Pol. J. Environ. Stud. Vol. XX, No. X (XXXX), 1-8
DOI: *10.15244/pjoes/201937* ONLINE PUBLICATION DATE: 2025-04-18

Original Research

Enhancement of Biogas Production through Phase Separated Anaerobic Co-Digestion of Cattle Manure with Fruit and Vegetable Waste

Maria Shoukat[1, 2], Atiq Ur Rehman[2, 3], Alam Khan[2], Moona Nazish[4]*, Irfan Ali[5], Mohammad Abul Farah[6], Samiullah Khan[2], Aamer Ali Shah[2], Shagufta Sanam[7], Mohamed Farouk Elsadek[8], Malik Badshah[2]**

[1]Clinical Medicine, School of Medicine and Population Health, University of Sheffield, Sheffield, S10 2TN, United Kingdom
[2]Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[3]Institute of Microbiology, Faculty of Veterinary & Animal Sciences, Gomal University, Dera Ismail Khan, KPK, Pakistan
[4]Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[5]Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan
[6]Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[7]Department of Microbiology, Shaheed Benazir Bhutto Women University, Peshawar, Pakistan
[8]Department of Biochemistry, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia

Received: 22 August 2024
Accepted: 16 February 2025

Abstract

In response to current energy challenges, anaerobic digestion (AD) is emerging as a viable and sustainable alternative to conventional fossil fuels. Phase separation evolves to mitigate process instability observed in single-stage AD, offering enhanced process stability and efficiency for biogas production. This study aims to comprehensively compare phase-separated and single-phase AD systems while incorporating cattle manure and fruit and vegetable waste (FVW) co-digestion to enhance process stability and biogas production. The two-stage and single-stage continuous AD systems were analyzed at an organic loading rate (OLR) of 3 $gVSL^{-1}day^{-1}$ with a hydraulic retention time (HRT) of 10 days and three retention periods (30 days) under uncontrolled environmental conditions (20-35ºC), which may fluctuate with seasonal changes, and controlled temperature (37ºC). The FVW and cattle manure were co-digested in a 1:1 ratio of volatile solids (VS) as a substrate. The two-stage AD system operated at 37ºC and exhibited the highest biogas yield (0.25 NL/gVS) at a steady state as compared to single-stage AD, including 0.113 NL/gVS, 0.178 NL/gVS, and 0.201 NL/gVS at environmental

*e-mail: mnazish@bs.qau.edu.pk
**e-mail: malikbadshah@qau.edu.pk

temperature (20-35ºC), controlled temperature (37ºC), and two-phase AD at environmental temperature (20-35ºC), respectively. The one-way analysis of variance (ANOVA) test revealed a significant effect of the controlled temperature and phase separation on biogas production, $F(3, 0) = 0.987$, $p<0.001$. Additionally, the total reduction in COD in the final effluent was notably higher in the two-stage anaerobic digester at 37ºC, showing an 85% reduction rate compared to single-stage anaerobic digesters with a mean difference of -6.51 (95% CI [-9.50, -3.52]) and SD = 1.88, $t(3) = -6.93$, $p = 0.006$, two-tailed. These results highlight the significant biogas productivity, improved process stability, and better effluent quality achieved through phase separation of the anaerobic digester at 37ºC. The estimated biogas potential from two-stage anaerobic co-digestion of cattle manure and FVW is 21.37 million m^3/day in Pakistan, representing significant prospects for implementing this technology as a renewable energy source.

Keywords: anaerobic digestion, phase-separated anaerobic digestion, hydraulic retention time (HRT), fruit and vegetable waste (FVW), cattle manure

Introduction

In Pakistan, the mismanagement of organic waste, particularly from the agricultural and livestock sectors, is a public health and environmental concern that requires sustainable waste management solutions. [1] Anaerobic digestion (AD) has emerged as a promising technology for organic waste treatment and the production of biogas and biofertilizers. However, traditional AD systems face the limitations of process stability and less productivity due to single-stage and mono-digestion approaches. Single-stage AD systems may not perform optimally, particularly under high organic loading rates or fluctuating feedstock conditions, resulting in process instability and reduced biogas production [2, 3]. Similarly, mono-digestion of particular feedstock, e.g., fruit and vegetable waste (FVW), can be challenging due to their high biodegradability and potential to produce acidic substances, potentially inhibiting the digestion process [4].

Recent developments in AD technology, such as phase separation and co-digestion of different substrates, offer potential advantages to address these limitations. Two-stage AD systems provide high process stability and efficiency by separating the hydrolysis and methanogenesis stages, optimizing biogas production [5, 6]. This phase separation helps to maintain a stable environment in reactors through pH control, VFA balance, and a high rate of methanogenesis. As a result, complex organic matter is converted into carbon dioxide, hydrogen, and VFAs in the acidogenic reactor, and easily biodegradable compounds are converted into carbon dioxide and methane in the subsequent methanogenic reactor. Furthermore, phase separation offers a better survival environment for maximal enzymatic activity in the microbial community.

Previous studies demonstrated that co-digestion techniques, such as mixing two or more substrates, improve system performance and biogas output by improving nutritional balance [7]. Co-digestion of FVW with cattle manure is a well-studied phenomenon that optimizes microbial activity and improves the growth rate of methanogens due to the high moisture content and readily biodegradable organic matter in FVW. In addition, it dilutes ammonia concentrations from cattle manure, thus reducing the toxicity in a bioreactor. This synergistic interaction enhances biogas production through co-metabolism and high biodegradability of organic matter [8-10]. This study aims to comparatively analyze the single-stage and two-stage AD systems under uncontrolled and controlled mesophilic temperature (37°C). It further aims to optimize the two-stage AD process at mesophilic temperatures and to estimate the biogas potential in Pakistan by using the results of a highly optimized process.

Materials and Methods

Experimental Setup

A single-stage and two-stage AD system was designed and operated with an organic loading rate (OLR) of 3 gVS $L^{-1}day^{-1}$, a flow rate of 0.5 L/day, and a hydraulic retention time (HRT) of 10 days. The first setup (S1) of single-stage AD was analyzed under uncontrolled environmental temperatures. This digester was operated in an open environment with seasonal temperature fluctuations ranging from 20-35ºC, while the second (S2) was maintained at a controlled temperature of 37ºC. In comparison, the two-stage AD was also studied at environmental (S3) and controlled temperature (S4) conditions.

Stainless steel reactors with a working volume of 5 L were designed with three openings for feeding, effluent removal, and N_2 sparging to create anaerobic conditions. To start the experiments, 3 L of a developed and degassed inoculum was added. After sealing, the reactors were flushed with nitrogen gas to establish anaerobic conditions. Daily feeding began with a slurry mixture of substrates in water to a final volume of 0.5 L, the flow rate for all single-stage anaerobic co-digestion reactors. No effluent was removed for the first 4 days to reach a working volume of 5 L. Afterward, 0.5 L of effluent was removed daily, maintaining the same feeding rate to keep a constant volume.

In two-stage AD, the partially digested effluent from the acidogenic reactor was used to feed the methanogenic reactor. Gases, including H_2, CO_2, and volatile fatty acids produced in the acidogenic reactor, were also transferred to the methanogenic reactor for methane production. The acidogenic and methanogenic reactors were connected via a catheter bag to transfer gases from the first reactor to the second. The methanogenic reactor was connected to 2-3 catheter bags to collect the total biogas. Volatile fatty acids (VFA) and alkalinity were examined weekly using titration [11]. The pH of both reactors was checked daily before feeding.

Determination of Biogas and Methane Contents

Biogas was collected in sterile catheter bags (2 L) attached to digesters through gas outlet openings. Once a steady state was achieved, methane levels in the biogas were determined by passing it through a 3 M sodium hydroxide (NaOH) solution. The NaOH solution acted as a cleaner, absorbing CO_2 and some H_2S from the biogas.

The solution was prepared and filled in 200 ml reagent bottles up to 100 ml. The bottles were sealed with rubber stoppers or cork with two metal tube openings (for inlet and outlet) to prevent air from getting in or out. One bottle opening was connected to the reactor to collect biogas, and the other to the gas-collecting catheter bag for methane capture. The entire reactor setup was then purged with nitrogen gas (N_2) for about 5 minutes to create anaerobic conditions in the reactors.

Analytical Methods

The total solids (TS) and volatile solids (VS) contents of cattle manure, FVW, and effluents were determined by oven drying at 105ºC for 24 hours, followed by ignition at 550ºC for 16 hours in a muffle furnace. Total Nitrogen (TN) contents were assessed using the Nitrogen (total) Cell Test, a spectrophotometric method [12]. Carbohydrate contents were analyzed using the phenol–sulfuric acid method [13], while proteins were assessed using the Lowry method [14]. Lipid concentrations were analyzed using a single-step method [15], and chemical oxygen demand (COD) was determined using a semi-automated colorimetry method [12].

Estimation of Total Volatile Fatty Acids and Alkalinity

The effluent's VFA and alkalinity were calculated every 7 days to check the reactor stability, and the results were determined according to APHA Standard Methods [12].

$$\text{Alkalinity (mg CaCO}_3\text{/l)} = A \times N \times 50000 \times \text{Sample Volume}$$

A = ml of acid consumed to titrate the sample from the actual pH to 4.3, N = Normality of the acid used (mg/L), VFA (mg/L) = B×N×50000×Sample Volume, B = ml of base (0.1 NaOH) used to titrate sample from pH 3.5 to 7, N = Normality of the base used (mg/L).

Statistical Analysis

A one-way analysis of variance (ANOVA) was employed to determine the difference in the mean levels of biogas production across all four anaerobic digesters operating in a steady state. Additionally, the one-sample T-test was performed to determine the difference in the COD of digesters' influent and effluent waste.

Results and Discussion

Properties of Substrate

The total solids (TS%) and volatile solids (VS%) of cow manure were measured at 22.12% and 15.61%, respectively, as shown in Table 1. Previous studies reported a TS range of 20 to 42% for cow manure, which can be attributed to differences in sample collection and handling procedures before the TS measurement. The presence of urine can notably increase the moisture content in manure, thereby reducing its TS content [16].

The FVW used in this study was collected from the main cafeteria of Quaid-i-Azam University, Islamabad. The TS and VS content of FVW were determined to be 11.21% and 10.53% of the total weight, respectively (Table 1). Although this study did not specifically measure TS values for individual food waste types, literature values were used to estimate TS for blended fruit and vegetable waste [17].

The total carbon content in FVW (52%) was found to be significantly higher than that in manure (26%), and a similar trend was observed for the total nitrogen content. The carbon-to-nitrogen (C/N) ratio, calculated as 16.3 for FVW and 25.6 for manure, provides essential insights into the biochemical characteristics of these waste materials, which are crucial for optimizing their management and utilization, as demonstrated by previous studies [18].

Biogas Production in Single-Stage AD at Different Temperatures

In the first setup (S1) of single-stage AD under uncontrolled environmental conditions, the maximum biogas production was observed at 1.78 NL/day after one retention time. A steady state was reached on the 21^{st} day, with total biogas production reaching 1.86 L and daily production stabilizing at 1.63 NL/day. The methane yield at steady state, determined by passing the gas through a 3 M NaOH scrubbing solution, was 1.27 L/day and 1.15 NL/day, yielding 0.077 NL/g VS, which represents approximately 68% of the total biogas

Table 1. Characteristics of the substrates.

Substrate	Cow manure	Fruit and vegetable waste (FVW)
TS%	22.12	11.21
VS%	15.61	10.53
VS of TS%	70.56	93.97
Moisture%	77.86	88.79
Total Nitrogen%	1.02	3.2
Total carbon%	26.13	52.2
C:N	25.61	16.3
Carbohydrates (g/L)	10.7	32.9
Proteins (g/L)	1.5	11.8
Lipids (g/L)	1.9	3.5

produced (Table 2). The observed decrease in biogas production due to temperature fluctuations highlights the sensitivity of methanogens to temperature changes. A direct correlation between temperature and biogas production was observed, where higher temperatures resulted in increased biogas production [19, 20].

In the second setup (S2) with a controlled temperature of 37ºC, biogas production increased significantly at the same OLR. Initial production was 1.8 L on the first day, gradually increasing to a steady state of 3.03 L/day and 2.67 NL/day at standard temperature and pressure (STP). The steady-state methane content was 1.99 L/day and 1.75 NL/day at STP, with a methane yield of 0.117 NL/g VS, which is notably higher than in S1. While the total methane content was slightly lower at 65.5%, the controlled temperature led to faster degradation and increased biogas production (Table 2). The enhanced biogas production at a controlled mesophilic temperature can be attributed to optimized microbial activity, particularly among methanogens, which are highly sensitive to temperature shifts, as supported by Singh et al. [21] and Wang et al. [22].

The substantial enhancement in biogas production at the controlled mesophilic temperature of 37ºC could be directly associated with optimizing microbial and enzymatic activity, predominantly among methanogens, which are highly sensitive to temperature fluctuations. The increase in biogas yield from 1.69 NL/day in S1 to 2.67 NL/day in S2 demonstrates the crucial role of temperature in promoting microbial metabolism. The increase in biogas production is correlated with the higher metabolic activity of methanogens at the optimal temperature of 37°C.

Overall, biogas production at standard temperature and pressure in single-stage digestion was 1.69 NL/day for S1 and 2.67 NL/day for S2, with yields of 0.113 and 0.178 NL/g VS per liter of reactor per day, respectively. This indicates a 36% increase in production under the controlled mesophilic temperature of 37°C compared to uncontrolled temperature. Previous studies also demonstrated an enhancement in biogas production under controlled mesophilic temperature [23, 24].

Biogas Production in Two-Stage AD at Different Temperatures

Two-stage AD significantly increased biogas production compared to single-stage AD at both environmental and controlled temperatures. At steady state, biogas production in the two-stage system was 3.42 L/day (3.01 NL/day), with a methane yield of 0.134 NL/g VS, constituting 66% of the total biogas produced. This represents a 44% increase in biogas yield compared to single-stage digestion at environmental temperature and an 11% increase compared to single-stage digestion at controlled temperature (Fig. 1(a-b)).

Under controlled temperature conditions of 37ºC, a gradual increase in biogas production was observed, reaching 4.2 L/day (3.74 NL/day) with a yield of 0.250 NL/g VS. Methane production was 2.84 L/day (2.5 NL/day), yielding 0.167 NL/g VS (Table 2). This represents a 55% increase over single-stage digestion at environmental temperature and a 29% increase compared to single-stage digestion at controlled temperature. An analysis of variance (ANOVA) confirmed a significant effect of controlled temperatures and phase separation on biogas production, $F(3, 0) = 0.987$, $p<0.001$, with a significant difference between groups ($M = 0.987$, $SS = 2.961$).

Table 2. Biogas production and COD reduction rate at steady state.

Experimental conditions	Setup	Biogas production (L/gVS)	Biogas yield (NL/gVS)	Methane yield (NL/gVS)	Methane contents %	COD Reduction g/L (%)
Single-stage anaerobic digestion at uncontrolled environmental temperature	S1	1.86	0.113	0.077	68	6.95 (67.5)
Single-stage anaerobic digestion at 37ºC	S2	3.03	0.178	0.117	65.5	6.37 (70.2)
Two-stage anaerobic digestion at uncontrolled environmental temperature	S3	3.42	0.201	0.134	66	7.1 (66.8)
Two-stage anaerobic digestion at 37ºC	S4	4.25	0.250	0.167	66.8	3.1 (85.4)

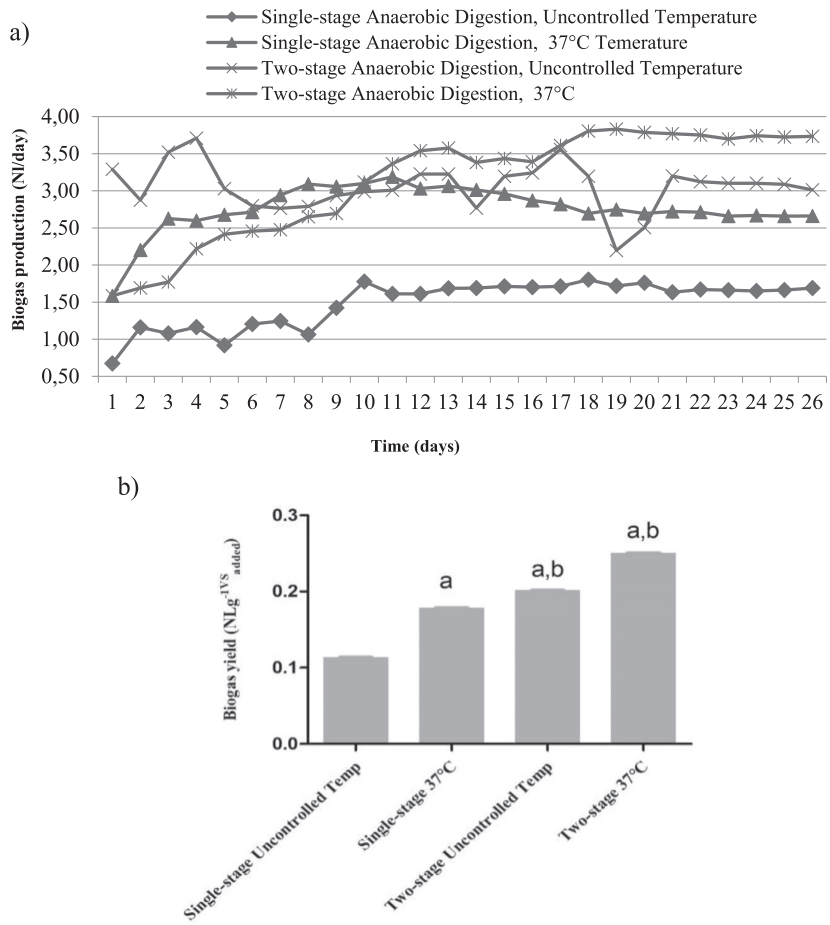

Fig. 1. a) Biogas production in different anaerobic digesters under environmental and controlled temperatures (37ºC), b) Biogas yield during anaerobic co-digestion of cattle manure with food and grocery waste under environmental and controlled temperatures (37ºC). * a = significantly different than the adjacent bar ($p<0.05$), and a, b = significantly different than all bars ($p<0.05$).

The two-stage system's observed increase in biogas production can be attributed to several factors**.** Phase separation enhances hydrolysis and acidogenesis in the first stage, creating optimal conditions for methanogenesis in the second stage. Previous studies demonstrated that the compartmentalization of the AD process allows more efficient substrate utilization and conversion of VFAs into methane [25, 26]. In addition, the microbial activity in the methanogenic reactor was improved due to optimal temperature [27-29].

The maximum biogas production observed at a controlled mesophilic temperature of 37ºC was 4.2 L/day, which correlates directly with the increased microbial activity facilitated by this temperature. It also correlates with the enzymatic activity of microorganisms, which is maximum at 37ºC and decreases with temperature fluctuation [30, 31].

Stability of Bioreactors in Single-Stage and Two-Stage AD

The bioreactors were continuously monitored to assess process stability by measuring pH, VFA, and alkalinity. In single-stage AD, VFAs were found to be significantly higher in concentrations, with values of 3200 mg/L at environmental temperature and 2250 mg/L at a controlled temperature (37ºC). At the same time, alkalinity levels were 1560 mg/L and 1500 mg/L, respectively (Table 3). The VFA-to-alkalinity ratio ranged from 1.5 to 2.1, indicating high VFA accumulation and low methanogenesis rates [32].

In two-stage digestion, VFA concentrations in the acidogenic reactor were high at environmental temperature and low at controlled temperature. VFA concentrations were significantly reduced in the methanogenic reactors, indicating effective conversion of intermediates into methane, thereby contributing to overall stability and efficiency. Previous studies also demonstrated that improved stability is likely due to the separation of acidogenesis and methanogenesis stages, which allow for the more effective control of process parameters [33].

Reduction in Total Volatile Solids (VS) and Chemical Oxygen Demand (COD)

Total reductions in VS and COD were more substantial in two-stage AD compared to single-stage

Table 3. Steady-state performance of digesters in terms of stability.

Conditions		VFA (mg/L)	Alkalinity (mgCaCO$_3$/L)	VFA/alkalinity
Single-stage anaerobic digestion setup				
Environmental temperature		3200	1560	2.1
Control temperature (37ºC)		2250	1500	1.5
Two-stage anaerobic digestion setup				
Environmental temperature	Acidogenic Reactor (R1)	3000	1500	2
	Methanogenic Reactor (R2)	1400	2000	0.7
Controlled temperature (37ºC)	Acidogenic Reactor (R1)	1500	1360	1.1
	Methanogenic Reactor (R2)	1250	1920	0.7

digestion. In single-stage reactors, VS reductions were 58% and 65%, while in two-stage digestion, reductions were 69% and 83%. Similarly, COD reductions were 67.5% and 70.2% at environmental and controlled temperatures, respectively, for single-stage digestion. In contrast, phase-separated digestion achieved COD reductions of 66.8% and 85.4% under similar conditions (Table 2). A one-sample t-test confirmed a significant difference in COD values between initial and post-digestion states, highlighting the enhanced efficiency of two-stage digestion at controlled temperatures.

Overall, phase-separated AD processes show greater efficiency in reducing organic matter, with optimal performance at controlled mesophilic temperatures, achieving up to 85% COD reduction and 83% VS reduction. These findings highlight the advantages of two-stage systems in enhancing stability and efficiency by separating acetogenesis and methanogenesis processes, also described by Ding et al. [34].

Estimation of Bioenergy Potential in Pakistan Using Two-Stage AD

The following calculations determined the approximate biogas potential in Pakistan from animal manure, specifically cattle and buffaloes, and FVW, utilizing the results of this study of biogas yield obtained by two-stage AD at a controlled temperature (37ºC).

a) Total manure by cattle and buffaloes in Pakistan

Approximately 92.54 million tons (30% of the total manure from all animals) [35].

Total generated manure = 768,000 tons/day = 768,000,000 kg/day

If we suppose the collection rate is about 70% with a 30% margin, then it is 768,000,000 × 0.70 = 537,600,000 kg/day.

According to this study's findings, the moisture content in cattle manure is approximately 77.86%, while the VS constitutes 70.56% of the TS; these values were used to calculate the TS and VS of the substrate.

TS = Weight before drying − Weight after drying
TS = 537,600,000 − (537,600,000×0.7786)
= 119,904,000 kg/day
VS = TS×VS of TS
VS = 119,904,000×0.7056 = 84,661,478.4 kg/day

b) Total FVW generated in Pakistan

Approximately 13.674 million tons (13,674,000,000 kg/year), of which almost 30% goes to waste due to transportation issues and mismanagement of temperature maintenance, etc. [36].

Total FVW wasted = 13,674,000,000 × 0.30 = 4,102,200,000 kg/year
If we suppose the collection rate is about 70% with a 30% margin
4,102,200,000×0.70 = 2,871,540,000 kg/year
= 7,867,074 kg/day

According to this study's findings, the moisture content of FVW is approximately 88.79%, while the VS constitutes 93.97% of the TS; these values were used to calculate the TS and VS of the substrate.

Total Solids = 7,867,074 − (7,867,074 × 0.8879) = 873,115.67 kg/day
VS = 873,115.67×0.9397 = 819,259.88 kg/day
Total biodegradable waste from manure and FVW
= 84,661,478.4+819,259.88 = 85,480,738.28 kg/day

c) Biogas and electricity production potential in Pakistan

The biogas yield in two-stage AD was 0.250 m³/kg VS with a methane content of approximately 66%; these values were used to calculate Pakistan's biogas and electricity generation potential.

Biogas potential = 85,480,738.28 × 0.250 = 21,370,184.57 m³/day
Methane gas potential = 21,370,184.57 × 0.66 = 14,109,723.22 m³/day

The energy potential from biogas can be calculated based on an energy content of 6.0 kWh per cubic meter of biogas [37]. For a daily biogas production of 21,370,184.57 m³, the total calorific energy produced amounts to 128,220,586.4 kWh/day. Considering a biogas-to-electricity conversion efficiency of 35% [38], the daily electricity generation potential would be 44,873,205.24 kWh/day or 44,873.21 MWh/day of electricity, highlighting the substantial energy production capacity from biogas in this context.

Pakistan's agriculture and livestock sectors produce large quantities of FVW and animal manure, which are suitable for biogas technology implementation. This technology's flexibility, from small-scale digesters in homes to large industrial facilities, makes it feasible for application across the country.

The study has the limitation of field validation of these experiments, which is attributed to the limited resources and time constraints faced during the research project; however, future studies could address this gap by incorporating field data and pilot-scale investigations. The Pakistani government should thoroughly assess the feasibility and applicability of two-stage anaerobic co-digestion technology in different regions where there is a need for alternative energy sources. This evaluation should include estimating local waste availability, infrastructure, and environmental conditions to ensure the technology is optimally suited to specific geographic and socio-economic conditions [39].

Conclusions

Two-stage anaerobic digestion (AD) significantly enhances biogas production and organic matter degradation at controlled mesophilic temperatures, offering more stable performance compared to uncontrolled conditions due to the improved enzymatic activity of methanogens. Maintaining digester stability is critical, with temperature, VFA, pH, and alkalinity being key factors. There is a need to study the long-term impacts of varying temperature regimes on microbial communities and biogas yields, alongside optimizing feedstock selection and pretreatment methods. Furthermore, microbial communities with high performance in the AD process should be considered for future studies.

Acknowledgments

The authors would like to express their sincere appreciation to the Researchers Supporting Project, number (RSP2025R349), King Saud University, Riyadh, Saudi Arabia.

Conflict of Interest

The authors declare no conflict of interest.

References

1. SAFAR K.M., BUX M.R., ASLAM U.M. Waste to energy: power generation potential of putrescible wastes by anaerobic digestion process at Hyderabad, Pakistan. Journal of Material Cycles and Waste Management. **20,** 1239, **2018**.
2. RASHEED R., YASAR A., WANG Y., TABINDA A.B., AHMAD S.R., TAHIR F., SU Y. Environmental impact and economic sustainability analysis of a novel anaerobic digestion waste-to-energy pilot plant in Pakistan. Environmental Science and Pollution Research. **26**, 26404, **2019**.
3. PARAJULI A., KHADKA A., SAPKOTA L., GHIMIRE A. Effect of hydraulic retention time and organic-loading rate on two-staged, semi-continuous mesophilic anaerobic digestion of food waste during start-up. Fermentation. **8** (11), 620, **2022**.
4. XIAO B., QIN Y., WU J., CHEN H., YU P., LIU J., LI Y.Y. Comparison of single-stage and two-stage thermophilic anaerobic digestion of food waste: Performance, energy balance and reaction process. Energy conversion and management. **156,** 215, **2018.**
5. EDWIGES T., FRARE L.M., ALINO J.H.L., TRIOLO J.M., FLOTATS X., DE MENDONÇA C.M.S.S. Methane potential of fruit and vegetable waste: an evaluation of the semi-continuous anaerobic mono-digestion. Environmental Technology. **41** (7), 921, **2018**.
6. ARELLI V., BEGUM S., ANUPOJU G.R., KURUTI K., SHAILAJA S. Dry anaerobic co-digestion of food waste and cattle manure: Impact of total solids, substrate ratio and thermal pre treatment on methane yield and quality of biomanure. Bioresource technology. **253**, 273, **2018**.
7. LI K., WANG K., WANG J., YUAN Q., SHI C., WU J., ZUO J. Performance assessment and metagenomic analysis of full-scale innovative two-stage anaerobic digestion biogas plant for food wastes treatment. Journal of Cleaner Production. **264**, 121646, **2020**.
8. ODEJOBI O.J., AJALA O.O., OSUOLALE F.N. Anaerobic co-digestion of kitchen waste and animal manure: A review of operating parameters, inhibiting factors, and pretreatment with their impact on process performance. Biomass Conversion and Biorefinery. **13** (7), 5515, **2023**.
9. LIU K., LV L., LI W., REN Z., WANG P., LIU X., ZHANG G. A comprehensive review on food waste anaerobic co-digestion: Research progress and tendencies. Science of the Total Environment. **878**, 163155, 2023.
10. JASIŃSKA A., GROSSER A., MEERS E. Possibilities and Limitations of Anaerobic Co-Digestion of Animal Manure – A Critical Review. Energies. **16** (9), 3885, **2023**.
11. LI D., SUN J., CAO Q., CHEN Y., LIU X., RAN Y. Recovery of unstable digestion of vegetable waste by adding trace elements using the bicarbonate alkalinity to total alkalinity ratio as an early warning indicator. Biodegradation. **30**, 87, **2019**.
12. HUE D.T.K. Development of Chemiluminescence Method for the Measurements of Chemical Oxygen Demand, Hypochlorite, Ammonium, and Urea in Water. Doctoral Thesis, Osaka Prefecture University, **2017**.
13. CHEN W., GAO L., SONG L., SOMMERFELD M., HU Q. An improved phenol-sulfuric acid method for the quantitative measurement of total carbohydrates in algal biomass. Algal Research. **70**, 102986, **2023**.
14. CHANG S.K., ZHANG Y. Protein analysis. Food analysis. 315, **2017**.
15. LYTRAS G., LYTRAS C., MATHIOUDAKIS D., PAPADOPOULOU K., LYBERATOS G. Food waste valorization based on anaerobic digestion. Waste and Biomass Valorization. **12**, 1677, **2021**.

16. PAGÉS-DÍAZ J., SÁRVÁRI-HORVÁTH I., PÉREZ-OLMO J., PEREDA-REYES I. Co-digestion of bovine slaughterhouse wastes, cow manure, various crops and municipal solid waste at thermophilic conditions: a comparison with specific case running at mesophilic conditions. Water Science and Technology. **67** (5), 989, **2013**.
17. LOPEZ V.M., FLORENTINO B., BARLAZ M.A. Chemical composition and methane potential of commercial food wastes. Waste Management. **56**, 477, **2016**.
18. SHAHBAZ M., AMMAR M., ZOU D., KORAI R.M., LI X. An insight into the anaerobic co-digestion of municipal solid waste and food waste: influence of co-substrate mixture ratio and substrate to inoculum ratio on biogas production. Applied Biochemistry and Biotechnology. **187**, 1356, **2019**.
19. LIU C., WACHEMO A.C., TONG H., SHI S., ZHANG L., YUAN H., LI X. Biogas production and microbial community properties during anaerobic digestion of corn stover at different temperatures. Bioresource Technology. **261**, 93, **2018**.
20. ZHANG S., MA X., SUN H., ZHAO P., WANG Q., WU C., GAO M. Response of semi-continuous anaerobic digestion of food waste to progressively increasing temperature: Methanogen community, correlation analysis, and energy balance. Industrial Crops and Products. **192**, 116066, **2023**.
21. SINGH R., HANS M., KUMAR S., YADAVY K. Thermophilic anaerobic digestion: an advancement towards enhanced biogas production from lignocellulosic biomass. Sustainability. **15** (3), 1859, **2023**.
22. WANG S., MA F., MA W., WANG P., ZHAO G., LU X. Influence of temperature on biogas production efficiency and microbial community in a two-phase anaerobic digestion system. Water. **11** (1), 133, **2019**.
23. CINAR S.Ö., CINAR S., KUCHTA K. Machine learning algorithms for temperature management in the anaerobic digestion process. Fermentation. **8** (2), 65, **2022**.
24. OKWU M.O., SAMUEL O.D., OTANOCHA O.B., BALOGUN P.P., TEGA O.J., OJO E. Design and development of a bio-digester for production of biogas from dual waste. World Journal of Engineering. **17** (2), 247, **2020**.
25. ATASOY M., OWUSU-AGYEMAN I., PLAZA E., CETECIOGLU Z. Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. Bioresource Technology. **268**, 773, **2018**.
26. ALIBARDI L., COSSU R. Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials. Waste management. **36**, 147, **2015**.
27. WESTERHOLM M., ISAKSSON S., LINDSJÖ O.K., SCHNÜRER A. Microbial community adaptability to altered temperature conditions determines the potential for process optimisation in biogas production. Applied Energy. **226**, 838, **2018**.
28. JANESCH E., PEREIRA J., NEUBAUER P., JUNNE S. Phase separation in anaerobic digestion: a potential for easier process combination? Frontiers in Chemical Engineering. **3**, 711971, **2021**.
29. CREMONEZ P.A., TELEKEN J.G., MEIER T.R.W., ALVES H.J. Two-Stage anaerobic digestion in agroindustrial waste treatment: A review. Journal of Environmental Management. **281**, 111854, **2021**.
30. SUDIARTHA G.A.W., IMAI T., CHAIRATTANAMANOKORN P., REUNGSANG A. Unveiling the impact of temperature shift on microbial community dynamics and metabolic pathways in anaerobic digestion. Process Safety and Environmental Protection. **186**, 1505, **2024**.
31. LI G., XU F., YANG T., WANG X., LYU T., HUANG Z. Microbial behavior and influencing factors in the anaerobic digestion of distiller: A comprehensive review. Fermentation. **9** (3), 199, **2023**.
32. PAN H., FENG Q., ZHAO Y., LI X., ZI H. Influence of Organic Loading Rate on Methane Production from Brewery Wastewater in Bioelectrochemical Anaerobic Digestion. Fermentation. **9** (11), 932, **2023**.
33. TANG S., WANG Z., LU H., SI B., WANG C., JIANG W. Design of stage-separated anaerobic digestion: Principles, applications, and prospects. Renewable and Sustainable Energy Reviews. **187**, 113702, **2023**.
34. DING L., CHEN Y., XU Y., HU B. Improving treatment capacity and process stability via a two-stage anaerobic digestion of food waste combining solid-state acidogenesis and leachate methanogenesis/recirculation. Journal of Cleaner Production. **279**, 123644, **2021**.
35. ARSHAD M., ANSARI A.R., QADIR R., TAHIR M.H., NADEEM A., MEHMOOD T., KHAN N. Green electricity generation from biogas of cattle manure: an assessment of potential and feasibility in Pakistan. Frontiers in Energy Research. **10**, 911485, **2022**.
36. MUNAWAR A., WAZDA M., MOEEN I.M., ASIM Y. Post-harvest losses in fruits and vegetables in Pakistan - AgriHunt - A Hunt for Agricultural Knowledge. Available from: https://agrihunt.com/articles/pak-agri-outlook/post-harvest-losses-in-fruits-and-vegetables-in-pakistan/ (accessed on 13 October 2024). **2018**.
37. SIDDIKI S.Y.A., UDDIN M.N., MOFIJUR M., FATTAH I.M.R., ONG H.C., LAM S.S., AHMED S.F. Theoretical calculation of biogas production and greenhouse gas emission reduction potential of livestock, poultry and slaughterhouse waste in Bangladesh. Journal of Environmental Chemical Engineering. **9** (3), 105204, **2021**.
38. SUHARTINI S., LESTARI Y.P., NURIKA I. Estimation of methane and electricity potential from canteen food waste. In IOP Conference Series: Earth and Environmental Science. **230**, 012075, **2019**.
39. FENG Y., SHOAIB M., AKRAM R., ALNAFRAH I., AI F., IRFAN M. Assessing and prioritizing biogas energy barriers: A sustainable roadmap for energy security. Renewable Energy. **223**, 120053, **2024**.