Anaerobic Biodegradability and Methane Potential of Crop Residue Co-Digested with Buffalo Dung

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ABSTRACT

ABD (Anaerobic Biodegradability) and BMP (Biochemical Methane Potential) of banana plant waste, canola straw, cotton stalks, rice straw, sugarcane trash and wheat straw co-digested with buffalo dung was evaluated through AMPTS (Automatic Methane Potential Test System). The substrates were analyzed for moisture, TS (Total Solids) and VS (Volatile Solids), ultimate analysis (CHONS), pH and TA (Total Alkalinity). The $BMP_{observed}$ during incubation of 30 days at the temperature of 37±0.2°C was 322 Nml CH_4/g VS_{add} for wheat straw followed by 260, 170, 149, 142 and 138 Nml CH_4/gVS_{add} for canola straw, rice straw, cotton stalks, banana plant waste and sugarcane trash respectively, whereas the maximum theoretical BMP was 481 Nml CH_4/gVS_{add} for cotton stalks, followed by 473, 473, 446, 432 and 385 Nml CH_4/gVS_{add} for wheat straw, banana plant waste, canola straw, rice straw and sugarcane trash respectively. The percentage ABD values were in the range of 68-30%. In addition to this, the effect of lignin content in the crop residue was evaluated on the ABD. The results of this study indicate that, the co-digestion of the crop residues with buffalo dung is feasible for production of renewable methane.

Key Words: **Anaerobic Biodegradability, Methane Potential, Automatic Methane Potential Test System, Crop Residue, Buffalo Dung.**

1. INTRODUCTION

Anaerobic Digestion (AD) involves disintegration of carbon-based material in molecular free oxygen (O_2) atmosphere. It results in formation of methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3) and other low molecular weight trace gases and carbon-based acids [1]. Formation of CH_4 through AD (bio-methane) is a clean and renewable source of energy. It can substitute fossil fuels and can decrease environmental pollution including acid rains and global warming [2]. Bio-methane is the most appropriate technique to convert agricultural biomass into a renewable energy source. An extensive array of crops and their residues, animal droppings and other different types of organic wastes can be used as feedstock for an AD [3-5]. Therefore AD has wide flexibility and can be modified to fulfill the precise requirements in management of agricultural farms [6]. Bio-methane production is often suggested in situations

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where animal dung is used as a major source of energy. The potential advantages of biogas from animal dung includes the replacement of an inefficient fuel with a more efficient and flexible one, the recovery of the fertilizer, which is lost if the animal dung is burned, and the benefits to public health (especially in reducing eye and lungs diseases) if the cleaner, less smoky biogas is used [7].

In addition to the animal waste, biogas can also be produced from the crop residue waste, which are being wasted or inefficiently used as reported by Sahito, et. al. [8]. The most important crop residues wasted in district Sanghar of Pakistan were banana plant waste, canola straw, cotton stalks, rice straw and sugarcane trash. These crop residues are either utilized conventionally for cooking, heating and backing bricks in brick kilns or being burnt into the agricultural fields and are wasted without getting any benefit from them. Such an open burning of crop residues can pollute environment [9-10]. Open burning of biomass is a source of greenhouse gases, including CO_2, CO, CH_4, nitrous oxide (N_2O) and nitrogen oxide (NO_x) [11]. It contributes aerosols and hydrocarbons to the atmosphere, because of the incomplete combustion process [12]. Biomass open burning is also the cause of lost nutrients and organic matter [13].

Biomass energy in crop residue and animal dung can be transformed into heat energy directly by adopting burning process or indirectly by first transforming them into secondary fuel as hydrogen, bio-methane, methanol or ethanol. Moreover, the heat can be utilized for space heating, cooking, and backing or for generation of stem followed by generation of electricity. Their transformation route depends on number of factors that includes, requirement of heat or steam, efficiency of the conversion process, physical system employed, process economy and its environmental consequences. Under most situations, bi-methane has been proven a perfect fuel. In comparison to the fossil fuels, bio-methane has very low environmental consequences and produces very low specific CO_2 per unit of electricity generated. Furthermore, due to CO_2 neutral behavior, there is an increasing trend towards use of bio-methane as vehicle fuel and for generating electricity [2].

Wet or dry AD processes can be employed to digest crop residues. In the agriculture management, it could be done by processing crop residues with animal dung. Controlled AD process not only provides renewable energy, but also decreases greenhouse gases and nitrogen oxides emissions and odor from animal dung management [14]. In anaerobic co-digestion of crop residues and animal dung, dung provides an extensive range of macro and micro nutrients and buffering capacity. The C/N (Carbon to Nitrogen) ratio of animal dung is low as compared to crop residues, while the co-digestion balances the C/N ratio and thus decreases the threat of ammonia inhibition [12].

The crop residues are lignocellulosic materials. They primarily comprise of three diverse polymers i.e. cellulose, hemicellulose and lignin. These polymers are allied with each other. Lignin is a most complex chemical compound, and a constitutional part of the plant's cell wall. It was first familiarized in 1819 and was derived from the Latin word "lignum", which means wood [16]. Lignin is the utmost copious renewable source of carbon on our planet, next to hemicellulose and cellulose. It enhances mechanical strength of the cell walls of wood and works as a cellulose fiber binder [17]. It decomposes gradually and virtually not yields net energy throughout its decomposition. Globally it plays an important function of atmospheric carbon sequestering. Because of high quantity available at a reasonable price, lignocellulosic biomass is an attractive renewable feedstock for production of biofuels [18-19]. As per biodegradability model, suggested by Chandler, et. al. [20], the maximum biodegradability of the lignin content crop residue under ideal condition would be 80%.

The design and construction of full-scale anaerobic digesters based on substrates from single or multiple sources requires a good understanding of the physical and biochemical characteristics of each individual substrate. BMP tests, i.e. anaerobic biodegradability assays, are used to determine the ABD and BMP of wastes or biomass, as well as the biodegradation rate under laboratory conditions. There are several methods exists for determining BMP of biomass. The elementary approach is to incubate a small amount of biomass with an anaerobic inoculum at a desired temperature and to measure the bio-methane production [21]. Generally methane is measured in terms of normalized volume and its percentage in total biogas production. Recently, Sambusiti, et. al. [22] have studied the effects of particle size on production of methane, in the same range 2, 1, 0.5 and 0.25mm particle sizes and shows that there are not any significant differences in terms of methane yields and kinetic constants.

The main objective of present study was to evaluate the suitability of co-digestion of crop residues, with buffalo dung. It involves the assessment of the six crop residues namely banana plant waste, canola straw, cotton stalks, rice straw, sugarcane trash and wheat straw for their BMP and ABD. For this crop residues were co-digested with buffalo dung in AMPTS and scrutiny them for higher methane production. In addition to this, the effect of lignin content in the crop residue was evaluated on the ABD.

2. METHODOLOGY

2.1 Preparation of Substrate

Six crop residues were collected from the fields of Hyderabad division. To obtain the representative crop residue sample, about 10-15 kg of each crop residue was taken from the field and dried at room temperature. The size of the dried crop residue samples were reduced by using hammer mill followed by the coffee grinder and brought up to the size of $\leq$1.0mm. The samples were placed in to the plastic bags for later determination of their characteristics and BMP.

2.2 Inoculum

Inoculum used in this study was the mixture of fresh buffalo dung obtained from the small animal farm located near MUET (Mehran University of Engineering & Technology), Jamshoro, Pakistan and effluent of mesophilic lab scale anaerobic digester treating buffalo dung and working at 37± 0.2°C. The ratio of buffalo dung and effluent on the basis of mass of volatile solids was set to 90:10 respectively.

2.3 Analytical Methods

MC (Moisture Content), TS, VS and TA were determined according to the Standard Methods [23]. The TA was determined by titration and distillation methods respectively for supernatant of the samples. The chemical composition (as % dry basis) of all crop residue and buffalo dung was determined with divanadium pentaoxide (V_2O_5) by flash combustion method using the CHNS analyzer (Thermo Scientific Flash EA series, USA). The values of C, H, N and S content for all samples were measured by instrument, whereas the % O content was determined as per difference basis. The pH was measured with a hydrogen ion sensitive electrode using a Lovibond, Senso Direct 150 pH meter.

The lignin in the crop residue samples were determined by acid delignification method, similar to Inari, et. al. [24] and Zhao, et. al. [25] with some modifications. Powdered crop residue of 500mg in terms of TS was placed in a 100ml Erlenmeyer flask containing 40ml of distilled water and then heated at 80°C in water bath. Subsequently 2ml of solution containing 15% of sodium chlorite ($NaClO_2$) and 0.1ml of acetic acid (CH_3COOH) were then added at every hour for five times. The mixture was filtrated on a Buchner funnel using vacuum pump and the residue was washed with distilled water. Subsequently, the residue was dried at the temperature of 105°C for the period of 24 hours to a constant mass. The loss of weight of crop residue was the measure of lignin content. For statistical significance lignin content was determined in triplicate and the average values were taken.

2.4 Preparation of Batch Assays

Batch assays were prepared by using AMPTS, which is a laboratory setup to measure methane production as the result from anaerobic digestion of biomass. It follows similar principle as the conventional methane potential tests. However, during the incubating period, its data logging of volume and flow of methane is fully automatic. Moreover, it is also compensating the gas pressure and temperature at standard values (101.325 kPa and 273.15K). The 500mL glass bottles were used as reactors and the BMP assays were performed at the temperature of 37± 0.2°C, which is most favorable temperature to methanogenic microorganisms [26]. The batch tests were accomplished as triplicate experiments for statistical significance. Each bottle was filled with 2g of VS of crop residue. The inoculum to substrate ratio was kept as 2.5 on the basis of grams of VS. After the inoculum and crop residue were added, each bottle was filled up to 400ml with tap water. Three bottles were filled as blanks, with only inoculum and tap water to quantify the production of bio-methane, forming from the inoculum alone. Moreover 1.5g of sodium hydrogen carbonate ($NaHCO_3$) was added in each bottle as buffer.

The bottles were sealed with hermetic rubber stoppers having 2 metal tubes (one for inert gas inlet and other for biogas exit) and 1 plastic tubing (for rotating shaft) and then by placing the plastic screw thread cap on the top. The bent stir rod of mixer was then connected to the DC motor attached with the plastic screw thread cap by carefully threading it into the Tygon tubing piece that is connected to the motor. The purpose of the mixer is to provide better contact of substrate and microorganisms. Afterwards all reactors were placed in the water bath and the motor connections were made in parallel and finally to the gas measuring device for getting electric power. One end of the metallic tubing of the reactor bottle was connected to the CO_2 separating bottle with the Tygon tubing, while the other was closed by installing plastic tubing clamps.

2.5 Setting CO_2 Separation Unit and Gas Volume Measuring Device

Methane can easily be separated by passing biogas from the solution of sodium hydroxide (NaOH) [27-28]. The 3M NaOH solution was used to absorb CO_2. Moreover, in NaOH solution 5ml of the 0.4% Thymolphthalein pH-indicator solution was also added. The prepared solution was then filled in 100ml CO_2 separating glass bottle up to 80ml. The bottles were closed with hermetic rubber stoppers having two metal tubes (one for inlet and second for exit) and then sealed by placing the plastic lid on the top, and screw until the thread on the bottle is no longer visible. One end of the CO_2 separating bottle was connected to the reactor bottle and the other to the gas measuring device with the Tygon tubing. The BMP assays were then flushed with Nitrogen gas (N_2) for the period of 5 minutes, to ensure the anaerobic condition in the head space of the reactors. The BMP assays were terminated after 30 days. The complete arrangement of the automatic methane potential test setup is shown in Fig. 1.

2.6 Theoretical and Observed Methane Potential

The usual representation of organic material is with its generalized formula i.e. $C_aH_bO_cN_d$. The $BMP_{theoretical}$ was calculated using Bushwell's Equation (1) [29], which assumes that the all the biodegradable organic material present in the substrate is converted to methane, CO_2 and ammonia.

$$C_aH_bO_cN_d + \frac{(4a-b-2c+3d)}{4}H_2O \rightarrow \frac{(4a+b-2c+3d)}{8}CH_4 + \frac{(4a-b+2c+3d)}{8}CO_2 + dNH_3 \qquad (1)$$

The observed methane gas production was obtained by using Equation (2), where $BMP_{observed}$ is the observed biochemical methane potential (NmL CH_4/g VS_{add}), $V_{ino\&sub}$ is the volume of methane produced by inoculum and substrate (NmL CH_4), V_{ino} is the volume of methane produced by inoculum alone (NmL CH_4) and mVS_{sub} is mass of volatile solids in substrate (g VS).

$$BMP_{observed} = \frac{V_{ino\&sub} - V_{ino}}{mVS_{sub}} \qquad (2)$$

2.7 ABD

The ABD was calculated by Equation (3), [30] in which $BMP_{observed}$ is observed biochemical methane potential, achieved at the end of 30 days BMP test and $BMP_{theoretical}$ is theoretical BMP, calculated from Equation (1).

$$ABD(\%) = \frac{BMP_{observed}}{BMP_{theoretical}} \times 100 \qquad (3)$$

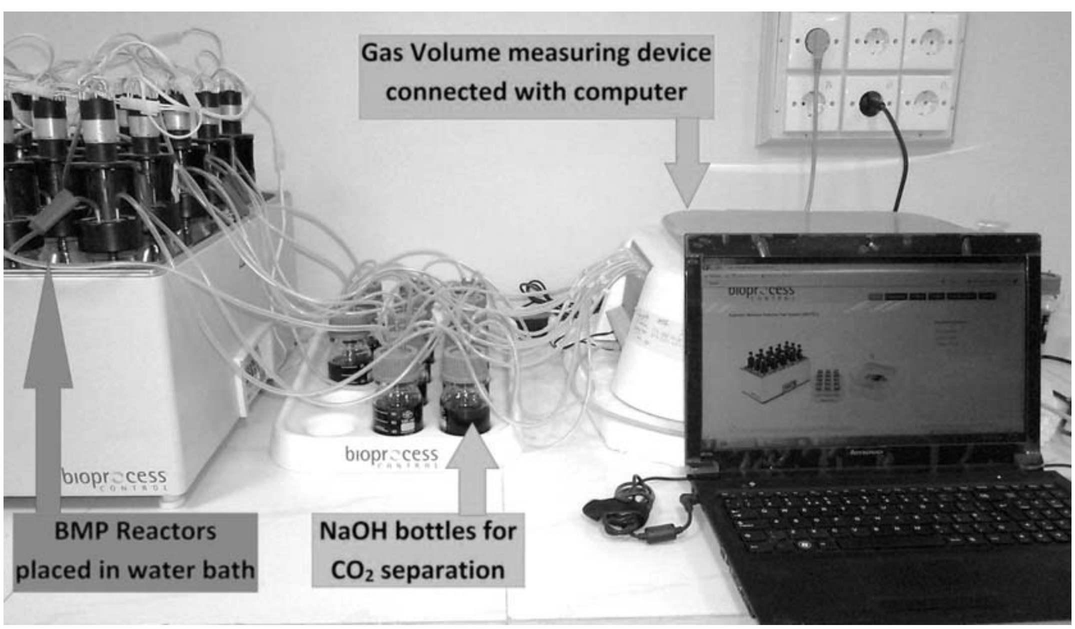

FIG. 1. AUTOMATIC METHANE POTENTIAL TEST SETUP

3. RESULTS AND DISCUSSION

3.1 Characterization of Substrates

The characterization of the substrates is an important step before they can be utilized in anaerobic digestion. The results of ultimate analysis, gravimetric analysis, lignin content and TA of the selected crop residues and buffalo dung is given in Table 1. Results show that the crop residues have substantial carbon content ranging from 39-45% on dry basis, whereas it is 38.6% for buffalo dung. Similarly crop residues have high VS ranging from 83-96% and buffalo dung has VS content of 71.8%. All the crop residues are acidic in nature having pH ranging from 5.3 (cotton stalks) to 6.8 (banana plant waste) and have low values of TA vary over the range of 100-1350mg $CaCO_3$/L for sugarcane trash and banana plant waste respectively, whereas the buffalo dung is alkaline in nature with pH value of 7.5 and TA of 1800mg $CaCO_3$/L.

3.2 Batch Assays

The flow rate of methane from crop residues and inoculum obtained at the incubation of 30 days at the temperature of 37± 0.2°C is shown in Fig. 2. It can be observed that the production of methane decreases remarkably after 20 days of incubation. The methane production within first 15 days of the test was ranging from 68-76%, whereas within 20 days it accounts from 85-91% of the total methane produced within 30 days of BMP test. The digester retention time is the key process design constraint that is selected to ensure that the microorganisms in the reactor have adequate time to grow and reproduce [30]. Simultaneously it is important for economic success to ensure that the digester is operated at the maximum rate of gas production. For the six crop residues the 20 days retention time may be kept.

3.3 Results of BMP and ABD

The BMP value is the quantity of bio-methane produced in a BMP assay and usually expressed in terms of per gram of VS [21], while the ABD is the ratio between observed and theoretical BMP values. Table 2 shows the theoretical and observed BMP values of all six crop residues along with their ABD, where BMP is represented in NmL CH_4/gVSadd. In addition to these, molar composition equations for biogas production for each crop residue are also stated.

TABLE 1. CHARACTERIZATION OF CROP RESIDUE AND BUFFALO DUNG

Characteristics	Banana Plant Waste	Canola Straw	Cotton Stalks	Rice Straw	Sugarcane Trash	Wheat Straw	Buffalo Dung
C (%TS)	40.74	43.44	45.02	37.64	40.69	42.68	38.62
H (%TS)	4.98	4.73	5.13	4.75	3.53	5.08	4.30
O (%TS)	35.99	39.75	37.91	36.95	41.23	35.83	40.12
N (%TS)	0.47	0.44	0.53	1.04	0.45	1.65	1.32
S (%TS)	0.11	1.02	0.44	0.23	0.14	0.25	0.15
Ash (%TS)	17.71	10.61	10.96	19.39	13.95	14.51	15.50
MC (%)	85.53	6.56	6.61	2.12	2.36	7.14	80.50
TS (%)	14.47	93.44	93.39	97.88	97.64	92.86	19.50
VS (%TS)	82.98	90.81	95.66	83.35	87.04	86.78	71.81
Lignin (%VS)	19.53	13.56	16.29	17.82	15.76	11.75	8.98
pH	6.80	5.40	5.30	6.00	5.40	5.50	7.50
TA (mg $CaCO_3$/L)	1350	650	167	550	100	500	1800

The maximum $BMP_{observed}$ was 322 Nml CH_4/gVS_{add} for wheat straw, which was 7% higher than reported by Nallathambi [3]. The second highest BMP observed was 260 Nml CH_4/gVS_{add} for canola straw, which was 8% higher than reported by Lehtomaki, et. al. [31]. The BMP obtained for rice straw was 170 Nml CH_4/gVS_{add}, whereas Chandra, et. al. [32] has achieved only 132.7 Nml CH_4/gVS_{add}. Cotton stalks produce 149 Nml CH_4/gVS_{add}, which was 56% higher than reported by Isic, [33]. Methane production from banana plant waste was 142 Nml CH_4/gVS_{add}, while Kalia, et. al. [34] achieved higher BMP as 267 Nml CH_4/gTS_{add}, by giving thermal treatment to banana plant waste. The BMP for sugarcane trash was observed as 138 Nml CH_4/gVS_{add}, in comparison to the 360 Nml CH_4/gTS_{add} achived by Chanakya, et. al. [35]. This higher methane production was due to the lower VS of substrate in BMP assays as 0.5g TS/L, if compared to 3.92g TS/L used in present study.

The maximum theoretical BMP was 481 Nml CH_4/gVS_{add} for cotton stalks, followed by 473, 473, 446, 432 and 385 Nml CH_4/gVS_{add} for wheat straw, banana plant waste, canola straw, rice straw and sugarcane trash respectively. The theoretical BMP is always higher than the observed, because the former accounts all organic matter (biodegradable and non-biodegradable) [36]. Moreover the ABD values for the selected crop residues were in the range of 68-30%, and were higher for wheat straw and lower for banana plant waste.

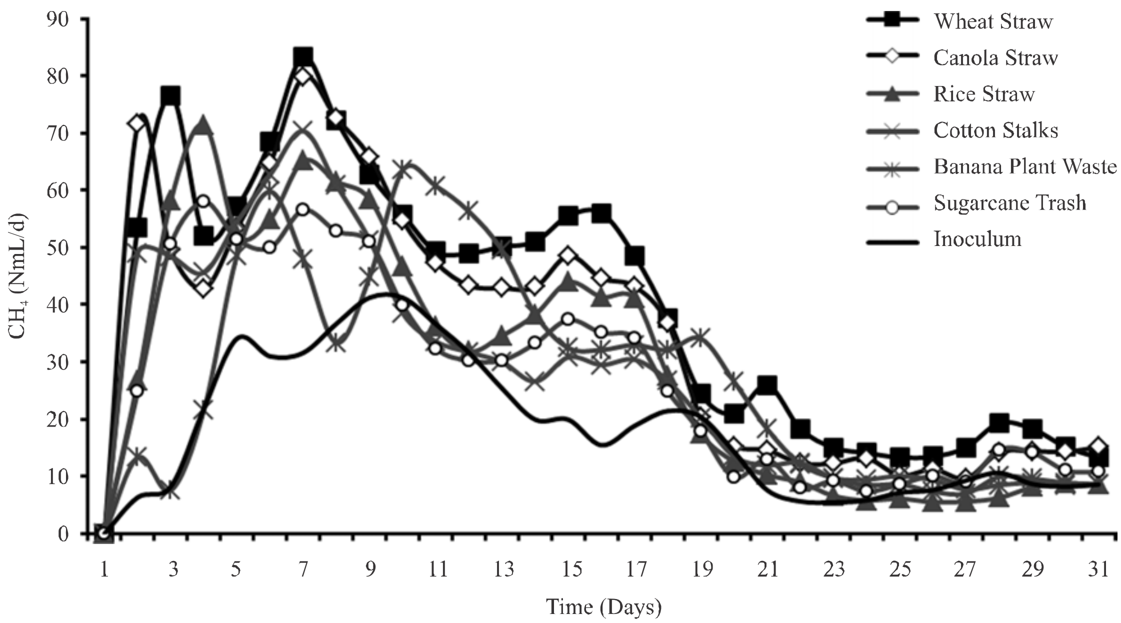

FIG. 2. METHANE FLOW RATE FOR CROP RESIDUES AND BUFFALO DUNG

TABLE 2. MOLAR COMPOSITION EQUATION FOR BIOGAS, BMP AND ABD VALUES OF CROP RESIDUES

No.	Name of Crop Residue	Molar Composition Equation for Biogas	$BMP_{theoretical}$ (NmL CH_4/gVS_{add})	$BMP_{observed}$ (NmL CH_4/gVS_{add})	ABD (%)
1.	Wheat Straw	$C_{30}H_{43}O_{19}+11H_2O \rightarrow 15CH_4+14.9CO_2+NH_3$	473	322	68.14
2.	Canola Straw	$C_{115}H_{149}O_{79}N+39H_2O \rightarrow 56CH_4+59.1CO_2+NH_3$	446	260	58.33
3.	Rice Straw	$C_{42}H_{63}O_{31}N+12H_2O \rightarrow 21CH_4+21.3CO_2+NH_3$	432	170	39.39
4.	Sugarcane Trash	$C_{105}H_{109}O_{80}N+39H_2O \rightarrow 46CH_4+59.6CO_2+NH_3$	385	138	35.76
5.	Cotton Stalks	$C_{99}H_{134}O_{63}N+35H_2O \rightarrow 50CH_4+48.8CO_2+NH_3$	481	149	31.02
6.	Banana Plant Waste	$C_{101}H_{147}O_{67}N+32H_2O \rightarrow 52CH_4+49.3CO_2+NH_3$	473	142	29.95

3.4 Relation between ABD and Lignin Content

Lignin and hemicellulose are not easily degraded under anaerobic conditions [37]. The percentage ABD based on the BMP test results and percentage lignin content in selected crop residues is illustrated on primary and secondary axis of Fig. 3. It is unevenly in agreement to the Moller et al. [4], that there is an opposite relation between the ABD and lignin content of the crop residues. The correlation between the percentage ABD and percentage lignin content of the selected crop residues is shown in Fig. 4. The coefficient of correlation (R^2) between ABD and lignin content was calculated as 0.79 and was higher than to 0.59 as reported by Tong, et. al. [38], whereas Chandler et al. [20] has reported a high R^2 value of 0.94. This shows that the production of methane, by the anaerobic degradation of lignocellulosic biomass cannot be predicted exclusively based on its lignin content.

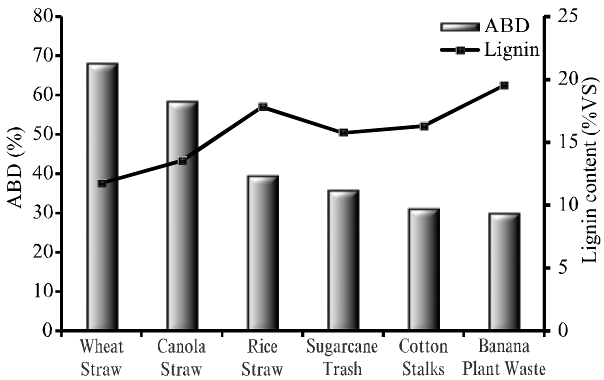

FIG. 3. LIGNIN CONTENT OF CROP RESIDUES AND THEIR ABD

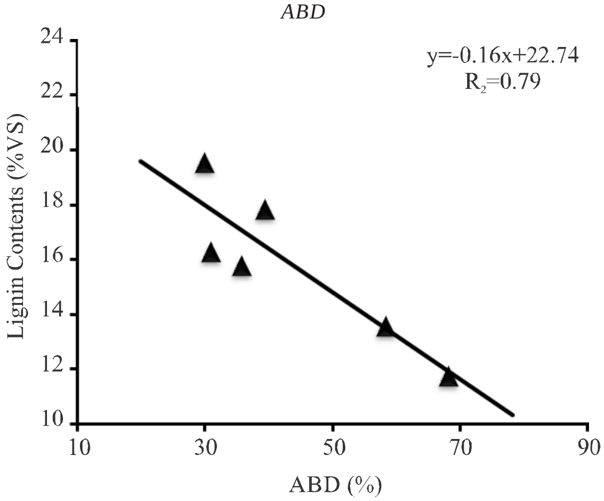

FIG. 4. CORRELATION BETWEEN ABD AND LIGNIN CONTENT OF CROP RESIDUES

4. CONCLUSIONS

The present results show that, the selected crop residues are acidic in nature and require some buffer, if used for anaerobic digestion. Buffalo dung can provide partial alkalinity if co-digested with crop residues. The 30 days BMP of selected crop residues were in the range of 322-138 Nml CH_4/gVS_{add} for wheat straw and sugarcane trash respectively, whereas the maximum theoretical BMP were in the range of 481-385 Nml CH_4/gVS_{add} for wheat straw and sugarcane trash respectively. Moreover, they have high percentage of VS content ranging from 83-96%, but have low percentages of ABD's ranging from 30-68%. The low percentages of ABD are because of the lingo-cellulose nature of the crop residues, as unevenly higher the lignin content, lower will be the biodegradability.

Furthermore, the anaerobic co-digestion of wheat straw, canola straw and rice straw with buffalo dung is feasible for production of methane, as they have substantial percentages of ABD ($\geq$40%) without any thermal, acidic or alkaline treatment. It is believed that methane production from wasted crop residues (canola straw and rice straw) and buffalo dung is a renewable and sustainable energy source, which can contribute in primary energy supply and agricultural waste minimization and decrease in environmental pollution.

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