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Characterization and evaluation of biogas generation of Arba Minch Town slaughterhouse wastewater, Ethiopia

Demisie Dawana and Kinfe Kassa *

Water Supply and Environmental Engineering Faculty, Arba Minch Water Technology Institute, Arba Minch University, Arba Minch, Ethiopia

*Corresponding author. E-mail: kinfe_k@yahoo.com

Abstract

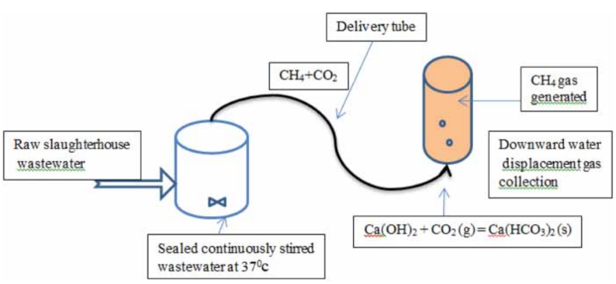
This study was carried out to characterize raw and treated Arba Minch town slaughterhouse wastewater and to assess its methane generation potential with lab-scale anaerobic batch reactors. The methane was collected by downward displacement of an alkaline water column. The methane generation potential of the slaughterhouse wastewater was 270.6 mL methane per gram of volatile solids at hydraulic retention time (HRT) of 20 days at 37 °C. The organic loading rate was 0.48 g $\frac{COD}{L.d}$ and the organic matter removal efficiency of the reactor was COD (93.5%), BOD_5 (88.5%), and TVS (94.7%). The result demonstrated that installation of a biogas reactor to treat slaughterhouse wastewater can recover methane, reduce pollutants and protect the environment. The result can be a demonstration for untreated slaughterhouse wastewaters in developing countries like Arba Minch Town to use anaerobic treatment and supplement their scarce energy options.

Key words: anaerobic digestion, Arba Minch, biogas measurement, bio-methane potential, slaughterhouse wastewater

Highlights

- It demonstrates the case for developing abattoir wastewater reuse.
- It shows how to generate energy, size for abattoirs and Environmental Agency.
- It shows the option of treatment for abattoir wastewater in developing countries.
- It demonstrates that abattoir buildings are polluting water bodies.
- The classical lab scale method can be used to study wastewater reuse where there is shortage of resources.

Graphical Abstract



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INTRODUCTION

Ethiopia has an estimated 60.39 million cattle population, 31.3 million sheep and 32.74 million goats (CSA 2018). In Ethiopia, besides slaughtering for ceremonies in villages, municipal slaughterhouses are administered by respective towns for local consumption and commercial slaughterhouses are mostly for export purposes. Commercial slaughterhouses are recent and have treatment facilities, but some are not properly functioning (Mulu & Ayenew 2015). Except in Addis Ababa, regional municipal slaughterhouses do not have any wastewater treatment facilities, so wastewater is released into the environment.

Slaughterhouse wastewater is very harmful: it causes de-oxygenation of rivers and contaminates groundwater (Sangodoyin & Agbawe 1992). Slaughterhouses and meat processing facilities generate a large volume of wastewater that contains organic matter measured as chemical oxygen demand (COD) from 500 to 15,900 mg L^{-1}, total nitrogen (50–841 mg/L) and orthophosphate (20–100 mg/L) (Bustillo-lecompte & Mehrvar 2015). The contributors to high COD are the stomach, faeces, fat and grease, undigested food, blood, suspended material, urine, loose meat, soluble proteins, excrement, manure, grit and colloidal particles (Farzadkia *et al.* 2016). Blood is a by-product of the slaughterhouses, representing up to 4% of the live animal weight or 6–7% of the lean meat content of the carcass (Bah *et al.* 2013). It is a problematic by-product of the meat industry because of the high volumes generated and its very high pollutant load when discarded untreated into the environment (Bah *et al.* 2013). Blood has a biological oxygen demand (BOD) of 150,000–200,000 mg/L (Verheijen *et al.* 1996). Moreover, residual blood, manure, cleaning, and sanitizing compounds are significant sources of phosphorous (USEPA 2002). Animal faeces and manure are sources of pathogens and other microorganisms in the sludge during biogas production (Islam *et al.* 2019).

Slaughterhouse wastewater is biodegradable, so it can be amenable to biological treatment. Anaerobic digestion processes have been proposed as a good treatment system to stabilize wastewaters with high to medium organic loads (Hickey *et al.* 1991). Anaerobic systems have high COD removal, low sludge production (5–20%) compared to aerobic systems, and fewer energy requirements with potential nutrient and biomethane recovery (Masse & Masse 2000). Anaerobic digestion reduces pathogens (Islam *et al.* 2019). Thus, the anaerobic digestion process is a common practice to increase the energy self-sufficiency of slaughterhouse wastewater (SWWT) (Bustillo-Lecompte & Mehrvar 2015). Optimal use of animal by-products can develop industries and create jobs and the wastewater benefits the societies by generating renewable energy while being treated (Afazeli *et al.* 2014).

However, slaughterhouse wastewater, which is a valuable resource of biogas, has not been efficiently used in developing countries like Ethiopia and is the cause of environmental hazard. In order to explore the mentioned advantages, bio-methane potential tests are used to measure the ultimate methane production and removal from slaughterhouse wastewater. Before construction of an anaerobic digestion plant, energy generation and sizing reactors, the technical and economic feasibility of the organic substrate is assessed by measuring the bio-methane potential (Holliger *et al.* 2016).

Therefore, the main aim of this work was to characterize, and determine the methane generation and pollutant removal efficiency of Arba Minch town slaughterhouse wastewater, Ethiopia, at a laboratory-scale anaerobic reactor under mesophilic conditions at a residence time of 20 days.

MATERIALS AND METHODS

Locations and site description

Experiments were carried out in Arba Minch, in the south of Ethiopia, located at N 0602′ E 37033′ at an elevation of 1,202 m.a.s.l. with a mean annual rainfall of 863.7 mm (local meteorology data). The location is characterized as a semi-arid climate with minimum and maximum average air temperature

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ranging between 17.4 °C and 30.5 °C. Arba Minch slaughterhouse slaughters on average 35 oxen per day, except Fridays and Wednesdays, and 55 oxen on holydays. The abattoir does not have a wastewater treatment system and it directly discharges its wastewater into the nearby River Kulfo. The composition of the wastewater includes stock pats, rumen, slaughter colloidal particles, meat, punch, dung, fat, grease, undigested grass, urine, and blood.

Sampling and analysis

Wastewater samples were collected from the slaughterhouse during slaughtering and from the experimental setup after digestion. A composite sample of wastewater was sampled by mixing equal volumes of wastewater collected at the beginning, middle and end of the slaughtering time. Triplicate samples of such mixtures were used for the experimental analysis. The quality of slaughterhouse wastewater is presented in Table 1. Most of the parameters were analyzed on the day of sampling; otherwise, they were stored at 4 °C in a refrigerator and analyzed as soon as possible. All the analysis was conducted according to APHA *et al.* (1999) unless otherwise stated.

Table 1 | Characteristics of the slaughterhouse raw wastewater and the batch anaerobically digested (biogas production) sludge at the same hydraulic and solid retention time (20 days) at 37 °C

Parameter	Raw wastewater $\bar{X} \pm$ SD	Treated wastewater, sludge ($\bar{X} \pm$ SD)
pH	7.40 ± 0.17	7.18±0.02
Conductivity (mS/cm)	8.21 ±0.05	3.31±0.03
Dissolved Oxygen (DO) (mg/L)	0.62 ±0.17	0.28±0.03
Biological Oxygen Demand (BOD) (mg/L)	10,984 ±293	1,265 ±0.6
Chemical Oxygen Demand (COD) (mg/L)	19,253 ±403	1,269.3±97.8
Ammonium Nitrogen (NH_4^+-N) (mg/L)	805 ±21	365.86 ± 18.0
Total Kjeldahl Nitrogen (TKN) (mg/L)	3,938 ±140	890.4±33.6
Total Solids (TS) (g/L)	67.7 ±2.9	4.0±0.2
Total Volatile Solids (TVS) (g/L)	48.045 ±6.2	2.5±0.1
Total Fixed Solids (TFS) (g/L)	19.61 ±3.4	1.5±0.2
Phosphate (PO_4^{3-}-P) (mg/L)	86.0 ±4.6	36.53±0.967
Sulfate (SO_4^{2-}) (mg/L)	1,214 ±243	346.4 ±12.3
Colour (Hazen Unit)	175,000	3,000
Turbidity (NTU)	1,600 ±100	293.33±50.33
Total Alkalinity (g/L as $CaCO_3$)	5.0 ±1.0	4.6±0.2
Total Coliform $\frac{CFU}{100ml}$	2.53×10^5	8.4×10^4
Faecal Coliform $\frac{CFU}{100ml}$	1.36×10^5	6.08×10^4

The results presented as Mean ± SD.

Water temperature, DO, conductivity and pH were measured using a portable HQ40d meter (Hach Co., USA) onsite and in the laboratory using the manufacturer's manual. Carbonaceous biochemical oxygen demand ($CBOD_5$) was incubated at 20°C after dilution with seeded and well-aerated dilution water and COD was measured by open reflux digestion and titration. Total solids (TS) were measured gravimetrically by using an evaporating dish at 103 °C and total volatile solids (TVS) and total fixed solids (TFS) after ignition at 550 °C. Turbidity of the samples was measured with Hach Model 2100A after calibration using the manufacturer's manual. Total Kjeldahl Nitrogen (TKN) was analyzed by digestion with strong sulphuric acid in the presence of a catalyst with Kjeldahl setup and ammonium nitrogen was distilled. Ammonium was measured using acid titration from the distillate.

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Phosphate (PO_4^3 -P) was measured spectrophotometrically at 690 nm after the filtered sample was mixed with molybdate and stannous chloride for blue colour development. The alkalinity of the sample was determined by titration with 0.02 N HCl solutions with methyl orange endpoint for total alkalinity (pH = 4.5). Sulfate (SO_4^{2-}) was determined by barium chloride precipitation and ignition at 800 °C after acidifying the sample with acid.

The concentration of total coliforms and faecal counts were conducted using membrane filtration methods after incubating at 37 °C and 44 °C, respectively.

REMOVAL EFFICIENCY

To evaluate the removal efficiency of the pollutants in the anaerobic wastewater, treatment was calculated as per the equation below.

$$\text{Percent Removal} = \frac{(C_{inf.} - C_{eff.})}{C_{inf.}} \times 100$$

where,

C_{inf} = the concentration of the raw influent;
C_{eff} = effluent of the biogas system.

Experimental setup and operation

The batch anaerobic digestion of slaughterhouse wastewater (SHWW) (Figure 1) was carried out with a working volume of 2 litres of completely mixed waste sample and the inoculum operated at

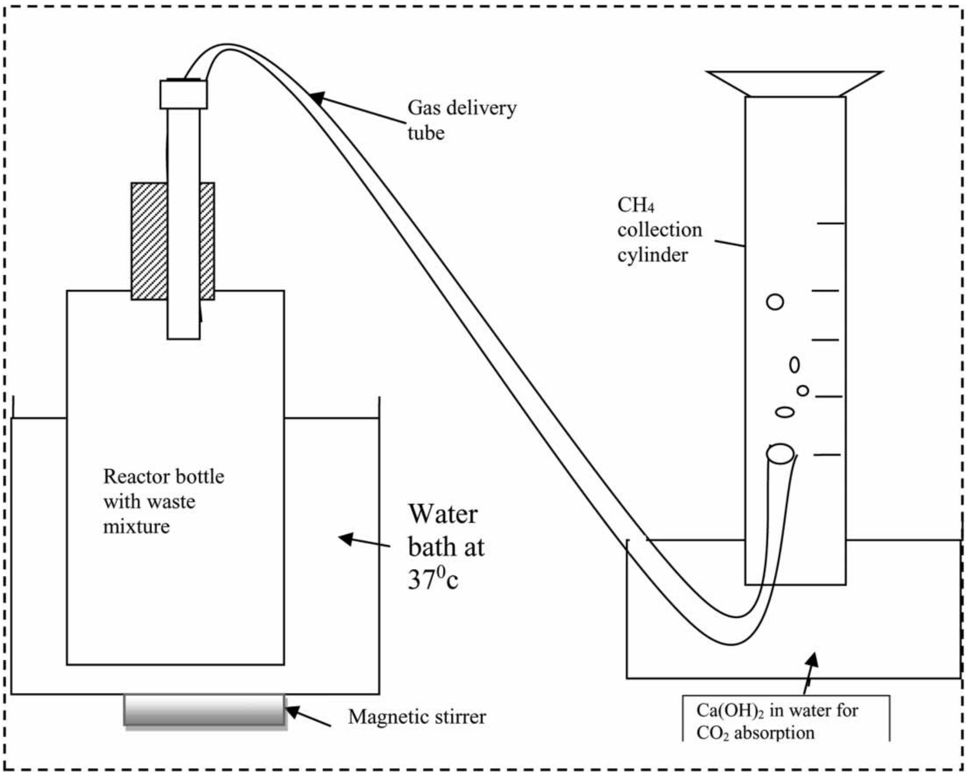

Figure 1 | Schematic diagram of lab-scale experimental set up for slaughterhouse waste and inoculum batch reactor at Arba Minch University, Water Quality Laboratory.

37 ± 1 °C (mesophilic conditions) at a hydraulic retention time (HRT) of 20 days and at the organic loading rate (OLR) of 0.48 g COD/L.d. The HRT was selected based on anaerobic digestion of other organic wastes (Thapa 2012).

The inoculum was collected from a household level biogas plant that receives municipal wastewater (20%) and animal manure (80%). The inoculum was incubated at mesophilic temperature (37 ± 1 °C) for 5 days to eliminate background gas. To eliminate the background gas production, a similar amount of inoculums was added in a separate reactor without adding raw material (Angelidaki *et al.* 2009). The inoculums had a volatile solid (VS) content of 42.33 (g/L) and a pH of 7.9.

Figure 1 shows the methane gas produced from the reactor passed through a delivery tube and was collected and measured in an inverted measuring cylinder by displacing water mixed with calcium hydroxide (0.1 M $Ca(OH)_2$) and phenolphthalein indicator (pink colour). The carbon dioxide produced was absorbed by the alkaline solution and the exhaustion of the base was monitored by the indicator. Phenolphthalein changed color when $Ca(OH)_2$ was exhausted or CO_2 was not absorbed (Mel *et al.* 2014).

Results and discussion

Overview

In this section, first the characteristics of the raw wastewater that was used for methane generation and the sludge after 20 days are presented in Table 1, then the removal efficiency of the system is discussed; finally, the methane production results and their discussion are presented.

Characteristics of the raw and treated slaughterhouse wastewater

pH, conductivity, and colour

Referring to Table 1, the pH value of the untreated slaughterhouse wastewater sample was 7.4, within the range of 6.0–9.0 is within the tolerance limits for discharge of slaughterhouse industries wastewater into surface water (World Bank 1999).

Methanogenic microorganisms prefer slightly alkaline or neutral pH conditions (5.5–8.5) (Calicioglu *et al.* 2018). Therefore, the wastewater was used for anaerobic digestion without pH adjustment.

The conductivity of the raw wastewater was 8,200 μS/cm, which was forty times more than the conductivity of the Arba Minch town water supply, 200 μS/cm. The high value in the wastewater and observed during the slaughter process indicates the slaughterhouse indiscriminately discharged blood and urine along with the washing water. Besides, curing and pickling contributes to high conductivity values. On the contrary, in relatively modern slaughterhouses in Addis Ababa and Modjo in Ethiopia, the conductivity measured was between 1,251 and 1,614 μS/cm (Mulu & Ayenew 2015). The low values of the conductivity in the modern slaughterhouse might indicate they separate the urine and blood, use excess water and dilute the wastewater. The indiscriminate release of blood and urine in the Arba Minch slaughterhouse is also indicated by the high colour of the wastewater, which was 175,000 Hazen units. The current untreated release of wastewater into the river affected the colour and conductivity unacceptably.

Total solids, total volatile solids and turbidity

As in Table 1, the concentration of TS, TVS, TFS and turbidity are 67,657, 48,045, 19,620 in mg/L and 1,600 NTU, respectively. The high value of the solids is due to the indiscriminate discharge of waste and sorting of the solid waste and no preliminary treatment being used to screen the solid waste from the SHWW. In the wastewater, various by-products were observed: animal faeces, soft tissue, fats, and soil from hides and hooves were the components. Discharge of wastewater with this composition can be the cause of a long-term demand for oxygen because of the slow hydrolysis rate of the organic fraction of the material. The loading of a high concentration of solids in streams leads to the degradation of water quality and causes problems to aquatic organisms (Kjelland *et al.* 2015).

Dissolved oxygen, biological oxygen demand, and chemical oxygen demand

From Table 1, the average values of COD and BOD_5 are 19,253 and 10,984 mg/L, respectively, and the values are in parallel to TVS. Processing of gut has a significant impact on the quantity and quality of wastewater generated (World Bank 1999). High BOD and COD results in slaughterhouse wastewater quality depend on the degree of separation of blood and other by-products (Mittal 2004). The COD value in this study is comparable with the result reported by Padilla-Gasca & López (2010) which ranged from 5,000 to 20,000 mg/L. It is also reported that blood contributes to a high organic load, with 150,000 mg/L to 200,000 mg/L of BOD and 375,000 mg/L of COD (Tritt & Schuchardt 1992). Therefore, the high BOD and COD values obtained in this study are mainly attributed to blood generated in slaughtering operations.

The ratio of BOD/COD for the raw slaughterhouse wastewater was 0.57, which indicates the wastewater is highly amenable to biochemical treatment. This number is comparable to those presented by Tchobanoglous *et al.* (2003), who stated that the typical values for the BOD/COD ratio of untreated municipal wastewater are usually in a range from 0.3 to 0.8. According to them, when the ratio is equal to 0.5 or greater, the wastewater can be treated by biological means. If the ratio is below 0.3, either the wastewater may have some toxic components or the wastewater is not favorable for biological treatment. On this account, it was determined the slaughterhouse wastewater was successfully digested anaerobically because the organics in the wastewater could be easily accessed by anaerobic microbes. So, the Arba Minch SHWW can be easily treated by the anaerobic system and this is important for biogas production as well as waste stabilization.

The lower DO of 0.6 mg/L is because of the high organic matter consumption by bacteria. When the wastewater is released into the River Kulfo, as is being done now, it depletes the DO values of the river from the usually recorded 6–7 mg/L; the impact will be serious in the dry season. In aquatic ecosystems, except during the daytime active photosynthesis period, DO is usually a critical factor and at times it may cause anoxia and death of aquatic organisms (Watson *et al.* 2015).

Total nitrogen, phosphate, sulphate and pathogens

From the analysis in Table 1, the total nitrogen and phosphate concentrations in the SHWW were 3,938.2 mg/L and 86 mg/L, respectively. This value was found to be much higher than the World Bank (Table 2) maximum value of 10 mg/L for nitrogen and 5 mg/L for phosphate for a discharge

Table 2 | Effluents from meat processing and rendering industry (mg/L, except pH and bacteria)

Parameter	Maximum value
pH	6–9
BOD	50
COD	250
TSS	50
Oil and grease	10
Nitrogen (total)	10
Total phosphorous	5
Coliform bacteria	400 MPN/100 mL

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into surface water (World Bank 1999). Discharge of such wastewater may cause eutrophication of the receiving water bodies. Excessive algae growth and subsequent dying off effects and mineralization of these algae may lead to the death of aquatic life because of oxygen depletion (USEPA 2002). The phosphate value obtained in this study was similar to the value obtained by Mulu & Ayenew (2015) from abattoir wastewater, 67.3 mg/L.

As indicated in Table 1, sulphate concentration is 1,214. Sulphate is present in industrial wastewaters (Tchobanoglous *et al.* 2003). This value is tremendously high and might be attributed to by-products of dressed animals having a protein nature, since sulfur is a constituent of some proteins. Sulphate-reducing bacteria compete with methane-generating bacteria for COD and decrease the methane yield (Paulo *et al.* 2015).

The microbiological characteristics of wastewater are of fundamental importance in the control of diseases caused by pathogenic organisms. The total coliform bacteria (TC) and faecal coliform bacteria (FC) values were $2.53\times10^5$and 1.36×10^5, respectively. The result is comparable with a similar study in Addis Ababa (Kara abattoirs) (Mulu & Ayenew 2015), which found 4.40×10^6 total coliforms and 1.35×10^6 faecal coliforms. The presence of indicator bacteria shows the possible public health threat associated with inadequately treated SHWW (Bustillo-Lecompte & Mehrvar 2015).

As a summary, the analysis results of the pollutant concentrations at the SHWW were very significant to cause pollution if they are directly discharged into Kulfo River untreated. Therefore, a treatment technology (anaerobic treatment) that treats wastewater and recovers valuable products is very attractive (Bustillo-Lecompte & Mehrvar 2017). Tchobanoglous *et al.* (2003) explained the waste can be easily treatable by biological means if the BOD: COD ratio is more than 0.5; in this study, the ratio was 0.57. Complete mix anaerobic digesters were tested at the lab scale and the treatment results and gas generation are reported in the next sections. Although the C:N ratio is low in our raw wastewater analysis, some anaerobic digestion studies have demonstrated that C/N ratios as low as 10–20 had good results, likely due to the biodegradability of the carbon (Lin *et al.* 2019).

Effluent quality and removal efficiency of the anaerobic digestion

The suspended growth process of anaerobic treatment is used for industrial wastewater treatment (Tchobanoglous *et al.* 2003). The batch complete mix anaerobic digester was used at lab scale in the treatment of SHWW. When the retention time of the waste (solid and hydraulic retention time) are the same, in the range of 15–30 days, sufficient safety for operation and process stability is provided (Tchobanoglous *et al.* 2003). In this experiment, 20 days were selected. Table 1 shows the effluent quality of the SHWW after anaerobic digestion at 37 °C.

From the results, most of the parameters have been reduced at a substantial level and the efficiency is summarized in Figure 2. In this particular experiment, although the removal was high, the effluent needed additional treatment in series as preliminary, post anaerobic treatment to ensure its safe disposal to the environment or for reuse.

Table 2 shows the World Bank (1999) effluent discharge quality requirement from meat processing and rendering industries to directly discharge into surface waters. In order to achieve such levels, separation of product (by-product) from wastes at each stage is essential for maximizing product recovery and reducing waste loads.

Methane generation from lab-scale anaerobic reactor

Average daily gas production from the reactors during the experiment is shown in Table 3.

Figure 3 presents the daily methane gas generated from the experimental setup at standard temperature and pressure (STP) (0 °C and 1 atmosphere).

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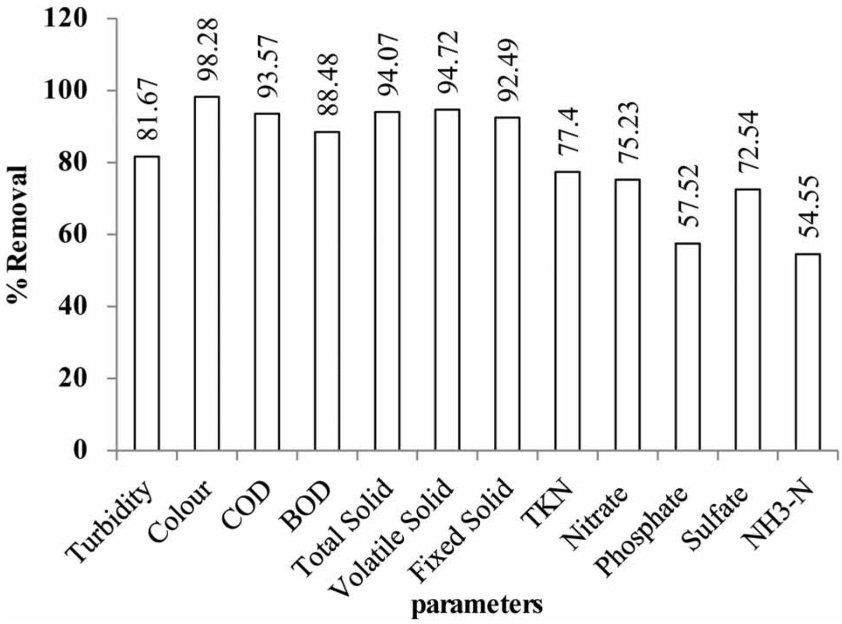

Figure 2 | Overall removal efficiency of the wastewater parameter after anaerobic digestion.

Table 3 | Daily volume of methane gas generated in the reactor from 29/6/2017 to 17/7/2017

Days	Volume of Gas/ day ($\bar{X}$ + SD), (mL/d)	Temperature (°C) of the gas collection	The volume of methane at standard temperature and pressure (STP) (mL/d)
1	330 ± 42.4	23	267.4
2	2,070 ± 14.1	22	1,683.0
3	2,780 ± 113.1	22	2,260.0
4	4,215 ± 545	23	3,415.0
5	1,950 ± 71	22	1,585.2
6	860 ± 198	23.5	696.0
7	745 ± 78	22	606.0
8	540 ± 184	21	441.0
9	750 ± 212.1	23	608.0
10	355 ± 347	23	288.0
11	230 ± 99	23	186.3
12	240 ± 226.3	22	195.1
13	160 ± 57	22	130.1
14	140 ± 14.1	23	113.4
15	150 ± 28.3	21	122.4
16	180 ± 99	22	146.3
17	150 ± 14.1	23	122.0
18	100 ± 28.3	23	81.0
19	57.5 ± 3.5	23	47.0
20	17.5 ± 3.5	23	14.1

The experimental result was the average of two sets of measurements ($\bar{X}$ + SD).

The daily methane production in Figure 3 shows there was a very short lag phase. Gas production increased sharply from the 2nd day and continued until it reached a peak value of 3,415 mL/d on the 4th day. The gas production decreased drastically and reached 113.4 mL/d on the 14th day. After the

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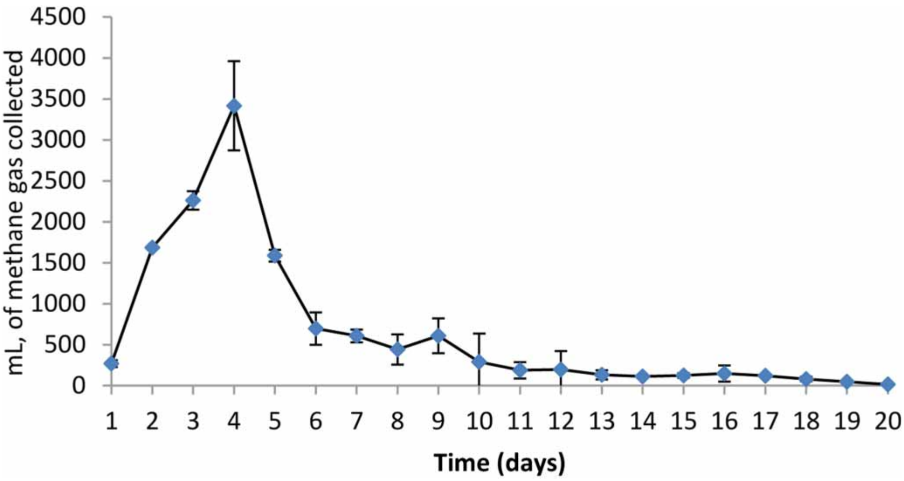

Figure 3 | The average (from two reactors in parallel) daily volume of methane gas generated from Day 1 to Day 20 at STP. The experiment was conducted at 37 °C.

14th day, there were fluctuations in the gas production until it reached stability, approximately 14.1 mL/d on the 20th day of operation. The highest gas production in one day was 3,415 mL from 2 litres of slaughterhouse wastewater taken in this experiment.

The cumulative gas production, with a time of digestion for the digester, is shown in Figure 4. The total methane gas production within 20 days' digestion time was 13 litres at STP. According to this finding, the corrected biomethane potential was 270.6 mL CH_4/g TVS (270.6 mL of methane gas per 1 gram volatile solids). The biomethane potential yield of slaughterhouse wastewater in this study was in agreement with the Filer *et al.*(2019) report that methane yield for different test materials ranges from about 250 ml–350 mL per gram of volatile solids.

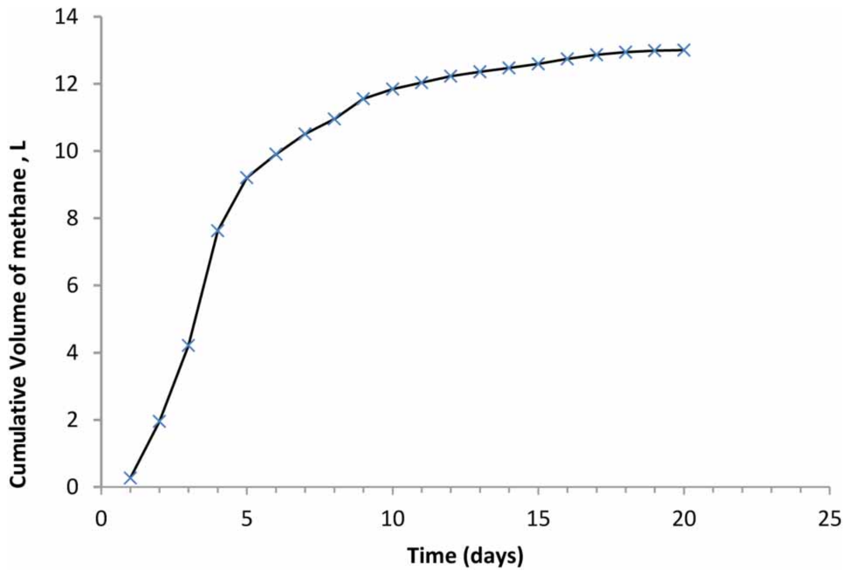

Figure 4 | Cumulative methane gas production at STP.

In general, the production of methane might have been hindered by the presence of high lipid content and ammonia in the reactor, which limited the methane-producing bacteria, since the slaughterhouse wastewater has a low C:N ratio. Co-digestion of slaughterhouse wastewater with other carbon substrates is required to change the carbon-nitrogen ratio and improve volumetric methane

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productivities (Banks & Heaven 2013). The cow dung disposed of along with the municipal solid waste can be a potential source for carbon and reduces the solid waste transport cost for Arba Minch town where the slaughterhouse is located.

CONCLUSIONS

According to the results, the slaughterhouse of Arba Minch town releases untreated wastewater to the environment with a COD of 19,253 mg/L. The lab-based anaerobic reactor demonstrated an average removal of TKN, BOD_5, COD, TS, TVS, and turbidity of 77.4, 88.5, 93.6, 94.1, 94.7, and 81.7%, respectively. The biomethane generation rate was 270.6 ml CH_4 per gram of volatile solids. From the study, it is possible to apply anaerobic digesters to treat slaughterhouse wastewater in combination with relevant treatment facilities before and after the digester to meet effluent standards for safe discharge to the environment and production of an energy source.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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