MICRO-AERATION-ENHANCED ANAEROBIC DIGESTION FOR THE STABILIZATION OF COFFEE-PROCESSING WASTEWATER

by

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(Under the Direction of Joseph G. Usack)

ABSTRACT

Coffee processing wastewater (CPW) contains high organic loads and inhibitory compounds such as caffeine. This study assessed micro-aeration-enhanced anaerobic digestion (MA-AD) for CPW treatment, using oxidation-reduction potential controlled oxygen dosing to compare anaerobic and micro-aerobic regimes. Both MA-AD and conventional anaerobic digestion (AD) achieved similar reductions in total and soluble chemical oxygen demand (COD) and solids (>66 % and >86 %, respectively). However, MA-AD resulted in higher turbidity and total suspended solids, likely due to oxygen-induced floc disruption. pH remained stable (6.8–7.1), though MA-AD showed increased acidification. Caffeine degradation was faster under MA-AD (>85 % in 28 h), while long-term removal rates were similar. Methane production was lower under MA-AD (up to 43 % reduction), likely due to oxygen inhibition of methanogens. MA-AD shows promise for enhancing hydrolysis and removal of recalcitrant compounds in CPW, though further optimization is needed to improve methane output.

INDEX WORDS: Anaerobic digestion, Micro-aeration, Coffee Processing Waste-water, Recalcitrant compounds

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DEDICATION

This Thesis is dedicated to God: The Author and Finisher of my Faith in whose bosom my earthly Father is currently resting. Continue to rest in peace, Baba.

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The journey so far looked like it wouldn't be completed, but here today, I am writing the final thesis. I would like to thank my Major Professor, Dr. Usack, for believing in me and challenging me to take on tasks I never thought I could. I do appreciate it. Also, I would like to thank my committee members for their insights and contributions to my work. Special appreciation to Samuel Ogundipe for analyzing the caffeine samples and to Elizabeth for feeding the reactors and running some analyses. Also, I would like to thank my lab mates for their help and support so far since day one and my Family for always encouraging me never to give up. Finally, my appreciation to God, my Father, for keeping me alive to submit this thesis. It is a dream come true!

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CHAPTER ONE

1. INTRODUCTION

The global coffee industry serves as a cornerstone of agricultural economies across more than 70 countries, supporting the livelihoods of over 125 million people and contributing significantly to international trade and gross domestic product (Ponte, 2002). Despite its economic importance, coffee production presents numerous environmental and sustainability challenges, particularly in relation to waste management. Among the most pressing issue is the treatment of coffee processing wastewater (CPW), a byproduct of the wet method used to remove pulp from coffee cherries. CPW is generated in large volumes during post-harvest processing and is characterized by high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), and the presence of recalcitrant compounds such as tannins, caffeine, polyphenols, and organic acids (Chen et al., 2018; Ijanu et al., 2020). These constituents present a high pollutant load and exhibit antimicrobial and phytotoxic properties, complicating biological treatment processes.

When discharged untreated or inadequately treated, CPW poses serious environmental risks. It depletes oxygen in receiving water bodies, disrupts aquatic ecosystems, encourages the proliferation of anaerobic bacteria, and results in offensive odors and water discoloration (Figueroa Campos, 2022; Singh et al., 2021). Moreover, CPW can contaminate groundwater resources, adversely affecting human health and agricultural productivity. In many coffee-producing regions, limited infrastructure and regulatory oversight exacerbate the environmental burden, highlighting an urgent need for effective, low-cost, and sustainable wastewater treatment technologies.

Anaerobic digestion (AD) is widely considered a viable option for CPW treatment due to its capacity for organic load reduction and concurrent biogas production. However, conventional anaerobic systems often perform sub-optimally when treating CPW due to its acidic nature, and the presence of inhibitory substances that suppress methanogenic microbial consortia (Ijanu et al., 2020; Qiao et al., 2013). These factors collectively result in extended lag phases, low methane yields, and incomplete degradation of complex organic molecules, limiting the viability of AD as a standalone treatment option.

To address these limitations, recent innovations have explored micro-aeration as a supplemental strategy to enhance the AD process. Micro-aeration-assisted anaerobic digestion (MA-AD) involves the controlled introduction of small amounts of oxygen into the anaerobic reactor, promoting the activity of facultative and microaerophilic microorganisms capable of degrading otherwise recalcitrant compounds (Fu et al., 2023; Magdalena et al., 2022). This approach has demonstrated promising outcomes in treating various high-strength and inhibitory waste streams, including those from food, lignocellulosic, nitrogen-rich materials, and poultry industries (Li et al., 2024; Song et al., 2020; Zhan et al., 2023). Improvements reported include enhanced COD removal, increased methane production, accelerated startup times, and enrichment of microbial diversity, particularly of hydrolytic and acidogenic populations (Ding et al., 2024; Shrestha et al., 2017). However, despite its successful application in other sectors, the potential of MA-AD for CPW treatment remains largely unexplored.

Given the complex composition of CPW and its inhibitory nature, we hypothesize that MA-AD will improve the degradation of recalcitrant compounds and significantly increase biogas yield when compared to conventional AD. This study aims to systematically evaluate the performance of MA-AD in treating CPW by investigating its effect on key treatment parameters, including organic load removal (e.g., COD), biogas production, and the breakdown of specific recalcitrant compounds such as tannins and caffeine.

By finding the effects of micro-aeration on process performance and microbial dynamics, this work aims to contribute to the development of a more efficient and resilient wastewater treatment strategy for the coffee industry. Ultimately, the findings are expected to support the implementation of sustainable wastewater management practices that align with circular economy principles and environmental conservation goals in coffee-producing regions.

CHAPTER TWO

2. REVIEW OF LITERATURE

Coffee Waste-Wastewater Management: Traditional Methods, Modern Alternatives and the Role of Micro-aeration.

Coffee has been consumed for over a millennium and is currently the most widely consumed beverage globally, with annual consumption surpassing 400 billion cups (Mussatto et al., 2011). Coffee belongs to the Rubiaceous family and the *Coffea* genus, which comprises more than 70 species. Despite this diversity, two species dominate global coffee production. Arabica coffee (*Coffea arabica*) accounts for approximately 75% of global coffee production and is renowned for its superior flavor profile. In contrast, Robusta (*Coffea canephora*) represents the remaining 25% of production and is characterized by a more acidic taste, increased disease resistance, and better adaptability to varied growing conditions (Belitz et al., 2009; Etienne, 2005; Mussatto et al., 2011).). Coffee is cultivated in about 80 countries and is the second most traded commodity worldwide, after petroleum. It is primarily consumed as an infusion of roasted and ground coffee beans, making it a staple beverage across cultures and economies (du Café, 1997; Mussatto et al., 2011).

2.1 Coffee Processing Methods

The first step in coffee processing is the extraction of green coffee beans from the harvested cherries, which contain layers of fruit pulp and mucilage. Three primary processing methods are commonly used: dry processing (natural processing), wet processing (washed processing), and semi-dry processing (honey method) (Febrianto & Zhu, 2023; Huch & Franz, 2015; Shen et al., 2023). The choice of processing method significantly influences the final flavor and quality of the coffee and often depends on environmental conditions, resource availability, and regional traditions (Banti & Atlaw, 2024; Idago & Cruz, 2011).

2.1.1 Dry Processing

Dry processing is the simplest and least expensive method, widely used in regions such as West Africa and Brazil (Banti & Atlaw, 2024; Clarke, 2012). It involves drying whole coffee cherries with their pulp and mucilage intact. The berries are first cleaned to remove dirt, leaves, and other debris, then spread under the sun for approximately 10–25 days, depending on the weather (Febrianto & Zhu, 2023; Shen et al., 2023). Regular raking prevents fermentation and ensures uniform drying. However, because the beans remain in contact with the pulp throughout the process, there is a possibility of chemical translocation, which can affect the final flavor profile (Banti & Atlaw, 2024).

2.1.2 Wet Processing

In wet processing, coffee cherries are first de-pulped to remove the outer exocarp, and then the beans are subjected to submerged fermentation for 12–36 hours (Febrianto & Zhu, 2023; Huch & Franz, 2015; Shen et al., 2023). This fermentation step helps break down the mucilage, which is later washed away before drying the beans for 5–10 days. The washed process is known for producing cleaner-tasting coffee with enhanced acidity and clarity, as it eliminates many unwanted flavors (Banti & Atlaw, 2024). However, this method requires significant amounts of freshwater, making it more feasible in regions with abundant water resources (Banti & Atlaw, 2024; Murthy & Naidu, 2012). Additionally, it generates large volumes of wastewater, which can pose environmental concerns if not properly managed (Banti & Atlaw, 2024; Rattan et al., 2015).

2.1.3 Semi-Dry Processing (Honey Method)

The semi-dry or honey processing method is a hybrid of the wet and dry processes. In this method, coffee cherries are de-pulped but retain part of their mucilage while drying (Febrianto & Zhu, 2023; Shen et al., 2023). Unlike fully washed coffee, semi-dry processing does not involve fermentation tanks; the mucilage is left to dry naturally or removed mechanically (Zhang et al., 2019).

2.2 Physicochemical Composition of CPW

CPW is a high organic strength effluent with a complex composition that varies depending on the processing method, coffee variety, and environmental conditions. It is characterized by high COD and BOD due to the presence of sugars, proteins, and other organic matter (Deepa et al., 2002). BOD levels can reach up to 20 $g \cdot L^{-1}$, whereas COD levels may exceed 50 $g \cdot L^{-1}$ (Rattan et al., 2015). The wastewater is typically acidic, with pH values ranging from 3.5 to 4.4, primarily due to the presence of organic acids and polyphenols released during coffee fermentation (Beyene et al., 2014; Selvamurugan et al., 2010). Additionally, CPW contains high concentrations of suspended solids from coffee pulp residues, mucilage, and organic debris, along with significant levels of TSS, including dissolved carbohydrates and proteins. Beyond these organic components, CPW also contains recalcitrant compounds such as tannins and caffeine, which can be difficult to degrade due to their antimicrobial properties (Shanmugam & Gummadi, 2021). The seasonal nature of coffee processing leads to fluctuations in wastewater composition, further complicating its management. A detailed breakdown of these physicochemical characteristics is provided in **Table 1**.

Table 1: Physicochemical Composition of CPW

Parameter	Values	References
pH	3.50-4.40	Beyene et al. (2014); Rattan et al. (2015)
COD ($g{\cdot}L^{-1}$)	9.30-50.00	Cruz-Salomón et al. (2017); Rattan et al. (2015)
BOD ($g{\cdot}L^{-1}$)	0.47-20.00	Kebede et al. (2010); Rattan et al. (2015)
TDS ($g{\cdot}L^{-1}$)	1.00-3.90	Fier and Maloney (2017); Kebede et al. (2010)
TSS ($g{\cdot}L^{-1}$)	2.39-2.82	Fier and Maloney (2017); Genanaw et al. (2021)
TVS ($g{\cdot}L^{-1}$)	8.20	Cruz-Salomón et al. (2017); Genanaw et al. (2021)
Turbidity (NTU)	1.48-45.00	Cruz-Salomón et al (2017); Getahun et al., (2024)
Total Phenol ($g{\cdot}L^{-1}$)	0.05-0.28	Cruz-Salomón et al. (2017); Oliveira and Bruno (2013)

COD (Chemical oxygen demand), BOD (Biological oxygen demand), TDS (Total dissolved solids), TSS (Total suspended solids), TVS (Total volatile solids).

2.3 Environmental Impact of CPW

Waste management remains a significant environmental challenge in many countries, where improper industrial and agricultural waste disposal leads to severe environmental degradation. One of the most affected sectors is water resources, particularly freshwater reservoirs, which suffer from organic pollution due to inadequate waste disposal practices (Woldesenbet et al., 2014). Among agro-based industries, coffee processing plants are notable contributors to water pollution, particularly in coffee-producing countries (Kanu & Achi, 2011; Woldesenbet et al., 2014)

2.3.1 Water Pollution from Coffee Processing

The coffee industry, particularly wet coffee processing, generates vast amounts of wastewater that contain high organic loads, suspended solids, and acidic effluents. This wastewater, which results from pulping, fermentation, and washing of coffee beans, is commonly discharged untreated into nearby water bodies, leading to severe ecological and health impacts (Chen et al., 2018; Ijanu et al., 2020; Woldesenbet et al., 2014). It is estimated that 5–15 liters of water are required to process 1 kg of clean green coffee beans, depending on the pulping process and fermentation intensity (Haddis & Devi, 2008; IARC, 1991; ICO, 2011). The discharge of untreated wastewater leads to the depletion of dissolved oxygen in water bodies, resulting in aquatic life mortality, proliferation of harmful microorganisms, and the degradation of water quality, making it unsuitable for domestic or industrial use (Figueroa Campos, 2022; Roa et al., 2000; Singh et al., 2021).

2.3.2 Impact on Human Health

The environmental pollution caused by CPW also has direct implications for human health. Residents living near coffee processing plants often experience health issues such as nausea, stomach pain, eye and skin irritation, and respiratory problems due to exposure to contaminated water and air (Amare et al., 2023; Ijanu et al., 2020). The decomposition of organic matter in the wastewater releases foul odors, attracting flies and other disease-carrying insects, further increasing the risk of health hazards (Ijanu et al., 2020; Qasim et al., 2020).

2.3.3 Environmental Challenges Posed by Coffee Byproducts

Apart from wastewater, solid byproducts such as coffee pulp and mucilage also contribute significantly to environmental pollution. These byproducts constitute about 40% of the wet weight of fresh coffee fruit and are often disposed of by dumping into rivers or piling up on agricultural land (da Silveira et al., 2020; Woldesenbet et al., 2016). The indiscriminate disposal of coffee pulp leads to soil and water contamination, affecting local biodiversity and agricultural productivity. The acidic nature of coffee pulp can alter soil pH, reducing soil fertility and harming crops. Furthermore, the fermentation of coffee pulp generates heat and acidic byproducts, which can negatively impact plant growth (Navia et al., 2011; Woldesenbet et al., 2014).

2.4 Traditional Methods for CPW Management

Traditional methods for managing CPW have long been employed, primarily focusing on natural and low-cost treatment approaches. These methods include lagooning, land application, and composting, each varying in effectiveness depending on local environmental conditions and resource availability. While these approaches have been widely used, their limitations, such as prolonged treatment time, inefficiency in removing recalcitrant compounds, and potential environmental risks, necessitate the exploration of more sustainable and technologically advanced alternatives. This section provides an overview of conventional wastewater management strategies in coffee processing, evaluating their advantages, limitations, and environmental impact.

2.4.1 Lagooning

Lagooning, also known as pond-based treatment, involves storing wastewater in open ponds where microbial activity facilitates the breakdown of organic matter over time. This process can be aerobic, anaerobic, or facultative, depending on the design and aeration conditions (Calabrò et al., 2024). It is one of the most commonly used methods due to its simplicity and low operating costs (Loganath & Senophiyah-Mary, 2020). However, it requires large land areas and long retention times to achieve adequate treatment (Loganath & Senophiyah-Mary, 2020). Additionally, a major challenge with the system is that during the rainy season, which typically comes after the harvest, can lead to pond overflows, allowing contaminants to seep into nearby water bodies and contaminate them (Rattan et al., 2015).

Furthermore, anaerobic lagoons without mixers often struggle to digest substrates with high suspended solids, such as CPW and dairy manure. These lagoons typically had a higher solids retention time than hydraulic retention time (HRT) and operated under ambient temperature conditions. Their efficiency and biogas production are significantly influenced by climatic factors and geographical location (Harris & McCabe, 2020). Under unfavorable conditions, these factors can contribute to the production of methane and hydrogen sulfide, leading to increased greenhouse gas emissions and odor issues (Loganath & Senophiyah-Mary, 2020). Moreover, improper pond construction can result in leachate infiltration, contaminating groundwater and posing risks to nearby communities (Aderemi et al., 2011).

2.4.2 Land Application

Land application of CPW serves as an effective strategy for irrigation and soil conditioning in some coffee farms. Under favorable conditions, this method can be more efficient and less energy-intensive compared to conventional wastewater treatment systems such as the activated sludge process, trickling filters, and aerated lagoons (Hansen & Cheong, 2019). Various techniques are employed for land application, including irrigation, surface ponding, groundwater recharge through injection wells, and subsurface percolation (Show, 2008). CPW provides agricultural benefits by supplying essential nutrients such as nitrogen and potassium, which enhance soil fertility (Lawson, 2021). It also supports waste recovery (Mittal, 2006) and improves soil structure (Masse & Masse, 2001) making it a sustainable alternative for resource utilization. However, excessive application of untreated or partially treated CPW can lead to soil acidification, microbial imbalances, and plant toxicity due to the high concentration of organic acids and polyphenolic compounds (Mittal, 2006). Furthermore, land application poses the risk of nitrate leaching and groundwater contamination, which can have detrimental effects on local water supplies (Venglovsky et al., 2006). This method is only feasible in regions with sufficient land availability and proper soil conditions, limiting its scalability.

2.4.3 Composting

Composting is a biological process that naturally breaks down organic matter in an oxygen-rich environment, facilitated by microorganisms such as bacteria and fungi producing CO_2, NH_3, H_2O, organic acids, and heat (Bernal et al., 2009). It involves mixing solid residues from coffee wastewater with organic materials such as sawdust or agricultural waste to produce compost. This technology has emerged as an effective approach for managing coffee by-products by recycling and converting them into nutrient-rich compost with minimal pathogenic microorganisms (Papafilippaki et al., 2015; Sanasam & Talukdar, 2017). This method offers a sustainable solution, as the compost produced can be used as fertilizer to enhance both the quantity and quality of agricultural yields (Edgerton, 2009) while also contributing to the conservation of natural resources (Zhao et al., 2018) and improving soil structure and fertility (Sayara et al., 2020). However, composting coffee waste presents several challenges, including the potential increase in soil salinity, which has been reported to delay plant germination (Adamcová et al., 2017; Carballo et al., 2009; Pérez-Gimeno et al., 2016). The process is time-intensive, often requiring several months to achieve complete decomposition (Pace et al., 1995). Additionally, excessive compost application in confined areas can lead to nutrient leaching, particularly during autumn and winter, increasing nitrate concentrations in soil and potentially contaminating surface and groundwater (Jorge-Mardomingo et al., 2015). Furthermore, large-scale composting operations demand significant space and labor, posing limitations for many coffee producers (Trujillo-Gonzalez et al., 2024).

2.5 Modern Approaches for CPW Management

Modern approaches to CPW treatment have emerged as more sustainable and effective solutions to address the environmental challenges posed by traditional methods. These advanced technologies, including photo-fenton reaction, ozonation, and AD, offer significant improvements in the efficiency and speed of wastewater treatment while enabling resource recovery. This section explores these cutting-edge methods, highlighting their potential to overcome the limitations of conventional strategies and contribute to a more sustainable coffee processing industry.

2.5.1 Photo-Fenton Reaction

The Photo-Fenton reaction involves the catalytic decomposition of hydrogen peroxide (H_2O_2) in the presence of ferrous ions (Fe^{2+}) under ultraviolet or visible light, generating hydroxyl radicals (OH) that effectively degrade organic pollutants (Tokumura et al., 2008). This method is particularly effective in breaking down recalcitrant compounds, reducing COD, and improving biodegradability (Gomes de Barros et al., 2020). Studies have demonstrated that the Photo-Fenton process can reduce the concentration of phenolic compounds by 97.83 % of COD commonly found in CPW (San juan-Garisado et al., 2024) and up to 100% removal of caffeine (Yamal-Turbay et al., 2012). Additionally, it operates at relatively mild conditions, making it a viable alternative to conventional treatment methods (Tokumura et al., 2008). However, limitations such as pH sensitivity, iron sludge formation, and the cost of H_2O_2 must be considered for large-scale implementation (Tokumura et al., 2008; Yamal-Turbay et al., 2012). Furthermore, this process is only efficient during sunny days and becomes less effective under low-light conditions. As a result, it is most viable in tropical regions with abundant sunlight but may not be practical in other areas. Additionally, its implementation requires a high level of technical expertise, which most coffee farmers and processors may not possess (Tokumura et al., 2008).

2.5.2 Ozonation

Ozonation is a widely recognized advanced oxidation process that utilizes ozone (O_3) as a powerful oxidant for degrading organic pollutants in wastewater. Ozone reacts with organic matter through direct oxidation or indirect pathways via hydroxyl radical (OH) generation, leading to the breakdown of complex and recalcitrant compounds commonly found in CPW (Rekhate & Srivastava, 2020). This method has been shown to significantly reduce COD and phenolic compounds and improve the biodegradability of CPW for subsequent biological treatment (Amaral-Silva et al., 2016). The efficiency of ozone-based treatment can be enhanced by integrating additional processes. For instance, combining ozone with ultraviolet (UV) radiation, known as the O_3/UV process, improves oxidation efficiency (Emam, 2012). Similarly, incorporating H_2O_2 generates more hydroxyl radicals, intensifying contaminant degradation (Barry et al., 2014). The use of catalysts further accelerates the oxidation reactions (Rekhate & Srivastava, 2020) while activated carbon enhances ozone adsorption and decomposition (Nawrocki, 2013). Additionally, sonolytic ozonation, which involves ultrasonic waves, has been shown to improve ozone reactivity (Mischopoulou et al., 2016). Studies indicate that combining O_3 treatment with others can achieve greater removal of color and aromatic compounds while operating under relatively mild conditions (Alfonso et al., 2021; Hussain et al., 2020; Pires & Momenti, 2009).

Despite its effectiveness, combining ozone with some other treatments has some limitations, including high energy consumption, short ozone half-life, and the potential formation of toxic byproducts such as bromate in bromide-containing waters (Das et al., 2024; Silva &

Jardim, 2006). Additionally, the need for continuous ozone generation and controlled operational parameters may pose economic and technical challenges for large-scale CPW treatment (Das et al., 2024; Derco et al., 2015).

2.5.3 Anaerobic Digestion (AD)

AD is a biological treatment process in which microorganisms break down organic matter in the absence of oxygen, producing biogas (mainly methane and carbon dioxide) (del Agua et al., 2015; Zhou et al., 2024). It is often considered a promising approach for CPW treatment due to its potential for energy recovery. However, the AD of CPW faces several challenges. The presence of inhibitory compounds such as caffeine, tannins, and phenolic acids can reduce microbial activity, leading to inefficient degradation of organic matter (Gyadi et al., 2024b). Furthermore, conventional AD reactors exhibit low degradation rates for complex organic compounds, leading to incomplete wastewater treatment (Gomes de Barros et al., 2020). Even with the application of high-rate anaerobic reactors such as upflow anaerobic sludge blanket reactors, AD alone is often inadequate for achieving complete organic matter removal. A significant fraction of recalcitrant compounds, such as lignin, tannins, and humic acids, persists in the treated effluent due to their resistance to biological degradation (Figueroa Campos, 2022; Gomes de Barros et al., 2020; Oliveira & Bruno, 2013; Péerez et al., 2007; Villa-Montoya et al., 2017). Additionally, the process is highly sensitive to operational conditions such as pH fluctuations, temperature variations, and HRT, which can impact its stability and performance (Uddin & Wright, 2023; Wang et al., 2019). To address these limitations, several strategies have been investigated to improve AD efficiency for CPW and other waste streams, including co-digestion with other substrates, pre-treatment methods, and process modifications.

2.5.3.1 Co-digestion

Co-digestion involves supplementing CPW with additional organic waste to balance nutrient composition and enhance microbial activity (Chen et al., 2018; Selvamurugan et al., 2010). Studies have demonstrated that mixing CPW with animal manure, food waste, or agricultural residues improves biogas production and process stability (Hagos et al., 2017; Mata-Alvarez et al., 2000). The benefits of co-digestion include an improved carbon-to-nitrogen ratio, which counteracts CPW's low nitrogen content and supports microbial growth (Xie et al., 2018). Additionally, the addition of easily degradable substrates enhances methane yield and energy recovery while also diluting recalcitrant compounds such as tannins, caffeine, and polyphenols, thereby reducing their inhibitory effects (Astals et al., 2014; Jagadabhi et al., 2008; Kunatsa & Xia, 2022; Mata-Alvarez et al., 2011; Shah et al., 2015). However, it also presents several challenges, including substrate variability, which can lead to nutrient imbalances and process inhibition. High-fat or protein-rich waste streams may cause ammonia accumulation, foaming, or microbial inhibition, negatively affecting digestion performance (Hagos et al., 2017).

2.5.3.2 Pre-treatment

Pre-treatment methods have also been explored to enhance AD efficiency by breaking down recalcitrant compounds in CPW before digestion. These methods can be categorized into chemical, biological, and physical pretreatments.

Chemical pretreatment methods, such as alkaline addition and ozonation, improve substrate accessibility by breaking down complex structures. Alkaline pretreatment modifies the acetate structure of feedstock, making it more available for hydrolytic enzymes (Karp et al., 2015). Ozonation, a unique chemical approach, requires no additional reagents except ozone, thereby reducing chemical residues. These methods offer advantages such as odor-free processing and pathogen elimination (Atelge et al., 2020). Additionally, ionic liquid pretreatment interacts with lignocellulosic biomass, fragmenting it into smaller components, thereby increasing the active surface area for enzymatic action in AD (Atelge et al., 2020).

Biological methods, such as enzymatic hydrolysis, employ specific enzymes to degrade polyphenolic compounds and enhance biodegradability. Enzymes such as lipase, cellulase, α-amylase, endo-xylanase, dextranase, and protease are commonly introduced into the AD reactor to accelerate organic matter breakdown (Burgess & Pletschke, 2008; Wawrzynczyk et al., 2008). Additionally, aerobic pretreatment involves microbial consumption of organic material, producing CO_2, H_2O, and nitrate, which subsequently enhances the availability of organic matter for AD microorganisms. This approach facilitates faster stabilization of conditions, promoting efficient methane production (Atelge et al., 2020; Fitzgerald, 2013).

Physical pretreatment methods enhance substrate degradation without adding chemicals, enzymes, or microbial agents (Atelge et al., 2020). Mechanical pretreatment increases surface area, bulk density, and porosity, improving microbial-substrate interactions (Atelge et al., 2020; P. O'Dwyer et al., 2008). Thermal pretreatment, involving high-temperature exposure, enhances the solubilization of organic matter, leading to improved methane yields (Zhou et al., 2015). Ultrasonic pretreatment, primarily used for sewage sludge treatment, induces sludge disintegration through hydro-mechanical shear forces and hydroxyl radical oxidation, further improving digestibility (Atelge et al., 2020; Banu & Kavitha, 2017). Despite their advantages, pretreatment methods present certain drawbacks. Chemical pretreatments often require a high quantity of reagents, leading to increased costs and potential inhibitory effects on microbial activity (Atelge et al., 2020; Cesaro & Belgiorno, 2014; Harris & McCabe, 2015; Montgomery & Bochmann, 2014). Physical pretreatment methods require significant energy inputs, which depend on the desired degree of material breakdown. In many cases, the energy demand surpasses the potential energy output of the material, making the process costly and less feasible for large-scale applications (Brodeur et al., 2011). Biological pretreatment, on the other hand, while cost-effective and operated under mild conditions, is limited by slow reaction rates and extended processing times, making it less efficient compared to other treatment methods (Brodeur et al., 2011; Sun & Cheng, 2002).

2.5.3.3 Two-stage Digestion

Beyond co-digestion and pre-treatment, process modifications in AD systems have been proposed to improve treatment efficiency. Two-stage digestion, where hydrolysis and acidogenesis occur in a separate reactor from methanogenesis, has been shown to enhance process stability (Hagos et al., 2017). Compared to a single-stage reactor (i.e., conventional AD), the two-stage system offers several advantages: (i) the ability to handle higher loading rates, (ii) high potential for pathogens controlling, and (iii) increased volatile solids and COD reduction efficiencies (Blonskaja et al., 2003; Bouallagui et al., 2005; Hagos et al., 2017; Riau et al., 2012). However, several studies have reported drawbacks, which include: (i) hydrogen accumulation, which can inhibit acidogenic bacteria; (ii) the possible disruption of interdependent nutrient requirements for methanogenic bacteria; and (iii) technical complexity and high start-up cost (Hagos et al., 2017; Ueno et al., 2007; Wang & Zhao, 2009).

While traditional methods for CPW management are widely used, they present significant drawbacks regarding efficiency, environmental impact, and operational feasibility. Lagooning and land application risk water contamination and require large land areas, while composting is time-intensive and does not address the liquid fraction of wastewater. AD provides an opportunity for biogas recovery but is hindered by the presence of inhibitory compounds that affect microbial performance. These limitations highlight the need for more advanced and sustainable wastewater treatment strategies, such as MA-AD, which has shown promise in improving the degradation of recalcitrant compounds and enhancing overall wastewater treatment efficiency.

2.6 Micro-aeration-enhanced AD

Micro-aeration, a controlled process of introducing low levels of oxygen (typically ranging from 0.005 to 5 L $O_2 \cdot L^{-1}_{reactor} \cdot day^{-1}$) into anaerobic systems, has been widely applied in wastewater treatment and is now gaining attention for CPW treatment (Nguyen & Khanal, 2018). Air or oxygen can be introduced into AD process in various ways, including a single dose, intermittent (pulse-mode) dosing, or continuous supply. This can be applied at different stages of the process, such as pre-treatment, during digestion, or post-digestion, to enhance overall performance (Girotto et al., 2018). During the pretreatment phase, oxygen is typically introduced in a single pass directly into the sludge or substrate before formal experiments. Given the air-tight nature of anaerobic reactors, many researchers prefer this approach as it simplifies operation by reducing the need for continuous oxidation-reduction potential (ORP) regulation (Li et al., 2024). To achieve the desired ORP, oxidizing gases such as air or oxygen are flushed into the reactor. However, as the reaction progresses, facultative microorganisms consume the available oxygen, requiring intermittent micro-aeration throughout the experiment to maintain appropriate ORP levels (Li et al., 2024). A persistent micro-aeration strategy is considered ideal but requires strict control to avoid disrupting microbial activity. Automated systems equipped with pH and ORP sensors help regulate pH, temperature, and aeration frequency, ensuring stable long-term operation (Li et al., 2024). The most effective and reliable method for standardizing microaerobic conditions across different studies is by utilizing ORP as a control parameter. ORP provides a measurable indicator of the system's oxidative state, and the ORP electrode can accurately detect even minor variations in oxygen concentration within the aqueous phase, ensuring consistency and comparability in research findings (Nguyen & Khanal, 2018). Khanal et al. (2003) & Yin et al. (2016) utilized ORP as a key parameter for controlling micro-aeration in various applications, including enhancing volatile fatty acid (VFA) production (−100 to −200 mV) and sulfide removal (−275 to −265 mV). The mechanism of micro-aeration in anaerobic systems includes enhanced hydrolysis and acidogenesis, where small amounts of oxygen stimulate facultative and hydrolytic bacteria, leading to improved breakdown of complex organic matter (Nguyen & Khanal, 2018). It also aids in sulfide oxidation by allowing limited oxygen to convert hydrogen sulfide toxic byproduct of sulfate-reducing bacteria, into less harmful compounds, thereby reducing methanogen inhibition and improving methane production (Krayzelova et al., 2015).

2.6.1 Application of Micro-aeration in various AD systems

Micro-aeration has been successfully applied in various AD systems, demonstrating its potential to enhance process stability, improve biogas production, and optimize microbial interactions. In sewage sludge digestion, it improves biogas production and reduces process instability (Barati Rashvanlou et al., 2020) similarly, in food waste digestion, it enhances hydrolysis and prevents acidification (Li et al., 2024). Furthermore, micro-aeration has proven beneficial in the digestion of lignocellulosic and nitrogen-rich substrates. In poultry litter and wheat straw digestion, controlled oxygen introduction resulted in increased methane yields (Zhan et al., 2023). In chicken manure digestion, micro-aeration has been shown to enhance methanization while simultaneously reducing hydrogen sulfide accumulation (Song et al., 2020). Additionally, in AD of corn straw, micro-aeration accelerated the release of soluble humic acids, enhancing electron transfer and overall efficiency (Zhu et al., 2022). Beyond substrate-specific applications, micro-aeration has been shown to influence microbial interactions within AD systems. Morais et al. (2024) reported that micro-aeration can stimulate facultative anaerobic and aerobic bacteria, which can help sustain syntrophic consortia under low-oxygen conditions. These successes highlight the potential of micro-aeration as a viable strategy for treating CPW and provide a strong rationale for its further investigation.

2.6.2 Factors affecting micro-aeration-based AD system

The effectiveness of micro-aeration depends on factors such as reactor type, oxygen dosing strategy, inoculum characteristics, and substrate properties (Nguyen, 2018). This section provides an overview of various micro-aeration methods and their impacts on AD performance.

2.6.2.1 Micro-Aeration in Different Reactor Types

Micro-aeration performs differently in various reactor configurations. In continuous stirred-tank reactors (CSTR), micro-aeration is primarily used to control the accumulation of VFA, but its effectiveness is relatively low. In contrast, upflow anaerobic sludge blanket reactors benefit from micro-aeration by improving methane production and removing sulfide, making its effectiveness moderate. Leached bed reactors, designed to enhance hydrolysis, show high efficiency under micro-aeration conditions. Similarly, in acid-phase reactors of two-stage systems, micro-aeration significantly enhances hydrolysis and VFA production**.**

2.6.2.2 Micro-Aeration Methods

Micro-aeration can be implemented using different techniques, each with distinct advantages. Gas-phase injection is highly effective for removing hydrogen sulfide (H_2S) from the biogas stream. Liquid-phase injection, which involves dissolving oxygen into the liquid medium, enhances hydrolysis, reduces dissolved sulfide, promotes VFA production, and helps regulate

VFA accumulation. However, its overall effectiveness ranges from low to moderate. Single oxygen injection, typically applied at the beginning of the digestion process, has a high impact on hydrolysis and VFA production. However, intermittent or continuous injection, used to stabilize VFA levels, has a lower effectiveness in comparison.

2.6.2.3 Influence of Inoculum Characteristics

The type and characteristics of microbial inoculum significantly affect the efficiency of micro-aeration. A low biomass concentration can reduce start-up time but has limited effectiveness in sustaining long-term stability. On the other hand, a high biomass concentration helps regulate VFA accumulation and enhances hydrolysis, making it highly effective. Microbial diversity also plays a role; while low-diversity biomass can still effectively enhance hydrolysis and regulate VFA accumulation, high-diversity biomass does not provide additional advantages under micro-aeration conditions.

2.6.2.4 Impact of Substrate Characteristics

The nature of the substrate used in AD also determines the success of micro-aeration. Recalcitrant substrates, which are more difficult to degrade, benefit the most from micro-aeration due to improved hydrolysis. In contrast, easily biodegradable substrates lead to increased VFA production and methane yield but show only moderate improvement with micro-aeration. The Organic loading rate (OLR) also plays a key role; low OLRs have a minimal enhancement in hydrolysis, whereas high OLRs benefit from micro-aeration by preventing excessive VFA accumulation.

2.6.3 The potential of micro-aeration for the treatment of CPW.

Micro-aeration plays a critical role in enhancing hydrolysis during AD by stimulating enzymatic activity and promoting biomass growth (Zhu et al., 2022). Studies have shown that a controlled oxygen supply can significantly boost hydrolysis efficiency, particularly for carbohydrates and proteins. Johansen and Bakke (2006) observed a 50% improvement in hydrolysis under limited aeration, though excessive oxygen led to reduced methane production. Similarly, Charles et al. (2009) reported increased cellulase and protease activity during pre-aeration, contributing to better substrate breakdown. However, Nguyen et al. (2007) reported that the effectiveness of micro-aeration depends on precise control of oxygen levels to avoid excessive oxidation and ensure optimal hydrolytic performance. Micro-aeration has proven effective in reducing VFA buildup in anaerobic reactors, thereby enhancing their stability (Nguyen et al., 2019). Botheju et al. (2010) observed an 80% reduction in VFA level when introducing 2.5% oxygen load in a semi-continuous reactor, with further reductions at higher oxygen levels. Similarly, Simon (2003) found that initial aeration in a three-stage aerobic-anaerobic-aerobic system prevented digester souring, improving methanogenesis. However, Joss et al. (1999) & Tang et al. (2004) reported that VFA levels remained unchanged under limited aeration, suggesting that the effects of oxygen depend on the balance between VFA production by acidogens and their consumption through aerobic respiration and methanogenesis. Additionally, the effect of oxygen on biogas production depends on several factors, including hydrolysis rate, biomass concentration, HRT, and OLR (Botheju et al., 2010). While controlled micro-aeration can enhance methane yield, excessive oxygenation may lead to reduced methane production due to aerobic substrate consumption (Botheju et al., 2010; Shen & Guiot, 1996). For instance, Botheju et al. (2010) observed that introducing limited oxygen (0–16% headspace oxygenation) in anaerobic batch reactors improved methane yield, but exceeding the optimal level resulted in methanogen inhibition and substrate accumulation.

Micro-aeration can be used to improve the treatment of CPW by introducing small amounts of oxygen into the AD process. Micro-aeration enhances microbial activity, effectively breaking down recalcitrant compounds and reducing COD. This accelerates the treatment process and minimizes the environmental impact of CPW, offering a more sustainable and efficient solution for the industry. Compared to other methods, micro-aeration requires limited infrastructure modifications, making it a cost-effective and practical choice for facilities. It boosts microbial efficiency without extensive system overhauls, offering scalability, reduced energy input, and lower operational costs.

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CHAPTER 3

MICRO-AERATION-ENHANCED ANAEROBIC DIGESTION FOR THE STABILIZATION OF COFFEE-PROCESSING WASTEWATER

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ABSTRACT

Coffee processing wastewater (CPW), a byproduct of agro-industrial operations, contains high organic loads alongside recalcitrant and potentially inhibitory compounds such as caffeine and tannins. This study evaluated the performance of micro-aeration-enhanced anaerobic digestion (MA-AD) for the treatment and valorization of CPW. Oxygen was intermittently introduced via oxidation-reduction potential (ORP)-controlled dosing, allowing for comparative assessment across anaerobic and micro-aerobic redox regimes. While both conventional anaerobic digestion (AD) and MA-AD achieved comparable reductions in total and volatile solids, total chemical oxygen demand (COD), and soluble COD (>66% and >86%, respectively), MA-AD exhibited significantly higher total suspended solids (TSS) concentrations and turbidity in later phases, likely due to oxygen-induced floc disruption and particulate release. pH profiles indicated a shift toward increased acidification under MA-AD, without compromising process stability, with both reactors stabilizing between pH 6.8–7.1. Caffeine degradation was accelerated under MA-AD in the first phase (>85% removal in 28 h), though long-term efficiency converged with the control. Methane production was consistently lower in MA-AD (up to 43% reduction), attributed to the oxygen sensitivity of methanogens and possible substrate competition. These results underscore the importance of oxygen dose regulation, redox control, and microbial adaptation in optimizing MA-AD performance. The findings support MA-AD as a promising strategy for enhancing hydrolysis and partial removal of recalcitrant compounds in CPW, though further refinement is required to sustain biogas quality and yield at scale.

3.0 Introduction

Coffee is one of the most widely traded global commodities, with trade volumes expected to exceed 10.49 million tons in 2024/2025 (USDA, 2024). Globally, it ranks as the second most valuable commercial product after oil, with significance across diverse economies and sectors (Ponte, 2002). The coffee processing industry plays a substantial role in driving economic growth at both national and international levels (Rattan et al., 2015). Also, about one-third of the global population consumes coffee, reflecting its pervasive socio-cultural impact. As the global coffee market expands, consumer expectations for fair and sustainable sourcing have also increased. However, coffee production practices frequently do not align with these expectations in many countries, largely due to limited regulatory oversight, inadequate incentives, gaps in knowledge, and a lack of resources needed to support sustainable practices (Dadi et al., 2018). Brewing practices in high-consumption regions such as the United States, the United Kingdom, and Europe underscore the resource intensity of coffee preparation. Typical brewing requires 42, 48, and 57 grams of roasted ground coffee per liter of water, respectively (IARC, 1991; ICO, 2011). Similarly, coffee processing wastewater (CPW), which is a by-product of coffee production and processing, is characterized by a high organic load (i.e., high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Chen et al., 2018). CPW contains a large amount of organic matter, including proteins, sugars, caffeine, tannins, and polyphenolic compounds. These compounds, particularly tannins, polysaccharides, and their mixtures, contribute to the dark coloration of the effluent due to the presence of melanoidins, which are resistant to biological degradation (Cárdenas et al., 2009; Ijanu et al., 2020; Péerez et al., 2007). When released into aquatic environments, these substances promote the formation of anaerobic conditions, fostering the proliferation of harmful bacteria. This bacterial activity depletes dissolved oxygen levels, disrupts aquatic food chains, and generates toxic byproducts, posing severe ecological risks to aquatic ecosystems (Singh et al., 2021). Furthermore, contamination of drinking water sources by these bacteria can lead to significant health risks for humans, such as skin irritation and gastrointestinal disturbances (Figueroa Campos, 2022).

In addition, CPW contains a high concentration of suspended solids, which increases turbidity in water bodies (Ijanu et al., 2020; Novita et al., 2012). This turbidity contributes to opacity, blocking light penetration, and reducing photosynthesis in aquatic plants, further disrupting the balance of aquatic ecosystems (Ijanu et al., 2020; Péerez et al., 2007; Takashina et al., 2018; Tokumura et al., 2006). These factors collectively make CPW a significant environmental pollutant, requiring advanced treatment methods to mitigate its impact on aquatic ecosystems. As global coffee consumption increases, addressing wastewater management has thus become essential to comply with rising environmental standards and minimize the industry's ecological footprint.

Traditional treatment methods often struggle with CPW’s complex and highly variable nature, necessitating innovative approaches such as anaerobic digestion (AD) and micro-aeration-assisted anaerobic digestion (MA-AD) (Ijanu et al., 2020). AD is a widely adopted biological process for treating high-strength organic wastewater, including agricultural, food processing, and industrial effluents (del Agua et al., 2015; Zhou et al., 2024). AD involves the breakdown of organic matter by microorganisms in the absence of oxygen, producing biogas composed primarily of methane (50-65%) and carbon dioxide (35-50%) (Angenent et al., 2022; Zhou et al., 2024). The conversion of organic substrates into biogas proceeds through four stages: hydrolysis, acidogenesis, acetogenesis, and, finally, methanogenesis (Rehman et al., 2019). This process is known for its ability to reduce the organic load of wastewater while simultaneously generating renewable energy in the form of biogas. However, the efficiency of AD is influenced by various factors, which can either improve or inhibit the process. These factors include the composition of the substrate, organic loading rate, mixing conditions, presence of inhibitory compounds, temperature, pH levels, carbon-to-nitrogen ratio, microbial diversity, and retention time (Gyadi et al., 2024a). The high levels of inhibitory compounds in CPW, such as phenols, caffeine, and tannins, can impede microbial activity, leading to lower biogas yields and incomplete degradation of organic matter (Figueroa Campos, 2022). In addition, CPW's low alkalinity, acidic pH, and lack of essential nutrients and trace elements further complicate its treatment through AD (Ijanu et al., 2020). Several studies have reported low biogas yield, COD removal, and accumulation of volatile fatty acids (VFA) during mono-digestion of CPW. Qiao et al. (2013) further reported reactor failures under such conditions. However, Du et al. (2020) noted that reactor failure could be mitigated and biogas production enhanced by supplementing trace minerals. Chen et al. (2018) & Selvamurugan et al. (2010) reported co-digestion as a strategy to stabilize the AD process of coffee residue. Still, the effectiveness in breaking down biologically recalcitrant compounds remains almost the same as the mono-digestion of CPW. Even with high-rate anaerobic reactors such as upflow anaerobic sludge blanket reactors, AD alone is often insufficient for achieving complete organic matter removal. A considerable portion of recalcitrant compounds, such as lignin, tannins, and humic acids, remain in the treated effluent, as they are highly resistant to biological degradation (Bruno & Oliveira, 2013; Campos et al., 2013; Gomes de Barros et al., 2020; Péerez et al., 2007; Villa-Montoya et al., 2017).

Furthermore, anaerobic microbiomes show inherent limitations in degrading recalcitrant compounds, particularly those with aromatic ring structures, due to the absence of oxygen as a potent electron acceptor and the lack of robust enzymatic mechanisms present in aerobic microbiomes. Aerobic systems, by contrast, utilize oxygen and powerful enzymes to cleave such molecular bonds, making them significantly more effective in breaking down these complex compounds (Ortiz-Ardila et al., 2024). Non-biological oxidative methods have, however, proven effective. For example, Tokumura et al. (2006) reported nearly complete decolorization of coffee processing effluent using the photo-fenton process (UV/Fe^{2+}/H_2O_2). Similarly, Takashina et al. (2018) demonstrated the effectiveness of a combined ozone and UV treatment (O_3/UV), achieving 98% removal of caffeine and 99% degradation of colorizing compounds in synthetic coffee wastewater. Furthermore, Yamal-Turbay et al. (2012) reported 100% removal of caffeine using the photo-fenton method combined with hydrogen peroxide. However, these methods, while effective, are often associated with high operational costs and technical complexity due to the need for specialized equipment, energy-intensive processes, and chemicals (Gogate & Pandit, 2004). Therefore, to overcome the challenges associated with conventional anaerobic systems, aerobic systems, and non-biological oxidative methods, relatively low-cost modifications, such as the introduction of micro-aeration, need to be explored.

Micro-aeration offers a cost-effective and sustainable alternative, leveraging natural microbial processes with minimal infrastructure modifications and lower energy requirements, making it more practical and accessible, particularly in resource-limited settings (Rajagopal & Goyette, 2024). Unlike traditional AD processes, which operate under strict anaerobic conditions, micro-aeration allows for the controlled dosing of oxygen at low levels to promote the growth of facultative microorganisms that augment the breakdown of particulate and recalcitrant compounds. Studies have also shown that micro-aeration can enhance biogas yield, COD removal, promote microbial diversity, and reduce sludge production in the treatment of various organic waste streams (Ding et al., 2024; Fu et al., 2023; Xu et al., 2021). Additionally, it has been shown to prevent acidification, enhance hydrolysis, mitigate process instability, reduce hydrogen sulfide accumulation, and stimulate microbial interactions, further optimizing AD performance (Rashvanlou et al., 2020; Li et al., 2024; Morais et al., 2024; Song et al., 2020; Zhan et al., 2023; Zhu et al., 2022). Furthermore, Fu et al. (2023); Magdalena et al. (2022); Nguyen and Khanal (2018); Shrestha et al. (2017) reported enhanced degradation of various recalcitrant compounds using micro-aeration in other AD applications, but to the best of our knowledge, no research has yet evaluated its effectiveness for treating coffee residues.

The synergy between micro-aeration and AD offers a promising solution for the treatment of CPW. This hybrid approach can address the limitations of conventional anaerobic reactors, such as slow degradation rates and sensitivity to recalcitrant compounds, by creating a more robust microbial community. Integrating micro-aeration in full-scale anaerobic reactors poses technical challenges, including maintaining stable conditions and achieving uniform oxygen distribution, which is critical for consistent treatment performance (Botheju & Bakke, 2011). Factors such as the dosage and timing of oxygen injection, reactor configuration, and microbial community dynamics play a crucial role in determining the efficiency of the process (Botheju & Bakke, 2011; Chen et al., 2020). Achieving the right balance between anaerobic and aerobic conditions is essential, as excessive oxygen can inhibit methanogenesis and shift the microbial community towards aerobic degradation pathways. At the same time, too little oxygen may have no significant effect. Thus, understanding the interactions between oxygen levels, microbial activity, and degradation of complex organic matter is critical to successfully implementing this technology at scale.

This study aims to address these knowledge gaps by evaluating the performance of MA-AD in treating CPW. Specifically, the study focuses on optimizing oxygen dosage and assessing the impact of micro-aeration on biogas yield, COD reduction, and the degradation of inhibitory compounds. By providing insights into the underlying mechanisms and operational conditions, this research seeks to contribute to the broader goal of sustainable wastewater management in the coffee industry.

3.1 Materials and Method

3.1.1 Reactor Set-up

The reactors consisted of two benchtop fermenters (BioFlow3100, New Brunswick Scientific Co., New Jersey, USA), each with a 1.5-L vessel and an operational volume of 1 L. Each reactor was outfitted with a headplate that included a sampling port, a gas sparger, an oxygen-reduction potential (ORP) probe (Sensorex, ORP probes, Garden Grove, California, USA), an influent inlet, and an effluent outlet. The reactors were set up as described by Usack et al. (2012) and operated as continuously stirred tank reactors (CSTR) using an overhead stirrer (Fisherbrand Compact Digital, Waltham, Massachusetts, USA) integrated with an ORP controller (Oakton 220 pH/ (ORP)/temperature controller, Vernon Hills, Illinois, USA). A 3-L water bath (Fisher Scientific, Hampton, New Hampshire, USA) maintained the

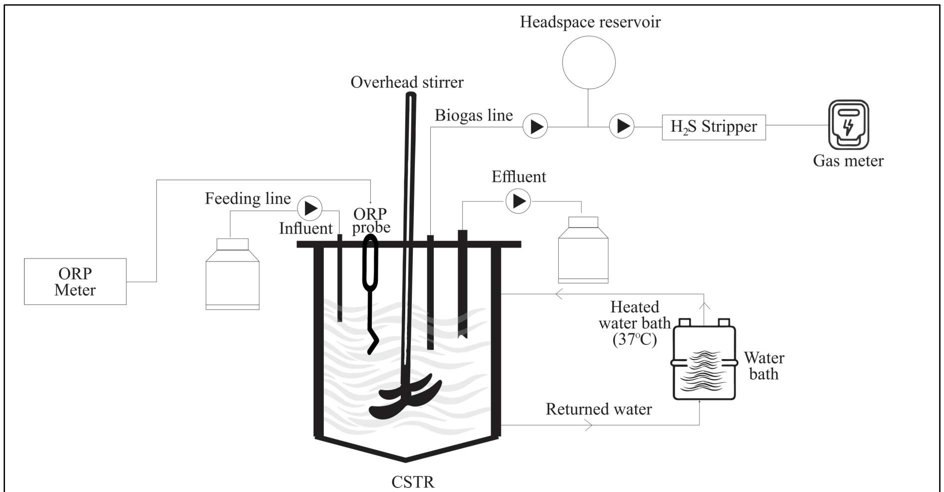

Figure 1: AD Reactor Set-up

reactor temperature at 37 ±1°C. The effluent was pumped out using a peristaltic pump (Masterflex®, Vernon Hills, Illinois, USA) for further analysis. Biogas production was monitored with integrated flow meters (BPC Instruments, Mobilvägen, Lund, Sweden). The reactors were set up as illustrated in **(Figure 1 and 2)** to provide: 1) a control reactor system and 2) an experimental reactor system for micro-aeration. The reactors were allowed to reach pseudo-steady-state operation before initiating the experimental phase of the study. During the experimental phase, one reactor was sparged with oxygen to induce microaerobic conditions, while the other was supplied with 100% nitrogen gas (N_2) to maintain AD conditions. The ORP in the micro-aeration reactor was regulated using integrated ORP controllers following the guidelines of Nguyen and Khanal (2018).

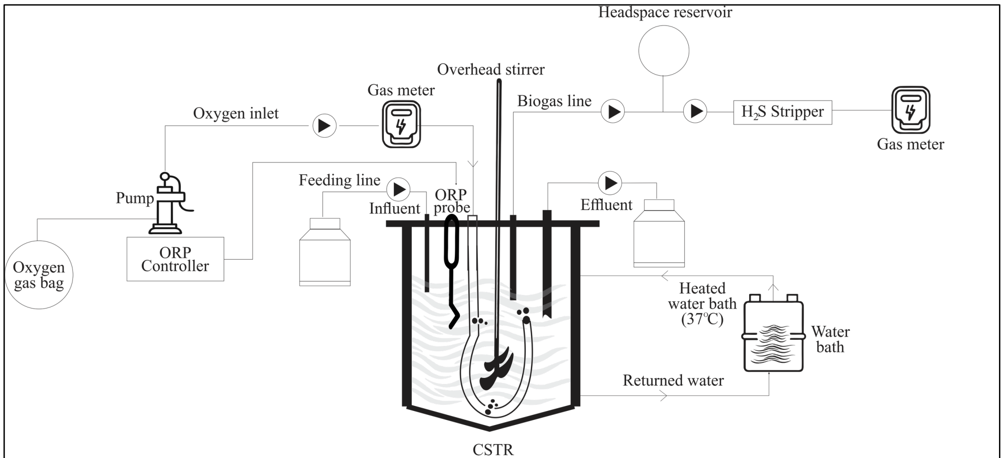

Figure 2: MA-AD Reactor with custom-made ORP Controller

3.1.2 ORP-controlled micro-aeration system

In this study, the reactor was controlled by an ORP controller (Oakton 220 pH/ORP/temperature controller, Vernon Hills, Illinois, USA) to maintain micro-aerobic conditions by targeting an ORP of +20 mV above the baseline anaerobic ORP during each phase. Micro-aeration was achieved by injecting oxygen at a controlled flow rate of 10 mL·min^{-1} using a pump. The daily oxygen dosing volume was tracked using integrated flow meters (BPC Instruments, Mobilvägen, Lund, Sweden), which measured the oxygen flow before it entered the reactor, ensuring precise and accurate daily oxygen measurements. The ORP probe was highly sensitive to dissolved oxygen (DO), detecting even small amounts, with DO concentrations as low as 0.1 $mg \cdot L^{-1}$ being measurable. As oxygen was injected, the ORP increased, indicating a shift towards a more oxidative environment. The ORP-controlled micro-aeration system was fully automated, with oxygen injection cycles triggered when the ORP fell below the target value and ceasing once the target ORP was achieved. This system maintained stable micro-aerobic conditions in the reactor, with consistent fluctuations of ORP around the target set point. The ORP setpoint values and the average oxygen dosing amount during each experimental stage are presented in **Table 2**.

Table 2: The Operational Parameters of Micro-Aeration during the Experiment

Phase	**Set ORP (mV)**	**Dosing techniques**	**Average Oxygen dosing ($mL \cdot O_2 \cdot day^{-1} \cdot L_{reactor}^{-1}$)**	**Gas type**	**Duration (Days)**
Steady-State	-490	NA	NA	NA	160
First	-470	Continuous	28.70 ± 1.03	Air	129
Second	-450	Continuous	100.70 ± 6.90	Oxygen	70

3.1.3 Inoculum and CSTR Operation

Spent coffee grounds (SCG) were obtained from a dining hall at the University of Georgia, Athens, USA, steeped for 48 hours at room temperature, and filtered to remove solid particles before being used as a model CPW substrate. The two reactors were inoculated with anaerobic digestion sludge (ADS) sourced from a wastewater treatment plant (Gwinnett, Georgia, USA). Reconstituted whole milk powder (RWM) was used in place of milk processing wastewater as a co-digestion substrate to provide additional organic content. The final substrate was a mixture containing per liter: SCG, 700 mL; ADS, 285.6 mL; RWM, 6.9 g; yeast, 1 g; 7.2 mL of mineral stock; and 7.2 mL of trace element solution. The mineral stock contains the following (in $g \cdot L^{-1}$):

$FeCl_2 \cdot 4H_2O$, 370; $MgCl_2 \cdot 6H_2O$, 120; KCl, 86.7; NH_4Cl, 26.6; and $CaCl_2 \cdot 2H_2O$, 16.7. The trace element solution consists of (in $g \cdot L^{-1}$): $COCl_2 \cdot 6H_2O$, 2; $MnCl_2 \cdot 4H_2O$, 1.33; H_3BO_3, 0.38; $ZnSO_4 \cdot 7H_2O$, 0.29; $Na_2MoO_4 \cdot 2H_2O$, 0.17; and $CuCl_2 \cdot 2H_2O$, 0.18. The reactors were maintained at a constant temperature of 37 °C and operated with a hydraulic retention time of 40 days at an organic loading rate of 0.6 $gCOD \cdot L^{-1} \cdot day^{-1}$. The influent composition was a mixture comprising 20% SCG, 60% RWM, and 20% ADS based on COD. The substrate compositions are shown in (**Table 3**).

Table 3: Substrate characterization data for SCG, ADS, and RWM.

Parameters	Units	SCG	ADS	RWM
COD	$g \cdot L^{-1}$	4.00 ± 1.09	8.42 ± 0.59	1.64 ± 2.41
TS	%	0.34 ± 0.05	3.03 ± 0.10	97.44 ± 0.01
TVS	%	0.22 ± 0.04	2.30 ± 0.65	86.31 ± 0.22
pH	-	5.70 ± 0.20	6.20 ± 0.42	6.70 ± 0.12
TDS	%	0.13 ± 0.01	0.34 ± 0.01	N/A
TVDS	%	0.10 ± 0.00	0.16 ± 0.00	N/A
TSS	%	0.17 ± 0.00	2.10 ± 0.05	N/A
TVSS	%	0.14 ± 0.03	1.4 ± 0.03	N/A

COD (Chemical oxygen demand), TS (Total solids), TVS (Total volatile solids), TDS (Total dissolved solids), TVDS (Total volatile dissolved solids), TSS (Total suspended solids), TVSS (Total volatile suspended solids).

3.1.4 Analyses:

Samples collected from the reactors were analyzed for pH, total solids (TS), COD, and volatile solids (VS) following standard methods (Rice et al., 2012). Headspace gas samples were taken weekly to assess biogas composition (i.e., CH_4, CO_2), utilizing a gas chromatograph equipped with a flame ionization detector (SRI 8610 C, Torrance, California, USA). Nitrogen served as the carrier gas, with an inlet and detector temperature maintained at 110°C and a constant oven temperature set at 40°C. The concentrations of individual VFA were determined using gas chromatography with a flame ionization detector (Agilent 6890, Santa Clara, California, USA) according to the procedure described by Usack et al. (2014) with minor modifications. Hydrogen was used as the carrier gas, with the inlet temperature set to 200°C and the detector temperature set to 275°C. Individual VFA species were separated using a fused silica capillary column (NUKOL, 15 m × 0.53 mm × 0.50 μm film thickness; Supelco Inc., Bellefonte, Pennsylvania, USA). The initial temperature was maintained at 70°C for 2 minutes, followed by an increase of 12°C per minute until reaching 200°C, at which point it was held for an additional 2 minutes. Turbidity was measured using a turbidity meter (HI 98703, Hanna Instruments, Woonsocket, Rhode Island, USA), following the manufacturer's standard operating procedure. Caffeine was extracted using a liquid-liquid extraction method (Vandeponseele et al., 2021). The supernatants from centrifuged samples underwent three chloroform extractions, followed by solvent evaporation using a Rotavapor system. The residue was resolubilized in HPLC-grade water, filtered (0.45 μm), and analyzed via HPLC (1100, Agilent Technologies Santa Clara California, USA). The mobile phase was water/methanol (65/35, v/v) with 1 % acetic acid, a 1 $mL \cdot min^{-1}$ flow rate, and a 20 μL injection volume. Caffeine was detected at 280 nm and quantified via calibration curves.

3.1.5 Statistical Analysis

Statistical differences between the AD and MA-AD reactor were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's Honest Significant Difference (Tukey's HSD) post-hoc test for pairwise comparisons. All analyses were conducted in R software (version 4.3.1). The stats package was used for ANOVA, and the agricolae and multicomp packages were employed for post-hoc tests and multiple comparisons. Data sets for methane yield, TS, VS, COD, and pH were assessed for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively. Where assumptions were not met, appropriate transformations or non-parametric equivalents were applied. All statistical tests were performed at a significance level of $\alpha = 0.05$. Data are presented as mean ± standard deviation unless otherwise stated.

3.2 Results and Discussion

3.2.1 Reactor Stabilization and Microbial Adaptation in Micro-aerated Digestion

The pH levels in both the AD and MA-AD reactors were monitored throughout the study to assess the impact of micro-aeration on reactor stability. During the start-up phase, both reactors stabilized around a baseline pH of 7.08 ± 0.02 **(Figure 3)**, indicating that the AD process was functioning normally under both conditions (Usack et al., 2012) with no significant differences in pH observed between the two reactors. Initially, air was used as the dosing gas to induce micro-aeration in the experimental reactor. However, this reduced the CO_2 partial pressure in the headspace between Day 160 and Day 290, leading to an increase in pH. This shift in pH was driven primarily by physical and chemical changes in the gas/liquid CO_2 equilibrium, not by biological processes. The pH change did not result in increased VFA or methane production. Upon replacing air with pure oxygen gas on Day 280, a drop in pH was observed in the MA-AD reactor compared to the AD reactor. This decrease in pH below baseline levels can be attributed to increased microbial activity under micro-aerobic conditions, which promotes the production of organic acids by facultative microorganisms, as reported by Girotto et al. (2018). The shift from air to pure oxygen likely created more favorable conditions for the growth of acid-producing microorganisms (Zhang et al., 2005) that thrive in micro-aerobic environments.

The increased microbial activity in the micro-aerated reactor may also have contributed to the breakdown of more complex organic compounds, potentially improving the treatment efficiency of the reactor by promoting the conversion of recalcitrant substances into simpler, biodegradable forms. These findings align with those of Canul Bacab et al. (2020), who reported similar shifts in microbial dynamics under oxygen-limited conditions. This process likely led to the formation of non-VFA organic acids or acidic byproducts such as lactic acid, succinic acid, or other intermediates that were not measured. VFA concentrations remained stable at 364.8 ± 49.4 mg·L^{-1} in the MA-AD reactor and 383.9 ± 88.2 mg·L^{-1} in the AD reactor. Despite these pH shifts, the MA-AD reactor adapted over time, stabilizing at 6.8 ± 0.2 by the second phase (after Day 290) without significant acid accumulation or process instability. Additionally, the low OLR (0.6 gCOD·L^{-1}·day^{-1}) employed during this study might have played a role in maintaining the reactor stability, limiting excessive acid accumulation, and allowing for the gradual adaptation of

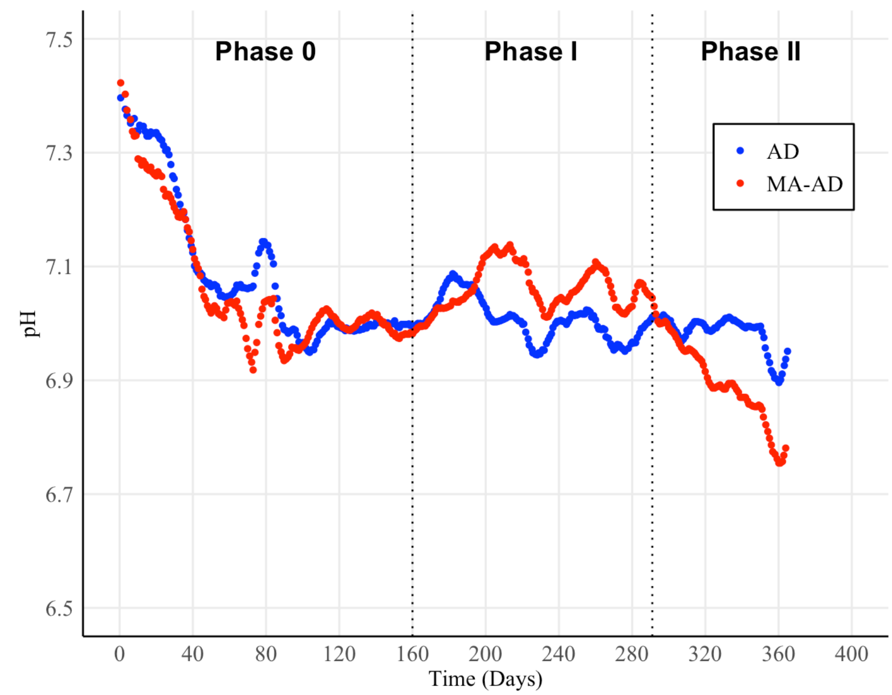

Figure 3: pH Variation over Time for AD and MA-AD

microbial communities to micro-aerated conditions (Nguyen, 2018). This observation is consistent with the findings of Fu et al. (2023) who reported that the role of micro-aeration in promoting or reducing VFA accumulation is closely linked to oxygen loading and OLR. Despite the observed shifts in pH during Phase 0, the total COD removal efficiency of the AD reactor (65.1 ± 17.6 %) and MA-AD reactor (68.2 ± 15.5 %), and soluble COD removal efficiency of the AD reactor (85.58 ± 12.02 %) and MA-AD reactor (88.2 ± 7.5 %) remained consistent across both reactors with no significant difference ($p = 0.607$ for total COD, $p = 0.279$ for soluble COD). This consistency suggests that the shift in acid production pathways due to micro-aeration did not enhance or impair the degradation of organic matter. Additionally, the COD removal efficiency from the experimental phases was analyzed separately, and no significant difference was observed ($p = 0.413$ for total COD, $p = 0.719$ for soluble COD, further confirming the stability of both reactors in terms of organic matter degradation. This could further indicate that the acids and metabolites produced under micro-aerobic conditions were not readily biodegradable, or their contribution to COD reduction was minimal, resulting in no significant difference in the overall COD removal efficiency. Furthermore, the micro-aerated reactor's ability to maintain stability under these altered conditions aligns with the work of Duarte et al. (2024), who reported similar benefits of limited oxygen exposure on anaerobic processes without disrupting overall reactor performance.

3.2.2 Solids Removal Efficiency in Micro-aerobic Conditions

In this study, TS and VS removal were evaluated under both AD and MA-AD conditions. A significant difference in TS removal efficiency was observed between the two reactors, with the MA-AD reactor showing an approximately 5.7 % lower reduction compared to the AD reactor **(Figure 4)**. On the other hand, the removal of VS did not show any significant difference between the two reactors, indicating that micro-aeration did not enhance the breakdown of the organic volatile fraction of CPW. The reduction in TS removal efficiency in the MA-AD reactor may be attributed to several factors, including an increase in biomass content resulting from heightened cellular activity potentially stimulated by micro-aerobic conditions (Diak et al., 2013b), as well as altered microbial dynamics. These shifts in microbial communities under limited oxygen exposure may have favored biomass accumulation over effective degradation of particulate matter, thereby reducing overall solids removal efficiency. Previous studies have reported that micro-aeration can alter the microbial community structure by enhancing the growth of facultative microorganisms that thrive under low-oxygen conditions (Morais et al., 2024), which could result in different metabolic pathways for the degradation of solids. These pathways, while potentially enhancing other metabolites or acid production, may not be as efficient at breaking down the bulk solids, including the more recalcitrant fractions of the solids. Specifically, Romero et al. (2021) reported that micro-aeration did not enhance TS removal from AD treatment of sewage sludge from municipal water resource recovery facilities.

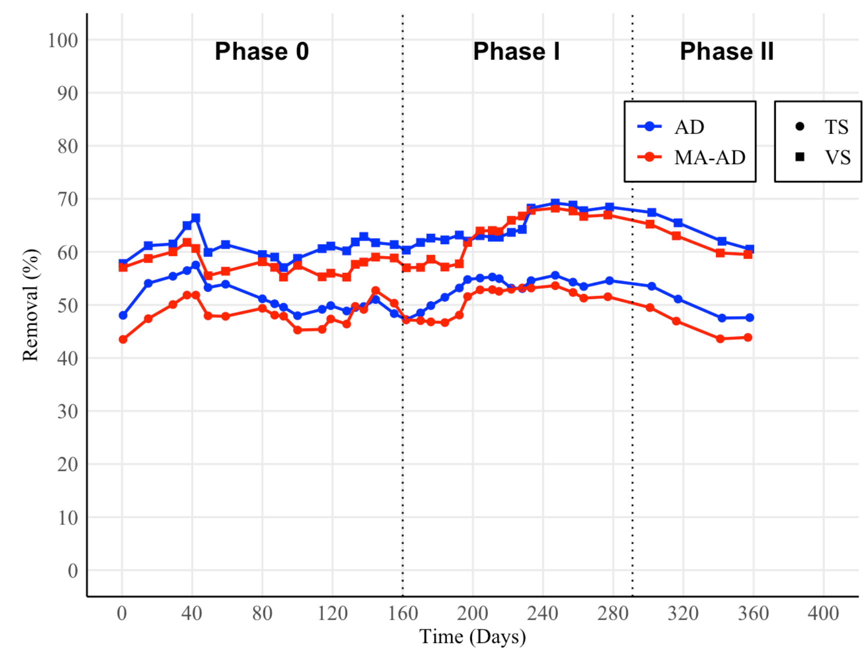

Figure 4: Total and Volatile Solids Removal in AD and MA-AD

Furthermore, this suggests that micro-aeration did not improve the breakdown of the more biodegradable volatile components in the organic matter. A plausible explanation is that micro-aeration is realized only when high concentrations of less degradable VS are present and not the more easily degradable VS. These results are consistent with findings from Diak et al. (2013a), who reported that micro-aeration did not enhance the removal of TS and VS under AD of primary sludge under septic tank conditions. The lack of significant improvement in VS reduction despite enhanced microbial activity under micro-aerobic conditions further supports the idea that the removal of biodegradable VS is not solely dependent on oxygen as an electron acceptor. Similarly, Ijanu et al. (2020) highlighted that recalcitrant compounds, such as caffeine present in CPW, are not easily degraded under either anaerobic or micro-aerobic conditions. These compounds may hinder the degradation process, resulting in a lack of significant differences in VS removal despite enhanced microbial activity in the MA-AD reactor.

3.2.3 Effects of Micro-aeration on Turbidity Reduction and Effluent Clarity

The total soluble solids (TSS*) concentrations in the MA-AD reactor were notably higher compared to the AD reactor **(Figure 5)**. Specifically, in Phase I, the MA-AD reactor exhibited a TSS* concentration of 5.1 ± 0.2 $g \cdot L^{-1}$, while the AD reactor had 4.6 ± 0.1 $g \cdot L^{-1}$. In Phase II, the TSS* concentrations were 5.8 ± 0.1 $g \cdot L^{-1}$ for MA-AD and 4.7 ± 0.3 $g \cdot L^{-1}$ for AD. These differences were statistically significant ($p < 0.05$). The elevated TSS levels in the MA-AD reactor suggest complex interactions between suspended solids and microbial communities under micro-aerobic conditions. The limited oxygen introduced likely promoted the growth of facultative anaerobes, which thrive in low-oxygen environments. This microbial shift may have influenced the flocculation and aggregation of solids, enhancing their retention within the reactor (Liu et al., 2023). In addition to these biological factors, physicochemical mechanisms may have contributed to the observed increase in TSS* under micro-aerobic conditions. Efendi et al. (2023) reported that increased aeration time and airflow in wastewater treatment systems resulted in a progressive increase in TSS, strongly correlated with dissolved oxygen (DO) levels. The study attributed this trend to the reaction between DO and dissolved metal ions, particularly Fe^{2+}, forming insoluble $Fe(OH)_3$ precipitates that increase measured TSS. In the present study, Fe^{2+} was introduced as part of the trace mineral supplement in the feed. Although dosed at relatively low concentrations, the oxidative environment created by micro-aeration likely facilitated the conversion of Fe^{2+} to Fe^{3+}, followed by precipitation as $Fe(OH)_3$, thereby contributing directly to the elevated TSS values observed in the MA-AD reactor. This provides a plausible mechanistic explanation for the significant difference in TSS* between the MA-AD and AD reactors in both treatment phases. However, Zouari and Al Jabiri (2015) reported contrasting results, noting that micro-aeration improved the digestibility of TSS by enhancing the breakdown of complex organic matter, thus preventing its accumulation in sludge. However, such outcomes may be highly system-dependent, varying with substrate composition, oxygen exposure regimes, and microbial consortia. Furthermore, Jenicek et al.

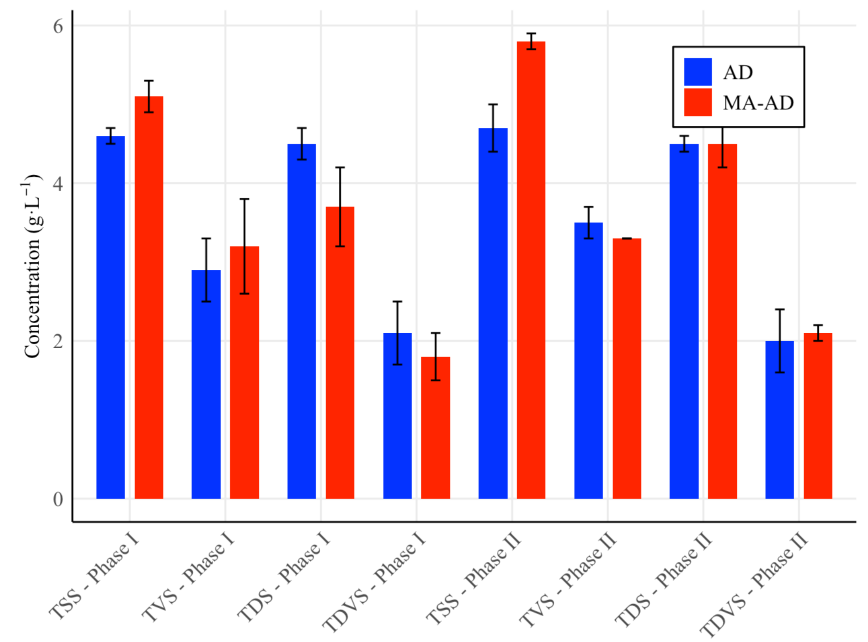

Figure 5: TSS, TVSS, TDS, and TDVS levels across treatment phases

(2011) observed no significant differences in TSS or VSS concentrations during the micro-aerobic treatment of digested sludge, further suggesting that micro-aeration may not universally enhance solids degradation. In contrast, the removal efficiencies of total dissolved solids (TDS), total volatile dissolved solids (TVDS), and total volatile suspended solids (TVSS) show no statistically significant differences between the AD and MA-AD reactors across both treatment phases. For example, in Phase II, TDS concentrations were 4.5 $g \cdot L^{-1}$ and 3.7 $g \cdot L^{-1}$, with TDVS values of 2.1 $g \cdot L^{-1}$ and 1.8 $g \cdot L^{-1}$, respectively. TVSS levels were 2.9 $g \cdot L^{-1}$ and 3.2 $g \cdot L^{-1}$ in the AD and MA-AD reactor, respectively. Similarly, in Phase II, TDS values were 4.5 $g \cdot L^{-1}$ in both reactors, TDVS was 2.0 $g \cdot L^{-1}$, and 2.1 $g \cdot L^{-1}$, and TVSS was 3.5 $g \cdot L^{-1}$ and 3.3 $g \cdot L^{-1}$, in AD and MA-AD reactor respectively. These closely aligned values across systems and phases suggest that micro-aeration did not significantly enhance the microbial breakdown of either dissolved or particulate VS. This finding supports previous reports that strictly anaerobic conditions may be more effective for degrading VS due to the dominance of specialized obligate anaerobes that metabolize VFA and other organics (Appels et al., 2008). In summary, while micro-aeration influenced the behavior of suspended solids, as is evident from the elevated TSS* in the MA-AD reactor, it did not provide a measurable advantage in removing either dissolved or suspended VS. These results emphasize the need for carefully calibrated oxygen input when designing or modifying AD reactors, especially when treating substrates rich in volatile organic matter.

The impact of micro-aeration on turbidity was evaluated by monitoring changes in Nephelometric Turbidity Units (NTU) across two treatment phases. Under strictly AD conditions, turbidity values were recorded at 2143 NTU in Phase I and 2030 NTU in Phase II **(Figure 6)**. In contrast, under MA-AD conditions, turbidity increased to 2402 NTU in Phase I and significantly more in Phase II, reaching 3146 NTU. These findings indicate that the effect of micro-aeration on turbidity is influenced by both the redox conditions and the aeration rate applied during each phase. In Phase I, the reactor was maintained at a less reducing environment (+20 mV relative to baseline) with lower oxygen input, whereas Phase II was operated closer to +40 mV, requiring a higher rate of oxygen injection to sustain the desired ORP. The pronounced increase in turbidity observed in Phase II may reflect the combined effects of increased oxygen availability (Zhang et al., 2019) and higher redox potential, both of which could alter microbial metabolism and destabilize floc structures. The substantial rise in turbidity under these

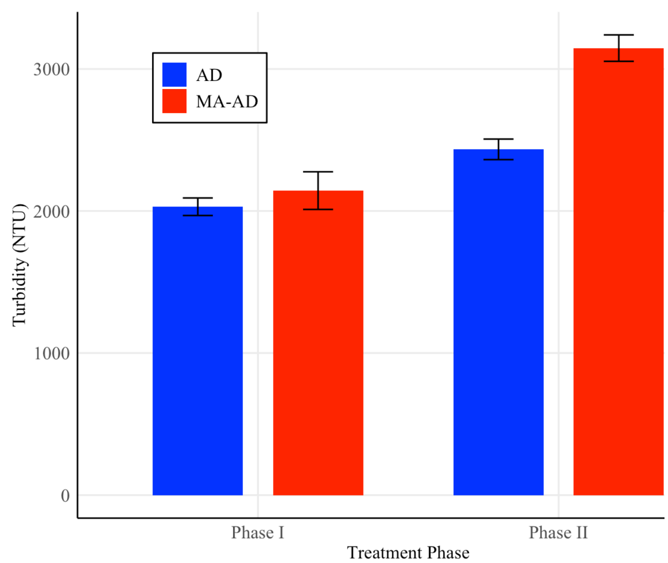

Figure 6: Turbidity Levels across the Treatment Phases

conditions suggests that greater oxygen exposure and redox potential may have disrupted floc integrity or enhanced the release of fine particulate matter into suspension. It is also plausible that elevated microbial metabolic activity partially degraded solids into smaller, light-scattering particles, contributing to increased turbidity. In addition, physicochemical reactions under micro-aerobic conditions may also have contributed to turbidity. For instance, studies have shown that the introduction of air into hydrogen sulfide-rich biogas leads to the formation of insoluble sulfur precipitates, which cause turbidity in water systems (Tang et al., 2004). This supports the hypothesis that micro-aeration may trigger similar precipitation pathways in AD, particularly in the presence of sulfur- or metal-containing ions. In wastewater with high organic loads and complex compounds such as CPW, the effect of micro-aeration on turbidity removal may be limited unless specific factors, such as aeration position and oxygen content, are optimized (Zhang et al., 2019).

3.2.4 Methane Yield in Micro-aerobic Digestion

Methane production was consistently higher in the AD reactor across all phases of the study. During the stabilization period (Phase 0), the AD produced approximately 100.0 ± 16.7 $mL \cdot CH_4 \cdot gCOD^{-1} \cdot L^{-1} \cdot d^{-1}$, while the MA-AD yielded a comparable 101.9 ± 21.5 $mL \cdot CH_4 \cdot gCOD^{-1} \cdot L^{-1} \cdot d^{-1}$ **(Figure 7)**, indicating near-equal performance. This relatively low methane productivity in both the AD and MA-AD reactor, compared to AD of other readily degradable substrates such as food waste, reflects the limited effectiveness of co-digestion alone in overcoming the recalcitrant characteristics of CPW. Studies on the AD of food waste, including co-digestion with substrates such as cattle manure, paper waste, and water hyacinth, have reported significantly higher methane yields, typically ranging from 388 to 607 $mL \cdot CH_4 \cdot gVS^{-1} \cdot L^{-1}$ (Kim & Oh, 2011; Marañón et al., 2012; Oduor et al., 2022; Zhang et al., 2013), highlighting their higher biodegradability. In contrast, Widjaja et al. (2017) reported that co-digesting coffee pulp without appropriate pretreatment does not significantly enhance methane yields due to the persistence of lignocellulosic and polyphenolic compounds. This implies that co-digestion may not be a sufficient strategy on its own to mitigate the inhibitory effects of such compounds in CPW**;** pretreatments may be essential to improve biodegradability and enhance methane recovery.

The methane production in the MA-AD reactor dropped to 77.5 ± 13.7 $mL \cdot CH_4 \cdot gCOD^{-1} \cdot L^{-1} \cdot d^{-1}$ in Phase I, a 23.9% reduction relative to 102.3 ± 12.1 $mL \cdot CH_4 \cdot gCOD^{-1} \cdot L^{-1} \cdot d^{-1}$ in the AD reactor. This decline became more pronounced in Phase II, where methane production in the MA-AD reactor decreased further to 62.8 ± 6.9 $mL \cdot CH_4 \cdot gCOD^{-1} \cdot L^{-1} \cdot d^{-1}$, representing a 43.5% decrease relative to the control reactor, which generated 111.2 ± 8.3 mL·CH_4·$gCOD^{-1}$·L^{-1}·d^{-1}. The progressive suppression of methane yield under micro-aerobic conditions can be attributed to the well-established oxygen sensitivity of methanogenic archaea. These organisms are obligate anaerobes, and their enzymatic systems are irreversibly inhibited by even trace amounts of oxygen (Botheju & Bakke, 2011; Girotto et al., 2018). The presence of oxygen alters redox balance and suppresses methanogenic pathways that are critical for converting VFA and hydrogen into methane. In addition to direct oxygen toxicity, several studies have shown that the introduction of oxygen into anaerobic reactors can shift the microbial community in favor of facultative and aerobic bacteria (Nguyen & Khanal, 2018). While such organisms may enhance the hydrolysis and acidogenesis stages of digestion, they compete directly with methanogens for substrates, particularly VFA. This substrate competition, if not properly managed, can significantly reduce methane production (Nguyen, 2018; Yoda et al., 1987). As reported by Nguyen et al. (2019) effective micro-aeration demands careful regulation of oxygen loading to maintain the delicate balance between facultative bacteria and obligate anaerobes. In the context of this study, the suppression of methane production may also be linked

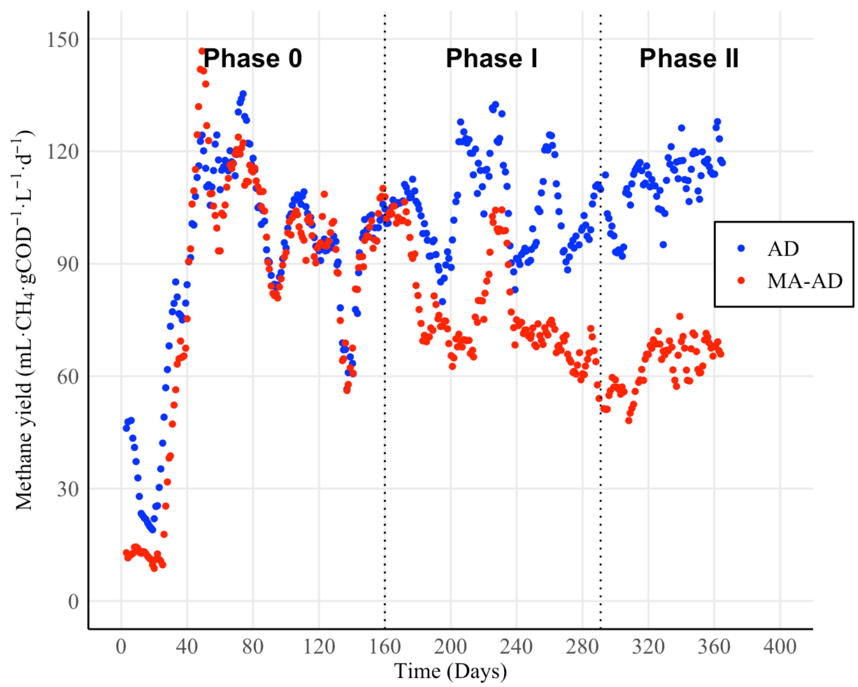

Figure 7: Methane Yield for AD and MA-AD Reactors.

to the chemical complexity of the feedstock. Although CPW comprised only 20% of the total COD in the co-digestion mix, the presence of recalcitrant compounds such as polyphenols, tannins, and caffeine may have contributed to localized or synergistic inhibitory effects on methanogenesis, particularly when combined with oxygen exposure in the MA-AD reactor. Methanogens are especially vulnerable to polyphenolic compounds, and even low concentrations can significantly impair their activity (Puchalska et al., 2021). Teng et al. (2024) investigated the effects of tea-derived polyphenols on methane production in cattle and found that even low doses suppressed methane output by altering the diversity and structure of the enteric microbial communities. This provides mechanistic support for the hypothesis that the polyphenolic content of CPW could inhibit methanogenesis, even at relatively low inclusion levels in the feedstock. In support of these findings, literature reports on the AD of coffee-related wastes reflect similar methane yields, albeit with some variation based on substrate type, operational conditions, and pre-treatment strategies. For instance, Sousa et al. (2021) reported a yield of 341 $mL \cdot CH_4 \cdot g^{-1} \cdot VS^{-1}$ during the co-digestion of coffee waste and glycerol, where the high energy potential of the co-substrate likely enhanced overall efficiency. In a related study, Qiao et al. (2015) reported a methane yield of 285 $mL \cdot CH_4 \cdot g^{-1} \cdot COD^{-1}$ under thermophilic conditions (55°C)**.** The elevated temperature likely contributed to the observed methane yield by accelerating metabolic activity and enhancing the hydrolytic and methanogenic capacity of the microbial consortia. Similarly, Neves et al. (2005) observed methane yields ranging from 240 to 280 $mL \cdot CH_4 \cdot g^{-1} \cdot VS^{-1}$ for five different coffee substitute by-products co-digested with excess sludge under mesophilic conditions. Notably, one of the by-products, composed entirely of barley, initially demonstrated poor methane productivity with a yield of just 20 $mL \cdot CH_4 \cdot g^{-1} \cdot VS^{-1}$. However, when the barley waste was subjected to alkaline hydrolysis pre-treatment and co-digested with kitchen waste, methane yields improved markedly to 200–360 mL·CH_4·g^{-1}·VS^{-1}. This significant enhancement shows the role of substrate conditioning and synergistic co-digestion in improving methane recovery from lignocellulosic and polyphenol-rich feedstocks, such as those derived from coffee-processing residues. Operational factors also played a role in the observed results. Between Days 130–150, methane production was abnormally low in both reactors due to construction-related adjustments that required intermittent reactor shutdown and reconfiguration. These data were excluded from the final methane yield analysis to preserve the integrity of inter-phase comparisons. A further factor that may have contributed to the observed suppression of methane production is the progressive increase in oxygen demand within the MA-AD reactor. Initially, volumetric oxygen dosing was maintained at approximately 28.7 ± 1.1 mL·O_2·day^{-1}·$L_{reactor}^{-1}$, but over time, the required oxygen input rose to as high as 100.7 ± 6.9 mL·O_2·day^{-1}·$L_{reactor}^{-1}$ **(Figure 8)** to maintain target redox conditions. This substantial increase in oxygen dosage could indicate either a shift in microbial community oxygen consumption or reduced oxygen transfer efficiency. In either case, the elevated oxygen levels may have exacerbated the inhibition of obligate methanogenic archaea, as sustained or excessive oxygen exposure is known to suppress methane-forming pathways and disrupt anaerobic syntrophy. Additionally, oxygen introduction

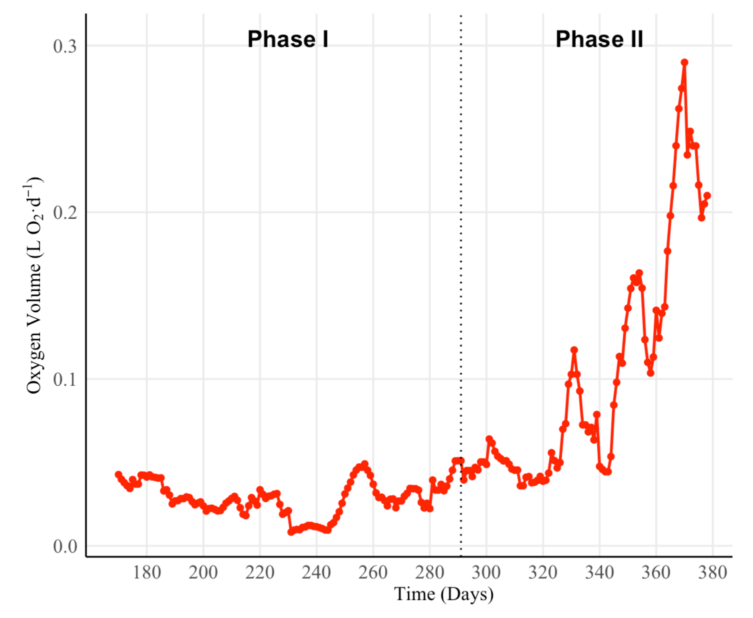

Figure 8:Oxygen dosing rate for the MA-AD Reactor

can favor the proliferation of facultative and aerobic microorganisms, leading to increased competition for key intermediates such as VFA, which are otherwise consumed by methanogens. This competition further reduces the availability of substrates for methane production and may hinder the stability of the methanogenic stage. Overall, while micro-aeration can stimulate microbial activity and accelerate the breakdown of complex organics, it may prove counterproductive when methane generation is the primary objective (Nguyen, 2018). The introduction of oxygen can disrupt the anaerobic microbial balance, inhibit methanogens, and dilute biogas quality through nitrogen intrusion. These effects are particularly critical in systems where biogas yield and composition are a priority. In contrast, when the operational focus shifts toward intermediate products such as VFA or enhanced hydrolysis, micro-aeration can be an effective and economical enhancement strategy (Nguyen, 2018). Ultimately, optimizing aeration intensity and controlling redox dynamics are essential for aligning system performance with specific treatment goals.

3.2.5 Degradation of Recalcitrant Compounds under Micro-aerobic Conditions

This study investigated the degradation dynamics of a polyphenolic and recalcitrant compound, caffeine, under strictly AD and MA-AD conditions, with a focus on understanding the influence of oxygen exposure and redox shifts on degradation efficiency.

3.2.5.1 Caffeine Degradation Performance

Caffeine degradation was evaluated over two independent spiking stages using an initial concentration of ~200 $mg \cdot L^{-1}$ of caffeine in the reactors. The reactors were operated under ORP-controlled conditions, with oxygen supplied as needed to the MA-AD reactor based on the set ORP. In the first degradation phase, the MA-AD reactor showed faster caffeine degradation compared to the AD reactor. Within 28 hours, caffeine concentrations decreased by >85 % in the MA-AD reactor, while the AD reactor achieved approximately 75 % removal **(Figure 9a).**

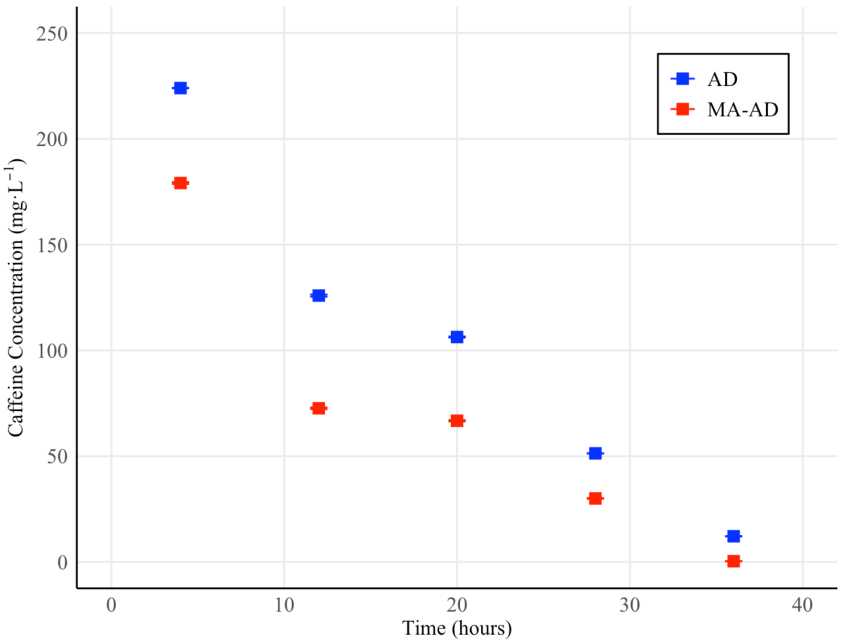

Figure 9a: Caffeine Degradation Curve across Treatment Phases

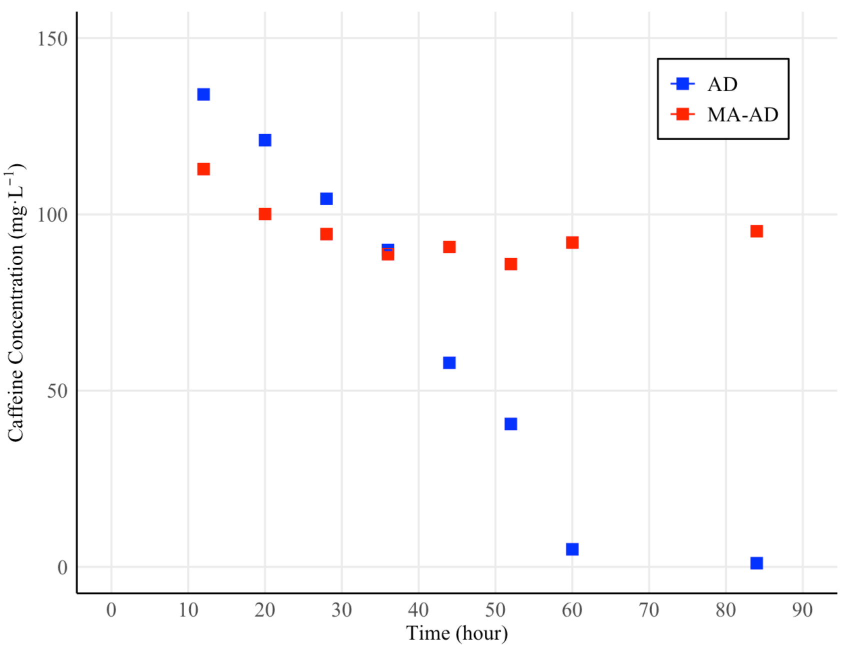

Figure 9b: Caffeine Degradation Curve across Treatment Phases

This initial enhancement is likely due to the stimulation of facultative microbial activity, which has been shown to accelerate the breakdown of aromatic compounds under oxygen-limited conditions (Aydin et al., 2025). Facultative microorganisms capable of co-metabolizing caffeine through oxidative demethylation may have been enriched under these conditions, contributing to the observed advantage. However, by 36 hours, both reactors had reached near-complete degradation (~90 %), indicating convergence of long-term removal efficiency regardless of oxygen input. A similar trend was reported by Chen et al. (2018) who demonstrated that complete caffeine degradation can be achieved under strictly anaerobic conditions using an anaerobic membrane reactor, reporting a long-term removal efficiency of 87.5 ± 5.3 %. In their study, caffeine degradation followed a two-step methanogenic pathway: the initial hydrolysis of caffeine into intermediates, followed by conversion of those intermediates into VFA, and eventually CH_4 and CO_2. Notably, the transformation of hydrolysis products into VFA was identified as the rate-limiting step of the process. This supports the hypothesis that while micro-aeration can accelerate the early degradation stages via stimulation of facultative microbes, the overall removal efficiency is ultimately dictated by the performance of downstream anaerobic metabolic pathways. Thus, while micro-aeration can enhance the kinetics of initial degradation, particularly in systems dealing with recalcitrant compounds such as caffeine, optimizing microbial adaptation and maintaining balance across aerobic and anaerobic consortia is critical to sustaining long-term degradation performance. In the second caffeine dosing stage, both reactors were spiked again after reaching baseline levels. Initially, the MA-AD reactor once more demonstrated a faster degradation rate than the strictly AD reactor. By 28 hours, caffeine concentration in the MA-AD reactor had decreased to 94.4 ± 0.2 $mg \cdot L^{-1}$, compared to 104.4 ± 0.3 $mg \cdot L^{-1}$ in the AD reactor **(Figure 9b).** This early-phase degradation advantage supports earlier observations that micro-aeration may enhance the initial transformation of caffeine, likely due to the stimulation of facultative microbial populations capable of oxidative co-metabolism**.** Interestingly, in both degradation stages, the initial degradation in the MA-AD reactor was consistently more rapid, achieving over 50% removal within 12–20 hours. However, this performance advantage was not sustained. In Phase II, beyond 36 hours, the trend reversed. The AD reactor showed continued degradation, reaching near-complete removal (~1.05 mg·L^{-1}) by 84 hours, while caffeine levels in the MA-AD reactor stagnated between 85–95 mg·L^{-1} from 36 hours onward. This suggests that overcompensation in oxygen dosing, which was needed to maintain ORP setpoints, might have disrupted the anaerobic microbial pathways responsible for the second phase of caffeine degradation. Furthermore, throughout this stage, the volume of oxygen dosed into the MA-AD reactor increased markedly compared to earlier phases **(Figure 8)** as reported in Section 3.4. This increase may reflect shifts in microbial oxygen demand or reduced microbial responsiveness to redox setpoint control. Such excessive oxygenation could inhibit obligate anaerobes, impairing the conversion of caffeine intermediates into VFA and methane (Lu & Imlay, 2021). This observation is consistent with the proposed two-step methanogenic degradation mechanism of caffeine, where hydrolysis to intermediates is followed by a rate-limiting conversion to VFA and methane (Chen et al., 2018). In this context, micro-aeration appears to enhance the first step but may suppress the second due to elevated oxygen exposure and associated redox imbalance. Aerobic microorganisms, which generally exhibit greater metabolic efficiency and higher energy yields compared to anaerobic counterparts, their ability to degrade caffeine in the MA-AD reactor may have been constrained by several factors. The microbial community present may not have been fully adapted to aerobic caffeine degradation, requiring specific enzymes or co-metabolic conditions that were not prevalent in the MA-AD reactor. Furthermore, oxygen toxicity may have inhibited certain microbial groups that play a crucial role in caffeine breakdown, disrupting enzymatic pathways and leading to metabolic inefficiencies. Additionally, aerobic pathways may lead to the accumulation of intermediate metabolites that were not readily degraded under the given conditions, potentially inhibiting further microbial activity. These findings suggest the importance of oxygen dosing precision and microbial community acclimation in maintaining the effectiveness of micro-aerated systems for recalcitrant compound degradation.

3.3 Conclusion and Future Perspective

This study provides a comprehensive evaluation of MA-AD in treating CPW, focusing on its effects on reactor stabilization, solids removal, turbidity, methane yield, and the degradation of inhibitory compounds such as caffeine and tannins. The results demonstrate that while micro-aeration can enhance early-stage microbial activity, hydrolysis, and degradation rates of certain recalcitrant compounds, its long-term success depends on carefully balanced operational parameters. Critically, oxygen dosing emerges as a paramount factor in the effective operation of MA-AD reactors. When applied in low, controlled quantities, oxygen can stimulate facultative microbial activity, promote partial oxidation of complex substrates, and accelerate the initial breakdown of polyphenolic compounds such as caffeine. However, excessive oxygen often introduced unintentionally through overcompensated redox control, can disrupt anaerobic microbial consortia, inhibit key methanogenic pathways, and diminish methane yields. This highlights the importance of finely tuned ORP regulation and real-time monitoring to prevent oxygen overload. Furthermore, the trace mineral composition of the substrate plays a pivotal role in reactor behavior. In this study, the presence of Fe^{2+} and other micronutrients may have catalyzed physicochemical reactions such as floc formation and particulate precipitation, influencing turbidity and solids retention. These interactions underscore the interconnectedness between substrate chemistry and reactor performance, especially under shifting redox levels.

Inhibitory effects from phenolic-rich substrates such as CPW were also evident, with results suggesting that even at relatively low concentrations (20% of total COD) compounds such as tannins and caffeine can exert significant microbial stress, particularly under oxidative stress. The observed shifts in pH and microbial efficiency further reinforce the need for cautious substrate formulation and reactor adaptation. Pretreatment strategies, such as alkaline hydrolysis, enzymatic conditioning, or strategic co-digestion, may offer viable approaches to enhance substrate bioavailability and mitigate inhibition. As demonstrated in comparable studies, these methods can substantially improve methane yields and solids reduction when dealing with lignocellulosic or polyphenol-laden waste streams. Ultimately, the success of MA-AD hinges on the integration of operational control (oxygen and ORP), feedstock chemistry (trace elements and inhibitors), and process design (loading rate, mixing, and pretreatment). Future research should aim to define optimal oxygen loading thresholds, assess microbial community shifts with high-resolution sequencing, and deploy advanced analytical tools (e.g., LC-MS, metabolomics) to track degradation intermediates and byproducts. When appropriately managed, MA-AD holds significant promise for the efficient and stable treatment of complex industrial wastewater such as those generated in coffee processing.

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CHAPTER FOUR

4. CONCLUSION

This study demonstrated that MA-AD offers a promising approach to enhancing the treatment of CPW, particularly for the degradation of recalcitrant compounds such as caffeine. The findings demonstrated that micro-aeration could provide a short-term advantage in the degradation rate, as observed with the accelerated caffeine removal in the MA-AD reactor. However, the study also revealed several limitations and drawbacks associated with micro-aeration. Specifically, while the initial degradation was enhanced, methane production was lower in the MA-AD reactor compared to the control, indicating that oxygen exposure inhibited methanogenic activity, which is critical for methane production in anaerobic digestion systems. This suggests that while micro-aeration can improve the rate of compound degradation, its long-term impact on overall treatment efficiency measured by parameters such as COD reduction and solid removal remains limited.

Beyond technical performance, the findings of this study have broader relevance to sustainable food systems and food security. Poorly managed agro-industrial waste, such as CPW, can contribute to environmental degradation and compromise the health of surrounding ecosystems and communities. Developing effective, affordable treatment strategies MA-AD can reduce the pollution burden associated with food production and help maintain the integrity of natural resources essential for continued agricultural productivity.

In line with circular economy principles, MA-AD provides a pathway for resource recovery from waste streams while minimizing environmental harm. Although reduced methane yield remains a limitation, the approach supports cleaner production practices and more resilient food value chains. Future research and optimization could enhance the role of MA-AD in waste valorization strategies that align with both environmental sustainability and the long-term goals of food system stability and security.