



Evaluating the co-digestion effects on chicken manure and rotten potatoes in batch experiments

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Abstract

Anaerobic co-digestion of various organic substrates has been shown to improve biogas yield and methane percentage yield by maintaining carbon to nitrogen admirable ratio. Two types of substrates have been digested in this study with aim of evaluating influence of co-digestion on biogas and methane yield; the first one was rich in carbon namely rotten potatoes (RP), while the second one was nitrogen rich chicken manure (CM) in mono-digested as well co-digested manner. In present study experimental plan has been designed for RP, CM, M₁ (mixture of RP:CM ratio of 50:50) and M₂ (mixture of RP:CM ratio of 75:25) digestion at 37°C. Physicochemical analysis of inoculum and both substrates were evaluated for the determination of theoretical methane production and activity of inoculum. Mono and co-digestion of substrates yielded 291.0, 226.1, 304.5, 341.2 ml/g VS methane for RP, CM, M₁ and M₂ respectively. While theoretical calculated yields from CHNS values were 309.0, 285.6, 298.7, and 304.5 for RP, CM, M₁ and M₂ respectively. Percentage of methane in biogas composition increased from 55.53% (CM) to 65.30% methane (M₂) of biogas production. Acetate was in maximum proportion with respect to other VFAs in all regular inspection, its range varied from 1044.1, 840, 1098.45 and 1178.9 mg/l for RP, CM, M₁ and M₂ respectively. The results of co-digestion indicated considerable increase in both biogas and methane production.

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Introduction

Anaerobic digestion (AD) is broadly utilised for solid waste treatment but initially used for wastewater treatment since two decades ago. AD of solid waste materials is progressively utilized by nearby authorities, agro-modern organisations and farms to produce CH₄. In Europe, around 4 million tons for every year of Municipal Solid Waste (MSW) are managed by around 120 full-scale plants (De Baere, 2006). Unlike in waste water, solid waste contains high insoluble organic matter content and chemical oxygen demand (COD) and this digestion is considered as recycling of organic material to provide bioenergy. Chicken manure (CM) is a biodegradable semisolid substance and therefore can be employed to produce cheap energy. It has been reported that the daily chicken excretion ranges between 80 and 125 g (wet)/chicken; in which 20-25% of excreta includes total solids (TS) that is rich in nitrogen, and 55-65% comprises volatile solids (VS) of TS which are valuable source of energy (Moral *et al.*, 2005; Abouelenien *et al.*, 2009).

AD is considered to be an important and beneficial process in the production of CH₄ rich biogas, a potential source of renewable energy (Wanget *al.*, 2012a). Although anaerobic process is a well-known method for digestion of animal manure as cited by many researchers (Nishio and Nakashimada, 2007) however, due to economic and environmental concerns, studies elucidating CM/poultry manure (PM) digestion in anaerobic environment are limited (Abouelenien *et al.*, 2009). The higher nitrogen content, due to protein and amino acids of CM compared to manure from other farm animals, makes CM a difficult substrate for anaerobic digestion (Bujoczek *et al.*, 2000; Salminen and Rintala, 2002).

The significant characteristics that prevent or decrease the digestion of CM are (1) low carbon/nitrogen (C/N) ratio of the manure, and (2) high total ammonia levels, eliminating from the degradation of proteinaceous organic materials. Generally, the C/N ratio of CM ranges between 8 and 10, which is significantly lower than the desired range, i.e. 15-30 (Borowski *et al.*, 2014; Font-Palma, 2012; Moral *et al.*, 2005).

Similarly, presence of ammonium in the CM, which is released during the degradation of proteinaceous organic materials, can inhibit the conversion of organic materials to biogas (Dalkılıç and Ugurlu, 2015).

Co-digestion with a carbon-rich substrate is recommended as an alternative method to improve biogas production from low C/N proportioned wastes (Wang *et al.*, 2014). The method directly influences the biogas production by improving nutrients balance and C/N ratio, diluting toxic substances including ammonia without the addition of water and expensive chemicals which is a superior quality of a digested product, and lower the processing costs of several substrates in one establishment (Gelegenis *et al.*, 2007; Khalid *et al.*, 2011; Wang *et al.*, 2012b). It has been well-established that the C/N ratios of 25:1 or 30:1 can yield threefold cumulative biogas production levels as compared to 15:1 levels (Wang *et al.*, 2014; Wu *et al.*, 2010). Many studies have demonstrated positive results of co-digestion with many types of livestock waste including cattle manure, cattle slurries, hog wastes (Magbanua *et al.*, 2001), anaerobic sludge (AS) (Bujoczek *et al.*, 2000), fruit and vegetable wastes (FVW), buffalo manure with organic fraction municipal solid waste (OFMSW) (Esposito *et al.*, 2012a) and a mixture containing 40% dairy manure, 40% PM and 20% wheat straw (Wang *et al.*, 2012a). The majority of existing studies of co-digestion on biogas and CH₄ yield show that co-digestion results in improved production compared to what would be expected based on results from mono-digestion of the same materials (Mao *et al.*, 2015; Mata-Alvarez *et al.*, 2014).

In addition to this, environmental conditions such as pH, temperature, substrates type, TS and VS content of organic waste, hydraulic retention time (HRT) and acclimation periods etc. are also responsible for the regulation of biogas production during the anaerobic digestion especially under digestion of proteinaceous organic materials (Chen *et al.*, 2008). The main results of co-digestion studies demonstrate improved production of biogas and as well ratio of CH₄ in biogas as compared to mono-digestion of the same materials.

CM and RP were used as a substrate to explore the effect of co-digestion, as literature review showed the effect of co-digestion on biogas or CH₄ potential can vary strongly with biomass mix composition.

The objective aims to explore the influence of co-digestion on biogas and CH₄ yield from CM and RP, along with its comparison with the biogas and CH₄ with mono-digested CM and RP.

Materials and methods

Substrate and inoculum for AD

CM was collected from a poultry ranch located in the village's Faisalabad, Pakistan whereas RP was obtained from a local vegetable market of Faisalabad. CM was ground and diluted with water to $13.78 \pm 2\%$ TS using a laboratory blender. RP was cut and ground into 2-3 cm sizes. The active inoculum was taken from biogas digestion plant of the institute, located at NIBGE, Faisalabad, Pakistan. Inoculum showed high biodegradability on a continuous feed of agricultural solid biomass. Both substrates and inoculum were separately homogenised and afterward stored at 4°C for subsequent analysis. The chemical and physical characterization of the substrates and of the inoculum are listed in Table 1.

Experimental design

Anaerobic batch digestion assessments were carried out (in triplicates) at 37°C for 50 digestion days as described previously (Wang *et al.*, 2012c). The initial VS ratio of substrate to inoculum (S/I) was remained constant at 1:2 for the whole experimental set-up. Each flask had 1 L volume capacity and contained 500 mL working volume of total feed, including 200 mL of inoculum and a proper amount of VS substrate i.e. 6.6g VS in each setup flasks. The compositions in the experimental set-ups were as follows: first, two batch assay both substrate were digested separately, then mono-digested potential of substrates was assessed. In third batch M₁ (mixture of CM and RP) setup, ratio of CM and RP was 50:50, in batch 4 M₂ (mixture of CM and RP), RP and CM was 75:25. The C/N ratio was measured based on the elemental composition of the substrates (Shanmugam and Horan, 2009).

pH of the medium was buffered by NaHCO₃ solution in appropriate amount. Blanks were established in triplicate having composition of 200 mL inoculum and 300 mL distilled water to normalise the biogas production. All flasks were firmly sealed with rubber septa and screw caps to maintain anaerobic condition. The flasks were flushed with nitrogen gas for about 3 min to assure anaerobic conditions prior to starting the digestion tests. Subsequently, they were shaken manually twice a day for one minute and also before measuring daily biogas production. pH of all experiments was maintained in between 6.8 to 7.5 to obtain complete digestion as soon as possible.

Analytical techniques

TS, VS, pH, total Kjeldahl nitrogen (TKN) and total ammonium nitrogen (TAN) of substrates were determined in accordance with standard techniques (Apha, 2005). The volume of biogas was measured by water displacement method. An alkaline solution (2%NaOH) was used in water displacement method. A gas composition analysis was measured using gas analyser (GFM 4XX series). For volatile fatty acids (VFAs) analysis, samples were taken with intervals 4 to 6 days and centrifuged at 15000 rpm for 20 min and then, supernatant was filtered by the 0.45 mm cellulose acetate membrane following measurements by APHA standards (Apha, 2005).

The elemental composition (C, H, O, N, and S) of substrates was analysed by an elemental analyser CHNS/O analyser, PE 2400 Series II, Perkin Elmer, USA. Theoretical CH₄ has been calculated by using Buswell and Mueller equation 1 from elemental composition of substrates (Buswell and Mueller, 1952). This equation is also applied to co-substrate experiments by calculating the average of CHNS values. Anaerobic biodegradability (BD) of the substrate has been calculated based on experimental bio-methane potential (BMP_{exp}) and theoretical CH₄ yield (BMP_{ThAtC}) by equation 3 (Elbeshbishy *et al.*, 2012).

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

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

$$BD_{the} = \frac{BMP_{exp}}{BMP_{ThAIC}} \quad (3)$$

nitrogen content in TKN, TAN and elemental nitrogen in CM as compared to RP and inoculum, as several researchers reported nitrogen content is higher than other agricultural substrates, food waste, cow manure, and pig manure (Qiao *et al.*, 2011).

Results and discussion

Experimental and theoretical bio-methane potential

Physical analysis and elemental analysis of substrates and inoculum (Table 1) values have observed higher

Table 1. Physical characteristics and elemental composition of substrate and inoculum.

Constituent/Unit	CM average (n=3)	SD (±)	RP average (n=3)	SD (±)	Inoculum average (n=3)	SD (±)
TS (%)	Original 40.1 adjusted 13.78 g/L	0.55	Original 35.5 adjusted 13.84 g/L	0.03	5.55	0.63
VS (%)	9.1	0.66	9.0	0.01	4.13	0.32
TKN (mg/L)	7400	650	245	6	--	--
TAN (mg/L)	4025	230	200	13	--	--
pH	9.1	0.1	4.6	0.1	7.4	0.2
C %	36.1	2.57	44.9	3.74	40.1	3.43
H %	4.97	0.06	6.5	0.08	6.0	0.75
N %	4.12	0.30	0.7	0.04	1.25	0.12
O %	30.3	2.17	34.20	1.05	35.0	1.17
S %	0.84	0.10	nd	nd	nd	nd

RP, rotten potatoes; CM, chicken manure; TS, total solids; VS, volatile solids; TKN, total Kjeldahl nitrogen; TAN, total ammonium nitrogen; C, carbon; H, hydrogen; N, nitrogen; O, oxygen; S, Sulphur. All these values are average of triplicate experiments result.

These value of nitrogen is not suitable for CM mono-digestion. Experiments designed based on these physical characteristics of substrates. Results of analysis are given in the Table. 2 with the comparison of literature study and theoretical values based on Buswell formula. BMP-_{ThAIC} yield/g VS based on 100% conversion of substrate. BMP-_{ThAIC} also included non-decomposable ingredient of substrate

i.e. lignin etc. Ideally BMP-_{ThAIC} values should be higher than BMP_{exp} but analysis showed higher BMP_{exp} in co-digestion, yet mono-digestion has shown similar expecting observations i.e. lower values of BMP_{exp} than BMP-_{ThAIC}. Overall co-digestion has shown higher CH₄ volume and biogas production volume at any inspection days of digestion as compared to mono-digested experiments.

Table 2. BMP_{exp}, BMP_{ThAIC}, Ratio of CH₄/CO₂, Buswell Chemical formula.

Substrate	BMP _{exp} (ml/gVS) This study	BMP _{exp} (ml/gVS) Lit.Ref [^]	BMP _{ThAIC} (ml/g VS)	Buswell chemical formula	CH ₄ /CO ₂ Lit. Ref [^]	CH ₄ /CO ₂ BG _{exp} (Mean) ^s	CH ₄ /CO ₂ BG _{ThAIC}
RP	291.0	177-313 (Poulsen and Adelard, 2016) 230±5 (Karthikeyan and Visvanathan, 2012)	309.00	C _{74.83} H _{130.0} O _{42.75} N _{1.0}	1.43-1.12 (Poulsen and Adelard, 2016)	60.04/33.99	56.93/43.07
CM	226.06	282.16 (Esposito <i>et al.</i> , 2012b)195 (Abouelenien <i>et al.</i> , 2010)156.9 (Wang <i>et al.</i> , 2012b)	285.58	C _{10.22} H _{16.89} O _{6.44} N _{1.0}	40.08/nd (Wang <i>et al.</i> , 2012b)	55.53/30.05	51.25/48.75
M ₁	304.50	325 (Poulsen and Adelard, 2016)	298.75	C _{19.61} H _{33.32} O _{11.71} N _{1.0}	0.94	65.30/34.88	54.40/45.60
M ₂	341.22	232 (Poulsen and Adelard, 2016)	304.48	C _{32.04} H _{55.08} O _{18.70} N _{1.0}	2.59 (Poulsen and Adelard, 2016)	67.05/33.20	55.73/44.72
Inoculum	----		285.08	C _{37.43} H _{67.20} O _{24.50} N _{1.0}	---	65.22/35.00	55.08/44.92

Exp.* Experimental, Lit. Ref[^] Literature Reference, (Mean)^s has been taken after first seven days of incubation. BMP-_{ThAIC} -Bio-methane potential theoretical, BMP_{exp}.

This might be due to the fact that feasibility of mesophilic co-digestion of RP and CM which resulted into significantly increase in both CH₄ and biogas yield and volumetric. Observed value of co-digestion has shown positive result of higher cumulative CH₄ production as compared to mono-digestion which is in promise with earlier published literature. About 30% of co-digestion result enhancing its impact on methane yields, it depends on properly mixing of optimize composition ratio of substrate. The maximum BMP_{-ThAtC} yield/g VS depends on the type

of organic matter, carbohydrate, proteins, lipids, and VFAs (Angelidaki and Ellegaard, 2003). However, BMP_{-ThAtC} values have been calculated on the basis of elemental composition of substrates.

Cumulative methane

ADs were intended for evaluation the BMP of substrate under particular operational conditions chosen to perform the BMP tests. The cumulative methane production of experiments is shown in (Fig. 1).

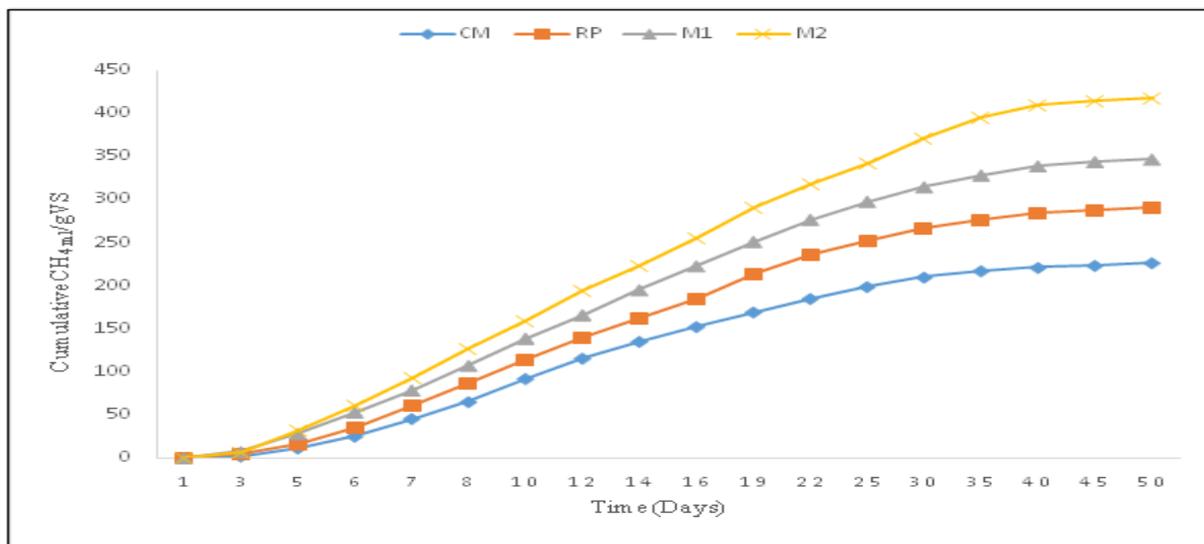


Fig. 1. Cumulative CH₄ production of CM, RP, M₁ and M₂ withincubation time of substrate at 37°C.

The highest CH₄ production was achieved in M₂ mixtures and lowest in CM i.e. 341.22 and 226.06 ml/g VS respectively. Order of methane of substrate from lower to higher is RP, CM, M₁ and M₂. It may be because of high biodegradability of M₂ due to RP in M₂ mixture has high biodegradable material that were not initially digested in stomach and CM mixing balanced the probably adequate proportion of nitrogen; while in M₁ and CM setups have higher nitrogen concentration that hinders the AD due to formation of toxicity like NH₃ (Callaghan *et al.*, 2002). RP produced methane very close to M₁ and lower than M₂ probably due to highly digestible materials in RP which resulted in accumulation of VFAs, high C/N ratio which is not favorable for AD and extent of biodegradability which did not include non-digestible material. BDele% calculated (using eq.3) 94.17, 79.15, 101.92 and 112.24 of RP, CM, M₁ and M₂ respectively.

BDele% of co-digestion was higher than total percentage, obviously showed increase in biodegradability of substrate in co-digestion. BDele% depends on BMP_{exp} and BMP_{-ThAtC}. Similarly, biogas production also showed similar pattern of production (Fig in supplementary material). These observed values did not show any comparison with previous data and also with theoretical values of substrates but showed similar behavior of production rate as presented in (Fig. 1). Results of co-substrate demonstrated the higher BMP_{exp} value than BMP_{-ThAtC}. This study enhanced the concept of CM using as favorite co-substrate due to its buffering capacity so it helps in maintaining the pH which are decreased due to temporary VFAs accumulation (Abouelenien *et al.*, 2016). Each BMP of substrates has compared with BMP values (Table 2) reported in literature and has observed variation pattern of BMP_{exp} data with literature data.

It is nearly impossible or morally inaccurate to directly compare with the literature data due to insufficient reported data of RP and CM co-digestion, and with other data studied under different experimental conditions. Nevertheless, results may differ because of different conditions and composition of RP and CM

relative soil condition, diet supplement, varying inoculum properties, operating temperature (Gou *et al.*, 2014), headspace volume of flasks (Zhang Cunsheng *et al.*, 2014) and finally S/I ratio (Abouelenien *et al.*, 2016).

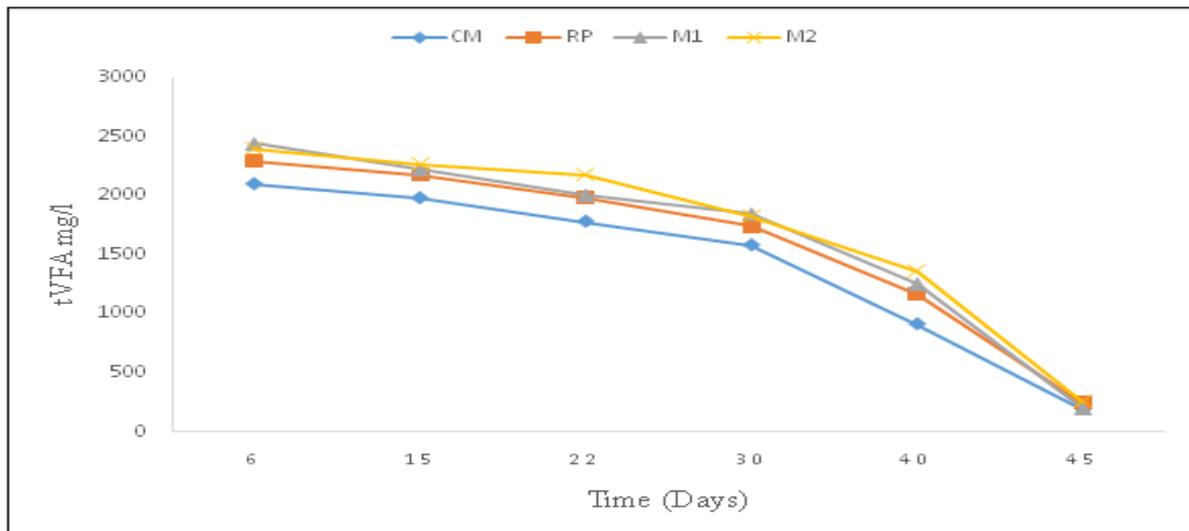


Fig. 2. tVFAs analysis of all VFAs production and consumption of CM, RP, M₁ and M₂ with incubation time of substrate at 37°C.

Biogas composition

Biogas composition of experimental literature and expected theoretical values are also enlisted in Table 2. As lot of prominent variation in composition of produced biogas (ratio of CH₄/CO₂) as reported in CH₄ potential for substrate. Biogas composition has higher CH₄ in co-digestion as well as mono-digestion as compared to literature data. Furthermore, CH₄ percentage increased in co-digestion than mono-digestion. Normally, biogas composition can be explained on the basis of degradability of substrates and production of high VFAs that fall the pH of medium. Thus the growth of methanogens was selectively reduced in response to acidic condition and methane reduced in the biogas composition. Conversely, addition of NaHCO₃ buffered acidity and maintained the methanogens growth and increase the capability of higher CH₄ production in both co-digestions than mono-digestions. Likewise, CM has nitrogen concentration in its composition, that produce NH₃ intermediate which may be neutralize

acidic pH, oppositely NH₃ cause inhibition in the range of 1500-3000 mg/L TAN, above pH 7.4 while concentrations above 3000 mg/L is toxic regardless of pH (Calli *et al.*, 2005). Moreover, mono-digestions has lower CH₄ than co-digestions; it may be due to NH₃ sensitivity in CM and acidic sensitivity in RP, co-digestion dilute the toxicity of ammonium which make less chance of any possible hindrance to methanation (Wang *et al.*, 2012b). However, RP biogas composition was very close to co-digestions due to buffer capacity. Here is difficult to find clear relationship between experimental and literature biogas composition but found nearly similar trend with theoretical composition.

Volatile fatty acids analysis

VFAs production from the digestion showed the accurate operation of digestion and stability needs appropriate transformation into end product i.e. CH₄ and CO₂. Analysis of VFAs has shown production of short acids namely acetic acid, propionic acid, butyric acid, and valeric acid.

These are the common intermediate products produced during digestion as reported (Buyukkamaci and Filibeli, 2004) however, Fig. 2 sets out the total of all VFAs (tVFAs) at different times of digestion and variation during digestion. Generally, initial concentration of tVFAs was higher and declined with incubation times in flasks as Fig. 2. These results also supported fluctuate behavior of VFAs by literature as initial concentration of VFAs are higher because it is produced in first three stages of AD i.e. hydrolysis, acidogenesis and acetogenesis, (De La Rubia *et al.*, 2009) and progressively decreased during methanogenesis i.e. VFAs consumption is started

(Buyukkamaci and Filibeli, 2004; Cysneiros *et al.*, 2012). tVFAs peak in Fig. 2 reached maximum for M₁, and M₂ is very close to tVFAs peak of M₁ however RP showed little bit low tVFAs peak and it can be explained by less digestibility and volume of biogas. CM has the lowest peak of tVFAs, therefore a low value of tVFAs that designates low digestibility, BMP and vice versa for other substrates. Commonly the substrate, which is difficult to digestible, has lower VFAs production. Among all acids, acetic acids play an ascendant role in biogas production (Zhang Ruihong *et al.*, 2007) and two times more effective than propionate.

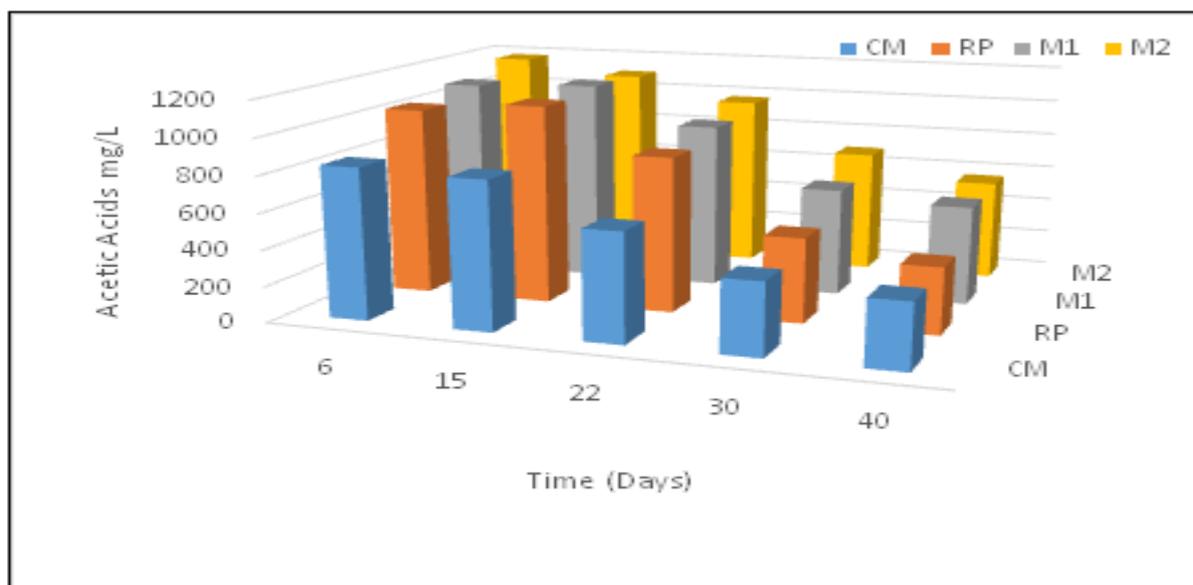


Fig. 3. Acetic acids production and consumption of CM, RP, M₁ and M₂ with incubation time of substrate at 37°C.

The most effective order is acetate > butyrate > valerate > propionate (Elefsiniotis and Wareham, 2007). Acetate production (39-44%) relative to other VFAs components was maximum in all inspected days. Acetate range varied from 1044.1, 840, 1098.45, & 1178.9 mg/l for RP, CM, M₁ and M₂ respectively after six days of incubation and was also similar in other inspections as showed in Fig 3. Acetic acids concentration was much lower to average reported value (5,431 mg/L) of acetic acids (Lee *et al.*, 2015). Similarly, its critical concentration is 0.8 g/L, above this concentration causes inhibition of AD (Buyukkamaci and Filibeli, 2004). It may be because of using active and adaptive inoculum, which has probably short lag periods for methanogenesis stage.

Besides, active inoculum used up VFAs regularly and risk of AD inhibition reduced due to accumulation of VFAs. This is also not possible to compare data with literature data due to variation in operational parameters of AD. The results of VFAs and biogas analysis of co-digestion are encouraging for not only improving biogas production but also stabilizing the digestion system. VFAs analysis showed acetate accumulation as a major component of the media with other components it consumed (Acetate 100% degradation) as cited in other finding (Abouelenien *et al.*, 2016). It creates the impression that the digestibility of substrate, stopped and consuming of VFAs into biogas and CH₄. CM digestion reinforced the above discussion that it has low values of VFAs

and relatively low acetate concentration and therefore less digestibility of CM as compared to other observed values. Therefore, values of VFAs production are showing a direct relationship with digestibility as well as biogas and CH₄ production.

Conclusion

It can be concluded that feasibility of mesophilic co-digestion of RP and CM significantly increased both CH₄ and biogas yield and percentage of CH₄ in biogas composition compared to the mono-digestion of RP and CM. The highest BMP_{exp} of 341.22 mL/g VS was acquired at RP:CM ratio of 75:25, and 226.06 mL/g VS was attained in CM digestion and 65.30% and 55.53% CH₄ of biogas composition was obtained respectively. Acetate was maximum in all digestion analysis and in all regular inspection. It ranges varies from 1044.1, 840, 1098.45, & 1178.9 mg/l in RP, CM, M₁ and M₂, respectively and shows relation with digestibility. CM has less digestibility so low Value of VFAs production. Digestibility of substrates show direct relationship with CH₄ production, its percentage and VFAs production. As VFAs production are also respectable gauges for accurate functioning of anaerobic digestion.

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