INTERACTIONS OF OPERATIONAL PARAMETERS FOR BREWERY WASTEWATER TREATMENT USING AEROBIC GRANULAR SLUDGE

by

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B.Eng., London South Bank University, 2018

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF APPLIED SCIENCE
IN
ENGINEERING

UNIVERSITY OF NORTHERN BRITISH COLUMBIA

May 2025

Abstract

With more stringent environmental regulations on wastewater effluent quality, efficient and sustainable treatment solutions for the brewing industry are increasingly critical. This study evaluated the impact of influent chemical oxygen demand (COD) and hydraulic retention time (HRT) on the treatment of high-strength brewery wastewater using the aerobic granular sludge (AGS) biotechnology. A 2^2 full factorial experimental design with a replicated center point was utilized to assess the effects of COD (3000, 4000, and 5000 mg/L) and HRT (10.67, 12.8, and 16 h) on the removal efficiencies of COD, ammonia, and phosphorus. Regression analysis was conducted using Design Expert software and visualized through scatter plots to model the relationships between these operational parameters and the responses (treatment performance). The results show consistently high COD removal efficiency, exceeding 99% across all experimental runs with minimal variation. Due to this consistency, the selection of effective conditions focused only on ammonia and phosphorus removal performance, with the highest efficiencies achieved at an HRT of 16 h. Specifically, Run 1 (COD: 2900 ± 180 mg/L; HRT:16 h) achieved 96% ammonia removal and 96% phosphorus removal, while Run 2 (COD: 5050 ± 140 mg/L; HRT: 16 h) achieved 98% ammonia removal and 90% phosphorus removal. Regression analysis revealed key trends, where ammonia removal efficiency showed a strong positive relationship with HRT, but a negligible relationship with influent COD. Phosphorus removal efficiency exhibited a moderate positive relationship with HRT, and a weak negative relationship with influent COD. COD removal efficiency showed a negligible relationship with HRT, and a weak negative relationship with influent COD. Additionally, pH was observed to influence nutrient removal, particularly ammonia, as it affects microbial activity in AGS systems. Variations in pH likely contributed to the incomplete nitrification and nitrite buildup, indicating that pH

should be a critical factor for optimization in future studies. These findings underscore the dominant role of HRT in enhancing nutrient removal and provide a foundation for improving AGS system efficiency in treating brewery wastewater. Future studies should focus on validating the results using real brewery wastewater, assessing long-term AGS stability, controlling the pH to enhance nitrification, and exploring resource recovery from AGS sludge to enhance sustainability.

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Glossary

AGS	Aerobic Granular Sludge
BOD	Biochemical Oxygen Demand
CAS	Conventional Activated Sludge
COD	Chemical Oxygen Demand
CBOD	Carbonaceous Biochemical Oxygen Demand
GAOs	Glycogen-Accumulating Organisms
GDM	Gravity-driven Membrane
HRT	Hydraulic Retention Time
IFAS	Integrated Fixed-film Activated Sludge
MFC	Microbial Fuel Cells
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
OLR	Organic Loading Rate
PAOs	Phosphorus-Accumulating Organisms
pH	Potential of Hydrogen
SBR	Sequencing Batch Reactor
SRT	Solids Retention Time
SVI	Sludge Volume Index
TN	Total Nitrogen
TSS	Total Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket
VFAs	Volatile Fatty Acids

Acknowledgements

I extend my deepest gratitude to my supervisor, Dr. Oliver Iorhemen, for his unwavering guidance, support, and expertise throughout this research. His insights have profoundly shaped my understanding of environmental engineering and sustainable wastewater treatment, fostering diligence and resilience in overcoming challenges.

I am immensely grateful to my committee members, Dr. Deborah Roberts, Dean of the Faculty of Science and Engineering at UNBC, and Dr. Rania Hamza, Associate Professor at Toronto Metropolitan University, for their invaluable feedback, encouragement, and academic support, which have significantly enriched this work.

Special thanks to the faculty, staff, and laboratory team at UNBC's School of Engineering for their technical assistance and resources. I am also grateful for the camaraderie and thought-provoking discussions shared with my colleagues. I sincerely appreciate the contributions of Mitacs intern Mohamed Meryeh and undergraduate student Rika Rao, whose support in the lab was instrumental to this research.

Finally, I express my deepest gratitude to my family for their unwavering encouragement. Special thanks to my mother, whose wisdom and constant support have been a guiding light throughout my academic journey. To my husband, whose belief in me, patience, and endless support have been my greatest source of strength, this achievement would not have been possible without you.

Dedication

This thesis is dedicated to my family, whose unwavering support and encouragement have been the foundation of my academic journey. To my mother, who instilled in me the values of perseverance and lifelong learning, and to my mentors, whose dedication to environmental sustainability continues to inspire me.

A special dedication to my husband, whose constant encouragement and belief in my potential gave me the confidence to embark on this master's journey. His unwavering support, patience, and motivation, especially during the most challenging moments, have been invaluable. His belief in my growth and success pushed me to strive for excellence.

I also extend my gratitude to Mitacs intern Mohamed Meryeh and undergraduate student Rika Rao for their invaluable support in the lab. Their dedication and assistance played a crucial role in the successful execution of my research.

Additionally, I am grateful to my friends and colleagues, whose insightful discussions and shared experiences enriched my learning process.

With sincere appreciation, I dedicate this work to all those who have guided, supported, and inspired me along the way.

Contributions

The following scholarly contributions resulted from this MASc work:

1. Soliman, R., Hamza, R.A., Iorhemen, O.T. (2024). Biofilm-based hybrid systems for enhanced brewery wastewater treatment – A review. *Journal of Water Process Engineering.* 58: 104763.

Details of contributions from the candidate and co-authors are listed below:

1. In this publication, the candidate conducted an extensive review of the relevant literature on biofilm-based hybrid systems for enhanced brewery wastewater treatment. This review involved identifying gaps in existing studies, critically evaluating published methodologies, and synthesizing findings to provide a comprehensive overview of biofilm-based technologies for wastewater treatment. Dr. Rania Hamza provided valuable guidance during the manuscript preparation phase, offering substantial input in terms of revisions, corrections, and improving the overall quality of the manuscript. Dr. Oliver Iorhemen worked closely with the candidate to structure and develop the paper from scratch, contributing significantly to the conceptual framework and methodology and advising on the manuscript structure and content, ensuring the clarity and precision of the final paper.

Chapter 1 Introduction

1.1. Background

Industrial wastewater management has become a critical environmental and public health concern due to rapid industrialization and urbanization. Among various industrial sectors, the brewing industry is a significant contributor to wastewater pollution, characterized by high organic loads, elevated nutrient concentrations, and variable flow rates. Brewery wastewater, with its elevated chemical oxygen demand (COD), suspended solids, and complex composition, poses a significant challenge for conventional treatment methods. Effective treatment is crucial to mitigate its environmental impact and comply with stringent discharge regulations. Studies have highlighted the detrimental effects of brewery effluents on aquatic ecosystems due to the high organic content and other pollutants (di Biase et al., 2020). The aerobic granular sludge (AGS) biotechnology has emerged as a transformative solution for high-strength industrial wastewater. Unlike traditional activated sludge systems, AGS forms compact, dense microbial granules with exceptional settling properties, high biomass retention, and enhanced treatment in small reactor footprints. AGS systems are particularly advantageous for simultaneous removal of organic matter, nutrients, and other contaminants, making them suitable for complex wastewater streams such as those from breweries. For municipal wastewater, AGS has been shown to outperform conventional treatment systems in terms of treatment efficiency, energy consumption, and operational simplicity, providing a more sustainable solution for industrial applications (Gao et al., 2011; Khan et al., 2015).

Existing research on AGS has demonstrated its efficacy in treating municipal and various industrial wastewater streams, including paper mill and food industry effluents (Schwarzenbeck et al., 2005; Abdullah et al., 2011; Morais et al., 2016; Stes et al., 2020; Silva et al., 2023). However, its application to brewery wastewater is limited. Studies have shown that varying organic loading rates and COD:N:P (COD to nitrogen to phosphorus) ratios can significantly impact granular sludge development and nutrient removal performance in AGS systems for high-strength wastewater treatment (Bruin et al., 2004; Hamza et al., 2019).

The brewery industry is a significant contributor to industrial wastewater pollution. Thus, effective treatment of brewery wastewater is crucial to mitigate environmental impact and comply with stringent discharge regulations. The AGS biotechnology presents a promising solution for treating high-strength industrial wastewater. Studies have confirmed that AGS can effectively treat brewery wastewater, achieving high removal efficiencies for both COD and nitrogen compounds, which are typically challenging to treat in traditional systems (di Biase et al., 2020).

However, the optimal operational conditions for AGS in treating brewery wastewater, particularly in terms of COD and hydraulic retention time (HRT), remain underexplored. The varying compositions and flow fluctuations in brewery wastewater add to the complexity, necessitating detailed research to enhance AGS systems. This research aims to fill this knowledge gap by investigating the effects of varying COD and HRT on the performance of AGS systems treating brewery wastewater. The findings of this research will have significant implications for the sustainable management of brewery wastewater. By improving AGS biotechnology, it is possible to achieve higher treatment efficiency, reduced operational costs, and minimized environmental impact. This research will contribute to the development of more effective and environmentally friendly wastewater treatment solutions, supporting the brewing industry's sustainability goals.

1.2. Research objective

The primary aim of this research project was to examine the impact of operational parameters, specifically influent COD and HRT, on the treatment performance of high-strength brewery wastewater using AGS biotechnology. The study seeks to assess the effects of varying COD levels and HRT on the removal efficiencies of COD, ammonia, and phosphorus. Utilizing a 2^2 full factorial experimental design with a replicated center point, the research sought to explore the relationships between these operational variables and treatment outcomes. The specific objectives were:

a) To determine the effect of COD concentration and HRT on pollutant removal from brewery wastewater.

b) To determine the optimal conditions for enhanced treatment of brewery wastewater.

The findings are intended to provide a robust understanding of AGS system behavior under different conditions, serving as a foundation for future investigations into its application for industrial wastewater treatment.

1.3. Organization of the thesis

This thesis is structured into five chapters. Chapter 1, the current chapter, begins by addressing the urgent need for effective and sustainable brewery wastewater management, given its high organic loads, nutrient concentrations, and variability, which pose significant environmental challenges. This chapter sets the stage for the research by framing its objectives to systematically evaluate these parameters, aiming to enhance AGS efficiency and promote sustainable industrial wastewater treatment practices.

Chapter 2 provides a thorough literature review on brewery wastewater treatment, establishing the foundation for this thesis. It characterizes brewery wastewater, emphasizing the demand for robust treatment strategies. The chapter critically evaluates conventional methods like electrocoagulation and chemical coagulation, advanced biological processes such as moving bed biofilm reactors and integrated fixed-film activated sludge (IFAS), and emerging technologies including gravity-driven membrane (GDM) filtration and microbial fuel cells (MFC), alongside up-flow anaerobic sludge blanket (UASB) reactors, addressing their advantages, limitations, and future research directions. AGS is identified as a promising technology.

Chapter 3 details the methodologies used in this research, covering the experimental setup, feed wastewater composition, experimental design, analytical methods, and statistical analyses.

Chapter 4 presents the results and discussion on adjusting COD and HRT for improving AGS system performance for brewery wastewater treatment. It analyzes six experimental runs, focusing on COD removal and nutrient reduction, with statistical tools such as scatter plots assessing main effects. The findings help identify optimal operational conditions, enhancing efficiency and reducing costs. The discussion evaluates the impact of these improvements on system performance and effluent quality, concluding with practical recommendations for sustainable wastewater treatment in the brewing industry.

Chapter 5 concludes the research by summarizing the key findings and highlighting the primary takeaways. It also outlines the study's limitations and recommendations for future research.

Chapter 2 Literature Review

2.1. Overview

Brewery wastewater management is a significant issue in the brewing industry, as breweries generate large volumes of wastewater of high organic content from various brewery processes such as cleaning, bottling, and fermentation. The treatment of brewery wastewater requires innovative approaches to efficiently remove organic compounds, nutrients, and other contaminants while minimizing energy consumption. The amount of brewery wastewater produced varies based on the production capacity of a brewery, which typically ranges from 3 to 10 L of wastewater per litre of beer produced (Nancharaiah & Kiran Kumar Reddy, 2018a). The global beer production in 2020 was estimated to be 190.8 billion litres (Statista, 2023). According to the Brewers Association, approximately 70 % of the water used by breweries is discharged as effluent (Brewers Association, 2014). Brewery wastewater poses unique challenges due to its complex composition and distinct characteristics, making its treatment difficult. Understanding the composition and properties of brewery wastewater is crucial for developing appropriate treatment strategies.

2.2. Characteristics of brewery wastewater

The characteristics of brewery wastewater are detailed in Table 2.1. By examining the numbers, we can gain insights into the specific challenges associated with brewery wastewater treatment and design targeted approaches to address them. The main contaminants in brewery wastewater include ethanol, sugars, proteins, and yeast cells (Singh et al., 2019). Brewery wastewater poses environmental risks due to its complex composition, including high levels of COD and biochemical oxygen demand (BOD) (Enitan-Folami et al., 2015; Swain, 2019; Badria, 2022). These high COD levels can lead to oxygen depletion in receiving water bodies resulting in detrimental environmental effects such as the formation of "dead zones" impacting biodiversity and ecosystem health if discharged untreated (Diaz & Rosenberg, 2008; Enitan-Folami et al., 2014, 2015; Metcalf & Eddy, 2014). Additionally, brewery wastewater contains high levels of nutrients and suspended solids. If discharged into the environment untreated, brewery wastewater can have adverse effects on human and environmental health such as eutrophication of water bodies (Cloern, 2001; Rabalais & Turner, 2003; Diaz & Rosenberg, 2008; Likens, 2009; Ashraf et al., 2021). Eutrophication leads to decreased oxygen levels due to reduced photosynthetic activity, resulting in hypoxic or anoxic conditions (Swain, 2019), and thus resulting in mass fish kills and disrupting the entire food chain (Anderson et al., 2002; Rabalais & Turner, 2003). Harmful algal blooms (HABs) can also release toxins into the water, posing risks to swimmers, recreational users, other uses of the water as well as aquatic life (Backer et al., 2010). The toxins produced by HABs can cause a range of health effects, including gastrointestinal illnesses, neurological disorders, and even fatalities in severe cases (Anderson et al., 2002; Backer et al., 2010; Hallegraeff, 2010). Furthermore, the presence of solids in brewery wastewater can lead to surface water pollution, habitat alteration, and potential harm to aquatic life, necessitating effective solids management to mitigate these adverse environmental impacts, ensuring the safeguarding of aquatic ecosystems and water quality (Metcalf & Eddy, 2014; Goldammer, 2022). Moreover, the heavy metals in brewery wastewater such as cadmium, chromium, lead, and iron can exceed World Health Organization limits, posing significant risks to humans and the environment, necessitating effective wastewater treatment to minimize heavy metal release (Yeole et al., 2012; Arimieari & Akari, 2020; Jebesa et al., 2024).

The treatment of brewery wastewater involves some preliminary units followed by the main treatment unit. Pretreatment processes are crucial for enhancing the main brewery wastewater treatment process by removing or reducing undesirable components, thus making it amenable to the main treatment process. Common pretreatment processes include screening, equalization, and primary clarification (Rytwo et al., 2011; Brewers Association, 2014; Latessa et al., 2023). Screening is a mechanical process that removes large solids and debris, preventing potential blockages and ensuring smoother operation of subsequent treatment stages (Rytwo et al., 2011; Brewers Association, 2014; Latessa et al., 2023). Equalization serves to mitigate sudden surges in organic and hydraulic loads by evening out fluctuations in wastewater flow and load as well as enhancing system stability without altering the chemical composition of the wastewater (Brewers Association, 2014). Primary clarification removes settleable solids, resulting in a clearer supernatant. However, clarification does not significantly impact the concentration of dissolved or colloidal constituents, necessitating further biological processes to address remaining organic and nutrient loads (Brewers Association, 2014; Metcalf & Eddy, 2014).

Various methods have been used as the main treatment method for brewery wastewater, including physical, chemical, and biological treatment systems. Among these, biological methods have gained popularity due to their sustainability and cost-effectiveness. Unlike physical and chemical methods that often require significant energy and chemical input, biological technologies leverage microorganisms to remove contaminants, aligning with environmental and economic goals. Biological processes can completely convert organic compounds into less harmful end products such as CO_2 and H_2O. Since they are cost-effective and environmentally friendly, biological methods are a preferred choice for brewery wastewater treatment. To enhance the treatability of brewery wastewater by biological processes, it is essential to keep a desired COD:N:P ratio. To ensure sufficient nutrients for biomass growth, the COD:N:P ratio of 100:5:1 is conventionally used for aerobic systems. However, other studies reported higher COD:N:P ratios for high-strength organic wastewater treatment including 100:1.1:0.4, 100:2.8:1 and 100:2.5:0.3 (Hamza et al., 2018, 2019).

Table 2.1: Characteristics of brewery wastewater

Parameter	Range	Sources	References
Organic Compounds (mg/L)	COD: 800 - 7,000	Brewing byproducts (e.g., spent grains), malt residues, and yeast metabolism.	(Brewers Association, 2014; Enitan-Folami et al., 2014, 2015, 2015; Swain, 2019; Badria, 2022)
	BOD: 300 - 3,500	Organic components in brewing ingredients, such as sugars and proteins.	
	COD:BOD ratio: 2.0:1.0 – 2.7:1.0		
Nutrients (mg/L)	Ammonia: 50 – 500	Yeast metabolism, protein degradation, and amino acids from brewing ingredients	(Metcalf & Eddy, 2014; Enitan-Folami et al., 2015, 2015; Lu et al., 2019; R. Singh et al., 2019; Swain, 2019; Ashraf et al., 2021)
	Nitrate: 10 – 100	Nitrogen conversion and nitrification processes in wastewater	
	Organic nitrogen: 20 – 200	Organic nitrogen compounds in brewing ingredients, such as amino acids	
	Total Kjeldahl Nitrogen 25 – 80		
	Phosphorus 10 – 50	Phosphorus from brewing additives, cleaning agents, and grain residues	
Solids (mg/L)	Suspended solids: 500 - 3,000	Solid particles such as grain husks, yeast, and precipitated solids	(Metcalf & Eddy, 2014; Goldammer, 2022; Solutions, 2022)
	Total Solids (TS): 5100 – 8750	Residues from grain, brewing additives, and byproducts	
	Total Suspended Solids (TSS): 2901 – 3000	Suspended particles in the wastewater	
	Total Dissolved Solids (TDS): 2020 – 5940	Dissolved salts and sugars in the wastewater	
Heavy Metals	Cadmium (Cd), Chromium (Cr), Lead (Pb), and Iron (Fe)	Can be present in the raw materials used for brewing, such as grains and water, or they can be introduced using additives, chemicals, or from the corrosion of metal pipes, tanks, or other infrastructure within the brewery.	(Yeole et al., 2012; Arimieari & Akari, 2020; Jebesa et al., 2024)
pH	3.5 – 9.0	pH fluctuations due to fermentation, cleaning, and discharge water quality	(Janhom et al., 2009; Metcalf & Eddy, 2014; Swain, 2019; Mehrvar & Johnson, 2020; Badria, 2022; H. Su et al., 2023)
Temperature (ºC)	18 – 40	Temperature influenced by brewery operations and location	(Rao et al., 2007; Metcalf & Eddy, 2014; Jaiyeola & Bwapwa, 2016; Lu et al., 2019; Badria, 2022; H. Tian et al., 2023)

2.3. Treatment technologies for brewery wastewater

2.3.1. Electrocoagulation and chemical coagulation

Electrocoagulation (EC) and chemical coagulation (CC) are chemical treatment technologies that have garnered increasing attention as effective approaches for managing high-strength industrial wastewater, such as brewery (Swain et al., 2020; Akinnawo et al., 2023; Bashir & Sin, 2023). EC operates by applying a direct electric current through sacrificial metal electrodes (typically aluminum or iron), leading to the in-situ formation of metal hydroxide coagulants that destabilize and aggregate contaminants in the wastewater. CC, in contrast, involves the addition of chemical coagulants such as aluminum sulfate (alum), to promote aggregation of suspended solids and colloidal particles and subsequent sedimentation. The integration of EC and CC in a combined system (either EC followed by CC or CC followed by EC) offers the potential to optimize treatment efficiency while maintaining operational cost-effectiveness (Zaleschi et al., 2012; Swain et al., 2020).

Swain et al. (2020) evaluated the performance of EC, EC-CC, and CC-EC configurations for the treatment of real brewery effluent. The results demonstrated that combined processes significantly improved treatment outcomes compared to individual EC or CC processes. The EC-CC system, when operated at optimal conditions of 5 W applied power and 20 min electrolysis time, achieved removal efficiencies of 74% for reactive phosphorus, 76% for total phosphorus (TP), 26% for COD, and 85% for TSS. The enhanced removal is attributed to the synergy between the electrochemical destabilization of contaminants and the additional coagulation effect provided by chemical additives.

Interestingly, the CC-EC configuration showed superior COD removal performance under certain conditions, particularly when the brewery wastewater contained a higher proportion of particulate organic matter. This highlights the importance of wastewater composition in determining the most effective treatment sequence. Both EC-CC and CC-EC systems proved capable of reducing over-strength discharge parameters to levels that can result in significant reductions in sewer surcharge fees for breweries (Zaleschi et al., 2012).

While the combination of EC and CC shows promising treatment performance, several challenges and limitations still exist. One of the primary concerns is the energy requirement associated with the EC process, particularly at higher applied voltages or longer treatment times (Martínez-Villafañe et al., 2009; Özyurt et al., 2018; Medina-Collana et al., 2023). Although EC-CC systems demonstrated higher pollutant removal efficiency, the energy costs associated with operating the system contribute significantly to overall treatment expenses (Swain et al., 2020). Additionally, electrode consumption in the EC process leads to sludge generation, which requires appropriate handling and disposal, potentially adding to operational complexity and cost (Kobya et al., 2007; Uludag-Demirer et al., 2020; Ebba et al., 2021).

Another limitation is the variable effectiveness of the system depending on the wastewater characteristics. For example, EC has been shown to be less efficient at removing soluble COD fractions, which are prevalent in brewery wastewater (Enitan-Folami et al., 2015; Eyvaz, 2016). Moreover, increasing treatment time or power beyond optimal levels may result in reduced performance due to phenomena such as electrode passivation, oversaturation of metal hydroxide ions, or re-stabilization of particles due to charge reversal (AL-Rubaye et al., 2024; Al-Ajmi et al., 2025). These operational complexities necessitate careful process control and optimization (Ingelsson et al., 2020; Swain et al., 2020).

Future research should aim to further optimize operational parameters such as current density, electrode spacing, and initial pH to enhance the efficiency and cost-effectiveness of EC and EC-CC systems. Investigating alternative electrode materials or electrode designs that are more resistant to passivation and consume less energy could improve system durability and reduce costs. Additionally, the development of integrated hybrid systems that combine EC/CC with biological treatment or membrane filtration could offer enhanced pollutant removal while mitigating the limitations associated with standalone EC or CC processes. There is also a need to explore the environmental impact and management of sludge generated during treatment, including its potential reuse or valorization. Moreover, full-scale pilot studies and life-cycle cost assessments are essential to validate laboratory findings and provide practical insights into the scalability and sustainability of EC-CC systems in industrial settings. Finally, advancing automation and real-time monitoring technologies could support more precise control of treatment parameters, leading to more consistent performance and improved adaptability to variable wastewater characteristics.

2.3.2. Moving bed biofilm reactor

The moving bed biofilm reactor (MBBR) technology has gained prominence in brewery wastewater treatment due to its unique attributes that enhance treatment efficiency and flexibility. MBBR systems feature floating biofilm carriers, typically constructed from plastic, which offer a substantial surface area for the attachment and activity of diverse microorganisms, allowing them to efficiently treat pollutants present in brewery wastewater (Javid et al., 2013; Boyle, 2019). The high surface area-to-volume ratio within MBBRs promotes microbial activity and mass transfer, leading to effective removal of a range of pollutants, including organic matter, nitrogen, and phosphorus (Javid et al., 2013; Boyle, 2019).

One of MBBR's distinctive advantage is its adaptability to the frequent load fluctuations observed in brewery wastewater treatment. Brewery operations often experience daily variations in wastewater flow and composition due to batch processing, cleaning cycles, and seasonal shifts in production (Boyle, 2019; Santos et al., 2020; Zhang et al., 2020). MBBR systems are designed to maintain stability against such fluctuations, ensuring continuous and reliable treatment performance. This adaptability is particularly valuable for brewery wastewater treatment, where maintaining efficient pollutant removal during peak production periods is essential (Boyle, 2019). The MBBR technology offers several advantages, including enhanced treatment efficiency due to large active surface area, stability against load fluctuations, reduced sludge production, energy efficiency, adaptability, and space efficiency. It is efficient for the removal of organic matter, nitrogen, and phosphorus; and its biofilm carriers maintain stability under varying conditions (Boyle, 2019; Santos et al., 2020; Jang et al., 2023). The reduced sludge production of MBBR aligns with sustainability goals, and its minimized energy consumption and adaptability make it a versatile choice for diverse treatment scenarios (Guo et al., 2019).

MBBRs have demonstrated high efficiency in brewery wastewater treatment, achieving over 90% COD and ammonia removal, approximately 85–99% TSS removal, 60–90% Total Kjeldahl Nitrogen removal, and 60–97% phosphorus removal. These results highlight MBBRs as a reliable solution for treating high-strength industrial effluents (di Biase et al., 2017; Amenorfenyo et al., 2019; bishopwaterstg, 2022). The effectiveness of nutrient removal in MBBRs can indeed vary depending on specific influent characteristics and system design (Manyuchi & Chikwama, 2016; Boyle, 2019). Achieving stringent nutrient removal targets, especially for phosphorus, may require additional treatment steps. To consistently meet these targets, MBBRs often need optimization, as nutrient removal can be influenced by various factors, including influent nutrient concentrations, system design, and operational parameters (Javid et al., 2013; Boyle, 2019; di Biase et al., 2019).

In addition to the limited nutrient removal that requires additional treatment steps, MBBR has more limitations. This includes the potential for media clogging, the need for regular maintenance, and higher capital energy and operational costs with a larger footprint (Boyle, 2019; Zhang et al., 2020). It also faces challenges in handling refractory compounds, preventing biofilm aging and detachment, and managing hydraulic issues (Jiang et al., 2018; Saini et al., 2023; M. Ding, 2024). Achieving consistent nutrient removal may require optimization, and the presence of hard-to-degrade compounds can impact overall efficiency. Careful strategies are necessary to mitigate biofilm aging and hydraulic irregularities (Boyle, 2019; di Biase et al., 2019).

The MBBR technology generally requires more energy compared to IFAS. This is because MBBR relies on continuous and rigorous mixing of liquid in the reactor to maintain suspension of solid media, which consumes more energy (Ali et al., 2021). On the other hand, IFAS requires less energy as it utilizes fixed media and does not require as much mixing. The specific energy consumption values mentioned in this study for both MBBR and IFAS are IDR 6880 kWh/d for MBBR and IDR 6507 kWh/d for IFAS, based on an average wastewater discharge of 0.09 m^3/s (Ali et al., 2021).

2.3.3. Integrated fixed-film activated sludge

The integrated fixed-film activated sludge (IFAS) is a wastewater treatment technology that combines suspended growth and attached growth processes in a single reactor. The IFAS system consists of a suspended growth activated sludge process with the addition of fixed media, which provides a surface for biofilm growth. The biofilm on the media enhances the treatment capacity of the system by increasing the effective surface area available for microbial activity (X. Tian & Delatolla, 2019; Gallardo-Altamirano et al., 2021). The IFAS system has several advantages for brewery wastewater treatment. It can effectively remove organic matter and nutrients. Studies have shown that IFAS systems can achieve up to 95 % removal efficiency for COD, 90 % for BOD, and 80 % for total nitrogen (TN) (Gallardo-Altamirano et al., 2021). (Sriwiriyarat, 2002; Fazelipour et al., 2021; González-Tineo et al., 2022; Jin et al., 2023)

IFAS can also handle load fluctuations and is stable since the biofilm on the media provides a buffer against changes in influent wastewater characteristics and quantity, allowing the system to maintain stable performance even during peak loads or shock loads. This implies that the system can handle fluctuations in the incoming wastewater flow rate and organic loading without compromising the treatment efficiency (Tian & Delatolla, 2019; Waqas et al., 2023). The potential of IFAS to reduce the environmental impact of brewery wastewater treatment and be integrated with other treatment processes makes it a promising technology for the future of wastewater treatment (Bashar et al., 2018; Waqas et al., 2023).

One limitation is the potential for clogging or fouling of the fixed media due to the accumulation of solids or biofilm growth. This can reduce the effective surface area available for microbial activity and decrease treatment efficiency. Regular maintenance and cleaning of the media may be necessary to prevent clogging and maintain optimal performance (Singh & Kazmi, 2016; Waqas et al., 2023). Another limitation is the potential for oxygen transfer limitations, which can occur if the dissolved oxygen (DO) levels are too low or if the aeration system is not functioning properly. This can lead to reduced treatment efficiency and increased energy consumption. Regular monitoring of DO levels and adjustments to aeration rates or oxygen transfer mechanisms are necessary to maintain optimal DO concentrations for efficient treatment performance and to avoid oxygen limitation and excessive energy consumption (Onnis-Hayden et al., 2011; Arias et al., 2018). Finally, the IFAS system may require a larger footprint compared to other treatment technologies due to the addition of fixed media. This may be a consideration when selecting a treatment technology for a specific site or application (Singh & Kazmi, 2016; Waqas et al., 2023).

2.3.4. Gravity-driven membrane filtration

Gravity-driven membrane (GDM) filtration is an emerging water treatment technology that utilizes ultrafiltration membranes under ultra-low pressure, relying solely on gravity as the driving force. Initially developed for drinking water treatment, GDM has been increasingly explored for wastewater applications due to its ability to maintain stable flux without frequent cleaning or backwashing (Pronk et al., 2019; Wu et al., 2017). The core principle of GDM filtration lies in biofilm formation on the membrane surface, which stabilizes flux while enabling microbial degradation of organic matter. This self-sustaining mechanism makes GDM highly energy-efficient compared to conventional pressure-driven membrane systems. Recent research has demonstrated its applicability in treating greywater, rainwater, industrial effluents, and particularly brewery wastewater, which is characterized by high organic loads and microbial contaminants (A. Ding et al., 2017; Barambu et al., 2021). Despite its advantages, limitations such as fouling, slow filtration rates, and the incomplete removal of dissolved contaminants necessitate further research to optimize its performance and broaden its industrial application.

The effectiveness of GDM filtration in treating wastewater is largely dependent on biofilm dynamics and feedwater composition. Studies have shown that GDM can achieve high removal efficiencies for turbidity, bacteria, and suspended solids, making it a viable option for decentralized treatment systems (Pronk et al., 2019; Wang et al., 2017). In the context of brewery wastewater, GDM has demonstrated stable filtration performance while removing up to 99.99% of bacteria and significantly reducing turbidity (Barambu et al., 2021; Toran et al., 2021; Stoffel et al., 2023). However, the removal of dissolved organic matter is limited, as conventional ultrafiltration membranes primarily target particulate and microbial contaminants. Biofilm activity on the membrane surface plays a crucial role in organic degradation, but its efficiency varies based on water quality parameters such as DO levels and nutrient availability. Furthermore, while GDM exhibits stable flux over extended operational periods, factors such as feedwater composition and membrane material influence overall performance, necessitating tailored system designs for industrial applications (Wang et al., 2017; Pronk et al., 2019; Qin et al., 2019).

Despite its potential, GDM filtration faces several challenges when applied to brewery wastewater treatment. One major drawback is its limited ability to remove dissolved organic pollutants and heavy metals, requiring additional post-treatment for effluent compliance with discharge regulations (Xiang et al., 2022; Ma et al., 2023). Another challenge is membrane fouling, which, although stabilized by biofilm activity, can still reduce long-term permeability, especially when treating high-strength wastewater such as brewery effluent (Du et al., 2019; Li et al., 2025; Pronk et al., 2019). The slow filtration rates associated with gravity-driven operation also limit the scalability of GDM for large industrial facilities (Pronk et al., 2019; Stoffel et al., 2023; Lee et al., 2025), as it may not meet high-volume wastewater treatment demands. Additionally, while GDM significantly reduces operational costs compared to conventional ultrafiltration systems, the initial investment in membrane modules and infrastructure remains a financial barrier for widespread adoption (Pronk et al., 2019; Hube et al., 2022).

To enhance the feasibility of GDM for wastewater treatment, future research should focus on hybrid treatment approaches that integrate GDM with advanced oxidation processes, adsorption techniques, or biological treatment methods to improve the removal of dissolved contaminants (Pronk et al., 2019). Additionally, optimizing biofilm management strategies, such as intermittent aeration or dynamic flux control, could mitigate fouling while maintaining long-term operational stability (Du et al., 2020). The development of advanced membrane materials with antifouling properties, including hydrophilic coatings or novel polymeric compositions, is another promising research avenue to extend membrane lifespan and enhance filtration efficiency. Furthermore, economic feasibility studies comparing GDM with traditional treatment technologies are necessary to evaluate cost-effectiveness and identify scenarios where GDM can provide a competitive advantage in industrial wastewater treatment. Addressing these challenges through targeted research will be critical for expanding the applicability of GDM filtration in brewery wastewater management and other industrial sectors.

2.3.5. Microbial fuel cell

Microbial fuel cells (MFCs) are a promising bioelectrochemical technology that facilitates the simultaneous treatment of wastewater and generation of electricity. In MFCs, electrogenic microorganisms oxidize organic matter in the anode chamber, releasing electrons and protons. The electrons are transferred via an external circuit to the cathode, where they combine with protons and an electron acceptor—commonly oxygen—forming water and completing the circuit (Singh & Sharma, 2010). MFCs offer multiple advantages including direct electricity generation, operation at ambient temperatures, reduced need for aeration, and potential integration into decentralized wastewater treatment systems (Brunschweiger et al., 2021).

Brewery wastewater is rich in organic matter, particularly carbohydrates, proteins, and residual fermentation byproducts, making it a suitable substrate for MFC applications. Singh and Sharma (2010) demonstrated that MFCs can achieve up to 94% COD removal efficiency and generate a maximum current of 10.89 mA under optimal conditions (pH 7), particularly when supplemented with easily degradable substrates such as glucose. Their study highlighted the role of electroactive microbial consortia and biofilm development in sustaining electricity generation and COD degradation.

Similarly, Brunschweiger et al. (2021) analyzed the feasibility of MFC integration in brewery wastewater treatment by benchmarking energy recovery requirements against conventional treatment technologies. Their findings indicated that MFCs could theoretically replace energy-intensive aerobic stages or serve as a pre-treatment step. Achieving COD removal benchmarks was feasible, although a longer HRT may be needed. MFCs were shown to potentially reduce energy costs, especially in small breweries where compact and stackable systems could replace or complement existing infrastructure.

Despite the promising potential of MFCs in wastewater treatment, several limitations currently hinder their large-scale application in brewery wastewater treatment systems. One of the main challenges is the relatively low energy recovery efficiency compared to conventional anaerobic treatment systems (Waller & Trabold, 2013; Do et al., 2018; Niju & Priyadharshini, 2023). While MFCs offer the advantage of direct electricity generation, studies have shown that their energy output often remains insufficient to fully offset the energy demands of the treatment process. For instance, Brunschweiger et al. (2021) reported that even under ideal conditions, the realistically achievable energy efficiency from MFCs was approximately 4.5% in certain scenarios, whereas the required benchmark to compete with existing systems was significantly higher. Moreover, MFCs typically require longer HRT to achieve satisfactory COD removal levels, which limits their practicality in high-throughput industrial settings such as breweries (Yamane et al., 2021; Sorgato et al., 2023).

Operational challenges also present notable drawbacks. Electrode fouling, biofilm overgrowth, and membrane degradation can adversely impact system performance over time, requiring frequent maintenance and potentially leading to operational instability (Ezziat et al., 2019; Noori et al., 2019; Pasternak et al., 2022). Additionally, the high initial costs associated with MFC construction, particularly for materials such as proton exchange membranes and specialized electrode components, can deter implementation, especially in small-scale facilities. Furthermore, system complexity, including the need for fine-tuned environmental control and biofilm management, adds another layer of difficulty in integrating MFCs into conventional treatment infrastructures (Borja-Maldonado & Zavala, 2022; Jalili et al., 2024; Ramirez-Nava et al., 2021; Zhang & Angelidaki, 2016).

To overcome these limitations and facilitate the wider adoption of MFCs in brewery wastewater treatment, further research is required across multiple domains. One critical area involves the development of advanced, cost-effective electrode materials with enhanced durability, conductivity, and biofouling resistance. Improving electron transfer efficiency through the use of innovative nanomaterials or bio-compatible coatings could significantly enhance overall system performance (Hernández-Fernández et al., 2015; Malik et al., 2023). Moreover, a deeper understanding of the microbial communities responsible for electricity generation is essential. Targeted research on microbial ecology and the engineering of robust electrogenic consortia could improve biofilm stability and electron transfer rates by 20–30%, particularly under varying wastewater compositions and environmental conditions typical of brewery effluent (Tan et al., 2021; Dwivedi, Huang, Wang, et al., 2022).

Another promising direction lies in the design of modular and scalable MFC configurations that can be easily integrated into existing wastewater treatment facilities. Hybrid systems that combine MFCs with traditional anaerobic digesters or aerobic polishing units may offer synergistic benefits, maximizing energy recovery while ensuring compliance with discharge regulations

(Dwivedi et al., 2022; Roy et al., 2023; Tan et al., 2021). Additionally, advancements in automation and real-time process control technologies can help optimize operating conditions such as pH, temperature, and substrate concentration, thereby improving system efficiency and reliability (Priya et al., 2022; Tsekouras et al., 2022). Finally, comprehensive techno-economic analyses and life-cycle assessments are needed to better understand the long-term viability and environmental impact of MFCs compared to conventional treatment methods. Such insights will be crucial for informing industry adoption and policy development in the context of sustainable wastewater management (Apollon, 2023; Roy et al., 2023).

2.3.6. Upflow anaerobic sludge blanket reactors

The upflow anaerobic sludge blanket (UASB) reactor is a high-rate anaerobic treatment system that has been effectively utilized for the treatment of brewery wastewater (Cronin & Lo, 1998; Rao et al., 2007; Ahn & Park, 2008). Due to its ability to efficiently degrade organic matter and produce biogas, UASB technology is a viable alternative to conventional aerobic treatment methods, which often suffer from excessive sludge production and energy-intensive operation.

The treatment performance of UASB reactors for brewery wastewater has shown significant advancements in recent studies. A pilot-scale UASB reactor seeded with 29 g VSS/L from municipal anaerobic sludge achieved a 95% COD reduction at an OLR of 7 kg COD/m^3·d and an HRT of 3.5 d, demonstrating the critical role of high seed sludge concentration in optimizing treatment efficiency (Öktem & Tufekci, 2006). In contrast, a lab-scale UASB operating at a higher OLR of 12 kg COD/m^3·d and a shorter HRT of 4 h achieved 89.1% COD removal, suggesting that modern systems can maintain high performance even under intensified conditions, though seed sludge details were not specified (Dutta et al., 2018). These results underscore the influence of seed sludge concentration and OLR on COD reduction, with inoculation levels of 10–

30% reactor volume (e.g., 20–60 g VSS/L) enabling COD removals exceeding 90% at OLRs of 12–20 kg COD/m³·d (Mainardis et al., 2020).

Co-digestion of brewery wastewater with other organic waste streams, such as dairy wastewater, has gained attention for enhancing treatment stability and biogas yield. While specific brewery-dairy co-digestion studies in UASB reactors remain limited, broader research supports its potential. For instance, co-treating sewage with microbial biomass in a UASB increased methane yield by 25% (from 156 to 211 NL CH_4/kg VS), indicating improved energy recovery with mixed substrates (Mainardis et al., 2020). Dairy wastewater alone has been treated with 97% COD removal at an OLR of 33 kg COD/m³·d, suggesting that its high biodegradability could complement brewery wastewater's carbohydrate-rich profile (Lier et al., 2015). Additionally, co-digestion of food waste with industrial matrices in UASB systems achieved over 90% COD removal at OLRs exceeding 20 kg COD/m³·d, highlighting stability and biogas enhancement potential, though further investigation into brewery-dairy combinations is still needed (Collivignarelli et al., 2021).

Despite its advantages, the UASB system has several limitations that can impact its efficiency and long-term performance. One of the primary challenges is the prolonged start-up period, which can range from two to eight months, depending on inoculum quality and wastewater characteristics (Díaz-Gómez et al., 2022; Suneeth Kumar Saragur, Indra Mani Mishra, 2023). This is largely due to the time required for the development of a stable granular sludge bed, which is essential for maintaining high treatment efficiency (Cronin & Lo, 1998; Dutta et al., 2018; Lier et al., 2015). Additionally, brewery wastewater is known for its variability in composition, which can lead to fluctuations in organic loading rates. These variations pose a challenge in maintaining process stability, as sudden changes in influent characteristics can disrupt microbial activity and lead to operational inefficiencies (Smetana & Grosser, 2024).

Another critical limitation is the necessity of proper seed sludge acclimation. Without an adequate acclimation period, reactors may experience low methane production and excessive sludge washout, particularly during the initial stages of operation (Cronin & Lo, 1998; Marzuki et al., 2021). Furthermore, while UASB reactors are highly effective in reducing COD levels, they have limited capability in removing nitrogen and phosphorus, which are essential for meeting stringent discharge regulations. As a result, post-treatment methods such as aerobic treatment or advanced oxidation processes are often required to achieve complete wastewater purification (Smetana & Grosser, 2024). Additionally, sludge disintegration and flotation may occur under suboptimal conditions, leading to biomass loss and reduced reactor efficiency. The presence of hydrogen sulfide (H_2S) in the biogas stream also poses challenges, as it contributes to odor issues and potential corrosion of infrastructure (Borzacconi et al., 2008; Smetana & Grosser, 2024).

To address these challenges and enhance the performance of UASB reactors in brewery wastewater treatment, several research areas require further exploration. Optimizing the acclimation process is crucial for reducing start-up times, and future studies should investigate different types of seed sludge and pre-treatment strategies to accelerate the formation of stable granules. Additionally, co-digestion with other organic waste streams, such as dairy wastewater, has shown potential in improving treatment stability and methane yield. However, research on the co-digestion of brewery and dairy wastewater in UASB reactors remains limited, and further studies are needed to assess its feasibility and long-term performance (Ambekar & Khan, 2024; Smetana & Grosser, 2024).

Another promising research direction is the integration of UASB reactors with post-treatment technologies to enhance overall effluent quality. Coupling UASB with aerobic or physicochemical treatment methods could significantly improve nitrogen and phosphorus removal, addressing a key limitation of anaerobic digestion. Additionally, investigating additives or operational modifications to enhance granule formation could help mitigate sludge washout issues. Understanding microbial dynamics within UASB reactors is also essential for optimizing reactor performance, particularly in response to fluctuations in wastewater composition. Further research into the microbial communities involved in anaerobic digestion could provide insights into process stability and efficiency under varying operational conditions (Kovacik et al., 2010; Zhang et al., 2020; Smetana & Grosser, 2024).

Finally, exploring nutrient recovery strategies could contribute to more sustainable wastewater management practices. Developing methods to recover nitrogen and phosphorus from UASB effluent would not only improve environmental compliance but also support resource recovery and circular economy approaches in wastewater treatment. By addressing these research gaps, UASB technology can be further optimized to provide a cost-effective and environmentally sustainable solution for brewery wastewater treatment.

2.3.7. Expanded granular sludge bed

The Expanded Granular Sludge Bed (EGSB) reactor is a modification of UASB reactor, designed to enhance the treatment of dilute wastewater streams through improved hydraulic mixing and higher upflow velocities (Yan et al., 2010; Cruz-Salomón et al., 2019). In brewery wastewater treatment, EGSB technology excels at removing organic pollutants, even at lower temperatures. Research using a full-scale Biobed® EGSB reactor showed COD removal efficiencies exceeding 90% at an OLR of 10–15 kg COD/m^3·d with upflow velocities of 6–10 m/h, adaptable to 20°C (Zoutberg & Frankin, 1996). Studies suggest that a 225.5-L pilot-scale EGSB could achieve over 80% COD removal at an OLR of 12.6 g COD/L/d, an HRT of 1.2 h, and a Vup of 7.2 m/h at 20°C, maintaining efficiency due to enhanced mixing and robust sludge (Cronin et al., 2018; Esposito et al., 2020).

However, the performance of EGSB reactors is significantly influenced by operational conditions, particularly temperature. When the temperature was lowered stepwise from 30°C to 12°C, COD removal efficiency declined from 73% to 35%, mainly due to reduced microbial activity and issues related to sludge retention. At lower temperatures, the acidified fraction of the influent COD increased, but the conversion of volatile fatty acids (VFAs) to methane was significantly hindered. These findings highlight the importance of maintaining optimal temperature conditions for stable reactor performance in brewery wastewater treatment (Kato et al., 1999; Xing et al., 2009; Castilla-Archilla et al., 2021).

Despite its advantages, EGSB reactors face several operational challenges when treating brewery wastewater. One key limitation is the high susceptibility to sludge washout, particularly when operating at high upflow velocities. The expansion of the sludge bed, coupled with gas production, can lead to biomass loss, ultimately reducing reactor efficiency (Cruz-Salomón et al., 2019). In the pilot-scale study, significant sludge washout occurred when the OLR exceeded 16.5 g COD/L/d, leading to a sharp drop in COD removal efficiency. Additionally, fluctuations in brewery wastewater composition can complicate reactor stability, as variations in influent organic matter can affect microbial adaptation and process performance (Kato et al., 1999; Xu et al., 2013; Jiménez et al., 2018; Pérez-Pérez et al., 2018). Another critical drawback of EGSB reactors is their limited ability to degrade propionate at lower temperatures. During the experiments, a shift in ethanol fermentation pathways resulted in increased propionate accumulation, which negatively impacted COD removal efficiency. This suggests that EGSB systems may require longer adaptation periods for microbial communities to effectively metabolize complex organic substrates under fluctuating conditions. Furthermore, as with other anaerobic systems, EGSB reactors exhibit low nutrient removal efficiencies, necessitating post-treatment steps to meet discharge standards (Kato et al., 1999; Lettinga et al., 1999; Collins et al., 2006; Yoochatchaval et al., 2008; Ozgun et al., 2012; Wu et al., 2019).

To improve the application of EGSB reactors in brewery wastewater treatment, further research should focus on optimizing sludge retention strategies. Enhancing granule stability through the use of biofilm carriers or modifications in reactor design could mitigate sludge washout issues and improve long-term reactor performance (Cruz-Salomón et al., 2019). Additionally, exploring the impact of different seed sludge sources on reactor acclimation and performance under varying organic loads could provide valuable insights into microbial dynamics. Another key research area involves the integration of EGSB technology with complementary post-treatment methods to enhance overall effluent quality. Combining EGSB reactors with aerobic treatment or membrane filtration could improve nitrogen and phosphorus removal while maintaining high COD removal efficiencies. Moreover, investigating the potential for co-digestion of brewery wastewater with other organic waste streams could enhance process stability and biogas yield, further increasing the sustainability of anaerobic treatment systems (Zhang et al., 2022).

Finally, understanding the microbial community dynamics within EGSB reactors under varying operational conditions could provide valuable insights into process optimization. Advanced microbial analysis techniques, such as metagenomics and stable isotope probing, could help identify key microbial populations involved in brewery wastewater degradation, ultimately leading to improved reactor performance and stability. By addressing these research gaps, EGSB technology can be further optimized to provide a robust and energy-efficient solution for brewery wastewater treatment.

Finally, understanding the microbial community dynamics within EGSB reactors under varying operational conditions could provide valuable insights into process optimization. Research shows that microbial shifts, influenced by factors like COD and temperature, affect EGSB performance, as seen in studies tracking community changes in industrial reactors treating brewery-like wastewaters (Bialek et al., 2012; Zhang et al., 2023). Advanced microbial analysis techniques, such as metagenomics and stable isotope probing, can identify key populations driving brewery wastewater degradation, with metagenomics revealing methanogenic pathways and SIP pinpointing active degraders like sulfate-reducers and methanogens (Delforno et al., 2017; Berry & Loy, 2018). By addressing these research gaps, EGSB technology can be optimized for robust and energy-efficient brewery wastewater treatment, as evidenced by high COD removal rates linked to microbial stability in full-scale systems (Lin et al., 2020).

2.3.8. Aerobic granular sludge

The AGS biotechnology has garnered substantial attention within the realm of wastewater treatment, particularly for its profound capabilities in addressing the intricate challenges inherent to brewery wastewater. With its distinct configuration characterized by compact and densely packed microbial aggregates, AGS stands as a transformative approach, exhibiting remarkable efficacy in the simultaneous removal of organic matter and nutrients (Li et al., 2014; Nancharaiah & Kiran Kumar Reddy, 2018b). This innovative biotechnology offers an array of intricate advantages, rendering it a promising solution for sustainable and efficient brewery wastewater treatment. The outstanding features of AGS, notably its exceptional settling ability and high biomass concentration in the reactor, engender a pivotal role in its operational capability. The intricate and self-immobilized microbial aggregates in AGS systems provide the necessary capability to remove brewery wastewater pollutants efficiently, resulting in high quality of the treated effluent. This paradigmatic approach adeptly addresses the complex nature of brewery wastewater, facilitating the simultaneous removal of organic matter, nutrients, and heavy metals (Di Iaconi et al., 2007; Gao et al., 2011; Alves, 2017; Nancharaiah & Kiran Kumar Reddy, 2018b).

The treatment of high-strength wastewater using AGS in a lab-scale sequencing batch reactor (SBR) was reported (Fortunato et al., 2020). The AGS bioreactor, operated at a volumetric exchange ratio (VER) of 50%, HRT of 16 h, and DO concentration of 2 mg/L, consistently achieved high removal efficiencies for ammonium nitrogen (NH_4^+-N) exceeding 95%, total nitrogen (TN) at 90%, TP at 76%, and COD up to 91%. Studies show that AGS systems under similar conditions—such as DO of 2 mg/L and high VER—can attain >96% NH_3-N, 90% COD, and significant TN and TP removal, with adjustments in HRT enhancing nutrient removal (Świątczak & Cydzik-Kwiatkowska, 2018). The granulation process also increased mixed liquor suspended solids (MLSS) concentrations to 15–16 g/L and improved settling characteristics, with SVI values dropping to 40–50 mL/g, reflecting enhanced biomass retention and settleability. These findings underscore the system's effectiveness in comprehensively treating wastewater.

The complex nature of the granular matrix allows for numerous pollutant removal pathways, including biological degradation, adsorption, and precipitation. The multifaceted microbial consortia provide the necessary microenvironments for metabolic transformations, converting pollutants into byproducts (Li et al., 2014; Wei et al., 2014; Vleeschauwer et al., 2021; Jiang et al., 2022; Ran et al., 2022). The combination of these processes constitutes an all-encompassing strategy, endowing AGS with improved treatment efficacy.

The advantages of AGS biotechnology are outlined below:

a) **High biomass concentration and compact reactor design:** The AGS biotechnology exhibits higher biomass concentration (8000–15,000 mg/L) compared to the CAS process (1500–4000 mg/ L) (Abdullah et al., 2013; Hamza et al., 2022). This, in turn, allows for a compact reactor design with a smaller spatial footprint, effectively optimizing land utilization (Hamza et al., 2022; Świątczak & Cydzik-Kwiatkowska, 2018; Weber et al., 2007). This compactness not only bolsters treatment capacity per unit volume but also presents an ideal solution for scenarios constrained by land availability, a prevalent consideration in modern wastewater treatment facilities.

b) **Enhanced settleability and reduced sludge production:** The dense and tightly packed configuration of the granules imparts an innate settling capability, facilitating rapid and efficient solids-liquid separation. This distinct attribute culminates in improved clarification processes and diminished sludge production, addressing a critical concern in wastewater treatment operations (Boavida-Dias et al., 2022; Miyake et al., 2022).

c) **Lower cost and energy consumption:** AGS technology can have lower energy consumption compared to many traditional wastewater treatment processes such as conventional activated sludge. However, whether it has lower energy consumption than other treatment processes depends on various factors, including the specific design, operational conditions, and the treatment goals. AGS is generally recognized for its energy efficiency due to factors such as enhanced settling properties, and lower excess sludge production (Miyake et al., 2022). The AGS process offers several cost and energy-saving advantages compared to CAS. AGS technology addresses the key bottlenecks of CAS (Nancharaiah & Sarvajith, 2019), including high land footprint, high energy requirements for biomass recirculation, and biomass-water separation issues. AGS simplifies the process design by achieving both biological treatment and solids-liquid separation in a single treatment tank, eliminating the need for secondary clarifiers and separating anoxic and aerobic compartments. This significantly reduces the land footprint of AGS-based wastewater treatment plants, with estimations showing a reduction of up to 75 % compared to conventional AS plants (Bengtsson et al., 2019). Additionally, AGS plants have lower capital and operational costs, with energy demand reported to be 30–48 % lower than CAS plants. These economic benefits, along with better treatment performance, make AGS process a cost-effective and energy-efficient alternative for sustainable wastewater treatment (Nancharaiah & Sarvajith, 2019).

Bengtsson et al. compared the AGS biotechnology to various wastewater treatment processes in terms of cost and energy consumption. The AGS process was found to have a smaller footprint (40–50 % less) and required 23 % less electricity compared to CAS. When compared to other compact treatment options such as IFAS and MBR, the AGS process had significantly lower electricity usage (35–70 % less). This indicates the cost and energy efficiency of AGS-based processes. However, the energy consumption of AGS systems can vary depending on various factors such as the size of the system, operational parameters, and the specific wastewater treatment processes involved (Bengtsson et al., 2019).

d) **Resilience to shock loads and influent fluctuations:** AGS displays an exceptional capacity to withstand shock loads and influent variations, underpinned by its inherent stability and robust microbial consortium. This resilience ensures uninterrupted and consistent treatment performance, particularly valuable in dynamic operational environments (Abdullah et al., 2013; Sarvajith & Yarlagadda, 2021). The application of AGS for winery wastewater treatment was studied (Basset et al., 2014). The AGS system demonstrated excellent granule settleability, high biomass retention which contributed to their effectiveness in treating winery wastewater, and the ability to handle shock loadings and inhibitory and toxic agents which makes AGS a suitable technique for treating winery wastewater. The system was efficient in handling sudden changes in influent COD concentration, achieving low effluent COD concentrations after abrupt changes. It also showed high removal efficiencies for a wide range of influent COD concentrations (1–6 g COD/L). Additionally, a performance comparison of activated sludge and AGS for the removal of metalloid and nutrients from wastewater after 100 d of operation was conducted at laboratory-scale (Sarvajith & Yarlagadda, 2021). The AGS-based system showcased impressive resilience against shock loads and influent fluctuations, maintaining consistent treatment performance. Specifically, three identical SBRs were used. The disturbances involved removing half of the volume of settled biomass in the reactors, resulting in an instantaneous doubling of the food-to-microorganisms (F/M) ratio. Fluctuations in reactor performance were observed during the disturbance stage, including changes in suspended solids concentration, solids retention time (SRT), and F/M ratio. Overall, the reactors showed similar trends and maintained stable performance throughout the experiment (Sarvajith & Yarlagadda, 2021). The granular structure, coupled with the diverse microbial consortium, contributed to the system's adaptability. This case study demonstrated AGS's capacity to withstand the dynamic operational conditions inherent in brewery wastewater treatment, ensuring reliable and uninterrupted pollutant removal.

e) **Superior removal efficiencies for organic matter and nutrients:** The diverse microbial community thriving within AGS granules enables the simultaneous degradation of a wide range of organic compounds, including recalcitrant pollutants (Fierro et al., 2023). This versatility results in high organic matter degradation rates. Additionally, AGS demonstrates an impressive capability for nutrient removal, particularly nitrogen and phosphorus, owing to its stratified granular structure that provides the necessary microenvironments (oxic, anoxic, and anaerobic layers) for concurrent removal of organic matter and nutrients (Abdullah et al., 2013; Świątczak & Cydzik-Kwiatkowska, 2018; Miyake et al., 2022; Hamza et al., 2022; Fierro et al., 2023). The AGS biotechnology has been variously applied for high-strength wastewater treatment. In a related study focused on wastewater treatment, Zhao et al. (2014) observed remarkable performance from AGS. The lab-scale system achieved outstanding organic matter removal rates exceeding 90 % when employed in a granular membrane bioreactor (Zhao et al., 2014). It also demonstrated exceptional nutrient removal efficiencies, with nitrogen removal rates exceeding 90 % in both SBR and GMBR. Moreover, AGS displayed efficient phosphorus removal, with removal rates surpassing 90 % in GMBR. These findings collectively underscore the efficacy of AGS in effectively eliminating pollutants from wastewater (Zhao et al., 2014).

In another study, AGS was used to treat high strength wastewater from a dairy plant. The study reported impressive removal efficiencies, with 90 % removal of total COD, 80 % removal of TN, and 67 % removal of TP achieved at a VER of 50 %. These results highlight the effectiveness of AGS in treating high strength organic wastewaters containing nutrients (Adav et al., 2008).

A study by Di Biase et al. (2012) investigated the use of AGS to treat anaerobically pretreated brewery wastewater at different loading rates (Show et al., 2012). The study found that stable granulation was achieved within two weeks and the size of the granules increased according to the organic loading rate (OLR) applied. The results indicated that low C: N:P and F/M ratios were favorable to achieve stable aerobic granules in the long term. The carbon removal rate was load-independent in the range examined (TCOD removal >80 %), whereas TN removals were inversely proportional to the OLRs. Overall, a longer aeration reaction time with a lower OLR was beneficial to granular structure, which exhibited a compact and defined architecture. Performance results within the other conditions studied further indicated that the microbial community and its complex functionality in nutrient removal was efficient at operational parameters of OLR at 0.8 ± 0.2 kg- kg TCOD/m^3·d and F/M ratio at 0.5 ± 0.2 kg TCOD/m^3·d (Show et al., 2012).

Another study by De Vleeschauwer et al. (2021) investigated the treatment of brewery wastewater with AGS operating at different OLRs between 1.2 and 1.5 kg TCOD/m^3·d. The study found that the AGS system was able to remove COD, TN, and TP effectively. The results indicated that the AGS system was stable and robust, with a high COD removal efficiency of 95 % and a TN removal efficiency of 80 % (Vleeschauwer et al., 2021).

2.4. Research gaps relating to AGS for brewery wastewater

Despite the advancements in AGS biotechnology, its application to brewery wastewater is limited. Brewery effluents, characterized by high COD levels ranging from 1,000 to 8,000 mg/L, pose a significant challenge due to their high organic content, low nutrient balance, and seasonal variability in production. Current research has primarily focused on municipal wastewater and industrial effluents with moderate COD levels; however, studies addressing brewery wastewater at COD levels of 3,000 mg/L and above remain scarce (Biase et al., 2020; Smetana & Grosser, 2024).

One of the critical factors influencing AGS performance in brewery wastewater treatment is the COD concentration in the influent. COD determines the organic load and treatment capacity of the system. High COD levels can lead to excessive production of extracellular polymeric substances (EPS), which are essential for granule formation but may also hinder the process by overwhelming microbial communities and causing incomplete degradation of organic matter (Ferraz et al., 2014). Conversely, low COD levels may lead to insufficient substrate for microbial growth, reducing treatment efficiency (Ferraz et al., 2014). Therefore, carefully regulating COD levels is essential for ensuring effective treatment and minimal production of toxic by-products.

Similarly, HRT plays a crucial role in AGS system performance. The duration for which wastewater remains in the reactor influences the balance between substrate availability and microbial growth. Studies indicate that an optimal HRT enhances pollutant removal by allowing sufficient time for microbial activity while maintaining granular sludge stability and preventing biomass washout (Wang et al., 2021b). Variations in cycle numbers (e.g., 4, 5, and 6 cycles/d) significantly impact microbial dynamics, substrate uptake, and granule stability. Research has shown that adjusting HRT to appropriate values enhances biological filtration efficiency, influencing both nutrient removal and microbial activity levels within AGS systems (Chys et al., 2017). However, few studies have systematically evaluated HRT for brewery effluents, making this an area ripe for innovation (Stes et al., 2018; Biase et al., 2020).

While AGS biotechnology has demonstrated exceptional efficiency in treating various wastewaters, its application to brewery wastewater, particularly in adjusting COD and HRT parameters, remains limited. Addressing these operational gaps will contribute to the development of robust guidelines for AGS applications in brewery effluents (Stes et al., 2018; Biase et al., 2020).

Chapter 3 Materials and Methods

3.1. Feed wastewater

Synthetic wastewater was prepared for all experiments using high-purity chemicals of more than 90% purity to simulate the necessary composition and achieve a total COD to nitrogen to phosphorus ratio of 100:5:0.5. This COD:N:P ratio is based on tests conducted with samples collected from Deadfall Brewing Company in Prince George, Canada (Table A1), and it also aligns with literature on high strength wastewater treated using AGS (Hamza et al., 2018). Sodium acetate anhydrous (>99%) and sodium propionate (99%) were utilized as carbon sources, while ammonium chloride (NH_4Cl) served as the nitrogen source. Potassium phosphate dibasic (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) provided phosphorus. Additionally, calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), and iron (II) sulfate ($FeSO_4 \cdot 7H_2O$) were included to supply essential micronutrients to support microbial growth and activities within the system. This composition ensured the synthetic wastewater closely mimicked actual wastewater.

The composition of the wastewater for total COD concentration of 1000 mg/L was as follows: sodium acetate anhydrous, 0.938 g/L; sodium propionate, 0.208 g/L; NH_4Cl, 0.19 g/L; K_2HPO_4, 0.0225 g/L; KH_2PO_4, 0.0195 g/L; $CaCl_2 \cdot 2H_2O$, 0.035 g/L; $MgSO4 \cdot 7H_2O$, 0.013 mg/L; $FeSO_4 \cdot 7H_2O$, 0.01 mg/L; and, micronutrients stock solution, 1 mL/L. A micronutrient stock solution containing (in g/L): H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl2$, 0.03; $MnSO_4.H_2O(NH_4)_6$, 0.05; $Mo_7O_{24}.4H_2O$, 0.05; $AlCl_3$, 0.05; $CoCl_2.6H_2O$, 0.05 and $NiCl_2$, 0.05, was used (Tay et al., 2002).

The synthetic wastewater was prepared every 3 d, as this is the duration required for the wastewater to be completely consumed by the system. Additionally, an extra 5 L was prepared each time to ensure the bioreactor did not run empty before the replacement process was complete.

This extra volume helped prevent interruptions, especially when the hoses could not reach the very bottom of the bioreactor.

3.2. Seed sludge

Granules were cultivated in another bioreactor, and after maturation, the granules were transferred into the three bioreactors for the experimental runs. The seed granules had both 5- and 30-min sludge volume index (SVI) of 32 mL/g which resulted in an SVI_5/SVI_{30} ratio of 1 (indicating successful granulation). The seed sludge had MLSS concentration of 11,900 mg/L. The bioreactor from which the seed granules were withdrawn was operated with feed wastewater containing a COD concentration of 400 mg/L, 19 mg/L of ammonia-nitrogen concentration, and 5.5 mg/L of phosphate (PO_4-P) concentration. The bioreactor was operated at room temperature (21 ± 2°C).

3.3. Experimental design and setup

3.3.1. Experimental design

A 2^2 full factorial experimental design with a replicated center point was employed to determine the impact of COD and HRT on the treatment of brewery wastewater in AGS systems. The study sought to evaluate the individual and interactive effects of these parameters on the treatment of brewery wastewater using the AGS biotechnology.

Total COD and HRT were selected for their significant influence on microbial dynamics, pollutant removal efficiency, and system stability. The SRT was maintained at 8 d. Each experimental run spanned three SRT cycles, equivalent to 24 d, allowing the microbial community to adapt to the imposed conditions. This approach minimized fluctuations and ensured consistent system performance.

Total COD levels were set at 3,000 mg/L, 4,000 mg/L, and 5,000 mg/L to simulate typical brewery wastewater characteristics and test the system's capacity to handle varying organic loads. These levels fall within the range of COD concentration for brewery effluent (Hamza et al., 2018), supporting the study's relevance to real-world brewery wastewater characteristics.

HRT was set at 10.67 h, 12.8 h and 16 h, to evaluate its effect on substrate degradation. These HRTs correspond to operating the SBR at 4, 5, and 6 cycles per day, respectively. This design allows for the assessment of the optimal balance between treatment efficiency and operational practicality. By selecting HRTs that span this range, the study captures a broad spectrum of operational conditions.

These parameters were critical for determining the system's capacity to achieve effective pollutant removal under different operational conditions.

3.3.2. Setup and operation

The experiments were performed in three 5 L AGS bioreactors constructed from acrylic (Plexiglas) material, with a diameter of 9 cm and a height-to-diameter (H/D) ratio of 7 The bioreactors had a working volume of 4 L and operated in SBR mode. Detailed operational parameters of the SBR setup are provided in Table 3.1. At the base of the bioreactors, air diffusers (AS4, Pentair, Sanford, US) were positioned to create air bubbles at a superficial up-flow velocity of 2 cm/s. Impact-resistant panel-mount air flowmeters (5079K27, McMaster-Carr, Cleveland, USA) were used to measure the air velocity. Peristaltic pumps (BT100-2J, Longer Pump, China) were used for feed inlet and effluent withdrawal from the bioreactors. Effluent was withdrawn through port #8 (Figure 3.1) situated at the reactor's mid-height, achieving a VER of 38%. A schematic of the experimental arrangement is presented in Figure 3.1.

Table 3.1: SBR operational conditions

Run #	COD concentration (mg/L)	Feeding time (min)	Aeration time (min)	Settling time (min)	Decanting time (min)	Idle time (min)	Cycle time (h)	HRT (h)
1	2900 ± 180	60	275	15	8	2	6.0	16.0
2	5050 ± 140	60	275	15	8	2	6.0	16.0
3	2900 ± 180	60	155	15	8	2	4.0	10.7
4	3900 ± 100	60	203	15	8	2	4.8	12.8
5	3900 ± 100	60	203	15	8	2	4.8	12.8
6	4900 ± 120	60	155	15	8	2	4.0	10.7

In this study, the center point was replicated twice to estimate pure error and detect curvature in the response surface. Replicating the center point provided a practical means to assess experimental error and model adequacy without exceeding available resources. This approach aligns with standard practices in experimental design, where center point replication is utilized to test for lack of fit and ensure model reliability (Veza et al., 2023; JMP Statistical Discovery, 2024).

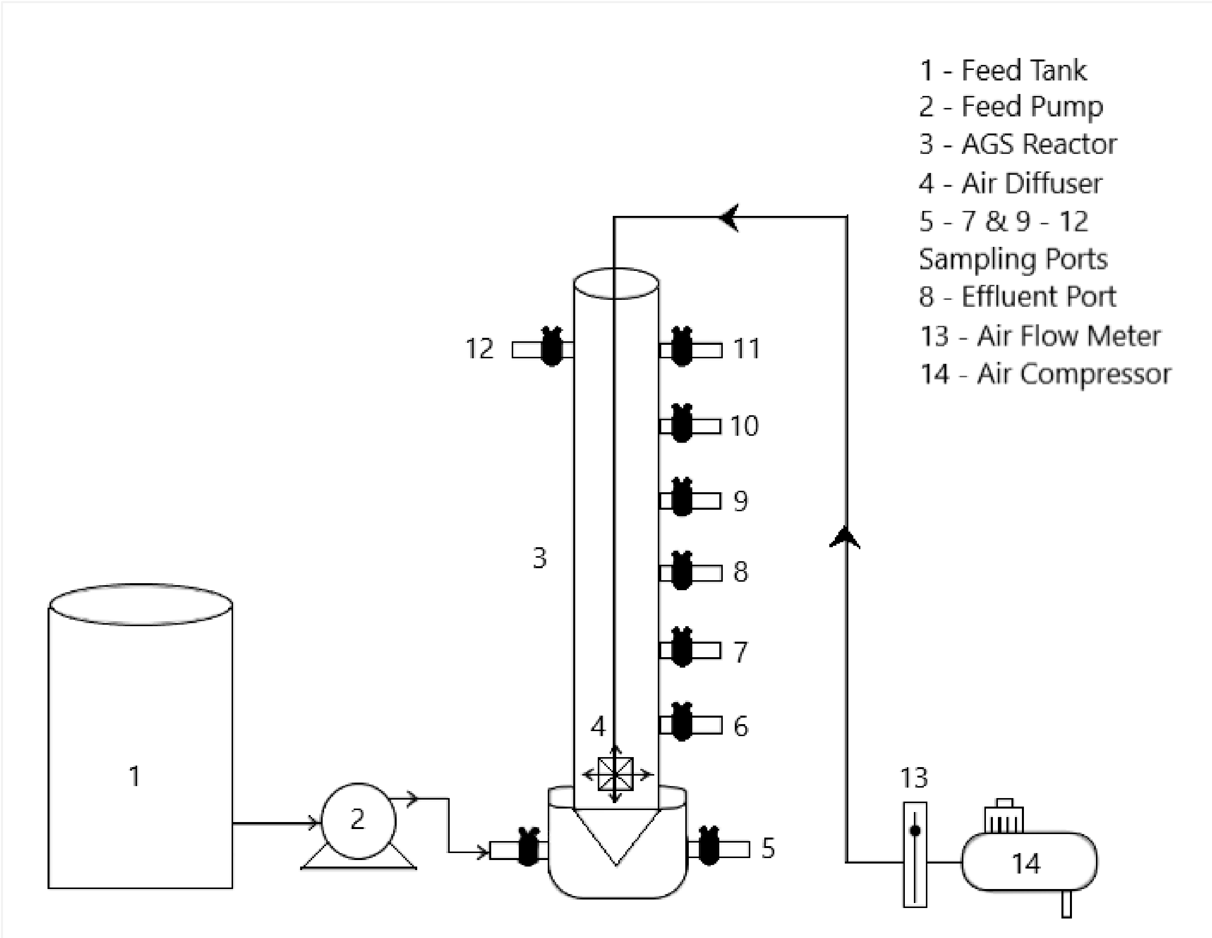


Figure 3.1: Schematic diagram of the experimental setup

3.4. Analytical methods

The analytical methods were divided into two categories: biomass characteristics and wastewater characteristics. Biomass characteristics included MLSS, MLVSS, SVI_5, and SVI_{30}, which were measured to assess biomass growth and sludge settleability. Wastewater characteristics comprised COD, ammonia, nitrite, nitrate, phosphate, pH, and TSS, evaluated to determine treatment efficiency. Measurements were taken twice a week in triplicates on days 4, 7, 15, 18, 21, and the final day of the run, day 24, to ensure consistent monitoring across all six bioreactor runs.

3.4.1. Biomass characteristics

Biomass characteristics, including MLSS, mixed liquor volatile suspended solids (MLVSS), effluent TSS, and 5- and 30-min SVI, were measured using standard methods of water and wastewater (Lipps et al., 2023). The procedures are explained as follows:

i. MLSS and MLVSS:

Method 2540 D was used to determine the MLSS concentration of the bioreactor mixed liquor. A 10 mL composite sample of the mixed liquor was collected from ports 5, 6, 7, and 8 of the bioreactors (Figure 3.1), during the aeration phase. The sample was subsequently filtered through a pre-weighed standard glass-fiber filter. After filtration, the filter containing the retained residue was dried at 105°C for about 12 h in an Isotemp™ 500 Series Economy Lab Oven (Fisher Scientific, Canada). The samples were cooled in a desiccator (CG122612, Chemglass Inc, Canada). Samples were taken in triplicate. The increase in the filter's weight represented the total suspended solids (Lipps et al., 2023). MLSS was calculated using Equation (1):

$$mg\ total\ suspended\ solids/L = \frac{(A-B)\times 1000}{sample\ volume, ml} \quad \ldots\ldots \quad (1)$$

Where:

A: The weight of the filter paper plus dried residue (mg).

B: The weight of the filter paper (mg).

Method 2540 E was used to determine the MLVSS concentration. The dried sample from the MLSS step was then subsequently ignited in Thermolyne™ small benchtop muffle furnace (Fisher Scientific, Canada) at 550°C for 15 min to burn off the organic portion (volatile solids). The remaining inorganic residue was then cooled in a polycarbonate desiccator (Chemglass Inc, Canada) and weighed (Lipps et al., 2023). MLVSS was calculated using Equation (2):

$$mg\ volatile\ suspended\ solids/L = \frac{(A-D)}{sample\ volume, ml} \quad \ldots\ldots \quad (2)$$

Where:

A: The weight of filter paper plus dried residue (after the sample has been dried at 105°C), mg

D: The weight of filter paper after ignition, mg

ii. SVI:

The SVI was determined using standard method 2710D (Lipps et al., 2023). Both SVI_5 and SVI_{30} were determined to assess the sludge settleability. A 1 L well-mixed sample of the mixed liquor was collected from the bioreactor during the aeration phase at designated points and allowed to settle in a 1-L graduated cylinder for 5- and 30-min periods, respectively. The SVI was then calculated as follows:

$$SVI\ (mL/g) = \frac{settled\ sludge\ volumet\ (mL/L) \times 1000}{suspended\ solids\ concentration\ (mg/L)}$$

3.4.2. Wastewater characteristics

i. COD:

Total COD was determined using the COD digestion vials, high range (HR) and low range (LR) by the United States Environmental Protection Agency (USEPA) Reactor Digestion method (Hach Method 8000, US EPA) which involved using pre-prepared COD digestion reagent vials, which contained a strong oxidizing agent (potassium dichromate), sulfuric acid, and catalysts.

The HR COD vials have a detection limit in the range: 20 – 1500 mg/L. Influent samples were diluted by a factor of 4 by adding 1.5 mL of deionized water to 0.5 mL of the sample prior to analysis (Hach, 2015a). The LR COD vials which were used for the determination of COD concentration of the effluent samples had a detection limit of 3–150 mg/L. No dilution was required for these samples (Hach, 2015b).

After samples were prepared, the vials were tightly sealed, mixed by gentle inversion, and then placed into a Hach digester (DRB200, Hach, Canada) which was pre-heated to a temperature of 150ºC and allowed to stay in the digester for 2 h. After digestion, the vials were allowed to cool to approximately 120°C in the digester before further cooling to room temperature in a test tube rack. Spectrophotometric analysis was then performed at 620 nm for HR samples and 420 nm for LR samples in a Hach spectrophotometer (DR3900, Hach, Canada). The COD concentration was determined by measuring the change in absorbance, which corresponded to the reduction of dichromate ions ($Cr_2O_7^{2-}$) to chromic ions (Cr^{3+}).

All necessary safety precautions were followed, including handling corrosive and toxic reagents with appropriate protective equipment, and disposing of reacted samples as hazardous waste per regulatory requirements.

ii. Ammonia-nitrogen (NH_3-N):

Ammonia-nitrogen (NH_3-N) concentrations in both influent and effluent samples were determined using the Hach Ammonia Nessler Method 8038. For the analysis, a 25 mL sample was prepared by diluting 0.5 mL of the filtered sample (filtered using 0.45 µm filter) with 24.5 mL of deionized water. To prevent interference from calcium and magnesium ions, three drops of mineral stabilizer were added, followed by three drops of polyvinyl alcohol dispersing agent to enhance the sensitivity of the test by dispersing the color-forming complex. Finally, 1 mL of Nessler reagent was added to the mixture a pipette, which reacted with ammonia to form a yellow-colored complex, with the intensity proportional to the ammonia concentration. The sample was allowed to react for 1 min before being transferred to a 10 mL cell, which was then placed into the cell holder of a Hach spectrophotometer (DR3900, Canada). The spectrophotometer was set to program 380 N (Ammonia, Ness) and the NH_3-N concentration of the sample was read from the display screen.

iii. Nitrite-nitrogen (NO_2-N):

Nitrite-nitrogen (NO_2-N) concentrations in the effluent were determined using colorimetric analysis with a test kit employing the Diazotization method 8507 and Hach NitriVer®3 powder pillows for low-range nitrite (0.002 – 0.300 mg/L NO_2-N). For the analysis, a 10 mL sample was prepared by mixing 0.4 mL of the filtered effluent sample (filtered using 0.45 µm filter) with 9.6 mL of deionized water. The contents of one NitriVer®3 nitrite reagent powder pillow were added to the sample, which was then mixed to dissolve. The mixture was allowed to stand for 20 min to allow for reaction. In the presence of nitrite, a pink color developed after the reaction. After 20 min, the content was poured into a sample cell and placed in a Hach spectrophotometer (DR3900, Canada). The spectrophotometer program was set to program 371N (Nitrite LR PP) and the NO_2-N concentration read from the display screen.

iv. Nitrate-nitrogen (NO_3-N):

Nitrate-nitrogen (NO_3-N) in the effluent was analyzed using the Cadmium Reduction Method (Method 8039), employing NitraVer® 5 nitrate reagent powder pillows, designed for concentrations between 0.3 to 30 mg/L NO_3-N. The sample preparation involved preparing both the actual effluent samples and a blank for instrument calibration. A 10 mL blank sample was prepared using deionized water to zero the instrument. For the actual effluent samples, 0.4 mL of the filtered effluent (filtered through a 0.45 µm filter) was diluted with 9.6 mL of deionized water, resulting in a 10 mL prepared sample for analysis. The content of one NitraVer® 5 nitrate reagent powder pillow was then added to the sample and thoroughly mixed to dissolve the content of the powder pillow completely. After mixing, the mixture was allowed to stand for 1 min for reaction.

Then the solution was left for 5 more min. The resulting amber color indicated the presence of nitrate, with the intensity directly correlating to the nitrate concentration. The sample was then placed in Hach spectrophotometer (DR3900, Canada) and the program set to program 355 N to obtain the concentration of NO_3-N in mg/L. The final result was recorded after zeroing the instrument with the blank sample, ensuring accurate measurement.

v. Phosphate (PO_4-P):

Phosphate (PO_4^{3-}–P) concentrations in the effluent were analyzed using Method 8048, employing PhosVer® 3 powder pillows for reactive phosphorus (orthophosphate) determination. A 10 mL sample was prepared in a sample cell by mixing 0.4 mL of the filtered effluent sample (filtered using 0.45 µm filter) 9.6 mL of deionized water, to which the content of one PhosVer® 3 Phosphate Reagent Powder Pillow were added. The mixture was thoroughly mixed to dissolve the contents of the powder pillow completely. After mixing, the mixture was allowed to react for 2 min. A blue colour developed in the presence of phosphate. After the 2-min reaction time, the content was poured into a sample cell and placed in the Hach spectrophotometer (DR3900, Canada) and the program set to program 490 P. Prior to placing the sample in the spectrophotometer, it had been previously zeroed using a blank sample. The spectrophotometer then provided the PO_4^{3-}–P concentration of the sample, reported in mg/L PO_4^{3-}P.

vi. pH:

The pH of both the influent and effluent samples were measured twice a week using DPH 7011 Digital pH Meter (General Tools and Instruments, New York, USA). The pH was measured by inserting the pH probe into the sample and taking the reading that displayed on the meter's screen.

vii. TSS:

Method 2540 D was used for effluent TSS determination. During the decanting phase, 1.5 L of effluent was decanted within 8 min. This 1.5 L was then collected and thoroughly mixed. A 10 mL sample was subsequently taken from the well-mixed effluent and filtered through a pre-weighed standard glass-fiber filter paper. The filter with the retained residue was then dried to a constant weight at 105°C for a minimum of about 12 h in an Isotemp™ 500 Series Economy Lab Oven (Fisher Scientific, Canada). The samples were cooled in a polycarbonate desiccator (Chemglass Inc, Canada). The samples were taken in triplicate. The increase in the weight of the filter represents the total suspended solids, calculated using the same formula as MLSS (Lipps et al., 2023).

3.5. Statistical analyses

Statistical analyses, including descriptive statistics (mean and standard deviation), scatter-based regression analysis, and factorial design interpretation, were conducted using Design Expert software to determine the significance of the main effects and interactions among the experimental factors. The means used in the regression analysis are from pseudo-replicates, three replicates taken from each test at the same sampling time, representing steady-state data collected from days 10–15 until the end of the cycle on day 24. Linear scatter plots were generated to evaluate trends in COD, ammonia, and phosphorus removal efficiencies. These plots provide a visual assessment of the relationship between operational parameters and treatment performance. Parameter selection and performance assessment were performed using the 2^2 factorial designed experiments to identify conditions that favor higher removal efficiencies within the tested ranges.

Chapter 4 Results and Discussion

4.1.Overall bioreactor performance

Table 4.1 provides a summary of the bioreactor and effluent characteristics across all six bioreactor runs. The means reported are from pseudo-replicates, three replicates taken from each test at the same sampling time, representing steady-state data collected from days 10–15 until the end of the cycle on day 24. The effluent COD concentrations ranged from 4.8 mg/L in Run 3 to 16 mg/L in Run 2. MLSS and MLVSS values varied, reflecting differences in biomass retention, with higher values in runs with longer HRTs of 16 h (Runs 1 and 2; MLSS: 15,600 and 15,000 mg/L, respectively). TSS and SVI results indicated that shorter HRTs, such as in Run 6 (TSS: 870 mg/L, SVI_5: 70 mL/g), led to poorer sludge settling characteristics and higher biomass washout.

The OLR ranged from 4.4 kg COD/m^3·d to 11 kg COD/m^3·d, highlighting the impact of influent COD and HRT on organic loading. To maintain an SRT of 8 d, wasting rates were set at 460 mL/d in Runs 1 and 2 and 500 mL/d in Runs 4 and 5. In contrast, no sludge wasting was applied in Runs 3 and 6 due to significant biomass washout caused by poor settling. As a result, the SRT in these two runs decreased to 6 d, as reflected by the elevated effluent TSS concentrations of 630 mg/L and 870 mg/L, respectively.

Nutrient concentrations, including ammonia, nitrite, nitrate, and phosphate, varied based on operational conditions. Runs 1, 2, 4, and 5, with SRTs of 8 d, achieved high ammonia (94–98%) and phosphorus (74–96%) removal. Run 3, with an SRT of 6 d, achieved 90% ammonia and 69% phosphorus removal, reflecting partial washout of nitrifiers and PAOs under hydraulic stress (HRT 10.67 h). Run 6 (SRT 6 d) showed similar ammonia removal (90%) but lower phosphorus removal (58%), aggravated by a higher OLR (11 kg COD/m^3·d).

This demonstrates the AGS system's resilience in retaining functional granules despite the washout, though nutrient removal efficiency decreased with lower SRTs in Runs 3 and 6, where the target SRT of 8 d could not be achieved due to excessive biomass loss. The Ammonia Loading Rate spanned 0.2 kg NH_3-N/m^3·d to 0.6 kg NH_3-N/m^3·d, indicating varying nitrification demands across the runs. The pH remained relatively stable across all runs, typical for aerobic systems.

Table 4.1: Summary of steady-state effluent characteristics across all six bioreactor runs

Parameters	Bioreactor experimental runs					
	Run 1 (COD: 2900 ± 180 mg/L, HRT: 16h)	**Run 2** (COD: 5050 ± 140 mg/L, HRT: 16h)	**Run 3** (COD: 2900 ± 180 mg/L, HRT: 10.67h)	**Run 4** (COD: 3900 ± 100 mg/L, HRT: 12.8h)	**Run 5** (COD: 3900 ± 100 mg/L, HRT: 12.8h)	**Run 6** (COD: 4900 ± 120 mg/L, HRT: 10.67h)
Effluent TCOD[a] (mg/L)	13 ± 3	16 ± 3	5 ± 1.8	6 ± 1	7 ± 1	13 ± 1
MLSS[b] (mg/L)	15,600 ± 724	15,000 ± 124	8,910 ± 61	13,300 ± 1,220	13,500 ± 1,840	12,400 ± 527
MLVSS[c] (mg/L)	13,500 ± 699	13,000 ± 208	7,000 ± 92	11,400 ± 1,066	11,500 ± 1,700	10,400 ± 1,450
Effluent TSS[d] (mg/L)	40 ± 33	90 ± 5	630 ± 25	13 ± 2	11 ± 2	870 ± 24
SVI_5[e] (mL/g)	40 ± 3	42 ± 1	90 ± 1	39 ± 3	37 ± 6	70 ± 2
SVI_{30}[f] (mL/g)	34 ± 1	38 ± 1	68 ± 1	32 ± 2	29 ± 4	40 ± 5
pH[g]	9.31 ± 0.04	9.43 ± 0.05	9.21 ± 0.10	9.13 ± 0.20	9.14 ± 0.20	9.11 ± 0.15
Influent $NH_3 - N$[h] (mg/L)	150 ± 2	250 ± 2	150 ± 2	200 ± 2	200 ± 2	250 ± 3
Effluent $NH_3 - N$[h] (mg/L)	6 ± 0	7 ± 1	15 ± 2	12 ± 1	12 ± 2	25 ± 3
$NO_2 - N$[i] (mg/L)	7.6 ± 0.6	0.1 ± 0.0	2.1 ± 0.1	1.0 ± 0	1.0 ± 0.0	0.1 ± 0.0
$NO_3 - N$[j] (mg/L)	16 ± 2	3 ± 1	17 ± 1	14 ± 2	14 ± 2	9 ± 2
Influent $PO_4 - P$[k] (mg/L)	16 ± 1	24 ± 2	16 ± 1	19 ± 2	19 ± 2	24 ± 2
$PO_4 - P$[k] (mg/L)	1 ± 0	3 ± 0	5 ± 0	4 ± 1	5 ± 1	12 ± 4
OLR[l] (kg COD/m³·d)	4.4	7.6	6.5	7.3	7.3	11
Ammonia Loading Rate (kg NH_3-N/m³·d)	0.2	0.4	0.3	0.4	0.4	0.6
SRT[m] (d)	8	8	6	8	8	6

[a] Total Chemical Oxygen Demand, [b] Mixed Liquor Suspended Solids, [c] Mixed Liquor Volatile Suspended Solids, [d] Total Suspended Solids, [e] Sludge Volume Index at 5 min, [f] Sludge Volume Index at 30 min, [g] Potential of Hydrogen, [h] Ammonia Nitrogen, [i] Nitrite Nitrogen, [j] Nitrate Nitrogen, [k] Phosphate Phosphorus, [l] Organic Loading Rate, [m] Sludge Retention Time.

4.2. COD removal

The effluent COD concentrations across the six experimental runs (Run 1 to Run 6) exhibited notable variations, as shown in Figure 4.1, which can be attributed to the different influent COD concentrations and HRT applied during the experiments.

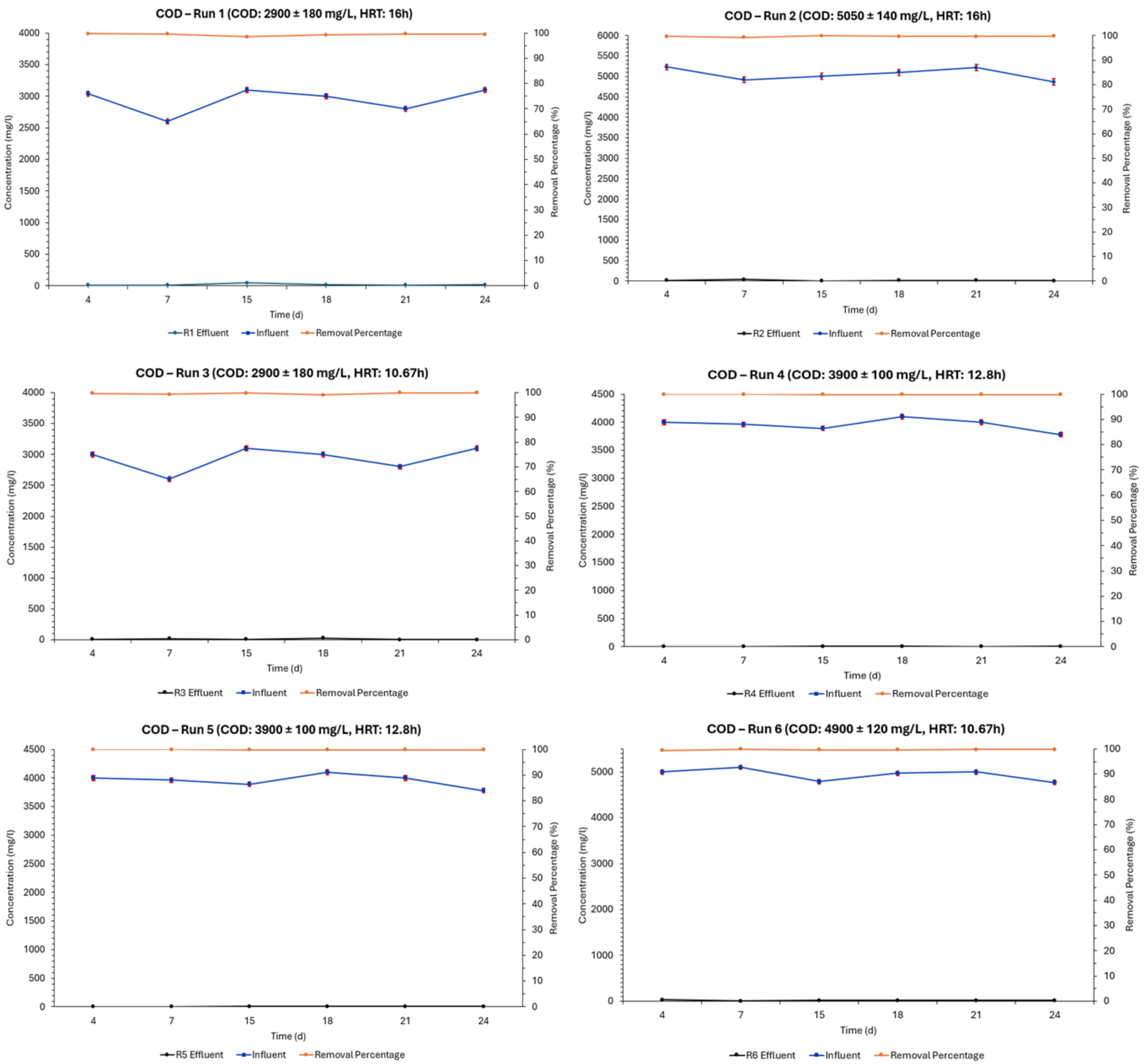


Figure 4.1: COD removal trends across the six experimental runs (Run 1 to Run 6). The graphs display influent and effluent COD concentrations, and COD removal efficiency over time.

During Run 1 (COD: 2900 ± 180 mg/L, HRT: 16 h), the AGS system achieved effluent COD concentrations of 13 ± 3 mg/L, resulting in 99.6% removal efficiency. These findings are typical of AGS systems where high organic loading rates are effectively led by dense granular biomass, which enhances microbial activity and COD degradation through simultaneous aerobic and anoxic processes within the granule structure (Yarlagadda et al., 2019). The extended HRT of 16 h likely maximized contact time between the wastewater and the microbial consortia, such as *Meganema* and *Thauera*, known for organic degradation, pushing removal efficiencies beyond the 90–96% commonly reported for lower COD influents (Świątczak & Cydzik-Kwiatkowska, 2018; Iorhemen et al., 2019). Furthermore, the system's ability to maintain stability and high biomass retention under such conditions aligns with observations in full-scale and pilot-scale AGS setups treating high-strength wastewaters, where granulation improves settling and pollutant removal (Campos et al., 2021; Zou et al., 2022)

In Run 2 (COD: 5050 ± 140 mg/L, HRT: 16 h), a similar high removal efficiency of 99.68% was observed, with an effluent COD of 16 ± 3 mg/L. This high efficiency underscores the AGS system's effectiveness for high-strength brewery wastewater, achieving near-complete organic removal, as seen in AGS systems reducing COD from ~5000 mg/L to <50 mg/L at HRTs of 12–24 h (M. Pronk et al., 2015; Świątczak & Cydzik-Kwiatkowska, 2018). The 16-h HRT supported robust aerobic degradation, with effluent COD of 16 ± 3 mg/L aligning with stable AGS performance reporting 10–20 mg/L residuals (Iorhemen et al., 2019). These results demonstrate the AGS bioreactor's reliability in treating high-COD wastewater under the tested conditions.

In Run 3 (COD: 2900 ± 180 mg/L, HRT: 10.67 h), the effluent COD concentration was 5 ± 1.8 mg/L, with steady-state values observed from day 15 to 24, yielding a COD removal efficiency of 99.8%. This rapid stabilization and near-complete removal reflect the AGS system's robustness for brewery wastewater, consistent with steady-state COD reductions to <50 mg/L within 10–20 days at HRTs of 12–20 h (M. Pronk et al., 2015; Świątczak & Cydzik-Kwiatkowska, 2018). Although the removal efficiency was still very high (99.8%), it is slightly lower than Run 2's 99.7% because the shorter HRT of 10.67 h reduced microbial contact time, leading to a higher residual COD, as shorter HRTs limit degradation efficiency (Rosman et al., 2014; Iorhemen et al., 2019). This aligns with literature reporting decreased COD removal with reduced HRT due to insufficient exposure for aerobic processes (Rosman et al., 2014).

For both Runs 4 and 5 (COD: 3900 ± 100 mg/L, HRT: 12.8 h), the mean effluent COD concentration was 6 ± 1 mg/L and 7 ± 1, respectively, with steady-state values observed from day 15 until end of run at day 24. These two runs, being the replicate and center points, showed similar performance, possibly due to the microbial community adapting to the organic load effectively and stabilizing earlier than most of the other runs. The steady-state performance in these runs indicates a favorable balance between COD concentration and HRT, which has been previously reported to enhance granule formation and improve organic matter removal efficiency (Pirsaheb et al., 2017; Sanchez-Sanchez et al., 2023).

In Run 6 (COD: 4900 ± 120 mg/L, HRT: 10.67 h), the mean effluent COD concentration was 13 ± 1 mg/L, with a peak observed at Day 4 (30 mg/L). This early peak suggests the AGS system was adjusting to the high COD load, as seen in initial acclimation phases with effluent COD spikes before stabilization (Adav et al., 2008; Świątczak & Cydzik-Kwiatkowska, 2018). However, as the system adapted, COD concentrations dropped, reaching a steady state from Day 15 to the end of the run, achieving a COD removal efficiency of 99.7%. This exceptional efficiency highlights the AGS system's capacity to treat brewery wastewater, reducing COD from ~5000 mg/L to <20 mg/L, consistent with reported AGS performance at HRTs of 8–12 h (Pronk et al., 2015; Iorhemen et al., 2019). These high removal rates, exceeding 95% even for high-strength effluents, underscore AGS's effectiveness and align with literature on aerobic granular systems managing similar organic loads.

Across all six runs, the AGS system consistently achieved COD removal efficiencies of 99.6% to 99.9%, reducing influent COD ranging from 2900 ± 180 mg/L to 5050 ± 140 mg/L to effluent concentrations of 5–16 mg/L, despite HRTs varying from 10.67 to 16 h. These very high removal rates, sustained across moderate to high organic loads typical of brewery wastewater, reflect the AGS system's exceptional microbial degradation capacity, aligning with reported efficiencies of >90–95% in similar aerobic systems (Pronk et al., 2015; Iorhemen et al., 2019). The ability to maintain near-complete removal at shorter HRTs (10.67 h) and stabilize by Day 15 underscores AGS's adaptability and reliability, even as COD increased from 2900 ± 180 mg/L (Run 3) to 4900 ± 120 − 5050 ± 140 mg/L (Runs 6 and 2), corroborating its suitability for high-strength wastewater treatment (Kebede, 2018; Świątczak & Cydzik-Kwiatkowska, 2018). This consistent performance highlights AGS as an effective biotechnology for achieving stringent effluent standards in industrial applications.

The effluent COD concentrations, ranging from 5 to 16 mg/L across all six experimental runs, meet the effluent quality standards outlined in the Government of Canada's Wastewater Systems Effluent Regulations under the Fisheries Act (SOR/2012-139), which stipulate a maximum carbonaceous biochemical oxygen demand (CBOD) of ≤25 mg/L, averaged over a year, quarter, or month. Direct CBOD measurements were not performed; however, a conservative estimation was derived from the measured COD values using a COD-to-BOD ratio, a critical parameter in wastewater characterization (Environment and Climate Change Canada, 2025). The ratio of 1.5–2 is well-documented for brewery wastewater, reflecting its predominantly biodegradable organic content (Kothiyal, 2018). The lower bound of 1.5 was selected to ensure a conservative estimate, generating higher CBOD values and thus providing a robust validation that the maximum effluent COD remains within regulatory limits. Applying this ratio, the effluent COD of 5–16 mg/L corresponds to an estimated CBOD range of 3.3–10.7 mg/L, substantially below the ≤25 mg/L threshold. This outstanding performance affirms the AGS system's robust capacity to meet federal organic load requirements, establishing it as a highly effective and reliable technology for treating high-strength brewery wastewater in Canada, with significant potential for broader industrial wastewater management applications.

The analysis of these results highlights the critical role of HRT in determining the overall performance of the AGS system. High COD concentrations can enhance microbial degradation, while shorter HRTs, on the other hand, may not allow sufficient microbial contact time for optimal organic matter degradation. These findings are consistent with the literature (Hamiruddin et al., 2021; Wang et al., 2021a), where similar experimental conditions have been reported to influence COD removal efficiency in AGS systems. The steady-state performance observed in Runs 4, 5, and 6 suggests that a balance between OLR based on COD concentration and HRT is crucial for achieving stable and efficient treatment.

4.3. Nutrient removal

4.3.1. Ammonia removal

For ammonia-nitrogen, the graphs in Figure 4.2 display the influent ammonia concentration, ammonia removal, and the corresponding removal percentage. Figure 4.2 also presents the concentrations of nitrate-nitrogen and nitrite-nitrogen in the effluent.

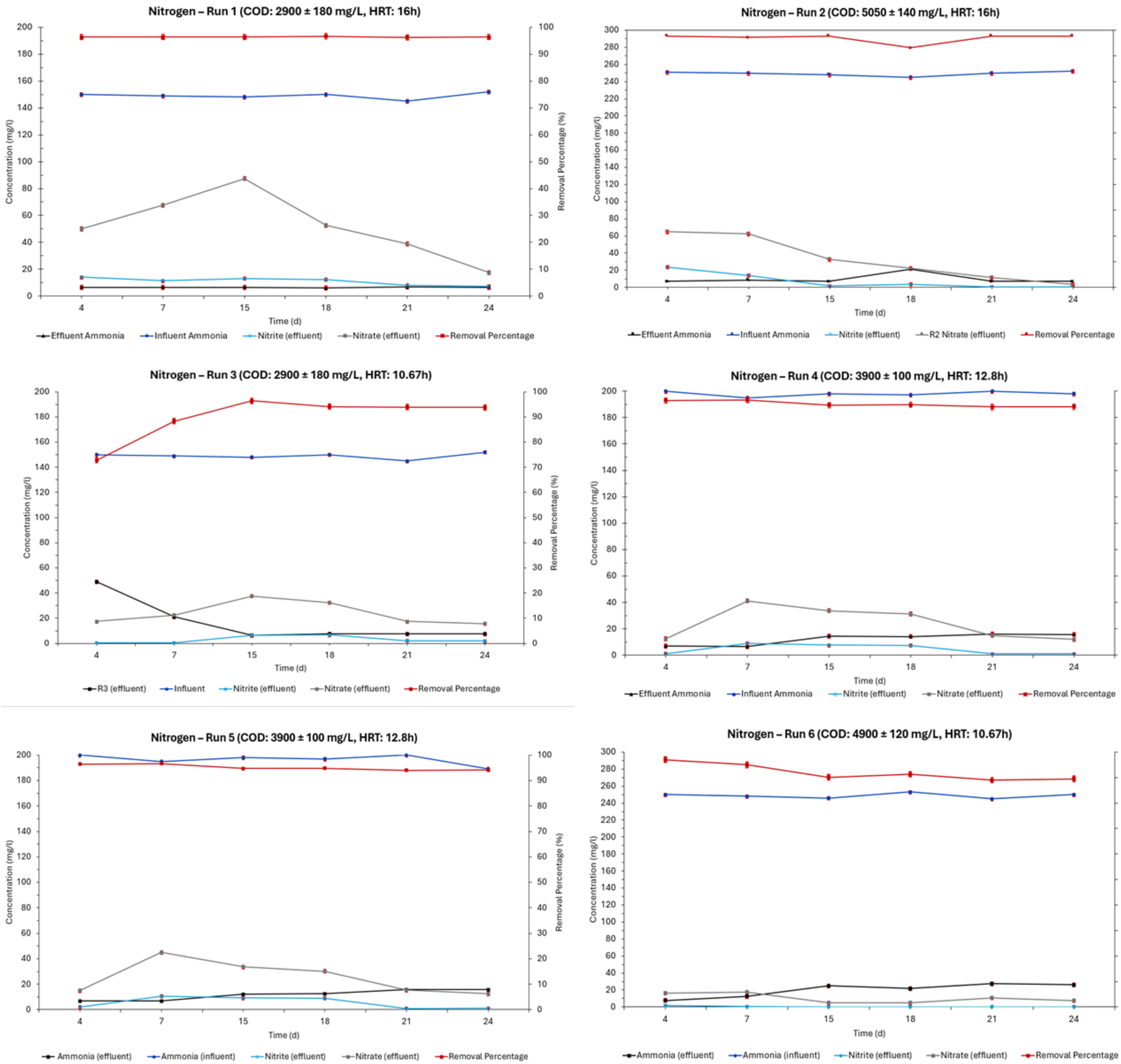


Figure 4.2: Nitrogen removal trends across the six experimental runs (Run1 to Run6). The graphs display influent and effluent ammonia concentrations (mg/L), nitrite and nitrate concentrations (mg/L) in the effluent, and ammonia removal efficiency over time (d).

In Run 1 (COD: 2900 ± 180 mg/ mg/L, HRT: 16h), the system demonstrated a high ammonia removal efficiency of 96%, reducing influent levels from 150 ± 2 mg/L to effluent concentrations of 6 ± 0.2 mg/L. Concurrently, nitrite levels decreased from 14 mg/L on day 4 to 7 mg/L on day 24, and nitrate levels also dropped from 50 mg/L on day 4 to 18 mg/L on day 24. The effluent pH was consistently high at 9.31 ± 0.04. While the high ammonia removal might initially suggest effective nitrification and denitrification, the elevated pH is a critical factor. High pH can inhibit both AOB and NOB, impairing nitrification (Shourjeh et al., 2021). In this run, the high pH likely led to significant ammonia stripping, a physicochemical process where ammonium ions (NH_4^+) are converted to gaseous ammonia (NH_3) and volatilized into the atmosphere. This process is strongly favored at pH values above 9 (Kim et al., 2003). The shift in equilibrium towards NH_3 at alkaline pH is well-established, and the subsequent volatilization is enhanced by aeration in AGS systems (Metcalf & Eddy, 2014).

The decrease in effluent nitrite and nitrate levels over time could indicate some denitrification. However, the high pH might have limited the supply of nitrate (the substrate for denitrification) due to inhibited nitrification (Kim et al., 2003). Additionally, while denitrifiers are less sensitive to high pH, the elevated pH could have enhanced the activity of other heterotrophic bacteria, increasing competition for organic carbon and potentially limiting denitrification efficiency (Michaud et al., 2006; Feng et al., 2023).

Furthermore, a portion of the removed ammonia was likely assimilated into biomass, contributing to the high MLSS values observed (up to 15,600 mg/L). In AGS systems, the dense granular structure promotes the retention of biomass (Adav et al., 2008), and under nitrogen-limiting conditions (due to inhibited nitrification), heterotrophic bacteria efficiently assimilate ammonia for growth (Metcalf & Eddy, 2014). The readily available COD in brewery wastewater would have further fueled this assimilation process.

Overall, the high ammonia removal achieved in Run 1 of the AGS system treating brewery wastewater under alkaline pH conditions was likely the result of a combination of three primary mechanisms. The dominant process was likely ammonia stripping, a physicochemical phenomenon that is significantly enhanced at the elevated pH of 9.31 (EPA, 2000). The high aeration intensity inherent to AGS systems would have further facilitated the volatilization of the gaseous ammonia formed. While some simultaneous nitrification and denitrification (SND) might have occurred, it was likely limited due to the inhibitory effects of the high pH on nitrifying bacteria and potential competition for organic carbon between denitrifiers and other heterotrophic microorganisms (Kim et al., 2003). Finally, a significant portion of the removed ammonia was also likely assimilated into the substantial microbial biomass present in the AGS reactor, contributing to the observed high MLSS values. The elevated pH in Run 1 was a key factor that shifted the dominant nitrogen removal pathways away from conventional nitrification, favoring ammonia stripping and influencing the microbial community dynamics within the AGS.

In Run 2 (COD: 5050 ± 140 mg/L, HRT: 16 h), the system maintained a consistent ammonia removal efficiency of 97%, with a temporary decrease to 93% on day 18. The system achieved ammonia reduction from 250 ± 2 mg/L in the influent to 7 ± 1 mg/L in the effluent. The effluent pH was even higher than Run 1, at 9.43 ± 0.05. Similar to Run 1, the extremely high pH would have strongly promoted ammonia stripping, likely the primary mechanism for ammonia removal (EPA, 2000; Metcalf & Eddy, 2014). The elevated COD in this run might have further enhanced heterotrophic activity, leading to increased competition for organic carbon and potentially limiting denitrification (Michaud et al., 2006; Albina et al., 2019; Lan et al., 2022).

The decrease in effluent nitrite levels from 24 mg/L on day 4 to 0.1 mg/L on day 24, and nitrate levels from 65 mg/L on day 4 to 4 mg/L on day 24 could suggest that some SND occurred. However, the high pH would have significantly inhibited nitrification, limiting the production of nitrate for denitrification (Albina et al., 2019). Furthermore, recent studies emphasize the importance of considering pH effects on the entire microbial community, including denitrifiers. Ammonia assimilation into biomass likely also contributed to ammonia removal and the high MLSS. The high COD provided ample carbon for heterotrophic growth, which would have been accompanied by nitrogen uptake (Bryson et al., 2022; Gu et al., 2023).

In Run 3 (COD: 2900 ± 180 mg/ mg/L, HRT: 10.67 h), the system achieved a steady-state ammonia removal efficiency of 90% from day 18 onward, with ammonia concentrations decreasing from 150 ± 2 mg/L in the influent to 15 ± 2 mg/L in the effluent. However, unlike Runs 1 and 2, effluent nitrite concentrations increased over time, from 0.3 mg/L on day 4 to 2 mg/L on day 24, while nitrate levels remained low, and the effluent pH in this run was 9.21 ± 0.10.

Again, the high pH would have significantly contributed to ammonia removal via stripping (EPA, 2000). However, the increasing nitrite levels strongly suggest incomplete nitrification. The shorter HRT in this run likely exacerbated the inhibitory effect of the high pH on NOB, preventing the efficient oxidation of nitrite to nitrate (Zhang et al., 2018; Shourjeh et al., 2021; Su et al., 2023). This indicates that nitrification, already hindered by the alkaline conditions, was further compromised by the reduced contact time of 10.67 h. While some ammonia was likely assimilated into biomass, the accumulation of nitrite points to a breakdown in the nitrification process, rather than efficient SND (Zhang et al., 2018; Shourjeh et al., 2021).

In Runs 4 and 5 (COD: 3900 ± 100 mg/L, HRT: 12.8 h), a steady state was achieved from day 15 onward, with consistent ammonia removal percentages around 94% where ammonia dropped from 200 ± 2 mg/L in the influent to 12 ± 1 mg/L and 12 ± 2 mg/L in the effluent, respectively. Nitrite and nitrate concentrations in Run 4 and Run 5 followed a similar trend, initially starting at low levels, rising mid-process, and then declining back to near their original values. In Run 4, nitrite levels rose from 1.2 ± 0 mg/L on day 4 to 9.3 ± 0 mg/L on day 7, before declining to 1 mg/L by day 24. A similar trend was observed for nitrate, which increased from 13 mg/L on day 4 to 41 mg/L on day 7, then dropped back to 12 mg/L on day 24. Likewise, in Run 5, nitrite levels increased from 2 mg/L on day 4 to 11 mg/L on day 7, before decreasing to 1 mg/L on day 24. The nitrate levels followed a comparable pattern, rising initially before declining from 15 mg/L to 13 mg/L by the end of the experimental period. While the effluent pH was 9.13 ± 0.20 and 9.14 ± 0.20, respectively.

Elevated influent COD levels typically exceeding 500 mg/L, can significantly impact nitrogen removal in wastewater treatment systems (Phanwilai et al., 2020). High COD concentrations provide substantial organic matter, which can enhance heterotrophic bacterial activity. This increased activity leads to competition for oxygen and other essential resources, such as biodegradable organic carbon sources with nitrifying bacteria. This competition can suppress the activity of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, resulting in lower nitrification rates and, consequently, relatively stable nitrite and nitrate concentrations. A study by Phanwilai et al. (2020) observed that at higher COD loading rates, anoxic COD removal became limited by the nitrite/nitrate supply from the aerobic reactor, affecting overall nitrogen removal efficiency. Additionally, Song et al. (2020) reported that the limited bioavailability of carbon sources, despite high COD levels, coupled with high DO concentrations, adversely affected nitrogen removal efficiencies in full-scale step-feed municipal wastewater treatment plants. Moreover, the still-alkaline pH in these runs would have resulted in significant ammonia stripping, and the fluctuating nitrite and nitrate levels suggest that nitrification and denitrification were unstable, likely due to the inhibitory effect of the high pH on nitrifying bacteria (Metcalf & Eddy, 2014; Zhang et al., 2018). Ammonia assimilation into biomass likely contributed to ammonia removal, but the extent is difficult to quantify without specific measurements.

It is also critical to note that balancing organic loading and retention times is crucial to ensure that nitrifying and denitrifying bacteria can perform their respective functions efficiently, thereby preventing the accumulation of intermediate nitrogen compounds such as nitrite (NO_2^-). Nitrite accumulation can occur when conditions are not optimal for complete nitrification and denitrification, leading to potential issues in water quality (Zhou et al., 2023).

In Run 6 (COD: 4900 ± 120 mg/L, HRT: 10.67 h), the ammonia removal efficiency was 89%, which was slightly lower than the 94% observed in Runs 4 and 5. This reduction can be attributed to the combined effects of high influent COD of 4900 ± 120 mg/L, shorter HRT of 10.67 h, and elevated pH levels of 9.11 ± 0.15. High influent COD levels provide substantial organic matter, which can enhance heterotrophic bacterial activity. This increased activity leads to competition for oxygen and other resources with nitrifying bacteria, potentially suppressing the activity of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (Kim et al., 2003). Such competition can result in lower nitrification rates and, consequently, reduced ammonia removal efficiency. A study by Mirhoseini et al. (2010) observed that at higher COD loading rates of 601 mg/L, anoxic COD removal became limited by the nitrite/nitrate supply from the aerobic reactor, affecting overall nitrogen removal efficiency.

Additionally, the shorter HRT of 10.67 h may not provide sufficient time for complete nitrification and subsequent denitrification processes, especially under high COD conditions. Insufficient HRT can limit the contact time between microorganisms and substrates, hindering the complete oxidation of ammonia to nitrate and the reduction of nitrate to nitrogen gas. This limitation can lead to the accumulation of intermediate nitrogen species and relatively unchanged nitrite and nitrate levels. Research by Li et al. (2012) indicated that a decrease in HRT from 30 to 5 h increased specific ammonium-oxidizing and nitrate-forming rates, suggesting that shorter HRTs can affect nitrification activities and population dynamics.

Furthermore, the elevated effluent pH in Run 6 likely promoted ammonia stripping. At higher pH levels, the equilibrium between ammonium (NH_4^+) and free ammonia (NH_3) shifts towards NH_3, which is volatile and can be removed through air stripping (EPA, 2000). While optimal ammonia stripping typically occurs at pH values above 10.8, significant volatilization can still occur at lower pH levels, especially under conditions of high aeration or turbulence (Guštin & Marinšek-Logar, 2011; Kinidi et al., 2018). These findings underscore the importance of carefully managing operational parameters to balance physical and biological mechanisms for effective ammonia removal.

However, despite the substantial ammonia removal efficiency, the effluent ammonia-nitrogen concentrations of 6–15 mg/L across all runs, measured under effluent conditions of pH 9.11–9.40 and temperature 22°C, result in un-ionized ammonia levels that exceed Canadian standards (Environment and Climate Change Canada, 2025). At 22°C (pKa ≈ 9.075), the un-ionized fraction ranges from 52.0% to 67.9%, yielding 3.12–10.19 mg/L un-ionized ammonia. Adjusted to the regulation's 15°C (pKa ≈ 9.25), the fraction is 42.0–58.5%, resulting in 2.52–8.78 mg/L, significantly surpassing the <1.25 mg/L limit stipulated by the Wastewater Systems Effluent Regulations (SOR/2012-139) (Environment and Climate Change Canada, 2025). This non-compliance is primarily attributable to the elevated effluent pH, which amplifies the un-ionized ammonia fraction. A reduction in pH to 7.8 at 22°C would lower the fraction to 4.8%, producing compliant levels of 0.29–0.72 mg/L, highlighting the need for pH optimization to meet federal standards.

4.3.2. Phosphorus removal

The phosphorus plots shown in Figure 4.3 illustrate the influent and effluent phosphorus concentrations, along with the phosphorus removal efficiencies, across the six experimental runs. The influent phosphorus concentrations are represented as consistent baselines, while the effluent concentrations display trends over the experimental period.

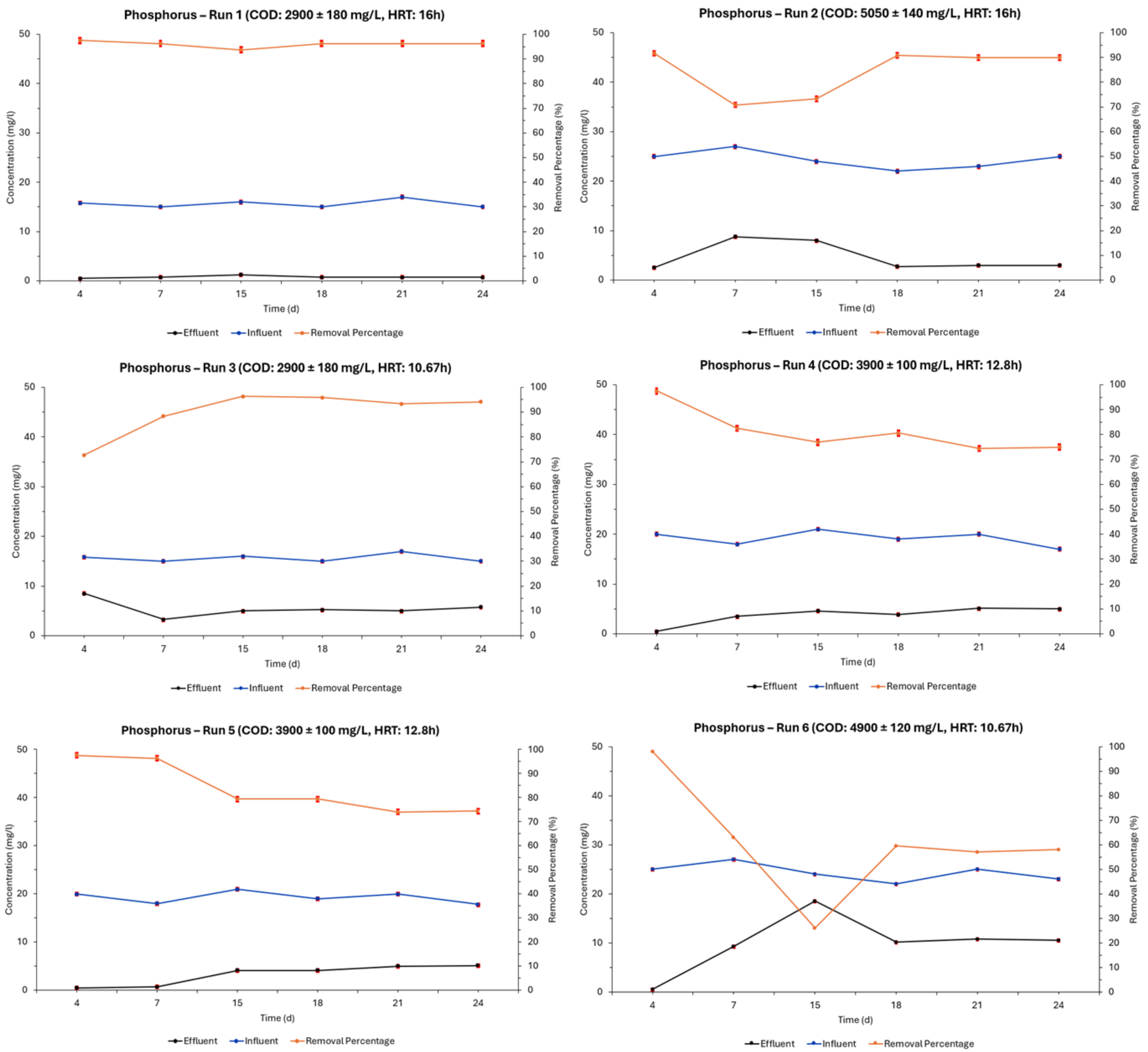


Figure 4.3: Phosphorus removal trends across the six experimental runs (Run 1 to Run 6). The graphs display influent and effluent phosphorus concentrations (mg/L), and phosphorus removal percentages over time (d).

In Run 1, the combination of 2900 ± 180 COD concentration and an extended HRT (16 h) allowed for effective phosphorus removal of 96% where it decreased from 16 ± 1 mg/L concentration in the influent to 1 ± 0 mg/L in the effluent. The 16h HRT provided ample time for phosphorus-accumulating organisms (PAOs) to uptake phosphorus during the aerobic phase. The 2900 ± 180 COD level supplied sufficient volatile fatty acids (VFAs), particularly acetate and propionate present in the synthetic feed, which are preferred carbon sources for PAOs, enhancing their activity and phosphorus uptake efficiency. Studies have demonstrated that these VFAs are effectively utilized by PAOs, leading to improved phosphorus removal performance (Oehmen et al., 2007).

In Run 2, the phosphorus removal percentage was 90%, slightly lower than the 96% observed in Run 1. Despite the higher COD concentration in Run 2 (5050 ± 140 mg/L), which can provide more VFAs, excessive concentrations may promote the growth of glycogen-accumulating organisms (GAOs), which compete with PAOs for VFAs. In biological wastewater treatment systems, the increased concentration of COD can lead to heightened competition among microbial communities, particularly heterotrophic bacteria. These heterotrophs utilize readily available organic carbon sources, which are also essential for the growth and activity of PAOs. This competition can inhibit PAO activity by depleting these critical resources, thereby affecting the efficiency of phosphorus removal processes (Domańska et al., 2023). Additionally, the presence of high COD levels can favor the growth of glycogen-accumulating organisms (GAOs), which also compete with PAOs for similar carbon sources. GAOs can outcompete PAOs under certain conditions such as temperature, SRT and carbon source availability, leading to reduced phosphorus removal efficiency (Stokholm-Bjerregaard et al., 2017). Therefore, managing COD concentrations is crucial to maintaining a balanced microbial community that supports optimal PAO activity and efficient phosphorus removal in wastewater treatment systems. 16-h HRT still provided adequate time for biological phosphorus removal processes, but the trade-off with higher COD concentrations impacted the final removal efficiency in Run 2.

In Run 3, the AGS system achieved a moderate efficiency of 69%, despite the shortest HRT (10.67 h). The influent COD concentration of 2900 ± 180 mg/L provided sufficient bioavailable carbon, primarily in the form of VFAs, to support phosphorus release and uptake by PAOs. However, the shorter HRT compared to Runs 1 and 2 likely limited the duration of the anaerobic and aerobic phases required for effective phosphorus uptake and storage by PAOs. This moderate efficiency reflects the adaptability of the microbial community, as the available carbon helped to mitigate some of the challenges posed by the reduced retention time. A study by de Kreuk et al. (2004) noted that short HRTs, such as 10.67 h, can constrain phosphorus removal in AGS systems by limiting the contact time for PAOs, often leading to moderate efficiencies unless COD and other conditions are carefully balanced. Thus, Run 3 underscores the importance of appropriately balancing HRT and COD to improve phosphorus removal in AGS systems, particularly under constrained hydraulic conditions.

Runs 4 and 5, conducted under identical conditions, achieved phosphorus removal efficiencies of 75% and approximately 74%, respectively. The 3900 ± 100 COD concentration supported microbial activity and provided sufficient carbon sources for phosphorus removal processes. However, the HRT of 12.8 h, though reasonable, may have been insufficient to allow complete phosphorus uptake by microorganisms, which could explain the slightly lower removal efficiency compared to Runs 1, and 2. The consistent performance across these runs highlights the balance between carbon availability and retention time in determining phosphorus removal efficiency. Studies indicate that shorter HRTs can limit the microbial activity required for complete phosphorus uptake, while moderate COD levels can support the removal process by enhancing the availability of bioavailable carbon (Abdoli et al., 2024; Benzing et al., 2020; Zhao et al., 2021).

Run 6 achieved a phosphorus removal efficiency of approximately 58%, the lowest among all runs. Despite the high influent COD concentration (4900 ± 120 mg/L) providing ample carbon sources, the system's shorter HRT of 10.67 h likely limited the time available for phosphorus uptake by microorganisms. In contrast, Run 3, with the same HRT but a lower influent COD concentration of 2900 ± 180 mg/L, achieved a higher phosphorus removal efficiency of 69%. This suggests that while high COD concentrations can supply the necessary carbon for phosphorus removal, excessively high COD levels may lead to competition for oxygen and other resources, potentially inhibiting phosphorus uptake. Additionally, the shorter HRT may not provide sufficient time for effective phosphorus removal, regardless of COD concentration. These findings align with studies indicating that both COD concentration and HRT are critical factors influencing phosphorus removal efficiency in wastewater treatment systems (Zhao et al., 2021). Additionally, a study by Wang et al. (2018) demonstrated that increasing the influent COD concentration can enhance phosphorus removal efficiency, but only up to a certain point. Beyond this threshold, the system may become overloaded, leading to decreased removal efficiency (Wang et al., 2009).

4.3.3. pH

Table 4.2 presents the influent and effluent pH of the AGS systems across all six experimental runs.

Table 4.2: Influent and effluent pH of the AGS system for all six experimental runs.

Time (d)	**Feed** (2900 ± 180 mg/L COD)	**Feed** (3900 ± 100 mg/L COD)	**Feed** (5050 ± 140 mg/L COD)	**Feed** (4900 ± 120 mg/L COD)	**Run 1** (COD: 2900 ± 180 mg/L, HRT: 16h)	**Run 2** (COD: 5050 ± 140 mg/L, HRT: 16h)	**Run 3** (COD: 2900 ± 180 mg/L, HRT: 10.67h)	**Run 4** (COD: 3900 ± 100 mg/L, HRT: 12.8h)	**Run 5** (COD: 3900 ± 100 mg/L, HRT: 12.8h)	**Run 6** (COD: 4900 ± 120 mg/L, HRT: 10.67h)
4	7.95	7.56	7.56	7.19	9.31	9.39	9.26	9.46	9.56	9.34
7	8.48	8.55	8.55	8.39	9.36	9.44	9.30	9.34	9.30	9.30
15	8.50	8.53	8.10	8.25	9.36	9.50	9.20	9.00	9.00	9.00
18	8.20	8.30	8.46	8.10	9.28	9.48	9.23	9.00	9.00	9.00
21	7.00	7.05	8.43	8.52	9.25	9.38	9.06	9.00	9.00	9.00
24	8.40	8.46	8.34	8.63	9.30	9.40	9.20	9.00	9.00	9.00

Feed pH fluctuated between 7.00 and 8.60, depending on the day the measurement was taken (Table 4.2). On the day the synthetic feed was initially prepared, the pH was typically between 7.00 and 7.50 for higher COD concentrations (2900 ± 180 to 5050 ± 140 mg/L). By day 2, the pH increases to approximately 8.0, and by the last day, day 3, it can rise up to 8.5. This gradual increase in pH is a natural consequence of the microbial and chemical processes occurring in the system over the three-day period before the feed is replenished. The observed pH change reflects the degradation of organic material and the accumulation of alkaline by-products, which are common in biological wastewater treatment, especially when high organic loads are present, such as in brewery wastewater. The feed composition, which includes organic compounds like sodium acetate and propionate, as well as ammonia from NH_4Cl, also plays a role in this pH increase, as the breakdown of organic acids (Such as: Formic Acid, Lactic Acid, and Butyric Acid) and the volatilization of ammonia contribute to a rise in pH over time (Rochette et al., 2013).

The fluctuations in feed pH are mirrored in the effluent pH, which consistently remains between 9.00 and 9.40 throughout the experiment. The higher pH of the effluent is typical in biological treatment processes, where microbial activity results in the production of alkaline by-products, such as minor ammonia, even when ammonia concentrations are not significant. This increase in pH observed in the effluent indicates that the AGS system is effectively degrading the organic load while shifting the effluent toward a more alkaline environment. Although the ammonia concentration in the effluent remains low, the presence of ammonia and other metabolic by-products can contribute to an increase in pH, particularly if denitrification is occurring alongside nitrification. In biological treatment systems, microbial metabolism generally leads to changes in pH due to the release of alkaline compounds. Specifically, while nitrification tends to lower pH by consuming alkalinity, denitrification generates alkalinity, which can counteract this effect and lead to an increase in pH (Qian et al., 2019; Barillo, 2019). Therefore, the observed pH increase in this system may be a result of the balance between nitrification and denitrification processes, with denitrification playing a significant role in buffering the system and raising the pH (Qian et al., 2019).

This phenomenon is consistent with findings in wastewater treatment literature. For instance, a study on AGS treatment of piggery wastewater observed that the aeration process led to the stripping of carbon dioxide, resulting in the effluent pH reaching 9.00 by the end of the operation (Zhou et al., 2024). This indicates that the AGS system effectively degrades the organic load while shifting the effluent toward a more alkaline environment (Zhou et al., 2024).

Overall, the observed pH fluctuations in both the feed and effluent reveal the dynamic nature of the AGS system and the influence of microbial activity on the treatment process. The steady increase in pH in the feed over the 3-d period, coupled with the corresponding rise in effluent pH, highlights that the AGS system is effectively handling the organic load while promoting an alkaline environment in the effluent. These findings reinforce the notion that microbial processes in the AGS system are effectively degrading the organic pollutants, with the resulting by-products influencing the pH dynamics of the system. Additionally, the variability in feed pH, dependent on which day the measurement is taken, further emphasizes the need for regular monitoring to capture the changing dynamics of synthetic wastewater in a treatment system.

pH is a critical operational parameter in wastewater treatment systems, significantly influencing the efficiency of various biological and chemical processes. In AGS systems, maintaining an optimal pH range is essential for achieving effective removal of organic matter and nutrients. Fluctuations in pH can disrupt microbial activity, alter chemical equilibria, and affect the overall treatment performance. This section provides a comprehensive overview of how pH levels can affect nitrification, denitrification, and carbon cycling in wastewater treatment systems.

Nitrification, the aerobic oxidation of ammonia to nitrate, is highly sensitive to pH variations. This process is mediated by two primary groups of autotrophic bacteria: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). While the optimal pH range for nitrification is frequently cited as 7.5–8.0, recent studies have demonstrated that the actual optimum can vary significantly depending on microbial community composition and environmental adaptation. For example, certain strains of Nitrosomonas (AOB) and Nitrobacter (NOB) have exhibited nitrifying activity at pH values approaching 9.5 in saline and alkaline systems (Ni et al., 2023). Furthermore, it has been shown that microbial acclimation to pH stress can lead to shifts in community structure, allowing for some degree of sustained nitrification even under suboptimal conditions (Rooyen et al., 2021). These findings highlight the importance of microbial plasticity and emphasize the need to evaluate pH impacts within the specific microbial and operational context of each wastewater treatment system (Yue et al., 2023).

Nonetheless, elevated pH levels generally exert inhibitory effects on both AOB and NOB activities, particularly when pH increases are abrupt or exceed microbial tolerance thresholds. At higher pH, the equilibrium between ammonium (NH_4^+) and free ammonia (NH_3) shifts towards NH_3, which is known to be inhibitory to AOB activity. This inhibition is attributed to the disruption of cellular processes, including membrane permeability and enzyme function. Moreover, high pH conditions can lead to the accumulation of free nitrous acid (FNA), which has been shown to exert a stronger inhibitory effect on NOB compared to AOB. Such conditions often result in nitrite accumulation and reduced nitrification efficiency, especially in systems without prior microbial adaptation (Rooyen et al., 2021; Yue et al., 2023).

Denitrification, the stepwise reduction of nitrate (NO_3^-) to nitrogen gas (N_2), is primarily facilitated by heterotrophic bacteria and is generally less sensitive to pH fluctuations compared to nitrification. The optimal pH range for denitrification is broader, typically between 6.5 and 8.5, providing a more stable operational window for wastewater treatment processes (Feng et al., 2023). However, extreme pH conditions can still significantly impact denitrification rates and efficiency. Elevated pH levels, typically above 8.5 and especially in the range of 9.0 to 10.5 can adversely affect the activity of denitrifying bacteria and shift microbial community structures, potentially favoring alkali-tolerant denitrifiers over more efficient but pH-sensitive strains. For instance, high pH conditions have been shown to inhibit the expression of key denitrification genes such as nirK, nirS, and nosZ, resulting in decreased denitrification performance and the accumulation of nitrate (Pan et al., 2023). These effects are particularly pronounced in systems lacking sufficient microbial acclimation to alkaline condition. In addition to its direct enzymatic effects, elevated pH can also influence denitrification indirectly by altering carbon dynamics within the microbial ecosystem. At higher pH levels, certain non-denitrifying heterotrophic bacteria may exhibit enhanced metabolic activity, which increases competition for readily biodegradable organic carbon (Michaud et al., 2006; Anderson et al., 2018; Msimango et al., 2024; Pan & Xu, 2025). As denitrifiers depend on this carbon as an electron donor, such competition can limit their efficiency, particularly in high-COD wastewater environments where multiple microbial groups are actively utilizing available carbon sources (Feng et al., 2023).

Moreover, high pH conditions can lead to the accumulation of intermediate nitrogen species, such as nitrite (NO_2^-), due to the imbalance in the activity of denitrifying enzymes. This accumulation not only indicates incomplete denitrification, but also poses potential toxicity risks to aquatic life (Pan et al., 2023). In summary, while denitrification exhibits a broader pH tolerance compared to nitrification, maintaining pH within the optimal range is crucial for efficient nitrogen removal. Understanding the interplay between pH, microbial community dynamics, and carbon availability is essential for optimizing denitrification processes in wastewater treatment systems.

Furthermore, pH exerts a significant influence on carbon cycling within AGS systems, primarily by affecting the activity and community composition of heterotrophic bacteria responsible for organic matter degradation (Morgan-Sagastume et al., 2008; Song et al., 2024). These bacteria, crucial for removing organic pollutants, exhibit varying pH optima, and deviations can shift the balance of microbial populations within the granules (Khanichaidecha et al., 2018; Bryson et al., 2022). For instance, extreme pH values can impact the formation and stability of AGS, with acidic conditions sometimes leading to filamentous granules and alkaline conditions potentially weakening granule structure (Rezasoltani et al., 2015). Maintaining an appropriate pH, generally around 7.0 for optimal performance, is vital for the thriving of heterotrophic bacteria in AGS, ensuring efficient degradation of organic matter and overall system stability (Bodle & Kirkland, 2024).

Moreover, pH in AGS systems can influence the production of extracellular polymeric substances (EPS) by heterotrophic bacteria, which are essential for granule formation and stability (Miranda et al., 2025). While the optimal pH range for most bacteria in AGS is generally near neutral (6.5-7.5), shifts towards more acidic or alkaline conditions can alter metabolic pathways and affect the efficiency of carbon removal (Khanichaidecha et al., 2018; Bryson et al., 2022). Therefore, careful monitoring and control of pH are crucial for maintaining a thriving heterotrophic community within AGS and ensuring effective carbon cycling in wastewater treatment.

4.4. Evaluation of treatment efficiency and statistical analysis

The evaluation utilized regression models to assess treatment efficiency. Linear regression analysis was conducted to describe the relationships between influent COD and HRT and the removal efficiencies for ammonia, phosphorus, and COD, suggesting a direct, proportional effect. These relationships are visually represented through scatter plots (Figures 4.4–4.6), where each plot includes the linear regression line and its equation, derived from the experimental data using Design Expert.

The data points in these models were based on steady-state averages for each of the six experimental runs, calculated from samples collected twice a week in triplicates on days 15, 18, 21, and 24, a period reflecting stable system performance. For each run, the triplicate measurements from these four sampling days were averaged to obtain a single representative value, resulting in six independent data points per response variable. This averaging ensures the data points are independent, as each run represents a distinct experimental condition with unique HRT and COD levels, satisfying the independence assumption of linear regression and addressing potential violations related to autocorrelation.

The experimental design matrix, developed using a 2^2 factorial design with a center point, enhanced the precision of the regression models. The high intercept values in the initial models reflected the tested experimental range (COD: $2900 \pm 180 - 5050 \pm 140$ mg/L, HRT: 10.67–16 h) rather than predictions at zero or extreme conditions. Design Expert prioritizes model accuracy within the studied range, making the scatter plots and regression lines reliable for assessing brewery wastewater treatment performance, though their applicability may be limited beyond the tested parameters.

4.4.1. Ammonia removal

4.4.1.1. Effect of HRT on ammonia removal

The scatter plot for ammonia (NH_3) removal efficiency versus HRT, shown in Figure 4.4a, exhibits a strong positive linear relationship, as evidenced by the regression equation $y = 1.337x + 76.004$ and R^2 value of 0.9387. Here, y represents ammonia removal efficiency (%), and x denotes HRT (h). The slope of 1.337 indicates that ammonia removal efficiency increases by approximately 1.34% for each additional 1 h of HRT within the tested range of 10.67 to 16 h. This positive trend underscores the critical role of HRT in facilitating simultaneous nitrification-denitrification (SND) in AGS systems, where extended retention times enhance microbial processes such as ammonia oxidation and nitrate reduction (Farazaki & Gikas, 2019).

The y-intercept of 76.004 represents the theoretical ammonia removal efficiency at an HRT of zero, but this value is an extrapolation outside the experimental range and not practically meaningful. Instead, it reflects the model's adjustment to the high baseline performance of the system, likely influenced by favorable conditions such as high influent COD (2900–5050 mg/L) and effluent pH (9.1–9.4) (Metcalf & Eddy, 2014).

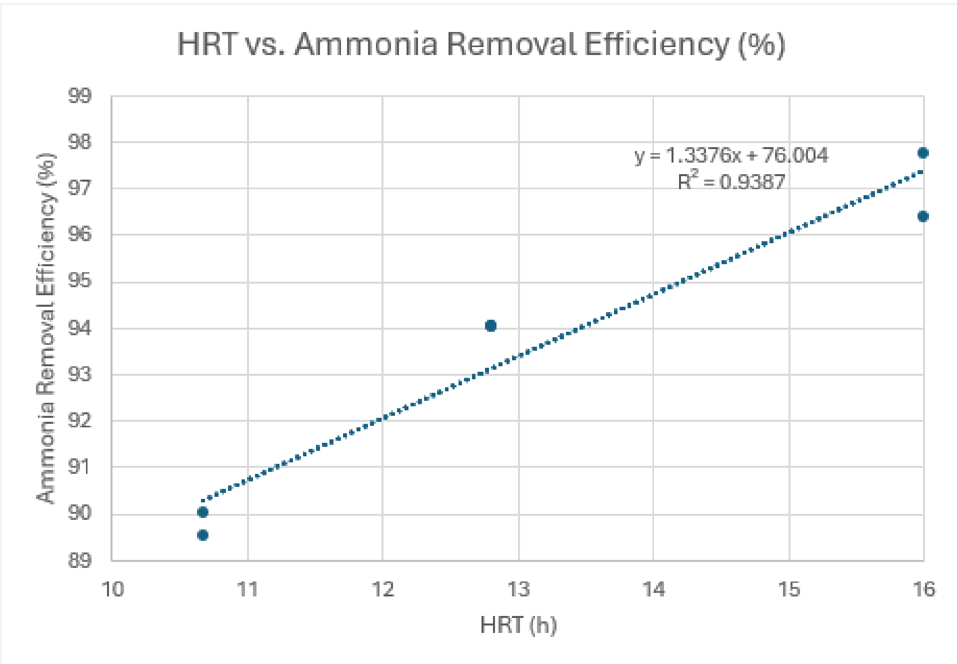
The R^2 value of 0.9387 indicates that 93.87% of the variability in ammonia removal efficiency is explained by its linear relationship with HRT, demonstrating a robust model fit. This high R^2 aligns with findings from AGS literature, where HRT is often a dominant factor in nitrogen removal under steady-state conditions (Gonzalez-Lopez & Gonzalez-Martinez, 2021; Qin et al., 2025). The remaining 6.13% of variability may be attributed to secondary factors such as influent COD or pH, which can influence SND efficiency in high-strength wastewater systems (Phanwilai et al., 2020). A regression analysis of the data yields a p-value of approximately 0.02 for the slope, confirming statistical significance at the 0.05 level (Montgomery & St, 2022), indicating that the observed relationship is unlikely to be due to random variation.

4.4.1.2. Effect of influent COD concentration on ammonia removal

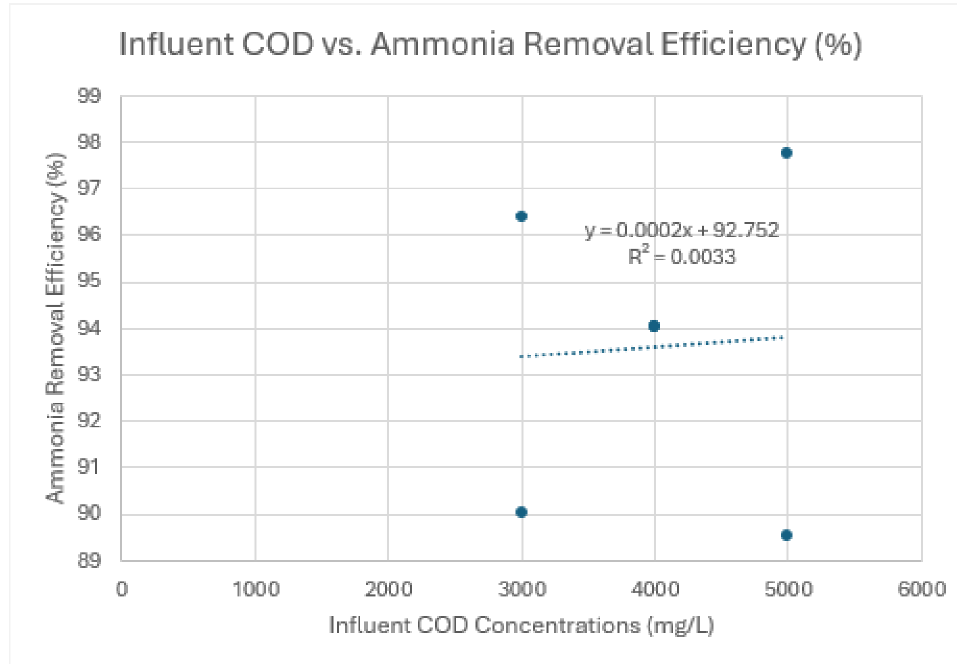
The scatter plot for ammonia removal efficiency versus influent COD concentrations (Figure 4.4b) shows a negligible linear relationship, as indicated by the regression equation $y = 0.0002x + 97.752$ and an R^2 value of 0.0033. In this model, y represents ammonia removal efficiency (%), and x denotes influent COD concentrations (mg/L) within the range of 2900 to 5050 mg/L. The slope of 0.0002 suggests an extremely small increase in ammonia removal efficiency (0.02%) for each 100 mg/L increase in influent COD, which is practically insignificant given the data scatter. This near-zero slope indicates that influent COD has minimal direct impact on ammonia removal efficiency under the tested conditions. The y-intercept of 92.752 represents the theoretical ammonia removal efficiency when COD is zero, but this is an extrapolation outside the experimental range and not relevant. Instead, it reflects the stable baseline ammonia removal efficiency (89%–97%) achieved across the COD range, suggesting that the AGS system maintains consistent nitrogen removal despite varying organic loads (Metcalf & Eddy, 2014).

The R^2 value of 0.0033 indicates that only 0.33% of the variability in ammonia removal efficiency is explained by its linear relationship with influent COD concentrations, signifying a very weak model fit. This low R^2 suggests that influent COD is not a primary determinant of ammonia removal efficiency in this system, consistent with findings that AGS systems can handle high organic loads without significant nitrogen removal inhibition, provided other parameters like HRT are adjusted (Song et al., 2020). The lack of a strong correlation may also reflect the system's ability to buffer COD variations through microbial adaptation or SND processes (Gonzalez-Lopez & Gonzalez-Martinez, 2021). A regression analysis yields a p-value of approximately 0.90 for the slope, indicating that the relationship is not statistically significant at the 0.05 level (Montgomery & St, 2022), further supporting the conclusion that influent COD has a negligible direct effect on ammonia removal efficiency within the tested range.

The comparison reveals that HRT has a substantial and statistically significant effect on ammonia removal, whereas influent COD concentrations have a negligible direct impact. This suggests that adjusting HRT is the primary strategy for enhancing ammonia removal in this AGS system treating brewery wastewater. However, the stable ammonia removal across the COD range indicates that the system can handle high organic loads, though careful monitoring of COD/N ratios may be necessary to avoid indirect competitive effects, particularly under extreme conditions (Song et al., 2020).



(a)



(b)

Figure 4.4. (a) HRT vs. Ammonia Removal Efficiency (b) Influent COD vs. Ammonia Removal Efficiency

4.4.2. Phosphorus removal

4.4.2.1. Effect of HRT on phosphorus removal

The scatter plot for phosphorus removal efficiency versus HRT (Figure 4.5a) displays a moderate positive linear relationship, as indicated by the regression equation $y = 5.5666x + 3.971$ and an R^2 value of 0.9175. In this model, y represents phosphorus removal efficiency (%), and x denotes HRT (h) within the tested range of 10.67 to 16 h. The slope of 5.5666 suggests that phosphorus removal efficiency increases by approximately 5.57% for each additional 1 h of HRT. This substantial increase highlights the significant influence of HRT on phosphorus removal, likely due to enhanced biological phosphorus removal (BPR) processes in the AGS system. Extended HRT provides greater opportunity for PAOs to uptake and store phosphorus under aerobic conditions, followed by release and uptake cycles, which are critical for efficient phosphorus removal (Oehmen et al., 2007). The y-intercept of 3.971 represents the theoretical phosphorus removal efficiency at an HRT of zero, an extrapolation outside the experimental range and not practically meaningful, but it reflects the model's adjustment to the low baseline phosphorus removal under minimal retention time.

The R^2 value of 0.9175 indicates that 91.75% of the variability in phosphorus removal efficiency is explained by its linear relationship with HRT, signifying a strong model fit. This high R^2 is consistent with studies on AGS systems, where HRT is a key parameter influencing phosphorus removal through BPR, particularly under steady-state conditions (Iorhemen et al., 2022). The remaining 8.25% of variability may be attributed to factors such as influent COD concentrations (2900–5050 mg/L), effluent pH (9.1–9.4), or the availability of volatile fatty acids (VFAs) for PAO activity (Metcalf & Eddy, 2014). A regression analysis of the data yields a p-value of approximately 0.03 for the slope, confirming statistical significance at the 0.05 level

(Montgomery & St, 2022), indicating that the observed increase in phosphorus removal with HRT is not due to random variation.

4.4.2.2. Effect of influent COD concentration on phosphorus removal

The scatter plot for phosphorus removal efficiency versus influent COD concentrations (Figure 3.1a) exhibits a weak negative linear relationship, as characterized by the regression equation $y = -0.0043x + 94.458$ and an R^2 value of 0.0765. In this model, y represents phosphorus removal efficiency (%), and x denotes influent COD concentrations (mg/L). The slope of -0.0043 indicates a slight decrease in phosphorus removal efficiency (0.43%) for each 100 mg/L increase in influent COD, suggesting a minor inhibitory effect. This negative trend may reflect competition between PAOs and heterotrophic bacteria for oxygen and carbon resources, where high COD levels favor heterotrophic growth, potentially reducing the efficiency of BPR (Oehmen et al., 2007; Phanwilai et al., 2020). The y-intercept of 94.458 represents the theoretical phosphorus removal efficiency at a COD concentration of zero, an extrapolation outside the experimental range and not practically significant, but it aligns with the high baseline phosphorus removal efficiencies (58%–96%) observed across the COD range.

The R^2 value of 0.0765 indicates that only 7.65% of the variability in phosphorus removal efficiency is explained by its linear relationship with influent COD concentrations, indicating a very weak model fit. This low R^2 suggests that influent COD is not a primary determinant of phosphorus removal efficiency, consistent with findings that AGS systems can maintain phosphorus removal stability across varying organic loads when other parameters are controlled (Song et al., 2024). The lack of a strong correlation may also be due to the system's ability to adapt through PAO activity, though high COD levels may still exert indirect effects via microbial competition (Iorhemen et al., 2022). A regression analysis yields a p-value of approximately 0.58

for the slope, indicating that the relationship is not statistically significant, further supporting the conclusion that influent COD has a minimal direct impact on phosphorus removal efficiency within the tested range.

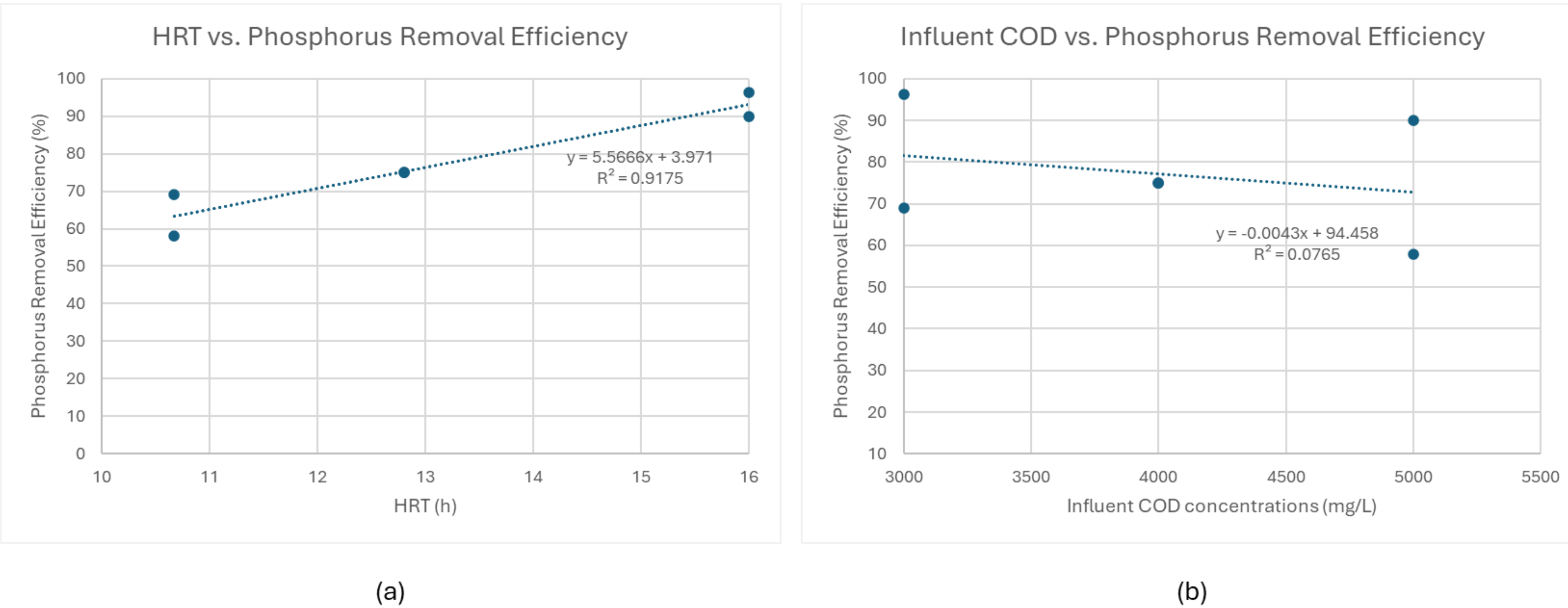


Figure 4.5: (a) HRT vs. Phosphorus Removal Efficiency (b) Influent COD vs. Phosphorus Removal Efficiency

4.4.3. COD Removal

4.4.3.1. Effect of HRT on COD removal

The scatter plot for COD removal efficiency versus HRT (Figure 4.6a) shows a negligible linear relationship, as indicated by the regression equation $y = -0.051x + 100.44$ and an R^2 value of 0.6011. The slope of -0.051 suggests a slight decrease in COD removal efficiency (0.51%) for each additional 1 h of HRT, which aligns with the unusual trend observed in the response analysis (Figure 4.8), where COD removal efficiency appears higher at lower HRT values. This counterintuitive trend, contrary to expectations that longer HRTs improve removal efficiency is likely due to the consistently high COD removal rates across all experimental runs (above 99%). When the system operates near its maximum capacity, variations in HRT have minimal impact, masking its true effect (Goletic & Imamović, 2010). For example, Runs 1 and 3 both achieve near 100% COD removal, reflecting the system's high efficiency regardless of retention time.

The R^2 value of 0.6011 indicates that 60.11% of the variability in COD removal efficiency is explained by its linear relationship with HRT, suggesting a moderate model fit. However, this moderate R^2, combined with the near-flat trendline, supports the response analysis finding that limited variability in COD removal efficiency (<99%) hinders the detection of HRT's actual influence. A regression analysis yields a p-value of approximately 0.22 for the slope, indicating that the relationship is not statistically significant, consistent with the response analysis's implication that statistical models may struggle to capture meaningful trends under such conditions.

4.4.3.2. Effect of influent COD concentration on COD removal

The scatter plot for COD removal efficiency versus influent COD concentrations (Figure 4.6b) exhibits a negative linear relationship, as characterized by the regression equation $y = 6E - 05x + 99.518$ and an R^2 value of 01255. The slope of 0.00006 indicates an extremely slight increase in COD removal efficiency (0.006%) for each 100 mg/L increase in influent COD, suggesting negligible influence at higher concentrations. This weak positive trend may reflect a minor adaptive response by heterotrophic bacteria to increased organic load, though the removal efficiency remains consistently high (<99%) across the tested range. The y-intercept of 99.518 represents the theoretical COD removal efficiency at a COD concentration of zero, an extrapolation outside the experimental range and not practical, but it closely aligns with the near-complete removal observed (Rittmann & McCarty, 2001).

The R^2 value of 0.1255 indicates that only 12.55% of the variability in COD removal efficiency is explained by its linear relationship with influent COD concentrations, signifying a very weak model fit. This low R^2 suggests that influent COD is not a primary determinant of COD removal efficiency within the tested range, and the minimal slope indicates that changes in organic load have little practical impact on removal performance, possibly due to the robust microbial capacity of AGS systems under these conditions (Phanwilai et al., 2020).

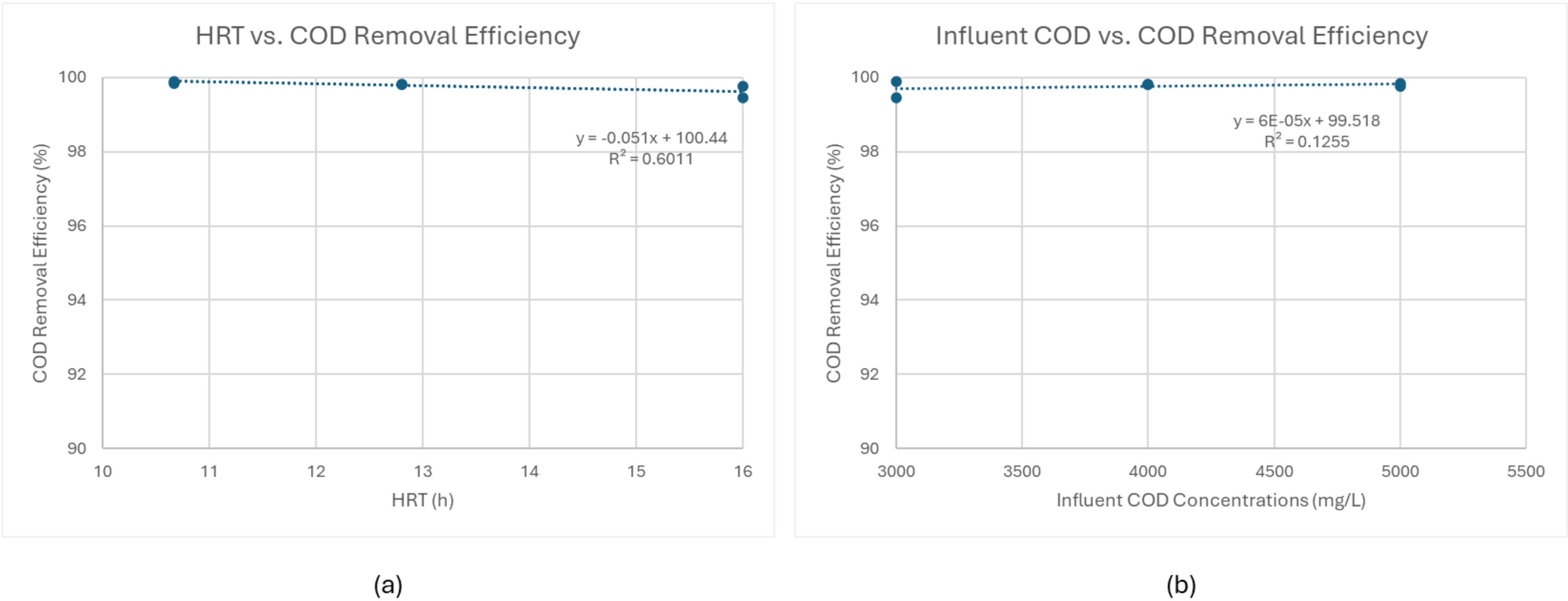


Figure 4.6: (a) HRT vs. COD Removal Efficiency (b) Influent COD vs. COD Removal Efficiency

4.4.4. Response analysis

To further validate the predictive performance of the models and to better understand the interactions between the studied factors, 3D surface plots and contour plots were generated. These visual representations allow for a more intuitive comprehension of how well the models align with the observed data and reveal the relationships between the independent variables (influent COD and HRT) and the dependent variables (ammonia, phosphorus, and COD removal). By illustrating the combined effects of these variables on the response, the contour plots provide critical insights into the enhancement process, highlighting regions of optimal performance. These graphical tools not only facilitate the interpretation of the regression model but also offer a clearer visualization of the model's accuracy and how different factor combinations influence ammonia, phosphorus and COD removal efficiencies.

i. Ammonia removal

The contour and 3D surface plots in Figure 4.7 visually demonstrate the interplay between influent COD concentrations and HRT in influencing ammonia removal efficiency within the brewery wastewater treatment system. The color gradient effectively represents removal efficiency, with higher removal efficiencies shown in red and lower efficiencies in blue. The plot reveals that ammonia removal efficiency increases significantly with longer HRTs, as indicated by the expansion of the red region in the upper region. This suggests that extending the HRT provides sufficient time for the microbial community to metabolize ammonia, thereby improving its removal. In contrast, the predominance of blue hues in the lower section of the plot reflects reduced removal efficiencies at shorter HRTs, emphasizing the limitations of achieving effective ammonia removal under these conditions.

As analyzed earlier, COD does not appear to have a significant impact on ammonia removal within the AGS system. In fact, HRT has a significant effect on nitrogen removal, regardless of the COD concentrations. The results indicate that HRT is the primary driver of ammonia removal efficiency, while variations in COD concentrations appear to have a minimal impact. This highlights the importance of adjusting HRT for effective nitrogen removal in brewery wastewater treatment systems. This linear relationship simplifies the process of operational modification, as it allows for precise modifications to HRT to achieve the desired ammonia removal outcomes. Ultimately, this analysis reinforces the critical role of HRT in enhancing ammonia removal, positioning it as a key operational parameter in maximizing treatment efficiency in brewery wastewater systems, irrespective of COD concentrations.

A study focused on the relationship between HRT and ammonia removal, noting that many studies have shown different HRTs (i.e., 3 – 12 h) have a significant impact on ammonia removal, as nitrifiers are slow-growing organisms. This confirms the importance of adjusting HRT to ensure sufficient time for nitrification processes to occur, thereby enhancing ammonia removal efficiency (Hasnain et al., 2016). Furthermore, another study published in the Egyptian Journal of Chemistry explored the impact of HRT on wastewater treatment using aerobic membrane reactors. The researchers investigated HRTs of 4, 6, 8, and 10 h and observed that the highest removal efficiencies for COD, TSS, and ammonia nitrogen were achieved at the longest HRT of 10 h. Specifically, they reported removal rates of 97.59%, 99.71%, and 90.54%, respectively. These findings further corroborate the importance of regulating HRT to enhance treatment performance (Qrenawi & Rabah, 2024).

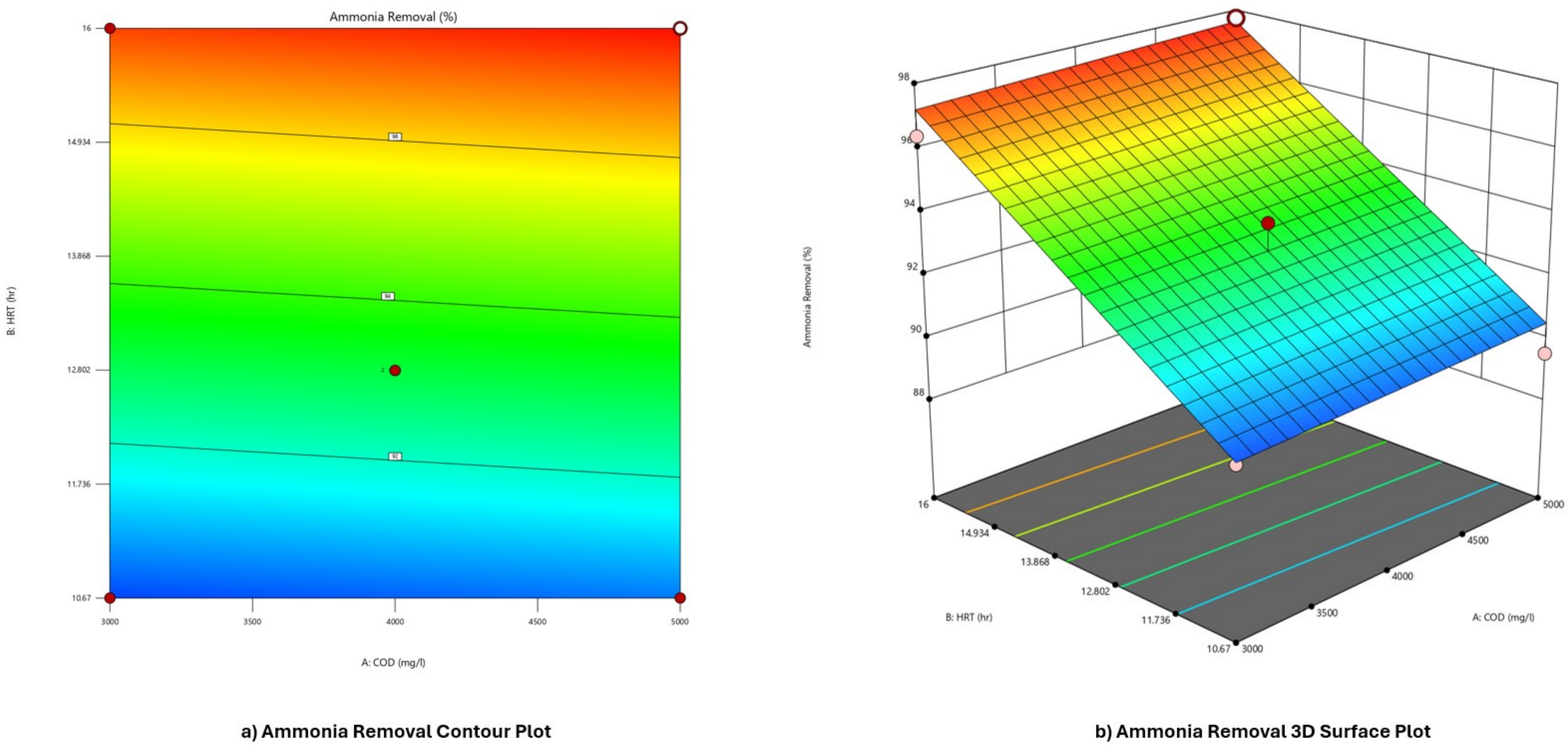


Figure 4.7: Ammonia Removal Contour and 3D Surface Plots

ii. Phosphorus removal

Figure 4.8 presents both contour and 3D surface plots, which illustrate the relationship between the independent variables, A-COD and B-HRT, and their effect on phosphorus removal. These plots clearly show a positive relationship between B-HRT and phosphorus removal, indicating that as HRT increases, phosphorus removal efficiency improves. In contrast, higher COD concentrations result in decreased phosphorus removal efficiency. The contour plot further highlights the optimal regions for phosphorus removal, revealing that higher levels of B-HRT and lower levels of A-COD are ideal for maximizing removal efficiency. The tightly packed contours in the upper-left corner of the plot emphasize this optimal combination, and both plots suggest that B-HRT has a more significant role in enhancing phosphorus removal when COD levels are lower.

When compared to the 3D surface plot, the contour plot serves as a simplified representation of the same data but with a focus on understanding specific removal levels. It is easier to pinpoint precise conditions for achieving target phosphorus removal percentages. Together, these two plots complement each other: the 3D surface plot provides a spatial view of the interaction between the factors, while the contour plot allows for a clearer interpretation of the regions corresponding to specific phosphorus removal percentages.

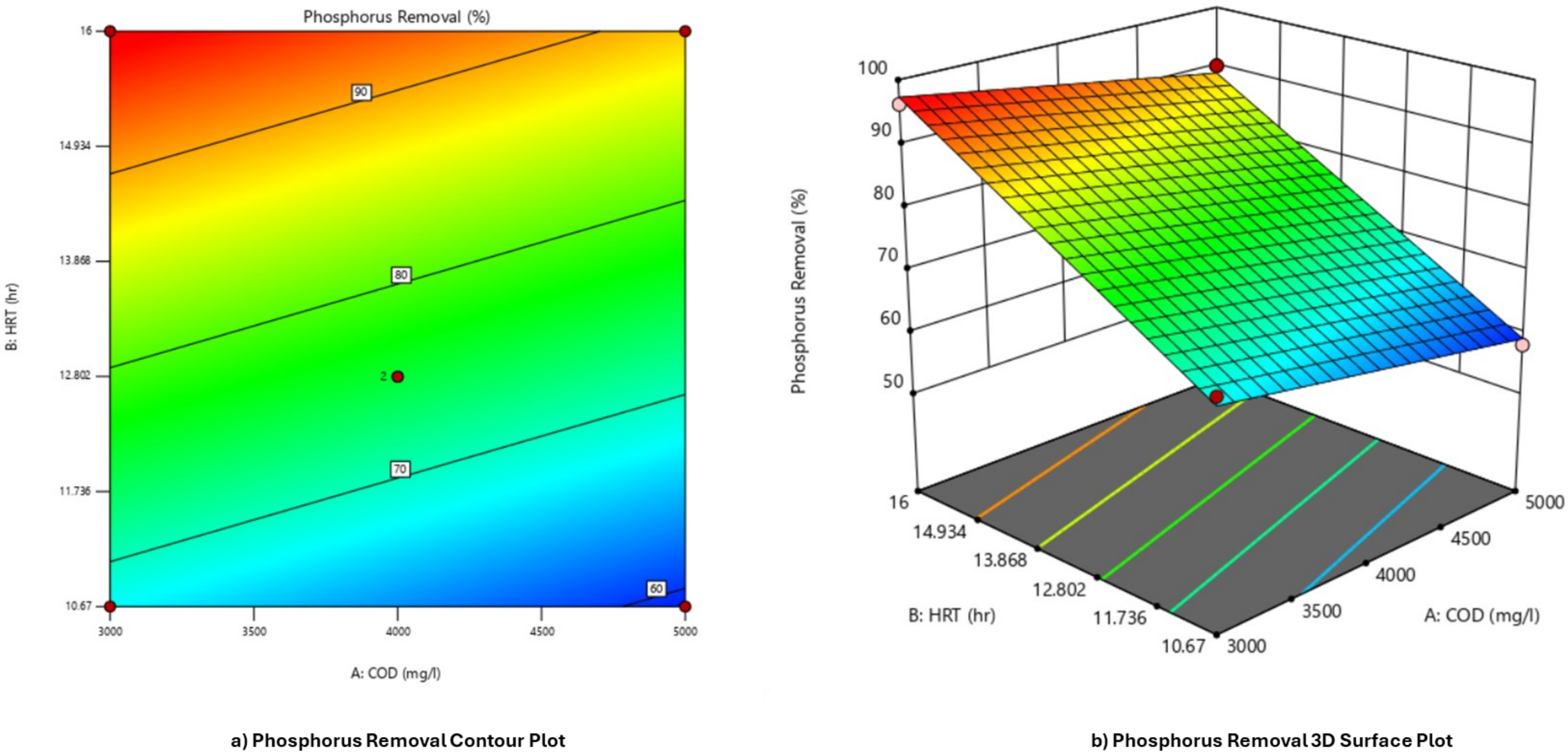


Figure 4.8: Phosphorus Removal Contour and 3D Surface Plots

iii. COD removal

The contour and 3D surface plots in Figure 4.9 indicate that COD removal efficiency increases at lower influent COD concentrations, which aligns with established findings in wastewater treatment research. Studies have shown that higher influent COD concentrations can lead to decreased COD removal efficiencies. For example, a study reported COD removal efficiencies of 95.5%, 90.3%, and 89.9% at influent COD concentrations of 836 mg/L, 2480 mg/L, and 3922 mg/L, respectively (Abdulgader et al., 2022).

Similarly, another study observed a decrease in COD removal efficiency from 94.81% to 89.35% when the influent organic load increased from 0.5 kg COD/($m^3 \cdot d$) to 1.0 kg COD/($m^3 \cdot d$) (Yang et al., 2018). These findings suggest that lower influent COD concentrations facilitate higher COD removal efficiencies, likely due to reduced organic loading, which allows microbial communities to more effectively degrade organic matter without being overwhelmed.

Furthermore, Figure 4.6 shows an unusual trend where COD removal efficiency appears to be higher at lower HRT values. This seems counterintuitive, as longer retention times are generally expected to improve treatment efficiency. However, this result is likely influenced by the consistently high COD removal rates across all experimental runs (above 99%). When a system operates near its maximum capacity, variations in influencing factors like HRT become less noticeable, making it harder to detect their actual effects.

In such cases, statistical models may struggle to capture meaningful differences or interactions, leading to misleading trends. This issue has been highlighted in wastewater treatment research, where limited variability in response data can affect the reliability of statistical interpretations (Bourget, 2023). To better understand the true relationship between HRT and COD removal, future experiments should include a wider range of COD concentrations and HRT values.

This would introduce more variability into the data, allowing for a more accurate assessment of how these factors influence treatment performance.

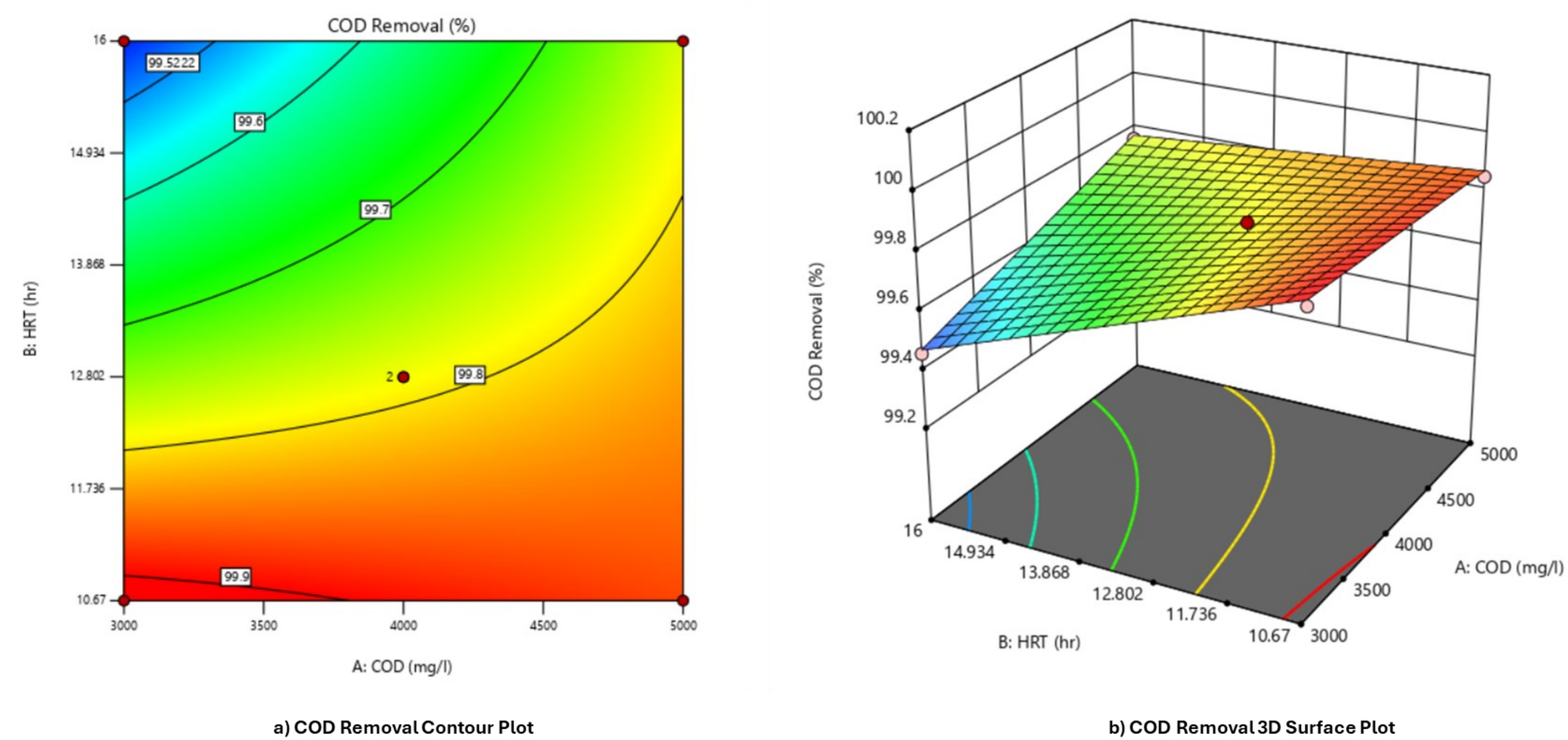


Figure 4.9: COD Removal Contour and 3D Surface Plots

Chapter 5 Conclusions and Recommendations

5.1. Conclusions

This study evaluated the performance of AGS for the treatment of high-strength brewery wastewater, focusing on the removal of COD, ammonia, and phosphorus. The study determined the impact of influent COD concentrations and HRT on the removal efficiencies of these contaminants. The results show exceptional COD removal across all runs, consistently achieving 99% efficiency with only minor decimal point variations. Thus, the optimal conditions were selected based on performance in terms of ammonia and phosphorus removal. The highest removals for both ammonia and phosphorus were observed in Run 1 (COD = 2900 ± 180 mg/L and HRT = 16 h) with removal efficiencies of 96.4% and 96.3%, respectively.

The results highlight the critical role of COD and HRT for high-strength wastewater treatment in AGS systems. A longer HRT is essential when high COD concentrations are involved to ensure adequate contact time for microbial processes to take place, facilitating the breakdown of organic matter and nutrient removal.

Regression analysis showed patterns, where ammonia removal efficiency displayed a robust positive correlation with HRT, yet showed little correlation with influent COD. Phosphorus removal efficiency demonstrated a noticeable positive correlation with HRT, alongside a slight negative correlation with influent COD. COD removal efficiency exhibited minimal correlation with HRT and a mild negative correlation with influent COD.

Findings from the present study not only show enhanced high-strength brewery wastewater treatment but also contributes to a deeper understanding of the relationship between operational parameters and the removal of organic matter and nutrient contaminants. These findings emphasize the potential of AGS biotechnology for sustainable, cost-effective wastewater treatment in the brewing industry where wastewater effluent quality poses significant challenges due to high organic loads and fluctuating nutrient concentrations. This research provides a solid foundation for more efficient and effective wastewater management strategies in the brewing sector.

5.2. Limitations of the study

While this research demonstrated promising results, it is important to acknowledge its limitations. These limitations include:

- The study employed synthetic high-strength wastewater, which, while useful for controlled experimental conditions, may not fully represent the complex and variable composition of real brewery wastewater. Actual brewery wastewater contains a wide range of organic compounds, salts, metals, suspended solids, and potentially inhibitory substances, which could influence the system's performance under real-world conditions (Enitan-Folami et al., 2015).
- The experiments were conducted in a controlled laboratory setting, which may not accurately simulate the dynamic and fluctuating conditions present in operational wastewater treatment plants. Variations in flow rate, temperature, and pollutant loads, which are typical in real-world applications, were not fully accounted for, potentially affecting the generalizability of the results (Uman et al., 2024).

5.3. Recommendations

Based on the results obtained from this thesis, it is imperative to further investigate the following research areas to further enhance the system's performance and sustainability:

- It is crucial to conduct further experiments using actual brewery wastewater to assess the system's performance in dealing with the complex and variable nature of real wastewater.

This would help validate the results obtained with synthetic wastewater and provide more accurate insights into the system's adaptability to industrial-scale conditions.

- Future studies should focus on evaluating the system’s response to shock loadings, including sudden increases in organic load or fluctuations in wastewater composition. Identifying and developing strategies to mitigate the negative impacts of such disturbances would be crucial for ensuring the long-term stability and efficiency of the AGS system under variable operational conditions.
- Investigating the potential for recovering valuable resources from AGS sludge presents an opportunity to add economic value to the treatment process. Techniques such as thermal, acid, or enzymatic hydrolysis could be explored for efficient recovery, and the recovered resources should be characterized for potential applications in various industries.
- Introducing an anaerobic phase into the treatment cycle can enhance nutrient removal efficiency. This phase allows for the selection of specific microorganisms, such as PAOs and GAOs, which are essential for efficient nutrient removal. Additionally, the anaerobic phase promotes the uptake of readily degradable and fermentable COD, contributing to the formation of stable granules in SBR.

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Appendices

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Appendix 2: Characteristics of brewery wastewater from Deadfall Brewing Company

Table A1 presents nutrient concentrations based on samples collected from Deadfall Brewing Company in Prince George, Canada in June 2023, September 2023, December 2023, and March 2024.

Table A1: Nutrient concentrations for Deadfall Brewing Company samples

Sampling Date	**COD (mg/L)**	**Ammonia (mg/L)**	**Phosphorus (mg/L)**
June, 2023	1000	50	5
September, 2023	1200	60	6
December, 2023	1050	53	5
March, 2024	1100	55	5