Full Length Research Paper

Two-stage anaerobic sequence batch digestion of composite tannery wastewater

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Received 13 September, 2017; Accepted 24 October, 2017

Most of the leather industries in Ethiopia discharge their effluent partially or without any treatment to the nearby water bodies. This creates a serious effect on aquatic biota and surrounding environment due to its high organic loading and chromium content. To minimize the effect, tannery wastewater should be treated before the effluent is discharged to the environment. Therefore, the main objective of the study was to use a two stage laboratory scale Anaerobic Sequence Batch Digester (Reactor) in order to investigate the potential of composite tannery wastewater to produce biogas. Two Stage Anaerobic Sequence Batch Digester was used because it has a conducive environment for microorganisms at different temperature. The present study characterized composite tannery wastewater with respect to biogas production parameters. Four sets of conditions were investigated: Mesophilic to mesophilic; thermophilic to thermophilic; mesophilic to thermophilic and thermophilic to mesophilic in the hydrolysis/acidification and methanogenesis stages (reactors), respectively. The Organic Loading Rate (OLR) was ranged between 9.58 and 10.28 kg COD/m\textsuperscript{3} day throughout the study. The highest volume of biogas (7232 ml) and content of methane (69.75\%) was in the thermophilic-thermophilic phase. The removal efficiency of total solid (TS) and volatile solids (VS) of all digesters were in the range of 52 to 69 and 58 to 81\%, respectively, treatment of composite tannery wastewater by a two stage Anaerobic Sequence Batch Digester (Reactor) (ASBR) produces high amount of methane at thermophilic - thermophilic phase and the lowest produced in mesophilic- mesophilic phase. Digesters in mesophilic-thermophilic ($D_3$) produced higher biogas and biogas quality than digesters with thermophilic- mesophilic ($D_4$) ones.

Key words: Anaerobic sequential batch reactor, wastewater to energy, tannery wastewater management.

INTRODUCTION

With a rapidly expanding human population and a growing trend of industrial development added with limited technological advancement, problems related to the management of industrial waste have become a
major problem in Ethiopia (Leta et al., 2004). In Ethiopia, there are more than 20 tanneries (EPA, 2003). Accumulation of large volumes of dried-sludge in treatment compound has become common (Leta et al., 2004). This has immediate public health implications, which are manifested as frequent outbreak of major epidemic diseases and also contributes to climate change as it releases greenhouse gases; methane and carbon dioxide (Abera, 2010).

The industrial strategic development plan of Ethiopia gives great emphasis to improve export-led products to join the international market in large-scale such as leather products. Emphasis has been given to ensure faster and sustained development of the industry sector in Ethiopia in the next five years of the Growth and Transformation Plan.

Industry zone development as the potentially suitable towns and cities of the country has been found as an irreplaceable measure to create favorable conditions for implementing industry development in the country. However, in Ethiopia most of the leather industries discharge their effluent without any treatment to nearby rivers (EPA, 2003; Leta et al., 2004). This creates a serious effect on aquatic biota and the surrounding environment.

The main objective of this study was to investigate the potential of composite tannery wastewater to produce biogas using a two stage laboratory scale Anaerobic Sequence Batch Digester (Reactor) (ASBR). Two stage ASBR was used because it has a conducive environment for micro-organisms at different temperature, and it reduces the effect of shock loadings to the methanogenic reactor, and increases the stability of the two phase system.

MATERIALS AND METHODS

Sample collection and preparation

Tannery wastewater samples were collected from Modjo Tannery, central Ethiopia using different size plastic bags every seven days for three months. The samples were collected from three different effluent lines which included the sulfur line; chrome line and general wastewater line. Five hundred cubic meter of wastewater is released from Mojo Tannery per day, from this, 160 m³ is from the sulfide line, 100 m³ is from chrome line and the rest 240 m³ is from general wastewater line in the ratio of 1.6:1:2:4, respectively. Using measuring cylinder based on the aforementioned ratio, the composite samples were prepared at Modjo Tannery, and for every seven days, 20 L composite sample was prepared and transported to the research lab at the Centre for Environmental Sciences, Addis Ababa University, and stored at 4°C in the refrigerator until it was added to the digester for treatment.

Experimental set up of the two-stage anaerobic sequential batch reactor (ASBR)

Two parallel anaerobic digestion systems consisting of four ASBRs in series were tested. The temperatures in this study were controlled at mesophilic condition (35±2°C), and at thermophilic condition (55±2°C). The two reactors in the first system were operated at the same temperature, D1:35°C and 35°C (mesophilic) and D2: 55°C and 55°C (thermophilic), respectively. The two reactors in the second system were at two different temperatures D3: 35°C and 55°C (mesophilic to thermophilic) and D4: 55°C and 35°C (thermophilic to mesophilic), respectively. Each reactor had a total liquid volume of 2.8 L. Totally eight reactors were prepared to observe the potential of composite tannery wastewater for production of biogas. In this experiment, each treatment was run in triplicates. The system was adapted from Dugba and Zhang (1999).

To create the anaerobic condition, the bottles were covered by rubber stopper having two hoses at the top and sealed with a gas kit maker to make oxygen free environment inside the digester. The two hoses on the top of the bottles had different purposes. In the first stage (acidogenic), the first hose was stretched up to the bottom of the solution enabling decanting of all the solution to the second stage (methanogenesis). While the second hose was placed above the solution for transferring the produced gas to gas collector plastic bag.

In a similar manner, the second phase (methanogenesis) reactors had also two hoses at the top. The first hose was immersed up to half-height of the reactor and used for filling of the solution from the first stage and decanting the solution. While the second hose was above the solution, and it channeled the produced gas to the plastic bag, which was used to collect the produced biogas (Figure 1).

The objective of the acidogenic reactor was to acidify the composite tannery wastewater in an effort to improve the performance of the methanogenic reactors. The acidogenic reactor was fed the composite tannery wastewater from Mojo Tannery.

Operation of the ASBR

The study was conducted for 90 days (3 months) in two different operational phases. The first phase at the start-up period of the ASBR was operated for 30 days from January 3/2013 to February 2/2013. This time was assigned for accumulation of biomass. During this period, the digester was operated in 24 h cycle mode, whereas 20 h was given for the reaction phase (T₁) and 3 h given for settling (T₂).

To have a good biomass settlement, the supernatant was manually decanted from the upper most of the reactor for 30 min with the help of pump drivers (PD 5206) at a speed of 606 rpm. Batch feeding was performed mechanically through the top of the reactor at the beginning of the next cycle for 30 min at the same speed as the substrate was decanted.

During the second phase, the ASBR was operated for 60 days (2 months) from February 3 to April 2, 2013, with a different cycle time from the first phase. The reactors were operated at a 48 h cycle mode, where 46 h was given for the reaction period (T₁), 1 h for settling (T₂) and the remaining 1 h was for filling and decanting, operated in the same way as in the first phase.

The organic loading rate (OLR) for the two-stage ASBR was adjusted to be steady. However, due to fluctuations in the composition of the raw wastewater, it was difficult to maintain a steady OLR, and the actual OLR was between 9.58 to 10.28 kg COD/m³-day throughout the study.

Chemical analysis

The characteristic of the composite tannery wastewater as feedstock for biogas production was carried out using its total solids
Figure 1. The two-stage ASBR setup.

### RESULTS AND DISCUSSION

#### Characterization of Modjo Tannery Composite wastewater in terms of biogas production parameters

The averaged values of TS, VS and C/N ratio of the feedstock are presented in Table 1. The average moisture content of the composite tannery wastewater was 91.94%. The average TS of the substrate was 8.06%, the appropriate amount of the feedstock as the most favorable percentage of TS for biogas production is in the range of 8 to 10% (Jurgen et al., 2009; Ituen et al., 2007). In general, the TS value for composite tannery wastewater in this study varied from 5.64 to 10.68%.

Out of the TS, VS and ash (fixed solids) content of the substrate were 73.23% and 26.77%, respectively. As VS is organic material that can be decomposed, it shows the potential for further digestion. This indicates that a large fraction of composite tannery wastewater is biodegradable, and thus it can serve as an important feedstock for biogas production (Table 1).

The average value of OC% and TN% in this study were 40.68 and 1.53, respectively. Methane yield and its production rates are highly influenced by the balance of carbon and nitrogen in the feeding material. The average C/N ratio in this experiment was 26.58, which is similar to the value 20:1 to 30:1 reported by Dahlman and Forst (2001). Pyle (1978) stated that optimum C/N ratio recommended for an anaerobic digester was 10 to 30. Generally, the balance of carbon and nitrogen of the

#### Biogas quantity and quality determination

The volume of biogas produced was measured when the supernatant was manually decanted and at the starting of the next cycle by directly measuring the volume of the gas collector bag. The sum of the total volume of the acidogenic and methanogenic reactors gave the total biogas produced by the system. Quality of biogas was measured by biogas analyzer weekly until the gas produced plateaued. The plastic bag which was filled by the biogas during measuring the volume was directly connected to the calibrated biogas analyzer, and the percentage of methane was displayed on the analyzer.

| Parameter          | Composite tannery wastewater |
|--------------------|------------------------------|
| Moisture content (%) | 91.94                        |
| TS (%)             | 8.06                         |
| VS (% based on TS) | 73.23                        |
| Ash (% based on TS)| 26.77                        |
| OC (%)             | 40.68                        |
| TN (%)             | 1.53                         |
| C/N ratio          | 26.58                        |
| pH                 | 9.49±1.15                    |

(TS), volatile solids (VS), total nitrogen (TN) and carbon and nitrogen contents by APHA 20E 4500 NB (1999) instruction.
Hydraulic retention time  

Figure 2. Average pH values in the acidogenic phase.

The composite tannery wastewater was good to be used for anaerobic digestion to produce biogas.

**Characteristics of digesters**

**pH**

The pH of each digester was maintained between 6.02 to 7.66. The findings for both reactors are shown in Figures 2 and 3.

**Acidogenic reactor**

In the first week of the startup period, the pH of the substrate in the reactor was between 9.49 to 8.13, in the following weeks the pH decreased. This is due to the formation of acids by acidogenic bacteria during the incubation period. Figure 2 shows the average result of the pH in acidogenic reactor.

As seen Figures 1 and 2, the pH of all the digesters showed a sharp decline at the beginning of the digestion period and kept declining up to the 45th day of fermentation. The decrease in pH is a function of the concentration of volatile fatty acids produced by the activity of hydrolytic acidogenic bacteria capable of degrading the feedstock in the first few days of incubation, bicarbonate alkalinity of the system, and the amount of carbon dioxide produced (Gomes and Speece 2003).

Nina et al. (2011) verified that the presence of fat can raise the formation of volatile fatty acids, leading to a fall in pH. The composite tannery wastewater from raw hide and skins processing at Modjo Tannery contains high amount of fat, therefore in this study there was no need of adding or adjusting substances like lime, ash or ammonia (pretreatment methods) as the gas producing bacteria was able to ferment the acid or alkali, and restore balance as reported by Saxon (1998).

Generally, the average value of the pH in the acidogenic reactor in this study was 6.02±0.51 almost similar to the value of 5.7 to 5.8 reported by Kasapgil et al. (1995) as best pH for the acid phase reactor. According to Antonopoulou et al. (2008), the optimum pH value for acid producing bacteria is from 5 to 6.

**Methanogenic reactor**

The methanogenic reactor was fed the acidified composite tannery wastewater from the acidogenic reactor. Figure 3 shows the average result of pH in the methanogenic reactor. As seen in Figure 3 after 21 days, the pH of the reactors increased which is an indication of the digestion of volatile acid and nitrogen compounds, and more methane being produced. The production of acids and its digestion continued up to 56 days of digestion, and the pH remained constant after the 56 days which is due to the presence of larger number of methanogenic bacteria in the reactor, and almost all the
acidic wastewater fed from the Acidic Reactor present could be digested to form methane and carbon dioxide gases. The pH of the methanogenesis reactor in this study ranged from 6.30 to 7.66, and with an average pH of 7.26±0.3 over the duration of the study. pH range of 6 to 7 is very suitable for optimum biogas production due to the normal functioning of methanogenic bacteria in this pH range (Ozmen and Aslanzadeh, 2009). According to Antonopoulou et al. (2008), the optimum pH in the reactor should be in the range of 6.5 to 8, as this is suitable for acetogens and methanogens. Generally, this study revealed that it is possible to produce biogas from composite tannery wastewater at pH range of 6.02±0.51 in acidogenic phase and 7.26±0.3 in methanogenic phase.

**Quantity and quality of biogas production**

Biogas production and its methane content were measured for about thirteen weeks of digestion period until the gas production became stable. The quantity and quality of biogas production for the two phase system were estimated by adding the daily biogas produced from the acidogenic and methanogenic reactors. Although the acidogenic reactor produced a fair amount of biogas, the methane content was fairly low (less than 10%), and therefore contributed very little to the overall biogas methane content.

**Quantity of biogas produced**

It was found that D2 (thermophilic-thermophilic) produced the highest volume of biogas (365 mL) in the first week of the digestion. During this period, the other digesters produced below 200 mL as indicated in Figure 4.

The average daily biogas production (volume) in almost all digesters increased persistently up to the six week and for D1 (mesophilic - mesophilic), the increment continued up to the eighth week. As the increase in temperature has a positive effect on biogas yield, most of the biogas was produced at thermophilic phase. Moreover, in the mesophilic range, the bacterial activity and growth decrease by one half for each 10°C drop below 35°C (Hulshoff, 1995). Thus, for a given degree of digestion to be attained, the lower the temperature, the longer is the digestion time.

There was a continual growth in biogas production with temperature increase as shown in Figure 4. Thus, the digesters in thermophilic temperature range produced more biogas and they reached their stable period within shorter time than the mesophilic ones. The highest values of the daily biogas production in each digester in their stable period were: 156, 699, 344 and 292 mL respectively from D1 to D4. D2 (thermophilic-thermophilic) produced highest amount of biogas and stable production within the six weeks, and D1 (mesophilic – mesophilic) produced the lowest amount of biogas and reached stable period within eight weeks. The acetogenic and methanogenic stages act as continuous reactors, which results in constant gas production (Chaudhary, 2008).

The average chemical oxygen demand (COD) after anaerobic digestion of composite tannery wastewater was 5100, 3550, 3850 and 4650mg/l, respectively from D1 to D4. Considerable removal efficiencies for COD were achieved (57.42, 70.36, 67.86 and 61.18%, respectively), recorded from D1 to D4. The main reason
for good removal of COD could be related to the presence of optimum environmental conditions such as temperature and pH required for anaerobic acetogenic and methanogenic bacteria. According to Metcalf and Eddy (1991), environmental factors that affect biological organic matter removal are pH and inhibitory substances.

The highest total biogas (7232 mL) was produced by D₂, and D₁ produced the least biogas (4153 mL). Digesters D₃ and D₄ produced 6375 and 5975 mL, respectively. The results of this study indicate that the highest total biogas production was in thermophilic-thermophilic relationship than the mesophilic-mesophilic. It is well known that the anaerobic biodegradation is faster in thermophilic reactors (Gómez, 2011). Idnani and Varadarajan (1974) observed that there was less gas production below 30°C, and the optimum temperature for biogas production was 40 to 50°C.

Further, the results of this study indicate that the mesophilic to thermophilic digesters produced high biogas than the thermophilic to mesophilic reactors. This is due to stability of microorganisms and change in temperature. Bolzonella et al. (2005) reported that thermophilic microorganisms are less stable than mesophilic ones, and Anonymous (1992) indicated that a stable temperature is very important to maintain gas production as the bacteria are very sensitive to changes in temperature.

**Quality of biogas produced**

The acidogenic reactor produced more biogas than the methanogenesis, of which the methane content was insignificant (less than 10%). Zhang et al. (2010) showed that during the two phase sludge anaerobic digestion, the sludge was hydrolyzed and acidified in the first phase, and then methane was produced in the second stage.

In connection to this, Ince (1998) and Kasapgil et al. (1995) reported methane percentages of 5 to 15 and 7 to 27 in acidogenic reactor. In this study D₂ in the acidogenic phase produced 22.4% of methane. This is due to high temperature (55°C) in the acidogenic phase. Due to the low methane content of the biogas from the acidogenic reactor and the high methane content in the biogas of the methanogenic reactor, it can be concluded that separation of the two phases was essential. Figure 5 shows the weekly biogas quality (Methane, CH₄) produced by each digester.

The quality of biogas produced by each reactor for four weeks of digestion period was below 50%. In this study, D₂ (thermophilic-thermophilic) reached stable production within six weeks whereas D₁ (mesophilic – mesophilic) reached its stable period within eight weeks. The growth rates of thermophilic methanogens are 2 to 3 times longer than those of mesophilic ones (Van Lier et al., 1993; Mladenovska and Ahring, 2000).

The percentage composition of methane was between 51 to 69 during the operational period. These values agree with the theoretical yield of 50 to 75% as suggested by Yadava and Hesse (1981). The highest cumulative biogas quality was produced by D₂ (69.75), and the lowest cumulative biogas quality was produced by D₁ (51.42). Digesters D₃ and D₄ produced cumulative

![Figure 4. Comparison of the average daily biogas production.](image-url)
biogas quality were 62.86 and 56.18, respectively. The thermophilic process is the most efficient in terms of organic matter removal and methane production (Zabranska et al., 2000; Ahring et al., 2003). The reason for this is that the growth rates of thermophilic methanogens are higher than those of mesophilic methanogens (Duan and Mao, 2006).

The results of this study shows the possibility of producing high quantity and quality biogas from composite tannery wastewater without addition of other co-digesters except starter (inoculants). The highest cumulative biogas 7232 mL and biogas quality 69.75% yield was recorded for the digester at thermophilic phase, which is represented by D$_2$ in this study.

Characteristics of the effluent

**Physical properties of the effluent**

The amounts of TS and VS that were retained in each of the digesters were measured. Table 2 shows the average values of percentages of TS and VS of the effluent of each digester.

![Figure 5. The weekly percentage of methane in biogas for each digester.](image)

**Table 2. The value of TS and VS after AD.**

| Treatment | TS (%) | %VS  | Removal efficiency of %TS | Removal efficiency of % VS |
|-----------|--------|------|---------------------------|---------------------------|
| D$_1$     | 3.79   | 30.56| 52.97                     | 58.27                     |
| D$_2$     | 1.76   | 13.87| 78.16                     | 81.05                     |
| D$_3$     | 2.47   | 18.32| 69.35                     | 74.98                     |
| D$_4$     | 3.37   | 25.47| 58.18                     | 65.21                     |

About 52.97, 78.16, 69.35 and 58.18% reduction of TS was observed for digesters D$_1$ to D$_4$, respectively. High and low removal efficiency of TS (%) was seen for D$_2$ (thermophilic-thermophilic) and D$_1$ (mesophilic-mesophilic). In mesophilic range, a considerable proportion of solid compounds are recalcitrant, which leads to poorer efficiencies in inorganic solids removal and methane production (Braber, 1995). As seen in Table 2, percent reduction in VS were 58.27, 81.05, 74.98 and 65.21, respectively for digesters D$_1$, D$_2$, D$_3$ and D$_4$. Similarly, high and low removal efficiency of VS was seen for D$_2$ (thermophilic-thermophilic) and D$_1$ (mesophilic-mesophilic).

According to Tsegaye et al. (2016), the relative higher removal efficiency of VS (%) than the TS (%) was a very good indication of high uptake rate of the organic fraction of total solids and the effectiveness of the anaerobic reactor. The ratio of VS/TS before digestion was always relatively higher than the ratio after digestion, which is an indication of the utilization of the organic fraction during the anaerobic digestion. The VS/TS ratio in this study before digestion was 9.08 while after digestion 8.06, 7.88, 7.41 and 7.55 were observed for digesters D$_1$ to D$_4$, respectively.
Table 3. Average pH of the effluent.

| Digester | D1  | D2  | D3  | D4  |
|----------|-----|-----|-----|-----|
| pH       | 7.18| 7.59| 7.61| 7.67|

**pH of the effluent**

The average pH value of the effluent for each digester is summarized in Table 3. The average pH value of the effluent varied from 7.18 to 7.67. The minimum and maximum pH accepted values for slurry was 6.0 and 8.5, respectively (Fokhrul, 2009). In addition, Williams (1998) reported the values lie in the range of the pH of the compost 6 to 7.

**Conclusion**

The current study demonstrated that composite tannery wastewater has a great potential for the production of biogas using two stage anaerobic sequential batch reactors under thermophilic conditions which create alternative source of renewable energy. Moreover, this system of managing wastewater significantly contributes towards resource-recovery and pollution management around tannery industry.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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