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Title of the thesis

Applying an Anaerobic Side Stream Reactor to Return Activated Sludge for Sludge Seduction

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Abstract

This study investigated the integration of an anaerobic side-stream reactor (ASSR) with a sequencing batch reactor (SBR) for enhanced sludge reduction and nutrient removal in municipal wastewater treatment. Two parallel laboratory-scale systems were operated: a conventional SBR as reference, and an SBR coupled with an ASSR as the experimental setup. Performance parameters including chemical oxygen demand (COD), nitrogen species, phosphorus concentrations, and observed sludge yield ($Y_{o\beta s}$) were monitored over a long-term operational period. The SBR-ASSR configuration achieved substantial sludge minimization, with a reduction in observed sludge yield of approximately 40–50% compared to the conventional SBR. This reduction was attributed to enhanced endogenous decay, cryptic growth, and prolonged anaerobic exposure in the ASSR. Organic and nitrogen removal efficiencies remained comparable in both systems, while phosphorus removal in the SBR-ASSR showed promising results initially but declined over time, likely due to phosphorus saturation and reduced volatile fatty acid (VFA) availability. These findings demonstrate that integrating an ASSR into SBR systems can effectively reduce sludge production while maintaining stable organic and nitrogen removal performance. However, further optimization is recommended to sustain long-term phosphorus removal and support full-scale implementation.

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1. Introduction and Literature Review

1.1. Background & Context

1.1.1. Sludge Management

Sludge management in municipal wastewater treatment plants (WWTPs) is a critical environmental and economic challenge worldwide. The production of sludge, a byproduct of wastewater treatment, has been increasing due to urbanization, population growth, and the expansion of sewerage systems (Spinosa, 2007). This section provides a comprehensive background on sludge management, including the amount produced in different regions, trends, impacts, costs, and future prospects.

Sludge production varies significantly across regions due to differences in wastewater treatment technologies, population density, and water usage patterns. According to recent studies, the global production of sludge is estimated to be in the millions of tons annually, with developed countries generally producing more sludge due to advanced treatment systems (Gusiatin et al., 2024a; Spinosa, 2007). Sludge production from wastewater treatment has surged globally, driven by urbanization and the expansion of treatment infrastructure. China has the world's second-largest sewage treatment capacity. Due to rapid urban development, the country treats over 210 million m^3/day of wastewater. As a result, sludge production has risen significantly, with an estimated 13 million tons of dry solids generated annually as of 2019. This underscores the growing challenge of sludge management in China (Zhao et al., n.d.). There also has been a shift towards incineration and deep dewatering technologies due to the phasing out of landfill disposal. This shift has led to a reduction in greenhouse gas (GHG) emissions, with CO2 emissions per ton of dry solids (DS) decreasing from 0.91 in 2019 to 0.67 in 2020 (Zhao et al., n.d.). In the United States, it is estimated that drinking water treatment plants (DWTPs) generate between 35–40 million metric tons of sludge annually, while municipal WWTPs contribute an additional 6.8–7.9 million metric tons each year (Krause & Bronstein, 2024). Across Europe, regulatory demands and disposal limitations pose additional challenges for wastewater treatment facilities. The average excess sludge production in Europe is estimated at approximately 9.7 million tons per year on a dry basis, highlighting the scale of the issue. (Campo et al., 2021; Collivignarelli et al., 2019). Globally, countries are increasingly adopting innovative technologies such as anaerobic digestion, thermal treatments (e.g., pyrolysis and gasification), and nutrient recovery to enhance the sustainability of sludge management. These approaches not only reduce sludge volume but also facilitate energy and resource recovery (Huang, 2024; Shaddel et al., 2019). Additionally, nutrient recovery technologies are being explored to reclaim valuable resources like nitrogen and phosphorus from wastewater streams, promoting sustainable fertilizer production and reducing environmental pollution (Śniatała et al., 2024; Z. Wang et al., 2024)

The improper management of sludge can have significant environmental and health impacts. Sludge contains organic matter, nutrients, and potentially harmful contaminants such as heavy metals and pathogens. If not treated properly, sludge can pollute soil, water, and air, posing risks to human health and ecosystems (Dentel & Qi, 2014; Di Giacomo & Romano, 2022). In terms of greenhouse gas emissions, sludge treatment and disposal contribute significantly to the overall carbon footprint of wastewater treatment. Incineration generally results in the highest direct greenhouse gas (GHG) emissions among sludge treatment methods. However, landfilling can also lead to substantial GHG emissions, particularly due to methane release, which has a much higher global warming potential than CO_2. If not properly captured or flared, methane from landfills may contribute even more to climate change over the long term (Yoshida et al., 2018; EPA, 2021). Innovative technologies such as anaerobic digestion and thermal conversion offer more sustainable alternatives, with reported GHG reductions of 0.09-0.46 t CO_2-eq/t DS compared to conventional methods (Hu et al., 2024).

The cost of sludge management plays a critical role in shaping the selection of treatment and disposal strategies in wastewater treatment plants (WWTPs). In systems utilizing the activated sludge process, the treatment of excess sludge can contribute up to 40–60% of the total operating costs, highlighting its significant economic burden (Arif et al., 2020). Common sludge disposal methods include landfilling, incineration, and agricultural reuse. However, landfilling has become increasingly unviable due to escalating land costs and stricter environmental regulations (Wei et al., 2003). While incineration can reduce sludge volume by as much as 95%, it involves substantial capital investment, consumes non-renewable energy, and often faces public opposition (Semblante et al., 2014a).

The reuse of sludge as fertilizer or soil conditioner offers a promising approach for resource recovery. Nonetheless, it frequently requires long-distance transport to agricultural end users and raises environmental concerns due to the potential presence of heavy metals and toxic organic micropollutants, which can accumulate in soil and enter food chains (Clarke & Smith, 2011; Semblante et al., 2014b). Consequently, modern wastewater management increasingly favors sludge minimization over traditional treatment, as it reduces the burden and cost of downstream handling, stabilization, transport, and disposal.

Regionally, these dynamics vary. In China, sludge treatment costs have risen due to shifts toward more sustainable practices, with incineration emerging as a preferred, albeit costlier, option (Zhao et al., n.d.). In the United States, efforts to quantify national sludge management costs are hampered by a lack of standardized and transparent reporting systems (Krause & Bronstein, 2024). Meanwhile, in Europe, sludge management costs differ considerably between countries. In Italy, for instance, increasingly stringent regulations have driven up the cost of transporting sludge to other regions for treatment, reinforcing the need for localized and diversified treatment approaches (Campo et al., 2021).

The future of sludge management lies in the adoption of sustainable and innovative technologies that prioritize resource recovery and environmental protection. Anaerobic digestion, for instance, is widely used for biogas production, offering both energy recovery and GHG emission reduction benefits (Dentel & Qi, 2014; Di Giacomo & Romano, 2022). Similarly, hydrothermal processes and wet oxidation are emerging as promising technologies for sludge treatment, offering significant reductions in GHG emissions and improved resource recovery (Hu et al., 2024). In addition to these technologies, the concept of a circular economy is gaining traction in sludge management. This approach emphasizes the recovery of valuable materials such as nutrients (e.g., struvite), humic substances, and biochar, which can be used in agriculture, energy production, and environmental remediation (Gusiatin et al., 2024; Mulopo, 2024).

1.1.2. Role of Iron Addition in Sludge Reduction and Nutrient Removal

Iron-based additives have gained attention as a practical enhancement in municipal wastewater treatment, contributing to nutrient removal, sludge reduction, and micropollutant degradation. Iron compounds, including ferric (Fe^{3+}), ferrous (Fe^{2+}), zero-valent iron (Fe^0), and ferrate (Fe^{6+}), function through chemical precipitation, redox reactions, and microbial stimulation (Liu et al., 2018). Each form exhibits distinct reactivity, influencing treatment efficiency and operational outcomes. Understanding these distinctions is essential for selecting the appropriate iron species based on treatment objectives, such as phosphorus removal, nitrogen cycling, or sludge volume reduction (Liu et al., 2018; Wilfert et al., 2015).

Ferric iron (Fe^{3+}), typically dosed as ferric chloride ($FeCl_3$), is widely used for chemical phosphorus removal. Upon hydrolysis, it forms ferric hydroxide [$Fe(OH)_3$], which binds phosphate and organic matter, aiding their removal via sedimentation. A Fe/P molar ratio of approximately 0.4 is generally sufficient to achieve effluent phosphorus concentrations below 1.0 mg/L. This mechanism not only improves phosphorus control but also contributes to the removal of colloidal particles and enhances sludge settleability (MISHIMA & NAKAJIMA, 2011; Plevri et al., 2023).

Ferrous iron (Fe^{2+}), often added as ferrous sulfate ($FeSO_4$), supports redox-driven nutrient removal and sludge minimization. Acting as an electron donor under anoxic conditions, Fe^{2+} facilitates denitrification and supports microbial communities involved in nitrogen cycling, especially under low-carbon conditions. Additionally, Fe^{2+} can influence the microbial consortia by promoting the growth of specific denitrifiers, which further enhances biological nitrogen removal efficiency. This has proven particularly beneficial in systems where carbon supplementation is limited or costly (Cheng et al., 2024; Gao et al., 2024).

Zero-valent iron (Fe^0) offers abiotic catalytic effects, enhancing denitrification and electron transfer. In bimetallic forms (e.g., Fe^0/Cu), it has shown potential to improve nitrogen gas selectivity and sludge dewaterability (Fu et al., 2014; Junyapoon*, 2005). These properties make Fe^0 an attractive option for advanced oxidation and integrated treatment systems, especially where complex organic contaminants are present. The presence of metallic surfaces also facilitates direct interspecies electron transfer (DIET), which can stimulate syntrophic microbial interactions and improve reactor stability (Jafari & Botte, 2021).

Ferrate (Fe^{6+}), applied as sodium or potassium ferrate, acts as a powerful oxidant capable of removing phosphorus and degrading various micropollutants. Dosing at approximately 7.5 mg/L has been shown to be effective in achieving both goals. Ferrate's strong oxidative capacity enables it to target refractory compounds, pharmaceuticals, and endocrine-disrupting chemicals while simultaneously contributing to pathogen inactivation. This makes it a promising candidate for tertiary treatment applications (Y. Lee et al., 2009; Munyengabe & Zvinowanda, 2019).

Iron's redox flexibility also influences microbial metabolism. Fe^{2+} can stimulate denitrifiers, while Fe^{3+} can act as an oxidant in biological systems. These reactions contribute to improved biological nitrogen removal and reduced sludge yield (Cheng et al., 2024; Ding et al., 2023). Iron-chitosan composites have also demonstrated potential to enrich functional bacteria, like Paracoccus, thereby supporting partial nitrification-denitrification (Luan et al., 2024). This biochemical synergy underscores the importance of iron in enhancing microbial diversity and system resilience.

In systems such as anammox reactors, moderate Fe^{2+} levels (0.06-0.08 mM) support the growth of key organisms like Candidatus Brocadia sinica, though concentrations beyond this range may inhibit performance. The dual role of Fe^{2+} as a micronutrient and electron donor reinforces its importance in maintaining balanced redox conditions and optimal microbial activity in anaerobic environments (Wu et al., 2023).

In Oxic-Settling-Anoxic (OSA) systems, ferric iron addition (up to 16.05 mg/L) has been linked to up to 87% sludge reduction, likely through enhanced floc disruption and increased microbial uncoupling. Similarly, iron dosing in SBRs integrated with ASSRs has shown synergistic improvements in both nutrient removal and sludge reduction (Sabet et al., 2023). These integrated approaches demonstrate the potential for iron to facilitate not only pollutant removal but also resource recovery and energy savings in modern wastewater treatment.

Recommended dosage ranges vary by application: 20–30 mg/L for $FeCl_3$ (P removal), 10–20 mg/L for $FeSO_4$ (denitrification, sludge reduction), 0.01-4 g/L for Fe^0 (methanogenesis), and around 7.5 mg/L for ferrate (Cheng et al., 2024; Y. Lee et al., 2009; Tian et al., 2020). Selection of appropriate dosing strategies must consider influent characteristics, reactor configuration, and microbial ecology to optimize treatment outcomes.

However, excessive iron, particularly Fe^{3+} over 60 mg/L, may inhibit enzymes like nitrite oxidoreductase and destabilize microbial processes in systems such as SNDPR or anammox. Therefore, careful selection of iron form and dosing strategy is essential to optimize treatment efficiency while avoiding adverse effects. Long-term monitoring and process modeling can aid in fine-tuning iron application to sustain system performance and ensure regulatory compliance (Cheng et al., 2024).

1.2. Importance of the Study

Biological wastewater treatment processes, while effective for pollutant removal, generate large volumes of waste activated sludge (WAS), leading to substantial environmental and economic challenges. Regions like Europe and China produce millions of tons of sludge annually (Bianchini et al., 2016; Zhao et al., n.d.), and sludge handling can comprise over 50% of total operating costs at wastewater treatment plants (Kacprzak et al., 2017). Conventional sludge treatment methods, such as incineration or land application, are either costly or environmentally unsustainable (Han et al., 2021; Cheng et al., 2018), prompting a need for more efficient and integrated sludge reduction solutions.

Anaerobic Side-Stream Reactors (ASSRs) have emerged as a promising in-process strategy capable of reducing sludge production by up to 80% with lower energy and capital requirements compared to post-treatment technologies (Corsino et al., 2023; Yagci et al., 2015). However, the underlying mechanisms, particularly the effects of iron dosing, organic loading, and sludge cycling, are not yet fully understood (Chon, Rome, Kim, Park, et al., 2011a; Ferrentino et al., 2016).

This study aims to evaluate and compare the overall performance of a conventional Sequencing Batch Reactor (SBR) and an SBR integrated with an ASSR, focusing primarily on sludge reduction but also assessing COD, nitrogen, and phosphorus removal. In a subsequent phase, ferric chloride ($FeCl_3$) will be added to investigate its influence on sludge yield and nutrient removal. By analyzing operational parameters and microbial responses, this research seeks to clarify the mechanisms driving performance improvements, ultimately supporting the design of more cost-effective and sustainable wastewater treatment systems.

1.3. Previous Relevant Research

Sludge management and nutrient removal remain critical challenges in municipal wastewater treatment, prompting research into diverse technologies that reduce sludge yield while maintaining or improving removal efficiencies for nitrogen, phosphorus, and organic matter. The following sections summarize key findings from recent and influential studies on chemical, physical, biological, and integrated sludge reduction strategies.

Oxic-Settling-Anaerobic (OSA) and Anoxic-Oxic-OSA Systems: The OSA process has been studied as a low-energy, in-line configuration for minimizing sludge production by exposing a portion of sludge to anaerobic conditions outside the main reactor. Semblante et al., 2014 investigated an SBR-OSA configuration at lab scale using real wastewater. The system achieved an observed sludge yield (Yobs) of 0.16 g SS/g COD with a solids retention time (SRT) of 10 days and achieved 10% sludge reduction and COD and NH_4^+-N removal efficiencies of 88% and 89%, respectively. Romero Pareja et al., 2018 evaluated the combination of anoxic and OSA systems and reported sludge reductions ranging from 23.5% to 32.5%, with enhanced total nitrogen (TN) removal of up to 69.9%.

Anaerobic Side-Stream Reactor (ASSR) Systems: The ASSR strategy integrates an anaerobic reactor into the sludge return loop, targeting degradation of extracellular polymeric substances (EPS) and promoting endogenous decay. Chon et al., 2011 operated a lab-scale SBR-ASSR system fed with a 50:50 mixture of real and synthetic wastewater, achieving a Yobs of 0.159 g SS/g COD, with 14.5% sludge reduction and >90% COD removal at an SRT of 10 days. Park and Chon (2015) reported greater sludge reduction (34.4%) with a similar SRT and Yobs of 0.27 g SS/g COD. Goel & Noguera, 2006 combined an EBPR SBR with an ASSR using synthetic wastewater, achieving a Yobs of 0.19 g SS/g COD and sludge reduction between 16-33%.

A comprehensive review by Chon et al., 2011 emphasized that anaerobic treatment in side-stream reactors can degrade iron-bound and base-extractable EPS, which are resistant to conventional aerobic digestion. In parallel, Chon et al., 2011 found that aerobic degradation of solubilized materials continued in the main reactor, leading to a split pathway of sludge reduction, half in the ASSR, half in the main SBR.

SIPER Process (Sludge Reduction, Inorganic Solids Separation, Phosphorus Recovery, and Enhanced Nutrient Removal): Developed in China, the SIPER process integrates sludge reduction and nutrient recovery. Yan et al., 2013 reported COD, TN, TP, and NH_4^+-N removal efficiencies of 92.7%, 75.5%, 95.3%, and 98.2%, respectively, with a Yobs of only 0.103 g VSS/g COD. A follow-up study (Yan et al., 2013b) achieved an even lower Yobs of 0.096 g VSS/g COD by recovering internal carbon sources. Yan et al., 2013 showed that integrating alkaline-treated sludge in a side-stream reactor improved TN and TP removal by 19.6% and 23.6%, respectively.

Comparative Review of Sludge Reduction Technologies: Q. Wang et al., 2017 categorized sludge reduction technologies into in-line treatments (e.g., free nitrous acid, metabolic uncouplers), end-of-pipe methods (ultrasonic, thermal hydrolysis), and emerging systems like anaerobic membrane bioreactors (AnMBRs) and SANI®. Reported sludge reductions ranged from 10% to nearly 100%, depending on the technology. Nutrient removal efficiencies were often >75% when integrated strategies were employed.

Iron-Based Enhancements: Iron addition, particularly ferric chloride ($FeCl_3$), has shown promise in supporting phosphorus precipitation and enhancing sludge hydrolysis. Yagci et al., 2015 demonstrated sludge reductions up to 87% in an OSA system dosed with Fe^{3+} under fast-feeding conditions. Similarly, Cheng et al., 2024 found that ferrous iron (Fe^{2+}) enhanced denitrification and nitrogen removal under low-carbon conditions, while supporting sludge disintegration. These enhancements are increasingly integrated into hybrid treatment systems to boost both nutrient and solids management efficiency.

Configuration	**Scale**	**Wastewater Type**	**Yobs (g SS/g COD)**	**SRT (d)**	**Sludge Reduction (%)**	**COD Removal (%)**	**NH4-N (%)**	**NO3-N (%)**	**PO4-P (%)**	**Reference**
SBR-OSA	Lab	Real wastewater	0.16	10	10	88	89	–	0	Semblante et al., (2017)
SBR-ASSR	Lab	Real wastewater	0.27	10	34.4	>90	–	–	–	Park & Chon, (2015)
SBR-ASSR	Lab	50% real, 50% synthetic	0.159	10	14.5	91	–	–	–	(Chon, Rome, Kim, Park, et al., 2011a)
SBR (EBPR)-ASSR	Lab	Synthetic wastewater	0.19	10	16–33	–	–	–	–	Goel & Noguera, (2006)
A+OSA	Pilot	Domestic WW	–	–	23.5–32.5	–	69.9	–	–	Romero Pareja et al., (2018)
SIPER	Pilot	Municipal WW	0.103	–	–	92.7	75.5	–	95.3	Yan et al., (2013)
SIPER + C-recovery	Pilot	Municipal WW	0.096	–	–	–	80	–	High	Yan et al., (2013a)
SIPER + Alk. Sludge	Pilot	Municipal WW	–	–	–	–	+19.6	–	+23.6	Yan et al., (2015)

1.4. Research Questions

This study aims to investigate the role of an Anaerobic Side-Stream Reactor (ASSR) integrated with a Sequencing Batch Reactor (SBR) in enhancing biological wastewater treatment performance. The key research questions addressed are:

- How does sludge recirculation between SBR and ASSR influence overall system efficiency?

This includes examining changes in chemical oxygen demand (COD), nitrogen, and phosphorus removal, as well as operational stability and effluent quality in both control SBR and SBR-ASSR systems.

- How does the ASSR configuration affect sludge reduction mechanisms?

The study explores whether prolonged anaerobic exposure in the ASSR promotes hydrolysis, endogenous decay, or microbial uncoupling, thereby enhancing sludge breakdown and minimizing waste activated sludge production.

- Can the SBR-ASSR system achieve improved nutrient removal while simultaneously reducing sludge production?

This question evaluates the potential synergy of combining aerobic and anaerobic processes to enhance nitrogen and phosphorus removal while achieving sustainable sludge minimization.

2. Research Objectives

The main goal of this research is to investigate how the Anaerobic Side-Stream Reactor (ASSR) helps to reduce overall sludge production in activated sludge systems. Based on Semblante et al., (2014b), the principle behind this is that circulating waste activated sludge (WAS) between an external anaerobic, substrate-limited chamber and the aerobic, substrate-rich main bioreactor triggers cryptic microbial growth, which leads to a reduction in biomass concentration.

Cryptic growth refers to biomass growth that occurs through the utilization of autochthonous (internally generated) substrates, and it can contribute to a decrease in overall biomass yield. While this strategy has been extensively studied in the context of sludge reduction, its practical application has been mostly limited to bench- or pilot-scale experiments. Implementation at large- or full-scale wastewater treatment plants (WWTPs) has been hindered by the high energy and chemical demands of typical supporting processes, such as ozonation, ultrasonication, milling, pyrolysis, and microwave treatment. These requirements undermine the environmental sustainability of cryptic growth-based sludge reduction approaches. Therefore, there is a clear need to explore cryptic growth technologies that function without external energy or chemical inputs (Guo et al., 2020).

The project aims to support the development of an ASSR system with an optimized batch-wise return to the main wastewater treatment plant. The ASSR will be operated in a way that either volatile fatty acids (VFAs) or biogas is accumulated. Recycling VFAs could enhance biological phosphorus and nitrogen removal in the mainstream reactor, while biogas production could improve the energy balance of the WWTP.

The expected benefits include reduced surplus sludge production, increased biogas or VFA recovery, and a move toward energy-positive WWTPs, even at smaller scales. Additionally, the waste sludge from these plants would be more stabilized, helping to lower methane emissions during handling and transport.

3. Materials and Methods

3.1. Experimental Setup

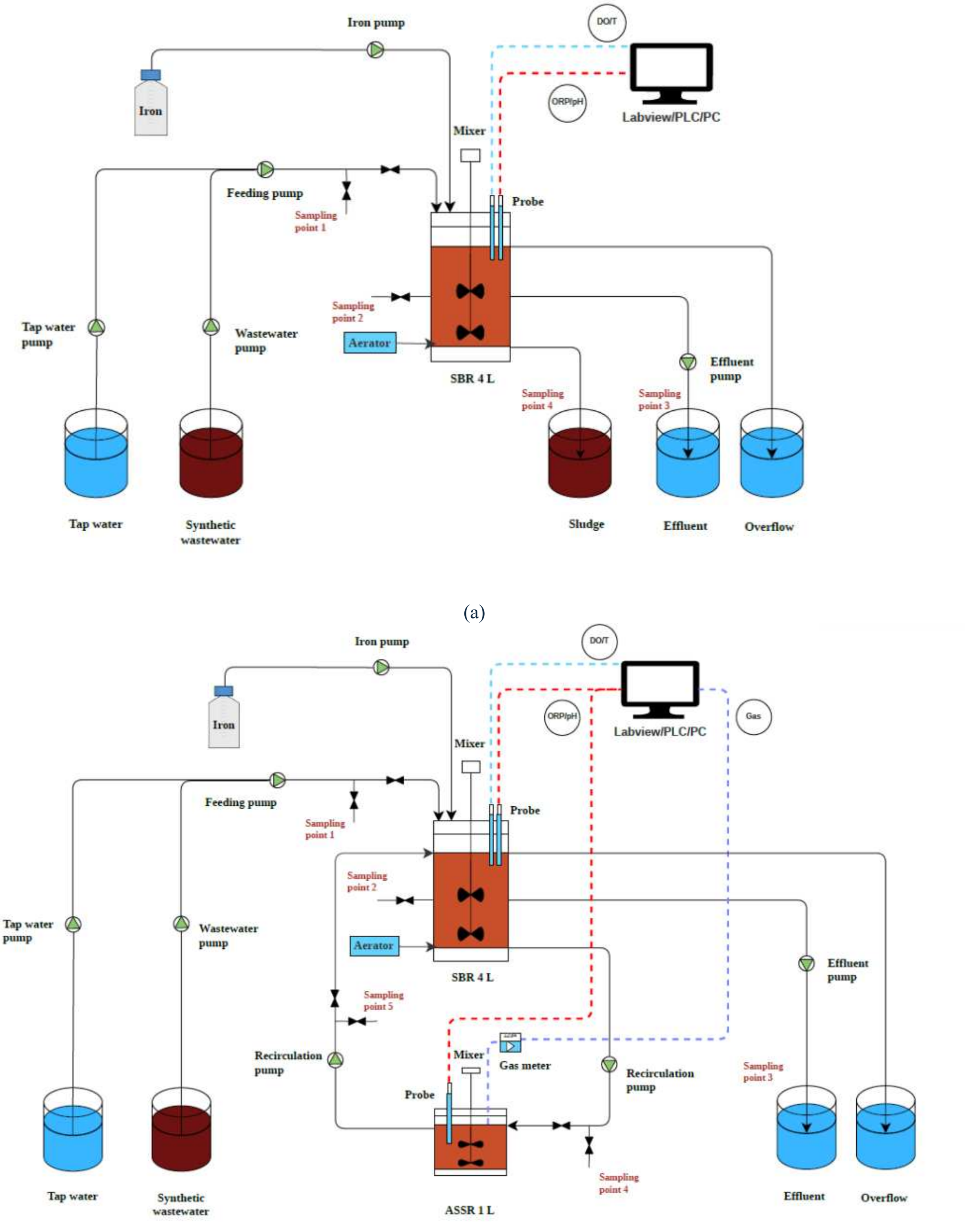
In this research, all experiments were carried out using synthetic wastewater, with pollutant concentrations based on the influent characteristics of the Harnaschpolder wastewater treatment plant (WWTP). The synthetic wastewater recipe was adapted from Gonzalez et al., (2021), as shown in *Table 1*. The feed solution was prepared with tap water, by thoroughly mixing carbon, nitrogen, and phosphate sources along with macro- and micronutrients.

Table 1. Synthetic wastewater prepared with tap water and its composition for 300 mg/L of COD [adapted from Gonzalez et al., (2021)]

Components	**Unit**	**Concentration (500 mg/L of COD)**
Carbon source		
• Cellulose	mg/L	42.33
• Egg white powder	mg/L	105.67
• Milk powder	mg/L	21.17
• Sodium acetate	mg/L	148
• Sodium propionate	mg/L	21.17
• Sodium bicarbonate	mg/L	317
• Lignin alkali	mg/L	10.5
• Humic acid sodium salt	mg/L	41.33
• Mix of fulvic and humic solution	mL/L	0.27
• Kitchen oil	mL/L	0.03
Nitrogen		
• NH_4Cl	mg/L	105.67
• Urea	mg/L	95.17
Phosphate		
• KH_2PO_4	mg/L	42.33
Macronutrient		
• $CaCl_2 \cdot 2H_2O$	mg/L	317
• $MgSO_4 \cdot 7H_2O$	mg/L	169
• $FeCl_3 \cdot 6H_2O$	mg/L	10.5
Trace element solution	mL/L	35.17
Trace element solution composition		
• $CoCl_2 \cdot 6H_2O$	mg/L	0.02
• $MnCl_2 \cdot 4H_2O$	mg/L	17.6
• $ZnCl_2$	mg/L	0.18
• H_3BO_3	mg/L	0.01
• $(NH_4)6Mo7O_{24} \cdot 4H_2O$	mg/L	0.04
• $NiCl_2 \cdot 6H_2O$	mg/L	0.18
• $Na_2SeO_3 \cdot H_2O$	mg/L	0.35
• $Na_2WO_4 \cdot 2H_2O$	mg/L	0.04
• $CuCl_2 \cdot 2H_2O$	mg/L	0.78

Two laboratory-scale systems were used in this research: (i) a sequencing batch reactor (SBR) (as the reference reactor) and (ii) an SBR with an ASSR (as the experimental reactor) (*Figure 1*). An SBR at laboratory scale was used because it did not require a large area, and all treatment processes were carried out in a single tank. In addition, the SBR could be adjusted to increase nutrient removal efficiency (phosphate and nitrogen) simultaneously by modifying the reaction process (Dutta & Sarkar, 2015). Before operating the system, a seeding and acclimatization process was conducted to stabilise the process. For the seeding phase, aerobic sludge from Harnaschpolder WWTP, Netherlands, was used as inoculum of the SBR with a concentration of approximately 5 g/L. Meanwhile, the ASSR was inoculated with waste activated sludge treated by an anaerobic thermophilic semi-continuous lab reactor with an SRT of 5 hours (~10 g/L). Then, the acclimatization process was carried out by measuring the COD in the effluent until a semi-steady state was reached, which was indicated by the removal efficiency not being significantly different between the two time checks.

The SBR was designed with a working volume of 4 L, of which half was filled with inoculum before feeding the wastewater. The total cycle time was 6 hours. Thus, the SBR was operated with a hydraulic retention time (HRT) of 12 hours. The operation cycle of each SBR consisted of 15 minutes of filling phase; 4.5 hours of reacting phase of which 60 minutes were anaerobic, 60 minutes aerobic, 45 minutes anoxic, 60 minutes aerobic, and 45 minutes anoxic; 60 minutes of settling phase; 10 minutes of decanting phase; and 5 minutes of idling phase. The SRT of the SBR was maintained at 10 days by regular sludge wastage. After that, 50 mL of settled sludge (~10 g/L) from the SBR was fed to the ASSR before being returned to the SBR. The ASSR had a working volume of 1 L. From the ASSR, 50 mL of mixed content was recirculated to the SBR to obtain a 5-day SRT. A shorter SRT in the anaerobic digester was typically promoted to achieve higher VFA production because it allowed less time for complete digestion of organic matter (I. S. Lee et al., 2011). The VFA was recirculated to the SBR to enhance phosphate removal (Coats et al., 2021). These reactors (reference and main reactor) were operated continuously until a semi-steady state was achieved before changing to the next stage. The agitator was installed in the SBR and ASSR, and the diffuser was also installed in the SBR to maintain the DO around 2 mg/L. Besides that, all reactors were assembled with pumps (Watson-Marlow 120 U/DV-220Du-323, USA), pH/ORP, and DO probes (Endress & Hauser, The Netherlands). The reactor system was maintained at room temperature (20–22°C). The summary of operational conditions of the SBR and ASSR can be seen in *Table 2*.



(a)

(b)

Figure 1. (a) Reference reactor and (b) SBR + ASSR

Table 2. Operational condition of SBR and ASSR

Reactor	**Condition**	**Value**
SBR	Volume	4 L
	HRT	12 h
	Ratio of the initial volume to the fill volume (Vo/Vf)	1
	Cycle	6 time/days (4 h/cycle)
	Phases:	
	- Fill	10 min
	- Anaerobic	50 min
	- Aerobic	60 min
	- Anoxic	45 min
	- Settle	60 min
	- Decant	10 min
	- Idle	5 min
	Settled sludge waste to ASSR/cycle	33.33 mL/cycle
	Decant effluent volume	33% (v/v)
	MLSS	~5 g/L
	SRT	10 days
	DO	~2 mg/L
	Temperature	20 – 22°C
	Dimension	Diameter: 12 cm
		Height: 36 cm
ASSR	Volume	2 L
	Mixed sludge returns to SBR/cycle	33.33 mL/cycle
	MLSS	~10 g/L
	SRT	5 days
	Temperature	20 – 22°C
	Dimension	Diameter: 10 cm
		Height: 26 cm

3.2. Sampling and Analytical Techniques

Several parameters were measured in the influent and effluent of both the SBR and ASSR systems (Table 3). Concentrations of COD, ammonium nitrogen (NH_4-N), nitrate nitrogen

(NO_3-N), orthophosphate (PO_4^{3-}-P), Fe^{3+}/Fe^{2+}, and sulfate (SO_4^{2-}) were determined using spectrophotometry-based test kits (Hach Lange LCK, Germany). Total suspended solids (TSS), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were monitored according to standard methods (APHA, 2012). Dissolved oxygen (DO), temperature, pH, and oxidation-reduction potential (ORP) were measured using a portable meter (Endress & Hauser, The Netherlands). Biogas production was monitored with biogas flow meters (Ritter Milligas Counter MGC-1-PMMA, Germany). The methane and carbon dioxide content of the biogas was analyzed using a gas chromatograph (GC) (Varian CP 4900, USA), while hydrogen concentration was measured using a micro gas chromatograph. Extracellular polymeric substances (EPS) and soluble microbial products (SMP) were extracted following the two-step method described by Le-Clech et al., (2006) and characterized based on carbohydrate and protein content. Volatile fatty acids (VFAs) were measured using a GC equipped with a flame ionization detector (FID) (Agilent 7890A, USA). The microbial community was analyzed based on 16S rRNA gene sequencing (Zhan et al., 2021). Oxygen uptake rate (OUR) measurements were performed using respirometry assays (Corsino et al., 2023).

3.3. Observed yield

Observed yield is widely recognized as a reliable indicator of sludge reduction (Saby et al., 2003; J. F. Wang et al., 2007). In this study, the observed sludge yield (Y_{obs}) and sludge reduction efficiency were calculated as the ratio of cumulative sludge generated to cumulative substrate consumed, according to Equation 1 (Chon, Rome, Kim, Park, et al., 2011a).

$$Y_{obs} = \frac{\Delta X_{SBR} V_{SBR} + \Delta X_{ASSR} V_{ASSR} + \Sigma(X_{SBR} Q_{SBR.waste} + X_{ASSR} Q_{ASSR.waste} + X_{eff} Q_{eff}).\Delta t}{\Sigma(S_{in} Q_{in} + X_{eff} Q_{eff})} \qquad \text{Equation 1}$$

where, ΔX_{SBR}, ΔX_{ASSR}, and Δt are the change of sludge (g TSS/L) in SBR and ASSR, and time (day), respectively. V_{SBR} and V_{ASSR} are the volumes of SBR and ASSR (L), respectively. Q_{SBR}, Q_{ASSR}, Q_{in}, and Q_{eff} are the flow rates of SBR, ASSR, influent, and effluent (L/d), respectively. S_{in} and S_{eff} are the substrate concentration of influent and effluent (g COD/L), respectively.

Table 3. Measured parameters during the experiment at every sampling point.

Sampling point	Parameters	Frequency of measuring
Influent (1)	COD NH_4-N NO_3-N PO_4^{3-}-P TSS	Twice a week
SBR (2)	MLSS MLVSS	Twice a week
	DO/Temperature pH/ORP	Everyday

Sampling point	Parameters	Frequency of measuring
	EPS	Once per stage*
	SMP	
	Microbial community	Once per stage*
	OUR	
Effluent (3)	COD	Twice a week
	NH_4-N	
	NO_3-N	
	PO_4^{3-}-P	
	TSS	
	Fe^{3+}/Fe^{2+}	
Influent of ASSR (4)	COD	Once a week
	NH_4-N	
	NO_3-N	
	PO_4^{3}-P	
	SO_4^{2-}	
	Fe^{3+}/Fe^{2+}	
ASSR (5)	MLSS	Twice a week
	MLVSS	
	pH/ORP/Temperature	Everyday
	Biogas	
	CH_4 and CO_2	Once per stage*
	EPS	
	SMP	
	Microbial community	Once per stage*
	OUR	
Effluent of ASSR (5)	COD	Once a week
	NH_4-N	
	NO_3-N	
	PO_4^{3-}P	
	SO_4^{2-}	
	Fe^{3+}/Fe^{2+}	
	VFA	

* These parameters were measured in triplicate.

According to Ferrentino et al., (2016a), different studies have employed various approaches to determine the observed yield (Y_{obs}). For example, Chudoba et al., (1992), J. Wang et al., (2008), Torregrossa et al., (2012) calculated Y_{obs} using mass balance methods based on daily solids production and substrate consumption. In contrast, researchers such as Chon, Rome, Kim, & Park, (2011a, 2011b), Zhou et al., (2015), and Coma et al., (2013) utilized graphical evaluations based on cumulative data. This latter method is considered more representative of actual conditions, as it incorporates variations in both solids and substrate concentrations throughout the entire experimental period.

4. Results and Discussion

4.1. Parameters

This study focuses on evaluating parameters relevant to both sludge reduction and organic and nutrient removal. For sludge reduction, the primary metric assessed is the observed sludge yield (Y_obs), calculated for both control and experimental systems. To assess treatment performance, concentrations of Chemical Oxygen Demand (COD), nitrogen species (e.g., NH_4^+-N, NO_3^--N), and phosphorus (PO_4^{3-}-P) were monitored across the control SBR and the integrated SBR-ASSR reactors.

Tabel 1 presents the average influent characteristics of the raw wastewater supplied to both systems, serving as the baseline for evaluating removal efficiencies and operational outcomes.

Tabel 1. Average influent characteristics of wastewater supplied to SBR and SBR-ASSR

Table 4. Average influent characteristics of wastewater supplied to SBR and SBR-ASSR

Parameter	Value for SBR-ASSR	Value for SBR
Total COD [mgTCOD L^{-1}]	458.1 ± 108.9	456.3 ± 80.6
NH_4–N [mgNH_4-N L^{-1}]	42.9 ± 16.5	44 ± 12.9
NO_2–N [mgNO_2-N L^{-1}]	0.0078 ± .0098	0.009 ± 0.006
NO_3–N [mgNO_3-N L^{-1}]	1.5578 ± 0.75	1.6721 ± 0.684
PO_4^- [mgPO_4^- L^{-1}]	2.305 ± 1.063	2.435 ± 0.893
TSS [mgTSS L^{-1}]	280	280
VSS [mgVSS L^{-1}]	200	180

Both the reference SBR and the experimental SBR-ASSR systems were operated under the same hydraulic retention time (HRT) and followed the same cycle structure, ensuring comparability of operational conditions. Excess sludge was purged daily from main SBR system at a volume of 200 mL, corresponding to 33 mL per 4-hour cycle, to sustain a stable total suspended solids (TSS) concentration in the mixed liquor.

4.1.1. Sludge Reduction

*Figure 2*a and b show the trends of cumulative sludge production over time in each experimental setup. The excess sludge production in the SBR-ASSR system was lower compared to SBR control, indicating that sludge minimization was achieved successfully. Specifically, the sludge production was lowered by approximately 44% without considering substrate consumed. The cumulative biomass production was plotted against the cumulative organic matter removed in order to evaluate the observed sludge yield (Y_{obs}) of the biological compartment referred to the SBR control and SBR-ASSR systems. The slope of the linear regression curves for each experimental period was determined. In the reference SBR, the Y_{obs} was equal to 0.45 g TSS g^{-1} COD. The Y_{obs} of a small WWTP ranges between 0.30 - 0.45 g TSS g^{-1} COD, without considering the contribution of the sludge post-treatment (i.e. anaerobic or aerobic digestion) (Ferrentino et al., 2021). Thus, the Y_{obs} referred to the reference SBR was in the range of reported values of typical domestic wastewater. The same evaluation of the Y_{obs} was performed for SBR-ASSR system. The Y_{obs} for this system was 0.27 g TSS g^{-1} COD, indicating a 40% reduction in the sludge generated based on consumed substrate.

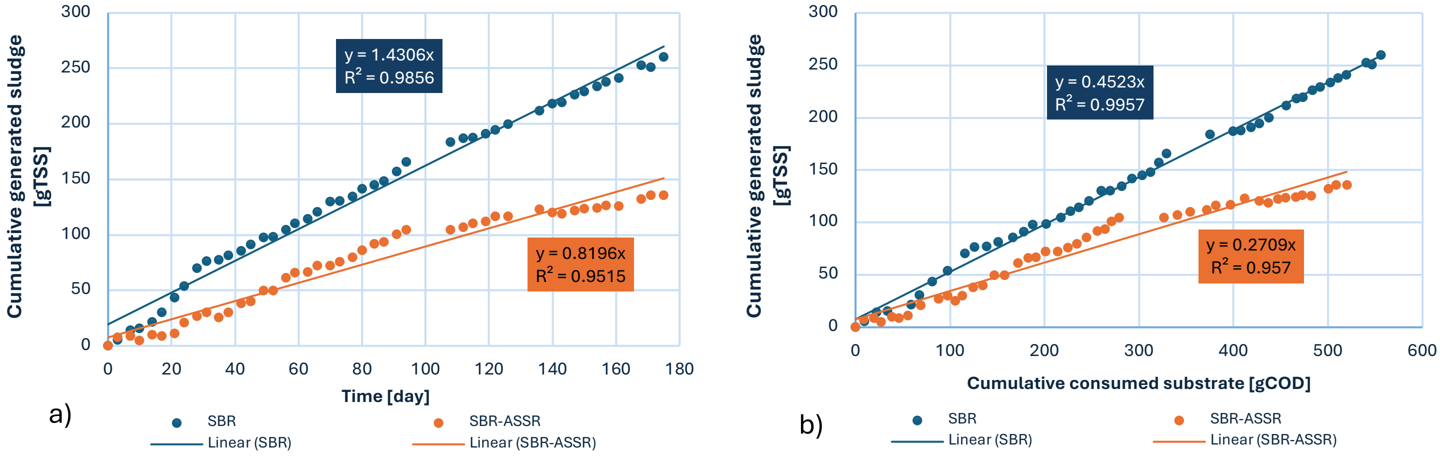


Figure 2. Cumulative sludge generation vs time a) cumulative sludge generation vs cumulative consumed substrate b

*Figure 3*a depicts the average daily sludge production rate (g TSS d^{-1}) against time (days). In both the control SBR and the SBR-ASSR systems, the sludge production rate undergoes a pronounced “startup” period before settling into a more consistent, steady-state value. During the first several weeks, fluctuations in the daily g TSS d^{-1} reflect the dynamic adjustments of the microbial community, sludge age, feast-famine cycling in the SBR, and the new anaerobic conditioning in the ASSR all drive transient spikes and dips in biomass yield as populations shift and acclimate.

As each system moves past this acclimation phase, the rates plateau, around day 50-60, indicating that a balance has been reached between substrate uptake, endogenous decay, and sludge wasting. In the reference SBR the steady-state rate stabilizes at a higher level of approximately 1.64 g TSS g^{-1} COD, reflecting the continual net growth of activated sludge. In contrast, the SBR–ASSR system settles at a lower, more consistent sludge production rate of around 0.94 g TSS g^{-1} COD, as the anaerobic side-stream, with its limited substrate, encourages cryptic growth and endogenous decay. When this treated sludge returns to the main reactor, there is less net biomass buildup.

*Figure 3*b, illustrating cumulative sludge production versus cumulative substrate consumption, provides insight into the biomass yield of both the control SBR and the SBR-ASSR systems when both systems reached a more stable operational state after day 60. Analyzing data reveals that the observed sludge yield (Y_{obs}) for the SBR-ASSR system decreased to 0.19 g TSS g^{-1} COD, compared to 0.44 g TSS g^{-1} COD for the control SBR. This indicates a significant reduction in sludge production for the SBR-ASSR system during the steady-state period.

The marked decrease in observed sludge yield (Y_{obs}) during steady-state operation of the SBR-ASSR system can be explained by several interlinked biological phenomena. Under the strictly anaerobic conditions of the ASSR, endogenous decay becomes predominant, as microbial cells consume their own biomass to meet maintenance energy requirements. This cryptic growth process leads to cell lysis and the release of intracellular organic compounds, thereby reducing net sludge production in the main reactor. Moreover, the ASSR environment selectively favours

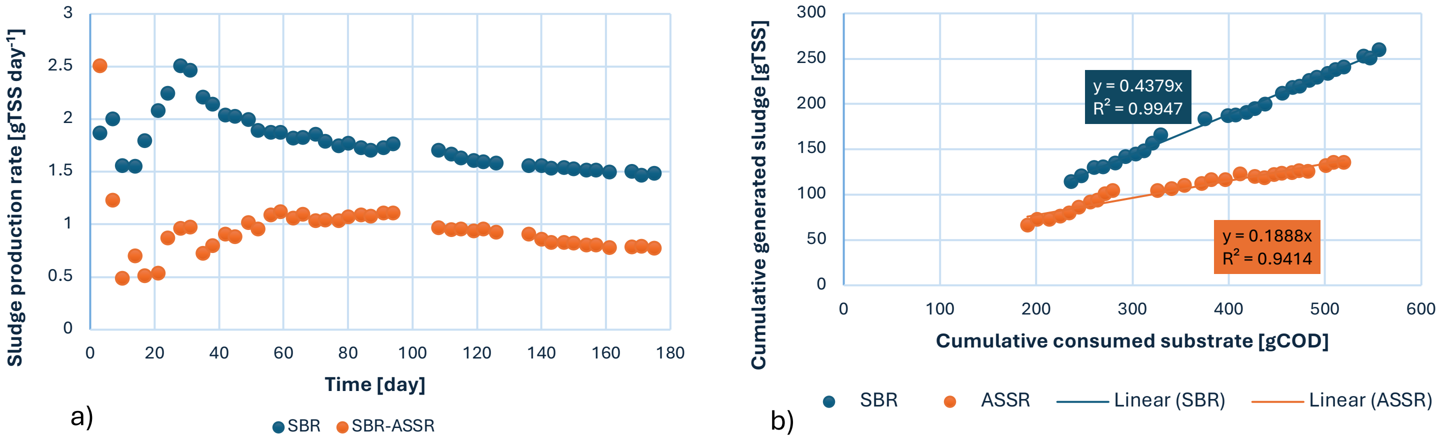
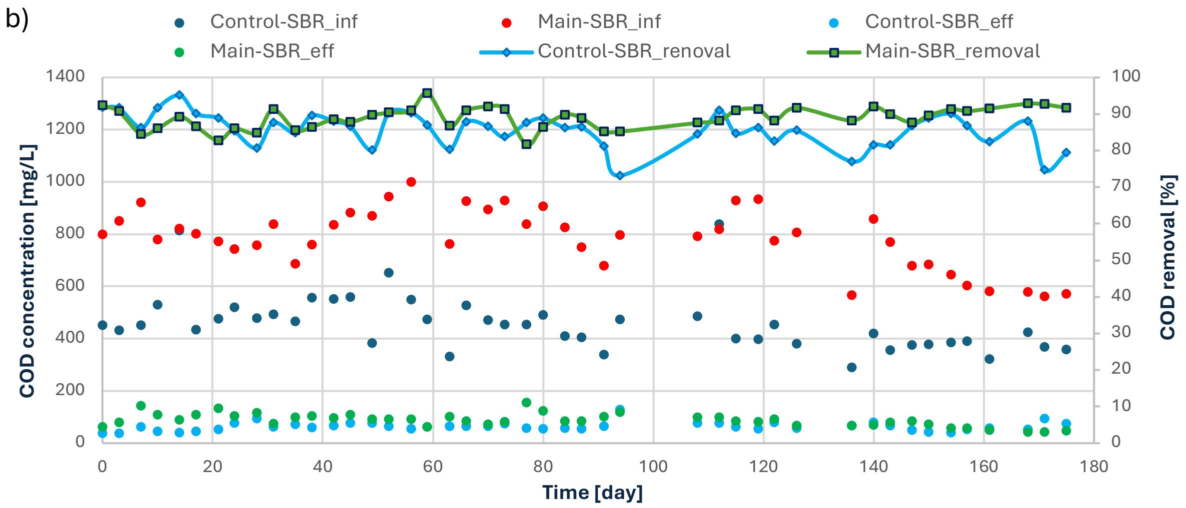


Figure 3. Sludge production rate versus time a) Cumulative Sludge generation vs cumulative consumed substrate (steady-state) b)

The marked decrease in observed sludge yield (Y_{obs}) during steady-state operation of the SBR-ASSR system can be explained by several interlinked biological phenomena. Under the strictly anaerobic conditions of the ASSR, endogenous decay becomes predominant, as microbial cells consume their own biomass to meet maintenance energy requirements. This cryptic growth process leads to cell lysis and the release of intracellular organic compounds, thereby reducing net sludge production in the main reactor. Moreover, the ASSR environment selectively favours slow-growing heterotrophic populations, such as denitrifying phosphate-accumulating organisms and sulfate-reducing bacteria, which utilize available organic carbon for metabolic activity without contributing significantly to new biomass synthesis. Additionally, the imposed sludge interchange ratio increases these effects by increasing the fraction of sludge exposed to anaerobic, substrate-limited conditions, further promoting maintenance metabolism over growth and driving down the steady-state sludge yield (Ferrentino et al., 2018).

4.1.1.1. COD and Nutrients Removal



The control SBR and SBR-ASSR system performances were monitored for 175 days shows the characteristics of influent and effluent in both SBR and SBR-ASSR systems. As shown in *Figure 4*, the two systems were almost effective in COD removal, with a consistent average total COD removal efficiency of 85.7 ± 4.6 and 80.5 ± 6% which is similar to previous research (Ferrentino et al., 2018). In the integrated SBR-ASSR system, the overall COD removal efficiency is slightly lower than in the control SBR, primarily due to continuous sludge recirculation and the absence of excess sludge discharge. In this research, the ASSR is designed not for COD removal but for sludge reduction via hydrolysis, fermentation, and cryptic growth under anaerobic stress. These processes convert particulate organic matter into soluble COD (sCOD), which is only partially metabolized within the ASSR due to the dominance of slow-growing microorganisms adapted to anaerobic conditions, rather than fast-acting aerobic degraders (*Table 5*). As a result, sCOD accumulates and is recirculated to the main SBR, increasing the organic load. Interestingly, the main SBR unit in the SBR-ASSR setup exhibits higher average total COD removal efficiency, 88.9% compared to the reference SBR, 85.7%, likely due to the enhanced biodegradability of the sCOD originating from the ASSR (*Figure 4*b). Soluble COD is more easily degraded during the aerobic phase, where active microbial oxidation occurs, potentially aided by fermentative and slow-growing bacteria returned from the ASSR. According to Ning et al., (2024), anaerobic conditions in the ASSR favour the growth of microorganisms such as Dechloromonas and Candidatus_Competibacter, which contribute not only to sludge reduction through cryptic growth but also to improved downstream biodegradation. Therefore, while the SBR-ASSR system experiences a slight overall reduction in COD removal efficiency due to internal recycling, it simultaneously enhances sludge reduction and achieves higher local treatment performance in the main SBR through microbial and substrate dynamics (Do & Nguyen, 2022; Ferrentino et al., 2018; Ning et al., 2024).

Table 5. Influent and effluent sCOD concentrations for ASSR

Day	**sCOD influent [mg L^{-1}]**	**sCOD effluent [mg L^{-1}]**
0	53	174
38	44.8	210
108	44	224

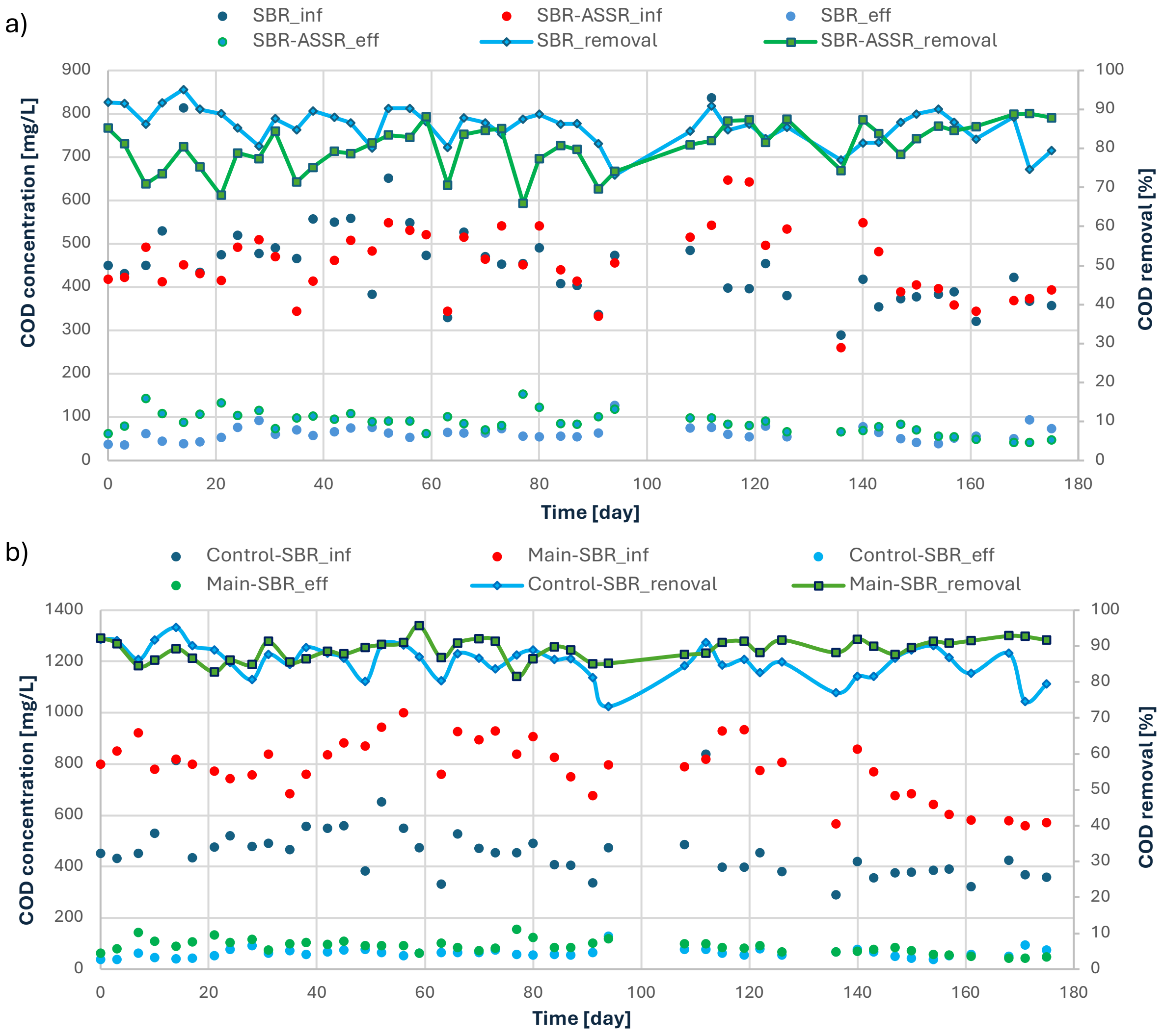


Figure 4. Influent and effluent COD concentrations, and corresponding removal percentages, for the control SBR and SBR-ASSR systems a) Influent and effluent COD concentrations, and corresponding removal percentages, for the control SBR and main SBR systems b)

*Figure 5*a and b present the concentrations of influent and effluent ammonium (NH_4^+-N), along with effluent nitrite (NO_2^--N) and nitrate (NO_3^--N) in the control SBR and SBR–ASSR systems, respectively. Both configurations exhibited comparable performance in ammonium removal, achieving average efficiencies of 81.6% in the SBR and 79.2% in the SBR–ASSR. These results indicate that the nitrification process was well established and stable across both systems during the experimental period. However, beginning around day 136, a gradual increase in NO_3^--N concentrations was observed in the effluents of both reactors. For the SBR, the average nitrate concentration rose from approximately 1 mg/L to 3 mg/L, while in the SBR–ASSR, it increased from around 2 mg/L to 4 mg/L. This upward trend suggests a deterioration in the denitrification process during the later stages of operation.

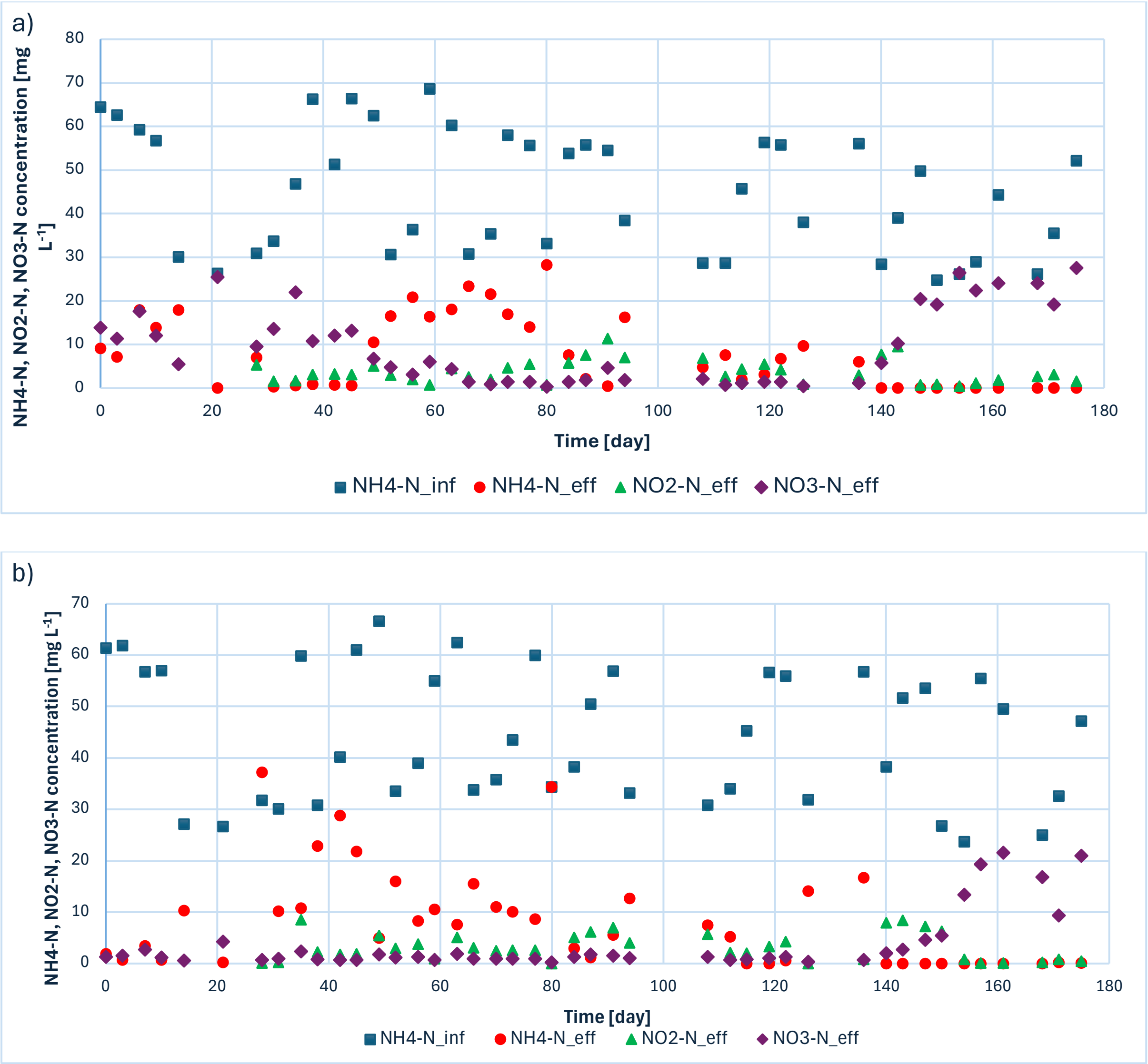


Figure 5. Concentrations of influent and effluent ammonium (NH_4^+-N), along with effluent nitrite (NO_2^--N) and nitrate (NO_3^--N) in the control SBR a) concentrations of influent and effluent ammonium (NH_4^+-N), along with effluent nitrite (NO_2^--N) and nitrate (NO_3^-).

The rise in effluent nitrate was attributed to insufficient carbon availability for complete denitrification. As the availability of biodegradable organic matter, particularly readily biodegradable COD, decreased, denitrifying microorganisms lacked the electron donors necessary to reduce nitrate to nitrogen gas. This hypothesis was supported by targeted experiments in which additional carbon in the form of sodium acetate was introduced at the start of the anoxic phase. Cycle measurements conducted under these amended conditions demonstrated an immediate improvement in denitrification performance, confirming that the observed nitrate accumulation was linked to COD limitation.

As shown in *Figure 6*b, during the initial weeks of operation, from the start of the experiment until approximately day 31, the phosphorus removal performance of the SBR–ASSR system was unstable. The removal efficiency fluctuated significantly, reflecting a system still undergoing microbial adaptation and process stabilization. This is a typical pattern in biological nutrient removal systems, where the establishment of key microbial populations such as polyphosphate-accumulating organisms (PAOs) requires time to reach functional dominance. From day 31 to day 73, the system demonstrated improved stability. During this period, the concentration of phosphate (PO_4^{3-}) in the ASSR effluent rose steadily from approximately 133 mg/L to 241 mg/L (*Figure 6*c). Despite this increase, the phosphorus removal efficiency remained high, averaging around 87.7%. This suggests that during this phase, PAOs were actively cycling phosphorus, releasing it under anaerobic conditions in the ASSR and re-uptaking it aerobically in the SBR. Between days 73 and 87, the phosphate concentration in the ASSR effluent remained relatively stable, averagely 246.7 mg/L. However, after day 70, the overall removal performance began to decline. While a few days still showed acceptable phosphorus removal, most exhibited negative removal efficiencies, indicating net phosphate release to the effluent. This deterioration in performance may be attributed to phosphorus saturation within the biomass. In the absence of sludge wasting, phosphate that is taken up by polyphosphate-accumulating organisms (PAOs) during the aerobic phase is not physically removed from the system but instead remains stored intracellularly. Over time, as intracellular polyphosphate reaches its storage limit, PAOs lose their capacity to take up additional phosphate. This leads to phosphate being released without subsequent uptake, particularly under the anaerobic conditions maintained in the ASSR (Do & Nguyen, 2022; Xia et al., 2013).

Another parameter that appears to have influenced phosphorus removal performance is the concentration of volatile fatty acids (VFAs) in the effluent of the ASSR (*Figure 6*d). VFAs serve as the primary carbon source for PAO metabolism, fueling phosphate release and PHA (polyhydroxyalkanoate) storage during the anaerobic phase. Until day 84, VFA concentrations in the ASSR effluent averaged approximately 34.4 mg/L, with observed values ranging from 0 to a maximum of 97 mg/L. During this same period, especially between day 31 and day 80, the phosphorus removal efficiency remained high. However, after day 84, VFA concentrations dropped to zero, coinciding with the onset of unstable and negative phosphorus removal efficiency. The absence of VFAs inhibits the key metabolic pathways of PAOs. Without sufficient carbon input, PAOs cannot generate the energy required to release phosphate anaerobically or to store carbon as PHA, both of which are prerequisites for successful phosphate uptake during the subsequent aerobic phase. This metabolic disruption contributes directly to the observed decline in phosphorus removal (Oehmen et al., 2007).

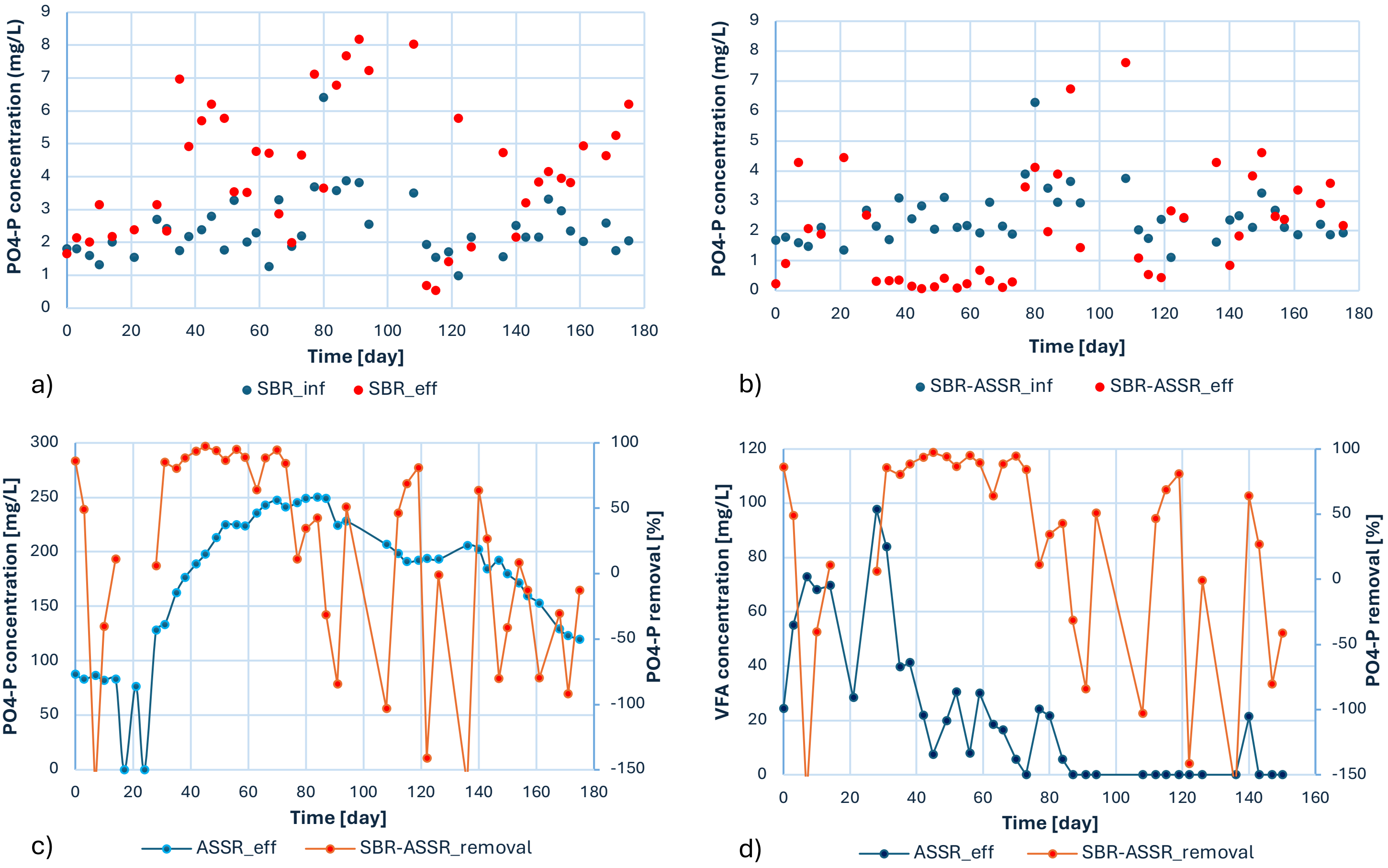


Figure 6. Influent and effluent PO4-P concentration of reference SBR vs time a) Influent and effluent PO4-P concentration of SBR-ASSR vs time b) effluent PO4-P concentration of ASSR and PO4-P removal efficiency in SBR-ASSR system vs time c) effluent VFA concentration of ASSR and PO4-P removal efficiency in SBR-ASSR vs time d)

In contrast to the SBR-ASSR configuration, the control SBR system exhibited consistently poor phosphorus removal performance throughout the experimental period (*Figure 6*a). The removal efficiency was predominantly negative, indicating that phosphate concentrations in the effluent often exceeded those in the influent. This net phosphate release suggests that the enhanced biological phosphorus removal (EBPR) process was not successfully established under the conditions applied in the control reactor.

Several factors may have contributed to the failure of phosphorus removal in the control SBR. One of the primary issues appears to be unfavourable microbial competition. Enhanced biological phosphorus removal relies on the enrichment of polyphosphate-accumulating organisms (PAOs), which cycle phosphorus by releasing it under anaerobic conditions and subsequently re-absorbing it in the aerobic phase. However, glycogen-accumulating organisms (GAOs) compete for the same carbon sources, particularly volatile fatty acids (VFAs), but do not contribute to phosphorus removal. Under certain operational conditions, GAOs may outcompete PAOs, especially in systems with low VFA availability, suboptimal pH, or limited anaerobic contact time. This microbial imbalance can suppress PAO activity and lead to inefficient or even negative phosphorus removal, as has been widely reported in EBPR studies (Mino et al., 1998; Oehmen et al., 2007). In addition to microbial competition, cycle imbalances in the SBR operation may have impaired the EBPR process. The standard cycle used in the control SBR involved 50 minutes of anaerobic, 60 minutes of aerobic, and 45 minutes of anoxic phases. While this structure provides all three redox environments, the relatively short anaerobic phase may not have allowed sufficient time for PAOs to uptake VFAs and release phosphate effectively. If the PAOs cannot accumulate polyhydroxyalkanoates (PHAs) during the anaerobic phase, they will be unable to uptake phosphate during the aerobic phase, resulting in net phosphorus release. Furthermore, the inclusion of an anoxic phase after the aerobic stage, without additional carbon input, may have created unfavourable conditions for both denitrification and phosphate uptake, compounding the removal inefficiency (Gnida et al., 2020).

5. Conclusions and Recommendations

This research investigated the performance of an integrated SBR-ASSR system compared to a conventional SBR in terms of sludge reduction and nutrient removal. The integrated SBR-ASSR system demonstrated significant sludge minimization compared to the reference SBR, as reflected by lower observed sludge yields. The reduction of biomass production was associated with enhanced endogenous decay and cryptic growth within the anaerobic side-stream reactor, where sludge was exposed to substrate-limited conditions that favored cell lysis and maintenance metabolism.

While COD removal efficiencies remained high in both configurations, a slightly lower overall COD removal was observed in the integrated system due to the continuous recirculation of partially hydrolyzed organic matter from the ASSR. Nevertheless, the increased soluble COD from the ASSR was effectively treated in the aerobic phase of the main SBR, supporting stable treatment performance.

Nitrogen removal efficiencies were generally comparable across both systems, with consistent ammonium removal over the experimental period. However, a gradual increase in nitrate concentrations toward the end of the operation period indicated incomplete denitrification, likely caused by insufficient carbon availability as biodegradable COD decreased over time.

Phosphorus removal in the SBR-ASSR system was initially promising due to improved VFA availability from the anaerobic side-stream. Over time, however, performance declined, likely due to phosphorus saturation within the biomass and a drop in available VFAs. In contrast, the control SBR consistently struggled with phosphorus removal, likely because of competition between PAOs and GAOs and insufficient anaerobic contact time for effective EBPR activity.

Overall, integrating the ASSR provided clear benefits for sludge reduction while maintaining comparable organic and nitrogen removal performance to a conventional SBR. However, phosphorus removal performance showed limitations over longer-term operation, highlighting the need for further optimization.

Recommendations for future work include:

1. Optimize Anaerobic Conditions in ASSR

Future work should optimize the sludge interchange ratio and anaerobic retention time to further enhance cryptic growth while avoiding excessive accumulation of soluble organics that might burden downstream treatment.

2. Control Carbon Availability for Denitrification

Strategies such as external carbon dosing or optimizing VFA production in the ASSR should be explored to ensure sufficient electron donors for complete denitrification, preventing nitrate accumulation during prolonged operation.

3. Address Phosphorus Saturation

To prevent phosphorus saturation within the biomass, periodic controlled sludge withdrawal or alternative phosphorus harvesting methods should be investigated, preserving EBPR functionality over the long term.

4. Investigate Microbial Community Dynamics

Additional research employing microbial community analysis is recommended to better understand shifts in functional populations (e.g., PAOs, GAOs, denitrifiers) under the imposed operational conditions and to inform strategies for their selective enrichment.

5. Mathematical Modelling

Developing and validating mathematical models of the SBR-ASSR process could help predict performance under varying loading rates, sludge ages, and recirculation regimes, supporting robust process control and scaling to full-scale applications.

6. References

Bianchini, A., Bonfiglioli, L., Pellegrini, M., & Saccani, C. (2016). Sewage sludge management in Europe: A critical analysis of data quality. *International Journal of Environment and Waste Management*, *18*(3), 226–238. https://doi.org/10.1504/IJEWM.2016.080795

Campo, G., Cerutti, A., Lastella, C., Leo, A., Panepinto, D., Zanetti, M., & Ruffino, B. (2021). Production and Destination of Sewage Sludge in the Piemonte Region (Italy): The Results of a Survey for a Future Sustainable Management. *International Journal of Environmental Research and Public Health 2021, Vol. 18, Page 3556*, *18*(7), 3556. https://doi.org/10.3390/IJERPH18073556

Cheng, Y., Lu, C., Gao, S., Koju, R., Li, H., Zhu, Z., Hu, C., & Qu, J. (2024). Synchronous in-situ sludge reduction and enhanced denitrification through improving electron transfer during endogenous metabolisms with Fe(II) addition. *Water Research*, *255*. https://doi.org/10.1016/J.WATRES.2024.121472

Chon, D. H., Rome, M., Kim, H. S., & Park, C. (2011). Investigating the mechanism of sludge reduction in activated sludge with an anaerobic side-stream reactor. *Water Science and Technology*, *63*(1), 93–99. https://doi.org/10.2166/WST.2011.015

Chon, D. H., Rome, M., Kim, Y. M., Park, K. Y., & Park, C. (2011a). Investigation of the sludge reduction mechanism in the anaerobic side-stream reactor process using several control biological wastewater treatment processes. *Water Research*, *45*(18), 6021–6029. https://doi.org/10.1016/J.WATRES.2011.08.051

Chon, D. H., Rome, M., Kim, Y. M., Park, K. Y., & Park, C. (2011b). Investigation of the sludge reduction mechanism in the anaerobic side-stream reactor process using several control biological wastewater treatment processes. *Water Research*, *45*(18), 6021–6029. https://doi.org/10.1016/J.WATRES.2011.08.051

Chudoba, P., Morel, A., & Capdeville, B. (1992). The case of both energetic uncoupling and metabolic selection of microorganisms in the OSA activated sludge system. *Environmental Technology*, *13*(8), 761–770. https://doi.org/10.1080/09593339209385207

Clarke, B. O., & Smith, S. R. (2011). Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environment International*, *37*(1), 226–247. https://doi.org/10.1016/J.ENVINT.2010.06.004

Coats, E. R., Deyo, B., Brower, N., & Brinkman, C. K. (2021). Effects of anaerobic HRT and VFA loading on the kinetics and stoichiometry of enhanced biological phosphorus removal. *Water Environment Research*, *93*(9), 1608–1618. https://doi.org/10.1002/WER.1544

Collivignarelli, M. C., Abbà, A., Miino, M. C., & Torretta, V. (2019). What Advanced Treatments Can Be Used to Minimize the Production of Sewage Sludge in WWTPs?

Applied Sciences 2019, Vol. 9, Page 2650, *9*(13), 2650. https://doi.org/10.3390/APP9132650

Coma Bech, M., Rovira, S., Canals, J., & Colprim Galceran, J. (2013). *Minimization of sludge production by a side-stream reactor under anoxic conditions in a pilot plant.*

Corsino, S. F., Carabillò, M., Cosenza, A., De Marines, F., Di Trapani, D., Traina, F., Torregrossa, M., & Viviani, G. (2023). Insights on mechanisms of excess sludge minimization in an oxic-settling-anaerobic process under different operating conditions and plant configurations. *Chemosphere*, *312*, 137090. https://doi.org/10.1016/J.CHEMOSPHERE.2022.137090

Dentel, S. K., & Qi, Y. (2014). Management of Sludges, Biosolids, and Residuals. *Comprehensive Water Quality and Purification*, 223–243. https://doi.org/10.1016/B978-0-12-382182-9.00049-9)

Di Giacomo, G., & Romano, P. (2022). Evolution and Prospects in Managing Sewage Sludge Resulting from Municipal Wastewater Purification. *Energies*, *15*(15), 5633–5633. https://doi.org/10.3390/EN15155633

Do, K. U., & Nguyen, V. L. (2022). Wastewater treatment by Sequencing Batch Reactor (SBR) without releasing excess sludge. *Current Developments in Biotechnology and Bioengineering: Advances in Biological Wastewater Treatment Systems*, 253–269. https://doi.org/10.1016/B978-0-323-99874-1.00001-4

Dutta, A., & Sarkar, S. (2015). Sequencing Batch Reactor for Wastewater Treatment: Recent Advances. *Current Pollution Reports*, *1*(3), 177–190. https://doi.org/10.1007/S40726-015-0016-Y/TABLES/2

Ferrentino, R., Langone, M., & Andreottola, G. (2021). Sludge reduction by an anaerobic side-stream reactor process: A full-scale application. *Environmental Challenges*, *2*, 100016. https://doi.org/10.1016/J.ENVC.2020.100016

Ferrentino, R., Langone, M., Merzari, F., Tramonte, L., & Andreottola, G. (2016). A review of anaerobic side-stream reactor for excess sludge reduction: Configurations, mechanisms, and efficiency. *Critical Reviews in Environmental Science and Technology*, *46*(4), 382–405. https://doi.org/10.1080/10643389.2015.1096879

Ferrentino, R., Langone, M., Villa, R., & Andreottola, G. (2018). Strict anaerobic side-stream reactor: Effect of the sludge interchange ratio on sludge reduction in a biological nutrient removal process. *Environmental Science and Pollution Research*, *25*(2), 1243–12536. https://doi.org/10.1007/S11356-017-0448-6/FIGURES/7

Fu, F., Dionysiou, D. D., & Liu, H. (2014). The use of zero-valent iron for groundwater remediation and wastewater treatment: A review. *Journal of Hazardous Materials*, *267*, 194–205. https://doi.org/10.1016/J.JHAZMAT.2013.12.062

Gao, Y., Yan, X., & Huang, X. (2024). Fe3+ Augmentation Enhancing the Nitrogen and Phosphorus Control in Denitrification Biofilter Based on Water Supply Sludge:

Performance and Microbial Characteristics. *Journal of Environmental Chemical Engineering*, *12*(5). https://doi.org/10.1016/J.JECE.2024.114002

Gnida, A., Zubrowska-Sudoł, M., Sytek-Szmeichel, K., Podedworna, J., Surmacz-Górska, J., & Marciocha, D. (2020). Effect of anaerobic phases length on denitrifying dephosphatation biocenosis - A case study of IFAS-MBSBBR. *BMC Microbiology*, *20*(1), 1–12. https://doi.org/10.1186/S12866-020-01896-3/FIGURES/5

Goel, R. K., & Noguera, D. R. (2006). Evaluation of Sludge Yield and Phosphorus Removal in a Cannibal Solids Reduction Process. *Journal of Environmental Engineering*, *132*(10), 1331–1337. https://doi.org/10.1061/(ASCE)0733-9372(2006)132:10(1331)/ASSET/3DF1823E-3F64-4C4B-8C6E-32860878D480/ASSETS/IMAGES/LARGE/5.JPG

Gonzalez, A., van Lier, J. B., & de Kreuk, M. K. (2021). The role of growth media on composition, bioconversion and susceptibility for mild thermal pre-treatment of waste activated sludge. *Journal of Environmental Management*, *298*, 113491. https://doi.org/10.1016/J.JENVMAN.2021.113491

Guo, J. S., Fang, F., Yan, P., & Chen, Y. P. (2020). Sludge reduction based on microbial metabolism for sustainable wastewater treatment. *Bioresource Technology*, *297*, 122506. https://doi.org/10.1016/J.BIORTECH.2019.122506

Gusiatin, M. Z., Kulikowska, D., & Bernat, K. (2024). Municipal Sewage Sludge as a Resource in the Circular Economy. *Energies 2024, Vol. 17, Page 2474*, *17*(11), 2474. https://doi.org/10.3390/EN17112474

Hu, D., Jiang, P., Chen, Y., Gao, F., & Liu, S. (2024). Sustainable Sludge Management in China: Quantifying GHG Emissions and Exploring Its Reduction Strategies. *Processes*, *12*(7), 1481–1481. https://doi.org/10.3390/PR12071481

Huang, X. (2024). The Promotion of Anaerobic Digestion Technology Upgrades in Waste Stream Treatment Plants for Circular Economy in the Context of “Dual Carbon”: Global Status, Development Trend, and Future Challenges. *Water 2024, Vol. 16, Page 3718*, *16*(24), 3718. https://doi.org/10.3390/W16243718

Jafari, M., & Botte, G. G. (2021). Electrochemical treatment of sewage sludge and pathogen inactivation. *Journal of Applied Electrochemistry*, *51*(1), 119–130. https://doi.org/10.1007/S10800-020-01481-6/TABLES/2

Junyapoon*, S. (2005). Use of Zero-Valent Iron for Wastewater Treatment. *CURRENT APPLIED SCIENCE AND TECHNOLOGY*, *5*(3), 587–595. https://li01.tci-thaijo.org/index.php/cast/article/view/145560

Krause, M. J., & Bronstein, K. E. (2024). Estimating national sludge generation and disposal from US drinking water and wastewater treatment plants. *Journal of Cleaner Production*, *453*, 142121. https://doi.org/10.1016/J.JCLEPRO.2024.142121

Le-Clech, P., Marselina, Y., Stuetz, R., & Chen, V. (2006). Fouling visualisation of soluble microbial product models in MBRs. *Desalination (Amsterdam)*, *199*(1–3), 477–479. https://doi.org/10.1016/J.DESAL.2006.03.110

Lee, I. S., Parameswaran, P., & Rittmann, B. E. (2011). Effects of solids retention time on methanogenesis in anaerobic digestion of thickened mixed sludge. *Bioresource Technology*, *102*(22), 10266–10272. https://doi.org/10.1016/J.BIORTECH.2011.08.079

Lee, Y., Zimmermann, S. G., Kieu, A. T., & Von Gunten, U. (2009). Ferrate (Fe(VI)) Application for Municipal Wastewater Treatment: A Novel Process for Simultaneous Micropollutant Oxidation and Phosphate Removal. *Environmental Science & Technology*, *43*(10), 3831–3838. https://doi.org/10.1021/ES803588K

Liu, H., Chen, Z., Guan, Y., & Xu, S. (2018). Role and application of iron in water treatment for nitrogen removal: A review. *Chemosphere*, *204*, 51–62. https://doi.org/10.1016/J.CHEMOSPHERE.2018.04.019

Mino, T., Van Loosdrecht, M. C. M., & Heijnen, J. J. (1998). Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research*, *32*(11), 3193–3207. https://doi.org/10.1016/S0043-1354(98)00129-8

MISHIMA, I., & NAKAJIMA, J. (2011). Application of Iron Electrolysis to Full-Scale Activated Sludge Process for Phosphorus Removal. *Journal of Water and Environment Technology*, *9*(4), 359–369. https://doi.org/10.2965/JWET.2011.359

Mulopo, J. (2024). A systematic overview of current advancements for chemical, material, and energy production using sewage sludge for industrial ecology and sustainability transition. *Environmental Sustainability*, *7*(1), 5–29. https://doi.org/10.1007/S42398-023-00301-9

Munyengabe, A., & Zvinowanda, C. (2019). Production, characterization and application of Ferrate(VI) in water and wastewater treatments. *Brazilian Journal of Analytical Chemistry*, *6*(25), 40–57. https://doi.org/10.30744/BRJAC.2179-3425.RV-19-2019

Ning, X., Hu, J., Yue, J., Tang, T., & Zhang, B. (2024). Microbial community structure of an anaerobic side-stream coupled anoxic–aerobic membrane bioreactor (AOMBR–ASSR) for an in-situ sludge reduction process. *Bioprocess and Biosystems Engineering*, *47*(7), 1027–1037. https://doi.org/10.1007/S00449-024-03019-Y/FIGURES/5

Oehmen, A., Lemos, P. C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L. L., & Reis, M. A. M. (2007). Advances in enhanced biological phosphorus removal: From micro to macro scale. *Water Research*, *41*(11), 2271–2300. https://doi.org/10.1016/J.WATRES.2007.02.030

Park, C., & Chon, D.-H. (2015). High-Rate Anaerobic Side-Stream Reactor (ASSR) Processes to Minimize the Production of Excess Sludge. *Water Environment Research*, *87*(12), 2090–2097. https://doi.org/10.2175/106143015X14362865227355

Plevri, A., Barka, E., Noutsopoulos, C., & Mamais, D. (2023). Enhancing the Performance of AnMBR Treating Municipal Wastewater at a High Organic Loading Rate with Iron Addition. *Energies*, *16*(7), 3069–3069. https://doi.org/10.3390/EN16073069

Romero Pareja, P. M., Aragon Cruz, C. A., Quiroga Alonso, J. M., & Coello Oviedo, M. D. (2018). Incorporating the oxic-settling-anaerobic (OSA) process into an anoxic–oxic system for sewage sludge reduction and nutrient removal. *Environmental Progress and Sustainable Energy*, *37*(3), 1068–1074. https://doi.org/10.1002/EP.12784;PAGE:STRING:ARTICLE/CHAPTER

Sabet, H., Moghaddam, S. S., & Ehteshami, M. (2023). A comparative life cycle assessment (LCA) analysis of innovative methods employing cutting-edge technology to improve sludge reduction directly in wastewater handling units. *Journal of Water Process Engineering*, *51*, 103354. https://doi.org/10.1016/J.JWPE.2022.103354

Saby, S., Djafer, M., & Chen, G. H. (2003). Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling-anoxic activated sludge process. *Water Research*, *37*(1), 11–20. https://doi.org/10.1016/S0043-1354(02)00253-1

Semblante, G. U., Hai, F. I., Ngo, H. H., Guo, W., You, S. J., Price, W. E., & Nghiem, L. D. (2014a). Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: Performance, mechanisms, and implications. *Bioresource Technology*, *155*, 395–409. https://doi.org/10.1016/J.BIORTECH.2014.01.029

Semblante, G. U., Hai, F. I., Ngo, H. H., Guo, W., You, S. J., Price, W. E., & Nghiem, L. D. (2014b). Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: Performance, mechanisms, and implications. *Bioresource Technology*, *155*, 395–409. https://doi.org/10.1016/J.BIORTECH.2014.01.029

Semblante, G. U., Phan, H. V., Hai, F. I., Xu, Z. Q., Price, W. E., & Nghiem, L. D. (2017). The role of microbial diversity and composition in minimizing sludge production in the oxic-settling-anoxic process. *Science of The Total Environment*, *607–608*, 558–567. https://doi.org/10.1016/J.SCITOTENV.2017.06.253

Shaddel, S., Bakhtiary-Davijany, H., Kabbe, C., Dadgar, F., & Østerhus, S. W. (2019). Sustainable sewage sludge management: From current practices to emerging nutrient recovery technologies. *Sustainability (Switzerland)*, *11*(12). https://doi.org/10.3390/SU11123435

Śniatała, B., Al-Hazmi, H. E., Sobotka, D., Zhai, J., & Mąkinia, J. (2024). Advancing sustainable wastewater management: A comprehensive review of nutrient recovery products and their applications. *Science of The Total Environment*, *937*, 173446. https://doi.org/10.1016/J.SCITOTENV.2024.173446

Spinosa, L. (2007). Wastewater Sludge: A Global Overview of the Current Status and Future Prospects. *Water Intelligence Online*, *6*(0), 9781780402154–9781780402154. https://doi.org/10.2166/9781780402154

Tian, T., Zhou, K., Xuan, L., Zhang, J. X., Li, Y. S., Liu, D. F., & Yu, H. Q. (2020). Exclusive microbially driven autotrophic iron-dependent denitrification in a reactor inoculated with activated sludge. *Water Research*, *170*, 115300. https://doi.org/10.1016/J.WATRES.2019.115300

Torregrossa, M., Di Bella, G., & Di Trapani, D. (2012). Comparison between ozonation and the OSA process: analysis of excess sludge reduction and biomass activity in two different pilot plants. *Water Science and Technology*, *66*(1), 185–192. https://doi.org/10.2166/WST.2012.153

Wang, J. F., Jin, W. B., Zhao, Q. L., Liu, Z. G., & Lin, J. K. (2007). [Performance of treating wastewater and anti-shockloading in oxic-settling-anaerobic (OSA) process for minimization of excess sludge]. *Huan Jing Ke Xue= Huanjing Kexue*, *28*(11), 2488–2493. https://europepmc.org/article/med/18290471

Wang, J., Zhao, Q., Jin, W., & Lin, J. (2008). Mechanism on minimization of excess sludge in oxic-settling-anaerobic (OSA) process. *Frontiers of Environmental Science and Engineering in China*, *2*(1), 36–43. https://doi.org/10.1007/S11783-008-0001-4/METRICS

Wang, Q., Wei, W., Gong, Y., Yu, Q., Li, Q., Sun, J., & Yuan, Z. (2017). Technologies for reducing sludge production in wastewater treatment plants: State of the art. *Science of The Total Environment*, *587–588*, 510–521. https://doi.org/10.1016/J.SCITOTENV.2017.02.203

Wang, Z., Skerlos, S. J., & Novak, P. J. (2024). Nitrogen and Phosphorus Recovery from Anthropogenic Liquid Waste Streams. *Annual Review of Environment and Resources*, *49*(1), 281–307. https://doi.org/10.1146/ANNUREV-ENVIRON-112320-082121/CITE/REFWORKS

Wei, Y., Van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. (2003). Minimization of excess sludge production for biological wastewater treatment. *Water Research*, *37*(18), 4453–4467. https://doi.org/10.1016/S0043-1354(03)00441-X

Wilfert, P., Kumar, P. S., Korving, L., Witkamp, G. J., & Van Loosdrecht, M. C. M. (2015). The Relevance of Phosphorus and Iron Chemistry to the Recovery of Phosphorus from Wastewater: A Review. *Environmental Science and Technology*, *49*(16), 9400–9414. https://doi.org/10.1021/ACS.EST.5B00150/SUPPL_FILE/ES5B00150_SI_001.PDF

Wu, T., Zhong, L., Pang, J. W., Ren, N. Q., Ding, J., & Yang, S. S. (2023). Effect of Fe3+ on the nutrient removal performance and microbial community in a biofilm system. *Frontiers in Microbiology*, *14*, 1140404. https://doi.org/10.3389/FMICB.2023.1140404/BIBTEX

Xia, C.-W., Ma, Y.-J., Zhang, F., Lu, Y.-Z., & Zeng, R. J. (2013). *A Novel Approach for Phosphorus Recovery and No Wasted Sludge in Enhanced Biological Phosphorus Removal Process with External COD Addition*. https://doi.org/10.1007/s12010-013-0575-6

Yagci, N., Novak, J. T., Randall, C. W., & Orhon, D. (2015). The effect of iron dosing on reducing waste activated sludge in the oxic-settling-anoxic process. *Bioresource Technology*, *193*, 213–218. https://doi.org/10.1016/J.BIORTECH.2015.06.109

Yan, P., Guo, J. S., Wang, J., Chen, Y. P., Ji, F. Y., Dong, Y., Zhang, H., & Ouyang, W. juan. (2015). Enhanced nitrogen and phosphorus removal by an advanced simultaneous sludge reduction, inorganic solids separation, phosphorus recovery, and enhanced nutrient removal wastewater treatment process. *Bioresource Technology*, *183*, 181–187. https://doi.org/10.1016/J.BIORTECH.2015.02.070

Yan, P., Ji, F., Wang, J., Fan, J., Guan, W., & Chen, Q. (2013a). Evaluation of sludge reduction and carbon source recovery from excess sludge by the advanced Sludge reduction, Inorganic solids separation, Phosphorus recovery, and Enhanced nutrient Removal (SIPER) wastewater treatment process. *Bioresource Technology*, *150*, 344–351. https://doi.org/10.1016/J.BIORTECH.2013.10.038

Yan, P., Ji, F., Wang, J., Fan, J., Guan, W., & Chen, Q. (2013b). Pilot-scale test of an advanced, integrated wastewater treatment process with sludge reduction, inorganic solids separation, phosphorus recovery, and enhanced nutrient removal (SIPER). *Bioresource Technology*, *142*, 483–489. https://doi.org/10.1016/J.BIORTECH.2013.05.071

Zhao, G., Tang, J., Zhou, C., Wang, C., … X. M.-P. J. of, & 2022, undefined. (n.d.). A Megacity-Scale Analysis of Sludge Management and Carbon Footprint in China. *Pjoes.ComG Zhao, J Tang, C Zhou, C Wang, X Mei, Y Wei, J XuPolish Journal of Environmental Studies, 2022•pjoes.Com*. https://doi.org/10.15244/pjoes/143510

Zhou, Z., Qiao, W., Xing, C., Wang, C., Jiang, L. M., Gu, Y., & Wang, L. (2015). Characterization of dissolved organic matter in the anoxic–oxic-settling-anaerobic sludge reduction process. *Chemical Engineering Journal*, *259*, 357–363. https://doi.org/10.1016/J.CEJ.2014.07.129