Biogas production from anaerobic co-digestion using kitchen waste and poultry manure as substrate—part 1: substrate ratio and effect of temperature

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Abstract
The rapidly declining fossil fuels are no longer able to meet the ever-increasing energy demand. Moreover, they are considered responsible for greenhouse gas (GHG) emission, contributing to the global warming. On the other hand, organic wastes, such as kitchen waste (KW) and poultry manure (PM), represent considerable pollution threat to the environment, if not properly managed. Therefore, anaerobic co-digestion of KW and PM could be a sustainable way of producing clean and renewable energy in the form of biogas while minimizing environmental impact. In this study, the anaerobic co-digestion of KW with PM was studied to assess the rate of cumulative biogas (CBG) production and methane percentage in four digester setups (D1, D2, D3, and D4) operated in batch mode. Each digester setup consisted of five parallely connected laboratory-scale digesters having a capacity of 1 L each. The digester setups were fed with KW and PM at ratios of 1:0 (D1), 1:1 (D2), 2:1 (D3), and 3:1 (D4) at a constant loading rate of 300 mg/L with 50 gm cow manure (CM) as inoculum and were studied at both room temperature (28 °C) and mesophilic temperature (37 °C) over 24 days. The co-digestion of KW with PM demonstrated a synergistic effect which was evidenced by a 16% and 74% increase in CBG production and methane content, respectively, in D2 over D1. The D3 with 66.7% KW and 33.3% PM produced the highest CBG and methane percentage (396 ± 8 mL and 36%) at room temperature. At mesophilic condition, all the digesters showed better performance, and the highest CBG (920 ± 11 mL) and methane content (48%) were observed in D3. The study suggests that co-digestion of KW and PM at mesophilic condition might be a promising way to increase the production of biogas with better methane composition by ensuring nutrient balance, buffering capacity, and stability of the digester.

Keywords Co-digestion · Kitchen waste · Poultry manure · Biogas · Degradation rate · C/N · Mesophilic temperature

1 Introduction
KW is an unavoidable and massively produced waste from everyday cooking activities of our daily life. The quantity of KW is increasing day by day due to the increase of populations and changing of food habit [1]. In developing countries, KW is normally disposed off with other solid waste to the dustbin. Local government authorities, e.g., city corporations or municipal authorities, collect them from the bin and dump in low land without maintaining proper landfilling systems [2, 3]. These scenarios are worst in the rural areas of those countries [4]. From the last decade, poultry industry has become a vital source of protein and a profitable business sector. As a result, poultry farms are increasing rapidly around the world. For instance, in the USA, the number of restaurant job increased by 84% from 2010 to 2018 where the restaurants are mostly grown based on chicken meat [5]. Apart from meeting the increasing demand of chicken meat, the chicken farms are also generating PM proportionally, of which only a tiny fraction is applied in the fishpond and the remaining are dumped in the waste bin with other solid waste. If the manure is not properly managed, they can pose serious threats to the environment including malodour,
prevalence of pest and pathogen, water pollution, and GHG emission [6–8].

Daily per capita waste generation in Bangladesh was estimated as 0.41 kg in 2005 and increased to 0.56 kg in 2014 [9, 10]. The most recent projection made by World Bank shows that the waste generation will reach 0.75 kg per capita by 2025 [11]. In Bangladesh, the generated KW and PM are thrown with other waste such as plastics, paper, glass, metals, ceramics, lather, rubber, rags, grass, wood, textile, and jute in the municipal bin and termed as municipal solid waste (MSW) [12, 13]. KW holds the major share in MSW, and the quantity is around 70%, whereas PM has the share only less than 3% [14, 15]. Since the KW and PM are rich in carbon and nitrogen content [14, 16], their continuous degradation by the action of indigenous microorganism facilitates the formation of CO₂, CH₄, N₂O, and other gases in tiny amount [14] that continue to be released to the environment from the waste dumping sites. According to the Intergovernmental Panel on Climate Change (IPCC)’s fifth assessment report [17], CH₄ and N₂O are 28 and 265 times more potential as GHG compared to CO₂. In addition, heavy metals present in MSW get leached out to the groundwater and cause serious water pollution, threatening aquatic and human life. With the groundwater being a major source of drinking water in Bangladesh, its pollution make people vulnerable to several debilitating waterborne diseases [18, 19].

To reduce the GHG emission and groundwater pollution, researchers are striving to search an alternative sustainable treatment route for MSW rather than landfilling. MSW has an energy content of 8.71 MJ/kg [14] which can be extracted by thermal treatment, e.g., incineration and gasification or biological treatment like anaerobic digestion (AD). During the incineration of MSW in a boiler, energy is recovered as heat in the form of steam or super-heated water which is used for heating, cooling, or electricity generation purposes [20]. However, incineration of MSW is associated with the emission of gaseous pollutants, such as SO₂, SO₃, NO, NO₂, NO₃, HCl, and Cl₂ [21–24]. Moreover, due to the presence of plastics and lather in MSW, dioxins and furans are also present in the fly ash produced from incineration [25]. Therefore, to ensure the environmental safety, the exhaust gas from incineration plant needs to be treated appropriately which is costly, ultimately increasing the cost of overall energy extraction process from MSW through incineration. On the other hand, MSW typically contains moisture content of 50.56% [19]. Hence, to extract energy as syngas through gasification, the moisture content of MSW needs to be reduced to less than 10% to ensure higher process efficiency by preventing slugging formation [26–30]. However, it requires implementation of thermal drying which is energy intensive. Though the exhaust emitted from gasification of MSW contains GHG within the permissible limit and the ash contains only the extractable heavy metals [26–30], the drying unit adds additional cost to the process of energy extraction, making it economically unfeasible.

AD is a biochemical process where microorganisms break down the biodegradable material of MSW in the absence of oxygen that releases the chemical energy contained within the MSW as biogas having methane (55–65%) and CO₂ (25–45%) as major component [31, 32]. Thus, AD has the potential to reduce the amount of KW by producing bioenergy in the form of methane. However, AD of KW alone comes with some limitations. Because of its having easily biodegradable organics and high C/N ratio [33], decomposition of KW causes rapid accumulation of volatile fatty acids (VFAs). The subsequent acidification of the digester leads to lower buffering capacity and thereby lower biogas production because of the inhibition of methanogenic activity at low pH [34, 35]. To overcome this difficulty, buffer materials need to be added to ensure digester stability and better efficiency in methane production [36]. An organic buffer material could be animal manure for which the buffering capacity originates from the release of ammonia during the degradation of organic nitrogen present in the form of protein and urea [37]. Again, the produced ammonia in excess can act as an inhibiting agent against biogas-producing bacteria [38]. Hence, co-digestion of organic wastes with animal manure in proper ratio is practiced to take the advantage of easy access to organics and improved buffering capacity from organic waste and animal manure, respectively [39]. The benefits of co-digestion also includes improved biogas as well as methane yield, economic advantage derived from sharing the same equipment by different substrates, and easier management of mixed wastes, etc. [40]. Co-digestion of some animal manures, such as cattle manure [36] and pig manure [41] with KW, have been studied, and a synergistic effect in terms of improved biogas production and digester stability has been reported. However, there is little or no information regarding the co-digestion of KW and PM, even though the composition of PM showed lower C/N than dairy manure [42]. Hence, it is expected that the addition of PM to KW would increase the stability of the digester which would be reflected in better CBG production with enriched methane content.

Therefore, the aim of this study was to compare the performance of AD of KW alone with the co-digestion of KW and PM, and also to assess the effect of biomass ratio in co-digestion. Moreover, the same studies done at room temperature (28 °C) were compared with that of at mesophilic temperature (37 °C). The efficiency of the digestion process was investigated on the basis of biomass degradation rate, biogas production, and methane percentage.
2 Materials and method

2.1 Substrate and inoculum collection

KW was collected from different student dormitories of Shahjalal University of Science and Technology, Sylhet, 3114, Bangladesh. Bangladesh poultry farm situated at Madina Market, Sylhet, provided us with PM. Cow manure (CM) was used as a source of inoculum and was taken from a village nearby the university.

2.2 Substrate processing and characterization

KW was immediately subjected to a manual sorting process to get rid of non-degradable materials such as plastic bags and metallic canes. Afterward, the KW was cut into small size to get it in reduced size for ensuring efficient biogas production [43]. Then these wastes were mashed into pest by using a kitchen blender. The homogenized KW was stored in a fridge at −20 °C before starting anaerobic digestion. The collected PM was filled with various waste such as chicken feather, jute rope, and part of brick. They were also separated from the PM manually. However, the collected cow dung was fresh and free of foreign materials and was used directly as solid inoculum without any processing. The composition of substrates, such as total solids (TS), volatile solids (VS), total nitrogen (TN), total carbon (TC), and pH, was analyzed following standard methods from the American Public Health Association (APHA) [44]. The main characteristics of the substrates (KW and PM) are detailed in Table 1.

2.3 Fabrication of digester

A plastic bottle having a capacity of 1 L was used to construct the digester. A sample collector pipe was inserted through the upper face of the digester reaching up to the bottom to collect slurry for routine test. A gas collector pipe placed at the top of the digester was used to collect the gas for further analysis. The gas flow was controlled by a manually operated valve inserted in the gas collector pipe. All the openings of the bottle were made air-tight using M-seal to maintain anaerobic condition. The pictorial view of digester is shown in Fig. 1.

2.4 Experimental setup

A number of such five digesters were connected parallelly to build each of the four digester setups (D1, D2, D3, and D4), totalling to a digester volume of 5 L and working volume of 4 L during each experiment. The total volume of gas was measured by water displacement method [45]. A schematic diagram of experimental setup is illustrated in Fig. 2.

In total, two sets of experiment were conducted. In the first set of experiment, four digester setups D1, D2, D3, and D4 were filled with KW and PM (wet weight basis) at ratios of 1:0, 1:1, 2:1, and 3:1, respectively. All the digesters were charged with the same amount of inoculum CM of 50 gm at the top of the substrate and were operated at room temperature (28 °C) by simply putting in the laboratory at ambient condition. The same experiment was replicated for mesophilic temperature of 37 °C by keeping the digesters in a digital thermostatic water bath that constituted the second set of experiment. The temperature was monitored on a regular basis by inserting a thermometer through the thermometer injector. Any variation in temperature was mitigated by adjusting the knob of the water bath to the desired temperature. However, in both set of experiments, the organic loading was maintained at 300 gm per L volume of the digester and a retention time of 24 days was allowed for all the digesters. At every 2 days interval, 5.0 mL slurry was collected from each digester through the sample collector pipe with a syringe. Then the slurry was centrifuged in a tabletop centrifuge (DSC-200A-2, Digisystem Laboratory Instruments Inc., Taiwan) and the pH of the supernatant

| Characteristic parameter | KW     | PM     |
|-------------------------|--------|--------|
| TS (wet basis, %)       | 65.03  | 54.90  |
| VS (wet basis, %)       | 57.11  | 48.75  |
| TC (dry basis, %)       | 63.71  | 46.55  |
| TN (dry basis, %)       | 1.81   | 4.90   |
| C/N                     | 35.20  | 9.5    |
| pH                      | 4.80   | 8.3    |

Fig. 1  Pictorial illustration of the digester
solution was measured via electrochemical method using a digital pH meter. The produced gas was also measured at 2 days’ interval. The ratio of substrates, amount of substrates and inoculum, and C/N in different digester setups are depicted in Table 2. All the measurements were carried out in triplicate and the average values were taken for further explanation.

### 2.5 Degradation rate measurement

To calculate the degradation rate, total produced gas from a digester was measured and divided by the amount of substrate loaded to the corresponding digester as depicted in Eq. 1 [46].

\[
\text{Degradation rate} = \frac{\text{total amount of gas produced from a digester}}{\text{total amount of substrate loaded}}
\]  

#### (1)

### 2.6 Methane content measurement

Biogas samples were analyzed for methane content using a gas chromatograph (Shimadzu GC-2014, Japan) system equipped with a stainless steel column packed with Unibeads C (80/100 mesh) and a thermal conductivity detector (TCD). The temperature of the injection port, column, and detector was maintained at 120, 60, and 150 °C, respectively. Helium was used as carrier gas with a flow rate of 50 mL/min. The analyses were carried out in triplicate and the results were reported as average on dry basis.

### 3 Result and discussion

#### 3.1 Synergistic effect

Co-digestion of KW with PM demonstrated a synergistic effect in terms of CBG production due to the rapid decomposition of the KW and buffering capacity of the PM. D1 (loaded with only KW) yielded 312 ± 9 mL of CBG while D2 (loaded with equal amount of KW and PM) produced 362 ± 13 mL over the entire experimental period of 24 days at room temperature of 28 °C (Table 3 and Fig. 3). Hence, D2 produced 16% more biogas than the D1 at the same temperature of 28 °C and with the same amount of inoculum 50 g CM. This increase in gas production might be attributed to the synergistic effect of co-digesting KW and PM in a single digester. This synergistic effect comes from the combination of the desirable characteristics of the two substrates: the easy biodegradability of KW required for increased availability of substrates to be converted into biogas and the increase in alkalinity by PM required to keep the pH close to the neutral value that is prerequisite for survival of methanogenic bacteria [39, 47]. Figure 4 illustrates...
that the drop in pH was higher in D1 due to the rapid degradation of KW that might have released more VFAs [48]. Whereas in D2, the acidity was neutralized to some extent by the ammonia released from PM decomposition that kept the pH at higher value than that of the D1. The KW contains high VS content [41] that undergo rapid hydrolysis during digestion to cause serious acidification of the digester that inhibits the activity of methanogens, resulting lower gas production [34]. In contrast, the decomposition of proteins and urea in PM generates alkaline compounds like ammonia and ammonium ions that add a buffering capacity to the digester. The added buffering capacity by PM stabilize the system and lower the negative impact of VFAs on methanogens and thus can contribute to higher gas production [52]. However, several authors also investigated the effect of PM on different lignocellulosic waste biomasses and observed a significant increase in biogas production. Borowski et al. [53] observed a 1.5 times higher production of biogas from municipal sewage sludge when co-digested with 30% PM. In another study, sole digestion of wheat straw experienced a biogas potential of 317.5 ± 31.3 mL/g VS, whereas co-digestion of the same with the PM gave 389.7 ± 24.7 mL/g VS biogas [42].

### 3.2 Biogas production at different biomass ratios

Biogas production was studied at different biomass ratios to achieve the optimum substrate ratio in co-digestion of KW and PM. Change in substrate ratios has impact on the daily biogas production and the pH of the digesters. The daily biogas production at room temperature and the corresponding pH change in four digester setups (D1 to D4) are shown in Figs. 3 and 4.

All the digesters displayed a similar trend in biogas production as well as in change of pH. In general, the gas...
production (Fig. 3) increased during the first couple of days that might be due to the degradation of soluble carbohydrates available in KW. Afterward, it decreased sharply due to drop in pH owing to possible accumulation of VFAs that led to instability in the growth of microorganism. This phenomenon can also be attributed to the difficulty in biological degradation of complex compounds after the conversion of easily degradable organic fractions [54, 55]. Then gas production increased again due to the gradual acclimation of the microorganisms to the treatment media followed by a permanent cease in gas production because the microorganisms could not sustain the environment below the neutral pH [34]. Several studies reported that the optimal pH for methanogenesis is about 7.0, whereas for hydrolysis and acidogenesis, it is between 5.5 and 6.5 [56, 57]. The pH (Fig. 4) of all digesters dropped initially at a steady pace up to 6 to 10 days and then maintained a plateau value until the gas production is stopped. The pH drop in D1 was at a higher rate than the others, which may be due to the rapid VFA production from decomposition of KW alone, i.e., high nutrient to microorganism ratio [36], and fell to the plateau value, around 3.1, after 6 days. In other digesters, the pH drops were slower due to the alkaline effect stemming from the PM decomposition, with D3 being the lowest pH drop experiencing digester. It took 10 days for D3 to fall to pH 3.8 and remained at that value over the rest of the period. The pH in D2 and D4 were somewhere in between that of the D1 and D3. 

During the co-digestion of KW and PM at room temperature (28 °C), the CBG (Table 3) increased from 362 ± 13 mL in D2 to 396 ± 8 mL in D3 and then dropped to 360 ± 15 mL in D4. This indicates that the CBG increased with increasing amount of KW up to twofold of PM in D3 and then decreased for a threefold of PM in D4. Compared to the pH (about 3.8) of the digester setup D3 at plateau condition (as shown in Fig. 4), the pH of D2 and D4 are (around 3.5) far away from the neutral value, which might led to more instability in the later digester setups and consequently lower gas production. In D4, alkaline effect of 25% PM was not sufficient to decrease the negative impact of VFAs from 75% KW. In contrast, the lower gas production in D2 might be attributed to the inhibitory effect of high concentration of ammonia from increased amount of PM which was 50% [58]. Moreover, the amount of KW had a significant impact on the duration of biogas production which plummeted from 20 days for 50% KW in D2 to 12 days for 75% KW in D4 (Fig. 3). However, the peak production was observed as 150 mL in D2 at the second day of digestion. In spite of that, D3 with 67.7% KW and 33.3% PM proves to be the optimum ratio in terms of CBG production, sustained biogas production, and moderate inhibitory effect from PM. This percentage of PM is believed to have provided balanced nutrient for the anaerobic microorganisms and enhanced buffering capacity to the digester through ammonia production. In the presence of ammonia, it has been reported that the gas production occurred due to the activity of hydrogenotrophic methanogens since acetotrophic methanogens are more sensitive to the ammonia level [59].

3.3 Effect of temperature on biogas production

Temperature plays a significant role in the anaerobic digestion process by controlling the rate of microbial metabolism in anaerobic environments [60, 61]. Findings of several studies indicate that there is a positive relationship between the temperature and the production of biogas [62]. There are mainly three different temperature ranges in which AD can be operated: psychrophilic (<30 °C), mesophilic (30–40 °C), and thermophilic (50–60 °C) [63]. Though thermophilic AD has been reported to produce higher biogas and give better quality digestate [64], it has also been observed that this type of AD suffer from instability. The fluctuation arises due to inhibition from high amount of ammonia release during organic nitrogen removal [65] and higher accumulation of VFAs due to slow metabolic and growth rate of acetoclastic methanogens [66, 67]. Moreover, thermophilic digestion is not an economically viable option due to its requiring large energy input for heating the system and associated operational difficulties [68]. Several authors also suggested mesophilic temperature range for optimum biogas production [61, 69]. Considering economic feasibility and process stability, mesophilic temperature of 37 °C was applied to the digesters to investigate the effect of temperature on biogas production.

Daily gas production (Fig. 5) and the associated pH change (Fig. 6) during the mesophilic digestion showed a similar trend to that of at room temperature (described in Sect. 3.2). The gas production (Fig. 5) increased initially and then followed a period of declination which was continued by a steady growth and then a final drop until it stops. The pH of all digesters (Fig. 6) observed a gradual drop initially up to 6 to 10 days and then hovered over that constant value until the cessation of gas production. The highest pH drop was observed in D1 with 100% KW and the lowest pH drop was observed in D3 with 66.7% KW. D3 achieved the highest peak production of 390 mL on the 10th day of digestion and is also the digester yielding highest CBG which is 920 ± 11 mL. However, all the digester setups gave higher CBG than those at the room temperature, as delineated in Table 3 and Fig. 7.

3.3.1 .

The percentage increase in CBG production was 66.26%, 69.61%, 132%, and 25.56% for D1, D2, D3, and D4, respectively. Liu et al. [70] also observed a greater efficiency in biogas production from the co-digestion of animal waste and
agricultural waste in the mesophilic temperature range. The reason can be attributed to the enhancement of the anaerobic degradation of the complex organic matters facilitated by the increased hydrolysis rate [71, 72]; a peak production on the 10th day can be considered as a testimony to this phenomenon. The substrate degradation rate was higher in mesophilic condition than the room temperature as compared in Fig. 8. It has been demonstrated that the activity and growth
rate of microbes increase by up to 50% for a temperature rise of 10 °C within the mesophilic range [73]. However, digester setup D2 containing high amount of protein (50% PM) experienced lower biogas production than D3 because of the increased inhibition from free ammonia due to shift in NH$_3$/NH$_4^+$ equilibrium at high temperature [62]. On the other hand, lower biogas production in D4 was observed due to acidic environment from the increased hydrolysis of organics [74] in 70% KW; the lowest pH of D4 among the co-digesters corroborates this assertion.

### 3.4 Methane percentage observation

Methane is the prime energy source in biogas, and the higher is the methane content, the better is the biogas quality. Table 4 compares the methane percentage in the produced biogas at room temperature (28 °C) and mesophilic temperature (37 °C). It can be seen that, irrespective of the temperature, biogas from D1 had the lowest methane percentage. This can be attributed to the acidification of the digest from rapid hydrolysis of readily available organic matters (100% KW) that led to lower growth and activity of methanogens. A similar phenomena was observed by Li et al. [36] while digesting KW alone and reported that higher food to microorganism ratio caused lower methanogenic activity. However, replacement of some KW with PM increased the methane percentage significantly. It is noted that PM is highly rich in undigested protein content [75]. Kafle and Kim [76] reported that the presence of protein in one substrate could facilitate the methane production. This is mainly due to the synergistic effect of co-digestion stemming from proper C/N ratio and increased buffering capacity [51]. At room temperature, D2 obtained 33% methane-containing biogas, while for D3 and D4, it was 36% and 31%, respectively. Methane content was highest in D3 because of the enhanced buffering capacity provided by the ammonia degraded from protein in PM. Moreover, the 33.3% PM in D3 provided a balanced nutrient for anaerobic bacteria. However, D2 experienced less methane content owing to inhibition from excess ammonia produced from 50% PM. Excess ammonia also inhibits methane production by facilitating accumulation VFAs since acetotrophic methanogens are more sensitive to ammonia [59]. In contrast, in D4, the lower percentage of methane might be attributed to poor neutralization of the VFAs by insufficient amount of ammonia produced from 25% PM.

However, at 37 °C, the methane percentages increased significantly and were above 40% in all the digester setups except D1. Typically, methane percentage in biogas that is exploitable for energy usage ranges from 40 to 75% [58]. Heavy acidification and lower pH in D1 from increased degradation of KW alone played an inhibitory effect on methanogens. Surge in methane percentage can be attributed to the increased acetate and propionate degradation efficiency with the increasing temperature [77]. Moreover, higher hydrolysis rate, microbial growth, and activity at mesophilic temperature range were also responsible for augmented methane percentage. The result of this study is consistent with the findings of Wang et al. [78] who observed an increase in methane percentage at mesophilic temperature when co-digested dairy manure, PM, and rice straw.
4 Conclusion

AD is a promising approach to curtail the ever-increasing load of KW in an environment friendly way through the production of clean energy such as biogas. However, the digestion of KW alone experiences VFA accumulation due to the easy biodegradability of the organics leading to pH drop — an environment hostile for methanogenic bacteria that cause lower CBG production with lower methane content. This study found that the co-digestion of KW with PM can improve the biogas production by enhancing the buffering capacity of the digester stemming from the ammonia released by the decomposition of organic nitrogen in PM. Compared to the mono-digestion of KW, a 16% increase in biogas production was observed when 50% KW was replaced by PM during the co-digestion process. However, the co-digestion of KW and PM showed considerable dependency on substrate ratio and process temperature. At room temperature (28 °C), the highest CBG production of 396 ± 8 mL was observed in digester setup D3 with a KW and PM ratio of 2:1, which can be attributed to the balanced nutrient and pH stability in the digester. At mesophilic condition (37 °C), CBG production of all the digesters increased due to the increase in hydrolysis of organics and activity of microbes. Among the digester setups, D3 yielded the highest biogas of 920 ± 11 mL which was 132% higher than that of the room temperature. The methane percentage in the produced biogas also followed the similar trend of CBG production in terms of dependency on substrate ratio and process temperature. Digester setup D3 was found to produce biogas with highest methane percentage of 36% and 48% at 28 °C and 37 °C, respectively. Therefore, the present study suggests that anaerobic co-digestion of KW and PM in proper ratio could be a sustainable way to mitigate the environmental problem and energy crisis simultaneously.

4.1 Limitation of the work

Due to the current COVID-19 pandemic situation that forced the laboratory to be remained off until further notice, we could not conduct and include experimental data regarding the changes in VFAs and COD values during the digestion period.

Declarations

Conflict of interest The authors declare no competing interests.

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