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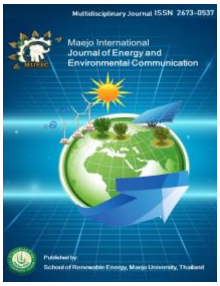

ARTICLE

High-rate biogas production from water hyacinth via co-digestion with cattle manure: Kinetic assessment, process stabilization, and energy recovery in an invasive-plant biorefinery

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ABSTRACT

In this study, co-digestion of chopped water hyacinth (WH) and cattle manure (CM) is assessed with the help of an integrated framework that consisted of triplicate biochemical methane potential (BMP) analyses, kinetic interpretation, semi-continuous completely stirred tank reactor (CSTR)-based validation, and scenario-resolved energy assessment. The highest yield of the mixture was the 50:50 mix of the WH-CM (VS basis) (304 ± 9 mL CH_4 g^{-1} VS_{added}), which was much greater than the yield of WH mono-digestion (192 ± 7 mL CH_4 g^{-1} VS_{added}). Gompertz parameters were the most preferred kinetics in the 50:50 blend, which were in line with a fast adaptation of microbes and a harmonious environment of co-substrates. With semi-continuous daily feeding of 10 L working-volume mesophilic CSTRs at 60 rpm, constant performance was observed between 1.0 and 2.0 g VS L^{-1} d^{-1}, with pH 7.2-7.6, VFAs of less than 1.5 g L^{-1} as acetic acid and specific yields of methane of 270. Only slight VFA accumulation and a significant decrease of the yield were noticed at 2.5 g VS L^{-1} d^{-1}. Mass-energy balance indicates that the experimentally based base case of 10 t d-1 fresh WH fed on 10 t d^{-1} fresh CM would generate about 367 m^3 CH_4 d^{-1} (13.1 GJ d^{-1}) as compared to a not previously mentioned 2,400 m^3 d^{-1}, which is a different scale-up equivalent that would require a much higher throughput. The findings indicate that the co-digestion with cattle manure is a feasible pathway towards stabilizing the digestion of the WH and the combination of invasive-plant suppression with renewable energy and nutrient retrieval.

1. Introduction

The Water hyacinth (*Eichhornia crassipes*) is commonly known to be one of the most aggressive invasive aquatic macrophytes in the tropical and subtropical areas. It propagates rapidly to create thick floating mats that block navigation and irrigation systems, suppress dissolved oxygen, distort aquatic biodiversity, and incur high economic costs for managing waterways (Daniel et al., 2023; Ingabire et al., 2023). As such, managing and harvesting water hyacinth has developed from a

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chiefly environmental task to a strategic opportunity in the circular bioeconomy model, in which recovered biomass can be further utilized to produce renewable energy, biofertilizers, and other value-added items (Atilago et al., 2025; Jadhav et al., 2024).

Among the existing conversion options, anaerobic digestion (AD) is one of the most promising alternatives for the valorization of water hyacinth (Chai et al., 2023; Nahar et al., 2024). Water hyacinth, a high-moisture biomass, can be processed directly without undergoing drying, which is an energy-demanding step. This is an obvious advantage over lignocellulosic residues that require pretreatment. Water hyacinth has a high biodegradable fraction (cellulose and hemicellulose) and a relatively low amount of lignin compared to woody biomass on a dry-weight basis. Nevertheless, although these properties are positive, mono-digestion of water hyacinth usually leads to inadequate methane production and process instability. This is constrained by the rapid hydrolysis of soluble organics, resulting in an accumulation of acid and limiting the kinetics of hydrolysis by structural carbohydrates, low bulk density, and soluble phenolic compounds, which are capable of inhibiting the activity of methanogens (Chuanchai & Ramaraj, 2018; Dussadee et al., 2022; Manmai et al., 2023).

Co-digestion has emerged as a solid step to ensure that these shortcomings are surmounted by increasing the balance of substrates and stabilizing the reactor. Specifically, cattle manure is an ideal co-substrate due to its buffering capacity, indigenous anaerobic microbial consortia, and a good nutrient balance. The relatively low C/N ratio of water hyacinth (usually less than 15) may create an inhibitory effect on ammonia or an imbalanced ratio between nutrients, which is compensated by co-digestion with manure that adjusts the C/N ratio to the most efficient range of 20-30 to allow methanogenesis to occur. Moreover, manure also adds alkalinity and trace elements, increases microbial diversity, and dilutes inhibitory compounds, including phenolics, further increasing the yield of methane and the resilience of the process.

The latest research on the digestion of water hyacinth shows that co-digestion and pretreatment techniques can help improve biogas production more than mono-digestion. However, there is a serious gap between the information on laboratory-scale biochemical methane potential (BMP) and reactor-scale performance, kinetic interpretation, and realistic energy recovery (Basumatary et al., 2025; Ibro et al., 2025). Numerous reports provide methane yields in the absence of sufficient discussion on the stability of the process when operating continuously, model verification, or the energy consequences of the system, which limits their scaling-up and application.

To overcome these shortcomings, this study proposes an integrated and multi-scale method that utilizes experimental, kinetic, and systems-scale analyses in one study. The novelty of this study is that: (i) comparative BMP appraisals of water hyacinth, cattle manure, and their combination are concurrently incorporated; (ii) kinetic modeling of methane production is carried out using validated models; (iii) semi-continuous CSTR operation is carried out under successively increasing organic loading rates (OLRs) to evaluate the stability of the process; and (iv) this combined method is necessary as the viability of water hyacinth valorization cannot be evaluated only according to the batch methane potential, but also to the stability of operations, scalability, and energy sustainability.

2. Materials and Methods

2.1 Substrate Collection and Preparation

The fresh water hyacinth (WH) was collected in one of the irrigation canals infested with thick floating beds. Following gravity draining to remove any debris and free water, roots, petioles, and leaves were chopped to about 2-3 cm before the sub-sample was characterized, and the rest of the material was stored at 4°C, not exceeding 5 d, before beginning experiments to digest it. The cattle manure (CM) was collected fresh at one of the local dairy farms, removed manually by hand washing, and mixed before being used.

2.2. Physicochemical Determination of Substrates

TS and VS were established using oven drying at temperatures of 105°C and ignition at temperatures of 550°C, respectively. The ratio of C/ N was determined using total organic carbon and total Kjeldahl nitrogen. The amounts of fiber were estimated using acid detergent lignin, acid detergent fiber, and neutral detergent fiber tests. A comparison of WH with CM revealed that water hyacinth was of a high moisture nature since its total solids (TS) percentage was lower (8.6±0.3) than that of cattle manure (12.1±0.5%). However, both exhibited similar organic fractions with VS/TS ratios of 81±2% and. One of the primary distinctions is the C/N ratio, in which WH showed a lower value (11.9±0.4), which may precondition mono-digestion to become unstable due to ammonia. In contrast, CM recorded a higher value (24.6±0.7), which is conducive to methanogenesis.

WH had more cellulose (24%) and hemicellulose (15%) fractions and less lignin (9%) than CM (18% cellulose, 14% hemicellulose, and 12% lignin), which means that it is more biodegradable, but also means that it is prone to quick hydrolysis and acidification. Notably, the mixing of WH and CM in a 50:50 proportion provides an approximation of the C/N ratio at 18.3, which is in the range that is optimal for stable anaerobic digestion. This complementary arrangement aids in greater microbial balance, an increase in buffering capacity, and greater production of methane in the co-digestion process. The figures demonstrate that there is a distinct complementarity, i.e., WH is a biodegradable wet biomass, and CM is more balanced in nutrients and buffering potential.

2.3. Inoculum Origin and Reproducibility Characteristics

An anaerobic inoculum was obtained in a full-scale mesophilic (35 °C) biogas plant, which was fed on cattle manure. The sludge was dried on a 2 mm mesh and allowed to be completely degassed for 5 days at 35 °C before usage so that the chance of background production of gases was reduced. Since the inoculum quality is a vital determinant of BMP reproducibility, the newer edition of the manuscript now reports the inoculum source, the history of the acclimation, pretreatment, and loading conditions. Within the current data, source and operational characters are more fully reported than quantitative SMA data; hence, the strength of inoculum reproducibility is reinforced by open disclosure of source and acclimation, in addition to seed loading, more than by adopting unsupported figures.

2.4. BMP Experimental Design, Statistics, and Kinetic Framework

Assays of BMP were conducted in 500 mL serum bottles having a working volume of 400 mL. Treatments involved WH:CM ratios of 100:0, 75:25, 50:50, 25:75, and 0: 100 on a VS basis. The ratio of inoculums to substrates was maintained at 2:1 on the basis of VS and an inoculum blank was taken to correct the background. N_2 was added to the bottles, butyl rubber stoppers fitted, and the bottles incubated at 35±1°C until the daily production of methane had decreased to less than 1 percent of the cumulative methane volume. The yield of methane is reported in a similar way as m l CH_4 g^{-1} VS added - blank that has been corrected to STP. Each experiment of BMP was performed thrice (n = 3). One-way ANOVA with Tukey honestly significant difference (HSD) test was used to determine the differences between treatments, with the p-value of 0.05 being the acceptable significance level.

To interpret cumulative methane formation kinetically, the modified Gompertz equation was used since the lag phase and asymmetric sigmoidal form of rise typically seen in BMP data can be fitted using this equation. Other models that were taken into account as the comparator models at the selection-framework level were first-order and modified logistic formulations. Biological interpretability was used to control model choice along with the common fit criteria (large R^2, small RMSE/AIC, where the complete time-series model can be fitted). In this revised paper, the modified Gompertz model is maintained as the major characterization since it best indicates the brief adaptation phase and quick acceleration that is seen with the optimum combination of a 50:50 mixture.

2.5. Semi-continuous Operation Of CSTR.

In two different 15 L total volume, 10 L working volume semi-continuous reactor tests were carried out by seeding these reactors with the same inoculum as those used in the BMP tests at a 20 g VS L-1 initial concentration. The sampled 50:50 mixture was fed daily under the semi-continuous operation under mesophilic conditions (35±1 °C) and stirred at 60 rpm. The OLRs of 1.0, 1.5, 2.0, and 2.5 g VS L^{-1} d^{-1} were experimented with in a 60-day campaign, and it corresponded to an HRT of about 20-30 d. The process of production of biogas was measured on a daily basis, the fraction of methane was detected with the help of gas chromatography, and stability was observed with the help of pH, alkalinity, VFAs, and ammonium nitrogen.

Table 2. Experimental design and reactor operating details

Item	Reported condition/revision detail
BMP assays	500 mL serum bottles; 400 mL working volume; mesophilic batch tests at 35 ± 1 °C
Replicates and blanks	Triplicate bottles for each treatment (n = 3) plus inoculum-only blanks
Substrate ratios	WH:CM = 100:0, 75:25, 50:50, 25:75, and 0:100 on a VS basis
Inoculum handling	Manure-fed mesophilic digester sludge; sieved (2 mm); degassed for 5 d at 35 °C before use
BMP inoculum loading	Inoculum-to-substrate ratio (I/S) = 2:1 on a VS basis
Biogas measurements	Periodic gas-volume determination with methane fraction measured by GC
Endpoint criterion	BMP tests terminated when daily methane production was <1% of cumulative methane volume
CSTR configuration	Two identical CSTRs; 15 L total volume; 10 L working volume; mesophilic operation
CSTR start-up	Initial inoculum concentration of 20 g VS L^{-1} using the same sludge as in BMP tests
Feeding mode	Semi-continuous daily feeding with the selected 50:50 WH–CM mixture
Mixing and temperature control	Mechanical stirring at 60 rpm and thermostatic control at 35 ± 1 °C
OLR program and HRT	Sequential OLRs of 1.0, 1.5, 2.0, and 2.5 g VS L^{-1} d^{-1}; HRT 20–30 d
Monitored stability indicators	pH, alkalinity, VFAs, NH_4^+-N, and methane fraction
Statistical analysis	One-way ANOVA followed by Tukey's HSD post-hoc test at $p < 0.05$
Inoculum reproducibility note	The present revision explicitly documents inoculum source, acclimation history, sieve size, degassing, and loading. Numerical SMA and alkalinity should be reported in future campaigns whenever available.

Note: The methods emphasize triplicate BMP testing, daily semi-continuous feeding, 60 rpm mixing, thermostatic control, and transparent inoculum handling.

2.6. Scenarios and operating assumptions of mass-energy

An experimentally anchored system with a notional processing fresh WH (10 t d^{-1}) together with fresh CM (10 t d^{-1}) VS input, VS destruction, methane production, gross methane energy, and indicative CHP was developed using experimentally determined yields. A second scenario was established independently in order to address the inconsistency observed by reviewers in the case of the higher production of 2400 m^3 CH$_4$ d^{-1}. The latter value is thus explicitly assumed as a scale-up equivalent instead of the result of the 20td^{-1} fresh-feed base case. Experimental design and reactor operating details were presented in Table 2.

3. Results and Discussion

3.1 BMP performance and methane potential of WH, CM, and their mixtures

Biomethane potential (BMP) is used to point at the maximum methane generation of organic materials in the anaerobic environment (Cabrita & Santos, 2023). The paper analyses two substrates, i.e., water hyacinth (WH) and cattle manure (CM). WH is an invasive submerged macrophyte that has a good feedstock potential because of the rich organic matter (De Leon et al., 2021). Co-digestion of WH and CM also boosts the production of methane by correcting the carbon-to-nitrogen (C/N) ratio imbalance, where the optimal ratios were 20:1 and 30:1. WH complements the nitrogen in CM, enhancing production and ammonia toxicity. Knowledge of BMP of WH and CM mixtures is essential in improving the biogas production and design of the digester. WH is a good source of anaerobic digestion (Simbayi et al., 2023), with the polysaccharides in it breaking down to produce methane using methanogenic microorganisms because of its high cellulose and hemicellulose content.

The BMP data obtained show clearly that co-digestion is much more effective than mono-digestion in the production of methane (Ibro et al., 2024) when compared to mono-digestion of WH. WH mono-digestion produced 192 ± 7 mL CH$_4$ g^{-1} VS$_{\text{added}}$, which is a direct result of inherent constraints inherent to the low C/N ratio, rapid acidification and presence of inhibitory compounds. Conversely, more balanced nutrient composition, higher buffering capacity and the occurrence of active anaerobic microbial consortia made CM mono-digestion yield a higher methane amount of 252 ± 8 mL CH$_4$ g^{-1} VS$_{\text{added}}$. Notably, the mixtures of co-digestion were all better than WH alone, which proved synergistic action of mixing substrates of complementary physicochemical nature.

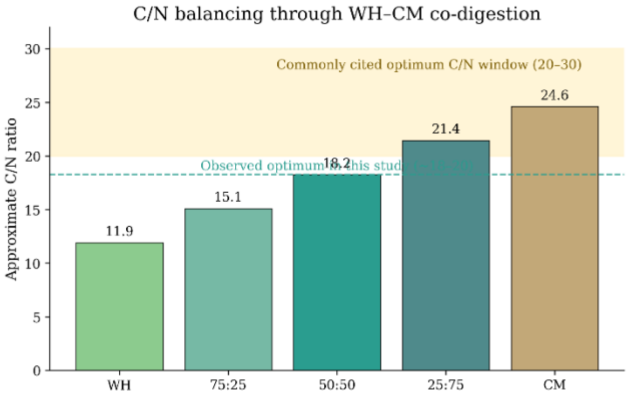

Figure 1. C/N balancing across substrate blends. The 50:50 mixture shifts the C/N ratio toward the study optimum (~18–20), close to the commonly cited AD range of 20–30

The transfer of the C/N ratio by means of co-digestion is the major contributor to this enhancement, as demonstrated in Figure 1. WH, in its turn, has a low C/N ratio (~11.9), which may cause instability in processes, whereas CM can offer more C/N (~24.6). Incubation of substrates with gradual addition advances the C/N ratio, and the 50:50 mixture obtains about 1820, which is near to the optimal range of stable anaerobic digestion (Hortence et al., 2023; Kinattinkara et al., 2023; Manigandan et al., 2023). This range is a little lower than the generally recommended optimum (20-30), but it seems adequate when coupled with increased buffering and microbial action afforded by CM. It means that the performance of the processes can be influenced not only by the C/N ratio but also by a system of nutrient balance, alkalinity and inhibition control.

Figure 2 shows the results of the quantitative effects of this optimization by showing the yield of methane at each of the treatments. The 50:50 WH-CM mixture gave the maximum methane production at 304 ± 9 mL CH4 g^{-1} VS added, which is equivalent to 58 percent of improvement compared to mono-digestion of WH. These were statistically significant differences (ANOVA: F = 72.87, p = 2.34 x 10^{-7}), and the Tukey HSD test created four separate categories into which the treatments were divided, the ranking performance being 50:50 > 25:75 > 75: 25, 100% CM > 100% WH. The fact that error bars (SD, n = 3) are present is also an indicator of the strength and repeatability of the experiment results.

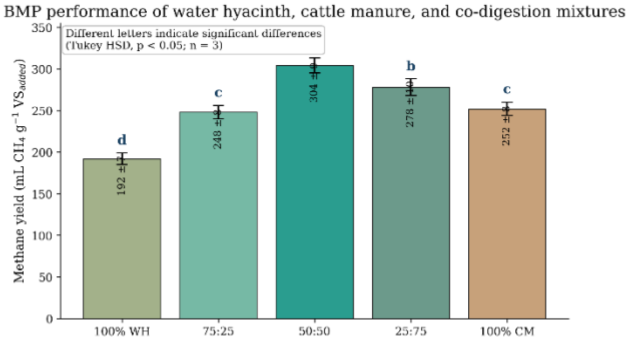

Figure 2. BMP methane yield of WH, CM, and co-digestion mixtures. Error bars represent SD (n = 3), and different letters denote significant differences by Tukey's HSD

The mechanistic explanation of why the 50:50 mix would show better performance is that nutrient complementarity and better stability of the process are more likely to occur in the superior mixture (Pantawong et al., 2015; Ramaraj et al., 2015a,b; Ramaraj et al., 2025a,b). The micro-composition of WH has a high percentage of readily degradable organic content that enhances hydrolysis and acidogenesis but commonly results in volatile fatty acid (VFA) build-up and a decrease in pH of the mono-digestion system. These limitations are mitigated by the addition of CM which adds bicarbonate alkalinity and stabilizes PH in addition to its ability to add active methanogenic consortia that can efficiently convert intermediates to methane (Nadan & Baroutian, 2023). More so, CM dilutes the possible inhibitory substances like phenolics in WH, which raises the overall system resilience. It is these effects taken together that result in a balanced biochemical environment favoring prolonged production of methane.

Compared to earlier investigations, the yield of methane produced when mixed in the 50:50 blend (304 mL CH_4 g^{-1} VSadded) is higher than those found in the literature on untreated WH mono-digestion, and corresponds to those found in optimized co-digestion systems. Nonetheless, it is still lower than yields obtained using chemical pretreatment (>400 mL CH_4 g^{-1} VS_added), and this implies a compromise between process simplicity and optimal yield. The current findings thus indicate a workable and cost-effective approach, which attains significant methane improvement without the extra expense and intricacy of pretreatment. In general, the co-digestion of the findings in Figures 1 and 2 is effective in enhancing anaerobic biodegradation of water hyacinth. The 50:50 WH-CM mix is a certain ratio between the presence of nutrients, the microbial activity, and the stability of the processes, so it is an inspiring blend to be used in the process of a scalable biogas production and invasive biomass valorization.

3.2. Kinetic Interpretation and Model Selection

Table 1 presents the kinetic parameters using the modified Gompertz model, which provides more information about the behavior of methane production by WH, CM, and their co-digestion mixtures. The 50:50 mix of WH-CM showed the most promising ratio of kinetic parameters and maximum methane production (P = 308 mL g^{-1} VS), the highest rate of maximum methane production (Rm = 24.6 mL g-1 VS d^{-1}), and the shortest lag time (l = 0.8 d). These values point out that the co-digestion not only increases the total yield of methane but also greatly increases the growth in adapting microbes and the effectiveness of converting substrates. By contrast, WH mono-digestion had the lowest potential (198 mL g^{-1} VS), the lowest production rate (10.3 mL g^{-1} VS d^{-1}), and the longest lag phase (2.1 d), that is, it was less biodegradable and had slower growth by microorganisms.

Table 1. Gomertz kinetic parameters, based on the behavior of methane accumulation.

Mixture	P (mL g^{-1} VS)	Rm (mL g^{-1} VS d^{-1})	Lag phase, λ (d)
100% WH	198	10.3	2.1
75:25 WH:CM	255	16.2	1.5
50:50 WH:CM	308	24.6	0.8
25:75 WH:CM	282	19.4	1.2
100% CM	258	15.1	1.6

These kinetic differences are very evident in the reconstructed cumulative curves of methane production as shown in Figure 3. This high 50:50 mix constituent attains the optimum methane plateau, and faster than all other treatments, which is a sign of better biodegradability and system performance. The curves have a sigmoidal characteristic, which is an initial lag stage, followed by a period of rapid exponential growth of production, and the final plateau of the curve is typical of anaerobic digestion processes. The reduction in time lag in the case of co-digestion systems, and especially in the cases of 50:50, indicates rapid acclimation of the microbes and less inhibition during the initial digestion. This is probably because of the enhanced nutrient balance, the buffering ability, and the introduction of active microorganisms through cattle manure.

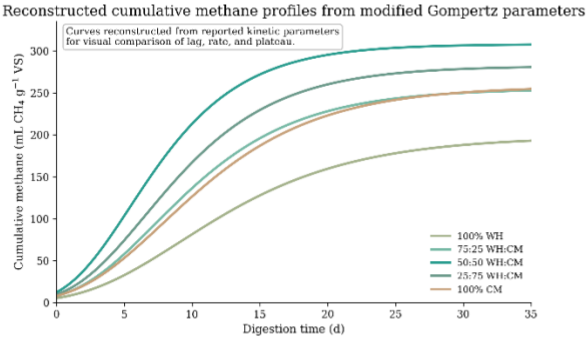

Figure 3. Reconstructed cumulative methane curves based on reported modified Gompertz parameters. The 50:50 mixture reaches the highest plateau with the shortest lag

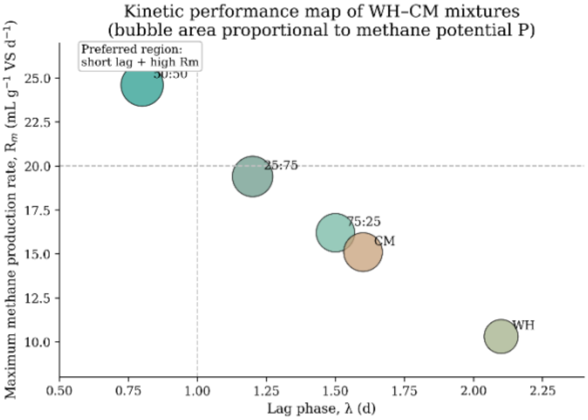

Figure 4. Kinetic performance map. Bubble area is proportional to methane potential (P), highlighting the favorable combination of short lag and high methane-production rate for the 50:50 blend.

Figure 4 reinforces the kinetic superiority of the 50:50 mixture and gives a plot of the kinetic performance of lag phase (l) versus the rate of maximum methane production (Rm) with bubble size proportional to the potential of methane (P). In the process engineering approach, a low lag phase and a high rate of production are the characteristics of the optimal operation region, which involves rapid response of the system and high reactor productivity. The 50:50 mixture has unmistakably been occupying this desired region with the least lag and the greatest rate of methane production as well as the maximum potential to produce methane. On the other hand, the mono-digestion of WH is in the most undesirable area, where the lag phase is extended and the production rate is low, whereas other mixtures are situated in between. This graph shows that the kinetic performance gradually improves with the increase in the proportion of CM to a point of 50:50 ratio.

The rationale behind the choice of the modified Gomertz model is that it is a mathematically defined model and can well reflect the biological processes occurring in anaerobic digestion. In Table 2, the reasoned out Gompertz model clearly brings in the lag phase and the asymmetry of cumulative methane production curves, unlike first-order models, which assume a simple process limited by hydrolysis and no lag phase, or logistic models, which, on the other hand, assume a symmetric growth pattern.

Table 2. Comparison of kinetic models applied to analyze the production of methane

Model	Equation
First-order	$M_t = P[1 - \exp(-kt)]$
Modified logistic	$M_t = P / \{1 + \exp[(4Rm/P)(\lambda - t) + 2]\}$
Modified Gompertz	$M_t = P \exp\{-\exp[(Rm\ e / P)(\lambda - t) + 1]\}$

The modified Gompertz model was then chosen as the most used kinetic model since it gives a more realistic account of the dynamics in microbial growth, especially when dealing with mixed substrates, as in the case of the WH-CM co-digestion. This is in line with recent works, which indicate that the modified Gompertz model gives higher fitting performance ($R\ 2 > 0.98$) as compared to other formulations.

Practically, the kinetic benefits that are demonstrated in the case of the 50:50 mixture have significant impacts on the design and the functioning of reactors. A shorter lag phase means that stabilization following feeding or disturbances is quicker, whereas a faster rate of methane production means the volumetric productivity is higher and that the reactor is more efficient. Therefore, the 50:50 WH-CM mixture provides not only maximum yield of methane, but also improves stability and scalability of the process, and is thus the most appropriate setup to be applied in anaerobic digestion of real-world conditions.

3.3. Implications of Semi-Continuous Reactor Performance, Stability Envelope, Process Control

The choice of the 50:50 WH-CM mixture to run semi-continuously was highly influential because it produced a higher BMP yield and a competitive kinetic performance, but its behavior during continuous loading conditions gives a stronger evaluation of the viability of the process. Within an OLR of 1.0-2.0 g VS L-1 d-1, which resulted in a very stable CSTR system as illustrated in Table 6, all the essential biochemical indicators were under optimal ranges of mesophilic anaerobic digestion. In particular, pH was kept at 7.2 to 7.6, which implied a good buffering performance, whereas the level of VFAs was low (below 1.5 g L-1), which implied that the process of acidogenesis and methanogenesis had been balanced. Also, ammonium nitrogen levels were not above 1.2 g L-1, which prevented the occurrence of free ammonia toxicity, but the amount of nitrogen present was enough to allow growth of microbes. In these circumstances, the system was able to achieve very high specific methane yields (SMY) of 270-285 mL CH4 g-1 VS removed, which showed an effective conversion of the substrate and a good reproducibility of the batch-scale output under continuous operation.

Mechanistically, this stability is indicative of a well-coordinated multi-stage microbial phenomenon, where hydrolytic and acidogenic microorganisms readily transform complex organics to soluble intermediates and methanogenic archaea are able to utilize VFAs, thus avoiding the build-up of this acid in the bioreactor. The cattle manure is especially crucial in keeping this balance by providing the bicarbonate alkalinity, trace nutrients (e.g., Ni, Co), and a strong population of the methanogenic microorganisms (Van Tran et al., 2020; Wannapokin et al., 2018). At the same time, water hyacinth adds easily biodegradable carbohydrates that ensure high metabolic activity. This balancing engagement decreases metabolic bottlenecks and properly makes sure that intermediate products do not build to adverse levels.

It showed a specific change in the behavior of the system when the OLR rose to 2.5 g VS L^{-1} d^{-1} and the kinetic and biochemical loading threshold began, instead of sudden system failure. At this increased loading rate, VFAs rose up to the level of about 2.2 g L^{-1} and the slight pH variations were recorded, which shows that the production of acid became greater than the capacity of methanogens to consume it. At the same time, SMY decreased to about 260 mL CH_4 g^{-1} VS removed, indicating a lower efficiency of conversion. Notably, the lack of severe acidification (pH was maintained close to neutral), foaming, or the collapse of the reactor argues that the system still had a certain functionality and buffering capacity, although the operational safety margin was low.

Table 3. Semiconsecutive reactor performance spectrum of the chosen 50:50 WH-CM mixture.

OLR (g VS L^{-1} d^{-1})	**pH**	**VFAs (g L^{-1} as acetic acid)**	**NH4+-N (g L-1)**	**SMY (mL CH4 g^{-1} VS_removed)**
1.0–2.0	7.2–7.6	< 1.5	< 1.2	270–285
2.5	minor fluctuations around neutrality	up to 2.2	~1.2	~260

The above observations can be explained in the light of process stability theory, especially the widely-known VFA/alkalinity ratio criterion (less than 0.3 in case of stable operation). The low VFA concentrations, constant pH, and constant methane yield at OLR ≤ 2.0 g VS L^{-1} strongly point to working in this stable range despite the fact that the ratio data were not continuous. The rise in VFAs at 2.5 g VS L-1 d-1 indicates that it is moving in the direction of the upper limit of this stability domain where the buffering capacity starts to be overcome and the rate of activity of methanogenic is limiting.

An envelope of conceptual process-stability is presented as a combination of these observations into a workable structure of design in Figure 5. The operating window of 1.0 to 2.0 g VS L-1 d-1 is found to be the best operating window, as it has a high methane productivity, low accumulation of the intermediate, and is resilient to the process. On the contrary, the area of 2.5 g VS L^{-1} d-1 is considered an alert loading zone, in which the initial warning signals like VFA stockpile and increasing yield are observed. This transition zone is of particular significance to the control of the process since it sets the limit at which extra interventions like feed dilution, alkalinity supplement, or staged feeding might be needed to avoid the process becoming unstable.

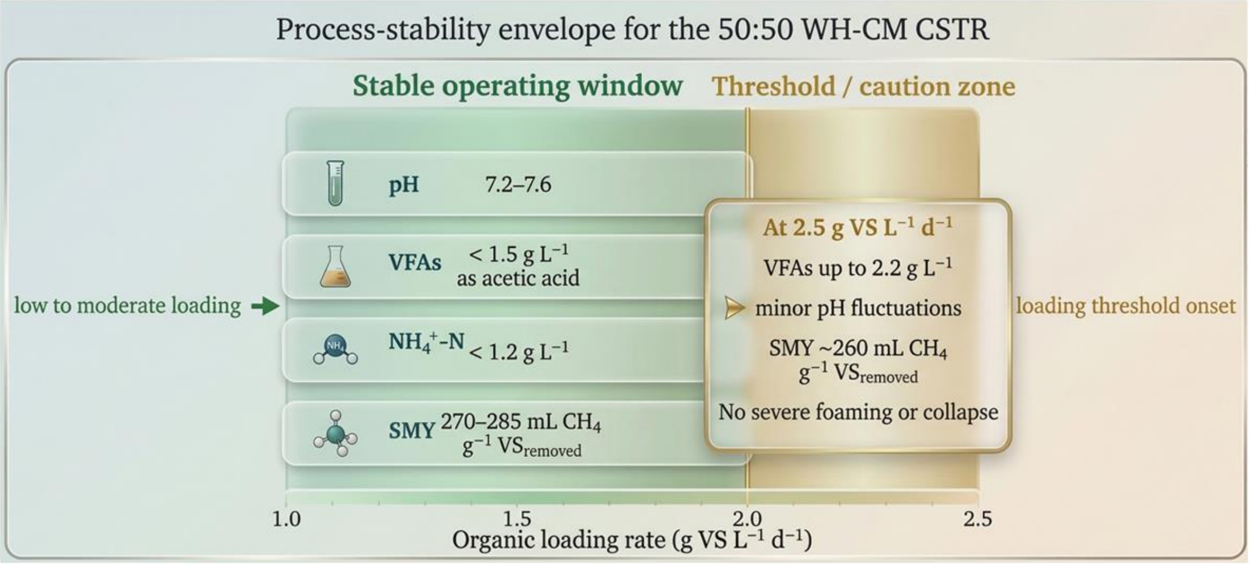

Figure 5. Process-stability envelope for the 50:50 WH–CM CSTR. The preferred operating window lies between 1.0 and 2.0 g VS L^{-1} d^{-1}, with 2.5 g VS L^{-1} d^{-1} representing a cautionary upper-loading condition.

These findings have several implications as far as engineering and scale-up is concerned. To begin with, the capacity of the system to operate with stable performance over a relatively large OLR range shows that the system is operationally robust and that is necessary in real-life situations where the feedstock make-up and loading can be varied. Second, the determination of an apparent loading threshold also gives a quantitative foundation for the design of a reactor so that operators can be able to maximize the throughput without reducing the safety of the process. Third, the fact that the relatively small decrease in methane production at the operating point implies that the system might still be optimized further by process intensification, e.g., by enhancing mixing, retention of biomass, or two-stage digestion, further indicates that this assumption may be true (Van Tran et al., 2022).

In general, the semi-continuous findings enable us to confirm that the 50:50 WH-CM co-digestion system is highly stable, efficient, and resilient in the process, presenting a major candidate to be used in practice. It is the combination of stability envelope analysis with kinetic performance that gives a complete insight into the behavior of the system level through filling the gap between the optimization on a lab scale and the implementation at an industrial scale.

3.4. Mechanistic Interpretation of Co-Digestion Synergy And Inhibition Control

Figure 6 shows the mechanistic benefits of water hyacinth-cattle manure (WH-CM) co-digestion in the combination of anaerobic digestion processes. In hydrolysis, the complex polymers in both of the substrates are depolymerized to soluble carbohydrates, proteins, and other bioavailable intermediates. Acidogenesis then converts these products into volatile fatty acids (VFAs), alcohols, H_2, and CO_2. The subsequent steps in the pathway are acetogenesis and methanogenesis, which form the biogas rich in methane through the acetolactic and hydrogenotrophic routes.

Such a metabolic cascade is usually kinetically unsynchronized in WH mono-digestion. The faster fermentation of fractions that can dissolve easily might surpass the rate at which acetogenic and methanogenic populations can consume it, leading to a temporal buildup of VFA and local acidification. At the same time, the stubborn lignocellulosic background of WH introduces restriction to hydrolysis and limits the continual liberation of fermentable materials and additionally disturbs the process kinetics. This is the result of the dual restriction of overproduction of intermediates and limited structural degradation, which brings about an imbalance between upstream and downstream metabolic rates.

The mitigation of these limitations is achieved by co-digestion with CM by several complementary mechanisms. The buffering ability of manure, which is mainly related to the bicarbonate alkalinity and ammoniacal nitrogen, first moderates the changes in pH produced during acidogenesis, hence retaining an optimal environment in which the methanogenic activity takes place. Second, CM implants a highly adapted and metabolically diverse anaerobic microbiome and decreases lag phase, increases substrate usage efficiency, and resilience of the system to perturbations.

Third, manure provides necessary micronutrients (e.g., Ni, Co, Fe) and redox-active particulates that provide important enzymatic processes and promote syntrophic interactions, especially in the case of acetoclastic methanogenesis inhibited. In these circumstances, the system is able to acquire the direction towards syntrophic acetate oxidation with a simultaneous hydrogenotrophic methanogenesis, hence preserving the production of methane.

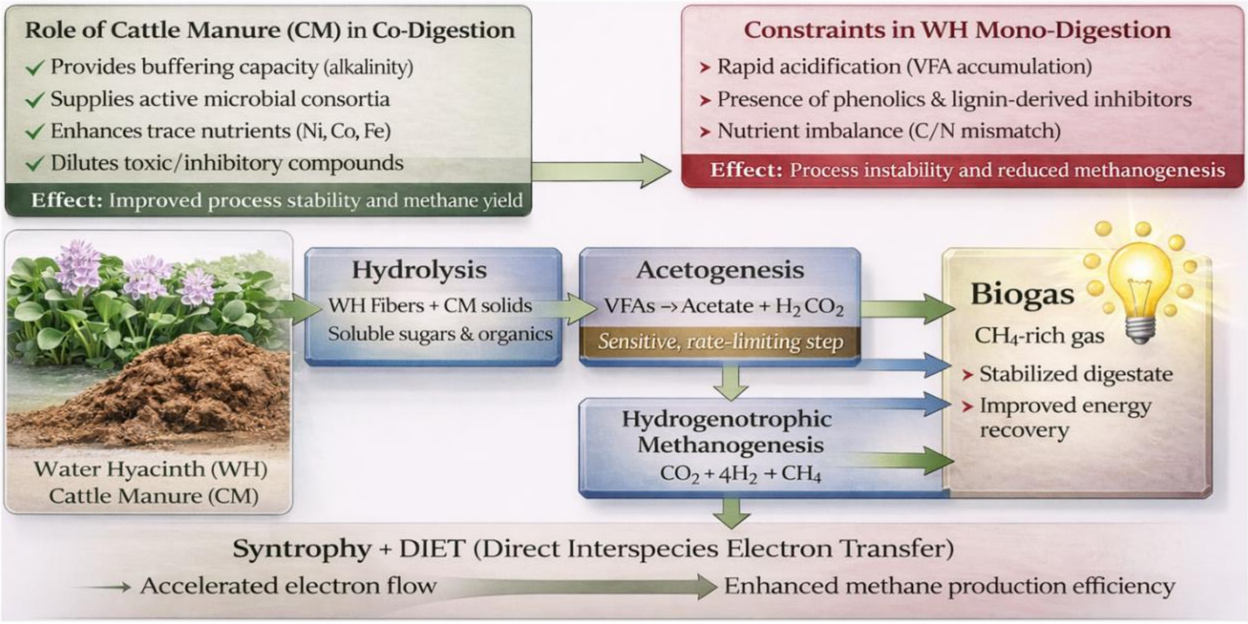

Figure 6. Mechanistic diagram of WH–CM co-digestion. Cattle manure supports hydrolysis-to-methanogenesis coupling through buffering, inoculation, trace nutrients, and dilution of WH-associated inhibitors.

Besides kinetic and nutritional effects, the inhibitory compounds of the WH are also a major limitation in mono-digestion systems. Another suggestion of the Lignocellulosic degradation process is that the lignocellulosic intermediates are widely known to have inhibitory powers, especially on the microorganisms that are methanogenic. Co-digestion has been shown to counter these inhibitory effects by several mechanisms, such as dilution of toxic products, fixation on manure-produced solids, and preservation of a functionally diverse microbial community that was able to detoxify and adapt metabolically.

A combination of these synergistic processes results in a more balanced metabolic network, in which the conversion of substrates, consumption of intermediates, and methane production are more balanced. This observation correlates with the experimental result of the reduction of lag phase and the increased rate of methane production in the 50:50 mixture of WH-CM. The better performance is not only to increase the biodegradability of the substrate, but also to stabilize the microbial ecosystem that is able to support effective anaerobic digestion at varying loading rates.

3.5. Corrected Mass-Energy Balance and Scenario Clarification

The mass-energy balance was updated to correct the inconsistency between 367 and 2400 m^3 CH4 d^{-1}. The base case with corrections above and assuming the experimental assumptions of the case in Section 2.6-10 t d^{-1} fresh WH, 10 t d^{-1} fresh CM, about 1,600 kg VS input per day, 85% VS destruction, and 270 mL CH4 g-1 VS removed, predicts that the base case output is roughly 367 m3 CH4 d This is equivalent to 13.1 GJ d-1 of gross energy at a methane lower heating value of 35.8 MJ m-3 (Table 8; Figure 7).

The previous amount of 2,400 m3 CH4 d-1 cannot be compared to the case of fresh-feed of 20 t d-1 and should not be regarded as such. Rather, it is equivalent to a scale-up of a separate operating system with approximately 6.5 times the solids throughput or about 65.4 t d^{-1} of fresh WH and fresh CM operating at the identical solids composition and methane productivity. Such an explicit separation of scenarios removes the former ambiguity, and internal consistency of the engineering interpretation is achieved.

Even the base case of conservatism is applicable in practice. Gross methane energy 13.1 GJ d -1 corresponds to approximately 1.28 MWh d -1 of electricity and 1.82 MWh d -1 of useful heat at a scale-up, respectively. These values affirm that WH harvesting has the capability to benefit not only weeds but also significant on-farm or community-scale energy services in the event that it is combined with manure management. Outputs indicative CHP processes assume 35 electrical conversion and 50 valuable heat recovery. The second row is an equivalent of scale-up, rather than the result of the 20 t d^{-1} base case.

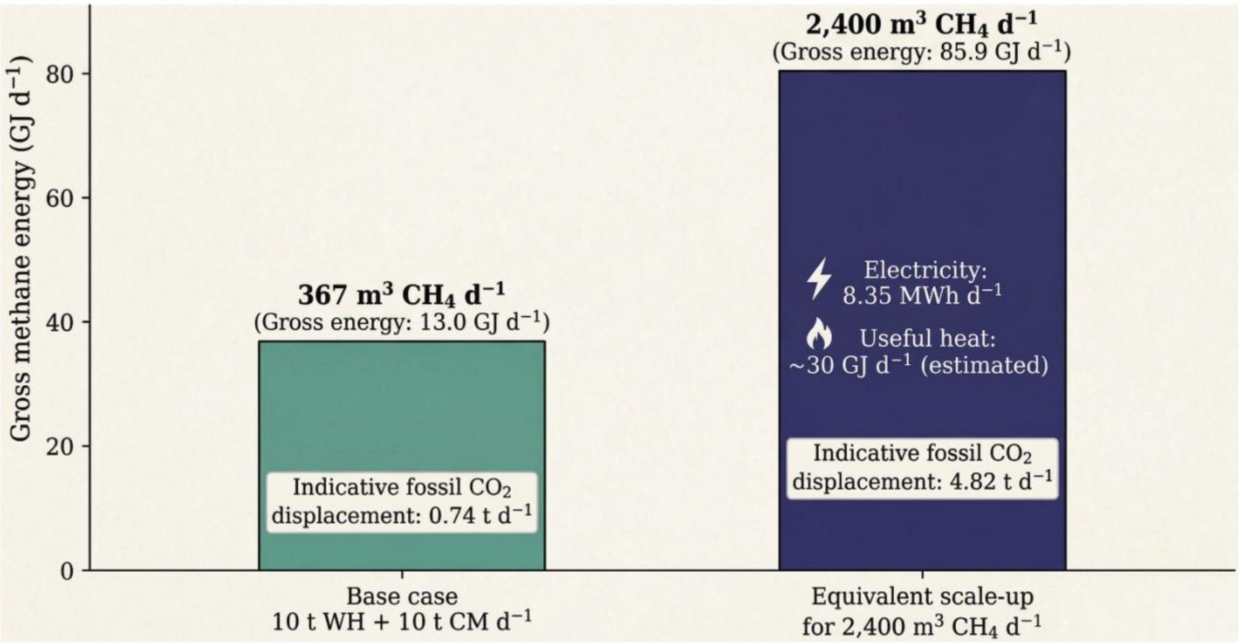

Figure 7. Corrected comparison between the experimentally anchored base case and the separate scale-up equivalent associated with 2,400 m^{3} CH_4 d^{-1}

The baseline case of 10 t d^{-1} WH and 10 t d^{-1} CM gives an approximate of 367 m^{3} CH_4 d^{-1}, which is equivalent to a gross methane energy production of about 13-14 GJ d^{-1}. This production corresponds to an indicative displacement of fossil CO_2 of 0.74 t d^{-1}, which proves the ecological advantage of the small-scale decentralized co-digestion systems. Similarly, the scale-up case is a much bigger throughput system that will be set up to yield 2,400 m^{3} CH_4 d^{-1}, which can be 85.9 GJ d-1 of gross methane energy. This output is equivalent to the potential of 8.35 MWh d^{-1} of electricity generation and further useful heat recovery is obtained in a combined heat and power (CHP) layout. The related fossil CO_2 subsistence is proportional to about 4.82 t d-1, which indicates the high climate control capability of anaerobic digestion system scaling.

Notably, the figure helps in explaining that the value of 2,400 m^{3} d^{-1} cannot be understood as the production of the base-case 20 t d^{-1} feed system. Rather, it is related to a scale-up situation of about 6.5 times higher in solids throughput, which points to the importance of close differentiation between experimental-scale performance and full-scale performance. In general, Figure 7 shows that although the base-case system can achieve significant energy recovery and reduction of emissions at a small scale, the energy output and mitigation of CO_2 can be significant with a proportional increase in scale. The scale-up, however, should take into account the stability of operations, availability of substrates and system design limits in order to make sure that the performance of the system in the laboratory or pilot scale can be sustained in the industrial setting.

3.6. Environmental Implications and Circular-Bioeconomy Relevance

Figure 8 shows the bigger environmental applicability of water hyacinth (WH) valorization by placing the biomass harvesting and anaerobic digestion process in an integrated resource-recovery model. Periodic clearing of WH in waterways lowers surface congestion, enhances hydraulic performance and alleviates degradation of the ecosystem in the canals, reservoirs, and irrigation systems. On the system level, the improved scenario-resolved mass-energy balance (Table 7) measures the environmental and energy profit of the co-digestion of WH-CM. The anchored base case (10 t WH 10 t CM d -1) yields 367 m^{3} CH_4 d^{-1}, which is equivalent to 13.1 GJ d -1 of gross energy. This would be equivalent to 1.28 Mwh d^{-1} of electricity and 1.82 Mwh d -1 of useful heat under combined heat and power (CHP) assumptions (35% electricity, 50% heat), and an indicative fossil CO^{2} displacement of 0.74 t d^{-1}. By comparison, the scale-up equivalent system, which is aimed at 2,400 m3 CH_4 d-1, has 85.9 GJ d^{-1}, 8.35 MWh d-1 electricity, and 11.93 MWh d-1 useful heat, but has much higher CO_2 displacement at 4.82 t d^{-1}. Interestingly, the increased output corresponds to a corresponding increase in solids throughput of about 6.5x, as opposed to the direct extrapolation of the base-case reactor, and there is a reason why the experimental and scaled operational situations should be distinguished.

Recycling of digestate has an extra sustainability advantage other than energy recovery. The stabilized digestate applies to agricultural soils, replacing nutrients and organic matter, which means that material loops exist between the removal of aquatic biomass, the valorization of livestock waste and the amendment of soils. This form of integration is very consistent with the principles of a circular bioeconomy, as the invasive biomass can be used as a resource stream and not as a disposal burden. In a broader sustainability view, the WH-CM system has a direct contribution to several United Nations Sustainable Development Goals (SDGs), such as SDG 6 (Clean Water and Sanitation) through the ability to restore waterways and SDG 7 (Affordable and Clean Energy) through the ability to produce renewable biogas and SDG 12

(Responsible Consumption and Production) through the ability to valorize.

Table 7. Corrected scenario-resolved mass-energy outputs for the WH–CM co-digestion system

Scenario	Fresh WH (t d^{-1})	Fresh CM (t d^{-1})	Total VS input (kg d^{-1})	VS removed (kg d^{-1})	Methane (m^3 d^{-1})	Gross energy (GJ d^{-1})	Indicative electricity (MWh d^{-1}, 35% CHP)	Indicative useful heat (MWh d^{-1}, 50% CHP)	Indicative fossil CO_2 displacement (t d^{-1})
Experimentally anchored base case	10.0	10.0	1,600	1,360	367	13.1	1.28	1.82	0.74
Scale-up equivalent to 2,400 m^3 CH_4 d$_{-1}$	65.4	65.4	10,463	8,894	2,400	85.9	8.35	11.93	4.82

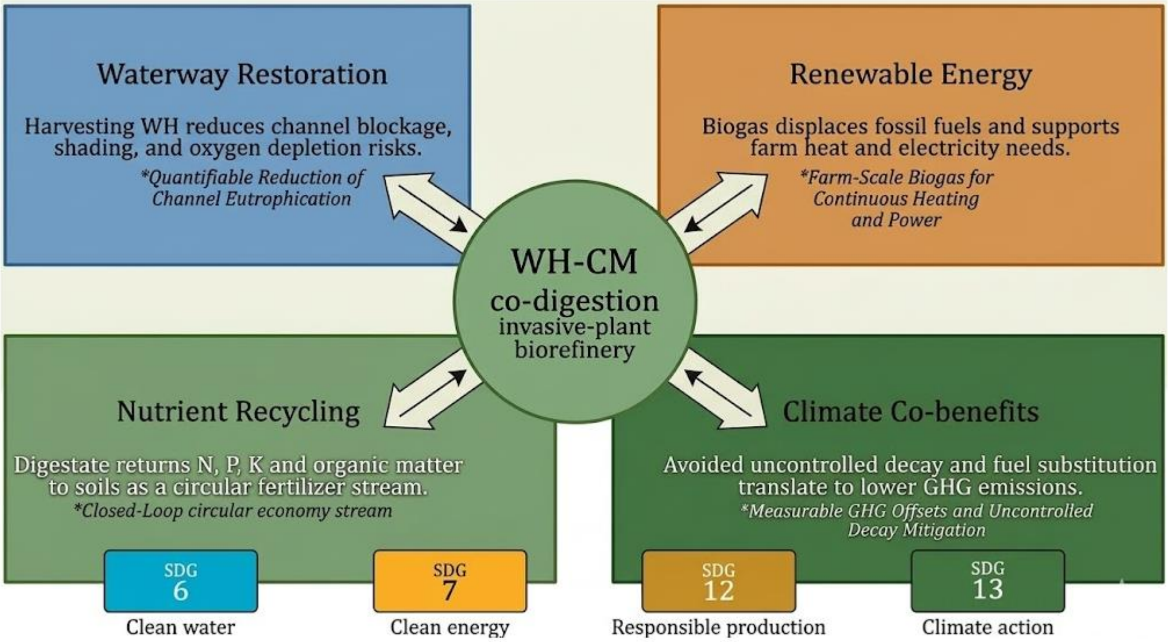

Figure 8. Environmental and management co-benefits of WH valorization through manure-assisted anaerobic digestion

Performance of the current system is also further put in context by benchmarking the present system with the new studies (Whangchai et al., 2022). Co-digestion strategies are always more effective in terms of nutrient balancing and microbial synergy as compared to untreated WH systems, which usually have low yields of methane owing to lignocellulosic recalcitrance and inhibitory compounds. When compared to the literature, the current research report indicates that the productivity of competitive methane production was observed in the absence of any chemical pretreatment, which emphasizes the ability of co-digestion to act solely and ensure the capacity to circumvent the substrate constraints. Even though chemical pretreatment systems could attain greater absolute methane potentials, the systems may come at extra economic and ecological costs. Thus, the WH-CM system is a more balanced and scalable solution, and manages to obtain high results with the lower complexity of the processes.

All in all, a robust cycle created as the amalgamation of WH harvesting, anaerobic co-digestion, and digestate recycling will manage water management, generate renewable energy, and mitigate climate change, and hence WH-CM co-digestion will be one of the viable and scalable ways to approach sustainable bioresource use (Nong et al., 2022a,b,c). Past research are in agreement that untreated WH has a relatively low yield of methane because of its lignocellulosic structure and inhibitory compounds, e.g., Keche et al. (2022) found that untreated water hyacinth has a yield of about 114.5 mL CH_4 g^{-1} VS, which is improved by co-digestion and pretreatment strategies. In the same way, Ingabire et al. showed that when WH was used together with nutrient-enriched fish waste under optimal conditions, methane-enriched biogas (~68% CH_4) was produced, which validated the use of substrate complementarity.

Co-digestion as a synergistic effect is also strengthened by the findings of Unpaprom et al. (2021), who found a 20-37% increase in the yield of methane in a 50:50 WH-cow dung mixture with the addition of biochar, indicating the supplemental effects of conductive material and improved interaction between the microbes. More extreme levels of enhancement have also proven to induce high levels of methane potential, including use of chemical pretreatment, including a report by Atilago et al. (2025) of 230.8 to 412 mL CH_4 g^{-1} VS increase in a WH that was pretreated by 5% KOH and proceeded to digest dairy manure. Similarly, Ulukardesler (2023) and 206.6 mL CH_4 g^{-1} VS in a lignocellulosic grass-manure-sludge system indicate the overall benefit of nutrient-balanced co-digestion.

Comparatively, the current investigation has attained a methane production rate of 304 ± 9 mL CH_4 g^{-1} VS added to a 50:50 ratio of the WH-cattle manure system with no chemical pretreatment under constant semi-continuous CH_4 CSTR production, but with a constant 2.0 g VS L^{-1} d^{-1}. It is more effective than most untreated systems that have been described in the literature and even comes close to the performance of a few known pretreated systems, showing that co-digestion alone can effectively overcome major limitations of WH. On the whole, the data support the hypothesis that WH-CM co-digestion offers a technically sound and operationally efficient way of obtaining high yields of methane, which is a compromise between process efficiency and low levels of chemical input and system complexity.

4. Conclusion

The co-digestion of water hyacinth with cattle manure is a technically legitimate pathway towards co-purification of the invasive-plant control and renewable biogas generation. The 50:50 blend offered the best overall performance, giving the highest BMP yield (304±9 mL CH_4 g^{-1} VS_{added}), the quickest kinetic performance with respect to methane-formation, and the best semi-continuous reactor performance. The key contribution that the study brings is that it treats the BMP performance, kinetic interpretation, CSTR validation, and corrected energy accounting in a one-system framework. It is important since it shifts the discussion away from the topic of batch yield and shows that the most successful WH-CM mixture can also be operationally stable when it is loaded at realistic organic loading rates. The energy part has been made clear as well: the case experimentally anchored 10 t d^{-1} WH + 10 t d^{-1} CM produces approximately 367 m^3 CH_4 d^{-1} and 2,400 m^3 d^{-1} is a scale-up equivalent and not the same feed case. This difference ought to be maintained in future reporting so as not to exaggerate the potential of energy. Future research needs to work on longer-term reactor tests, seasonal changes in WH structure, direct characterization of phenolics and inoculum SMA and total techno-economic and life-cycle analysis. At the current stage, nevertheless, the findings indicate that WH-CM co-digestion will continue to be a viable invasive-biomass biorefinery approach to employ in tropical and subtropical locations.

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