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Optimization of Operating Parameters for Two-Phase Anaerobic Digestion Treating Slaughterhouse Wastewater for Biogas Production: Focus on Hydrolytic–Acidogenic Phase

Dejene Tsegaye *, Mohammed Mazharuddin Khan and Seyoum Leta

Center for Environmental Science, College of Natural and Computational Science, Addis Ababa University, Addis Ababa P.O. Box 1176, Ethiopia
* Correspondence: dejene.tsegaye@aau.edu.et

Abstract: In a two-phase anaerobic digestion process, enhanced biogas production and organic pollutant removal depend on the stability and performance of the hydrolytic–acidogenic and methanogenic phases. Additionally, the hydrolytic–acidogenic phase is a rate-limiting step, which calls for the further optimization of operating parameters. The objective of this study was to optimize the operating parameters of the hydrolytic–acidogenic reactor (HR) in the two-phase anaerobic digestion treating slaughterhouse wastewater. The experiment was carried using bench-scale sequential bioreactors. The hydrolytic–acidogenic reactor operating parameters were optimized for six different hydraulic retention times (HRTs) (6–1 day) and organic loading rates (OLRs) (894.41 ± 32.56–5366.43 ± 83.80 mg COD/L*day). The degree of hydrolysis and acidification were mainly influenced by lower HRT (higher OLR), and the highest values of hydrolysis and acidification were 63.92% and 53.26% at an HRT of 3 days, respectively. The findings indicated that, at steady state, the concentrations of soluble chemical oxygen demand (SCOD) and total volatile fatty acids (TVFAs) decrease as HRT decreases and OLR increases from HRTs of 3 to 1 day and 894.41–1788.81 mg COD/L*day, respectively, and increase as the HRT decreases from 6 to 4 days. The concentration of NH_4^+-N ranges from 278.67 to 369.46 mg/L, which is not in the range that disturbs the performance and stability of the hydrolytic acidogenic reactor. It was concluded that an HRT of 3 days and an ORL of 1788.81 mg COD/L*day were selected as optimal operating conditions for the high performance and stability of the two-phase anaerobic digestion of slaughterhouse wastewater in the hydrolytic–acidogenic reactor at a mesophilic temperature. The findings of this study can be applicable for other agro-process industry wastewater types with similar characteristics and biowaste for value addition and sustainable biowaste management and safe discharge.

Keywords: organic loading rate; optimal condition; hydraulic-acidogenic phase; slaughterhouse wastewater; two-phase anaerobic digestion

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1. Introduction

The diverse ecosystems that support human life have deteriorated recently due to industrialization, urbanization, and population growth throughout the world. The mismanagement of agro-industrial wastewater and overuse of water create maximum stress on freshwater bodies such as rivers, lakes (lotic and lentic), seas, and oceans, as well as a decrease in the quality of aquatic ecosystem services [1–4].

Agro-processing industries such as slaughterhouses produce typical wastewater with high values of biochemical oxygen demand (BOD), chemical oxygen demand (COD), biological organic nutrients (nitrogen and phosphate), which are insoluble, slowly biodegradable solids, pathogenic and non-pathogenic viruses and bacteria, and parasite eggs [5–7]. Furthermore, it is high in protein and quickly putrefies, causing an environmental pollution problem. This revealed that slaughterhouses are among the most environmentally polluting agro-processing industries [4,8–11]. The sources of the wastewater are mainly from different activities such as holding and washing animals, bleeding out, skinning, carcass washing, cutting and boning, and the cleaning of rooms. Moreover, it may also contain particles of skin and meat, rumen fluid content, urine, manure, blood, and other pollutants that originate from floor washing. Due to this, it is rich in both soluble and insoluble organic compounds and comprises large quantities of putrefactive and bulky sludge that requires special handling or treatment [8]. As a result, the major environmental issue associated with slaughterhouse wastewater is the large amount of organic matter or suspended solids and liquid waste released into the environment, with its complex nature and pungent odor [9,12,13].

Slaughterhouses and the meat processing sector are growing in Ethiopia along with the rest of the globe; however, they are still far smaller than they ought to be given the country's resource potential [14]. Recently, there are more than thirty-five export and two hundred and ninety-six municipal slaughterhouses [15–20]. Between 2015 and 2020, the amount or the volume of wastewater generated by the meat processing industry (slaughterhouses) in Ethiopia and around the world was 95.31 million and 41.53 billion m^3, respectively (Figure 1), with predicted steady growth at the global level, necessitating treatment facilities for a sustainable and safe discharge. The main driving forces for the increase in wastewater from meat processing industries or slaughterhouses are urbanization, meat demand, economic growth, and the transition of the economy from an agricultural-based to an agro-industrial development strategy (especially in developing countries). Moreover, the transition from an agricultural-based economy to an agro-industrial development strategy in Ethiopia additionally contributes to an increase in industrial growth and, consequently, in the wastewater production. Moreover, recent research findings have indicated that the annual global wastewater production reached about 360–380 $\times$ 10^9 m^3 and will increase at a rate of 24% and 51% in the years 2030 and 2050, respectively [15,19–21]. All this indicates a market opportunity of wastewater treatment from slaughterhouse-processing industries, in particular agro-processing industries, primarily in developing countries such as Ethiopia.

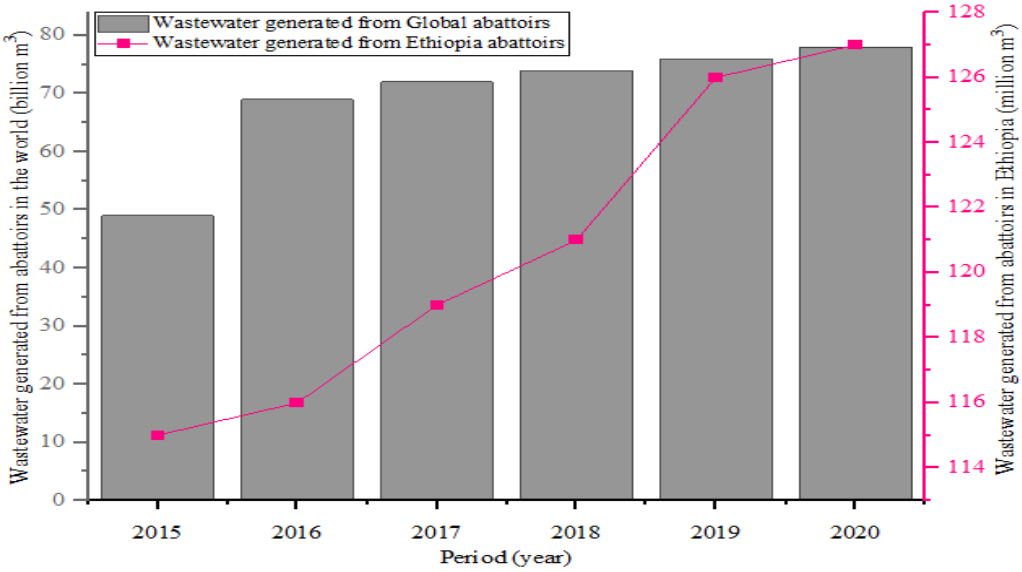

Figure 1. Volume of wastewater from slaughterhouses in Ethiopia and in the world (2015–2020).

A previous report showed that 90% of agro-industries in Ethiopia discharge their effluent directly to nearby water bodies or the environment, thereby putting these ecosystems at risk [22]. This affects the biological diversity of aquatic ecosystems and disrupts the fundamental web of our life support systems, on which a wide range of sectors, from urban development to food production and industry, depend. Furthermore, wastewater treatment facilities at agro-industries in Ethiopia can be taken as nonexistent; moreover, if there are any, they are mismanaged, i.e., the facilities are there, but the functionality, efficiency, and sustainability are questionable [23].

Anaerobic digestion (AD) represents an efficient treatment alternative for wastewater with high biodegradable organics, sufficient alkalinity, and adequate phosphorous, nitrogen, and micronutrient concentrations for bacterial growth with no toxic compounds such as those from a slaughterhouse [5,11,24]. Anaerobic digestion is a sequence of metabolic steps involving consortiums of several microbial populations to form a complex metabolic interaction network resulting in the conversion of organic matter into methane (CH_4), carbon dioxide (CO_2), and other trace compounds [25]. During the anaerobic digestion process, complex organics such as proteins, lipids, and polysaccharides are hydrolyzed to intermediates such as amino acids, fatty acids, and sugars by enzymes [26]. The above intermediates are then degraded further to volatile fatty acids (VFAs) using acidogens. The efficiency of anaerobic digestion is highly dependent on the characteristics of the waste, the reactor configuration, and other operational parameters [27,28]. In traditional single-stage anaerobic digestion practice, all the hydrolysis, acidogenesis, acetogenesis, and methanogenesis take place in the same or one reactor. In such reactors, acidogens grow faster and are less sensitive to pH variations than methanogens or acetogens, resulting in the accumulation of volatile fatty acids, a lowering of pH, and the suppression of methanogens, resulting in reactor failure or instability [29].

Two-phase separation prevents VFA accumulation, pH drops, and the separation of acidogenesis and methanogenesis bacteria, allowing the feedstock to degrade more efficiently [30–34]. In general, the separation of hydrolytic–acidogenic and methanogenesis phases in anaerobic digestion processes results in shorter retention times, better stability, and higher biogas or methane production rates than single-phase anaerobic digestion processes [35]. Furthermore, phase separation allows for the optimization of the organic loading rate (OLR) and hydraulic retention time (HRT) based on the needs of each phase's microbial consortiums and can prevent the imbalance caused by groups of anaerobic bacteria in single-phase reactors [29,36]. In the two-phase system itself, the hydrolysis reactor is characterized by the formation of excess volatile fatty acids and ammonia that eventually result in system instability [37]. Furthermore, the hydrolytic–acidogenic phase is identified as a rate-limiting step, implying that the operating parameters should be optimized [38]. Recently, the hydrolysis–acidogenesis phase as well as overall process performance enhancement were studied by scholars. As a result, the following major techniques have been used to improve the performance of the hydrolysis–acidogenesis phase: varying temperature and HRT [39], changing reactor temperature, pH, and HRT [40], solid content and HRT [41], reaction time variation and pH optimization using the response surface method [42], micro-aeration [43], the introduction of potential co-substrates such as sugar press mud, dairy wastewater, and municipal fruit and vegetable wastes [44–46], semi-continuous feeding mode and gas transfer [47], and phase separation [48] for biogas production using a two-phase system. Though systematic operational parameter optimization of the anaerobic digestion process has been studied in the pertinent literature, studies on the hydrolytic–acidogenic phase in two-phase anaerobic digestion fed with slaughterhouse wastewater alone remains rare. Therefore, to maximize the gas yield, avoid reactor instability, and achieve the best degradation of the substrate, the optimization of the hydrolysis reactor operating parameters in terms of OLR and HRT for optimum total volatile fatty acid (TVFA), soluble chemical oxygen demand (SCOD), and ammonia concentration is crucial.

Therefore, this study was envisioned to optimize the operating parameters of a hydrolytic–acidogenic reactor (HR) for the two-stage anaerobic digestion of slaughterhouse wastewater at mesophilic temperatures.

2. Materials and Methods

2.1. Source of Feedstock

The slaughterhouse wastewater was taken from an organic export abattoir found in Modjo, Ethiopia. Eight hundred to one thousand two hundred sheep and goats (each) were slaughtered at this slaughterhouse per day, and a total of 400-L of water per sheep or goat was used. Almost an equivalent amount of wastewater was discharged into the nearby Modjo River, especially increasing the pollution load on Koka Lake, the destination of the Modjo River. The composite slaughterhouse wastewater was collected in an acidified 20-L polyethylene plastic jerrycan and transported to the Center for Environmental Science Laboratory, where it was stored at 4 °C until applied to the reactor. The experiment was carried out using a bench-scale reactor at the College of Natural Sciences of Addis Ababa University.

Rumen contents (cud) from the same slaughterhouse were used as the source of the inoculums for the purpose of the present study. A 1:1 ratio of the inoculums/cud on a volume basis was used as a source of the crucial microbes for the feedstock (wastewater) to kick off the hydrolysis/acidogenesis system.

2.2. Experimental Setup (Digester Design) of the Laboratory Scale Digester

The optimization of the hydrolytic–acidogenic phase was carried out using a 40-L galvanized metal reactor (digester) with a working volume of 36 L and a 4-L gas-space. The reactor was sealed with a gasket maker to create an anaerobic condition and tensioning bolts to support the sealing. The temperature of the reactor was maintained at 37.5 °C using hot water circulated from the thermostat water bath (cu-420, Hangzhou West Tune Trading Co., Ltd, Zhejiang, China). A clean water pump (inGCO Inc., Zhejiang, China) was used to circulate the hot water to maintain the reactor temperature at 37.5 °C. The pipes used for hot water circulation were composed of stainless steel pipes inside the reactor and $\frac{3}{4}$ PPR pipe for the extension of the pipe outside the reactor.

The reactor had wastewater feeding and discharging, level regulation, and sludge discharging ports with a control valve at each port. Figure 2 shows the detailed laboratory scale experimental system setup.

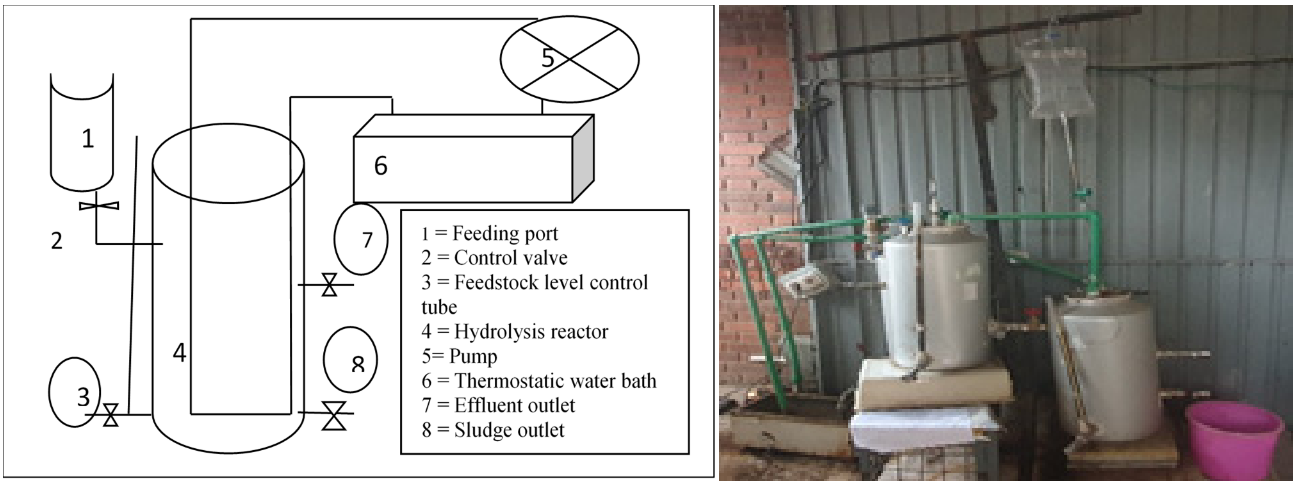

Figure 2. Schematic diagram (**left**) and photo (**right**) of the bench-scale hydrolytic–acidogenic reactor setup.

2.3. Operating Conditions

For the optimization of the operating parameters of HRT and OLR in the hydrolytic–acidogenic phase, a 40-L total volume reactor was established at laboratory scale. To maintain an anaerobic condition in the HR, inert gas (nitrogen gas) was sparged before starting the experiment. The detailed operating condition of the HR is presented in Table 1.

To initiate the system, the reactor was fed with a 1:1 ratio of inoculums to slaughterhouse wastewater on a volume basis. The system was acclimatized by gradually increasing the amount of wastewater fed per day until the desired working volume was reached. As shown in Table 1, the optimal OLR and HRT conditions for the hydrolytic–acidogenic process were determined by comparing process performance at six different HRTs (6, 5, 4, 3, 2, and 1 day) at a mesophilic temperature of 37.5 °C using the methods described in [45,49]. The short HRT and the pH did not allow the activity of the methanogens. The methanogens bacteria dominance indicator biogas production was also followed but no significant biogas production was observed. The reactor efficiency parameters considered during the optimization were TCOD, SCOD, TVFA (the key parameters as it is the main acid phase product reflecting the organic matter that has been hydrolyzed), and NH_4^+-N (an inhabitant of the reactor or system).

Table 1. Slaughterhouse wastewater characteristics and operating condition of hydrolytic–acidogenic reactor at different HRT/OLR.

Characteristics of Slaughterhouse Wastewater		Operating Condition		
Parameter (Unit)	**Mean Value ± SD**	**HRT (Day)**	**OLR (mg COD/L. Day)**	**Flow Rate (L/Day)**
pH	7.06 ± 0.30	6	894.41	6
Salinity (ppm)	1208.98 ± 43.48	5	1073.27	7.2
EC (µS/cm)	1346.00 ± 46.88	4	1341.61	9
Resistivity (Ω)	458.46 ± 16.75	3	1788.81	12
TDS (ppm)	1170.74 ± 40.84	2	2683.22	18
ORP (mV)	−80.50 ± 18.13	1	5366.43	36
TVFA (mg/L)	816.60 ± 38.67			
TCOD (mg/L)	5366.43 ± 83.80			
SCOD (mg/L)	4842.21 ± 83.81			
NH_4^+-N (mg/L)	338.40 ± 58.13			

At each OLR, the values of the parameters under study (TVFA, NH_4^+-N, TCOD, and SCOD) were evaluated at steady state conditions. The steady state conditions were assumed to be achieved when the concentrations of the parameters under study were within 10% variation, and ten (10) consecutive readings were taken for each parameter after realization of the steady state conditions.

2.4. Degree of Acidification

Degree of acidification is a parameter used to measure the degree of success of acid fermentation, which represents the amount of solubilized matter converted to VFAs. It was quantified using Equation (1).

$$DA\ (\%) = \frac{Sf}{Si} \times 100 \quad (1)$$

where *DA* represents the degree of acidification, *Si* is the initial feedstock concentration expressed in mg/L of COD, and *Sf* is the net VFA produced (final-initial), expressed as the theoretical equivalent of the COD concentration in mg/L. The COD equivalent of each VFA is acetic acid (1.066), butyric acid (1.816), propionic acid (1.512), valeric acid (2.036), and caproic acid (2.204) [50].

The DH defines the degree of solubilization of organic matter and calculated according to [51].

$$DH\ (\%) = \frac{Ss}{Si} \times 100 \quad (2)$$

where *Ss* is the effluent SCOD measured and *Si* represents the initial TCOD (tCODi).

2.5. Analytical Methods

The physico-chemical characteristics of the raw slaughterhouse wastewater, effluent from HR, were analyzed for the various parameters as follows: chemical oxygen demand (COD), total Kjeldahl nitrogen (TN), and ammonium nitrogen (NH_4^+-N) were characterized using the standard method as described by the American Public Health Association (APHA) (2012). Total alkalinity and total volatile fatty acid (TVFA) were analyzed using the titration methods as described by APHA (2012). The parameters, such as oxidation reduction potential (ORP) and pH, were analyzed using a pH meter (JENWAY, Manchester, UK). Resistivity, salinity, electrical conductivity (EC), and total dissolved solids (TDS) were analyzed using a multimeter (EUTECH Instruments, Madrid, Spain).

2.6. Data Analyses

The data generated from the analysis during the study were entered into the MS Excel spreadsheet based on the objectives set for further statistical analysis. The statistical analysis for mean, standard deviation, correlation, and one-way analysis of variance (ANOVA) performed at a 5% level of significance tracked by a post hoc test was also performed using the Excel statistical package to compare the mean difference of the parameters at HRTs of 6, 5, 4, 3, 2, and 1 days, and the Origin 2022 software was used to draw graphs. Prior to the one-way ANOVA analysis for the mean comparison, the two basic assumptions of ANOVA, normality, and homogeneity of variances were considered and checked using the real dataset by Shapiro–Wilk or using histogram and Levene's tests, respectively, at a 5% level of significance. All the sample analysis values for the parameters under study were taken in at least triplicate to ensure the reproducibility of the experiment.

3. Results and Discussions

3.1. Characteristics of Feedstock (Organic Export Abattoir Wastewater)

The main characteristics of the organic export abattoir effluent are presented in Table 1. As indicated in Table 1, the COD value (mean $\pm$ SD) of slaughterhouse wastewater was 5366.43 $\pm$ 83.80 mg/L, of which about 60–90.24% (4842.21 $\pm$ 83.81 mg/L) was in soluble form and the other in suspended particulate matter. The BOD/COD ratio of the wastewater of 0.46 also indicated the biodegradability of the wastewater. The average concentrations of TVFA and NH_4^+-N were 816.60 $\pm$ 38.67 and 338.40 $\pm$ 58.13 mg/L, respectively. This high organic matter content in terms of COD is mainly due to the grease and fat, blood, manure, and indigested food content in the slaughterhouse wastewater, which was also the case in the previous research [52,53]. Mulu and Ayenew [10], Ren et al. [54], and Worku and Leta [1] reported the COD values of the slaughterhouse as 4752.66 $\pm$ 1156.27 mg/L and 6942.59 $\pm$ 152.98–7079.69 $\pm$ 226.89 mg/L, respectively. Furthermore, slaughterhouses and meat-processing facilities produce a huge volume of wastewater with organic matter measured as COD between 500 and 15,900 mg/L [52], TVFA between 435 and 1197 mg/L [55], and NH_4^+-N between 186 and 6407 mg/L [1,46]. This high concentration of organic matter necessitates a greater amount of oxygen to be oxidized into carbon dioxide and water, which may contribute to an increase in the COD and BOD of the receiving water body [56].

The average pH value of the organic export abattoir effluent was nearly in the neutral pH range (6.80–7.39). The temperature and ORP ranged from 28.9 to 30.5 °C and −62.50 to −101.10 mV, respectively. The EC, TDS, salinity, and resistivity of the slaughterhouse wastewater used as feedstock during the study ranged between 1345 and 1970 ppm, 1160 and 1688 ppm, 1210 and 1628 ppm, and 290.90 and 425.00 Ω, respectively, which can be attributed to the NH_4^+-N, SO_4^{-2}, and NO_3^--N ions dissolved in the SHWW, which is also consistent with the previous finding [11].

3.2. Effect of HRT/OLR on Reactor Stability and Performance Indicator Parameters

The digestion of the anaerobic process begins with the microbial hydrolysis of the feedstock material to break down insoluble polymers such as carbohydrates, proteins, and fats and make them available for the microorganisms. Once absorbed, these insoluble organic polymers undergo microbial degradation, which results in the production of soluble sugars. Under optimal waste treatment conditions, anaerobic digestion is much more susceptible than aerobic digestion for the same degree of factor devotion, according to Chen et al. [57]; moreover, under optimal waste treatment conditions, anaerobic digestion is much more susceptible than aerobic digestion for the same degree of factor devotion.

As a result, the current study evaluated the hydrolysis phase reactor stability parameters based on the breakdown of large molecules into the accumulation of intermediates: VFA, alkalinity, SCOD, and NH_4^+-N. Table 2 shows the average stability and performance indicator parameters' concentration or values of hydrolytic–acidogenic reactor.

Table 2. The average stability and performance indicator parameters' concentration of hydrolytic–acidogenic reactor at steady state for different HRT and OLR.

	HRT (Day)					
Parameters	**6**	**5**	**4**	**3**	**2**	**1**
pH	6.81 ± 0.14	6.34 ± 0.17	6.00 ± 0.18	5.78 ± 0.32	5.77 ± 0.25	5.60 ± 0.27
Salinity (ppm)	1784.67 ± 12.26	1784.27 ± 71.04	1785.27 ± 18.54	1650.40 ± 12.22	1538.33 ± 16.04	1710.00 ± 16.65
EC (µS/cm)	1934.47 ± 13.43	1964.80 ± 80.26	1950.20 ± 19.85	1809.73 ± 12.07	1674.07 ± 17.68	1835.87 ± 14.18
Resistivity (Ω)	292.23 ± 16.00	289.43 ± 12.49	296.43 ± 29.83	318.01 ± 22.49	341.66 ± 41.01	313.17 ± 32.41
TDS (ppm)	1697.27 ±11.48	1726.93 ± 53.74	1702.00 ± 17.20	1576.47 ± 11.60	1469.80 ± 17.75	1602.33 ± 13.24
ORP (mV)	81.21 ± 3.36	82.58 ± 3.49	81.69 ± 5.25	82.25 ± 6.54	78.59 ± 3.21	79.03 ± 3.15
TVFA (mg/L)	1084.83 ± 14.37	1006.42 ± 30.35	1155.92 ± 16.20	1176.50 ± 81.66	1006.42 ± 30.35	996.75 ± 14.60
TCOD (mg/L)	4924.47 ± 25.79	4799.33 ± 37.49	4913.67 ± 22.79	4934.6 ± 25.24	4721.07 ± 67.35	4821.73 ± 30.37
SCOD (mg/L)	2324.80 ± 25.16	2359.00 ± 40.79	2483.73 ± 47.72	3430.2 ± 80.44	3106.87 ± 72.65	2084.4 ± 71.00
NH_4^+-N (mg/L)	278.67 ± 47.25	281.20 ± 8.79	319.08 ± 40.21	369.46 ± 11.28	369.33 ± 51.75	346.42 ± 40.67

3.2.1. The Effect of OLR/HRT on Salinity, EC, TDS, and Resistivity

Salinity and TDS are the buffering capacity enhancers in the anaerobic digestion system. The average values are shown in Table 2, and the variations are indicated in Figures 3–5 for the parameters under study at different HRTs. The average (mean ± SD) salinity, TDS, EC, and resistivity of the HR ranged from 1785.27 ± 18.54 mg/L at HRT of 4 day to 1538.33 ± 16.04 mg/L HRT of 2 day; 1726.93 ± 53.74 mg/L HRT of 5 day to 1469.80 ± 17.75 mg/L at HRT of 2 day; 1964.80 ± 80.26 µS/cm at HRT of 5 day to 1674.07 ± 174.68 µS/cm at HRT of 2 day; and 341.66 ± 41.01 Ω at HRT of 2 day to 289.43 ± 12.49 Ω at HRT of 5 day, respectively. The correlation statistical analysis of pH, ORP, EC, TDS, salinity, and resistivity was also computed. Table 3 indicates the correlation matrix of some optimized parameters in the present study. Accordingly, pH, ORP, salinity, EC, and TDS have strong positive and negative correlations with each other and resistivity, respectively (Table 3).

Table 3. Correlation matrix of some parameters for hydrolysis–acidogenesis reactor.

Parameters	**pH**	**ORP**	**EC**	**TDS**	**Salinity**	**Resistivity**
pH	1					
ORP	0.99	1				
EC	0.9	0.94	1			
TDS	0.86	0.91	1	1		
Salinity	0.9	0.93	0.99	0.98	1	
Resistivity	−0.87	−0.9	−1	−0.99	−0.98	1

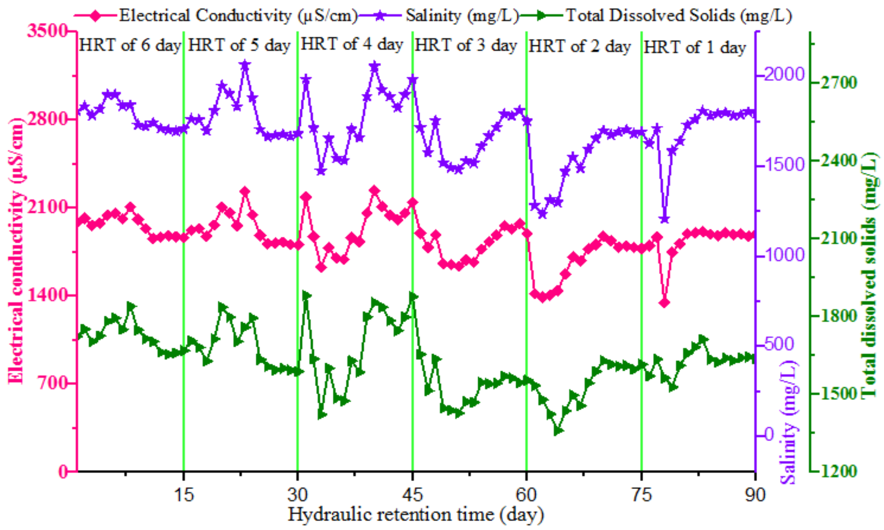

Figure 3. Variation in TDS, salinity, and EC of hydrolytic–acidogenic reactor at different HRT.

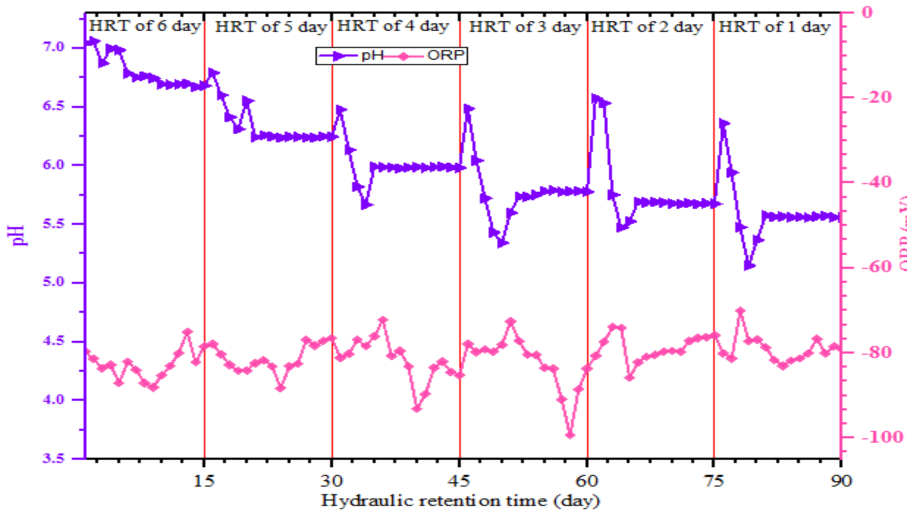

Figure 4. Variations in pH and ORP of hydrolytic–acidogenic reactor at different HRTs.

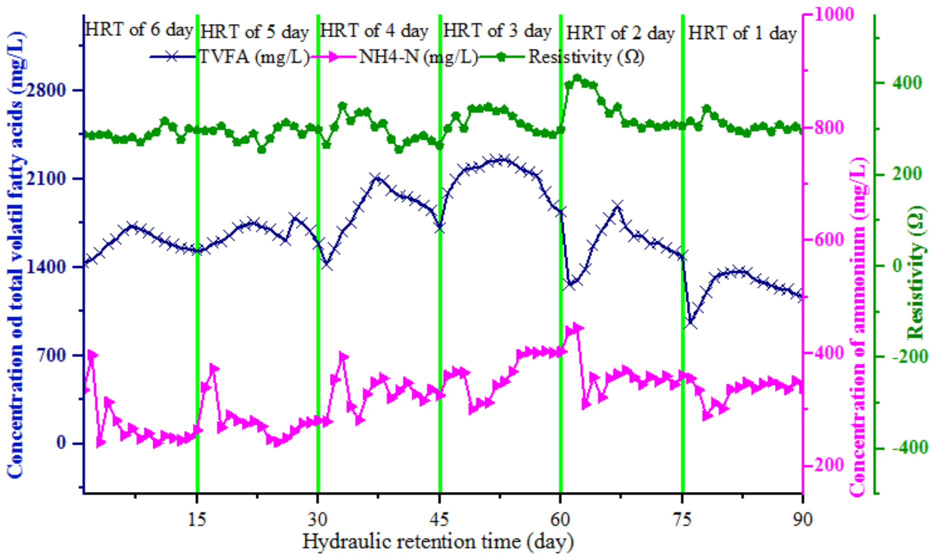

Figure 5. Variation of resistivity, NH_4^+-N, and TVFA of hydrolytic–acidogenic reactor at different HRTs.

3.2.2. The Effect of OLR/HRT on pH

The average values and trends in pH variation of the HR during the study period at different OLRs and HRTs are shown in Table 2 and Figure 4, respectively. As indicated in Table 2 and Figure 4, the pH was influenced by the reactor operational conditions (OLR and HRT). Accordingly, the average pH values decrease as HRT decreases from 6–1 day at the respective OLR, and the variation in mean pH was significant ($p < 0.05$); however, there was no significant difference (p-value = 0.84) between the mean pH of 2 and 3 days of HRT. Furthermore, during the HR optimization process, an overall pH range of 7.06–5.15 was observed, with OLR and HRT ranging from 894.41 to 5366.43 mg COD/L*day and 6–1 day, respectively. Specifically, the pH ranges for HRT at 6, 5, 4, 3, 2, and 1 day were 7.06–6.67, 6.79–6.24, 6.49–5.68, 6.49–5.34, 6.57–5.47, and 6.36–5.15, respectively. Furthermore, as indicated in Figure 4, pH values above six were recorded during the startup of the reaction period, decreased, and gradually increased until the system attained a relative steady state for all the OLRs and corresponding HRTs. As was revealed from Table 2, the mean pH values at an HRT of 5–1 were in the range of 6.34 ± 0.17 to 6.00 ± 0.27, which is within the optimum range (5.2–6.3) for the hydrolytic–acidogenic consortium of bacteria except for the HRT of 6 days [58]. A pH range of 5.25–6.11 in a hydrolytic–acidogenic reactor with high TVFA and SCOD production was reported [59,60], which is consistent with the findings of this study; moreover, even an HR operating at a constant pH value of 5.7 can support the maintenance and growth of hydrolytic–acidogenic bacteria [61], which will enhance the production of TVFA. Moreover, in the hydrolytic–acidogenic phase, pH values between 5.0 and 6.0 are have also been reported as the optimal range, though it depends on the target products and feedstock [62,63].

The decrease in pH during the start or acidification phase was likely due to the VFA intermediates, lactate, and ethanol, produced from the degradation of the organic matter in the slaughterhouse wastewater used as a feedstock. Alkaya and Demirer [64] and Subasi and Demirar [65] also reported similar trends in pH during the acidification of sugar beet processing wastes. Furthermore, Berhe and Leta [45] reported pH values ranging from 7.98 to 4.90 during the optimization of HR operating conditions for the anaerobic co-digestion of tannery and dairy wastewater at different HRTs and OLRs.

ORP is a measure used for intracellular metabolism controlled with redox balance and electron transfer, hence expressing the condition of biochemical reactions. The optimal ORP value for methane-reducing bacteria is above −230 mV, and an ORP value above −280 mV is inhibitory to sulfate-reducing bacteria [66]. The optimal ORP for a hydrolytic–acidogenic reactor, which is a function of pH, is between −75 mV and −250 mV [67]. This study result shows that the ORP values are within a range that is suitable for optimum methane production at all HRTs (Table 2).

3.2.3. Effect of HRT/OLR on TVFA Production

In anaerobic digestion with separate hydrolysis and methanogenesis phases, the VFA concentration in the HR is the main indicator of system stability. The average TVFA concentrations at steady state conditions for the HR during all HRTs are shown in Table 2. The average concentrations of TVFA produced during the optimization of the system were 1084.83 ± 14.37, 1006.42 ± 30.35, 1155.92 ± 16.20, 1176.50 ± 81.66, 1006.42 ± 30.35, and 996.75 ± 14.60 at HRTs of 6, 5, 4, 3, 2, and 1 days; OLRs of 894.41, 1073.27, 1341.61, 1788.81, 2683.22, and 5366.43 mg/L of COD, respectively. As shown in Figure 5 and Table 2, the OLRs and HRTs have an effect on the TVFA concentration in the reactor. The TVFA concentration increased with an increase in OLR from 894.4 to 1788.81 mg of COD/L*day; moreover, as HRT decreased from 6 to 3 days, the increased OLR resulted in the fast production of a high-quality intermediate product such as TFVA via a consortium of bacteria in the HR. This may be attributed to the large amount of biodegradable organic matter in the slaughterhouse wastewater. The decrease in TVFA concentration as HRT decreases from 3 to 1 days or OLR increases from 1788.81 to 5366.43 mg COD/L*day may be attributed to the washout of consortiums of bacteria in the HR, which is also consistent with the previous report [68].

The trends of the VFA produced at all HRTs and corresponding OLRs during the experimental period are presented in Figure 5. As was indicated in Figure 6, the TVFA concentration during the reaction course shows an increasing trend at startup and becomes nearly stable after the 6th day of the reaction period for each HRT under study. This may be due to the fact that microorganism consortiums usually take time to start their metabolic activity before becoming fully efficient.

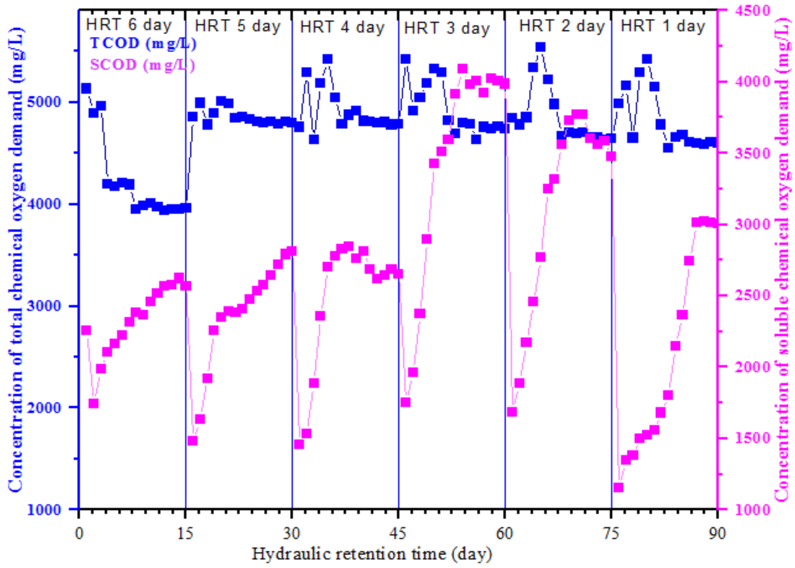

Figure 6. Variation trends of TCOD and SCOD of hydrolytic–acidogenic reactor at different HRTs.

Berhe and Leta [45], Demirel et al. [69], and Lim et al. [38] also reported that the concentration of TVFA decreased as HRT increased, but that further increases in HRT would not increase the production of TVFA. Moreover, for the HR operating at optimum condition, TVFA values ranging from 2800 to 4453 mg/L as $CaCO_3$ were reported during the two-phase anaerobic co-digestion of tannery and dairy wastewater [45], slaughterhouse waste, and municipal fruit and vegetable waste [44]. The acidic pH (5.57) of the reactor favors the high TVFA production in the HR reactor in the two-phase AD process than alkaline conditions [70–72]. The high TVFA produced in HR is a crucial precursor for methane production as well as for organic pollutant removal, and it speeds up the methanogenesis phase because methanogens use the TVFA immediately, as has been noted [40]. In the two-phase AD process, the high TVFAs produced in the HR are the main methanogenic consortium of the bacteria carbon source, and the biogas produced [44] at the same time is an indicator of the better performance of the HR. It was also reported that increasing the temperature increased the hydrolysis rate constant, with the maximum found at 37.5 ± 0.3 °C, while increasing the temperature to 45 °C decreased the constant rate [49,73].

3.2.4. The TCOD and SCOD Production at Different HRT

TCOD and SCOD were also among the parameters taken into consideration in the present study to optimize the performance of the HR. As presented in Table 2, the average HR effluent TCOD was 4924.47 ± 25.79, 4799.33 ± 37.49, 4913.67 ± 22.79, 4934.60 ± 25.24, 4721.07 ± 67.35, and 4821.73 ± 30.38 at HRTs of 6, 5, 4, 3, 2, and 1 days and OLRs of 894.41, 1073.27, 1341.61, 1788.81, 2683.22, and 5366.43 mg/L of COD, respectively. As presented in Table 2, the average SCOD was 2324.80 ± 25.16, 2359.00 ± 40.79, 2483.73 ± 47.72, 3430.20 ± 80.44, 3106.87 ± 72.65, and 2084.40 ± 71.00 at HRTs of 6, 5, 4, 3, 2, and 1 and OLRs of 894.41, 1073.27, 1341.61, 1788.81, 2683.22, and 5366.43 mg/L of COD, respectively. The ANOVA followed by a post hoc test revealed that the variation in mean SCOD was significant at $p < 0.05$, except for HRTs of 5 and 6 days ($p = 0.33$). The mean variation of the TCOD concentration was insignificant between each HRT and OLR at $p < 0.05$, statistically indicating that it was not an influential factor of the HR stability and performance at each HRT or OLR.

The trend of TCOD and SCOD concentrations during the experimental period at each HRT and OLR is presented in Figure 6. The TCOD concentrations for all HRT fluctuated at the start of the reaction period and stabilized after the 7th day (Figure 6). As depicted in Figure 6, at each OLR or HRT, the SCOD concentration shows a steady increase with reaction time. This may be due to the fact that the microorganism consortiums had acclimatized and were acting at their optimal condition; increasing the fermentation performance also increases fast solubilization, as observed at an HRT and an OLR of 3 days and 1788.81 mg of COD/L. With a three-day HRT and an OLR of 1788.81 mg COD/L*day, the highest TCOD (mg/L) and SCOD (mg/L) levels were obtained. As a result, an HRT of 3 days at an OLR of 1788.81 mg COD/L was chosen as the optimal HRT and OLR for HR. Previous findings also indicate that feedstock with a high SCOD concentration is the precursor for high biogas yield and an indicator of HR performance [74,75] when used as a feedstock for a methanogenesis reactor. Furthermore, HR serves as a buffer tank in the two-phase AD process, as does high SCOD production, which is easily converted to TVFA and then to methane in the methanogenesis phase [35,67,72,76,77].

3.2.5. Degree of Acidification

In this study, the extent of acidification was assessed using the degree of acidification, and their acidification performances were also compared and depicted in Figure 7. Increases in OLR from 894.41 to 1342.61 mg/L of COD increased the DA from 17.17 to 57.26%, and then increasing beyond this resulted in a decrease in DA (Figure 7). The minimum and maximum acidification were achieved for the TCOD loading rates of 894.41 mg/L and 1341.61 mg/L, respectively, and the influent SCOD of 2354.71 mg/L. In general, DA results ranging from 17.17 to 57.26% obtained in this study are within the range of previously studied research. The DA value of the current study was in the range of the value reported (20–60%) by Burak Demirel and Yenigun [78] in their study on the anaerobic digestion of dairy wastewater but higher than the assumed optimum DA value reported (40–50%) for anaerobic digester process stability by [79]. Bouallagui [80] reported a DA range of 38.9–4.4% in HR at an HRT of 3 days in their study of two-phase anaerobic digestion of a fruit and vegetable waste mixture. The maximum DA value (57.26%) obtained in the present study is consistent with the value reported by Berhe and Leta [45], which was 55.5% at optimal conditions in their study of two-phase anaerobic co-digestion of tannery and dairy wastewaters, focusing on the effect of operational parameters on the performance of the hydrolytic–acidogenic phase.

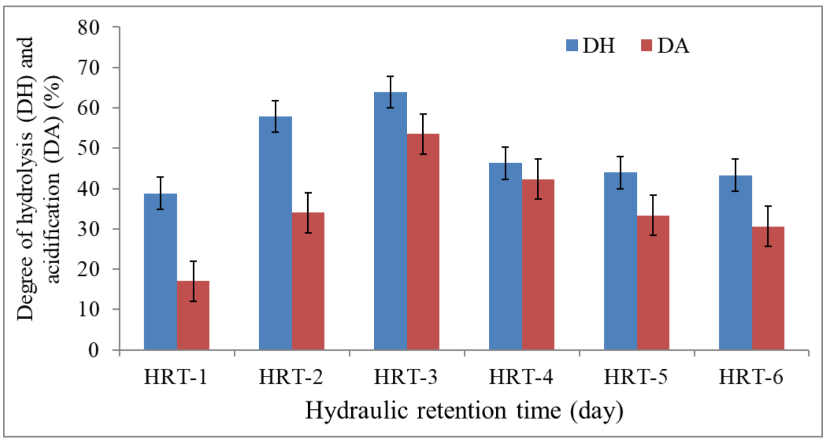

Figure 7. DH and DA of the hydrolytic–acidogenic reactor at different HRT.

3.2.6. Ammonium Nitrogen Production at Different HRT

In reality, feedstocks such as slaughterhouse wastewater containing high nitrogen can frequently pose problems for the process stability of anaerobic digesters. The average NH_4^+-N concentration of the effluent of the HR during the optimization of the two-phase anaerobic digestion is presented in Table 2. The NH_4^+-N produced during the hydrolytic–acidogenic phase of AD is mostly in the form of nitrogenous compounds, mostly proteins that were hydrolyzed into amino acids and further degraded into ammonia. The produced NH_4^+-N during hydrolysis has a significant role in buffering the digester, stimulating microbial growth, and stabilizing the hydrolysis process [81]. It is also a preferred nitrogen nutrient for methane-forming bacteria; however, when present in high values, it will cause reticence in the anaerobic process [82,83]. The concentrations of NH_4^+-N produced were high at the start of the reaction periods and gradually decreased and reached a steady state almost after the 4th day of the reaction period (Figure 5) for all HRTs. As indicated in Table 2, the highest and lowest mean NH_4^+-N values were observed at the HRTs of 3 days (369.46 11.28 mg/L) and 6 days (278.67 47.25 mg/L), while the highest and lowest NH_4^+-N values were observed at HRTs of 2 and 6 days, and on the 2nd (501 mg/L) and 10th (241 mg/L) days of the reaction course period, respectively (Figure 5). Sossa et al. [84] investigated ammonium inhibition on an anaerobic film enriched by methylaminotrohic methane-producing Archaea and reported that 848.8 mg/L were the maximum inhibitory ammonia values on the activity of methanogenic bacteria. Different scholars reported different lowest NH_4^+-N inhibition levels. Angelidaki and Ahring [85], Braun et al. [86], and Speece [87] reported that NH_4^+-N concentration inhibition in the anaerobic digester starts at 5000, 8500, 14,000, and 400 mg/L, respectively. The results of the present study indicate that the concentration of NH_4^+-N reported during the optimization process does not adversely disturb the performance and stability of the reactor process.

Table 4 shows the selected values for the parameter, indicating the digester stability at the optimization of the HR in the two-phase anaerobic digestion of slaughterhouse wastewater. Therefore, as indicated in Table 4, the optimum values for most of the stability indicating parameters were obtained at an HRT of 3 days and an OLRT of 1788.81 mg of COD/L*day.

Table 4. Summary of the mean values for the parameters indicating the stability of the reactor at optimum working condition (HRT and OLR) of hydrolytic–acidogenic reactor.

S/n	Stability and Performance Indicator Parameter	Concentration
1	pH	5.78 ± 0.32
2	SCOD (mg/L)	3430.20 ± 80.44
3	NH_4^+-N (mg/L)	369.46 ± 11.28
4	TVFA (mg/L)	1176.50 ± 81.66
5	DH (%)	63.92
6	DA (%)	57.26
7	HRT (day)	Three
8	OLR (mg COD/L. day)	1788.81
9	Flow rate (L/day)	12

4. Conclusions

In this study, operating parameters such as the HRT and OLR were optimized to establish the suitable operating condition for the HR of the two-phase anaerobic digestion of slaughterhouse wastewater. The findings indicated that, at steady state, the concentration of SCOD and TVFA decreased as HRT increased or OLR decreased from 3 to 1 day HRT and increased as HRT decreased from 6 to 4 days HRT. The SCOD, TVFA, and pH values at optimal condition were 3430.20 ± 80.44, 1176.50 ± 81.66, and 5.57, respectively. Furthermore, the concentration of NH_4^+-N reported in the present study during the optimization process of all HRTs did not adversely disturb the performance and stability of the HR process. As a result, it can be concluded that an HRT of 3 days at an OLR of 1788.81 mg of

COD/L*day was an optimal operating condition for the HR at a mesophilic temperature, maintained constant using hot water circulated from a thermostatic water bath during the two-phase anaerobic digestion of slaughterhouse wastewater. The findings of this study can be applicable for other agro-process industry wastewater types with similar characteristics and biowaste for value addition and sustainable biowaste management and safe discharge. Further optimization of the process using statistical and mathematical tools, and the dominant microorganisms responsible for the hydrolysis and acidogenesis, should be investigated.

Author Contributions: All authors have made an obligatory intellectual involvement to this study. D.T. designed the study, conducted the experiments, collected, analyzed, drafted the manuscript, and interpreted the data, and wrote the draft manuscript. S.L. participated in the study design, supervised the experiment, revised the manuscript critically, and provided comments and suggestions for the whole work. M.M.K. conceptualized and supervised the work, revised the primary manuscript, edited the manuscript, and critically revised, provided pertinent comments and suggestions as well as the approval of final manuscript for publication. All authors read, approved the final manuscript agreed to be accountable for all aspects of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

APHA: American Public Health Association; BOD: biological oxygen demand; COD: chemical oxygen demand; DA: degree of acidification; DH: degree of hydrolysis; DO: dissolved oxygen; EC: electrical conductivity; EPA: environmental protection authority; HRT: hydraulic retention time; NH4-N: Nitrogen ammonium; OLR: organic loading rate; ORP: oxidation reduction potential SCOD: soluble chemical oxygen demand; SHWW: slaughterhouse wastewater; TCOD: total chemical oxygen demand; TDS: total dissolved solid; TKN: total nitrogen; TSSs: total suspended solids; TVFAs: total volatile fatty acids; VFAs: volatile fatty acids.

References

1. Worku, Z.; Leta, S. Anaerobic Digestion of Slaughterhouse Wastewater for Methane Recovery and Treatability. *Int. J. Sustain. Green Energy* **2017**, *6*, 84–92.
2. Minnesota Pollution Control Agency. Nutrients: Phosphorus, Nitrogen Sources, Impact on Water Quality—A General Overview. *Encycl. Environ. Change* **2008**, *3*, 1–2.
3. Environmental Protection Agency (EPA). Effluent Limitations Guidelines and New Source Performance Standards for the Meat and Poultry Products Point Source Category. In *Proposed Rule*; EPA: Hong Kong, China, 2002.
4. Community Development Research (CDR). *Ethiopia Methane Emissions from Agricultural Waste Country Resource Assessment*; Global Methane initiative: Addis Ababa, Ethiopia, 2011.
5. Dyan, M.O.; Putra, G.P.; Budiyono, B.; Sumardiono, S.; Kusworo, T.D. The effect of pH and operation mode for COD removal of slaughterhouse wastewater with Anaerobic Batch Reactor (ABR). *Waste Technol.* **2015**, *3*, 7–13.
6. Tritt, W.P. The anaerobic treatment of slaughterhouse wastewater in fixed-bed reactors. *Bioresour. Technol.* **1992**, *41*, 201–207. [CrossRef]
7. Nweke, C.; Igbokwe, P.; Nwabanne, J. Anaerobic Digestion of Slaughterhouse Wastewater: A Kinetics Study. *Open J. Renew. Energy Sustain. Dev.* **2014**, *1*, 71–80.
8. Widiasa, B.S. Study on Slaughterhouse Wastes Potency and Characteristic for Biogas Production. *Int. J. Waste Resour.* **2015**, *1*, 4–7. [CrossRef]

9. Mittal, G.S. Treatment of wastewater from abattoirs before land application—A review. *Bioresour. Technol.* **2006**, *97*, 1119–1135. [CrossRef] [PubMed]
10. Mulu, A.; Ayenew, T. Characterization-of-Abattoir-Wastewater-and-Evaluation-of-the-Effectiveness-of-the-Wastewater-Treatment.doc. *Int. J. Sci. Eng. Res.* **2015**, *6*, 1026–1040.
11. Padilla-Gasca, E.; López-López, A.; Gallardo-Valdez, J. Evaluation of Stability Factors in the Anaerobic Treatment of Slaughterhouse Wastewater. *J. Bioremediat. Biodegrad.* **2011**, *2*, 114.
12. Dawana, D.; Kassa, K. Characterization and evaluation of biogas generation of Arba Minch town slaughterhouse wastewater, Ethiopia. *Water Pract. Technol.* **2020**, *15*, 899–909.
13. Abdallah, M.; Greige, S.; Beyenal, H.; Harb, M.; Wazne, M. Investigating microbial dynamics and potential advantages of anaerobic co-digestion of cheese whey and poultry slaughterhouse wastewaters. *Sci. Rep.* **2022**, *12*, 10529.
14. U.S. EPA. *Technical Development Document for the Final Effluent Limitations Guidelines and Standards for the Meat and Poultry Products Point Source Category (40 CFR 432)*; U.S. EPA: Washington, DC, USA, 2002.
15. Ritchie, H.; Rosado, P.; Roser, M.; Meat and Dairy Production. Our World Data. 2017. Available online: https://ourworldindata.org/meat-production (accessed on 18 February 2023).
16. Eshetie, T. Meat production, consumption and marketing tradeoffs and potentials in Ethiopia and its effect on GDP growth: A review. *J. Nutr. Health Food Eng.* **2018**, *8*, 17–24.
17. Shafi, M.W. Reviw on application of hazard analysis critical control point in Ethiopian slaughtering plant. *Int. J. Vet. Sci. Res.* **2021**, *7*, 40–56. [CrossRef]
18. BCaD Consulting Management PLC. *Value Chain Study on Meat Processing Industry in Ethiopia: 'Strengthening the Private Sector in Ethiopia' Project Finance by the Danish Embassy in Ethiopia Input*; Addis Ababa Chamber of Commerce and Sectoral Associations (AACCSA): Addis Ababa, Ethiopia, 2015; pp. 1–37.
19. Philipp, M.; Jabri, K.M.; Wellmann, J.; Akrout, H.; Bousselmi, L.; Geißen, S.U. Slaughterhouse wastewater treatment: A review on recycling and reuse possibilities. *Water* **2021**, *13*, 3175.
20. Burkhart, K.; Bernhardt, C.; Pelton, T.; Schaeffer, E.; Philips, A. *Water Pollution from Slaughterhouses (Three Quarters of U.S. Meat Processing Plants That Discharge into Waterways Violated Their Permits, 2016–2018)*; Environmental Integrity Project: Washington, DC, USA, 2018; pp. 1–47.
21. Jones, E.R.; Van Vliet, M.T.H.; Qadir, M.; Bierkens, M.F.P. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. *Earth Syst. Sci. Data* **2021**, *13*, 237–254.
22. Leta, S.; Assefa, F.; Dalhammar, G. Characterization of tannery wastewater and assessment of downstream pollution profiles along Modjo River in Ethiopia. *Ethiop. J. Biol. Sci.* **2003**, *2*, 157–168.
23. Haddis, A.; de Geyter, A.; Smets, I.; Van der Bruggen, B. Wastewater management in Ethiopian higher learning institutions: Functionality, sustainability and policy context. *J. Environ. Plan. Manag.* **2014**, *57*, 369–383. [CrossRef]
24. Salom, M.; Oliveira, V.; Alves, M.M.; Pereira, C.; Carvalho, M.; Mesquita, D.P. Volatile Fatty Acids (VFA) Production from Wastewaters with High Salinity—Influence of pH, Salinity and Reactor Configuration. *Fermentation* **2021**, *7*, 303.
25. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99*, 4044–4064. [CrossRef] [PubMed]
26. Angelidaki, I.; Ellegaard, L.; Ahring, B.K. Applications of the anaerobic digestion process. In *Biomethanation II*; Advances in Biochemical Engineering/Biotechnology; Springer: Berlin/Heidelberg, Germany, 2003; Volume 82, pp. 1–33.
27. Lindorfer, H.; López, C.P.; Resch, C.; Braun, R.; Kirchmayr, R. The impact of increasing energy crop addition on process performance and residual methane potential in anaerobic digestion. *Water Sci. Technol.* **2007**, *56*, 55–63. [CrossRef]
28. Siegert, I.; Banks, C. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochem.* **2005**, *40*, 3412–3418. [CrossRef]
29. Koutrouli, E.C.; Kalfas, H.; Gavala, H.N.; Skiadas, I.V.; Stamatelatou, K.; Lyberatos, G. Hydrogen and methane production through two-stage mesophilic anaerobic digestion of olive pulp. *Bioresour. Technol.* **2009**, *100*, 3718–3723. [CrossRef]
30. Yunqin, L.; Dehan, W.; Lishang, W. Biological pretreatment enhances biogas production in the anaerobic digestion of pulp and paper sludge. *Waste Manag. Res.* **2010**, *28*, 800–810. [CrossRef] [PubMed]
31. Maharaj, I.; Elefsiniotis, P. The role of HRT and low temperature on the acid-phase anaerobic digestion of municipal and industrial wastewaters. *Bioresour. Technol.* **2001**, *76*, 191–197. [CrossRef]
32. Elefsiniotis, P.; Oldham, W.K. Influence of pH on the acid-phase anaerobic digestion of primary sludge. *J. Chem. Technol. Biotechnol.* **1994**, *60*, 89–96. [CrossRef]
33. Elefsiniotis, P.; Oldham, W.K. Anaerobic acidogenesis of primary sludge: The role of solids retention time. *Biotechnol. Bioeng.* **1994**, *44*, 7–13. [CrossRef] [PubMed]
34. Elefsiniotis, W.K.; Oldham, P. Effect of HRT on Acidogenic Digestion of Primary Sludge. *J. Environ. Eng.* **1994**, *120*, 645–660. [CrossRef]
35. Mahmod, S.S.; Azahar, A.M.; Luthfi, A.A.I.; Abdul, P.M.; Mastar, M.S.; Anuar, N.; Takriff, M.S.; Jahim, J.M.D. Potential Utilisation of Dark-Fermented Palm Oil Mill Effluent in Continuous Production of Biomethane by Self-Granulated Mixed Culture. *Sci. Rep.* **2020**, *10*, 9167. [CrossRef]
36. Ghosh, S.; Buoy, K.; Dressel, L.; Miller, T.; Wilcox, G.; Loos, D. Pilot- and full-scale two-phase anaerobic digestion of municipal sludge. *Water Environ. Res.* **1995**, *67*, 206–214. [CrossRef]

37. Cuetos, M.J.; Gómez, X.; Otero, M.; Morán, A. Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochem. Eng. J.* **2008**, *40*, 99–106. [CrossRef]
38. Lim, S.-J.; Kim, B.J.; Jeong, C.-M.; Choi, J.; Ahn, Y.H.; Chang, H.N. Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. *Bioresour. Technol.* **2008**, *99*, 7866–7874. [CrossRef]
39. Castellucci, S.; Cocchi, S.; Allegrini, E.; Vecchione, L. Anaerobic digestion and co-digestion of slaughterhouse wastes. *J. Agric. Eng.* **2013**, *44*, 526–530. [CrossRef]
40. Wang, Q.; Liang, Y.; Zhao, P.; Li, Q.X.; Guo, S.; Chen, C. Potential and optimization of two-phase anaerobic digestion of oil refinery waste activated sludge and microbial community study. *Sci. Rep.* **2016**, *6*, 38245. [CrossRef] [PubMed]
41. Ponsá, S.; Ferrer, I.; Vázquez, F.; Font, X. Optimization of the hydrolytic–acidogenic anaerobic digestion stage (55 °C) of sewage sludge: Influence of pH and solid content. *Water Res.* **2008**, *42*, 3972–3980. [CrossRef] [PubMed]
42. Lin, C.Y.; Chai, W.S.; Lay, C.H.; Chen, C.C.; Lee, C.Y.; Show, P.L. Optimization of hydrolysis-acidogenesis phase of swine manure for biogas production using two-stage anaerobic fermentation. *Processes* **2021**, *9*, 1324. [CrossRef]
43. Bacab, F.C.; Gamboa, E.E.; Espinoza, J.E.R.; Leal-Bautista, R.M.; Tussell, R.T.; Maldonado, J.D.; Canché, B.C.; Alzate-Gaviria, L. Two phase anaerobic digestion system of municipal solid waste by utilizing microaeration and granular activated carbon. *Energies* **2020**, *13*, 933. [CrossRef]
44. Hailu, A.M.; Asfaw, S.L.; Tegaye, T.A. Effect of carbon-rich-waste addition as co-substrate on the performance and stability of anaerobic digestion of abattoir wastewater without agitation. *Bioresour. Bioprocess.* **2020**, *7*, 44. [CrossRef]
45. Shifare, B.; Seyoum, L. Two Phase Anaerobic Co Digestion of Tannery Wastewater and Dairy Wastewater: Effect of Operational Parameters on Performance of Hydrolytic–Acidogenic Step. *Int. J. Sustain. Green Energy* **2017**, *6*, 1–9.
46. Anyango, B.N.; Wandera, S.M.; Raude, J.M. Abattoir Wastewater Treatment in Anaerobic Co-Digestion with Sugar Press Mud in Batch Reactor for Improved Biogas Yield. *Water* **2022**, *14*, 2571. [CrossRef]
47. Chakraborty, D.; Karthikeyan, O.P.; Selvam, A.; Palani, S.G.; Ghangrekar, M.M.; Wong, J.W.C. Two-phase anaerobic digestion of food waste: Effect of semi-continuous feeding on acidogenesis and methane production. *Bioresour. Technol.* **2022**, *346*, 126396. [CrossRef]
48. Van, D.P.; Fujiwara, T.; Tho, B.L.; Toan, P.P.S.; Minh, G.H. A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. *Environ. Eng. Res.* **2020**, *25*, 334. [CrossRef]
49. 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* **2022**, *8*, 620. [CrossRef]
50. Yilmaz, V.; Demirer, G.N. Improved anaerobic acidification of unscreened dairy manure. *Environ. Eng. Sci.* **2008**, *25*, 309–317. [CrossRef]
51. Demirer, N.; Chen, S. Effect of retention time and organic loading rate on anaerobic acidification and biogasification of dairy manure. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 1381–1387. [CrossRef]
52. Bustillo-Lecompte, C.F.; Mehrvar, M. Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *J. Environ. Manag.* **2015**, *161*, 287–302. [CrossRef] [PubMed]
53. González-Fernández, C.; Mahdy, A.; Ballesteros, I.; Ballesteros, M. Impact of temperature and photoperiod on anaerobic biodegradability of microalgae grown in urban wastewater. *Int. Biodeterior. Biodegrad.* **2016**, *106*, 16–23. [CrossRef]
54. Ren, J.; Yuan, X.; Li, J.; Ma, X.; Zhao, Y.; Zhu, W.; Wang, X.; Cui, Z. Performance and microbial community dynamics in a two-phase anaerobic co-digestion system using cassava dregs and pig manure. *Bioresour. Technol.* **2014**, *155*, 342–351. [CrossRef]
55. Tsegaye, D.; Leta, S. Optimization of operating parameters for biogas production using two—Phase bench—Scale anaerobic digestion of slaughterhouse wastewater: Focus on methanogenic step. *Bioresour. Bioprocess.* **2022**, *9*, 1–16. [CrossRef]
56. Abdullahi, A.; Kadarman, N.; Hassan, A.; Madobi, I.S. Negative Impact of Abattoir Activities and Management in Residential Neighbourhoods in Kuala Terengganu, Malaysia. *Int. J. Public Health Sci.* **2015**, *4*, 124–130.
57. Chen, S.; Zhang, J.; Wang, X. Effects of alkalinity sources on the stability of anaerobic digestion from food waste. *Waste Manag. Res.* **2015**, *33*, 1033–1040. [CrossRef] [PubMed]
58. Dobre, M.F.; Nicolae, F. Main factors affecting biogas production—An overview: Letters, Romanian Biotechnological. *Rom. Biotechnol. Lett.* **2014**, *19*, 9283–9296.
59. Funk, J.A.; Funk, J.A. *TigerPrints Evaluation of Two-Phase Anaerobic Digestion for Increased Sludge Handling Capacity and Energy Production at the F. Wayne Hill Water Resources Center Evaluation of Two-Phase Anaerobic Digestion for Increased Sludge Handling Capacity and Energy*; Clemson University: Clemson, SC, USA, 2021.
60. Yu, H.G.; Fang, H.H.P. Acidogenesis of dairy wastewater at various pH levels. *Water Sci. Technol.* **2002**, *45*, 201–206. [CrossRef] [PubMed]
61. García-Depraect, O.; Castro-Muñoz, R.; Muñoz, R.; Rene, E.R.; León-Becerril, E.; Valdez-Vazquez, I.; Kumar, G.; Reyes-Alvarado, L.C.; Martínez-Mendoza, L.J.; Carrillo-Reyes, J.; et al. A review on the factors influencing biohydrogen production from lactate: The key to unlocking enhanced dark fermentative processes. *Bioresour. Technol.* **2020**, *324*, 124595. [CrossRef] [PubMed]
62. Menzel, T.; Neubauer, P.; Junne, S. Role of microbial hydrolysis in anaerobic digestion. *Energies* **2020**, *13*, 5555. [CrossRef]
63. Chatterjee, B.; Mazumder, D. Role of stage-separation in the ubiquitous development of Anaerobic Digestion of Organic Fraction of Municipal Solid Waste: A critical review. *Renew. Sustain. Energy Rev.* **2019**, *104*, 439–469. [CrossRef]
64. Alkaya, E.; Demirer, G.N. Anaerobic acidification of sugar-beet processing wastes: Effect of operational parameters. *Biomass Bioenergy* **2011**, *35*, 32–39. [CrossRef]

65. Eylem Dogan Subasi, G.N.D. Phased Anaerobic Digestion of Organic Fraction of Manucipal Solid Waste. *Int. J. Adv. Sci. Eng. Technol.* **2017**, *5*, 313–314.
66. Duangmanee, T. *Micro-Aeration for Hydrogen Sulfide Removal from Biogas*; Iowa State University: Ames, IA, USA, 2009.
67. Sukphun, P.; Sittijunda, S.; Reungsang, A.; Atta-Obeng, E. Volatile Fatty Acid Production from Organic Waste with the Emphasis on Membrane-Based Recovery. *Fermentation* **2021**, *7*, 159. [CrossRef]
68. Jiang, J.; Zhang, Y.; Li, K.; Wang, Q.; Gong, C.; Li, M. Volatile fatty acids production from food waste: Effects of pH, temperature, and organic loading rate. *Bioresour. Technol.* **2013**, *143*, 525–530. [CrossRef] [PubMed]
69. Demirel, B.; Neumann, L.; Scherer, P. Microbial community dynamics of a continuous mesophilic anaerobic biogas digester fed with sugar beet silage. *Eng. Life Sci.* **2008**, *8*, 390–398. [CrossRef]
70. Castilla-Archilla, J.; Heiberger, J.; Mills, S.; Hilbig, J.; Collins, G.; Lens, P.N.L. Continuous Volatile Fatty Acid Production from Acid Brewery Spent Grain Leachate in Expanded Granular Sludge Bed Reactors. *Front. Sustain. Food Syst.* **2021**, *5*, 664944. [CrossRef]
71. Al-sulaimi, I.N.; Nayak, J.K.; Alhimali, H.; Sana, A. Effect of Volatile Fatty Acids Accumulation on Biogas Production by Sludge-Feeding Thermophilic Anaerobic Digester and Predicting Process Parameters. *Fermentation* **2022**, *8*, 184. [CrossRef]
72. Cheah, Y.K.; Vidal-Antich, C.; Dosta, J.; Mata-Álvarez, J. Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH. *Environ. Sci. Pollut. Res.* **2019**, *26*, 35509–35522. [CrossRef] [PubMed]
73. Donoso-Bravo, A.; Retamal, C.; Carballa, M.; Ruiz-Filippi, G.; Chamy, R. Influence of temperature on the hydrolysis, acidogenesis and methanogenesis in mesophilic anaerobic digestion: Parameter identification and modeling application. *Water Sci. Technol.* **2009**, *60*, 9–17. [CrossRef] [PubMed]
74. Zhang, L.; Lee, Y.W.; Jahng, D. Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. *Bioresour. Technol.* **2011**, *102*, 5048–5059. [CrossRef]
75. Zhang, Y.; Banks, C.J. Co-digestion of the mechanically recovered organic fraction of municipal solid waste with slaughterhouse wastes. *Biochem. Eng. J.* **2012**, *68*, 129–137. [CrossRef]
76. Carvalheira, M.; Duque, A.F. From Food Waste to Volatile Fatty Acids towards a Circular Economy. In *Fermentation—Processes, Benefits and Risks*; Laranjo, M., Ed.; InTech: London, UK, 2021; p. 22.
77. Tesfaye, S. *Evaluation of the Treatability and Biogas Production Potential of Slaughter House Wastewater in Anaerobic Sequential Batch Reactor*; Addis Ababa University: Addis Ababa, Ethiopia, 2014.
78. Demirel, B.; Yenigun, O. Anaerobic acidogenesis of dairy wastewater: The effects of variations in hydraulic retention time with no pH control. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 755–760. [CrossRef]
79. Karlsson, A.; Björn, A.; Yekta, S.S.; Svensson, B.H. *Improvement of the Biogas Production Process*; Biogas Research Center, Linköping University: Linköping, Sweden, 2014.
80. Bouallagui, H.; Touhami, Y.; Cheikh, R.B.; Hamdi, M. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. *Process Biochem.* **2005**, *40*, 989–995. [CrossRef]
81. Garcia-Peña, E.I.; Parameswaran, P.; Kang, D.W.; Canul-Chan, M.; Krajmalnik-Brown, R. Anaerobic digestion and co-digestion processes of vegetable and fruit residues: Process and microbial ecology. *Bioresour. Technol.* **2011**, *102*, 9447–9455. [CrossRef]
82. Mehta, C.M.; Jensen, P.D.; Batstone, D.J. Pilot Scale Resource Recovery from Cattle Paunch Waste. In Proceedings of the IWA International Symposium on Waste Management Problems in Agro-Industries, Kochi, Japan, 24–26 November 2014.
83. Nielsen, H.B.; Ahring, B.K. Effect of tryptone and ammonia on the biogas process in continuously stirred tank reactors treating cattle manure. *Environ. Technol.* **2007**, *28*, 905–914. [CrossRef]
84. Sossa, K.; Alarcón, M.; Aspé, E.; Urrutia, H. Effect of ammonia on the methanogenic activity of methylaminotrophic methane producing Archaea enriched biofilm. *Anaerobe* **2004**, *10*, 13–18. [CrossRef] [PubMed]
85. Angelidaki, I.; Ahring, B.K. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. *Water Res.* **1994**, *28*, 727–731. [CrossRef]
86. Braun, R.; Huber, P.; Meyrath, J. Ammonia toxicity in liquid piggery manure digestion. *Biotechnol. Lett.* **1981**, *3*, 159–164. [CrossRef]
87. Speece, R.E. Anaerobic biotechnology for industrial wastewater treatment. *Environ. Sci. Technol.* **1983**, *17*, 416A–427A. [CrossRef]

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