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Enhancing biogas production by anaerobic co-digestion of pretreated agricultural and horticultural waste

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Abstract
Biogas production through anaerobic digestion is a resource effective way of managing the large volume of organic waste generated, which allows for simultaneous waste utilization and energy generation. In this study biogas production technologies from different agricultural as well as horticultural feedstock was used along with the pretreatment with efficient cellulose degrading bacterial isolate DKW showed maximum cellulose degrading ability as 1.375 mm (0.362 mg/ml) when compared to other isolates, a comparative experimental analysis between different types of biomass residues used for biogas production as estimation of biological oxygen demand (BOD) (HW-T4-M1-C2: 134 mg l^{-1}), chemical oxygen demand (COD) (HW-T3-M1-C1: 113 g l^{-1}), cellulose (AW-T2-M1-C2: 16.56%), pH (HW-T4-M1-C2: 8.48), Nitrogen (HW-T3-M1-C1: 1.48%), Phosphorus (HW-T4-M1-C2: 1.96%) and Potassium (HW-T3-M1-C1: 0.94%) during the anaerobic fermentation process. The comparison is achieved using as a parameter the biogas quality and quantity which are produced variable quantity at different intervals, using available agricultural and horticultural waste material.

Keywords: Anaerobic digestion technology, pretreatment, cellulose, feedstock, BOD, COD

1. Introduction
Agricultural and Horticultural crop residues are generated in large quantities and constitute an abundant but underutilized source of renewable biomass in agriculture. The amount of crop residues available in India is estimated to be approximately 620 million tons. These are the largest potential feedstock, and wide varieties of these wastes can be used as sources of biomass energy. Scenarios have shown that the energy demand will increase during this century by a factor of two or three, as a result of the population growth and energy consumption per capita. At the same time, concentrations of greenhouse gases (GHGs) in the atmosphere are rising rapidly, the fossil fuel derived CO_2 emissions being the most important contributor (Peter, 2010) [23].

Anaerobic digestion and biogas production are a promising means of achieving multiple environmental benefits and producing an energy carrier from renewable resources. Replacing fossil fuels with biogas normally reduces the emission not only of greenhouse gases, but also of nitrogen and sulfur oxides, hydrocarbons, and particles (Borjesson and Berglund, 2006) [6]. No negative or limited environmental side effects are observed because biogas can be produced from all types of "green" biomass (Busch *et al.* 2009) [7]. Anaerobic co-digestion strategies have become the development trend of biogas engineering (Maranon *et al*. 2012 and Amon *et al*. 2007) [20, 2]. Methane can be obtained by anaerobic digestion of different organic matters including industrial residues, agricultural wastes, industrial wastewaters and municipal solid wastes. Biological pre-treatment were mainly focused on the use of pure cultures, such as anaerobic bacteria, fungi, and Actinomycetes, which were able to degrade lignocelluloses. Different technologies are developed presently for the energy recovery of all types of wastes, one of them being the anaerobic fermentation process, which has as the main product the biogas, considered a CO_2 neutral gaseous fuel. However, biological treatment, particularly anaerobic, is often the best and/or most cost-effective alternative for economical pretreatment (Hansen and Cheong, 2007) [13].

However, most of the lab research and engineering project only stay on the two kinds of material mixed fermentation (5~7). Therefore, this study was aimed to reveal the enhancement of biogas production by pretreated agricultural and horticultural wastes along with cow dung.

Under mesophilic condition (37±1 ^{0}C), a bench-scale experiment based on the anaerobic co-digestion process of this ternary mixtures was conducted in a fed-batch single-phase reactor. The biogas production performance and the nutritional status was analyzed separately in order to assess the degradability of cellulosic waste material and the effect of pretreated microbial consortia on agricultural and horticultural wastes at different intervals.

2. Material and methods

The material and the methods adopted during the course of an investigation are presented in this chapter. The analysis was conducted in the Department of Agricultural Microbiology and Bio-energy, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad.

2.1 Isolation and identification of cellulose degrading bacteria

Isolation of cellulolytic bacteria cellulolytic bacterial strains were isolated from soil by using serial dilutions and pour plate technique. The medium used for isolation of cellulolytic bacteria contains 1.0% peptone, 1.0% carboxymethyl cellulose (CMC), 0.2% K_2HPO_4, 1% agar, 0.03% $MgSO_4.7H_2O$, 0.25% $(NH_4)_2SO_4$ and 0.2% Gelatin at pH 7 for 48 hours of incubation at 30 ^{0}C (Yin *et al.,* 2010) [32]. Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 40C for further identification and screening for cellulase production.

2.2 Screening of cellulolytic bacteria

Pure cultures of bacterial isolates were individually transferred in CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1% congored and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis (Andro *et al.,* 1984) [3]. The bacterial colonies having the largest clear zone were selected for identification and cellulase production in the submerged system.

2.3 Cellulase activity test for the isolates

Cellulase activity was assayed using dinitrosalicylic acid (DNS) reagent (Miller, 1959) [21] by estimation of reducing sugars released from CMC solubilized in 0.05 M phosphate buffer at pH 8 (Bailey *et al.,* 1992) [5]. The culture broth was centrifuged at 14000×g for 10 min at 4 ^{0}C and the clear supernatant served as crude enzyme source. The crude enzyme was added to 0.5 ml of 1% CMC in 0.05 M phosphate buffer and incubated at 50 ^{0}C for 30 min. After incubation, the reaction was stopped by the addition of 1.5 ml of DNS reagent and boiled at 100 ^{0}C in a water bath for 10 min. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using a glucose calibration curve (Shoham *et al.,* 1999) [5]. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1 µmol of glucose per minute under standard assay conditions.

2.4 Procedure for pretreatment

First, 1 g dry ground corn straw powder was poured into a118 ml serum glass bottle, after which 10 ml distilled water was added into the bottle. After autoclaving for 120 min, the bottles were inoculated with the complex microbial agents in aseptic conditions. A 0.01% (w/w) dose of the complex microbial agents was used in our study. Each of 20 bottles was then covered and sealed with a plastic film. A sponge plug was inserted into the middle of the film to sparge air while preventing airborne microorganisms from entering the bottle. The control bottle was not inoculated with complex microbial agents. It only contained 1 g dry ground corn straw and 10 ml distilled water. The bottles were placed in an incubation chamber. Samples were obtained on the 0th, 5th, 10th, 15th, and 20th day of incubation for composition determination, chemical analyses, and biochemical methane potential (BMP) assay.

2.5 Reactor set-up

Biogas production was studied in the lab with four treatments and three replications each and with 250 grams cattle dung, 500 grams substrate and 1000 ml water (1:2:5). A completely recycled anaerobic glass bottle made from a cylindrical column of borosilicate glass with a total volume of 3 L was utilized in the study. The glass bottle was blanketed with a cork borer to avoid entry of direct sunlight and escape of process heat. Reactor system for anaerobic fermentation with arrangement for feed, recirculation and biogas measurement is made by using a 1-liter container (Measuring cylinder).

2.6 Reactor operation

The maize straw and cattle dung slurry were fed to the reactor from the top by a one-way funnel and the equal quantity of the reactor digestate was withdrawn for the physicochemical analysis. The complete recycle was done to obtain complete mixing/agitation of the reactor digestate. The controlled up flow pattern of maize straw with cattle dung slurry through the reactor renders stratification of the phases such as hydrolysis, acidogenesis and methanogenesis. Such a pattern of single-phase reactor operation provides advantages of the two-phase reactor.

2.7 Inoculum

Cattle dung slurry along with some cellulose degrading bacterial consortium was used as a source of inoculum since rumen of cattle dung contains the anaerobic microbial population. The cow dung slurry was prepared by mixing water in 1:2:5 proportions and sieved to remove coarse particles. The cow dung slurry and the straw were mixed in 1:2 proportion and the mix was poured in the reactor. The reactor content was mixed thoroughly by 100% recirculation from the outlet (top) to the inlet (bottom) of the reactor with the manual stirring process.

2.9 Estimation of pH in the slurry samples

The pH of the slurry samples was determined in 1:2.5 substrate: water suspension by using digital pH meter (Systronics µ pH system361) (Jackson, 1973) [15].

2.10 Estimation of total Nitrogen content in the slurry samples

Total nitrogen in slurry samples was estimated by a modified Kjeldahl method using sulphuric and salicylic acid mixture. One gram of slurry sample was taken into 100 ml conical flask, 30 ml of sulphuric acid - salicylic acid mixture and 0.5 g of sodium thiosulfate was added mixed well and kept aside for half an hour and digested on the flame. After 30 min of digestion, one gram of copper sulfate and 10 g of potassium sulfate was added, digestion was continued till the colorless solution obtained. The digested material was washed with distilled water and the only supernatant liquid was transferred

to a beaker. From that beaker, the solution was transferred to Kjeldahl flask. 50 ml of 4% boric acid taken into 250 ml conical flask to which two drops of the mixed indicator was added and kept at the flask at the receiving end of distillation set in such a way that the receiving end immersed into the solution. Few Zn pieces, little quantity of paraffin and 120 ml of 40% NaOH was added to the Kjeldahl flask and immediately mouth of the flask was closed. The distillation continued till no more ammonia was evolved at the receiving end of the distillation set. At the end of distillation, the tip of receiving end was washed with distilled water, contents of the flask were cooled and titrated against 0.01 N H_2S0_4 till blue color changed to pinkish red color.
Burette reading was noted and nitrogen% was calculated as:

Weight of the plant sample taken = 0.1 g
Blank titre value = B ml of 0.01 N H_2SO_4
Sample titre value = S ml of 0.01 N H_2SO_4
Actual titre value = (S-B) ml
1000 ml of 1 N H_2SO_4 = 14 g N
(S-B) ml of 0.01 N H_2SO_4 = $\frac{(S-B)0.01 \times 14}{1000}$
Present in 0.1 g plant sample
100 g of plant sample contains = $\frac{(S-B)\ 0.01 \times 14 \times 100 \text{ g of N}}{1000 \times 0.1}$
= (S-B) × 0.14% of N

2.11 Estimation of total Phosphorus content in the slurry samples
Total phosphorus content in slurry samples was determined by perchloric acid digestion method using Barton's reagent as described by Jackson (1967) [14]. One gram of the slurry sample was taken into 100 ml conical flask and 12-15 ml of tri acid mixture was added (Nitric acid: Sulphuric acid: Perchloric acid at 9:2:1). The mouth of the flask was covered with a funnel. The contents were digested over a sand bath until a clear solution was obtained. The filtrate was collected and 5 ml was taken into 25 ml volumetric flask and 5 ml of Barton's reagent was added and volume made up to 25 ml with distilled water. The yellow color was developed in 30 minutes and the intensity of color was measured in a photoelectric colorimeter using blue filter (470 nm). The color will be stable for 24 h. A standard curve was prepared and the concentration of phosphorus in the solution was deduced from that value and the percentage of phosphorus in the sample was calculated.

Concentration of phosphorus in coloured solution = X ppm
i.e., 1 ml of coloured solution contains = X µg P
50 ml of coloured solution contains = 50 × X µg P
Which is present in 5 ml of the diluted digest
100 ml of diluted plant digest contains 50 × X × $\frac{100}{5}$ = X × 1000 µg P
Which is obtained from 1 g sample
100 g of the sample consists of = X × 1000 × $\frac{100}{1}$
= X × 10^5 × 10^{-6}% of P
= X × 0.1%

2.12 Estimation of total Potassium content in the slurry samples
The tri-acid extract was directly aspirated to the flame photometer to estimate the total potassium content (Systronics flame photometer 128) by Jackson (1967) [14]. 5 ml of tri-acid extract was taken into 25 ml volumetric flask and volume made up to the mark with distilled water. The concentration of K in the solution was measured using flame photometer. A standard curve was prepared and the concentration of K in the solution was deduced from that value and the percentage of K in the sample was calculated. Amount of K present in the sample (% of K) =

The concentration of K in the sample = X ppm
1 ml of the sample = X µg of K
100 ml of the sample = ?
= 100 × X µg of K
1 g of sample = 100 × X µg of K
100 g of sample = ?
= $\frac{100}{1}$ × 100 × X µg of K
= X × 10^4 × 10^{-6} g K
= X × 0.01%

2.13 Biological Oxygen Demand (BOD)
Biological Oxygen Demand was determined by measuring oxygen concentration in the slurry sample, idometrically before and after incubation in the dark at 20 ^{0}C for 5 days by the colorimetric method (APHA, 1992) [4]. BOD bottles were taken and filled with the sample and stopper was placed. Then the stopper was removed and 1 ml of each manganous sulphate and alkaline potassium sulphate were added. Precipitation was dissolved by adding 2 m1 of sulphuric acid. Then the whole content was transferred into a conical flask. Few drops of the starch indicator were added and titrated against sodium thiosulphate solution till the initial blue colour turned tocolorless. The titre value obtained gives dissolved Oxygen ($D.O_1$)

Dissolved Oxygen ($D.O_1$) = $\frac{(V1N \times 8 \times 1000)}{V2-V3}$

Volume of titrant (ml) = V_1

The volume of sampling bottle after placing the stopper (ml) = V_2
Volume of manganous sulphate and potassium sulphate solution added (ml) = V_3

Other sets of BOD bottles were prepared the same as above and incubated in BOD incubator for 5 days. After 5 days the content from BOD bottles were transferred into conical flasks and titrated similarly. The dissolved oxygen content $D.O_5$ was determined.

BOD = ($D.O_1$ - $D.O_5$) × dilution factor.

2.14 Chemical Oxygen Demand (COD)
Chemical Oxygen Demand was determined by taking centrifuged slurry samples and refluxed with a known amount of Potassium dichromate in sulphuric acid medium and excess of dichromate was titrated against ferrous ammonium sulphate. The amount of dichromate consumed was proportional to the oxygen required to oxidize the organic matter. Chemical oxygen demand was determined by the closed reflux colorimetric method (APHA, 1992) [4].

COD= $\frac{(B-T) \times N \times 1000 \times 8}{\text{The volume of the sample (ml)}}$

2.15 Estimation of cellulose
Cellulose content was estimated by the method of Uppdegraff (1969) [28]. A hundred milligrams of pretreated cellulose

wastes were added with 5 ml of Nitric reagent and boiled and cooled. It was centrifuged at 5000 rpm for 5 min. The pellet was washed with distilled water. 10 ml of 67% sulphuric acid was added. One ml of the sample was diluted to 100 ml. To 1 ml of each diluted solution, 10 ml of freshly prepared ice cooled anthrone reagent was added and boiled in a boiling water bath for 10 min at 100 ^{0}C. After that absorbance was recorded at 600 nm.

2.16 Measurement of Gas production

The biogas production readings were taken on an alternate day by water displacement method with the measuring jar.

2.16.1 Estimation of methane percentage using gas chromatography

Methane percentage in the biogas was estimated by using gas chromatography (Bruker-450) with a Flame Ionization Detector (FID) temperatures were maintained at 300 ^{0}C in the detector, 75 ^{0}C in the injector and 50 ^{0}C in the oven. The column used was porapak Q. The gas flow in the column was maintained as 60 ml min^{-1}.

3. Results

3.1 Isolation, screening and cellulase activity

Cellulose degrading bacteria were isolated from different soil samples and waste samples [Soil samples of farmer fields, Domestic Kitchen Waste (DKW) and Sewage Water Sample (SWS)] by using CMC agar medium (Plate 1). Among all the samples of soil, DKW and SWS samples four different efficient cellulose degrading bacteria were screened based on morphological and biochemical characteristics these isolates are also tested for their efficient cellulase degrading capacity by qualitative and quantitative studies. Among the four isolates DKW showed maximum cellulase activity by formation of solubilisation zone of 1.375 mm (0.362 mg/ml) (Fig 1 & 2) as when compared to PS-1 has 1.250 mm (0.353 mg/ml), BCS-5 has 1.175 mm (0.354) and SWS has showed 1.125 mm (0.350 mg/ml) (Table 1). Gautam *et al*. (2012) studied the diversity of cellulolytic microbes and biodegradation of municipal solid waste by a potential strain.

Table 1: Different isolates of soil and waste samples and their cellulase activity

S. No	Sample	Isolate. No	Cellulase activity zone (mm)	Cellulase activity (mg/ml)
1	Soil	PS-1	1.250	0.353
2	Soil	BCS-5	1.175	0.354
3	Domestic Kitchen Waste	DKW	1.375	0.362
4	Sewage Water Sample	SWS	1.125	0.350

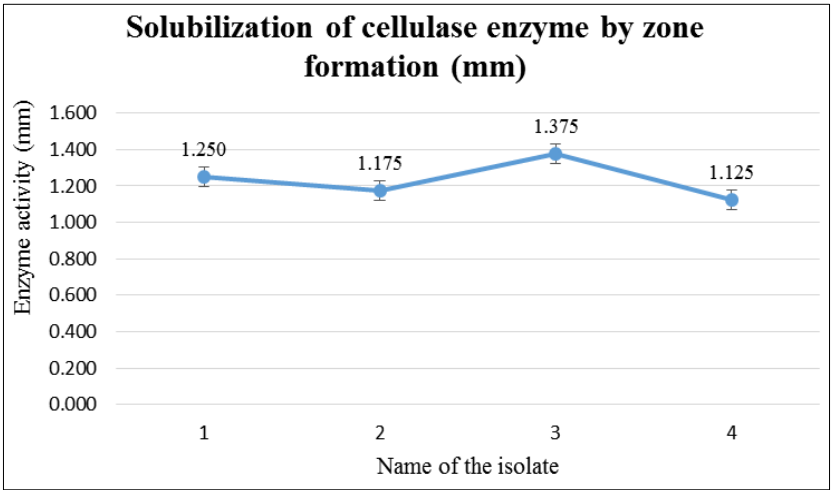

Fig 1: Qualitative estimation of cellulase enzyme activity

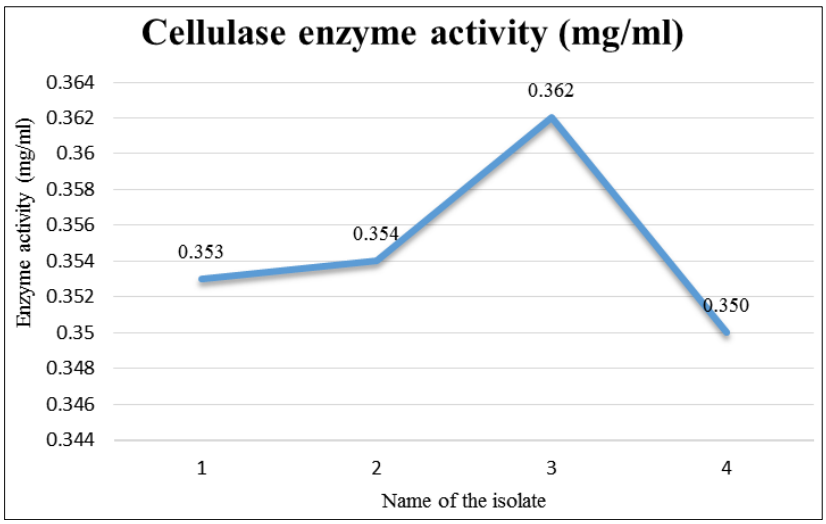

Fig 2: Quantitative estimation of cellulase enzyme activity

CCRA- Control plate CCRA- Control plate with bacteria

Plate 1: Isolation of cellulose degrading bacterial isolates

Table 2: Operational parameters during the experiment along with the production of biogas from agricultural and horticultural waste

Treatments	Biogas production (%)					BOD (mg l^{-1})	COD (g l^{-1})	Cellulose (%)	pH	Nitrogen (%)	Phosphorus (%)	Potassium (%)
	0th day	15th day	30th day	45th day	60th day							
AW-T_1-M_1-C_1	550.00 (57.51)	720.30 (58.12)	980.20 (59.02)	580.00 (46.20)	550.50 (16.48)	101	110	16.00	8.19	0.76	0.17	0.72
AW-T_2-M_1-C_2	680.30 (21.45)	700.20 (22.08)	950.30 (20.79)	620.30 (19.56)	580.00 (15.06)	118	109	16.56	8.26	0.98	0.19	0.78
HW-T_3-M_1-C_1	500.60 (51.74)	580.30 (54.30)	500.20 (57.56)	420.00 (45.08)	380.30 (34.12)	121	113	15.85	8.14	1.48	1.06	0.94
HW-T_4-M_1-C_2	490.20 (51.52)	600.20 (53.96)	580.00 (54.13)	500.30 (44.96)	450.00 (35.15)	134	110	15.32	8.48	1.28	1.96	0.89

*The table values with in the brackets indicate the composition of methane percent of different treatments
AW- Agricultural waste;
AW-T_1-M_1-C_1- Biogas production without pretreatment
AW-T_2-M_1-C_2- Biogas production with pretreatment
HW- Horticultural waste;
HW-T_3-M_1-C_1- Biogas production without pretreatment
HW-T_4-M_1-C_2- Biogas production with pretreatment

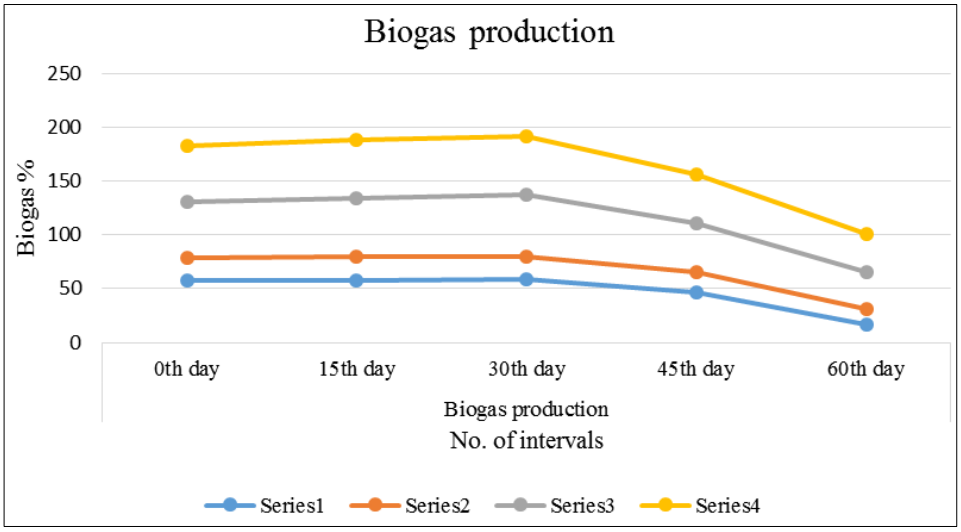

Fig 3: Production of biogas from agricultural and horticultural waste

3.2 Biogas production from Agricultural and Horticultural waste enriched with and without cellulose degrading bacteria

All the biogas production units with four treatments and three replications were set on the same day with 250 grams cow dung, 500 grams substrate and 1000 ml water (1:2:5 ratio). The results of biogas production revealed that the end of the 7th day in AW-T_2 (Biogas production with pretreatment) 680.30 ml of biogas was released followed by (550.00 ml) in AW-T_1 (Biogas production without pretreatment) (Fig 3). At the end of the 15th day, 720.30 ml of biogas was released in AW-T_1 (Biogas production without pretreatment) and the lower amount was observed in HW-T_1 (Biogas production without pretreatment) (580.30 ml). At the end of the 30th day in AW-T_1 (Biogas production without pretreatment) more amount of biogas was evolved (620.30 ml). At the end of 60th day highest gas production was observed in AW-T_2 (Biogas production with pretreatment) (580.00 ml). Based on the water displacement readings more biogas evolved in AW-T_1 (Biogas production without pretreatment) at 15th day (550.00

ml), 30th day (980.20 ml), AW-T_2 (Biogas production with pretreatment) having more biogas evolution at 7th day (680.30 ml), 45th day (620.30 ml) and 60th day (580.00 ml) (Table 2). The above results were similar to those of Vikrant and Shekar. (2013) who studied on the anaerobic digestion of horticulture waste for the production of biogas with a combination of the mixed inoculum was used for biogas production at 37 ^{0}C in the laboratory (small scale) reactor and results were obtained as in between 10 to 150 ml during the process of anaerobic digestion. In the above result methane (CH_4) in the four treatments was similar to that of Ziganshin *et al.* (2013) [33] who conducted an experiment was anaerobic digestion in laboratory scale biogas reactors fed with different agricultural waste materials and obtained the results of methane (CH_4) (57.50, 51.70 and 44.20% in different biogas reactors).

3.3 Biological oxygen demand (BOD)

BOD in the agricultural waste among all the treatments was found to be highest in the treatment HW-T_4 (134 mg l^{-1}) at the end of the experiment. Among treatments on par, results were observed with the treatments of HW-T_3 (121 mg l^{-1}) and AW-T_2 (181 mg l^{-1}) and AW-T_1 (110 mg l^{-1}) (Table. 2). BOD content in different treatments was similar to the results of Kavya *et al.* (2015) [18] who studied that variations in BOD and COD at various stages of biogas production using different agricultural wastes and recorded the BOD (151.75 g l^{-1}, 80.14 g l^{-1} and 76.26 g l^{-1}) in different treatments of agricultural waste digestion process. The similar kind of results was noticed in the study conducted by Sinha and Bharambe (2008) [27] where, they have conducted an experiment and recorded biological oxygen demand as 63.5, 45.8 and 83.2 mg l^{-1} in different treatments.

3.4 Chemical oxygen demand (COD)

COD in the agricultural waste among all the treatments were found to be highest in the treatment HW-T_3 (113 g l^{-1}) at the end of the experiment. Among treatments on par, results were observed with the treatments of AW-T_1 (110 g l^{-1}) and HW-T_4 (110 g l^{-1}) and AW-T_2 (109 g l^{-1}) (Table. 2). The similar kind of results was noticed in the study conducted by Sinha and Bharambe (2008) [27] where, they have conducted an experiment and recorded chemical oxygen demand as 112, 128, and 132 g l^{-1} in different treatments. In the present study COD content in the horticultural waste was higher when compared to the results of Jimenez *et al.* (2015) [17] who studied that A new organic matter fractionation methodology for organic wastes: bioaccessibility and complexity characterization for treatment optimization and recorded the COD (56.90, 79.50 and 98.20 g l^{-1}) in the digested sludge of agricultural waste in different treatments.

3.5 Cellulose

In all the days of intervals, AW-T_2 (16.56%) treatment processes highest cellulose content. Among treatments on par, results were observed with the treatments of AW-T_1 (16%) and HW-T_3 (15.85%) and HW-T_4 (15.32%) (Table. 2). Lin *et al*. (2014) [19] who demonstrated that comparison of solid-state anaerobic digestion with effluent from liquid anaerobic digestion. The cellulose percent in the substrates were estimated as 19.1% (in yard trimmings), 12.2% (in leaves), and 23.8% (in the grass). The variation in the percentage of cellulose present in the agricultural waste substrate between different treatments at different intervals may be due to variation in chemical characteristics of substrates used and organisms involved in degradation. Similar kind of result was noticed in the study by Chen *et al*. (2015) [9] where they have recorded cellulose content was 25.61 percent. The results of Shankarappa *et al*. (2015) [24] who studied the biological pretreatment of agro-residues with ligninolytic fungi for delignification and recovery of cellulose and hemicellulose was showed cellulose content was 0.32% in corn stover, 0.33% in a corn husk.

3.6 pH

Slightly acidic pH values recorded in HW-T_4 (8.48), AW-T_2 (8.26), AW-T_1 (8.19) and lowest pH was recorded in the treatment HW-T_3 (8.14) (Table. 2). Ideal pH for obtaining best biogas was 8.0-8.5 this was varied from substrate to substrate and also different from the complete process of the complete period. pH in the agriculture waste was similar to the results of Yangyang *et al*. (2016) [31] who demonstrated that anaerobic digestion of corn stover in a laboratory scale reactor at mesophilic conditions (35 ^{o}C). The pH in the substrates was estimated as 7.4, 7.5, 7.7 and 8.1 in agriculture waste at different treatments (corn stover digestion). The variation in pH between different treatments at different intervals of biogas production may be due to variation in chemical characteristics of substrates used and organisms involved in degradation as this was noticed in the study conducted by Chandrashekara *et al*. (2011) [8] where, they have recorded more pH (7.4) compared to biogas production produced by without inoculums (6.5) and also with microbial consortium (6.8). Ideal pH for obtaining best biogas production was 7.1-8.3 this was varied from substrate to substrate and also different from the complete process of the anaerobic digestion process. pH in the horticultural waste was similar to the results of Abubakar *et al.* (2012) [1] who investigated the effectiveness of cow dung for biogas production and the pH of cow dung was in between 7.40 and 7.70 and during the anaerobic digestion, there were variations between 6.12-7.70. The variation in pH between different treatments at different intervals of biogas production may be due to variation in chemical characteristics of substrates used and organisms involved in degradation as this was noticed in the study conducted by Chandrashekara *et al*. (2011) [8].

3.7 Total Nitrogen

N content during anaerobic digestion of agricultural waste among all the treatments highest nitrogen content was recorded in the treatment HW-T_3 (1.48%) and on par results were also recorded in the treatments HW-T_4 (1.28%), AW-T_2 (0.98%) and lowest nitrogen content was recorded in the treatment AW-T_1 (0.76%) (Table. 2). The similar kind of results was noticed in the study conducted by Wani *et al*. (2013) [30] where they have recorded more N% in cow dung (2.03%) observed in the present experiment is similar to the results as 1.95% and 1.91% respectively. The percentage of highest N content in the present work as compared with the results presented by Jeyabal *et al.* (2001) [16] who studied the composition of nutrients like N, P and K in biogas digested slurry and obtained the N (1.65%). The similar kind of results was noticed in the study conducted by Chandrashekara *et al*. (2011) [8] where they have recorded more nitrogen content without inoculum (1.36%) and also with the microbial consortium (1.54%). The percentage of higher N per cent in the present work as compared higher results compared to the work presented by Daicy *et al.* (2014) [10] who studied horticultural waste as substrates for cutting rooting and

growth of rosemary and estimated the composition of nutrients like N (0.14 and 0.15%) and (0.15 and 0.17%).

3.8 Total Phosphorus

Among all the treatments P content was found significantly highest in the treatment HW-T_4 (1.96%) and least was found in AW-T_1 (0.17%). Among all the treatments on par results were found in the treatments HW-T_3 (1.06%) and AW-T_2 (0.19%) (Table. 2). The percentage of highest P% in all the treatments from the present work as compared with the results presented by Jeyabal *et al.* (2001) [16] who studied the composition of nutrients like N, P and K in biogas digested slurry recorded as P (0.70, 0.76 and 1.20%). The similar kind of results was noticed in the study conducted by Chandrashekara *et al*. (2011) [8] where they have recorded more phosphorus content in the as (1.90%) compared to without inoculum (1.36%) and also with the microbial consortium (1.54%). The similar kind of results was noticed in the study conducted by Singh and Nain (2014) [26] where they have conducted an experiment microorganisms in the conversion of agricultural wastes.

3.9 Total Potassium

The available potassium in the agricultural and horticultural waste among all the treatments was found to be on par in HW-T_3, HW-T_4 (0.94 and 0.89%) and AW-T_2, AW-T_1 (0.78 and 0.72%). Among all the treatments K content was found to be highest in the treatment HW-T_3 (0.94%) followed by HW-T_4 and least was found with the treatment AW-T_1 (Table. 2). In the results obtained as K percent in the treatments was more (0.94, 0.89, 0.78 and 0.72%) compared to the observations of Patil *et al.* (2011) [22] was studied and it obtained 0.22% nitrogen and 0.20% phosphorus. The variation in the available potassium content between different treatments at different intervals may be due to variation in chemical characteristics of substrates used and efficiency of organisms involved in degradation as similar kind of results were noticed in the study conducted by Chandrashekara *et al*. (2011) [8] where they have recorded more available potassium content. The percentage of higher P per cent in the present work as compared with the results presented by Jeyabal *et al.* (2001) [16] who studied the composition of nutrients like N, P and K in biogas digested slurry recorded as P (0.70, 0.76 and 1.20%) in cow dung, biodigested slurry and sugarcane pressmud. The nutrient states of higher P per cent in the present work as compared higher results compared to the work presented by Daicy *et al.* (2014) [10] who studied horticultural waste as substrates for cutting rooting and growth of rosemary and estimated the composition of nutrients like P (0.03 and 0.04%) and (0.02, 0.03 and 0.04%) in different treatments. In the above result P in the treatments were similar to the observations of Ewemoje *et al.* (2016) [11] who investigated the nutrients level recorded the P in between 0.81 and 0.90%. In the results obtained as K% in the observations of Daicy *et al.* (2014) [10] was studied the horticultural waste as substrates for cutting rooting and growth of rosemary and estimated the composition of nutrients and obtained 1.74 and 1.95% of potassium, 1.55, 1.57 and 1.62% of potassium in different treatments. The variation in the available potassium content between different treatments at different intervals may be due to variation in chemical characteristics of substrates used and efficiency of organisms involved in degradation as similar kind of results were noticed in the study conducted by Chandrashekara *et al*. (2011) [8] where they have recorded more available potassium content without inoculum (0.42%) and also with microbial consortium (0.51%). In the above result K in the treatments were less to the observations of Ewemoje *et al.* (2016) [11] who investigated the nutrients level and recorded the K in between 3.98 and 4.45%.

4. Discussion

Biogas production resumption time was longer with longer low-temperature duration and increased rapidly, then decreased slightly when the temperature was restored in the low-temperature duration of 12 h and 24 h. The delay in recovery was presumably due to the slow degradation of relatively low methane-yielding cellulosic materials. The products resulting from fermentation require an additional transformation before being able to produce methane. It is here that intervene the acetogenesis reducing bacteria and the sulfate-reducing bacteria, producing hydrogen sulfide (H_2S). The ultimate phase during which two types of methanogens bacteria take over: the first ones (acetogenesis) reduce methane acetate, CH_4 and bicarbonate. The second ones reduce methane bicarbonate. Rises in the methane content of biogas as a result of a decrease in the bioreactor temperature. The increase in the quality of biogas is attributed to the raised solubility of carbon dioxide at the lower temperature cycle.

The ideal biological oxygen demand (100-110 mg l^{-1}) was important for maintaining anaerobic conditions and production of methane under particular bioreactor by using agricultural was as a substrate. Dissolved oxygen depletion is most likely to become evident during the anaerobic digestion, the microbial population explosion in response to a large amount of organic material. If the microbial population deoxygenates the water, however, that lack of oxygen imposes a limit on population growth of microbial organisms. The ideal chemical oxygen demand (145-150 g l^{-1}) was important for maintaining anaerobic conditions and production of methane under particular bioreactor by using agricultural was as a substrate. The increasing COD indicated oxygen required to an oxidized substance to carbon dioxide and water. As a result of this, COD values tend to be greater than BOD values. COD values can be vastly greater if large amounts of biologically resistant organic matter are present.

Ideal cellulose content was 36-39% cellulolytic microorganisms can establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions. The time of maximum cellulose degradation began when the digested material reached the thermophilic (53-63 ^oC) phase, and this increase in the rate of cellulose degradation coincided with the emergence of the actinomycetes as a major population.

More increase in phosphorus in different treatments is probably due to mineralization and mobilization of phosphorus due to the microbial population. During organic matter decomposition by the microorganisms is the major mechanism for solubilization of insoluble phosphorus, which subsequently results in an increase in phosphorus content. The degradation rate of the organic matter decreases gradually as the progress because of the reduction in available carbon sources, and synthesis reactions of the new complex and polymerized organic compounds (humification) prevail over mineralization during the maturation phase.

5. Conclusion

Samples were collected from different sources i.e. Student farm, farmer's fields, forest soil, horse dung dump, domestic kitchen waste, municipal solid waste, sewage water and cow dung. They were further tested qualitatively and quantitatively for cellulase activity. Among thirty isolates, four bacterial isolates were having maximum cellulase activity. Finally, these four isolates were selected for pretreatment of agricultural (Maize straw) and horticultural waste (Banana fruit waste) for biogas production under anaerobic condition after enriching the substrates with cellulose-degrading cultures. Biological pretreatment with complex microbial agents proved to be an efficient method to improve biodegradability to enhance biogas production of agricultural waste and horticultural waste. Compared to untreated controls the pretreated agricultural and horticultural waste yielded higher manurial value and given more biogas production. The enhanced biogas production was attributed to the improved biodegradability of the straw and fruit waste as indicated by increased TS and VS reductions and a shortened digestion time. By the pretreatment of agricultural waste, horticultural waste was easily degraded by enriched cultures and their enzyme activities. The N, P and organic carbon% increased in all the treatments of horticultural waste compared to agricultural waste. Considering the characteristics of the high moisture solid waste of agricultural and horticultural waste, anaerobic digestion represents a feasible and effective method to convert the waste to biogas fuel. The agricultural waste was found to be the best in biogas production as compared to horticultural waste. Horticultural waste was comparatively better in terms of N, P, K and organic carbon%.

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