Analysis of agricultural substrates for nutritive values and biomethane potential

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Bio-methane from agricultural waste has enough potential to compete with other sources of energy. This study aims to examine the bio-methane potential of numerous agricultural wastes, including cotton waste, wheat bran, lentil straw, barley straw, rice bran and peanut peels straw with the aim to produce renewable energy and solve waste disposal issues. The proximate, ultimate and chemical composition analyses were performed to predict the theoretical bio-methane potentials *in silico*. However, the potential was experimentally assayed at mesophilic conditions. Moreover, elemental and lignin based biodegradability of substrates have also been determined. The methane contents in biogas are in the range 57–64% and the yield varied from 216.3 (barley straw) to 317.6 (cotton waste) ml/g volatile solids. These results indicate that higher biodegradability of substrates resulted in higher methane production. The prediction of bio-methane potential from chemical composition, elemental composition and organic fraction were not as fit accurately as being assessed for methane potential. It merely provided the extent of biodegradability. During digestion, volatile fatty acids were produced, viz. acetic acid (58–63%), butyric acid (28–32%), propionic acid (6–13%) and converted into methane but limited concentrations of intermediate acids indicated similar microbial consortium in all digestions. Hence, it was also concluded that the lignin and hemicellulose content played a limiting role in digestion and posed negative impact on biogas production.

Keywords: Acid detergent fibre, acid detergent lignin, anaerobic digestion, neutral detergent fibre, volatile fatty acids.

PAKISTAN is an agricultural country with more than 60% of agricultural land producing large number of agricultural goods and waste residues. These wastes have been globally recognized as a promising source of renewable energy. Bioenergy has been gaining interest over the past decade as it is cheap and eco-friendly (reduced 60% greenhouse gases) and is well acknowledged as a second generation biofuel. Agricultural waste substrates (AWSs) do not require any special input for growing. More than 50% of AWSs are reported to have been used in energy production in Europe[1]. Hence, AWSs should be utilized for energy production in developing countries like Pakistan, where huge quantities of agricultural wastes are available around the year.

Anaerobic digestion (AD) is a prominent and economically favourable (waste-to-energy) technique for the production of bio-methane from AWSs[1]. AD converts organic matter into biogas via four consecutive stages, i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the initial stages, organic compounds are converted into simple monomers, volatile fatty acids (VFAs) and hydrogen (H_2) and finally these intermediate products are converted into CH_4 in methanogens[2]. Typical composition of biogas is 55–80% CH_4 and 20–45% CO_2, with other impurities such as H_2 and H_2S (traces)[3]. A number of reports illustrate that bio-methane potentials (BMPs) ranged from 200 to 450 ml/g volatile solids (VS) of different AWSs[1,4]; e.g. 20–360 ml/g VS for barley waste[5], 270–350 ml/g VS[6] for grasses, 202–410 ml/g VS for maize waste[7], 67–230 ml/g VS for sugar beet waste[6]. The produced biogas can be used without modification in power gas engines for combined heat and power[3,8].

A variety of different agricultural wastes are being produced in Pakistan including, cotton waste (CW), wheat bran (WB), lentil straw (LS), barley straw (BS), rice bran (RB) and peanut peels straw (PPS). Therefore, they can be used for energy and power generation. This study aimed at examining the various locally produced agricultural wastes for biomethane potential by BMP assays. In addition, nutritional composition of substrates and their effect on BMP were determined. The various BMP predication methods used in this study were ultimate analysis BMP (BMP_{CHNO}), stoichiometry BMP_{OFC} by organic fraction and $BMP_{NDF,ADF,ADL}$ from neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin content (ADL).

Experimental procedure

Substrate and inoculum

Indigenous agriculture wastes such as CW, BS, LS, WB, RB and PPS were collected, dried and ground with a laboratory blender to a fine powder (size ranging from 0.2 to 0.3 cm). The active and developed inoculum was taken

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from the biogas plant, located at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.

Analytical methods

Total solids (TS), VS and ash were determined by gravimetric method[9]. The elemental composition (C, H, O, N, and S) of substrates was analysed by an elemental analyser CHNS/O analyser (PE 2400 Series II, Perkin Elmer, USA). NDF, ADF and ADL were determined by modified methods of Van Soest[10]. Hemicellulose (HC) was calculated by the difference of NDF and ADF (HC = NDF – ADF) while cellulose were determined from the difference of ADF and ADL (cellulose = ADF – ADL). Crude fibre content (CF) was determined by modified Maynard method of food analysis[10]. Crude protein (CP) was estimated by multiplying nitrogen content with 5.7 (i.e. N × 5.7). The nitrogen content was determined by CHNS/O analyser while 5.7 is a protein factor used to calculate CP of agricultural waste[11]. Total carbohydrates were measured by anthrone method. Theoretical CH_4 yields were predicted by using the following Buswell and Mueller eqs (1) and (2) based on the elemental composition of substrates[12]

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right) H_2O \rightarrow \left\{\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right\} CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right) \times CO_2 + cNH_3. \quad (1)$$

$$BMP_{CHNO}\ (\text{ml/g VS}) = \frac{22.4 \times 1{,}000 \times (n/2 + a/8 - b/4 - 3c/8)}{12n + a + 16b + 14c}. \quad (2)$$

Organic fraction composition (OFC), i.e. carbohydrates and CP values were used to calculate BMP_{OFC} by eq. (3)[13]

$$BMP_{OFC} = 415 \times \%\ \text{carbohydrates} + 496 \times \%\ \text{proteins} + 1014 \times \%\ \text{lipids}. \quad (3)$$

Similarly, eq. (4)[14] was used for calculating $BMP_{NDF,ADF,ADL}$

$$BMP_{NDF,ADF,ADL} = 5.4729 \times NDF\% - 4.0630 \times ADF\% - 1.2422 \times ADL\%. \quad (4)$$

Elemental biodegradability (BD_{ele}) of the substrate was estimated by eq. (5)[15]

$$BD_{ele} = \frac{BMP_{exp}}{BMP_{CHNO}} \times 100. \quad (5)$$

Similarly, the experimental biodegradability (BD_{exp}) was determined by using eq. (6) based on initial and final values of VS[16]

$$BD_{exp}VS = \frac{VSi - VSf}{VSi} \times 100. \quad (6)$$

Bio-methane potential assay

For assessment of BMP_{exp} at mesophilic temperature and batch conditions, bottles of 250 ml volume (Schott Duran, Germany) were used and 100 ml prepared media was added in each bottle. The media was prepared according to Angelidaki[17]. A 2% VS of substrate and 10 ml inoculum was poured in the bottles. Each bottle was flushed with N_2 and CO_2 mixture (80/20% by volume) for 5 min and was tightly closed to maintain anaerobic conditions. These bottles were incubated at 37°C for 35–40 days and vortexed twice a day. The CH_4 production was measured on a daily basis during the 1st week, alternate days in the 2nd week and then after every two or three days in the 3rd, 4th and 5th week of incubation.

Biogas analysis

Biogas volume was measured through water displacement method by alkaline solution (2% NaOH)[17]. A gas composition analysis was measured using gas analyser (GFM 4XX series, UK). The sample was withdrawn regularly for VFA analysis. The individual short chain volatile fatty acids (SCVFAs) were determined after 6 days, 15 days and 30 days of experiment by APHA standards[18]. The total volatile fatty acids (tVFAs) was determined[19] by eq. (7) at regular time intervals.

$$\text{tVFA mg/l} = \frac{N \times V \times 5000}{A}, \quad (7)$$

where A is the volume of sample in ml, N the normality of acid used (0.1N H_2SO_4) and V is the volume of acid used in attaining end point pH = 4.3.

Biodegradability (BD) based on lignin BD (BD_{LB}) is calculated by the model described by Chandler[20] using eq. (8)

$$LB\% = (0.83 - (0.028 \times Xi)) \times 100, \quad (8)$$

where B is the biodegradable fraction of VS and Xi is initial lignin concentration of VS.

Statistical analyses

All statistical analyses were performed using Excel of MS 2016. One-way ANOVA was applied on average of CH_4 yields of AWSs.

Table 1. Ultimate analyses of substrate along with physical properties

	CW	BS	LS	WB	RB	PPS
Components	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C%	56.5 ± 2.3	53.2 ± 2.7	52.9 ± 1.6	48.4 ± 2.1	42.0 ± 0.8	40.2 ± 0.6
H%	3.9 ± 0.4	3.6 ± 0.4	4.6 ± 0.3	5.3 ± 0.7	5.1 ± 0.2	5.6 ± 0.5
N%	0.13 ± 0.05	0.41 ± 0.2	1.2 ± 0.1	0.3 ± 0.03	0.7 ± 0.2	1.3 ± 0.2
S%	0.32 ± 0.4	0.08 ± 0.03	0.08 ± 0.02	0.06 ± 0.03	0.05 ± 0.03	0.07 ± 0.02
O%	39.2 ± 2.8	42.7 ± 3	41.2 ± 1.9	45.9 ± 1.8	52.2 ± 0.6	52.8 ± 0.9
Moisture %	15.3 ± 1.2	18.3 ± 2.1	19.1 ± 1.0	12.7 ± 0.8	11.8 ± 2.4	9.7 ± 0.4
VS%	75.4 ± 2.3	52.7 ± 1.9	57.7 ± 2.2	69.6 ± 1.5	66.9 ± 1.6	64.3 ± 1.9
FS% (Ash)	4.3 ± 0.2	5.7 ± 0.5	7.6 ± 0.5	4.4 ± 0.3	5.7 ± 0.3	2.4 ± 0.3
TS%	79.7 ± 2.1	58.4 ± 1.5	65.3 ± 2.6	74.02 ± 1.1	72.6 ± 1.3	66.7 ± 1.8

The ultimate analyses of substrates included CHNSO contents and ash contents.

Table 2. Main chemical composition of substrates

Components	CW	BS	LS	WB	RB	PPS
NDF%	73.3 ± 0.8	72.3 ± 1.6	63.3 ± 0.9	43.2 ± 0.9	29.1 ± 0.6	38.9 ± 2.2
ADF%	50.4 ± 2.4	50.3 ± 2.7	32.6 ± 1.6	11.8 ± 0.4	13.3 ± 1.0	17.4 ± 1.9
ADL%	8.6 ± 0.8	14.0 ± 0.9	8.4 ± 0.2	3.4 ± 0.3	4.3 ± 0.6	7.7 ± 0.6
HC%	22.9 ± 3.1	22.0 ± 2.2	30.6 ± 1.8	31.4 ± 1.2	15.8 ± 0.4	21.5 ± 3.9
Cellulose%	41.7 ± 1.9	36.3 ± 2.1	24.2 ± 1.4	8.3 ± 0.7	8.9 ± 1.6	9.7 ± 2.3
CF%	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.3	1.2 ± 0.2	1.4 ± 0.3	1.3 ± 0.1
CP%	0.7 ± 0.3	3.1 ± 1.0	6.8 ± 0.6	1.6 ± 0.2	3.8 ± 1.2	7.3 ± 1.2
Carbohydrates%	77.8 ± 4.1	65.3 ± 1.6	69.0 ± 3.9	72.1 ± 4.8	61.1 ± 5.2	60.6 ± 0.4

Results

Ultimate and proximate analysis

In the ultimate analyses, carbon content varied from 40.2% to 56.5% in the order CW > BS > LS > WB > RB > PPS while hydrogen content varied from 3.6% to 5.6% and oxygen varied from 39.2% to 52.9% in the reverse order of carbon, i.e. PPS > RB > WB > LS >BS > CW. Nitrogen and S were low. VS and TS content varied from 52.7% to 75.4% and 58.4% to 79.7% respectively, in the order CW > LS > PPS > RB > WB > BS (Table 1).

Chemical compositions

The chemical compositions of AWSs used in this study are listed in Table 2, along with their distinctive properties. The NDF content varied between 29.1% (RB) and 75.6% (BS) in the order BS > CW > LS > WB > PPS > RB. The ADF content varied between 11.7% (WB) and 55.1% (BS) in the order BS > CW > LS > PPS > RB > WB. The ADL content varied between 3.4% (WB) and 8.7% (CW) in the order CW > LS > PPS > BS > RB > WB. HC content varied from 15.8% to 31.4% in the order WB > LS > CW > BS > PPS > RB. Cellulose content varied with a large difference from 8.4% to 41.7% in the order CW > BS > LS > PPS > RB > WB. Carbohydrate content varied from 60.7% to 77.8% in the order CW > WB > LS > BS > RB > PPS.

Biogas analysis

The CH_4 contents in biogas composition were in the range 57–64%; an average of CH_4 content in the 3rd and 4th week are given in Table 3. The CH_4 contents were not constant throughout the process and increased gradually from 40% (first week) to 50–60% (second and third week) and finally reached a peak of 65–67%. The rate of production was highest in the first and second week of AD and gradually reduced in remaining weeks of AD. Maximum CH_4 production per day is given in Table 3. This production was noted between the 6th and 9th day of AD. The cumulative BMP_{exp} is shown in Figure 1 and actual rate of CH_4 production is given in Figure 2.

BMP_{exp}, BMP_{CHNO}, BMP_{OFC} and $BMP_{NDF,ADF,ADL}$ assay

BMP_{exp} varied from 216.3 (BS) to 317.6 (CW) ml/g VS. The order of BMP_{exp} was CW > WB > LS > RB > PPS > BS (Table 4), and statistical non-significant differences were found in the cumulative CH_4 values ($0.204 > \alpha = 0.05$). The predicted BMPs are shown in Table 4. Empirical formulae have been deduced from ultimate analysis of AWSs. BMP_{CHNO} was the maximum BMP when compared to other predicted methods and the order was CW > LS > BS > WB > RB > PPS, varying from 340.7 to 484.3 ml/g VS. BMP_{OFC} was lesser than BMP_{CHNO} and varied from 272.5 to 326.5 ml/g VS. The

Table 3. Characteristics of AD of different substrates

Analysis	CW	BS	LS	WB	RB	PPS
BD_{exp}% VS[1]	76.0	65.5	70.0	74.0	68.5	67.5
LB%[2]	58.7	43.7	59.3	73.5	70.9	61.6
BD_{ele}[3]	65.6	49.4	59.4	68.1	77.9	75.0
tVFA mg/l at 6th day	2250 ± 22.3	1700 ± 12.5	1925 ± 26.1	2100 ± 18.6	1900 ± 20.9	1755 ± 14.9
tVFA mg/l at 12th day	900 ± 12.4	876 ± 20.1	785 ± 14.2	800 ± 17.6	750 ± 18.2	720 ± 16.0
t VFA mg/l at 30th day	130 ± 6.5	126 ± 8.3	200 ± 9.7	176 ± 12.3	180 ± 10.8	190 ± 11.6
CH_4%	63 ± 3.2	57 ± 2.8	60 ± 2.9	63 ± 3.4	64 ± 3.1	60 ± 2.5
Max. CH_4 ml/g VS[4]	27.1 ± 2.3	20.1 ± 3.5	28.1 ± 1.3	31.0 ± 2.7	23.9 ± 2.5	22.7 ± 4.1

[1]Biodegradability experimentally calculated based on VS; [2]Biodegradable fraction based on lignin, BD_{exp} VS; [3]Elemental biodegradability; [4]Maximum daily CH_4 ml/g VS.

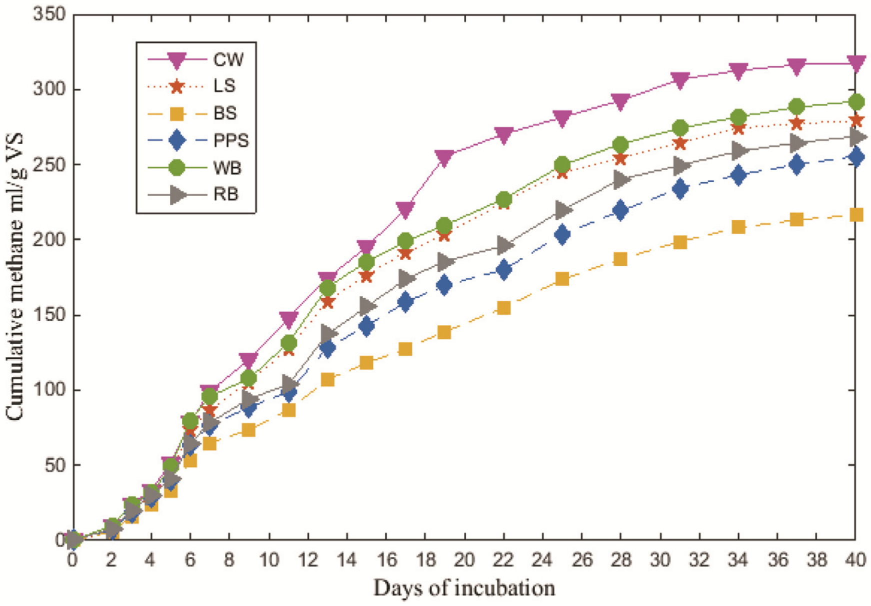

Figure 1. Cumulative CH_4 ml/g VS of agricultural waste substrates.

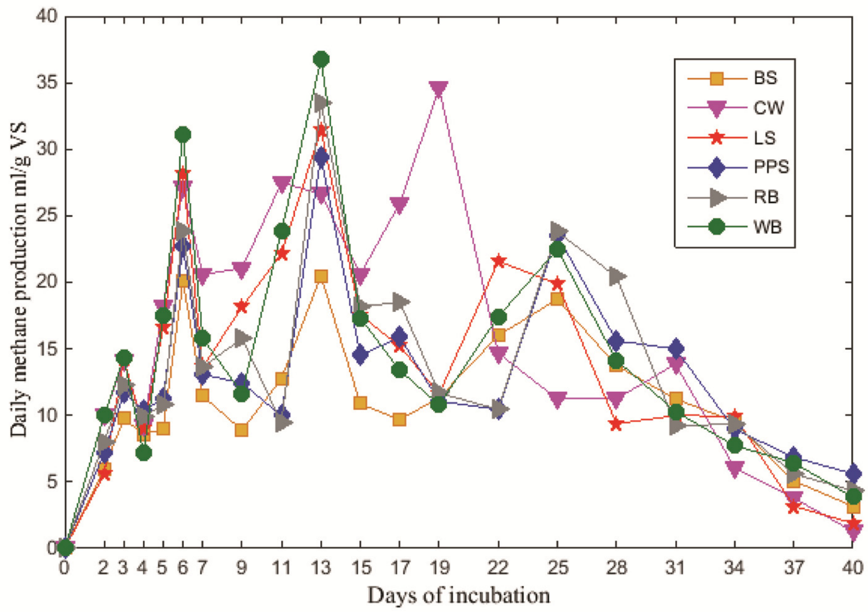

Figure 2. Rate of CH_4 production of agricultural waste substrates.

Table 4. Empirical formulae, theoretical BMPs based on elemental, chemical and compositional, experimental BMP of substrates

		ml/g VS			
AWSs	Empirical formula	BMP_{CHNO}[1]	BMP_{OFC}[2]	$BMP_{NDF,ADF,ADL}$[3]	BMP_{exp}[4]
CW	$C_{4.71}H_{3.87}O_{2.47}N_{0.01}$	484.3	326.5	185.6	317.6 ± 12.4
BS	$C_{4.43}H_{3.63}O_{2.67}N_{0.03}$	438.0	286.3	173.8	216.4 ± 5.6
LS	$C_{4.41}H_{4.56}O_{2.58}N_{0.09}$	469.7	292.6	203.8	279.2 ± 4.5
WB	$C_{4.03}H_{5.33}O_{2.88}N_{0.02}$	428.9	306.9	184.7	292.0 ± 5.6
RB	$C_{3.50}H_{5.10}O_{3.26}N_{0.05}$	344.9	272.5	99.8	268.8 ± 8.8
PPS	$C_{3.35}H_{5.56}O_{3.31}N_{0.09}$	340.7	288.6	132.6	255.6 ± 3.5

[1]BMP_{CHNO} was calculated from CHNO values determined from CHNS analyser; [2]BMP_{OFC} was calculated from carbohydrates and protein contents only; [3]$BMP_{NDF,ADF,ADL}$ was calculated from NDF, ADF and ADL values; [4]Experimentally determined.

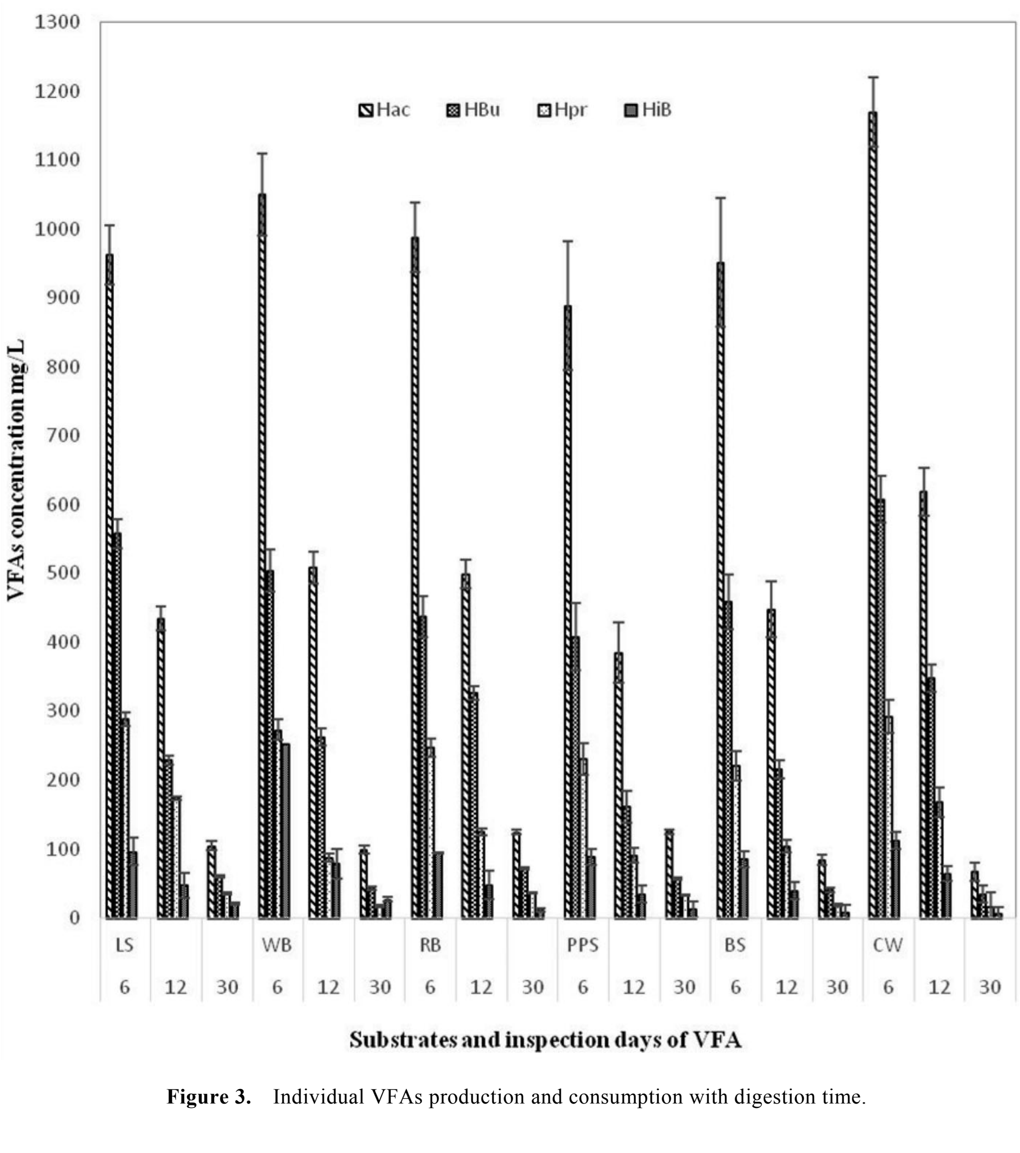

Figure 3. Individual VFAs production and consumption with digestion time.

order of BMP_{OFC} was CW > WB > BS > LS > RB > PPS. $BMP_{NDF,ADF,ADL}$ was minimum in all these predicted BMPs, and varied from 99.8 to 203.8 ml/g VS in the order LS > CW > WB > BS > PPS > RB.

BD analysis

BD_{ele} content varied from 49.4 to 77.9 and the order was RB > PPS > WB > CW > LS > BS. BD_{exp} content varied from 65.5% (BS) to 76.0% (CW) and the order was CW > WB > LS > RB > PPS > BS. LB% varied from 43.7 (BS) to 73.4 (WB), and the order was WB > RB > BS > PPS > LS > CW as shown in Table 3.

VFAs analysis

The concentration of total volatile fatty acids (tVFA) is shown in Table 3. The analysis of AD showed the following order of tVFA in the first inspection: CW > WB > LS > RB > PPS > BS, i.e. 2250, 2100, 1925, 1900, 1755 and 1700 mg/l respectively. After the end of the second week (15th day), its concentration reduced to the following order: CW > BS > WB > LS > RB > PPS, i.e. 900, 876, 800, 785, 750 and 720 mg/l respectively. In the final reading, i.e. 30th day, its concentration further reduced to the following order: LS > PPS > RB > WB > CW > BS, i.e. 200, 190, 180, 176, 130 and 126 mg/l respectively. Individual analysis of VFAs revealed the presence of acetic acid (HAc), propionic acid (HPr), butyric acid (HBu) and isobutyric (HiB) acid in tVFA as shown in Figure 3. HAc was higher (58–63% of tVFA) in all inspected results of all AWSs as compared to HPr, HBu and HiB. However, HBu also remained higher at 28–32% of tVFA and HPr remained at 6–13% as compared to the other two acids.

Discussion

Ultimate, proximate and chemical analysis

A lot of variations in proximate, ultimate and chemical compositions were reported even within the same species of agricultural biomass. Generally, dry matters are in the range 15–25.5% of agricultural biomass while ash and organic matters contents are in the range 9.2–16.5% and 83–92% of dry matter respectively. Similarly, CPs, NDF, ADF, ADL, HC and cellulose are in the range 8–20%, 41.1–76.5%, 21.2–50%, 2.4–10.2%, 12–30% and 13.7–40% of dry matter respectively[21,22]. In this study, all these characteristics were found within the range of reported data. However, slight variations of these results found in the literature might be due to variations in biomass growth parameters like growth and environmental (soil, plant maturity stage and seasonal difference) conditions. As previously reported, the variations in these parameters have an influence on the chemical compositions[23]. Studies have mentioned that the ripening of crops has reduced the concentrations of NDF, ADF, CP and ADL[24,25]. The insight image of chemical compositions of AWSs enabled to predict incubation time and BMP. Among proximate analyses, VS is more important because only VS fractions were digestible. The elemental composition majorly comprised of carbon, hydrogen, nitrogen and oxygen which was an important factor for successful operation of the process because it not only predicted nutritional requirements of microbial growth but also helped in balancing carbon to nitrogen ratio. The ultimate values of AWSs were found in the broad ranges of carbon, hydrogen, oxygen, nitrogen and sulphur, i.e. 36.3–46.7%, 4.6–5.6%, 33.7–46.8%, 0.2–0.7 and 0.01–0.17% respectively[26]; only slight variations were found in carbon content. The reason for variation in results of ultimate compositions was similar to the variation as discussed above for the chemical compositions[23]. These ultimate and chemical compositions predicted the maximum BMP and BD, based on 100% conversion of biomass into gas[12]. All these variations in the properties have shown different BD of AWSs.

Biogas analysis

Generally, CH_4 contents range was reported around 60–70% and 70–80% for albizia and food wastes respectively[27]. However, an overall CH_4 content range was reported around 55–80% in biogas for AWSs[3]. The CH_4 content ranges of this study were in line with literature. Although pre-adopted inoculum was used, no significant improvements in CH_4 content were observed and it mainly depended on the methanogenic activity and its numbers. The CH_4 contents were initially low due to acclimation period needed by methenogens; but using pre-adopted inoculum acclimation periods were reduced and sustainability was achieved rapidly[27]. However, there were slight variations in the CH_4 content which might be due to the differences in substrate compositions. Overall it depended upon the lignin content and nature of the inoculum[28]. The cumulative Figure 1 does not represent whole story of biogas production; but actually, a lot of variation was observed in the rate of biogas as shown in Figure 2. Initial production rate was higher due to higher amount of VS content which degraded gradually. Consequently, its production rate was decreased further in the first two weeks and ultimately stopped when digestible AWSs were used up.

BMP_{exp}, BMP_{CHNO}, BMP_{OFC} and $BMP_{NDF,ADF,ADL}$ assay

The BMP_{exp} has been reported lower than predicted BMPs, because of recalcitrant carbon, which is not considered as recalcitrant in stoichiometric calculations[15,29]. This non-biodegradable constituent also reduced the BD of other chemical components such as HC and celluloses[29]. These predicted BMPs cannot be used as a gauge of BMP, but are helpful in assessing the BD of AWSs, also called as BD index[1]. The other reason for higher values of predicted BMPs than BMP_{exp} was due to some components of AWSs in the BMP assay which were also utilized for microbial growth and metabolism, but not considered in stoichiometric calculation[29]. The comparison within predicted BMPs showed that BMP_{CHNO} has the highest value because it was only based on CHNO values irrespective of being degradable or non-degradable. Whereas in BMP_{OFC} lipids were not included in calculations, as AWSs may have minor or no lipids in their composition. However, lipids if present, have given higher BMP_{exp} as compared to protein and carbohydrates[13]. Surprisingly, $BMP_{NDF,ADF,ADL}$ prediction has shown lower values than both predicted BMPs and BMP_{exp}, because lignin has been considered as a strictly recalcitrant component. The BMP assay showed broad BMP range of 20–360 ml/g VS for barley waste[5], 270–350 ml/g VS for grasses[6], 202–410 ml/g VS for maize waste[7]. However, overall BMPs ranging from 200 to 450 ml/g VS of AWSs are reported[1,4]. The variations in BMPs data as well as BD data were due to difference in chemical compositions in AWSs. Among all chemical components, lignin has been considered as a rate limiting component for BD. In this study, CW has shown highest BMP_{exp}, i.e. 317.6 ml/g VS and highest BD which indicated that lignin was loosely linked with carbohydrates and provided soluble carbohydrates irrespective of its content in other subjected AWSs. Moreover, highest BMP_{exp} might be due to balancing of carbon to nitrogen ratio (C/N) of the medium which can also be explained by higher BD_{exp} than stoichiometry calculated BD. The BMP_{exp} of CW was much higher than reported range of cotton stalk, i.e. 78–149 ml/g VS[30], which might be due to the use of optimized inoculum and medium that maximized digestion of CW. The BMP_{exp} of BS was found in line with BMP_{exp} range as in reported data[5] as mentioned above, i.e. 216.3 ml/g VS and less than CW. It might be explained by higher content of lignin (14.0%) that reduced the BD. The BMP_{exp} of LS and PPS was 279.2 and 255.6 ml/g VS respectively. No BMP_{exp} data was found in the literature to compare results. Hence it was ideal to compare with CW because of similar ADL content (only ADL is responsible for controlling BD and hence used for comparison). However, their BMP_{exp} is much less than CW. This might be due to high HC content in LS which is recalcitrant to some extent and reduces the BD[31]. But PPS has lower BMP than LS. It also has lower HC content, which might be explained on the basis of lack of proper microflora for AD. No doubt inoculum was constant in all analysis but composition of AWSs selected the growth of microflora in the digester. Wheat and rice wastes have shown broad range of BMP_{exp} data in the literature, i.e. 202–243 ml/g VS of wheat straw[32] and 156.6 ml/g VS of rice bran[26]. However, BMP_{exp} of WB (292.0 ml/g VS) and RB (268.7 ml/g VS) was higher than reported data because of lower ADL content in WB and RB that increased BD. Generally, BMP_{exp} variations were found in the literature due to different AWSs compositions and AD operational parameters. The BMP_{exp} and BD of this study were higher than previously reported data. This was because AWSs have intact VS which was not digested previously in the stomach or composting. It was also higher since activity of inoculum was maintained by addition of essential mineral and vitamins in the medium. The BD_{exp} was higher than the predicted BD, because BD_{exp} also considered the utilization of AWSs components into microbial growth. BD_{ele} was a ratio of BMP_{exp}/BMP_{CHNO} and the LB was based on lignin content only which predicted maximum of 83% BD of AWSs with 0% lignin content[20].

VFA analysis

During AD, VFAs production and conversion (into CH_4 and CO_2) was an indicator of the accurate operation. Analysis of individual VFAs showed concentrations of HAc higher than HPr, HBu, HiB and HVa. It was due to conversion of other VFAs into HAc[33]. The HAc concentration above 800 mg/l was critical but did not effect AWSs digestion due to the use of sodium bicarbonate ($NaHCO_3$). The percentages of individual VFA in tVFAs were nearly close in all AWSs digestion processes which indicated that similar microflora and metabolic pathways were present in the conversion of AWSs into CH_4. Initially, tVFA concentration was higher due to higher rate of hydrolysis, lower rate of methanogensis and increased pH which might affect the AD process[33]. However, tVFA concentration was much lower than inhibition concentration of tVFA, i.e. 4000 mg/l (ref. 34) and pH was balanced by addition of buffer in the medium. Moreover, lignin content in AWSs reduced the digestion and produced VFA in moderate rate[28].

Conclusion

BMP_{exp} of AWSs was observed as 317.6, 292.0, 279.2, 268.8, 255.6, 216.4 ml/g VS in the order CW > WB > LS > RB > PPS > BS. The same order of BD_{exp} indicated that both these properties were directly proportional to each other. The active inoculate has not improved the CH_4 content but attained sustainability rapidly in the range 57–64% which increased the BMP_{exp} and biodegradability of agricultural substrates. The BMP_{exp} variations among AWSs were due to different chemical compositions and biodegradability based on chemical composition especially (lignin and HC content). Theoretically predicted BMPs were higher than the BMP_{exp} except $BMP_{NDF,ADF,ADL}$. AD produced higher Hac (58–63%) as compared to HBu (28–32%) and HPr (6–13%) in all AWSs. The initial concentrations of VFAs were higher and gradually reduced by conversion into CH_4. The percentages of individual VFAs were very narrow and indicated the similar pathways of digestion in all assays. This study also showed that similar kind of microbial consortia in digestion process produced similar intermediates.

Conflict of interest: All authors declare that there are no conflicts of interest.

1. Wall, D. M., O'Kiely, P. and Murphy, J. D., The potential for biomethane from grass and slurry to satisfy renewable energy targets. *Bioresour. Technol*., 2013, **149**, 425–431.
2. Bernet, N. and Béline, F., Challenges and innovations on biological treatment of livestock effluents. *Bioresour. Technol*., 2009, **100**, 5431–5436.
3. Murphy, J. D. and Power, N. M., An argument for using biomethane generated from grass as a biofuel in Ireland. *Biomass. Bioenergy*, 2009, **33**, 504–512.
4. Nizami, A. S. and Murphy, J. D., Optimizing the operation of a two-phase anaerobic digestion system digesting grass silage. *Environ. Sci. Technol*., 2011, **45**, 7561–7569.
5. Neves, L., Oliveira, R. and Alves, M., Anaerobic co-digestion of coffee waste and sewage sludge. *Waste Manage*., 2006, **26**, 176–181.
6. Gunaseelan, V. N., Biochemical methane potential of fruits and vegetable solid waste feedstocks. *Biomass. Bioenergy*, 2004, **26**, 389–399.
7. Weiland, P., Production and energetic use of biogas from energy crops and wastes in Germany. *Appl. Biochem. Biotechnol*., 2003, **109**, 263–274.
8. Appels, L. *et al.*, Anaerobic digestion in global bio-energy production: potential and research challenges. *Renew. Sust. Energ. Rev*., 2011, **15**, 4295–4301.
9. Spellman, F. R., *Handbook of Water and Wastewater Treatment Plant Operations*, CRC Press, 2013.
10. Faithfull, N. T., *Methods in Agricultural Chemical Analysis: A Practical Handbook*, Cabi, 2002.
11. Labconco, C., *A Guide to Kjeldahl Nitrogen Determination Methods and Apparatus*, Labconco Corporation, Houston, TX, USA, 1998.
12. Buswell, A. and Mueller, H., Mechanism of methane fermentation. *Ind. Eng. Chem*., 1952, **44**, 550–552.
13. Lesteur, M., Bellon, V., Gonzalez, C., Latrille, E., Roger, J., Junqua, G. and Steyer, J., Alternative methods for determining anaerobic biodegradability: a review. *Process Biochem*., 2010, **45**, 431–440.
14. Nurk, L., Bühle, L. and Wachendorf, M., Degradation of fibre and non-fibre fractions during anaerobic digestion in silages of maize, sunflower and sorghum-sudangrass of different maturities. *Bioenergy Res*., 2016, **9**, 720–730.
15. Raposo, F. *et al.*, Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J. Chem. Technol. Biotechnol*., 2011, **86**, 1088–1098.
16. Nielfa, A., Cano, R. and Fdz-Polanco, M., Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnol. Rep*., 2015, **5**, 14–21.
17. Angelidaki, I. *et al.*, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water. Sci. Technol*., 2009, **59**, 927–934.
18. Apha, A., WEF, *Standard methods for the examination of water and wastewater*, 2005, **21**, 258–259.
19. APHA, L., Clesceri, A. and Greenberg, A., Eaton. *Standard Methods for the Examination of Water and Wastewater (20th ed.),* American Public Health Association, Washington, DC, 1998.
20. Chandler, J. A., Jewell, W. J., Gossett, J., Van Soest, P. and Robertson, J., In Predicting methane fermentation biodegradability, Biotechnol. Bioeng. Symp. (United States), Cornell Univ., Ithaca, NY, 1980.
21. Das, L. K., Kundu, S., Kumar, D. and Datt, C., Fractionation of carbohydrate and protein content of some forage feeds of ruminants for nutritive evaluation. *Vet. World*., 2015, **8**, 197–206.
22. Das, L. K., Kundu, S., Kumar, D. and Datt, C., Assessment of Energy Content of some Tropical Concentrate Feeds of Ruminants Using Model of National Research Council-2001. *Ind. J. Sci. Technol*., 2014, **7**, 1999–2006.
23. Carpita, N. C., Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Plant Biol*., 1996, **47**, 445–476.
24. Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K. and Gruber, L., Biogas production from maize and dairy cattle manure – influence of biomass composition on the methane yield. *Agric. Ecosyst. Environ*., 2007, **118**, 173–182.
25. Ferreira, G. and Mertens, D. R., Measuring detergent fibre and insoluble protein in corn silage using crucibles or filter bags. *Anim. Feed Sci. Technol*., 2007, **133**, 335–340.
26. Chandra, R., Takeuchi, H. and Hasegawa, T., Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renew. Sust. Energ. Rev*., 2012, **16**, 1462–1476.
27. Ge, X., Matsumoto, T., Keith, L. and Li, Y., Biogas energy production from tropical biomass wastes by anaerobic digestion. *Bioresour. Technol*., 2014, **169**, 38–44.
28. Luostarinen, S., Luste, S. and Sillanpää, M., Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. *Bioresour. Technol*., 2009, **100**, 79–85.
29. Triolo, J. M., Pedersen, L., Qu, H. and Sommer, S. G., Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. *Bioresour. Technol*., 2012, **125**, 226–232.
30. Sahito, A. R., Mahar, R. and Brohi, K. M., Anaerobic biodegradability and methane potential of crop residue co-digested with buffalo dung. *Mehran Univ. Res. J. Eng. Technol*., 2013, **32**, 509–518.
31. Lehtomäki, A., Huttunen, S., Lehtinen, T. and Rintala, J., Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresour. Technol*., 2008, **99**, 3267–3278.
32. Giordano, A.; Cantù, C. and Spagni, A., Monitoring the biochemical hydrogen and methane potential of the two-stage dark-fermentative process. *Bioresour. Technol*., 2011, **102**, 4474–4479.
33. Ali, S., Shah, T. A., Afzal, A. and Tabbassum, R., Evaluating the co-digestion effects on chicken manure and rotten potatoes in batch experiments. *Int. J. Biosci*., 2017, **10**, 150–159.
34. Siegert, I. and Banks, C., The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochem*., 2005, **40**, 3412–3418.

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