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In situ Physicochemical and Microbial Changes During Kitchen Refuse Biogasification

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Abstract: The kitchen refuse evaluated for biomethane showed a potential of 32l/kg dry wt. with the highest percent methane content of 63, demonstrating kitchen refuse as a good biomethanation candidate. Physical factors like, texture, size and the substrate proximates affect biomethanation. A 50 µm resolution SEM degradation pattern study gave a micro-level understanding of the gradual physical changes during biomethanation. The substrate degradation pattern signified the actual consumption and decomposition of the material. An appreciably high methane yield compared to substrates like agro-wastes and cow-dung is attributed to the low C/N ratio of the fed substrate. An increase in the percent easy-to-degrade organic input can ensure a good amount of quality biogas. Methane and VFA correlated well, wherein the methane percentage increased while the VFA value decreased. A better biomethanation was associated with the acclimatised inocula and wetness of the sample. Promising biomethanation-mediating microbes with diverse enzymatic activities indicate at the possibility of formulating a niche-specific effective microbial consortium. While formulating effective microbial consortia for enhanced biomethanation, bacterial groups with enzymatic activities such as amylase, cellulase, lechithinase, and lipase were successfully isolated and screened. Elemental analyses of the digestate established its safety as manure.

Key words: biogas, digester, digestate, kitchen refuse, microbial consortium, microscopy.

Introduction

Evolving civilisation is in urgent need of alternative energy sources, and biogas fits in suitably. Initiated with cattle dung as the substrate material, the technology can potentially accommodate almost all organic substrates with a biomethanation potential, such as the refuses from cattle-shed, municipality, commercial activities, forests, or even households, including that from the kitchen. There is a growing need to fine-tune the technology, and broaden its use [5]. Ideally, biogas has 54-80 % CH_4, 20-45 % CO_2 and traces of H_2, N_2, H_2S, etc. [4]. Methane is the simplest of all hydrocarbons and works as an excellent fuel (pure CH_4 has 9,100 kcal/m^3 energy), and biogas roughly has 4,713kcal/m^3 (4.698 kWh electricity equivalents) energy at 1 Atm. pressure. It is carbon-neutral and emits

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lower NO_x and CO_x than diesel and petrol [18]. Biogas has advantages over conventional vehicular fuel – 1.33-1.87m^3 and 1.5-2.1m^3 are petrol and diesel litre equivalents, respectively.

Biogasification, anaerobic digestion of organic substrates, is a 4-stage process, *viz*., hydrolysis, acidogenesis, acetogenesis and methanogenesis, occurring simultaneously and interdependently [6]. Hydrolysis is a combined physical and enzymatic degradation (mesophilic microbial fermentation) of the substrates, which is followed by the acidogenesis wherein various catabolic enzymatic processes lead to the production of an array of organic acids. Acetogens like *Acetobacter* and *Syntrobacter* set in acetogenesis almost concurrently with acidogenesis, where all these organic acids channelise into acetate. These convert the hydrolysed and catabolic organic acid products into H_2, CO_2 and acetate. In methanogenesis, during biogasification, the acetogenic end-products are biotransformed into CH_4 largely by facultative anaerobic methanogens like *Methanobacter* and *Methanococcus* [18,25]. Physicochemical factors like particle size, pH, temperature, organic loading, VFA, etc. and operational parameters like the loading rate and retention time [8] influence the biogasification efficiency. The critical BMP parameters like inoculum to substrate ratio, total to headspace volume ratio, substrate pH, substrate preparation/ quality, headspace pressure, and the gas flow measurement system employed can all be important [17].

Numerous substrates have been evaluated for their biogasification potential. More complex and longer the polymers in the substrate, the longer shall it take to convert to VFA, and thereby lower shall be the methane potential. Household wastes have high water and biological activities, and thus degrade easily [3]. Owing to its high soluble sugars, starch and proteins contents [10], the household kitchen refuse is a suitable biogasification candidate. Though an array of good methane potential sources are reported, relevant parameters such as the elemental constituents, microbial load, potential enzymatic sources and the substrate-level submicroscopic physical changes in them have been feebly reported. Often, finding the complete set of this information for a particular study is still scarce.

Biotransformation of solid organic wastes into biogas has been more relevant in the recent past [9]. Biogasification is an applied biotechnology to maximise the organic waste-to-wealth attributes. The digestate thus obtained is mineral-rich manure that helps reduce the synthetic/chemical fertiliser demand. Sufficiently inexpensive sugar-rich streams from renewable organic lignocellulose biomass, thus, could provide a spectrum of fuels and chemicals, replacing petroleum and other fossil feedstock [28].

A batch BMP test gives an idea on the cumulative (total accumulated) CH_4 produced from the substrate from when it goes into the reactor until fully degraded. The method has many benefits including its scalability, and performing multiple tests in parallel. These provide a scope to use different substrate combinations to compare their performances in parallel. However, as the test is done at a smaller scale, the role of the moderating compounds is difficult to examine, and the quality of the degestate deposits may be ill-characterised.

KIIT University, Bhubaneswar houses 25-30,000 students and the disposal of the food leftovers and the kitchen refuse is a critical environmental issue to address. The present work was an attempt to estimate the behavior of anaerobic digestion comparing the quantity (BMP) and quality of the biogas (% CH_4), elemental constituents (heavy metals) of the digestate, microbial load and composition, microscopic degradation pattern and related physicochemical parameters.

Materials and methods

Sample collection and preparation

Kitchen refuse substrate for the study was collected from the hostel canteens of KIIT University, which largely had the potatoes peelings, cooked rice leftovers, cucumbers, cabbage and carrot. The substrate was manually differentiated, pulverised and ground to a particle size of about 10mm. Fresh cattle dung was added as a natural inoculum @ 1:3, followed by water @ 1:1.5 of total mixture. The final substrate appearance and the analysed mean percent composition are presented in Fig. 1, and the

physicochemical status in Table 1. The mixture was then put into specially-designed batch bioreactors (Fig. 2).

Bioreactor design and set-up

A set of fabricated laboratory-scale batch bioreactors of 2.0 l capacity with a working volume of 1.75 l having two openings at the top-one for the collection of gas and other for sampling was designed (Fig. 2) and operated at 40°C. The openings were tightly closed with rubber septa and screw caps. The headspace of each reactor was purged with Nitrogen gas for about five minutes to ensure anaerobic conditions. The substrate in the digester was mixed manually by shaking it vigorously for a minute prior to sampling.

Physicochemical parameters

A known weight of the sample (W_s) was dried in an oven at 105°C for 24h and the final weight (W_{DM}) was recorded to determine the dry matter (DM). The DM content was calculated as % DM = 100 × W_{DM}/W_s. The conductivity, TDS and salinity were measured using a digital portable water/soil analysis kit (VSI 07, VSI Electronics Pvt Ltd, India). Other physical parameters such as the TDS (2540-C), TVS (2540-B) were estimated as per the APHA [1].

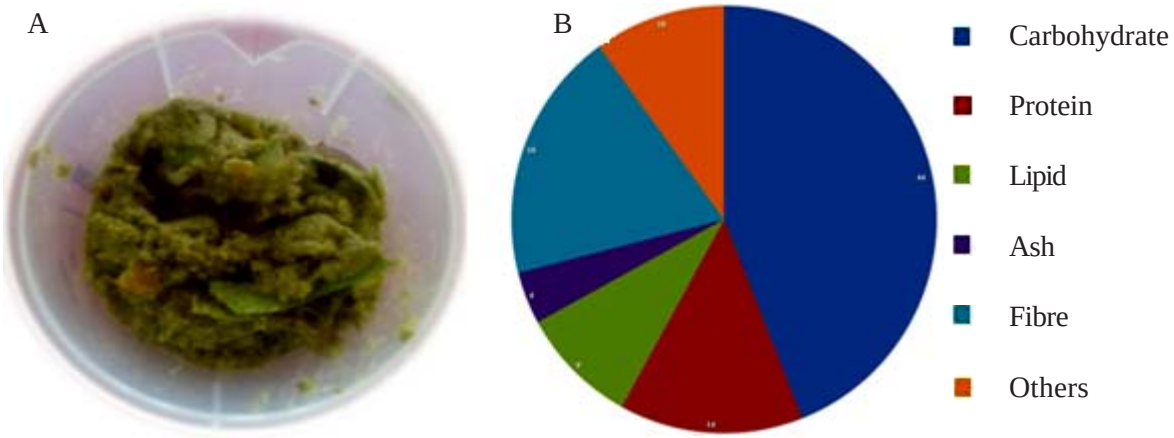

Fig. 1. The kitchen refuge substrate*: its A: physical processing; B: proximate composition
*The % composition was confirmed by analysing samples from three different hostel kitchens (of KIIT University) in triplicates

Table 1. The physicochemical status and the percent mean composition of the substrate

Parameter	Unit	Values
pH		4.1
Moisture	%	81.0
Dry matter	%	19.0
TDS	ppm	2370
COD	ppm	162
Total volatile solids	% DS	95.3
Proteins	% DS	14.4
Nitrogen	%	1.83
Carbohydrates	% DS	44.9
Organic carbon	%	28.0
Lipids (fats)	% DS	9.6
Ash	% DS	4.2
C/N ratio	-	15.3

Fig. 2. The digester set-up to study the biomethanation potentials

The pH, nitrate (4500-B), phosphate (4500-P), carbonate (Alkalinity2320), COD (Sec.5220) and organic carbon (Sec 5310), and carbohydrate, protein, lipids, and ash were estimated as per the standard procedures [1].

Volatile fatty acid (VFA)

The VFA was measured titrimetrically [30] for mainly acetate VFA. The sample was filtered and centrifuged at 5000 rpm for 6min, and the pH was recorded. To a 20.0 ml of this, 0.1M HCl was added to adjust the pH to 4.0. The sample was heated on a hot plate (100°C) for 3min, cooled, and 0.1M NaOH was added till the pH was 7.0. The quantities of HCl and NaOH solutions consumed in the process were recorded. The total VFA content was calculated as mg/l acetic acid = Volume of NaOH titrated X 87.5.

Microbiological studies

The weekly sample was collected from the digester aseptically and was serially diluted with normal saline solution (0.875 % NaCl), and pour-plated on nutrient agar media (Peptic digest of animal tissue-5.000 g/l, Sodium chloride-5.000 g/l, Beef extract-1.500 g/l, Yeast extract-1.500 g/l, Agar-15.000 g/l, pH 7.4±0.2 at 25°C) [27]. The cell counts were recorded after 48 h incubation [10].

Isolated single bacterial colonies were picked from the pour plate and streaked to the new agar plate, repeating the step twice. The bacterial culture was examined under the light microscope for purity. The pure cultures of the isolates were screened, including for the different enzymatic activities [13].

Electron microscopy

Periodically withdrawn digester samples were freeze-dried, labeled, stored in close containers, and subjected to scanning electron microscopy (HITACHI S-3400N, Germany) to observe its gradual physical changes with time [2,31].

Elemental analyses

The samples were subjected to atomic absorption spectrometry [20] for elemental analyses. 1.0 g of the periodically withdrawn sample was acid-digested with concentrated HCl followed by appropriate dilutions prior to the analyses (PerkinElmer Model AA-400).

Qualitative and quantitative CH_4 estimation

The biogas volume formed anaerobically was collected through a simple water displacement method and then cumulative amount was calculated. The biogas was analysed using an on-line gas chromatograph (Nucon, 5700, equipped with push-fill type of sample injector) to quantify the methane, CO_2 and H_2S [33] Porapak QS column (length 2.0m; # 80-100 or 149-177µ) was used with a TCD detector at 200 mA. The oven, injector and the detector temperature were maintained at 40, 90 and 120°C, respectively. Hydrogen with a flow-rate of 35 ml/min was the carrier gas. The average values of the duplicate readings were recorded.

Statistical analyses

To know the brevity of the data, the average means of the triplicate data were subjected to the relevant statistical analyses, *viz*., standard deviation on MS-Excel, and the graphs prepared (GraphPad Prism Ver. 5.00; San Diego, California, USA).

Results and discussion

Physicochemical and proximate status of substrate

The physicochemical status of the raw kitchen refuse and the final digestate are listed in Table 2. A 17 % increase in the total moisture content and an appreciable 17.8 % decrease in the total dry matter (19-1.2 %) of the digestate were attributable to the dissolution of the solids during biogasification. The digestate salinity (6.9 ppt) confirmed its acceptability as manure, agreeing with Lefebvre *et al*. [15]. An increase from 129 to 163 ppm of the phosphate content of the digestate justifies its utility as fertiliser. The organic carbon reduced from 28-3.6 %, while the carbonate contents increased by 69.0 ppm. Strongly influenced by carbonate, bicarbonate, ammonia, phosphate and VFA, the alkalinity showed an overall 150.0 ppm increase.

The COD values increased rather narrowly (by 34.0 ppm) suggesting that the digestate was chemically rich, as also opined by Hill and Baier [12], despite the depleting organic contents. Further, the heavy metals suitability has been analysed under the elemental analyses results, later.

The pH indicates the biodigester health and methane quality. Anaerobiosis of organic substrate produced fermentative organic acid products as VFA, which led to an initial drastic pH reduction from 6.5 to 4.8 by the 7th day (Fig. 3A). The pH increased to 5.8 between the 2nd and 3rd week, attributable to VFA utilisation (Fig. 3B) by the acetogens followed by the methanogens, corroborating with the observations of Strik *et al*. [26].

Fig. 3B indicates the steadily increasing VFA production pattern for which there is an ease of methane production in a batch process, though may not be in a sustainable way. A continuous

Table 2. The physicochemical status of the substrate (0th day) and the digestate (28th day)

Parameters	Units	Raw	Digestate
Moisture	%	81.0	98.0
Dry matter	%	19.0	1.2
TDS	ppm	2370	1890
Conductivity	mmhos/cm	3.40	3.30
Salinity	ppt	7.3	6.9
Alkalinity	ppm	350	500
Carbonate	ppm	300	369
Phosphate	ppm	163	129
Nitrogen	%	1.83	1.92
Nitrite	ppm	84.0	45.0
Organic carbon	%	28.0	3.6
COD	ppm	162	196
C/N ratio	-	15.3	1.87

increase till 7.7 g/l by the 2nd week is ascribable possibly to an initial methanogens population below the threshold level. The VFA was maximal by the 14th day agreeing with the observations in municipal solid wastes, of Shanmugam and Horan [23]. A threateningly high VFA accumulation might render the digester unstable by threatening the very survival of methanogens [16]. By the 21st day, the VFA concentration (6.1g/l) fell to a more acceptable level, attributable to their slow *albeit* steady utilisation by the methanogens.

The qualitative methane production (Fig. 3C) increased simultaneously with a peak and then decrease in the VFA concentration (Fig. 3B) by the 14th day. The VFA concentration reduced by the 21st day when the methane production was at its peak, confirming that it was a multi-stage bioprocess. The high methane production by the 21st day (65%; Fig. 3C) is attributable to the possibly actively multiplying methanogens. Methane production ceased by about the final day as the total organic carbon possibly reduced drastically to a sub-minimal level. Gradual reduction of methane (23%; Fig. 3D) could be due to the limiting substrate in a batch processing ultimately resulting in a system collapse [7].

Estimating CH_4 production

The weekly methane production gas chromatographs are presented in Fig. 4, and the daily and cumulative methane productions are illustrated in Fig. 5. Methane production (3.09 l/kg dry wt.) was the maximum on the 13th day and the production almost ceased by the 28th day. Although the methane production was noticeable from the 7th day onwards (Fig. 3C), the recorded quality of the biogas in terms of percent methane content was the best by the 21st day (Fig. 3C).

Gupta *et al.* [9] reported 55-57% methane content for the 1:1 for mahua oil-seed cake and cattle dung combination, and Satyanarayan *el al.* [22] reported a 66 % methane content for the 1:3 mustard oil-seed cake and cattle dung, supporting the present findings.

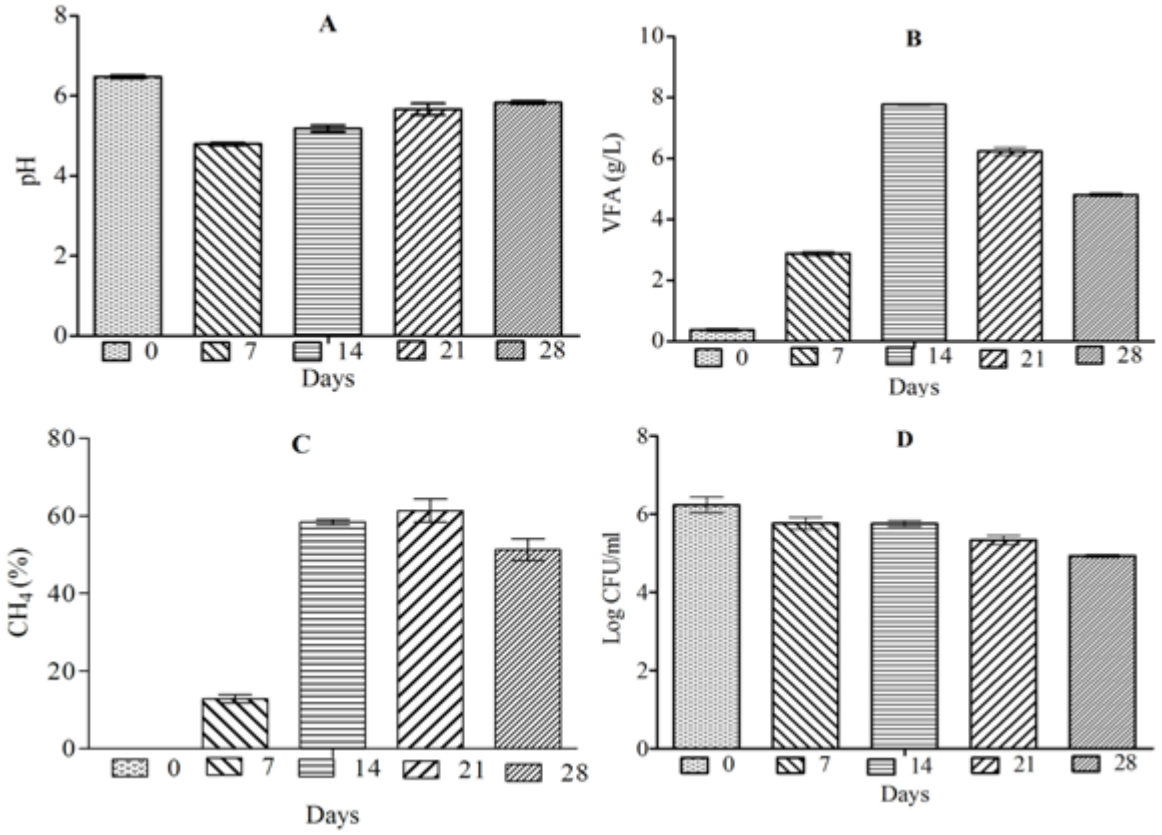

Fig. 3. The pH (A), VFA (B), % CH_4 (C) and microbiological counts (D) during the study

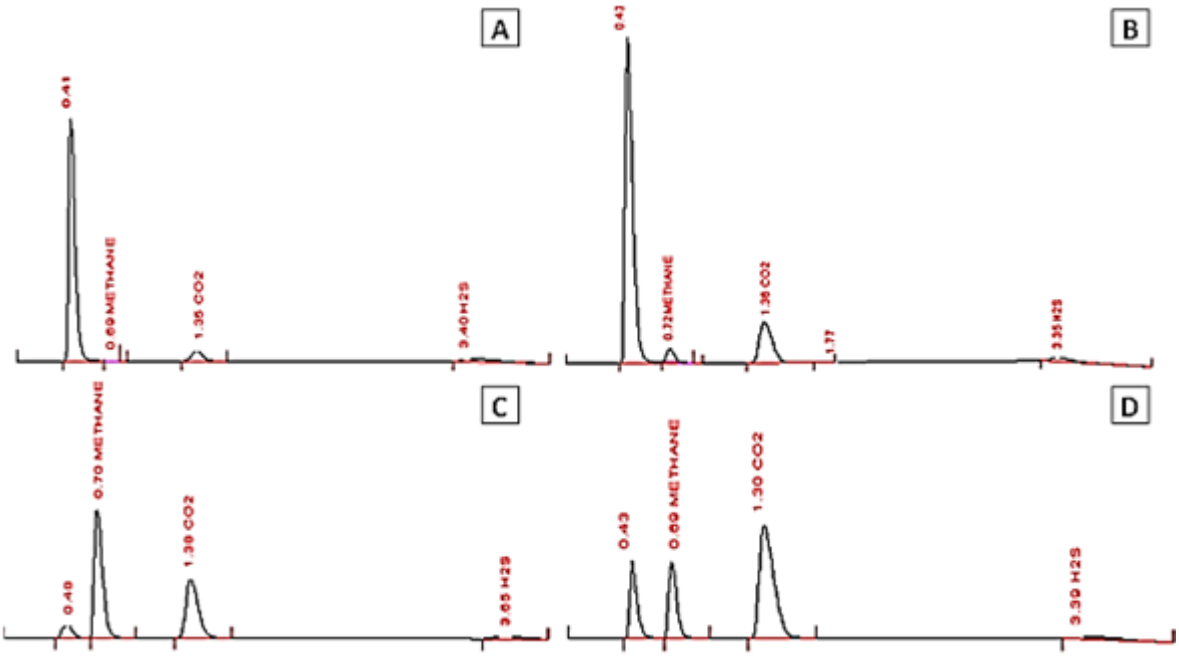

Fig. 4. The GC graphs of the gas samples for analysis of CH_4, CO_2 & H_2S concentrations on the 7th (A), 14th (B), 21st (C) and 28th (D) days during the study period

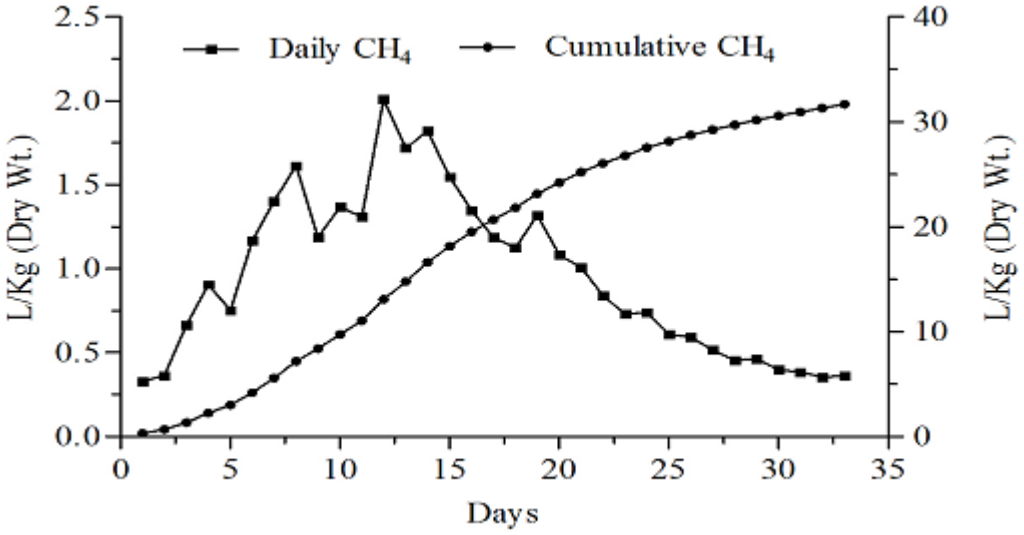

Fig. 5. Daily and cumulative methane production

Microbial consortia

An initial healthy microbial consortium is attributable to the varied and nutritionally rich organic material. The high microbial population of 5.76 X 10^6 CFUs/ml during the initial days dwindled till the end of the study (Fig. 3D). It was noticed that their numbers and composition declined, attributable to the limiting substrates, and an *in situ* physicochemical alteration allowing only the specialised microbes to flourish.

Out of the pure isolates, 28 were randomly chosen and tested for the amylase, lipase, cellulase and lecithinase activities (Table 3; Fig. 6). Amylase-positive isolates outnumbered the others, presumably due to the starch-richness of the refuse. Many exhibited diverse substrate utilisation abilities, predicting their potential usefulness in bioprocess applications.

Table 3. Screening of enzymatic activities of bacteria isolated from the digestate

Isolated strain	Cellulase	Amylase	Lecithinase	Lipase (Tween 80)
DS 01	+	++	+	+++
DS 02	+++	++	+	-
DS 03	+	+++	+	+++
DS 04	++	+	-	++
DS 05	+++	+	+	++
DS 06	+	+++	-	-
DS 07	-	+	+	-
DS 08	+++	+	-	++
DS 09	-	++	-	++
DS 10	++	+++	-	-
DS 11	+++	++	+	++
DS 12	++	+	+	-
DS 13	+++	+	+	++
DS 14	-	+++	++	-
DS 15*	++	+++	+	++
DS 16	++	-	++	-
DS 17	+	++	-	+++
DS 18	-	+	-	-
DS 19	+	-	-	-
DS 20	-	++	-	+
DS 21	++	+++	+	-
DS 22	+	+++	-	+
DS 23	+	-	-	+
DS 24	-	-	-	-
DS 25	-	-	++	-
DS 26	-	+++	-	++
DS 27	-	-	+	-
DS 28	+	+++	++	-

* This isolate was identified for further studies

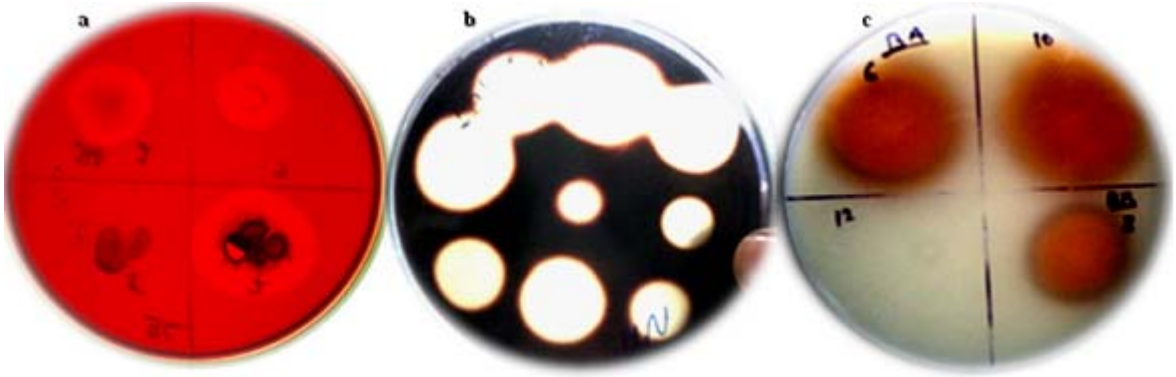

Fig. 6. Enzyme activities of digestate isolates (a: Cellulase; b: Amylase; c: Lecithinase)

Along with the various physical and chemical factors, formulating a microbial consortium of the isolates with the desired enzyme activity for the various substrates employed is the key to enhanced biomethanation [29].

Elemental analysis

The kitchen refuse comprising of the vegetable waste matter reportedly contains metal ions including heavy metals [32]. To confirm the quality of the digestate as manure from the heavy metals perspective, metals profile of the raw substrate along with its subsequent biodegraded forms was analysed (Fig. 7), wherein it was observed that the concentrations of nickel (1.0-3.0 ppm) and iron (22.0-50.0 ppm) gradually increased. This could possibly be due to factors like, possible less participation of the metals in the biotransformation, the pH fluctuating in a large range, and active microbial enzymatic transformations. All these help release the metal ions and accumulating in the digestate. The decreasing particle size evidenced in Fig. 8 might have facilitated the process further. Okeh *et al*. [19] reported enhanced digester performance and biogas yield with rice husk substrate when heavy metals like Ni^{2+}, Cu^{2+}, Zn^{2+} were added.

Electron microscopy

SEM analyses of the biogasifying substrate (Fig. 8) showed the constant disappearance of the initial sharp corrugation on the surface (Fig. 8a) as evidenced by a rough and corroding surface later (Figs. 8b-f), attributed to the enzymatic activities coupled with severely fluctuating pH.

There was no coarse granular surface initially (Figs. 8a,b) which slowly appeared later attributable to the surface activity of invading microbes and acidolysis, resulting in the degradation [28]. It was interesting to observe that the minute oval elevations on the raw substrate surface (Fig. 8a; 100 µm scale), progressively disappeared in Fig. 8c through Fig. 8e, gradually forming into pits (Fig. 2.8f). Wei *et al*. [31] had a similar observation in a wastewater study.

Conclusion

The biomethane potential of kitchen refuse was evaluated and found to be 32 l/kg dry wt. and the highest methane percentage recorded was 63 %, two points less than the values reported from the same lab in an earlier communication. The texture, size and the composition of the substrate are a few of the deciding physical factors in bio-methanation. A 50 µm resolution SEM study on the degradation pattern gave a micro-level under-standing of the physical changes during the gradual biomethanation. The percent maximum level of methane was 63, which is good enough to demonstrate that the kitchen refuse is a good bio-

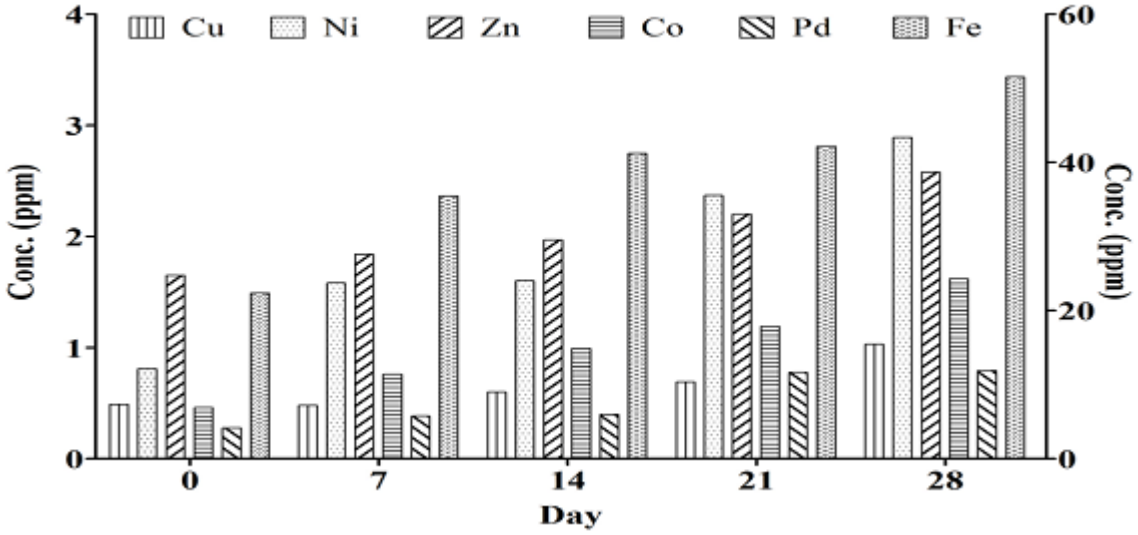

Fig. 7. Elemental analyses at different time intervals
Pd and Fe values are according to the right hand side Y-axis, and others according to left handed Y-axis

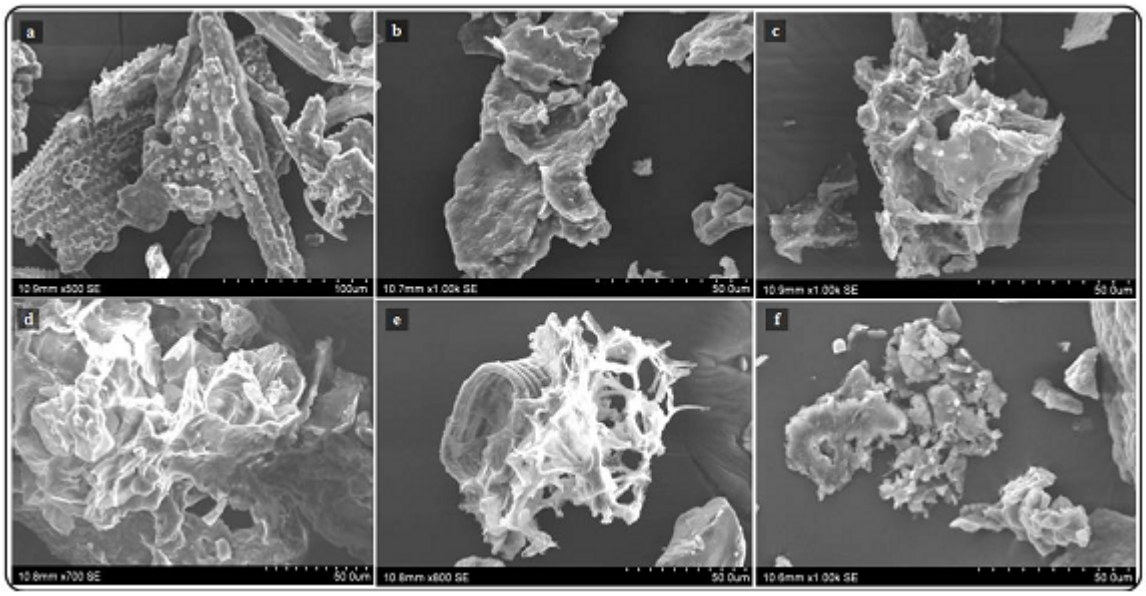

Fig. 8. SEM Images showing gradual degradation
(a: raw substrate; b: 1st day; c: 7th day; d: 14th day; e: 21st day; and f: 28th day).
All images (except 'a' at 100 μm) are at 50 μm scale

methanation candidate. Browne and Murphy [5] opined that a better biomethanation was associated with the acclimatised inocula and wetness of the sample. Present study also made an attempt to enhance biomethanation successfully by employing microbial enzymatic process. Such promising microbes having diverse enzymatic activities that mediate biomethanation indicate at the possibility of formulating a niche-specific effective microbial consortium.

The pH varied satisfactorily providing an optimal biomethanation condition. Still, a good buffering system seems necessary in a kitchen refuse fed biodigester due to the observed alarmingly high VFA. Since both pH and VFA are interdependent, *prima facie* it is hard to determine the factor for increased biogasification, although it may be the pH which directly or indirectly controls the various other undergoing processes. A good correlation between methane and VFA was observed, wherein the methane percentage increased while the VFA value decreased, agreeing with Siegert and Banks [24].

Elemental analyses of the digestate substantiated that it was a good candidate for safe manure. A well-studied substrate degradation pattern signifies the actual composition of the material and the way the various organic substrates can be subjected to easy decomposition, as also studied and discussed by Salehian *et al.* [21]. Applying the biogas slurry (digestate) in Arsenic contaminated paddy fields, Jia *et al.* [14] reported a significant alteration in the behavior of As in soil-rice system and an enhanced As accumulation in the rice plants.

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