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Biochemical methane potential of *Tagetes* Flowers

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ABSTRACT

Marigold (*Tagetes*) is an annual flower and bloom over the long season. Mostly, in India, it is used for worship of god in temple and at home. After that it is thrown outside on road or in rivers. Present study is an attempt to find out the Biochemical Methane Potential (BMP) of *Tagetes* flower to produce biogas and digested slurry. The experiment was carried out in 20 l capacity anaerobic digesters. The flower waste was blended with cattle dung in three different ratios of 25%, 50% and 75% and control set with flower (100% flower) and cattle dung (100% cattle dung) were put without blending. Care was taken to keep the total solids concentration in each digester at 8%. Biogas production was measured along with environmental temperature for 137 days by water displacement method and the proportion of methane in biogas was measured by Gas chromatography. The pH, organic carbon, nitrogen, phosphorus and potassium contents of digestion mixtures before and after anaerobic digestion were measured following standard methods. To understand the microbial activities total viable count in digested slurry has also been measured at 0 and 137 days. Since Biochemical methane potential has direct relationship with total volatile solids content so it was also measured at 0 and 137 days of digestion. Data were analysed statistically. Results show that among blending treatments highest Biochemical Methane Potential (317.27 ml CH4/g TVS used) was recorded in treatment receiving 25% flower waste blend with 75% cattle dung. This treatment also shows highest reduction in total volatile solids (51.91 g% at 137 day over 0 day) content. With progress in time the digestion materials become neutral to alkaline in pH in all the treatments except *Tagetes* flowers control. Content of total organic carbon decreased significantly with time whereas available nitrogen, available phosphorus and available potassium content increased significantly in all the treatments at 1% level of significance. Total viable counts were also increased significantly with time in all the treatments except *Tagetes* flowers control. It can be concluded that *Tagetes* flowers has good biochemical methane potential and can be used successfully to produce methane and nutrient rich digested slurry. This study will certainly achieve a significantly high economic value in big temples having bulk volume of *Tagetes* flower collection.

Key words: *Anaerobic digestion, Biogas, Cattle dung, Tagetes*

Introduction

Biogas is the renewable energy source that can be generated by anaerobic digestion of biodegradable waste. In India, the total biogas production was 2.07 billion m^3 (2014-15), which is very less compared to its estimated potential of 29-48 billion m^3 on that year (Mittal *et al.*, 2018).

Ample literature is available regarding the anaerobic digestion of different waste materials alone or their co-digestion (Deepanraj *et al.*, 2015; Monteiro *et al.*, 2011; El-Mashad and Zhang, 2010). Research studies mention the effectiveness of anaerobic digestion as alternative and efficient technology, which combines bio-fuel production and sustainable waste management (Bharathiraja *et al.*, 2018). Utilization of methane serves the two-fold purpose of fulfillment of energy needs and also a reduction in the methane emission into the atmosphere (Al-Mamun and Shuichi, 2015).

Biochemical Methane Potential (BMP) assay has widely accepted standard protocol to measure the biodegradability and methane potential of different feedstock in anaerobic digestion (Labatut *et al.*, 2011; Raposo *et al.*, 2011; Feng *et al.*, 2013).

Marigold flower (*Tagetes erecta* L.) grown for business purposes in Mexico, Peru, Ecuador, Spain, India and China (Sivel *et al.*, 2014). In the Chennai city, Southern part of India, around 1.925 ton per day flower waste has been generated from five temples, dumping of such offering in plastic bags in nearby river create detrimental effect on life of water body (Waghmode *et al.*, 2018). Temple waste flowers are type of biomass materials and easily available locally. Large amounts of flowers are used for various cultural ceremonies, which are considers as waste after use and discarded into wasteland because there is no appropriate method of disposal. Some of the disposal practices pollutes the environment severely and making highly unhygienic (Elango *et al.*, 2018). Although the flower waste degradation through anaerobic digestion has been studied by various researcher for biogas production (Deepanraj *et al.*, 2015; Sing and Bajpai, 2014; Kumar and Swapnavahini, 2012) but present study is focused to find out the Biochemical Methane Potential (BMP) value of flower waste. The broad objective of this study was to find out the best ratio of cattle dung and *Tagetes* flowers to get high BMP. In addition, the change in nutrients contents and microbial count during digestion period was also investigated.

Materials and Methods

Materials

Flower wastes were collected from the Nana Ambaji, Giyod and Jaxani Mandir, Sadra temples in Gandhinagar district of Gujarat. The cattle dung for the study has been collected from the animal shed of Gujarat Vidyapith, Sadara, District-Gandhinagar, Gujarat.

Experimental setup

To find out the BMP of *Tagetes* flowers alone and in co-digestion with cattle dung following five treatment combinations were formulated- T1 = 100 % Cattle dung, T2 = 100% *Tagetes* Flower, T3 = 25% *Tagetes* Flower + 75% Cattle dung, T4 = 50% *Tagetes* Flower + 50% Cattle dung and T5 = 75% *Tagetes* Flower + 25% Cattle Dung. Each treatment was replicated twice. Biomethanation was carried out in a 20L capacity plastic bottle, mostly used for mineral water supply to home, remained connected with a glass bottle of 2L capacity to work as gas holder. Gas holder further remains connected with a 2L capacity glass bottle for water displacement.

Experimental sets were arranged in laboratory following Random Number Table (Gomez and Gomez, 1984). In each set *Tagetes* flower waste and cattle dung were added as per Table 1 to achieve the targeted treatment combinations. The experiment was run at 8% (w/v) Total solids (TS) concentration in digester. To achieve it the TS of cattle dung and *Tagetes* flower were find out as 20% and 22% respectively. The working volume of digester was also kept constant for all the treatments as 15L and remaining space was left for accumulation of biogas. Now to fill the digesters till 15L capacity calculated amount of distilled water (Table 1) was added. As an inoculum digested slurry of running biogas plant of our institute was also added @10% v/v in all the digesters (Table 1). Materials were mixed well. In gas holder and water displacement bottle 2L colored and acidified (pH-2) distilled water was added to measure the level of water sharply. A 15 cm rule was also pasted on outer surface of gas holder bottle to measure the amount of gas produced accurately. All the joints were made anaerobic by vacuum grease. All the experimental sets were kept at room temperature for a total of 137 days.

Analytical Methods

Biochemical Methane Potential

The amount of biogas produced in digesters was measured daily at 9:00AM by water displacement method. Level of colored water in gas holder bottle was set at zero. Now as the biogas produced in digester enters into gas holder bottle its water level goes down due to the pressure of biogas and simultaneously a similar amount of water rises in water displacement bottle. At 9:00AM of next day the water level in gas holder bottle is noted down at the rule pasted on it and converted in milliliter gas produced per day, followed by reset the water level in gas holder at zero for next day reading. The value of

Table 1. Experimental material used in each treatment

Treatments	Cattle dung (Kg)	Flower (Kg)	Inoculum (L)	Volume make up (L)
T1	6.0	0	1.5	15
T2	0	6.0	1.5	15
T3	4.5	1.5	1.5	15
T4	3.0	3.0	1.5	15
T5	1.5	4.5	1.5	15

Note: T1=100 % Cattle dung, T2=100% Flower, T3=25% Flower + 75% Cattle dung, T4= 50% Flower + 50% Cattle dung T5=75% Flower + 25% Cattle Dung

average daily biogas production was obtained by dividing the total biogas production during 137 days by 137.

Methane content in Biogas

The Methane content of produced biogas was measure at different interval with Gas chromatography method. The methane content of the biogas was determined using made of Perkin Elmer, model Auto System XL gas chromatographs with flame ionization detector. The capillary column was packed with BP-5. Injector, oven and detector temperatures were 100 °C, 45°C and 150 °C respectively. The pure methane (99.5%; 30kg/cm^2) for standard run was procured from Vadilal Gases Limited, Baroda. The nitrogen carrier gas flow was 30 ml/min. Methane yield were calculated by dividing the corrected methane volume by the weight of used volatile solids to each bottle (Dahunsi *et al.,* 2016; Edward *et al.,* 2015).

Physico-chemical and microbial analysis of Digested slurry

The physico-chemical parameters of cattle dung and Flower waste like pH (Richards *et al.,* 1954), Temperature (Celsius thermometer), Total Solids and Total Volatile Solids (Greenberg *et al.,* 1992), Total organic carbon (Walkley and Black, 1934), Available Nitrogen (Subbiah and Asija, 1956), C/N ratio, available phosphorus (Olsen *et al.,* 1954) and available potassium (Jackson, 1967) were measured as per the standard protocols. For the enumeration of total viable microorganism, the Nutrient agar media (Himedia) was used and spread plate technique was perform by serial dilution. For microbial analysis, 1 gm of the sample was homogenized with 9 mL of sterile distilled water. After mixing, serial dilution was made from 10^{-1} to 10^{-9} for culturing in nutrient agar media (Islam *et al.,* 2019).

Statistical Analysis

Data were subjected to One-Way Analysis of Variance (One-Way ANOVA) to determine whether there was a significance difference in treatments or not using MS Excel (2010) computer software program of Microsoft Inc.

Results and Discussion

Physiochemical characterization of digesting materials

Table 2 shows the data regarding physico-chemical properties of cattle dung and flowers. Flowers contain higher total solids, and hence higher total volatile solids than cattle dung. Total solids and total volatile solids are important parameters to decide the organic loading rate (Krido *et al.,* 2009). Flowers are weakly acidic due to presence of higher number of aromatic compounds in its structure that impart acidic pH while dissolving in water (Rios *et al.,* 2008).

Flowers are also richer in nutrients like total and available nitrogen and phosphorus whereas cattle

Table 2. Physiochemical characterization of Cattle dung and Tagetes Flower waste

Parameters	Cattle dung	*Tagetes* Flower
Total Solids (%)	20.00	22.00
Total Volatile solids (%)	13.94	16.00
pH	7.20	6.51
Total Organic Carbon (%)	34.18	32.97
Total Nitrogen (%)	1.12	1.82
Available Nitrogen (%)	0.14	0.25
C/N Ratio	30.52	18.12
Phosphorous (%)	0.24	0.36
Potassium (%)	1.35	1.14

dung contains higher organic carbon and potassium. Physio-chemical characterization of organic material before the anaerobic digestion gives better idea to select the parameter during digestion process (Abdelgadir *et al.*, 2014).

Biogas production

Daily biogas production along with corresponding environmental temperature is presented in Figure 2 to 6. In the treatment T1, where the cattle dung alone was used as control substrate, daily biogas production was ranged between 300 and 1000 ml/day till 35th day of experimentation afterwards it shows a sharp increase and reaches maximum (4218.5 ml/day) on 92nd day of experimentation after which ups and downs were observed in it but it remains higher than 2000 ml/day (Figure 1). It is clear from the data that biogas production is in line with environment temperature. Among all the variables, temperature is the most significant variable to regulate the microbial metabolism rate in anaerobic condition (Tian *et al.*, 2018). The Experiment was conducted at ambient temperature and during the whole study range of temperature was between minimum 16.55 °C to maximum 38.90 °C. Inhibition of methane production in psychrophilic condition was due to a limitation of the activity of acetoclastic bacteria (Bouallagui *et al.*, 2004). Here the average biogas production was 2126.40 ml/day for 137 days age of digester.

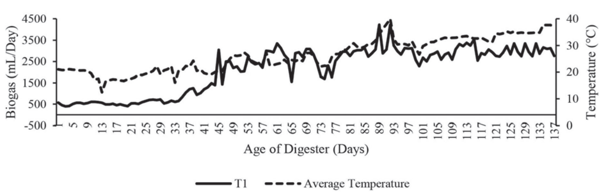

Fig. 1. Biogas production from cattle dung alone (Treatment T1)

In contrast to cattle dung control (T1), flower waste control (T2) shows a great fluctuation in daily biogas production with a maximum value of 1300.0 ml/day on 26th day age of digester and average of 483.75 ml biogas/day (Figure 2).

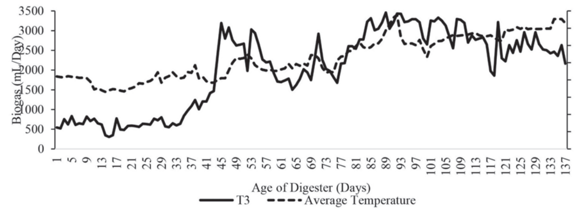

Fig. 3. Biogas production from 25% Flower waste Marigold and 75% Cattle dung (Treatment T3)

As the 25% cattle dung is being substituted with *Tagetes* flowers (T3) the amount of biogas production again rise and reaches a maximum value of 3458 mL/day on 89th day age of digester, with an average of 2027.05 mL biogas/day (Figure 3).

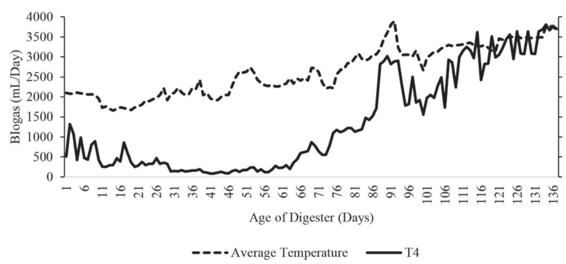

Fig. 4. Biogas productions from the mixture of 50% Flower waste and 50% cattle dung (Treatment T4)

At equal proportion of *Tagetes* flowers and cattle dung (T4) maximum biogas production was recorded as 3802.50 ml/day on 134th with average value of 1329.94 mL biogas/day (Figure 4).

When the proportion of flowers is further increased to 75% (T5) the value of maximum biogas production slightly decreased to the level of 3640

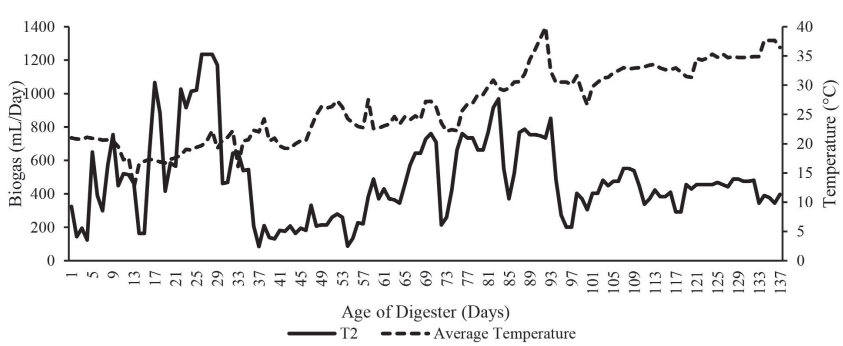

Fig. 2. Biogas productions from Flower waste Marigold alone (Treatment T2)

ml/day on 136th day age of digester with an average of 1258.38 mL biogas/day (Figure 5).

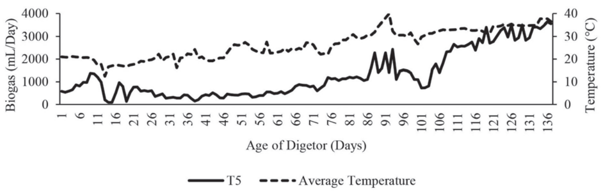

Fig. 5. Biogas production from 75% Flower waste Marigold and 25% Cattle dung (Treatment T5)

Data clearly reflects that daily biogas production varies in all the treatments. Biogas production depends on several factors like pH, TS, TVS (Dobre *et al.*, 2014). Data further reveals that average daily biogas production per day follows the trend of T1>T3>T4>T5>T2 which concludes an inverse relationship between proportion of flower waste and biogas production. As the proportion of flower wastes is increased, amount of biogas produced decreased this may be due to higher organic carbon and slow nutrient release characteristic of cattle dung. In the earlier study it has been found when flower waste was mixed with food waste for biogas production, the cumulative biogas production was decrease from 12.27L to 9.25L when the flower waste increase from 70% to 100% (Kulkarni and Ghanegaonkar, 2019a).

Methane content in biogas varied between 59.38 (T2) and 66.13 (T5) which is in line with the observations recorded previously (Kulkarni and Ghanegaonkar, 2019b).

When biogas production and its methane content is calculated based on total volatile solids consumption than they follow the same trend of T1>T3>T5>T4>T2 which is slightly different from that of average daily biogas production. Biochemical Methane Potential and methane yield were higher in T5 than T4 whereas average per day biogas production was higher in T4 than T5.

Biochemical methane potential of solid organic wastes ranged between 200-500 mLCH4 g-1 TVS, due to the use of non-standardize inoculums (Hansen *et al.*, 2004).

Physio-chemical and microbial properties of Digested slurry

pH

During the biodegradation study of Marigold flower waste, a significant variation in pH of anaerobic digested material was observed from 0 to 137 days. At 0th day, except treatment T1 all the treatments had slightly acidic pH ranged between 5.75 (T5) and 6.60 (T3) (Table 4). At 137th day age of digester, except treatment T2 with pH 5.06, all the treatments have neutral or alkaline pH. Acidic pH during anaerobic digestion of Rose flower residues was also reported earlier (Kumar and Swapnavahini, 2012).

Totals Volatile Solids

During biomethanation process, the total volatile solids decreased in all the treatments and ranged

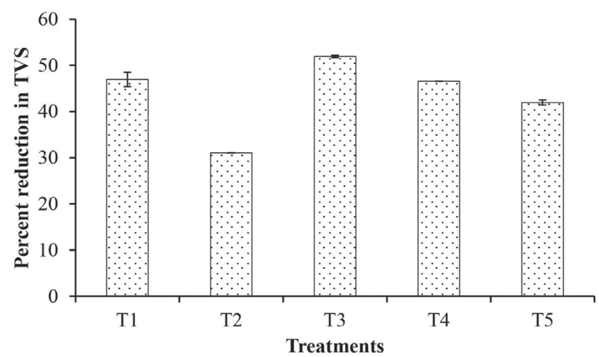

Fig. 6. Percentage reduction in TVS content of treatments 137 day over 0 day age of digester.

Table 3. Biochemical Methane Potential of *Tagetes* Flower waste

Treatments	Biochemical Methane Potential (mL g^{-1} TVS used)	Average Methane (%)	Methane Yield (mL CH4 g^{-1} TVS used)
T1	563.65	64.13	361.44
T2	191.66	59.38	113.80
T3	495.73	64.00	317.27
T4	341.80	65.88	225.16
T5	353.08	66.13	233.47

Note: T1=100 % Cattle dung, T2=100% Flower, T3=25% Flower + 75% Cattle dung, T4= 50% Flower + 50% Cattle dung T5=75% Flower + 25% Cattle Dung between 31.04 (T2) and 51.91% (T3) (Figure 7). The Total volatile solids are one of the important components of digesting material utilized by microorganisms and get converted in methane (Morosini *et al.*, 2016).

Table 4. Variation in pH of digesting material at different age of digester

Treatments	Age of digester (Days)	
	0	137
T1	7.25	7.67b
T2	5.95ab	5.06
T3	6.60c	7.72b
T4	6.30bc	7.02a
T5	5.75a	7.18a
SEM (±)	0.14	0.14
CD (5%)	0.34	0.34
CD (1%)	0.51	0.52

Note: Same letters in a column indicate the treatments are non-significantly different between themselves at p<0.01 *(T1=100 % Cattle dung, T2=100% Flower, T3=25% Flower + 75% Cattle dung,* T_4*= 50% Flower + 50% Cattle dung T5=75%* Flower + 25% Cattle Dung), SEM= Standard Error of Means; CD= Critical difference

Total Organic Carbon, Available Nitrogen, Available Phosphorous and Available Potassium

Data presented in Table 5 reveals that during biomethanation a significant change is observed in contents of total organic carbon and plant available nutrients. On one hand the contents of total organic carbon decreased whereas on the other hand plant nutrients content increased significantly in all the treatments after biomethanation. Nutrients of digesting materials are utilized by microorganisms for their growth and metabolism and hence their content is changed (Ofoefule *et al.*, 2010). Although all the treatments differ among themselves for nutrients contents but maximum content of total organic carbon and available potassium was found in T2 whereas maximum available nitrogen and phosphorus was recorded in T3.

Total Viable Count (TVC)

The total viable count (TVC) of facultative anaerobic microorganism also varies with time (Table 6). The source of these microorganisms in digesting material is cattle dung and inoculum that's why a minuscule number was found in T2 comprises only flower

Table 6. Total Viable Count (TVC) (CFU/g) during anaerobic digestion of cattle dung and flower waste with different treatments (x 106)

Treatments	Age of digester (Days)	
	0	137
T1	139.00a	748.00
T2	0.0290a	0.0053
T3	369.00	800.50
T4	19.00a	43.65a
T5	11.10a	76.05a
SEM (±)	40.97	11.27
CD (5%)	100.25	27.58
CD (1%)	151.87	41.79

Note: Same letters in a column indicate the treatments are non-significantly different between themselves at p<0.01 (T1=100 % Cattle dung, T2=100% Flower, T3=25% Flower + 75% Cattle dung, T4= 50% Flower + 50% Cattle dung T5=75% Flower + 25% Cattle Dung), SEM= Standard Error of Means; CD= Critical difference

Table 5. Variation in Total Organic Carbon, Available Nitrogen, Available Phosphorous and Available Potassium (%)

Treatments	Total Organic Carbon		Available Nitrogen		Available Phosphorous		Available Potassium	
	0	137	0	137	0	137	0	137
T1	2.66a	1.83a	0.030a	0.199b	0.057ab	0.074a	0.185b	0.225a
T2	5.56c	3.61d	0.072c	0.091a	0.073c	0.086ab	0.225c	0.250a
T3	3.50ab	2.22ab	0.040ab	0.615	0.068c	0.090b	0.146ab	0.176
T4	4.27b	2.66bc	0.049b	0.130ab	0.045a	0.053	0.093a	0.115
T5	4.69bc	3.02cd	0.072c	0.097a	0.066bc	0.076a	0.200bc	0.250a
SEM (±)	0.33	0.20	0.005	0.022	0.004	0.004	0.018	0.011
CD (5%)	0.82	0.49	0.012	0.053	0.009	0.009	0.044	0.026
CD (1%)	1.24	0.74	0.018	0.080	0.014	0.013	0.066	0.039

Note: Same letters in a column indicate the treatments are non-significantly different between themselves at p<0.01 (T_1=100 % Cattle dung, T_2= 100% Flower, T_3=25% Flower + 75% Cattle dung, T_4= 50% Flower + 50% Cattle dung T_5=75% Flower + 25% Cattle Dung), SEM= Standard Error of Means; CD= Critical difference waste and inoculum. A close view of data reveals that at 0 day of experiment although the TVC count differs with treatments but statistically all the treatments were at par among themselves except T4. After biomethanation their count increased in all the treatments compared to that of 0 day, except T2 and here the count was significant among treatments except T3 and T5 which were found at par among themselves. A change in microbial load is due to nutrients availability and favorable conditions of biomethanation as reported earlier (Ofoefule *et al.*, 2010).

Conclusion

It can be concluded from the data that *Tagetes* flowers has Biochemical Methane Potential and they alone can be converted successfully into biogas but if they are mixed with cattle dung in 1:3 ratio (25% *Tagetes* flowers and 75% cattle dung) than their Biochemical Methane Potential and biogas yield increased. Methane content in produced biogas was also remains higher than 60%.

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