Thermal And Chemical Pre-Treatments Of Cow Dung And Poultry Litter Enhance Biogas Production In Batch Fermentation

Animut Assefa, Meseret C. Egigu, Ameha Kebede

Abstract: Low degradability of substrates is one of the factors that hinder the production of biogas. With the aim of maximizing biogas yields from cow dung (CD) and poultry litter (PL), a series of experiments were carried out under mesophilic conditions at 38 ºC using batch digester operating for 21 days hydraulic retention time (HRT). Temperature pre-treatment at 60 and 80 ºC and chemical pre-treatment with NaOH (0.45 g, 1.35 g and 2.25 g) were applied as a pre-treatment. Cumulative biogas production and VS reduction from anaerobic digestion of 80 ºC pre-treated substrate was 46.3% and 26.1% higher than the control, respectively. However, thermal pre-treatment at 60 and 80 ºC did not show statistically significant difference in biogas production. Biogas yields of substrates that received 0.45 g, 1.35 g, and 2.25 g of NaOH increased biogas production by 0.03%, 21% and 56% over that of the control, respectively. Overall results indicated that the biogas yield and VS and TS reduction can be enhanced through thermal and chemical pre-treatments prior to anaerobic digestion.

Keywords: Anaerobic digestion**,** biogas**,** chemical pre-treatment, temperature pr- treatment, total solids, volatile solids

1. INTRODUCTION

As far as fuel is concerned, the rural population in developing countries including Ethiopia heavily depend on biomass mainly in the form of fire wood. It accounts for about 94.7% of the total energy supply [1]. The dependence on fossil fuel and forest resources as primary energy source has led to global climate change, environmental degradation and human health problems. Therefore, environmentally friendly renewable energy source is a key to curb these problems. Biogas, which consists mainly of methane is one of such alternative renewable energy source produced through anaerobic digestion of organic matter by various specialized groups of bacteria in several successive steps [2]. Biogas technology represents one of a number of village-scale technologies that offer the technical possibility of obtaining energy from organic wastes. Apart from energy, this technology offers many opportunities. In Ethiopia for example, households with at least four cattle and access to water can install a biogas plant, which then help them reduce the daily work load, mainly of women that spend collecting fire wood for energy source [3]. As a clean energy source it reduces air pollution, while the digested residue (effluent) is serving as organic fertilizer [4].

- *Animut Assefa; M.Sc. student in the Department of Biology, Haramaya University, Ethiopia*
- *Meseret C.Egigu: Corresponding author, Assistant professor in the Department of Biology, Haramaya University, Ethiopia. Email: swamy12in@yahoo.co.in*
- *Ameha Kebede: Associate Professor in the Department of Biology, Haramaya University, Ethiopia.*

The National Biogas Program (NBP) of Ethiopia, which aimed to establish 14000 biogas plants between 2008 and 2012 in different parts of the country, utilizes manure (cow dung) as the feedstock for biogas production [3]. However, the efficient use of this technology is limited by the low degradability of manure, which is only in the range of 30–43% [5]. Biogas yield, however, can be improved by increasing the rate of hydrolysis of lignocellulose through different mechanisms [6]. For example, pretreatment of organic substrates (corn stover) with NaOH results in more biogas yield than untreated substrate [7, 8]. Thermal pre-treatment commonly between 60 and 180 ºC has also been evaluated to help biogas production [9]. Thermal pre-treatments, which are considered as low temperature treatments (below 100 ºC) were found to enhance biogas production [10, 9]. Cow dung is not the only source of biogas. Ethiopia ranks first in cattle from Africa. Apart from cattle husbandry, farmers also raise considerable number of poultry with their wastes not used for biogas production. Some studies have shown that mixing different substrates in some proportions will result in more biogas production than sole digestion. This study was therefore conducted with the objectives of (i) identifying the optimum mix ratio of cow dung and poultry manure yielding high amount of biogas and (ii) to evaluate the effects of thermal and chemical pre-treatments on biogas yield of the optimum substrate mix.

2. MATERIALS AND METHODS

2.1. Substrates Collection and Preparation

Two types of lignocellulosic biomass, cow dung (CD) and poultry manure (PM) obtained from Haramaya University animal farm were used in this study. The two substrates were mixed in a 4:1 ratio of CD: PM. This mix ratio was selected as optimal substrate (OS) due to its high biogas yielding nature based on preliminary identification of the optimum mix ratio to yield biogas [14]. Fresh rumen fluid used as a starter of anaerobic digestion was obtained from the nearby slaughterhouse at Haramaya University. The fluid was filtered through a cloth of 0.5 mm sieve diameter to separate solid content from slurry and starved for a week at 38 ºC to remove easily degradable volatile solid (VS) prior to using as inoculums [15, 16] .

2.2 Thermal and chemical pre-treatments and digestion of the optimal substrate

The optimal substrate (hereafter called substrate) was added in 0.5 L flasks (digesters) for thermal pre-treatments. Two temperature levels, 60 and 80 ºC were selected to pre-treat the substrate. After covering the flasks with plastic film, they were treated with temperatures of 60 and 80 ºC for 3 hours by keeping in water bath with intermittent gentle shaking to ensure the homogeneity of temperatures in the flasks [10]. Substrate that was not exposed to these temperatures, but left under room temperature was considered as not thermally pre-treated (control). After pre-treatments, all the slurries were kept in a refrigerator at 4 ^{o}C for 24 h. Then, 100 mL of inoculums (rumen fluid) were added and the total solid was adjusted to the recommended level (8%) using appropriate amount (104.5 mL) of distilled water [11]. For chemical pre-treatments, 0.45 g, 1.35 g, and 2.25 g of 6N NaOH were added into substrates in 0.5L digesters. Then the solutions were mixed for 1 hr. using rotary shaker to attain pH levels of 9, 11, and 13, respectively. The control digester received no NaOH [12]. After chemical pre-treatment, the pH of all treatments was reduced to neutral (pH ~7.0) by adding appropriate amount of 6N H_2SO_4 [13]. After 24 hours of stay in a refrigerator, equal amount of inoculums (100 mL) were added to the slurry and the TS was adjusted to 8% by adding distilled water as mentioned above.

2.3 Digester arrangement and measurement of biogas and other parameters

Anaerobic digestion was done in plastic bottles (0.5 mL) arranged randomly in three replications for bench-scale experiments. For this, three plastic bottles containing the slurry, acidified brine solution and empty bottle to collect brine solution were arranged in order (1st to 3rd) on the table. The three containers were interconnected with a plastic tube having a diameter of 1 cm. The tube connecting the first bottle to the second was fitted just above the slurry in the first bottle to help gas collection. Thus, the biogas produced by fermentation of the slurry was driven from the first bottle to the second bottle that contained a brine solution so as to displace a volume of the brine solution equivalent to the volume of biogas produced [17]. The lids of all digester were sealed tightly using super glue in order to control the entry of oxygen and loss of biogas. The temperature of all digesters was maintained at 38 °C by keeping in oven (EED115), which represents mesophilic condition. The digesters were randomly arranged on a table in the lab in three replications.

2.4 Physico-chemical characterization of pre-treated substrates before and after anaerobic digestion, and biogas yield measurement

Substrates were analysed for total solids (TS), volatile solids (VS) and pH before and after AD process based on the Standard Methods for the Examination of Water and Wastewater [18]. Also the carbon content of the substrates was obtained from volatile solids data using an empirical equation reported by [19].

2.7. Data Analysis

Data were first checked for their normality. Data that were not normally distributed were log-transformed and thereafter subjected to analysis of variance (one-way ANOVA) using SPSS for Windows 16.0 (SPSS; Chicago, IL, USA). Fishers Least Significant Difference (LSD) was used to investigate statistical significance between the different treatments, where as paired samples T-test was used to investigate statistical significance within a treatment. Difference between means was considered statistically significant at $P<0.05$.

3. RESULTS

3.1. Physico-chemical characteristics of the thermally and chemically pre-treated substrate before and after AD

No significant difference was observed between the substrates assigned to the different temperature levels and NaOH concentrations before AD with respect to pH and organic carbon content. Similarly, the pH value of substrates of the different levels of both treatments did not significantly vary after AD. However, percent organic carbon showed significant difference between the different levels of temperature and NaOH treatments after AD (Table 1). When compared to the initial, the pH of the content increased in all treatments after AD. However, percent organic carbon decreased after AD (Table 1).

Table 1

PHYSICO-CHEMICAL FEATURES OF BLENDED CD AND PM AT 75%: 25% RATIO FOR THERMAL AND CHEMICAL PRE-TREATMENT TESTS BEFORE AND AFTER AD (VALUES ARE MEAN ± SE, N=3). MEANS FOLLOWED BY DIFFERENT SMALL LETTERS IN COLUMN ARE SIGNIFICANT AT 0.05 PROBABILITY LEVEL FOR PAIRED SAMPLES T-TEST WITHIN TREATMENT. MEANS FOLLOWED BY DIFFERENT CAPITAL LETTERS IN ROW ARE SIGNIFICANTLY DIFFERENT AT 0.05 PROBABILITY LEVEL FOR BETWEEN TREATMENTS (ONE-WAY ANOVA). NOTE: IN ROWS STATISTICAL COMPARISONS OF TEMPERATURE AND NAOH TREATMENTS ARE INDEPENDENT.

	Treatment						
	Temperature			**NaOH**(g)			
Parameter	Control	60 ºC	80 ºC	0	0.45	1.35	2.25
Initial pH	6.94±0.01^{aB}	7.70±0.04^{bA}	7.71±0.04^{aA}	6.94±0.01^{bB}	7.23±0.03^{aA}	7.3±0.05^{bA}	7.2±0.06^{aA}
Final pH	8.03±0.09^{aA}	8.07±0.09^{aA}	7.97±0.03^{aA}	8.03±0.09^{aA}	8.03±0.09^{aA}	8.2±0.15^{aA}	8.1±0.15^{aA}
% initial organic C	9.31±0.13^{aA}	9.31±0.12^{aA}	9.35±0.08^{aA}	9.31±0.13^{aA}	9.4±0.04^{aA}	9.27±0.14^{aA}	9.1±0.21^{aA}
% final organic C	4.42±0.01^{bA}	2.26±0.05^{bB}	2.00±0.04^{bC}	4.42±0.01^{bA}	4.21±0.07^{bB}	3.42±0.03^{bC}	1.64±0.02^{bD}

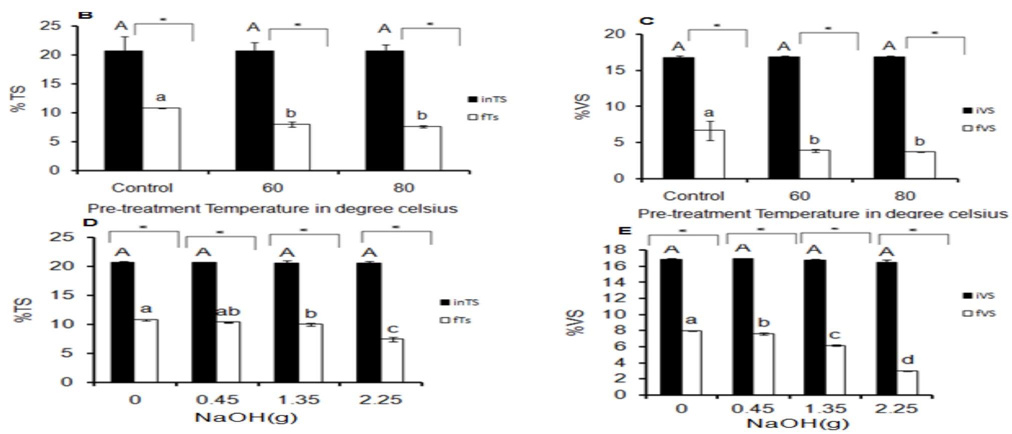
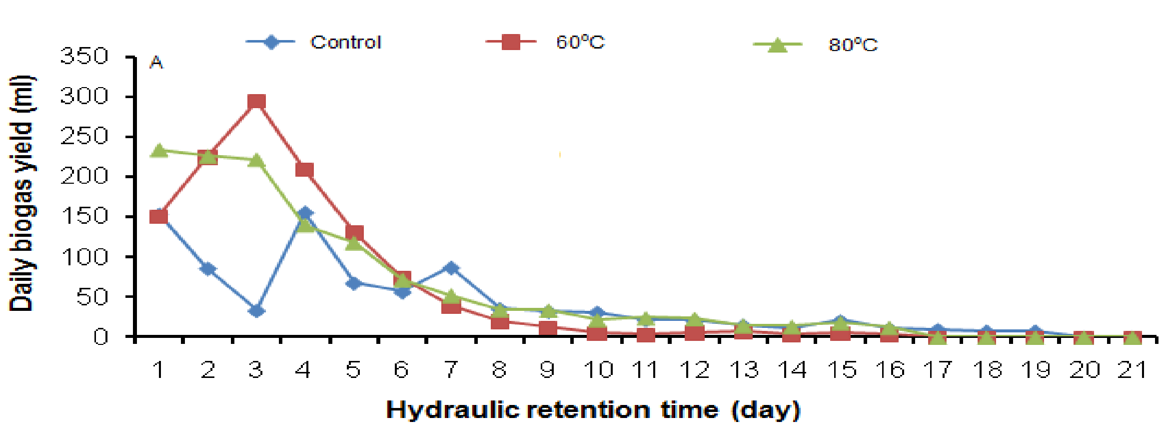


***Fig. 1**. Values of TS and VS: for thermally pre-treated substrates (B and C, respectively) and for NaOH pre-treated substrates (D and E, respectively). Black bar represents values measured before AD while open bars are for after AD. Capital letters represent differences between %TS or VS of the substrate under different temperature and NaOH pre-treatments before AD while small letters represent that of after AD. Asterisk (*) shows there is significant difference in % TS or VS between before and after AD. VS=Volatile Solids, TS=Total solids*

3.2 Daily and cumulative biogas yield of thermally and NaOH pre-treated substrates

Initially at day 1, the average daily gas production of the control and 60 ºC pre-treated substrates was the same. However, that of 80 ºC substrate was higher than the control and 60 ºC pre-treated substrate. The trend of gas production showed that gas production for 60 and 80 ºC pre-treated samples ceased after 17 days while that of control continued up to 21 days (Fig.2A). No gas production was observed in NaOH pre-treated substrates during the first and second days of fermentation. However, gas production commenced during the third and fourth days and became superior to the control before ceasing on day 21 (Fig.2B).



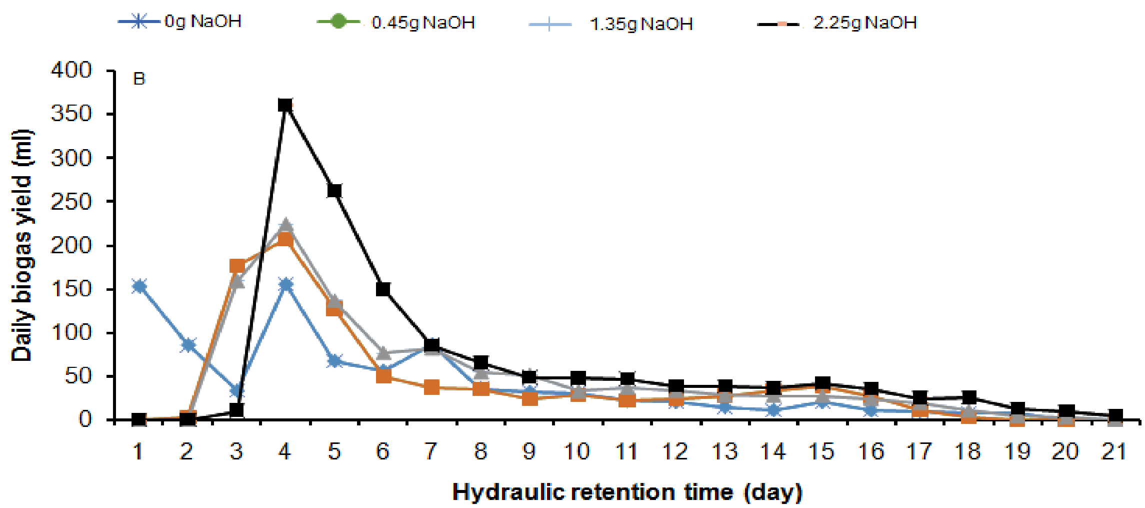


***Fig. 2**. Daily mean biogas yield profile of thermally pre-treated substrate (A) and NaOH pre-treated substrate (B).*

The cumulative biogas yield of substrates pre-treated with 60 and 80ºC was significantly higher than that of control. However, there was no significant difference between 60 and 80ºC pre-treated substrates in cumulative biogas yield (Fig.3A). The cumulative biogas production from NaOH pre-treated samples was significantly higher than untreated sample, though no significant difference was seen between the control and substrate that received 0.45 g of NaOH (Fig. 3B).

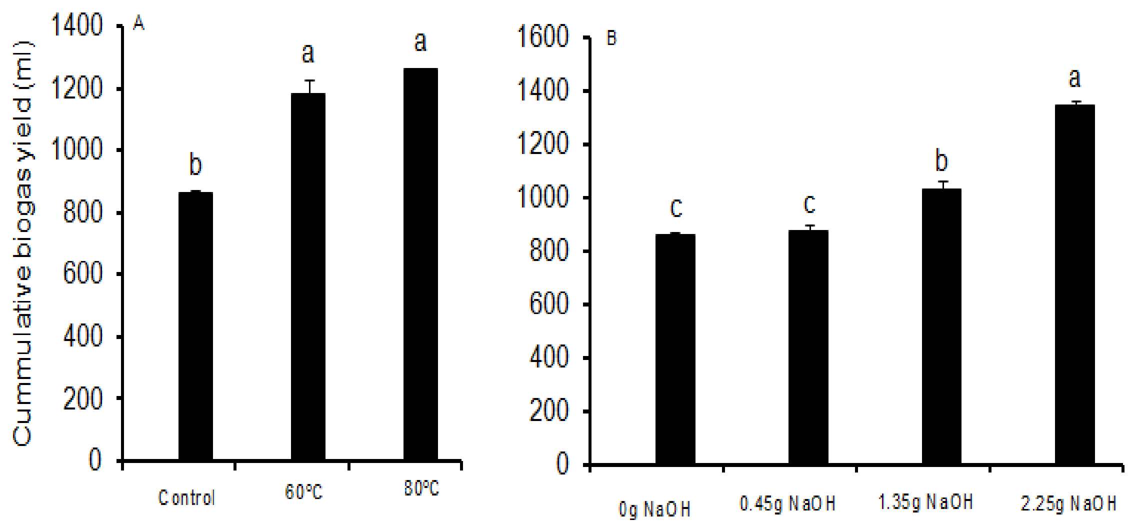


***Fig. 3.** Cumulative biogas yield of thermally pre-treated substrates (A) and NaOH pre-treated substrates (B).*

4. DISCUSSION

4.1. Effect of temperature and NaOH pre-treatments on the physico-chemical characteristics of the substrates and biogas yield

Before inoculation and digestion, the pH value of temperature and NaOH treated substrates was about neutral. The pH value of the rumen fluid used in this experiment was also almost neutral (pH=7.53). Optimal pH for biogas production is reported to be neutral[20]. The pH value before digestion showed slight increase with temperature and NaOH concentrations (*P*<0.05). This may be explained by solubilization of compounds such as proteins due to thermal and alkali pre-treatments [21,22,23]. After AD, the pH values of all treatments were found to be alkaline which might also be due to the buildup of ammonium compounds, $(NH_4)_2CO_3$, for example [24]. Under all temperature and NaOH treatments, percent organic carbon significantly decreased after AD, and the extent of decrement was found to increase with temperature and NaOH concentrations. Decrement in organic carbon shows effective degradation process during anaerobic digestion [25]. Increased consumption of organic carbon at higher temperature levels and NaOH concentrations may therefore be ascribed to its utilization by bacteria for various metabolic requirements including biogas production . High biogas yield obtained in this experiment under higher temperature and NaOH concentration treatments supports the same. Before AD, there were no significant differences between temperature treatments in TS and VS. However, both TS and VS were significantly reduced after AD, and percent reduction increased with temperature. Similar trend was also noticed in the case of NaOH treatments. This result suggests that high temperatures and high concentrations of alkali compounds facilitate the decomposition of substrate for bacteria to act on during anaerobic digestion [26, 27,28,23,29, 30]. Reduction in TS and VS could be an indication of the utilization of substrate by bacteria for different metabolic activities that may also be reflected in biogas production [23]. In this experiment, compared to the control, great reduction of TS and VS after AD under higher temperature and NaOH treatments corresponded to high biogas yield, suggesting temperature and alkali treatments will increase the degradability of substrates so as to make materials ready for an efficient anaerobic digestion. On day one, the average daily gas production of the control and 60 ºC treatments was the same. However, that of 80 ºC treated substrate was higher than the control and 60 ºC treated substrates. This indicates that the 80 ºC pre-treated samples were more easily accessible to hydrolytic bacteria at the early stage of digestion. Biogas production for 60 and 80 ºC pre-treated samples ceased after 17 days while that of control continued up to 21 days. Thus, pre-treatment does not only yield greater amount of biogas, but it also reduces hydraulic retention time needed for AD [28]. For thermally (60 and 80 ºC) pre-treated samples greater than 66% of biogas were measured within 4 days, suggesting the availability of more organic material for microbes within this time period. In the case of NaOH treatment, gas production was not observed during the first two days. This could probably be due to the addition of 6N H_2SO_4 to maintain the pH at neutral. Addition of sulphuric acid results in by-products such as 5-hydroxylmethylfurfural (5-HMF) and furfural [31]. Though these by-products do not inhibit methane production, the methanogenic microorganisms may require a period of adaptation to start methane production [32, 33]. However, gas production commenced during on the third or fourth day and became superior to the control before it ceased on the 21 day, which may be due to lack of the necessary nutrients from the digesters [34].

5. CONCLUSION

Cumulative biogas production and volatile solid reduction from anaerobic digestion of 80 ºC pre-treated substrate, for example, was 46.3% and 26.1% higher than that of control, respectively. Likewise, pre-treatment with 0.45 g, 1.35 g, and 2.25 g of NaOH increased cumulative biogas yield by 0.03, 21 and 56% over that of control, respectively. Overall, this experiment revealed that thermal and NaOH pre-treatments of the substrate mix obtained from poultry litter and cow dung in 1:4 ratio enhance VS reduction and biogas production**.**

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