UNIVERSITY OF MANITOBA

Domestic wastewater treatment for biological phosphorus removal by integrated fixed-film activated sludge sequencing batch biofilm reactor IFAS-SBBR

by

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Abstract

Phosphorus is considered a limiting nutrient with respect to eutrophication of surface water bodies and of great concern to governments regulating wastewater treatment plant effluents. Issues associated with eutrophication include increased algal biomass, decreased water transparency, low dissolved oxygen (DO) levels, increased fish mortality and more frequent incidences of toxic phytoplankton. The current phased research investigates phosphorus removal from medium strength domestic wastewater using an integrated fixed biomass in an activated sludge sequencing batch biofilm reactor (IFAS-SBBR) owing to concurrent nutrient removal. Research findings include phosphorus uptake and release correlation coefficients of 0.339 and 0.877 for the AS-SBR and IFAS-SBBR respectively favoring the IFAS reactor. This is further supported by acetic acid utilization data showing a correlation coefficient of 0.593 and 0.987 for the AS-SBR and IFAS-SBBR respectively. The anaerobic mass fraction may have promoted concurrent nutrient removal by extending the anaerobic stage 30% to 120 minutes, promoting settled sludge in delaying anaerobic mixing, and when considering anaerobic sublayers of the IFAS-SBBR. Taken together, acidogenic co-fermentation of rbCOD is implicated since the current research found nearly complete phosphorus removal with or without an adequate supply of influent VFAs.

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"Perfection is Achieved Not When There Is Nothing More to Add, But When There Is Nothing Left to Take Away" Antoine de Saint-Exupery.

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Abbreviations

AGS: Aerobic Granular Sludge

AOB: Ammonia Oxidizing Bacteria

ATP: Adenosine Triphosphate

BOD: Biochemical Oxygen Demand

BNR: Biological Nutrient Removal

CFSTR: Continuous-Flow Stirred Tank Reactor

CFP: Continuous Flow Plant

COD: Chemical Oxygen Demand

DI: Deionized

DO: Dissolved Oxygen

EBPR: Enhanced Biological Phosphorus Removal

EPS: Extracellular Polymeric Substances

FIA: Flow Injection Analysis

F:M: Food to Microorganism Ratio

FNA: Free Nitrous Acid

GAO: Glycogen Accumulating Organisms

HRT: Hydraulic Retention Time

HPLC: High Purity Liquid Chromatograph

IFAS: Integrated Fixed-film Activated Sludge

MLSS: Mixed Liquor Suspended Solids

MLTSS: Mixed Liquor Total Suspended Solids

Abbreviations

MLVSS: Mixed Liquor Volatile Suspended Solids

NOB: Nitrite Oxidizing Bacteria

OHO: Ordinary Heterotrophic Organisms

ORP: Oxygen Reduction Potential

PAO: Phosphate Accumulating Organisms

PFR: Plug Flow Reactor

PHA: Polyhydroxyalkanoates

rbCOD: Readily Biodegradable Chemical Oxygen Demand

SBR: Sequencing Batch Reactor

SCVFA: Short Chain Volatile Fatty Acids

sCOD: Soluble Chemical Oxygen Demand

SRT: Solids Retention Time

SVI: Sludge Volume Index

TN: Total Nitrogen

TP: Total Phosphorus

TSS: Total Suspended Solids

VFA: Volatile Fatty Acids

VSS: Volatile Suspended Solids

WAS: Waste Activated Sludge

WRRF: Water Resource Recovery Facility

Introduction

In recent years it has become evident that "the old ways just won't cut it" with respect to municipal wastewater treatment systems. Environmental and process design engineers, with few exceptions, are expected to design cost effective nutrient removal solutions with emphasis on nitrogen and phosphorus. Phosphorus is considered a limiting nutrient with respect to eutrophication of surface water bodies and of great concern to government regulators. Issues associated with eutrophication include increased algal biomass, decreased water transparency, low dissolved oxygen (DO) levels, increased fish mortality and more frequent incidences of toxic phytoplankton (Carey & Migliaccio, 2009).

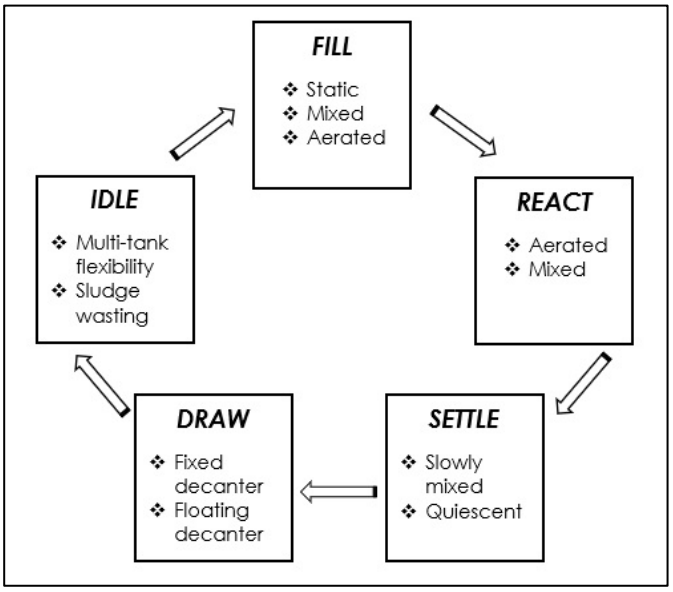
Wastewater treatment plants (WWTPs) traditionally receive municipal wastewater for purification by physical and biochemical systems. In recent years, general scarcity of natural resources has prompted consideration of wastewater as a renewable resource. To this end WWTPs are now viewed as water resource recovery facilities (WRRFs) from which valuable products like chemicals, nutrients, bioenergy and purified water can be harvested. For example, technologies for phosphorus removal from wastewater traditionally include physicochemical precipitation supplemented since the 1990s with enhanced biological phosphorus removal (EBPR) (Lizarralde et al., 2019).

Sequencing batch reactor (SBR) technology is ideally suited to incorporating alternating anaerobic and aerobic stages associated with biological phosphorus removal (BPR). In the anaerobic stage, phosphorus is released into the bulk liquid by a group of microorganisms called phosphorus accumulating organisms (PAOs). As a result, high soluble phosphorus concentrations occur in this stage. The phosphorus release is typically accomplished by an appreciable consumption of soluble organic substrates and will not occur unless oxygen and oxidized nitrogen are both absent. The aerobic stage that follows, reduces bulk solution soluble phosphorus concentration to less than 1 mg L^{-1}. In a typical SBR tank, treatment is divided into five discrete time periods: Fill, React, Settle, Draw,

and Idle. Overall control of the system is accomplished with level sensors and a timing device or microprocessor.

Figure 1

Different stages of SBR operation cycle



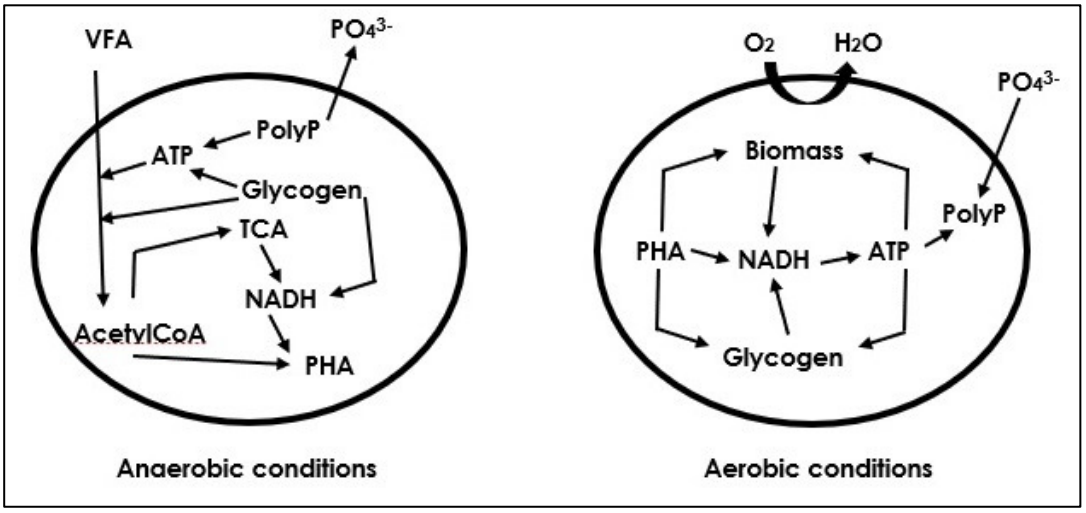
The sequencing batch reactor operation stages depicted in Figure 1, demonstrate the versatility of the SBR system to treating wastewater. A cycle begins with the fill stage by receiving raw wastewater. Variations include ***Static Fill*** (high F:M, no mixing or aeration, suitable for biological phosphorus removal). ***Mixed Fill*** (mixing or organic influent with biomass, anoxic environment for denitrification). ***Aerated Fill*** (aeration to begin reactions occurring in the react stage). Desired biochemical reactions occur during the react stage. ***Aerated React*** (aerobic reactions initiated during aerated fill are completed, nitrification). ***Mixed React*** (anoxic condition for nitrogen removal and anaerobic conditions for phosphorus removal). The ***Settle*** stage is when microorganisms are separated from treated effluent under gravity. During the ***Draw*** stage or decant, treated effluent is discharged. Lastly, the ***Idle*** stage is time allotted between discharge and fill, intended to provide operational flexibility and to waste biomass.

SBR waste activated sludge release typically occurs once per treatment cycle and may take place during one of the stages as follows. Wasting can occur near the end of the react, or during settle, draw, or idle. Waste activated sludge (WAS) release is used for controlling sludge age, also known as solids retention time (SRT). WAS wasting is also how phosphorus leaves the SBR system each cycle (i.e., settled biosolids). In support of the claim that the release and consumption of phosphorus is biologically mediated, phosphorus containing volutin granules (i.e., intracellular storages of complexed inorganic polyphosphate, poly-P), have been determined to increase and decrease in the aerobic and anaerobic stages respectively (Manning & Irvine, 1985).

Phosphorus accumulating organisms (PAOs) have the ability to take up wastewater short chain volatile fatty acids (SCVFAs) under anaerobic conditions, and store them as polyhydroxyalkanoates (PHAs). The energy required for this transformation is mainly obtained from the hydrolysis of poly-P, Figure 2. In the aerobic stage, soluble phosphorus is taken up and stored again as poly-P, but in excess of metabolic requirements (termed luxury uptake).

Figure 2

Metabolic pathways of PAOs under aerobic and anaerobic conditions; polyhydroxyalkanoates (PHAs); and polyphosphate (PolyP)



Integrating attached fixed film biomass to the biochemical activated sludge floc (IFAS) has numerous advantages. When the approach is applied to a sequencing batch reactor (SBR), it results in a sequencing batch biofilm reactor (SBBR) or alternatively an IFAS-SBBR, in a sink-source application, for available chemical oxygen demand (COD) oxidation. Biofilm can be desirable to augment suspended floc activated sludge reactors when high biochemical enrichment is required, independent of hydraulic load and sludge settling character. Batch reactors treat substrate by cycling through a high load (fill) stage followed by a low substrate concentration (react) stage. Together the stages are called a feast-famine regime. The regime embodies microbial uptake and storage of soluble COD (feast) followed by metabolic utilization of stored substrate (famine) during the react stage. The SBBR can function as a sink-source system when influent substrate and nutrients becomes integral to the biofilm by means of adsorption, ion exchange, or absorption processes (Wilderer & McSwain, 2004).

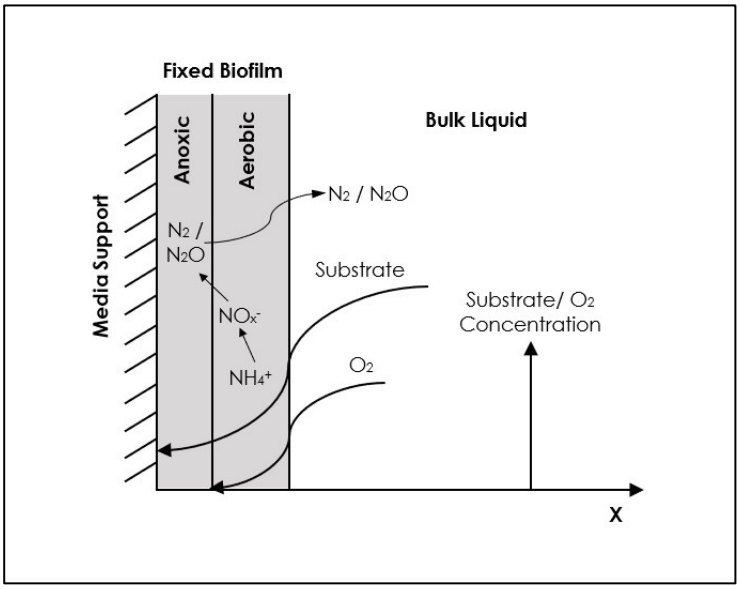
Enhanced biological phosphorus removal (EBPR) requires sufficient influent carbon in the form of either short-chain volatile fatty acids (SCVFAs) or readily biodegradable COD (rbCOD) that could be converted into VFAs in the anaerobic zone. Fermentation of mixed liquor, anaerobically, may be attainable to augment the influent soluble carbon and achieve reliable phosphorus removal. Many of the statements about the reliability of the EBPR process ignore the fact that about 8 mg L^{-1} of VFAs are required to remove 1 mg L^{-1} of phosphorus and if not available, phosphorus removal will suffer. Some rbCOD can be converted to VFAs by fermentation in the anaerobic zone thus a better measure of the potential of a plant to remove phosphorus is to ensure a rbCOD:P ratio of greater than 14 (Barnard & Kobylinski, 2014).

Research found that during the aeration stage, at the low influent ammonium (NH_4^+-N) concentrations, the SBBR removed NH_4^+-N, however, only a small amount of nitrate (NO_3^--N) was measured. This result might be caused by the concurrent nutrient removal. Even though sufficient dissolved oxygen (DO) concentration was maintained in solution, the inner layer of biofilm may be kept anoxic because of the oxygen diffusion

limitation into biofilm was occurring. The external aerobic biofilm thickness can be about 1mm, providing nitrifiers in this biofilm layer with adequate dissolved oxygen concentrations, whereas the denitrifiers are preferentially active in deeper internal biofilm with very limited dissolved oxygen concentrations, Figure 3. At the low influent ammonium concentration, the released and phosphorus uptake amounts in SBBR were lower than those in SBR because the biofilm in SBBR might experience diffusion limitation that could affect P removal.

Figure 3

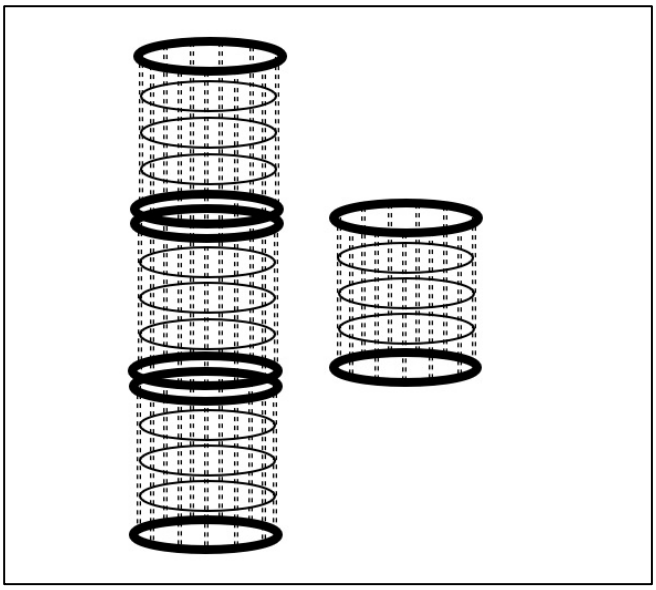
Schematic of fixed biofilm showing dual limiting concentration nutrient profiles



Fixed media supporting the biomass of the IFAS-SBBR comes in many varieties. For the research, the media used is similar in function to the polyvinyl chloride or polyethylene rope or ribbon-type media product consisting of plastic fibres netted into continuous strands, looped in rows, along a plastic ribbon, Figure 4. The ribbon is attached to a cage-frame and wound in a spiral of loops resulting in a rope-like appearance. Since the media is not self-supporting, it is mounted on a fixed lattice or frame and bolted to the reactor cover underside, ensuring fixed biomass is fully immersing.

Figure 4

Cage-frame type support and polyvinyl chloride or polyethylene rope or ribbon-type media for integrated fixed film activated sludge application



Volatile fatty acids (VFAs), shown in Figure 2, uptake per released phosphorus increases linearly with the influent readily biodegradable (rbCOD) concentration. The polyhydroxyalkanoates (PHAs) accumulation, internal to the microorganisms, is still the major factor in the promotion of phosphorus release. More importantly, the accumulated PHAs would be completely utilized for phosphorus uptake although only extrinsic biofilm can produce and accumulate PHAs. The effect of PHAs accumulation and COD utilization on the biofilm phosphorus removal process is consistent with the effect on suspended culture performing EBPR process. Although the mechanism of COD utilization for PHAs formation is similar, it maybe that its reaction in a suspended culture is more direct and quicker than that in a biofilm culture. In other words, the biofilm could quickly adsorb the substrate on the biofilm's surface, but the adsorbed substrate was not capable of being transformed rapidly to PHAs (Chiou & Yang, 2008).

Fixed biomass detachment and attachment is a random process caused by local instabilities within the physical biofilm structure in combination with external forces

including shear forces caused by fluid flow or random collisions of particles. As a result, biofilm thickness will vary from location to location on fixed media and from time to time. Detachment processes fall into four categories distinguished as, abrasion, erosion, sloughing and predator grazing. While rates of detachment and attachment are given as a function of many different parameters including biofilm thickness, shear stress, growth rate, and density, most rate expressions have one feature in common. That is, they lead to a constant biofilm thickness under constant operating conditions (Eberhard Morgenroth & Wilderer, 2000).

Hypothesis and Objectives

Hypothesis

Phosphorus removal treatment for domestic wastewater should be enhanced by integrated fixed biomass in an activated sludge sequencing batch biofilm reactor IFAS-SBBR, owing to concurrent nutrient removal, when contrasted with a conventional activated sludge sequencing batch reactor AS-SBR.

Objectives

- Explore nutrient removal, under quasi stable conditions, using wastewater quality parameters including chemical oxygen demand, oxidation reduction potential, pH, and dissolved oxygen.
- Improve nutrient removal by the integration of acidogenic co-fermentation of influent soluble carbon anaerobically, to augment VFAs for enhanced biological phosphorus removal.
- Explore nutrient removal, through wet acid digestions of mixed liquor suspended solids, both bulk and fixed biomass, tracking fate-and-effect of the biological phosphorus removal.
- Develop nutrient removal, under anoxic conditions, by denitrifying phosphorus accumulating heterotrophs with a range of influent wastewater carbon-to-nitrogen and carbon-to-phosphorus ratios.

Literature Review

Sequencing Batch Reactor (SBR)

Sewage purification in the late 19th century involved chemical precipitation or filtration, or a combination of the two. Activated sludge systems began in the USA and England with simple experiments in which air was blown into the basins containing wastewater. The expectation for oxidation of contaminants failed because the experiments did not adequately recognize the requirement for higher concentrations of suspended microorganisms. Students Ardern and Lockett at Lawrence Experimental Station, Manchester, England, demonstrated activated sludge treatment by not discarding biological humus or the deposited solids formed during the cycle aeration of sewage in a fill-and-draw system (Wilderer et al., 2001).

Specifically, their earlier laboratory experimental work treating raw sewage from Manchester showed that 5-weeks of continuous aeration was needed for complete nitrification. Ardern and Lockett (1914), then decanted the clear supernatant, added a second sample of raw sewage, and aerated the sample "in contact with the original deposited matter" until nitrification was again completed (as quoted in Wilderer et al., 2001, p.8). This process was repeated many times. Their findings where that as the deposited matter increased, the time required for each succeeding oxidation gradually diminished until eventually it was possible to completely oxidize a fresh sample of raw sewage within 24-hours, calling the technology "activated sludge" (Wilderer et al., 2001).

Ardern and Lockett made observations on a number of factors regarding aeration. They apparently where reflecting on what was either concurrent or simultaneous nitrification and denitrification when they noted the aeration intensity had a marked effect on the nitrogen balance. They linked ammonia removal efficiency to both aeration cycle times and sludge mass; in their statement, (Wilderer et al., 2001) suggested that, "Better effluents were obtained, without an increased expenditure of total volume of air, by using higher proportion of sludge" (p.8).

The typical operation protocols, Table 1, proposed by Ardern and Lockett (1914) are reflected; in their statement, Wilderer et al. (2001) state that, "From the point of view of practicality, when working on the fill-and-draw system, the proportion of 1 volume of sludge to 1 volume of sewage should not be exceeded, mainly on the account of the difficulty of settlement of the sludge" (p.8).

Table 1

Proposed draw-and-fill SBR protocols

Operation	Time for 20% Sludge (min.)	Time for 40% Sludge (min.)
Fill	60	40
React	240	120
Settle	120	120
Draw	60	40

In the USA, full scale variable-volume activated sludge treatment appears to have first been used in 1915 in Milwaukee (Wisconsin). In 1915-1916 fill-and-draw systems were tested in Brookland (New York), Chicago (Illinois), Cleveland (Ohio), and Houston (Texas), in the USA. Virtually all of the USA full-scale fill-and-draw systems placed into operation between 1914 and 1920 were converted to continuous flow systems, despite observations at the time that for the same level of treatment, twice the time is required in the continuous sewage liquor as opposed to fill-and-draw system treatment (Wilderer et al., 2001).

Three major reasons were given by Ardern in 1927 for the switch from fill-and-draw to continuous flow systems (Wilderer et al., 2001). They were:

- High dissipation of energy during the draw (high discharge flow rate relative to that of the influent).
- Clogging of coarse bubble diffusers resulting from repeated settlement of the sludge on the diffusers, and

- Increased operator attention resulting from the need to switch valves and clean diffusers.

Although multiple-tank SBR facilities and vast improvements in aeration devices and control systems have now eliminated these concerns, periodic process remained dormant in the USA until 1940s and in Europe until 1959 (Wilderer et al., 2001).

The SBR is a time-oriented, periodic process that can be designed and operated to simulate virtually all conventional continuous-flow activated sludge systems. Mathematically, the SBR models as a continuous-flow stirred tank reactor (CFSTR) followed by a plug-flow reactor (PFR), which is the ideal configuration in terms of tank volume requirements for conventional continuous-flow activated sludge systems. The inherent unsteady-state nature of the time-based SBR can be magnified by judiciously alternating aerobic, anoxic, and anaerobic conditions. In this way organisms ordinary selection pressures, associated with natural variation in the wastewater, can be minimized and the desired organism distribution can be enriched and maintained (Irvine & Ketchum, 1989).

Essentially, the difference between the SBR and a conventional continuous-flow activated sludge system is that each SBR tank carries out functions such as equalization, aeration, and sedimentation in time rather than in space sequence. One advantage of time sequence used in the SBR, is flexibility of operation. The total time in the SBR is used to establish the size of the system and can be related to the volume of a conventional continuous-flow facility. As a result, the fraction of time devoted to a specific function (i.e., stage) in the SBR is equivalent to some corresponding selector tank (i.e., aerobic, anoxic, anaerobic) in a space-oriented system. In the conventional-flow activated sludge facility, the selector tank volumes are fixed and cannot be shared or redistributed as easily as with the sequencing batch reactor (Irvine & Ketchum, 1989).

Fill and React in a typical SBR may have several possible different phases based on aeration and mixing policies. The SBR can employ one or more sets of tanks, with each set having a common cycle. Theoretically, there is no limit to the size of each tank or the

number of tanks in a set. A single tank SBR system would be unusual for a normal domestic wastewater application, but not at all that uncommon for day schools, amusement parks, and industries which operate 8 to 20 hours in a day with little or no flow generated during the remaining hours (Irvine & Ketchum, 1989).

Activated Sludge vs Fixed Biomass

There are many ways to characterize biological wastewater treatment systems. Perhaps the most common characterization distinguishes fixed-film systems, in which organisms grow attached to surfaces, from activated sludge systems, in which the organisms grow in suspension. In both cases, mixed, rather than pure, cultures of microorganisms convert (oxidize) contaminants present in wastewaters to new cell mass, carbon dioxide, water, and other end products which depend upon the nature of the contaminants and the organism distribution present (Irvine & Ketchum, 1989).

A variation on the activated sludge SBR is the sequencing batch biofilm reactor (SBBR), which is a combination of suspended and attached growth biomass. Biofilm grows at a solid-fluid interface by attachment to the support material. It provides an opportunity to slow growing microorganisms to proliferate, irrespective of the hydraulic retention time (HRT), and sedimentation characteristics of the bio-aggregates. The selection of support material and its size depend on the characteristics of the wastewater and the treatment objectives. The reactor may be packed with the support material or it may be suspended in the reactor fluid. A typical SBBR cycle has fill, react, and draw stages only. Plug flow conditions exist an SBBR. The time required for washing of the support media may be considered analogous to the settling time of an SBR. Due to excessive head loss and sloughing off risk, the SBBR systems are unsuitable for influent with high TSS and when excessive microbial growth is expected (Dutta & Sarkar, 2015).

A key distinguishing characteristic of the fixed biomass reactor is the microorganisms live on a biofilm attached to a surface. This implies that electron donors, and electron acceptors, and all other nutrients must be transported to the microorganisms within the biofilm by diffusional and other mass transport processes. It is the necessity to

consider the combined effects of mass transport and reaction that makes designing for biofilm systems different, and more complex, than suspended growth systems (Grady et al., 2011).

Substrate concentration will always be lower in the biofilm than the bulk fluid. Furthermore, because of consumption, the substrate concentration will continue to drop with depth in the biofilm. In order for the consumption to continue, substrate must be transported from the bulk fluid to the liquid-biofilm interface by molecular and turbulent diffusion. It must also be transported within the biofilm. The net effect is to cause a substrate concentration profile that looks something like Figure 3. In this instance, the observed substrate consumption rate depends on the rate of mass transport external to and within the biofilm.

External mass transfer is generally assumed by idealizing the substrate concentration profile in the bulk liquid as shown inf Figure 3. The variation in substrate concentration is restricted to the hypothetical stagnant liquid film thickness through which substrate must be transported to reach the biofilm. Consequently, the substrate concentration throughout the remaining fluid, (i.e., the bulk liquid phase), is constant. All resistance to mass transfer from the bulk fluid to the biofilm is assumed to occur in the stagnant liquid film, boundary layer.

The growth and substrate utilization kinetics described for the suspended growth process relates to the dissolved substrate concentration in the bulk liquid. For attached growth processes, substrate is consumed within a biofilm. Depending on the growth conditions and the hydrodynamics of the system, the biofilm thickness may range from 100 microns to 10 mm. A stagnant liquid boundary layer (diffusion layer) separates the biofilm from the bulk liquid that is flowing over the surface of the biofilm or is mixed outside of the fixed film. Substrates, oxygen, and nutrients diffuse across the stagnant liquid layer to the biofilm, and products of biodegradation from the biofilm enter the bulk liquid after diffusing across the stagnant liquid layer. Substrate concentration decreases with biofilm depth as the substrate is consumed and diffuses into the biofilm layers. As a result, the

process is said to be diffusion limited. Substrate and oxygen concentrations within the film are lower than the bulk liquid concentration and change with biofilm depth and the substrate utilization rate. The overall substrate utilization rate is less than would be predicted based on the bulk liquid substrate concentration (Tchobanoglous et al., 2003).

Biofilm systems are distinguished from activated sludge systems by the fact that the mass flux of material between the bulk liquid and the microbial aggregate is one-dimensional. Transport processes proceed mainly perpendicular to and from the surface (substratum) to which the biofilm adheres. Thus, diffusion limitations are common in biofilm systems. As a result, only a fraction of the biofilm can contribute to the overall metabolic processes (Wilderer et al., 2001).

Quantitatively, the active fraction of the biofilm is affected by the following:

- The concentration of substrates, electron donors and electron acceptors in the bulk liquid.
- The actual metabolic rates within the biofilm.
- The thickness of the biofilm.
- The thickness of the concentration boundary layer at the biofilm fluid interface which by itself depends on the hydrodynamic conditions in the bulk fluid and the morphology of the biofilm.

Additionally, low inlet concentrations and dilution effects as a result of high volumetric recycle rates (i.e., required to achieve high filter velocities and thus enhanced mass flux into the biofilm) can decrease the treatment efficiency of a biofilm system. In general it can be assumed that the efficiency of biofilm reactors increases with decreasing biofilm thickness and with increasing surface area of the biofilm support material relative to the volume of the reactor (Wilderer et al., 2001).

In recent years, researchers have demonstrated bench scale Phosphate recovery using fixed media biomass apparatus arranged as a trickling filter. The research employed biological phosphorus removal by circulated readily biodegradable carbon (rbCOD)

substrate (anaerobic, feast) recovery solution, alternately with synthetic (5.0 mg PO_4^{3-}-P L^{-1}) wastewater feed (aerobic, famine) through a fixed biomass reactor. The repeating 16-hour feast-famine cycle continuing for 250 research days. By day 100 of research, the recovery solution concentration increased to 100 mg PO_4^{3-}-P L^{-1}, remaining above this concentration for the remainder of study. Phosphorus contents of the fixed biofilm measured on day 150 were 87.4 and 77.5 mg P g^{-1} (dry weight) at the ends of aerobic and anaerobic stages. The difference of these weights is believed to denote the phosphate quantity assimilated or released by the fixed biofilm phosphorus accumulating organisms (PAOs) during each cycle accounting for only 11% of the biomass mean phosphorus content. This suggests that most of the phosphate remains with the biofilm at the end of the anaerobic stage (Kodera et al., 2013; Zhang et al., 2021).

Published research has found mechanisms of in situ sludge reduction associated with different biomass carrier materials. Physical and chemical properties of carriers influenced sludge reduction but only in a small way compared with sludge reduction caused by energy-uncoupling metabolism. This uncoupling was explained as follows, biofilm shedding, results from excessive accumulation of suspended solids in the carriers. The article described a slight increase in MLSS from first-media type reactor compared with the alternative second-media type reactor with variations in MLSS from slow increase, followed by a decrease, and finally an increase (Wang et al., 2018).

The article went on to say, biofilms in the first-media reactor were not as stable as those in second-media reactor. The attached biofilm could fall off under shear by the bulk water flow in the aeration stage, inducing an increase in the MLSS concentration and the content of decayed microorganisms. It was believed, the thickness of the biofilm could increase transmission resistance and restrict the transfer of external carbon sources to the biofilm interior, resulting in a fasting environment at the bottom of the biofilm for second-media reactor inducing the attached microorganisms to secrete more EPS, and EPS hydrolysis could provide the extra carbon source for the internal microbes in the biofilm. Because of this, alternating fast/feast environment occurring through the biofilm structure

was created in second-media reactor, augmenting the energy-uncoupling metabolism inducing in situ sludge reduction. The composite carrier, second-media, produced fluid separation and could intercept suspended matter which contributed to biofilm formation and biofilm lysis to release dissolved organic matter, The energy-uncoupling metabolism, sludge decay, and enrichment of slow-growth bacteria all contributed to in situ sludge reduction in second-media reactor (Wang et al., 2018).

Further research studied phosphorus removal biofilm grown in a lab-scale submerged biofilm system, SBBR. Alternated anaerobic and aerobic conditions were used to obtain an enriched PAO culture. The column was packed with ceramic balls having diameters between 0.5 and 0.8 cm. The column was fed medium strength synthetic wastewater. Air flow factors, included biofilm adhesion and required oxygen for PAOs, both fixed and in solution, was considered when deciding dissolved oxygen (DO) concentration in the bulk solution. Theoretically, high DO would enhance phosphorus uptake by PAO, however, the biofilm was detached at air flow rates associated with high DO. The mechanism of substrate biological transformation was linked to biological activity in the accumulation of PHAs (Chiou & Yang, 2008).

Biological Phosphorus Removal (BPR)

Biological phosphorus removal was first proposed in 1955, when it was observed that activated sludge could take up phosphorus as a concentration beyond that required for normal growth of the organisms. Progress from the ensuing years was published at two conferences held in 1982, the International Association on Water Pollution Research (IAWPR) Post Conference on Phosphorus Removal in Biological Treatment Processes, Pretoria, South Africa, and a US Environmental Protection Agency (EPA) Workshop on Biological Phosphorus Removal in Municipal Wastewater Treatment, Annapolis, Md. (Manning & Irvine, 1985).

Enhanced biological phosphorus removal, now an integral component of many treatment plants, uses a special group of organisms known as polyphosphate accumulating organisms (PAOs) that, under alternating anaerobic and aerobic conditions, incorporate the

influent phosphorus into the cell mass. The sludge is subsequently removed during sludge wasting. Under anaerobic condition, PAOs take up available carbon such as short chain volatile fatty acids (SCVFAs) and store them in the form of polyhydroxyalkanoates (PHAs), see Figure 3. The energy for this process is obtained mainly through hydrolysis of the intracellular stored poly-P, resulting in the release of ortho-phosphate into the bulk liquid. Under aerobic or anoxic conditions, PAOs are able to take up excess phosphorus from the surrounding bulk liquid, while growing new biomass, and replenishing glycogen by using stored internal PHAs as the energy source (Dutta & Sarkar, 2015).

The phosphate release in the anaerobic stage is less than that absorbed in the aerobic or anoxic stage; the net removal of phosphorus can be achieved through wasting sludge which is enriched in poly-P. Sequencing batch reactors (SBRs) can achieve alternating anaerobic and aerobic conditions by controlling the operational process, and consequently, biological phosphorus removal using SBRs has drawn increasing attention worldwide. The P-removal efficiency as high as 90% has been reported in SBR, whereas in conventional activated sludge systems, maximum efficiency achieved tends to be 10-20% (Dutta & Sarkar, 2015).

Another group of organisms, known as glycogen accumulating organisms (GAOs), biochemically resemble and compete with PAOs in their metabolism. GAOs have no contribution to the P-removal and their proliferation is known to cause P-removal failures in reactors. Finding optimal conditions that favor PAOs over GAOs are necessary to success in biological P-removal. The controlling parameters include pH, temperature, and more importantly, substrate type. Lower temperature may favor PAOs. Increasing pH is believed to provide an advantage to PAOs over GAOs, and optimum pH is believed to be between 7.2 and 8.0 for effective GAO control. A high COD-to-phosphorus ratio such as above 40 in raw wastewater help to achieve low effluent phosphorus concentration and high process stability in full-scale plants. The form of COD is also a critical factor for selection of PAOs. If the influent COD has a sufficient portion of short chain volatile fatty acids (SCVFAs) or readily biodegradable (rbCOD) that can be fermented into VFAs, PAOs

can outcompete GAOs and achieve low phosphorus levels in effluent (Dutta & Sarkar, 2015).

Published bench scale research utilized biomass from laboratory and full-scale enhanced biological phosphorus removal (EBPR) water resource recovery facilities (WRRFs), for batch testing conducted with reactors operated as sequencing batch reactors (SBRs). The reactors were fed with a mixture of VFA-rich fermenter liquor and raw, unfiltered wastewater influent. Despite some nitrification, quality EBPR performance was sustained. The adverse effects of nitrate on EBPR are well known. All reactors were mixed with magnetic stir bars and operated at room temperature without pH control. The EBPR systems were enriched with both PAOs and GAOs, with relative fractions consistent with past research. Batch testing explored the potential effects of anaerobic HRT, and VFA:VSS ratio on EBPR performance. Longer anaerobic HRTs did not appear to induce excess aerobic P removal. Conversely, higher VFA:VSS ratios imposed in the batch tests yielded greater effluent P concentrations (Coats et al., 2021).

The published article went on to say, given that anaerobic P release was significantly impacted by VFA:VSS ratio, results indicate that the biomass retained "extra" metabolic energy capacity (i.e., poly-P) to take up VFAs, which aided in aerobic P removal. The ratio of aerobic P removed to anaerobic P release was not adversely affected by the VFA:VSS ratio or the anaerobic HRT. Anaerobically synthesized PHA reserves, which are metabolically linked to increased aerobic P removal, increased with higher VFA:VSS ratios. Providing more VFAs increased intracellular PHA concentrations, and the biomass responded by accumulating more P aerobically. Under increasing VFA loads, the reactors, assumed to be under pseudo-steady state poly-P reserves, hydrolyzed more poly-P to generate additional energy as adenosine triphosphate (ATP) an organic compound that drives many processes in living cells. ATP energy in this instance is to uptake and catabolize substrate (Coats et al., 2021).

While "low" effluent P concentrations were not maintained, these results suggest that PAOs will adapt to dynamic VFA loading to sustain EBPR. Anaerobic P release occurs

without PHA synthesis. This observation indicates that some of the catabolized VFAs are not used directly for PHA synthesis but instead are metabolized through the tricarboxylic acid (TCA) cycle, to provide necessary nicotinamide adenine dinucleotide (NADH) for energy, cell maintenance, and NADH to support PHA synthesis, Figure 2.

Another laboratory research article exploring functional PAOs describes sequencing batch reactor (SBR) with 0.5 L working volume inoculated with sludge from a WWTP. The SBR was operated with 8 h cycles. A synthetic medium containing hydrolyzed casein protein (i.e., dairy substitute) and yeast extract was fed during a 105-day acclimation period. Thereafter, the yeast extract was removed from the feed, and the reactor operated for an additional 9-months period. *Tetrasphaera*-related PAOs assimilate a wide range of carbon sources, such as casamino acids, to perform aerobic P uptake. Casein protein is mainly comprised of casamino acids and small peptides; thus, this wider complexity was considered to be advantageous to select a wider group of *Tetrasphaera*-related PAOs. Both PAO groups, *Tetrasphaera* related organisms and *Accumulibacter*, were identified in the enriched culture. The glycogen accumulating organisms (GAOs) represented <1% of the biomass. The culture was mainly *Tetrasphaera*, comprising a volume fraction of over 60% of the total bacterial community. *Accumulibacter* was also detected in this culture, with an average volume fraction close to 20% (Marques et al., 2017).

The article went on to say, upon comparing the results from this *Tetrasphaera*-enriched culture with typical *Accumulibacter* behavior, the culture displayed comparable levels of intracellular P, glycogen degradation and P-release, while much lower PHA production was found. This agrees with previous studies, which state that most *Tetrasphaera*-related organisms are not able to produce and oxidize PHA except in filamentous species and *Tetrasphaera japonica*. PHA synthesis and oxidation was therefore assumed to be performed only by *Accumulibacter* during the anaerobic and aerobic stage, respectively. In this way, it was concluded that the *Tetrasphaera*-related organisms are the main group responsible for P removal (approximately 80%) in this

culture when fed Casein protein. Furthermore, the researchers found that 90% of *Tetrasphaera*, and not *Accumulibacter*, were responsible for amino acid and glucose consumption, while *Accumulibacter* likely survived on *Tetrasphaera* sourced fermentation products. This result is of significance since close to 30% of the chemical oxygen demand (COD) in domestic wastewater influents are composed of proteins and amino acids. These results suggest that *Tetrasphaera*-related organisms can contribute substantially towards P-removal in EBPR plants (Marques et al., 2017).

Combined SBR with BNR

With ever increasing treated effluent water quality regulations, it has become inevitable to include tertiary treatment units for nutrient removal from wastewater apart from the conventional pollutants like chemical oxygen demand (COD), biological oxygen demand (BOD), and suspended solids and pathogens. As SBR-based treatment plant can easily address this requirement without addition of any new infrastructure, only by optimizing the sequence of aerobic, anoxic, and anaerobic stages during the different stages of the SBR process (Dutta & Sarkar, 2015).

During the fill stage, the SBR receives the raw wastewater that comes in contact with the active biomass left inside the tank at the end of the previous cycle. There are three variations which may be incorporated or combined, depending on the wastewater characteristics, the target organics and biological nutrient removal: static fill, mixed fill, and aerated fill, Figure 1. During static fill, influent wastewater is added to the biomass present in the SBR without mixing, resembling almost a plug flow reactor (PFR) situation creating a high food-to-microorganism (F:M) ratio. This is similar to a selector compartment used in an activated sludge plant (ASP) promoting the growth of floc-forming bacteria by suppressing the filamentous ones, which provides good settling characteristics for the sludge. Additionally, static fill conditions create a “feast”-like situation in which phosphorus accumulating organisms (PAOs) are favored (Dutta & Sarkar, 2015).

The react stage is intended for the completion of the biological reactions responsible primarily for the degradation of organics. The react stage is often designed to

provide a high degree of nutrient removal. Process air is the primary controlling factor which when turned on or off, provides any combination of anaerobic, anoxic, or aerobic conditions. Designs may include conversion of ammonia-nitrogen to nitrite-nitrogen and ultimately to nitrate-nitrogen, a process known as nitrification. Anoxic conditions when provided can achieve denitrification, a process in which nitrate-nitrogen is converted into nitrogen gas. Anaerobic feed conditions, absent of oxygen, nitrite, and nitrate, will create a "feast" stage that promotes phosphorus removal (Dutta & Sarkar, 2015).

During the settle stage the entire reactor acts as a batch clarifier, without any inflow or outflow. In a continuous flow process (CFP), on the contrary, the quiescent settling is often impaired by the continuous inflow and outflow of liquid, given rise to inefficient settling that may cause poor effluent quality. The draw stage uses a decanter, either fixed or floating, to decant (i.e., discharge) the treated supernatant after the settlement of the biomass (i.e., settle stage) generated from the react stage. Finely, the idle stage is the time between draw and fill stage. The need for such a stage is often necessitated when there are several reactors operating in parallel operation, acting as a buffer in time. During this stage, mixing of the biomass to condition the reactor contents, and wasting of excess sludge, may be taken up, depending on the operating strategy. The entire cycle time spans the duration between beginning of fill and end of idle stage for a single tank SBR system (Dutta & Sarkar, 2015).

It is expected that nitrification, denitrification, and enhanced biological phosphorus removal (EBPR) should often take place concurrently in an SBR. For concurrent nutrient removal, the interaction among processes, if not optimally controlled, may give rise to the failure of the treatment plant. Among the reactants and intermediate products, toxicity of nitrite and its acidic counterpart, free nitrous acid (FNA), is important as they are known to provide a competitive advantage to GAOs over PAOs in the EBPR systems. They are a key selection factor in the PAOs/GAOs competition, severely inhibiting PAO activity at a concentration as low as 2 mg L^{-1} nitrite-N and complete inhibition at 6 mg L^{-1} nitrite-N. Early studies pointed to disruption to phosphorus removal under nitrate-rich conditions in

the anaerobic stage. This observation was later improved to suggest the disruption results from the consumption of volatile fatty acids by denitrifying non-polyphosphate heterotrophs, and inhabitation of PAOs by nitrite, as a result of incomplete denitrification (Dutta & Sarkar, 2015).

Control strategies are another area were SBRs shine compared with continuous flow plants (CFP). In addition to better effluent quality, in terms of COD and nutrients, better control of filamentous bacteria as well as low energy consumption exemplify the SBR advantage. Over the past 30 years, control technologies for the SBR process have continually evolved, leading to the development of a wide variety of control systems to offset any complexity of the SBR process. The classical SBR fixed time cycle, that could not adapt to varying influent compositions, has been replaced by integrated real-time logic controls. Optimization through real-time controls is now able to adapt and optimize under varied influent conditions. Precise real-time process control requires feedback on at least the start and end of various biological reactions taking place within the SBR (Dutta & Sarkar, 2015).

Interpreting Data Plots

Real-time monitoring of direct parameters such as chemical oxygen demand (COD), total suspended solids (TSS), nitrite, nitrate, ammonium, and phosphorus may not be sufficiently accurate with currently available technology. Online monitoring of indirect parameters such as pH, dissolved oxygen (DO), and oxidation reduction potential (ORP) can successfully indicate the reaction processes that occur during carbon and nitrogen removal in SBR processes. ORP has been demonstrated to correlate directly with nitrification rates and other biological reactions in anoxic conditions. In normal condition, ORP is positive and increases during aeration stage and negative during anoxic stage. The normal range of values of ORP is 0 to 50 mV in aerobic stage and 0 to -300 mV in anoxic stages. In the anoxic stage the, ORP has a continuous dropping profile with respect to time; a steep drooping profile, known as nitrate knee, occurs that signifies the end of

denitrification so that it is safe to stop anoxic stage and start the next step (Dutta & Sarkar, 2015).

The pH profile increases during denitrification and decreases during the nitrification reaction. There are two important breakpoints in the pH profile with respect to time:

- Ammonia valley: As nitritation produces acid, pH tends to decrease gradually at the beginning of nitrification. When all the ammonia has been oxidized to produce peak nitrite concentration, there is no further acid production due to ammonia conversion. pH profile shows an associated minimum which is known as the ammonia valley.
- Nitrate apex: During the anoxic stage, the pH rises and produces a continuously rising profile. A maximum is reached when all available nitrate is converted to nitrogen gas, indicating an end of denitrification stage. Nitrate apex exactly corresponds with the nitrate knee as observed through the ORP profile.

Researchers debate that pH profile is the best indicator of the changes in the microbial life-signs occurring inside an SBR reactor. However, the background alkalinity present in the wastewater often provides a strong buffering capacity that minimizes noticeable variations in the pH profile (Dutta & Sarkar, 2015; Puig et al., 2004).

The literature review found a number of deficiencies in the published research that the current research will work toward resolving. More research is need regarding fundamentals of integrated biofilm formation and evaluation of the physicochemical properties of a biofilm for an SBBR reactor. Anoxic nitrogen and phosphorus removal, by denitrifying PAOs, requires further exploration using varied C/P or C/N ratios. Little is known about the SBBR retained flocculated and biofilm biomass and the relationship of the two fractions as weakly-attached biomass is readily dislodged to contribute flocs to the bulk liquid, especially in response to physical disturbances. Integration of acidogenic co-fermentation of soluble carbon anaerobically, needs to be further explored as it can potentially augment influent VFAs benefiting biological phosphorus removal.

Methods and Materials

Two bench scale, clear acrylic, covered reactors Figure 5 were maintained during the five Phased investigation treating medium strength synthetic municipal wastewater as defined in Metcalf and Eddy (Tchobanoglous et al., 2003). Synthetic wastewater concentrate was prepared fresh on alternate days and batched with tap water three times daily corresponding with the 8-hour treatment cycle. Aeration was provided by an array of stone diffusers, positioned at the reactor bottom, oriented to provide optimum self-cleaning and oxygen transfer. The, 19.4 cm diameter by 30.0 cm height, 8.5 litre reactors operated at a working volume of 7.5 litres. Reactor covers included a series of openings to accommodate feed, waste, discharge, sampling, and a four-sensor array. The two reactors, activated sludge sequencing batch reactor, AS-SBR (Control), and integrated fixed film activated sludge biofilm reactor, IFAS-SBBR (Research), did not include pH or temperature control and operated between 21^{o}C +/- 1^{o}C ambient temperature, and 7.5 pH to 8.5 pH.

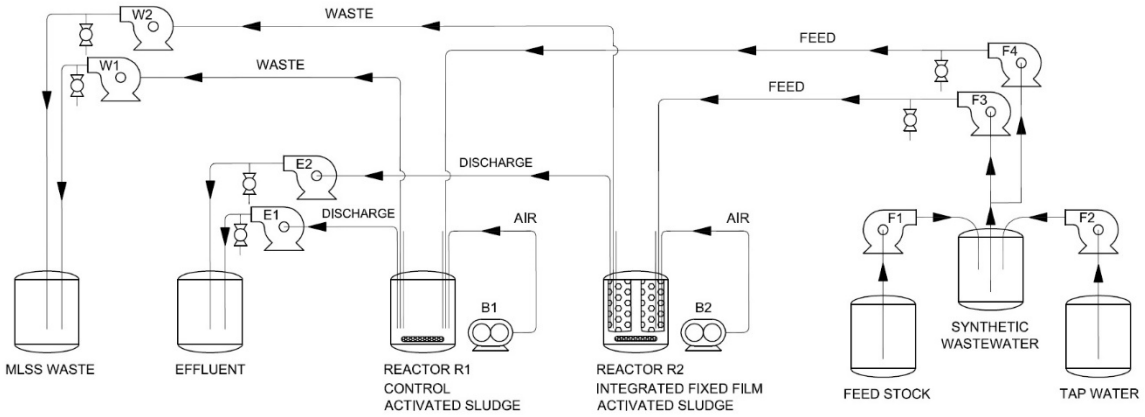
Both reactors underwent three 8-hour treatment cycles daily controlled using seven Control Company traceable controllers. Treatment cycles began with a 15-minute anoxic feed stage. The cycle continued with 75-minute anaerobic mixing, 303-minute aerobic mixing, 2-minute MLSS waste, 40-minute settle, 30-minute discharge, and 15-minute idle stage. Investigated enhancements for phosphorus removal included extending the anaerobic stage length and delaying the anaerobic mixing during the later phases of investigation. Each SBR reactor was supported on a 17.5 cm magnetic stirrer, operated at selected speeds from 300 to 600 rpm.

Seven Cole Parmer peristaltic pump/drive/controller sets with flow rates ranging from 3.5 ml min^{-1} to 350 ml min^{-1}, were used to transport all fluids. Each SBR included a pH/ORP/DO/Temp sensor array (Osorno Enterprises Inc. Winnipeg, Canada) housed in a 5.0 cm PVC tube passed through the reactor cover. An industrial grade human machine interface (HMI) connected to the sensors processed and displayed data. The HMI displayed real-time data measured every second and logged every minute. The HMI displayed real-

time plots and sensor measurements while also generating downloadable monthly csv data files, and daily, weekly, and monthly plot images as png files.

Figure 5

Control (AS-SBR) and research (IFAS-SBBR) reactor setup



The IFAS-SBBR included three fixed film media each of 6.0 cm diameter by 20 cm submerged length in vertical orientation, bolted from the reactor cover, and arrayed around the reactor inside diameter directly above several aeration diffuser stone. The media used is similar in function to the polyvinyl chloride or polyethylene products consisting of plastic fibres netted into continuous strands, looped in rows, along a 6 mm wide plastic ribbon, Figure 4. The plastic loops result a rope-like appearance. Since the media is not self-supporting, it is mounted on a cage-frame fixed and bolted to the reactor cover underside, and fully immersed.

A gap of approximately 2.0 cm between the media outside face and inside wall face of the reactor permitted bulk mixed liquor suspended solids (MLSS) and air to flow to pass around the fixed biofilm. The fixed film shaft was overlain with 15 layers of black 5.0 cm plastic fibre netting (ULINE S-6580BL) fixed in place with nylon zip ties. The wetted surface area of the fixed film media was approximately equivalent to AnoxKaldnes™ K1 media at approximately 20% reactor volume. The diffuser stones being positioned to

provide self-scour and process air directly to the fixed biomass while also supplying oxygen to the bulk fluid mixed liquor.

Three times daily, each reactor discharged 5.0 litres of treated effluent which was replaced with 5.0 litres of fresh synthetic wastewater for an exchange ratio of 66%. In order to accommodate the fixed media with biomass, the IFAS SBR decanted effluent down to 2.0 litre reactor volume while the Control SBR decanted to 2.5 litre reactor volume. Influent water quality analysis shows slight concentration differences between reactors owing to the different dilutions.

Average influent feed strength based on weekly samples was 402 mg L^{-1}, 47.3 mg L^{-1}, and 11.3 mg L^{-1} for COD, NH_4^+-N, and PO_4^{3-}-P respectively. The recipe included sodium bicarbonate, at 760 mg L^{-1}, to ensure sufficient alkalinity for nitrification.

The synthetic municipal wastewater feed recipe, Table 2, was based on (Smolders et al., 1994). The basic composition of the feed was:

Table 2

Synthetic municipal wastewater recipe concentrations

Component	Phase 1-2	Phase 3	Phase 4-5
	mg L^{-1}	mg L^{-1}	mg L^{-1}
Yeast Extract	760	760	760
NH_4Cl	460	155	460
KH_2PO_4 $3H_2O$	**300	155	300
$MgSO_4$	90	90	90
$CaCl_2$	185	185	185
EDTA	155	155	155
Trace Minerals	0.55	0.55	0.55

Note. ** December 13, 2019 bumped to 900 mg L^{-1}

The trace mineral solution contained: $ZnSO_4 \cdot 7H_2O$: 0.12 g L^{-1}, $CoCl_2 \cdot 2H_2O$: 0.15 g L^{-1}, $MnCl_2 \cdot 4H_2O$: 0.12 g L^{-1}, $CuSO_4 \cdot 5H_2O$: 0.03 g L^{-1}, $NaMoO_4 \cdot 2H_2O$: 0.12 g L^{-1}, H_3BO_3: 0.15 g L^{-1}, KI: 0.03 g L^{-1} and $FeCl_3 \cdot 6H_2O$: 1.50 g L^{-1}. Potassium phosphate concentration was varied during the early and later investigation Phases.

Analytical Methods

Samples for nutrient analysis were passed through 1.0 µm paper filter and concentrations of phosphorous as orthophosphate (PO_4^{-3}-P) and nitrogen as ammonium (NH_4^+-N), nitrite (NO_2^--N) and nitrate (NO_3^--N) were measured by flow injection analyzer (FIA), Quick Chem 8500, LACHAT Instruments). Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were performed according to Standard Methods 2540 D (Eaton et al., 2005). Soluble chemical oxygen demand (COD) samples were passed through 1.0 µm paper filter. The DR 2800 Spectrophotometer by Hach Company was used to measure COD according to Standard Methods 5220 D (Eaton et al., 2005). The COD results in mg L^{-1} were correlated to the mg of O_2 oxidized per liter of sample.

The fixed biomass of the IFAS-SBBR was removed alternate weeks by a vigorous rinse using deionized water (DI). The captured biosolids was than characterized by solids analysis and by wet digestions for total phosphorus. Therefore, an alternate SRT for the IFAS should include the weighted average of the 7.5-day MLSS SRT plus a 14-day fixed biomass SRT.

Mixed liquor total phosphorus was assessed by wet digestions using H_2SO_4 and heat, followed by pH neutralization. The filtered digestion samples were run through the FIA to measure PO_4^{-3}-P. In addition, high purity liquid chromatography (HPLC) samples from later phase kinetic studies measured flux in anaerobic volatile fatty acids (VFAs). Also later in the phased investigations, sludge volume index (SVI) and food to mass ratio (F:M) were calculated and monitored in support of the research.

Implementation

Conventional waste activated sludge (WAS) from the City of Winnipeg West End Water Pollution Control Centre, practicing biological phosphorus removal, was sourced for seeding the reactors (i.e., 2000 ml WAS per reactor). Following start up, effluent solids, nitrogen and phosphorus removal gradually improved over 3 to 4 solids retention times (SRTs) as did sludge volume index (SVI) and reactor stability. Each reactor when stabilized wasted 1000 ml MLSS per day. Accounting for effluent solids, generally between 0.5 and 1.5 g $week^{-1}$, the Control SBR SRT was 6.5 days while the IFAS SRT was 7.3 days. The hydraulic retention time (HRT) using 5-litre exchange per treatment cycle equates to 12-hours. Average MLSS for the stabilized process was MLSS (Control): 2765 mg L^{-1}, MLVSS (Control): 2079 mg L^{-1}, MLSS (IFAS): 2009 mg L^{-1}, and MLVSS (IFAS): 1508 mg L^{-1}.

Aeration diffuser arrays at the bottom of each reactor maintained MLSS dissolved oxygen levels between 4.0 and 7.0 mg L^{-1} by delivering 2.0 L min^{-1} air flow, distributed across the reactor bases to best promote self-scour and deliver process oxygen. Diffuser aeration stones were removed bi-weekly, cleaned, tested and returned to maintain consistent air flow. Sensor arrays were removed with the alternate day feeding cycle, cleaned, checked and returned to maintain consistent measurements.

Sludge age was controlled for both reactors by wasting a volume of MLSS each cycle. MLSS waste occurred at the end of the aerobic mixing or “react” stage when the air was stopped for 5-minutes but the mixing continued allowing for a representative MLSS waste. The IFAS SBR required an additional step involving a surface rinse of the fixed media biomass with the bi-weekly reactor cleaning. During the reactor cleaning, the IFAS media were rinsed using a jet of DI water from 2 litre, pump-pressurized, hand garden sprayer. The procedure was able to remove approximately 95% fixed biomass from the fixed media. The rinsed biomass with DI water measured 1000 ml, was sampled to estimate TSS, VSS, and total phosphorus (TP).

The bench scale investigations proceeded as five phases with Phase 1 dedicated to equipment start up. Phase 2 and Phase 3 are linked in that air flow (and mixing) balanced for process air and fixed biomass self-cleaning would sustain dissolved oxygen concentration at a level to support anoxic conditions. In this manner, anoxic conditions are assumed for denitrifying phosphorus accumulating heterotrophs at a range of influent carbon-to-nitrogen and carbon-to-phosphorus ratios.

Leading to Phase 4, influent phosphorus and nitrogen concentrations started as medium strength as described above, before transitioning incrementally lower to achieve the 100:10:1 ratio for carbon to nitrogen to phosphorus, a condition of integrating acidogenic co-fermentation of influent soluble carbon anaerobically, to augment VFAs for enhanced biological phosphorus removal. In two steps, the anaerobic stage length increases from 90 minutes to 120 minutes followed by a delay of anaerobic mixing by 30 minutes. Nitrogen gas sparge for kinetic study anaerobic phase (with or without mixing) serves to lower dissolved oxygen marginally to approximately 0.30 mg L^{-1} benefiting acidogenesis.

Phase 4 and Phase 5 are linked through the incremental return of influent nitrogen and phosphorus to medium strength established and wet acid digestions of mixed liquor suspended solids, both bulk and fixed biomass, for tracking fate-and-effect of the biological phosphorus removal. Kinetic studies are a source for fate-and-effect data of phosphorus tracking through the reactors. Phase 5 further tests treatment response by discontinuing influent feed over a short period (less than one week) to investigate the reactors as they move to recovery following restoration of the influent feed.

Results and Discussion

The following paragraphs will present results and discussion from laboratory scale investigations conducted to examine, phosphorus removal usefulness of an integrated fixed film activated sludge sequencing batch biofilm reactor (IFAS-SBBR), compared with a conventional activated sludge sequencing batch reactor (AS-SBR) under quasi stable operation.

Wastewater Influent Characteristics

Characterization of the influent wastewater is represented in Table 3 below. The most revealing Phase 1-2 characteristic is the influent phosphorus bump on December13, 2019 from 20 g/L to 60 g/L. Interestingly, influent ammonium and alkalinity also showed increases in conjunction with the phosphorus concentration increase. This finding, though interesting, hasn't been fully explored. Unfortunately, the real-time pH sensors were not reading correctly during Phase 1-2 and the sensors recorded values consistently higher than normal during this period. The condition was corrected by Phase 3 with sensor recording values which generally cycled between 7.5 pH and 8.5 pH.

Table 3:

Average influent characteristics by phase

	COD	**NH_4^+-N**	**PO_4^{-3}-P**	**TN**	**TP**	**Alkalinity**
Average	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Phase 1-2	n/a	33.6	13.2	87.3	14.9	411
			P "bump"		P "bump"	
Phase 1-2	n/a	53.6	21.0	132	46.0	621
Phase 3	342	46.4	18.2	n/a	n/a	372
Phase 4	320	45.4	13.6	78.3	13.7	362
Phase 5	335	46.1	8.25	63.5	30.5	351

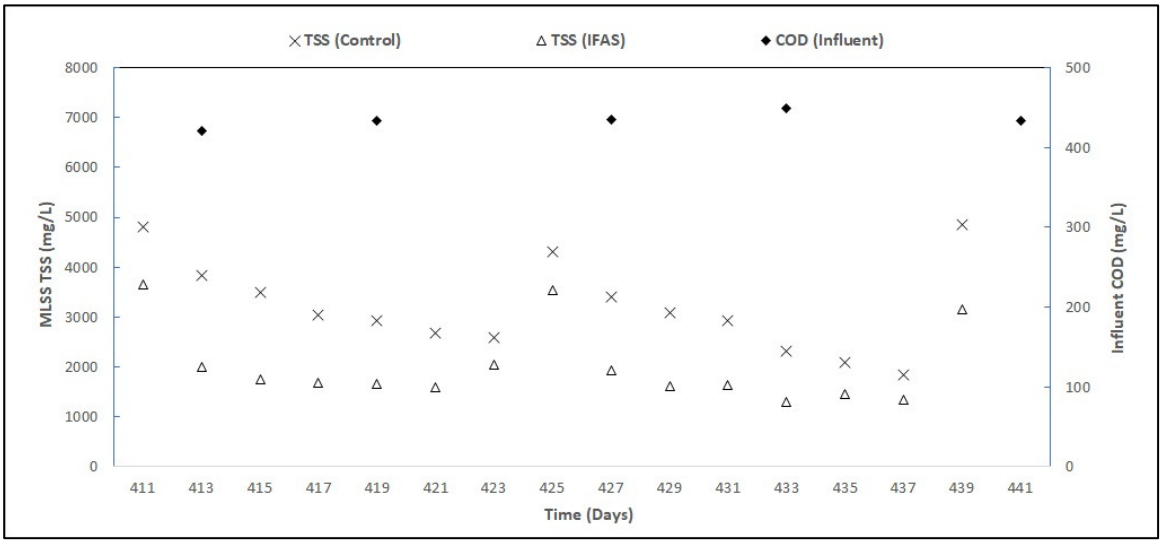
Stable Operation Defined

Biochemical processes such as wastewater treatment, involve a myriad of interrelated and interdependent processes such as carbon oxidation, nitrogen oxidation, and phosphorus uptake and release. Accurately measured (i.e., measured each second, logged each minute) parameters including pH, ORP, DO and temperature can provide interpretations for biochemical processes in turn providing a window into the microbial oxidation of carbon, nitrogen, and phosphorus removal. With so many moving parts, figuratively not literally, it can be challenging for an inexperienced researcher to know what parameters to track and when reactor process stability is realized.

The current research initially used TSS and VSS as a surrogate for defining when process stability is achieved. This approach though reasonable, isn't practical for research that includes bi-weekly reactor cleanings. Normal probability is used together with 90% and 95% confidence level estimates to gauge stability. This approach suggests that for the wastewater treatment research being conducted, approximately two-months of solids analysis data (i.e., alternate day sampling) will yield a 95% confidence level and that the process likely is stabilized. Bi-weekly weekly reactor cleanings require a novel approach.

Figure 6

Total suspended solids IFAS-SBBR showing bi-weekly rinse



The reactor cleanings are essential to know definitively the character of the fixed media biomass, and for removing biofouling from the aeration diffusers. Additionally, purging the fixed media biomass provides for mixed liquor suspended solids (MLSS) estimated concentrations on a two-week cycle. The reactor cleaning causes a periodic blending of the fixed biomass, released from reactor walls and floor, with the bulk liquid MLSS resulting in occasionally high MLSS measurements. Over the ensuing days, the measured bulk MLSS concentrations gradually diminish as the biofilm again adheres to the reactor wall and floor. This cycle is reflected also in other effluent nutrient concentrations including NH_4^+-N, NO_2^--N, and NO_3^--N. Stated another way, the volatile solids of the biomass, that adheres to the walls, is actively working to treat wastewater but is no longer represented in the MLSS measurement and analysis. Figure 6, is representative of the TSS and VSS concentrations during late phases of the research.

The research found that by and large, ammonium and chemical oxygen demand (i.e., soluble carbon) are the two nutrients most effectively oxidized and removed from the wastewater. That being said, ammonia oxidizing bacteria (AOBs) seemed to prosper but not so much nitrite oxidizing bacteria (NOBs). For this reason, the research shows extended periods of full nitrification to nitrate, and roughly an equal amount of time with the accumulation of nitrite with only nominal levels of measured nitrate.

Influent COD concentrations are readily oxidized, and generally COD is the first nutrient achieving quasi-steady state removal. The section below on Stable Operation provides tabular removal efficiencies for ammonium, phosphorus, and soluble COD in Table 4, for the Control and IFAS reactors respectively. The table illustrates average sCOD removal efficiencies for the Control (AS-SBR) and Research (IFAS-SBBR) reactors to be 95%. Removal efficiencies of the influent ammonium nitrogen concentrations is as well quite good. Table 4 illustrates average NH_4^+-N removal efficiencies for the AS-SBR and IFAS-SBBR reactors with supporting correlation coefficient data.

Stir bar failure, disrupting Phase 3 treatment process, include November 26, 2020 when the AS-SBR stir bar dislocated, knocking out a diffuser stone. The upset caused PO_4^{-3}-P removal to drop from 93% to 72% before recovering in January to 96%.

Early April progressive IFAS stirrer failure, continued to April 17, 2021, shown as PO_4^{-3}-P removal drops from 93% to 80% before recovering in May to 87% and later in June to 92%.

With regards to phosphorus removal efficiencies, average PO_4^{3-}-P removal efficiencies for the AS-SBR and IFAS-SBBR reactors to be 93% and 91% respectively. The results presented above support the assumption of effluent ammonium and sCOD as surrogates for reactor stability.

Table 4

Onset of stable treatment process by 3-SRTs (21-days) with correlation coefficient

	Phase 1-2		**Phase 3**		**Phase 4**		**Phase 5**	
AS-SBR	NH_4^+-N	sCOD	NH_4^+-N	sCOD	NH_4^+-N	sCOD	NH_4^+-N	sCOD
	93%	94%	95%	76%	97%	98%	93%	97%
	95%	99%	94%	96%	97%	100%	95%	100%
	92%	97%	95%	97%	96%	98%	93%	98%
Avg	93%	97%	95%	90%	97%	99%	94%	98%
R^2	0.318		0.214		0.250		0.893	
IFAS-SBBR	NH_4^+-N	sCOD	NH_4^+-N	sCOD	NH_4^+-N	sCOD	NH_4^+-N	sCOD
	88%	95%	96%	74%	97%	95%	96%	97%
	94%	97%	96%	93%	96%	96%	93%	100%
	92%	100%	96%	98%	95%	97%	93%	98%
Avg	91%	97%	95%	88%	96%	96%	94%	98%
R^2	0.318		0.898		0.999		0.571	

At this point removal efficiency, stated as a percentage, of ammonium, phosphorus, and soluble carbon removal is established as the criteria best suited to define the onset of reactor process stability. That being said, data outliers need to be identified and accounted for in the calculation of removal efficiency. Given that three of the five identified research Phases use seed from fresh waste activated sludge and two begin with process mechanical improvements, each of the Phases are viewed independently with respect to identifying a point when process stability has been achieved.

The Table 4 is a composite of removal efficiencies for defining the onset of stable treatment process. From the precent removal data, effluent criteria corresponding with stable reactor process is similar for the Control and IFAS reactor. A minimum operation duration of 3-SRTs (i.e., about 3-weeks) is assumed in conjunction with the three effluent parameter removal efficiencies. Interpreting from the tables, stable operation can ideally occur when effluent parameters equal or surpass 98%, and 95% for NH_4^+-N, and sCOD respectively. In other words, as the removal efficiencies reach 90% or greater, the reactors are assumed quasi stable, for the purpose of the current exploratory research.

Reactor Startup

During late 2019 and early 2020 the current wastewater treatment research being conducted at the University of Manitoba, Environmental Engineering Laboratory halted first by the onset of aerobic granular sludge (AGS) and again by the global Covid-19 pandemic. The initial treatment run extended October 2019 to January 2020. The following treatment run extended February 2020 to March 2020. Collectively, these runs are referred to as Phase 1-2. The reactors aeration diffuser design change between the first and second phases provides more diffused air and improved air distribution, following the January 2020 formation of AGS. It is believed that the asymmetric diffuser design may be contributing to hydrodynamic shear, one of several factors implicated in the formation of AGS (Beun et al., 1999; Hamiruddin et al., 2021).

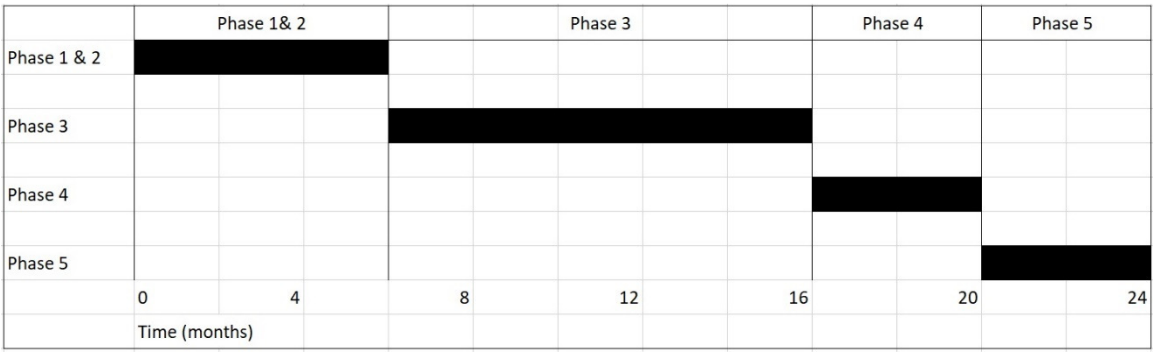
The treatment startups described in the previous paragraph use seed waste activated sludge (WAS), 2000 ml per reactor, from the City of Winnipeg, West End Water Pollution Control Centre (WEWPCC). The WEWPCC is a biological nutrient removal (BNR) facility designed to biologically remove phosphorus. This means that the WAS arrives with phosphorus accumulation organisms (PAOs) already established.

A global pandemic closure of the Engineering Laboratory from April to July 2020 occurred. Near the end of the summer research at the Engineering Laboratory was introduced again with public health regulations. The current wastewater treatment research resumed during August 2020 with seed WAS from the WEWPCC.

The third treatment phase extended from August 2020 to May 2021 and was defined primarily by frequent magnetic stirrer failure of one or the other reactor generally frustrating research progress until new replacement stirrers arrived and defining a "reset" of the wastewater research and a new fourth research run, Figure 7.

Figure 7

Research schedule timeline by phases



Research phases four and five extended from June 2021 to December 2021 and did not require new WAS seed but are defined as changes to operating conditions mostly related to stirring conditions. As mentioned above, new replacement stirrers define the end of phase three and the start of phase four. The new stirrers should provide improved process stability and for this reason mixing increased beyond the initial mid-range of 350 rpm and taken up to 600 rpm for a period before reducing to 500 rpm.

Unfortunately, even with the new replacement stirrers operating at 500 rpm, intermittent stir bar failure continues to occur. Eventually, it was recognized that the glass plate inserts, to prevent stir bar wear of the reactor floor, in fact float slightly and potentially rotate almost invisible to the eye but enough to destabilize and fail the magnetic stir bar. The glass plate inserts were removed September 2021, the mixing speed reduced to 300 rpm and this event defined the beginning of phase five, the last and final investigation of the wastewater treatment research.

The fourth research phase extended from June 2021 to August 2021 with efforts to align influent carbon, nitrogen, and phosphorus concentrations that agree with a published minimum ratio of 100:10:1 for C:N:P (Meng et al., 2020; Ni et al., 2021) and thereby establish a lower limit influent nutrient concentration. Other enhancements involve variations in air flow, to lower average mixed liquor dissolved oxygen concentration, for testing denitrification potential. The fourth phase dataset of mixed liquor total phosphorus measurements are by wet digestion acid method, a procedure analogous to Standard Methods 4500-P H (Manual Digestion and Flow Injection Analysis for Total Phosphorus) (Eaton et al., 2005).

The fifth and final research phase from September 2021 to December 2021 focuses mostly on anaerobic stage characteristics, and is defined by trouble free stirring and reasonable effluent quality. The anaerobic characteristics sought are related to acidogenic formation of short chain volatile fatty acids (SCVFAs) from readily biodegradable chemical oxygen demand (rbCOD) for the benefit of phosphorus accumulating organisms (PAOs). The engineering building, housing the environmental laboratory scheduled a power outage in September 2021, prompting the use of nitrogen gas for anaerobic sparge and mixing, rather than mechanical mixing. This approach passing nitrogen gas through the aeration diffuser system, applied to several subsequent kinetic studies, including one scheduled the day of the outage. This anaerobic mixing approach set a marginally lower mixed liquor dissolved oxygen concentrations and lower oxidation reduction potential readings under anaerobic conditions during the kinetic study. Nitrogen gas mixing led to

new observations related to phosphorus release and VFAs uptake. In conjunction with nitrogen gas sparging, subsequent kinetic study investigations further explore the above characteristics by way of staged or delayed anaerobic sludge mixing.

As well, sensitivity to influent VFAs concentrations is explored, using either fresh batched feed or two-day old feed, to test the corresponding anaerobic peak phosphorus release and release rates in response to supplied and endogenously produced VFAs. Samples from kinetic studies undergo high purity liquid chromatography (HPLC) analysis to yield influent VFAs and anaerobic stage MLSS VFAs concentrations.

The Table 4 results make use of the reactor "stable operation" as defined to estimate at what point during each of the phases the reactors achieve stability. It is further assumed that upon achieving the effluent NH_4^+-N, PO_4^{-3}-P, and sCOD removal values the stable condition is maintained.

pH, ORP, and DO Monthly Average by Phase

The pH and ORP parameters, as biochemical indicators of process changes, may be more responsive than dissolved oxygen as a means of identifying or assessing treatment process performance and general healthiness. This observation will be tested further in the section discussing reactor response to influent feed disruption. As described in the section on interpreting data plots, employing pH and ORP data plots and/or rate changes to trace the activated sludge oxidation and reduction of nitrogen has been researched by others.

The following paragraphs and data from Table 5 and Table 6, will contrast records from real-time sensor data across investigation phases against observed or apparent process changes. Included in the records are air flow manipulations intended to enhance overall treatment effectiveness, as detailed below:

Phase 1 provides for asymmetric air diffusers operating at full air flow between 4.5 L min^{-1} for most of the phase. Early attempts are made at changing air flow as a tool to enable 1) self-scour of fixed media, 2) augment aerobic mixing, and 3) entrain process air. Bulk liquid dissolved oxygen (DO) remined near saturation even with air flow ranging

between 2.0 L min^{-1} to 4.0 L min^{-1}. Higher flow rates are maintained for most of Phase 1. Near the middle of January aerobic granular sludge (AGS) formed in the research reactor followed shortly by red worms. By the end of January, the AGS is fully formed to the point that the fixed media biomass is completely displaced by AGS. From first observation, it took 7-days, or 1-SRT, to fully develop the aerobic granules.

Phase 2 from mid-February extending to late in March, until the global Covid-19 pandemic shuttered research. Phase 2 develops and employs an alternate aeration diffuser system replacing the original asymmetric air stone. The alternate system uses a symmetric diffuser stone array that more evenly distributed air flow from the base of the reactors. In conjunction with the new diffusers, a lower air flow regime of 1.5 L min^{-1} is explored, down from 4.0 L min^{-1}, in an effort to moderate maximum reactor DO, previously over 6.5 mg L^{-1}. The expectation was to further promote denitrification already evident from the fixed biomass reactor.

Phase 3 from reactor startup in August air flow is held at 2.0 L min^{-1} an average DO of 5.5 mg L^{-1} and 5.0 mg L^{-1} for the Control and IFAS reactors respectively.

Phase 3 from mid-September the IFAS-SBBR develops filamentous organisms with corresponding reduced performance. At this point, settled waste activated sludge (WAS) from AS-SBR is used to seed the IFAS reactor, and air flow is bumped from 2.0 L min^{-1} to 4.0 L min^{-1} producing average dissolved oxygen of 7.0 mg L^{-1} for both reactors.

Phase 3 from mid-January onward, in conjunction with new stone diffusers, air flow is reduced from 4.0 L min^{-1} to 2.0 L min^{-1} as can be seen from the February averages dissolved oxygen.

Phase 4 from mid-July air flow is reduce from 2.0 L min^{-1} to 1.5 L min^{-1}. By August the flow is restored to 2.0 L min^{-1}, though the average dissolved oxygen for this period remains near 4.5 mg L^{-1} for the IFAS-SBBR and 5.5 mg L^{-1} for the AS-SBR. This air flow condition remains throughout Phase 4 and Phase 5.

Table 5

AS-SBR monthly averages pH, ORP, and DO records

Sensor Records	AS-SBR	AS-SBR	AS-SBR	AS-SBR	AS-SBR	AS-SBR	AS-SBR
Monthly Averages	pH unitless	pH unitless	pH unitless	ORP mV	ORP mV	DO mg L^{-1}	DO mg L^{-1}
Phase 1-2	Avg.	Max.	Min.	Avg.	Min.	Avg.	Min.
2020/01	8.21	8.76	7.20	10	-272	5.65	0.25
2020/02	8.31	9.00	7.16	103	-478	6.38	0.25
2020/03							
Phase 3							
2020/08	8.36	9.22	1.19	26	-389	4.63	0.23
2020/09	8.31	9.22	2.78	-10	-335	6.07	0.25
2020/10	8.26	9.39	5.82	7	-247	6.85	0.26
2020/11	8.18	9.02	5.02	35	-255	6.27	0.27
2020/12	8.33	9.20	6.30	55	-275	6.55	0.26
2021/01	8.21	10.0	3.27	-4	-307	6.12	0.00
2021/02	8.12	8.89	5.64	-32	-286	5.46	0.17
2021/03	8.05	9.07	6.69	-30	-315	4.55	0.27
2021/04	8.10	9.32	7.11	-41	-325	3.94	0.48
2021/05	8.14	8.91	6.65	-31	-289	4.76	0.60
Phase 4							
2021/06	8.15	8.77	5.66	-30	-289	5.18	0.61
2021/07	7.80	10.5	4.40	-40	-377	5.68	0.34
2021/08	7.52	8.32	4.96	-65	-331	5.89	0.46
Phase 5							
2021/09	7.61	8.24	6.91	-114	-380	5.67	0.44
2021/10	7.61	8.51	5.11	-120	-339	5.35	0.43
2021/11	7.80	8.81	6.09	-129	-346	5.74	0.41
2021/12	7.63	8.67	6.28	-165	-373	5.00	0.40

Table 6

IFAS-SBBR monthly averages pH, ORP, and DO records

Sensor Records	IFAS-SBBR	IFAS-SBBR	IFAS-SBBR	IFAS-SBBR	IFAS-SBBR	IFAS-SBBR	IFAS-SBBR
Monthly Averages	pH unitless	pH unitless	pH unitless	ORP mV	ORP mV	DO mg L^{-1}	DO mg L^{-1}
Phase 1-2	Avg.	Max.	Min.	Avg.	Min.	Avg.	Min.
2020/01	8.21	10.0	0.37	-18	-469	6.37	0.01
2020/02	8.37	9.00	7.16	79	-478	6.27	0.25
2020/03							
Phase 3							
2020/08	8.54	9.13	1.54	-133	-488	4.86	0.04
2020/09	7.90	8.97	-10.0	4	-427	6.73	0.04
2020/10	8.44	9.20	7.49	3	-294	6.25	0.31
2020/11	8.36	90.2	6.74	17	-262	6.00	0.34
2020/12	8.39	9.39	6.84	11	-266	6.33	0.34
2021/01	7.09	10.0	1.19	1	-274	6.31	0.01
2021/02	8.28	9.05	6.48	-16	-327	6.15	0.10
2021/03	8.25	8.84	7.01	-19	-353	5.39	0.23
2021/04	8.20	8.87	7.08	-36	-394	3.27	0.22
2021/05	8.25	9.49	7.31	-19	-329	4.40	0.23
Phase 4							
2021/06	8.30	9.07	6.99	-25	-330	4.64	0.31
2021/07	8.06	10.5	4.55	-55	-360	5.06	0.33
2021/08	7.79	11.5	5.51	-101	-348	3.89	0.13
Phase 5							
2021/09	7.70	8.37	6.91	-104	-382	5.26	0.22
2021/10	7.65	17.2	6.13	-118	-403	4.55	0.24
2021/11	7.69	8.80	6.82	-111	-402	4.60	0.23
2021/12	7.69	8.66	6.63	-156	-381	4.06	0.22

Phase 5 data reflects the extension of the anaerobic stage from 90 minutes to 120 minutes. As well, Phase 5 saw the implementation of delayed anaerobic stage mixing which delays mixing following the feed stage by 30 minutes. Taken together, the above two enhancements (extending anaerobic stage, and mixing delay) reduces average dissolved oxygen (DO) and oxidation reduction potential (ORP). Table 5, Table 6 and Table 16 demonstrates that the enhancements produce improved denitrification verified by reduced effluent nitrite (NO_2^--N) and nitrate (NO_3^--N) values across both reactors over Phase 4 and Phase 5.

The average ORP reduction may be associated also with phosphorus release values of the IFAS-SBBR demonstrated during Phase 5 kinetic studies (Barnard et al., 2017). Intermittent stirring system failures persists until Phase 4 which was defined by the implementation of new magnetic stirring equipment. Satisfaction quickly reverted as stirring failures continue with the new equipment. Noting that from the start of Phase 1 research, glass plates cut and fit at the base of the reactors, provide stir bar wear prevention. On the assumption that the glass plates, are "floating" they are removed from both reactors in August, this decision proves accurate. In conjunction with the change, mixing speed is reduced.

Early in August, Phase 4 underwent a reduction in influent ammonium and phosphorus with intention to align the influent carbon, nitrogen, and phosphorus ratio with something approaching theoretical ideal 100:10:1 (C:N:P). Both reactors fully oxidize influent ammonium to nitrate. Following the change, both reactors fully oxidize influent ammonium only to nitrite.

Phase 5 in mid-September reactors sustain four days (16^{th} to 20^{th}) without feed concentrate in the batch feed, Figure 8 to Figure 13. An electrical cable failure caused this condition. It appears that endogenous respiration sustained both reactors on water only (no feed concentrate) and hydrolyzed biomass for this interval.

Kinetic Study Investigations (Phase 4)

Between May and August 2021 corresponding with Phase 4, five kinetic study nutrient removal investigations demonstrate the progressive enhancement of phosphorus removal in the IFAS-SBBR relative to the AS-SBR. Two of the studies 2021-06-04 and 2021-07-02 isolate the fixed media biomass by siphoning MLSS from the reactor leaving only fixed biomass to treat the influent synthetic wastewater. Improved mixing provides for lower air flow rate and lower mixed liquor dissolved oxygen across both reactors testing denitrification under quasi anoxic mixed liquor conditions. Quasi anoxic denitrification is accompanied with a range of influent carbon-to-nitrogen ratios and carbon-to-phosphorus ratios.

Reactor MLSS samples across one 8-hour treatment cycle and from both reactors' trace through the anoxic and anaerobic feed stage followed by the aerobic react stage. Most evident in the plots is the rise (feast) and fall (famine) of phosphate concentration during the anaerobic and aerobic stages respectively. Residual nitrate carries over from the previous treatment cycle to be immediately denitrified anaerobically in the presence of rbCOD entering with the fresh feed.

The kinetic studies of Phase 4 demonstrate the influence of varied influent VFAs concentration on the anaerobic release of phosphorus. In essence there are two configurations presented, they are fresh influent feed with less than 40 mg L^{-1} VFAs and 2-day old influent feed with upwards of 190 mg L^{-1}. As noted, whether the VFAs enter with the influent or not, average influent total phosphorus measured at about 31 mg L^{-1} and effluent total phosphorus measured in the order of 1.0 mg L^{-1} represent a net reduction of 30 mg L^{-1} of phosphorus. Literature suggests that 8 mg L^{-1} of VFAs are required to remove 1.0 mg L^{-1} of phosphorus or in this case 240 mg L^{-1} of VFAs. This suggests that in order for the phosphorus to be removed, under current conditions of fresh batched influent feed, VFAs need to be formed anaerobically by acidogenesis from readily degradable rbCOD. Stated another way, many of the statements about the reliability of the EBPR process

ignores the fact that about 8 mg L^{-1} of VFA is required to remove 1 mg L^{-1} of phosphorus and if not available, phosphorus removal will suffer (Barnard & Kobylinski, 2014).

Nutrient removal plots for the Phase 4 kinetic studies found in Appendix A, are described in greater detail below:

Kinetic Study, 2021-05-21 (2-day old feed, influent total VFAs = 140 mg L^{-1}); (only Acetic Acid measured)

- COD removal both reactors stable with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was 75 mg L-1 and 100 mg L-1 for the Control and IFAS reactors respectively.
- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N appear essentially equal between reactors.
- Nutrient PO_4^{3-}-P removal, is more or less the same not favoring the Control SRB over the IFAS-SBBR. The IFAS showed a marginally higher release which resulted in a slightly longer period of phosphorus uptake under aerobically.

Kinetic Study, 2021-06-04 (2-day old feed, influent total VFAs = 90 mg L^{-1}); (Formic, acetic, and propionic acid shown. Lesser concentrations of isobutyric, butyric, isovaleric, and valeric acids not shown). Attempt at separating IFAS fixed media contribution from MLSS nutrient removal. The IFAS SBR MLSS was siphoned out and refrigerated for the kinetic study, leaving only the fixed media to treat the synthetic wastewater feed. Following the study, the MLSS was returned for the subsequent feed cycle.

- COD removal AS-SBR (Control) typical reduction from 300 to 50 mg L^{-1} by the end of anaerobic stage. By contrast the IFAS reactor reduced COD from just under 300 to just above 100 mg L^{-1} by the end of the anaerobic stage. It is noteworthy

that the removal to the and if the anaerobic stage is similar to the previous study for both reactors.

- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N was typical for the Control SBR with ammonium oxidation to nitrite and then to nitrate providing nearly complete nitrification by the end of the aerobic stage. By contrast the IFAS fixed media was able only to oxidize 20%, or 6 mg L^{-1} influent ammonium to nitrate by the end of the aerobic stage. Considered another way, the weighted average of fixed biomass VSS by volume represents approximately 30% but only the outer 2 or 3 mm would actively participate in the treatment. It may be that more than two weeks of fixed biomass growth are necessary to colonize the fixed biomass with nitrifier microbes.
- Nutrient PO_4^{3-}-P removal, was typical for the Control SBR releasing about 60 mg L-1 during the anaerobic stage, followed by near complete uptake during the aerobic stage. By contrast the IFAS reactor showed a muted release of 15 mg L^{-1} during the anaerobic stage followed by perhaps 6 mg L^{-1} uptake during the aerobic stage leaving about 23 mg L^{-1} in the effluent. It should be noted that the phosphorus release is approximately 18% of the 85 mg L^{-1} observed for the full IFAS reactor. Again, this value is meaningful given the quantity of surface fixed microbes actively participating in the treatment.

Kinetic Study, 2021-07-02 (fresh feed, influent VFAs = 20 mg L^{-1}); (Formic, acetic, and propionic acid shown. Lesser concentrations of isobutyric, butyric, isovaleric, and valeric acids not shown). Attempt at separating IFAS fixed media contribution from MLSS nutrient removal. The IFAS SBR MLSS was siphoned out and refrigerated for the kinetic study, leaving only the fixed media to treat the synthetic wastewater feed. Following the study, the MLSS was returned for the subsequent feed cycle.

- COD removal AS-SBR (Control) good reduction from 220 to 50 mg L^{-1} by the end of anaerobic stage. By contrast the IFAS reactor reduced COD from just under 250

to just above 100 mg L^{-1} by the end of the anaerobic stage, exceptional for only fixed biomass.

- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N was typical for the Control SBR with ammonium oxidation to nitrite and then to nitrate providing nearly complete nitrification by the end of the aerobic stage. By contrast the IFAS fixed media was able only to oxidize a small amount of influent ammonium to nitrate by the end of the aerobic stage. Ammonium was largely unchanged with basically no nitrite or nitrate formed.

- Nutrient PO_4^{3-}-P removal, was typical for the Control SBR releasing about 40 mg L^{-1} during the anaerobic stage, followed by near complete uptake during the aerobic stage. By contrast the IFAS reactor showed a muted release of 20 mg L^{-1} during the anaerobic stage followed by perhaps 10 mg L^{-1} uptake during the aerobic stage leaving about 25 mg L^{-1} in the effluent. It should be noted that the phosphorus release is approximately 20% of the 85 mg L^{-1} observed for the full IFAS reactor. Again, this value is meaningful given the quantity of surface fixed microbes actively participating in the treatment.

Kinetic Study, 2021-07-16 (2-day old feed, influent total VFAs = 173 mg L^{-1}); (Formic, acetic, and propionic acid shown. Lesser concentrations of isobutyric, butyric, isovaleric, and valeric acids not shown).

- COD removal both reactors very good with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was 50 mg L^{-1} for both reactors having started with about 300 mg L^{-1} for the Control and IFAS reactors.

- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N was typical for the Control SBR and the IFAS reactor with ammonium oxidation to nitrite and then to nitrate providing nearly complete nitrification by the end of the aerobic stage.

- Nutrient PO_4^{3-}-P removal, was typical for the Control SBR releasing about 40 mg L^{-1} during the anaerobic stage, followed by near complete uptake during the aerobic stage. By contrast the IFAS reactor showed a strong release of 50 mg L^{-1} during the anaerobic stage followed by perhaps near complete uptake during the aerobic stage.

Kinetic Study, 2021-08-27 (2-day old feed, influent total VFAs = 196 mg L^{-1}); (Formic, acetic, and propionic acid shown. Lesser concentrations of isobutyric, butyric, isovaleric, and valeric acids not shown).

- COD removal both reactors very good with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was 75 mg L^{-1} while the IFAS reactor was 100 mg L^{-1} having started with about 275 mg L^{-1} for the Control and IFAS reactors.

- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N was typical for the Control SBR and the IFAS reactor with ammonium oxidation to nitrite and then to nitrate providing nearly complete nitrification by the end of the aerobic stage.

- Nutrient PO_4^{3-}-P removal, was low for the Control SBR releasing about 20 mg L^{-1} during the anaerobic stage, followed by near complete uptake during the aerobic stage. By contrast the IFAS reactor showed a strong release of 42 mg L^{-1} during the anaerobic stage followed by perhaps near complete uptake during the aerobic stage.

Kinetic Study Investigations (Phase 5)

Between October and December 2021 corresponding with Phase 5, five kinetic study nutrient removal investigations are conducted to demonstrate progressive enhancement of phosphorus removal in the IFAS-SBBR relative to the AS-SBR. Extending the anerobic mass fraction proceeded in two steps. Initially with extending the anaerobic phase from 90 minutes to 120 minutes, followed by implementing staged mixing by delaying anaerobic mixing by 30 minutes. Anaerobic mass fraction is understood to influence acidogenic co-fermentation of influent soluble carbon for VFAs augmentation.

Tracking fate-and-effect of the biological phosphorus removal include wet acid digestions of mixed liquor suspended solids, both bulk and fixed biomass throughout phase 5.

Reactor MLSS samples across one 8-hour treatment cycle and from both reactors' trace through the anoxic and anaerobic feed stage followed by the aerobic react stage. Most evident in the plots is the rise (feast) and fall (famine) of phosphate concentration during the anaerobic and aerobic stages respectively. Residual nitrate carries over from the previous treatment cycle to be immediately denitrified anaerobically in the presence of rbCOD entering with the fresh feed. Ammonium remains relatively stable through the anaerobic stage before plunging (i.e., nitrification) to nearly zero during the aerobic stage. Aerobically, autotrophs *Nitrosomonas,* AOB and *Nitrobacter*, NOB, oxidize ammonium to nitrite, followed by the oxidization of nitrite to nitrate respectively. The fascinating thing to watch is the nitrate plot rise in sync with the nitrite plot to the point ammonium is exhausted. Forward from that point, the nitrate plot continues to ascend to a maximum concentration plateau, while the nitrite plot begins to descend to zero. As Appendix B plots demonstrate, the mixed liquor rdCOD concentration is substantially or completely depleted by PAOs by the end of the anaerobic (feast) stage. What remains is particulate COD going into the aerobic stage.

Heterotrophs use organic compounds for electron donor and carbon for cell synthesis. Given that stabilization of organic matter is premier for biochemical operations, heterotrophs predominate. Chemoautotrophic bacteria (autotrophs) use inorganic compounds for electron donor and carbon dioxide as carbon source for cell synthesis. Through the attachment of biofilm within the reactors, slow growing autotrophs (i.e., nitrifiers) are established providing nitrification and if thick enough, the biofilm may also support anoxic layers capable of denitrification. Of course, with the IFAS-SBBR supporting comparatively more fixed biomass, denitrification is typically higher in the research reactor when compared with the control reactor (López-Palau et al., 2012).

Nutrient removal plots for the Phase 5 kinetic studies found in Appendix B, are described in greater detail below:

Kinetic Study, 2021-10-22 (fresh feed, influent VFAs = 32 mg L^{-1}); (nitrogen sparge and mixing)

- COD removal both reactors stable with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was equal between reactors.
- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N appear essentially equal between reactors.
- Nutrient PO_4^{3-}-P removal, more specifically anaerobic release, is showing a stark difference in favor of the IFAS-SBBR. This augmented release may be attributable to phosphorus accumulating organisms releasing phosphate held in the fixed biomass.

Kinetic Study, 2021-11-05 (fresh feed, influent VFAs = 20 mg L^{-1}); (nitrogen sparge and mixing)

- COD removal both reactors stable with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was equal between reactors.
- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N appear essentially equal between reactors.
- Nutrient PO_4^{3-}-P removal, more specifically anaerobic release, is showing a stark difference in favor of the IFAS-SBBR. This augmented release may be attributable to phosphorus accumulating organisms releasing phosphate held in the fixed biomass.

Kinetic Study, 2021-11-19 (fresh feed, influent VFAs = 40 mg L^{-1}); (nitrogen sparge and staged anaerobic mixing)

- COD anaerobic removal better for AS-SBR, by the aerobic stage both reactors stable with equal COD removal equal between reactors.
- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N appear essentially equal between reactors.
- Nutrient PO_4^{3-}-P removal, more specifically anaerobic release, is showing a stark difference in favor of the IFAS-SBBR. This augmented release may be attributable to phosphorus accumulating organisms releasing phosphate held in the fixed biomass.

Kinetic Study, 2021-12-03 (2-day old feed, influent VFAs = 190 mg L^{-1}); (staged anaerobic mixing, no sparge)

- COD removal both reactors stable with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was equal between reactors.
- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N were essentially equal between reactors.
- Nutrient PO_4^{3-}-P removal, more specifically anaerobic release, is showing a stark difference in favor of the IFAS-SBBR. This augmented release may be attributable to phosphorus accumulating organisms releasing phosphate held in the fixed biomass.

Kinetic Study, 2021-12-31 (fresh feed, 0.23 g L^{-1} sodium acetate, influent VFAs = 114 mg L^{-1}); (staged anaerobic mixing, no sparge)

- COD removal both reactors stable with the lower initial value of the AS-SBR (Control) owing to dilution by a slight larger volume. By the end of the anaerobic stage COD was equal between reactors.

- Nutrient removal NH_4^+-N, NO_2^--N, and NO_3^--N were essentially equal between reactors.
- Nutrient PO_4^{3-}-P removal, more specifically anaerobic release, is showing a stark difference in favor of the IFAS-SBBR. This augmented release may be attributable to phosphorus accumulating organisms releasing phosphate held in the fixed biomass.

Influent Feed Adjustment to Theoretical

One of the essential parameters that influence wastewater treatment is the ratio of carbon to nitrogen to phosphorus, Table 7. Moreover, the carbon-to-nitrogen (C/N) ratio can influence functional microorganisms, including autotrophic ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), as well as heterotrophic species. Also, it has been established that C/N significantly influences the enhanced biological phosphorus removal (EBPR), (i.e., C/N $\leq$ 6 favors P release during final settling) (Mannina et al., 2020).

In the case of the current research, reducing ammonium nitrogen from the initial medium strength feed resulted in lost nitrite oxidation to nitrate. This condition continued through most of phase 4 and phase 5. It was phase 1-2 when the IFAS-SBBR formation of nitrite was stimulated to full nitrification with the introduction of an influent phosphorus increase from 20 g L^{-1} to 60 g L^{-1}. The assumption being at the lower nitrogen and phosphorus level, phosphorus limiting conditions may play a factor in nitrification.

The influent C/P ratio is particularly important since it has been correlated with EBRP performance and stability. Published research studies with EBPR systems operated at different C/P ratios showed that polyphosphate limitation, at higher ratios of C/P, coincides with switching of polyphosphate metabolism to glycogen metabolism in the system. However, whether it resulted from the associated increases in abundance of glycogen accumulating organisms (GAOs) over phosphorus accumulating organisms (PAOs) and/or actually phenotypic changes in PAOs remain unclear (Majed & Gu, 2020).

Table 7

Influent feed adjustment to theoretical 100:10:1 (C:N:P)

Phase 4-5	**COD**	**NH4-N**	**PO4-P**			
Date	mg L^{-1}	mg L^{-1}	mg L^{-1}	**C:**	**N:**	**P**
6/10/2021	423	53.6	21.8	100	13	5
6/24/2021	404	55.2	21.4	100	14	5
7/8/2021	396	54.8	13.6	100	14	3
7/22/2021	380	47.2	13.0	100	12	3
8/5/2021	386	42.4	11.4	100	11	3
8/19/2021	396	39.9	9.60	100	10	2
9/2/2021	383	43.9	9.80	100	11	3
9/16/2021	370	43.8	9.02	100	12	2
9/30/2021	391	38.1	5.51	100	10	1
	New Yeast			New Yeast		
10/14/2021	413	44.6	7.96	100	11	2
10/28/2021	417	43.9	9.19	100	11	2
11/11/2021	421	44.4	8.03	100	11	2
		N “bump”			N “bump”	
12/1/2021	449	55.3	8.54	100	12	2
12/23/2021	394	55.1	9.24	100	14	2

A published investigation of EBPR systems with a carbon feed mixture (i.e., acetate, propionate and amino acids) warranted further study. The investigation as described adds NH_4Cl to maintain a stoichiometric requirement of nitrogen for growth (i.e., C:N:P of 100:5:1). The sludge retention time (SRT) and hydraulic retention time (HRT) of the system described were maintained at 7 days and 12 h respectively, at a range reported to allow good EBPR performance (Majed & Gu, 2020).

Some rbCOD (i.e., including extracellular polymeric substances) can be converted to VFAs by acidogenic fermentation in the anaerobic zone thus a better measure of the potential of a system to remove phosphorus is to ensure rbCOD:P ratio of greater than 14

(Barnard and Kobylinski, 2014). The current research maintained a soluble carbon to phosphorus ratio on the order of 14.

Investigate Response to Feed Disruption

In September 2021 between the 16th and 20th, feed to the reactors was disrupted by the failure of a batch pump. Several observations stand out from Figure 8 to Figure 13, these plots are based on detailed sensor data, and trace several days of reactor performance leading up to and following the feed disruption. Starting with, both reactors abruptly retuned to near normal readings, when the feed was restored. The exception being Control DO, which apparently didn't detect the reduced biochemical activity. This would appear to be a fault of the Control DO sensor, until observing Table 8, which also shows no response from the Control SVI (30). This characteristic should be studied further in the future. Meanwhile, the rise in IFAS DO (and SVI) indicates reduced biochemical activity that appears to have diminished the MLSS settling characteristics.

Staying with Figure 8 to Figure 13, the IFAS average and median ORP values are basically the same, suggesting a biochemical stability, not seen in the other plots. Following reactor cleanings on the 14th and 28th, there appears to be only a slight ORP rise, which is surprising, especially for the IFAS which has the fixed media biomass nearly completely removed for analysis.

Average IFAS DO is a full 1.0 mg L^{-1} lower than the Control DO, it is believed that this DO differential is a contributing factor in the IFAS denitrification yielding lower total effluent nitrate and nitrite. Average IFAS pH was only moderately higher than the Control pH before the feeding disruption, with both reactor pH basically the same following feed restoration. The only parameter that shows a reduction across both reactors following the feed restoration is ORP. It is believed that the skew toward lower ORP (max, med, avg, min) is related to the sensors having been purged of biomass coating during the prolonged starvation. This natural oxidation, "sensor cleaning", returned accuracy to the ORP readings, when the feed was restored.

Figure 8

Batch feed disruption, control pH Plot

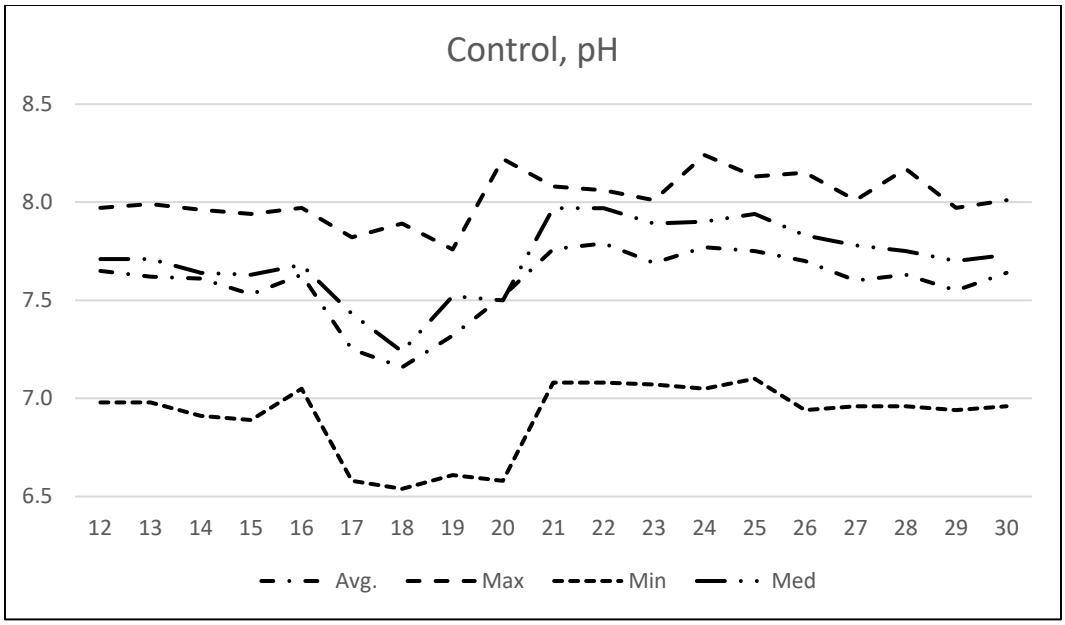


Figure 9

Batch feed disruption, IFAS pH Plot

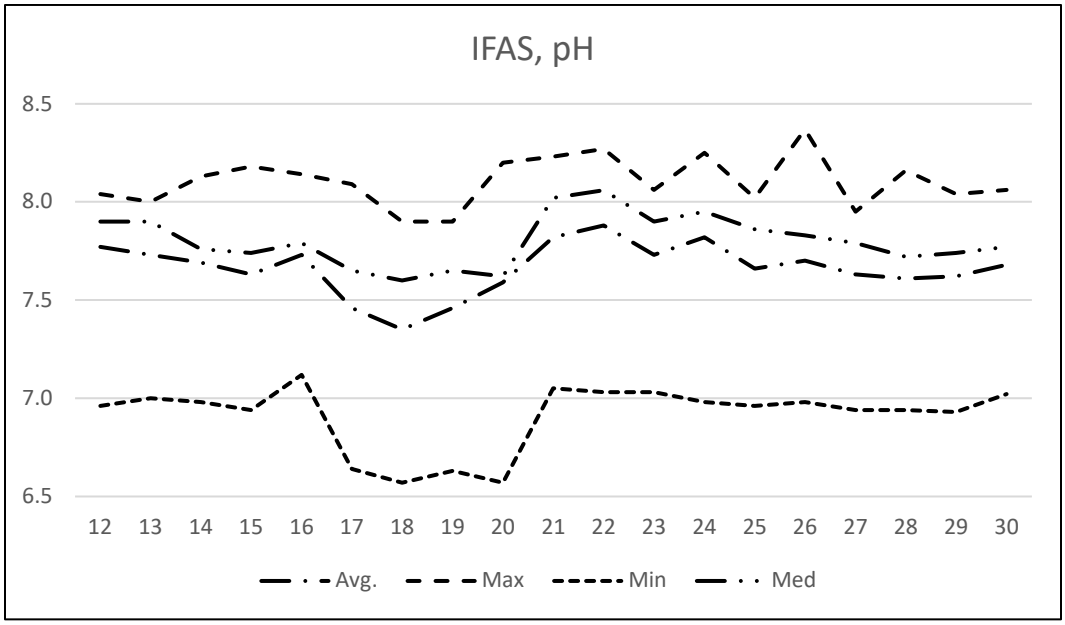


Figure 10

Batch feed disruption, control ORP Plot

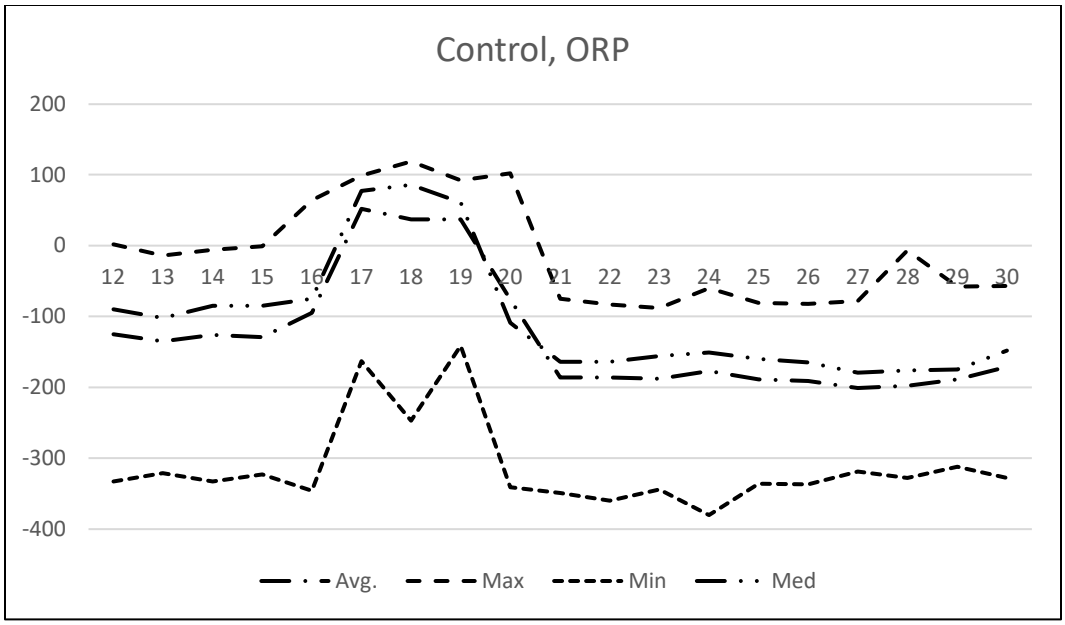


Figure 11

Batch feed disruption, IFAS ORP Plot

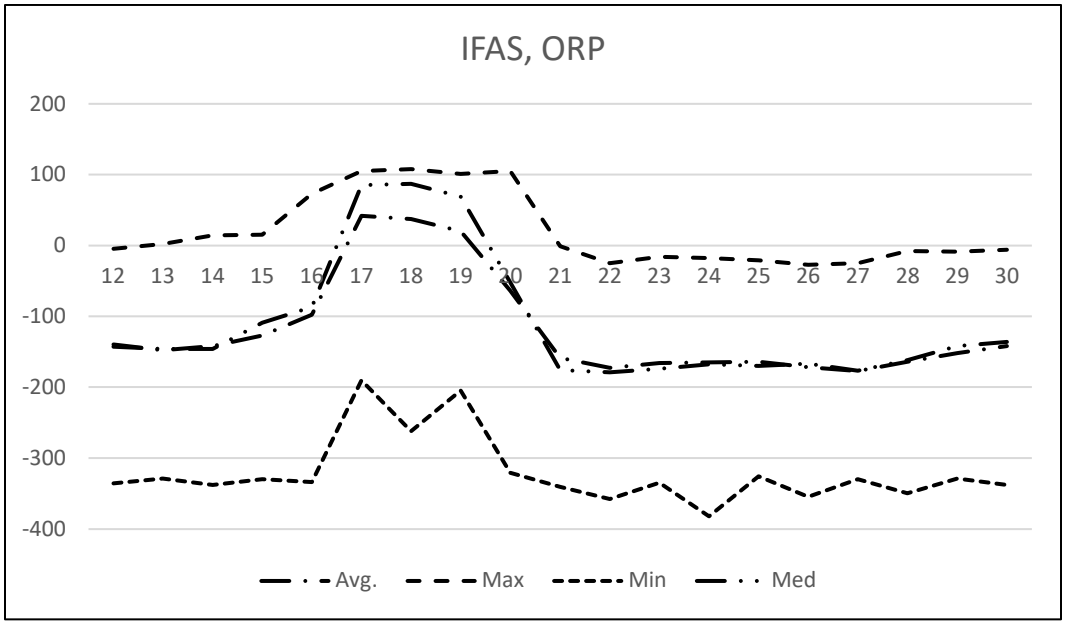


Figure 12

Batch feed disruption, control DO Plot

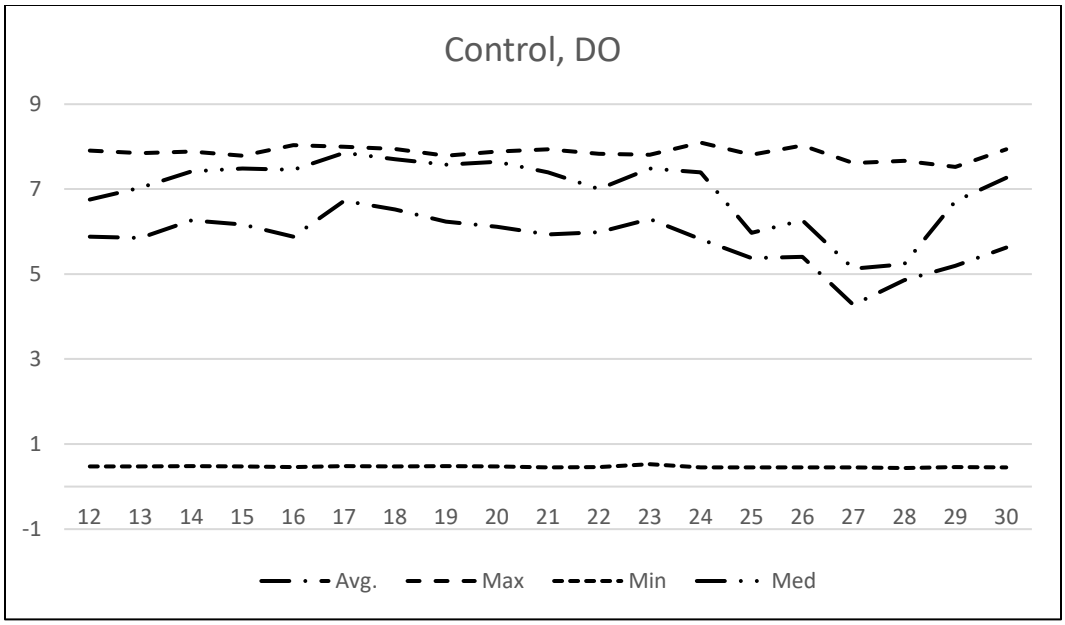


Figure 13

Batch feed disruption, IFAS DO Plot

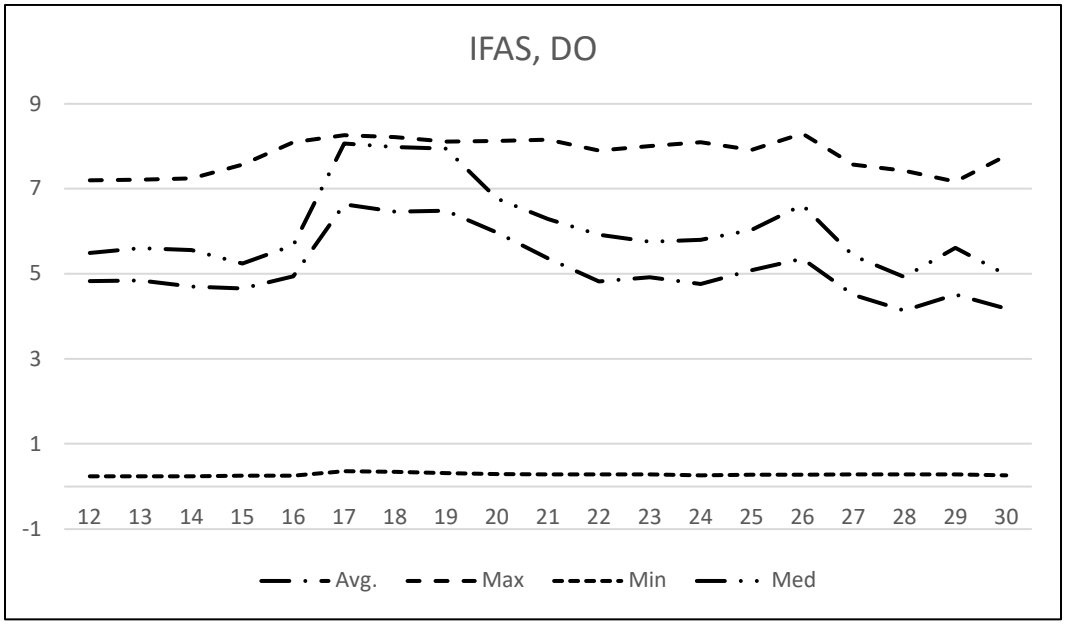


Table 8

Effluent quality, P-release, and SVI for September feed study

mg L^{-1}	Control	Control	Control	SVI(30)	IFAS	IFAS	IFAS	SVI(30)
Date	NH_4^+-N	PO_4^{-3}-P	P-release	ml g^{-1}	NH_4^+-N	PO_4^{-3}-P	P-release	ml g^{-1}
12	0.62	0.23	n/a	34	2.26	2.25	n/a	96
14	1.77	0.21	27.7	n/a	2.25	1.99	51.7	n/a
16	1.67	0.14	24.9	38	1.14	0.22	45.5	90
18	**1.55	0.59	n/a	42	**0.75	0.17	n/a	127
20	1.42	0.77	29.9	44	0.08	0.34	38.5	139
22	19.8	1.88	29.7	32	15.6	3.69	37.7	86
24	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
26	11.9	0.12	n/a	27	5.67	0.20	n/a	75
28	12.3	0.16	32.4	n/a	2.54	0.16	62.2	n/a
30	5.04	0.00	25.4	35	1.21	0.12	53.9	76

Note: ** data averaged from two adjacent data points.

Table 8 is provided in conjunction with Figure 8 to Figure 13 related to the four-day feed disruption. The disruption did bode well for the IFAS effluent phosphorus which appears lower when the feed was restored. That said, both reactors effluent phosphorus reduced to below the detectable limit shortly after. This characteristic may be attributable to the onset of aerobic granular sludge in both reactors, that continued through to the end of the investigations.

Two SRTs (14-days) following the feed disruption, there is an onset of aerobic granular sludge (AGS) in both reactors. Initially, the AGS represents about 30% of the mixed liquor (MLSS) at which point the waste activated sludge (WAS) stage is modified by starting the waste pump at the same time as the air stopped, defined as the end of the aerobic stage. Previously, the waste pump starts one minute after the end of the aerobic stage. It may be that this delay provides selective pressure for the heavier AGS granules

while washing out MLSS floc. By mid-October AGS presence is reduced moderately to 3%-5% of the mixed liquor continuing for the AS-SBR to the end of Phase 5. However, later in December the IFAS-SBBR AGS grew larger and within a few days very much overtakes the floc in the IFAS reactor as demonstrated by the SVI (5) equaling the SVI (30).

There is no reason to suggest from the current research, that aerobic granules could form as they did, given that none of the commonly cited parameters including short settling time, and high hydrodynamic shear are present. Among strategies to promote AGS, strong hydraulic selection pressure (HSP) from both short settling time, and short hydraulic retention time (HRT) combine with high organic loading rate (OLR), is considered the most effective approach resulting in aerobic granulation. Settling time and hydraulic retention time (HRT) are two controlled hydraulic selection pressures implicated in the formation of aerobic granulation (Liu et al., 2016).

Investigate Response to Staged Mechanical Mixing

November 2021, staged mixing is implemented for both reactors influencing anaerobic mass fraction for acidogenesis and enhanced conversion of readily biodegradable COD to volatile fatty acids (VFAs). This is supplementary to extending the duration of the anaerobic phase from 90 minutes to 120 minutes using the same rational. This approach, first applied during the November 5, kinetic study, includes nitrogen gas sparge for quasi mixing following the feed stage onward through the anaerobic stage. However, at about 30 minutes into the anaerobic stage, the mixers are turned on for 2 minutes which mobilize a quantity of settled sludge from below the air diffusers (i.e., source of nitrogen gas sparge). The result, an uncharacteristic drop in ORP, already well below -300 mV down to -400 mV, initially evident for the IFAS, and later to a lesser degree for the Control reactor as well. Reality being consumption of VFAs is extremely rapid and VFAs introduced by acidogenesis within the reactors will most certainly be consumed immediately and are not measurable for the current research.

Published literature links lower ORP values that are below about –300 mV to the growth of organisms like *Tetrasphaera* that thrive under those conditions. *Tetrasphaera* can ferment higher carbon forms, and produce VFAs, to the benefit of other PAOs while also taking up phosphorus under anoxic conditions (Barnard et al., 2017).

The following observations are based on Figure 14 to Figure 19, detailed sensor data plots that trace reactor performance leading up to and following the implementation of staged mixing. From November 6th onward, the anaerobic mix stage is delayed by 30 minutes following reactor feed. From the plots of the Control pH and IFAS pH, the Control reactor appears unusually dynamic compared with the IFAS for no explicable reason. In fact, the IFAS pH is exceptionally stable, especially the average and median, throughout the observed period.

With respect to plots showing Control ORP and IFAS ORP, both plots trend lower at the end of the period compared with the start. The Control ORP plot appears relatively uniform when contrasted with the IFAS ORP. There appears to be a rise in the IFAS ORP between the November 5th and November 19th, kinetic studies. It may be that the first study caused a disruption to the process that corrected itself following the second study.

This apparent divergence in IFAS ORP may be supported by the P-release data shown in Table 9, which displays an increase from 42.2 mg L^{-1} (i.e., influent phosphorus plus released poly-P) to 59.5 mg L^{-1} before moderating back to 43.9 mg L^{-1}. It should be noted as well that the Control reactor follows a similar but muted ORP pattern. Further, both reactors display increases following a kinetic study which normalized within a week.

Dissolved oxygen (DO) plots for both reactors seem relatively uniform with a small bump in the IFAS apparently associated with the November 5th kinetic study. Average and median Control DO are trending down over the period, but remain higher than the IFAS. The plots, as seen previously, demonstrate that the IFAS DO is consistently lower than the Control DO, assumed to be a contributing factor in the denitrification displayed by the IFAS SBR reactor.

Figure 14

Reactor sensitivity to staged mixing, control pH Plot

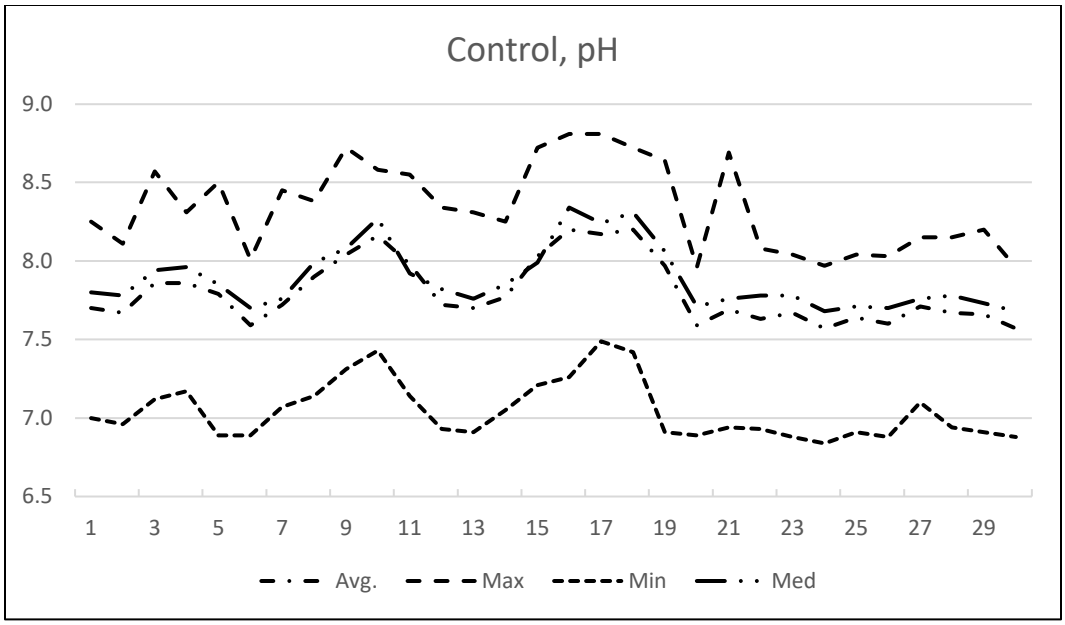


Figure 15

Reactor sensitivity to staged mixing, IFAS pH Plot

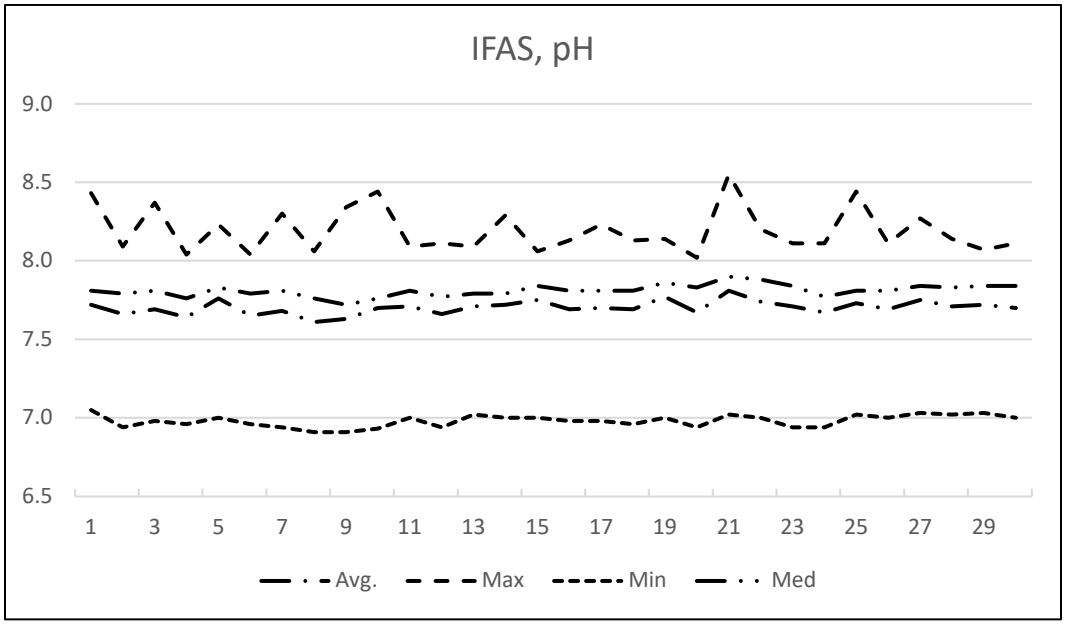


Figure 16

Reactor sensitivity to staged mixing, control ORP Plot

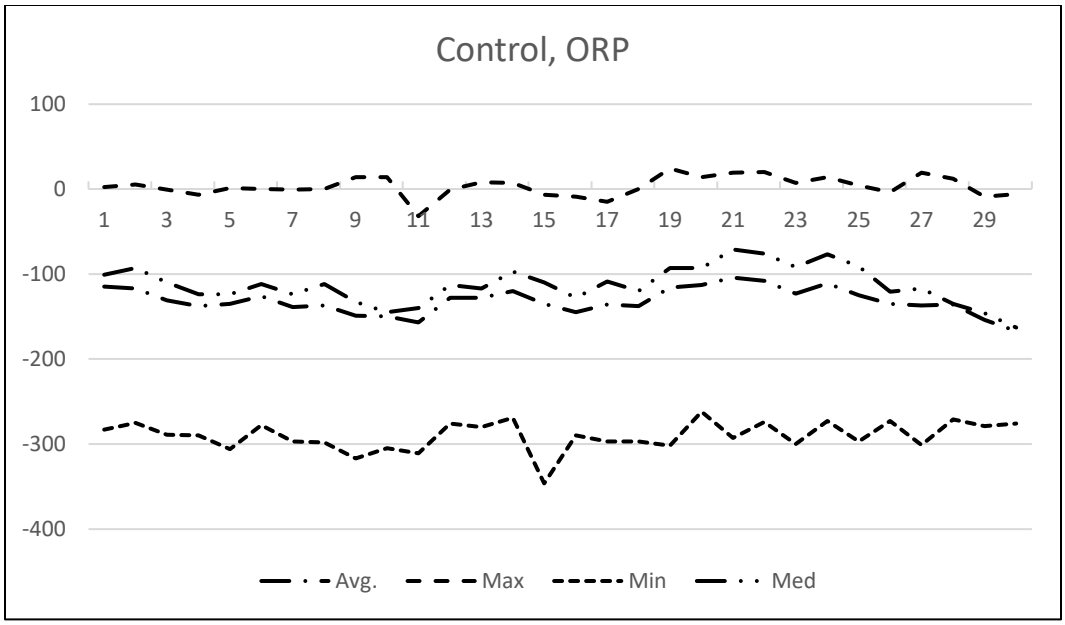


Figure 17

Reactor sensitivity to staged mixing, IFAS ORP Plot

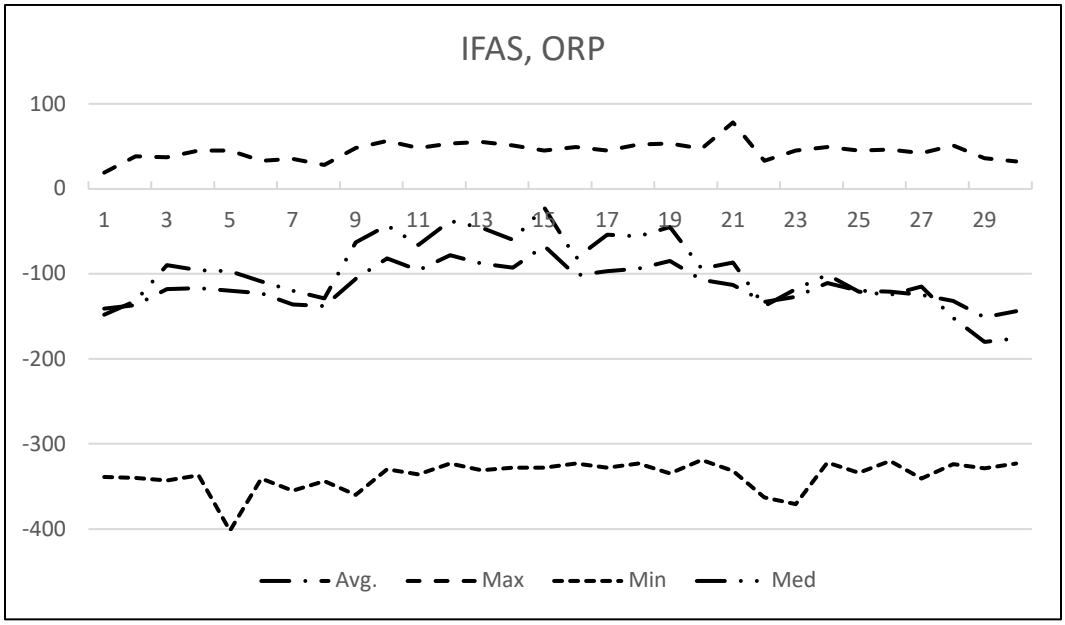


Figure 18

Reactor sensitivity to staged mixing, control DO Plot

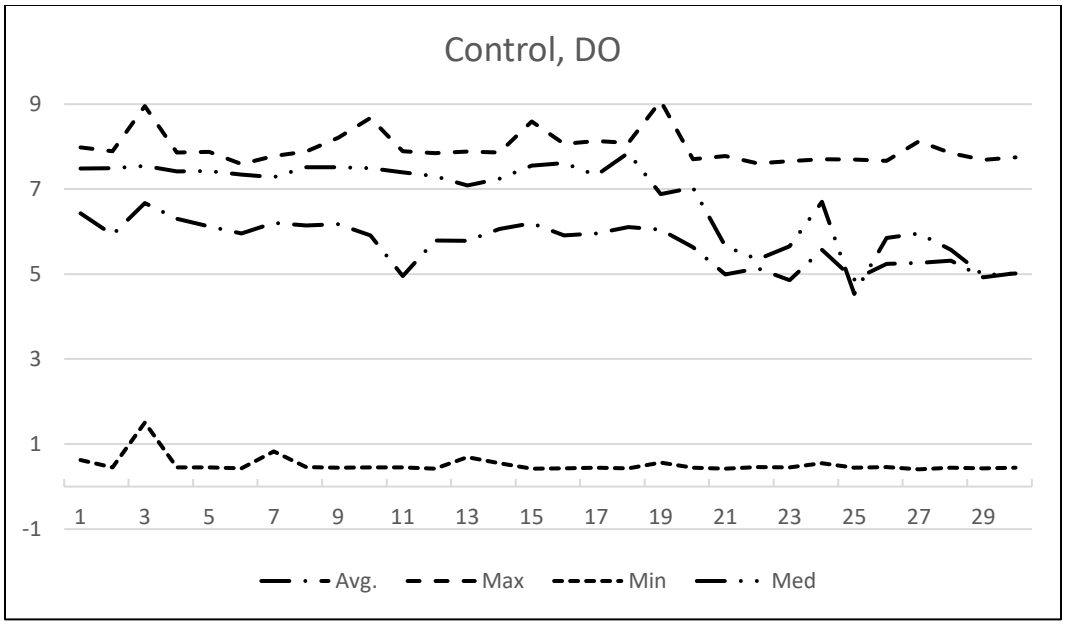


Figure 19

Reactor sensitivity to staged mixing, IFAS DO Plot

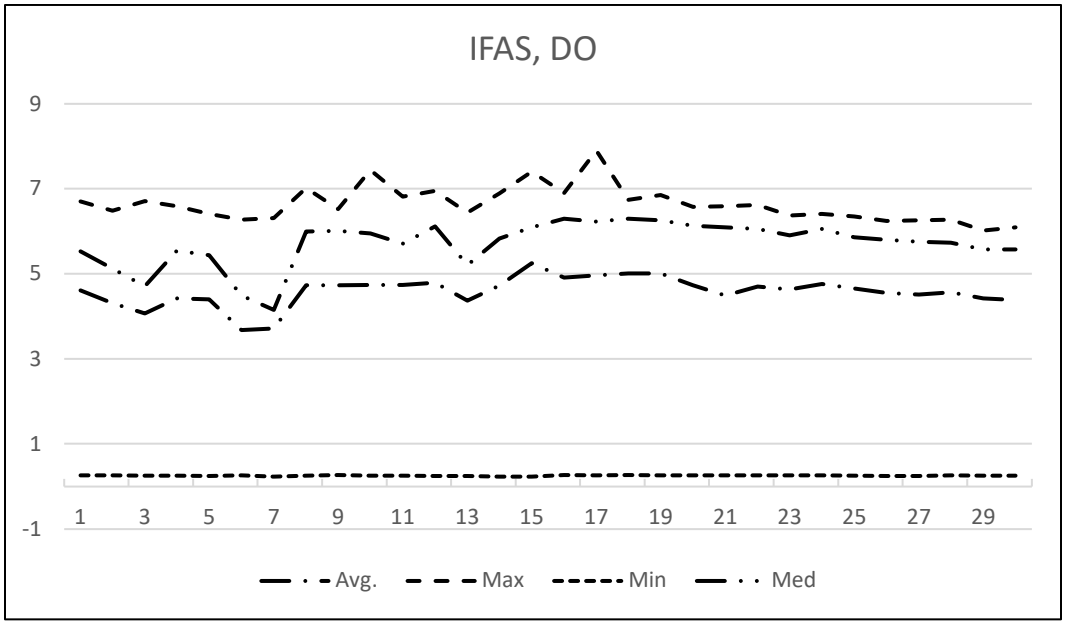


Table 9

Effluent quality, P-release, and SVI for November mixing study

mg L^{-1}	Control	Control	Control	SVI(30)	IFAS	IFAS	IFAS	SVI(30)
Date	NH_4^+-N	PO_4^{-3}-P	P-release	ml g^{-1}	NH_4^+-N	PO_4^{-3}-P	P-release	ml g^{-1}
1	0.49	0.00	18.9	48	0.13	0.00	23.0	83
3	0.53	0.00	21.7	50	0.24	0.00	42.2	85
5	Kinetic	Study			Kinetic	Study		
7	0.29	0.00	n/a	48	0.59	0.00	n/a	83
9	1.33	0.00	26.4	n/a	0.61	0.27	59.5	n/a
11	2.04	0.00	21.3	60	1.12	0.00	51.0	90
13	0.23	0.25	n/a	51	0.19	0.00	n/a	86
15	0.66	0.00	15.4	56	0.22	0.00	43.9	89
17	0.80	0.00	14.6	51	0.45	0.00	47.9	84
19	Kinetic	Study			Kinetic	Study		
21	0.38	0.00	n/a	46	0.23	0.00	n/a	69
23	1.22	0.09	15.6	39	0.56	0.21	61.6	42
25	2.23	0.00	14.1	50	1.16	0.00	44.9	78
27	0.28	0.00	n/a	49	0.27	0.00	n/a	81
29	0.65	0.00	14.3	44	0.37	0.19	44.6	73

Investigate Response to Influent Ammonium Concentration Increase

Related to bringing the C:N:P ratio back from theoretical 100:10:1 and closer to values from earlier in the research, influent ammonium concentration is increased twice during December 2021. The first increase to 20 mg L^{-1} (from 10 mg L^{-1}) is December 9th and the second to 30 mg L^{-1} on December 17th. This step is primarily associated with restoring nitrification lost when ammonium was reduced. The expectation for restoring nitrification was not realized however, potentially related to phosphorus being limited.

As described in the previous section, AGS that forms in both reactors during October is later moderated on both reactors to 3%-5% of the mixed liquor. However, later in December the IFAS-SBBR AGS grew in size overtaking the mixed liquor floc which may have been influenced by the influent ammonium concentration. The IFAS process change to AGS is not evident in the data measurements, however the Control reactor does demonstrate a declining minimum ORP trend between the start and end of December.

Detailed sensor data from the week prior to the influent ammonium increase, and extending four weeks to the end of the month are presented in Figure 20 to Figure 25, plots from which the following observations are based.

Starting with the Control pH plot, again the Control pH does not appear as well behaved as the IFAS pH plot. The pH range appears between a low of 6.9 pH and a high of 8.2 pH, values considered typical of activated sludge. For the period, the Control reactor average pH appears as 7.6 pH. The more stable readings of the IFAS reactor pH appear marginally higher ranging from a low of 7.0 pH to a high of 8.1 pH. For the period, the IFAS reactor average pH appears as 7.75 pH, possibly being indicative of denitrification.

Moving to ORP plots, the Control reactor shows a declining trend toward -375 mV at the end of the period from -300 mV at the start of the period. This may be indicative of increased microbial (anaerobic) synergies in response to influent ammonium. That being said, ammonium is oxidized by autotrophs in the aerobic stage. Heterotrophs have the capacity to switch from aerobic function to anoxic function, so microbial augmentation would have started in the aerobic stage before subsequently influencing the anaerobic stage. Maximum and average ORP for the Control reactor hovered about -10 mV and -150 mV respectively.

The IFAS ORP shows a response to the initial influent ammonium increase, and a more defined response to the second increase. Minimum ORP hovered around -350 mV throughout much of the period. The IFAS maximum and average ORP showed a response to both the first and second ammonium bumps. Maximum ORP decreased to -10 mV from 10 mV following the firsts bump, increasing to 50 mV following the second bump, before

returning again to negative ORP territory by the end of the period. Average ORP for the IFAS reactor remained about -220 mV before and after the first ammonium bump, increasing to -100 mV following the second bump, before returning again to -220 mV territory by the end of the period.

This departure in IFAS ORP plot in response to the influent ammonium bump is in some way supported by P-release data shown in Table 10, which displays an increase following the first bump, from 45.0 mg L^{-1} (i.e., influent phosphorus plus released poly-P) to 61.1 mg L^{-1} before moderating back to 55.8 mg L^{-1}. This occurred again following the second influent ammonium bump. It should be noted as well that the Control reactor follows a similar but muted ORP pattern. Effluent ammonium also shows a small increase following both bumps as shown in Table 10.

Dissolved oxygen (DO) plots for both reactors appear relatively uniform over the period with the influent ammonium bumps basically going unnoticed. As seem previously, the IFAS DO tracks the Control reactor and remains on average of 1.0 mg L^{-1} lower than the Control reactor. The plots again demonstrate that the IFAS DO is consistently lower than the Control DO, assumed to be a contributing factor in the denitrification displayed by the IFAS-SBBR reactor.

Figure 20

Reactor sensitivity to influent ammonium, control pH Plot

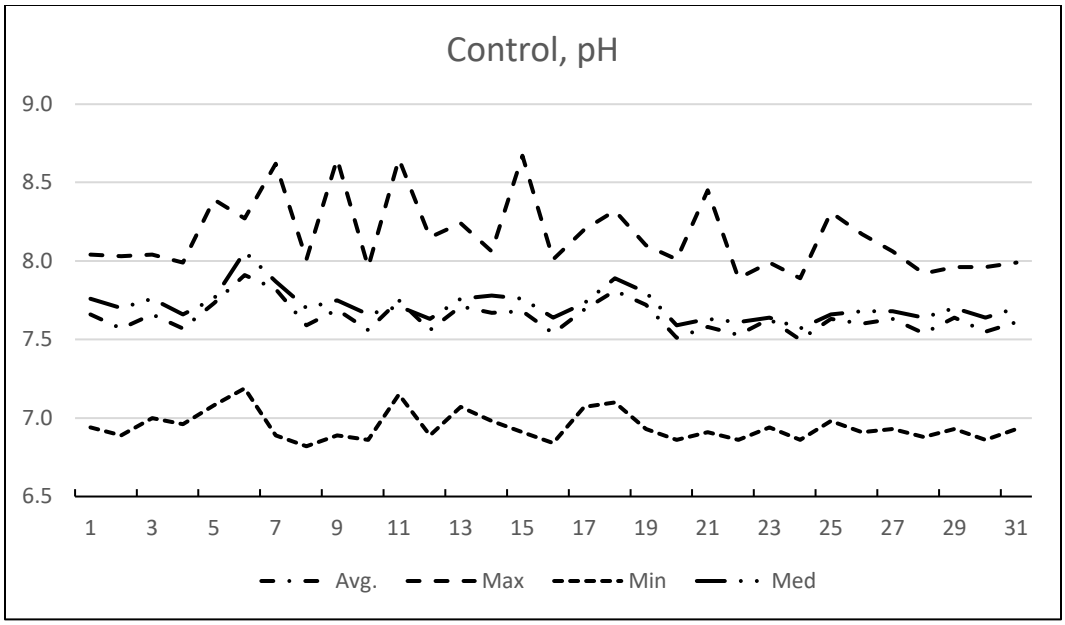


Figure 21

Reactor sensitivity to influent ammonium, IFAS pH Plot

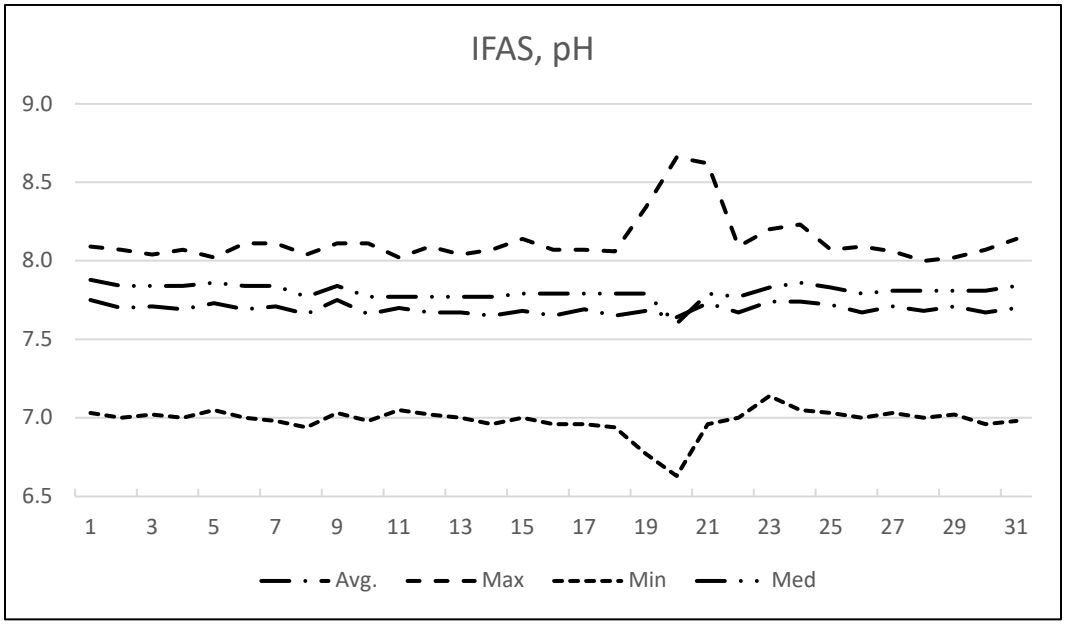


Figure 22

Reactor sensitivity to influent ammonium, control ORP Plot

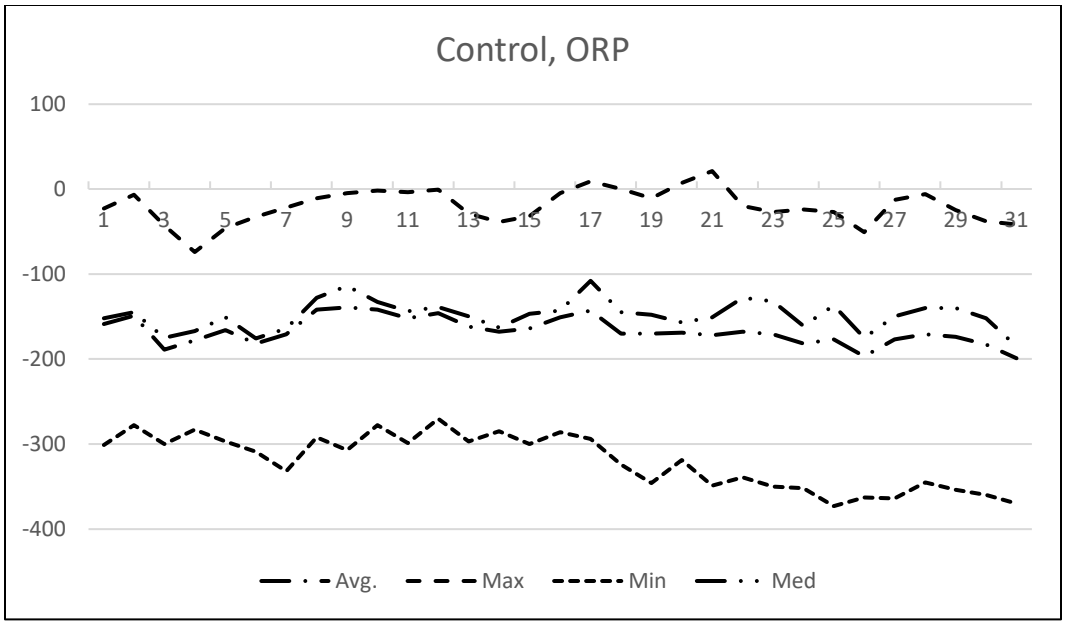


Figure 23

Reactor sensitivity to influent ammonium, IFAS ORP Plot

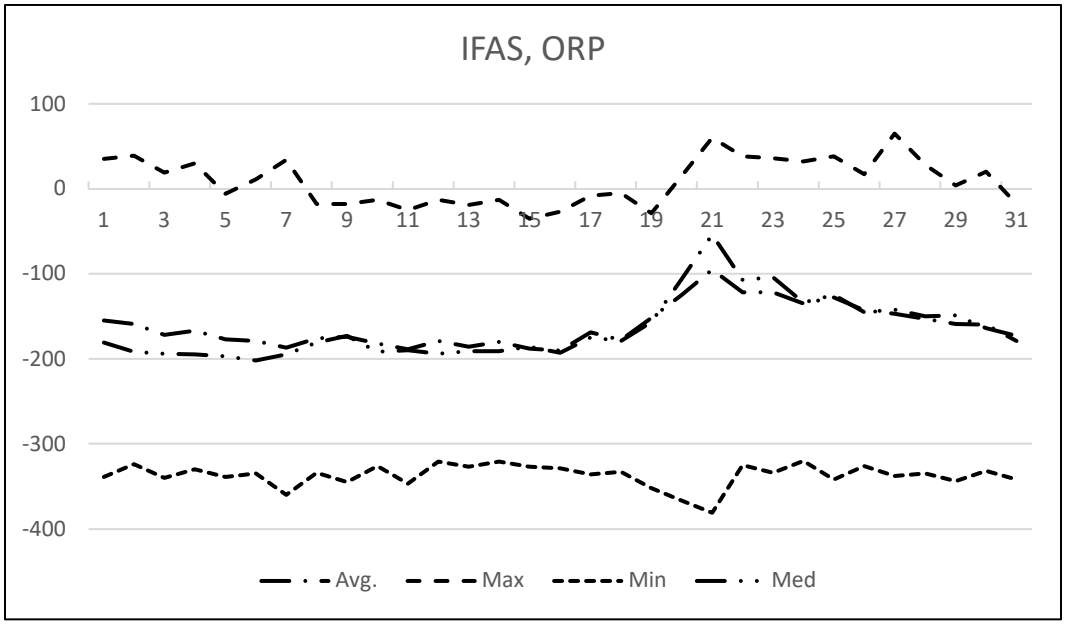


Figure 24

Reactor sensitivity to influent ammonium, control DO Plot

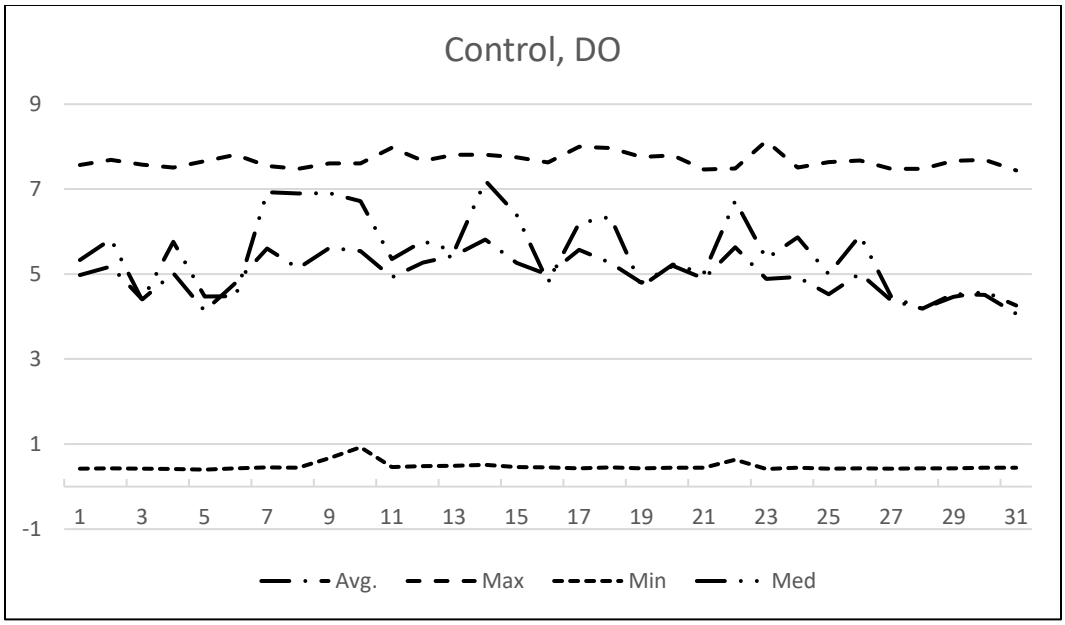


Figure 25

Reactor sensitivity to influent ammonium, IFAS DO Plot

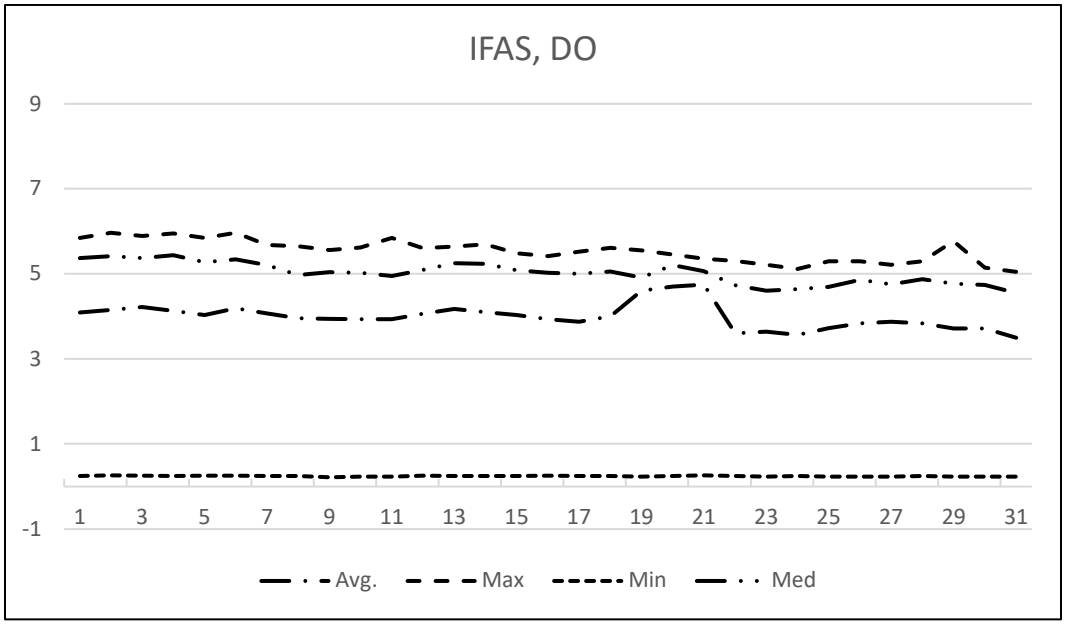


Table 10

Effluent quality, P-release, and SVI for December ammonium study

mg L^{-1}	Control	Control	Control	SVI(30)	IFAS	IFAS	IFAS	SVI(30)
Date	NH_4^+-N	PO_4^{-3}-P	P-release	ml g^{-1}	NH_4^+-N	PO_4^{-3}-P	P-release	ml g^{-1}
1	0.68	0.00	12.3	52	0.42	0.00	45.0	77
3	Kinetic	Study			Kinetic	Study		
5	0.27	0.00	n/a	49	0.29	0.00	n/a	75
7	0.36	0.00	23.7	n/a	0.70	0.43	61.1	n/a
9	1.10	0.00	20.4	45	0.58	0.02	52.3	76
11	0.41	0.37	n/a	43	0.39	0.35	n/a	66
13	0.50	0.00	19.8	44	0.41	0.00	59.7	66
15	0.50	0.00	19.6	48	0.42	0.00	69.3	70
17	0.49	0.07	17.5	45	0.12	0.19	55.8	72
19	0.12	0.00	n/a	44	0.13	0.08	n/a	62
21	0.44	0.00	22.5	n/a	0.22	0.15	68.5	n/a
23	0.45	0.00	19.5	46	0.23	0.05	52.0	74
25	0.42	0.00	n/a	n/a	0.27	0.26	n/a	n/a
27	1.66	0.00	21.3	48	0.60	0.25	58.1	63
29	1.73	0.00	22.2	45	1.01	0.25	72.9	73
31	Kinetic	Study			Kinetic	Study		

Ammonium Oxidation Rates

Ammonium is a key nutrient of municipal wastewater that must be effectively removed by aerobic oxidation. Oxidation rates for ammonium removal are tabulated for the various phases and found to nearly represent published research values in the order of 3.0 mg NH_4^+-N (g VSS h)$^{-1}$ (Furumai et al., 1999). The Table 11 below provides investigation results from a series of Phase 5 kinetic studies. Influent ammonium concentrations were provided as well to distinguish the dilution intrinsic to the IFAS-SBBR

reactor. The dilution is a consequence of the displaced volume of the fixed media plus fixed biomass. Stated another way, the Control SBR decanted treated effluent leaving 2500 ml of supernatant and settled sludge. Conversely, the Research SBBR decanted treated effluent leaving 2000 ml of supernatant and settled sludge, the 500 ml difference is to accommodate the volume of the fixed biomass when the reactors each received 5000 ml of influent feed. In this way, both reactors at 8.5 litres, provide a working volume of 7.5 litres.

Table 11

Ammonium oxidation rates, phase 5 kinetic studies with correlation coefficient

Phase 5	**Control**	**(Feed, time = 0)**	**IFAS**	**(Feed, time = 0)**
Date	mg-NH4-N g-VSS-h $^{-1}$	mg L^{-1}	mg-NH4-N g-VSS-h $^{-1}$	mg L^{-1}
9/24/2021	2.03	24.8	4.13	25.4
10/8/2021	2.35	16.2	3.55	19.5
10/22/2021	2.03	17.0	3.14	20.6
11/5/2021	2.04	17.5	4.01	20.6
11/19/2021	1.62	19.2	4.02	22.8
12/3/2021	4.59	34.6	8.39	37.6
12/31/2021	9.94	62.3	19.3	66.9
Average	3.51	27.4	6.65	30.5
R^2	0.959		0.999	

To some degree the rate at which nitrite and nitrate was produced, especially between research phases, could suggest that ammonium in the mixed liquor was possibly removed by other means. One candidate considered is ammonia nitrogen stripping from the aerobic stage due to turbulence from the vigorous mixing. However, this is speculation and guidance from the USEPA suggests $10.5 < pH < 11.5$ a requirement for ammonia stripping which was not the case for the current research given $6.5 < pH < .8.5$ as the operating range. A correlation coefficient of the data suggests nearly identical results, further diminishing any speculation of significant nitrogen stripping.

Phosphorus Uptake/Release Rates

Contrasting Control (AS-SBR) and Research (IFAS-SBBR) ortho-P uptake and release, from Table 12, it becomes evident from recent investigations that for essentially the same process conditions, the IFAS-SBBR may be enhanced by the biochemical augmentation of a fixed biomass. It should be noted that the September, October, and November study data correlates with (1) Anaerobic stage N_2 sparge, (2) Fresh batch feed, and (3) K_2HPO_4 $3H_2O$ at 10 g L^{-1}. For the December studies, the potassium phosphate was bumped to 20 g L^{-1}, and the 12/3/2021 feed was 2-days old, while 12/31/2021 feed was fresh batched with 0.23 g L^{-1} sodium acetate, impacting the influent VFAs. A correlation coefficient of the data suggests preferential IFAS uptake and release assuming several factors ranging from anoxic phosphorus uptake within the biomass, to anaerobic mass fraction related acidogenesis and VFAs formation favoring the IFAS-SBBR PAOs.

Table 12

Phosphorus uptake/release rates, phase 5 kinetic studies with correlation coefficient

Phase 5	**Uptake**	**Release**	**Uptake**	**Release**
Date	mg-PO4-P g-VSS-h $^{-1}$	mg-PO4-P g-VSS-h $^{-1}$	mg-PO4-P g-VSS-h $^{-1}$	mg-PO4-P g-VSS-h $^{-1}$
	Control	Control	IFAS	IFAS
9/24/2021	7.59	9.91	19.4	17.4
10/8/2021	4.33	4.77	15.0	12.9
10/22/2021	11.2	6.58	18.6	16.7
11/5/2021	10.0	4.05	20.0	14.4
11/19/2021	5.72	2.88	15.8	16.8
12/3/2021	7.57	4.99	22.5	34.3
12/31/2021	12.82	11.5	30.1	53.3
Average	8.46	6.38	20.2	23.7
R^2	0.339		0.877	

Published values for EBPR maximum specific uptake and release mg PO_4^{-3}-P (g VSS-h)$^{-1}$ vary widely.

- 3.8 to 6.5 mg PO_4^{-3}-P (g VSS-h)$^{-1}$ uptake; 5.3 to 7.4 mg PO_4^{-3}-P (g VSS-h)$^{-1}$ release (Jabari et al., 2016).
- 6–21 mg PO_4^{-3}-P (g VSS-h)$^{-1}$ uptake; 5.0 to 32 mg mg PO_4^{-3}-P (g VSS-h)$^{-1}$ release (Zaman et al., 2021).
- 18.8 mg PO_4^{-3}-P (g VSS-h)$^{-1}$ (primary release); 1.8 mg PO_4^{-3}-P (g VSS-h)$^{-1}$ secondary release (Danesh & Oleszkiewicz, 1997).

Acetic Acid Utilization

Table 13 contrasts control (AS-SBR) and research (IFAS-SBBR) ortho-P release as a function of acetic acid uptake.

Table 13

Acetic acid utilization, phase 5 kinetic studies with correlation coefficient

Phase 5	**Δ PO4/Δ Hac**		**Δ PO4/Δ Hac**	
Date		mg-PO4 mg-Hac^{-1}		mg-PO4 mg-Hac^{-1}
	Control	Control	IFAS	IFAS
9/24/2021	1.35/1.87	0.72	3.40/5.31	0.64
10/8/2021	2.50/2.90	0.86	3.70/4.14	0.89
10/22/2021	3.64/4.87	0.75	2.87/9.46	0.30
11/5/2021	2.63/3.65	0.72	4.47/3.34	1.34
11/19/2021	0.32/3.65	0.09	0.58/3.34	0.17
12/3/2021	5.54/66.3	0.08	25.6/40.0	0.64
12/31/2021	25.0/69.9	0.36	73.0/90.0	0.81
Average		0.51		0.68
R^2	0.593		0.987	

From Table 13 it becomes evident using recent investigations that for essentially the same process conditions, the IFAS-SBBR may be enhanced by the fixed biomass. A correlation coefficient of the data suggests favorable acetic acid utilization assuming several factors ranging from anoxic phosphorus uptake within the biomass, to anaerobic mass fraction related acidogenesis and VFAs formation favoring the IFAS-SBBR PAOs.

It should be noted that the September, October, and November study data correlates with (1) Anaerobic stage N_2 sparge, (2) Fresh batch feed, and (3) K_2HPO_4 $3H_2O$ at 10 g L^{-1}. For the December study, the potassium phosphate was bumped to 20 g L^{-1}, and the 12/3/2021 feed was 2-days old, while 12/31/2021 feed was fresh batched with 0.23 g L^{-1} sodium acetate, impacting the influent VFAs. Noting also that 2-day synthetic feed influent VFAs (170 to 190 mg L^{-1}) verses fresh synthetic feed influent VFAs (20 to 40 mg L^{-1}). The reactors reasonably agree with the published values, 0.026 – 0.760 mg PO4 released per mg-Hac taken up (Smolders et al., 1994).

Total Phosphorus and VSS to TSS Ratio

Contrasting Control (AS-SBR) and Research (IFAS-SBBR) biosolids total phosphate (TP) Table 14, provides Phase 5 TP data established using biomass wet digestions followed by filtered sample measurements as PO_4^{-3}-P (from FIA). Further, TP is related to the calculated VSS TSS^{-1} ratio from solids analysis, Standard Methods (Eaton et al., 2005). As enhanced phosphorus removal develops, in the reactors, the inorganic phosphorus gradually increases the TSS mass or numerator value resulting in a reduced ratio.

Published research has suggested the indicators to be considered when aiming for EBPR at low SRT values were as follows. Low sludge retention time (SRT), though 3 days or less may lead to treatment process washout. Other common indicators: i) increase of the ratio of VSS TSS^{-1} tending to 1.0, ii) decrease of phosphorus to carbon P:C ratio, iii) decrease of the VSS, iv) decrease of the P-release and P- uptake and v) COD not being

completely consumed under anaerobic conditions and part of it being transferred to the aerobic stage. It has been suggested that the P-uptake capacity is lost before the P-release capacity, so an increase of effluent P could indicate that the system may be headed to failure. This is in agreement with the hypothesis that PHA oxidation kinetics are limiting in the aerobic metabolism of PAO (Chan et al., 2017).

Table 14

Volatile solids relation, phase 5 kinetic studies with correlation coefficient

Phase 5	**mg-TP g-VSS^{-1}**	**VSS TSS^{-1}**	**mg-TP g-VSS^{-1}**	**VSS TSS^{-1}**	**mg-TP g-VSS^{-1}**	**VSS TSS^{-1}**
Date	Control	Control	IFAS	IFAS	Fixed	Fixed
9/24/2021	50	80.7%	57	81.5%	41	86.5%
10/8/2021	54	79.1%	50	78.2%	35	85.0%
10/22/2021	56	80.4%	54	80.5%	53	82.1%
11/5/2021	72	80.1%	60	78.4%	39	85.8%
11/19/2021	45	79.5%	49	78.6%	35	86.1%
12/3/2021	52	79.1%	56	77.6%	40	84.4%
12/31/2021	47	78.5%	66	73.6%	38	84.3%
R^2	0.138		0.313		0.563	

The Figure 26 demonstrates, the inverse relationship between TP and VSS TSS^{-1} ratio, that as the total phosphorus of the solids increased the VSS per TSS ratio declines as noted by the correlation coefficient $R^2 = 0.5023$ accompanying the trendline in the figure.

Figure 27 and Figure 28 provide some insight into the fate and effect of phosphorus moving through the reactors. The source of the data is twofold. First, reactor influent and effluent samples were tested in duplicate to measure total phosphorus (TP) concentrations using HACH test kits, TP845 (PO_4^{3-}-P range, 2-20mg L^{-1}). Second, mixed liquor suspended solids samples underwent wet digestions in duplicate, at 25x dilution, followed

by filtration and FIA analysis for PO_4^{3-}-P measurement, being equivalent to TP, of the MLSS sludge.

Figure 26

Trendline showing VSS to TSS ratio vs total phosphorus

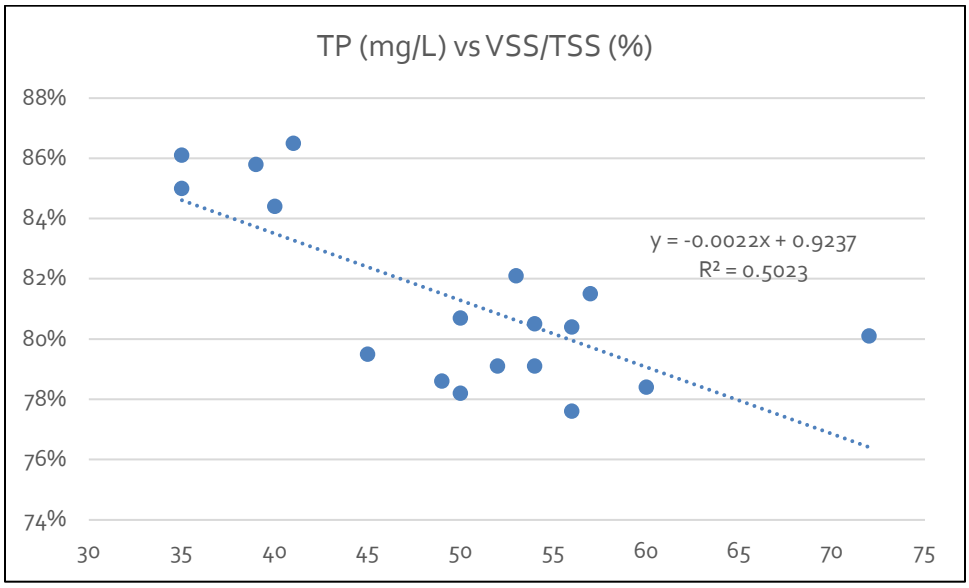


Figure 27

Tracing fate and effect of phosphorus (AS-SBR)

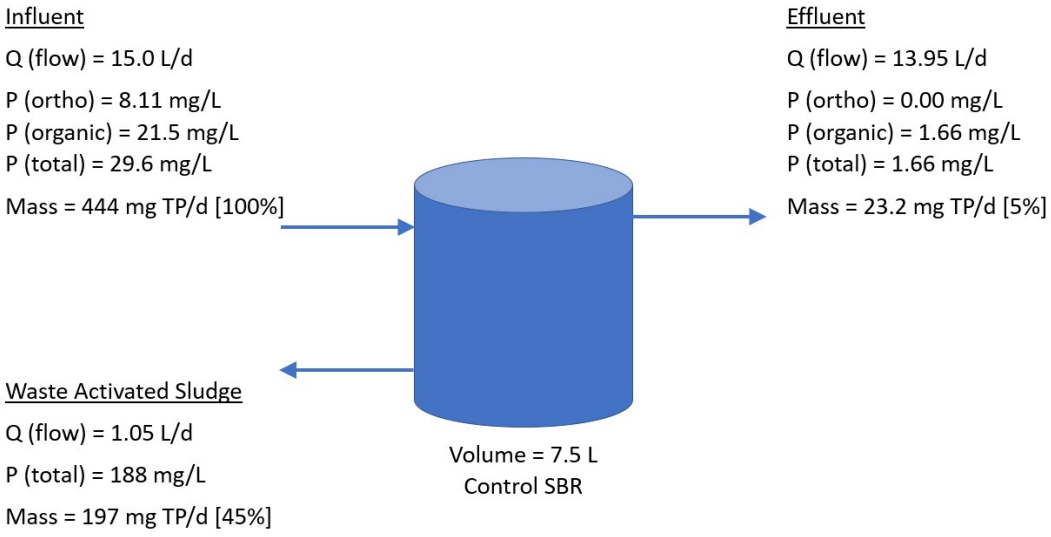


Figure 28

Tracing fate and effect of phosphorus (IFAS-SBBR)

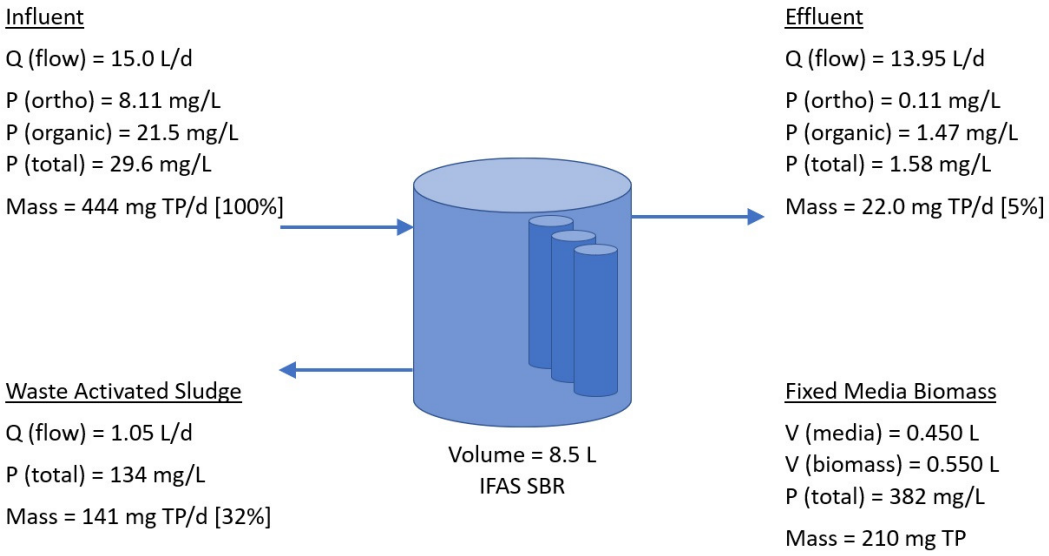


Figure 29

Tabulated fate and effect for tracing of biosolids phosphorus

Based on TP845 Hach 2-20 mg/L PO4-P				
10/22/2021	Raw Influent	29.3 mg/L		PO4 Inf.Avg.
	duplicate	29.8 mg/L		8.11 mg/L
	average	29.6 mg/L		Oct8-Oct30
	Control Effluent	1.54 mg/L		PO4 Inf.Avg.
	duplicate	1.77 mg/L		0.00 mg/L
	average	1.66 mg/L		Oct8-Oct30
	IFAS Effluent	1.51 mg/L		PO4 Inf.Avg.
	duplicate	1.65 mg/L		0.11 mg/L
	average	1.58 mg/L		Oct8-Oct30

10/26/2021			VSS, mg/L	mg-P/g-VSS
Control (1:25 dilution)	188	TP (mg/L)	3343	56
Control Duplicate		**Control**		
IFAS (1:25 dilution)	134	TP (mg/L)	2510	53
IFAS Duplicate		**IFAS**		
Fixed (1:25 dilution)	382	TP (mg/L)	7217	53
Fixed Duplicate		**Fixed**		
Standard (17.4 mg/L)	20.2	high	Standard Adjusted	

10/26/2021	**Control SBR**							
29.6	mg TP/L	Influent	15.00	L/d	443	mg P/d		
1.66	mg TP/L	Effluent	13.95	L/d	23.1	mg P/d	5%	
188	mg TP/L	WAS	1.05	L/d	197	mg P/d	45%	
					223	mg P/d	remaining in reactor	50%
10/26/2021	**IFAS SBR**							
29.6	mg TP/L	Influent	15.00	L/d	443	mg P/d		
1.58	mg TP/L	Effluent	13.95	L/d	22.0	mg P/d	5%	
134	mg TP/L	WAS	1.05	L/d	141	mg P/d	32%	
					281	mg P/d	remaining in reactor	63%

There were three wet digestion products, 1) AS-SBR MLSS, 2) IFAS-SBBR MLSS, and 3) IFAS-Fixed Biomass "MLSS". The fixed biomass "MLSS" was achieved because the media underwent a near complete rinse/flush bi-weekly using deionized (DI) water such that following the rinse, the total "MLSS" volume was 1000 ml. This allowed the TP to be reported on a mg L^{-1} scale, and the product was wasted and not returned to the IFAS-SBBR.

The phosphorus tracing had three measured and calculated components as seen in Figure 29. At the top left of Figure 29 were the HACH TP measurements from the Friday, Kinetic study (i.e., October 22), paired with averaged effluent phosphorus, for the time horizon in question (i.e., Oct8-Oct30). Next, on the top right is the FIA results from the wet digestions conducted the Tuesday (i.e., October 26) following the Friday kinetic study. The results from the top feed into the bottom tableau of Figure 29. These results were than used to populate Figure 27, and Figure 28 graphically showing the fate and effect of phosphorus moving through, or retained by the two systems.

MLTSS, MLVSS, and Sludge Production

The average mixed liquor total suspended solids (MLTSS) and average mixed liquor volatile suspended solids (MLVSS) concentrations for the AS-SBR and IFAS-SBBR reactors, by experimental phases, are presented in Table 15 assuming quasi-steady state conditions. Values for average F:M, and sludge production are also shown when available.

Experimental results show the IFAS-SBR to have a lower VSS solids concentration than the AS-SBBR. Accounting for biosolids harvested from the fixed media, and adding this to the bulk MLVSS of the IFAS-SBBR reactor, yields a total MLVSS of 98% compared with the control reactor MLVSS. This suggests two things. First, the method for sampling fixed media biomass was unable to extract 100% of the VSS biosolids. Second, given the same influent feed conditions, the two reactors essentially generate similar VSS as they each stabilize the same organic substrate concentrations.

Table 15 demonstrates further the lower total solids concentration of the IFAS-SBBR over the AS-SBR representing an enhancement of reduced sludge generation and correspondingly less sludge for dewatering and disposal in the context of a full-scale treatment plant.

Table 15

Average reactor solids concentrations by phase with correlation coefficient, sludge production, and food-to-microorganism ratio

mg L^{-1}	**Phase 1-2**		**Phase 3**		**Phase 4**		**Phase 5**	
AS-SBR	MLTSS	MLVSS	MLTSS	MLVSS	MLTSS	MLVSS	MLTSS	MLVSS
Avg.	2808	2066	2517	1729	2397	1755	2793	2198
Med.	2800	2040	2480	1730	2410	1693	2717	2107
Avg.R^2	0.855							
kg d^{-1}			0.0020		0.0018		0.0023	
F:M			0.227		0.209		0.173	
IFAS-SBBR	MLTSS	MLVSS	MLTSS	MLVSS	MLTSS	MLVSS	MLTSS	MLVSS
Avg.	1587	1281	1933	1361	2021	1483	1858	1451
Med.	1720	1403	2007	1390	2240	1627	1680	1337
Avg.R^2	0.690							
kg d^{-1}			0.0014		0.0014		0.0016	
F:M			0.272		0.222		0.288	

Sludge volume index (SVI) is a simple and highly effective measure of the mixed liquor propensity to settle and compact. It is speculated that the higher IFAS-SBBR SVI(30) or 30-minute SVI might be related to microbial filaments extending beyond the outer surface of the flocs. Filaments are the skeleton which together with extracellular polymeric substance (EPS) glue, holds the floc and microorganisms together. Longer than necessary filaments projecting into the bulk liquid from the flocs will negatively impact

settling. The correct filament length, and the descending floc will essentially comb fine suspended particles from the water column as the flocs settle, thereby adding volume to the settled sludge, and helping to purify while clarifying.

One of the characteristics of the IFAS-SBBR system over the AS-SBR is the unique ability to keep slowly growing nitrifying bacteria in the bioreactor, by separating hydraulic retention time (HRT) with solids retention time (SRT). Stated another way, the age of the sludge can be much older (i.e., long SRT) in the IFAS owing to the volume of fixed biomass. By Phase 4 and Phase 5 of the investigations, the fixed biomass of the IFAS-SBBR was being removed alternate weeks by a vigorous rinse using deionized water (DI). The captured biosolids were characterized by solids analysis, and wet digestions for total phosphorus.

Therefore, an alternate weighted SRT for the IFAS should include the 7.5-day MLSS SRT plus a 14-day fixed biomass SRT. On average about 500 ml of sludge was removed from the fixed media translating into a MLSS concentration of about 7100 mg L^{-1}. Using Table 15 as a guide, it is possible to take the estimated IFAS MLVSS concentration at 1500 mg L^{-1} and supplement it with the estimated fixed biomass MLVSS of (factored for the reactor volume, 500 ml / 7500 ml) 7100 mg L^{-1} and compare this with the estimated AS-SBR MLVSS of 2000 mg L^{-1}. The full IFAS MLVSS would be 1500 + 475 = 1975 mg L^{-1} or 98% of the AS-SBR. Now this is really quite close considering that not all of the fixed biomass could be removed and analyzed. The result does however suggest that for the same influent feed concentration, or substrate, the two batch reactors produce approximately the same quantity of organic life.

Activated sludge zone-settling velocity, and compacted volume may relate to the sludge volume index (SVI). SVI and zone-settling rate are common measures to quantify the settling characteristics of activated sludge (Tchobanoglous et al., 2003). For instance, an SVI = 150 ml g^{-1} as often considered the dividing line between activated sludge bulking and non-bulking (Grady et al., 2011). It is quite evident from the current investigations that

the SVI range is identifiably lower than that found at full sized treatment plants. The SVI(30) or 30-minute SVI provides a measure of how effectively the sludge will settle.

Food to mass ratio (F:M) represents a direct measure of the amount of food available for use per unit mass of microorganisms in the reactor mixed liquor. F:M generally dictates the type of microbial diversity in the aeration reactor and therefore the expected effluent quality from the treatment process. Activated sludge values $0.2 < F:M < 0.5$ represent conventional systems, and $0.05 < F:M < 0.20$ can be expected from extended aeration activated sludge systems owing to older microorganisms.

Interestingly, the IFAS reactor maintains a higher sludge volume index (SVI) in the order of 70 ml g^{-1}compared with 35 ml $g^{-1}$for the control reactor, with both reactors well below the 100 to 150 ml g^{-1} value commonly published. Both reactors settled readily, though the research reactor tends to settled fine floc quicker producing a clearer supernatant when compared with the control reactor. Sludge settling generally indicates a healthy process with one notable exception. Following Phase 3 startup, the research reactor started to overproduce filamentous bacteria as observed under microscopic examination. It maybe that the food to mass (F:M) ratio was low, as filamentous microbes will out compete heterotrophs for food under low substrate conditions. Rather then, make a change to feed strength, daily for one-week (i.e., 1-SRT), approximately 150 ml of settled waste sludge from the control reactor was used to seed the research reactor. This approach proved successful and by the following week both reactors sustained a healthy mixed liquor.

The lower rows of Table 15, demonstrates less sludge generated by the IFAS-SBBR research reactor, compared with the AS-SBR control reactor. This may be attributed to relatively older sludge of the IFAS-SBBR. It is suggested that sludge retention time (SRT) higher than 8 days is required to perform nitrification, working with fixed biofilm reactor it is possible to operate at higher SRT values thus reducing the sludge production, while also improving nitrogen removal processes. In contrast, running a shorter SRT, reduces external energy input while also maximizing the excess sludge production, if for instance biogas production is a necessary criteria (Mannina et al., 2020).

Denitrification, Alkalinity, and NOx Differential

Over the various research Phases, air flow is adjusted to manipulate mixing energy and to supply process air for COD removal, nitrification, denitrification and phosphorus removal. It is found that the necessity to strip off surplus biomass from the fixed media required a minimum air flow of 2.0 L min^{-1} that corresponded with average reactor dissolved oxygen of between 5.0 mg L^{-1} and 6.0 mg L^{-1} somewhat limiting the capacity for the AS-SBR to denitrify nitrogen compared with the IFAS-SBBR as demonstrated in Table 16.

Reactor pH during the phased investigations is not regulated but allowed to fall and rise based on the stages, from anaerobic (i.e., 6.5 pH, low range) to aerobic (i.e., 8.5 pH, high range). The exception regarding pH occurred later during Phase 3 of the investigations. It was believed at the time, that moving the influent feed concentration to an ideal ratio of C:N:P of 100:10:1 would provide a base condition for subsequent research phases. As the influent ammonium is reduced from 30 g L^{-1} to 20 g L^{-1} and finely 10 g L^{-1} the reactor pH reduced by approximately 0.5 pH across both reactors accordingly, impairing nitrification and causing nitrite to accumulate in both reactors. This condition persisted until Phase 4 when influent ammonium concentration is restored to 30 g L^{-1}.

Table 16 further demonstrates the IFAS-SBBR capacity to denitrify NOx compared with AS-SBR effluent, across all phases of the investigations. Alkalinity required for nitrification amounts to 7.07 g (7.17 g, without accounting for ammonia going to cellular nitrogen) alkalinity as $CaCO_3$ per gram of NH_4^+-N oxidized and denitrification recovers about half of the alkalinity used in nitrification, Metcalf and Eddy (Tchobanoglous et al., 2003). Higher effluent alkalinity, as Table 16 demonstrates for the IFAS reactor, is indicative of enhanced denitrification. A correlation coefficient of the data suggests preferential IFAS denitrification most notably during phase 1 to phase 4 assuming factor related to conditions favorable to anoxic phosphorus uptake within the biomass.

Table 16

Average reactor nitrification by phase with correlation coefficient

mg L^{-1}	**Phase 1-2**		**Phase 3**		**Phase 4**		**Phase 5**	
AS-SBR	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Avg.	2.00	**32.0**	0.60	**26.4**	1.30	**24.9**	16.6	8.10
Med.	0.90	**32.2**	0.60	**26.7**	0.40	**25.1**	19.2	6.00
*Sum	34.0		27.0		26.2		24.7	
R^2	0.205		0.000		0.132		0.128	
IFAS-SBBR	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Avg.	17.9	10.2	1.20	**23.5**	3.80	**18.7**	11.4	8.10
Med.	18.6	8.00	0.50	**24.1**	2.30	**21.1**	12.6	7.00
*Sum	28.1		24.7		22.5		19.5	
R^2	0.832		0.481		0.593		0.044	

Note. * Sum of the average nitrite and nitrate

In most systems, nitrification and denitrification may cause a detrimental impact on EBPR due to availability of nitrite and nitrate in the recycle which enters anaerobic zone, potentially leading to process failure. Anaerobic availability of electron acceptors such as nitrate and nitrite sparks denitrification by ordinary heterotrophic organisms (OHO) that can potentially out-compete PAOs for nutrients (Izadi et al., 2020). It is reported that concurrent nitrification and denitrification in the aerobic zone is observed in the IFAS process. The denitrification in the aerobic zone is accomplished in the anoxic zone of biofilm layers. At a full scale USA WWTP, the amount of oxidized nitrogen is denitrified between 30% and 88% in the wastewater treatment process in which the aerobic zones are installed with IFAS fixed media (Sriwiriyarat et al., 2008).

As mentioned, the reactor systems are operated at the moderate SRT of 7.5 days, at the HRT of 12 h and at an ambient temperature of 21 °C. In addition, complete nitrification is not always achieved across all Phases and for both reactors. Phase 1-2 demonstrated full nitrification in the control reactor. The IFAS reactor formed nitrite and comparatively small amount of nitrate, suggesting nitrite oxidizing bacteria (NOB) inhibition.

Figure 30

IFAS nitrite reversal following influent P-bump

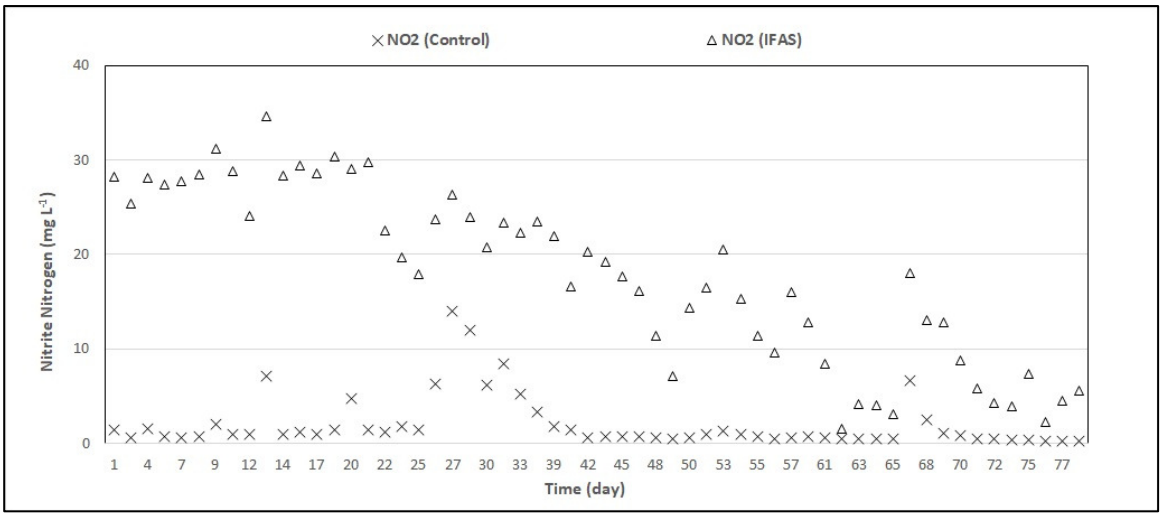
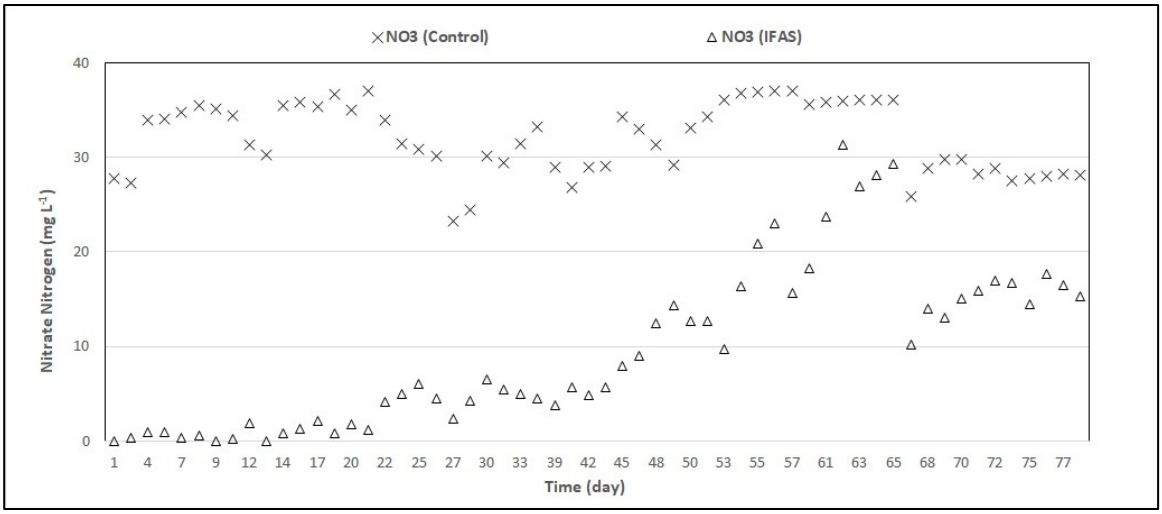


Figure 31

IFAS nitrate reversal following influent P-bump



It may be that insufficient NOBs in the IFAS-SBBR system is a cause of the failed oxidization of nitrite to nitrate nitrogen, suggesting possibly NOBs are out competed by heterotrophs for substrate. Another explanation for elevated mixed liquor nitrite levels is partial denitrification of nitrate to nitrite. Although the source of the NOB inhibition is not fully realized, the incomplete nitrification condition reverses following the Phase 1 (December 13, 2019) bump in influent phosphorus. Within two SRTs of the influent phosphorus bump, Figure 30, Figure 31, full nitrification is restored to the IFAS reactor. Supplementary influent phosphorus, potentially supplies needed energy, basically stimulating organism growth in the nitrifier population.

To achieve biological nitrogen removal the usual pathway is ammonia oxidation to nitrate and subsequent reduction to nitrogen gas. The processes of nitrification and denitrification are combinations of several microbial conversions during which a number of intermediate products are produced. During normal operation those intermediates do not accumulate to significant levels. One intermediate in nitrogen removal that accumulates in some cases is nitrite. Further, both incomplete nitrification and incomplete denitrification can cause nitrite accumulation. Nitrite is a toxic compound that can inhibit denitrifying bacteria. Once denitrification is significantly inhibited more and more nitrite will accumulate during the anoxic stages eventually causing complete breakdown of denitrification (E. Morgenroth et al., 2000).

Mass Based Stoichiometric nitrification equation (Grady et al., 2011), **Step 1**:

Half-reaction nitrification and typical yield values, for *Nitrosomonas*, using NH_4^+:

$$NH_4^+ + 2.457\ O_2 + 6.716\ HCO_3^- \Rightarrow 0.114\ C_5H_7O_2N$$
$$+ 2.509\ NO_2^- + 1.036\ H_2O + 6.513\ H_2CO_3$$

Note: the alkalinity (HCO_3^-) used during the oxidation of ammonia to nitrite.

Mass Based Stoichiometric nitrification equation, **Step 2**:

Half-reaction nitrification and typical yield values, for *Nitrobacter*, using NO_2^-:

$NO_2^- + 0.001\ NH_4^+ + 0.014\ H_2CO_3 + 0.003\ HCO_3^-$

$+ 0.339\ O_2 \Rightarrow 0.006\ C_5H_7O_2N + 0.003\ H_2O + 1.348\ NO_3^-$

Note: the small amount nitrifying biomass produced.

Mass Based Stoichiometric Equation

Combining **Step 1** and **Step 2** half-reactions reveals the overall stoichiometry as:

$NH_4^+ + 3.300\ O_2 + 6.708\ HCO_3^- \Rightarrow 0.129\ C_5H_7O_2N$

$+ 3.373\ NO_3^- + 1.041\ H_2O + 6.463\ H_2CO_3$

Note: the amount of alkalinity (HCO_3^-) going to neutralize H^+ ions (carbonic acid, H_2CO_3) not creating new biomass, during the oxidation of ammonia nitrogen to nitrite nitrogen (Grady et al., 2011).

During most of the experimental phases, the AS-SBR system forms nitrate in excess of what the IFAS-SBBR produces. It is understood that the fixed biomass provides treatment layers (i.e., oxygen diffusion limits) from outside to inside aerobic, anoxic, and anaerobic. Dissolved oxygen diffuses only a short distance into the fixed biomass providing conditions for different nutrient removal. The anoxic layer containing nitrite and nitrate but little or no dissolved oxygen may be responsible for providing denitrification. It is noted that the fixed biomass treatment layers operate symbiotically such that substrate/waste from one layer diffuses radially in all directions. In this way nitrate and nitrite from the aerobic layer diffuses into the anoxic layer to be denitrified in the absence of oxygen.

Summary and Conclusions

The current research demonstrates wastewater phosphorus removal enhanced by the IFAS-SBBR system owing to a concurrent nutrient removal contribution of the integrated fixed biomass. This is found to be true by way of phosphorus uptake and release data showing a correlation coefficient of 0.339 and 0.877 for the AS-SBR and IFAS-SBBR respectively. The enhancement is further supported by acetic acid utilization data showing a correlation coefficient of 0.593 and 0.987 for the AS-SBR and IFAS-SBBR respectively. Similarly, contrasting the VSS TSS^{-1} ratio with MLSS total phosphorus wet acid digestions, using correlation coefficient data found 0.138, 0.313, and 0.563 respectively for the three MLSS products AS-SBR, IFAS-SBBR, and IFAS fixed biomass.

By extending the anaerobic stage from 90 minutes to 120 minutes, and later promoting settled sludge by delaying the start of anaerobic mixing, the anaerobic mass fraction was extended which also implies the anaerobic sublayers of the IFAS-SBBR. It is the anaerobic mass fraction that may have promoted concurrent nutrient removal by the integration of acidogenic co-fermentation of influent soluble carbon anaerobically, to augment VFAs for enhanced biological phosphorus removal. The current research found nearly complete phosphorus removal with or without a supply of influent VFAs, given to mean that rbCOD underwent anaerobic co-fermentation.

Anoxic conditions in promoting denitrifying phosphorus accumulating heterotrophs for a range of influent wastewater C:N and C:P ratios are implicit in two sets of data from the current research. The first as detailed above regarding TP wet acid digestions contribution by the IFAS fixed biomass. The second is related to the denitrification capacity of the IFAS-SBBR as demonstrated by the average effluent nitrite and nitrate correlation coefficient data for phase 1-2 to phase 5. They are 0.205, 0.000, 0.132, 0.128 respectively for the AS-SBR, and 0.832, 0.481, 0.593, and 0.044 for the IFAS-SBBR respectively.

Engineering Significance

Recognizing that laboratory scale wastewater treatment research findings don't easily scale up to full scale wastewater treatment solutions, there is much to be learned from bench scale biological nutrient removal. At times during the current research, the expected performance of the integrated fixed film activated sludge (IFAS-SBBR) system, over the control SBR, simply wasn't evident. If effluent quality was the sole deciding factor of phosphorus removal, then the relative simplicity of the conventional SBR reactor would certainly benefit small to medium sized wastewater treatment plant operations. However, in the case of wastewater treatment plants, lower sludge production, from an IFAS-SBR, could translate into cost savings related to sludge dewatering and final disposal.

The IFAS-SBBR demonstrated the capacity to removal nutrients as well as the control SBR while maintaining a lower mixed liquor concentration. This fact alone would be worth hundreds of thousands of dollars in sludge disposal savings from the typical operational budget of small to medium sized mechanical SBR treatment plants. Now add the fact that the SBBR can function as a sink–source system when influent waste constituents are stored onto biofilm by means of adsorption, ion exchange, or absorption processes. In this way, high loads of degradable substrates, toxic and non-readily degradable substances are removed quickly from the bulk liquid. As the react phase continues, substrate is hydrolyzed, and some is metabolized by biofilm organisms. Desorption from the biofilm returns substrate to the bulk liquid to be further metabolized by organisms. (Wilderer & McSwain, 2004).

The repeated onset of aerobic granular sludge may hold potential regarding phosphorus recovery. About 82% of mined phosphorus in the world is used in agriculture, 7% is used to make animal feed. The remaining 11% of the mined phosphorus is used in the production of pharmaceuticals, oils, and textiles. The issue of when the deposits will be depleted is the subject of much discussion (Cieslik & Konieczka, 2017). Granular sludge grown with a calcium-phosphate core may potentially be viable for smaller EBPR plants, given residues of suitable purity, yielding a safe fertilizer amendment (Melia et al., 2017).

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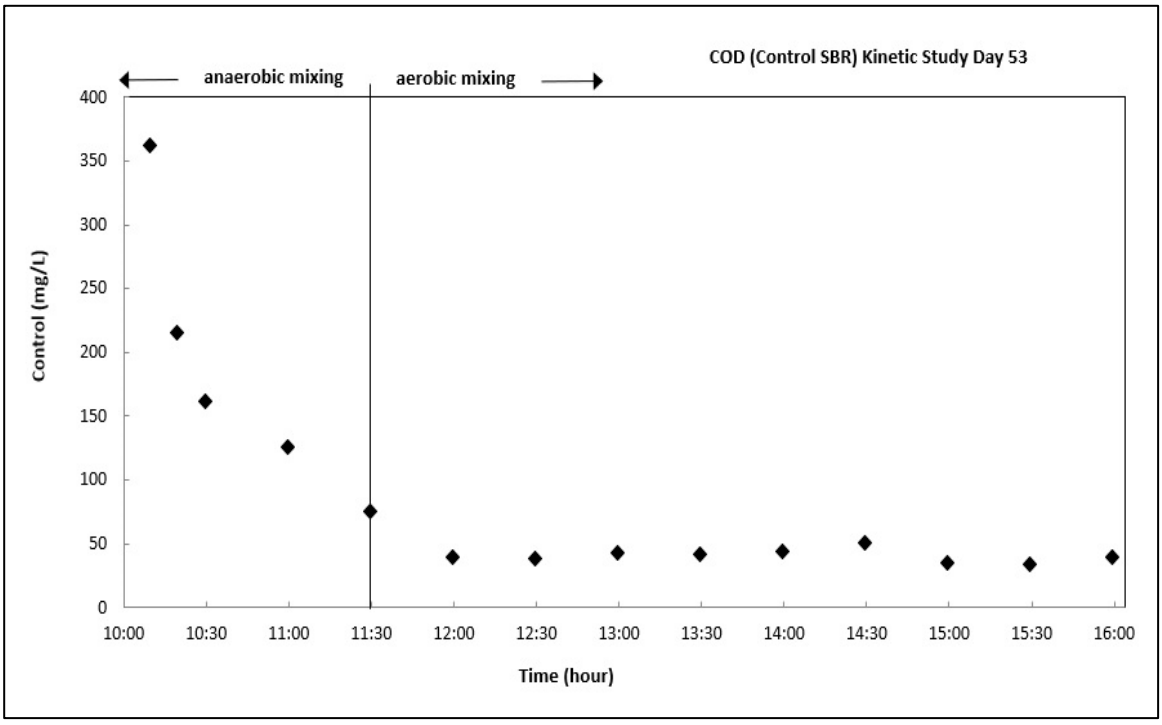
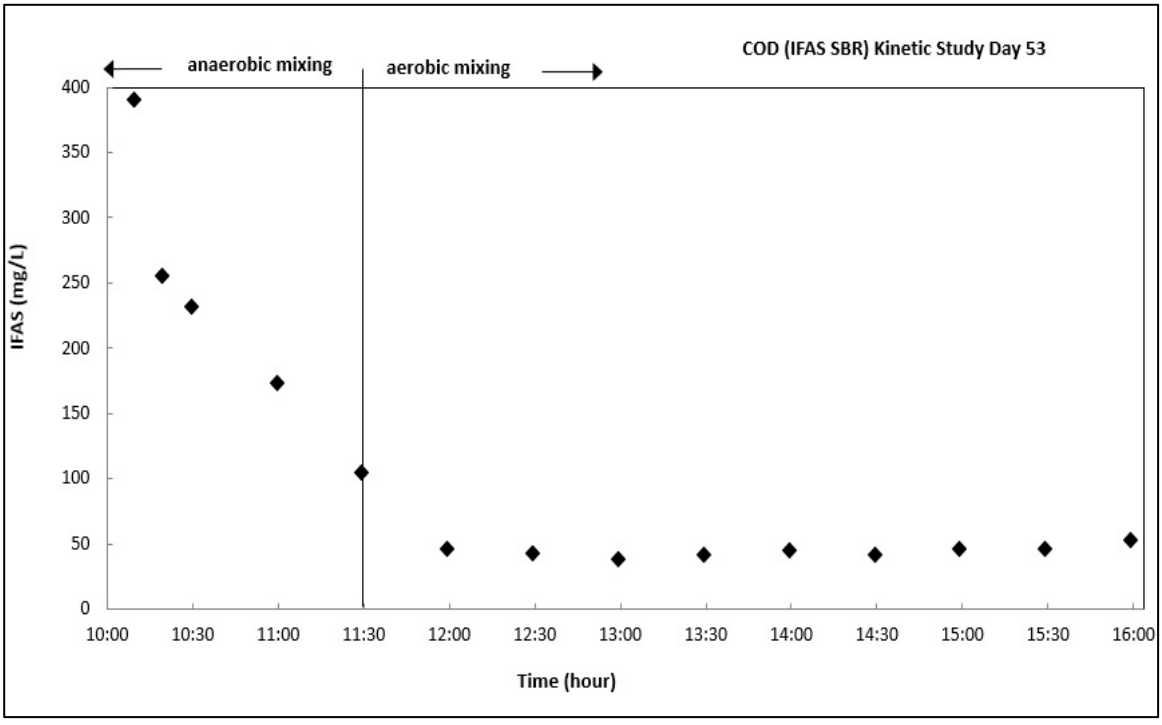
Appendix A

Kinetic study investigations (Phase 4), nutrient removal plots for AS-SBR (Control) and IFAS-SBBR:





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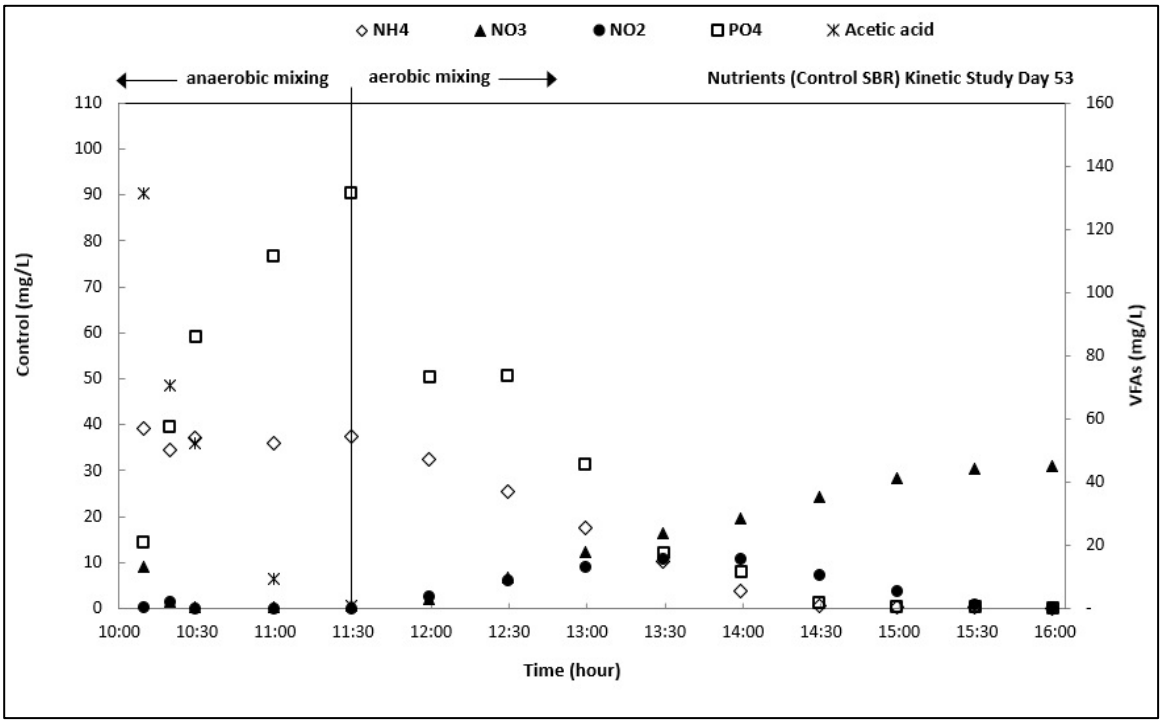

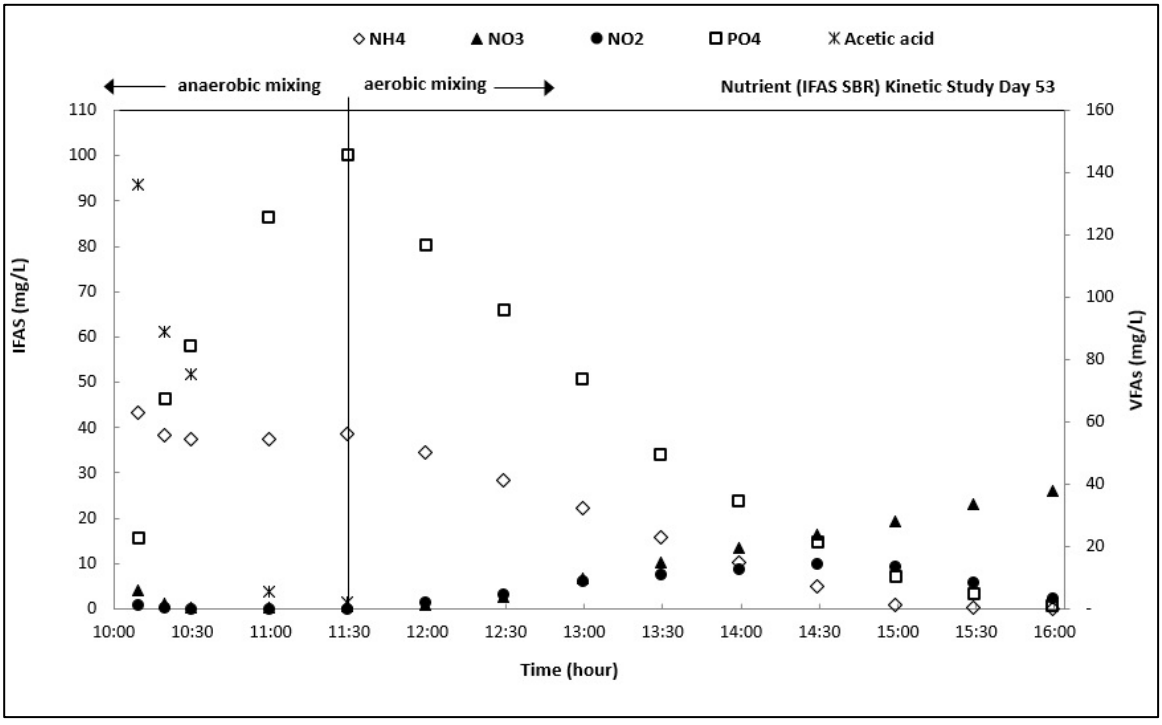

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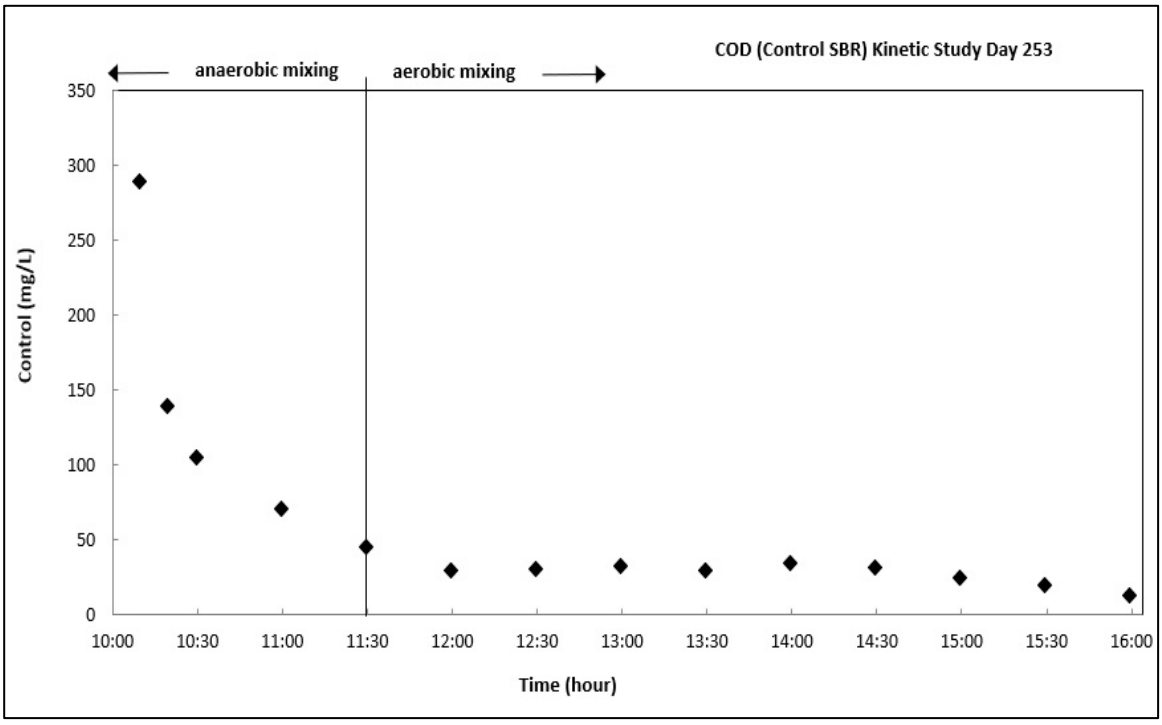

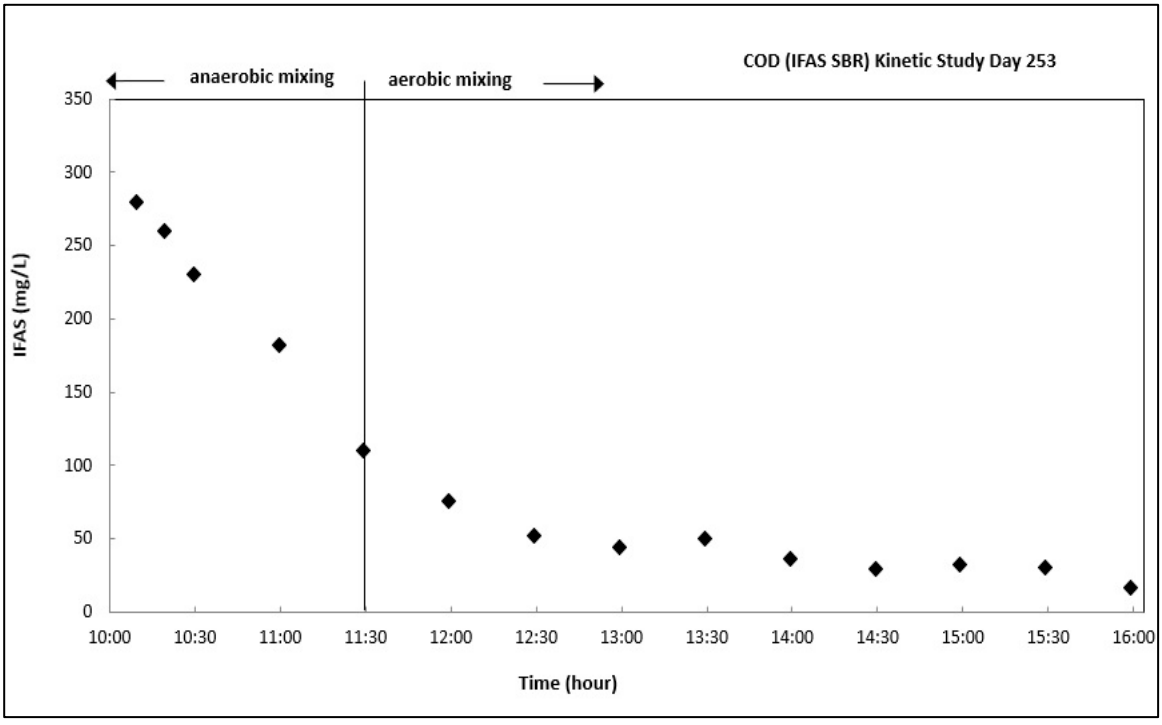

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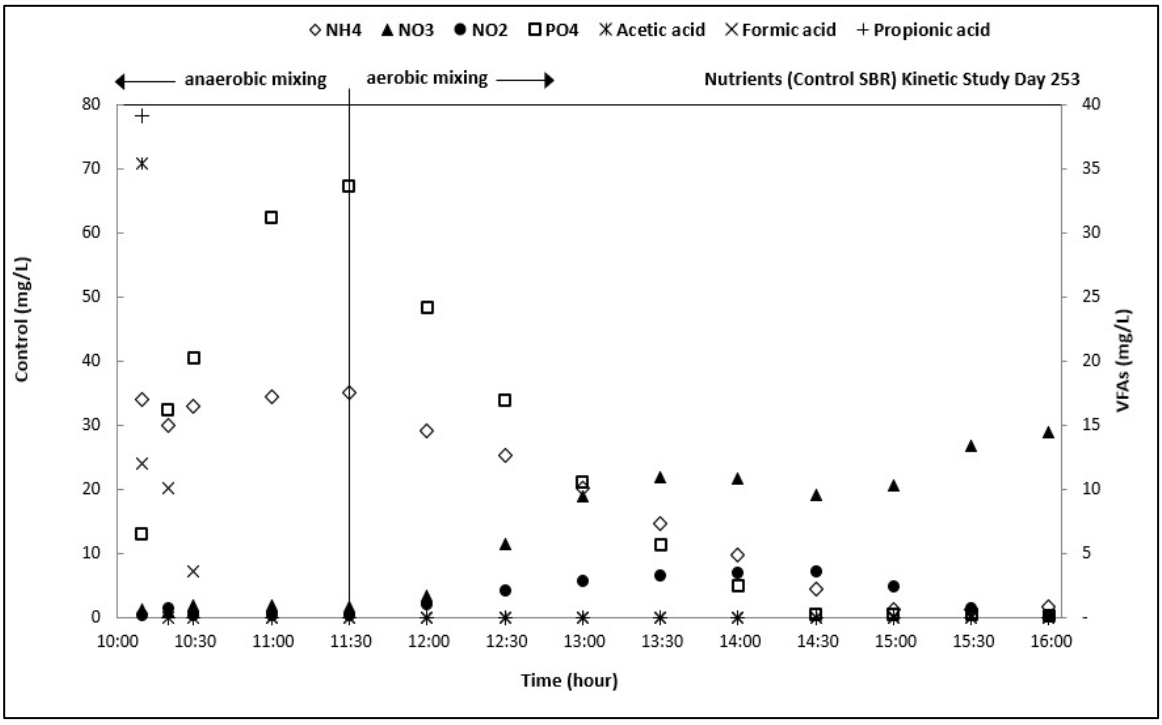

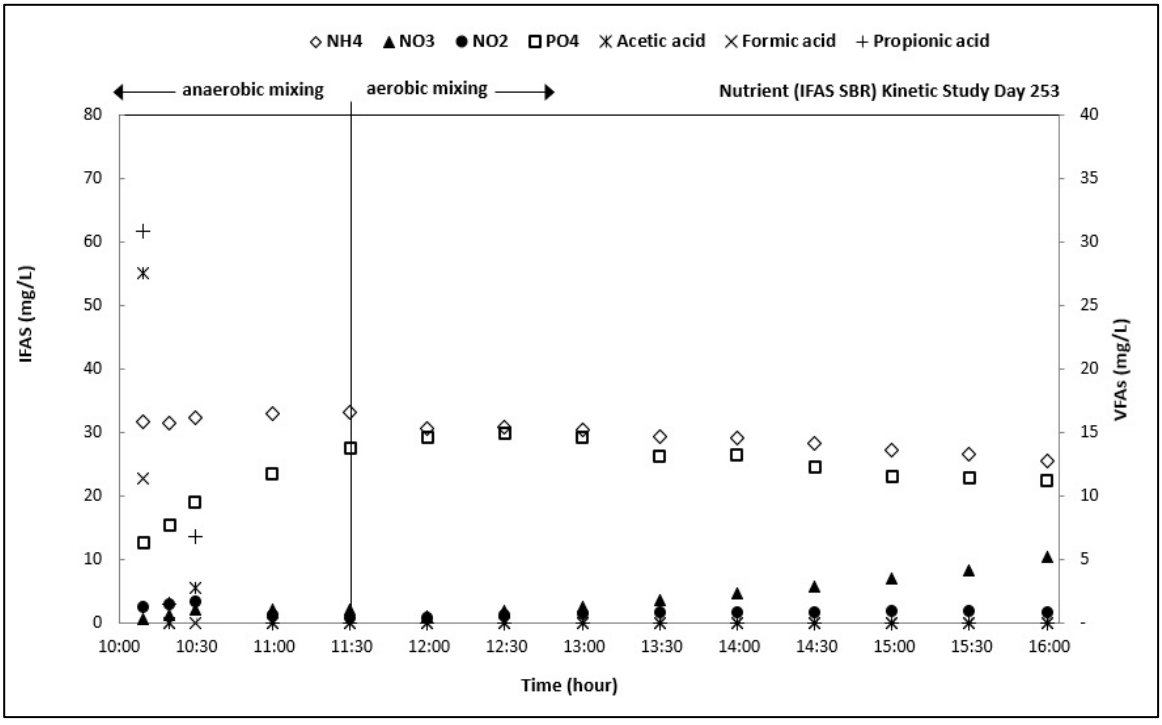

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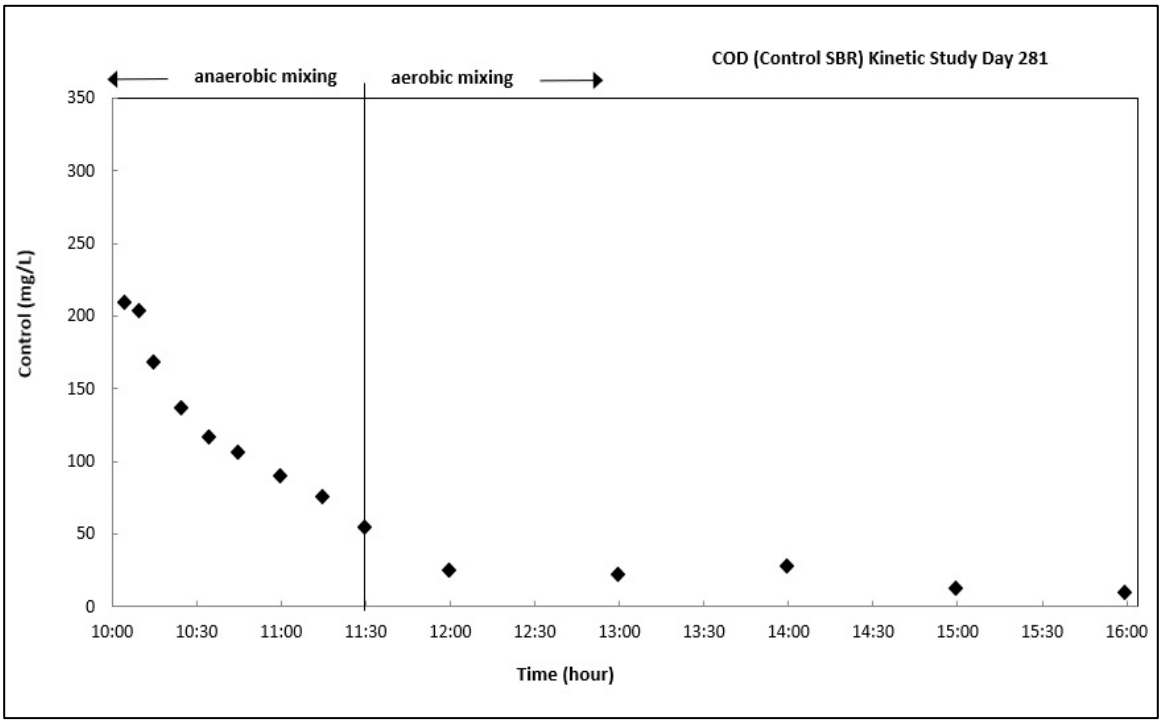

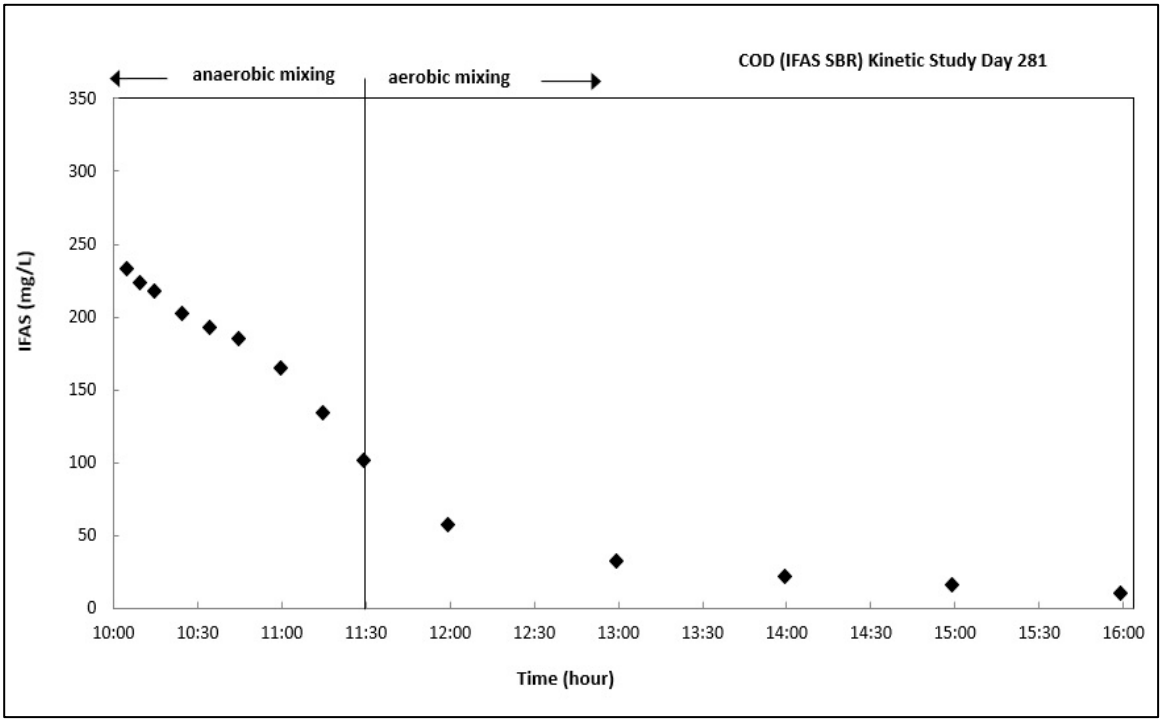

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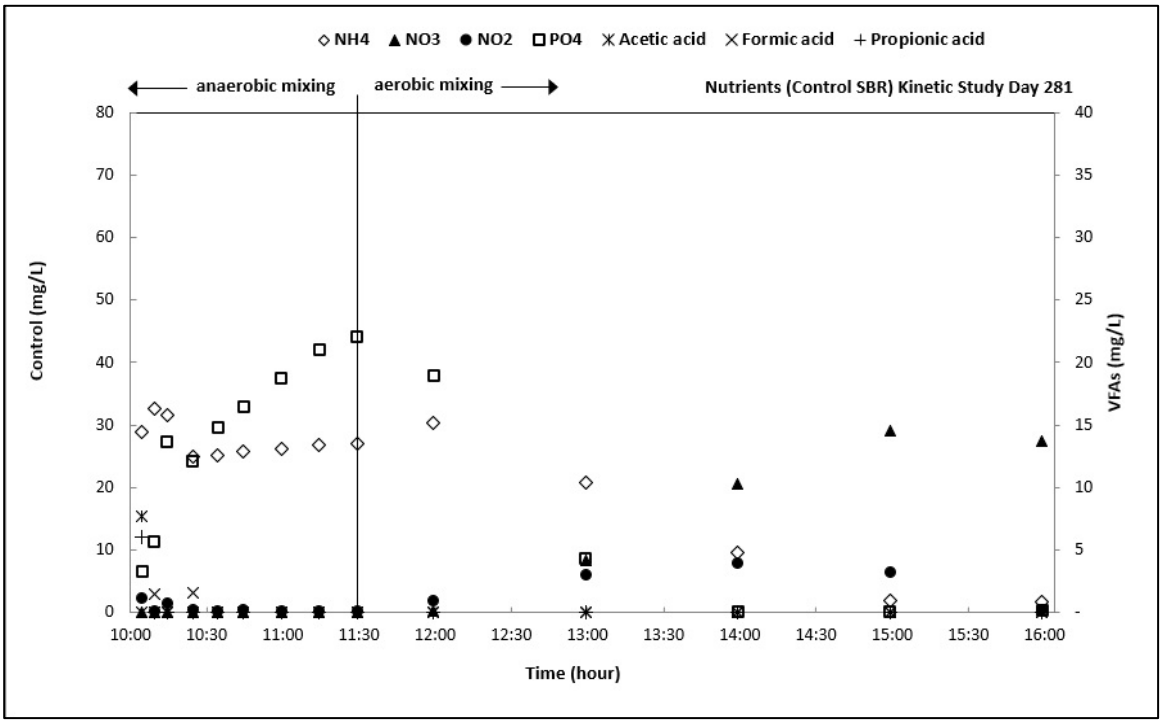

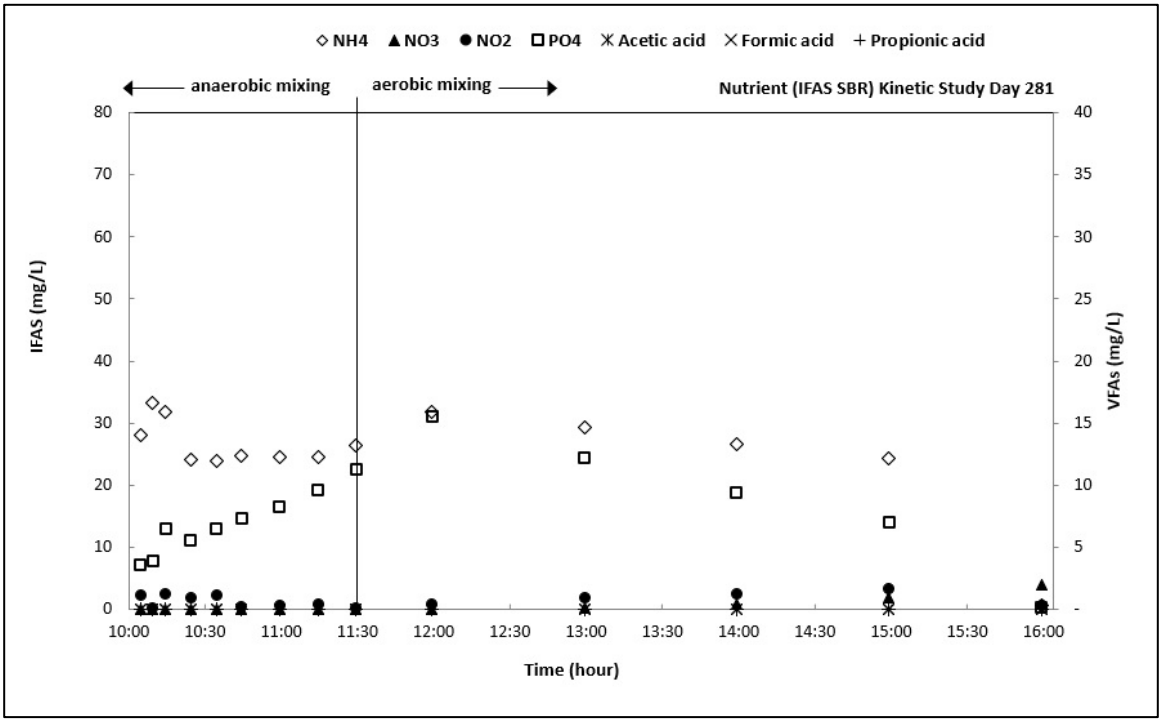

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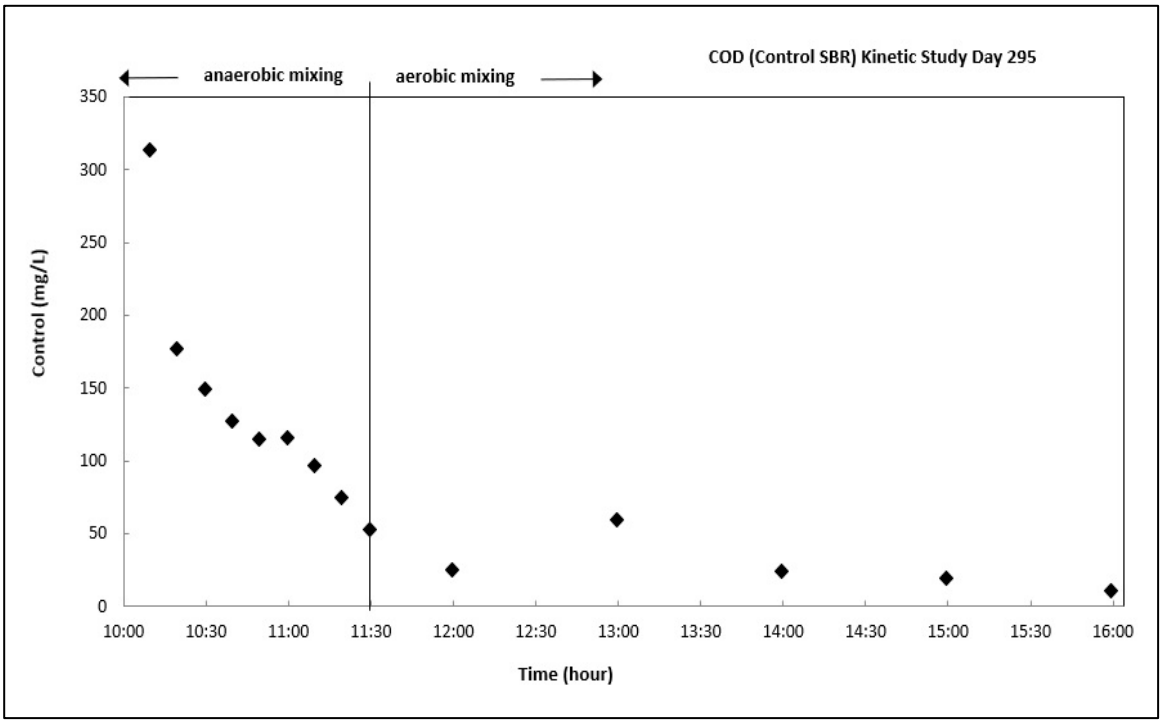

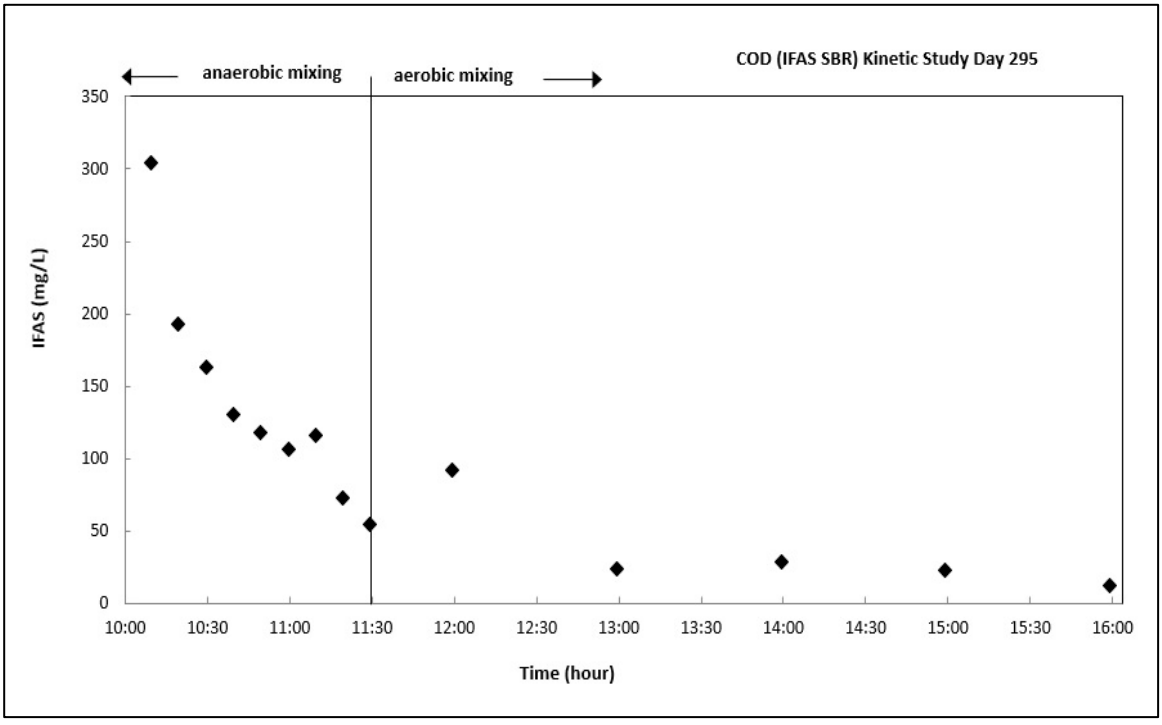

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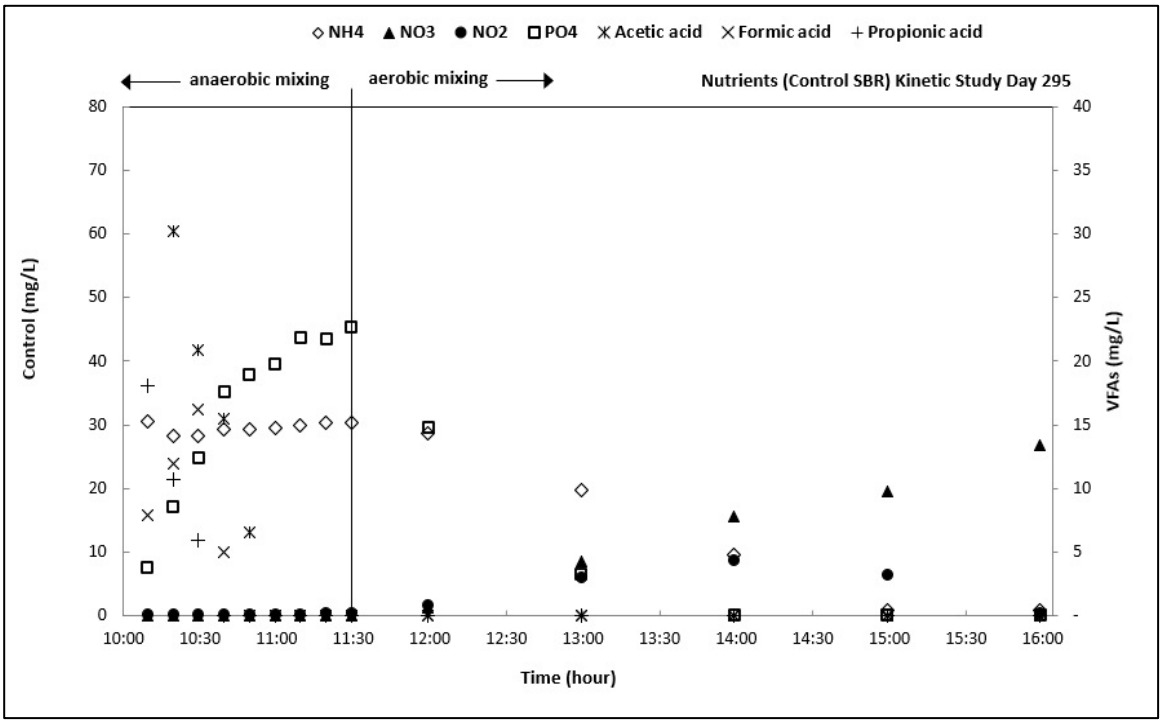

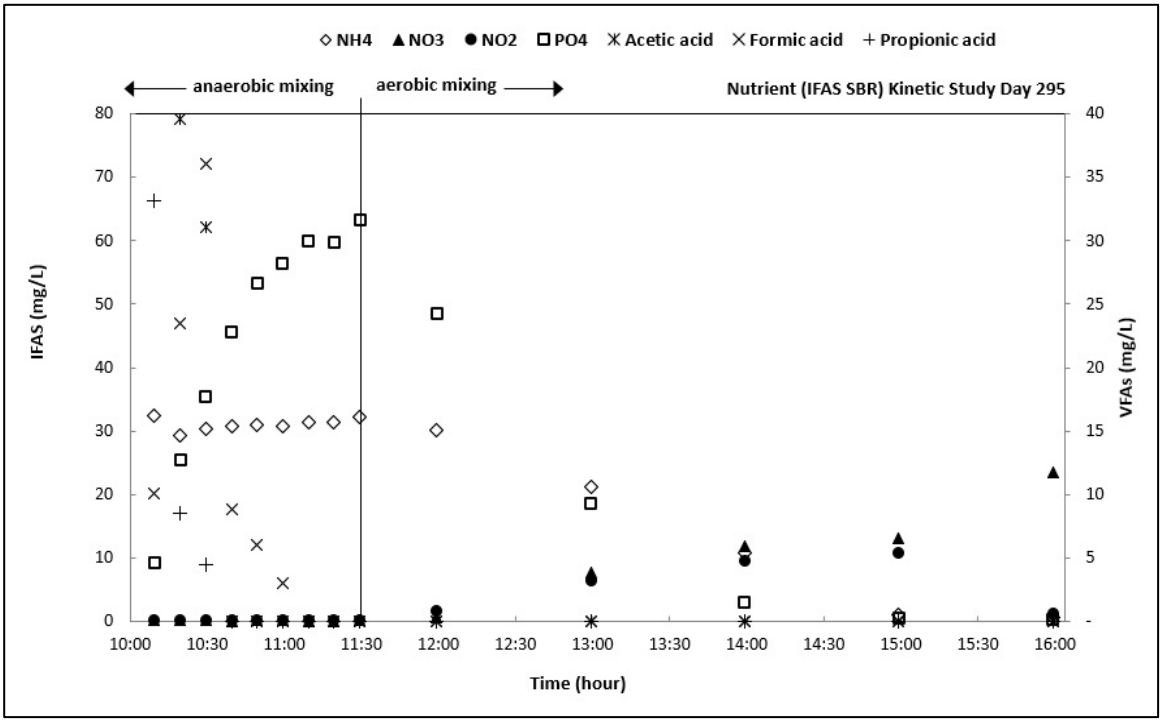

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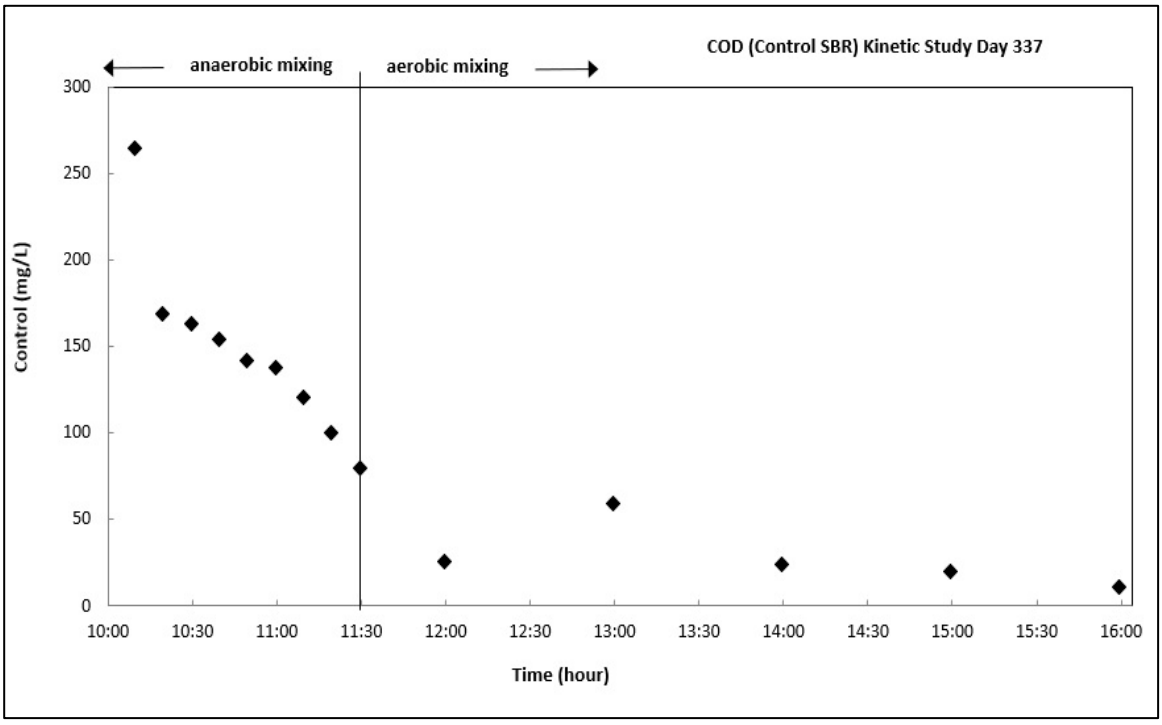

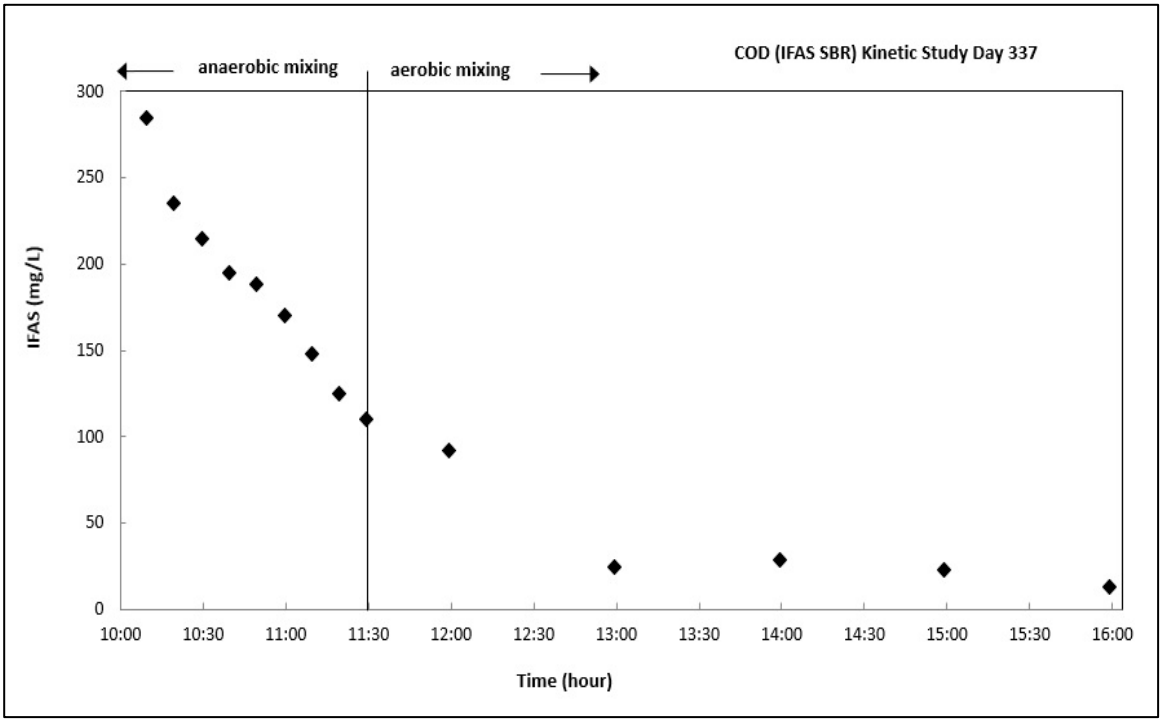

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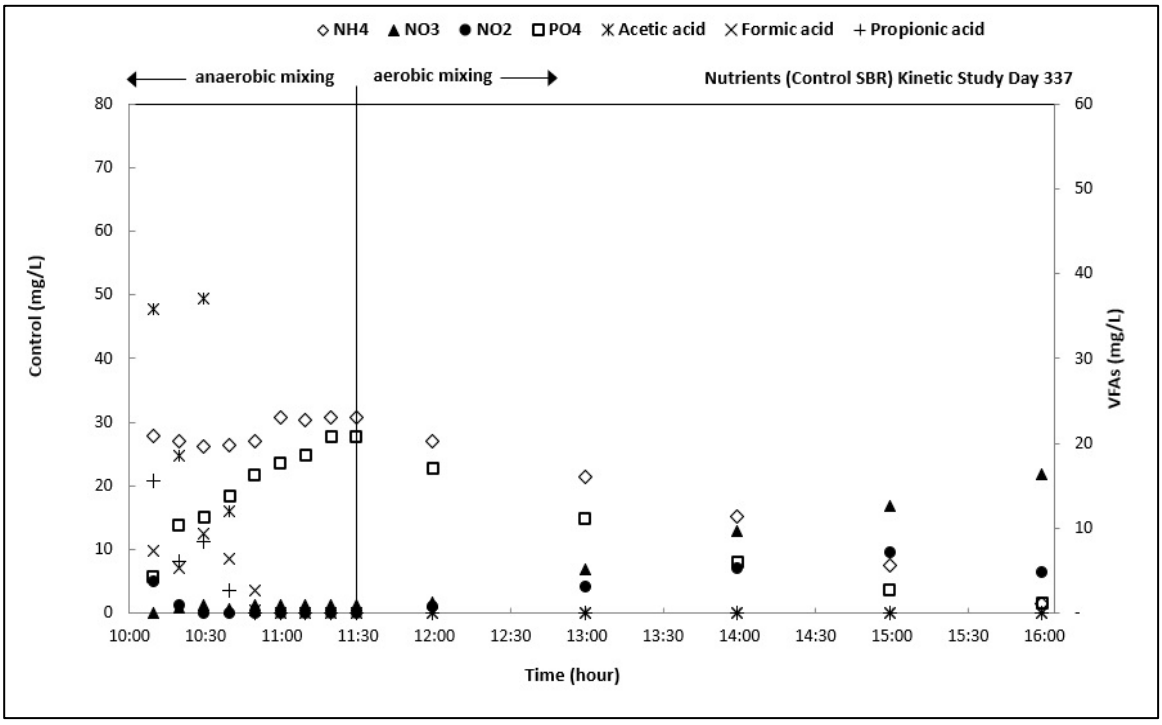
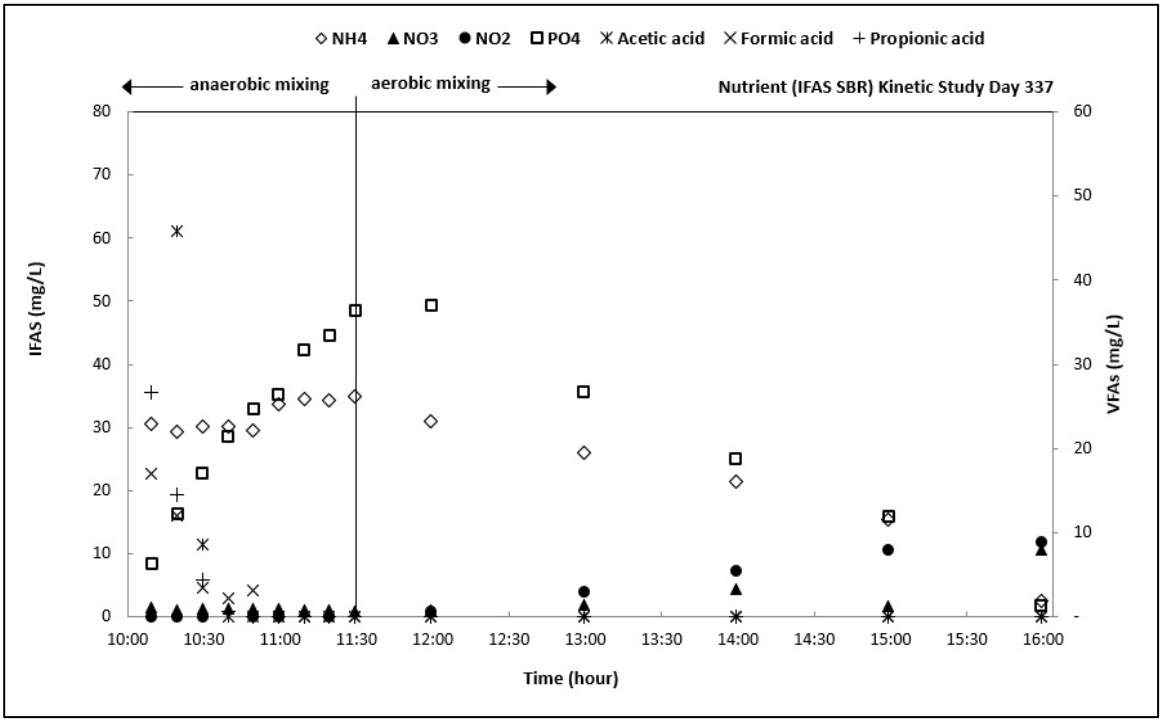
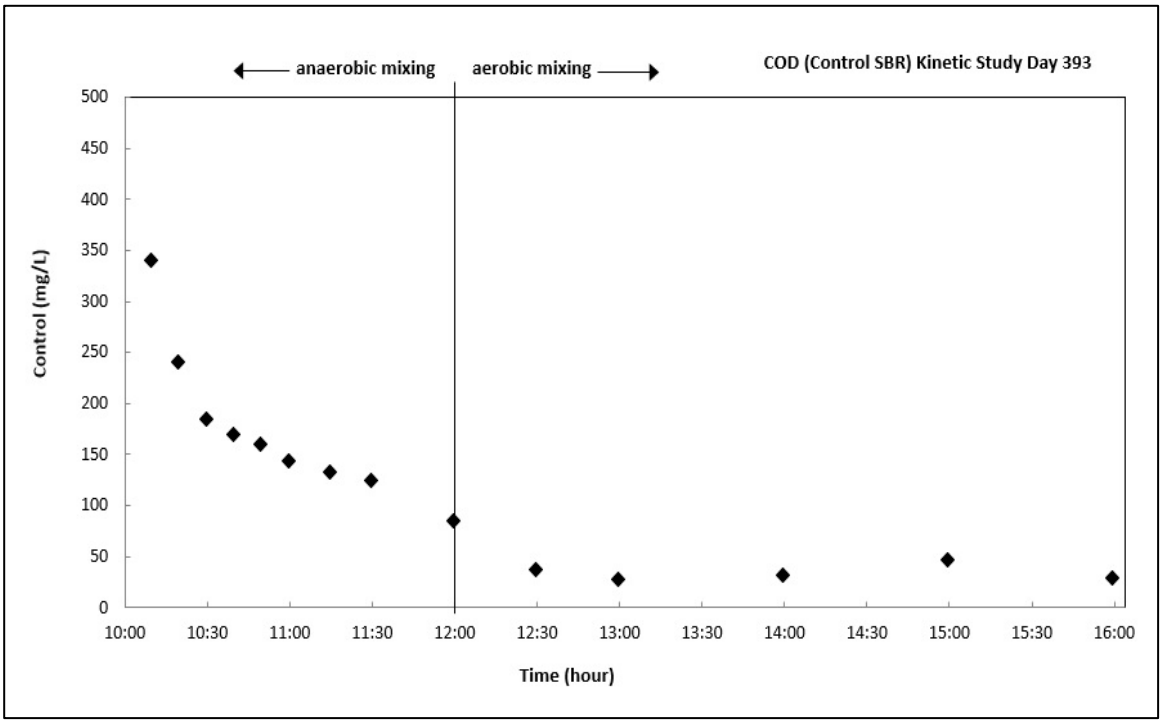


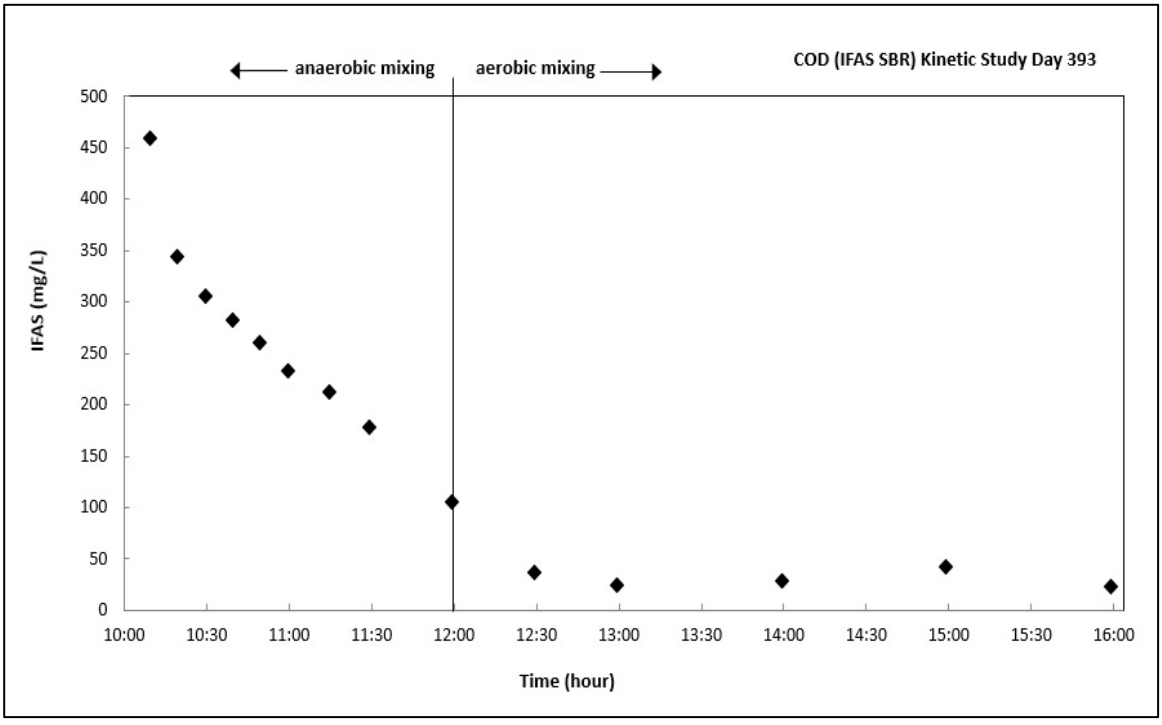


2021-08-27 KINETIC STUDY NUTRIENT REMOVALS

Appendix B

Kinetic study investigations (Phase 5), nutrient removal plots for AS-SBR (Control) and IFAS-SBBR:





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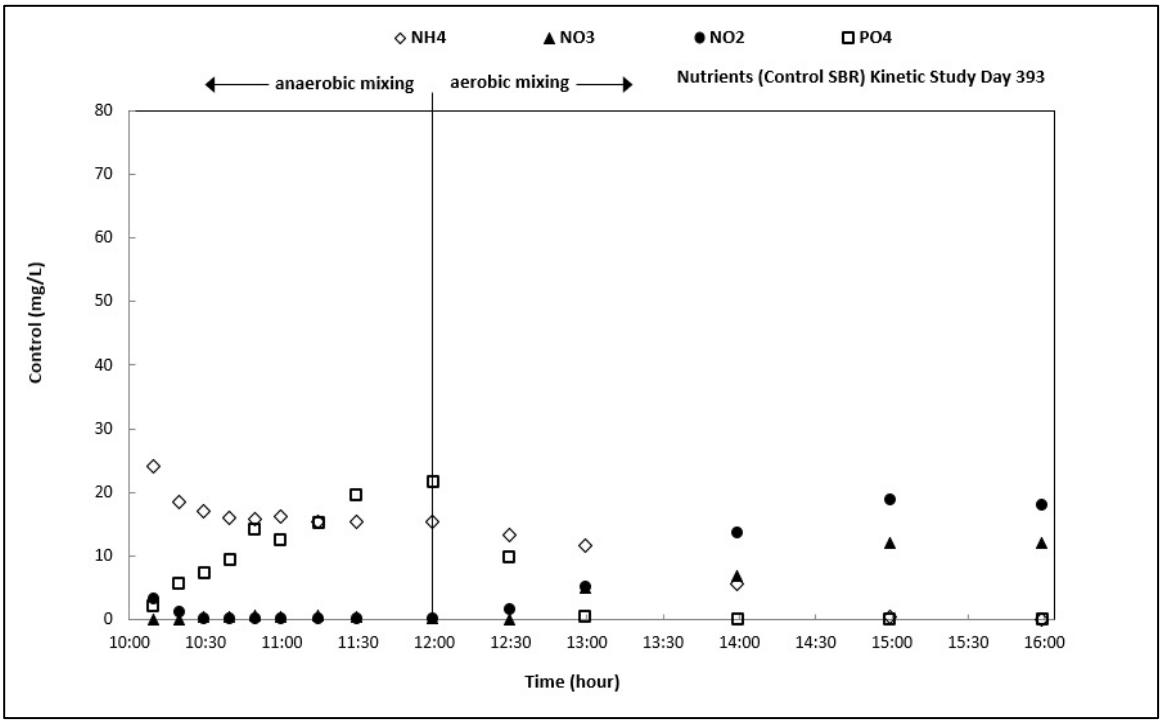

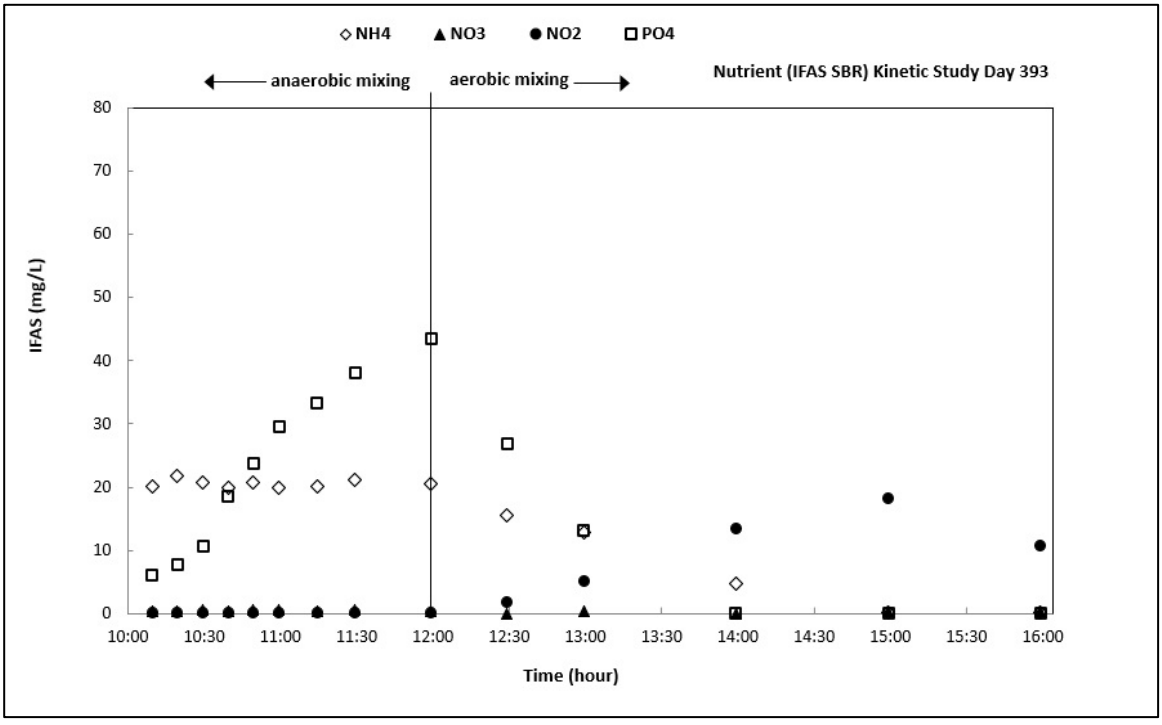

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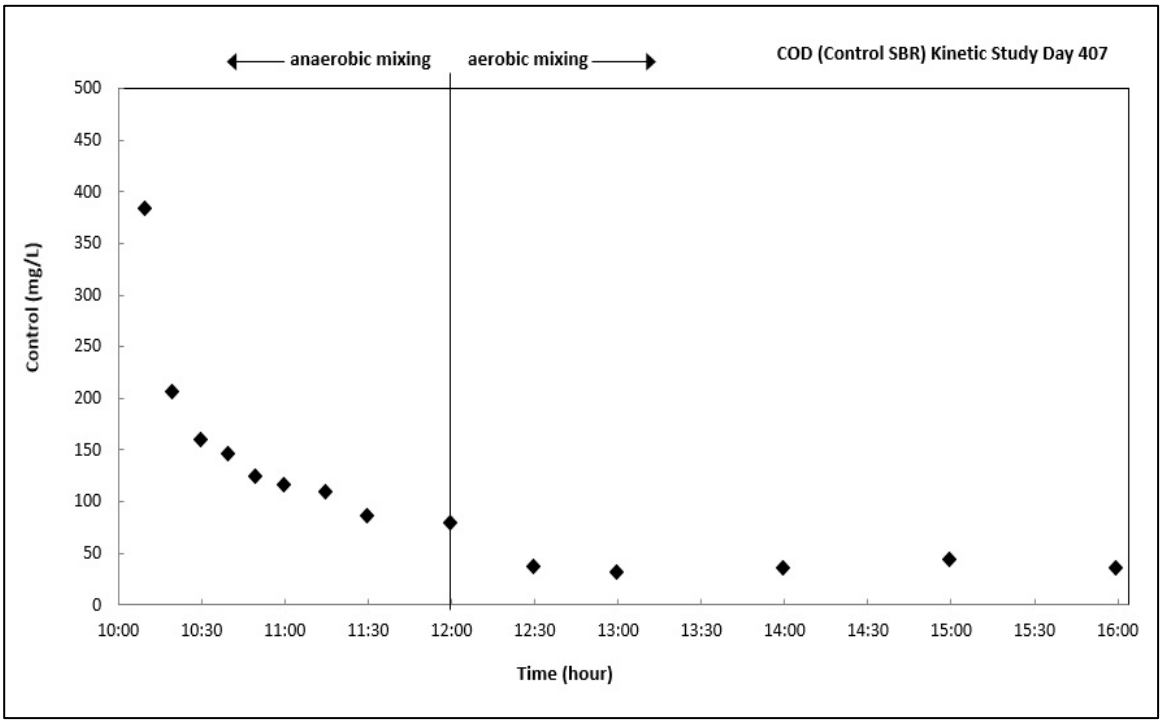

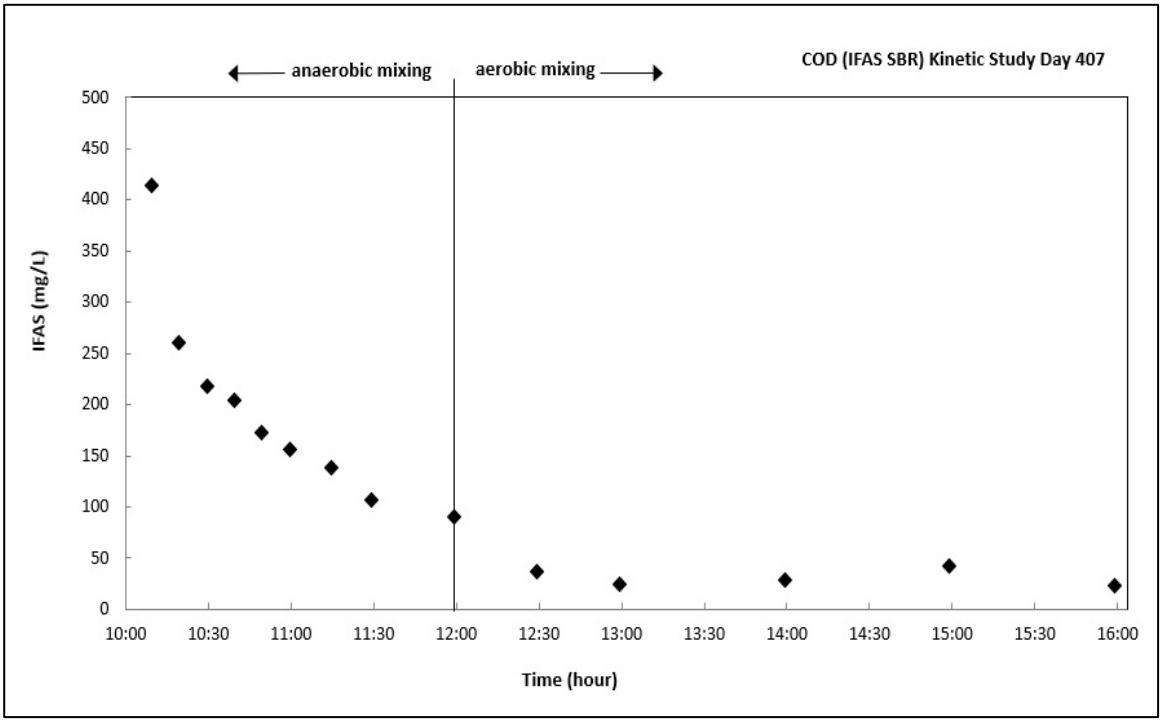

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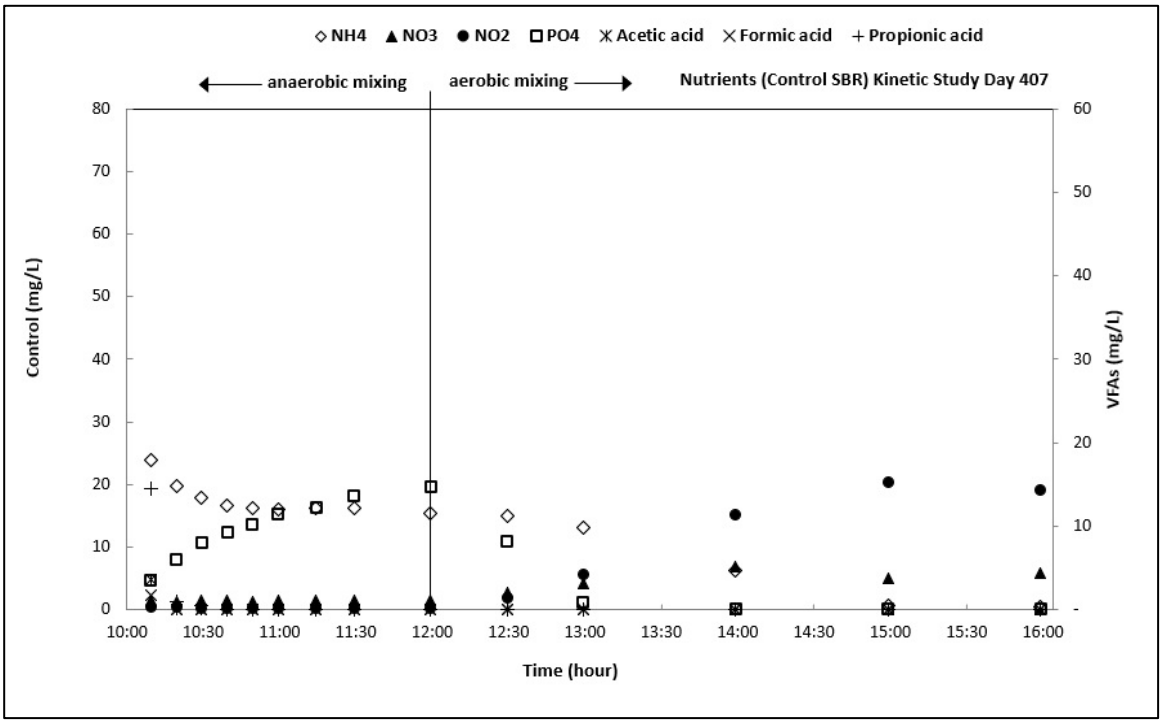

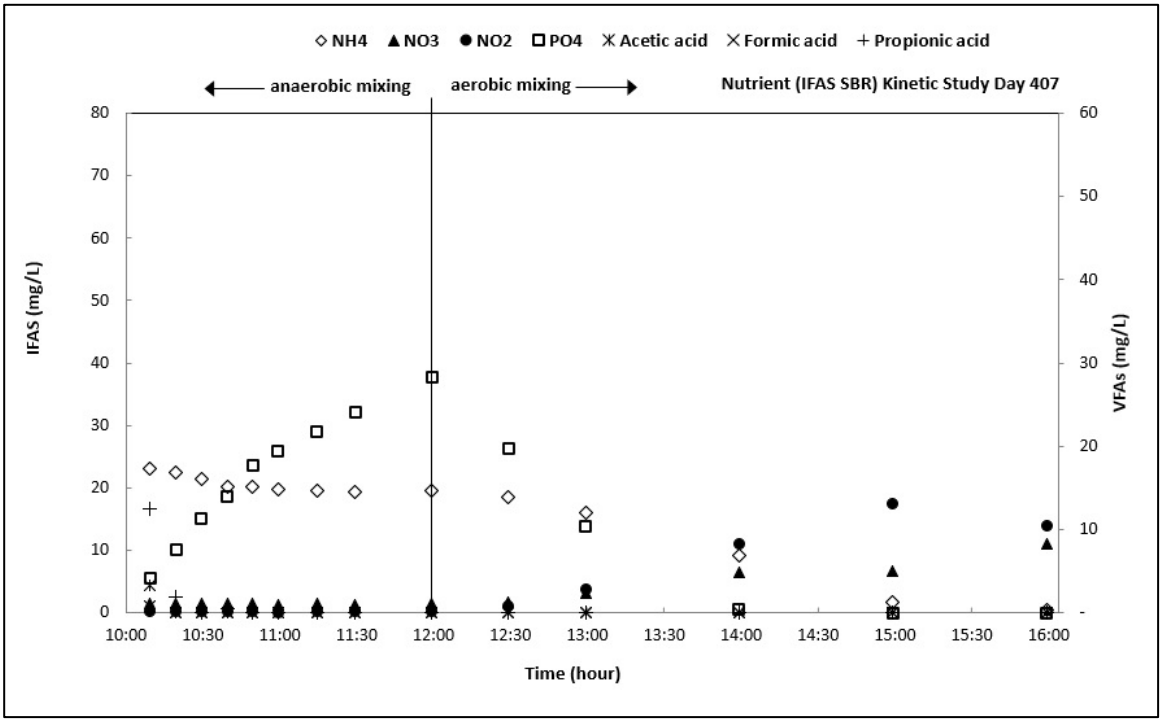

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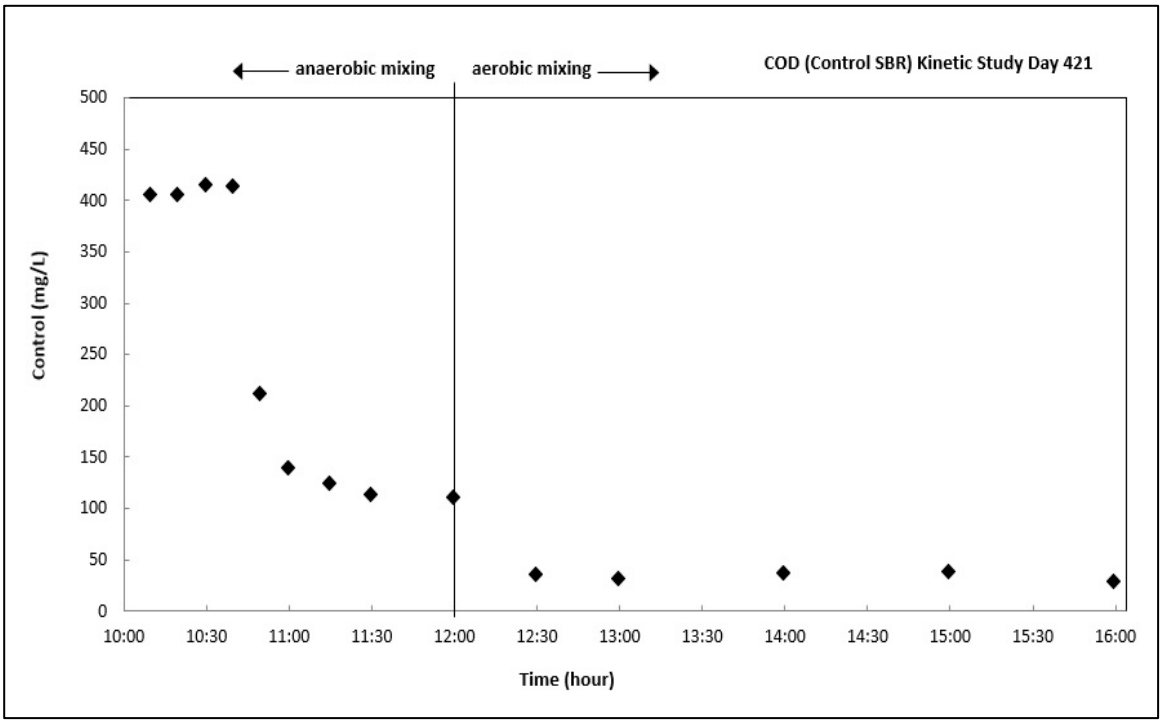

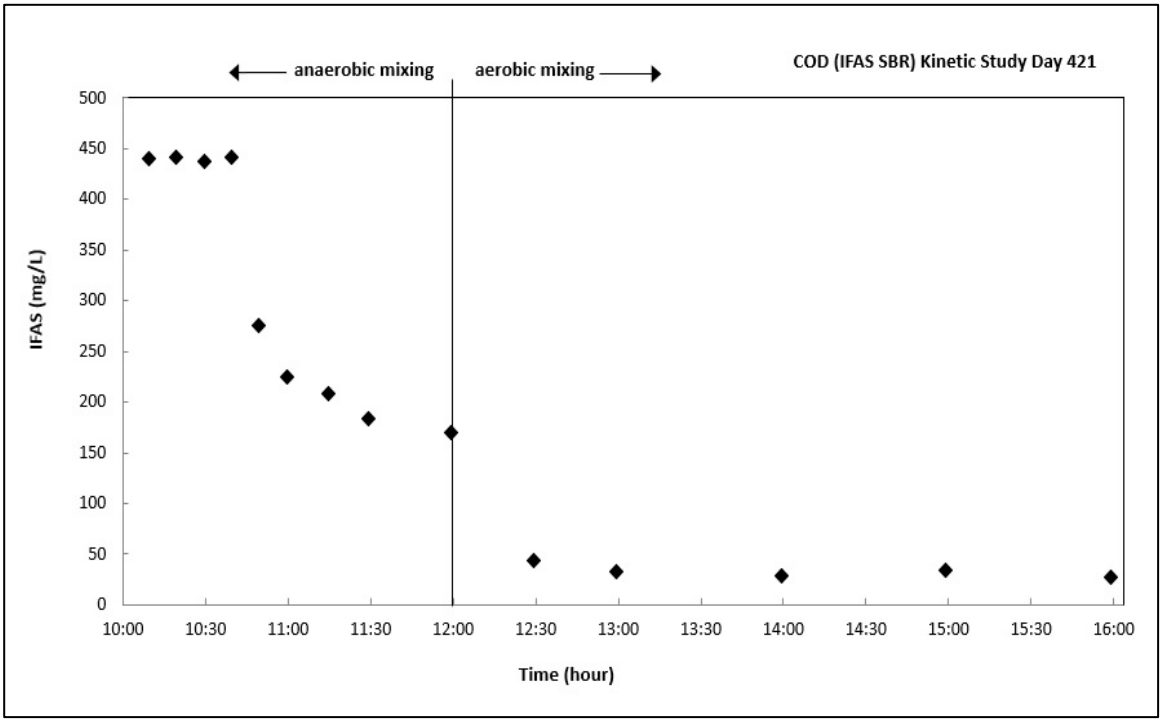

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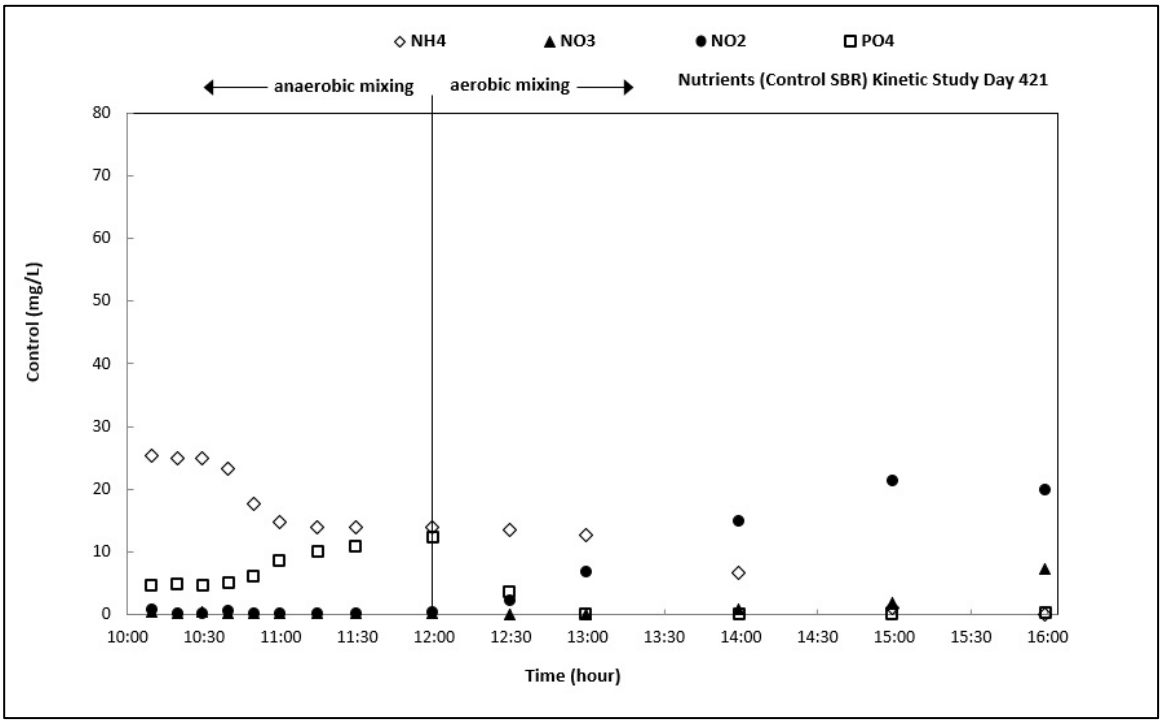
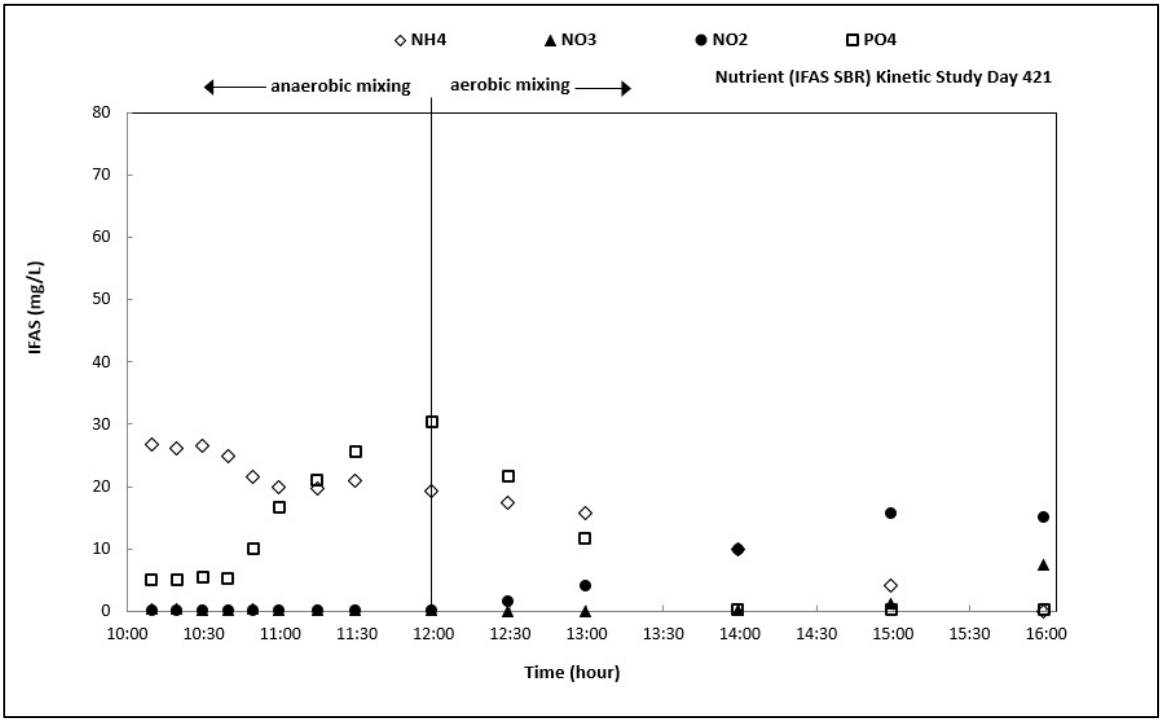
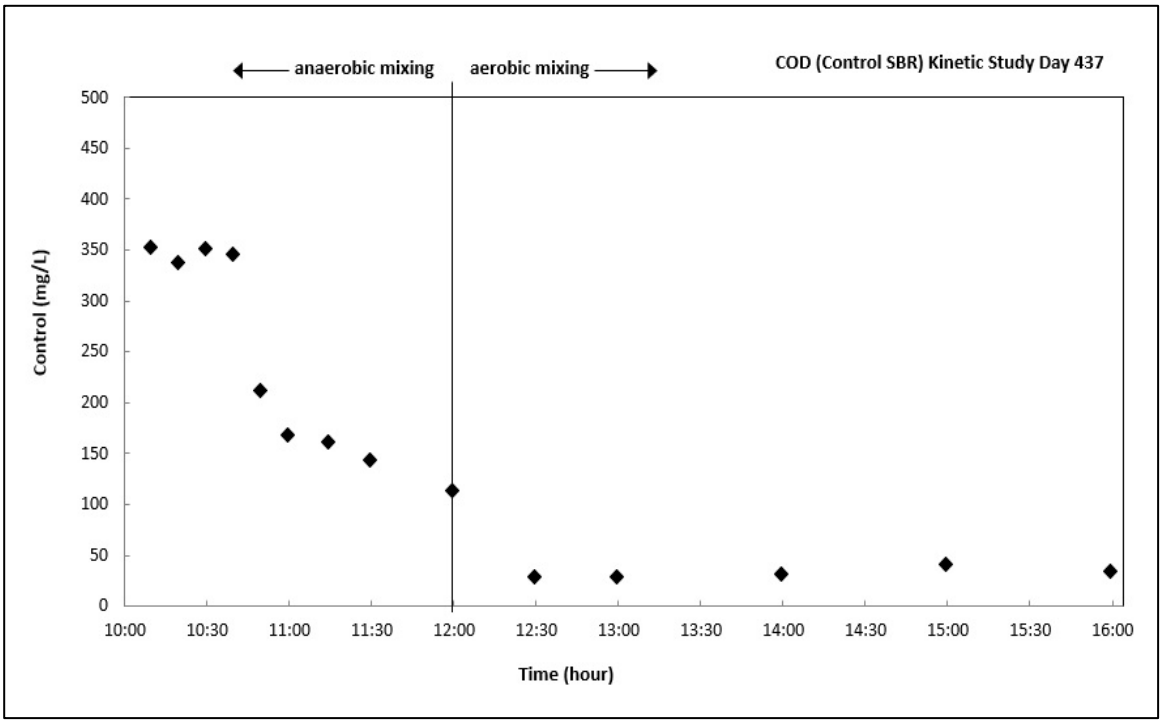
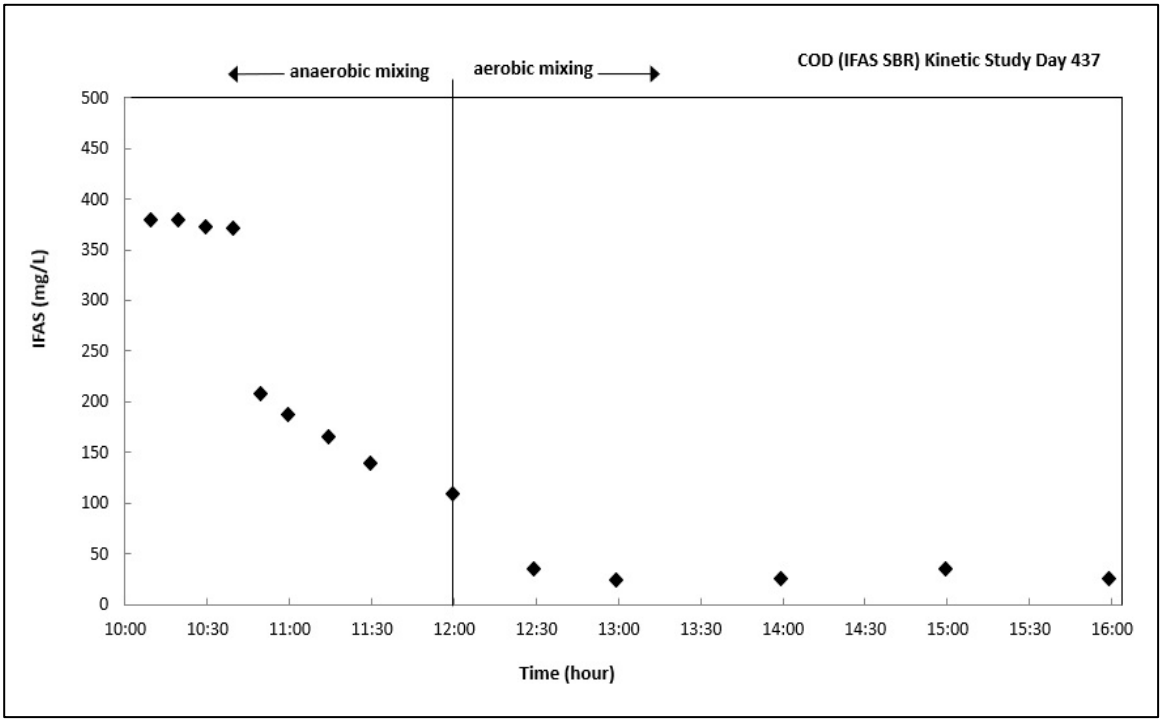


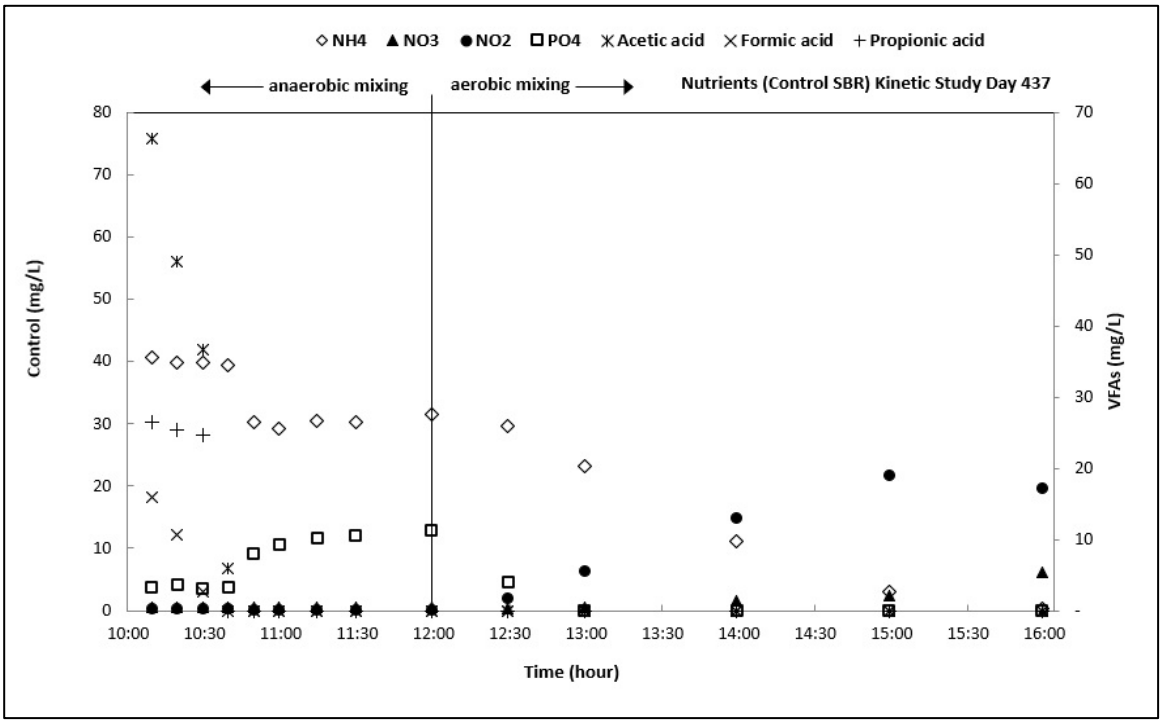


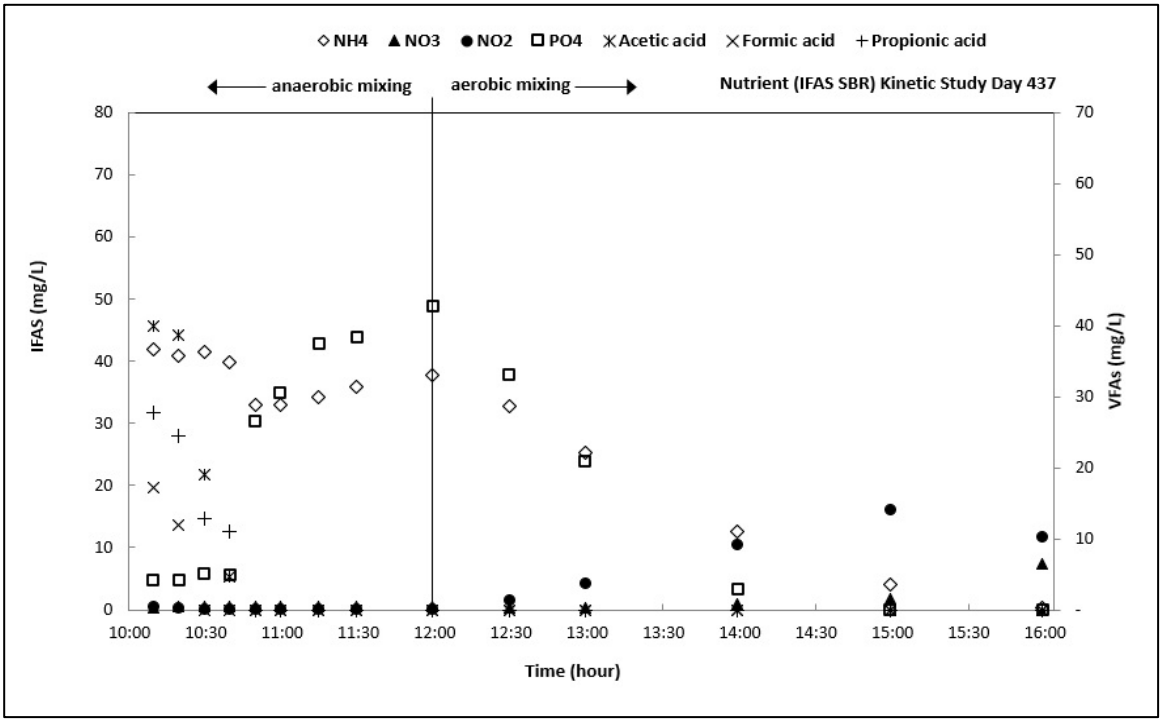
2021-11-19 KINETIC STUDY NUTRIENT REMOVALS



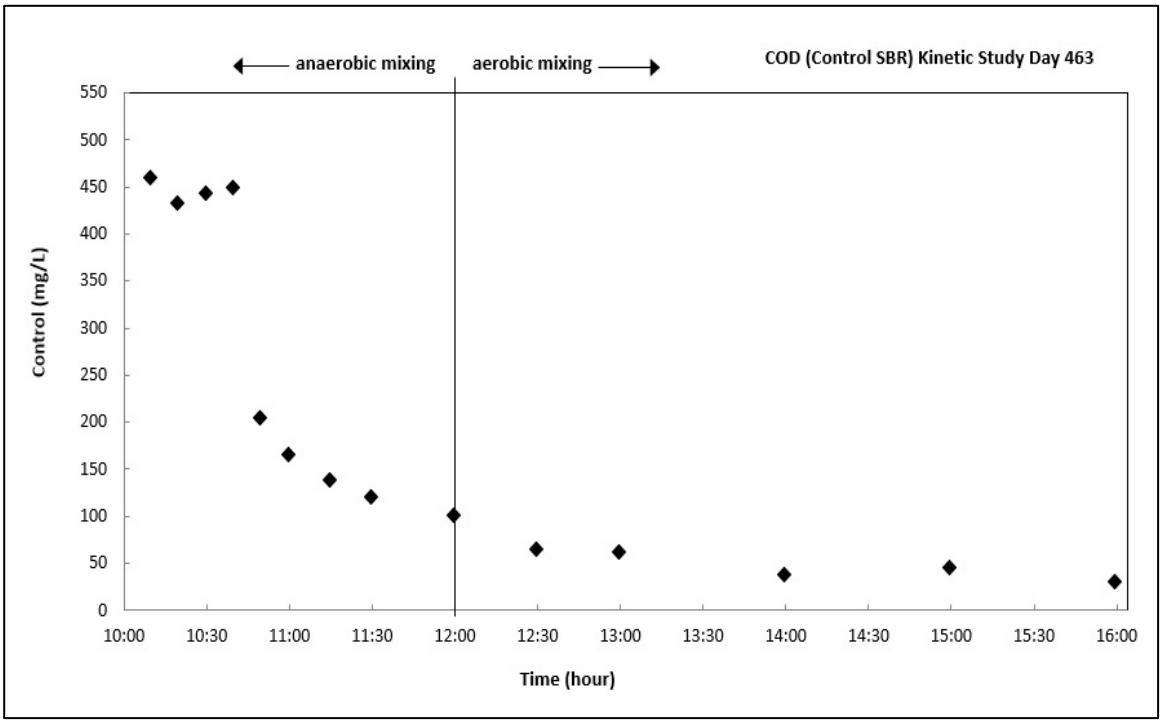


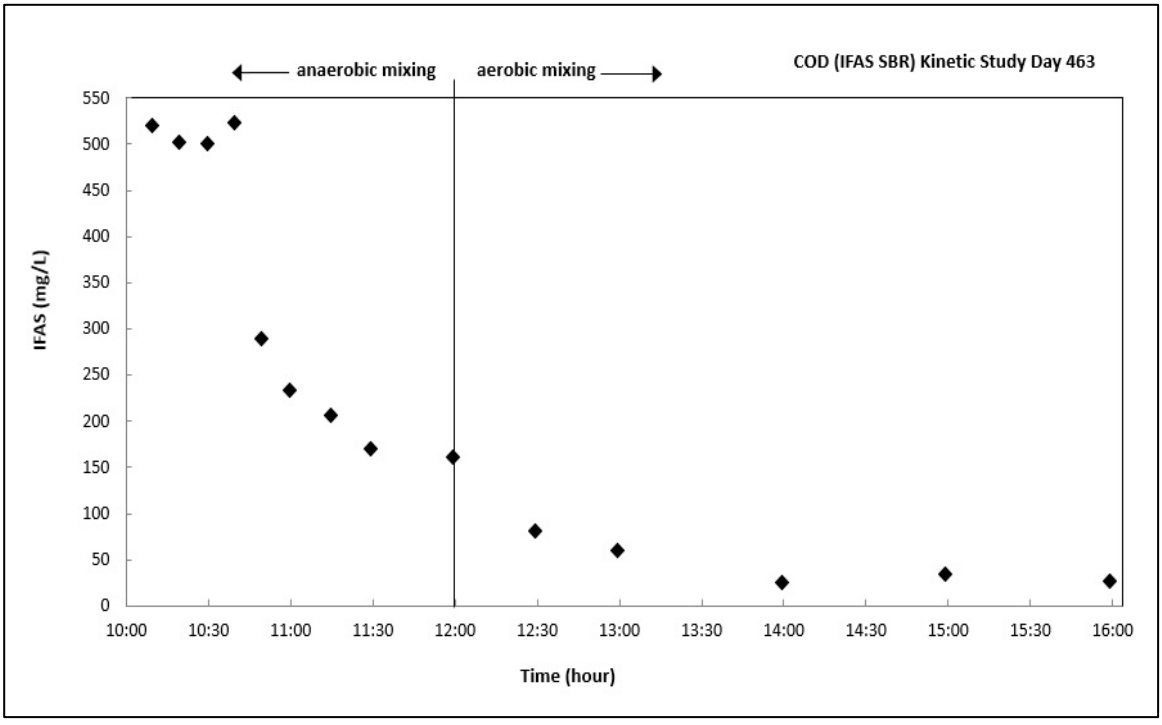
2021-12-03 KINETIC STUDY COD REMOVALS



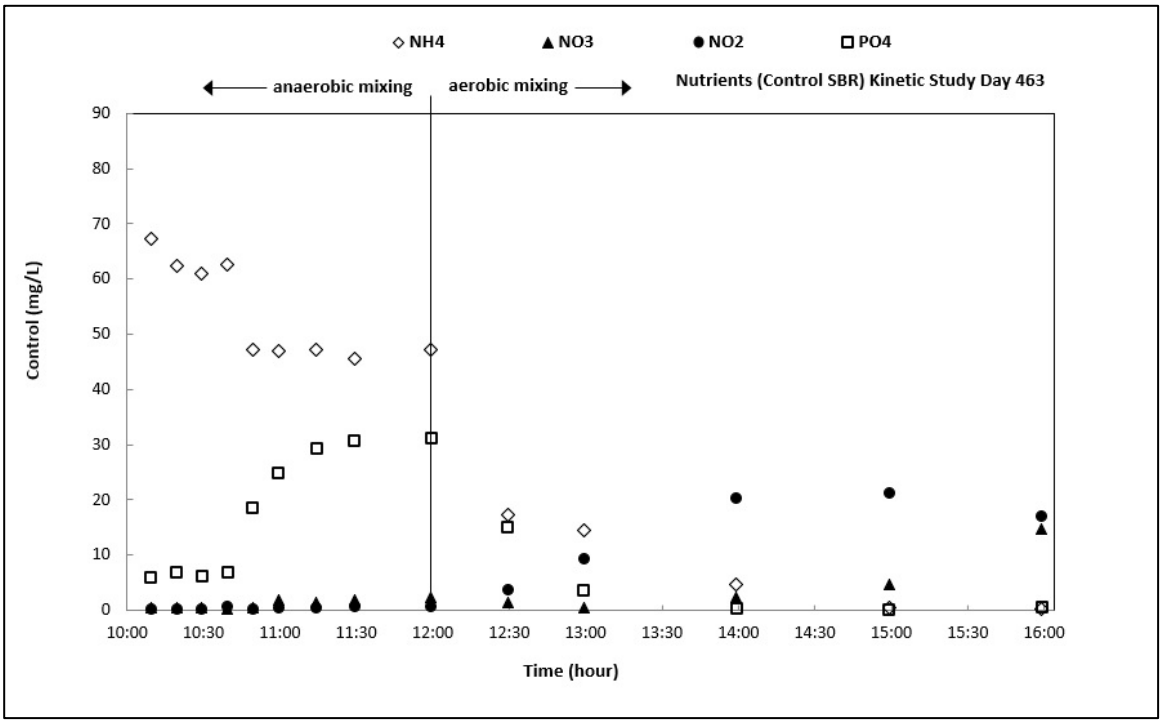


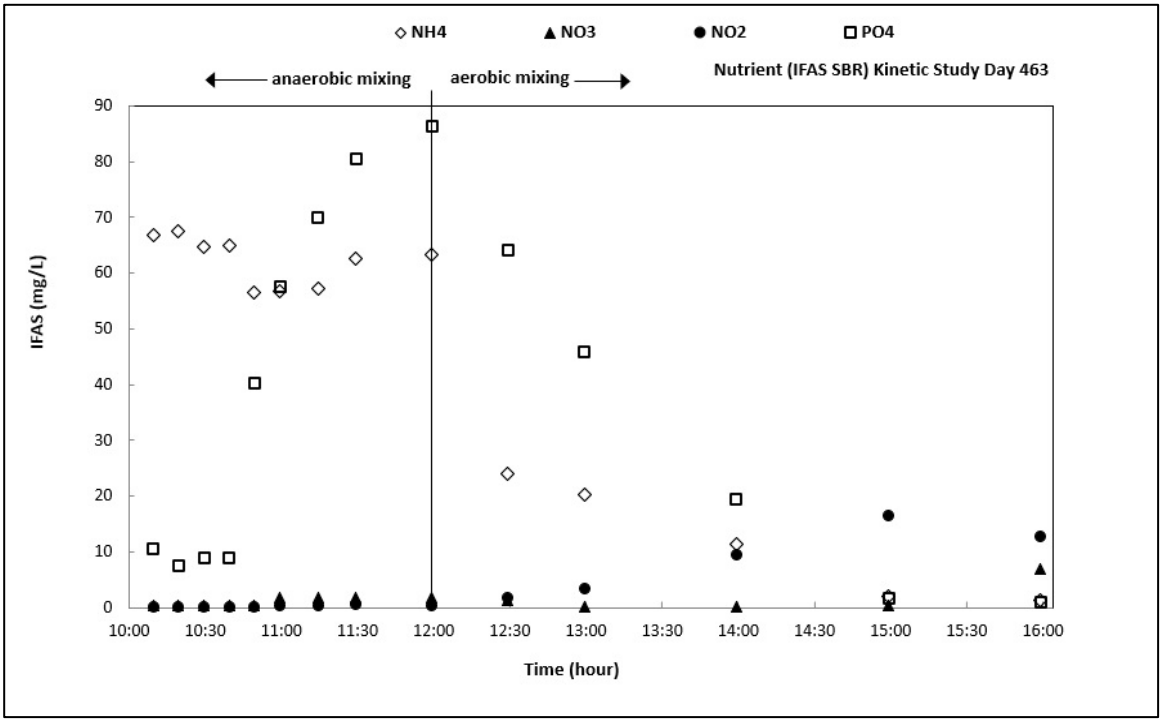
2021-12-03 KINETIC STUDY NUTRIENT REMOVALS





2021-12-31 KINETIC STUDY COD REMOVALS





2021-12-31 KINETIC STUDY NUTRIENT REMOVALS