Enhancement Methane Generation by Co-Digestion of Cow Dung and Grass (*Cynodon dactylon*) with Activated Sludge

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Anaerobic co-digestion (AD) of *Cynodon dactylon* grass and cow dung was conducted with four different composition levels. To increase lignocellulose digestibility and methane yield, grass was pretreated with NaOH and thermally-modified bentonite was used to inhibit the ammonia production. The highest cumulative methane yield obtained from treatments C (0.40:0.60), D (0.60:0.40), and B (0.20:0.80) was 427 mL/ g.VS, 333 mL/g.VS, and 303 mL/g.VS, respectively, over a digestion period of 40 days at a mesophilic temperature. Treatment C showed an 89.7% increase in the methane yield with respect to the control, followed by treatment D, which recoded a 48% increase, and treatment B at a 34.6% increase. A modified Gompertz model was used to explain the methane generation scenario. The process stability parameters of the anaerobic co-digestion system, such as pH, chemical oxygen demand (COD), electrical conductivity (EC), total solids, total and free ammonia contents, and volatile solids removal were explained in depth in this study.

Keywords: Biomass; Biogas; Co-digestion; Grass; Activated sludge

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INTRODUCTION

Food and energy are the basic needs of mankind. Due to the rapid increase in the world population and the high dependence on fossil fuels, developing countries have had a significant part in greenhouse gas emissions and environmental pollution (Park *et al.* 2018). Due to the limited resources of oil, gas, coal, and the abrupt fluctuation in their prices, developing countries have serious problems in their energy sectors (Bhoite and Vaidya 2018). In the past decade, nations have moved towards renewable energy sources to fulfil their energy requirements (Li 2018). In recent years, renewable energy sources have gained importance due to the abundance of available raw materials, access in remote areas, control of environmental pollution, good performance, reduction of biomass waste, energy transfer, and low costs (Hes *et al.* 2017; Kannah *et al.* 2017; Ravi *et al.* 2018). Due to the increase in population, industrial development, and great dependence on fossil fuels for their energy needs, Pakistan has faced serious energy crises during last decade (Zuberi *et al.* 2015).

Fortunately, Pakistan has a huge potential of renewable energy sources, such as biogas (*e.g.*, generation of agricultural waste, municipal solid waste, animal manure, poultry waste, and energy crops), solar energy (*e.g.*, solar thermal and solar photovoltaic), biofuels (*e.g.*, biodiesel and bioethanol), wind energy, ocean or tidal energy, mini and micro hydroelectric, and geothermal energy sources (Sheikh 2010; Shaukat *et al.* 2016; Arshad *et al.* 2018). Through the effective management and utilization of these energy sources, Pakistan can produce an enormous amount of renewable energy to meet its maximum energy demand (Ghafoor *et al.* 2016).

Approximately 60% of the population of Pakistan lives in rural areas that use biomass for cooking and heating, which has a negative impact on the environment (Rafique and Ahmad 2018). In addition, approximately 46% of the rural population in Pakistan has access to electricity, and the remaining population lives without basic energy requirements (Valasai *et al.* 2016). These rural areas are rich in both human and renewable energy sources (Kamran 2018). Due to the large population of the livestock sector, 652 million kg/day of animal manure is produced from buffalo and cattle. By collecting, handling, and using this manure as a feedstock for biogas production, 16.3 million m^3 of biogas can be produced daily (Sheikh 2010; Ghafoor *et al.* 2016). Grasslands have an important place in world agriculture, covering approximately 26% of the world's land area (Feng *et al.* 2017). Grass grows along rivers, roads, railways, and in other public places, such as parks, schools, universities, *etc.* (Haryanto *et al.* 2018; Tedesco and Daniels 2018). For this reason, it is difficult to measure the hectares of available grass (Meyer-Aurich *et al.* 2016). Recently, the consideration of grass as a raw material has increased considerably due to its low water consumption and the ability to cultivate it on land not useful for other crops (Richter *et al.* 2011; Wall *et al.* 2016). A study that utilized cow dung and grass silage with different C/N ratios achieved a 185 mL/g.VS methane yield from a ratio of 1:1 (Prapinagsorn *et al.* 2017). The effect of harvesting period, drying and ensiling conservation methods on biogas yield from grass reported in previous studies (Chiumenti *et al*. 2017; Chiumenti *et al*. 2018).

Different studies have been carried out to enhance methane yield and the stability of the anaerobic digestion process. Co-digestion of macroalgae and sugar industry waste was performed in batches in an up-flow bioreactor, which resulted in a maximum of 375 mL of biogas with 40% methane content and 114 mL/g.VS of biogas with 75% methane content, respectively (Karray *et al.* 2017). In another study, organic municipal waste and fruit and vegetable waste were digested with increasing composition rates, which resulted in a 141% and 43.8% increase in the methane yield with respect to their mono-digestion, respectively (Pavi *et al.* 2017). Additionally, it has been reported that the 152 mL/ g.VS and 198.85 mL/ g.VS methane yields were obtained from the co-digestion of sheep dung with corrugated paper and the co-digestion sheep dung with office paper, respectively (Li *et al.* 2018). Goose manure and corn stover mixed with four different composition levels recorded 92.1% methane increments with respect to the control (Hassan *et al.* 2017). The complex structure of plant tissue and its inherent forces do not allow them to easily become biogas due to the abundance of nitrogen, creating a higher concentration of volatile fatty acids that stop methanogenesis and result in a lower methane production (Bustillo-Lecompte and Mehrvar 2017; Biswas *et al.* 2018). Thus, the best route to improve biogas production is the anaerobic co-digestion (AD) process (Hassan *et al.* 2016b). Thermo-chemical pre-treatment for different crops is beneficial for the improvement of methane because treating the biomass can increase the hydrolysis stage, which will result in a greater production of methane (Bhatnagar *et al.* 2018; Nagler *et al.* 2018). Corn cob pretreated with alkali and enzymatic hydrolysis showed a 23% increment in methane yield compared with raw corn cob (Pérez-Rodríguez *et al.* 2017). A comparative study conducted by using lemon grass, cow dung, and chicken droppings separately through the pre-fermented technique showed an inclined behavior compared with their raw digestion (Korai *et al.* 2016). Anaerobic digestion of grass is reported less compared with other agricultural wastes, and no study was found that dealt with the co-digestion of cow dung and thermally NaOH pretreated *Cynodon dactylon* grass.

The objective of this study was to access the biogas production of cow dung and *C. dactylon* grass through AD. The methane yield of co-digestion of cow dung and *C. dactylon* grass was quantified with the stability of the AD process with this substrate and the effect of thermochemical pretreatment.

EXPERIMENTAL

Materials

Cow dung collection

Cow manure was collected from a dairy farm near the Agricultural Engineering Department of Bahauddin Zakariya University Multan (30° 11' 44" North, 71° 28' 31" East) Pakistan. Coarse materials, such as plastic bags, stone, wood chips, and other impurities, were extracted from this cow dung. Then, the cow dung was diluted with tap water and was mixed to obtain homogeneity throughout the sample. The composition parameters of the cow dung are represented in Table 1.

Table 1. Chemical Characteristics of Substrates

Parameter	Cow Dung	*C. dactylon* Grass	Sludge
pH	7.2	ND*	7.2
TS* (g/kg)	152.4	3.1	7.7
VS* (g/kg)	38.4	1.3	7.3
COD* (mg/L)	6733	ND*	9030
TN (%)	1.3	0.91	3.6
Protein (g/kg)	7.8	5.46	21.6

*ND = not determined, TS = total solids, VS = volatile solids, COD = chemical oxygen demand and TN=Total nitrogen

C. dactylon grass preparations

Grass was collected from the Agricultural Engineering Department in Punjab, Pakistan, then sun dried over 2 months. After it was dried in an oven for 24 h and cut into pieces of 2 cm to 3 cm, these pieces were ground and sieved through a 1 mm mesh to obtain homogeneity in the feedstock. This physical pre-treatment was done to improve the surface area of the substrate by reorganizing the cellular structure. After that, grass powder was placed in the refrigerator for a day. This herb powder was used for the thermochemical pretreatment and as a composition parameter of the grass (Table 1).

Pre-treatment of grass

To improve the yield of methane, increase the digestibility of lignocellulose biomass, and reduce accumulation of volatile fatty acids, the pre-treatment method of alkali with a thermal effect was adopted. For the chemical pre-treatment of grass, 100 g samples were taken in a flask and mixed with distilled water before being placed in the oven for 20 min at 80 °C to avoid biological deformation. Then, 7.5 g of NaOH was put in the flask and the sample was homogenized and by adding water to make the volume in the flask exactly 100 mL. For the thermal pre-treatment, this grass sample was autoclaved for 20 min at 100 °C and 0.01 MPa. Due to this pretreatment of the grass, the intermolecular bond between the lignin and hemicellulose was loosened and water could easily enter the cell wall due to the maximum available surface area.

Bentonite preparation

To obtain the best results, bentonite was heated at 300 °C for 2 h in a muffle furnace and placed in the desiccator to cool. Bentonite is added to the cow dung to prevent the accumulation of ammonia, which inhibits the production of biogas (Angelidaki and Ahring 1992). In this experiment, co-digestion and the addition of bentonite to cow manure were comparatively analyzed for better performance.

Sludge activation

Anaerobic sludge was collected from a large-scale biogas plant near Bahauddin Zakariya University in Multan, Pakistan. That biogas plant was fed with animal manure at room temperature. The stones and other impurities were collected from the sludge before it was placed in an anaerobic digester, and 2 g/L of glucose was added daily for one month (Table 1). After one month, the addition of glucose ceased, and the sludge remained until biogas production stopped. Inoculum was vaccinated through injector which is used on daily basis with required glucose dose.

Methods

Experimental anaerobic test design

The batch test was designed with 1L glass bottles having 700 mL of working volume. The correspondent grams of the substrate were calculated and fed to the reactors according to the co-digestion designed. The design had four different ratios of grass to dung composition digester: 0:100, 0.20:0.80, 0.40:0.60, and 0.60:0.40 on a mass basis represented by A, B, C, and D, respectively. The digestor C had thermochemical pretreated grass, whereas digestor D had bentonite as treatment. The digestor A worked as control. Each bottle had two ports at the top, one for gas sampling, to find the gas composition in the AD process as biogas composition, and one port to extract a liquid sample to find parameters such as pH, chemical oxygen demand, electrical conductivity, and solid content ratio. Each test was performed in triplicate for the validation of the results. In a reactor, activated sludge was anaerobically digested alone to evaluate the exact methane production from cow dung and grass (*Cynodon dactylon*). The sludge was digested to find out the exact value of biogas production from all the tests. All tests were performed at 37 °C (mesophilic temperature). The generation of biogas was quantified *via* a water displacement mechanism and the gas container had a port to add water to find the exact amount of biogas production. All the anaerobic reactors were manually stirred daily to inhibit the accumulation of solids. The biogas was measured daily, and other parameters such as pH, chemical oxygen demand, electrical conductivity, and solid contents were measured at three-day intervals. In each digester, the activated sludge and distilled water were added so that the working volume of each digester was exactly 800 mL. The measurement of biogas was designed for both criteria for the daily and cumulative scenarios. The measurement mechanism correlated with the proportion of volatile solids (VS) that were added and with the daily and cumulative production of biogas. The total ammonia nitrogen (TAN) concentration was determined during the experiment with total nitrogen analyzer (Lianhua Technological Company, Ltd, China), while free ammonia nitrogen FAN was calculated by using Eq. 1 (Hassan *et al.* 2017).

$$FAN = TAN\left[1+\frac{10^{-pH}}{10^{-(0.09018+\frac{2729.92}{TK})}}\right]^{-1} \tag{1}$$

In Eq. 1, FAN (g/L) is the free ammonia contents and TAN (g/L) is the total ammonia nitrogen contents. The CODs removal was calculated using the equation referred by (Hassan *et al.* 2017). The VS removal (%) was estimated by Eq. 2 (Hassan *et al.* 2017).

$$VSrmoval\% = \left[1-\frac{VS_{digestrate}\times\left(100-VS_{feed}\right)}{VS_{feed}\times\left(100-VS_{digestrate}\right)}\right] \tag{2}$$

For the measurement of methane content, the modified Gompertz model was applied. This model assumes that cumulative methane production is the function of hydraulic retention time. The modified Gompertz equation can be presented as follows,

$$P = a\times e^{-e\left[\frac{U\times e}{A}(\lambda-t)+1\right]} \tag{3}$$

where *P* is the cumulative production of methane in the digestion period in days in mL/g.VS, *A* is the potential yield of methane in mL/g.VS, *U* is the maximum methane production rate in mL/g.VS.day, *e* is the constant (2.718), λ is the period of the delay phase (days), and *t* is the experimental digestion period in days. The constants *A*, *U*, and λ were solved using the curve fitting tool in MATLAB version 8.3.0.523 (R2014a; MathWorks, Natick, MA, USA).

RESULTS AND DISCUSSION

Anaerobic co-digestion is a complex and relatively sensitive process that depends on many parameters. For the continuous production of biogas and the stability of the process, each parameter should be within the prescribed limit. A small fluctuation in a single parameter results in the instability of the entire AD process (Park *et al.* 2018). In the first digester, the methane yield for cow dung, indicated by line A, is shown in Fig. 1. In this digester, the initial value recorded was 11 mL/g.VS. After that, the methane production increased during the first three days and then declined possibly due to the free ammonia accumulation in the reactor. After that, it increased, resulting in a zigzag profile for the first and second week of the experiment. This was found in the second digester and is shown in line B in Fig.1. The three peaks describe the excellent performance for this design.

In the third digester, the initial value registered for the methane yield was 16 mL/g.VS (Fig. 1). In the first week of experimentation, two peaks are shown that describe the thermal and chemical pre-treatment of the grass, and the hydrolyzed bond between lignin and hemicellulose. The microorganisms were handled so as not to perform additional work and so that an additional yield of the digester, in which NaOH was used, was obtained. Additionally, the second and third week showed a zigzag behavior to quantify the methane yield.

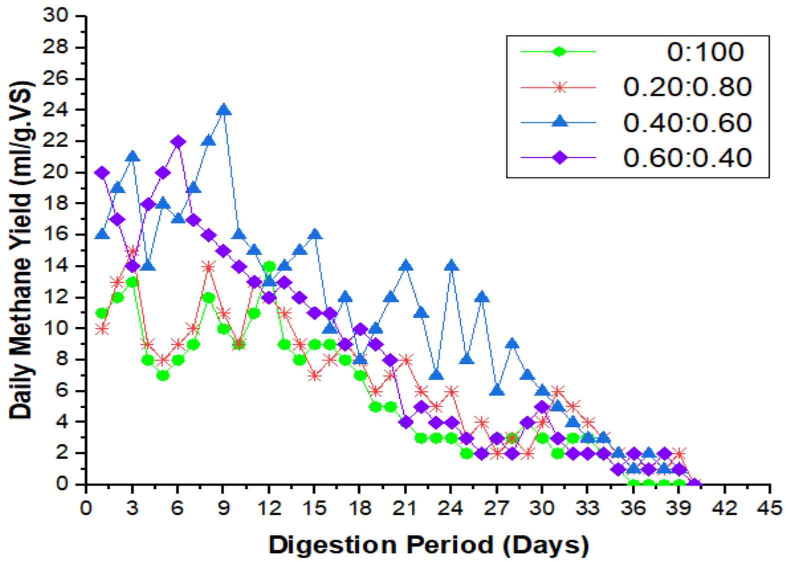

Fig. 1. Profile of daily methane production

Due to the thermo-chemical pre-treatment of dry grass, most of the methane was obtained in the first 20 d. In this digester, the maximum methane yield was obtained on the eighth day (22 mL/g.VS) of the experiment. The overall methane yield obtained from this digester was the highest among all treatments. This maximum yield showed that the co-digestion of cow manure and pre-treated grass had good results and that the pre-treatment was effective for this type of AD process. In the fourth digester, bentonite was added with the co-digestion of grass and cow dung, as shown in Fig.1.

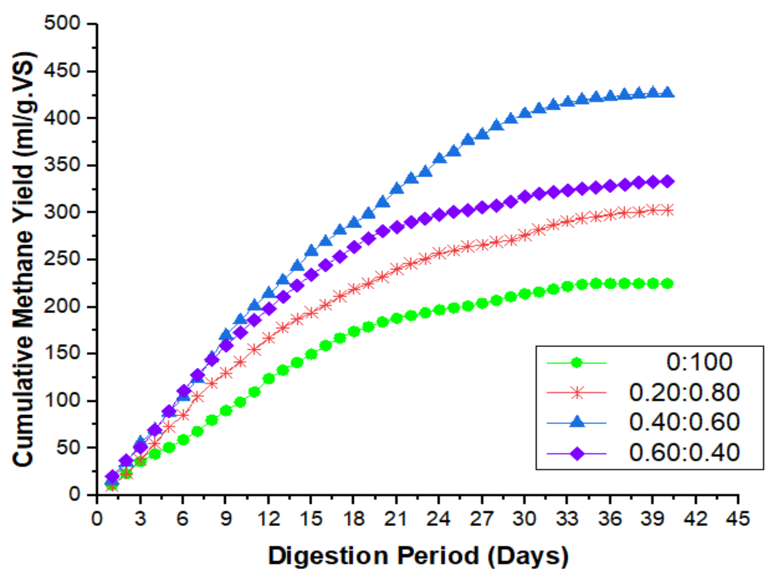

Fig. 2. Profile of cumulative methane yield

Due to the addition of bentonite, the methane content in the biogas produced by this digester was high because of the limited production of intermediate byproducts. On the first day of digestion, this digester recorded an initial methane value of 20 mL/g.VS. In the first week of the experiment the highest possible value was obtained due higher acetic acid production from this digester. The highest methane yield was recorded on the sixth day (22 mL/g.VS). Overall, the highest yield was obtained by this digester after treatment C.

The cumulative methane yield, that is, the sum of daily methane yield, is represented in Fig. 2. The cumulative yields obtained from treatments A, B, C, and D were 225 mL/g.VS, 303 mL/g.VS, 427 mL/g.VS, and 333 mL/g.VS, respectively. As shown in Fig. 2, the highest yield was obtained from treatment C, followed by treatments D, B, and A, respectively.

The methane yield obtained from treatment C was 89.7% higher with respect to the control. Treatment D showed a 48% increment with respect to the reference. Treatment B recorded a 34.6% increase in its methane yield with respect to the control digester methane yield. It is clear from Fig. 2 that the thermochemical treatment resulted in the highest methane and biogas yields. This was followed by the bentonite addition, which resulted in better yields after the co-digestion of cow dung and grass, which additionally had a good scenario regarding their yield. Overall, all treatments were in the range which was designed to run for this AD system. One previous study resulted in 72.9% biogas enhancement while using 2% NaOH pre-treatment at 20 °C for 3 d, reducing the digestion time by 34.6% (Erden and Filibeli 2010). Corn stover was digested with anaerobic sludge, and a 66% methane increment was found in the biogas composition (Hassan *et al.* 2016a). Therefore, the present study decreased the pre-treatment time and improved the digestion stability, lignocellulose digestibility, and methane contents.

Chemical oxygen demand (COD) is an important parameter in the anaerobic digestion system (Fig. 3). During the initial stage of this process, some derivative products are produced as short and long chain monomers and fatty acids, proteins, alcohol, glucose, and peptides. Chemical oxygen demand is the measure of production of these and their conversion into useful products. During the AD process, the conversion of biomass by bacterial activity is performed with the production of the above-mentioned products, which constitute an important reason to increase the COD in the digester (Selvankumar *et al.* 2017).

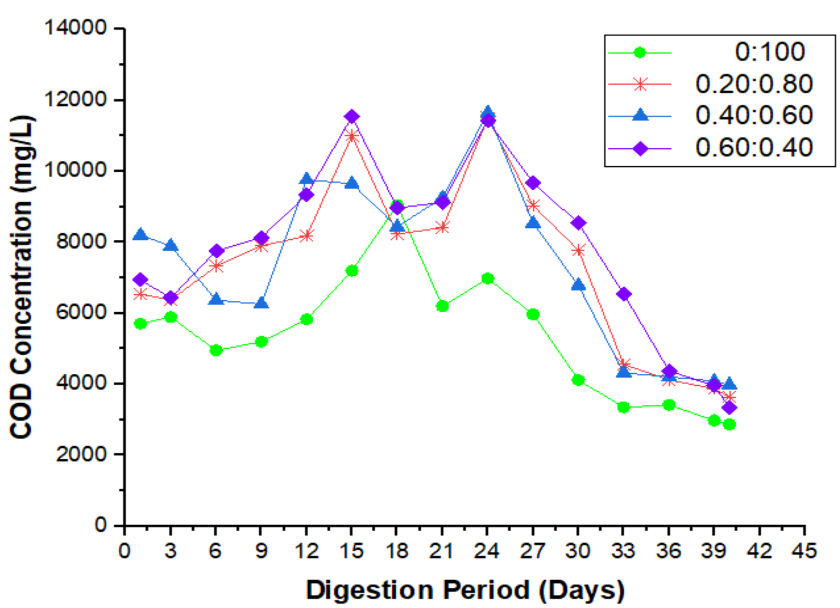

Fig. 3. COD behaviour in the AD process

In this study, a large amount of variation in the COD value was observed. The minimum value of 2870 mg/L was recorded for grass and a maximum of 11648 mg/L was recorded for treatment C. In general, all fluctuating values were in accordance with the range given by different authors for this AD system. The intermediate product that was converted into biogas was produced by the action of microorganisms and was given a real value of COD in the digester. The COD in digester C ranged from 3990 mg/L to 11650 mg/L, and showed the highest value on day 24 and the lowest on the last day of digestion.

Co-digested goose manure and corn stover show a COD profile that ranges below 18000 mg/L (Hassan *et al.* 2017). Higher COD values indicate enhanced anaerobic digestibility of the substrate and ultimately, a higher methane production (Li 2018). In the early stages of AD, substrate macromolecules were hydrolyzed with anaerobic sludge that resulted in the production of acetates, total volatile fatty acids (TVFAs), and intramolecular products (Arshad *et al.* 2018).

The capacity to direct the digestion process resulted in the pH value decreasing due to the decomposition of the substrate *via* bacterial activities. During the AD process, the pH value differs with the bacterial activity; the pH value for acidogenesis should range from 5 to 7, and in methanogenesis, the pH value should range from 7 to 8 (Amon *et al.* 2007). For continuous biogas generation, a stable pH value should be maintained so that the microorganism works in a better environment, thus improving the stability of the AD process. The methane contents in yielded biogas was improved, it reached a maximum value, and then it decreased gradually to some extent. The VS was degraded about 42.8% in this system. Thus, co-digestion could boost VS removal efficiency by 9.9%. The VS removal has very close correlation with methane yield. Therefore, percentage of VS removal was improved as the cumulative methane production was increased.

Figure 4 shows the pH values of all treatments. This figure shows that the pH values of all treatments were in the prescribed range that was given by previous studies. The initial pH values were 7.6, 7.5, 7.8, and 7.6 for treatments A, B, C, and D, respectively.

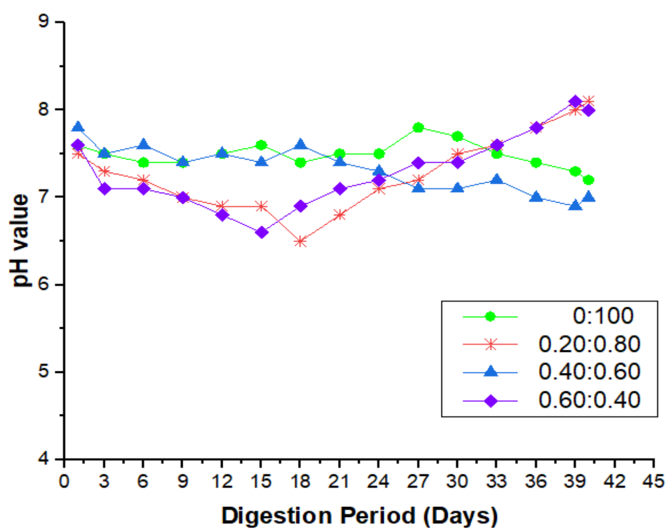

Fig. 4. Profile of pH behavior

A maximum pH of 8.1 was recorded by treatments A and C on days 40 and 39 of the experiment, respectively, throughout the digestion period of all treatments. In contrast, the pH of 6.5 recorded in treatment B was the lowest value for this AD system. Fluctuations with respect to digestion time were shown by all treatments. This aspect should lead towards the bacterial activity of the whole digestion system. A study conducted on co-digested oil refinery waste and chicken manure found that the anaerobic digestion process should take place in the pH range of 5 to 8.5 (Mehryar *et al.* 2017). Another study found the same scenario for pH behavior as was found by the current study (Ravi *et al.* 2018).

There was a direct relationship between the concentration of soluble salt and electrical conductivity (EC) in the AD system. Figure 5 describes the EC scenario in this study for all treatments used in this system. Another reason for the high concentration of these salts was that, due to this effect, the moisture content in the cell of the microorganism decreased below the optimum level and the condition for dehydration was deactivated. As a result, the methanogens that are strongly recommended to produce biogas in that situation process stop functioning and all parameters stop working properly (Al-Masri 2001).

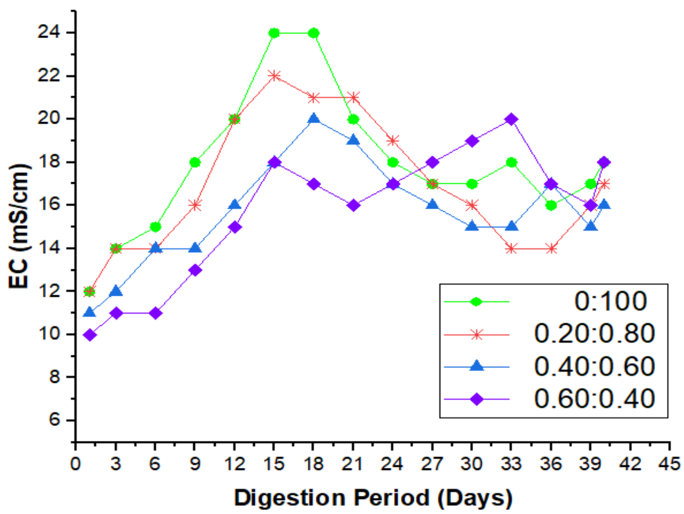

Fig. 5. EC value for AD process

The transport process of the microorganisms stops because of the higher total ammonia and free ammonia content of the substrate that stopped the further degradation of the biomass (Hassan *et al*. 2016a). Therefore, EC was the most important parameter in the sense of bacterial activity and constantly producing biogas. In this study, the value of EC (Fig. 5) was in accordance with the prescribed range that resulted from previous studies. The EC range for this study was 10 mS/cm to 24 mS/cm. The highest value resulted from treatment A, which was recorded on day 15 of the experiment, and the lowest was for digester D on the first day of the AD period. In all digesters, this value fluctuated with respect to time, but in treatment A this variation was large with respect to the other treatments. This may have been due to low bacterial activity.

Anaerobic digestion downstream characteristics

Animal waste was considered as consisting of high total ammonium nitrogen content due to indigested protein and uric acid production during anaerobic digestion. The total ammonia nitrogen (TAN) and free ammonia (FAN) profile are shown in Figs. 6 and 7, respectively. The co-digestion of cow dung and grass played an important role to lower the production of TAN and FAN during the AD process.

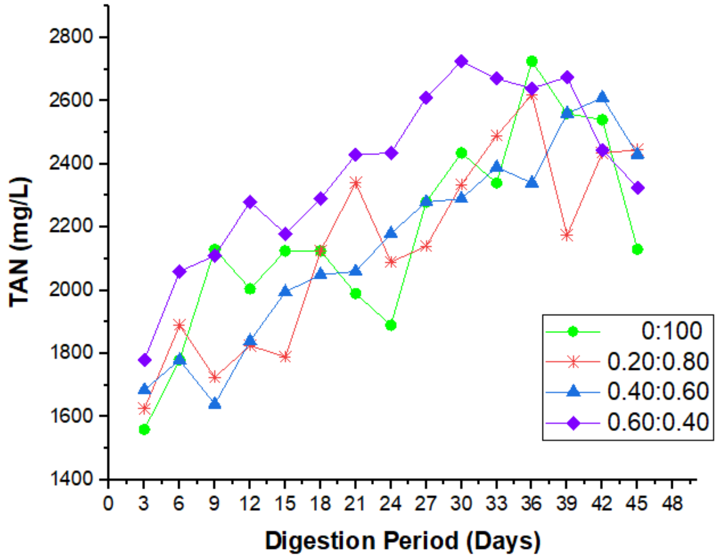

Fig. 6. TAN value for AD process

The maximum value of TAN and FAN generation during this study were 2725 mg/L on 33rd day and 48 mg/L and 3rd day respectively. The TAN value recorded slight increases in the first and second weeks and showed an increasing trend in the third week of digestion. In the last week it showed decreasing behavior. FAN generation started with high values, but in the first and second week its showed a decreasing trend during the digestion process. In the third and fourth week it showed a slight increase in production during processing. The maximum TAN value for digesters A, B, C, and D were as 2720, 2620, 2610, and 2725 mg/L respectively. TAN and FAN values correlated with previous studies.

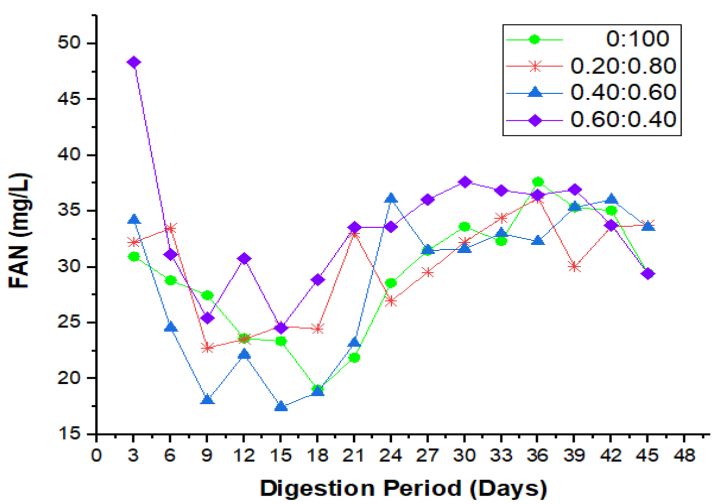

Fig. 7. FA value for AD process

Basically, anaerobic co-digestion is the phenomenon of conversion of organic matter into methane and carbon dioxide with the help of microorganisms. Pretreatment and co-digestion techniques were involved in better volatile solid consumption rate that accounted high yield of methane gas. In present study highest volatile solid removal rate was recorded for digester C which is 66.23% (Fig. 8). This phenomenon also reveals that highest methane yield recorded for this digester. The volatile solid removal rate for digesters A, B and D were 50.41%, 54.48% and 62.01% respectively. The minimum volatile solid removal rate was achieved from digester A which is taking as control which also confirms that lowest yield was obtained from this treatment.

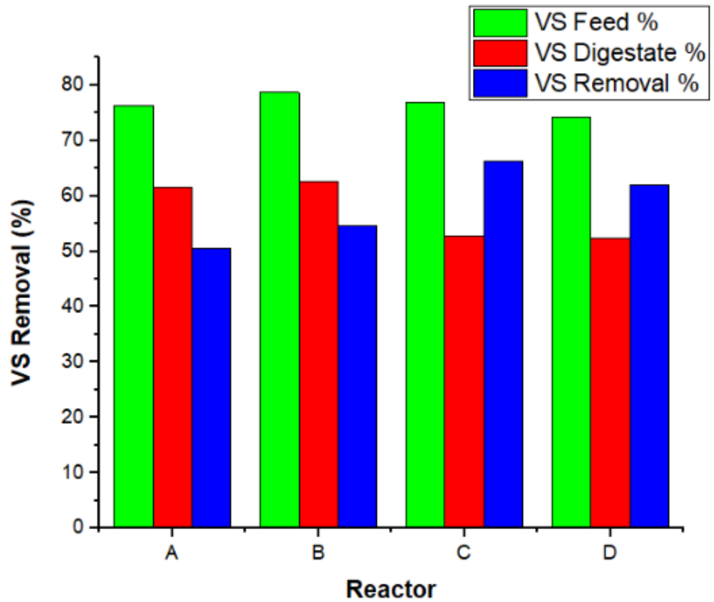

Fig. 8. VS removal value for AD process

CONCLUSIONS

The anaerobic co-digestion (AD) technique was adopted in this study for methane enhancement by using *C. dactylon* grass and cow dung with activated sludge. There were four different digesters, which were designed to obtain the specific goals of this study. The highest methane yield was recorded for treatment C, which was 427 mL/g.VS with 90% increment as compared with control. After that, treatment D showed 48%, and the last digester showed 34.6% methane enhancement with respect to the control. This methane increment showed good stability of the AD process and an effective microbial community.

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