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Feasibility Study of Anaerobic Co-digestion of Slaughterhouse Wastewater with Sugar Press mud for Biomethane Recovery

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Abstract— Slaughterhouse wastewater (SHWW) is another most-explored proteinous substrate for biomethane recovery. However, inhibitory compounds associated with its anaerobic monodigestion (AMoD) limit its practical and industrial applications. In this regard, anaerobic codigestion (ACoD) is effectively applied to optimize system stability and methane (CH_4) yield by allowing toxic compound dilution. Therefore, the current study investigated the influence of sugar press mud (SPM) as a co-substrate on the treatment performance of AD. The ACoD process was semi-continuously conducted in lab-scale continuous stirred tank reactors (CSTRs) at varied hydraulic retention times (HRTs) under mesophilic conditions (37.0±1.0°C). Subsequently, the proportions of SHWW and SPM to produce CH_4 were optimized in batch experiments at different mixing ratios. The addition of SPM enhanced CH_4 yield and VS removal by 69.1% and 62.4%, respectively, at an optimum mixing ratio of 80%SPM:20%SHWW and 15 days (d) HRT. Moreover, the addition of SPM improved the AD process stability, as verified by the decreased ammonium nitrogen (NH_4^+-N) concentration. Results suggested that ACoD can be used as an alternative method for the treatment of these organic agrowastes for bioCH_4 recovery. Furthermore, repeating the study under thermophilic conditions to examine the stability of the AD process in practice would also be a fruitful area for further work.

Keywords— Anaerobic Digestion, Co-Digestion, Co-substrate, Mesophilic Condition, Slaughterhouse Wastewater.

I. INTRODUCTION

MEAT sector in the agro-processing industry has received considerable critical attention as it contributes immensely to high-strength wastewater generation [1]. Meat processing plants that automate carcass dressing consume more water and produce an effluent with high protein and lipid-based organic matter content. A key issue is the safe disposal of this wastewater that is associated with increased risk of disease-causing microbes; a serious environmental hazard and a threat to human health [2]. Unfortunately, modern slaughterhouses pose a challenge to sustainably and adequately treating such organic waste. Likewise, the sugar processing industry faces management challenges associated with handling of the resultant SPM. It is generated in substantial quantities ranging approximately at a rate of 0.01 to 0.07 tons per ton of ground sugarcane [3]. Therefore, there is an urgent need to address the safety problems caused by SPM composts as they emit obnoxious smell creating a nuisance to residents proximate to the sugar factory. Moreover, toxic gases sulphur dioxide (SO_2) and sulphur trioxide (SO_3) emitted on burning their briquettes pollute the environment [4].

To date, a large and growing body of literature has investigated the anaerobic mono-digestion (AMoD) of cattle SHWW [5, 6, 7, 8]. Nonetheless, AMoD of SHWW is associated with the volatile fatty acids (VFAs) accumulation, and/or ammonia (NH3) inhibitions [9, 10] and operational challenges such as sludge flotation; digester foaming; and pipe obstructions [11]. Fortunately, co-digestion is one means of feasible option to overcome such drawbacks[12]; since it delivers the required macro and micronutrients nutrients, adjusts pH, improves buffer capacity and biodegradability, and widens the microbial consortia involved in the AD process and increases biogas yield [13, 14].

Nonetheless, literature has highly recommended both feedstocks for biomethanation [15, 8, 4]. However, their digestibility is of great concern. For instance, SHWW exhibit inhibitory compounds (i.e. VFAs and NH3) while presence of wax and problem of fast acidification affect operation performance of SPM biogas plants. Furthermore, SPM contains a high amount of ash that may elevate concentrations of mud within the CSTR, resulting in a higher OLR that can possibly inhibit the AD process [16]. Therefore, both wastes indicate suitability as a co-substrate to one another.

Several scholars (Table I) also evaluated the co-digestion of abattoir waste with other feedstocks. In general, all authors observed that ACoD of slaughterhouse waste gave better results than AMoD. On the contrary, Monou et al. [17] reported negative improvement for ACoD of abattoir wastewater with potato processing wastewater. The authors credited the results to poor buffering capacity and low pH of abattoir wastewater. Nonetheless, ACoD of SHWW with SPM is a possible solution and offers an efficient remedy, to solving environmental

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pollution and energy recovery from these two organic wastes. Whilst extensive research has been carried out on AD, studies on the ACoD of SHWW with SPM as a co-substrate in optimization of AD process performance are limited. This paper, investigated the performance and stability of ACoD of SHWW with SPM using lab-scale continuous CSTRs at varied HRTs under mesophilic condition (37.0 ± 1.0 °C).

TABLE I
PERFORMANCE OF ANAEROBIC CO-DIGESTED SLAUGHTERHOUSE WASTES WITH DIFFERENT SUBSTRATES

Reference	Co-digested feedstock	Operation Conditions	Improvements
[2]	SHWW; WMS	Batch;CSTR;37±1 °C;HRT18d,13.5, 11d; OLR 1.5 kg VS/m^3 d	50%CH_4 increase
[18]	Poultry SHWW; sewage sludge	Batch;34±1°C; HRT 50d, 42d	63%VSremoval ;88%COD reduction
[15]	PD; SPM	Batch;CSTR;20-45 °C ; HRT 20d	29%CH_4 increase
[19]	OMW; SHWW	Batch and ASBR; 37±1°C; OLR 10 g COD/L/day; HRT 20d	Reactor degraded 10 g COD/L/day
[20]	SHWW; RP	CSTR; 35 and 55±1°C; 1.0 and 1.5kg VS/m^3 day OLRs; 50 d HRT	262–572mL CH_4/g VS
[21]	SHWW; OFI	CSTR; 38±1°C; OLR 64 g VS L^{-1} day^{-1}	57%CH_4 increase
[22]	FVW; AWW	Single-stage ASBR; 35 and 55±1°C;20 d, 10d HRT; 2.56 g VS l^{-1} day^{-1} OLR	75% more CH_4 yield
[17]	PPWW; RPS	Batch ; mesophilic temp; HRT 22 d	72%VS removal; 35 mL daily CH_4 yield; 32% max CH_4
[23]	AWW; FVW	Unstirred two-staged ASBR; 38±0.2 °C	70.26%CH_4 increase;57.11 %VS reduction

[a] SHWW=Slaughterhouse Wastewater, SPM=Sugar Press Mud, AWW=Abattoir Wastewater, FVW=Fruit and Vegetable Waste, WMS=Waste Mixed Sludge, OMW=Olive Mill Wastewaters, PD=Poultry Droppings, SHWs=Slaughterhouse Wastes, OFI=Opuntia fícus-indica, PPWW=Potato Processing Wastewater, RPS=Raw Pig Slurry, RP= Rendering Plant, CSTR=Continuously Stirred Tank Reactor, OLR=Organic Loading Rate, HRT=Hydraulic Retention Time, VS=volatile solids, ASBR= Anaerobic Sequencing Batch Reactors.

II. MATERIALS AND METHODS

A. *Seed sludge and substrates*

The SHWW samples were collected from a cattle abattoir in the outskirts of Juja town in Kiambu County, Kenya. While, the SPM was collected from Busia Sugar Industry (BSI), in Busia County located in western Kenya. Samples of SPM were collected while in their fresh state directly from the production line, packed and transported in a cool box to Juja, within 24 hours. In the laboratory, the SPM was pre-processed to reduce the particle size and increase surface area for ease of feeding and further, fasten the biodegradation process. For easy feeding into the reactors, SPM was sieved through 0.42-mm sieve and dissolved in distilled water to 6% total solids (TS). Mixture of 10% distilled water and 90% anaerobic sludge obtained from an active mesophilic (37.0 ± 1.0 °C) biogas digester treating dairy manure was used as inoculum. Until feeding, all the feedstocks were labeled, sealed, and refrigerated at 4 °C to minimize undesirable fermentation processes. Table III under results and discussion section summarizes the raw SHWW, SPM, mixed feedstocks and inoculum physicochemical characteristics, from the analyses undertaken.

B. *Impact of SPM addition as a Co-Substrate*

The influence of SPM as a co-substrate on the performance of AD was studied by codigesting SHWW with SPM in different mixing ratios. These proportions were tested in batch experimental set-up under mesophilic condition (37±1.0 °C) for 66 days. The BMP tests were prepared according to the procedure used by Anyango et al. [24]. The objective was looking for the mixing ratio with the optimum performance. Proportioning of these feedstocks in the optimum 20%SHWW:80%SPM mix ratio was undertaken on a weekly basis and refrigerated at 4 °C awaiting feeding.

The continuous AD experimental setup consisted of two control reactors (with inoculum only) and three paired test digesters (with 20%SHWW: 80%SPM mix). The 80% SPM: 20% SHWW mixture was used since it presented optimum CH_4 potential, based on results obtained by Anyango et al. [24] for batch experiments. The purpose of having control digesters was to examine the comparative benefits or drawbacks of co-digestion for AD process stability and performance. Each bioreactor was acclimatized separately without addition of the feedstock using the inoculum. The result of this experiment was used to evaluate the influence of the addition of co-substrate (SPM) on the organic degradation and CH_4 production of the SHWW. It also provided the optimum mix proportion with the best performance measured by the highest reduction of volatile solids (VS) and CH_4 yield.

C. *The Experimental Setup and Operation Procedures of the CSTR*

The experiment was carried out in a semi-continuous mode using CSTRs with working volume of 800 ml. Each digester was made entirely of glass and sealed using a polyethylene cap (air tight) and wrapped in parafilm. Two ports were fitted at the top of each digester such that one was used for feeding and sampling while the other acted as a biogas outlet from headspace of the each reactor to biogas collector bags as shown in Fig. 1. Every digester headspace was purged for roughly 2-3 minutes with 99.9% pure nitrogen.

Fig. 1 CSTRs laboratory set-up in the incubator

The bioreactors were operated at a total retention time of 90 days (45 days for the 15 HRT, 30 days for the 10 HRT and 15 days for the 5 HRT) (Table II) and constant mesophilic temperature (37±1.0 °C) maintained via a temperature-controlled incubator (Model IB-01E/11E/21E, Lab Companion, Joe Tech Co., Ltd., South Korea). Calculated amount of pre-characterized 20%SHWW:80%SPM mix loads (ml/day) (Table II) were manually fed on a daily basis for five days that is from Monday through to Friday after unloading the same volume via a 100 mL plastic syringe. On Mondays and Fridays, the reactors were fed double the daily volume to compensate for the lack of feed during the weekend. The produced biogas was collected in 1.0 L biogas collector bags that were connected to each reactor. The reactors' performance and stability was verified by periodic sampling of influents and effluents of the CSTRs. This was followed with an analysis of the following control parameters during digestions assays: gas volume, pH, TS, VS, ammonium nitrogen (NH_4^+-N) concentration, COD and biogas composition. Fig. 2 depicts the schematic illustration of the anaerobic codigestion process.

TABLE II
OPERATING CONDITIONS AND PARAMETERS DURING CSTR EXPERIMENT

Days	OLR ($gVSL^{-1} d^{-1}$)	HRT (days)	SPM:SHWW mix ratio	Flow rate (ml/day)	Temperature
45	2.9	15	80:20	53.33	37±1.0 °C
30	4.3	10	80:20	80	37±1.0 °C
15	8.6	5	80:20	160	37±1.0 °C

D. Analytical Methods

Before feeding into the CSTRs, the homogenized inoculum and substrates were characterized in triplicate and the resultant mean values recorded accordingly. The TS and volatile solids (VS) were determined according to Standard Methods for Examination of Water and Wastewater [25]. For COD analysis, the closed reflux technique was used. The pH readings were taken from the samples directly via a portable pH meter (pH3210, Germany). The Nessler method was used to measure NH_4^+-N concentration and was determined using a Shimadzu UV-VIS-1800 spectrophotometer (DR 2500, Hach, USA). The pre-processed feedstocks (SHWW and SPM) were characterized for Carbon (% C), hydrogen (% H), Oxygen (O %) and nitrogen (% N) contents using elemental analyzer (EA 1112 Flash CHNS/O-analyzer). The composition and volume of the biogas were measured using gas analyzer (Geotechnical instrument (UK) Ltd, S/N: BM14068) and airtight syringe, respectively.

The daily produced biogas volume from each reactor was measured using a gas-tight syringe, and then converted to the volume under standard temperature and pressure (STP, 0 oC and 101 kPa). The biogas content was analyzed for CH_4 (CH_4, %), and carbon dioxide (CO_2, %) using a gas chromatography (GC 7890 A, Agilent, Santa Clara, CA 95051, USA) fitted with a thermal conductivity detector, and a stainless-steel column (13803-U, Sigma–Aldrich, Saint Louis, MO, USA). The splitless inlet, oven, and TCD detector temperatures were all kept at 60, 70, and 200 °C, respectively. The CH_4 and CO_2 were measured by a dual wavelength infrared cell with a reference channel. The certified gases CH_4 (60, 15.01%) and CO_2 (40, 15.01%) were used to calibrate the gas analyzer. Argon gas was used as the carrier gas in the GC, while nitrogen was used as the makeup gas. The GC was calibrated using standard gases consisting of CH_4 (60%) and CO_2 (40%) on a volume basis (v/v).

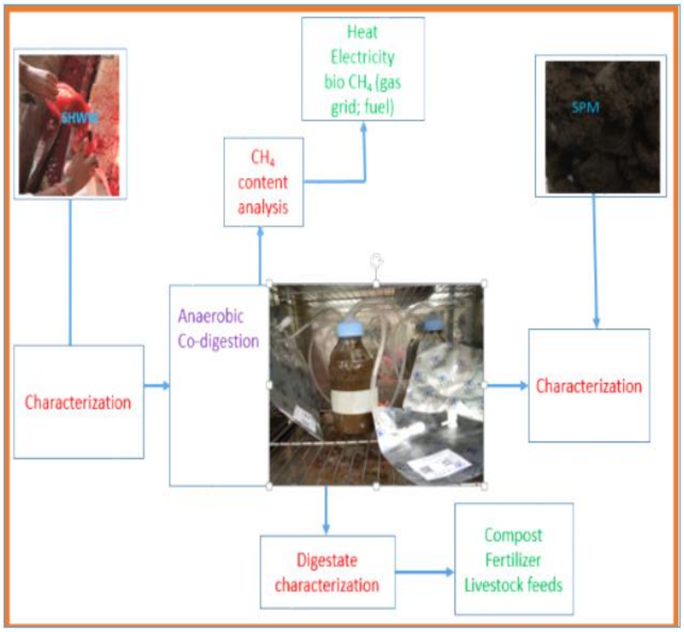

Fig. 2 Schematic illustration of the anaerobic codigestion process

III. RESULTS AND DISCUSSION

A. Substrates and Inoculum Characteristics

The performance and stability of the biodigestion process are highly influenced by substrate characteristic, operating parameters, and an array of different microbial groups, and their functions [23]. Results of the average values (mean ± SD) of physicochemical characteristics of the raw SHWW, SPM, 80% SPM:20% SHWW mixture and inoculum are presented in Table III according to Anyango et al. [24].

A keen look at Table III clearly shows that the feedstocks of interest in the current study are suitable for AD. For instance, according to Jeung et al. (2019), the VS/TS% of both substrates (90%), is more suitable for AD. Additionally, the sampled SHWW, in particular, contained blood, which resulted in a high COD concentration (16 gL^{-1}). Overall, COD degradation was not attractive even for an 80% SPM: 20% SHWW reactor. One such scenario may imply that supplementary treatment is necessary immediately after AD so that the effluent can be unloaded into the surroundings in compliance with the applicable standards.

TABLE III
CHARACTERIZATION OF FEEDSTOCKS AND THE INOCULUM (MEAN ± SD)

Parameter	SPM	SHWW	Inoculum	MIX80:20
TS (%)	6.3±0.3 (5.1 ±0.3)	3.5±0.3 (2.4±0.3)	7.1±0.3 (3.1±0.3)	6.2±1.5 (4.5±1.5)
VS (%)	5.7±0.6 (2.5±0.6)	3.2± 0.3 (1.5± 0.3)	6.3±0.3 (2.4±0.3)	4.3±0.8 (1.4±0.8)
VS/TS (%)	90	91	90	70
VS removal (%)	60	53	62	67
pH	5.41±0 (7.76±0)	8.06±0 (8.34±0)	7.2±0 (7.69±0)	7.2±0 (8.10±0)
TCOD ($g\ l^{-1}$)	7.36±0 (5.16±9)	16±0.1 (12±6.1)	15.0±0.1 (11.0±9.1)	10.8±0.1 (8.3±6.1)
(%)COD removal	30	25	27	23
NH_4^+-N ($mg\ l^{-1}$)	1300±3.3 (1205±0.3)	6407±5.5 (4208±0.5)	1097±8.7 (674±0.7)	5521±7.3 (2426±0.3)
C (%)	27.28±0.2	32.62±0.1	/	/
H (%)	16.51±0.6	17.88±0.7	/	/
O (%)	1.37±0.7	2.45±0.4	/	/
N (%)	1.04±0.4	3.38±0.3	/	/
C/N ratio	26.23	9.65	/	/

[b]Notes: n = 3, the first values refer to the substrates and mixtures before AD, the second values in brackets refer to the respective digestate after AD.

The C/N ratio is a crucial element for AD. However, SHWW exhibited a very low C/N ratio (9.65). Nonetheless, the best C/N ratio, efficient for microbial metabolic activities and adequate to sustain system operation and satisfy nutrient and energy needs for cell growth, ranges from 20 to 30 [16, 12] Consequently, SHWW is a problematic substrate for biogas plants due to perceived NH_3 inhibition and an unbalanced C/N ratio [5].

Another vital factor in AD is pH with ideal range of 6.5 to 8.2 for methanogenesis [26]. In that regard, the current investigation recorded pH values of 8.06 and 5.41 for SHWW and SPM, respectively. The pH level for SHWW remained above 8.0 for almost the entire process due to the relatively higher NH_4^+-N concentration (6407 mg/L) (Table III), which was caused by the degradation of the proteins in SHWW. These findings imply the possibility of NH_3 inhibition during the AD of SHWW.

In contrast, the SPM reactor had a low initial pH because of its acidic nature. Consequently, it did not recover fully despite having a C/N ratio (26.23) well within the allowable threshold. This implies that the buffering capacity of the system was insufficient to keep a pH level within the satisfactory limits for AD. However, according to Qamar et al. [27], pH adjustment through alkali treatment can maintain the stability of the process. As for the 80% SPM: 20% SHWW mix ratio, the initial pH significantly increased from 7.2 to 8.10 in the effluent due to the consumption of VFAs, thus indicating the presence of a buffer effect that maintained optimal AD conditions.

B. Evaluation and performance of the co-digestion Process

The performance of the co-digestion processes was evaluated in terms of gas (quality and quantity) production and VS reduction for the different HRTs monitored as presented in Table IV. The highest daily biogas was obtained in 15 d HRT with an average value of 350.8 mL/g-VS. However, the biogas composition remained constant throughout the HRT trial (at about 50–65% of CH_4 and 50–35% of CO_2). Increase in HRT increased CH_4 yield probably due to the sufficient contact time to allow for the substrate degradation by microbial population. However, the values here obtained were much lower than those reported by other authors on the AD of SHW [28, 21]

The addition of the SPM as co-substrate to SHWW AD enhanced the biogas yield by 69.1% at the optimum HRT of 15 d. Moreover, the addition of carbon-rich co-substrate (SPM) to nitrogenous substrate SHWW) could have led to more suitable C/N ratio. Furthermore, an increase in the OLR resulted in a decrease in biogas production (Table IV). This is attributed to the fact that increasing OLR reduces contact between the substrate and methanogens. However, such a problem might be reduced by adequate mixing [23]. Biogas production is influenced by the production of inhibitory compounds during the digitation process [10].

For instance, reactors operating at 5 d HRT exhibited the lowest biogas potential of about 255.36 mL/g-VS, signifying possible inhibition that hindered complete degradation of the organic material. This could also be due to the highest ammonium nitrogen (NH_4^+-N) concentration (8333 mg/l) as shown in Table IV. Progressive increase in HRT, lead to sufficient contact time with microorganisms. As a result, methane production is improved due to balanced C: N ratio that in turn cause a reduction in NH_4^+-N concentration. Furthermore, it could be attributed to either nutrient deficiency or insufficient contact between bacteria substrate due to lack of proper mixing; consequently, inhibiting methanogenesis process. Similarly, Hejnfelt & Angelidaki [29] reported HRT of less than 3 days to be very low in completely mixed systems as it could cause washout of active biomass, as methanogens are assumed to have longer generation times of several days. Therefore, in this work, the ACoD of SPM and SHWW under semi-continuous operation presented possible inhibitory problems. This is similar to what Cuetos et al. [28] reported during the ACoD of maize and poultry blood.

Volatile solids' (VS) reduction is an indirect measurement of organic matter utilization in the AD process and used to monitor digester's performance. During the AD process, VS are degraded to a certain extent and converted into biogas. The degree of stabilization is often expressed as the percent reduction in VS [23]. The average VS reduction of reactors at 5 d HRT, 5 d HRT and 5 d HRT was 45.4%, 53.9%, and 62.4%, respectively. This VS reduction was found almost consistent with the biogas production rate. Again 15 d HRT obtained maximum VS reduction of about 62.4%, which clearly shows a good condition of the AD process indicating that the decomposition of biodegradable organic matter was fast proceeding. VS conversion to biogas in the other reactors operating at 10 and 5 d HRT, respectively, was hindered probably due to the generation of inhibitory substances. Nonetheless, the increased VS removal efficiency in overall experiment indicates an exponential growth of bacteria which in turn yielded favourable results. Longer HRT particularly in high lipid wastewater, promote scum reduction, forming the potential of a system and better VS reduction [15]. Therefore, in present study, performance of reactor at HRT of 15 d was found to be very much efficient at retaining biomass.

C. *Process Stability of the Anaerobic Digestion of SHWW*

AD process stability depends on the buffering capacity of the digester contents. Alkalinity is an important parameter that measures bioreactors buffering capability to neutralize the increased acid from the acidogenesis. High alkalinity values indicates that the methanogenic digesters have a greater capacity to resist pH changes [30]. pH is an important parameter in the AD process. Consequently, in the current investigation, the pH level of each digester was stabilized in between 7.82 and 8.09 that reflects a stable system. A massive pH change was not experienced throughout the experiment due to the good buffering capacity achieved through codigestion.

According to previous studies, a neutral pH of around 6.7–7.6 is preferred for the effective operation of an anaerobic reactor [22]. In this study, the pH of the reactors remained within the working rage (7.82–8.09) for all HRTs. Also, NH_4^+-N is very toxic to methanogenic bacteria and inhibits their growth when its concentration is within the inhibition level [6, 31]. However, in this study, NH_4^+-N values were much lower than toxic limits reported in literature for digestion of nitrogenous wastes [28]. The NH_4^+-N level in 15 d HRT of 5334mg/l increased to 6763 mg/l at10 d HRT, and finally to 8833 mg/l at 5 d HRT. Moreover, reactors which operated at shorter HRT of 5 d experienced a high level of NH_4^+-N levels attributed to the degradation of the nitrogenous organics in the SHWW. Consequently, a reduced rate of biogas production in reactors operating at 10 d HRT and 5 d HRT was observed due to the accumulation of a relatively high level of NH_4^+-N concentration (Table IV).

TABLE IV

PERFORMANCE OF CSTRS AT DIFFERENT HRTS

	Parameter	Unit	Control		15 HRT	10 HRT	5 HRT
Duration		Days			1 to 45	46 to 75	76 to 90
OLR		mL/d			53.3	80	160
				Influent	Effluent	Effluent	Effluent
Removal efficiency	pH		7.79±0.0	7.34±0.0	8.09±0.	7.96±0.1	7.82±0.1
	TS	%	72.18±0.1	69.31±0.1	40.96±2.3	56.47±1.4	62.23±4.5
	VS	%	69.91±0.2	42.3±0.3	15.9±4.1	19.50±3.2	23.11±6.2
	COD	g/L	15±0	32.96±0	8.64±2.4	10.24±4.1	16.64±3
	NH_4^+-N	mg/L	6162±2.3	9387±3.1	5334±2.0	6763±1.3	8333±1.1
Gas Production	CH_4 Production	mL/g-VS	108.4±3.0		350.8±3.3	305.76±5.1	255.36±4.0

IV. CONCLUSION

The current study aims to ascertain the effect of the inclusion of SPM as a co-substrate in AcoD with SHWW at different HRTs. The research findings revealed that codigestion improved biogas yield by 69.1%, VS removal by 62.4%, and remarkable reduction in NH_4^+-N concentration at an optimal 15-day HRT. The current findings clearly support the relevance of ACoD. The possibility of SPM effectively counteracting the possible NH_3 inhibition in SHWW is one implication of this. The current study looked into the mesophilic ACoD of SHWW with SPM in a continuous feeding mode. The lack of automated feeding, agitation, and gas measuring mechanisms, however, limits the study. Despite its limitations, the study sheds new light on the HRT required for optimal bioenergy recovery from the respective agrowastes. Finally, repeating the study under thermophilic conditions to investigate the stability of the AD process in practice would be an interesting area for future research. As a result, there is a clear need for policies to encourage commercial production and distribution of bioCH4.

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