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Anaerobic Digestion of Pineapple Waste for Biogas Production and Application of Slurry as Liquid Fertilizer Carrier for Phosphate Solubilizers

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ABSTRACT

Background: Pineapple peel wastes was seasonal which comprised of peels and rags. Their disposal posed a serious environmental pollution. Since pineapple peel was rich in cellulose, hemicellulose and other carbohydrates it was found to be a potential substrate for methane generation by anaerobic digestion.

Methods: Here pineapple peel and pulp wastes were collected. The hydraulic retention time of biogas (HRT) was monitored regularly for nine days and at three days intervals in gas collection bladders (Hans Seamless latex valve bladders). The slurry collected was periodically treated with phosphate solubilizers- *Providencia rettgeri,* a bacterial solubilizer and *Meyerozyma gullerimondi,* an yeast solubilizer. The biometric parameters of *Ananas comosus* was tested after slurry application. The germination per cent of *Passiflora edulis* were also calculated.

Result: The best combination of biogas slurry with maximum manorial content for phosphate solubilizers were treatment with cow dung and fruit waste in the ratio 1:2 with high amount of magnesium: 0.0037%, followed by 0.075 N and 0.00054% P which was selected for biometric observations for plants. Pineapple waste were good source for making biogas and slurry obtained could be utilized as carriers for phosphate solubilising liquid fertilizers.

Key words: Biogas, Liquid fertilizer, Phosphate solubilizer, Pineapple waste.

INTRODUCTION

Waste management in fruits and vegetable refining factories was one among the challengeable jobs around the world. Scientists had centred their attention on the utilization of pineapple waste preferably for biogas and fibre production (Upadhyay *et al.,* 2013). Some of these wastes had been used in industrial applications like gas generations (Mbuligwe and Kassenga, 2004). Bio-methanation of fruit wastes was a simple waste treatment as it both adds energy in the form of methane and also results in a highly stabilized effluent fertilizer with almost neutral pH and odourless property (Bardiya *et al*., 1996). Rani and Nand (2004) reported that different treatments of pineapple peels gave biogas yields ranging from 0.41-0.67 mg/kg volatile solids with methane content of 41-65%. This proved pineapple pulp could be used successfully for biogas production. The use of pulp and peel together gave higher biogas production (Pimjai *et al.,* 2012). The performance of biogas production depends on biomass chemical composition as well as standardized conditions of feed concentration, hydraulic retention time, pH and temperature (Boe, 2006; Turovskiy and Mathai, 2006; Rehm *et al*., 2000).

Bio-methanation of fruit wastes was the best suited waste treatment as it both adds energy in the form of methane and also results in a highly stabilized effluent with almost neutral pH and odorless property (Bardiya *et al*., 1996). They utilized pineapple waste for the production of methane using semi-continuous anaerobic digestion which could produce up to 1682 ml/day of biogas with methane content of 51% in maximum.

The lowest possible HRT for banana peel was 25 days, resulting in 36% substrate utilization and with maximum gas production of 0.76 vol/day, while pineapple processing digestors could be operated at 10 days HRT, with 58% substrate utilization and maximum gas production rate of 0.93 vol/day (Bardiya *et al.,* 1996). Gas production over 3 days incubation period was found to be the highest with cow manure followed by orange rind, with the lowest value for papaya peel and banana skins (Inthapanya *et al.,* 2013). In this experiment, we had screened the efficient treatment with cow dung and biogas slurry for maximum methane gas production. The slurry was developed as liquid fertilizer

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carrier for phosphate solubilizer- *Providencia rettgeri,* a bacterial solubilizer and *Meyerozyma gullerimondi,* a yeast solubilizer.

MATERIALS AND METHODS

Anaerobic digestor

The biogas plant was installed at the household premises at Mannuthy, Thrissur. The work was carried out in Pineapple Research station Vazhakulam, Ernakulam and Nehru Arts and Science College, Coimbatore in year 2019 to 2020. The floating drum biogas plant of 0.5 m^3 (Plate 1) capacity was used for anaerobic digestion of pineapple substrate. The substrates were added through the inlet pipe. The gas produced inside the digestor was collected in gas holder and the bottom of the gas holder was dipped into the substrates to create an anaerobic condition. The gas collected in the gas holder was used daily through gas outlet. When substrates got completely digested, slurry flowed through slurry outlet. Regular feeding had done with the slurry at the rate of 1 litre per day to the biogas plant.

Inoculation of phosphate solubilizers

The phosphate solubilizers selected were *Providencia rettgeri,* a bacterial solubilizer and *Meyerozyma gullerimondi,* a yeast solubilizer isolated from rhizosphere soil of pineapple plants from Vazhakulam, Ernakulam district, Kerala. The ratio of 6×10^4 CFU/ml of slurry was the inoculum used for both organisms. The fermentation was carried out for overnight.

Regular monitoring of biogas plant

During the anaerobic digestion period, hydraulic retention time (HRT) and daily temperature inside the digester, volume of gas produced and quantity of slurry generated were determined regularly. Hydraulic Retention Time (HRT) was defined as the maximum time taken by the substrates for maximum gas production. The daily temperature of the biogas unit was noted by using digital thermometer for the entire period of study.

The gas volume was recorded every day. The gas produced was measured and used for burning the stove. The increase in height of the gas holder was recorded daily and volume of gas was calculated using the formula,

$$V= \pi r^2h$$

Where

V denotes volume, r denotes radius of gas holder and h denotes height increased after gas production. The slurry output from the digester was also measured daily for all the treatments using measuring cylinder (Enaboifo and Adadu, 2020).

Analysis of biogas and biogas slurry

The gas produced during the first three days was discarded for a stabilized biogas production. Biogas samples were collected after nine days at three days intervals in gas collection bladders (Hans Seamless latex valve bladders) and analysed. The Biogas composition in laboratory test (CH_4, CO_2, H_2, H_2S and O_2) was measured using an automated gas analyser according to Brettschneider *et al.* (2004). The slurry generated in each treatment were tested for profiling the quantity of macronutrient and micronutrients present in it using FESEM-EDX and Elemental mapping.

Biometric observations of seedlings

Biometric observations were recorded biweekly for three months. Plant height, number of leaves per plant, plant girth was recorded. Vigour index was also calculated from the biometric observations (Kaur and Phutela, 2014).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) (Panse and Sukhatme, 1985) using statistical package 'MSTAT-C' package (Freed, 2006). Wherever the F test was significant (at 5% level) multiple comparison among the treatments were done with Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Composition of biogas

The optimum combination of cow dung and pineapple waste for maximum gas production was standardized using completely randomized design with five treatments and three replications for a period of 18 months. All the six treatments were done separately on all five weeks of 6 months from October 2018 to June 2020 at Mannuthy, Thrissur, Kerala. The readings were taken every week.

The highest methane content of 60.49% was recorded in T_4 which was on par with T_5 (63.81%) and T_3 (60.29%) and was significantly higher than T_1 (50.00) and T_2 (46.65). From the results it is clear that co-digestion of cow dung with fruit waste increased the methane content in 1:1.5 ratio. With the increase of pineapple fruit waste proportion with cow dung as 1:2 methane generations decreased to 50.86% (Fig 1a). It was evident that CO_2 concentration varied significantly between the treatments. It was found to be highest in T_2 (50.86%) and the lowest in T_4 (32.00%). The recorded CO_2 concentration in T_6, T_1, T_3, T_5 were 45.37, 37.48, 37.31 and 34.47 respectively (Fig 1a).The major nutrients and heavy metal composition of biogas slurry and substrate was shown in Table 1.

Hydraulic retention time (HRT) and volume of gas generated

The hydraulic retention time (HRT) was minimum (15 days) in the treatment T_3 (cow dung + fruit waste, 1:0.5) followed by T_4 (cow dung + fruit waste, 1:1) with 17 days. The highest HRT of 28 days was observed in treatment T_2 (fruit waste alone) whereas the treatments T_1 (cow dung alone) and T_5 (cow dung + fruit waste, 1:1.5) was recorded 23 days and 19 days. The HRT of T_6 was 25 days (Fig 1b). The volume of gas was maximum (0.43 m^3/day) in the treatment T_4 (cow dung + fruit waste, 1:1) which was followed by T_3 (cow dung + fruit waste, 1:0.5) with 0.41 m^3/day. The lowest volume of 0.29 m^3/day was observed in T_2 (fruit waste alone) whereas the treatments T_1 (cow dung alone) and T_5 (cow dung + fruit waste, 1:1.5) was recording 0.35 m³/day and 0.39 respectively and the total volume in the treatment T_6 was 0.36 m³/day (Fig 1 b).

Total quantity of slurry

The quantity of biogas slurry generated in each treatment was analysed. The T_6 treatment recorded the highest quantity of slurry (96 L) followed by T_5 (78 L), T_4 (62 L), T_3 (57 L) and the treatments T_1 and T_2 were on par recording 40 L (Fig 1c). The nutrients present in the slurry was recorded using FESEM-EDX. The FESEM analysis and EDAX for elemental analysis of the crude extracts and its biofertilizers *i.e.*, biogas slurry and biogas substrate were shown in Fig 2 and Fig 3.

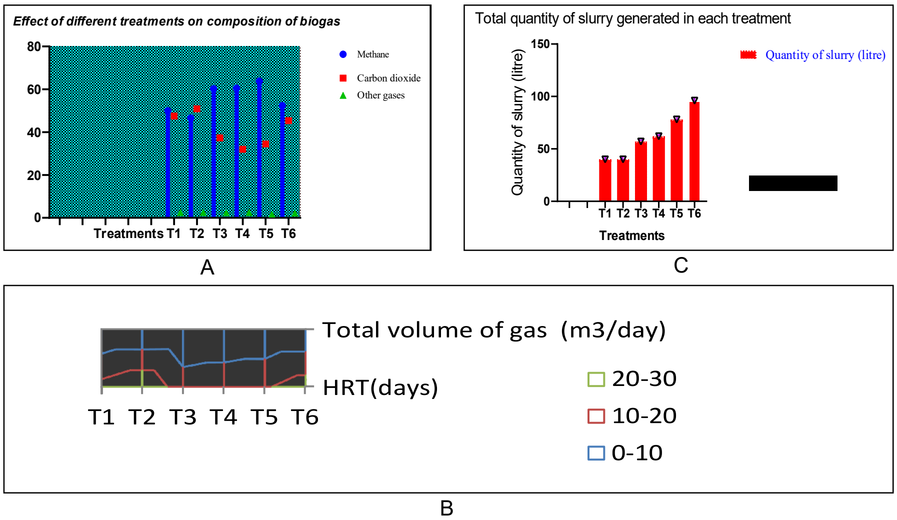

Fig 1: A. Graph showing the different treatment on composition of biogas; B. Graph showing hydraulic retention time and volume of gas as influenced by diffe rent treatments; C Graph showing the quantity of slurry generated in each treatment.

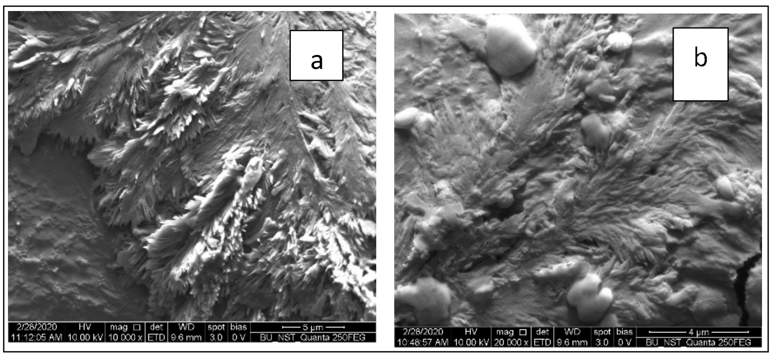

Fig 2: The FESEM Analysis of liquid biofertilizers and their crude extracts biogas slurry (a); biogas substrate(b).

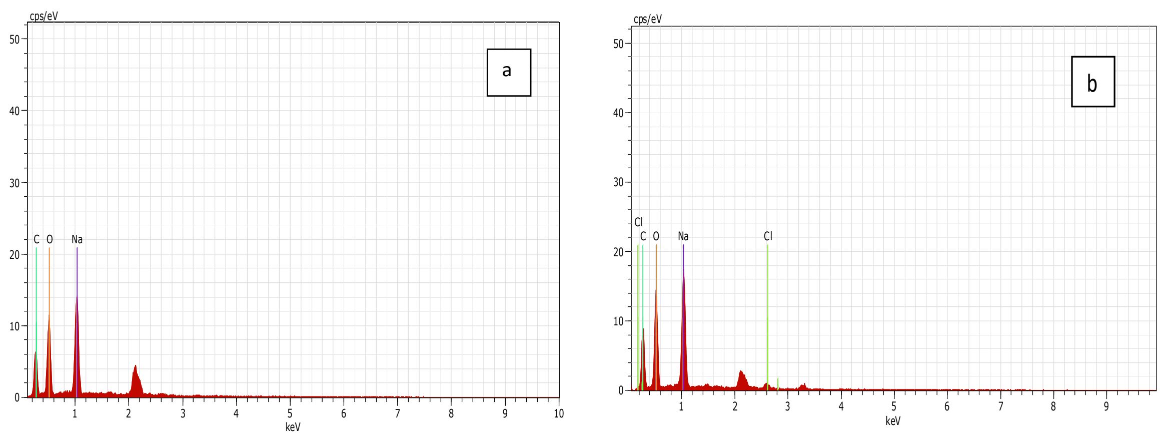

Fig 3: FESEM-EDX spectra for elemental analysis of the crude extracts and its biofertilizers. (a): Biogas slurry BF; (b): Biogas substrate.

Ananas comosus (Pineapple) and _Passiflora edulis_ (Passion fruit)

The germination studies of biogas slurry were done alone in passion fruit seedlings since tissue culture pineapple variety, MD_2 was selected to determine the biometric observations like length and leaf number of the plant and weight of the fruit. This MD_2 pineapple plants treated with biogas slurry at 15,30,45,60 DAP was shown in fig 4a, 4b, 120 DAP in 4c, 365 DAP in 4d. The germination percent was recorded higher for treatment, T_3 (49.42%) which had pre-soaking with biogas slurry having maximum manurial value (12 h) (Table 3, Fig 5a). The minimum per cent (40.20%) was observed for storage in shade. The coating treatments also followed the pre-soaking treatments but the effects were comparatively less. The passion fruit seeds pre-soaked with pineapple fruit waste slurry recorded highest vigour index (197.68). Coating with biogas slurry also showed marginal improvement (177.44) compared to gober gas slurry (Table 3, Fig 5a).

The length of pineapple plants (MD_2) was calculated for 365 days with different batches of different plants. The highest plant height (85.33 cm) was observed for pre-soaking treatment with biogas slurry (Table 4, Fig 5b). The lowest height was recorded for pineapple plants coated with gober gas slurry (69.33 cm). The plant stored in shade as control without any treatments recorded 82.33 cm length after 365 days.

The effect of treatments on number of leaves of pineapple plant were significant for 180 days after planting and found to be non-significant there after. The highest

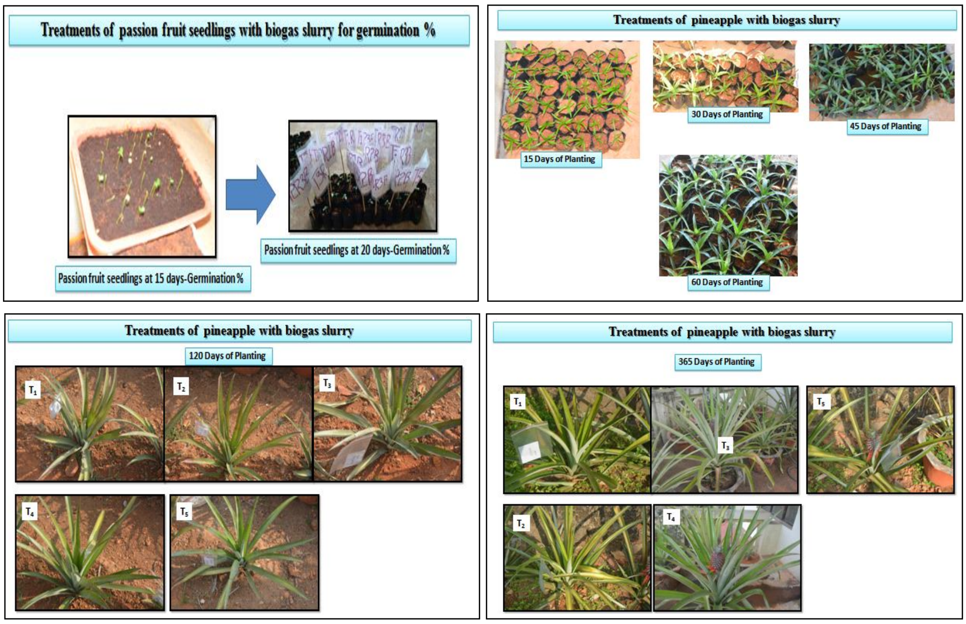

Fig 4: a. Germination % of passion fruit seedlings; b. Pineapple MD_2 plants treated with biogas slurry (15,30,45,60 DAP); c. Pineapple MD_2 plants treated with biogas slurry (120 DAP); d. Pineapple MD_2 plants treated with biogas slurry (365 DAP).

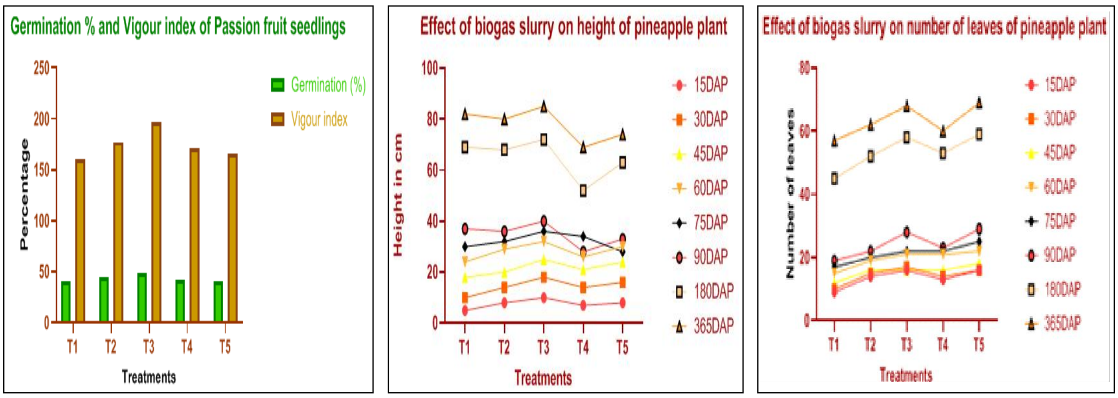

Fig 5: a. Graph showing the germination % and vigour index of passion fruit seedlings; b. Graph showing the effect of biogas slurry on the height of pineapple plant; c. Graph showing the effect of biogas slurry on the number of leaves of the pineapple plant.

Table 1: Major nutrients and heavy metal composition of biogas slurry and substrate.

Samples	C%	H%	N%	S%	Cl (ppm)	Cu (ppm)	Fe (ppm)	Mg (ppm)	Mn (ppm)	Zn (ppm)	PO_4 (ppm)	K (ppm)	Cr (ppm)	Ni (ppm)	Pb (ppm)
Biogas slurry	0.06	19.66	0.07	ND	19.79	0.130	3.509	37.57	0.154	BDL	5.424	ND	2.41	BDL	BDL
Biogas substrate	0.03	8.76	0.01	ND	7.98	ND	0.35	37.57	0.154	BDL	4.2	ND	1.64	BDL	BDL

Table 2: Elemental analysis (EDAX) of crude extracts and its bio-fertilizer.

Extracts	Elemental composition (%)																					
	C		O		Na		N		Cl		P		S		K		Zn		Ca		Mg	
	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%
Biogas slurry	67.88	73.79	32.12	26.21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
Biogas substrate	40.2	50.59	28.12	11.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd

nd: Not detected; W%: Weight per cent; A%: Atomic per cent; C: Crude extract; B: Liquid biofertilizer.

Table 3: Effect of biogas slurry on germination % and vigour index of seedlings of passion fruit.

Treatments	Germination (%)	Vigour index
T_1	40.20^e	160.8^e
T_2	44.36^b	177.44^b
T_3	49.42^a	197.68^a
T_4	42.76^c	171.04^c
T_5	41.73^d	166.92^d
CD (0.05)	0.559	0.919

number of leaves was recorded in T_5 at 180 and 365 days after planting (Table 5, Fig 5c). Among the treatments, the plants treated with pineapple waste biogas slurry were significant throughout and found to be the best, recording the maximum number of leaves. The lowest number of leaves was observed for control plant in shade, T_1.

The average weight of the fruits obtained were also calculated, the maximum fruits were obtained in the case of pre-soaking treatment with biogas slurry (Table 6). The average weight obtained was 1.28 kg for this treatment. The lowest value obtained for control plant, 1.02 kg.

Anaerobic production of methane from pineapple waste

Pineapple peels had been found to be promising feed for biogas generation, since they were rich in carbohydrates and proteins. Their disposal possessed a serious environmental pollution problem. Since pineapple peel was rich in cellulose, hemi-cellulose and other carbohydrates, it was found to be a potential substrate for methane generation by anaerobic digestion (Rani and Nand, 2004). Among the fruit waste, pineapple generate enormous amount of fermenting waste particularly its core and peel, henceforth this fruit waste was selected for producing methane in pilot scale digester (Malik *et al.*, 2001). The experiment was conducted for a month and the maximum amount of methane was generated in treatment with 1:1.5 composition of cow dung and pineapple fruit waste. The nutritional value (NPK) was high in slurry obtained from treatment having 1:2 ratio of cow dung and fruit waste. This slurry (1:2 treatment) generated was applied as foliar and drenching to pineapple (MD_2)- *Ananas comosus* var L. plants itself to study their height, leaf number and average weight of the fruit. In recent studies, pineapple waste and cow dung were used in raw form for methane gas generation (Hamzah *et al.,* 2020), here we had utilized cow dung slurry instead and maximum production of methane gas was achieved in 1:1 ratio of slurry application. Similar studies were done by Mugerwa., (2018) where 2:1 ratio of pineapple waste and cow dung slurry generated highest methane content of 64% whereas here highest of 63.41% of methane gas was obtained from 1:1 ratio of pineapple waste and cow dung slurry.

Effect of sustainable liquid fertilizer made from environmental waste to crops

The plant *Ananas comosus* (pineapple) was usually treated with chemical fertilizers like Urea, potash *etc*. Fungicides

Table 4: Effect of biogas slurry on height of the *Ananas comosus* plant.

Treatments	Height of the plant (cm)							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	180 DAP	365 DAP
T_1	5.1[d]	10.31[d]	18.03[e]	24.08[e]	30.17[d]	37.23[b]	69.76[b]	82.33[b]
T_2	8.0[b]	14.0[c]	20.06[d]	29.07[c]	32.36[c]	36.60[c]	68.56[c]	80.00[c]
T_3	10.0[a]	18.06[a]	25.80[a]	32.04[a]	36.06[a]	40.20[a]	72.00[a]	85.33[a]
T_4	7.3[c]	14.1[c]	21.00[c]	26.02[d]	34.33[b]	28.26[e]	52.66[e]	69.33[e]
T_5	8.3[b]	16.2[b]	24.00[b]	30.07[b]	28.36[e]	33.06[d]	63.87[d]	74.67[d]
CD (0.05)	0.644	0.460	0.157	0.021	0.589	0.609	1.046	1.485

Table 5: Effect of biogas slurry on number of leaves of *Ananas comosus* plant.

Treatments	Number of leaves							
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	180 DAP	365 DAP
T_1	9.97[e]	10.97[c]	12.23[b]	15.00[c]	17.66[c]	19.00[b]	45.66[c]	57.33[c]
T_2	14.31[b]	15.0[ab]	16.55[a]	19.66[b]	20.66[bc]	22.00[b]	52.00[b]	62.00[b]
T_3	16.46[a]	17.0[a]	16.15[a]	21.00[ab]	22.66[ab]	28.00[a]	58.00[ab]	69.33[a]
T_4	13.33[b]	14.66[b]	16.58[a]	21.33[ab]	22.00[b]	23.33[b]	53.33[ab]	60.00[bc]
T_5	16.55[a]	16.94[a]	18.27[a]	22.66[a]	25.33[a]	29.33[a]	59.33[a]	68.00[a]
CD (0.05)	1.649	2.049	3.42	2.93	3.080	4.578	6.03	3.787

Table 6: Effect of biogas slurry on average weight of *Ananas comosus* fruit from treatments.

Treatments	Average weight of fruit in kg
T_1	1.02[c]
T_2	1.21[b]
T_3	1.28[a]
T_4	1.22[b]
T_5	1.25[b]
CD (0.05)	0.030

T_1- Storage in shade (Control).
T_2- Coating with biogas slurry having maximum manurial value.
T_3- Presoaking with biogas slurry having maximum manurial value (12 h).
T_4- Coating with gober gas slurry from treatment 1.
T_5- Presoaking with gober gas slurry of treatment 1 (12 h).

like Phytrax, SAAF, Bavistin and hormonal treatments like Ethephon for uniform inflorescence. Comparatively, we had designed a recyclable organic fertilizer from pineapple waste for pineapple plants. The core and peel waste biogas slurry were applied to pineapple plants. Pre-soaking with the slurry regulates the transport of water to seed which acts as water reservoir (Aswathy and Sushama, 2015). In this way slurry soaking might had improved germinating ability of seeds. In a current study it showed that pre-treatment of pineapple peel using the alkali enhanced enormous lignin reduction and enhanced biogas production (Dahunsi, 2019), similarly palm oil mill effluent containing various microbial consortium were used for anaerobic fermentation of pineapple pulp and peel waste for biogas production at a range of (40.5 to 70.1%) (Aziz, 2017; Azouma *et al.,* 2018). Likewise, in this research we had fermented pineapple waste using cow dung slurry and phosphate solubilizers for methane gas production and had a recovery percentage ranging from (46.65 to 63.49%).

CONCLUSION

Production of biogas from *Ananas comosus* waste (peel and core) were done and the treatment, T_5 with 1:1.5 ratio of cow dung and pineapple fruit waste resulted in 63.81% methane and 34.47% carbon dioxide which was closely followed by T_4(60.49%, 32.0%) having cow dung + pineapple fruit waste in ratio 1:1. Throughout the study period the volume of gas was generated in the order $T_5>T_4>T_3>T_6>T_1>T_2$. The temperature inside the digester was always found to be higher compared to the atmospheric temperature. The HRT reduced from 23 days to 19 day by co digestion of pineapple fruit waste with cow dung in 1:15 ratio as compared with either of the substrate. Similarly, with biogas slurry (T_6=1:2 ratio of cow dung and fruit waste), the germination % (49.42) and vigour index (197.68) was studied with *Passiflora edulis* (Passion fruit seedlings) and were recorded highest in pre-soaking treatment, T_3 with biogas slurry having maximum manurial value (12 h); So, the growth rate order for passion fruit seedlings in terms of germination is $T_3>T_2>T_4>T_5>T_1$. In addition to this, Seedling height (85.33 cm), Leaf number (69.33) of *Ananas comosus* (Pineapple) was more in presoaking treatments of both fruit waste and gober gas slurry. For leaf the growth rate order was $T_5>T_3>T_2>T_4>T_1$ and for height the growth was reported as $T_3>T_1>T_2 >T_5>T_4$. This data infers the treatments of pre-soaking with fruit waste slurry or gober gas slurry with phosphate solubilizers- *Meyerozyma gullerimondi,* a yeast solubilizer and *Providencia rettgiri,* a bacterial solubilizer induces maximum growth rate to the crop.

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