-4].

Treatment of wastewater includes physical, chemical, and biological processes in a full-scale treatment plant before discharging into a water body. WWTP and related facilities are designed to prevent effluent from affecting the receiving waterbodies adversely. Biological wastewater treatment relies on microorganisms to break down and remove organics from the wastewater. Microorganisms consume organic matter and nutrients present in the wastewater for their growth, thus, remove these components from wastewater. Biological treatment includes aerobic and anaerobic processes. The aerobic process happens in presence of oxygen, while anaerobic refers to a process in which oxygen is absent. The conventional activated sludge (CAS) system is an aerobic biological process which facilitates oxidation of organic matter and reduction of other pollutants like nitrate, phosphate, etc. The CAS system commonly includes an

aeration tank for biological degradation and secondary sedimentation tank to separate the sludge from treated wastewater. Aerobic granular sludge is an innovative biological wastewater treatment technology alternative to the present activated sludge process in industrial and municipal wastewater treatment systems [5-7].

Aerobic granular sludge (AGS) consists of dense self-immobilized microbial granules that have compact microbial structures and higher settling velocities. AGS can perform the simultaneous removal of C, N, P, and other pollutants in a single sequencing batch reactor [8]. It has a robust granular structure with a diameter of 2-4 mm which benefits the treatment system by ensuring rapid settling and producing less sludge for disposal [9]. Extracellular polymeric substances (EPS) have a significant influence on the formation and stable operation of aerobic granular sludge in the field of wastewater. EPS is a mixture of a complex high-molecular-weight bio-polymers surrounding bacterial cells, mainly composed of polysaccharides (PS), proteins (PN), humic-like substances, nucleic acid, lipids, and glycoproteins. EPS acts like a gel, making the granules compact, and can sorb organics and other pollutants on its surface [10-13].

To achieve the structures and qualities of AGS mentioned above, wastewater must be treated in a sequencing batch reactor (SBR). SBRs use a fill-and-draw system for wastewater treatment. The difference between the SBR system and the CAS system is that the SBR performs equalization, biological treatment, and secondary clarification in a single reactor using a timed sequence whereas CAS system requires different aeration and secondary sedimentation tanks [14]. It is the SBR operation that forms the compact granules. Detailed information about SBR operation, functions, and benefits will be explained in the next chapter literature review. With the compact granular structure and the EPS matrix of AGS achieved by the operation of SBR, it may

be possible to remove complex organic contaminants like PFAS from wastewater while also removing conventional contaminants such as C, N, and P.

Perfluoro alkyl substances (PFAS) are man-made chemicals which are widely used, long-lasting in the environment, and less biodegradable due to strong chemical bonds. The major sources of PFAS contamination in drinking water, groundwater, and soils include industrial sites, landfills, consumer products, fire response sites, and effluent coming from wastewater treatment plants [15-17]. PFAS has some detrimental effects on human health and animals [16, 18, 19]. Because of their widespread use and their persistence in the environment, many PFAS are found in the blood of people and animals all over the world [9, 20, 21]. So, it is essential to remove PFAS from wastewater to an acceptable level [22]. For this project, two different types of PFAS, perfluorooctanoic acid (PFOA) and perfluoro octane sulfonic acid (PFOS), were tested in a laboratory SBR. Among all the compounds in the PFAS family, PFOA and PFOS have been found ubiquitously in the environment. All the fluorochemicals may ultimately degrade to PFOA and PFOS [23]. These two compounds are mostly used as surfactants, and fluoropolymers [24].

In this project, two SBRs (control and test SBR) were set up to cultivate aerobic granular sludge. Both SBRs were operated with eight cycles a day. Each cycle consisted of four phases-fill, aerate, settle, and draw. Wastewater with typical concentrations of C, N, and P circulated through different phases in the reactors to feed the granular sludge. After 350 days of continuous operation, a known concentration of PFAS was added to one of the reactors (test reactor) via the influent wastewater. Based on some previous research about EPS biosorption [10, 25], it was expected that the EPS of the granules would capture the PFAS compounds and remove a significant amount of PFAS from wastewater.

Three different research questions will be addressed in this thesis: 1) To what extent can the aerobic granular sludge reduce the PFOA and PFOS concentration in synthetic wastewater? 2) How does PFOA and PFOS contamination change the physical properties of the granules? and 3) How does exposure to PFOA and PFOS influence the efficiency of conventional wastewater treatment? This thesis consists first of literature reviews in Chapter 2 focusing on detailed information about PFOA, PFOS and their treatment mechanisms, their effects on physical properties of granules, conventional nutrient removal, the role of AGS in wastewater treatment and its benefits, and SBR operations. Experimental methods are then described in Chapter 3. Chapter 4 consists of results and discussion of the results' relevance and applicability. In Chapter 5, conclusions and outcomes of the project are presented.

CHAPTER TWO

LITERATURE REVIEW

Perfluoro Alkyl Substances (PFAS)Introduction of Fluorinated Substances

“Fluorinated substances” is a group of organic and inorganic substances that contains at least one fluorine (F) atom and possesses different physical, chemical, and biological properties. [26, 27]. A specific subgroup of fluorinated substances with a perfluoroalkyl group in their structure is known as per and poly-fluoroalkyl substances (PFAS). The general term for perfluoroalkyl group is C_nF_{2n+1} which has one or more carbon (C) atoms bonded with a fluorinated aliphatic chain. In the group of perfluoroalkyl compounds, most hydrogen (H) atoms are substituted by F atoms [27, 28]. Besides the fluorinated alkyl chain, the substance also contains a terminal functional group such as carboxylate, sulphonamide, phosphonate, sulfonate, or alcohol [24].

PFAS are known as emerging contaminants containing more than 5,000 human-made, fluorinated, organic chemicals [23, 29]. Since 1950, PFAS have been widely used in numerous industrial and commercial applications [26, 27, 30]. New PFAS compounds are being synthesized by industry to replace those that have been banned, and individual PFAS compounds can break down into shorter chain variants. Thus, overall number and scope of PFAS compounds is difficult to ascertain with confidence. The carbon-fluorine (C-F) bond present in PFAS structure is one of the strongest chemical bonds and make PFAS tough to break and resistant to thermal, chemical, and biological degradation [31]. While C-F bond in PFAS makes them

persistent and hard to remove from the environment, the same bond makes PFAS attractive for industrial use as a surfactant or surface protector [32].

Classification of PFAS

Major PFAS compounds are depicted in (Figure 1). The most common group of perfluoroalkyl substances are perfluoroalkyl acids (PFAAs). PFAAs are generally broken into two groups, long chain ($C \geq 8$) and short chain ($C < 8$), based on number of fluorinated carbon atoms present in their chemical structure, the fully or partially fluorinated alkyl chain.

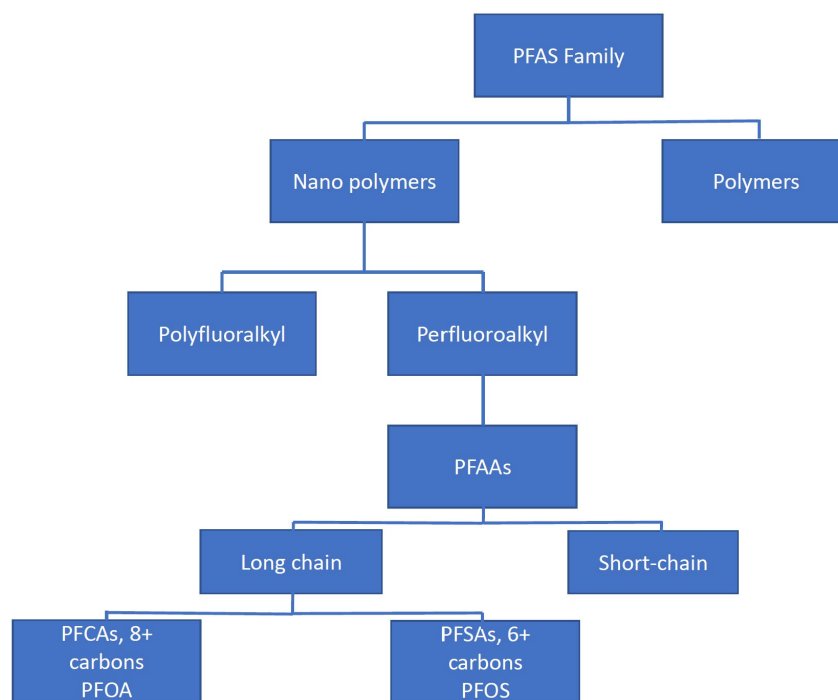


Figure 1: Overview of major PFAS family (per- and polyfluoroalkyl substances), including: PFAAs (perfluoroalkyl acids), 8 or more carbon PFCAs (perfluoroalkyl carboxylic acids/perfluoroalkyl carboxylates), PFOA (perfluorooctanic acid), 6 or more carbon PFSAs (perfluoroalkane sulfonic acids/perfluoroalkyl sulfonates), PFOS (perfluorooctane sulfonate/perfluorooctane sulfonic acid) [33].

Short-chain PFAS are considered as alternatives to long-chain PFAS. Though short-chain PFAS are assumed to have a lower bioaccumulation potential, they have other properties of

concern due to their high mobility in soil and water. This results in a fast distribution to water resources, thus, fast contamination of drinking water resources [34]. Short chain PFAS are reported to have higher potential for long range transport than long-chain PFAS [35]. The short-chain PFAS exposure to environment will be a long-term phenomenon, since there will be continuous and poorly reversible background concentrations of short-chain PFAS [22, 36-39]. Due to their low adsorption potential, a conventional activated carbon filter is a less effective method for short-chain PFAS removal [40].

Most common long chain PFAAs are perfluoroalkyl carboxylic acids (PFCAs, $C_nF_{2n+1}CO_2H$, ($n \geq 7$)), such as perfluorooctanoic acid, (PFOA) and per-fluoro-octane sulfonic acids (PFSAs, $C_nF_{2n+1}SO_3H$, ($n \geq 8$)), such as perfluoro octane sulfonate, (PFOS) [24]. PFCAs and PFSAs are the concern of international, domestic, and local scientific study and regulation due to their adverse impacts on the environment and human health. Long-chain PFAS are becoming regulated under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) and other international regulations due to their persistency, and bio-accumulative and toxic properties. Based on the sorption tendency of long-chain PFAS on activated carbon filters, it is expected that AGS can remove significant amount of long chain PFAS from wastewater by sorption [40]. Considering the wide use, toxic properties, and possibility for removal of long chain PFAS, detailed information about PFOA and PFOS are presented in the next section.

Perfluorooctanoic Acid (PFOA). Perfluorooctanoic acid is a perfluorinated carboxylic acid which is also known as C8 due to the presence of 8-carbon in PFOA structure (Figure 2). PFOA are produced and used worldwide as different industrial and commercial products. PFOA

has the chemical formula $C_8HF_{15}O_2$ with a molar mass of 414.07 grams per mol; a melting point of 40 to 50°C and a boiling point of 189°C.

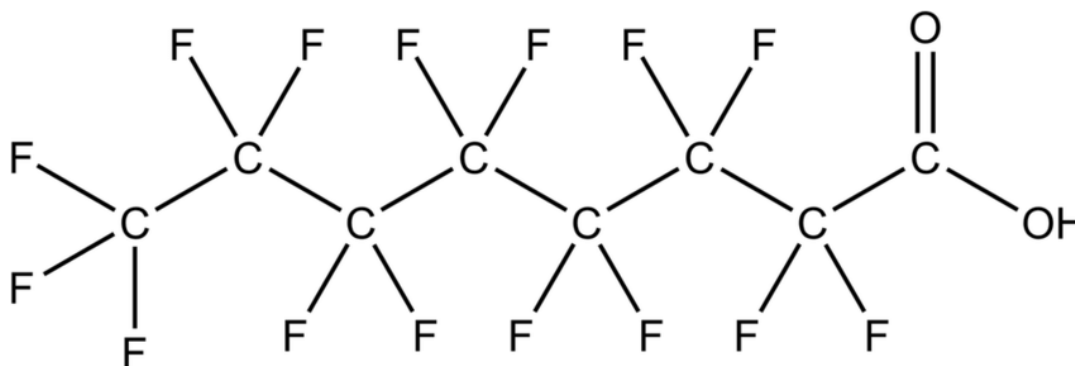


Figure 2: Chemical composition of PFOA [41].

The chemical structure of PFOA consists of a perfluorinated, *n*-octyl "tail group" and a carboxylate "head group". The hydrophilic head group in combination with the hydrophobic and lipophobic tail group make PFOA an effective surfactant [42]. With the surfactant property, PFOA creates a surface with lower surface tension and ensures its extensive use in industry [32]. PFOA is used in several industrial applications, including carpeting, upholstery, apparel, floor wax, textiles, firefighting foam. PFOA can also form as a breakdown product from a variety of precursor molecules. Due to the bio-persistence in the environment and harmful effects on human beings, PFOA is extensively studied to understand fate and transport in soil and aquatic environments. Although PFOA has been phased-out in use by food and drug administration (FDA) since 2016, there still remains a large amount of PFOA in underground water and soil systems. It is reported that PFOA ranged from 18 to 241 ng/g dry weight in sludge from wastewater treatment plants in New York, USA [43-47].

Perfluoro Octane Sulfonic Acid (PFOS). Perfluoro octane sulfonic acid (PFOS) also has the 8-carbon chain structure and a sulfonic acid functional group (Figure 3). The chemical formula of PFOS is $C_8HF_{17}O_3S$ with a boiling point of $133^{\circ}C$. The molar mass of PFOS is 500.13 grams per mol. PFOS is an exceptionally stable compound in the environment due to the C-F bond. Although of low acute toxicity, PFOS has captured much concern for its pervasiveness and environmental impact. PFOS is highly water-soluble and easy to transport in the aquatic environment. The hydrophobic perfluoroalkyl chain and hydrophilic sulfonate functional groups are the reasons for the characteristic surfactant behavior of PFOS and provide the tendency to be adsorbed on different solid–water interfaces [45, 48-50].

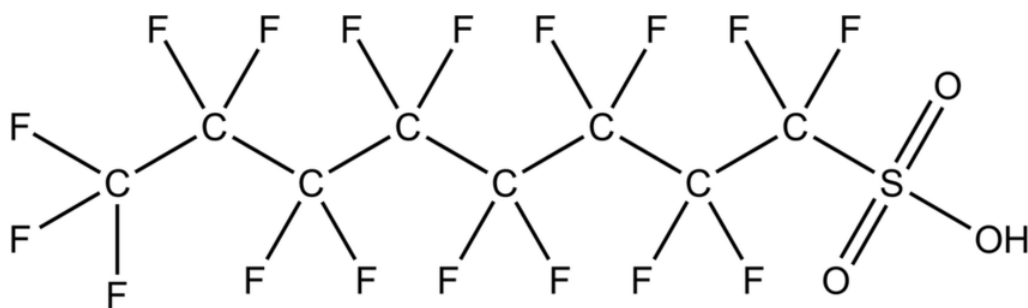


Figure 3: Chemical composition of PFOS [51].

PFOS has been recognized as a global contaminant and was designated as a new member of persistent organic pollutants (POPs) under the Stockholm Convention in 2009.

Sources and Uses of PFOA and PFOS

The perfluorinated or polyfluorinated compounds are anthropogenic in nature which means that they are manufactured and released into the environment due to human and industrial activities [52]. Due to their strong C–F bond strength (485 kJ/mol), PFAS are remarkably resistant to thermal, chemical, and biological degradation. Some of the documented PFAS uses

are in hydraulic fluids, biocides, construction products, fire-fighting foams, household products, wetting and mist-suppressing agents, surfactants for oil and natural gas recovery enhancement, polymerization agents, low-friction bearings and seals, insulators, cables, wires, protective coating materials, nonstick coatings, surgical patches, cardiovascular grafts, implants, carpets, leather products, and textiles. PFAS are manufactured and extensively used in a variety of chemical, pharmaceutical, and other consumer products due to their unique surfactant properties. PFAS may be released to the environment during the processes of production, usage, and disposal of these customer products [17, 24]. The main risk pathway to human health is the food chain, drinking water, and dietary intake [15, 22, 38, 53].

Transport Pathways

PFAS are widely detected in soil and aquatic environments. PFAS enter the environment via the usage of customer products and their disposal. The unique combination of physical and chemical properties and the hydrophobic and lipophobic groups make PFAS resistant to friction and extremely suitable as water and oil repellants. Another primary transportation path is effluent of most WWTP which generally have low removal efficiencies for PFAS. Several biological remediation techniques (e.g., aerobic/anaerobic digestion, biological filtration) applied during wastewater treatment influence the transformation of PFAS precursors in WWTP, ultimately increasing the concentrations of PFAS in effluents. Also, biosolids from secondary treatment are a significant source of PFAS emission into the environment [19, 54-57]. High concentrations of conventional PFAS in WWTP effluents (> 100 ng/L) and different degraded PFAS (concentrations up to some 100 ng/L) have also been detected in WWTPs. The conventional WWTP may remove longer-chain PFAS from wastewater via adsorption onto sludge, but

WWTP also facilitates the formation of shorter-chain PFAS during treatment. Since the short-chain PFAS are highly mobile and more persistent, they can be detected in all ecosystems and can be equally toxic as their longer-chain counterparts. The application of biological deodorization can also cause a rise in the PFAS concentration in the influent wastewater to WWTP [58-64].

Motivation

Excessive PFAS compounds in the environment pose hidden health risks to living organisms. The bioaccumulation and biomagnifying of PFAS compounds in organisms and across many trophic levels cause acute ecotoxicity. High levels of PFAS can lead to health threats, including metabolic and organ dysfunction, behavior modulation, immunosuppression, cancers, endocrine patterns disruption, and malformation of organs during development. Studies have reported that several acute as well as chronic human diseases, such as thyroid, asthma, anxiety, obesity, pediatric allergies, hyperuricemia, peroxisome proliferation, kidney disorders, liver damage, and cardiovascular diseases, may be associated with PFAS exposure [16, 18, 19, 61, 65-67].

Regulation

The previous guidelines by the US Environmental Protection Agency (EPA) (2016) set a limit of 70 parts per trillion (ppt) for both PFOS and PFOA in drinking water. The new EPA health advisories (2022) decrease that by more than thousandfold. The new limit, a non-enforceable maximum contaminant level goal (MCLG), for PFOS is 0.02 ppt; for PFOA, the MCLG is 0.004 ppt. Considering the toxicity of these compounds, the EPA wants the limits to be as close as possible to zero [68]. However, the large number of PFAS applications, the chemical

diversity of PFAS structures, and limited existing remediation approaches pose challenges to any regulatory body. Regulating only a subset of PFAS has led to their replacement with other members of the class with similar hazards. In 2019, EPA assembled a master list of 6,330 PFAS compounds from several existing lists. The total number of PFAS may be even larger, as some PFAS compounds lack chemical abstracts service (CAS) numbers. Many new PFAS compounds are produced as a degradation product of other PFAS. Specific members of the PFAS class have been regulated by several authoritative bodies. For example, perfluoro octane sulfonic acid (PFOS) and its salts are listed as persistent organic pollutants (POPs) in Annex B of the Stockholm Convention whereas perfluorooctanoic acid (PFOA) and its salts and related compounds are listed in Annex A. Annex A eliminates the production and use of listed chemicals while Annex B restricts the production and use of listed chemicals. Several PFAS are listed in the European Chemicals Agency's (ECHA) registration [69].

Aerobic Granular Sludge (AGS)

Aerobic granular sludge (AGS) technology has gained interest during the past two decades in wastewater treatment due to its multiple benefits over the conventional activated sludge (CAS) process. AGS consists of growing biomass in granules (biofilm) without any carrier material. Granules comprise diverse microorganisms embedded in an extracellular hydrogel matrix. Dissolved oxygen (DO) is consumed by the bacteria in the outer layer before it can diffuse into the center of the granules, creating an oxygen gradient across the biofilm. The relatively high DO at the surface and low DO inside the granules allows several different biochemical reactions to take place simultaneously (Figure 4) [70-73]. In conventional WWTP where biomass grows in flocs, different units and recycling are required to perform the aerobic

and anaerobic conversions necessary for complete wastewater treatment. However, in AGS, anaerobic and aerobic reactions can occur within the same granule due to the presence of oxygen gradient in the granule's multilayer structure. Nitrifiers grow in the outer layer of the granules where DO concentration is high while denitrifiers and, phosphate accumulating organisms (PAOs) develop inside the granules under anaerobic and anoxic conditions [74, 75]. The technology is based on SBR mode operation which includes a cycle of feeding under anaerobic conditions followed by an aeration period, a settling period, and an effluent discharge. The influent is fed at the bottom of the reactor in a plug-flow regime with the subsequent effluent withdrawal [76]

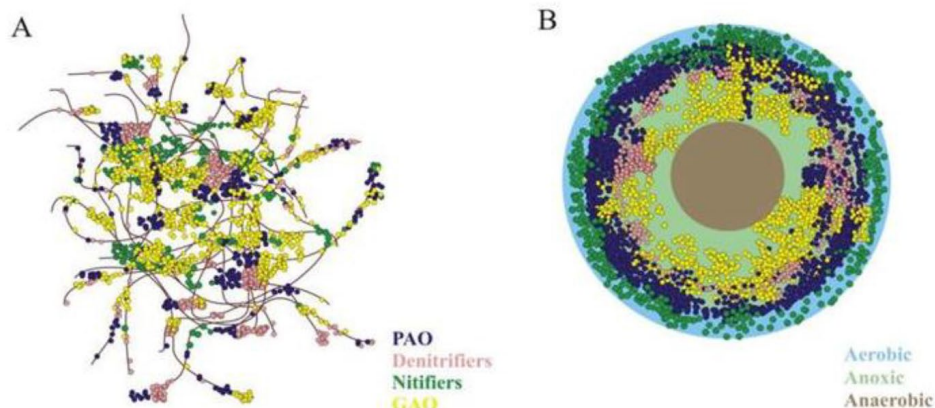


Figure 4: Microbial distribution in A) a sludge flocs and B) a heterotrophic aerobic granule [77].

Thus, AGS can offer simultaneous removal of C, N, P, and other pollutants in a single sludge system. The Nereda® technology is based on aerobic heterotrophic granules that are able to remove simultaneously C, N, and P in one unit [78].

Benefits of AGS System

The conventional activated sludge (CAS) process is a commonly used wastewater treatment technology with significant drawbacks, including poor settling properties of CAS flocs, limited biomass concentrations in aeration tanks, separate aeration/settling tanks, high energy consumption, and large surface area requirements. The CAS system produces large amounts of waste sludge, thus, requires additional cost for sludge management and disposal [73, 75]. Compared to the flocs growing in CAS systems, AGS contains several advantages for wastewater treatment with their dense and robust structure (Table 1). AGS is faster in settling than CAS and allows the use of high hydraulic loads to reactors without wash out of active biomass [9]. The high biomass retention enhances the performance of the AGS reactor to treat wastewater. The compact structure and high settling velocity of AGS reduce construction costs and land requirements by eliminating the need for additional facilities to separate biomass from wastewater [74, 79-81].

Table 1: Comparison of physical properties between full-scale AGS and CAS

Parameter	Aerobic granular sludge (AGS)	Conventional activated sludge (CAS)
SVI (mL/g)	10-15* [82]	100-300# [83]
TSS (mg/L)	18,000-22,000 [82]	3,000-6,000 [84]
VSS (mg/L)	15,000-18,000 [82]	2,500-4,500 [84]
SRT (day)	30-40 [82]	10-15[84]

* SVI measured after 5 minutes settling.

SVI measured after 30 minutes settling.

Granulation

Aerobic granules are biofilms containing different microorganisms, with average size of 2-4 mm [85]. Granulation is a process of transformation from flocculent sludge to dense, self-immobilized granular sludge under special operational conditions. Granulation is a multistep process where changes in physicochemical and biological parameters take place. There are four steps involved in the process of granulation (Figure 5). The first stage is initiated by cell-to-cell attachment contributed by different forces such as hydrodynamics, diffusion, gravity and contact among cells [86]. The second stage includes physical, chemical, and biochemical forces to produce aggregates. The attached cells gradually develop into microaggregates of microorganisms by attaching to new cells [87]. In the third stage, cells secrete extracellular polymeric substances (EPS) which acts as a biological glue and makes up the hydrogel structure of the aggregates. The EPS matrix is composed of polysaccharides, proteins, nucleic acids lipids, carbohydrates, phospholipids or humic substances and plays an important role in granule formation and structural stability. During the last stage, hydrodynamic shear forces contribute to granule maturation by promoting round, dense, and compact granules [74]. Dense biofilms contain oxygen gradients across the granule and provides aerobic, anoxic environments for simultaneous removal of C, N and P [75, 88].

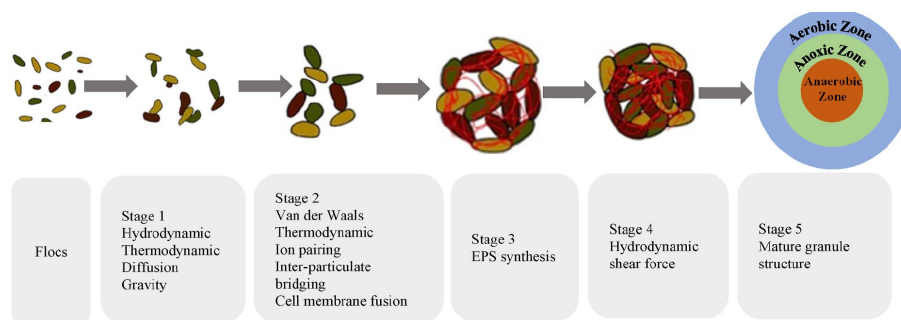


Figure 5: Aerobic granular sludge granulation process [73].

Factors Affecting Granulation. There are several environmental and operational parameters which can affect the granulation process for AGS specifically. These parameters are hydrodynamic shear force, selection of granules, dissolved oxygen (DO) concentration, sludge retention time (SRT), hydraulic retention time (HRT), settling time, seed sludge, substrate composition, organic loading rate (OLR), and reactor configuration [74, 89, 90]. A brief description of significant parameters which can be controlled in real WWTP is given below:

The hydrodynamic shear force is one of the important factors that has positive impacts on granulation. The shear force resulting from the upflow gas velocity in the reactor has significant influence on sludge settling velocity and size of granules. Higher upflow gas velocity provides higher shear forces on the granules and ensures dense and compact granules which improves settling velocity. However, too high shear force results in granule disintegration and low shear force would be unfavorable for granulation [91]. AGS system should have balanced shear force resulting from a gas velocity of approximately 1.2 cm/sec to ensure proper granulation and to avoid granule disintegration [92].

The sludge settling time is another important parameter for granulation. Short settling time contributes to granulation by keeping fast-settling biomass in the reactor and wasting suspended sludge with effluent. During reactor start-up, it is typical to begin with a long settling time (10-15 minutes) and gradually reduce it as granulation occurs. A settling time that is too short causes washout of biomass and produces poor treatment efficiency since there is insufficient biomass available to convert the substrate. Moreover, short settling time allows more left-over suspended sludge to remain in the reactor which could hinder granulation process. For a lab-scale reactor, AGS can be cultivated with settling time of less than 15 min [93].

Sludge retention time (SRT) is the average amount of time the granules stay inside the reactor. Selecting which granules to waste, or remove from the reactor, for maintenance of a suitable SRT is an important factor for granulation. Relatively older and larger granules from the bottom of the reactor need to be wasted regularly. Removing older granules maintains the maturation process of new and highly active granules inside the reactor, thus, improves nutrient removal efficiency. Too long SRT will lead to aged and less active sludge prone to disintegration under exposure to high shear force [94]. Too short SRT reduces nitrogen removal efficiency because slow growing ammonia oxidizing bacteria get washed out [95]. Therefore, the selection of appropriate granules and SRT is necessary for the structural stability of AGS and high performance of the treatment process.

There are some other parameters such as C/N ratio, pH, temperature etc. The recommended value of C/N for desired granulation is 100:10 [96]. pH and temperature are important factors in the microbial growth process. The pH range of 7-7.5 [97, 98] and temperature range of 20–25°C [8] are recommended to achieve dense and compact structure of granules. It is also reported that granulation can occur at moderate to low temperatures such as 8-15°C. But at low temperatures (8°C), time for granulation was twice of time needed at 20°C [99]. Granulation was also improved by stepwise increasing of salinity at low temperature (12°C) [100].

Sequencing Batch Reactor

The sequencing batch reactor (SBR) is a fill and draw sludge system for wastewater treatment (Figure 6). In this system, wastewater is added to a single “batch” reactor, treated to remove undesirable components, and then discharged. SBR offers equalization, aeration, and

clarification in a single batch reactor [101]. The unit processes of the SBR and CAS systems are the same. The difference between the two technologies is that the SBR uses a single reactor for equalization, biological treatment, and secondary clarification by using a timed control sequence whereas, CAS system requires several tanks for these unit processes [102].

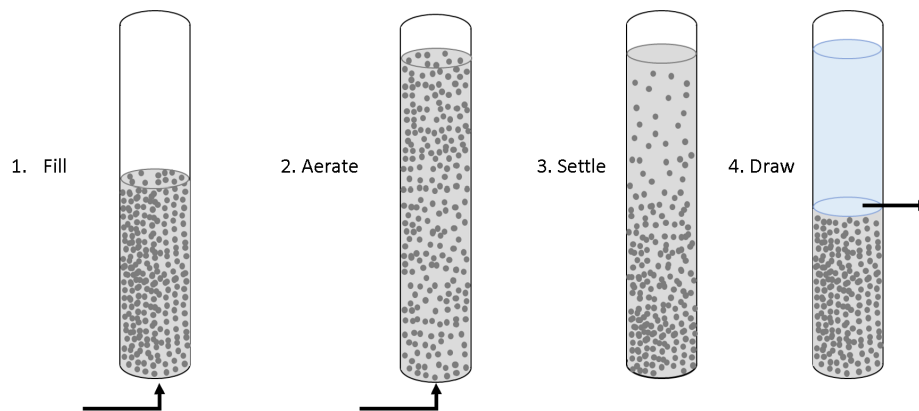


Figure 6: Cycle of sequencing batch reactor operation.

During the fill phase, the reactor receives the raw wastewater through the settled bed of active biomass left inside the reactor at the end of the previous cycle. Influent wastewater is added anaerobically to the biomass already present in the SBR without mixing, resembling plug flow. This feeding strategy creates a high food to microbe (F/M) ratio and promotes the growth of granule-forming bacteria by suppressing the filamentous ones. The F/M ratio is the amount of substrate provided relative to the quantity of microorganisms present in the reactor. When substrate is abundant, the microbes uptake the carbon anaerobically and store it for later use. The aeration phase is intended for the completion of the biological reactions including the complete degradation of organics. Further, the aeration phase is often designed to provide a high degree of nutrient removal as well. The treatment is controlled by air, either on or off, to produce

anaerobic, anoxic, or aerobic conditions due to oxygen gradients in the biofilm granules. During the settling phase, the entire reactor tank acts as a batch clarifier, without any inflow or outflow. The draw phase is used to discharge treated wastewater after the settlement of the biomass generated after the reaction (aeration) phase.

Role of EPS

The extracellular polymeric substance (EPS) matrix is an important component of AGS and plays a major role in granule formation and structural stability of granules. Usually, the EPS are composed of polysaccharides, proteins, nucleic acids lipids, carbohydrates, phospholipids or humic substances. Microbial cells and polysaccharides are most abundant at the outer edge of the granules, while the center is mostly composed of proteins and dead or inactive cells [74]. Protein at the center has a role in the formation and stability of AGS [12, 74]. EPS also protects cells from shock loads and toxic compounds such as antibiotics or phenolic compounds [10]. The capacity bio-adsorption of AGS to remove PFAS can also be provided by the EPS content [103].

Since PFAS are nonbiodegradable, sorption is found to be predominant way for PFAS removal in WWTP. The capacity of PFAS adsorption is highly dependent on sludge properties. Several studies have reported that PFAS can be removed from wastewater by the activated sludge process via adsorption onto the bio-sludge [104]. The enhanced biological phosphorus removal (EBPR) sludge (86%) and aerobic granular sludge (AGS) (85%) have the highest adsorption capacity for PFOA whereas anaerobic sludge has lower removal percentage (73%) compared to EBPR and AGS [103]. The largest distribution co-efficient (K_d) value (528-806 L/kg) of EBPR sludge among all these three sludges refers to the highest affinity of PFOA to EBPR sludge [103]. Besides protecting the cells from toxic compounds and forming microbial

aggregates, EPS plays a significant role to remove micropollutant in biological wastewater treatment processes via adsorption [105]. The EPS matrix contains a wide range of binding regions and sites, including amine, carboxy, and hydroxyl groups. The interaction between EPS and PFAS reveals the importance of proteins in binding PFAS onto sludge surface. The aromatic and amide groups of protein can attract the C–F chains and carboxylic/sulfonic head of PFAS via hydrophobic interaction and electrostatic attraction. Besides proteins, other compounds such as humic substances may contribute in PFAS adsorption [13, 25].

Granule Application

AGS offers the possibilities for removing C, N, P, non-biodegradable organic compounds (PFAS), micropollutants such as pharmaceuticals and personal care products, recalcitrant or toxic xenobiotic compounds, and metal ions from wastewater [106, 107].

Nutrient Removal

Biological wastewater treatment by AGS can provide C, P, and N removal in a single reactor [8, 108, 109]. Simultaneous removal of C, N, and P can be achieved by AGS due to the presence of different redox zones (aerobic, anoxic, and anaerobic zones) within granules multilayered structures. Multiple kinds of microbes such as nitrifiers, denitrifiers, and phosphorus accumulating organisms (PAOs) reside within those multilayers of granules [108]. The DO gradient results from diffusional limitations meaning most DO gets consumed by the bacteria in the outer layer of granules. Low DO gets transferred inside the granules due to higher DO consumption at outer layer of the granules. This low oxygen transfer creates the anoxic zone inside the granules which ensures proper denitrification and phosphorus uptake inside the

granules [109]. For simultaneous nitrification and denitrification (SND), the oxygen penetration depth is controlled by the oxygen concentration in the bulk liquid. A desirable SND efficiency was obtained at DO of 1.5-2.5 mg/L [106, 110, 111].

Nitrogen removal by aerobic granular sludge is divided into two different processes: nitrification in the outer layer, and denitrification inside the granules. Nitrification is the oxidation of ammonia (NH_3) by ammonia-oxidizing bacteria (AOB) and production of nitrite (NO_2^-) from ammonia. Nitrite-oxidizing bacteria (NOB) convert the nitrite to nitrate (NO_3^-). Denitrification occurs inside the granules where the anoxic environment prevails (Figure 7). Denitrification produces nitrogen gas (N_2) from nitrate [8, 108]. Complete nitrogen removal requires both aerobic and anoxic conditions for ammonium nitrification and denitrification of nitrate or nitrite, respectively. Thus, a combination of high and low DO is required for achieving complete nitrogen removal. Nitrification and denitrification are performed simultaneously under the same reactor operating conditions due to the presence of different microbes (nitrifying, and denitrifying populations) in multilayered structure of granules [112-114]. A DO concentration between 1.5-2.5 mg/L during the aeration phase and granules approximately 1.5-2 mm in diameter have been shown to reliably produce the necessary oxygen gradients and redox conditions within the granules for complete nitrogen removal [8, 115].

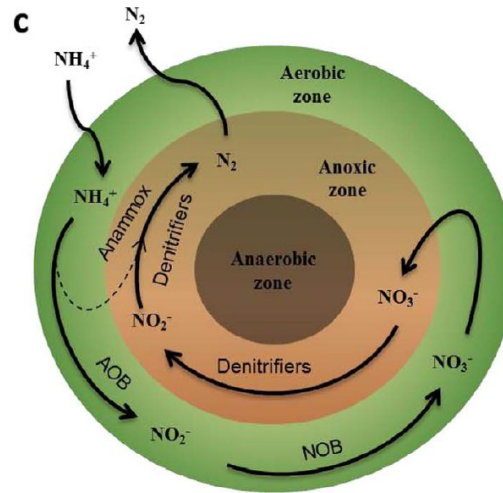


Figure 7: Multilayered structure and the simultaneous nitrification and denitrification in aerobic granular sludge. Upper aerobic zone (green), middle anoxic zone (light brown) and inner anaerobic zone (dark brown) are depicted [74].

Removal of phosphorus (P) is achieved by PAOs in aerobic granular sludge and the favorable conditions for PAOs are alternating anaerobic feeding and aeration periods, and low dissolved oxygen concentrations (1.5-2 mg/L) [116]. The PAOs uptake carbon and release phosphate during anaerobic feeding phase. These microorganisms have the capability to store carbon in the form of intracellular polyhydroxyalkanoates (PHA). The PHA is used as electron source for denitrification [108]. During aeration phase, PAOs consume the phosphate for their further growth, thus, remove P from wastewater. *Candidatus Accumulibacter phosphatis* is a key PAO responsible for enhanced bio-P removal (EBPR) capabilities of AGS [117]. The glycogen-accumulating organisms GAOs are undesirable organisms for EBPR. GAOs compete with PAOs for substrate thereby reducing overall P removal by PAOs. A combination of carbon source, pH and temperature is required for PAOs to win the competition between GAOs and PAOs, resulting proper P removal from wastewater. Low temperature (10°C) or moderate temperature

(20°C) with combination of acetate and propionate as carbon source are favorable for PAOs growth [118].

Carbon source is important both for N and P removal by AGS since carbon acts as electron donor. Carbon is the energy source for PAOs and gets removed by PAOs in anaerobic feeding phase. For full-scale system, availability of volatile fatty acids (VFAs) in the influent has a positive impact on biological phosphorus removal. VFAs require less reducing power and less P- release to be uptaken, resulting more likely C removal by PAOs [116]. The removal of C for lab-scale system depends on the variety of carbon used as substrate. The COD removal rate is higher for sole sources of carbon like only acetate [106, 108, 109, 114].

There are some factors affecting simultaneous nutrient removal by AGS. Factors influencing SND include structure, size, density and concentration of sludge flocs, dissolved oxygen (DO), hydraulic retention time (HRT), sludge retention time (SRT), carbon/ nitrogen (C/N) ratio, settling time, temperature and pH [111]. For SND in a lab reactor, the suitable values of influencing parameters are HRT of 10-12 h, DO concentration of 1.5-2.5 mg/L, C/N ratio of 10, pH of 7.5 ± 0.3 , temperature of 20-30 °C [119, 120] and a balanced settling time to avoid the washout of AOB. A balanced settling time allows retaining well settling granules and prevents losing of AOB (slow growing) to maintain proper nitrification [110]. A suitable F/M (0.2-0.5 for CAS) will ensure sufficient substrate to the microbes which remove the nutrients [121, 122].

CHAPTER THREE

MATERIALS AND METHODS

Sequencing Batch Reactor Set Up

Two laboratory-scale aerobic granular sludge reactors were used in this project. Each reactor had a working volume of 3.4 L (Figure 8) with a cycle exchange ratio of 53%. The internal diameter and height of the reactors were 6.35 cm and 152 cm respectively. The HRT was 5.7 hours [123]. The substrate load was $1.2 \text{ kg COD/m}^{-3}\text{d}^{-1}$. Influent was added at the bottom of the reactors and effluent was discharged at a port positioned at a distance of 55 cm from the bottom. There was a sampling port placed at 45 cm from the bottom of the reactor. Air was introduced through an aeration stone placed at the bottom of the reactors with a upflow air velocity of 70-78 m/h. The air flow rate was controlled by a mass-flow controller (MFC) (Brooks, 30 SLPM, Nitrogen gas and Model: 5850S). Two MFCs directed nitrogen gas and compressed air to reactors to ensure the air flow rate of 4.5-5 L/min during the aeration phase. A gas recycle pump (UN86KNP, KNF Neuberger. Inc) captured the gas produced inside the reactors during aeration and redirected to the reactors via MFCs. Dissolved oxygen (DO) of 1.5-2 mg/L was measured throughout the aeration phase by a DO probe (Vernier Optical DO Probe) inserted at a distance of 50 cm from the bottom. The temperature inside the reactors was measured by temperature probe (Vernier Stainless Steel Temperature Probe) placed at 51 cm from the bottom. The experiment was performed at a temperature of 20-22°C [78, 82, 106, 112, 121, 122, 124-128]. A pH probe (Vernier pH Sensor) was positioned at 50 cm from the bottom to measure the pH continuously. The pH was maintained at 7.0 ± 0.3 using 1M NaOH and 1M HCl.

Acid and base solutions were injected to reactors at a distance 47 cm from the bottom by acid and base pump (Model: 7543-01, Cole Parmer). Dissolved oxygen (DO), pH, and temperature were monitored continuously by LabView program (Version 18.0.1f4).

The reactors were operated as a sequencing batch reactor (SBR) with one cycle of 3 hours (180 min) having 100 min of influent filling, 70 min of aeration, 5 min of sludge settling, and 5 min of effluent withdraw. During the fill phase, influent was added to reactor by one water pump and one C and N/P media pump (Model: 7524-50, Masterflex). Then the aeration phase introduced air to reactors to facilitate proper reactions. After aeration, five min settling time was allowed for AGS to settle down. Then treated wastewater was withdrawn for five min via effluent discharge port. At the beginning, when sludge was less dense, the settling time was 15 min. During start-up, a two second pulse of nitrogen gas was inserted at the bottom of the reactors every five minutes during the fill phase to prevent short-circuiting and ensure distribution of substrate among all granules. After around 240 days of operation, AGS became compact and took only 5 min to settle. Then influent wastewater was added to the reactors without mixing, resembling a plug flow regime [78, 82, 121, 126-128].

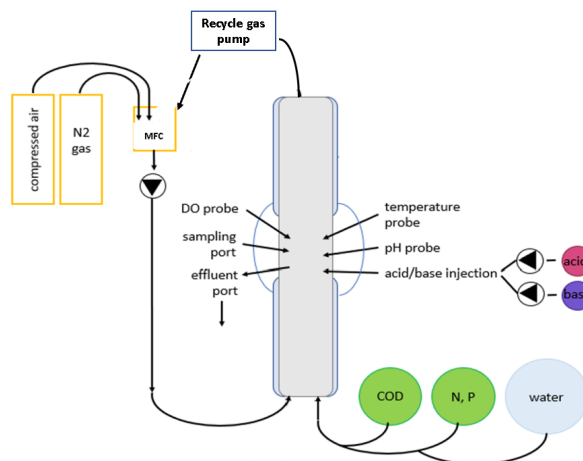


Figure 8: Schematic representation of SBR adapted from image by Dr. Catherine M Kirkland.

Seeding Aerobic Granular Sludge

Aerobic granular sludge was collected from a full-scale wastewater treatment plant (WWTP) and stored for several months in refrigerator (3-4°C). These granules broke up into flocs later. Both reactors were started with those floccular sludge and cultivated into aerobic granules using synthetic wastewater as feed [82, 112, 126, 129, 130].

Media Preparation and Loading Rate

Two concentrated media solutions were diluted with deionized water (DI water is purified water with a shallow ion content) and mixed in the influent to produce the synthetic wastewater. The carbon source was provided by C media, while a separate nutrient media provided nitrogen and phosphorus for the bacteria. Separating the C and N/P media prevented the contamination of the media while in use. After mixing all the chemicals in DI water to prepare the media, they all were autoclaved at 123°C for 2 hours. The chemicals used to produce concentrated media are shown (Table 2) below:

Table 2: Chemical composition of concentrated media/substrate.

	Name	Formula	MW (g/mol)	mM
C medium	Sodium acetate trihydrate	$\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$	136.079	65.1
	Magnesium sulfate heptahydrate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.466	3.7
	Potassium chloride	KCl	74.5513	4.8
N/P medium	Ammonium chloride	NH_4Cl	53.491	35.2
	Potassium phosphate dibasic	K_2HPO_4	174.2	2.2
	Potassium phosphate monobasic	KH_2PO_4	136.086	4.4
Trace element solution	Ethylenediamine tetra acetic acid (EDTA)	$\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$	292.24	171.1
	Zinc sulfate heptahydrate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	287.54	76.5
	Calcium chloride	CaCl_2	110.99	49.9
	Manganese chloride tetrahydrate	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	197.91	25.6
	Iron sulfate heptahydrate	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	278.01	17.9
	Ammonium molybdate tetrahydrate	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	1235.86	0.9
	Copper (II) sulfate pentahydrate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	249.68	6.3
	Cobaltous chloride hexahydrate	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	237.93	6.8

In one cycle of 180 min, 1300 mL of DI water, 150 mL of C medium and 150 mL of N/P medium were added to the reactor. The trace element solution was prepared separately and mixed with N/P medium before adding to the reactor. Each liter of N/P medium required 10 mL of trace element [78, 82, 112, 121, 125-128, 130, 131].

Table 3: Concentrations of different media both in influent tubing and in reactor.

Media	Concentration in tubing (mg/L)	Diluted concentration in the reactor (mg/L)
Carbon	360 mg/L as TOC	199 mg/L as TOC
Nitrogen	59 mg/L as $\text{NH}_3\text{-N}$	31.5 mg/L as $\text{NH}_3\text{-N}$
Phosphorus	58 mg/L as PO_4^{3-}	31 mg/L as PO_4^{3-}

Concentrations of media in influent tubing and inside the reactor after dilution are shown in Table 3. Concentration in tubing means the concentration of media collected from influent tubing during the fill phase. Concentration in reactor is the theoretical concentration of the media after dilution with the remaining solution in the reactor following the previous cycle. Diluted concentration in the reactor was defined as influent concentration in the results showing removal of PFOA and PFOS by AGS in Chapter Five. To ensure an expected concentration of PFOA/PFOS inside the test reactor, influent concentration for PFOA/PFOS was measured from the diluted concentration in the reactor. For the results presenting conventional nutrient removal efficiency, concentration in tubing was defined as influent concentration. This is the typical way to present the influent concentrations of nutrient (C, N, P) in raw wastewater.

Stock solutions of pentadecafluorooctanoic acid (PFOA) ($C_8HF_{15}O_2$) and pentadecafluorooctane sulfonic acid potassium (PFOS) ($CF_3(CF_2)_7SO_3K$) with purity >98.0% from Milipore Sigma were used. The stock solution was made by mixing 50 mg of both PFOA and PFOS salts into 500 mL 100 % methanol. This produced a stock solution containing 100 mg/L each PFOA and PFOS. The stock solution was stored in HDPE bottle at 4°C [132]. After 350 days of operation, 30 mL of a mixed solution of both PFOA and PFOS in methanol with individual concentration of 5.3 mg/L was added to the reactor in each cycle with C and N/P media. The individual concentrations of PFOA and PFOS were expected to be 100 µg/L inside the reactor after dilution (influent concentration).

Analytical Methods and Sampling

Two different instruments were used to measure concentration of total carbon, nitrite, nitrate, and phosphate during the different phases of a cycle to monitor the removal of those

constituents. To measure the nutrient removal efficiency, laboratory standard methods were used. For carbon content, total organic carbon (TOC) was measured. TOC is the total amount of carbon from organic compounds in water. For TOC measurement, SKALAR Formacs^{HT} TOC/TN Analyzer was used. First a standard curve was developed with a set of standard TOC concentrations ranging from 10-500 mg/L. The chemical used for the standard solution was potassium hydrogen phthalate, 99.99%, acidimetric standard ($C_8H_5KO_4$) from Acros Organics. The actual carbon content in the synthetic and effluent wastewater is estimated using that standard curve. Around 8-10 mL sample filtered with Sartorius Minisart RC25 syringe filter (0.45 μ m, 25mm, sterile) was placed in disposable borosilicate glass tube (16 X 100mm, from Fisher Scientific) as a sample of SKALAR Formacs^{HT} TOC/TN Analyzer. In the TOC/TN analyzer, gas pressure was maintained at 300 KPa and sampling needle added 3M HCl to each sample. The temperature was checked above 800°C before analysis started.

Inorganic ions such as PO_4^{3-} , NO_3^- , NO_2^- were measured by high performance ion chromatography (IC) [8, 106, 108, 115, 133] using a Dionex ICS1100 Anion IC. The analytical column was AS22 (Dionex IonPac AS22 standard bore 250mm x 4 mm, Thermo Fisher #064141) with guard column AG22 (Dionex IonPac AG22 standard bore 50 mm x 4 mm, Thermo Fisher #064139). The mobile phase (Dionex AS22 eluent concentrate from Thermo Fisher #063965, 100X concentrate containing 0.45M carbonate/0.14M bicarbonate) containing 4.5mM sodium carbonate and 1.4 mM sodium bicarbonate was used for this instrument. Samples were filtered with Sartorius Minisart RC25 syringe filter (0.45 μ m, 25mm, sterile) and 0.6 mL samples placed in a 0.5 mL poly vial with filter cap (Thermo Scientific). In this instrument, a set of standard concentrations 50%, 25%, 12.5%, 6.25% and 3.13% of stock solution (Dionex Combined seven anions standard 1, Thermo Scientific #056933) were analyzed

to get individual standard curves for the ions of interest. Finally, the concentration of ammonia in different cycle phases was determined using Hach DR 3900 instrument and Hach ammonia test kit TNT 832 (2-47 mg/L $\text{NH}_3\text{-N}$). The sample preparation and measurement of carbon, nitrogen and phosphorus followed the standard methods [134]. For TOC and other anions measurement, samples were collected from reactors 2-3 times a week.

Total suspended solids (TSS) and volatile suspended solids (VSS) are important physical properties of granules that provide information about the quantity of active biomass inside the reactor. For TSS and VSS measurements, standard methods were used. A 20 mL sample was taken at the middle of the aeration phase and vacuum filtered using glass fiber filter (Hach, 47 mm, Cat No: 253000). Then it was placed in an oven for 24 hours at 105°C . The weight of empty filter (w_1) and weight of the filter dried in the oven at 105°C (w_2) were measured. Total suspended solids (TSS) $= \frac{w_2 - w_1}{\text{sample volume}}$ in mg/L. Then the filter was placed in the oven again for five hours at 550°C . After baking the sample, only the fixed, or inorganic, solids are left in the sample. The weight of these fixed solids (w_3) was measured and was subtracted from the dry weight of the filter to determine the organic fraction. Organics are volatilized during baking and thus are lost. The volatile suspended solids concentration is calculated as (VSS) $= \frac{w_2 - w_3}{\text{sample volume}}$ in mg/L [85, 135-137].

The sludge volume index (SVI) represents the settling capacity of granules with units of [L/g]. SVI is calculated based on the time that granules take to settle down during the settling phase in the SBR. Larger and more dense granules take less time to settle down by gravity, thus, settle faster than smaller and fluffier ones. The higher the SVI value, the slower the granules in

settling. The compactness of the granular microbial structure results in a good settling ability and the SVI_5 and SVI_{30} values for granules are usually below 60 mL/g [138, 139].

The volume of settled granules after 3 min, 5 min, and 10 min was measured to calculate the SVI value. Initially, SVI for 10 min (SVI_{10}) was measured when small granules took longer time to settle, when the settling phase duration was 15 min. Later, the settling time decreased to 5 min and the SVI_5 and SVI_3 were measured [82, 140-142]. Using the TSS value and the volume of settled granules after time t , the SVI_t was calculated by the equation given below:

$$SVI \text{ (L/g)} = \frac{\text{volume of settled granules (L)} \div \text{total volume in SBR (3.4 L)}}{TSS \left(\frac{g}{L} \right)}$$

To calculate SRT, the VSS of the reactor's granules, the VSS in the effluent, the effluent flow rate, the sludge waste rate, and the reactor's working volume were used [123]. SRT is the time that an average granule stays within a reactor. Maintaining an appropriate SRT ensures sufficient new granules in the reactor to facilitate better treatment. To retain new granules in the reactor, around 400 mL of old granules were wasted from the reactor every 9 days. To ensure the waste of old, larger granules, these 400 mL of granules were taken at the bottom of the reactor during the settling period. The SRT can be calculated by mass balance over the sludge (Figure 9) [143-147]. The equation used for SRT calculation is given below:

$$SRT \text{ (days)} = \frac{V_r(L) * VSS \left(\frac{mg}{mL} \right)}{Q_{eff} \left(\frac{mL}{day} \right) * VSS_{eff} \left(\frac{mg}{mL} \right) + Q_{sludge} \left(\frac{mL}{day} \right) * VSS \left(\frac{mg}{mL} \right)}$$

where,

V_r = working volume of reactor (L),

VSS = VSS of granules inside the reactor (mg/mL),

Q_{eff} = flow rate of effluent (mL/day),

VSS_{eff} = VSS of effluent sludge (mg/mL) and

Q_{sludge} = sludge waste rate (mL/day).

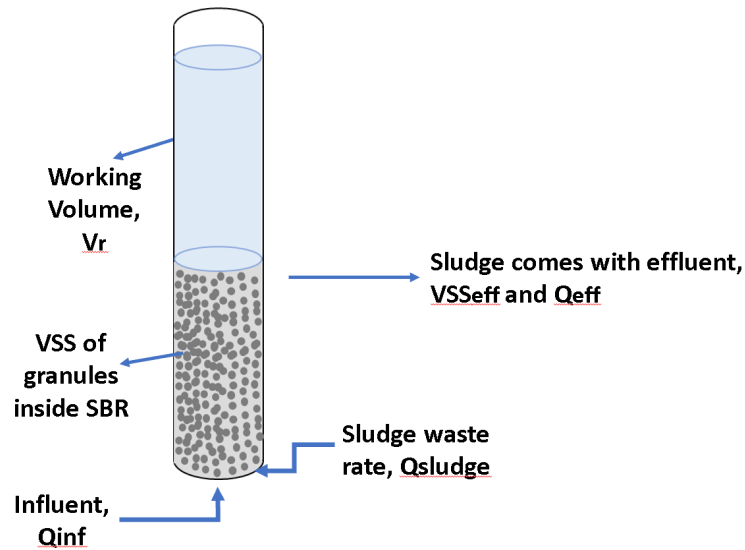


Figure 9: shows the mass balance required for SRT calculation for SBR [148].

To determine the granule size distribution, 100 mL of granules collected during the aeration phase was sieved with a series of sieves with different mesh sizes (1 mm, 0.5 μm and 0.2 μm). These sieve fractions together with 100 mL of unsieved sludge were dried at 105°C (Figure 10). Using the same procedures to determine the VSS mentioned above, the VSS of each fraction of granules was measured. The percentage of each size granules was measured by dividing the VSS of granules of certain size by the total VSS of all size granules [136, 137, 140]. All the sample preparations and measurements were done according to standard methods [149].

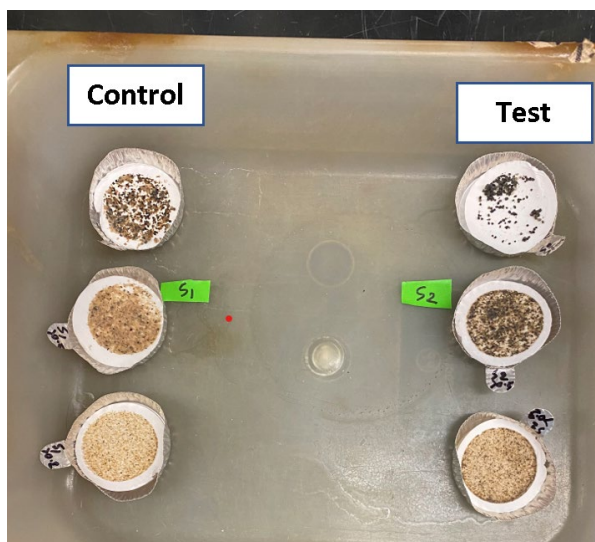


Figure 10: Different sieved granules fractions after drying at 105°C.

Solid Phase Extraction (SPE)

To extract the PFOA and PFOS from the liquid and sludge samples, the solid phase extraction process was used in this project. Solid phase extraction (SPE) is a technique designed for rapid, selective sample preparation, purification, and concentration prior to chromatographic analysis. In SPE, one or more analytes from a liquid sample are isolated by adsorbing onto a solid stationary phase before being extracted from the stationary sorbent with a selective mobile phase.

Wastewater samples from reactor were allowed to settle, and an aliquot of 200mL was carefully decanted into a HDPE bottle [150]. The sample of 200 mL containing both PFOA and PFOS was filtered through Glass-Microfibre Discs nitrocellulose filter (Hach) with a glass vacuum filter apparatus. A known mass, 100 μ L of 20 mg/L PFOA/PFOS stock solution was added to 100 mL of the filtered sample. This is called the pre-spiked sample. The remaining 100 mL is called the unspiked sample. These two pre-spiked and unspiked liquid samples were used

in solid phase extraction process to determine the percentage of PFOA and PFOS recovered during SPE [151, 152]. To prepare the sludge sample for SPE, standard methods were used [153].

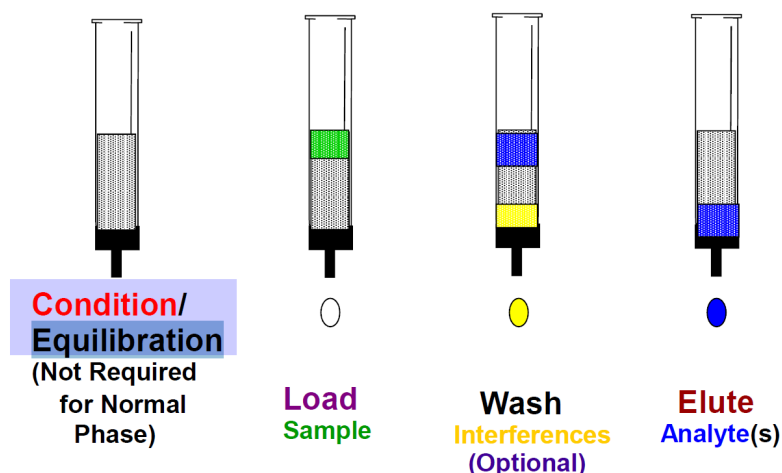


Figure 11: Sequence of SPE method step [154].

All the HDPE bottles and polypropylene tube connectors were cleaned with 100% methanol and Milli-Q water. First the glass vacuum manifold set up was placed with the Oasis WAX cartridges (6cc, 150mg, 30 μ m, Waters). A sequence of SPE method steps (Figure 11) was used. The cartridges were pre-conditioned with 10 mL 100% methanol and 10 mL Milli-Q water. A flow rate of 5 mL/min of sample was maintained through the cartridges. Then the cartridges were washed with 5 mL 2% formic acid. The cartridges were allowed to dry for 30 min before elution. The target fraction was eluted with 4 mL 100% methanol and 8 mL 5% ammonium hydroxide with a flow rate of 1 drop/sec. Finally, the total 12 mL eluent was transferred into 20 mL HDPE bottles and placed under nitrogen gas to be concentrated to final volume of 1 mL [23, 150, 151, 153, 155-159]. Using the method described above, 1 mL of concentrated samples were obtained both for pre-spiked and unspiked samples. A known mass, 100 μ L of 20 mg/L

PFOA/PFOS stock solution was added to the 1 mL concentrated unspiked sample. This is called the post-spiked sample [152, 160]. These pre- and post-spiked samples were used to calculate the recovery percentage (Figure 12) for PFOA and PFOS individually. Recovery percentage determines how successfully the SPE method isolates the analytes of interest. The recovery percentage for PFOA and PFOS was 86-94%, determined using the equation mentioned below:

$$\% RE^* = 100 \times \frac{\text{Response Extracted Sample (with analyte(s))}}{\text{Response Post-Extracted SPIKED Sample}}$$

Figure 12: Recovery of SPE procedure [161].

Liquid Chromatography (LC) and Mass Spectrometry (MS)

A method was developed to extract PFOA/PFOS both from aqueous and sludge phase using solid phase extraction (SPE) and liquid chromatography/mass spectrometry (UPLC with ESI Q-TOF-MS) that would provide the low detection limits necessary for accurately detecting and quantifying PFOA/PFOS in the samples from the test SBR. Ultra-performance liquid chromatography electrospray ionization - quadrupole time-of-flight with mass spectrometry (UPLC with ESI Q-TOF-MS) was used to quantify PFOA and PFOS in various samples. After SPE, liquid chromatography (LC) and mass spectrometry (MS) were used to separate and measure PFOA and PFOS in the concentrated samples. LC is used to separate individual components of a mixture. Mass spectrometry is an analytical tool to measure one or more components in mixed solution. Standard solutions both for PFOA and PFOS ranging from 1

$\mu\text{g/L}$ - 5 mg/L were analyzed with UPLC with ESI Q-TOF-MS to obtain the standard calibration curve both for PFOA and PFOS individually. The analytical standard solution for PFOA was 1 $\mu\text{g/mL}$ (Absolute Standard, Inc, Hamden, CT). For the PFOS analytical standard, 100 $\mu\text{g/mL}$ heptadecafluorooctane sulfonic acid solution in methanol (Sigma-Aldrich, Allentown, PA) was used. The detection limit for each PFOA and PFOS was 5 $\mu\text{g/L}$. For a typical 1 mL concentrated sample obtained by SPE, this detection limit concentration corresponds to a diluted concentration in the reactor of approximately 0.05 $\mu\text{g/L}$.

Concentrated samples obtained from SPE were placed in 2 mL amber glass vials (Thermo Scientific, Rockwood, TN) for UPLC with ESI Q-TOF-MS. Two mobile phases of water with 20 mM ammonium acetate (mobile phase A) and 100% LC grade methanol (mobile phase B) were used during liquid chromatography. The column Agilent Eclipse Plus C18 RRHD 1.8 μm , (2.1 X 100 mm) was used for LC. The gradient of mobile phase A was ramped down from 70% to 35% in 5 min and then to 5% at 7 min, held at 7% for 2 min. The gradient was again ramped from 5% to 70% at 9.10 min, held at 70% for 0.9 min. Gradient of mobile phase B was ramped from 30% to 65% at 5 min and then to at 95% at 97min, held at 95% for 2 min. The gradient was ramped down from 95% to 30% at 9.10 min, held at 30 % for 0.9 min [23, 150, 151, 153, 155-157, 159].

CHAPTER FOUR

RESULTS AND DISCUSSION

Two SBRs were run for 402 days, and the test reactor was dosed with PFOA and PFOS on day 350 and continuing for 52 days. A mixed PFOA and PFOS solution was added to the test reactor with the influent. The expected concentration of PFOA and PFOS in the reactor was 100 $\mu\text{g/L}$ after dilution with other media. While operating both reactors before and after dosing, some undesirable situations like losses of ammonia oxidizing bacteria (AOB) due to the presence of high pH and short SRT, lower denitrification rate due to lower carbon loading, etc were observed. The most important of these instances include an episode of high pH in the test reactor that lasted for two days approximately 14 days before dosing started and a period of low DO (0.1-0.9 mg/L) in both reactors during the last two weeks of dosing. These observations are addressed in more detail in Appendix A. After dosing started, aqueous and sludge samples were collected from the test reactor three days per week to measure concentrations of PFOA and PFOS in the influent, effluent and adsorbed to the sludge. ‘Influent’ here refers to the concentration of a given species after dilution in the reactor and is calculated from the measured concentration in the influent feed tubing. Effluent concentrations were measured directly from aqueous samples collected at the end of the cycle. ‘PFOA/PFOS sorbed on sludge’ refers to the mass of PFOA/PFOS adsorbed on the total amount of wet sludge present in the test reactor. To track the nutrient removal efficiency, concentrations of C, N, and P in influent, effluent, and at end of feeding phase (end of anaerobic) were measured. In the results showing concentrations of C, N, and P, influent refers to the measured concentration in the influent feed tubing. Concentrations at the end of feeding phase were measured directly from the aqueous samples

collected at the end of feeding phase. Effluent concentrations were measured directly from aqueous samples collected at the end of the cycle.

PFOS and PFOA Removal

The continuous removal of PFOS and PFOA within the dosing period (52 days) is shown in Figure 13. It can be clearly seen from the figure that the maximum removal efficiency was achieved within the first 10 days of dosing and after that period, steady state performance was achieved. The removal percentage for PFOS is higher than that of PFOA. So, it is clear that PFOS is more likely to be removed from wastewater via sorption on AGS.

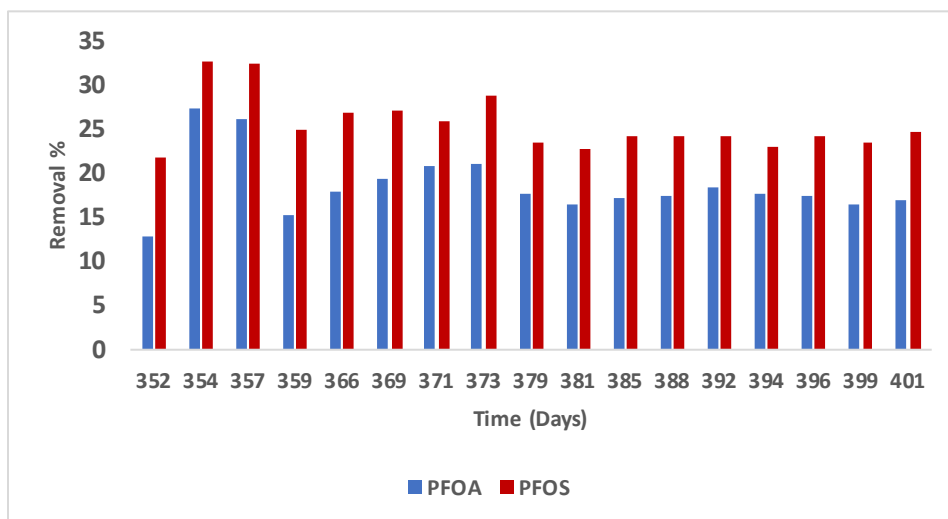


Figure 13: The removal of PFOA and PFOS individually over time by aerobic granular sludge.

PFOS and PFOA are nonbiodegradable, and adsorption is the main pathway for their removal in WWTP [103]. EPS of aerobic granular sludge plays an important role in the adsorption process. Among all components of EPS, exoproteins (PN) play a crucial role in binding PFOA and PFOS to EPS/sludge [162]. Hydrophobic interaction occurs among the aromatic and amide groups in exoproteins to keep the protein stable [163]. PFOA and PFOS

have low solubility due to their lower surface tension which indicates the hydrophobic interaction between PFOA/PFOS and water [164]. Then the positively charged aromatic and amide groups in exoproteins can attract the sulfonate and carboxylate heads of PFOS and PFOA respectively via electrostatic attraction [103].

PFOS Removal

The phase partitioning of PFOS in aqueous and sludge samples are presented in Figure 14. The total mass of PFOS in the test reactor's wastewater during a full cycle (influent), the mass in the effluent (effluent), and the mass on the solid sludge were measured (sorbed on sludge). Based on the mass of PFOS in the three different samples, the loss of PFOS by additional sources (e.g glass reactor, media tube) was estimated (additional losses). All data are presented as mass of PFOS to reflect the mass distribution of PFOS in different phases.

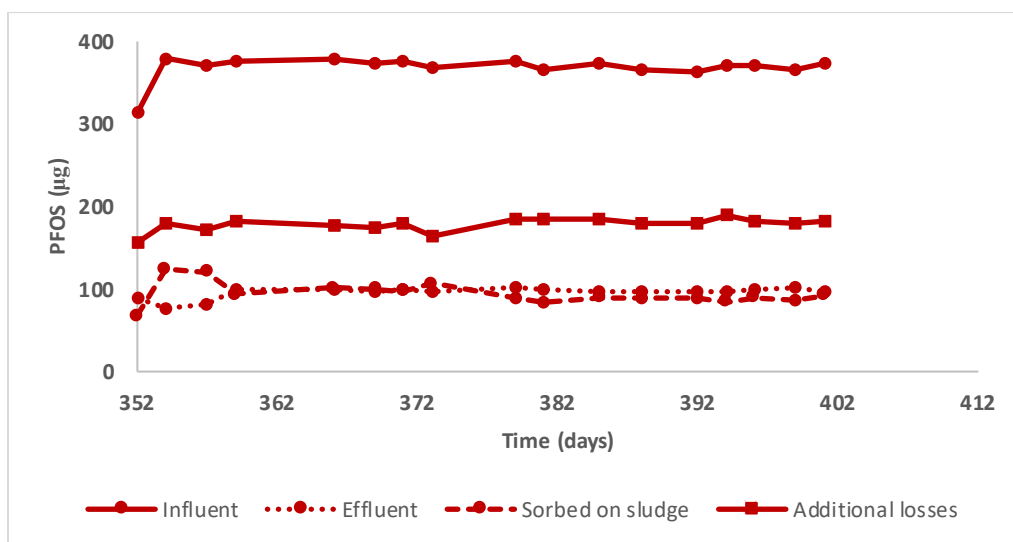


Figure 14: Mass of PFOS in influent (solid line), effluent (dotted line), sorbed on sludge (dashed line) and lost by additional sources (solid line with square marker) are presented.

The influent mass of PFOS (mass of PFOS in the test reactor after dilution during a full cycle) was 313 μg on day 352 of the experiment when dosing started. Then the mass increased from 313 μg to 379 μg on day 354 due to the presence of residual PFOS from the previous cycle. Throughout the period of dosing, the influent mass was around 366-372 μg . The maximum removal efficiency for PFOS was observed during first 7-10 days of dosing when the removal percentage was 33%. At maximum removal efficiency, around 120-124 $\mu\text{g/cycle}$ of PFOS was sorbed on the total amount of wet sludge in the test reactor. On day 366, the granules' performance reached steady state. During the steady state performance, a removal efficiency was achieved with removal percentages of 22-24%. During steady state, around 90-105 $\mu\text{g/cycle}$ of PFOS was removed from wastewater by AGS sorption. Around 100 $\mu\text{g/cycle}$ of PFOS was discharged with effluent at steady state. The remaining approximately 170 $\mu\text{g/cycle}$ of PFOS was not directly measured and may have been lost to other sinks like the sampling process and sorption on lab materials. Biodegradation is unlikely given the recalcitrant nature of PFAS compounds.

PFOA Removal

The phase partitioning of PFOA in aqueous and sludge samples are depicted in Figure 15. The total mass of PFOA in the test reactor's wastewater during a full cycle (influent), the mass in the effluent (effluent), and the mass on the solid sludge were measured (sorbed on sludge). Based on the mass of PFOA in three different samples, the loss of PFOA by additional sources (e.g glass reactor, media tube) was estimated (additional losses). The influent mass of PFOA (mass of PFOA in the test reactor after dilution during a full cycle) was 442 μg on day 352 of the experiment when dosing started. Then the mass increased from 442 μg to 594 μg on day 354 due

to the presence of residual PFOA from previous cycle. Throughout the period of dosing, the influent mass was around 397-400 μg . The maximum removal efficiency for PFOA was observed during first 7-10 days of dosing when the removal percentage was 28%. At maximum removal efficiency, around 140-165 $\mu\text{g}/\text{cycle}$ of PFOA was sorbed on total amount of wet sludge in the test reactor. On day 366, the granule's performance reached steady state. During the steady state performance, a removal efficiency was achieved with removal percentages of 16-17%. During steady state, around 70-85 $\mu\text{g}/\text{cycle}$ of PFOA was removed from wastewater by AGS sorption. The process (measurement of mass per unit mass of dry sludge and considering the VSS and volume of test granules) mentioned for PFOS was used to measure the total mass of PFOA adsorbed on the total wet sludge. Around 125-135 $\mu\text{g}/\text{cycle}$ of PFOA was discharged with effluent at steady state. The remaining approximately 180 $\mu\text{g}/\text{cycle}$ of PFOA was not directly measured and may have been lost to other sinks like the sampling process and sorption on lab materials.

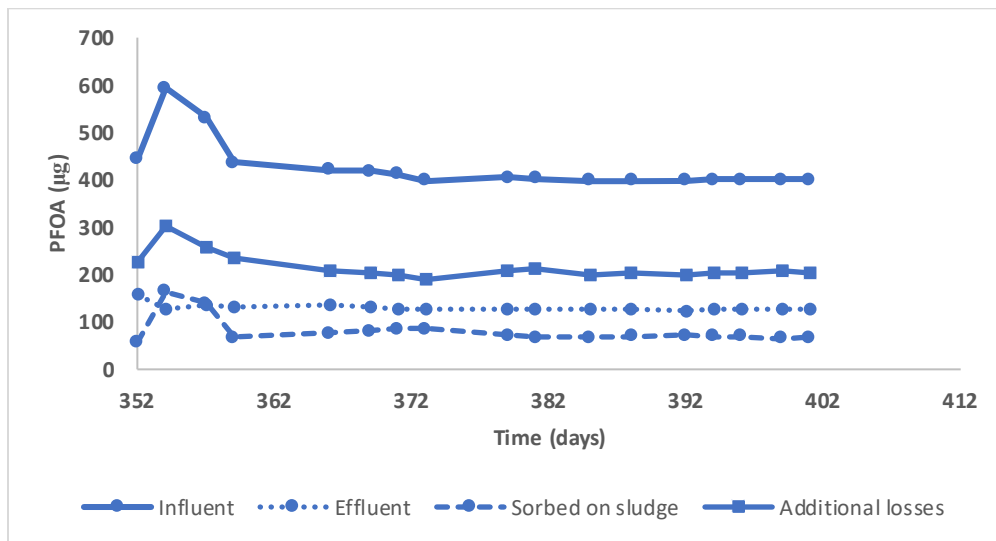


Figure 15: Mass of PFOA in influent (solid line), effluent (dotted line), sorbed on sludge (dashed line) and lost by additional sources (solid line with square marker) are shown.

AGS facilitated maximum sorption of 11 µg/g of dry sludge and steady sorption of 7-8 µg/g of dry sludge for PFOS. For PFOA, the maximum and steady sorption capacity of AGS were 15 µg/g and 6 µg/g respectively. Typical concentrations of PFOA/PFOS range from 6-1050 ng/L in most WWTPs in the United States [165, 166]. The sorption capacity of CAS for PFOA/PFOS ranges from 4-10 ng/g [167]. Thus, AGS would likely be effective to remove PFOA/PFOS in WWTP, since AGS demonstrated higher sorption capacity of 6-8 µg/g in the test reactor.

Maximum 33% of PFOS and 28% PFOA were removed from synthetic wastewater by AGS in the test reactor. Yang et al., (2019) confirmed around 32-36.4% removal of PFOA by adsorption of AGS. No aerobic biodegradation was observed [153]. The results obtained in this experiment are consistent with the results shown in the paper by Yang et al., (2019). The reasons for similar results may be use of synthetic water and AGS as well as, similar SBR operating conditions. Xie et al., (2022) found around 66-71% removal of PFOA in anaerobic digestion. Anaerobes showed the capacity to reduce PFOA via biochemical degradation and provided a high removal percentage. Around 36-61% of total removal was contributed by the adsorption of sludge [168]. Due to the difference in experimental set up and sludge type, Xie et al., (2022) got different results though the percentage of removal by adsorption is consistent with the results obtained in this experiment. To remove PFOA/PFOS from wastewater, there are other technologies such as, activated carbon adsorption, ion exchange resins, and high pressure membranes [169]. It is reported that the activated carbon filter (ACF) has the highest sorption capacity (73%) for PFOA [170]. The order of PFOA sorption capacity for activated carbon is ACF > PAC (powdered activated carbon (49%) > GAC (granular activated carbon) (43%) [170].

PFOS has higher sorption propensity than PFOA due to the presence of sulfonate head in PFOS's chemical structure [171]. In previous studies, PFOS has demonstrated higher sorption on lab materials, Gram-positive and Gram-negative bacteria, and activated carbon than PFOA [43, 170, 172, 173]. The sulphonic group in PFOS shows more hydrophobicity than the carboxyl group in PFOA [170]. The experimental results also showed higher removal percentage for PFOS than PFOA. Before dosing, the granules were non-contaminated and it is likely that most of the inner sorption sites in their porous structure were available [171]. After dosing started, PFOA/PFOS sorbed and saturated those sites, reducing the capacity for additional sorption. After obtaining steady state sorption performance, large granules started to break down. Due to the toxicity of PFOA/PFOS, AGS may secrete more EPS to protect themselves. The increased EPS can make the AGS structure loose and broken, affecting the settling performance negatively. Increased EPS production may facilitate the steady state sorption of PFOA/PFOS, thus, remove PFOA/PFOS at steady state [168, 174]. During dosing, an SRT of 34 days (Appendix A) was maintained to ensure continuous growth of new granules, thus more EPS for continuous sorption. To increase the PFOA/PFOS removal by AGS, more stable EPS content should be ensured. The maintenance of PN/PS (protein/polysaccharides) ratio of 4 and particle size of 0.5-1 mm results in higher content of exoprotein in EPS which will increase the removal efficiency [162].

For full-scale WWTP, pre-treatment of wastewater or re-use of sludge can increase the removal efficiency. Pre-treatment will reduce a portion of solids from wastewater, thus, improve the capacity of the further treatment units. Re-use of sludge containing VFA would ensure most resource recovery [175]. EBPR (enhanced biological phosphorus removal) sludge are also reported as most efficient for PFOA/PFOS removal (86%) and to have high content of VFA [116].

Effects of PFOA and PFOS Exposure on Granules Morphology

After PFOA/PFOS exposure in the test reactor, the granules physical properties were measured. The effect of PFOA/PFOS on granules morphology will be discussed in this section.

Size Distribution

Figure 16 presents the size distribution of granules from the control and test reactors over time. The percentage of different size granules in both reactors was measured throughout the operational period.

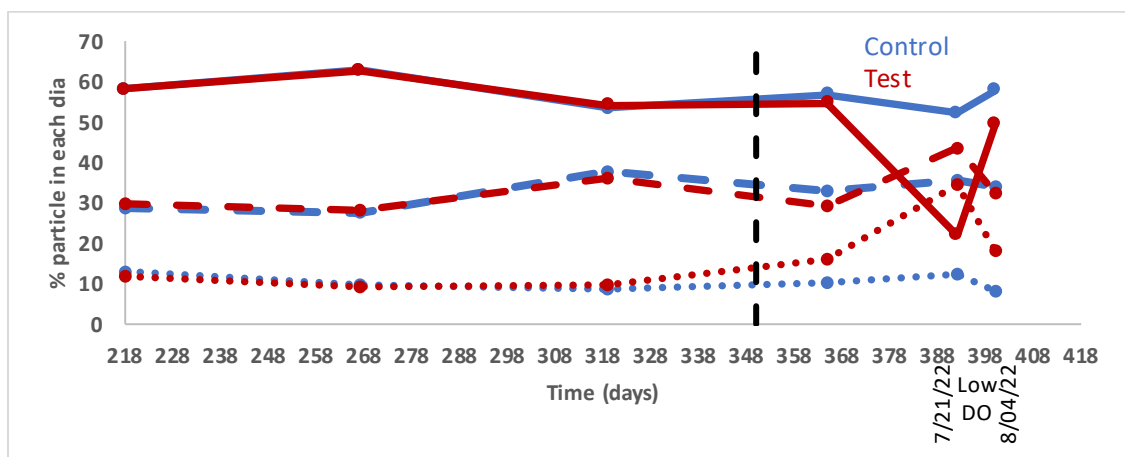


Figure 16: Size distribution of granules for both reactors before and after dosing. The granules diameter greater than 1 mm (solid line), diameter of 0.5-1 mm (dashed line) and diameter smaller than 0.5 mm (dotted line) are presented. The vertical dashed line marks the beginning of dosing PFOS and PFOA into test reactor.

Before dosing, both reactors had similar proportions of granules of each size range. Most granules (54-62%) had a diameter greater than 1 mm (larger granules). There were 28-36% granules with diameters ranging from 0.5 mm to 1 mm (medium granules) and 9-11% granules with diameters less than 0.5 mm (smaller granules). After dosing started, a gradual change was observed in the granule's size distribution for the test reactor. The number of larger granules

started to decrease from 54% on day 365 to 21% on day 392. At the same time, the number of medium and smaller granules increased from 29% to 43% and 11% to 34%, respectively. This situation indicated that at the beginning of dosing, PFAS exposure broke down the granules in the test reactor and made more smaller granules. But on day 391, the percentage of larger granules increased (49%), and percentages of medium and smaller granules decreased (32% and 18% respectively). The change in size of the test granules due to PFOA/PFOS exposure was recovered within one SRT of around 34 days of dosing period. During the last two weeks of dosing (days 388-402), both reactors experienced a low DO concentration (0.1-0.9 mg/L) during aeration. The low DO situation was created due to some mechanical issues in MFCs and operational errors in LabView program. But granules in the test reactor still recovered while having low DO. The control reactor also experienced a low DO between days 388-402. Figure 16 shows that the granules size in the control reactor was barely affected by low DO. The changes in physical properties caused by PFOA/PFOS will be presented in the next three results of SVI, TSS, and VSS of granules over time.

Sludge Volume Index

The sludge volume index (SVI) value is an indicator of the sludge settling characteristics. SVI values associated with short settling times (i.e. SVI₁₀-10 min settling) describe the fast-settling properties of aerobic granular sludge [176]. For AGS, the standard value of SVI₅, SVI₃₀ ranges from 40- 60 mL/g [138, 139].

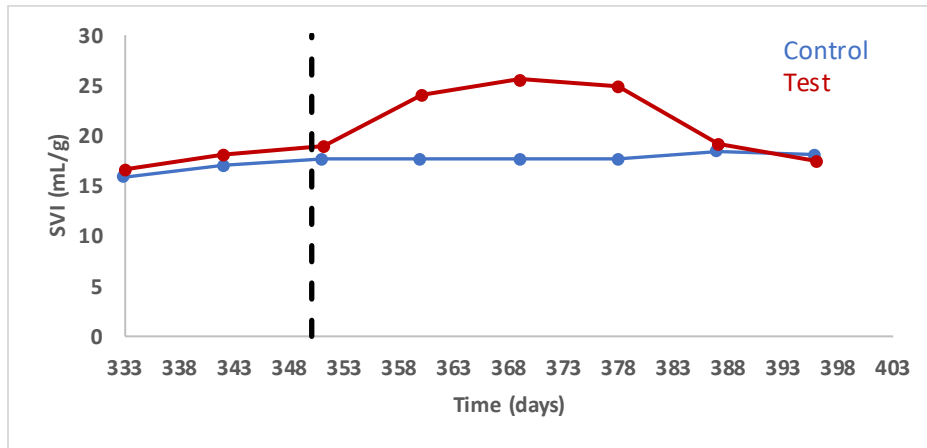


Figure 17: Change in SVI₃ value of AGS over time due to the presence of PFAS in test reactor. The SVI₃ for control (blue) and test (red) reactor are presented. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

Since both reactors had a steady state settling time of 3 min, the SVI₃ for granules was measured (Figure 17). The SVI₃ for both reactor's granules varied from 15-18 mL/g, indicating compact and dense granules were present in both reactors before dosing. After dosing, the SVI₃ value of the test reactor granules increased from 18 mL/g on day 351 to 25 mL/g on day 369. This increased value of SVI₃ in the test reactor indicates that the granules became less dense and were slower to settle. On day 378, the SVI₃ value of the test reactor again started to decrease and recovered to the initial value of 18 mL/g on day 396.

Both reactors experienced low DO (0.1-0.9 mg/L) during last two weeks of operation. The low DO period coincides with the recovery of the initial SVI₃ in the test reactor. It is reported that low DO (less than 1.1 mg/L) has strong negative effect on settleability of sludge, especially when COD loading is high (10-13 kg COD (kg MLSS)⁻¹ day⁻¹ [177]. There are other factors affecting SVI value such as, length of feeding period, SRT, and F/M ratio. Long SRT, low F/M ratio, and high COD loading decrease settleability of sludge whereas long feeding period favors the settling of sludge [178]. In this experiment, it appears that the SVI values of the

control and test granules were not negatively affected by low DO. The positive influence of the favorable operational conditions like long feeding period (100 min), balanced SRT (34 days), and low COD loading ($1.2 \text{ kg COD/m}^3 \text{ d}^{-1}$) appears to have outweighed any potential impacts of the low DO concentration.

Total Suspended Solids (TSS)

The total suspended solids (TSS) are the total amount of solids in the bulk fluid that can be trapped by a glass fiber filter (Hach, 47 mm, Cat No: 253000). Before dosing, the value of TSS of both reactors varied from 20-23 mg/mL (Figure 18). During dosing, the TSS value in the test reactor decreased from 19 mg/mL on day 352 to 12 mg/mL on day 387. On day 387, the TSS value in the test reactor again started to increase and reached the steady state value (20 mg/mL) on day 401. Throughout the dosing period, control reactor had a consistent TSS value of 18-20 mg/mL.

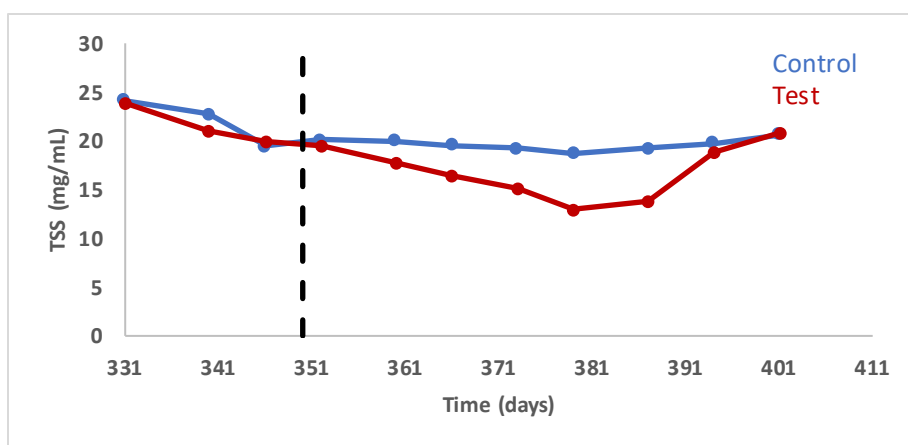


Figure 18: Effect of PFOF/PFOS on TSS of granules over time in both reactors. TSS value of control granules (blue line) and test granules (red line) are presented. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

Volatile Suspended Solids (VSS)

Volatile suspended solids (VSS) are the total amount of organic solids that are lost on combustion of the dry solids at 550°C. Volatile substances vaporize whereas nonvolatile substances do not. Before dosing, the value of volatile suspended solids (VSS) of both reactors varied from 15-19 mg/mL (Figure 19). The VSS value in the test reactor decreased from 15 mg/mL on day 352 to 9 mg/mL on day 373. On day of 379, the VSS value in the test reactor again started to increase and reached the steady state value (16.5 mg/mL) on day 394. The VSS value in the control reactor was around 14-16 mg/mL throughout the time of dosing.

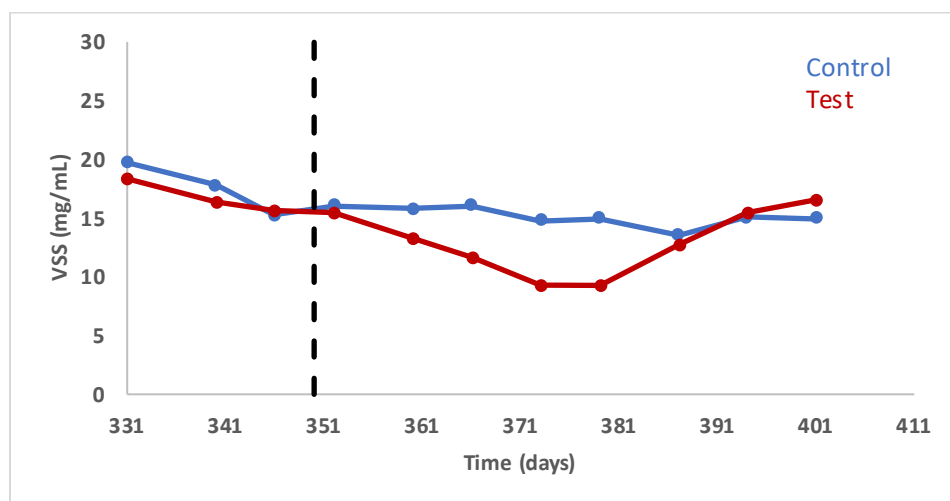


Figure 19: Effect of PFOF/PFOS on VSS of granules over time in both reactors. VSS value of control granules (blue line) and test granules (red line) are presented. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

The TSS and VSS in the test reactor decreased after dosing started and recovered within approximately one SRT. Though both reactors experienced low DO in last two weeks of operation, TSS and VSS values were hardly affected by low DO. Gonzalez et al., (2020) reported a small reduction in MLVSS and a measurable change in TSS after a two-day exposure of AFFF (aqueous film forming foam), a major source of PFAS in the environment, in WWTP. The

MLVSS recovered within 14 days after dosing [105]. The reduction in the test reactor's TSS value (Figure 18) and relatively longer time (34 days) for recovery in this experiment might be due to long term dosing (52 days) and different PFAS compounds. Yu et al., (2018) also found a reduction in suspended solids (SS) after adding PFOA to CAS and a recovery of SS after around 80 days which is consistent to the results obtained in this experiment [179]. It is reported that at the beginning of dosing, PFOA affected the microbial growth negatively and growth rate were slower than the decay rate. That might cause the reduction of SS after dosing started. Then microorganisms became adapted to the given concentration of PFOA after long term exposure which might be the reason for the recovery [179].

Effects of PFOA and PFOS Contamination on Conventional Treatment

To measure the impact of PFOA/PFOS exposure on conventional treatment, concentrations of C, N, and P in the influent, at the end of anaerobic feed phase and in the effluent were measured. 'Influent' here refers to the concentration of given species in the influent feed tubing. This is how influent concentration for nutrition (C, N, P) is typically defined in raw wastewater. Concentrations at the end of anaerobic feed phase and in effluent were measured directly from the samples collected at the end of anaerobic feed phase and at the end of cycle.

Carbon Removal

The influent and effluent concentration of total organic carbon (TOC) content in the samples collected from both reactors over time are shown in Figure 20. The fluctuation of influent TOC concentrations may be caused by substrate deterioration during the storage period. Before PFOA/PFOS addition, the influent and effluent concentrations varied from 313-290 mg/L

and 25-5 mg/L respectively. Before and after dosing, both reactors performed 93-99% removal of total organic carbon from the synthetic wastewater. The test reactor's influent and effluent concentrations of TOC were not significantly changed after being exposed to PFOA/PFOS. After PFOA/PFOS contamination, the test granules offered similar amounts of TOC removal as it did before dosing. Yu et al., (2018) found negative impact of PFOA, dosed at 20 mg/L on DOC (dissolved organic carbon) removal for CAS. It is reported that addition of PFOA negatively affected the microorganisms required to metabolize DOC, thus increased the DOC in effluent. Like SS, DOC removal also recovered after long term exposure [179]. Zheng et al., (2022) reported reduction in COD removal efficiency after adding PFOA at 2 mg/L for AGS [174]. In this experiment, no significant change in carbon removal was observed and the reason might be the low concentration (100 µg/L) of PFAS compounds used. Gonzalez et al., (2021) also did not find any measurable change in COD removal [105].

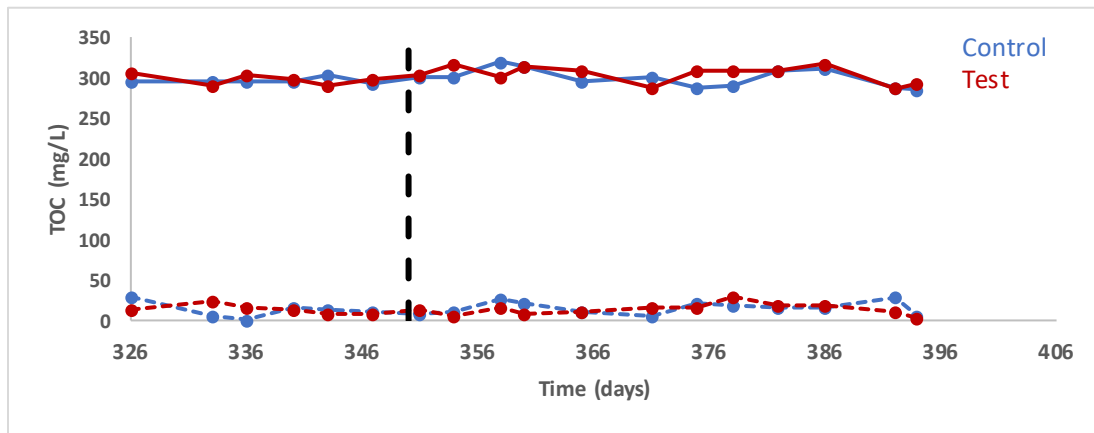


Figure 20: Effect of PFOA/PFOS contamination on carbon removal in both reactors over time. The influent concentration (solid line) and effluent concentration (dashed line) of carbon are presented for control (blue) and test (red) reactor. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

Phosphorus Removal

Typically, PAOs release phosphate (phosphorus) and uptake carbon during the anaerobic feeding phase. Phosphate is consumed by PAOs during the aeration phase, producing a net removal of P from the wastewater. The concentrations of phosphate at different phases for both reactors before and after dosing are presented in Figure 21. The influent concentration of phosphate was around 58-62 mg/L. At the end of the anaerobic phase, the concentration of phosphate was 650-780 mg/L. The effluent concentration of phosphate varied from 2-6 mg/L. Before and after dosing both reactors offered 95-99% phosphate removal. No significant change was observed in phosphate removal in the test reactor as compared to the control. Zheng et al., (2022) found decreased P removal after addition of 2 mg/L PFOA to AGS and the P removal efficiency was recovered quickly [174]. Gonzalez et al., (2021) found no significant reduction in P removal after adding AFFF to WWTP [105]. The reason for not observing significant impact in P removal in this experiment might be the low concentration (100 µg/L) of PFOA/PFOS.

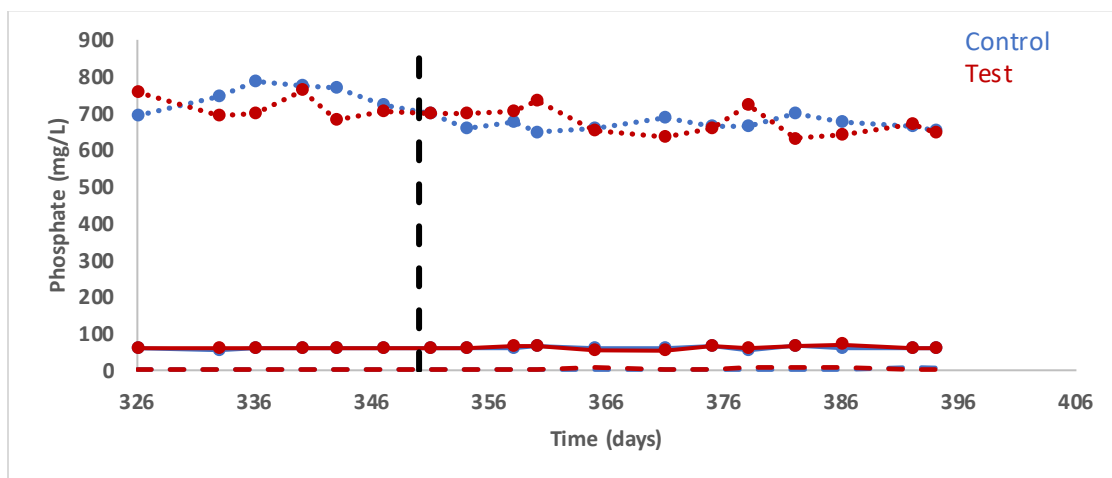


Figure 21: Effect of PFOA/PFOS contamination on phosphorus removal in both reactors over time. The influent concentration (solid line), concentration at the end of anaerobic phase (dotted line), and effluent concentration (dashed line) of phosphate are presented for control (blue) and test (red) reactor. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

Nitrification

Before dosing, the test granules were exposed to high pH due to some undesirable mechanical issues. The pH of the test reactor exceeded the set point of 6.7-7.3 and reached pH 10-11 between days 336-339. High pH affects nitrification negatively (Appendix A). Removal of nitrogen via ammonium oxidation in the presence of ammonium oxidizing bacteria (AOB) is presented in Figure 22. Before dosing PFOA and PFOS, ammonia concentration in the influent and at the end of the anaerobic feed phase were 58-61 mg/L and 19-22 mg/L respectively. Concentration of ammonia decreased from the influent to the end of anaerobic phase due to dilution inside the reactor Table 3. Volatilization of ammonia also might contribute to the decrease in ammonia concentration at the end of the anaerobic feed phase [180]. The anammox process (anaerobic oxidation of ammonium) might be another reason for decreasing ammonia concentration at end of anaerobic phase, though the presence of anammox bacteria was not confirmed in this study. Ammonia concentration in effluent was non-detectable in both reactors prior to day 336.

From day 340-371, however, ammonium oxidation was badly affected despite the recovery of the pH in the test reactor. High concentrations of ammonia were left in the effluent (20-22 mg/L). This excess ammonia in the effluent indicates that high pH likely affected AOB negatively, reducing nitrification. The rise of pH above 8 causes a shift of ammonium to ammonia in ammonium/ammonia equilibrium [181]. High concentrations of free ammonia in solution caused by high pH inhibit nitrification because free ammonia is toxic to bacteria. Dosing of PFOA/PFOS began on day 350 while the nitrification capacity was recovering.

A similar incident of poor nitrification due to high pH (Appendix A) occurred previously in the control reactor. At that time, nitrification recovered after approximately four weeks of

reactor operation. In this case, the test reactor required approximately five weeks for recovery of nitrification, with ammonia concentrations in the effluent falling below 1 mg/L after day 375 for the duration of the experiment. It appears that PFOA/PFOS exposure did not prevent the test reactor from recovering its full nitrification capacity, though the exposure may have extended the time required for recovery of AOB population. Zheng et al., (2022) found a little negative effect of PFOA on nitrification in AGS which supports the result of nitrification obtained in this experiment [174]. Gonzalez et al., (2021) and Yu et al., (2018) did not find any measurable change in nitrification due to short term AFFF exposure to WWTP and long term PFOA exposure to CAS, respectively [105, 179]. The reasons for different result obtained in this experiment might be the different PFAS compounds, length of dosing, and more than one PFAS compounds used.

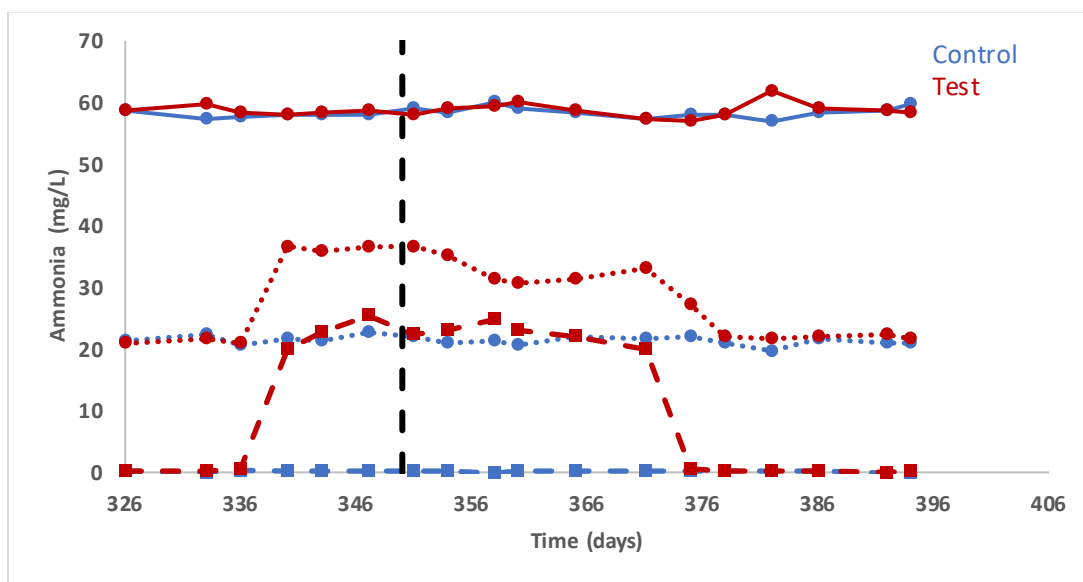


Figure 22: Effect of PFOA/PFOS contamination on nitrification in both reactors over time. The influent concentration (solid line), concentration at the end of anaerobic phase (dotted line), and effluent concentration (dashed line with square marker) of ammonia are presented for control (blue) and test (red) reactor. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

Denitrification

In the denitrification process, nitrate produced at the end of nitrification is converted to nitrogen gas by denitrifying bacteria. EPA has set the maximum contaminant level (MCL) of nitrate at 10 mg/L [182]. Before and after dosing, the concentration of nitrate in the test reactor effluent remained under the MCL (Figure 23). PFOA/PFOS exposure does not appear to have significant effect on denitrification in this experiment. Gonzalez et al., (2021) reported that denitrification does not appear to change after adding AFFF to WWTP [105]. Yu et al., (2018) confirmed that addition of 100 mg/L PFOA to CAS has detrimental effect on denitrification, whereas, 20 mg/L PFOA has no negative effect on denitrification [179].

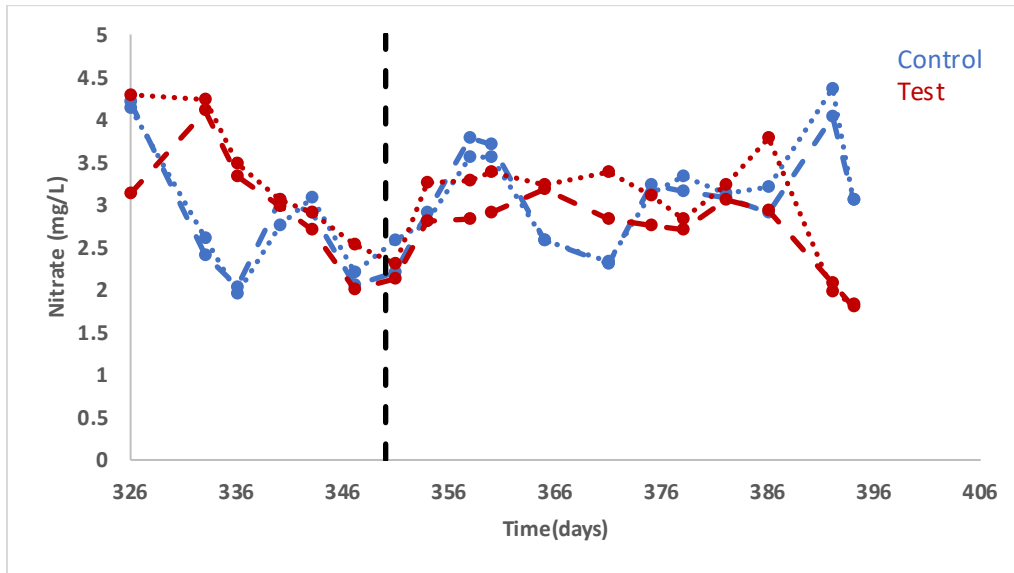


Figure 23: Effect of PFOA/PFOS on denitrification. Concentration of nitrate at the end of anaerobic phase (dotted line) and in effluent (dashed line) are presented. The vertical dashed line indicates the beginning of dosing PFOA/PFOS into test reactor.

CHAPTER FIVE

CONCLUSION

This project investigated the efficiency of AGS to remove PFOA/PFOS from synthetic wastewater and the effects of PFOA/PFOS exposure on granule's physical properties and conventional wastewater treatment. The results show that it is possible to remove a fraction of PFOA and PFOS from synthetic wastewater by AGS. The maximum removal efficiency for PFOS (100 µg/L) and PFOA (100 µg/L) obtained by AGS were 33% and 28% respectively and occurred within the first 7-10 days of dosing. After 13-14 days of dosing, the steady state performance was achieved with removal percentages of 22-24% for PFOS and 16-17% for PFOA. AGS facilitated maximum sorption for PFOS of 11 µg/g of dry sludge and steady sorption of 7-8 µg/g of dry sludge. For PFOA, the maximum and steady sorption capacity of AGS were 15 µg/g and 6 µg/g of dry sludge, respectively. The sorption capacity of CAS for PFOA/PFOS ranges from 4-10 ng/g [167], three orders of magnitude less than the findings of this study. The main pathway of removal is adsorption offered by EPS. PFOS has higher sorption propensity than PFOA due to the presence of sulfonate head in PFOS's chemical structure. PFOS has also demonstrated higher sorption on activated carbon, lab materials, and in other methods such as anaerobic digestion. Biodegradation is not a feasible pathway for removal because PFOA and PFOS are non-biodegradable.

PFOA/PFOS (100 µg/L) exposure affected the physical properties such as size of the granules, SVI, TSS, and VSS negatively. These properties recovered after approximately one SRT despite low DO during the last two weeks of operation. The negative effects of PFOA/PFOS exposure on granules' physical properties are also confirmed by other literature

sources. Granules may have adjusted to the toxicity of PFOA/PFOS after long term exposure, and thus, recovered after one SRT (34 days). More research should be done to understand the strategy for this recovery. PFOA/PFOS (100 µg/L) contamination had no measurable impact on removal of C and P or on denitrification despite low DO and unintentional high pH. Nitrification was affected by high pH in the test reactor. PFOA/PFOS exposure appears to have delayed the nitrification recovery. Other literature demonstrated some different results for other PFAS compounds and concentrations, dosing length etc.

This project is only focused on removal of PFOA/PFOS from synthetic wastewater. There would be many other PFAS compounds present in different environmental matrices including soils and biosolids. It is necessary to develop methods to remove other PFAS from different environmental matrices.

As a concern of this project, proper disposal of sludge contaminated with PFOA/PFOS should be ensured. Contaminated sludge can be incinerated and disposed to a site where ground water layer is much deeper than the ground surface to prevent the infiltration or percolation of PFOA/PFOS to ground water. To increase the PFOA/PFOS removal percentages by AGS, sorption of PFOA/PFOS on lab material and the stability of EPS sorption need to be analyzed. The operational parameters of SBR can be readjusted for better treatment efficiency.

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APPENDICES

APPENDIX A

UNDESIRABLE PHENOMENA IN REACTOR OPERATION

Effect of High pH on Nitrification

A negative effect of high pH on the nitrification process in the control reactor was observed (Figure A. 1). On day 128, due to an error by the pH sensor, excessive base solution was added to the control reactor. The pH level of the control reactor exceeded the set point 7.3 and reached pH 10-11. The granules were taken out of the reactor immediately and washed with DI water. Then the washed granules were placed again in the reactor. It took approximately 1-2 days for the pH to return to the set point of 6.7 -7.3. During this time, the granules also produced foam (Figure A. 1b). Due to the high pH, a low nitrification rate and high effluent ammonia concentrations were observed. Complete nitrification happened approximately after 4 weeks from the day of occurrence. High pH also made the granules floating and filamentous (Figure A. 1a). The SVI_{10} value changed from 12 mL/g to 17 mL/g.

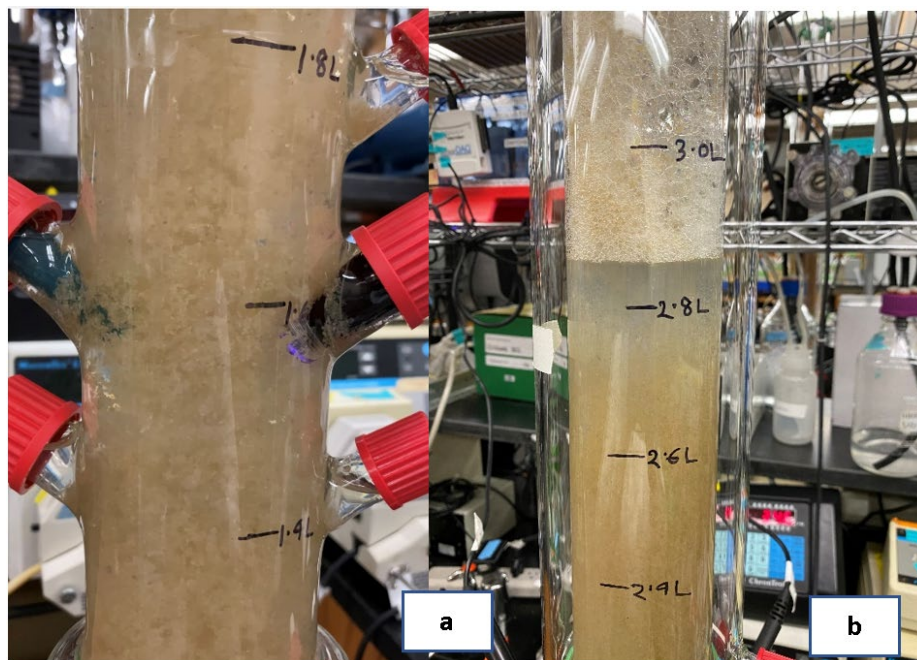


Figure A. 1: (a) Effect of high pH on granules structure: (b) Foam production by granules at high pH.

A pH range of 7-7.5 is favorable for granulation and the nitrification rate depends on granular structure, as well as pH [110, 111]. Antoniou et al. (1990) reported that the number, growth, and activities of nitrifying bacteria decreased due to the high pH. And due to lower number and poor performance of nitrifying bacteria at high pH, the nitrification rate became reduced (Figure A. 2) [183].

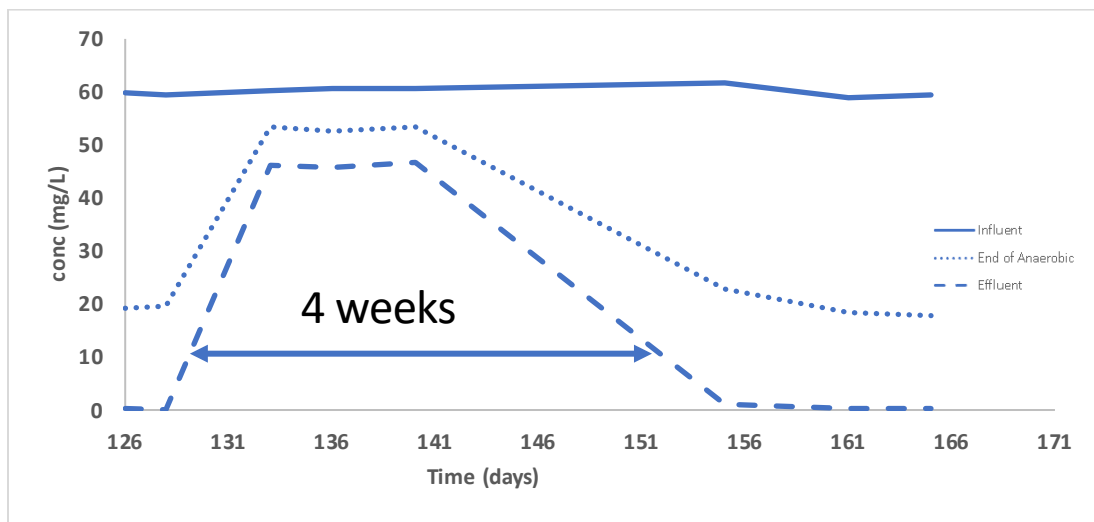


Figure A. 2: Concentration of ammonia in influent (solid line), effluent (dashed line) and at end of anaerobic phase (dotted line). High pH affected nitrification negatively over the period day 128-154.

Effect of Short SRT on Nitrification

The effect of short SRT on nitrification process is shown in Figure A. 4. Between days 231-245, SRT of the control reactor was maintained at 40 days. Then the value of SRT decreased from 40 to 22 days on day 253 (Figure A. 3). The purpose of short SRT was to ensure new granules in the reactor. The short SRT of 22 days was maintained until day 297. Within this short SRT period, a gradual reduction in the nitrification process was observed, continuing until day 319. During this period, high effluent ammonia concentrations were observed. At steady state,

the concentration of ammonia in effluent was very negligible. But after being affected by short SRT, the concentration of ammonia in effluent increased to 14-27 mg/L and continued till day 319. On day 308, a longer SRT of 39 days was provided to improve the nitrification. The nitrification process again reached steady state performance on day 322.

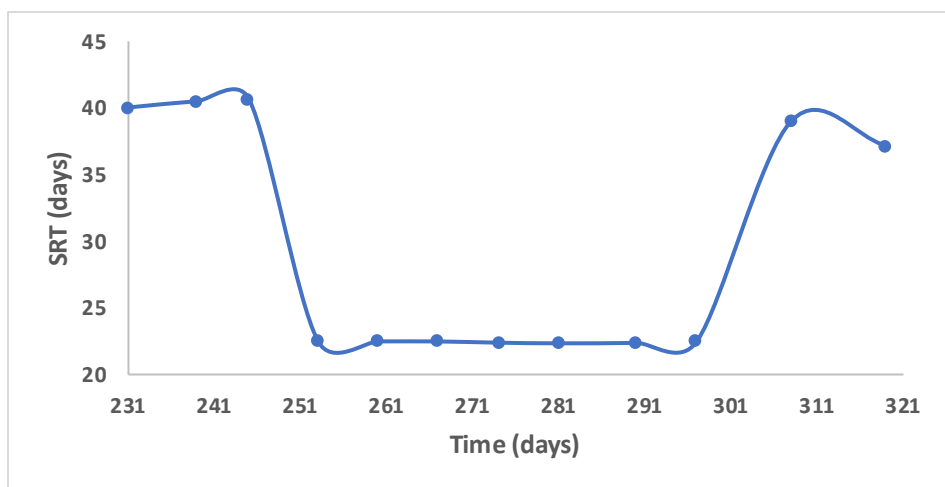


Figure A. 3: Different SRT determined in control reactor overtime to see the impact on nitrification.

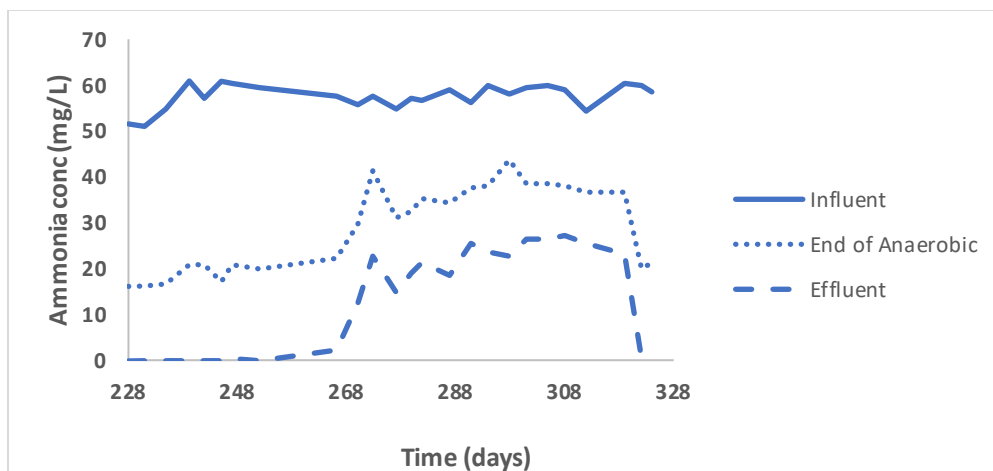


Figure A. 4: Concentrations of ammonia in influent (solid line), effluent (dashed line) and at the end of anaerobic phase (dotted line). Effect of short SRT on nitrification process is depicted.

Solid retention time is also called sludge age. This time indicates how long a granule stays inside a reactor. An optimal SRT greater than 15 days should be maintained to ensure new granules in the reactors. New granules will ensure better treatment since they would have higher activity [110]. When SRT was decreased from 40 days to 22 days, it caused washout of significant amounts of nitrifying bacteria. Nitrifying bacteria are slow growing microorganisms. They need a longer time to grow inside the reactor [95]. If the SRT is less than the time required for the growth of nitrifying bacteria, poor nitrification will take place due to the washout of nitrifying bacteria [95, 111]. The behavior of the control reactor can be explained this way where nitrifying bacteria were washed out due to the short SRT and thus, the nitrification process was affected in adverse way.

Effect of Carbon Loading on Denitrification

The significance of carbon loading to ensure proper denitrification process is presented Figure A. 5 . In the denitrification process, denitrifying bacteria take in nitrate and convert it to nitrogen gas. At steady state, the concentration of nitrate in effluent was 2-4 mg/L which is below maximum contaminant level (MCL) for nitrate of 10 mg/L set by EPA [184]. In the control reactor, poor denitrification was observed between days 228-238. At the end of the 3-hour cycle, the effluent containing 22-37 mg/L of nitrate proved improper denitrification in the control reactor. To improve this situation, the concentration of carbon in the substrate was increased from 160 mg/L to 350 mg/L (Figure A. 6). On day 239, complete denitrification occurred where effluent concentration of nitrate was 1.4 mg/L. This improvement in denitrification was achieved with the increased carbon loading as influent to reactor.

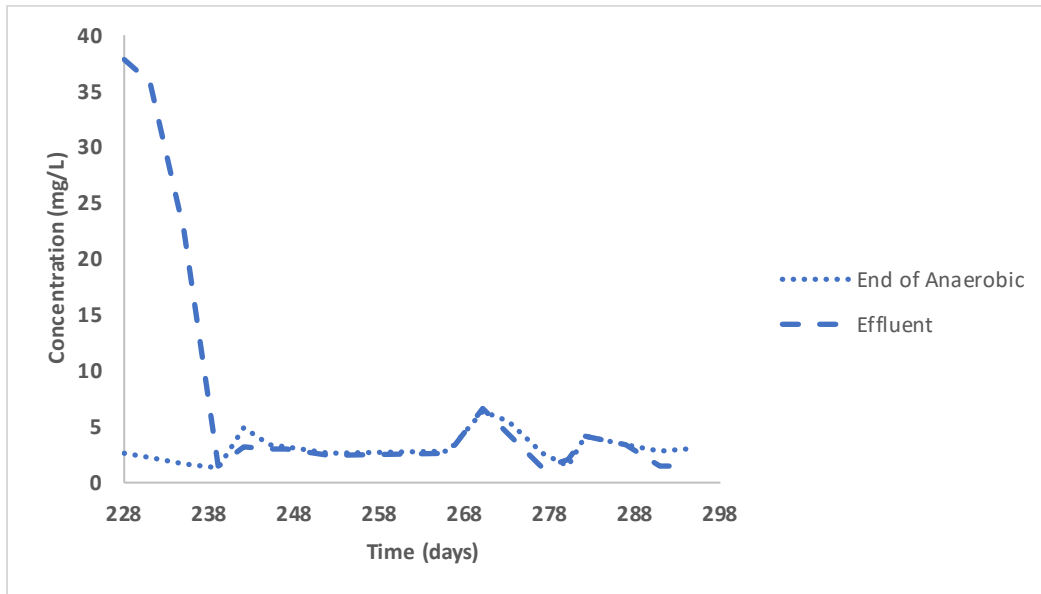


Figure A. 5: Concentration of nitrate at the end of anaerobic phase (dotted line) and in effluent (dashed line). Incomplete denitrification took place on day 228-238 in reactor and high concentration of nitrate left in effluent at the end of 3 hours cycle.

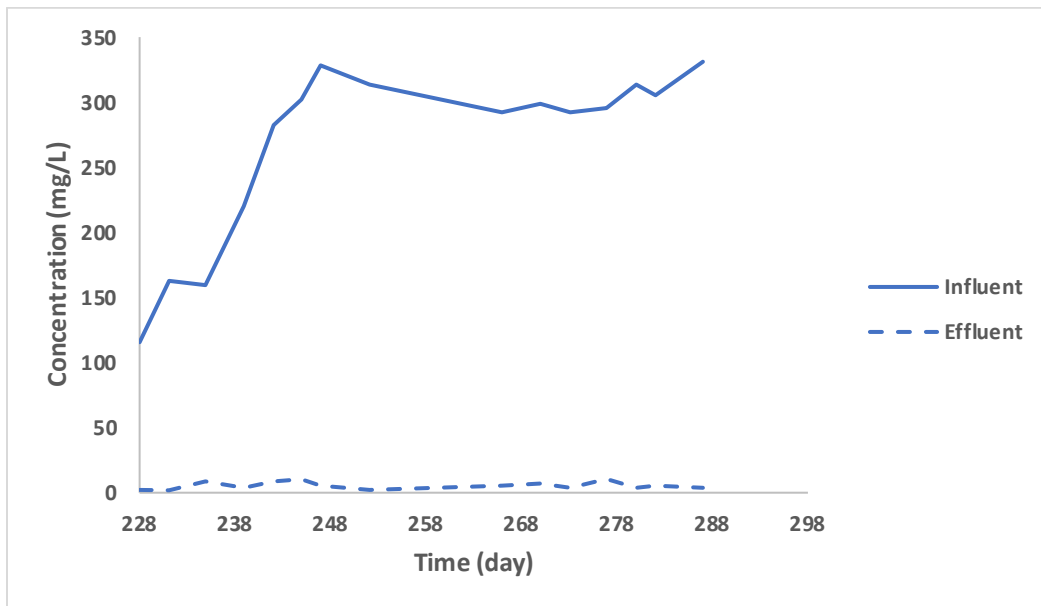


Figure A. 6: Carbon concentrations are presented in influent (solid line) and effluent (dashed line). Increase in carbon concentration offered better denitrification.

There are several factors affecting the performance of simultaneous nitrification and denitrification (SND) in reactor such as dissolved oxygen (DO), pH, C and N concentration,

SRT, organic loading [111, 185]. Among them, the carbon content (organic loading) is significant. An increase in organic loading to the reactor will accelerate the denitrification process. Hou et al. (2021) reported that a range of organic loading rate (1.5-9 kg COD/m³. d) helped keep long-term stability and nutrients removal efficiency of aerobic granules. Too low value of OLR can lead to the proliferation of filamentous bacteria in the granules, which makes the granular structure loose, porous, and less stable [86]. Lui et al (2015) found that AGS cultured under an OLR of 12 kg COD/m³. d can grow faster but gradually expand and disintegrate. On day 228-238, the OLR value was approximately 0.7 kg COD/m³. d in the control reactor which reduced the stability of granules and denitrification rate.