Research Article

Biochemical Methane Potential of Agro Wastes

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The focus of our work is on anaerobic digestion of locally available agro wastes like coconut oil cake, cashew apple waste, and grass from lawn cuttings. The most productive agro waste, in terms of methane yield, was coconut oil cake and grass. The results showed that the initial volatile solids concentration significantly affected the biogas production. The methane yield from coconut oil cake was found to be 383 ml CH₄/g VS and 277 ml CH₄/g VS added at 4 and 4.5 g VS/l. In case of grass the biogas production increased with increasing VS concentrations with methane yield of 199, 250, 256, 284, and 332 ml CH₄/g VS at 3, 3.5, 4, 4.5, and 5.0 g VS/l. For cashew apple waste single-stage fermentation inhibited biogas production. However, phase separation showed methane yield of 60.7 ml CH₄/g VS and 64.6 ml CH₄/g VS at 3.5 and 4.0 g VS/l, respectively. The anaerobic biodegradability of coconut oil cake was evaluated in fed batch mode in a 5 L anaerobic reactor at 4 g VS/L per batch, and the maximum methane yield was found to be 320 ml CH₄/g VS.

1. Introduction

In the light of rapidly rising costs associated with energy supply, waste disposal, and increasing concerns with environmental quality degradation, conversion of wastes to energy is becoming an economically viable solution [1]. Anaerobic digestion (AD) is a biological process in which bacteria break down organic matter producing biogas as the end product. AD is a sequence of chemical reactions during which organic matter is decomposed through the metabolic pathways of naturally occurring microorganisms in an oxygen depleted environment. AD is a promising technology which could effectively address the problem of waste disposal yielding valuable outputs like biogas and fertilizer. AD without any pretreatment but with energy recovery is the most attractive method for treating solid wastes [2]. AD of organic wastes and energy crops to produce methane would benefit society by providing a clean fuel from renewable feed stocks. This could substitute fossil fuel-derived energy and reduce environmental impact including global warming and acid rain [3]. Agro wastes and energy crops represent an important source of biomass that can serve as a substrate in anaerobic digestion, resulting in the production of renewable energy. The wastes

selected for our study are coconut oil cake (residue obtained after oil extraction), cashew apple waste (crushed apple waste obtained after extraction of “Feni”—“Feni” is a form of liquor i.e., distilled from the pure fermented juice of cashew apple without addition of spirit), and grass from lawn cuttings.

The direct comparison of biomethane production from different feedstocks is difficult as performance data for specific types are often produced under a wide variety of experimental conditions, for example, mixing regime, temperature, total solids, volatile, solids and hydraulic retention time. For this reason, it is better to compare feedstocks by their ultimate methane yield determined by the biochemical methane potential (BMP) assay [4–6]. Chynoweth et al. [7] studied the biochemical methane potential of a variety of feedstocks. Gunaseelan [8] reported the ultimate methane yields of several fractions of fruit and vegetable solid wastes, sorghum and Napier grass, using the BMP assay. Cavinato et al. [9] studied the codigestion of cattle manure with agro wastes and energy crops. Similar studies on biomethanization of energy crops were reported by Demirel and Scherer [10]. The purpose of this work was to study the anaerobic digestion of three different substrates, namely, coconut oil cake, cashew apple waste, and grass cuttings, in order to evaluate the

potential of anaerobic digestion as an alternative to the conventional solution-like composting and incineration while reducing the energy consumption of fossils fuels.

2. Materials and Methods

2.1. Feedstocks. Coconut oil cake and cashew apple waste were collected from local industries in and around Goa. Grass cuttings were from the lawns of our institute campus (BITS, Pilani, K. K. Birla Goa Campus). Cashew apple waste was collected from a cashew plantation located in Sancoale, Goa. Coconut oil cake was collected from a factory extracting coconut oil located in Cansaulim, Goa. And grass clippings were from the lawns of our institute campus.

Prior to use, the substrates were ground in a blender to give a fraction with particle size less than 2 mm and stored at 4°C until use.

2.2. Inoculum. Cow dung was used as inoculum with total solid content of 20 g/L. The inoculum was preincubated in order to deplete the residual biodegradable organic material present in it.

2.3. Analytical Methods

2.3.1. Total Solids (TS) Estimation. For total solids, a known amount of sample was transferred into a previously weighed crucible and dried at 105°C for 24 h. The increase in weight over that of the empty crucible represents the total solids [11].

2.3.2. Volatile Solids (VS) Estimation. For volatile solids estimation, the dried sample obtained after TS estimation was ignited in a muffle furnace at 550°C for 2 h. The weight lost on ignition represents the volatile solids [11].

2.3.3. Chemical Oxygen Demand. The chemical oxygen demand measurement is performed on fresh waste. Prior to use, the substrates were ground in a blender to give a fraction with particle size less than 2 mm. The substrate (1g) is then suspended in 1 L mL of distilled water, stirred on a magnetic stirrer for one hour, and the COD of the suspension is measured as described by Raposo et al. [12]. Briefly, 10 mL of suspension was digested with potassium dichromate and concentrated sulphuric acid at 150°C for 2 h in a COD Block Digestion Unit. After cooling, the digestate is titrated against ferrous ammonium sulphate (0.5 N) using 1, 10-phenanthroline as indicator.

2.3.4. Biochemical Methane Potential Assays. The biochemical methane potential (BMP) of the substrates was performed according to [5, 6]. The methane potential of the substrates was determined over the following range of VS, that is, 3.0, 3.5, 4.0, 4.5, and 5.0 g VS/L. The reactors were supplemented with nutrients, trace elements, and bicarbonate. Finally, the reactors were made up to the working volume 0.1 L with distilled water, and the headspace was flushed with nitrogen. A control without substrate was also set up to account for the endogenous biogas produced from the inoculum. All

the experiments were carried out in triplicates. The bottles were shaken manually once a day. Biogas production was measured using water displacement technique. Gas samples were taken periodically for composition analysis by gas chromatography using helium as carrier gas. The calculated biogas production is also corrected for blank biogas production.

One mL macronutrients, 0.8 mL micronutrients, and 5 mL of buffer from the above-mentioned stock solutions were added (Table 1).

2.3.5. Biogas Measurement. The biogas production was measured by water displacement setup [13]. A tube was used to connect the reactor with an inverted 250 mL graduated measuring cylinder immersed in a 1000 mL beaker filled with water. Biogas produced was collected in the graduated cylinder connected with a water reservoir which allowed volumetric biogas measurements at atmospheric pressure.

2.3.6. Gas Composition. Gas samples were taken periodically for composition analysis by gas chromatography. The samples were analysed with a gas chromatograph (GC-7610, Chemito) equipped with thermal conductivity detector. The carrier gas was hydrogen. The oven, injector, and detector temperatures were 80, 150, and 250°C, respectively.

2.3.7. Batch Reactors. A double-walled reactor of 3 L effective volume was used in this work. Temperature was maintained at 35°C by a water recirculation. Mixing was done by magnetic stirring.

Series of experiments were carried out at the same time. The reactor was seeded at a volatile solid (VS) concentration of 3.0 g/L with anaerobic sludge, taken from the outlet of an anaerobic pilot reactor treating food waste.

3. Results and Discussion

3.1. Selection of Feedstocks

3.1.1. Coconut Oil Cake. India is the third largest coconut producing country in the world with a cultivation area of about 1.78 million hectares [14]. In a conventional edible oil manufacturing mill, oil is extracted from dried copra (matured coconut Kernel) leaving behind a protein and lignocellulosic rich oil cake.

The advantage of using oil cakes as a substrate for biogas production is their cheaper availability throughout the year. Moreover, with increasing emphasis on cost reduction of industrial processes and value addition to agro-industrial residues, utilization of oil cakes as an energy source seems to be promising because of their higher energetic value. Typically coconut oil cake was used as cattle feed, but the dominant factor that affected coconut oil cake market was the situation of other oil cakes (meal), especially, soybean meal and sunflower meal. Also the reason for looking for alternative use of coconut oil cake rather than as cattle feed is the introduction of attractive incentives for green fodder

TABLE 1: Composition of solutions.

		(g/L)
Macronutrients		
NH ₄ Cl	g/L	26.6
KH ₂ PO ₄	g/L	10
MgCl ₂ , 6H ₂ O	g/L	6
CaCl ₂ , 2H ₂ O	g/L	3
Micronutrients		
FeCl ₂ , 4H ₂ O	g/L	2
CoCl ₂ , 6H ₂ O	g/L	0.5
MnCl ₂ , 4H ₂ O	g/L	0.1
NiCl ₂ , 6H ₂ O	g/L	0.1
ZnCl ₂	g/L	0.05
H ₃ BO ₃	g/L	0.05
Na ₂ SeO ₃	g/L	0.05
CuCl ₂ , 2H ₂ O	g/L	0.04
Na ₂ MoO ₄ , 2H ₂ O	g/L	0.01
Buffer		
NaHCO ₃	g/L	50

cultivation by Government of Goa, India. High incentives are given by the local government, that is, incentive under Perennial Fodder Cultivation at 370 USD per hectare area of land for the 1st year, 185 USD each per hectare area of land for the 2nd and 3rd years. This has resulted in decline in demand for coconut oil cake as cattle feed in Goa, India.

3.1.2. Cashew Apple Waste. Cashew is an important cash crop grown in India, on an area of 820,000 hectares which produced 539,000 tons of raw nuts in 2004-2005 [15]. The fruit consists of mainly the nuts containing an embryo (Kernel) and a false fruit commonly called as cashew apple. The nuts represent only 10% of the total fruit weight, and large amounts of cashew apples are lost in the field after nut removal [15]. Although cashew apples can be consumed as juice, jams, and other food stuffs, the cashew tree cultivation is an agricultural activity directed at the production of the cashew nuts. Due to its large availability and low cost, cashew apple has been studied as a substrate for fermentative and enzymatic processes for several applications [16–20]. As such, cashew apple is considered an agricultural residue rich in reducing sugars (fructose and glucose), vitamins, minerals, and some amino acids [21] and can be a suitable low cost substrate for anaerobic digestion studies.

3.1.3. Grass. Energy production from lignocellulosic biomass will be a major alternative to conventional energy sources. The efficient conversion of plant biomass to biogas remains a challenge because of the presence of recalcitrant and insoluble starting materials [22].

The characteristics of the different substrates selected are shown below in Table 2.

The TCOD/VS ratio close to unity indicates better biogas potential, and thus methanogenesis can be elucidated from COD/VS ratio [23].

TABLE 2: Characteristics of substrates.

Feedstock	TS %	VS %	VS/TS %	TCOD g/gVS	COD g/kg
Coconut oil cake	90.93	84	0.92	0.87	1.057
Cashew apple	21.8	21.2	0.97	0.9	1.077
Grass	56	53	0.95	0.4	2.3

FIGURE 1: Daily biogas production rates at different volatile solid rates.

3.2. Digestion of Coconut Oil Cake. The daily biogas production rate showed multiple peak values over time (Figure 1). Multiple peaks indicate the presence of multiple substrates within coconut oil cake or solid matrix of the coconut oil cake, and as a result, additional cellulose becomes bioavailable [24]. Coconut oil cake is rich in lignocellulosic content and protein [14]. Biogas production increased until the 30th day, and then there was slight increase until the 60th day. There was an initial lag phase for 5 days. The average biogas production was found to be 451 and 662 mL/g VS at 4 and 4.5 g VS/L, respectively (Figure 2). The percentage of methane in the biogas produced from coconut oil cake was found to be 55% which corresponds to 383 mL CH₄/g VS and 277 mL CH₄/g VS added, respectively. At lower 3.0 and 3.5 g VS/L as well as higher concentration of volatile solids (5 g VS/L) the reactors did not recover from the initial lag phase, and no significant amount of biogas was produced. Increasing the volatile solid loading rate decreased biogas production; this could be due to accumulation of intermediates as reported by Hansen et al. [5] for waste rich in fats and lipids. Coconut oil cake contains about 5-6% of oil in it even after extraction as the extraction of oil is only by mechanical extraction.

3.3. Digestion of Cashew Apple. The biogas production started from the first day but declined rapidly with no biogas produced at the end of 14 days of digestion (Figures 3 and 4).

FIGURE 2: Biogas yield for coconut oil cake at different volatile solid rates.

FIGURE 3: Biogas production rate for cashew apple waste.

The pH values in the digesters at the end of digestion time were below 6.0. Fruit and vegetable wastes tend to have low total solids and high volatile solids. The VS/TS ratio for cashew apple was very high 0.97 g VS/g TS. The rapid hydrolysis of the feedstocks might have led to acidification and consequent inhibition of methanogenesis, which is a major limitation of one-stage anaerobic digestion system [25].

3.4. Two-Stage Studies for Cashew Apple Waste. The substrate, that is, cashew apple waste, was kept in the hydrolytic stage in reactors at different loading rates by following the pH which indicates acidification of the reactor. Hydrolysis stabilized at pH 5.5 for OLR of 3.5–4.0 g VS/L after 15 days. After 15 days, the pH was adjusted to 7.2 so as to start the methanogenesis.

Maximum biogas 132 and 140 mL/g VS was produced at OLR of 3.5 and 4 g VS/L (Figure 5). The methane content of the biogas was 46% and 46.2% which corresponds to 60.7 mL

FIGURE 4: Biogas yield for cashew apple waste mL/g VS.

FIGURE 5: Biogas yield mL/g VS for cashew apple waste.

and 64.6 mL at 3.5 and 4.0 g VS/L, respectively, at the end of 25 days of digestion.

3.5. Digestion of Grass. The daily production rates and average biogas yield for digestion of grass waste are shown in Figures 6 and 7. The daily biogas production rate showed multiple peak value overtime. Multiple peaks are due to presence of multiple substrates within a single component as explained earlier. The daily biogas production rate was highly variable during the 25 days of digestion and then stabilized until the end of the experiment. As shown in Figure 7 the biogas yield increased with increasing volatile solid rates. Volatile solids analysis determines the total amount of organic matter in a substrate. With increasing VS, the amount of organic matter being added to the digester or the reactor increases, hence contributing to increasing methane yield per VS with increasing VS loading. The average biogas yields were 391, 490, 501, 557, and 651 mL/g VS, respectively. The percentage of methane in the biogas was found to be 51% which

FIGURE 6: Biogas production rate mL/g VS for grass.

FIGURE 7: Biogas yield mL/g VS for grass waste.

corresponds to 199, 250, 256, 284, and 332 mL $\text{CH}_4/\text{g VS}$. The results gave a biogas yield higher than that found in batch experiments with grass carried out by Liu et al. [26], who obtained a yield of 372 mL/g VS. Grass and leaves contain waxes and lipids that were not quantified. However, these compounds would not be as degradable as carbohydrates [22]. The percentage of methane in the biogas produced from grass was found to be 51%.

3.6. Fed Batch Studies for Coconut Oil Cake

3.6.1. Biogas Production. Based on the previous results, the anaerobic biodegradability of coconut oil cake was evaluated in fed batch mode in a 5 L anaerobic reactor. The substrate was added at 4 g VS/L/batch for all the batches. The volume of biogas produced was determined using water displacement

FIGURE 8: Methane yield for three sequential batches for Coconut oil cake.

method, and results obtained are presented in Figure 8 which represents the percentage of methane in the biogas produced with time during a typical batch. A significant lag phase was seen for all the batches during the start of each batch. The maximum biogas was produced between 5 and 13 days of digestion. The first cycle of fed batch was operated for 15 days after which there was no production of biogas. Following this, the second batch of substrate addition was made. The reactor was operated for three batches with feeding after indication of endogenous respiration. On all three accounts of substrate addition, the maximum methane yield was 320 mL/g VS. The results indicate that there is no accumulation of inhibiting chemicals [27] when operated in fed batch mode. This aspect can be taken into account for designing pilot scale anaerobic digester for coconut oil cake.

3.6.2. Methane Production Potential. The maximum biodegradability (B_o) is given as $\text{m}^3 \text{CH}_4$ produced per kg VS. The theoretical maximum methane production is $0.35 \text{ m}^3 \text{CH}_4/\text{kg COD}$, and one gram of VS for “biological” sludge is normally considered equal to 1.4 g COD [28]. Thus, the theoretical maximum methane production will be $0.49 \text{ m}^3 \text{CH}_4/\text{kg VS}$. Thus, the B_o for the substrates tested that is, coconut oil cake, and grass cuttings except for cashew apple were 0.36, and $0.33 \text{ m}^3 \text{CH}_4/\text{kg VS}$, respectively. As the methane production potential and hence maximum biodegradability of coconut oil cake were higher compared to grass cuttings it was taken for fed batch studies.

The local oil mill which processes coconuts handles about 500 kg of dried coconut copra per day, and after extraction of oil, about 200 kgs of coconut oil cake is available as waste residue whose market as cattle feed is on the decline. Based on the fed batch studies we will be able to produce 320 mL/g VS of methane or in other words 64000 dL/200 kg VS or 6400 L/200 kg of raw coconut oil cake (@ 10% TS). Therefore this mill could produce 6.43 of biogas per day which could replace firewood used for cooking for 6 families considering each family has 5-6 people and does cooking

thrice a day. The amount of biogas thus produced will also replace 355 kg of CO_2/m^3 of biogas.

4. Conclusions

The potentialities of three different residues as substrates for anaerobic digestion have been investigated in this study. For coconut oil cake biogas was produced only at 4 and 4.5 g VS/L. Coconut oil cake has a high potential for biogas production. However, they contain slowly biodegradable organic matter, and the loading rates should remain low to avoid any accumulation of slowly biodegradable solids in the digesters. The potential of cashew apple for anaerobic digestion is very low. For cashew apple as a substrate phase separation resulted in high process stability and significant biogas productivity compared with a single-stage reactor. The data obtained from this study would be used for designing large scale anaerobic digesters for treatment of agro waste. Our future work is focused on pilot scale anaerobic digestion of coconut oil cake in a 0.5-tonne horizontal plug flow reactor (already constructed).

Abbreviations

AD:	Anaerobic digestion
BMP:	Biochemical methane potential
TS:	Total solids
VS:	Volatile solids
COD:	Chemical oxygen demand
TCOD:	Total chemical oxygen demand
HRT:	Hydraulic retention time
OLR:	Organic loading rate.

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