



Biogas Production from Co-digestion of Cotton Yarn Waste and Human Urine

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Authors' contributions

This work was carried out in collaboration among all authors. Author MT designed the study, performed the experiment, wrote the protocol and wrote the draft of the manuscript. Authors MM and EON managed the analyses of the study. Author XB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the feasibility of producing biogas from anaerobic co-digestion of cotton yarn wastes (CY) and human urine (HU) using fresh cow dung as the inoculum.

Study Design: Anaerobic co-digestion of CY waste and HU and CY waste alone were done using batch reactors.

Place and Duration of Study: CY were collected from Rivatex Eastern Africa Limited, Eldoret, Kenya while fresh cow manure used as inoculum was collected from a farm at Moi University, Eldoret, Kenya. Human urine sample was collected in a clean sterile container at Moi University hostel, Eldoret, Kenya. The experimental set up and analyses were performed at Chemical and Process Engineering Laboratory, Moi University, Kenya between January 2020 and May 2020.

Methodology: CY, HU and fresh cow dung were subjected to physicochemical analysis. Batch anaerobic co-digestion of CY and HU, and CY alone were carried out under ambient temperature ($25 \pm 3^\circ\text{C}$) conditions for 95 days and 37 days, respectively.

Results: The CY contained 90.46% total solids, 77.12% volatile solids and 9.54% moisture content while the corresponding values for HU were 2.9%, 58.5% and 97.1%, respectively. CY had a high

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carbon to nitrogen ratio. The biogas yield from anaerobic co-digestion was 35.6% more than digestion of CY alone. The highest daily biogas production for co-digestion and digestion of CY alone were 330 mL and 386 mL on day 12 and 21, respectively. The total biogas yield when CY co-digested for 95 days was 10,125 mL which decreased to 6,519 mL without co-digestion after 37 days.

Conclusion: Our results showed that co-digestion produced more biogas than digestion of CY alone. Conclusively, the presence of HU during anaerobic digestion of CY enhanced the biogas production by more than 35.6% demonstrating that HU could be an effective waste for co-digestion of solid wastes such as CY. Further research should focus on monitoring parameters like temperature, buffering capacity and fatty acid levels to ensure optimal efficiency and maximum biogas yield.

Keywords: Batch reactor; textile waste; total solids; moisture content; carbon to nitrogen ratio.

1. INTRODUCTION

Since the beginning of industrial revolution, the required energy for developing industries has been extremely increasing worldwide. Population growth and promotion of living standards have always been one of the key drivers of increased energy demand and fiber consumption [1]. Globally, energy demand is rising steadily although fossil fuels are still dominating the energy market [2]. However, increasing world population along with depletion of fossil fuels reserves have resulted in an interest to gradually change from fossil energy to renewable energy [3]. Additionally, environmental pollution caused by dumping or landfilling of organic wastes in the environment is among the most crucial issues the world is facing today. Currently, textile wastes management involves reusing them as second-hand textiles, textile filling materials in industry, composting, landfilling, and direct burning [4]. Therefore, the annual global production of end life textile wastes is absolutely increasing, causing an increased interest in the impact of the disposed wastes on the environment. However, textile wastes are enriched sources of energy and materials. Textile wastes include wastes from streams of fiber, textile and cloth manufacturing processes, commercial services and consumption [5]. Textile wastes mainly consist of cotton and viscose fibers. Previous studies showed that cotton have a significant potential to be used as a substrate for the production of different bioenergy such as biogas [6,7]. However, cotton yarn wastes (CY) has a high carbon to nitrogen (C/N) ratio which is beyond the suitable range for biogas production. To stabilize the C/N ratio, CY have to be co-digested with another substrate with high nitrogen content such as human urine (HU) or animal manure [8].

Human urine consists primarily of water (91% to 96%), with organic solutes including urea, creatinine, uric acid, and trace amounts of enzymes, carbohydrates, hormones, fatty acids, and inorganic ions such as sodium, potassium, chloride, magnesium, calcium, ammonium, sulfates, and phosphates [8-10] (Table 1). The nutrient content of organic substrates determines their biogas quality and quantity. Macronutrient elements must be present in the substrate for microbial growth to occur [11]. Additionally, maximum biogas yield is dependent on adequate and efficient nutrient supply of microorganisms in the digester [11,12]. Biogas could be produced by anaerobic decomposition of any degradable organic wastes such cotton wastes, cow dung, and human wastes [13]. Biogas is composed mainly (60-70%) of methane and 30-40% of carbon dioxide [14]. The pH of urine is also favorable for biogas production by anaerobic digestion (AD) process [14,15]. The C/N ratio of the raw material(s) is/are an important factor for biogas production [16]. It is commonly recognized that a C/N ratio of 20-30:1 is acceptable [17]. Lack of nutrients required by methanogens causes AD failure [18]. AD is a complex biochemical process that involves sequential hydrolysis as a rate-limiting step, acidogenesis, acetogenesis, and methanogenesis [19,20]. The process is mediated by a diverse and complex microbial community which requires different optimum conditions to grow. AD is influenced by different factors such as pH, C/N ratio, temperature, concentration of free ammonia, total solids (TS), and volatile solids (VS) [21]. In addition, the process is sensitive to changes in pH, temperature, microcommunity composition, presence of inhibitory substrates and micropollutants. Therefore, an efficient AD process requires a delicate balance of microbial groups, substrate composition and optimum

operating conditions [22]. AD has been proven as an efficient technology for bioenergy production. The use of different feedstocks can influence the AD process stability [23]. This is because organic substrates vary in their physicochemical characteristics especially the C/N ratio [11].

Cotton yarn wastes provide nutrients while the addition of other organic wastes could increase the biogas production. Therefore, HU could have a potential to increase biogas production rate. Additionally, HU is a suitable substrate for co-digestion with CY so as to stabilize the C/N ratio and improve biogas production [24]. Residues left after digestion are good fertilizers rich in nitrogen, phosphorous and potassium [15,23]. In most cases, a stable AD process is maintained by the use of buffers [25]. However, for optimal C/N ratio and pH, diverse substrates are mixed for better biogas production [26]. More balanced macronutrients in anaerobic co-digestion process increases buffering capacity which could be sufficient to maintain AD stable [16,27]. On the other hand, anaerobic co-digestion of organic materials has gained acceptance because the process can remain stable and efficient without the use of chemical substances [28]. However, establishing the right feedstock combination for anaerobic co-digestion has a challenge and can significantly influence biogas production [29]. A different perspective on this will involve natural substances and waste materials with relevant or similar composition as the chemical buffers to provide an alternative option, increase nutrients and moisture content for stable AD [30-32].

Table 1. Physicochemical composition of human urine

| Components | Quantity | References |
|------------------|-----------|------------|
| Water (%) | 91-96 | [10,33] |
| Urea (g/L) | 9.3-23.3 | [9,34] |
| Nitrogen (%) | 80-90 | [16,34] |
| Ammonia (%) | 7 | [20,35] |
| C/N ratio | 2.58-4.8 | [35,36] |
| pH | 5.2-6.5 | [9,37] |
| Creatinine (g/L) | 0.67-2.15 | [9,38] |
| Potassium (g/L) | 0.75-2.62 | [39] |

There is no research in open literature that focused on CY AD to produce biogas. Isci and Demirer [6] studied the anaerobic treatability and methane generation potential of different cotton wastes in batch reactors. Their results indicated that cotton wastes can be treated anaerobically

and is a suitable substrate of biogas. Given its large potential for biogas production, cotton certainly merits more research attention for being used as a feedstock in digestion with various substrates. Rasel et al. [40] studied cotton (spinning, knitting and cutting) wastes proper utilization via biogas production. However, to the best of our knowledge, no previous study examined CY and HU as a potential substrate for biogas production. The objective of this research was therefore, to investigate the potential of producing biogas from CY and HU using batch anaerobic co-digestion process.

2. MATERIALS AND METHODS

The CY and HU were the substrate employed in this study. CY samples were collected from Rivatex Eastern Africa Limited, Eldoret, Kenya while the fresh cow manure used as inoculum was collected from a farm at Moi University, Eldoret, Kenya. Human urine sample was collected in a clean sterile container from a volunteer at Moi University hostel, Eldoret, Kenya. The volunteer did not take any drugs for 1 week prior to sampling period to avoid the effect of pharmaceutical loading on urine that could affect bacterial activities on biogas production. The CY were cut into small pieces using a pair of scissors to facilitate biodegradation. The cut samples were kept in the laboratory at ambient conditions for one week (Fig. 1). Measured 20% of total volume working reactor was used as inoculum. The inoculum was kept in a refrigerator at 4°C for two days and was used without any further treatment.

The physicochemical properties of CY, HU and inoculum were characterized before digestion and the mixtures loaded were prepared according to those characterizations. The TS, VS, and MC were analysed according to standard Methods 2540 [41] (Fig. 2). Kheldahl method was used to determine the total nitrogen content. Total carbon analysis was done using Walkey-Black potassium dichromate method [42,43]. The pH was analysed using pH-009(I) pen type pH meter. The experiment was carried out in batch type laboratory scale reactors at Chemical and Process Engineering Laboratory, Moi University, Kenya between January 2020 and April 2020. The digesters of 2 L total volume, 12 cm diameter and 25 cm height each made of aspiration plastic bottles were used for biogas production [4]. All the digesters with 75% working volume (1.5 kg) were run concurrently. These

digesters were closed with suitable rubber plugs and some holes were dispersedly drilled in the center of the plugs for water displacement and biogas collection. The flexible rubber piper and syringes were employed to pass water in and out of the conical flask (1000 mL) for displaced water measurements. The digesters were sealed and then arranged for the entire setup. The substrate were mixed with inoculum (needed only at initial stage), fed to the digesters and then digesters were closed. The batch digesters were buried in a bucket filled with saw dust at a depth of 30 cm to minimize temperature fluctuations during the day and at night.

The volume of biogas collected was measured by water displacement method daily for 37 days (single digestion) and 95 days for co-digestion. The operating parameters of the digester were controlled so as to enhance microbial activity and thus increase AD efficiency. Evaluation of process parameters was done periodically in order to assess the efficiency of the anaerobic treatment. The substrates were loaded at TS content of 30% for both co-digestion and digestion of CY alone. Here, the mixing ratio was 1:2.5 i.e CY to water and CY to HU. This was done to investigate the effect of HU on biogas production. The experiments were run in triplicate. The operating temperature was $25 \pm 3^\circ\text{C}$ which was good for biogas production [44]. The pH was maintained at 7.2 ± 0.4 . The following equations were used to determine the TS, VS and MC. Both TC and MC were calculated on a wet basis.

$$TS = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (1)$$

$$VS = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \quad (2)$$

$$MC = \frac{\text{Weight of wet sample} - \text{Weight of dry sample}}{\text{Weight of wet sample}} \times 100 \quad (3)$$

Where W_1 = Weight of crucible, W_2 = Weight of wet material and crucible, W_3 = Weight of dry material and crucible at 105°C in the oven, W_4 = Weight of material and crucible after ignition at 550°C .

The TS content was 35.943 g and mixed loading material were 343 g, 857 g and 857 g for CY, HU and water, respectively. Each digester was then filled with 300 g of fresh cow dung as inoculum to get constant quantity of working volume. The biogas produced was standardized according to DIN 1343 (standard conditions; temperature (T) = $^\circ\text{C}$ and pressure (P) = 1,103 bar) [45]. The biogas volume was normalized using equation 4 [45,46].

$$V_N = \frac{V \times 273 \times (760 - P_w)}{(273 + T) \times 760} \quad (4)$$

Where V_N = volume of dry biogas at standard conditions (mL), V = biogas volume recorded (mL), P_w = vapour pressure of water (mmHg), T = room temperature (K).

Normally, the digester gas is saturated with water vapor. Therefore, the water vapour pressure was calculated according to the modified Buck equation (equation 5) [45,47].

$$P_w = P(T) = 4.58445 \times \exp \left(\frac{18.678 - \frac{T}{234.5}}{\frac{T}{257.14 + T}} \right) \quad (5)$$

From which P is the vapour pressure in mmHg and T is the temperature at the ambient space ($^\circ\text{C}$).



Fig. 1. CY waste samples (a) bulk sample (b) cut into small pieces

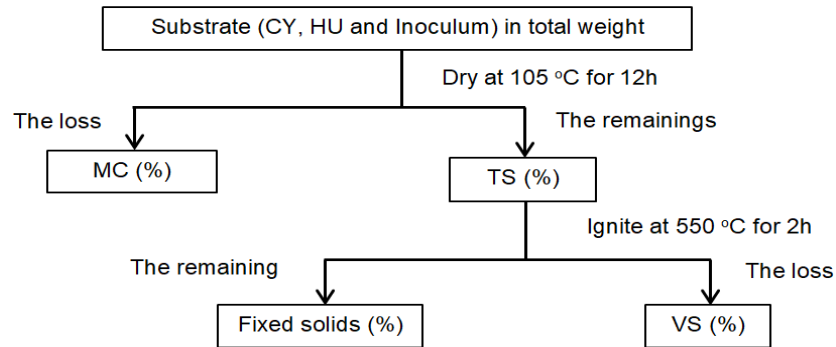


Fig. 2. Summary of the procedures followed in the analysis of CY, HU and inoculum

3. RESULTS AND DISCUSSION

3.1 The Physicochemical Characteristics of Substrates

The physicochemical characteristics of substrate and inoculum are given in Table 2. The characteristics of organic wastes determine the success of AD process (e.g. high biogas production potential and degradability). The pH in the digester ranged between 6.8 and 7.2. The TS and VS content were 90.46% and 77.12% for CY. These are in agreement with the suggested values for biogas production (70-95%) [48]. The TS and VS content of HU were 2.9% and 58.5% (Table 2). These results are within range of 2.4-3.4 and 49.5-62.5% of TS and VS respectively according to Colón et al. [35]. The MC were 9.54% and 97.1% for CY and HU, respectively. The MC result of HU agreed with the work of Dubey et al. [10] who reported that the MC of HU was in the range of 91-96%. The low MC of CY could have been due to its high solid content. The VS/TS ratio represents the degree of biodegradability of wastes. The VS/TS ratio was 85.25% for CY and this showed that a large fraction of it is biodegradable thus it can serve as a good feedstock for biogas production. The high VS/TS ratio of the substrate suggested the presence of sufficient amount of volatile organic substances in the feedstock to support fast hydrolysis process in AD. Gaur and Suthar [31] reported that biogas yield was directly related to the biodegradability of VS in the digester at the start of the process. Therefore, the appropriate level of VS could be mentioned in the digester at the beginning of the process. Thus, the feeding TS and VS were 35.943 g and 27.932 g. The TS content for feedstock in the digester was 30%.

Nitrogen content in the feedstock plays an important role in anaerobic process as it acts as

an important feed for microbial growth [49]. Unbalanced nutrients are regarded as an important factor limiting AD of organic wastes. The CY had high C/N ratio of 44.26. However, it has been previously reported that the optimum C/N ratio for AD of organic wastes is 20-30:1 [50]. Nevertheless, C/N ratios of the feedstocks are often much lower or higher than this [51]. To maintain the C/N ratio of the digester material at optimum levels, substrates with high C/N ratio can be co-digested with nutrient-rich organic wastes (low C/N ratio) like HU or animal manure [52]. The HU had a low C/N ratio of 4.56. This result was close to 4.8 reported by Zanta et al. [36]. Hence, co-digestion of the feedstock was done to improve the C/N ratio. Co-digestion is a cost-effective method that increases microbial activity to optimize AD. HU which was rich in nitrogen improved the C/N ratio of the feedstock, corroborating a previous observation [36].

3.2 Biogas Production

Biogas production during AD was calculated in order to evaluate the effect of HU on the AD of CY wastes. AD of CY alone (R_1) and co-digestion (R_2) started producing biogas in 4 hours. Gu et al. [53] reported that rapid production of biogas as in this study is due to the large amount of organic matter available in the digester. The fast production of biogas may also be associated with the capacity for adaptation to the AD process by microorganisms already present in the substrate. Biogas production increased until day 15 for co-digestion and day 10 for digestion of CY alone. Thereafter, it started decreasing until day 34 and day 69, respectively. Then, the production became slightly constant as shown in Fig. 3. Both digestion of CY alone and co-digestion showed similar trends of biogas production. They started with high production and sharply declined in 7 days.



Fig. 3. Full biogas set up

Table 2. Physicochemical characteristics of CY wastes, HU and inoculum

| Parameter | CY wastes | HU | Inoculum |
|-------------------|--------------|----------------|--------------|
| pH | 7.10 ± 0.20 | 6.10 ± 0.30 | 6.40 ± 0.20 |
| MC (%) | 9.54 ± 0.30 | 97.10 ± 0.40 | 90.64 ± 0.30 |
| TS (%) | 90.46 ± 0.20 | 2.90 ± 0.30 | 9.36 ± 0.20 |
| VS (%TS) | 77.12 ± 0.20 | 58.50 ± 0.30 | 85.64 ± 0.10 |
| Ash content (%TS) | 22.88 ± 0.30 | Not applicable | 14.36 ± 0.20 |
| C/N ratio | 44.26 ± 0.10 | 4.56 ± 0.20 | 21.50 ± 0.20 |

Values are presented as means ± standard deviations of triplicates.

The biogas production for the first days of digestion may be related to the easily biodegradable substrates that were present in the CY (high solid content, carbohydrates, proteins, and starch) [52,53]. Afterwards, the production began increasing up to the highest production volume.

The highest daily biogas production for R₁ and R₂ were 330 mL and 386 mL on day 12 and day 22, respectively. The average biogas yield for digestion of CY alone and co-digestion were 6,519 mL and 10,125 mL after 37 days and 95 days, respectively (Fig. 4). Biogas yield is closely related to the feedstocks characteristics (C/N ratio, pH, TS and VS), type of reactor, operation factors and microbial communities [54]. CY is a promising organic substrate for AD owing to its ease of digestibility. Nevertheless, the digestion of CY as the sole substrate can induce the accumulation of volatile fatty acids due to its high C/N ratio and there upon lead to bioreactor instability [55]. The high C/N ratio of CY can inhibit AD and lead the digester to a sour situation due to formation of more volatile fatty acids [56]. In general, CY is rich in

carbohydrates, has a high C/N ratio and is easily hydrolysable. Therefore, CY and HU complement each other as the latter is characterized by a low C/N ratio and low biogas production [57,58]. Further, it has adequate micronutrients, high moisture content and is alkaline [59].

3.3 Effect of Human Urine on Biogas Production

Analysis of results showed that the volume of biogas produced is important for controlling and monitoring the process of AD. A good biogas production reflects proper operation of the digester. The production of biogas in batch digester mode was recorded for 95 days of operation. This advance of AD could be justified by the effect of HU that had sufficient nutrients introduced into the digester [38]. This promoted rapid microbial growth and kept the microorganisms. It should be noted that the higher biogas production observed in batch mode is related to the increase in the methanogenic bacterial community [60]. Furthermore, the biogas production was continuous without interruption despite the

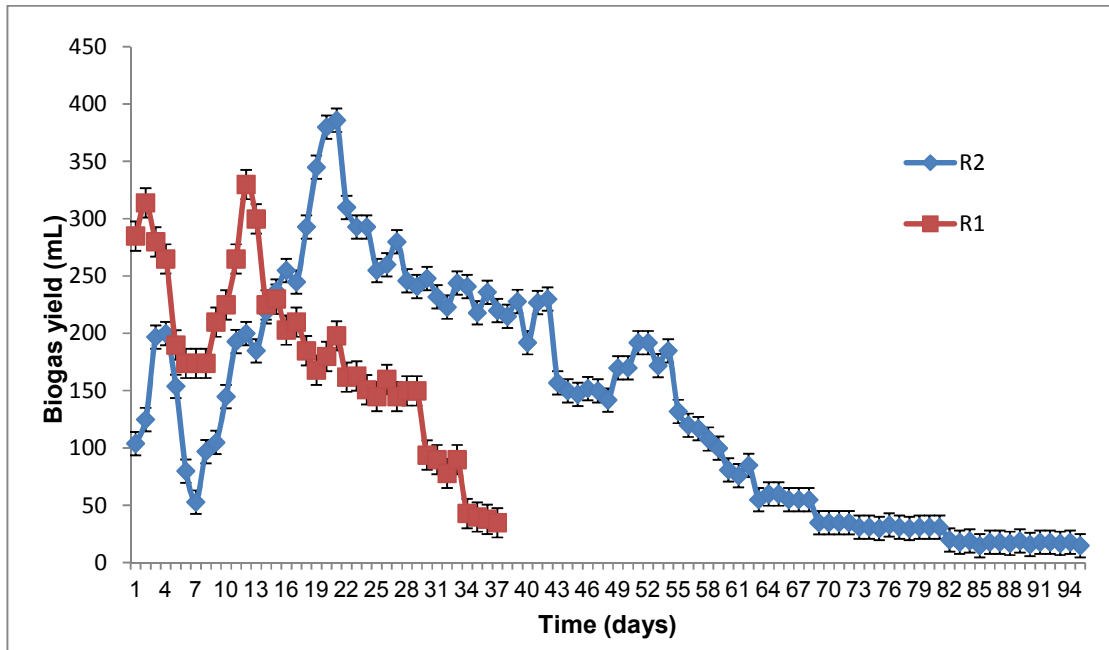


Fig. 4. Cumulative daily biogas production from anaerobic co-digestion and digestion of CY alone

differences in phases of loopbacks. This is linked to the maintenance of the methanogenic bacterial community in the digester. The average volumes of biogas produced were 6,519 mL and 10, 125 mL for digestion of CY alone and co-digestion. This explained the contribution of organic matter introduced by HU. The increase in the volume of biogas produced in this study shows that optimal biogas yield is produced by improving the nutritional composition of substrates. However, biogas production did not end at the same time. This could be because the carbon in the digesters were not equally degraded or converted to biogas through AD. This stability of the reaction medium despite the differences in phases of refeeding can be explained by the fact that the CY was buffered with HU in the digester [59]. This helped to stabilize pH which maintained the methanogenic bacteria responsible for the biogas production. According to previous authors [45,59], the resistance of methanogenic bacteria is closely related to the environment of the reaction process which is optimal for pH around neutral. The pH increased due to release of ammonium ion in the urine [61]. The suitable range of C/N ratio is 20-30:1 for maximum biogas yield and nitrogen is the main nutrient for anaerobic bacteria [62]. Carbon supplies energy while nitrogen is needed for building up the microbial cell structure [63]. The high C/N ratio means lack

of nitrogen while a low C/N ratio leads to increased biogas production [20]. The biogas production from co-digestion was 35.6% higher than that from digestion of CY alone. A similar finding was observed by Haque and Haque [16] who reported a 30% enhancement in production of biogas by addition of HU to cow dung. Manna and Mandal [57] reported also that the biogas production from predigested aquatic plant was increased with addition of HU as an enhancer.

4. CONCLUSION

The physicochemical characteristics of CY showed that it has potential for use as a substrate for biogas production. The CY had an average TS content of 90.46% and VS of 77.12% which are appropriate for biogas production. However, the C/N ratio of CY was 44.26, which was far higher than expected for AD. The physicochemical characteristics of HU showed that TS and VS were 2.9% and 58.5%, respectively. The biogas yield when CY was co-digested with HU was 35.6% higher than when digested alone. Furthermore, the anaerobic culture adapted to digest the substrate at ambient temperature ($25 \pm 3^\circ\text{C}$) in relative retention time of 37 and 95 days for digestion alone and co-digestion respectively. Further research should focus on monitoring parameters like temperature, buffering capacity or fatty acid

levels to ensure optimal efficiency and maximum biogas yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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