SUBSTRATE IMPACT ON BIOGAS PRODUCTION AND MANURIAL VALUE OF SLURRY

By

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(2012-11-158)

THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Substrate Impact on Biogas Production and Manurial Value of Slurry"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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ABBREVIATIONS

ATIC	Agriculture Technology Information Centre
Br	Bromine
cm	Centimetre
Co	Cobalt
CO_2	Carbon dioxide
CoH	College of Horticulture
Cr	Chromium
CRD	Completely Randomized Design
C	Carbon
C:N	Carbon Nitrogen ratio
Ca	Calcium
Cd	Cadmium
CH_4	Methane
^0C	Degree Celsius
Cu	Copper
dS m^{-1}	Deci Siemens per meter
E	East
EC	Electrical Conductivity
FAO	Food and Agriculture Organization
FCO	Fertilizer Control Order

Fe	Iron
FID	Flame Ionization Detector
FYM	Farm Yard Manure
g	Gram
GC	Gas Chromatography
H_2	Dihydrogen
H_2S	Hydrogen sulphide
ha	Hectare
HCl	Hydrochloric acid
HRT	Hydraulic Retention Time
K	Potassium
K_2O	Potassium oxide
KAU	Kerala Agricultural University
kg	Kilogram
kWh	Kilo watt hour
L	Litre
LPG	Liquified Petroleum Gas
m^3	Cubic metre
µg	Microgram
mg	Milligram
Mn	Manganese

Mo	Molybdenum
N	Nitrogen
N_2	Dinitrogen
Na	Sodium
NH_3	Ammonia
NH_4-N	Ammoniacal nitrogen
Ni	Nickel
O_2	Oxygen
OLR	Organic Load Rate
P	Phosphorus
P_2O_5	Phosphorus pentoxide
pH	Negative logarithm of hydrogen ion concentration
PoP	Package of Practices and Recommendations for Crops
S	Sulphur
Sc	Scandium
ts	total solids
VFA	Volatile Fatty Acids
vs	volatile solids
W	Tungsten
W/W	Weight by Weight

1. INTRODUCTION

1. INTRODUCTION

The demand for energy in the world is increasing with the growth of technology and urbanization. The major share of energy is contributed by fossil fuel which is a non renewable source of energy. Thus the availability of fossil fuels for the future generation is not predictable. Over exploitation and combustion of fossil fuels lead to emission of huge quantity of carbon dioxide which degrades the ozone layer leading to global warming.

Rise in the population has led to an increase in the quantity of wastes produced especially biodegradable wastes. World over ten million metric tons of biodegradable wastes are produced per annum. A large quantity of such wastes is heaped in public places which act as a medium for the multiplication of vectors and pathogens. Major part of these wastes is dumped into water bodies which cause nitrification of water bodies leading to eutrophication. It also increases the microbial population in water contaminating drinking water and causing epidemics. In addition to this, a large quantity of animal manure is produced every year in India especially in villages which can be utilised for the production of organic manure.

Safe disposal of garbage involves huge cost and requirement of high power which is not affordable for the developing nations. A safe and better technology named biomethanation (biogas production) can be adopted for waste disposal and energy production in our country. The anaerobic fermentation of bio wastes produces combustible gas (biogas) which is colourless and safer than liquid petroleum gas. This technology is cheaper than any other waste recycling technology. The slurry produced after anaerobic fermentation is good manure which can be used as a balanced nutrient source for agricultural crops.

Biogas production reduces the production of carbon dioxide by activating methanogenic bacteria by anaerobic fermentation. One major source of carbon dioxide production is usage of fossil fuels. The substitution of fossil fuels with biogas reduces the carbon dioxide emission and thus biogas technology reduces

the atmospheric pollution. Anaerobic fermentation of organic waste reduces the multiplication of pathogens and vectors, thus preventing epidemics and improving the public health sector.

During biomethanation, nutrients present in substrate will be converted to more available forms and thus the slurry after biomethanation is best suitable for application in crop field and enhances the plant yield. The biogas slurry builds up organic matter in soil which increases bulk density, water holding capacity and also acts as a nutrient sink. Adequate nutrient supply and better soil physico-chemical condition increase crop growth and yield. The anaerobic fermentation also reduces the germination capacity of weed seeds present in raw animal dung and thus it reduces the need the herbicide application. Hence biogas slurry is one of the good sources of nutrients for organic agriculture. The application of slurry is good for soil conditioning and also useful for land reclamation and restoration of nutrients. Biogas slurry can also be used as food material in aquaculture.

In rural areas biofuels such as fire wood, dung cakes etc. are used for cooking and heating purpose. The heat energy produced by the combustion of biogas is three times greater than direct burning of equal quantity traditional biofuels. As per World Health Organization globally about 1.5 million deaths per year are caused by smoke inhaled from fuel wood.

Biogas can be used as a substitute for fossil fuels and 5.663 m^3 biogas gives an energy equivalent to that of 4.546 L petrol. The usage of biogas in combustion diesel engines can reduce 80 per cent of diesel consumption. Biogas can substitute electricity to a certain extent, and on an average 6 kWh per m^3 of raw biogas and 10 kwh per m^3 of biomethane is produced.

A large range of substrates can be used for biogas production like animal manure, crop residue, wastes from food processing industry, domestic wastes, toilet waste, spend wash sewage, sludge etc. The major components of biogas are methane, carbon dioxide, hydrogen sulphide and traces of moisture. Based on the substrate, the composition of biogas varies.

Small reactors are very useful for waste recycling, energy production and also as a nutrient resource for agriculture in rural areas. In Kerala, the organic sources available in plenty for biogas production are poultry manure, goat manure, elephant dung, crop residues, biodegradable household wastes, cow dung etc. In this context, the present study entitled "Substrate impact on biogas production and manurial value of slurry" was undertaken with the following objectives

1. To analyse the composition of biogas produced from different substrates, and to characterise the biogas slurry.

2. To study the effect of different irrigation treatments using biogas slurry on soil characteristics, growth and yield of cowpea (*Vigna unguiculata*) var. Bhagyalakshmy.

3. To study the effect of different seed treatments with biogas slurry on germination, vigour and growth of cowpea (*Vigna unguiculata*) var. Lola seeds.

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. IMPORTANCE OF BIOGAS

There is a great deal of pressure in many parts of the world to ascertain how livestock wastes can be handled effectively. Livestock manures, like cow dung in the absence of appropriate disposal methods can cause adverse environmental and health problems such as pathogen contamination, foul odour, air borne ammonia, green house gases etc. (Harikrishnan and Sung, 2003).

The biological organic materials are renewable and can be recycled to produce biogas. The wastes that are usually disposed off, either into the sea, river or on the land as solid amendment materials support breeding of flies and cause health hazards to people living around the area, are converted into biogas by anaerobic fermentation (Ezeonu *et al.*, 2002). Biogas provides a renewable and environmentally friendly process that supports sustainable agriculture. It is one of the simplest sources of renewable energy and can be derived from sewage, liquid manure from hens, cattle and pigs, organic waste from agriculture and food processing. Additionally the byproducts of the digesters provide organic waste of superior quality (Arthur *et al.*, 2011).

The storage of manure makes significant contribution to global methane (CH_4) emissions. Anaerobic digestion of pig and cattle manure in biogas reactors before storing outside might reduce the potential methane emissions. The aerobic surface processes contributed significantly to CO_2 emission than anaerobic surface (Moller *et al.*, 2004). The introduction of food waste into anaerobic digestion brings a promising scenario of increased feed stock availability and over all energy production from anaerobic digestion (Ye *et al.*, 2015).

In recent years anaerobic digestion has become a prevailing choice for sustainable organic waste treatments all over the world. It is well suited for various wet biodegradable organic wastes of high water content (over 80%) yielding methane rich biogas for renewable energy production and use (Zupancic

and Grilc, 2012). Biogas technology could prevent pollution of soil and water and provide pathogen free digested sludge as a fertilizer for organic cultivation (Saseendran *et al.*, 2009).

Biogas is a gaseous mixture of methane, carbon dioxide, hydrogen sulphide and several other gases produced by anaerobic fermentation of organic material such as animal and human manure, leaves, twigs, grasses, industrial wastes etc. The presence of methane in biogas gave it the property of combustion (Mazumdar, 1982). This is the mixture of gas produced by methanogenic bacteria while acting upon biodegradable materials in an anaerobic condition. Biogas is mainly composed of 50 to 70 percent methane, 30 to 40 percent carbon dioxide (CO_2) and low amount of other gases. Biogas is about 20 percent lighter than air and has an ignition temperature in the range of 650^0C to 750^0C. It is an odourless and colourless gas that burns with clear blue flame similar to that of LPG gas (Sathianathan, 1975). Its calorific value is 20 MJ m^{-3} and burns with 60 percent efficiency in a conventional biogas stove (FAO, 1996).

2.2. BIOGAS PRODUCTION TECHNOLOGY

2.2.1. Method of Biogas Production

The biogas digester is initially filled with water until it overflows which creates an air lock with water in lower two third of the tank and air in the top one third, then charged with the manure water slurry. When the chamber is filled with manure slurry, the bacteria start decomposing the organic matter, and as the matter flows through the tank biogas begins to accumulate in the upper part of the digester (Rota *et al.*, 2012).

2.2.2. Maintenance of Good Biogas Production

The fresh cattle dung is mixed with water in the ratio of 1:1 on unit volume basis. The dilution to be maintained to the total solid varies from 7 - 10 per cent. A survey made by Biogas Sector Partnership (BSP), Nepal revealed that the farmers often over diluted the slurry (FAO, 1996). Before feeding the digester,

the excreta, especially fresh cattle dung has to be mixed with water at the ratio of 1:1 on unit volume basis. However, if the dung is in dry form, the quantity of water to be added has to be increased accordingly to arrive at the desired consistency of the inputs (ratio could vary from 1:1.25 to 1:2). In both cases, gas production was less than optimum (Gurung, 1996). When the dung was too diluted, the solid particles might have settled in the digester and when too thick, gas formed at lower part of digester was impeded to flow up through the substrate (Ituen *et al.,* 2007).

Proper mixing of digestate provided intermediate contact between microorganism and substrate when uniform temperature and uniform distribution of bacteria and volatile solids was maintained. It also minimized the sludge formation on the top of slurry which interfered with release of biogas. Thus scum formation at the bottom of digester reduced the effective volume of digester and obstructed the release of gas from digester contents unless it was broken up and removed periodically (Mital, 2007).

2.2.3. Substrates Used for Biogas Production

Various types of feedstock that can be used for the production of biogas are animal manure and slurries, crop residues, organic wastes from dairy production, food industries and agro-industries, wastewater sludge, organic fraction of municipal solid wastes and organic wastes from different sources. One main advantage of biogas production is the ability to use "wet biomass" types as feedstock, all characterised by moisture content higher than 60–70%. In recent years, a number of energy crops (maize and rapeseed) have been largely used as feedstock for biogas production in countries like Austria or Germany. Besides energy crops, all kinds of agricultural residues, damaged crops which are unsuitable for food or resulting from unfavourable growing and weather conditions could be used to produce biogas (Seadi *et al.,* 2008).

2.2.4. Co-fermentation of Substrate

The co-fermentation of plant mass with liquid manure enabled the stabilized process of biogas production due to the high buffering capacity of manure in the substrate and it limited dysfunctions caused by the higher ammonia contents (Khan *et al.*, 1994). Co-digestion generally resulted in improved biogas and methane yields compared to separate digestion (Schnurer *et al.*, 1999; Westerholm *et al.*, 2012). Livestock wastes are also substrates of interest but had one major disadvantage of low organic content coupled with low biodegradability (Vedrenne *et al.*, 2008). Consequently using manure in anaerobic digesters was relatively rare and co-substrates were often added to increase biogas production (Mata-Aluvarez *et al.*, 2000).

Biogas and methane production were significantly higher during the period of 0-7 days and to some extended degree above 20 days (Adelard, *et al.*, 2015). For single substrate, the bio-methane potential assay showed that kitchen waste had the highest methane yield of 352 l CH_4 kg vs^{-1} which was more than dairy manure alone (Ye *et al.*, 2015).

Crop residues represent another fraction of agricultural waste. Substantial quantities of unused stalks, straw and bark are produced from a variety of crops which could be used for energy generation, but they are poor substrate in terms of nitrogen and phosphate. Therefore, co-digestion of animal manure and crop residues could supply a proper C: N ratio for microorganisms. The optimal C: N ratio was 20–30:1 and excess N led to ammonia inhibition for digestion processes (Molnar and Bartha, 1998).

2.3. ANAEROBIC FERMENTATION PROCESS

Anaerobic fermentation is the biological process that takes place in the absence of air, and is a thermo-chemical process that transforms organic matter into biogas which comprises principally of methane and carbon dioxide. The reaction starts naturally in large heaps of organic matter like agricultural biomass.

The methane rates varied from 50-80 per cent according to the type of process and biomass used (Rota *et al.*, 2012). A number of stages were involved in the process. Initially organic materials were hydrolyzed by enzymes on to simple sugars, alcohols, peptides and amino acids. These were then converted to volatile fatty acids, hydrogen, carbon dioxide, water and methane. Methane forming bacteria converted fatty acid to methane, carbon dioxide and water (Mital, 2007).

Mazumdar (1982) reported that the process of anaerobic fermentation was catalysed by a consortium of micro organisms (inoculums) that converted complex macro molecules into low molecular weight compounds (methane, carbon dioxide and ammonia).

According to Straka *et al.* (2007), organically bound nitrogen in biomethanation was converted mostly to ammonia. The inhibition effect of ammonia was strongly related to pH value of the reactor as ammonia is toxic to methane producing bacteria. When there was high partial pressure of CO_2 produced ammonia was kept in aqueous phase. The problem was toxicity of free (non dissociated) ammonia to methane producing bacteria. The protein parts of substrate were easily degradable and produced biogas. The fast decomposition of protein could also increase ammonia concentration over limits of toxicity which was followed by rapid decline in biogas production.

The main action in anaerobic digestion was of butanoic fermentation of polymers. In the last degradation step, methane was mainly formed from the hydrogen and carbon dioxide or from acetate. In this step, ammonia might have caused inhibitory effect on the anaerobic process due to inhibition of acetate utilizing (aceticlastic) methanogens (Chen *et al.*, 2008).

Best and cheap method for biomethanation of substrates rich in nitrogen was co-fermentation with low nitrogen substrates achieving the C:N ratio higher than 20-40:1 w/w (Straka *et al.*, 2007). Shilpakar and Shilpakar (2009) reported that the C: N ratio ranging from 20 - 30:1 was considered as optimum for anaerobic digestion. In addition to providing complementary trace elements, co-digestion

also provided a more optimal C: N ratio and improved buffering capacity, and thus had positive synergetic effects on both gas production and process stability (Angelidaki and Ellegaard, 2003). The nutrient limitation could be overcome either by addition of trace elements (Demirel and Scherer, 2011) or by co-digestion with a nutrient-rich material, such as manure (Wu *et al.*, 2010). This anaerobic digestion released biogas while converting an unstable and nutrient rich organic substrate like manure into a more stable and nutrient rich material with a reduced pathogen load (Rota *et al.*, 2012).

As a result of the digestion process, a number of changes in the composition of slurry can be expected. These include a substantial reduction (upto 25%) in solid content and a consequential increase in ash content due to the conservation of minerals and reduced slurry carbon content. Increase in slurry pH upto 0.5 pH units and ammonium nitrogen (N) content (upto 25%) were also noted, though these changes were less consistent than the reduction in solid content and organic matter content, and might be transient or dependent on digester operating conditions and the analysis of the feedstock slurries (Smith *et al.*,2007).

The anaerobic digestion consisted of different stages viz., hydrolysis, fermentation, acetogenesis and methanogenesis all of which were performed by digestive group of microorganisms leading to overall degradation of complex organic compounds (Angelidaki *et al.*, 2011). Micro organisms achieved anaerobic digestion in two steps; the first was transformation of complex substances into intermediate compounds like acetic acid and hydrogen which became the food for the methanogenic micro organisms during the second step (Rota *et al.*, 2012).

2.3.1. Hydrolysis

In the initial hydrolysis stage, polymers such as carbohydrates, fat and proteins in the raw materials were hydrolysed by extracellular enzymes to monomeric fatty acids, simple sugars and amino acids (Dieter and Angelika, 2008).

Fantozzi and Buratti (2009) reported that the anaerobic digestion process was characterized by a series of biochemical transformations brought about by different consortia of bacteria. Firstly, organic materials of the substrate like cellulose, hemicellulose and lignin must be liquified by extracellular enzymes, then it was treated by acidogenic bacteria. The rate of hydrolysis depended on the pH, temperature, composition and concentration of intermediate compounds.

The first phase consisted of hydrolysis of the substrate into simple molecules such as fatty acids, simple sugars and alcohol causing a decrease in pH of the substrate up to the second phase of degradation, and the transformation product from the first phase was acetates (Zupancic and Grilc, 2012).

In most cases, biomass was made up of large organic compounds. For the microorganisms in anaerobic digesters to access the chemical energy potential of the organic material, the organic matter macromolecular chains should first be broken down into their smaller constituent parts. These constituent parts or monomers such as sugars were readily available to microorganisms for further processing. The process of breaking these chains and dissolving the smaller molecules into solution was called hydrolysis. Therefore hydrolysis of high molecular weight molecules was the necessary first step in anaerobic digestion. Simple molecules created through the acidogenesis phase were further digested by acetogens to produce largely acetic acid (or its salts) as well as carbon dioxide and hydrogen (Zupancic and Grilc, 2012).

2.3.2. Acetogenesis

In acetogenesis stage, the monomers were further degraded by acetogenic bacteria to hydrogen gas, carbon dioxide, alcohols, organic acids (including acetate), ammonia (NH_3) and hydrogen sulphide (H_2S). The soluble organic components including the products of hydrolysis were converted into organic acids, alcohols, hydrogen and carbon dioxide by acidogens (Chynoweth *et al.*, 2001). This phase was indicated by a rise in pH. In the last stage, the production of biogas from the products of the acetogenic phase was performed using

methanogenic bacteria. Each step of the biochemical process highlighted different bacterial populations (Martinez *et al.*, 2015).

Acetates and hydrogen produced in the hydrolysis could be used directly by methanogens. Other molecules such as volatile fatty acids (VFA's) with a chain length that was greater than acetate must first be catabolised into compounds that could be directly utilised by methanogens. The biological process of acidogenesis occurred where there was further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here volatile fatty acids were generated along with ammonia, carbon dioxide and hydrogen sulphide as well as other by-products. The third stage in anaerobic digestion was acetogenesis (Zupancic and Grilc, 2012).

2.3.3. Methanogenesis

In the final stage, strictly anaerobic methanogenic archaea earned methane mainly from CO_2 and H_2 (hydrogenotrophic methanogens) or acetate (acetotrophic methanogens), and also small amounts from dinitrogen (N_2), NH_4 and H_2S (Deublein and Angelika, 2008).

The products of the acidogenesis were converted into acetic acid, hydrogen and carbon dioxide. Methane was produced by methanogenic bacteria from acetic acid, hydrogen and carbon dioxide from other substrates of which formic acid and methanol were the most important (Chynoweth *et al.*, 2001).

The final stage of anaerobic digestion was the biological process of methanogenesis. Here methanogenic archaea utilise the intermediate products of the preceding stages and converted them into methane, carbon dioxide and water. These components made up the majority of the biogas released from the system. Methanogen besides other factors were sensitive to both high and low pH values and the best performed well between pH 6.5 and 7.2. The remaining, non-digestible organic and mineral material, which the microbes could not feed upon, along with any dead bacterial residues constituted the solid digestate (Zupancic and Grilc, 2012).

2.4. FACTORS AFFECTING ANAEROBIC DIGESTION

The process of biogas production depended upon parameters such as anaerobic condition during production, temperature in fermenter, pH value of substrate, uniformity and pressure in fermenter etc (Mursec *et al.*, 2009).

2.4.1. Importance of C: N Ratio in Biogas Production

The relationship between the amount of carbon and nitrogen present in organic materials is expressed in terms of C: N ratio. To achieve a stable and efficient biogas process, the nutrient composition of the substrate material is of great importance. While using straw for biogas production, the C:N ratio and level of trace elements are limiting factors. To obtain optimal microbial growth in a biogas reactor, the C: N ratio of the ingoing input material should be about 20 to 30:1 (Igoni *et al.*, 2008). If the C: N ratio was too high (>30), which was typically the case for many plant-based materials including straw (C:N ~ 100) resulted in nitrogen limitation of microbial growth and consequently low efficiency of the biogas process. The level of trace elements was also very important, as these were essential for the activity of many microorganisms and low levels had been shown to be a limiting factor during biogas production from plant-based material (Schattauer *et al.*, 2011).

Plant materials such as crop residues were difficult to digest than animal wastes (manures) because of difficulties in achieving hydrolysis of cellulosic and lignin constituents with abundant acidity in the biogas system leading to reduction and sometimes cessation of gas flammability or gas production (Ukpai and Nnabuchi, 2012). For good biogas production, plant biomass at a harvesting humidity over 45 per cent and with C: N ratio ranging from 20 to 30:1 is especially suitable (Herout *et al.*, 2011).

In microorganisms, biomass ratio of C:N:P:S was approximately 100:10:1:1. The ideal substrate C: N ratio was 20-30:1 and C: P ratio 150-200:1. The C: N ratio higher than 30 caused slower microorganism multiplication due to

low protein formation and thus low energy and structural material metabolism of microorganisms. Consequently lower substrate degradation efficiency was observed. On the other hand, the C:N ratio as low as 30:1 resulted in successful digestion. However, when substrate with low C:N ratios and high nitrogen were applied (that is often the case using animal farm waste) a possible ammonium inhibition should be considered (Zupancic and Grilc, 2012).

A C:N ratio ranging from 20 - 30:1 was considered optimum for anaerobic digestion. If the C: N ratio was very high, the nitrogen would be consumed rapidly by methanogens for meeting their protein requirements and would no longer react on the left over carbon content of the material. As a result, gas production would be low. On the other hand, when the C: N ratio was very low, nitrogen would be liberated and accumulated in the form of ammonia (NH_4). The NH_4 would increase the pH value of the content in the digester. A pH higher than 8.5 resulted in toxic effect on methanogen population. Animal waste, particularly cattle dung, had an average C:N ratio of about 24 (Zupancic and Grilc, 2012).

The recommended ratios of carbon and nitrogen for good biogas production was 10-30:1 and the C:N:P:S ratio were 600:15:5:3 (Schmidt *et al.,* 2014). Micro elements (trace elements) like iron, nickel, cobalt, selenium, molybdenum or tungsten were equally important for the growth and survival of the anaerobically digested micro organisms. Sufficient provision of nutrients and trace elements as well as too high digestibility of the substrate caused inhibition and disturbances in the anaerobic digestion processes (Shahidi and Janak-Kamil, 2001).

2.4.2. Other Elemental Ratios Influencing Biogas Production

As per Kayhanian and Rich (1995), the recommended optimum concentrations of micronutrients for biogas production were: Fe 100-50000 $\mu g\ kg.ts^{-1}$, Ni 5-20 $\mu gkg.ts^{-1}$, Co less than 1-5 $\mu g\ kg.ts^{-1}$, Mo less than 1-5 $\mu g\ kg.ts^{-1}$ and W less than 1$\mu g\ kg\ ts^{-1}$. As per the reports by Oechner *et al.,* (2008) the recommended micronutrient concentrations for optimum biogas production were Fe 750-5000 $\mu g\ kg.ts^{-1}$, Ni 4- 30 $\mu g\ kg.ts^{-1}$, Co 0.4 -1 $\mu g\ kg.ts^{-1}$,

Mo 0.05-16 µg $kg.ts^{-1}$, W 0.1-30µg $kg.ts^{-1}$. Mn 100-1500 µg $kg.ts^{-1}$, Cu 10-80 µg $kg.ts^{-1}$, Sc 0.5-4.0 µg $kg.ts^{-1}$ and Zn 30-400 µg $kg.ts^{-1}$.

Acidification process due to deficiency of Fe and Ni from methanogenic achaea, the addition of Fe and Ni at lower dosage during the third phase caused process stabilization. When a few days after Fe deplete to 5.85 mg l^{-1}, anaerobic process collapsed. In Fe depleted reactor, mainly propionic acid was accumulated upto more than 700 mg l^{-1} and acetic acid concentration only increased significantly after the collapse. In Ni depleted reactor, both acids were accumulated at significant levels at levels up to 1500 mg l^{-1} and decreased again after Ni addition (Shahidi and Janak-Kamil, 2001).

Schmidt *et al.* (2014) reported that the trace elements were to be augmented for the anaerobic digestion of wheat silage at high organic load rates. An impact of Fe and Ni deficiency occurred after two hydraulic retention times while Co and W seemed to affect the process on a long term (less than 7 hydraulic retention times). The depletion of Fe seemed to influence not only methanogenesis but propionate oxidizing bacteria as well.

Methanogenesis needed Fe, Co and Ni to make methane production feasible (Li *et al.*, 2014). The optimal H_2/CO_2 ratio of 3.45 - 3.7 produced gas with high calorific value (Jurgensen *et al.*, 2015).

In anaerobic digestion systems, a characteristic phenomenon was observed. Some substances which were necessary for microbial growth in small concentrations inhibited the digestion at higher concentrations. 100-200 mgl^{-1} sodium was optimum for biogas production and above 3500 mg l^{-1} it was inhibitory. Potassium content of 200-400 mg l^{-1} was optimum for biogas production and above 2500 mg l^{-1} it was inhibitory. When calcium is present in 100-200 mg l^{-1}, it was good for biogas production and beyond 2500 mg l^{-1}, it was inhibitory. Magnesium concentration between 75 and 150 mg l^{-1} was optimum for biogas production and beyond f1000 mg l^{-1}, it was inhibitory. Heavy metals also had stimulating effects on anaerobic digestion in low concentrations,

however higher concentrations were toxic. In particular, lead, cadmium, copper, zinc, nickel and chromium caused disturbances in anaerobic digestion process (Zupancic and Grilc, 2012).

Mineral ions, heavy metals and the detergents were some of the toxic materials that inhibited the normal growth of pathogens in the digester. Small quantity of mineral ions (e.g. sodium, potassium, calcium, magnesium, ammonium and sulphur) also stimulated the growth of bacteria while very heavy concentration of these ions had toxic effect. Similarly, heavy metals such as copper, nickel, chromium, zinc, lead, etc. in small quantities were essential for the growth of bacteria but their higher concentration had toxic effects. Likewise detergents including soap, antibiotics, organic solvents, etc. inhibited the activities of methane producing bacteria and addition of these substances in the digester should be avoided (FAO, 1996).

2.4.3. Temperature of Biogas Unit

Anaerobic digestion operated at a wide range of temperature between 5^0C-65^0C. Generally there were three widely known and established temperature ranges for operation *viz.*, psychrophilic (15-20^0C), mesophilic (30-40^0C) and thermophilic (50-60^0C). With increasing temperature the reaction rate of anaerobic digestion strongly increased. For instance with ideal substrate, thermophilic digestion could be approximately four times faster than mesophilic. However using real waste substrates, there were other inhibitory factors that influenced digestion, that made thermophilic digestion approximately two times faster than mesophilic digestion (Zupancic and Grilc, 2012).

The process of biomethanation was temperature dependent and slowed down considerably below 30^0C, optimum being 35-38^0C which was known as mesophillic range. Above this temperature, the process slowed down between 40 and 45^0C and resulted in a peak between 55^0C and 60^0C which was known as thermophilic range. When the temperature decreased by 11^0C from the ambient temperature, gas production was nearly half (Mital, 2007).

Operation temperature influenced the ammonium toxicity. It increased with increasing temperature and could be relieved by decreasing the process temperature. However, while decreasing the processing temperature to 50^0C or below, the growth rate of the theromophilic micro-organisms dropped drastically and the risk of the microbial population wash out occured due to a growth rate lower than the actual hydraulic retention time (Angelidaki *et al.*, 2004).

2.4.3.1. Thermophilic Digestion

Potential advantages of thermophilic processes were increased degradation rates of organic solids, higher methane yields and increased inactivation of pathogens (Buhr and Andrews, 1977).

The effect of temperature on biogas production in vegetable waste was moderate. The independent effect of pressure on biogas production seemed to be predominant as compared to the independent effect of temperature. Ambient temperature in biogas production also was significant compared to the independent effect of ambient temperature (Anuraja and Guruswamy, 1998).

An alternative strategy to reach high biogas production was to increase the operating temperature (Wilki *et al.*, 2000). The solubility of various compounds (NH_4, H_2, CH_4, H_2S and volatile fatty acids) also depended on the temperature. This was of great significance to materials which had an inhibiting effect on processes (Shahidi and Janak-Kamil, 2001).

Psycrophilic temperature was shown to be less efficient than mesophilic processes (Leven *et al.*, 2012). The anaerobic digestion processes usually run at 30-40^{0}C (mesophilic) or 50-60^{0}C (thermophilic) temperatures. Mesophilic temperatures were well documented to display good operating performance and to be less sensitive. High temperature resulted in high microbial activity and faster degradation of organic materials, allowing shorter hydraulic retention time and higher organic load rate. The conventional temperatures used for digestion were mesophilic (35-40^{0}C) and thermophilic (50-65^0 C) (Kim *et al.*, 2002).

The viscosity of the anaerobically digested substrate was inversely proportional to temperature. This means that the substrate with more liquid at high temperatures and diffusion of dissolved material was facilitated. In thermophilic operations high temperature resulted in faster chemical reaction rates and thus better efficiency of methane production, higher solubility and lower viscosity. The higher demand for energy in the thermophilic process was justified by the higher biogas yield. It was important to keep a constant temperature during the digestion process as temperature changes or fluctuations affected the biogas production negatively. Thermophilic bacteria were sensitive to temperature fluctuation of $\pm 1^0$C and required longer time for it to adapt to a new temperature in order to reach the maximum methane production. Mesophillic bacteria were less sensitive. Temperature fluctuations $\pm 3^0$C were tolerated without significant reduction in methane production (Shahidi and Janak-Kamil, 2001).

The hydrolysis rate of cellulose was also shown to be higher at thermophilic than at mesophilic temperature. On the other hand, thermophilic processes were typically less stable (Ge *et al.*, 2011). During digestion, nitrogen rich substances in thermophillc temperatures could be problematic due to relatively increased fraction of NH_4-N (Moestedt *et al.*, 2013)

Thermophilic digestion gave higher biogas and methane productivity than mesophilic and was able to operate at the higher organic load rate (OLR), where mesophilic digestion showed signs of instability. Thermophilic operation allowed higher loadings to be applied without loss of performance, and gave a digestate with superior dewatering characteristics and very little foaming potential. The thermophilic process could operate stably at this organic load rate (OLR) and recovered more than 68% of the calorific value than as methane (Shanti *et al.*, 2013).

The results showed that operation at 44^0C was the most successful strategy, resulting in 22% higher methane yield compared with the mesophilic reactor, despite higher free ammonia concentration. Furthermore, kinetic studies revealed

higher biogas production rate at 44^{0}C compared with 38^{0}C, while the level of hydrogen sulphide was not affected (Moestedt *et al.*, 2014).

2.4.4. Hydraulic Retention Time (HRT)

The time taken by the substrates for the maximum gas production is the hydraulic retention time and 70-80% of digestion got completed on hydraulic retention time (Tomor, 1995). Retention time (also known as detention time) is the average period that a given quantity of input remains in the digester to be acted upon by the methanogens. In a cow dung plant, the retention time is calculated by dividing the total volume of the digester by the volume of inputs added daily. Thus, a digester should have a volume of 50 - 60 times the slurry that is added daily. But for a night soil biogas digester, a longer retention time (70 - 80 days) was needed so that the pathogens present in human faeces were destroyed. The retention time was also dependent on the temperature and upto 35^{0}C, higher the temperature, the lower the retention time (Lagrange, 1979).

Anuraja and Guruswamy (1998) reported that the increasing trend in gas production was observed after eight days of fermentation and period and it continued upto 28th day of fermentation, there after it increased appreciably upto 56th day of retention period.

Hydraulic retention time varied according to temperature and substrate. The shorter hydraulic retention time was likely to face the risk of washout of active bacterial population while longer retention time required large volume of digester (Yadvika *et al.*, 2004).

Hydraulic retention time was correlated with the digester volume and the volume of substrates fed per unit time. A shorter hydraulic retention time provided a good substrate flow rate but lower gas yield. It was therefore important to adopt the hydraulic retention time to the specific decomposition rate of the used substrates, and it was possible to calculate the necessary digester volume (Mital, 2007).

An almost complete degradation of the input substrate was achieved when the hydraulic retention time was more than 100 days. It was reported that the residual methane yield was significantly correlated to the hydraulic retention time (r=-0.73) (Ruile *et al.*, 2015).

Higher gas production rate was obtained at shorter hydraulic retention time of 19 days. However, higher methane content of the biogas was obtained at longer hydraulic retention time of 27 days. Therefore enhancement of methane production from co-digestion could be achieved by significant operating hydraulic retention time (Ratanatamskul *et al.*, 2014)

2.4.5. Total Solid Concentration

The biomass as renewable source can be utilized for production of biogas which can be produced through anaerobic digestion of animal excreta and other agricultural wastes. The main raw material used for biogas production is cattle dung and most of the biogas plants installed in India are operated at 10% total solids concentration. To achieve this, equal quantity of water should be added with dung. The results indicated that on an average 203 litre of biogas kg^{-1} dry matter was produced from cattle dung at total solid concentration of 15 per cent in modified Janata biogas plant with an average methane content of 60% (Palled *et al.*, 2012).

At 10% total solid level, the cumulative gas production was maximum (345.8 L). The maximum per cent of methane was recorded in the gas produced from cattle dung at 10% total solid (Mathad *et al.*, 2013).

2.4.5.1. Volatile Solid Concentration

The weight of organic solids burned off when heated to about 538^0C is defined as volatile solids. The biogas production potential of different organic materials, can also be calculated on the basis of their volatile solid content. Higher the volatile solid content in a unit volume of fresh dung, the higher the gas production. One kg of volatile solids in cow dung would yield about 0.25 m^3 biogas (Sathianathan, 1975).

Anaerobic fermentation reduced the volatile solid ratio to total solid ratio, and volatile solids degradation efficiencies of the reactors were approximately 40-45% (Arioci and Kocra, 2015).

2.4.5.2. Volatile Fatty Acids

Van-Lier *et al.* (1993) reported that accumulation of propionic acid and decreased gas yield caused shorter hydraulic retention time which led to decline in specific methane production and process instability. A moderate increase in volatile fatty acid concentrations caused foaming. The increase in volatile fatty acid concentrations and reduced specific methane production clearly indicated decreasing efficiency of degradation. The increased volatile fatty acid concentration was a result of increased efficiency of hydrolysis and fermentation (Schmidt *et al.*, 2014). Lindorfer *et al.* (2008) reported that accumulation of volatile fatty acids finally led to process failure.

2.4.6. pH of the Digester

All the reactors performed stable process during starting phase with a pH of about 7.3 (Schmidt *et al.*, 2014). The pH value of the anaerobic digestion of substrate influenced growth of methanogenic microorganism and effected the dissociation of some compounds important for the anaerobic digestion process. Results showed that methane formation took place within a relatively narrow pH interval from about 5.0 to 8.5 with an optimum interval between 7.0 and 8.0. The foremost methanogenic bacteria and an acidogenic microorganism usually had lower value of pH (Mital, 2007). The value of pH could be increased by ammonia produced during degradation of proteins or by the presence of ammonia in the dead stream, while the accumulation of volatile fatty acid decreased the pH value (Schmidt *et al.*, 2014). The pH value inside the digester depended on the partial pressure of carbon dioxide and on the accumulation of alkaline and acid compounds in the liquid phase (Vilniskis *et al.*, 2011).

Anaerobic digestion under elevated pressure conditions led to decreasing pH values in the digestate due to augmented formation of carboxylic acid. The pressurized anaerobic filter had a major influence on the methane content of biogas produced. The higher NH_4 content led to higher pH value in the digester (Lemmer *et al.,* 2015). However, there was some evidence of ammonia inhibition probably due to the uncontrolled pH employed (Abubakar and Ismail, 2012).

In anaerobic digestion, pH was mostly affecting the methanogenic stage of the process. The pH optimum for the methanogenic microorganisms was between 6.5 and 7.5. If the pH decreased below 6.5, more acids were produced and that led to imminent process failure. In real digester systems with suspended biomass and substrate containing suspended solids, normal pH of operation was between 7.3 and 7.5. When pH decreased upto 6.9 already serious actions to stop process failure must be taken. When pH value decreased, CO_2 was dissolved in the reactor solution as uncharged molecules. With increasing pH value dissolved CO_2 from carbonic acid ionized and released hydrogen ions. At pH 4 all CO_2 was in form of molecules. At pH 13, all CO_2 was dissolved as carbonate. The centre point around which pH value swings with this system was at pH 6.5. With decreasing pH value, ammonium ions were formed with releasing of hydroxyl ions. With increasing pH value more free ammonia molecules were formed. The centre point around which pH value swings with this system was at pH 10 (Zupancic and Grilc, 2012).

The optimum biogas production was achieved when the pH value of input mixture in the digester was between 6 and 7. The pH in the biogas digester was also a function of the retention time. During the initial period of fermentation, large amounts of organic acids were produced by acid forming bacteria. The pH inside the digester decreased upto 5. This inhibited or even stopped the digestion or fermentation process. Methanogenic bacteria were very sensitive to pH and did not thrive below a value of 6.5. Later as the digestion process continued, concentration of NH_4 increased due to digestion of nitrogen which increased the pH value to above 8. When the methane production level was

stabilized, the pH range remained buffered between 7.2 and 8.2 (FAO, 1996).

2.4.7. Loading Rate

Loading rate is the amount of raw materials fed per unit volume of digester capacity per day. If the plant was overfed, acids would accumulate and methane production was inhibited. Similarly, if the plant was underfed, the gas production was low (FAO, 1996).

2.4.8. Microbial Activity and Population

Microbiological population showed that methanogenic archaea and syntrophic acetate-oxidising bacteria had responded to the new process temperature while sulphate-reducing bacteria were only marginally affected by the temperature change (Bardya *et al.*, 1996).

The microbial metabolism, processes depended on many parameters. Therefore, these parameters must be considered and carefully controlled in practice. Furthermore the environmental requirements of acidogenic bacteria differed from the requirements of methanogenic archaea. Provided that all steps of the degradation processes showed take place in one single reactor (one-stage process). Usually methanogenic archaea requirements must be considered with priority. Namely these organisms have much longer regeneration time, much slower growth and were more sensitive to environmental conditions other than bacteria present in the mixed culture (Zupancic and Grilc, 2012).

For hydrolytic and acidogenic bacteria, the optimum conditions were: temperature 25-35^{0}C, pH value of 5.2-6.3 and substrate C:N:P:S ratio of 500:15:5:3. But methanogenic bacteria required two temperature ranges *viz.*, mesophilic (30-40^0 C) and thermopilic (50-60^0 C). A pH range between 6. 5 and 7.5 and the substrate C: N: P: S ratio of 600:15:5:3 was more optimum for methanogenic bacteria. The presence of Ni, Co, Mo and Se were essential for good performance of methanogenic bacteria (Zupancic and Grilc, 2012).

2.5. COMPOSITION OF BIOGAS

Methane is most valuable component of biogas. If methane accounted for more than 60% of biogas it was considered to be a valuable fuel (Noyola *et al.*, 2006). Biogas consisted of 60 - 65% methane and remaining portions were carbon dioxide, traces of hydrogen sulphide, ammonia and other impurities which were toxic (Mital, 2007). Besides, biogas consistsed of NH_4, CO_2, H_2O, N_2, H_2S and O_2 (Cepanko and Baltrenas, 2011). Herout *et al.* (2011) reported that the highest methane content was observed from liquid beef manure and maize silage in the ratio of 60:40 when compared to biogas produced from maize silage, liquid beef manure, grass haylage and ray grain alone.

The gas obtained at the beginning had higher percent of carbon dioxide and other gas constituents. The percent of methane content recorded was maximum (63.0%) in vegetable waste substrate during the fifth week of the fermentation period. There after it varied appreciably from 63-55%. The average percent of methane content for the entire retention period was found to be 53.12% where as for control treatment the average methane (CH_4) content was 46.76% (Anuraja and Guruswamy, 1998). Thy (2003) opined that on an average, biogas contained 55-65 per cent methane, 35-45 percent carbon dioxide, 0-3 percent nitrogen, 0-1 percent hydrogen and 0-1 percent hydrogen sulphide.

The amount of carbon and nitrogen in nutrient sources affected the growth of microorganism and the biogas production. Qualitative analysis of biogas components for paddy chaff and water hyacinth indicated that the methane content was high for both whereas CO_2, H_2S and CO were found in variable proportion according to the source of organic wastes (Saravanan and Manikandan, 2012).

The general composition of biogas mixture depended upon the source of feed stock and the management of digestion process. The biogas typically composed of 50-70% methane, 30-40% carbon dioxide, 1-10% hydrogen, 1-3% nitrogen, 1% oxygen, carbon monoxide and traces of hydrogen sulphide (Wantanee and Sureelak, 2004).

The National Centre for Energy Research and Development, University of Nigeria, investigated the anaerobic digestion and generation of biogas from three types of wastes viz., cow dung, cowpea and cassava peeling. The result obtained from the gas production showed that the cowpea produced the highest methane content of 76.2% followed by cow dung with 67.9% methane content. Cowpea had the highest carbon dioxide content of 33.2% followed by cassava peeling with 32.2% of carbon dioxide content (Ukpai and Nnabuchi, 2012).

According to Parajuli (2011), gas chromatography (GC) was an optimal analytical tool for the analysis of components of biogas such as methane, carbon dioxide and hydrogen sulfide. The instrument had several advantages such as high resolution, high speed, high sensitivity and good quantitative results. Callaghan *et al.* (1999) used gas chromatograph fitted with a Porapak Q packed column to estimate the methane and carbon dioxide concentrations in the biogas.

The sulphate present in material was converted to hydrogen sulphide (H_2S) by sulphate reducing bacteria consuming organic material that would otherwise be destined for biogas production (Moestedt *et al.*, 2013). Production of hydrogen sulphide was negative as they have odours, which are toxic, corrosive and inhibitory for microorganisms involved in an anaerobic digestion (Schmidt *et al.*, 2014).

2.6. BIOGAS SLURRY

The use of biogas slurry as manure gives double advantage of biogas plant. The fibrous material, inorganic solids which could not be digested or converted into methane either settled down in the plant or come out with slurry liquid through outlet. This contained many rich and nutritive elements including nitrogen, phosphorus, potassium, iron and trace elements (Zn, Fe, Ni, Cu, Cd, Cr, Br, Ca, Na) (Gupta, 2007). These residues especially biogas slurry were a good source of plant nutrients and improved the soil properties (Garg and Kaushik, 2005).

Biogas slurry consisted of 93% water, 7% dry matter of which 4.5% was organic and 2.5% was inorganic matter. The percent NPK content in slurry on wet basis was 3.6, 1.8 and 3.6 respectively. In addition to major plant nutrients, it also provided micronutrients such as Zn and Cu (FAO, 1996).

The nitrogen in animal manure was normally available in an organic form but after passing through the fermentation process in a biogas digester it was changed by bacteria to inorganic form, mostly ammonia nitrogen (NH_4^+), which was easily soluble and utilized by crop plants (Nasir *et al.*, 2010).

2.6.1. Effect of Biogas Slurry in Soil

Slurry as such helped in maintaining soil fertility, soil pH and improving fodder production. The application of slurry to crop land was an alternative option to its disposal because the physical properties of soil were improved and nutrients were supplied by slurry (Mosquera *et al.*, 2000). Froseta *et al.*, (2013) noted that the use of digestate was effective in increasing soil aggregate stability.

Nitrogen and most other nutrients were preserved in the residues and could be used for manuring (Messe *et al.*, 2007). Organically bound N in manure and crop residues was mineralized to ammonium (NH_4^+) a soluble form of N so that plant roots could easily absorb (Moller and Stinner, 2009). Anaerobic digestion in a biogas plant resulted in a residue that differed profoundly from the raw materials fed to the process in having residues with a higher pH, lower contents of dry matter and total carbon (C). It also had higher proportion of ammoniacal or nitrate nitrogen to total N and a lower carbon to nitrogen C:N ratio. However there was generally no alteration in total nitrogen (N), potassium (K) and phosphorus (P) (Field *et al.*, 1984; Kirchmann and Witter, 1992). Therefore application of biogas residues could be expected to lead to different effects in arable soil compared with the use of regular organic fertilizers (Leven *et al.*, 2012; Engwall and Schnurer, 2002)

Ding *et al.* (2015) studied the effects of biogas slurry on the growth and quality of bean and soil fertility. The results showed that biogas slurry could not only increase the bean production, but also improved the nutritional quality of the bean. In addition, the physical and chemical characters of soil were also improved with increase of soil organic matter, N, K and other trace elements.

The fertilizers consistently stimulated a higher bacterial growth than the no-fertilizer control. The liquid digestate resulted in a level of bacterial growth higher or equal to that of mineral fertilizer while undigested slurry resulted in lower bacterial growth. Effects of these fertilizer on bacterial growth mirrored the effects on plant growth (Walsh *et al.*, 2012)

Factors that influenced nitrogen availability from slurry are its inorganic N content, digestion process (aerobic or anaerobic), C: N ratio, pH, the method and time of application, soil type and properties (Warman and Termeer, 2005). Due to the decomposition and breakdown of its organic content, digested slurry provided fast acting nutrients that easily entered the soil solution thus becoming immediately available to plants. They simultaneously served as primary nutrients for the development of soil organisms, e.g. the replenishment of microorganisms that are lost through exposure to air in the course of spreading the slurry over the fields. They also nourished actinomycetes that acted as organic digesting specialists in the digested sludge (Kossman and Ponitz, 1996). The addition of organic matter to slurry was useful for maintaining or increasing the organic substances or nitrogenous compounds in soil that decomposed slowly but steadily (Balsari *et al.*, 2005). The slurry produced by a biogas plant was considered to be an effective fertilizer and soil conditioner. Farmyard manure was not as rich in micro nutrients as biogas slurry (Gupta, 2007).

The interactions of slurries with soil, mainly due to soil pH and texture were relevant for adsorption, fixation, immobilization of ammonium and microbial nitrogen turn over process (Huijasmans and Mol, 1999).

2.6.2. Biogas Slurry and Seed Germination

Lakshman (1988) reported that higher bhindi vegetable yields were obtained by pelleting seed with biogas slurry. Presoaking seed treatment had become increasingly popular in telescopic period of germination besides enhancing its per cent vigour index. Biogas slurry was known to contain significant amounts of growth promoting substances (Chawala, 1986) which had a positive impact on the seed germination. Among the different duration of seed treatments, tested seed soaking for six hours had been found to be the most effective duration. Enhancing the seed treatment for 12 hours caused a reduction in germination. Similarly significant increase in root, shoot length and vigour index were observed when seeds were pretreated with biogas slurry for six hour duration.

After anaerobic fermentation, organic nutrients of these materials became more soluble in the soil with no decrease in nutritional values (Nasir *et al.*, 2010).

2.6.3. Biogas Slurry and Crop Response

The analysis of slurry showed that it was well digested and it could be used as enriched manure which was reflected in the improvement of nitrogen content in digested slurry. The improvement of nitrogen content in the digested slurry might be due to conversion of insoluble ammonium salt and break down of organic material in the digester resulting in increased nitrogen content (Srivastava *et al.*, 1993: Anuraja and Guruswamy, 1998). The effluent produced is an excellent fertilizer because of high concentration of ammonium. Slurry is one of the most environmentally sound organic fertilizers in use today. It did not pollute the atmosphere during its application and did not pose health hazards to the user and or to animals nearby (Rota *et al.*, 2012)

Islam *et al.* (2010) reported that the application of biogas slurry as nitrogen fertilizer stimulated the growth of maize fodder. Approximately 70 kg of slurry nitrogen was the optimum level for maize growth. Rahman *et al.* (2008) observed that approximately 67 kg of slurry N ha^{-1} was the optimum level for maize fodder

production. Increasing the level of slurry nitrogen presumably increased the availability of soil nitrogen than that of other macro and micro nutrients which might have enhanced meristematic growth and resulted in higher fodder yield. Beckwith *et al.* (2002) reported that crude protein content in cut grasses increased as the level of nitrogen in cattle slurry decreased the crude protein content 28 kg slurry N kg^{-1} which indicated that the excessive slurry N might have inhibited protein synthesis in maize fodder.

High P content of slurry might also have positively contributed to the biomass yield of maize. Rehman *et al.* (2008) observed that maize fodder biomass yield decreased in response to excessively high levels of slurry N. The ash content in maize might also vary due to individual mineral content in soil and slurry and the rate of biogas slurry application clearly affected NPK content in maize. The highest NPK and S content was obtained with 70kg slurry N ha^{-1}. Higher levels of biogas slurry reduced the yield and other fodder qualities which might be due to accumulation of some heavy metal in the slurry that reduced the yield and quality at higher levels (Islam *et al.*, 2010).

In the biogas slurry, element K was the most effective nutrient component due to its largest available fraction and highest mobility factor of 78.4. Ca and Mg could be viewed as potential nutrient sources because their mobility factor exceeded 60% (Neng-Min *et al.*, 2015).

Application of 50, 75 and 100% nitrogen through biogas slurry from poultry manure resulted in a significant increase in cob and stover yield of maize. The green fodder yields of cowpea which was grown on residual fertility were significantly influenced by biogas slurry produced from poultry manure. The soil fertility status of available major nutrients (N, P_2O_5 and K_2O) was found to be significantly higher in the treatment that received N through biogas from poultry manure and poultry manure (Shanti *et al.*, 2013).

It was also observed that the nitrogen, phosphorus and potassium contents of digested slurry (cattle dung after digestion) were 1.50, 1.40 and 0.48 per cent

respectively as compared to that of 1.26, 1.20 and 0.40 per cent respectively for fresh cattle dung which indicated that digestion of cattle dung at higher solid concentration also resulted in rich nutrient fertilizer (Palled *et al.*, 2012).

Biogas slurry proved to be of high quality organic manure compared to the farm yard manure as the digested sludge had more nutrients. However nutrients especially nitrogen were lost by volatilization when exposed to sunlight (heat) and by leaching due to rain (Karki *et al.*, 2005). Digested sludge contained organic nitrogen (mainly amino acids), minerals in abundance, and low molecular weight bioactive substances (hormones, humic acids, vitamins etc.) and could be used as organic fertilizer in seedlings. Use of the slurry also inhibited diseases and increased yields (Liu *et al.*, 2008).

Various slurry demonstrations were conducted by Tripathi and Mishra (2007) across India on two plots of equal size by sowing same crop. In addition to crop yields, the quality of vegetables such as size and shape were also observed. There were fewer weeds, low number of diseases and pests attack and improvement in soil physical and chemical properties of treatments where biogas slurry was applied.

Singh *et al.* (1995) in another study reported that lower yields of crops were produced by digested manure as a result of unavailability of N to crops at critical stages due to slow release rate. He also concluded that biogas slurry was better than organic manure and farm yard manure for obtaining a higher yield in pea, okra, corn and soybeans. In comparison with farm yard manure, the use of biogas slurry in combination with the recommended dose of fertilizer gave better yields.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

An investigation on 'Substrate impact on biogas production and manurial value of slurry' was conducted at the College of Horticulture, Vellanikkara, Thrissur ($76^0$11' E, $10^0$33' N, and 37.9 m above mean sea level). The objectives of the study were to analyze the composition of biogas as influenced by different substrates, and to analyse the biogas slurry generated from different substrates for nutrient composition, and to study the effect of biogas slurry on plant characteristics, seed germination and vigour index of vegetable cowpea.

3.1. Climatic Parameters

The climatic parameters were collected from the Department of Agricultural Meteorology, College of Horticulture, Vellanikkara, Thrissur. The average annual maximum temperature of the experimental site was 32.1^0C, the average minimum temperature was 23.3^0C and relative humidity was 74%. The average annual rainfall was 2777.5 mm and average sun shine hours was 5.4 h.

EXPERIMENT I

3.2. Methods of Biogas Production

Biogas was produced in $0.5m^3$ ordinary floating dome mobile biogas plant with 2 kg per day intake capacity (Plate 1). There were six treatments and three replicates per treatment and the experiment was laid out in completely randomized design (CRD) (Plate 3). Table 1 shows the details of different treatments used for the study. The materials for biogas production were collected from the different sources and are given in Table 2 (Plate 2).

Plate 1. Floating drum type biogas unit of 0.5 m^3 capacity

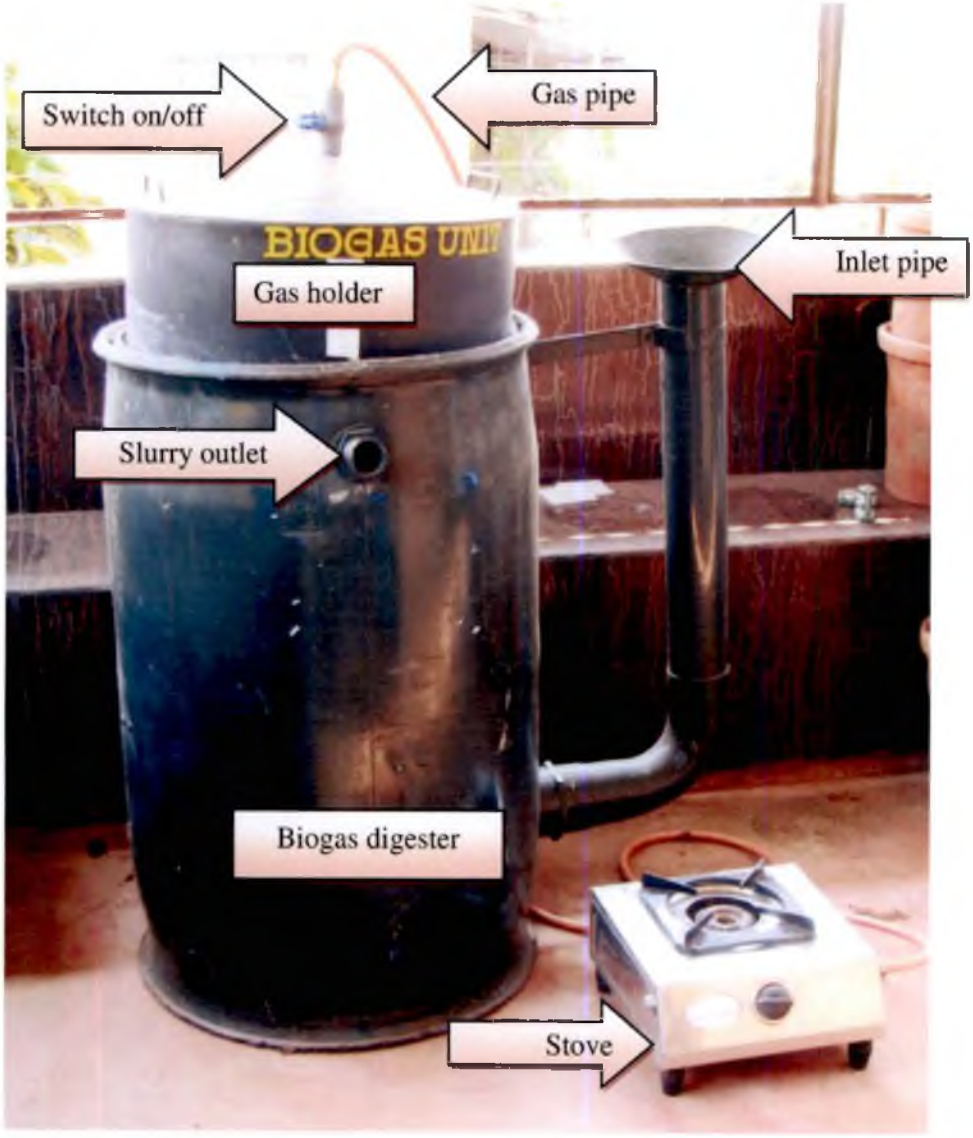


Table 1. Details of treatments for biogas production using different substrates

Treatments	Details
T_1	Cow dung + water (1:1)
T_2	Poultry manure + cow dung + water (1:1:2)
T_3	Goat manure + cow dung + water (1:1:2)
T_4	Biodegradable household waste + cow dung + water (1:1:2)
T_5	Elephant dung + cow dung + water (1:1:2)
T_6	Crop wastes from pulses + cow dung + water (1:1:2)

Table 2. Locations from where substrate collections were made for production of biogas

Sl.No.	Substrate	Source of collection
1	Cow dung	Vellanikkara, Thrissur
2	Poultry manure	Kerala Veterinary and Animal Science University Poultry farm, Mannuthy, Thrissur
3	Goat manure	Kerala Veterinary and Animal Science University Goat farm, Mannuthy, Thrissur
4	Pulse residue	College of Horticulture, Vellanikkara, Thrissur
5	Elephant dung	Peramangalam, Thrissur
6	Household wastes	Ladies Hostel, College of Horticulture.Vellanikkara, Thrissur

Plate 2. Substrates used for biogas production

Cow dung

Poultry manure

Goat manure

Biodegradable household waste

Elephant dung

Pulse residue

3.2.1. Biogas Experiment

Design : CRD

Treatments : 6

Replications : 3

From the second to sixth treatment, finely chopped specified substrate, cow dung and water were mixed in the ratio 1:1:2. In all treatments substrate combinations and water were taken on volume basis and mixed well thoroughly by hand mixing. For initial loading 200 litre of water loaded substrate is required for each biogas plant. Regular feeding was done according to the type of treatment. Treatment allocations @ 2l per day were done in all biogas plants.

3.2.2. Analysis of Substrate

The analysis of substrates was carried out on dry weight basis. After collecting the substrate, it was shade dried, followed by oven drying so that the substrate attained a constant weight before the analysis. The physical and chemical analyses of samples were carried out as per the procedures given in Table 3.

3.2.3. Analysis of Biogas

Hydraulic Retention Time (HRT) is the time taken for maximum gas production by measuring the height of gas holding drum. Hydraulic Retention Time was noted after the initial loading and the gas produced during the first 25 days were discarded in order to get a stabilized biogas production. After 25 days, biogas samples were collected at three days interval in gas collection bladders (Hans Seamless latex value bladders) and analyzed in the gas chromatography (Khoiyangbam *et al.*, 2004). The collection of biogas samples for analysis are presented in Plate 4. The gas chromatograph used for analysis of biogas was 'Thermo Scientific Trace 600 GC' with packed column injector (poropack-q) and Flame Ionization Detector (FID) with additional methanizer. By comparing with standard chromatogram, the carbon dioxide and methane content of samples were found out (Plate 5).

Table 3. Physico-chemical analysis of substrates used for biogas production

Parameter	Method	References
pH	Direct measurement using pH meter (substrate and water in 1:1 ratio)	Fertilizer Control Order (FCO), 1985
EC	Direct measurement using EC meter (substrate and water in 1:1 ratio)	
Organic carbon	Ashing method	
N	Microkjeldahl digestion and distillation	
P	Ashing- 25% HCl extract and spectrophotometry	
K	Ashing- 25% HCl extract- Flame photometry	
Ca, Mg, Fe, Mn, Zn and Cu	Ashing- 25% HCl extract- Atomic Absorption Spectrophotometry	

3.2.4. Daily Temperature of Biogas Unit

The daily temperature of the biogas unit was noted by using digital thermometer during the experiment period from 24.3.2014 to 26.02.2015. The observations were taken daily at 5.00 pm

3.2.5. Analysis of Biogas Slurry

Quantity of slurry generated after each intake of substrate (Plate 6) upto 24 hrs for all treatments were recorded. The pH and other chemical characteristics were found out. Standard procedures adopted for analysis of nutrient status of biogas slurry is given in Table 4.

Plate 3. Biogas production experiment at College of Horticulture

Plate 4. Biogas collection for analysis of composition

Table 4. Methods adopted for analysis of physico-chemical characteristics of biogas slurry

Parameter	Method	References
pH	Direct measurement using pH meter	Fertilizer Control Order FCO ,1985
EC	Direct measurement using EC meter	
Total N	Single acid digestion and distillation	
Organic carbon	Dry ashing method	
Nitrate nitrogen	MgO	
Ammoniacal nitrogen	Devarda alloy method	
P	Ashing- 25% HCl extract- spectrophotometry	
K	Ashing- 25% HCl extract- Flame photometry	
Ca, Mg, Fe, Mn, Zn, and Cu	Ashing- 25% HCl extract- reading taken in Atomic Absorption Spectrophotometry	

EXPERIMENT II

3.3. Pot Culture Experiment

Experimental design : CRD

Treatment : 9

Replications : 3

Variety : Bhagyalakshmy

The pot culture experiment was conducted at the College of Horticulture, Vellanikkara with 9 treatments and 3 replications (Plates 7 and 8). Garden soil and sand were mixed in 1:1 ratio and 3 kg of this mixture was filled in each pot. Twenty seven pots were filled with potting mixture and field capacity was attained by 0.5l per pot. After one week, germination of seeds was completed. Irrigation was continued till the second leaf emergence. After the second leaf emergence, treatments were started as given in Table 5.

Plate 5. Analysis of biogas composition using gas chromatography

Plate 6. Slurry obtained from different treatments

For treatments 4 to 9, the field capacity was maintained in the pots by application of slurry as per the treatment details. For treatments 1 and 2, the pots were maintained at field capacity using irrigation water. In the third treatment, pots were maintained at field capacity using supernatant solution of fresh cow dung slurry.

3.3.1. Biometric Observations of Plant

Biometric observations recorded were plant height, number of leaves per plant, number of branches per plant, number of pods per plant, number of seeds per pod, yield per plant and 100 seed weight.

3.3.2. Soil Analysis

Soil samples were collected and analysed for nutrient parameters before and after the experiment following standard procedures. The pH, EC, organic carbon, available nitrogen, available phosphorus, and available potassium, Ca, Mg, Fe, Mn, Zn and Cu were estimated before and after the pot culture experiment. The details of soil analyses are given in Table 6.

3.3.3. Plant Analysis

After the completion of crop harvest, the plants were uprooted, shade dried and oven dried still a constant weight was attained. The plants were ground to fine powder. The uptake of nitrogen, phosphorus and potassium by plants in each treatment was estimated. From this, sample required for plant analyses were taken. The standard procedures adopted for analysis are given in Table 7.

Plate 7. Initial stages of pot culture experiment.

Plate 8. Pot culture experiment during active flowering and fruiting stage

Table 5. Details of treatments in pot culture experiment

Treatments	Particulars
T_1	Absolute control (irrigated with irrigation water)
T_2	As per Kerala Agricultural University (KAU), Package of Practices Recommendations (Farmyard manure @ 20 kg ha^{-1} and 20:30:10 kg NPK ha^{-1}) water maintained at field capacity with irrigation water.
T_3	Unfermented cow dung slurry (cow dung and water in ratio 1:1)
T_4	Biogas slurry obtained from cow dung + water (1:1)
T_5	Biogas slurry obtained from poultry manure + cow dung + water (1:1:2)
T_6	Biogas slurry obtained from goat manure + cow dung + water (1:1:2)
T_7	Biogas slurry obtained from biodegradable household waste + cow dung + water (1:1:2)
T_8	Biogas slurry obtained from elephant dung + cow dung + water (1:1:2)
T_9	Biogas slurry obtained from crop wastes from pulses + cow dung + water (1:1:2)

Table 6. Details of soil analysis before and after pot culture experiment

Parameters	Methodology	Reference
pH	1:2.5 soil water suspension using pH meter	Jackson, 1958
EC	Direct measurement using EC meter (substrate and water in 1:2.5 ratio)	
Organic C	Wet oxidation	Walkley and Black, 1934
Available N	Alkaline permanganometry	Subbiah and Asija, 1956
Available P	Bray No.1 Extraction and estimated colorimetrically by reduced molybdate ascorbic acid blue colour method	Extraction (Bray and Kurtz, 1945) Estimation (Watanabe and Olsen, 1965)
Available K	Neutral normal ammonium acetate extraction followed by flame photometry	Jackson, 1958
Ca and Mg	Extraction with neutral normal ammonium acetate and estimation with atomic absorption spectrophotometery	Sims and Johnson, 1991
Fe, Mn, Zn and Cu	HCl extraction followed by Atomic Absorption Spectrophotometry	Sims and Johnson, 1991

Plate 9. Seed germination test using cowpea seeds.

Plate 10. Germinated seeds in different treatments.

Table 7. Standard procedures followed in plant and seed analyses

Parameters	Method	References
Total N	Single acid digestion (in the presence of copper sulphate and potassium sulphate) and Kjeldahl distillation method	Subbiah and Asija, 1956
Total P	Diacid extraction and spectrophotometry	Piper, 1966
Total K	Diacid extraction and flame photometry	Jackson, 1958
Ca, Mg, Fe, Mn, Zn, and Cu	Diacid extract - Atomic Absorption Spectrophotometry	Piper, 1966

3.3.4. Study of Seed Characteristics

The seeds obtained from different treatments were collected before uprooting of plants and shade dried. The germination per cent and vigour index were obtained. The uptake of nutrients by the seeds were found out using standard procedures (Table 7).

The nitrogen estimation of seeds was done and from this crude protein content was calculated by multiplying with 6.25.

EXPERIMENT III

3.4. Seed Treatment Studies

The cowpea seeds of var. Lola were purchased from Agriculture Technology Information Centre (ATIC), Mannuthy, Thrissur. The seeds were treated in six different slurries in three different ways to study the following:

a) effect of different biogas slurries on seed germination and vigour index.

b) best suitable method of seed treatment for seed germination and vigour index.

3.4.1. Methods for Seed Treatment

1) Seeds were soaked for 2 hours in biogas slurry.

   Seven separate sets of seeds were packed in cloth bag and soaked in six different biogas slurries for two hours. A control was maintained with distilled water.

2) Seeds dipped in biogas slurry.

   Separate sets of seeds were packed in cloth bag and dipped in six different biogas slurries and shade dried. A set control was maintained with distilled water.

3) Seeds were coated with biogas slurry

   Seven separate sets of seeds were mixed with 1% maida solution (Umarani *et al.*, 2014) and biogas slurry in 1:1ratio using 100g of seeds and dried under shade for 5 h. One set was maintained as control which was coated only with maida solution. The seed germination test of treated cow pea seeds are presented in Plate 9 and 10.

The treated seeds were kept for germination in Pseudomonas treated sand. Fourteenth day after sowing, germination per cent was recorded. The length of shoot and root were noted on 14^{th} day by pulling out the seedlings. The samples were washed and oven dried to record dry weight. The vigour index was calculated using the formula

Vigour index1 = Seedling length x Germination %

Vigour index 2 = Seedling dry weight x Germination %

(Abdul-Baki and Aandrason, 1973)

Table 8. Details of treatments for seed germination and percent vigour index.

Treatments	Details
TST_1	Seeds dipped in biogas slurry from cow dung and water in 1:1 ratio
TST_2	Seeds dipped in biogas slurry from cow dung, poultry manure and water combination in 1:1:2 ratio
TST_3	Seeds dipped in biogas slurry from cow dung, goat manure and water in 1: 1:2 ratio
TST_4	Seeds dipped in biogas slurry from cow dung, biodegradable household waste and water in 1:1:2 ratio
TST_5	Seeds dipped in biogas slurry from cow dung, elephant dung and water in 1:1:2 ratio
TST_6	Seeds dipped in biogas slurry from cow dung, pulse residue and water in 1:1:2 ratio
TST_7	Seeds dipped in irrigation water
TST_8	Seeds soaked in biogas slurry from cow dung and water in 1:1 ratio
TST_9	Seeds soaked in biogas slurry from cow dung, poultry manure and water in 1:1:2 ratio
TST_{10}	Seeds soaked in biogas slurry from cow dung, goat manure and water in 1:1:2 ratio
TST_{11}	Seeds soaked in biogas slurry from cow dung, biodegradable household waste and water in 1:1:2 ratio
TST_{12}	Seeds soaked in biogas slurry from cow dung, elephant dung and water in 1:1:2 ratio
TST_{13}	Seeds soaked in biogas slurry from cow dung, pulse residue and water in 1:1:2 ratio
TST_{14}	Seeds soaked in irrigation water
TST_{15}	Seeds coated with biogas slurry from cow dung and water in 1:1 ratio
TST_{16}	Seeds coated with biogas slurry from cow dung, poultry manure and water combination in 1:1:2 ratio
TST_{17}	Seeds coated with biogas slurry from cow dung, goat manure and water in 1: 1:2 ratio
TST_{18}	Seeds coated with biogas slurry from cow dung, biodegradable household waste and water in 1:1:2 ratio
TST_{19}	Seeds coated with biogas slurry from cow dung, elephant dung and water in 1:1:2 ratio
TST_{20}	Seeds coated with biogas slurry from cow dung, pulse residue and water in 1:1:2 ratio
TST_{21}	Seeds coated with one per cent maida solution

3.5. STATISTICAL ANALYSIS

Correlation studies of data were carried out by the method suggested by Panse and Sukatme (1978) using SPSS package. Analysis of variance in CRD was done using MSTATC package.

4. RESULTS

4. RESULTS

4.1. EXPERIMENT – I COMPOSITION OF BIOGAS AS INFLUENCED BY DIFFERENT SUBSTRATES

The results of the study conducted to elucidate 'Substrate impact on biogas production and manurial value of slurry' are presented in this chapter. For this we had selected six substrate for biogas production. Physico-chemical properties of the initial substrate are presented in Table 9.

4.1.1. Initial Analysis of Substrate

Table 9. Physico-chemical characteristics of different substrates used for the study

Characteristics	Substrates					
	Cow dung	Poultry manure	Goat manure	Household waste	Elephant dung	Pulse residue
pH	7.6	7.3	7.5	8.7	8.3	8.7
EC (dS m^{-1})	0.31	0.33	0.53	0.30	0.55	0.34
Organic carbon (%)	22.32	23.33	27.59	17.43	42.6	22.25
Total N (%)	0.74	1.03	0.97	0.74	0.93	0. 80
Total P (%)	0.33	0.22	0.43	0.45	0.23	0.30
Total K (%)	0.73	0.65	0.62	0.69	0.60	0.75
C:N	30:1	23:1	28:1	24:1	45:1	28:1
Total Ca (mg kg^{-1})	2581.6	779.1	2778.6	1217.8	1747.7	3104.0
Total Mg (mg kg^{-1})	158.8	158.5	144.8	148.4	153.8	150.1
Total Mn (mg kg^{-1})	200.9	224.0	225.3	81.8	35.7	157.1
Total Cu (mg kg^{-1})	24.3	34.6	38.1	25.8	41.5	15.9
Total Zn (mg kg^{-1})	36.3	45.6	19.1	27.6	21.8	18.7
Total Fe (mg kg^{-1})	352.5	367.1	410.6	437.8	226.6	443.3

4.1.2. Effect of Different Substrates on Composition of Biogas

In order to study the influence of different substrates on biogas composition, an experiment was carried out in completely randomized design with six treatments and three replications. The major gases namely methane (CH_4) and carbon dioxide (CO_2) were estimated using gas chromatograph (GC). Comparative evaluation of gas components from different substrates are presented in Table 10.

Table 10. Composition of biogas as influenced by different substrates

Gas composition / Treatment	Methane (per cent)	Carbon dioxide (per cent)	Other gases (per cent)
T_1	60.27 ^{b}	38.72^{b}	1.01
T_2	56.26 ^{c}	42.73^{a}	1.02
T_3	60.58 ^{b}	38.39^{b}	1.04
T_4	63.36 ^{ab}	35.60^{bc}	1.04
T_5	64.57 ^{a}	34.41^{c}	1.02
T_6	64.86 ^{a}	34.11^{c}	1.02
CD (0.05)	3.46	3.46	NS

Treatment effect was significant in case of methane and carbon dioxide production. Among the different treatments, methane (CH_4) content was significantly higher for T_6 (64.86%) which was on par with T_5 (64.57%) and T_4 (63.36%). Carbon dioxide (CO_2) content was significantly higher for T_2 (42.73%) and lowest carbon dioxide content was for T_6. The other gases were present only in trace quantities.

4.1.3. Daily Slurry Production, Temperature and Volume of Gas Generated

The influence of different treatments on daily slurry production, temperature and volume of gas generated are presented Table 11. The quantity of slurry generated after each intake did not show any significant difference among

treatments. The average slurry generation ranged from 4256.96 - 4539.14 ml. The average daily temperature ranged from 30.79 - 32.80 ^{0}C. The temperature inside digester was little greater than mean daily atmospheric temperature. The daily volume of biogas ranged from 34.09 m^3 to 34.43 m^3. There was no significant difference between treatments with respect to biogas production.

Table 11. The influence of different treatments on daily slurry production, temperature, volume of gas generated and hydraulic retention time

Treatment	Slurry production (ml day^{-1})	Temperature of biogas unit (^{0}C)	Volume of gas generated day^{-1} (m^3)	Hydraulic Retention time (days)
ST_1	4459.41	32.80^a	34.42	20.67^b
ST_2	4539.14	31.61^b	34.40	22.67^a
ST_3	4518.00	30.79^b	34.09	23.67^a
ST_4	4256.96	31.30^b	34.31	21.33^b
ST_5	4479.35	31.41^b	34.43	20.33^b
ST_6	4367.01	31.18^b	34.32	20.67^b
CD (0.05)	NS	1.14	NS	1.03

4.1.4. Hydraulic Retention Time

The data pertaining to hydraulic retention time are also depicted in Table 11. The lowest hydraulic retention time was recorded for T_5 (20.33 days) followed by T_6 and T_1 (20.67 days). The highest retention time was recorded for T_3 (23.67 days) which was on par with T_2 (22.67 days).

4.1.5. Analysis of Biogas Slurry

The chemical characterisation of slurry produced from different treatments is given in Table 12.

Table 12. The chemical characteristics of biogas slurry obtained from different treatments.

Treatment	pH	EC (dS m^{-1})	Moisture content (%)	Organic carbon (%) (oven dry basis)
T_1	7.83	0.34^{c}	99.42	25.43^{a}
T_2	7.90	0.34^{c}	96.89	19.84^{c}
T_3	7.97	0.71^{a}	99.14	24.95^{a}
T_4	8.20	0.33^{c}	99.20	22.88^{b}
T_5	7.93	0.46^{b}	99.55	22.69^{b}
T_6	8.20	0.35^{c}	99.70	21.39^{bc}
CD (0.05)	NS	0.08	NS	1.84

4.1.6. The pH of Biogas Slurry

The data pertaining to pH of biogas slurry is furnished in Table 12. The pH of biogas slurry ranged from 7.83 - 8.2. The different treatments did not show any significant effect on pH of biogas slurry.

4.1.7. Electrical Conductivity of Biogas Slurry

The data pertaining to electrical conductivity of biogas slurry is furnished in Table 12. The electrical conductivity was significantly higher for T_3 (0.71 dS m^{-1}) followed by T_5 (0.46 dS m^{-1}). The least electrical conductivity was recorded for T_4 (0.33 dS m^{-1}) which was on par with T_1 (0.34 dS m^{-1}), T_2 (0.34 dS m^{-1}) and T_6 (0.35 dS m^{-1}).

4.1.8. Moisture Content of Biogas Slurry

The data related to moisture content of biogas slurry was furnished in Table 12. The moisture content ranged from 96.89 - 99.70%. Statistical analysis revealed that there was no significant difference among the different treatments on biogas slurry moisture content.

4.1.9. Organic Carbon Content of Biogas Slurry

Organic carbon content was significantly higher in T_1 (25.43%) and was on par with T_3 (24.95%). This was followed by T_4 (22.88%), T_5 (22.69%) and T_6 (21.39%) which were on par. The least content of organic carbon was recorded in T_2 (19.84%) (Table 12).

4.1.10. Ammoniacal Nitrogen, Nitrate Nitrogen and Total Nitrogen Content of Slurry

Nitrate nitrogen is one of the available forms of nitrogen and the nitrate nitrogen content ranged from 0.33 - 0.04%. Significant effect was not noticed among different treatments.

Ammoniacal nitrogen is another form of available nitrogen. Significantly highest ammoniacal nitrogen content was recorded in T_4 (0.79%) followed by T_6 (0.77%) and they were on par which was followed by T_6 (0.77%), T_2 (0.66%), T_1 (0.560%) and T_5 (0.37%). The least contribution of ammoniacal nitrogen was from T_3 (0.07%). The ammoniacal and nitrate nitrogen concentrations of various treatments are depicted in Table 13.

Table 13. The contents of major nutrients and C:N ratio in biogas slurry (oven dry basis)

Treatment	Nitrate N (%)	Ammoniacal N (%)	Total N (%)	Total P (%)	Total K (%)	C:N ratio *
T_1	0.36	0.56^{c}	1.27^{b}	0.49^{b}	1.79	$20:1^{b}$ (4.47^{b})
T_2	0.11	0.66^{bc}	1.79^{a}	0.16^{e}	1.75	$11:1^{d}$ (3.27^{d})
T_3	0.04	0.07^{e}	1.75^{a}	0.24^{d}	1.48	$14:1^{c}$ (3.79^{c})
T_4	0.05	0.79^{a}	0.80^{e}	0.50^{b}	1.68	$29:1^{a}$ (5.38^{a})
T_5	0.04	0.37^{d}	0.83^{e}	0.79^{a}	1.56	$28:1^{a}$ (5.26^{a})
T_6	0.12	0.77^{ab}	1.70^{a}	0.37^{c}	1.87	$13:1^{c}$ (3.65^{c})
CD (0.05)	NS	0.12	0.16	0.09	NS	2.65

* Square root transformed data is given in bracket

Total nitrogen contents of biogas slurry generated from different treatments are given in Table 13. It showed that significantly higher total nitrogen content was for T_2 (1.79%) followed by T_3 (1.750%) and T_6 (1.70%) which were on par. The treatment, T_1 with total nitrogen content of 1.275% was significantly different from T_2. The total nitrogen content of T_5 (0.83%) and T_4 (0.70%) were on par.

4.1.11. Total Phosphorus Content of Slurry

The data regarding total phosphorus content in slurry are furnished in Table 13. Significantly highest phosphorus content was recorded for the slurry from T_5 (0.79%), followed by T_4 (0.50%), which was followed by T_6 (0.37%) and T_3 (0.24%). The least value for phosphorus was recorded for T_2 and is 0.16%. Statistical analysis revealed that all the treatments were significantly different from each other.

4.1.12. Total Potassium Content of Slurry

The data pertaining to total potassium content of slurry from various treatments are given in Table 13. The highest potassium content was recorded for T_6 (1.87%) and lowest for T_3 (1.48%).

4.1.13. C:N Ratio of Biogas Slurry

The C:N ratio of biogas slurry from various treatments are furnished in Table 13. The wide C:N ratio was recorded for T_4 (29:1) which was on par with T_5 (28:1), followed by T_1 (20:1). The treatment T_2 had relatively narrow C:N ratio (11:1) when compared to other treatments.

4.1.14. Calcium Content of Biogas Slurry

The data pertaining to total calcium content in slurry was recorded in Table 14. Calcium content was significantly highest for T_1 (720.26 mg kg^{-1}) followed by T_6 (624.90 mg kg^{-1}), T_4 (355.35mg kg^{-1}) and T_5 (314.56 mg kg^{-1}). The treatment T_3 (144.11 mg kg^{-1}) was found to contain the least calcium content among different treatments.

Table 14. Secondary and micronutrient content of biogas slurry obtained from different treatments (mg kg^{-1}).

Treatment	Ca	Mg	Zn	Cu	Mn	Fe
T_1	720.26^{a} *(26.81^{a})	12.15	13.73^{c} *(3.70^{c})	0.54	0.22^{a}	13.59
T_2	150.45^{d} *(12.17^{c})	11.96	32.143^{a} *(5.67^{a})	0.51	0.14^{b}	14.32
T_3	144.11^{d} *(11.97^{c})	12.13	21.88^{b} *(4.68^{b})	0.51	0.23^{a}	14.90
T_4	355.35^{c} *(18.84^{b})	12.08	31.987^{a} *(5.66^{a})	0.52	0.24^{a}	15.92
T_5	314.56^{c} *(17.74^{b})	11.50	7.74^{d} *(2.77^{d})	0.52	0.24^{a}	16.93
T_6	624.90^{b} *(24.98^{a})	11.72	6.64^{d} *(2.57^{d})	0.52	0.14^{b}	13.95
CD (0.05)	84.20	NS	3.04	NS	0.03	NS

*Within bracket square root transformed data

4.1.15. Magnesium Content of Biogas Slurry

The magnesium contents of biogas slurry are presented in Table 14. The magnesium content of slurry ranged from 11.50 - 12.15 mg kg^{-1}. The magnesium contents of slurry from different treatments were not statistically significant.

4.1.16. Zinc Content of Biogas Slurry

The zinc content of biogas slurry for various treatments are presented in Table 14. The zinc content of biogas slurry was significantly higher for T_2 (32.14 mg kg^{-1}) which was on par with T_4 (31.99 mg kg^{-1}) followed by T_3 (21.88 mg kg^{-1}) and T_1 (13.73 mg kg^{-1}). The least zinc content was observed in T_6 (6.64 mg kg^{-1}) which was on par with T_5 (7.74 mg kg^{-1}).

4.1.17. Copper Content of Biogas Slurry

The copper concentrations of biogas slurry from various treatments are presented in Table 14. The copper content in biogas slurry obtained from

different treatments ranged from 0.51 - 0.54 mg kg^{-1}. There was no significant effect of copper among different treatments.

4.1.18. Manganese Content of Biogas Slurry

The manganese concentrations of biogas slurry are furnished in Table 14. Significantly highest concentration for manganese was recorded for T_5 (0.24 mg kg^{-1}) which was on par with T_4 (0.24 mg kg^{-1}), T_3 (0.22 mg kg^{-1}) and T_1 (0.22 mg kg^{-1}). The lowest value was noted for T_6 (0.140 mg kg^{-1}).

4.1.19. Iron Content of Biogas Slurry

The data pertaining to iron content of biogas slurry are showed in Table 14. The iron (Fe) content in biogas slurry obtained from different treatments ranged from 16.93-13.58 mg kg^{-1}. There was no significant effect of treatments on iron concentration in biogas slurry.

4.2. EXPERIMENT II. POT CULTURE EXPERIMENT USING BIOGAS SLURRY

4.2.1. Physico-Chemical Characteristics of Soil Used for Pot Culture Experiment

Table 15. Physico- chemical characteristics of soil used for study

Parameter	Nutrient content
pH	5.73
EC (dS m^{-1})	0.17
Organic carbon (%)	0.68
Available N (mg kg^{-1})	68.61
Available P (mg kg^{-1})	2.15
Available K (mg kg^{-1})	39.46
Available Ca (mg kg^{-1})	27.7
Available Mg (mg kg^{-1})	1.12
Available Zn (mg kg^{-1})	2.6
Available Cu (mg kg^{-1})	1.62
Available Mn (mg kg^{-1})	9.73
Available Fe (mg kg^{-1})	12.96

4.2.2. Chemical Properties of Soil after Experiment

In order to find out the effect of biogas slurry treatments on chemical properties, the soil after pot culture experiments were analysed for pH, EC, organic carbon, primary, secondary and micronutrients.

Table 16. Effect of biogas slurry on chemical characteristics of soil

Treatment	pH	EC (dS m^{-1})	Organic carbon (%)	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
T_1	5.73	0.17	0.92^{de}	66.67^{e}	2.12^{d}	16.08^{d}
T_2	5.92	0.18	0.80^{e}	81.90^{bc}	2.58^{cd}	40.94^{c}
T_3	5.72	0.30	0.92^{de}	76.93^{c}	6.35^{b}	39.31^{c}
T_4	5.71	0.21	1.36^{cde}	109.23^{a}	8.11^{a}	44.13^{bc}
T_5	5.77	0.24	1.08^{cd}	93.76^{ab}	2.36^{cd}	67.20^{a}
T_6	5.63	0.23	1.20^{abc}	68.40^{c}	2.39^{cd}	63.50^{a}
T_7	5.72	0.25	1.52^{b}	68.70^{c}	2.30^{cd}	48.35^{b}
T_8	5.83	0.21	1.94^{a}	101.53^{a}	2.23^{c}	63.54^{a}
T_9	5.68	0.22	1.36^{bc}	108.20^{a}	7.64^{ab}	47.95^{b}
CD (0.05)	NS	NS	0.091	35.915	2.36	13.84

4.2.2.1. pH of Soil

The data pertaining to pH of soil are given in Table 16. The pH of soil ranged from 5.92 to 5.63. Statistical analysis revealed that there was no significant effect on soil pH by different treatments.

4.2.2.2. Electrical Conductivity of Soil

There were no significant differences among electrical conductivity of soils under different treatments. The highest value was recorded for T_3 (0.30 dS m^{-1}) and lowest electrical conductivity was recorded for T_1 (0.17 dS m^{-1}) (Table 16).

4.2.2.3. Organic Carbon Content of Soil

The organic carbon content was significantly highest for T_8 (1.9%) followed by T_7 (1.52%) which was on par with T_9 (1.40%) and T_4 (1.36 %) followed by T_6 (1.18%) which was on par with T_5 (1.08%). The least organic carbon content was recorded for T_2 (0.80%) (Table 16).

4.2.2.4. Available Nitrogen Content in Soil.

The available nitrogen content was significantly higher for T_4 (109.23 mg kg^{-1}) which was on par with T_9 (108.20 mg kg^{-1}), T_8 (101.53 mg kg^{-1}) and T_5 (93.76 mg kg^{-1}). This was followed by T_3 (76.93 mg kg^{-1}), T_2 (81.90 mg kg^{-1}), T_7 (68.70 mg kg^{-1}) and T_6 (68.40 mg kg^{-1}). The lowest content was noticed for T_1 (66.67 mg kg^{-1}).

4.2.2.5. Available Phosphorus Content in Soil

The data pertaining to available phosphorus is shown in Table 16. Significantly highest value for available phosphorus was recorded in T_4 (8.11 mg kg^{-1}) which was on par with T_9 (7.64 mg kg^{-1}) followed by T_3 (6.35 mg kg^{-1}). Lowest available phosphorus content was noticed in T_1 (2.10 mg kg^{-1}).

4.2.2.6. Available Potassium Content in Soil

The data pertaining to available potassium content in soil is shown in Table 16. Significantly available potassium content in soil was recorded for T_5 (67.20 mg kg^{-1}) which was on par with T_6 (63.50 mg kg^{-1}) and T_8 (63.54 mg kg^{-1}). The treatments T_7 (48.35 mg kg^{-1}), T_9 (47.95 mg kg^{-1}) and T_4 (44.13 mg kg^{-1}) were on par. The lowest value recorded for available potassium was for T_1 (16.08 mg kg^{-1}).

4.2.2.7. Calcium Content in Soil

The calcium content in soil are presented in Table 17. The available calcium content was significantly higher for T_5 (440.91 mg kg^{-1}) followed by

T_9 (269.62 mg kg^{-1}), T_1 (268.62 mg kg^{-1}), T_4 (267.45 mg kg^{-1}), T_6 (249.08 mg kg^{-1}) T_2 (244.21 mg kg^{-1}), T_3 (256. 62 mg kg^{-1}) and T_8 (249.66 mg kg^{-1}). The lowest calcium content was found in T_7 (233.25 mg kg^{-1}).

4.2.2.8. Magnesium Content in Soil

Significantly highest magnesium content was recorded in T_8 (185.66 mg kg^{-1}) which was on par with T_9 (179.75 mg kg^{-1}), T_3 (155.33 mg kg^{-1}) and T_4 (149.54 mg kg^{-1}) followed by T_1 (139.02 mg kg^{-1}). The least value for Mg in soil was recorded for T_6 (115.69 mg kg^{-1}) followed by T_7 (133.70 mg kg^{-1}) and T_2 (135.80 mg kg^{-1}) (Table 17).

Table 17. Effect of different treatments on available secondary and micronutrient status of soil (mg kg^{-1})

Treatment	Ca	Mg	Zn	Cu	Mn	Fe
T_1	268.62 ^{b}	139.02 ^{bc}	2.21 ^{e}	13.26 ^{d}	92.34	104.66 ^{c}
T_2	244.21 ^{bc}	135.58 ^{c}	3.99 ^{ab}	27.81 ^{c}	94.27	100.65 ^{c}
T_3	256.62 ^{bc}	155.33 ^{abc}	4.67 ^{a}	36.06 ^{b}	75.55	129.03 ^{b}
T_4	267.46 ^{b}	149.54 ^{abc}	4.20 ^{ab}	29.39 ^{c}	79.98	147.06 ^{a}
T_5	440.91 ^{a}	121.71 ^{c}	3.76 ^{bc}	37.02 ^{b}	86.94	147.54 ^{a}
T_6	249.08 ^{bc}	115.69^{c}	2.64 ^{de}	41.76 ^{a}	65.46	152.14 ^{a}
T_7	233.25 ^{c}	133.70 ^{c}	3.80 ^{bc}	37.15 ^{b}	77.94	163.59 ^{a}
T_8	249.66 ^{bc}	185.66 ^{a}	3.19 ^{cd}	41.94 ^{a}	70.20	152.64 ^{a}
T_9	269.62 ^{b}	179.75 ^{ab}	2.70 ^{de}	40.74 ^{a}	76.8	158.22 ^{a}
CD (0.05)	33.11	40.77	0.78	3.54	NS	17.15

4.2.2.9. Zinc Content in Soil

The zinc content was significantly higher for T_3 (4.67 mg kg^{-1}) which was on par with T_4 (4.20 mg kg^{-1}), T_2 (3.99 mg kg^{-1}), T_7 (3.80 mg kg^{-1}) and T_5 (3.76 mg kg^{-1}). The lowest zinc content was noted in T_1 (2.21 mg kg^{-1}) (Table 17).

4.2.2.10. Copper Content in Soil

The copper content in soil was significantly higher for T_6 and T_8 (41.94 mg kg^{-1}) which were on par with T_6 (41.76 mg kg^{-1}) and T_9 (40.74 mg kg^{-1}) followed by T_7 (37.15 mg kg^{-1}) which were on par with T_5 (37.02 mg kg^{-1}) and T_3 (36.06 mg kg^{-1}) followed by T_4 (29.39 mg kg^{-1}) and T_2 (27.81 mg kg^{-1}). The least value recorded for copper content in soil was for T_1 (13.26 mg kg^{-1}) (Table 17).

4.2.2.11. Manganese Content in Soil

The manganese content in soil ranged from 65.46-94.27 mg kg^{-1}. No significant difference was noted among the different treatments for manganese content in soil (Table 17).

4.2.2.12. Iron Content in Soil

The highest value obtained for iron content in soil was for T_7 (163.59 mg kg^{-1}) which was significantly higher and on par with T_9 (158.22 mg kg^{-1}), T_8 (152.64 mg kg^{-1}), T_6 (152.14 mg kg^{-1}), T_5 (147.54 mg kg^{-1}) and T_4 (147.06 mg kg^{-1}) followed by T_3 (129.03 mg kg^{-1}) and T_1 (104.66 mg kg^{-1}). The least iron content was recorded for T_2 (100.65 mg kg^{-1}) (Table 17).

4.2.3. Biometric Observations of Plants

Plant height, number of leaves per plant, number of branches per plant and number of pods per plant and cowpea seedlings in under pot culture experiment by conducted by different biogas slurry treatments are presented in Table 18.

4.2.3.1. Height of Plants

The height of plants in pot culture experiment ranged from 25.03 to 35.63 cm. Statistical analysis of data regarding the height of potted plants showed that the treatments applied did not have a significant effect on plant height (Table 18).

Table 18. Effect of different treatments on plant height, number of leaves, number of branches per plant and number of pods per plant in pot culture experiment

Treatment	Plant height (cm)	Number of leaves per plant	Number of branches per plant	Number of pods per plant
T_1	31.50	27.50^{e}	0.83	13.17^{e}
T_2	31.57	31.00^{a}	1.33	13.67^{e}
T_3	28.87	26.00^{e}	0.67	13.33^{e}
T_4	25.03	28.00^{b}	1.00	13.00^{e}
T_5	35.00	27.00^{d}	0.67	11.67^{d}
T_6	27.00	17.50^{h}	0.67	13.67^{e}
T_7	33.40	22.50^{f}	0.67	15.00^{b}
T_8	35.63	27.00^{d}	1.00	15.83^{a}
T_9	33.08	21.00^{g}	0.17	15.83^{a}
CD (0.05)	NS	0.165	NS	0.83

4.2.3.2. Number of Leaves per Plant

The data pertaining to number of leaves are tabulated in Table 18. The highest numbers of leaves was recorded for T_2 (31.00) followed by T_4 (28.00), T_1 (27.50), T_8 (27.00), T_5 (27.00), T_3 (26.00), T_7 (22.50) and T_9 (21.00). Lowest number of leaves was recorded for T_6 (17.50).

4.2.3.3. Number of Branches per Plant

The number of branches per plant in pot culture experiment ranged from 0.17 to 1.0. Statistical analysis revealed that there was no significant effect of treatment on number of branches produced (Table 18).

4.2.3.4. Number of Pods per Plant

The data regarding number of pods per plant are shown in Table 18. The highest number of pods per plant were obtained for T_8 (15.83) and T_9 (15.83) followed by T_7 (15.00), T_2 (13.67), T_6 (13.67), T_3 (13.33), T_1 (13.17) and T_4 (13.00). Least number of pods per plant was obtained for T_5 (11.67).

Table 19. Hundred seed weight, number of seeds per pod, plant dry weight and yield per plant in pot culture experiment.

Treatment	Hundred seed weight (g)	Number of seeds per pod	Plant dry weight (g)	Yield per plant (g)
T_1	8.43[d]	8.54[a]	10.33c	14.20[h]
T_2	8.25[e]	6.25[c]	12.77[bc]	15.00[g]
T_3	8.23[e]	9.30[a]	18.00[b]	25.08[c]
T_4	8.84[a]	6.66[bc]	12.77[bc]	17.64[f]
T_5	8.72[b]	7.70[abc]	24.80[a]	19.92[e]
T_6	8.79[a]	7.78[abc]	17.30[b]	28.44[b]
T_7	8.51[c]	8.33[ab]	17.40[b]	25.44[b]
T_8	8.37[d]	9.03[a]	16.33[b]	21.84[d]
T_9	8.12[f]	7.73[abc]	16.50[b]	35.40[a]
CD (0.5)	0.07	1.83	5.31	8.38

4.2.3.5. Hundred Seed Weight

The data pertaining to hundred seed weight is recorded in Table 19. The highest hundred seed weight was recorded for T_4 (8.84 g) which was on par with T_6 (8.79 g) followed by T_5 (8.72 g), T_7 (8.51 g), T_1 (8.43 g), T_8 (8.37g), T_2 (8.25 g) and T_3 (8.23 g). The least hundred seed weight was recorded for T_9 (8.12 g).

4.2.3.6. Number of Seeds per Pod

The data on number of seeds per pod is depicted in Table 19. It showed that the highest number of seeds was obtained for T_3 (9.30) which were on par with T_8 (9.03), T_7 (8.33), T_6 (7.78), T_9 (7.73) and T_5 (7.70). The least number of seeds per pod was recorded for T_2 (6.25) which was on par with T_4 (6.66).

4.2.3.7. Plant Dry Weight

The data pertaining to plant dry weight are given in Table 19. Significantly higher plant dry weight was recorded for T_5 (24.80 g) followed by T_3 (18.00 g), T_7 (17.4 g), T_6 (17.30 g), T_8 (16.33 g), T_9 (16.5 g), T_2 (12.77 g) and T_4 (12.77 g). The

least plant dry weight was recorded for T_1 (10.33 g).

4.2.3.8. Yield per Plant

Data regarding yield per plant are furnished in Table 19. The result indicated that the yield per plant was significantly higher for T_9 (35.40 g) followed by T_6 (28.44 g) which is on par with T_7 (25.44 g) followed by T_3 (25.08 g), T_8 (21.84 g), T_5 (19.92 g), T_4 (17.64g) and T_2 (15.00 g). The lowest yield per plant was recorded for T_1 (14.20 g).

4.2.4. Plant Analysis

4.2.4.1. Total Nitrogen in Plants

The nitrogen content was the highest for T_5 (0.63%) which was on par with T_6 (0.59%), T_4 (0.58%), T_2 (0.57%) and T_9 (0.57) followed by T_1 (0.27%), T_8 (0.57) and T_7 (0.27%). The lowest nitrogen content was recorded for T_3 (0.23%). The data related to nitrogen content was shown in Table 20.

Table 20. Effect of treatments in nitrogen, phosphorus and potassium contents in plants

Nutrients / Treatment	Total N (%)	Total P (%)	Total K (%)
T_1	0.27 ^{b}	0.33 ^{b}	2.92
T_2	0.57 ^{a}	0.46^{a}	3.23
T_3	0.23 ^{b}	0.29 ^{bc}	3.11
T_4	0.58 ^{a}	0.29 ^{bc}	3.09
T_5	0.63 ^{a}	0.27 ^{bc}	3.53
T_6	0.59 ^{a}	0.27 ^{bc}	2.34
T_7	0.27 ^{b}	0.22 ^{c}	3.25
T_8	0.27 ^{b}	0.07 ^{d}	3.52
T_9	0.57 ^{a}	0.04^{d}	3.26
CD (0.05)	0.24	0.111	NS

4.2.4.2. Phosphorus Content in Plants

The data pertaining to phosphorus content in plant are given in Table 20. The phosphorus content was significantly higher in T_2 (0.46%) followed by T_1 (0.33%), T_3 (0.29%), T_4 (0.29%), T_5 (0.27%), T_6 (0.27%), T_7 (0.22%) and T_8 (0.07%). T_9 (0.04%) showed the lowest phosphorus content in plants.

4.2.4.3. Potassium Content in Plants

The data on total potassium content of plants are shown in Table 20. No significant difference in potassium content could be obtained among the different treatments studied. The highest value was obtained for T_5 (3.533%) and least value obtained for T_6 (2.340%).

4.2.4.4. Calcium Content in Plants

The calcium content in plants ranged from 17.9 mg kg^{-1} to 28.25 mg kg^{-1}. Statistical data revealed that there was no significant effect for treatments on calcium content in plants (Table 21).

4.2.4.5. Magnesium Content in Plant

The highest magnesium content was recorded for T_4 (7.50 mg kg^{-1}) and the lowest magnesium content was recorded for T_3 (6.80 mg kg^{-1}) (Table 21).

4.2.4.6. Copper Content in Plants

The copper content in plants ranged from 1.15 to 1.75 mg kg^{-1}. The data related to copper content in plant are presented in Table 21. There was not any significant effect of treatments on copper content in plants.

4.2.4.7. Iron Content in Plants

The iron content in plants are presented in Table 21. The iron content of plants ranged from 6.53 to 20.25 mg kg^{-1}. Statistical analysis revealed that there was no significant effect of treatments on iron content in plants.

Table 21. Secondary and micronutrient content in plants (mg kg^{-1})

Nutrient (mg kg^{-1}) / Treatments	Ca	Mg	Cu	Fe *	Zn	Mn
T_1	28.18	7.24	1.40	15.15 (3.89)	1.6 ^{d}	4.72 ^{de}
T_2	28.25	7.39	1.32	15.69 (3.85)	4.65 ^{a}	9.77 ^{c}
T_3	28.16	6.80	1.35	20.25^{a} (4.46^{a})	3.44 ^{abc}	7.01 ^{d}
T_4	27.14	7.50	1.30	11.27^{a} (3.16^{a})	1.64 ^{d}	4.65 ^{de}
T_5	25.18	7.14	1.46	12.11^{a} (3.48^{a})	3.69 ^{ab}	4.09 ^{e}
T_6	26.50	7.17	1.64	38.42^{a} (5.99^{a})	1.88 ^{cd}	11.58 ^{c}
T_7	24.89	7.32	1.15	12.66^{a} (3.31^{a})	3.70 ^{ab}	19.50^{a}
T_8	17.91	6.90	1.75	6.53^{a} (2.55^{a})	2.55 ^{bcd}	16.30^{b}
T_9	25.47	7.14	1.54	15.15^{a} (3.77^{a})	2.71 ^{bcd}	4.75 ^{de}
CD (0.05)	NS	NS	NS	NS	1.75	2.73

*Within brackets are square root transformed data

4.2.4.8. Zinc Content in Plants

The zinc content in plants are shown in Table 21. Significantly higher zinc content was observed in T_2 (4.65 mg kg^{-1}) which was on par with T_5 (3.69 mg kg^{-1}), T_7 (3.70 mg kg^{-1}) and T_3 (3.44 mg kg^{-1}). The least value for zinc in plants was recorded for T_1 (1.60 mg kg^{-1}) and T_4 (1.64 mg kg^{-1}).

4.2.4.9. Manganese Content in Plants

The data pertaining to manganese content in plants are presented in Table 21. Significantly higher manganese content was recorded for T_7 (19.50 mg kg^{-1}) followed by T_8 (16.30 mg kg^{-1}), T_6 (11.58 mg kg^{-1}) and T_3 (7.01 mg kg^{-1}) which was on par with T_1 (4.72 mg kg^{-1}) and T_4 (4.65 mg kg^{-1}). The least manganese content was recorded for T_5 (4.09 mg kg^{-1}).

4.2.4.10. Total Nitrogen in Seed

The total nitrogen content in seeds ranged from 1.33 to 1.69%. Statistical data revealed that there was no significant effect for treatments on grain nitrogen content. The data related to nitrogen content in seeds are shown in Table 22.

4.2.4.11. Total Phosphorus Content of Seeds

The data related to total phosphorus content in seeds are recorded in Table 22. The phosphorus of grains ranged from 0.19 to 0.25%. The statistical analysis revealed that there was no significant effect for treatments on total phosphorus content in seeds.

4.2.4.12. Total Potassium Content of Seeds

The potassium contents of seeds are shown in Table 22. The potassium content of seeds ranged from 0.17 to 0.23%.

Table 22. Effect of different treatments on nitrogen, phosphorus and potassium content of cowpea seeds

Nutrient (%) / Treatment	Nitrogen	Phosphorus	Potassium
T_1	1.33	0.25	0.17
T_2	1.55	0.21	0.22
T_3	1.60	0.23	0.23
T_4	1.50	0.19	0.18
T_5	1.51	0.19	0.22
T_6	1.46	0.19	0.20
T_7	1.75	0.22	0.17
T_8	1.51	0.22	0.17
T_9	1.69	0.23	0.20
CD (0.05)	NS	NS	NS

4.2.4.13. Calcium Content in Seeds

The data pertaining to calcium content in seeds are presented in Table 23. The highest value for calcium in grains were recorded for T_8 (412.00 mg kg^{-1}) which was followed by T_5 (249.40 mg kg^{-1}) and T_6 (244.83 mg kg^{-1}) and least value for calcium were recorded for T_4 (153.17 mg kg^{-1}).

4.2.4.14. Magnesium Content in Seeds

The magnesium content in grains ranged from 3148.00 mg kg^{-1} to 3298.00 mg kg^{-1} (Table 23).

4.2.4.15. Micronutrient Content in Seeds

The highest zinc concentration in seeds was recorded for T_6 (93.70 mg kg^{-1}) which was on par with T_2 (85.800 mg kg^{-1}) followed by T_9 (76.17 mg kg^{-1}). The least value for zinc concentrations in seeds was noticed for T_1 (27.67 mg kg^{-1}).

Copper content of seeds are furnished in Table 23. The highest copper content was recorded for T_5 (229.00 mg kg^{-1}) which was on par with T_1 (198.33 mg kg^{-1}) and T_6 (198.00 mg kg^{-1}) followed by T_4 (182.00 mg kg^{-1}), T_8 (182.00 mg kg^{-1}), T_9 (181.50 mg kg^{-1}), T_2 (179.00) and T_7 (177.17 mg kg^{-1}). The least copper content was recorded for T_3 (160.67 mg kg^{-1}).

The highest manganese content was recorded for T_4 (283.67 mg kg^{-1}) which was on par with T_3 (278.50 mg kg^{-1}), T_8 (273.03 mg kg^{-1}), T_7 (271.50 mg kg^{-1}) and then T_1 (285.83 %). The least content of manganese in seeds was for T_2 (243.00 mg kg^{-1}).

The data pertaining to iron content in cowpea seeds are presented in Table 23 and highest iron content in seed was recorded for T_1 (430.17 mg kg^{-1}) which was on par with T_8 (475.33 mg kg^{-1}) which was followed by T_3 (384.00 mg kg^{-1}). The least value was recorded for T_6 (253.00 mg kg^{-1}).

Table 23. Effect of treatments on secondary and micronutrient contents in cowpea seeds (mg kg^{-1})

Nutrient (mg kg^{-1}) / Treatment	Ca	Mg	Zn	Cu	Mn	Fe
T_1	239.33^{bc} (15.26^{bc})	3220.33	27.67^{e} (5.26^{f})	198.33^{ab}	265.83^{abc}	430.17^{a} (20.73^{a})
T_2	193.33^{bcd} (13.79^{bcd})	3209.00	85.80^{a} (9.26^{ab})	179.00^{bc}	243.00^{cd}	362.17^{cd} (19.03^{bc})
T_3	183.07^{bcd} (13.25^{bcd})	3263.00	58.50^{c} (7.64^{d})	160.67^{c}	278.50^{ab}	394.00^{bc} (19.85^{ab})
T_4	153.17^{cd} (12.35^{cd})	3236.67	74.00^{b} (8.59^{c})	182.00^{bc}	283.67^{a}	380.33^{cd} (19.50^{bc})
T_5	249.40^{b} (15.79^{b})	3200.17	69.33^{b} (8.33^{c})	229.00^{a}	254.00^{bc}	374.83^{cd} (19.36^{bc})
T_6	244.83^{b} (15.62^{b})	3298.00	93.70^{a} (9.68^{a})	198.00^{ab}	243.50^{cd}	253.00^{e} (15.90^{d})
T_7	116.33^{d} (10.79^{d})	3272.83	48.09^{d} (6.93^{e})	177.17^{bc}	271.50^{abc}	280.50^{e} (16.745^{d})
T_8	174.00^{bcd} (13.17^{bcd})	3237.33	56.00^{cd} (7.48^{d})	182.00^{bc}	273.03^{ab}	425.33^{ab} (20.62^{a})
T_9	412.00^{a} (20.298^{a})	3184.00	76.17^{b} (8.73^{bc})	181.5^{bc}	218.57^{d}	357.5^{d} (18.89^{c})
CD (0.05)	87.65	NS	8.95	33.29	29.18	35.52

4.2.5. Seed Quality Analysis in Pot Culture Experiment

In order to find out the effect of biogas slurry treatment on seed qualities *viz.*, germination test, protein content, vigour index, seeds obtained from pot culture experiments from different treatments were taken. Following seed quality parameters were studied by taking the seeds from pot culture experiment.

4.2.5.1. Crude Protein Content in Seeds

The crude protein content of grains from pot culture are shown in Table 24. The crude protein content in seeds varied from 8.22 to 10.85%. Statistical analysis showed that there was no significant effect of treatments on crude protein content of seed.

4.2.5.2. Root Length of Seedlings

The data pertaining to the root length of seedlings are presented in Table 24. The root length of seedlings obtained from the seeds of pot culture experiment ranged from 7.97 to 11.73 cm. There was no significant effect of treatments on root length of seedlings.

4.2.5.3. Shoot Length

The data pertaining to shoot length of seedlings are given in Table 24. The shoot length was significantly higher for T_9 (22.38 cm) which was on par with T_1 (21.32 cm) and T_7 (20.53 cm) followed by T_2 (19.51 cm), T_8 (19.50 cm), T_5 (19.27 cm), T_3 (18.77 cm) and T_6 (17.10 cm). The least shoot length was noted for T_6 (17.10 cm).

4.2.5.4. Germination Per Cent of Seeds

The data regarding the germination per cent is shown in Table 24. The germination per cent of seeds from pot culture experiment ranged from 67.13to 76.93%. Statistical data showed that there was no significant effect of treatments on germination per cent of seedlings.

4.2.5.5. Seedling Dry Weight

The dry weights of seedling are shown in Table 24. The seedling dry weight of seedlings obtained from seeds of pot culture experiment ranged from 0.04 g to 0.05 g. Statistical analysis showed that there was no significant effect of treatments on seedling dry weight.

4.2.5.8. Vigour Index I

The highest vigour index was recorded for T_7 (2954.88) which was on par with T_9 (2924.66), T_5 (2934.28), T_1 (2679.96) T_8 (2525.84) and T_2 (2527.49) followed by T_3 (1895.98) and T_4 (1809.080). The least value obtained for vigour index one was for T_6 (1709.413) (Table 24).

4.2.5.6. Vigour Index II

The data obtained for vigour index II is recorded in Table 24. The vigour index I of seedling obtained from seeds of pot culture experiment ranged from 3.23 to 3.75. Statistical analysis revealed that there was no significant effect of treatments on vigour index II of seedlings.

Table 24. Analysis of seed quality, root length, seedling dry weight and shoot length in pot culture experiment

Treatment	Crude protein (%)	Root length (cm)	Seedling length (cm)	Germination (%)	Seedling dry weight (g)	Vigour index 1	Vigour index 2	Shoot length (cm)
T_1	8.22	9.49	30.81 ^{ab}	69.70	0.04^{a}	2679.96$^{a}_{b}$	3.24	21.32 ^{ab}
T_2	9.59	8.47	27.98 ^{c}	72.40	0.04^{a}	2527.49$^{a}_{bc}$	3.56	19.51 ^{bc}
T_3	9.94	11.73	26.89 ^{cd}	70.50	0.05^{a}	1895.98$_{bc}$	3.93	18.77 ^{cde}
T_4	9.32	9.44	26.84 ^{cd}	67.13	0.040^{a}	1809.08$_{bc}$	3.36	17.40 ^{de}
T_5	9.73	10.20	29.17 ^{bc}	75.00	0.05^{a}	2934.28$^{a}_{b}$	3.75	19.27 ^{cd}
T_6	9.03	7.97	25.07 ^{d}	68.13	0.04^{a}	1709.41^{c}	2.84	17.10 ^{e}
T_7	10.85	10.20	30.71 ^{ab}	76.93.	0.04^{a}	2954.88^{a}	3.85	20.53 ^{abc}
T_8	9.36	9.70	29.20 ^{bc}	69.87	0.04^{a}	2525.84$^{a}_{bc}$	3.23	19.50 ^{bc}
T_9	10.49	10.87	33.29 ^{a}	70.27	0.04^{a}	2924.66^{a}	3.25	22.38 ^{a}
CD (0.05)	NS	NS	2.65	NS	NS	390.36	NS	1.904

4.2.5.7. Seedling Length

The data related to seedling length of the seeds obtained from pot culture experiment are shown in Table 24. The highest seedling length was obtained for T_9 (33.29 cm) which was on par with T_1 (30.81 cm) and T_7 (30.71 cm) followed by T_5 (29.17 cm), T_2 (27.97 cm), T_3 (26.89 cm) and T_4 (26.84 cm). The least seedling length was recorded for T_6 (25.07 cm).

4.2.6. Total Uptake of Major Nutrients by Cowpea Plants

4.2.6.1. Total Nitrogen Uptake by Plants

The data pertaining to total nitrogen uptake are furnished in Table 25. The highest nitrogen uptake was recorded for T_9 (54.70 g plant^{-1}) which was on par with T_5 (46.51 g plant^{-1}), T_7 (43.35 g plant^{-1}) and T_8 (52.87 g plant^{-1}). This was followed by T_6 (39.81 g plant^{-1}) and T_3 (39.62 g plant^{-1}). Least nitrogen uptake was recorded for T_1 (20.66 g plant^{-1}).

Table 25. Uptake of nitrogen, phosphorus and potassium by plants

Treatment No.	Uptake of nutrient		
	N (g plant^{-1})	P (g plant^{-1})	K (g plant^{-1})
T_1	20.66^{e}	6.41^{c}	27.55
T_2	32.10^{de}	6.94^{c}	38.33
T_3	39.62^{bcd}	13.54^{a}	58.04
T_4	34.27^{cd}	7.52^{c}	35.19
T_5	46.51^{abc}	10.95^{ab}	53.87
T_6	39.81^{bcd}	9.35^{bc}	55.07
T_7	43.35^{abcd}	8.85^{bc}	45.54
T_8	52.87^{ab}	6.75^{c}	51.39
T_9	54.70^{a}	7.15^{c}	58.50
CD (0.05)	13.28	3.38	NS

4.2.6.2. Total Phosphorus Uptake by Plants.

The data related to total uptake of phosphorus are recorded in Table 25. The highest total phosphorus uptake was noted for T_3 (13.54%) which was on par with T_5 (10.95 g plant^{-1}) followed by T_7 (8.85 g plant^{-1}) and T_9 (1.32 g plant^{-1}) which were on par followed by T_6 (9.35 g plant^{-1}), T_7 (8.85 g plant^{-1}). The least phosphorus uptake was recorded for T_1 (6.41 g plant^{-1}).

4.2.6.3. Total Potassium Uptake by Plant

The data related to total uptake of potassium was recorded in Table 25. Highest total uptake of potassium was recorded in T_9 (58.5 g plant^{-1}). Total potassium was least for T_1 (27.55 g plant^{-1}).

EXPERIMENT III 4.3. EFFECT OF SLURRY ON SEED GERMINATION AND VIGOUR INDEX OF SEEDLINGS

In order to find out the effect of different seed treatment method using different biogas slurry, an experiment was conducted in completely randomized design with 21 treatments and three replications.

4.3.1. Germination Per Cent

The data regarding to germination per cent of seedlings from treated seeds are shown in Table 26. The highest germination per cent was observed for TST_{14} (96.88%) which was on par with TST_2 (96.88%), TST_6 (91.88%), TST_7 (88.75%), TST_{18} (88.75%), TST_1 (87.50%), TST_{20} (80.95%), TST_5 (80.63%), TST_9 (80.63%), TST_{19} (76.25%), TST_{21} (75.00%) and TST_{12} (75.00%). The least value recorded was for TST_4 (52.50%).

4.3.2. Vigour Index I

The data pertaining to vigour index I of seedlings from treated seeds are presented in Table 26. The highest value observed was for TST_{14} (4991.8) and was on par with TST_2 (4349.39) TST_7 (4081.37), TST_6 (3834.41) and TST_{18} (3818.44). The least value observed was for TST_4 (2311.64).

4.3.3. Vigour Index II

The highest value observed was for TST_{14} (6.07) which was on par with TST_{18} (5.67), TST_1 (5.37), TST_7 (5.33), TST_2 (5.32), TST_{11} (5.22), TST_9 (5.17), TST_5 (5.17), TST_6 (4.88), TST_8 (4.29), TST_{20} (4.20). The least value observed was for TST_{13} (2.87) (Table 26).

Table 26. Effect of different types of seed treatment on seedling characteristics

Treatment	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (g)	Germination (%)	Vigour index I	Vigour index II
TST_1	11.30	29.62 ^{b}	41.01	0.06	87.50^{abc}	3551.96 ^{bcd} *(59.59 ^{abcd})	5.37 ^{abc}
TST_2	14.67	30.34 ^{b}	45.02	0.06	96.88^{a}	4349.39 ^{ab} *(65.90 ^{ab})	5.32 ^{abc}
TST_3	15.7	30.56 ^{b}	46.33	0.05	65.63 ^{cde}	3105.23 ^{cde} *(55.09 ^{bcde})	3.39^{de}
TST_4	14.93	27.74 ^{bcd}	42.66	0.06	52.50^{e}	2311.64 ^{e} *(46.90 ^{e})	3.39 ^{de}
TST_5	12.70	30.52 ^{b}	42.79	0.06	80.63 ^{abcd}	3453.79 ^{bcde} *(58.501 ^{bcd})	5.17 ^{abcd}
TST_6	13.50	28.40 ^{bcd}	41.65	0.05	91.88 ^{ab}	3834.41 ^{abcd} *(61.77 ^{abcd})	4.88 ^{abcd}
TST_7	15.27	30.72 ^{b}	46.00	0.06	88.75 ^{abc}	4081.37 ^{abc} *(63.89 ^{abc})	5.33 ^{abc}
TST_8	16.40	28.87 ^{bcd}	45.71	0.07	64.05 ^{cde}	2857.41 ^{de} *(52.96 ^{cde})	4.29 ^{abcde}
TST_9	14.40	29.21^{bc}	43.58	0.06	80.63 ^{abcd}	3517.00 ^{bcde} *(59.14 ^{bcd})	5.17 ^{abcd}
TST_{10}	13.23	28.93 ^{bcd}	42.16	0.05	67.50 ^{bcde}	2844.13 ^{de} *(53.33 ^{cde})	3.50 ^{cde}
TST_{11}	15.69	28.54 ^{bcd}	44.24	0.07	70.63 ^{bcde}	3149.78 ^{bcde} *(55.87 ^{bcde})	5.22 ^{abcd}
TST_{12}	12.14	28.55^{bcd}	40.69	0.06	75 ^{abcde}	3009.25 ^{cde} *(54.50 ^{cde})	4.15^{abcde}
TST_{13}	15.76	25.88^{cd}	41.64	0.05	62.50^{de}	2632.00 ^{de} *(50.80 ^{de})	2.87 ^{e}
TST_{14}	22.63	28.85 ^{bcd}	51.53	0.06	96.88 ^{a}	4991.80 ^{a} *(70.65 ^{a})	6.07 ^{a}
TST_{15}	14.38	25.53 ^{d}	39.92	0.06	70.00 ^{bcde}	2829.91 ^{de} *(52.642 ^{de})	3.87 ^{bcde}
TST_{16}	11.75	34.48^{a}	46.23	0.06	68.81 ^{bcde}	3197.87 ^{bcde} *(56.01 ^{bcde})	3.71 ^{cde}
TST_{17}	12.11	30.31 ^{b}	42.42	0.05	68.95 ^{bcde}	2871.72 ^{de} *(53.59^{cde})	3.60 ^{cde}
TST_{18}	13.39	29.60^{b}	43.08	0.06	88.75 ^{abc}	3818.44 ^{abcd} *(61.75 ^{abcd})	5.67 ^{ab}
TST_{19}	15.43	30.74^{b}	46.19	0.05	76.25 ^{abcde}	3489.97 ^{bcde} *(59.02 ^{bcd})	4.06 ^{bcde}
TST_{20}	15.11	28.07 ^{bcd}	43.18	0.05	80.95 ^{abcd}	3464.56 ^{bcde} *(58.70 ^{bcd})	4.20 ^{abcde}
TST_{21}	16.38	29.17 ^{bc}	43.89	0.05	75.00 ^{abcde}	3277.77 ^{bcde} *(56.73 ^{bcde})	3.49 ^{cde}
CD (0.05)	NS	3.44	NS	NS	24.93	1206.13	1.92

*Within brackets square root transformed data were given

4.3.4. Seedling Length

The data pertaining to seedling of seedlings produced from different type of slurry treated seeds are recorded in Table 26. The seedling length of seedlings obtained from treated seeds are ranged from 39.92 to 51.52 cm. Statistical analysis revealed that there was not any significant effect of slurry treatment on length of seedlings.

4.3.5. Dry Weight of Seedlings

The dry weight of seedlings obtained from treated seeds ranged from 0.05 to 0.07g. There was no significant effect on dry weight of seedling (Table 26).

4.3.6. Root Length of Seedlings

The root length of seedlings obtained from treated seeds ranged from 11.39 to 22.63 cm. Statistical analysis revealed that there was no significant effect of slurry treatment on root length of seedlings (Table 26).

4.3.7. Shoot Length

The shoot lengths of seedlings from treated seeds were recorded in Table 26. The longest shoot was obtained for TST_{16} (34.48 cm). The shortest shoot length was observed in TST_{15} (25.53 cm).

5. DISCUSSION

5. DISCUSSION

An investigation on 'Substrate impact on biogas production and manurial value of slurry' was conducted at College of Horticulture to find out the composition of biogas produced from different substrates and to analyse the nutrient content of the slurry produced from these substrates. The effects of different types of slurry treatments on seed characters were also attempted. The results presented in previous section are discussed in this chapter.

5.1. EXPERIMENT I

5.1.1 Composition of Biogas

The methane content in biogas depends upon substrate and digestion conditions. The quantity and composition of biogas depends on the composition of the substrate. There is an enhancement of biogas production when co-digestion of organic waste was done with cow dung. In addition to this, co-digestion with materials containing high and low C:N ratio enhanced biogas production, with an increase in CH_4 content. The compositions of biogas from different treatments are presented in Figure 1.

In this experiment, the highest methane content was recorded for pulse residue and cow dung mixed in 1:1 ratio with equal quantity of water. It may be due to comparatively high alkaline pH (8.7) of substrate which favours bacterial decomposition and multiplication (Figure 2). The methane content of biogas produced from different treatments is presented in Figure 1. Among the different treatments, highest methane content was noticed in pulse residue by Ukpai and Nnabuchi (2012). High methane content was also reported by Abdulsalam *et al.*, (2012) when co-digestion of cow dung was done along with elephant dung. If methane content is above 60%, the biogas can be considered to be a valuable fuel (Vilniskis *et al.*, 2011).

Figure 1. Composition of biogas from different treatments

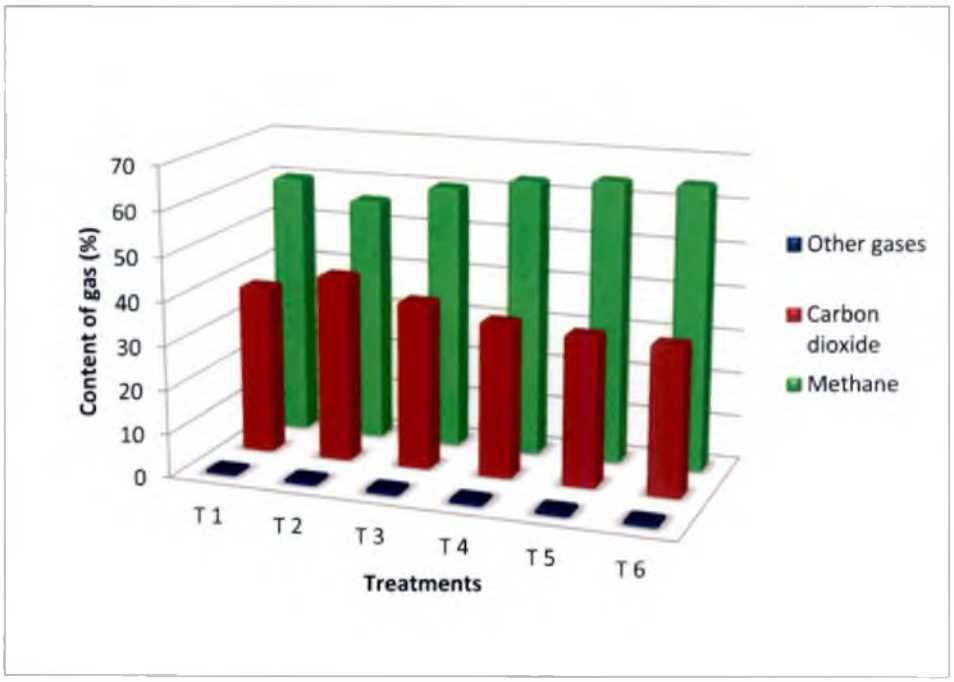
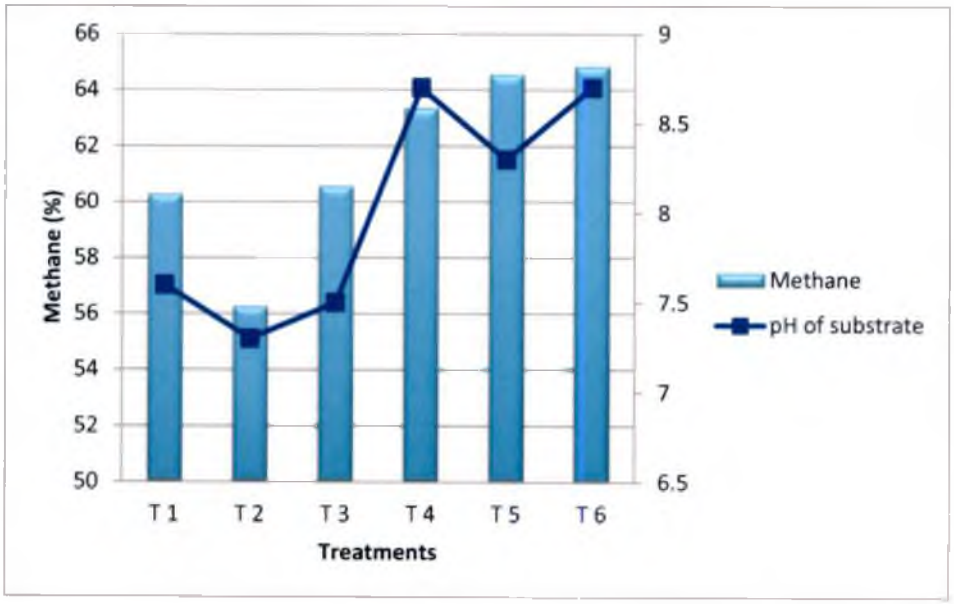


Figure 2. Effect of substrate pH on biogas methane content



The fresh and partially digested material yields excellent biogas production with high methane content. The pulse residue and cow dung, biodegradable household waste and cow dung combinations showed higher content of methane and lower content of carbon dioxide. The increase of methane in these treatments may be due to fresh materials used as substrate. Excreta of rumen animal (cow dung) are known to contain the native microbial flora that aid in faster biogas production. The C:N ratio was also reduced to optimum range by anaerobic digestion which favours the CH_4 emission. Co-digestion of wastes is one of the optimized techniques known to improve biogas production (Misi and Forster, 2004).

The methane content produced from cow dung, poultry manure and water in the ratio 1:1:2 was found to be less. The decrease may be due to the completely digested substrates such as poultry manure, cow dung and goat manure. Since these manures, contain high nitrogen content, it might lead to ammonia inhibition during methane production. In addition that the cows dung (rumen excreta) would acts as inoculum for anaerobic digestion.

5.1.2. Daily Temperature and Slurry Output

The daily temperature of biogas unit was slightly higher than the mean daily atmospheric temperature. The temperature inside the digester varied from 27°C to 33°C during biogas production from co-digestion of kitchen waste with cow dung in different proportion (Ravi *et al.*, 2013). Alfa *et al.* (2014) reported that during anaerobic digestion of poultry droppings, the temperature varied from 28^0C to 36.7^0C.

The atmospheric temperature had a negative effect on temperature inside the digester (Figure 3). This is also supported by the findings of Shejir (2014). Ravi *et al.* (2013) reported that the solar radiation was responsible for increasing the slurry temperature inside the digester, which influenced the rate of biogas production.

Figure 3. Daily temperature inside digester of different treatments and mean atmospheric temperature

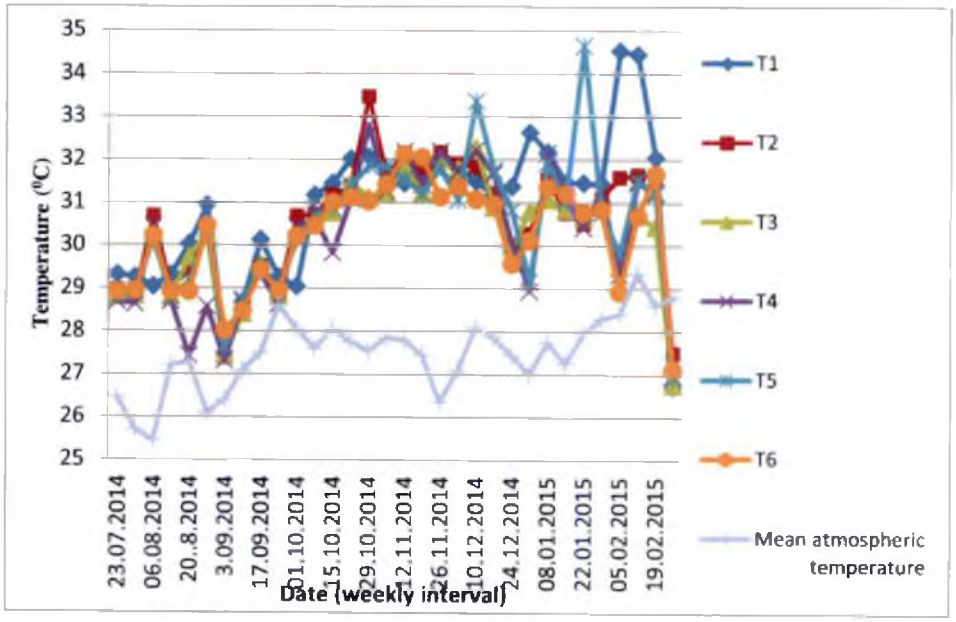
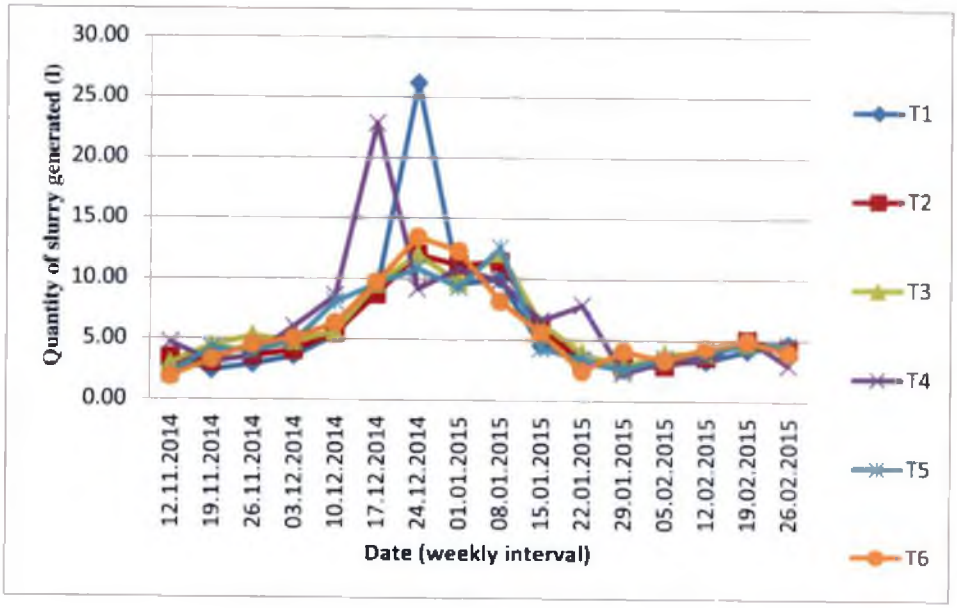


Figure 4. The effect of different treatments on production of slurry during winter months



During winter months, the slurry produced was higher in quantity inside the digester. In Kerala, December and January are winter months and during these months, the slurry production in all treatments was more when compared to other months (Figure 4). Slurry production is negatively correlated with rainfall and relative humidity of atmosphere. Similar results were also reported by Shejir (2014) and Khoiyangbam *et al.* (2004).

5.1.3. pH and Moisture Content of Slurry

The pH of slurry ranged from 7.83 to 8.20 and moisture content ranged from 96.89 to 99.70 percent. Biogas systems are highly pH dependent and methanogenesis takes place within pH range of 6.6 - 7.6 and in some cases up to 8.5 (Speece and McCarthy, 1964). The pH of poultry manure digestate reached up to pH 8.85 on the ninth day of digestion and it was maintained above 8.0 till the last day of study (Alfa *et al.*, 2014). The alkaline pH could be attributed to feed stock material used (Ojolo *et al.*, 2007; Ahamad *et al.*, 2009).

The increased moisture content and reduction in dry matter content during anaerobic digestion of raw slurry might be due to the breakdown of complex material into simple components during anaerobic digestion. During this process it produces water as byproduct. The reduction in solid content upto 20 percent from the feed stock was reported by Frost and Gilkinson (2011). The reduction in solid content upto 25 per cent was reported by Smith *et al.*, (2007).

5.1.4. Hydraulic Retention Time

The least hydraulic retention time was noticed for elephant dung and cow dung combination followed by pulse residue and cow dung combination. It might be due to the presence of higher organic carbon source in elephant dung for microbial attack and supply of required methanogens from cow dung and it act as nitrogen source which reduces the C:N ratio to a suitable range. Next low hydraulic retention time was recorded for cow dung alone. It might be due to small particle size and presence of larger amount of methanogenic bacteria in

substrate for fast action during anaerobic digestion process. The hydraulic retention time varied from 20.67 to 23.67 days. The retention time would vary according to substrate and digestion conditions. Ranade *et al.* (1990) reported that the digestion process was stable at 20-30 days of hydraulic retention time in the case of anaerobic digestion of market wastes.

The highest retention time was recorded for goat manure and cow dung combination and it might be due to the fact that time required for hydration and hydrolysis of goat manure was more because of its dry and compact nature. Moestedt *et al.* (2014) reported that high ammonia concentration and volatile fatty acids concentration increased the hydraulic retention time.

5.1.5. Organic Carbon Content of Biogas Slurry

The treatment having highest organic carbon content was recorded for biogas slurry produced from cow dung alone and treatment with goat manure and cow dung. Co-digestion with cow dung reduces the C:N ratio of substrate to a favourable range (20-30:1) for decomposition. But for cow dung alone, the C:N ratio was highest and rate of decomposition might be comparatively lower than other substrates. Organic carbon content in substrate might be conserved in slurry. For goat manure and cow dung combination, the retention time was more and decomposition rates were lesser. So the organic carbon in substrates might have retained in slurry due to low decomposition rates. The least organic carbon was recorded for poultry manure and cow dung combination. It might be due to high nitrogen supply during digestion period which led to fast decomposition of carbonaceous materials.

5.1.6. Ammoniacal Nitrogen, Nitrate Nitrogen and Total Nitrogen Content in Biogas Slurry

There was no significant difference among treatments in case of nitrate nitrogen and it ranged from 0.041 to 0.36 per cent. In anaerobic condition the content of nitrate nitrogen was less when compared to ammoniacal nitrogen.

The pH of substrate was positively correlated with nitrate nitrogen content in slurry and correlation value is (0.482*). Total nitrogen in substrate was negatively correlated with nitrate nitrogen content in slurry and correlation value is (-0.496*). Organic carbon content in substrate was negatively correlated with nitrate nitrogen content in slurry and correlation value is (-0.562*)

In the case of ammoniacal nitrogen, the highest content was recorded for biodegradable household waste and cow dung combination followed by pulse residue and cow dung combination. The least content of ammoniacal nitrogen was recorded in cow dung - goat manure combination (Figure 5). It might be due to decreased bacterial activity because of its hard nature and reduced hydration rates. Thus conversion rate of nitrogen to ammonia in goat manure was comparatively less.

The highest total nitrogen content was observed for cow dung and poultry manure combination followed by cow dung- goat manure combination and it was due to high nitrogen content in the substrates. The total nitrogen content in slurry was 73.78 % than that of initial substrate in cow dung and poultry manure combination and in case of goat manure cow dung combination it is 80.41 % than that of initial substrate. Frost and Gilkinson (2011) reported an increase of nitrogen content in biogas slurry upto 19 per cent than that of substrate.

The pH of substrate was positively correlated with the total and ammoniacal nitrogen in slurry. Total nitrogen in substrate was correlated with total nitrogen and ammoniacal nitrogen of slurry. In general, livestock manures supply surplus of nitrogen also and there was increase in digestate ammonia content as protein breakdown occurs in anaerobic digestion. Increase in ammoniacal nitrogen content in slurry was also reported by Smith *et al.,* (2007). The C:N ratio of substrate was correlated with total nitrogen of slurry.

5.1.7. Total Phosphorus Content in Slurry

The highest phosphorus content was recorded for slurry from elephant dung

and cow dung combination followed by household waste and cow dung combination (Figure 6). The least phosphorus content was recorded for poultry manure and cow dung combination and it might be due to the effect of C:N ratio of the substrate. The extractable phosphorus content is decreased in the anaerobic digestion. The reduction may possibly occur due to sorption on small particle surfaces (Field *et al.*, 1984).

The total potassium content in slurry ranged from 1.563 per cent to 1.872 per cent (Figure7). The nutrient status of slurry was enhanced after anaerobic digestion and it was made more available to the plant. Co-digestion also helped in increasing the nutrient availability.

The pH of substrate was positively correlated with total phosphorus content of slurry and correlation value is (0.522*). Organic carbon content in substrate was positively correlated with total phosphorus content in slurry and correlation value is (0.575*). C:N ratio of substrate was positively correlated with total phosphorus content of slurry and correlation value is (0.805**)

5.1.9. C: N Ratio of Biogas Slurry

The C: N ratio of biogas slurry obtained from different treatments ranged from 11-29:1 (Figure 8). The highest C:N ratio was recorded for household waste and cow dung combination which might be due to the presence of higher amount of vegetable seeds which cannot be fully digested by anaerobic digestion and biodegradable household waste was not uniform in terms of composition.

Generally biogas slurry had a reduced C:N ratio since the anaerobic digestion processes reduced the carbon content in substrate and converted it to carbon dioxide and methane and also increased the concentration of nitrogen. The lowest slurry C:N ratio was recorded for poultry manure and cow dung combination.

Figure 5. Ammoniacal and total nitrogen in biogas slurry from different treatments

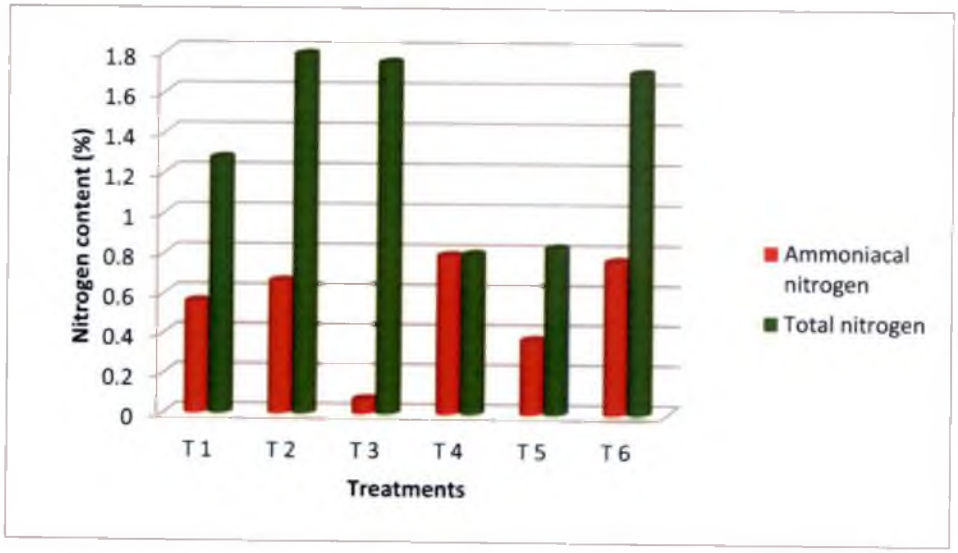


Figure 6. Total phosphorus content of slurry from different treatments

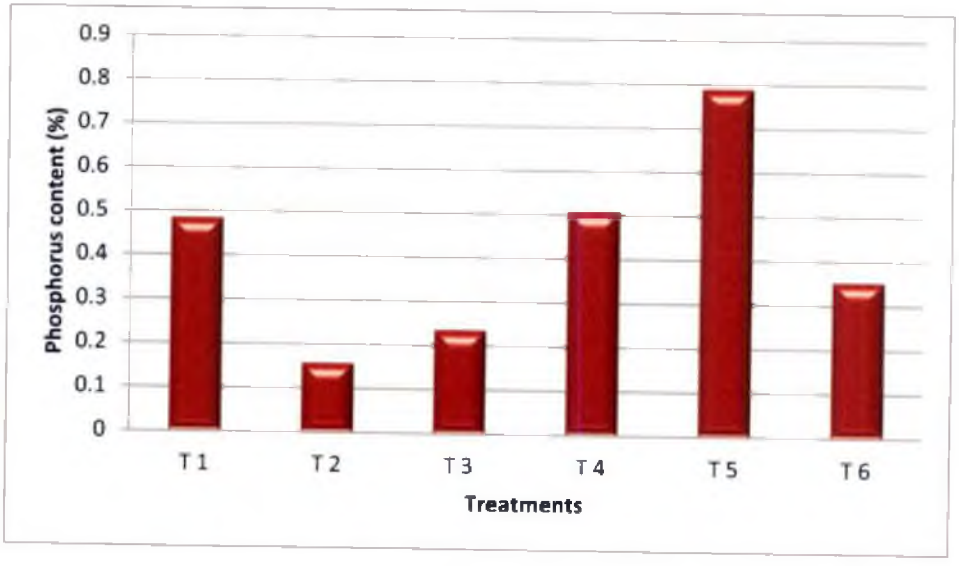


Figure 7. Total potassium content of slurry from different treatments

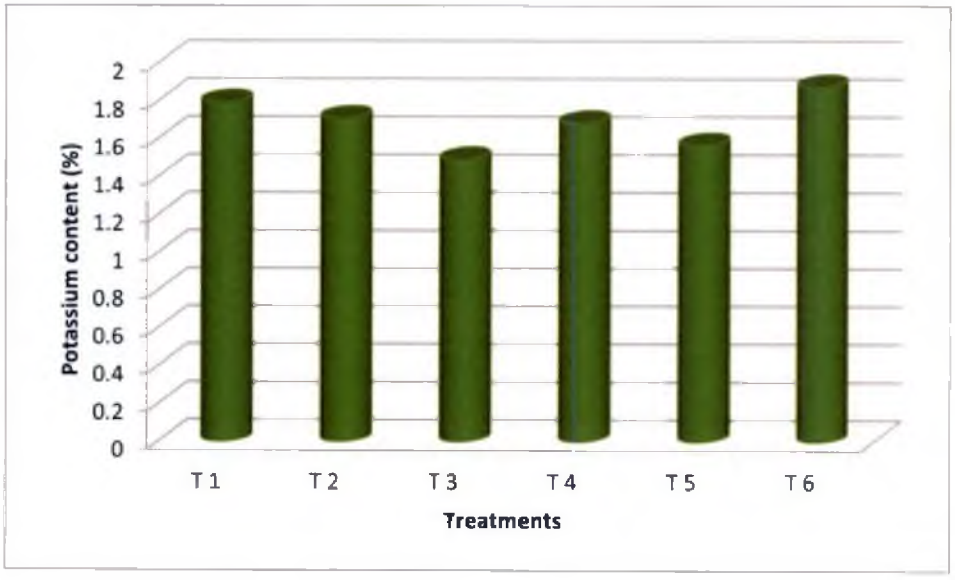
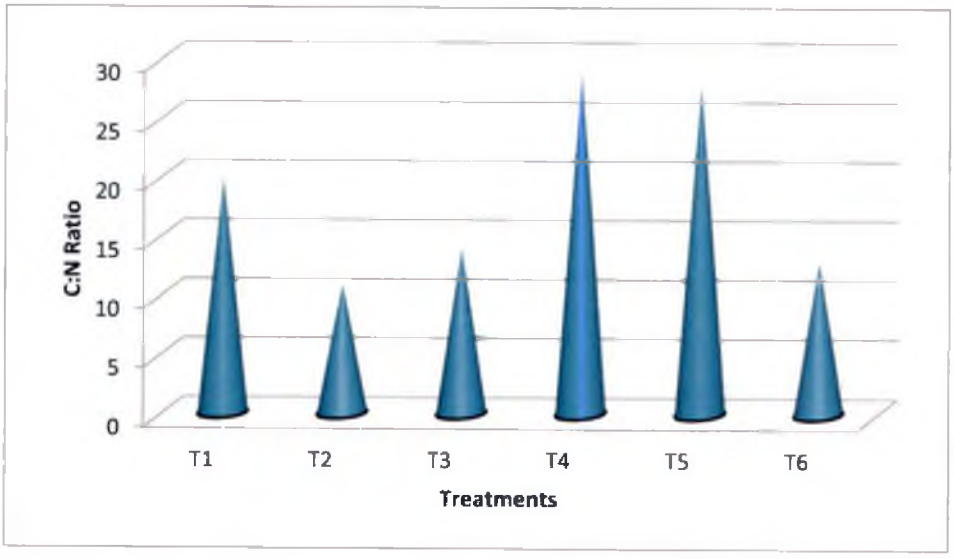


Figure 8. C:N ratio of biogas slurry from different treatments



The C:N ratio of substrate was positively correlated with C:N ratio of slurry and correlation value is (0.536*). Total phosphorus content slurry was positively correlated with C:N ratio of slurry correlation value is (0.709**)

5.10. Secondary and Micronutrient Content in Slurry

The highest calcium content was recorded for biogas slurry produced from cow dung alone and the lowest calcium content was recorded for biogas slurry produced from cow dung and goat manure combination. Total nitrogen in substrate was negatively correlated with calcium content slurry correlation value is (-0.809**). The total calcium and magnesium content in slurry decreased sustainability during anaerobic digestion (Callander and Barford, 1983). According to Field *et al.* (1984) the reduction in available calcium and magnesium fractions decreased in the aerobically digested effluent, possibly because of sorption on small particle surface.

The highest zinc content of biogas slurry was obtained from poultry manure and cow dung combination which was on par with biodegradable household waste and cow dung combination. The least zinc content was observed in pulse residue and cow dung. Organic carbon in substrate was correlated with Zn content in slurry. The manure pH strongly influenced the solubility of micronutrients (Hjorth *et al.*, 2010). Micronutrients may be involved in sorption to solid fraction, either biomass or inert suspended matter and formation of complexes in solutions with intermediates and product compounds produced during anaerobic digestion may lead to decrease in micronutrient concentration in biogas slurry (Callander and Barford, 1983; Chen *et al.*, 2008).

The highest concentration of manganese was recorded for elephant dung and cow dung combination which was on par with biodegradable household waste and cow dung combination and cow dung alone. The least manganese content was recorded for cow dung and pulse residue combination. The iron content of biogas slurry ranged from 13.58 mg kg^{-1} to 16.93 mg kg^{-1}. The micronutrient content of biogas slurry may vary according to substrate composition, decomposition rate

and types of microbes acting upon substrate. The complex reactions played an important role in bioreactors making a particular metal either more or less bioavailable (Callander and Barford, 1983).

EXPERIMENT II

5.2. POT CULTURE EXPERIMENT

5.2.1. Physico-Chemical Properties of Soil

The pH of soil ranged from 5.68 to 5.92 and EC ranged from 0.17 to 0.30 dS m^{-1}. The higher organic carbon content was shown in soil which was irrigated with biogas slurry when compared to irrigation without biogas slurry. The biogas slurry produced from poultry manure and cow dung combination was having significantly lesser amount of organic carbon and thus the soil irrigated with this slurry showed comparatively lesser amount of organic carbon than other slurry treated soils. The highest organic carbon content was recorded for soil irrigated with biogas slurry produced from elephant dung and cow dung combination and is 185.29 per cent more than organic carbon in initial soil sample and 110 percent more than that of control.

The organic carbon content of soil irrigated with water alone, as per KAU package of practices and recommendations (Figure 9) for crops and supernatant solution of cow dung slurry (cow dung and water in 1:1 ratio) showed lesser amount of organic carbon and this may be due to lesser amount of organic carbon content in the applied source.

Highest available nitrogen content was recorded for soil irrigated with biogas slurry produced from cow dung alone and cow dung and pulse residue combination. The lowest nitrogen content was recorded for control. The increase in the available nitrogen content was 59.20 per cent more than available nitrogen in initial soil sample and 63.83% more than control. The nitrogen in animal manure was normally available in organic forms but after passing through fermentation process it was converted to inorganic forms mostly to ammonia

(Nasir *et al.*, 2010) and it is one of the available forms of nitrogen which would increase the soil available nitrogen content. Effect of different treatments on available nutrient contents in soil are presented in Figure 10.

Highest phosphorus content in slurry was recorded for soil irrigated with biogas slurry produced from cow dung alone and the lowest phosphorus content was recorded for control. During anaerobic digestion most of the phosphorus was stored as polyphosphates and significant amount present in organic material was released (Alfa *et al.*, 2014) and this might have contributed to soil available fraction.

Organic carbon content in slurry was positively correlated with available phosphorus content in soil and correlation value is (0.593**). Calcium content in biogas slurry was positively correlated with available phosphorus content in soil and correlation value is (0.710**)

Available potassium content in soil was highest for poultry manure and cow dung combination. The lowest value for available potassium was recorded for control. The available primary nutrient content in soil is presented in Figure 8. Calcium content in biogas slurry was negatively correlated with available potassium (correlation value is -0.803**)

The highest calcium content was recorded for soil irrigated with biogas slurry produced from cow dung and poultry manure combination and the least calcium content was recorded for the soil irrigated with biogas slurry produced from biodegradable household waste and cow dung combination. The increase in calcium content was 61.68 per cent more than initial soil sample and 64.14 per cent more than control. Organic carbon content in slurry was negatively correlated with calcium content in soil and correlation value is (-0.662**).

Figure 9. Effect of different treatments on organic carbon content in soil

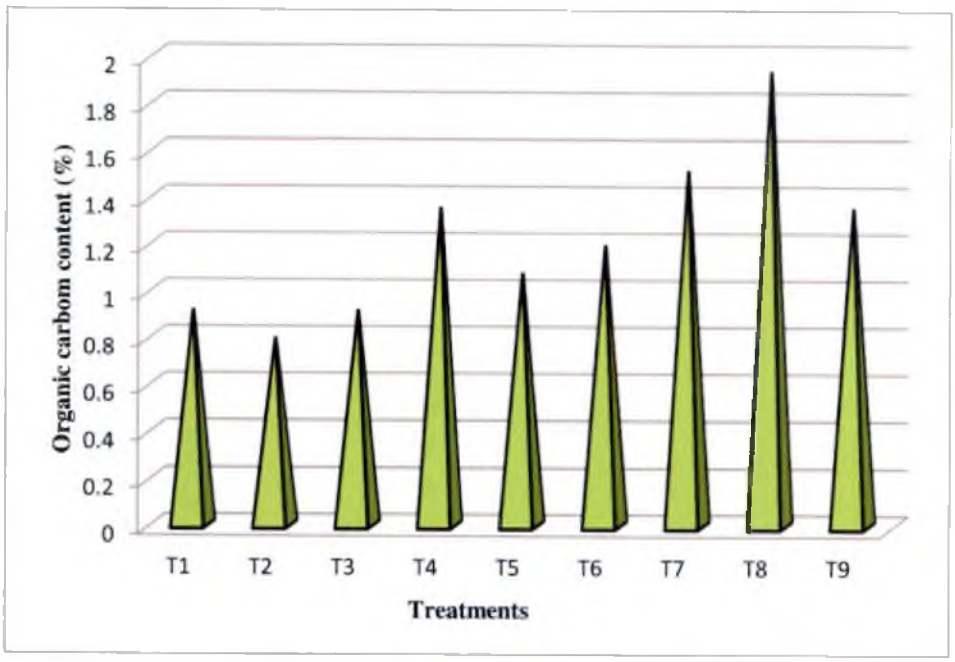
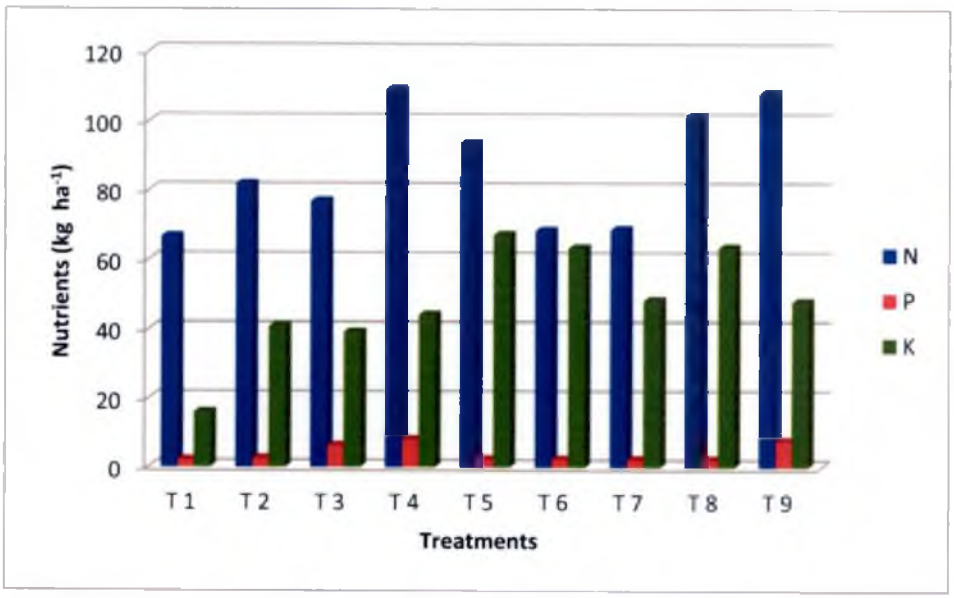


Figure 10. Effect of different treatments on available nutrients in soil



5.2.2. Secondary and Micronutrient Content in Soil

The highest magnesium content was recorded for soil irrigated with biogas slurry produced from elephant dung and cow dung combination which was on par with cow dung and pulse residue combination. Biogas slurry produced from cow dung alone and supernatant solution of cow dung and water in 1:1 ratio and least magnesium content was noticed in poultry manure and cow dung combination.

The highest zinc content was recorded for soil irrigated with supernatant solution of cow dung and water in 1:1 ratio which was on par with control and soil irrigated with biogas slurry produced from cow dung alone. The least zinc content was recorded for soil treated as per KAU Package of Practices and Recommendations for Crops 2011.

The highest copper content was recorded for soil irrigated with biogas slurry produced from cow dung and elephant dung combination which was on par with soil irrigated with cow dung and poultry manure biogas slurry and biogas slurry from cow dung and pulse residue. The least content was recorded for control. The iron content in soil which was treated with biogas slurry were showing comparatively higher amount of iron than other treatments. Soil from control and soil maintained as per KAU Package of Practices and Recommendations for Crops were shown significantly lesser amount of iron.

5.2.3. Biometric Observations of Plant

The plant height of cowpea ranged from 25.03 to 35.63 cm and it did not show any significant effect of treatments. The number of leaves per plant was highest for treatment where KAU Package of Practices and Recommendations for Crops was done. It may be due to the effect of mutual shading. The number of branches produced did not show any significant effect of treatments. The highest number of pods produced was in plants irrigated with pulse residue and cow dung combination. It might be due to higher uptake of nitrogen by the plants.

5.2.4. Yield Parameters of Plant

Biogas residues remaining after anaerobic digestion provide a valuable nutrient source, the fertilizer value of which has to be counted for improving nutrient use efficiency at field and farm. Greater hundred seed weight was recorded for plants irrigated with biogas slurry obtained from cow dung alone which was on par with bio gas slurry obtained from poultry manure and cow dung. This might be due to greater availability of nutrients for crop growth from this slurry. The yields obtained from different treatments are shown in Figure 11.

The pH of biogas slurry was negatively correlated with yield per plant and correlation value is (-0.547*). Total potassium content in slurry was positively correlated with yield per plant and correlation value is (0.582*)

The highest number of seeds per pod was obtained for the plants irrigated with supernatant solution of fresh cow dung. The highest plant dry weight was obtained for plants which were irrigated with cow dung and goat manure combination (Figure 12) and this biogas slurry was having highest total nitrogen content. In the case of plant dry weight influence of both phosphorus and potassium supply had greater effect. Similarly the increase in plant height and dry matter production was noticed when biogas slurry was applied to fodder maize by Nasir *et al.* (2010) and Islam *et al.* (2010). The increased level of nitrogen supply increased the plant uptake of nitrogen which enhanced meristematic growth, formation of enzymes and co-enzymes and also increased photosynthetic area. This might have caused increased uptake of other nutrients like phosphorus and potassium and thus enhanced plant dry matter production.

Available potassium content in soil was positively correlated with plant dry weight and correlation value is (0.518*). Calcium content in soil was positively correlated with plant dry weight and correlation value is (0.673**). Calcium content in biogas slurry was negatively correlated with plant dry weight and correlation value is (-0.563*). Organic carbon content in slurry was negatively correlated with plant dry weight and correlation value is (-0.522*).

Figure 11. Effect of different treatments on yield of cowpea

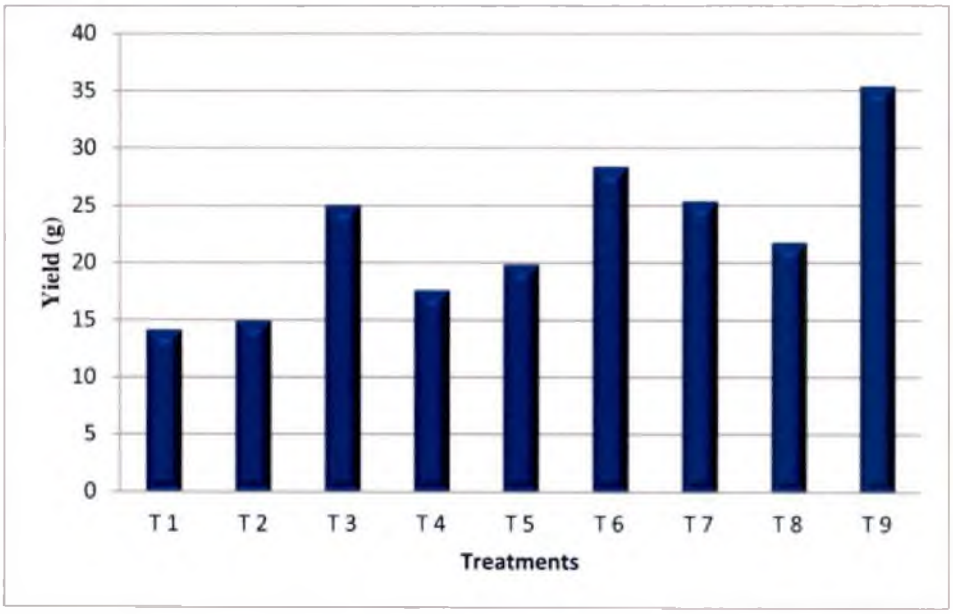
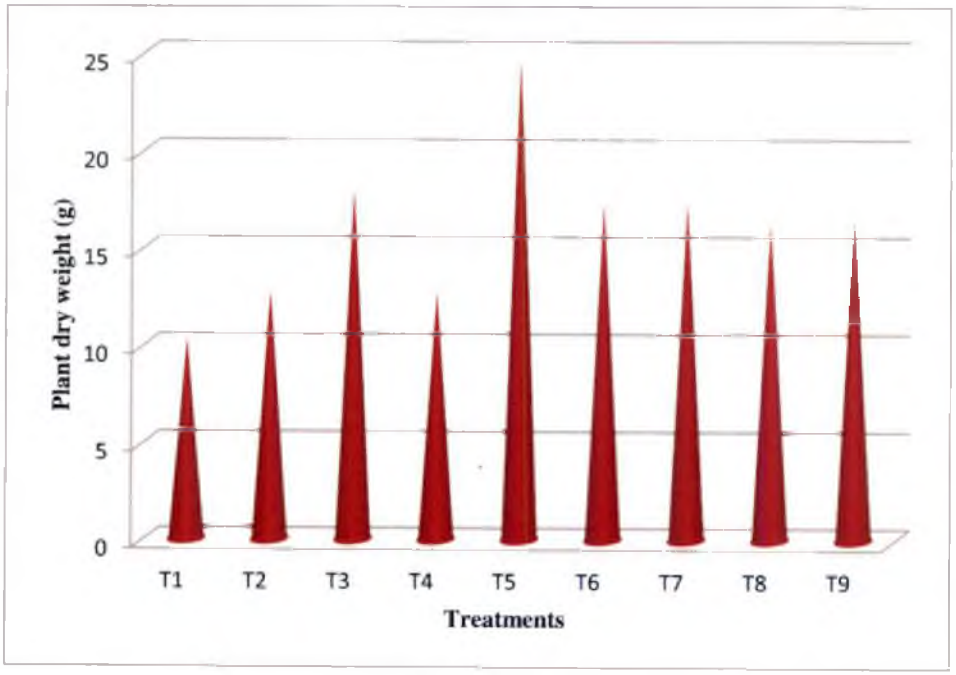


Figure 12. Effect of different treatments on dry weight of cowpea



Apart from all the application of biogas slurry, increased soil physical properties which might have helped for providing good growing condition and ultimately yield. A similar experiment conducted in maize fodder by Rahman *et al.* (2008) reported that other factors might also affect fodder plant height such as the genetic makeup of fodder, soil fertility, climatic conditions, quantity of daylight, light intensity, and season. Among these, genetic factors and soil fertility are more important.

Maximum shoot length, seedling length, and vigour index I was observed for seeds obtained from plants irrigated with biogas slurry which was produced from cow dung and pulse residue. This might be due to higher nutrient content in pulse residue and cow dung co-digested slurry. The least shoot length, seedling length and vigour index I were recorded for seeds of plants irrigated with biogas slurry produced from poultry manure and cow dung combination. Nutrient availability from poultry manure might be very slow. Nitrate nitrogen in slurry was positively correlated with vigour index I.

5.2.5. Major Nutrient Content of Plants

The nitrogen content of plants showed an increasing trend as in the case of total nitrogen content of slurry. The total nitrogen content in biogas slurry produced from poultry manure and cow dung combination; cow dung alone; goat manure and cow dung combination were comparatively higher than that of elephant dung - cow dung combination and bio degradable household waste - cow dung combination. The plant dry weights with respect to total nitrogen content of slurry are shown in figure 13. This might be due to higher levels of nitrogen in poultry manure and goat manure in combination with cow dung. The plants did not show a noticeable trend on ammoniacal, nitrate and total nitrogen content of biogas slurry even though the plants irrigated with slurry produced from cow dung and poultry manure combination showed the highest nitrogen content in plants. This slurry had higher ammoniacal nitrogen content. C: N ratio of substrate was negatively correlated with Mg content in plants and correlation value is (-0.483*)

Figure 13. Effect of total nitrogen content in slurry on nitrogen content in plants

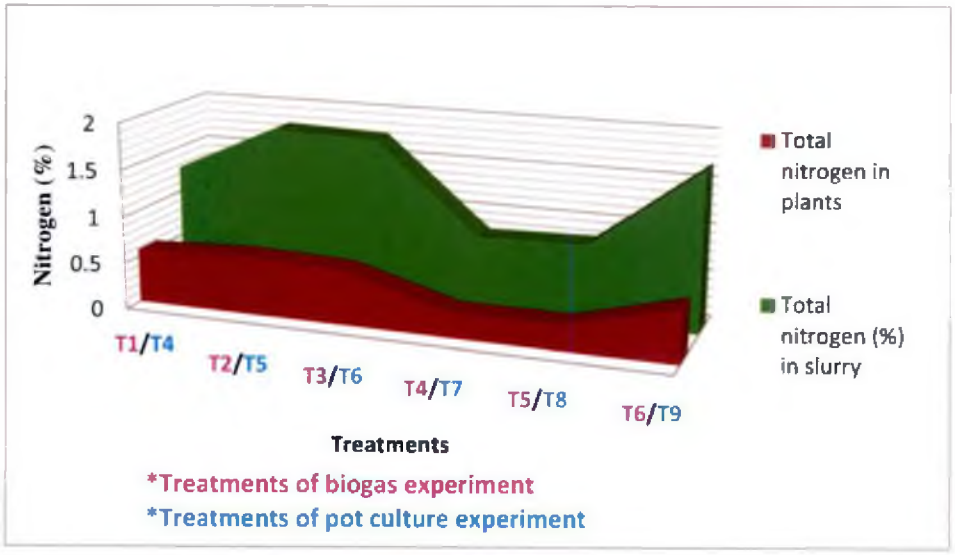
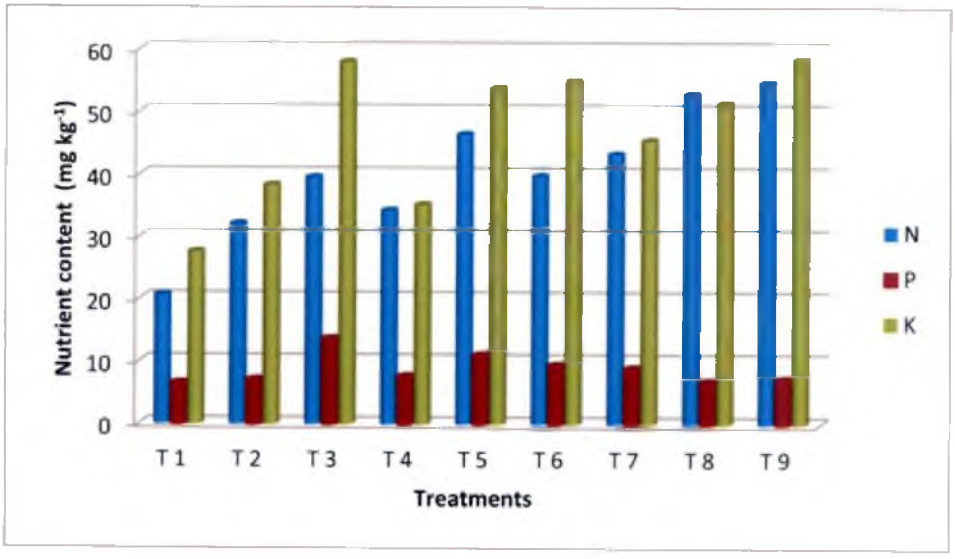


Figure 14. Total uptake of primary nutrients by plants



The nitrogen use efficiency of organic fertilizers depended on a number of factors such as nitrogen content, NH_4 to total nitrogen ratio, C: N ratio and stability of organic matter, the application time and technique as well as weather and site condition (Sorensen and Rubek , 2012).

The plants maintained as per KAU Package of Practices and Recommendations for Crops, 2011 recorded higher phosphorus content in plants. Both organic and inorganic nutrient supplies were provided.

5.2.6. Uptake of Major Plant Nutrients

The highest uptake of total nitrogen was recorded for the plants irrigated with biogas slurry produced from pulse residue and cow dung combination which was on par with all other plants irrigated with biogas slurry. The highest phosphorus uptake was recorded for plants irrigated with the supernatant solution of fresh cow dung slurry which was having comparatively higher phosphorus content than biogas slurry and the least phosphorus content was recorded for biogas slurry produced from elephant dung - cow dung combination. The uptake of major nutrients are presented in Figure 14.

The pH of biogas slurry was positively correlated with total up take nitrogen by plants and correlation value is (0.496*). C:N ratio of biogas slurry was negatively correlated with total uptake of phosphorus by plants and correlation value is (-0.539*).

Calcium content in soil was positively correlated with total uptake of phosphorus by plants and correlation value is (0.503*). Calcium content in soil was positively correlated with total uptake of potassium by plants and correlation value is (0.618**)

5.2.7. Micronutrient Content in Plants and Seeds

Calcium in plants ranged from 17.91to 28.25 mg kg^{-1}. Magnesium content in plants ranged from 6.80 mg kg^{-1} to 7.50 mg kg^{-1}. Calcium and magnesium content of plants are presented in Figure 15. Copper in plants ranged from

1.15 mg kg^{-1} to 1.75 mg kg^{-1}. Iron in plants ranged from 6.53 mg kg^{-1} to 38.42 mg kg^{-1}. Micronutrient content of plants are presented in Figure 16. Calcium content in biogas slurry was positively correlated with magnesium content in plants and correlation value is (0.488*). Available phosphorus content in soil was positively correlated with magnesium content in plants and correlation value is (0.598**)

The highest zinc content was recorded for plants irrigated with supernatant solution of cow dung. During anaerobic digestion process, the available zinc content was reduced. It might be due to immobilization of zinc. The highest manganese content in plants was recorded for biogas slurry from household waste and cow dung combination. Total nitrogen content in slurry was negatively correlated with manganese content in plants and correlation value is (-0.752**)

The highest calcium content in seeds was obtained for plants irrigated with biogas slurry produced from pulse residue and cow dung combination and the least value was observed for biodegradable household wastes and cow dung combination. The magnesium content in seeds ranged from 3184.00 mg kg^{-1} to 3200.17 mg kg^{-1}. Calcium and magnesium content of seeds are presented in Figure 17. C:N ratio of substrate was positively correlated with copper content in plants and correlation value is (0.491*)

The highest zinc content in grains was observed in plants irrigated with goat manure and cow dung combined biogas slurry and the least zinc content was recorded in control. Organic carbon content in slurry was negatively correlated with zinc content of plants and correlation value is (-0.513*). Plant dry weight was positively correlated with zinc content in plants and correlation value is (0.563*). Total uptake of potassium by plants was positively correlated with zinc content in plants and correlation value is (0.486*)

The highest copper content was recorded for seeds of plants irrigated with biogas slurry generated from poultry manure and cow dung. The least seed copper content was observed for seeds of plants irrigated with supernatant

Figure 15. Calcium and magnesium content of plants (mg kg^{-1})

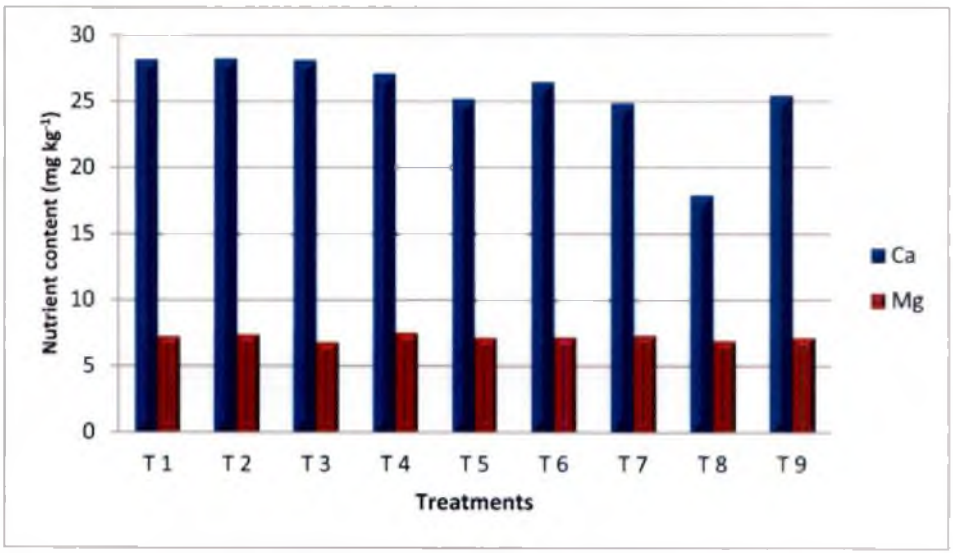


Figure 16. Micronutrient content of plants (mg kg^{-1})

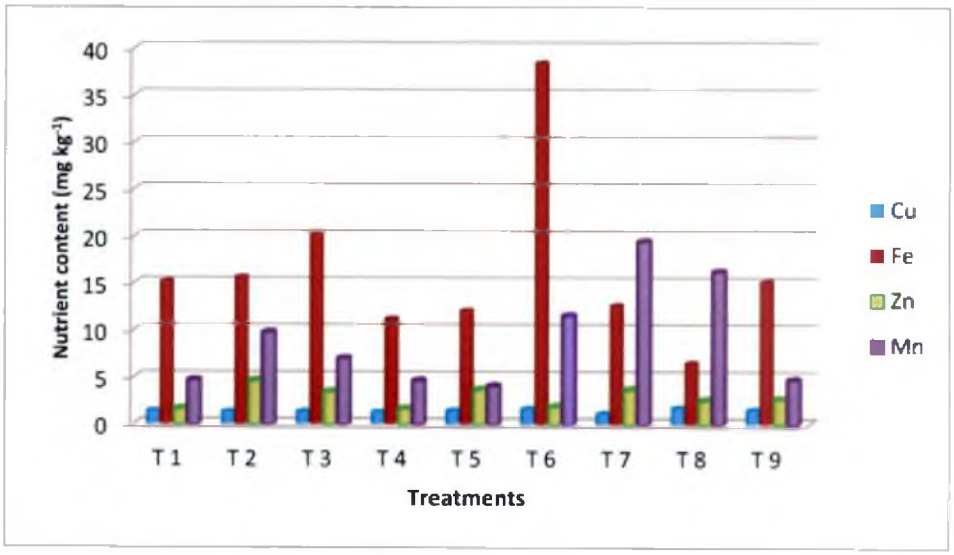


Figure 17. Calcium and magnesium content of seeds

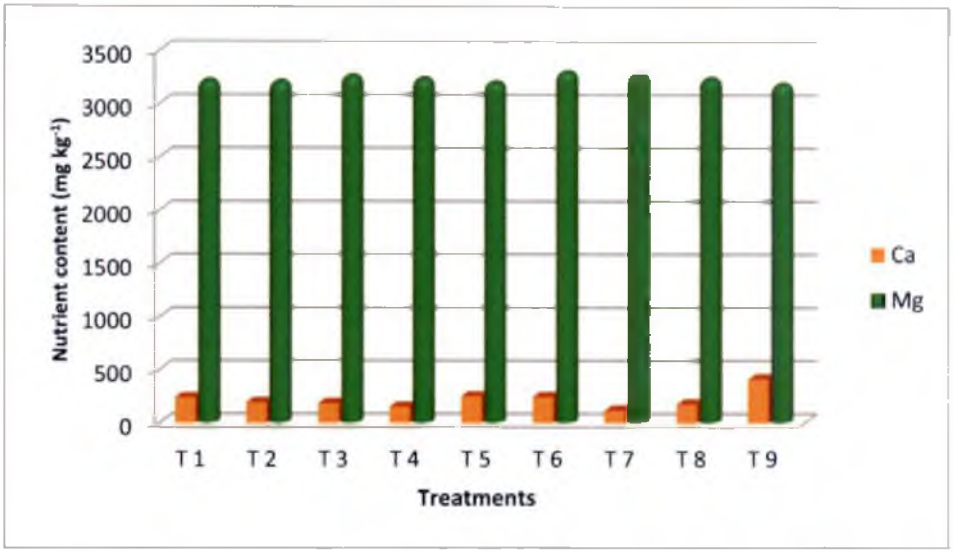
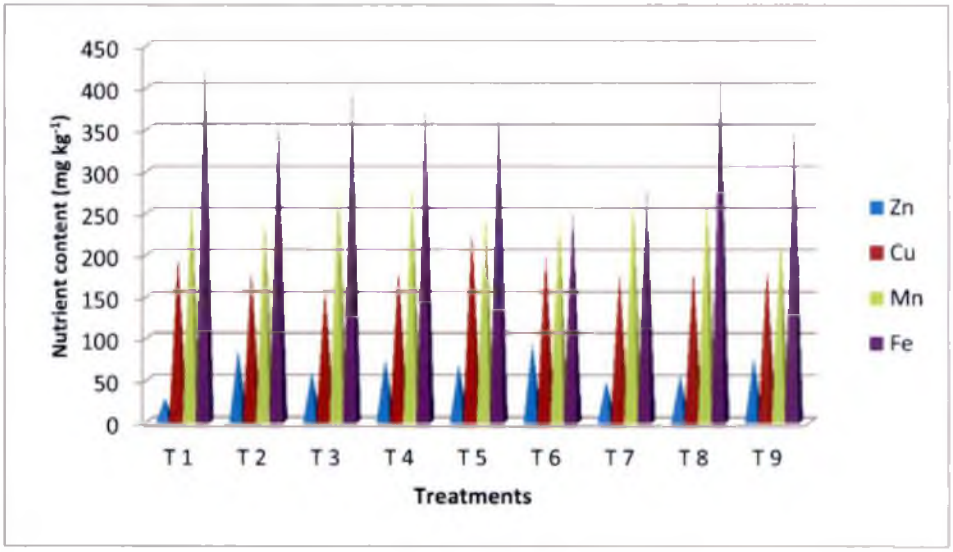


Figure 18. Micronutrient content of seeds



solution of fresh cow dung and water slurry. Highest manganese content in seeds were recorded for the plants irrigated with biogas slurry produced from cow dung alone and least manganese content was observed in seeds of plants irrigated with biogas slurry produced from pulse residue - cow dung combinations. Micronutrient content of seeds are presented in Figure 18.

The highest iron content was recorded for seeds from control treatment and least iron content was observed in the plants irrigated with biogas slurry produced from poultry manure and cow dung combination.

5.2.8. Crude Protein, Germination per Cent, Root Length, Seedling Dry Weight and Vigour Index II Of Seedling Obtained from the Seeds of Pot Culture Experiment

Crude protein content of seeds ranged from 8.22 to 10.84%. Germination per cent of seeds from pot culture experiment ranged from 67.13 to 76.93%. Root length of seedlings obtained from seeds of pot culture experiment ranged from 7.92 to 11.73 cm. Seedling dry weight of seedlings obtained from seeds of pot culture experiment ranged from 0.04 to 0.05 g. Vigour index II of seedlings obtained from seeds of pot culture experiment ranged from 2.48 to 3.93.

5.2.9. Seed Quality Analysis in Pot Culture Experiment

Maximum shoot length, seedling length and vigour index I was observed for seeds obtained from plants irrigated with biogas slurry produced from cow dung and pulse residue and least shoot length seedling length and vigour index I were recorded for seeds of plants irrigated with biogas slurry produced from poultry manure and cow dung combination.

5.3.1. EXPERIMENT III - SEED TREATMENT STUDIES

5.3.1. Effect of Slurry on Seed Germination and Vigour Index of Seedlings

The highest shoot length was recorded for seed coated with biogas slurry produced from cow dung, poultry manure and water in 1:1:2 ratio which was

supported by similar works of Shakuntala and Jagadeesh (1996) in sesamum, sunflower and safflower. Least shoot length was recorded for seeds coated with biogas slurry produced from cow dung alone.

Organic carbon content in slurry was negatively correlated with shoot length of seedling obtained from coated seeds and correlation value is (-0.524*). C:N ratio of biogas slurry was positively correlated with seedling dry weight obtained from dipped seeds and correlation value is (0.603**)

The highest germination per cent was recorded for the seeds soaked for two hours in water and seeds dipped in biogas slurry produced from cow dung alone. This might be due to more amount of growth promoting substances present in cow dung. Enzyme activity and degradation of proteins to amino acids were reduced due to dipping in slurry. The least germination per cent was observed in the seeds dipped in biogas slurry produced from household waste and cow dung combination. Moisture content in slurry was found to be correlated with germination per cent of seedlings obtained from soaked seeds. Total potassium content in slurry was positively correlated with germination percent of coated seeds and correlation value is (0.615**).

Vigour index I is the product of seedling length and germination per cent and vigour index II is the product seedling dry weight and germination per cent. The highest vigour index I and II were recorded for seedlings obtained from soaked seeds in water for two hours. Least vigour index I was recorded for seeds dipped in biogas slurry produced from household waste and cow dung combination and the least vigour index II was recorded for seeds soaked in biogas slurry produced from cow dung, pulse residue and water in 1:1:2 ratio.

Nitrate nitrogen content in slurry was positively correlated with vigour index II of seedlings obtained from biogas coated seeds and correlation value is (0.469*). C:N ratio of biogas slurry was positively correlated with vigour index II of seedlings obtained from coated seeds and correlation value is (0.504*)

5.3.2. Root Length, Seedling Dry Weight and Length of Seedlings Obtained from Treated Seeds

The root lengths of seedlings from treated seeds ranged from 11.30 to 22.67 cm and dry weight of seedlings from treated seeds ranged from 0.05 to 0.07g. The seedling lengths of plant treated seeds ranged from 39.62 to 51.52cm.

C:N ratio of biogas slurry was positively correlated with vigor index II of seedlings obtained from soaked seeds and correlation value is (0.473*). C:N ratio of biogas slurry was positively correlated with root length of seedlings obtained from coated seeds and correlation value is (0.576*).

6. SUMMARY

6. SUMMARY

The study on 'Substrate Impact on Biogas Production and Manurial Value of Slurry' was conducted at College of Horticulture, Vellanikkara. In order to find the substrate impact on biogas production, an experiment was conducted with six treatments and three replications. The treatments were biogas production using cow dung alone with equal quantity of water as control (T_1), cow dung with poultry manure and water in 1:1:2 ratio (T_2), cow dung with goat manure and water in 1:1:2 ratio (T_3), cow dung with biodegradable house hold waste and water in 1:1:2 ratio (T_4), cow dung with elephant dung and water in 1:1:2 ratio (T_5) cow dung with pulse residue and water in 1:1:2 ratio (T_6). Among these treatments, the highest methane content was obtained from cow dung with pulse residue and water in 1:1:2 ratio (T_9) and highest carbon dioxide content was obtained from cow dung and water in 1:1 ratio (T_2). The lowest hydraulic retention time was recorded for cow dung with elephant dung and water in 1:1:2 ratio (T_5). The highest organic carbon content was recorded for cow dung with poultry manure and water in 1:1:2 ratio (T_2). The ammoniacal nitrogen in the slurry was highest for the slurry produced from cow dung with biodegradable house hold waste and water in 1:1:2 ratio (T_4) and total nitrogen content was highest for cow dung with poultry manure and water in 1:1:2 ratio (T_3). The total phosphorus content in biogas slurry was highest for cow dung with elephant dung water in 1:1:2 ratio (T_5).

For the assessment of manurial value of slurry, a pot culture experiment with cowpea variety Bhagyalekshmy was conducted with nine treatments and three replications. The first treatment was control (T_1), in the second treatment cowpea plants were maintained as per Package of Practices and Recommendations of KAU (T_2), the third treatment was the plants irrigated with supernatant solution of fresh cow dung and water in 1:1 (T_3). In the fourth treatment (T_4), the pots were maintained at field capacity using biogas slurry produced from first treatment (cow dung and water in 1:1 ratio) of first experiment. In the fifth treatment (T_5), the pots were maintained at field capacity

using biogas slurry produced from second treatment of (cow dung, poultry manure and water in 1:1:2 ratio) first experiment. In the sixth treatment (T_6), the pots were maintained at field capacity using biogas slurry produced from third treatment of (cow dung, goat manure and water in 1:1:2 ratio) first experiment. In the seventh treatment (T_7), the pots were maintained at field capacity using biogas slurry produced from fourth treatment of (cow dung, biodegradable house hold waste and water in 1:1:2 ratio) first experiment. In the eighth treatment (T_8), pots were maintained at field capacity using biogas slurry produced from fifth treatment of (cow dung, elephant dung and water in 1:1:2 ratio) first experiment and in the ninth treatment (T_9) the pots were maintained at field capacity using biogas slurry produced from sixth treatment of (cow dung, pulse residue and water in 1:1:2 ratio) first experiment.

The highest soil organic carbon content in soil was recorded for the soil irrigated with biogas slurry produced from co-digestion of cow dung with elephant dung and water in 1:1:2 ratio. The highest available nitrogen and phosphorus content was recorded for the soil irrigated with biogas slurry produced from cow dung and water in 1:2 ratio. The highest available potassium content was recorded for the soil irrigated with biogas slurry produced from co-digestion of cow dung with poultry manure and water in 1:1:2 ratio.

The highest number of pods per plant and yield per plant was recorded for the plants irrigated with biogas slurry produced from pulse residue and cow dung combination. The highest number of seeds per pod was noticed for plants irrigated with supernatant solution of fresh cow dung slurry. The highest plant dry weight was observed for the plants irrigated with biogas slurry produced from poultry manure and cow dung combination.

The highest total uptake of nitrogen was recorded for the plants irrigated with biogas slurry produced from pulse residue and cow dung combination and highest total phosphorus uptake was done by plants irrigated with supernatant solution of fresh cow dung slurry. Total uptake of potassium by plants did not

show significant effect of treatments. The seeds obtained from the pot cultured plants were subjected to germination test and vigour index analysis. The highest seedling length and vigor index one was recorded for the seeds obtained from the plants irrigated with biogas slurry produced from pulse residue and cow dung combination.

In order to find an effective seed treatment method using various types of biogas slurry produced in first experiment, three methods were adopted *viz.*, coating of seeds with biogas slurry, soaking of seeds for two hours in biogas slurry and dipping of seeds in biogas slurry. One set of control was kept for each seed treatment method. Results indicated that the seeds soaked in irrigation water were found to be the best seed treatment than any other biogas slurry treatment.

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7. REFERENCES

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APPENDICES

APPENDIX 1

Weather parameters during the experiment

Weeks	Temperature (^{0}C)		Relative humidity (%)	Average sun shine hours (h)	Rainfall (mm)	Evaporation (mm)
	Maximum	Minimum				
24.03.2014	38.1	24.3	56.0	8.9	0.0	6.3
02.04.2014	36.3	25.9	71.0	7.1	0.7	4.8
09.04.2014	34.5	24.3	74.0	5.1	20.0	4.1
16.04.2014	35.2	25.8	73.0	8.4	3.5	4.5
23.04.2014	35.2	26.5	75.0	4.9	14.6	3.9
30.04.2014	35.0	25.0	72.0	5.6	68.4	4.1
07.05.2014	31.5	25.1	82.0	4.3	71.7	2.5
14.05.2014	33.2	25.0	75.0	7.6	0.0	3.8
21.05.2014	33.4	25.2	77.0	5.6	0.0	3.3
28.05.2014	32.7	25.2	81.0	5.4	27.5	3.5
04.06.2014	30.4	24.5	86.0	3.1	32.3	3.1
11.06.2014	30.7	23.8	86.0	2.4	13.2	2.6
18.06.2014	30.5	24.2	88.0	12.0	23.8	2.8
25.06.2014	31.1	24.6	83.0	4.6	23.9	3.5
02.07.2014	30.8	23.0	81.0	4.4	7.6	2.9
09.07.2014	28.1	22.8	91.0	0.2	34.8	2.2
16.07.2014	29.2	22.8	89.0	1.5	26.1	2.3
23.07.2014	29.8	23.1	89.0	0.7	27.7	2.5
30.07.2014	28.0	23.4	92.0	0.3	43.3	2.6
06.08.2014	28.4	22.5	88.0	6.4	22.7	2.3
13.08.2014	30.6	23.8	83.0	4.9	6.1	2.6
20.08.2014	31.0	23.6	84.0	5.4	21.4	3.6
27.08.2014	29.2	23.0	88.0	1.3	29.0	3.2

Contd...

Weeks	Temperature (^{0}C)		Relative humidity (%)	Average sun shine hours (h)	Rainfall (mm)	Evaporation (mm)
	Maximum	Minimum				
03.09.2014	29.8	23.0	85.0	3.4	9.5	2.5
10.09.2014	30.9	23.3	81.0	7.4	11.0	3.3
17.09.2014	31.5	23.5	80.0	7.3	1.8	3.1
24.09.2014	33.6	23.6	79.0	6.1	22.7	3.7
01.10.2014	32.4	23.7	82.0	4.4	19.7	3.1
08.10.2014	31.4	23.8	85.0	3.8	12.0	2.4
15.10.2014	32.2	23.9	77.0	3.8	16.0	3.2
22.10.2014	31.8	23.7	79.0	4.9	16.9	2.5
29.10.2014	32.0	23.1	81.0	4.4	10.3	2.3
05.11.2014	32.5	23.2	79.0	5.5	15.6	3.8
12.11.2014	32.2	23.4	70.0	7.8	18.4	4.1
19.11.2014	31.4	23.5	65.0	5.2	0.0	3.5
26.11.2014	30.3	22.4	61.0	2.3	0.0	3.5
03.12.2014	32.3	21.9	63.0	8.5	0.0	3.7
10.12.2014	32.4	23.8	68.0	6.4	7.8	3.3
17.12.2014	31.5	24.2	69.0	5.4	0.0	3.8
24.12.2014	31.6	23.3	65.0	5.0	1.6	3.5
01.01.2015	32.5	21.5	68.0	8.0	0.0	3.2
08.01.2015	32.0	23.5	59.0	8.7	0.0	4.5
15.01.2015	32.4	22.1	54.0	9.0	0.0	4.5
22.01.2015	32.9	23.0	55.0	9.3	0.0	5.1
29.01.2015	32.9	23.7	53.0	9.2	0.0	6.0
05.02.2015	33.6	23.2	52.0	8.3	0.0	5.9
12.02.2015	35.2	23.5	60.0	8.6	0.0	4.6
19.02.2015	35.0	22.2	48.0	6.9	0.0	6.1
26.02.2015	34.0	23.6	68.0	9.7	0.0	4.3

APPENDIX 11

Effects of different treatments on the temperature (^{0}C) inside the digester (weekly average)

Weekly	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
23.07.2014	29.31	28.96	28.80	28.69	28.80	28.91
30.07.2014	29.26	28.87	28.83	28.63	28.93	28.94
06.08.2014	29.04	30.69	30.27	30.44	30.14	30.21
13.08.2014	29.31	28.96	28.80	28.69	28.80	28.91
20..8.2014	30.03	29.44	29.73	27.43	29.14	28.91
27.08.2014	30.94	30.40	30.29	28.57	30.40	30.44
3.09.2014	27.77	27.39	27.46	27.31	27.63	28.01
10.09.2014	28.67	28.40	28.41	28.67	28.73	28.47
17.09.2014	30.13	29.53	29.54	29.34	29.54	29.43
24.09.2014	29.26	28.87	28.83	28.63	28.93	28.94
01.10.2014	29.04	30.69	30.27	30.44	30.14	30.21
08.10.2014	31.17	30.71	30.47	30.60	30.70	30.46
15.10.2014	31.44	31.17	30.77	29.83	30.87	31.00
22.10.2014	32.01	31.39	31.44	31.09	31.39	31.11
29.10.2014	32.05	33.46	31.11	32.80	31.84	31.03
05.11.2014	31.73	31.63	31.24	31.26	31.83	31.41
12.11.2014	31.44	32.16	31.84	32.19	31.56	32.11
19.11.2014	31.77	31.81	31.23	31.43	31.20	32.04

Contd...

Weekly	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
26.11.2014	31.93	32.17	32.00	32.19	31.81	31.13
03.12.2014	31.80	31.90	31.77	31.73	31.06	31.37
10.12.2014	31.50	31.84	32.27	32.21	33.36	31.07
17.12.2014	31.19	31.19	30.90	31.67	31.74	30.96
24.12.2014	31.37	30.03	29.97	30.06	30.83	29.57
01.01.2015	32.63	30.29	30.80	28.94	29.16	30.11
08.01.2015	32.17	31.54	31.09	32.16	31.91	31.36
15.01.2015	31.47	30.77	30.86	31.14	31.04	31.21
22.01.2015	31.46	30.51	30.66	30.41	34.64	30.76
29.01.2015	31.43	31.11	31.00	31.24	30.77	30.84
05.02.2015	34.53	31.59	29.50	29.33	29.83	28.91
12.02.2015	34.44	31.64	30.71	31.47	31.53	30.69
19.02.2015	32.04	31.63	30.44	31.26	31.20	31.66
26.02.2015	26.73	27.50	26.77	26.99	26.93	27.11

APPENDIX III

Quantity of slurry generated after each intake (weekly average)

Weekly	Quantity of slurry generated (ml)					
	T_1	T_2	T_3	T_4	T_5	T_6
23.07.2014	3575.0	3587.1	5089.3	5913.6	5267.9	4800.0
30.07.2014	3321.4	1064.3	3325.7	1435.7	1245.7	2278.6
06.08.2014	800.0	1081.4	2205.7	678.6	461.4	514.3
13.08.2014	1878.6	1492.9	2242.9	1871.4	1428.6	857.1
20..8.2014	2864.3	750.0	2878.6	2828.6	1147.1	3531.4
27.08.2014	2400.0	2985.7	2628.6	1782.9	2251.4	994.3
3.09.2014	1935.7	1978.6	1092.9	821.4	2250.0	1041.4
10.09.2014	2472.9	1378.6	218.6	391.4	427.1	1525.7
17.09.2014	1330.0	1642.9	1338.6	770.0	792.9	917.1
24.09.2014	1730.0	1207.1	1217.1	708.6	838.6	1371.4
01.10.2014	2871.4	2778.6	2728.6	2407.1	2264.3	2257.1
08.10.2014	2057.1	4164.3	3392.9	2814.3	4285.7	5635.7
15.10.2014	4314.3	6028.6	4228.6	5321.4	5907.1	3257.1
22.10.2014	5671.4	7285.7	5785.7	2821.4	3335.7	4057.1
29.10.2014	1714.3	2707.1	2792.9	2007.1	1707.1	1371.4
05.11.2014	3214.3	3228.6	3642.9	2928.6	2535.7	1642.9

Contd...

Weekly	Quantity of slurry generated (ml)					
	T_1	T_2	T_3	T_4	T_5	T_6
12.11.2014	3428.6	3428.6	2992.9	4678.6	2044.3	1864.3
19.11.2014	2400.0	3064.3	4392.9	3142.9	4250.0	3285.7
26.11.2014	2900.0	3607.1	5250.0	3571.4	3964.3	4535.7
03.12.2014	3585.7	4071.4	4785.7	6000.0	4785.7	5035.7
10.12.2014	5314.3	5500.0	5571.4	8571.4	8071.4	6285.7
17.12.2014	9000.0	8714.3	9571.4	22857.1	9571.4	9714.3
24.12.2014	26142.9	12000.0	12000.0	9142.9	10857.1	13428.6
01.01.2015	9571.4	11142.9	9714.3	10857.1	9285.7	12285.7
08.01.2015	10000.0	11428.6	12142.9	9857.1	12571.4	8142.9
15.01.2015	4500.0	6107.1	6357.1	6571.4	4285.7	5571.4
22.01.2015	3228.6	3214.3	3942.9	7857.1	3428.6	2428.6
29.01.2015	2571.4	2785.7	2785.7	2285.7	2571.4	4071.4
05.02.2015	3214.3	2928.6	3857.1	3214.3	3571.4	3357.1
12.02.2015	3285.7	3642.9	4000.0	3500.0	4028.6	4357.1
19.02.2015	4142.9	5000.0	4785.7	5142.9	4642.9	4928.6
26.02.2015	4714.3	4285.7	4214.3	2928.6	4642.9	4000.0

APPENDIX IV

Effect of different treatments on volume of gas generated (weekly average)

Weekly	Treatments					
	T1	T2	T3	T4	T5	T6
23.07.2014	45.57	45.73	45.89	45.89	45.57	45.90
30.07.2014	47.15	51.25	52.25	49.74	47.30	54.59
06.08.2014	65.70	64.20	64.84	62.37	63.90	64.43
13.08.2014	68.20	69.30	69.46	70.12	69.36	69.34
20..8.2014	67.34	67.23	67.16	68.12	68.32	67.21
27.08.2014	68.67	68.34	67.96	68.23	67.26	67.46
03.09.2014	71.96	67.87	67.00	65.95	66.67	72.90
10.09.2014	68.67	68.34	65.43	68.24	57.60	68.90
17.09.2014	68.34	67.96	68.36	67.96	69.30	69.30
24.09.2014	67.96	68.23	68.20	68.52	69.46	67.00
01.10.2014	68.23	67.26	68.52	68.36	70.12	68.52
08.10.2014	67.26	67.46	67.20	68.21	69.36	68.36
15.10.2014	67.46	68.21	68.36	68.05	69.34	59.93
22.10.2014	68.21	69.67	68.52	68.96	67.60	69.93
29.10.2014	66.46	67.40	68.67	66.70	68.50	71.96
05.11.2014	68.20	68.36	68.52	68.67	68.83	68.99
12.11.2014	68.21	67.74	68.89	66.15	68.94	66.95
19.11.2014	69.20	69.30	69.46	70.12	69.36	69.34
26.11.2014	68.20	68.36	68.52	68.67	68.83	68.05
03.12.2014	68.96	67.96	62.45	68.16	68.94	69.23
10.12.2014	51.30	68.52	68.36	68.20	67.89	68.05
17.12.2014	68.20	68.36	68.52	68.67	68.83	68.99

Contd...

Weekly	Treatments					
	T1	T2	T3	T4	T5	T6
24.12.2014	68.20	72.21	69.20	68.20	68.96	68.67
01.01.2015	67.89	68.05	68.20	68.36	68.52	68.67
08.01.2015	68.20	68.96	68.67	68.20	68.20	59.60
15.01.2015	68.05	68.83	68.67	68.52	68.36	68.20
22.01.2015	69.30	67.89	68.34	67.20	68.20	68.36
29.01.2015	69.46	68.05	67.96	68.36	68.21	68.20
05.02.2015	70.12	68.20	68.23	68.52	69.20	68.52
12.02.2015	69.36	68.36	67.26	68.67	68.20	67.20
19.02.2015	69.34	68.52	67.46	68.83	68.96	68.36
26.02.2015	68.54	68.67	68.21	68.05	68.67	68.52

SUBSTRATE IMPACT ON BIOGAS PRODUCTION AND MANURIAL VALUE OF SLURRY

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The growing population of the world increases energy demand and waste generation. Due to lack of proper recycling technology, large quantities of biodegradable wastes are being produced. Biogas production is a good technology for mitigating both the problems. The slurry produced after biomethanation is a good manure which provides balanced nutrition for crops and improves soil quality. There are large varieties of biodegradable wastes which can be used as feed stock for biogas production. The quality and quantity of biogas and slurry generated are based on the nature and composition of feed stock. In order to find out an efficient substrate from the available substrates in Kerala and to determine manurial value of different types of slurry, the present study was undertaken at College of Horticulture, Vellanikkara during 2012 – 2014.

To elucidate the impact of different substrates on biogas production, an experiment was laid out with three replications and six treatments *viz.*, cow dung alone and co digestion of cow dung with poultry manure, goat manure, biodegradable house hold waste, elephant dung, and pulse residue in 1:1 ratio with equal quantity of water. The biogas generated from different treatments were analysed for CH_4 and CO_2. The highest CH_4 production was recorded for the treatment combination of pulse residue with cow dung which was on par with cow dung and elephant dung combination, while the highest CO_2 was recorded in the biogas produced from poultry manure and cow dung combination. The hydraulic retention time recorded was lowest for elephant dung and cow dung combination.

The highest organic carbon content was recorded in the slurry generated from cow dung alone, which was on par with the combination of goat manure with cowdung. The highest ammoniacal nitrogen content was recorded for biodegradable house hold waste - cow dung combination followed by pulse residue - cow dung

combination. The highest total nitrogen content was observed for cow dung - poultry manure combination and cow dung - goat manure combination.

A pot culture experiment was conducted to find out the manurial value of the slurry obtained from the treatments for biogas production with three replications and six treatments. This experiment was done by irrigating the pots with the slurry obtained from the treatments along with absolute control, as per Package of Practices and Recommendations of KAU (both were irrigated with fresh water) and with fresh undigested cow dung slurry with cowpea (var. Bhagyalakshmy) as test crop. The highest number of pods per plant and the highest yield were obtained from the plants which were irrigated with biogas slurry produced from pulse residue and cow dung combination.

After harvest, the highest organic carbon content was noted in soil which was irrigated with biogas slurry produced from elephant dung and cow dung combination. The highest available nitrogen and available phosphorus content was recorded for soil irrigated with slurry produced from cow dung alone and cow dung - pulse residue combination. Available potassium content in soil was highest for in the soil which was irrigated with slurry produced from poultry manure and cowdung combination which was on par with slurry produced from elephant dung - cow dung combination.

The plants irrigated with the slurry produced from cow dung-pulse residue combination and cow dung-elephant dung combination had recorded highest uptake of total nitrogen. Plants raised from the seeds obtained from these treatments showed greater shoot length, seedling length and vigour index. However elaborate studies are necessary to monitor the hormones present in different types of slurry generated from different substrates.