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Bioprospecting Kitchen Refuse as a Suitable Substrate for Biogasification

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ABSTRACT: Conventionally, methane nonproducing organic substrates such as kitchen refuse (KR) are amenable as biogasifiers, similar or even better than that of the naturally biogasifying cow dung (CD) through process modification. Comparative physicochemical and biological analyses revealed that KR had no methanogen and was low on amylase and cellulase positive and total microbial counts. It was observed that the pH level lowered further when the KR alone was biogasified, attributable to the accumulating volatile fatty acids, which indicates the failure of the last and final step of biomethanation. Study of the raw and digested forms of KR, CD, and kitchen refuse fortified with cow dung (KC) revealed that there was a net percentage decrease in dry matter (70.00, 94.33, and 88.88, respectively), total dissolved solids (1, 1.5, and 1.5, respectively), and phosphate contents (12, 19, and 20, respectively), indicating an optimal microbial activity in all the substrates. Although digestion rate in CD was better than that in KR, KC exhibited an enhanced digestion rate over KR attributable to the process being facilitated by increased microbial counts; amylase-, cellulase-, and lipase-positive microbes; and methanogens. Furthermore, the active methanogens in CD inoculum (in KC) facilitated biomethanation by better utilizing the volatile fatty acids that ensured better stability in the pH level throughout. The cumulative biogas production values were 1281, 4448, and 3256 cm^3 in KR, CD, and KC, respectively. Methane production started by the seventh day in CD and KC and reached up to 63.65% and 53%, respectively, by the 21st day in batch operation. Thus, KR is a promising candidate for biogasification, thereby opening a plethora of opportunity to utilize the technology even in urban and periurban locations that are low on cattle resources albeit rich in other organic refuse. There is a necessity to estimate the biomethanation potentials of various other available organic refuse.

KEYWORDS: cow dung, kitchen refuse, kitchen refuse seeded with cow dung

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Introduction

Organic matter, such as carbohydrate, protein, or lipid, is composed of carbon in combination with elements such as hydrogen, oxygen, nitrogen, and sulfur. Anaerobic digestion of organic matter generates sludge of agricultural value as well as a mixture of gases primarily containing methane that can be used to generate heat and electricity.[1,2] Decomposer microbes break the complex carbon into smaller substances. Methane emission accounts for 16% of all global greenhouse gas emissions. Anthropogenic activities have been estimated to contribute to more than 60% of the global methane release. Mosier et al[3] reported a global average atmospheric methane concentration value of 1720 ppbv, which was more than double the preindustrial period value of 800 ppbv.

Dwindling cattle population is affecting the sourcing of the best natural biomethanation source, ie, cow dung (CD). Growing menace of municipal wastes in the urban and periurban localities could use biogas technology productively and effectively to manage the wastes. Tonnes of nutrient-rich kitchen refuse (KR) produced daily in households in cities and villages can be a promising and handy alternate substrate. However, the acidity buildup in this process due to the action of acid-fermenting bacteria such as the lactic acid bacteria and the absence of methanogens make it less competitive. Hence, pretreatments such as pulverization, pH adjustment, and seeding with microbes that are useful in biodigestion can increase its biomethanation potential. The present study was aimed at providing leads on the use of household KR as a potential biomethanation candidate.

Materials and Methods

Substrate. CD and KR were used in proportions to prepare three different experimental substrates. The first setup had KR and water, the second one had CD and water, and finally the third one had CD, KR, and water, all in equal proportion making the final volume to 1.2 L. The CD was collected from a local cattle shed. The KR, which primarily included potato, cucumber, and other vegetable peelings, was collected from the student hostel canteen of campus 11, KIIT University, Bhubaneswar, Odisha, India. This substrate was first differentiated and chopped using a knife and finally grinded in a mixer to ensure uniform small particle size to facilitate an optimal digestion rate.

Digester setup. The experimental setup consisted of 1.5 L capacity polypropylene bottles separately for each substrate, with a 10% headspace and tubing on the top for gas and sample collection. The contents in the bottles were mixed by shaking once daily in the morning to prevent formation of any dead space.

Microbiological study. Samples were carefully collected on weekly bases, serially diluted six times (10^6) with phosphate buffer solution, and the microbial diversity of different setups was studied. Samples were pour-plated on nutrient agar (HiMedia) and incubated overnight till 48 hours. The viable microbial counts in the substrates were determined by colony forming unit (CFU) count, a microbiological method to estimate the viable bacterial/fungal numbers. To ensure that the samples yielded CFUs in the range of 30–300, ten-fold dilutions were prepared, and the dilution series were plated in replicates over the chosen range of dilutions and incubated at 37°C till 48 hours. The counted CFUs were mathematically deduced to CFU per milliliter, factoring in the amount plated and its dilution factor. Morphologically different isolates were picked and streaked on nutrient plates to obtain pure cultures. These were then screened for cellulase and amylase activities. The various bacterial isolates thus obtained were subjected to microbial and enzymatic activity assays to confirm their roles in the biomethanation process.

Physicochemical analyses. The pH of the digester was recorded weekly. The dry matter (DM) content was determined by drying a known weight (W_s) in an oven at 105°C for 24 hours and measuring the weight after drying (W_{DM}). The DM was calculated as: DM (%) = $100 \times W_{DM}/W_s$. The conductivity and total dissolved solids (TDS) were measured using digital portable water–soil analysis kit (VSI Electronics Pvt. Ltd). The phosphate content was measured by standard protocols.[4]

The volatile fatty acid (VFA) was measured following the method of Hemakrishna et al,[5] with modifications. The weekly collected sample was centrifuged (3000 rpm, 5 minutes) and filtered. A total of 100 mL of this (or a suitably diluted one) was taken and titrated with 0.1 N HCl to pH 3.0 (A mL), boiled for 3 minutes in a 250 mL flask to remove the CO_2, cooled immediately, and again titrated with 0.1 N NaOH to pH 6.5 (B mL). The VFA values were calculated as[5]:

$$VFA\,(mg/l) = \frac{(B*100) - (A+100)*DF*60}{99.23}$$

Gas analyses. The pH was measured using a digital pH meter (SD Fine), and the produced gas was analyzed weekly in a gas chromatograph (Nucon 5700) to measure the quality and quantity of methane, CO_2, and H_2S. Porapak QS column of 2.0 m length and 80–100 meshes was used with thermal conductivity detector (TCD) detector at 200 mA. Hydrogen was used as the carrier gas with a flow rate of 35 mL/minute. The temperature was kept at 40, 90, and 120°C for the oven, injector, and the detector, respectively.

Results and Discussion

Microbial study.

Colony forming unit. Microbial hydrolysis of organic macromolecules like proteins, carbohydrates, and fats to amino acids, sugars, and fatty acids occur during the early part of the AD process.[6] Thus, it was important to study the microbial biocosm in the substrate. Figure 1 shows the microbial load in the experimental substrates in terms of CFUs. The CFUs progressively increased in KC and CD till the seventh day, and then, it gradually decreased, attributable to the modified ecological conditions including a drop in the pH due to the VFA accumulation. The CD had 8.1×10^5 and 13×10^5 CFUs/mL, and KC had 6.7×10^5 and 10.6×10^5 CFUs/mL, respectively, on the 0th and 14th day. The microbial load increased till the 7th day to 6.3×10^5 CFUs/mL in KR, gradually decreasing by the 14th day. It shows that the microbes multiplied faster in CD than in KR and KC, thus an obvious faster degradation rate in CD. KR had the least, whereas CD had the highest microbial load among all. Such study helped decipher the active microecology in methanation.

Enzymatic activities of different microbes. Table 1 shows the cellulase-, amylase-, and lipase-positive isolates from KR, CD, and KC during the study. The microbial profile in KR showed that three of the isolates were lipase positive, five were amylase positive, and four were cellulase positive. Three others were positive for amylase, cellulase, lipase, out of which KIIT VSKW003 was the best. The microbial profile in CD showed five cellulase positive, four amylase positive, and four lipase positive isolates. Isolate KIIT VSCD003 was the best in cellulase activity, KIIT VSCD004 was the best in amylase activity, and KIIT VSCD005 was the best in lipase activity. Swain and Ray[7] isolated an amylase- and cellulase-positive industrially important *Bacillus subtilis* strain from CD. As KC had a combined profile of KR and CD, it exhibited the best microbial profile. A majority of the isolates from KC were

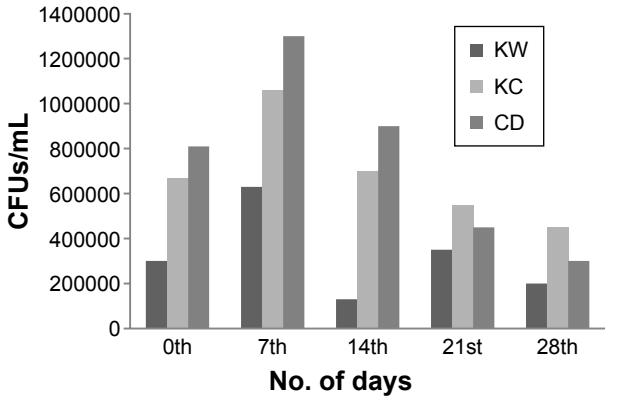

Figure 1. CFUs of different substrates: the number of CFUs in KC and CD progressively increased till the 7th day after which it gradually decreased. In KR, the microbial population increased till the 7th day and then decreased by the 14th day due to the accumulating VFA leading to a drop in pH.

Table 1. Screening for different enzyme activities of the microbial isolates from KR, CD, and KC during the study period.

SL. NO.	UNIDENTIFIED STRAIN	CELLULASE	AMYLASE	LIPASE
Kitchen Refuse (KR)				
1	KIIT VSKW001	+	+	+
2	KIIT VSKW002	+	+	+
3	KIIT VSKW003	+++	+++	++
4	KIIT VSKW004	++	–	–
5	KIIT VSKW005	–	+	–
6	KIIT VSKW006	–	++	
Cow Dung (CD)				
7	KIIT VSCD001	++	–	–
8	KIIT VSCD002	–	++	–
9	KIIT VSCD003	++	++	++
10	KIIT VSCD004	++	+++	+
11	KIIT VSCD005	++	++	+
12	KIIT VSCD006	++	–	+
Kitchen Refuse + Cow Dung (KC)				
13	KIIT VSKC001	+	+	+
14	KIIT VSKC002	++	++	++
15	KIIT VSKC003	+	+	+
15	KIIT VSKC004	++	+++	+
17	KIIT VSKC005	+	+	++
18	KIIT VSKC006	+++	+++	+++

positive for amylase, cellulase, and lipase, out of which KIIT VSKC006 had the best enzyme activity profile. The 16S rRNA sequence revealed that KIIT VSKW003 and KIIT VSKC006, respectively, were *B. subtilis* and *Bacillus tequilensis* strains.[8]

Physicochemical analyses.

Moisture and DM content. DM content indicates the amount of available nutrients in a substrate and also the digestibility of the substrate. The study revealed that all the substrates were digested well as the percent DM reduced in the digestates of all. The percent DM decreases were 70.00, 94.33, and 88.88, respectively, in KR, CD, and KC. Compared to KR, the digestion was better in CD, attributable to the differential microbial population on one hand and the particle size on the other. It is noteworthy that the CD is a semidigested material carrying a huge profusely active microbial consortium. The digestion rate in KR was enhanced compared to KR when seeded with CD (ie, in KC). The KR included carbohydrates, fats, proteins, vitamins, minerals, and antioxidants (eg, thiocyanate, anthocyanin, and quercetin). Carbohydrates, fats, and proteins, that provide energy in foods, make up 90% of the dry weight of a diet. Also, the water content in foods varies widely. For these reasons, knowing the amount of DM present in the substrate fed to the digester was necessary. Digestion reduces the total solids content and enhances the moisture content in the digestate when the substrate macromolecules break down. Such studies help studying the digestion rate and degree taking place in the digester.

pH. Digestion process has pH as an important determining factor, especially in microbial growth and overall digester performance. The pH initially falls due to the accumulating volatile acids in batch anaerobic digestion, but the acid environment soon reverts to near alkaline as methanogens use these volatile acids, thereby stabilizing the digester. Jain and Mattiasson[9] reported that the biomethanation efficiency was above 75% at a pH more than 5.0. The pH in CD and KC remained between 6.0 and 7.0 during the major study period, attributable to the active methanogens. However, it reduced greatly in KR to 5.8 (on the 7th day) and 4.4 (on the 14th day), and gas production completely stopped wherein the pH had to be manually adjusted to neutral by adding carbonate. Even further, the pH again dropped down to 5.8 (Fig. 2) on the 21st day possibly due to nontrigger of the final stage of biomethanation, attributable to the lack of methanogenic activity contributing to VFA accumulation. According to Sandberg and Ahring[10] and Ward et al,[11] anaerobic digestion occurs optimally at a pH 6.8–7.2, and a highly alkaline pH can result in disorganization of microbial activities leading to process failure.

Volatile fatty acids. One of the reasons for digestion failure, VFA concentration, is probably an important parameter to be monitored as a process performance indicator. VFAs encompass a group of six compounds, namely, acetate, propionate, butyrate, valerate, and caproate, of which acetate is predominant. According to Wang et al,[12] acetic acid and butyric acid concentrations of 2400 and 1800 mg/L, respectively, resulted in no significant inhibition of the activity of methanogens, while a propionic acid concentration of 900 mg/L resulted in significant inhibition of the methanogens. Figure 3 shows the VFA concentrations of different substrates during the study

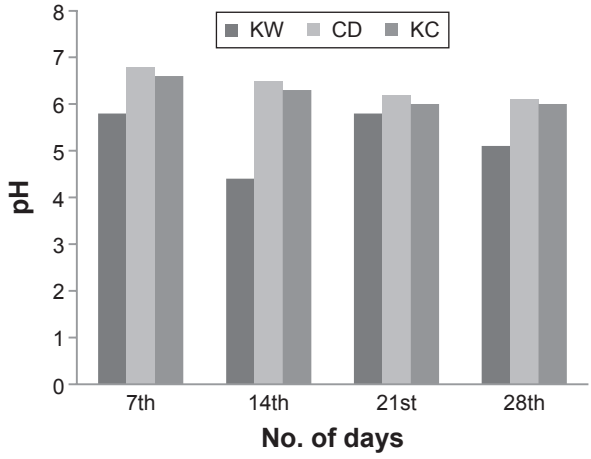

Figure 2. Weekly pH recordings of different substrates: pH of CD and KC was in between 6.0 and 7.0 throughout. In the case of KR, the pH values recorded on the 7th and 14th days were 5.8 and 4.4, respectively, the pH had to be neutralized by adding carbonate.

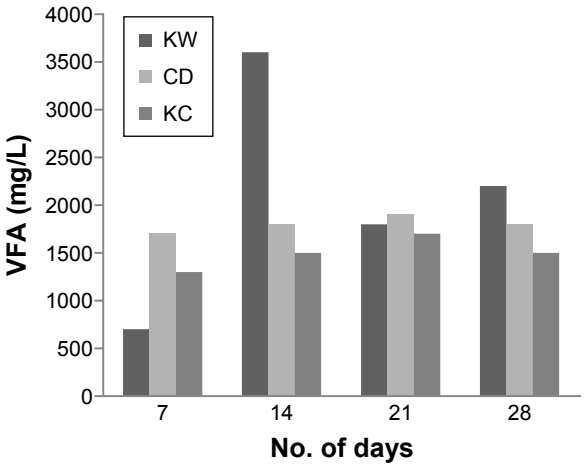

Figure 3. VFA concentrations of different substrates: VFA recorded was 3600 mg/L on the 14th day, a reason for which gas production stopped. In CD and KC, the VFA ranged between 1700–1900 mg/L and 1300–1700 mg/L, respectively.

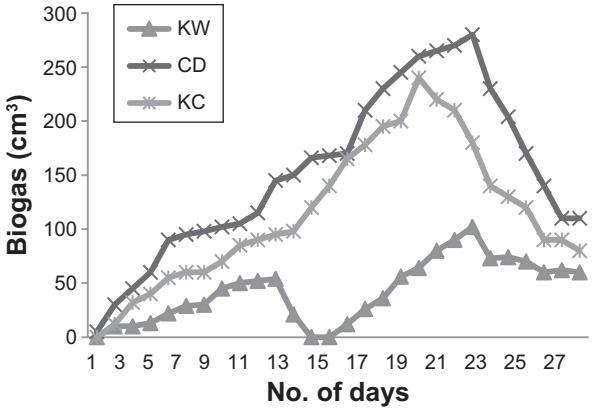

Figure 4. Biogas production potential of different substrates: KR, CD, and KC produced a total of 1281, 4448, and 3256 cm^3, respectively, of biogas in 30 days.

period. In the case of KR, the highest VFA recorded was 3600 mg/L on the 14th day, which is very high for a gasification system to hold, a possible reason why the gas production stopped. The VFA ranges were between 1700–1900 mg/L and 1300–1700 mg/L, respectively, in CD and KC; the VFA produced got utilized by the active methanogens. Siegert and Banks[13] considered that the VFA concentrations above 2000 mg/L inhibited cellulose degradation, while the same above 4000 mg/L feebly inhibited glucose degradation. The toxic effects of high VFA during anaerobic digestion have been reported by several authors, resulting in a drastic drop in pH. Acetate reportedly inhibited the propionate and butyrate degradation.[14]

TDS, conductivity, and phosphate. Conductivity is directly proportional to TDS. Conductivity increases with the increase in TDS. TDS, made up of inorganic salts and small amount of organic matter, represents the total concentration of dissolved substance in a solution. Common inorganic salts in a solution include cations such as calcium, magnesium, potassium, and sodium and anions such as carbonates, nitrates, bicarbonates, chlorides, and sulfates.

One way to measure TDS is to measure its electrical conductivity. The TDS concentration decreased after digestion in all the three substrates due to the biological reactions during the digestion as a result of which the conductivity also decreased. The study of such parameters helps study the degree or percentage of digestion during a batch processing. The TDS concentration was high in raw KC and the lowest in the digestate of KR. In KR, CD, and KC, the percentage decreases in TDS after digestion were 1, 1.5, and 1.5, respectively.

Phosphate is important in metabolism and facilitates microbial growth and multiplication. Its contents decreased with increasing digestion rate in all substrates indicating that it was used up by the active microbes. This could be a parameter that helps acknowledge the digestion rate. A high decrement in phosphate content was observed in CD and KC. The phosphate contents in KR, CD, and KC post digestion decreased by 12%, 19%, and 20%, respectively.

Quantitative and qualitative analysis of biogas.

Biogas- and biomethane-producing potential of different substrates. Figure 4 shows the amount of biogas produced daily from different substrates. KR started producing gas from the 7th day, but it dropped by the 14th day, possibly due to a decrease in pH and VFA accumulation. The gas production again started after adjusting the pH and continued till the 30th day. KR produced a cumulative biogas of 1281 cm^3, CD produced 4448 cm^3, and KC produced 3256 cm^3. Figure 5 graphically presents the methane percent produced from different substrates. KR did not produce any discernible methane, possibly due to the absence of methanogens. CD and KC produced methane from the 7th day (63.65%) and yielded a maximal amount by the 21st day (53%). Mohan and Gopalan[15] and Xavier and Nand[16] reported 60% and 62% methane from CD,

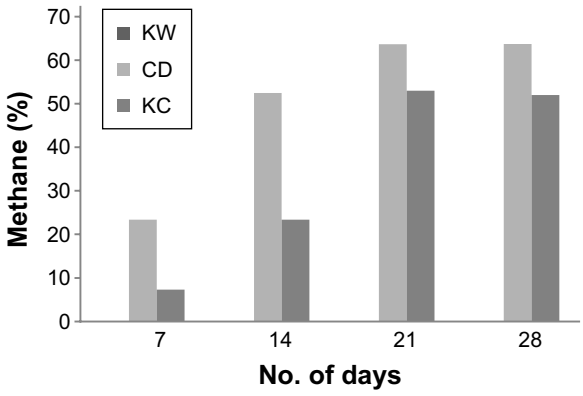

Figure 5. Biomethane potential of different substrates: CD and KC started producing methane from the 7th day (63.65%) and yielded a maximal amount by the 21st day (53%).
Note: KW did not produce methane at all.

respectively. Biomethanation increased when the KR was supplemented with CD, attributable to methanogens. It showed that CD played a significant role in biogasification.

Conclusions

From the above study, it is concluded that CD played a decisive role in biogasification. KR alone could not biogasify successfully. However, when it was supplemented with CD, there was a qualitative and quantitative increase in the microbial consortium; CD got the amylase, cellulase, and lipase positive bacteria and methanogens that facilitated an increase in the digestion rate (KC). Methanogens helped biomethanation, thereby stabilizing the pH. KC could produce biogas containing 53% methane by the 21st day. It proved that KR widely available at the household and industrial level could be a good biogas candidate, provided that it undergoes some physical pretreatment and is seeded with CD as an inoculum.

Author Contributions

Conceived and designed the experiments: VKM, SM and SKO. Analyzed the data: VKM and SM. Wrote the first draft of the manuscript: VKM. Contributed to the writing of the manuscript: VKM, SM, KN, BN and PKS. Agree with manuscript results and conclusions: VKM, SM, SKO, KN, PKS and BN. Jointly developed the structure and arguments for the paper: VKM and SM. Made critical revisions and approved final version: VKM and SM. All authors reviewed and approved of the final manuscript.

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