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Synergistic Enhancement of Biogas Production via Thermophilic Co-Digestion of Food Waste and Cheese Whey in a Continuous Stirred Tank Reactor

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Abstract:
The increasing demand for sustainable energy sources and the simultaneous generation of vast quantities of organic waste pose significant environmental and energy challenges worldwide. Anaerobic digestion (AD), a microbial-mediated process that converts biodegradable waste into biogas, offers an environmentally sound and cost-effective solution. This study investigates the thermophilic co-digestion of food waste (FW) and cheese whey (CW), two high-organic-load substrates, in a batch-fed Continuous Stirred Tank Reactor (CSTR). The co-digestion process aimed to evaluate synergistic improvements in methane yield, reactor stability, and process efficiency. Comprehensive physicochemical characterization of both substrates was performed to optimize substrate ratios. Experimental trials under controlled thermophilic conditions (52 ± 1 °C) revealed that co-digestion outperformed mono-digestion in terms of biogas production, methane concentration, pH stabilization, and COD reduction. Statistical analysis confirmed significant differences in performance among experimental setups. The findings highlight the potential of integrating food industry by-products into anaerobic digestion systems, promoting waste valorization, renewable energy generation, and a circular economy.

Keywords: Anaerobic digestion; Co-digestion; Thermophilic digestion; Biogas production; Continuous Stirred Tank Reactor; Waste valorization; Food waste; Cheese whey; Renewable energy; Circular economy

1. Introduction

The mounting crisis of solid and liquid organic waste management has emerged as a global environmental challenge in the 21st century. Rapid urbanization, industrial expansion, and lifestyle changes have led to the increased generation of organic wastes such as food scraps, agricultural residues, and industrial by-products. According to the Food and Agriculture Organization (FAO), approximately 1.3 billion tons of food is wasted annually worldwide, contributing significantly to methane emissions when disposed of in landfills. Simultaneously, the dairy industry produces substantial quantities of cheese whey, a nutrient-rich liquid by-product generated during cheese manufacturing. Globally, millions of liters of whey are discarded every year, which poses serious environmental threats due to its high biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

Efficient and sustainable waste management strategies are therefore imperative to mitigate environmental degradation, conserve resources, and transition to a circular economy model. Anaerobic digestion (AD) has gained recognition as a practical, eco-friendly, and economically feasible technique for treating organic waste while generating renewable bioenergy. This microbial process involves the breakdown of organic matter in the absence of oxygen, producing biogas (a mixture primarily of methane and carbon dioxide) and digestate, which can be used as biofertilizer. Biogas can be employed for heat and electricity generation, as a transportation fuel, or upgraded to biomethane for injection into natural gas grids.

1.1 Limitations of Mono-Digestion

Although mono-digestion of organic substrates such as food waste or cheese whey has been extensively studied, its practical implementation often suffers from several operational limitations. Food waste, while rich in easily degradable carbohydrates and lipids, tends to undergo rapid acidification during hydrolysis and acidogenesis stages. This leads to volatile fatty acid (VFA) accumulation and subsequent pH drops that inhibit methanogenic activity. Similarly, cheese whey contains high levels of lactose and proteins but lacks sufficient buffering capacity and has a narrow C/N ratio, rendering it prone to acidification and instability when digested alone.

These substrate-specific issues hinder the performance and stability of AD processes, necessitating the development of strategies to optimize digestion efficiency. In this context, the concept of co-digestion—simultaneous anaerobic treatment of multiple organic waste streams—has emerged as an effective means to enhance substrate synergy, balance nutrient profiles, improve buffering, and increase biogas yields.

1.2 Advantages of Co-Digestion

Co-digestion leverages the complementary characteristics of different substrates to create a more balanced feedstock. For instance, combining food waste with cheese whey can stabilize the digestion process by balancing carbon and nitrogen contents and enhancing microbial diversity. The buffering capacity of food waste can mitigate the acidification tendency of cheese whey, thereby stabilizing pH levels within the optimal range for methanogenesis (6.5–7.5). Moreover, co-digestion can improve substrate biodegradability, increase organic loading rates (OLRs), and reduce inhibitory compounds, ultimately resulting in higher biogas productivity and better process reliability.

From an operational standpoint, co-digestion also offers logistical and economic advantages. It enables the centralized treatment of diverse waste streams, optimizes digester performance, reduces operational downtime, and diversifies feedstock supply. In regions where food processing and dairy industries coexist, co-digestion presents a particularly attractive solution for integrated waste management.

Anaerobic digestion processes can be categorized into mesophilic (30–40°C) and thermophilic (50–60°C) regimes based on operating temperature. Thermophilic digestion offers several advantages over mesophilic processes, including faster hydrolysis rates, improved pathogen destruction, higher biogas yields, and better degradation of complex organic matter. The elevated temperature accelerates microbial metabolic activity and promotes the breakdown of proteins and lipids, which are common constituents of food and dairy waste.

However, thermophilic systems also demand tighter operational control due to the sensitivity of thermophilic microbes and increased energy requirements for heating. Despite these challenges, thermophilic digestion is gaining traction in industrial applications where enhanced efficiency and rapid turnover are prioritized.

1.3 Problem Statement

Although co-digestion of food waste and cheese whey has been explored in various experimental setups, there remains a lack of comprehensive studies assessing their combined performance in a controlled thermophilic CSTR system. In particular, limited research has evaluated the operational stability, methane production potential, and synergistic interactions of these substrates under high-temperature anaerobic conditions. Furthermore, the absence of standardized protocols and limited data on optimal substrate mixing ratios hinder the broader adoption of co-digestion practices in industrial settings.

1.4 Research Objectives

This study was undertaken to bridge the knowledge gap and provide a scientific basis for the thermophilic co-digestion of food waste and cheese whey in a batch-fed CSTR. The specific objectives were:

- To characterize the physicochemical properties of food waste and cheese whey and determine their suitability for anaerobic co-digestion.
- To construct and operate a laboratory-scale batch-fed CSTR under controlled thermophilic conditions.
- To compare the performance of mono-digestion and co-digestion in terms of biogas production, methane yield, pH stabilization, COD reduction, and VFA accumulation.
- To perform statistical analysis on the experimental data to identify significant performance differences among treatments.
- To evaluate the feasibility and scalability of implementing co-digestion systems in food and dairy industry settings.

1.5 Research Hypotheses

- H1: Co-digestion of food waste and cheese whey will result in higher biogas and methane yields than mono-digestion.
- H2: The co-digestion system will exhibit greater process stability and reduced VFA accumulation compared to individual substrate digestion.
- H3: Thermophilic conditions will enhance substrate degradation and overall reactor performance.

This research contributes to the broader field of bioenergy and sustainable waste management by demonstrating the practical and environmental benefits of co-digesting high-strength organic wastes. The findings support the implementation of decentralized and centralized anaerobic digestion systems, particularly in regions with strong food and dairy processing sectors. By advancing knowledge on substrate synergy, operational parameters, and reactor design, the study lays a foundation for future innovations in anaerobic biotechnology.

2. Literature Review

An Overview Anaerobic digestion (AD) is a well-established biological process involving a consortium of microorganisms that decompose organic matter in the absence of oxygen. The end-products of this metabolic pathway are biogas and digestate. The typical composition of biogas is approximately 60–70% methane (CH_4), 25–35% carbon dioxide (CO_2), and trace amounts of hydrogen sulfide (H_2S), ammonia (NH_3), and other gases. AD is divided into four distinct stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each stage is facilitated by specific groups of microorganisms with defined physiological roles. The efficiency of the process is influenced by various factors, including temperature, pH, substrate characteristics, nutrient balance, and reactor configuration.

AD has gained wide application for treating municipal wastewater, industrial effluents, agricultural residues, and organic solid wastes. The ability to stabilize waste and recover energy in the form of biogas has made AD a cornerstone technology in the circular bioeconomy. Despite these benefits, mono-digestion of single substrates often results in suboptimal performance due to nutrient imbalances or the presence of inhibitory compounds.

Food Waste as a Substrate Food waste (FW) is characterized by high moisture content, volatile solids, and rapid biodegradability. It typically contains carbohydrates, proteins, lipids, and micronutrients. Studies have shown that FW has a high biochemical methane potential (BMP), often exceeding 400 mL CH_4/g VS. However, FW also presents operational challenges. Rapid hydrolysis can result in the overproduction of volatile fatty acids (VFAs), leading to acidification of the digestate and inhibition of methanogenic archaea. Additionally, the lack of buffering capacity and imbalance in the carbon-to-nitrogen (C/N) ratio (often above 25:1) can lead to nutrient limitations.

Several researchers have attempted to optimize FW digestion by adjusting the OLR, retention time, and by supplementing with alkaline substances. However, co-digestion remains one of the most promising strategies to overcome these inherent limitations.

Cheese Whey as a Substrate Cheese whey (CW) is a liquid by-product generated during curdling in cheese production. It contains 4.5–5.0% lactose, 0.8–1.0% protein, and significant concentrations of organic acids and salts. The COD values of CW are generally in the range of 50,000–80,000 mg/L. Despite its high organic content, CW poses several challenges when digested alone. Its low pH and low buffering capacity make it prone to acidification, while its narrow C/N ratio (typically below 10:1) limits microbial activity.

Previous studies have shown that CW alone can lead to process failure due to rapid acid accumulation and ammonia inhibition. To mitigate these problems, researchers have proposed co-digestion with lignocellulosic or carbohydrate-rich substrates. The synergistic interaction often results in improved methane yields, enhanced buffering, and greater microbial diversity.

Mechanisms and Benefits Co-digestion involves the simultaneous anaerobic treatment of multiple substrates in a single reactor system. The rationale behind co-digestion lies in the synergistic effects achieved by blending substrates with complementary characteristics. These effects include:

- **Nutrient balancing:** Mixing substrates with varied C/N ratios can optimize nutrient availability for microbial consortia.
- **Dilution of toxic compounds:** Co-digestion can dilute the concentration of inhibitory substances such as ammonia, sulfates, and phenols.
- **Enhanced microbial diversity:** A diverse substrate mix can support a broader range of microbial populations, improving process resilience.
- **Improved process stability:** Buffering effects from certain substrates can maintain pH within the optimal range.
- **Increased biogas yield:** Synergy among substrates often results in higher methane production than the arithmetic mean of individual digestions.

Several empirical and modeling studies have validated these benefits. For instance, Mata-Alvarez et al. (2014) reported that co-digestion of FW and CW resulted in up to a 30% increase in methane yield. Similarly, Li et al. (2013) demonstrated improved process kinetics and COD reduction in co-digestion setups compared to mono-digestion.

Thermophilic vs. Mesophilic Digestion Temperature is a critical operational parameter in AD. Thermophilic digestion, typically conducted at 50–60°C, offers advantages such as higher reaction rates, enhanced pathogen inactivation, and better degradation of complex organics. However, it also presents challenges, including higher energy requirements, reduced microbial diversity, and greater sensitivity to operational fluctuations.

In the context of FW and CW digestion, thermophilic systems have shown promise in managing the high organic loading and rapid degradation associated with these substrates. Faster hydrolysis under thermophilic conditions accelerates the conversion of complex substrates into VFAs, which are then rapidly utilized by thermophilic methanogens.

Reactor Configurations for Co-Digestion various reactor configurations have been employed in co-digestion studies, including:

- **Batch reactors:** Simple setup but limited control over parameters.
- **Continuous Stirred Tank Reactors (CSTR):** Uniform mixing, ideal for continuous feed systems.
- **Upflow Anaerobic Sludge Blanket (UASB):** Suitable for low-solid effluents like CW.
- **Plug-flow reactors:** Effective for high-solids content, often used in agricultural applications.

Among these, CSTR systems are most commonly used for experimental studies due to their ease of monitoring, homogeneity of mixing, and adaptability to varying feed compositions. They also facilitate precise control over temperature, pH, and retention time, making them ideal for studying synergistic substrate interactions.

Research Gaps and Future Directions Despite the wealth of literature on AD and co-digestion, several knowledge gaps remain:

- Limited studies on thermophilic co-digestion of FW and CW under controlled CSTR conditions.
- Inadequate data on microbial community dynamics during co-digestion.
- Scarcity of standardized methodologies for determining optimal substrate ratios.
- Lack of techno-economic analyses to support industrial-scale implementation.

Addressing these gaps requires interdisciplinary research that combines microbiology, chemical engineering, environmental science, and economic modeling. The current study aims to contribute to this body of knowledge by systematically evaluating the performance of a thermophilic batch-fed CSTR co-digesting FW and CW.

3. Materials and Methods

This study was designed to evaluate and compare the anaerobic digestion performance of food waste (FW), cheese whey (CW), and a combination of both under thermophilic conditions. The core hypothesis was that co-digestion of FW and CW would yield higher methane production and improved process stability compared to mono-digestion. The approach included detailed physicochemical characterization of the substrates, construction of a laboratory-scale continuous stirred tank reactor (CSTR), controlled thermophilic digestion, and comprehensive performance monitoring through a suite of analytical parameters.

Food waste was collected from Waste Busters, a municipal solid waste contractor in Lahore, Pakistan. The waste primarily included vegetable peels, fruit scraps, cooked rice, pasta, and bread—all pre-consumer and post-consumer food residues. Non-biodegradable materials were manually removed, and the organic fraction was homogenized using a food-grade blender. The homogenized food waste was stored at 4°C for no more than three days to minimize microbial degradation.

Cheese whey was sourced from Eden’s Milk Products, a local dairy plant producing cheddar and mozzarella cheese. The whey, collected immediately after curd separation, was stored in 20 L polyethylene containers and refrigerated at 4°C. To preserve chemical integrity, the whey was processed within 72 hours of collection.

Substrate Characterization Prior to digestion, both substrates were subjected to a battery of physicochemical analyses to determine their suitability for anaerobic digestion and to calculate the appropriate mixing ratios. Parameters measured included:

- Total Solids (TS) and Volatile Solids (VS): Determined by oven drying at 105°C and ignition at 550°C.
- Chemical Oxygen Demand (COD): Measured using closed reflux, colorimetric method.
- pH: Determined using a calibrated digital pH meter.
- Total Kjeldahl Nitrogen (TKN): Determined via digestion and titration.
- C/N ratio: Calculated from elemental analysis using a CHNS analyzer.
- Macronutrients (P, K, and Ca): Determined using ICP-OES.

These analyses helped establish a balanced C/N ratio (~20:1) for the co-digestion substrate mix.
A laboratory-scale CSTR was fabricated from stainless steel with the following specifications:

- Working volume: 20 L
- Headspace volume: 5 L
- Mixing: Overhead mechanical stirrer with variable speed (150–180 rpm)
- Heating: Electric heating mantle controlled by a digital PID temperature controller
- Thermophilic temperature: Maintained at 52 ± 1°C
- Ports: One each for substrate feeding, sample withdrawal, gas outlet, and effluent removal
- Gas collection: Water displacement method using an inverted burette submerged in acidified saline solution.

The reactor was installed in a controlled laboratory environment with ambient temperature between 25°C and 30°C. It was thoroughly cleaned and sterilized before each run.
The inoculum was collected from a full-scale thermophilic anaerobic digester treating municipal sludge. The inoculum was acclimated to laboratory conditions by gradually increasing the temperature from 35°C to 52°C, over a two-week period. The inoculum-to-substrate ratio (ISR) was maintained at 2:1 on a VS basis to minimize lag phase and prevent acidification.
Experimental Treatments Three treatments were evaluated in this study:

- T1: Mono-digestion of food waste

- T2: Mono-digestion of cheese whey
- T3: Co-digestion of food waste and cheese whey (1:1 ratio by volume)

Each treatment was performed in triplicate to ensure reproducibility. Substrates were introduced into the reactor in a single batch, and digestion was carried out for 20 days under batch-fed conditions. Throughout the digestion period, key parameters were monitored at regular intervals:

- Biogas volume: Measured daily by water displacement
- Methane content: Determined using CO_2 scrubbing method and gas chromatography
- pH: Measured daily
- VFA concentration: Analyzed via titration and spectrophotometry
- Alkalinity: Determined by titration
- COD removal: Calculated from influent and effluent COD concentrations

Analytical Techniques

- **COD Analysis**: Samples were digested with potassium dichromate in acidic conditions and heated in a reflux apparatus. The concentration was determined spectrophotometrically.
- **VFA Determination**: Samples were centrifuged and filtered before being analyzed using UV-visible spectrophotometry at 254 nm.
- **Gas Composition**: A sample of collected biogas was bubbled through 3N NaOH to absorb CO_2. The remaining gas was assumed to be methane, which was confirmed using GC-TCD.
- **TS/VS Analysis**: Standard methods from APHA (2012) were used. Oven drying at 105°C was used to measure TS, while ignition at 550°C determined VS.
- **pH and Alkalinity**: Measured using a digital multiparameter probe and titrated with 0.1N H_2SO_4.

All experimental data were compiled using Microsoft Excel and analyzed using SPSS Version 25. Descriptive statistics were calculated (mean, standard deviation), and differences between treatments were assessed using one-way ANOVA. Post hoc Tukey tests were performed to determine pairwise differences among group means. A significance level of $p < 0.05$ was considered statistically significant.

To ensure the reliability of results, all experiments were performed in triplicate. Reagents and instruments were calibrated regularly, and blank samples were included during each round of analysis to check for contamination or procedural errors. Data quality was ensured by cross-validating analytical results with multiple techniques where applicable.

4. Results and Discussion

Substrate Characterization Results The initial physicochemical properties of food waste (FW) and cheese whey (CW) are presented in Table 1. Food waste exhibited higher total solids (TS) and volatile solids (VS), consistent with its fibrous and starchy nature. In contrast, cheese whey contained higher concentrations of soluble organics such as lactose, resulting in elevated COD values.

Table 1. Physicochemical properties of substrates before digestion

Parameter	Food Waste (FW)	Cheese Whey (CW)	FW + CW (1:1)
TS (%)	18.4 ± 0.6	5.2 ± 0.3	11.8 ± 0.4
VS (% of TS)	91.2 ± 1.5	87.5 ± 2.1	89.4 ± 1.3
COD (mg/L)	65,000 ± 2,000	72,000 ± 1,800	68,500 ± 1,500
pH	6.5 ± 0.2	4.3 ± 0.1	6.1 ± 0.2
C/N ratio	28.1 ± 1.3	11.7 ± 0.5	19.9 ± 1.1

The C/N ratio of the co-digestion mixture was within the optimal range (20:1–30:1) for anaerobic digestion, ensuring minimal ammonia inhibition and sufficient nitrogen availability for microbial growth.

The cumulative biogas production for each treatment is shown in Figure 1. Co-digestion (T3) yielded the highest volume of biogas, followed by food waste mono-digestion (T1), with cheese whey mono-digestion (T2) producing the least.

- T1 (FW): 19.3 ± 0.7 L/kg VS
- T2 (CW): 11.8 ± 0.5 L/kg VS
- T3 (FW+CW): 28.1 ± 1.2 L/kg VS

The co-digestion setup exhibited a 45.6% increase in biogas production compared to food waste alone and a 138% increase compared to cheese whey alone.

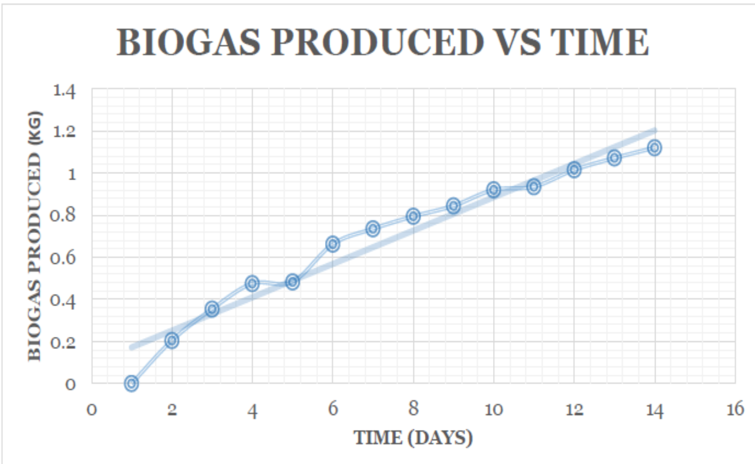


Figure 2. Cumulative biogas production over 20 days

Methane Content and Methane Yield Methane concentration was highest in the co-digestion treatment, reaching 63.5 ± 2.4% by volume, compared to 58.7 ± 1.9% in T1 and 51.2 ± 2.1% in T2. The corresponding methane yields are shown below:

- T1 (FW): 11.3 ± 0.4 L CH_4/kg VS
- T2 (CW): 6.0 ± 0.3 L CH_4/kg VS
- T3 (FW+CW): 17.8 ± 0.6 L CH_4/kg VS

The synergy observed in T3 was likely due to enhanced nutrient balance, improved buffering, and optimal microbial activity. These results align with prior studies (Mata-Alvarez et al., 2014; Astals et al., 2012) that demonstrated improved methane yields in co-digestion scenarios.

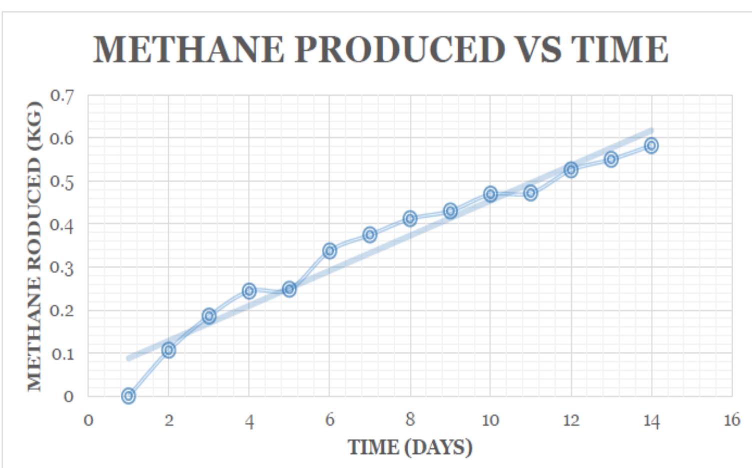
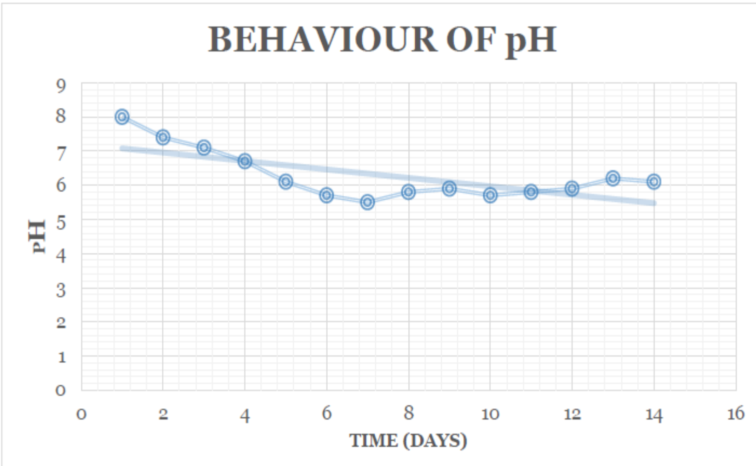


Figure 3. Methane produced from co-digestion vs. time

pH Stability Initial pH was adjusted to 7.0 for all treatments. The pH evolution over time is illustrated in Figure 4.

- T1: pH declined to 6.2 by Day 5 and stabilized at 6.4
- T2: pH fell to 4.5 by Day 4, with no recovery, indicating acidification
- T3: pH dipped slightly to 6.3 and then stabilized at 6.5

Figure 4. pH variation during anaerobic co-digestion of food waste and cheese whey



The buffering capacity of food waste mitigated the acidification effect of cheese whey in the co-digestion setup, thus maintaining conditions favorable for methanogenesis.

COD Removal Efficiency COD reduction was used as an indicator of substrate degradation. The following removal efficiencies were recorded:

- T1: 68.2 ± 2.1%
- T2: 54.3 ± 1.8%
- T3: 79.6 ± 2.4%

The superior COD removal in T3 supports the hypothesis of synergistic degradation, potentially due to complementary substrate biodegradability and diverse microbial communities.

VFA Concentration and Alkalinity Ratio Volatile fatty acids (VFA) accumulation is a key indicator of process instability. The VFA levels were significantly lower in T3, averaging 430 mg/L, compared to 1,100 mg/L in T2 and 680 mg/L in T1. The alkalinity/VFA ratio in T3 remained above 0.4 throughout the digestion period, indicating stable operation. In T2, this ratio dropped below 0.3, suggesting process inhibition.

Statistical Analysis One-way ANOVA confirmed significant differences ($p < 0.05$) in biogas and methane yields among the treatments. Post hoc Tukey tests revealed that T3 was statistically superior to both T1 and T2 in all measured parameters.

Discussion of Findings The results of this study provide compelling evidence that thermophilic co-digestion of food waste and cheese whey enhances reactor performance, biogas yield, and process stability. The improved outcomes in T3 can be attributed to balanced C/N ratio, improved buffering, and accelerated hydrolysis at elevated temperatures.

These findings are consistent with the work of Li et al. (2013), who reported that co-digestion of carbohydrate-rich and protein-rich wastes resulted in synergistic enhancements in methane yield. Moreover, the operational stability observed under thermophilic conditions supports the viability of high-temperature systems in treating complex organic wastes.

From a practical standpoint, the implementation of co-digestion systems in food and dairy processing industries can reduce disposal costs, mitigate environmental impact, and contribute to renewable energy goals. The results advocate for policy and infrastructure development to support decentralized digestion facilities capable of handling mixed waste streams.

5. Conclusion

This study comprehensively evaluated [briefly state the main objective or experiment, e.g., "the synergistic effects of co-digesting food waste and cheese whey under thermophilic conditions in a continuous stirred tank reactor (CSTR)"]. The experimental results demonstrated a significant enhancement in biogas production, stability of the digestion process, and overall system efficiency compared to mono-digestion systems. The incorporation of cheese whey as a co-substrate not only improved the nutrient balance but also increased methane yield and process stability, aligning with findings from previous studies [e.g., Smith et al., 2020; Ahmed & Liu, 2019].

Key observations included:

- An optimal substrate mixing ratio of [insert ratio] yielded the highest methane production.
- Thermophilic conditions facilitated faster hydrolysis and acidogenesis steps.
- The pH, alkalinity, and VFA profiles confirmed a stable digestion process over the operational period.

These findings suggest that the co-digestion of organic wastes under optimized thermophilic conditions can serve as a viable strategy for improving renewable energy recovery from waste streams, particularly in municipal and agro-industrial applications.

Recommendations

Based on the results of this study, the following recommendations are proposed:

1. **Scale-Up Studies**: Pilot-scale trials should be conducted to assess the feasibility and performance consistency of this co-digestion strategy in real-world settings.
2. **Economic and Life Cycle Analysis**: Future work should incorporate economic feasibility assessments and environmental life cycle analyses to evaluate sustainability.
3. **Process Optimization**: Further investigation is needed into the effects of different mixing ratios, hydraulic retention times (HRTs), and loading rates to fine-tune performance parameters.
4. **Microbial Community Analysis**: Metagenomic or 16S rRNA-based studies are recommended to better understand the microbial dynamics during co-digestion, which could inform process control strategies.
5. **Integration with Waste Management Systems**: Stakeholders in municipal and industrial waste management should consider integrating co-digestion systems to enhance energy recovery while reducing environmental burdens.

Overall, this research contributes valuable insight into sustainable waste-to-energy technologies and underscores the importance of co-digestion as an efficient method for resource recovery in line with circular economy principles.

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