SUITABILITY OF AZOLLA (*Azolla pinnata*) FOR BIOGAS SLURRY ENRICHMENT

By

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(2009-11-158)

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KERALA, INDIA

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THESIS

Submitted in partial fulfillment of the
requirement for the degree of

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Department of Soil Science and Agricultural Chemistry

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KERALA, INDIA

2012

DECLARATION

I hereby declare that the thesis entitled **"Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment"** 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.

Vellanikkara, **Bishnu Prasad Paudel**

Date: 07/03/2012 **(2009-11-158)**

CERTIFICATE

Certified that the thesis entitled **"Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment"** is a record of research work done independently by **Mr. Bishnu Prasad Paudel** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellanikkara,
Date: 07/03/2012

Dr. Jayasree Sankar S.
Chairperson, Advisory committee

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Bishnu Prasad Paudel (2009-11-158)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Soil Science and Agricultural Chemistry**, agree that the thesis entitled **"Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment"** may be submitted by **Mr. Bishnu Prasad Paudel**, in partial fulfilment of the requirement for the degree.

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Bishnu Prasad Paudel

ABBREVIATIONS

Ca	Calcium
cm	Centimeter
C:N	Carbon Nitrogen ratio
Cu	Copper
Fe	Iron
FYM	Farm yard manure
Fig	Figure
G	Gram
HCl	Hydrochloric Acid
HRT	Hydraulic Retention Time
K	Potassium
KAU	Kerala Agricultural University
kg	Kilogram
L	Litre
m^2	Square meter
m^3	Cubic meter
mg	Milligram
Mn	Manganese
N	Nitrogen
NH_4^+	Ammonium
OC	Organic Carbon
^0C	Degree Celsius
P	Phosphorus
pH	Hydrogen ion concentration
%	Percentage
S	Sulphur
TN	Treatment Number
TS	Total solid
V	Volume
Zn	Zinc

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Dedicated to beloved Laxmi, Sushant & Oshan

Introduction

1. INTRODUCTION

Anaerobic digestion is an important biological conversion process that converts biomass to methane in the absence of oxygen, popularly known as biogas, leaving a stabilized residue which makes high-quality, nitrogen-rich organic manure and soil amendment. Digestion of manure or other organic biomass in an anaerobic digester transforms part of the organically bound nutrients to a mineral form. This is significant for nitrogen, where the organic nitrogen is released as ammonium, which is readily available for the crops. It reduces the need for applying additional mineral nitrogen fertilizers and can decrease the ammonia volatilization and nitrate leaching, mitigating environmental impact.

Cattle manure which is largely burnt after drying in rural area household for cooking purpose can be used for biogas and slurry production which provides both fuel and high quality manure. Slurry production with co-digestion of azolla and cow dung can produce more methane gas and quality manure than cow dung alone (Das *et al.*, 1994). Though, azolla is used for dual cropping in rice field, its incorporation is labour intensive. So the use of azolla as a N biofertilizer and organic manure is very much limited in Kerala condition.

Anaerobic digestion of azolla with cow dung is another alternative for enriching the slurry with high N and K content and for narrowing C/N ratio for increased gas production. Azolla which fixes atmospheric N in symbiosis with the blue green algae (*Anabaena azollae*) can be harvested within a short period of time because of its fast multiplication rate. This feature enables its utilization as a substrate in biogas plant thereby reducing the quantity of cow dung. This combination would enhance gas production and enrich the slurry.

Though biogas slurry is used for crop nutrition, the acceptance and extent of use is much limited owing to the handling and application problems arising out of its liquid nature. In India, slurry is mostly dried and stored before using in the field. When fresh slurry is air-dried, the available nitrogen, particularly ammonium is lost by volatilization. Thus nitrogen content of air-dried slurry is less than that of air-dried dung (Gurung, 1996). Drying of digested slurry with suitable materials can be the best alternative to conserve nitrogen in the slurry and to produce quality manure.

Production and use of this nutritive organic preparation would help and promote quality agriculture by producing of healthy food for a healthy life. The present study is an earnest effort to investigate the potential of azolla in enriching biogas slurry and to enhance its handling property with greater application as crop manure.

The main objectives of the study were:

1. To explore the potential of azolla in augmenting biogas slurry
2. To obtain the suitable material to prepare enriched manure for field application
3. To evaluate the crop response to enriched manure

Review of Literature

2. REVIEW OF LITERATURE

2.1 Anaerobic digestion

Wilkie (2000) reported that anaerobic digestion was a natural process that converted biomass to energy. Biomass could be any organic material from plants, animals or their wastes. The anaerobic process removed a majority of the odorous compounds. Yadav and Hesse (1981) reported that biogas contained 50-70 per cent methane, 30-40 per cent carbon-dioxide, 5-10 per cent hydrogen, 1-2 per cent nitrogen and traces of hydrogen sulphide.

Mclnerny and Bryant (1981) reported that biogas process could be divided into three steps namely hydrolysis, acidification and methane formation. The first step involves the enzyme- mediated transformation of insoluble organic material and higher molecular mass compounds such as lipids, polysaccharides, proteins, fats, nucleic acids, etc. into soluble organic materials and is carried out by strict anaerobes such as *Streptococci*. In second step of acidogenesis, another group of microorganisms ferment the break-down products to acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid. In third step, these acetic acid, hydrogen and carbon dioxide are converted into a mixture of methane and carbon dioxide by the methanogenic bacteria (acetate utilizers like *Methanosarcina species* and *Methanothrix* species and hydrogen utilizing species like *Methanobacterium and Methanococcus*).

2.1.1 Factors responsible for anaerobic digestion

The degradation of organic substances is a complex process, involving (i) enzymatic hydrolysis and the formation of sugars, amino acids and fatty acids; (ii) acedogenesis of volatile fatty acids (VFAs) and (iii) methane (and CO_2) formation (Burton and Turner, 2003).

Potential hydrogen ion concentration (pH) of the digester

According to Hobson *et al.* (1981), a digester usually runs at a pH of 7- 7.2. It is optimum for methanogenic bacteria. The pH of the system is the function of bicarbonate alkalinity of the system, the fraction of carbon dioxide in the digested gas and concentration of volatile acids. The methanogenic group of bacteria is the most pH sensitive.

Deshpande *et al.* (1979) conducted an experiment with water hyacinth as an additive, mixed with cattle dung (1:1), to view the pattern of volatile fatty acid production, pH levels and the gas produced during the above fermentation. During the first stage of fermentation (1 to 13 days) there was an accumulation of volatile fatty acids liberated through the degradation of organic matter, resulting in a decrease in the pH of the fermenting slurry and in second stage of fermentation (13 to 28 days), pH was increased to 7.0, this pH of fermenting slurry was steady at 7.0 up to last day of retention time.

Raw material and C: N ratio

Shilpkar and Shilpkar (2009) reported that the C: N ratio ranging from 20-30:1 was considered as optimum for anaerobic digestion. They reported that in high C/N ratio, the nitrogen was consumed rapidly by methanogens for meeting protein requirements and no longer reacted with left over carbon content of the materials. As a result gas production was low. On the other hand, in low C/N ratio, nitrogen was liberated and accumulated in the form of ammonia which increased the pH within the digester. When the pH was more than 8.5, it was toxic to methanogenic bacteria. Karki and Dixit (1984) reported that the common substrates used for biogas production were cattle manure (24:1), pig manure (18: 1), poultry manure (10: 1), human excreta (8: 1) and vegetable waste (12-30: 1).

It was reported by Singh *et al.* (1983) that the acetate production was less in anaerobic digestion of cow dung which was the main reason for low gas production even though C/N ratio was 26: 1. Further, he also indicated that acetate played an important role during coordinated action of acidogenic and methanogenic microorganisms.

Dilution and consistency of inputs

Gurung (1996) reported that fresh cattle dung had to be mixed with water at the ratio of 1:1 on a unit volume basis. The dilution should be made to maintain the total solid from 7 to 10 per cent. When the dung was too diluted, the solid particles settled down into the digester and when too thick, gas formed at the lower part of digester was impeded to flow up through the particles. In both cases, gas production was less than the optimum. Iteun *et al*. (2007) found that the best dilution was with 1:1 ratio of substrate and water for better gas production which ensured 8 per cent of total solids.

Temperature of digester

Chawla (1986) reported three temperature zones i.e. thermophilic zone (above 45 ^{0}C), mesophilic zone (22- 45 ^{0}C) and psychrophilic zone (below 20 ^{0}C) during anaerobic digestion process. But effective and efficient anaerobic fermentation was carried out at both thermophilic and mesophilic temperature. However, 30- 35 ^{0}C was considered as the optimum temperature, because the rate of volatile acid produced became more or less proportional to its utilization by methanogenic bacteria. Maurya *et al*. (1994) and Desai and Madamwar (1994) reported that the different temperature ranges during which anaerobic fermentation could be carried out varied between psychrophilic (below 30 ^{0}C), mesophilic (30–40 ^{0}C) and thermophilic (50–60 ^{0}C) zones. However, anaerobes were most active in the mesophilic and thermophilic temperature range. The length of fermentation period was dependent on temperature.

Hydraulic Retention Time (HRT)

Tomar (1995) reported the time taken by the substrates for maximum gas production as the HRT and that 70-80 per cent digestion got completed on Hydraulic Retention Time (HRT). According to temperature and substrate, HRT varied. Under Indian condition, it varied from 30 – 60 days. He also reported that in Kerala, Hydraulic Retention Time (HRT) was 30 days**.** Human excreta, due to its high nutrient content, needed no more than 30 days retention in biogas plants (KVIC, 1983). The shorter HRT was likely to face the risk of washout of active bacterial population while longer retention time required a large volume of the digester. Hence, there was a need to reduce HRT for domestic biogas plants based on solid substrates (Yadvika, 2004).

2.2 Biogas slurry as an organic manure and soil amendment

During the anaerobic digestion process, organic compounds were broken down, firstly via acetogenic bacteria to methane precursors, largely volatile fatty acids and then to methane and other products via methanogenic bacteria. Under anaerobic conditions, 50 per cent organic forms of nitrogen (N) were converted into ammonium-N (NH_4-N) (Anon, 2006).

FAO (2007) reported that biogas slurry consisted of 93 per cent water and 7 per cent dry matter, of which 4.5 per cent was organic and 2.5 per cent was inorganic matter. The percentage of NPK content of slurry on wet basis was 0.25, 0.13 and 0.12 while on dry basis it was 3.6, 1.8 and 3.6 respectively. In addition to the major plant nutrients, it also provided micro-nutrients such as Zn, Fe, Mn and Cu. Janotti *et al*. (1986) and Goldstein (2000) concluded that during fermentation process of manures and other biomaterials, the NH_4^+ content and pH of the biogas slurry was increased, while dry matter content, C/N ratio and smell decreased in comparison to animal manures used as organic sources.

2.2.1 Effect of slurry on physic-chemical and biological properties of soil

The decline in soil fertility and productivity due to excessive soil erosion, nutrient run-off and loss of soil organic matter had stimulated interest in improving overall soil quality by the addition of organic matter (Bastian and Ryan, 1986). Increase in fertilizer use efficiency must be ensured to achieve sustainable production (Tolanur and Badanur, 2003 and Laxminarayana, 2006). Use of chemical fertilizers in combination with organic manure was found to be essential to improve the soil health (Bajpai *et al*., 2006).

Soil physical properties

Manure provided N, P, K, S and other nutrients besides serving as a soil conditioner by increasing organic matter and improving porosity and water-holding capacity (Safley *et al*., 1986; Eghball and Power, 1994 and Eghball *et al*., 2002).

Swift and Woomer (1993) reported that besides supplying plant nutrients, organic matter played an important role in enhancing the cation exchange capacity (CEC), improving soil aggregation, increasing water holding capacity of the soils, stabilizing its humus content and preventing the leaching of nutrients.

Soil chemical properties

Bulk of nitrogen was present in organic form and therefore total N content in soils was closely related to organic matter content, which in mineral soils could vary from traces to 20-30 per cent (Prasad and Power, 1997). Therefore total N content in soils could vary from traces to two per cent depending upon the C/N ratio of soil organic matter. Due to high temperature in tropic and subtropics, organic matter content in soil in these regions was much lower than in temperate region. Prasad (2007) reported that total N content in Indian soils (0-15 cm layer) varied from 0.02 – 0.1 per cent.

Gallert *et al*. (2003) opined that soil pH increased by 1.20 and 1.0 in 0-2 cm and 2-5 cm soil layers respectively two days after slurry application. Increasing pH from 1 to 2 units had been previously reported by them in the first few centimeters of soil amended with animal slurry. An increase in soil pH following slurry application could be partially attributed to the chemical composition and the dissociation of slurry carbonates (De la Rubia *et al.*, 2006).

Forster-Carneiro *et al.* (2007) reported that 2-6 days after the slurry addition, the pH values remained stable in the top 5 cm of the soil. The slight decline in the soil pH after day 6 could be attributed to the acidifying effects of NH_3 volatilization and nitrification. Most of the slurry-derived volatile fatty acids were decomposed four days after slurry application, indicating that these compounds are rapidly used by soil microbes (Perez *et al.,* 1997). Loss of N through nitrate leaching, microbial denitrification and NH_3 volatilization resulted in decreased supply of N to crops (Möller *et al.*, 2008). Several studies had shown that slurry might induce immobilization of soil N after application (Opperman *et al*., 1989 and Paul and Beauchamp, 1995). One of the factors affecting N immobilization was volatile fatty acid in the slurry (Kirchmann and Lundvall, 1993).

Forster-Carneiro *et al.* (2007) and Voca *et al*. (2005) reported that the initial soil NH_4 -N content was approximately 2.0 mg kg^{-1} but increased sharply following slurry application. At day 2 after slurry addition, the soil NH_4^+ content was significantly higher in the 0-2 cm soil layer due to the higher NH_4^+ content of the slurry. From days 2 to 8 after slurry application, the NH_4^+ concentration gradually decreased in the top 5 cm of the soil. The difference observed during the period (2 to 8 days) suggested that biological processes such as immobilization and nitrification significantly contributed to slurry NH_4^+ transformation after the NH_3 volatilization rate returned to a lower level and was mainly related to soil pH and the NH_3 concentration in the top 5 cm of soil.

Goutam *et al*. (1996) observed the methane emission from rice fields amended with urea, biogas slurry + urea and FYM + urea. The combined fertilization (FYM + urea) plot showed the maximum emission rate of 4.86 mg m^{-2} h^{-1} with a total load of 49.44 kg ha^{-1} and was 2.3 times higher than BSS + urea treated plot (22.08 kg ha^{-1}). The grain yield in urea, BSS + urea and FYM + urea plots were 3.34, 2.94, and 2.85 t ha^{-1} respectively. He suggested that biogas slurry was a preferred source over FYM by causing lesser environmental pollution without any significant reduction in grain yield.

Soil Biological Properties

Xianjun *et al.* (2011) reported that slurry application increased soil microflora and amounts of phosphobacteria, silicate bacteria, ammonifying bacteria, N-fixation bacteria and actinomycetes but the accumulation of fungi was significantly inhibited. Compared with the control treated by chemical fertilizers, the bacteria and fungi ratios of the soils treated with biogas slurry @ 168 kg ha^{-1} and 225 kg ha^{-1} increased by 142.7 and 202.3 per cent respectively. At the same level of slurry @ 225 kg ha^{-1}, the activities of soil enzymes viz., invertase, phosphatase and protease of the experimental groups also increased by 63.96, 137.61 and 139.66 per cent respectively.

2.3 Effect of slurry on crop

Bhattarai and Maskey (1988) reported that well digested biogas slurry alone could increase the crop production by 20 to 30 percent besides having a beneficial effect on soil conditions. The vegetables responsive to biogas slurry were potato, tomato, sweet potato, water-melon, radish, carrot, cauliflower, turnip, onion, garlic etc. Rice, sugarcane and jute also responded well to biogas slurry.

Karki (2001) opined that the highest yield of cabbage (69.6 t ha^{-1}) was produced with the application of full dose of recommended fertilizer together with 20 t ha^{-1} of slurry compost which was 36.2 per cent higher over the control. The second highest yield was recorded as a result of slurry compost treatment applied at 20 t ha^{-1}. It was 28.4 percent higher than the control. Likewise, there was not much difference in the yield of cabbage due to application of liquid slurry (18.4 per cent increment) and full dose of chemical fertilizer (19.6 per cent increment). He concluded that biogas slurry in liquid form produced 6.6 per cent higher yield than the FYM treatment. Similarly, slurry compost produced 11.06 per cent higher yield than the liquid slurry whereas mineral fertilizer produced 6.0 per cent lower than the slurry compost. Experiment conducted at Khumaltar on different vegetables and cereal crops clearly indicated that biogas slurry had a good effect on yield of vegetable crops. French bean recorded the highest yield increase (70 per cent), followed by maize (37.03 per cent), wheat (33.3 per cent), cauliflower (17.85 per cent), tomato and rice (10 per cent) (Bhattarai and Maskey, 1988).

Lakshmanan (1993) observed that soaking of wheat seeds for 6-12 hours in slurry and water before sowing resulted in significant increase in germination. In addition the mean germination time was reduced and the root length of seedlings was also increased consequent to soaking. He concluded that as slurry effluent contained soluble nutrients and numerous active substances capable of promoting metabolism of the seedlings, it holds promise as an effective seed coating medium.

2.4 Azolla as a substrate for slurry enrichment

2.4.1 Chemical composition of azolla

Singh (1979), Liu (1979) and Van Hove (1989) reported that the chemical composition of azolla varied not only according to species and ecotypes but also with the ecological conditions and phase of growth. They reported that azolla plant had 94.96 per cent moisture content and the range of chemical composition of *Azolla pinnata* in per cent on dry weight basis was ash 10.5, crude fat 3.0-3.36, crude protein 23.0-30.0, nitrogen 4.0-5.0, phosphorus 0.5-0.9, calcium 0.1-1.0, potassium 2.0-4.5, magnesium 0.5-0.65, manganese 0.11-0.16, iron 0.06-0.26, soluble sugars 3.4-3.7 and starch 6.5-6.54.

The nutrient composition of azolla was affected by the time or age of harvest, manner of drying and exposure to sunlight. It had been found that the sun dried azolla has higher nitrogen value than air dried (Van Hove, 1989). According to Herzalla *et al*. (2001), total carbohydrate content of azolla plants exposed to different dark and light condition showed no significant differences. They proved that *Azolla pinnata* had higher carbohydrate level than *A. microphylla* and *A. filiculiodes*.

Lumpkin and Plucknett (1980) determined that *Azolla pinnata* had a C/N ratio of 15:1. Reports indicated an increase in nitrogen recovery when azolla was incorporated into soil rather than allowed to decompose in water. Nitrogen in azolla was released slowly and its availability was only about 70 per cent of ammonium sulfate. Most of the studies had revealed that two thirds of the nitrogen in azolla was released after six weeks. Kanniyan & Somporn (1987) also reported that azolla species had a low C/N ratio ranging from 10.14-13.79:1. Nina (1999) reported that fast growing floating and submerged freshwater macrophytes were used commercially all over the world in aquaculture systems to produce protein rich feed for animals, green manure and biogas production.

2.4.2 Multiplication of azolla

Thomas (2008) reported that about 25 kg of fertile soil, 5 kg cow dung and 30 g of superphosphate per square meter were optimum for the growth of azolla. The fresh and pure culture of azolla was placed in water at the rate of 500 g per square meter. Subsequently, a mixture of 15 g superphosphate and one kg cow dung were added for each one square meter area in every 10 days.

Lumpkin and Plucknett (1980) found that azolla could survive within a pH range of 3.5 to 10, but optimum growth was in the range of 4.5 to 7. The relative growth rate was influenced by a direct relationship between light intensity and pH. The highest growth rate was achieved at high pH (9-10) at high light intensity and low pH (5-6) at low light intensity. Nitrogen fixation was optimal at pH 6.0 and temperature 20 ^{0}C. They further claimed that relative growth and nitrogenase activity was at a maximum at 50 per cent of full sunlight although the difference between growth at 50 per cent and 100 per cent sunlight was not significantly different. Heavy shading was known to decrease azolla growth to almost zero.

According to Van Hove *et al.* (1983), azolla spreads laterally until it covers the whole surface of the water. Its exponential growth rate is subjected to many environmental constraints. *Azolla pinnata* doubles its biomass in 3-5 days in lab situation and in 5-10 days in field condition. Alexander *et al.* (2009) reported that inoculation of fresh biomass of azolla @200 kg ha^{-1} could multiply faster and covered the rice field as a green mat in 2-3 weeks with 15-20 tones biomass accumulation. The cultivation of azolla not only supplied biomass and N, but also contributed K, P, Ca, S, Zn and Fe.

2.4.3 Azolla as a substrate for biogas production

Chowdhary *et al*. (1994) reported that powdered leaves of some plants and legumes (Gulmohar, *Leucacena leucocephala*, *Acacia auriculiformis*, *Dalbergia sisoo* and *Eucalyptus tereticonius*) stimulates biogas production between 18 to 40 per cent. .

Das *et al*. (1994) have studied the use of azolla on biogas production. They mixed azolla and cow dung in different proportion. The different ratios of cow dung and azolla tried were 1: 0.2, 1: 0.4, and 1: 0.6. They found that mixed residues containing cow dung and azolla in the ratio of 1: 0.4 could produce maximum quantity of biogas.

2.5 Post treatment of digested slurry

Biogas residue resulting from anaerobic digestion of organic waste had significant potential as a crop fertilizer and soil conditioner. However, the residue might not be a suitable for soil improvement in its basic form, owing to possible phytotoxicity (Poggi-Varaldo *et al*., 1999 and Wang, 1991), viscosity and odor (Smet *et al*., 1998), handling difficulty and expensive soil application approaches (Tchobanoglous *et al*., 2002). Therefore, further treatment was essential to enhance its applicability as a crop fertilizer (Abdullahi *et al.*, 2008).

Biogas residue displayed high water content, raising an issue of whether it should be dewatered and dried before application. On the other hand, upon drying, up to 90 per cent NH_4^+ might be lost as NH_3^+ (Rivard *et al.*, 1995) which would dramatically reduce the benefits of biogas residue as a crop fertilizer.

Gurung (1996) concluded that only 10 per cent of the total nitrogen in fresh dung was readily available for plant growth. On drying the fresh cow dung, approximately 30 to 50 per cent of the nitrogen escaped within 10 days while nitrogen escaping from digested slurry within the same period was only 10 to 15 per cent. Therefore, the value of slurry as manure was higher than when slurry was used after being stored and dried.

Toumela *et al*. (2000) reported that biogas residue could be further processed and stabilized in compost to reduce the risk of N loss. This resulted in a product of higher nutrient quality, as mineralized N was absorbed on humus fractions. Additionally, composting of the digested residue induced the degradation of resistant organic fraction, such as lignin, which were usually not completely degraded by anaerobic microorganisms. He concluded that the aerobic microbes present in compost transformed phytotoxic NH_3 into nitrates, resulting in an end-product with improved nutrient quality that was more suitable as a soil conditioner.

Suthar (2010) reported that after 15 weeks of vermicomposting of biogas slurry, an increased NPK status and a C/N ratio of less than 20 was recorded. The results suggested that vermitechnology could be a potential method to convert biogas slurry of domestic biogas plants into value-added products.

Materials and Methods

3. MATERIALS AND METHODS

This investigation to find out the "Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment" was carried out through three experiments as detailed below:

Experiment 1: Anaerobic digestion of substrates
Experiment 2: Preparation of enriched manure
Experiment 3: Crop response to enriched manure

3.1 Experiment – 1: Anaerobic digestion of substrates

The experiment was designed using floating drum biogas plants to determine whether azolla contributes to enrich biogas slurry and if so, the optimum ratio of cow dung and azolla to be used for such enrichment.

3.1.1 Materials used

Azolla (*Azolla pinnata*) was used as a substrate for co-digestion to enrich cow dung based biogas slurry. Azolla was multiplied in specially designed tanks in the vermicompost unit of College of Horticulture.

About 20 shallow cement tanks of 2.5 m x 2 m x 15 cm size, available at the vermicompost unit of College of Horticulture were used for mass multiplication of azolla. About 25 kg of fertile sieved soil, 10 kg cow dung and 60 g superphosphate were added and mixed in each tank. Water level was maintained at 10 cm depth. To this, azolla (Source: Mahima clusters, Kannara, Pattikkad, Thrissur) was placed at the rate of 500 g m^{-2}.

Azolla pinnata

Cow dung

Plate 1. Substrates used for anaerobic digestion

Plate 2. Mass multiplication of azolla at vermicompost unit

3.1.2 Anaerobic digester

The floating drum biogas plants of 0.5 m^3 capacity were used for anaerobic digestion of substrates. The main body of the biogas plant is the digester which holds substrates. The substrates are added through inlet pipe. The gas produced inside the digester is collected in gas holder and the bottom of gas holder must be dipped into the substrates to create an anaerobic condition. The gas collected in gas holder can be used daily through gas outlet. When substrates get completely digested, slurry comes out through slurry outlet (Plate 1).

3.1.3 Loading of substrates in digester

As per the treatments, the required quantities of cow dung and azolla were loaded in each treatment. The details are given in Appendix I.

The water content for each treatment was determined using the recommendation for better biogas production as reported by Ituen *et al.* (2007) to get a total solid (TS) of 8 per cent in the slurry input. Hence, the proportion of total solid to water was the same in all the slurry input treatments.

3.1.4 Technical details

Design	-	CRD (Completely Randomized Design)
Replications	-	3
Treatments	-	5
Capacity of Digester	-	0.5 m^3

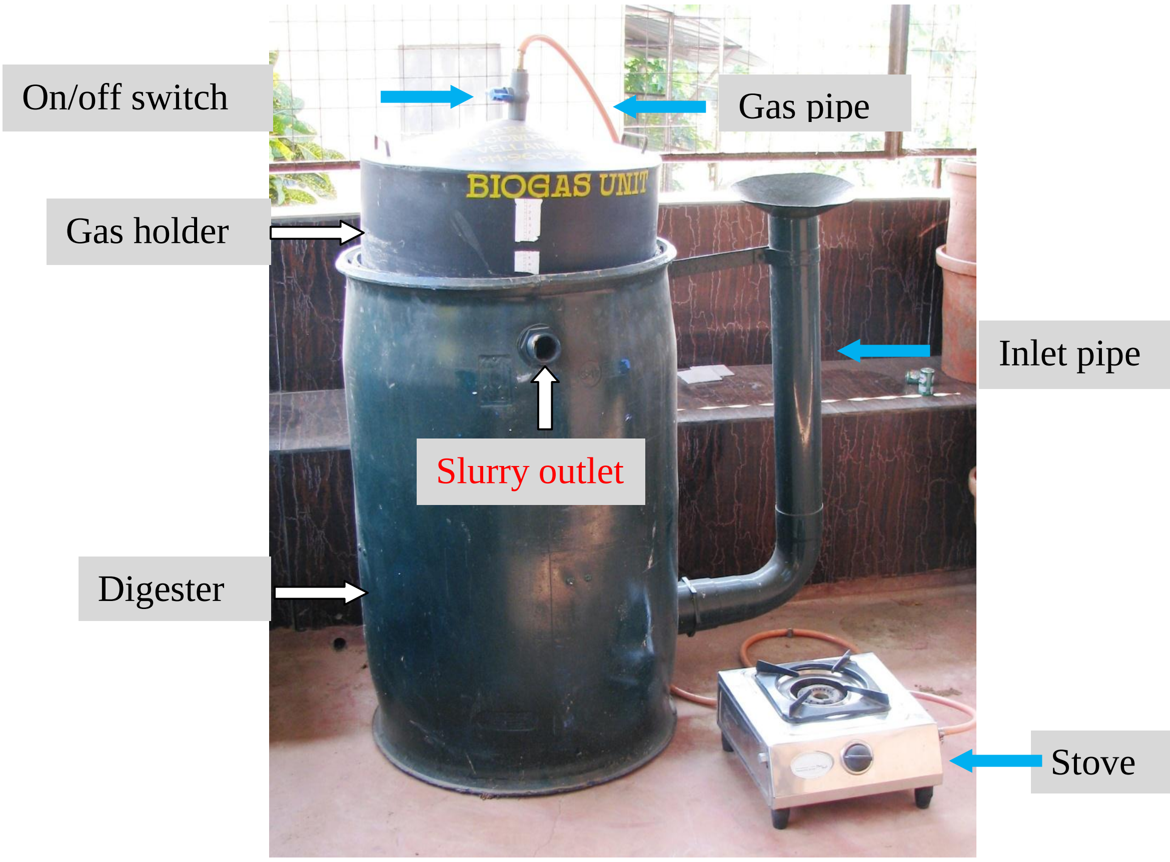


Plate 3. Floating drum biogas unit of 0.5 m³ capacity

Table 1. Treatment details of Experiment I

Treatment No.	Details	Cow dung /Azolla ratio
T_1	cow dung alone	1: 0
T_2	cow dung + azolla	1: 0.25
T_3	cow dung + azolla	1: 0.50
T_4	cow dung + azolla	1: 0.75
T_5	cow dung + azolla	1: 1

3.1.5 Biochemical composition of **azolla**

Before the initiation of the experiment, moisture per cent of azolla was determined and then it was analyzed for N, P, K, crude fat and crude fiber. The methodology adopted to determine the above parameters are detailed in Table 2.

Table 2. Composition of azolla- Methodology adopted

Parameter	Methods	Reference
Crude protein (N content X 6.25)	Sulphuric acid + catalyst (copper sulphate and sodium sulphate) digestion followed by Microkjeldahl distillation.	Jackson (1958)
P	Diacid Extract; Spectrophotometry	
K	Diacid Extract; Flamephotometry	
Crude fat	Petroleum ether extract by Soxhlet apparatus	Thimmaiah (1989)
Crude fiber	Hot diluted H_2SO_4 and NaOH	

Crude protein content was calculated by multiplying N with the factor 6.25.

The processing of azolla was done by washing the samples thoroughly using distilled water, packed into brown paper bags, dried in an an oven at 70c and powdered manually for analytical work.

3.1.6 Manurial value of cow dung

The moisture per cent of cow dung was determined by oven drying at 105 ^{0}C for 8 hours. Laboratory analysis was done for total N, P, K, carbon and pH. These parameters were determined using standard procedures as described in Table 3.

3.1.7 Digestion of substrates

During the anaerobic digestion period, Hydraulic Retention Time (HRT) and pH of digester were determined. HRT is defined as the time taken by the substrates for maximum gas production. Normally 70- 80 per cent digestion gets completed in the Hydraulic Retention Time (HRT). The gas volume was recorded everyday in each treatment. The daily temperature inside the digester was also measured in all treatments.

3.1.8 Measurement of gas

The gas produced in each treatment was measured and used for burning, every 24 hours. The increase in height of gas holder was recorded daily and volume of gas was calculated with the formula, $V= \pi r^2h$ where, V denotes volume, r denotes radius of gas holder and h denotes height increased after gas production.

3.1.9 Slurry analysis

The slurry was analysed for N, P, K, pH and carbon content using standard procedures as detailed in Table 3.


KERALA AGRICULTURAL UNIVERSITY
M.Sc. Project on
"Suitability of Azolla (Azolla pinnata) for slurry enrichment"
Design : CRD
Replications : 3
BIOGAS UNIT
R2T1
Cow dung alone
R2T2
Cowdung+Azolla
(1:0.25)
R2T4
Cowdung+Azolla
(1:0.75)
BIOGAS UNIT
BIOGAS UNIT
R1T5
Cowdung+Azolla
(1:1)


KERALA AGRICULTURAL UNIVERSITY
M.Sc. Project on
"Suitability of Azolla (Azolla pinnata) for slurry enrichment"
R2T5
Cowdung+Azolla
(1:1)
R3T5
Cowdung+Azolla
(1:1)

Table 3. Methods of slurry analysis

Parameter	Method	Reference
N	Microkjeldhal digestion and distillation	Jackson (1958)
P	Diacid Extract; Spectrophotometry	
K	Diacid Extract; Flamephotometry	
pH	Direct measurement using pH meter.	
C	Schollenberger's modified method	Schollenberger (1945)

3.2 Experiment 2: Preparation of enriched manure

This experiment was conducted to identify the best material which could be used to prepare enriched manure from enriched slurry.

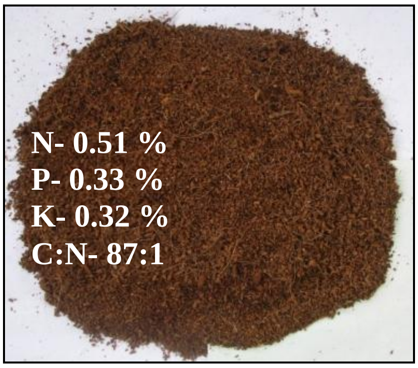
3.2.1 Materials used

The best two treatments obtained from the first experiment were mixed with solid materials for improving the handling properties. The materials used for mixing the slurry included saw dust, coir dust and powdered coconut leaves.

3.2.2 Method of mixing

The digested slurry and solid materials were mixed manually until the moisture per cent was reduced to the level for easy handling, approximately to 70 per cent.

Saw dust



Coir dust

Dried coconut leaves

Powdered coconut leaves

Plate 5. Solid materials used to prepare enriched manure

3.2.3 Technical details

Design	-	CRD (Completely Randomized Design)
Replications	-	4
Treatments	-	6
Solid materials	-	Saw dust, coir dust and powdered coconut leaves

Table 4. Treatment details of Experiment II

Treatment No.	Details of treatment
T_1	Enriched slurry I + saw dust
T_2	Enriched slurry I + coir dust
T_3	Enriched slurry I + powdered coconut leaves
T_4	Enriched slurry II + saw dust
T_5	Enriched slurry II + coir dust
T_6	Enriched slurry II + powdered coconut leaves

Highest nitrogen rich digested slurry was designated as Enriched slurry I
Second highest nitrogen rich digested slurry was designated as Enriched slurry II

3.2.4 N, P and K content of solid materials

The N, P and K content of saw dust, coir dust and powdered coconut leaves was determined before mixing with enriched slurry. The methodology adopted to determine the above parameters is detailed in Table 3.

3.2.5 Addition of solid materials

The quantity of solid materials required for mixing slurry obtained from the selected best two treatments to arrive at the moisture per cent of easy handling was recorded.

N- 3.44 %
P- 0.74 %
K- 2.93 %
C:N- 6.45:1

Enriched slurry

Enriched manure after mixing with coconut leaves

N- 4.1 %
P- 1.14 %
K- 3.89 %
C:N- 8.6:1

Enriched manure after mixing with coir dust

N- 3.74 %
P- 1.01 %
K- 3.23 %
C:N- 10.2:1

Enriched manure after mixing with saw dust

N- 3.53 %
P- 0.88 %
K- 3.12 %
C:N- 9.8:1

Plate 6. Enriched slurry before and enriched manure after mixing

3.2.6 Manurial value of enriched manure

The manurial value of enriched manure was determined by standard procedures as described in Table 3. (After mixing with solid materials, Enriched slurry I and II selected from first Experiment were designated as Enriched manure I and II).

3.3 Experiment 3: Crop response to enriched manure

This experiment was conducted to evaluate the response of rice crop (variety – Jyothy) to enriched manure.

3.3.1 Soil

The soil used for the study was laterite of the order ultisols belonging to Vellanikkara series. The soil was analysed for available N, P_2O_5, K_2O, pH and organic carbon before the initiation of the experiment. The methods employed for soil analyses are given in Table 5.

Table 5. Methods of soil analysis

Parameter	Methods	Reference
pH	1: 2.5 soil water suspension – pH meter.	Jackson (1958)
Organic carbon	Wet oxidation method	Walkley and Black (1934)
Available N	Alkaline permanganate method	Subbiah and Asija (1956)
Available P_2O_5	Bray's extractant (0.03 *N* NH_4F in 0.025 *M* HCL)	Bray and Kurtz (1945)
Available K_2O	Neutral normal ammonium acetate method using flame photometer.	Jackson (1958)

Plate 7a. Overall view of pot culture experiment

Plate 7b. Crop comparison between control and enriched manure II+NPK

3.3.2 Technical details

Design	-	CRD (Completely Randomized Design)
Replications	-	3
Treatments	-	7
Crop	-	Rice
Variety	-	Jyothy

Table 6. Treatment details of Experiment III

Treatment No.	Details of treatment
T1	Absolute control
T2	NPK+FYM (as per POP, KAU)
T3	FYM alone (as per POP, KAU)
T4	Enriched manure I alone (@ 5 tons ha-1)
T5	Enriched manure II alone (@ 5 tons ha-1)
T6	Enriched manure I (@ 5 tons ha-1) + NPK (as per POP, KAU)
T7	Enriched manure II (@ 5 tons ha-1) + NPK (as per POP, KAU)

3.3.3 Details of pot culture

Surface soil collected from 0-15 cm depth was dried and ground with a wooden mallet. About 10 kg of soil was transferred to the pots. Manures and fertilizers were added to the pots according to treatments (The details of manures and fertilizers applied are given in Appendix IV) and watered adequately. Before the initiation of experiment, a nursery was raised and maintained for 21 days. Three uniform sized seedlings were transplanted in each pot.

3.3.4 Soil analysis after harvesting of crop

Soil samples were collected and analysed after harvest for the available nutrients N, P_2O_5, K_2O, pH and organic carbon. The methods employed for soil analysis are given in Table 4.

3.3.5 Biometric observations

The biometrical characters such as height of the plant, number of tillers per pot, length of panicle, number of grains per panicle, grain and straw yield were recorded at crop harvest stage.

3.3.6 Plant analysis

Plant samples were collected by uprooting two plants from each treatment at harvest stage and fresh weight of the whole plant was recorded. The plant samples were analysed for the content of N, P and K. The methodology adopted to determine the above parameters are detailed below in Table 7.

Table 7. Methods of plant analysis

Parameter	Methods	Reference
Total nitrogen	Sulphuric acid + catalyst (copper sulphate and sodium sulphate) digestion followed by Microkjeldahl distillation.	Jackson (1958)
Total phosphorous	Nitric-perchloric acid digest (2:1) estimated colorimetrically by spectrophotometer.	Hesse (1971)
Total potassium	Diacid digestion method using flame photometer.	

3.3.7 Nutrient uptake study

Based on the uptake of different major nutrients by the crop, the total uptake of nutrients was computed.

3.4 Statistical analysis

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

Results

4. RESULTS

The results of the investigations conducted on "Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment" are presented in this chapter.

4.1 Experiment 1: Anaerobic digestion of substrates

4.2 Experiment 2: Preparation of enriched manure

4.3 Experiment 3: Crop response to enriched manure

4.1 Anaerobic digestion of substrates

4.1.1 Physico-chemical properties of substrates

The substrates used for anaerobic digestion were cow dung and azolla (*Azolla pinnata*) and their chemical compositions are detailed in Table 8 and 9 respectively.

Table 8. Manurial value of cow dung

S. N.	Parameters	Value
A.	Moisture (%)	79.00
B.	Dry matter (%)	21.00
1.	pH	6.29
2.	Nitrogen (%)	0.79
3.	Phosphorus (%)	0.47
4.	Potassium (%)	0.62
5.	Total carbon (%)	26.00
6.	C/N ratio	32: 1

Table 9. Chemical composition of azolla

S. N.	Parameters	Value (%)
A.	Moisture	94.00
B.	Dry matter	6.00
	Chemical composition on dry weight basis	
1.	Nitrogen	3.63
2.	Phosphorus	0.75
3.	Potassium	2.31
4.	Total carbon	51.54
	Biochemical composition on dry weight basis	
6.	Crude protein	22.68
7.	Crude fibre	19.1
8.	Crude fat	3.20

The content of moisture in cow dung and azolla were 79 per cent and 94 per cent respectively. The N, P and K content of cow dung was 0.79, 0.47 and 0.62 per cent and that of azolla 3.63, 0.75 and 2.31 per cent respectively.

The crude protein, crude fat and crude fibre of azolla were recorded as 22.68, 3.2 and 19.1 per cent respectively. Total carbon was recorded as 51.54 per cent in azolla and 26.0 per cent in cow dung. The C/N ratio of azolla was 14: 1 and that of cow dung 32: 1.

4.1.2 Temperature of substrates inside the digester of biogas plant (^{0}C)

The data on digester temperature during anaerobic digestion was recorded on daily basis for 30 days and the mean temperature at 5 days interval are furnished in Table 10. The daily atmospheric temperature during anaerobic digestion period is given in Appendix II.

Table 10. Temperature (^{0}C) inside the digester during the anaerobic digestion

Treatments \ Days	1-5	5-10	10-15	15-20	20-25	25-30
	Temperature, ^{0}C					
T_1 (cow dung alone)	30	31	31	32	31	29
T_2 (cow dung+azolla, 1: 0.25)	30	31	32	32	31	30
T_3 (cow dung+azolla, 1: 0.5)	31	32	33	34	31	30
T_4 (cow dung+azolla, 1: 0.75)	31	32	32	33	31	30
T_5 (cow dung+azolla, 1: 1)	30	31	32	32	30	30

The highest temperature of 34 ^{0}C was recorded in the treatment T_3 (cow dung+azolla, 1: 0.5) and the lowest of 29 ^{0}C in the treatment T_1 (cow dung alone). The temperature increased within 10 to 20 days of digestion in all treatments.

4.1.3 Hydraulic Retention Time (HRT)

The Hydraulic Retention Time (HRT) as influenced by different treatments is given in Table 11.

Table 11. Influence of different treatments on HRT of anaerobic digestion

Treatment No.	Treatment details	HRT (Days)
T_1	cow dung alone	26^a
T_2	cow dung+azolla (1: 0.25)	23^b
T_3	cow dung+azolla (1: 0.5)	20^c
T_4	cow dung+azolla (1: 0.75)	24^{ab}
T_5	cow dung+azolla (1: 1)	25^a

(Means with same superscript are non significant)

The Hydraulic Retention Time (HRT) was minimum (20 days) in the treatment T_3 (cow dung+azolla, 1: 0.5) followed by T_2 (cow dung+azolla, 1: 0.25) with 23 days. The highest HRT of 26 days was observed in treatment T_1 (cow dung alone) whereas 25 days of HRT was observed in T_4 (cow dung+azolla, 1: 0.75).

4.1.4 Effect of treatments on total volume of gas produced

The total volume of gas produced per kg of total solids is given below in Table 12.

Table 12. Influence of different treatments on total volume of gas, $m^3\ kg^{-1}$ TS

Treatment	Details	Volume of gas
T_1	cow dung alone	0.21^{c}
T_2	cow dung + azolla (1: 0.25)	0.23^{bc}
T_3	cow dung + azolla (1: 0.5)	0.29^{a}
T_4	cow dung + azolla (1: 0.75)	0.25^{b}
T_5	cow dung + azolla (1: 1)	0.24^{b}

(Means with different superscript are significantly different)

The total volume of gas was recorded maximum in treatment T_3 (cow dung+azolla, 1: 0.5) with 0.29 $m^3\ kg^{-1}$ TS whereas minimumof 0.21 $m^3\ kg^{-1}$ TS was observed in T_1 (cow dung alone). The treatment T_2 (cow dung+azolla, 1: 0.25), T_4 (cow dung+azolla, 1: 0.75) and T_5 (cow dung+azolla, 1: 1) recorded a gas volume of 0.23, 0.25 and 0.24 $m^3\ kg^{-1}$ TS respectively.

4.1.5 Effect of treatments on rate of gas production ($cm^3\ kg^{-1}$ of TS)

The effect of treatments on rate of gas production at two days interval is given in Table 13.

The treatment T_3 (cow dung+azolla, 1: 0.5) had the highest influence on rate of gas production during the initial stage of digestion. This treatment recorded 6.09 and 5.8 $cm^3\ kg^{-1}$ of TS on 14th and 12th day respectively. The treatment T_4 (cow dung+azolla, 1: 0.75) registered maximum gas production of 5 $cm^3\ kg^{-1}$ on 16th day whereas treatments T_2 (cow dung+azolla, 1: 0.25) and T_1 (cow dung alone) recorded the highest value of 4.6 $cm^3\ kg^{-1}$ on 18th day of anaerobic digestion.

Table 13. Rate of gas production as influenced by treatment combinations during anaerobic digestion

Treatment Combination	Rate of gas production ($cm^3\ kg^{-1}$ of TS) at an interval of two days														
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
T_1	0.21^{c}	0.21^{c}	0.42^{d}	0.84^{c}	1.05^{e}	1.68^{e}	3.15^{d}	4.2^{b}	2.94^{c}	1.89^{a}	1.05^{b}	0.84^{a}	0.63^{b}	0.63^{a}	0.42^{a}
T_2	0.23^{c}	0.46^{b}	0.46^{d}	1.15^{b}	1.61^{d}	2.3^{d}	3.22^{d}	4.14^{b}	4.6^{a}	1.84^{a}	1.15^{a}	0.69^{b}	0.69^{a}	0.46^{b}	0.23^{b}
T_3	0.29^{a}	0.58^{a}	1.16^{a}	1.74^{a}	4.35^{a}	5.8^{a}	6.09^{a}	4.06^{b}	2.03^{d}	1.16^{b}	0.58^{c}	0.58^{c}	0.29^{d}	0.29^{c}	-
T_4	0.25^{b}	0.5^{b}	0.75^{c}	1.25^{b}	2^{c}	3.5^{c}	4.25^{c}	5^{a}	3.75^{b}	1.25^{b}	1^{b}	0.5^{d}	0.5^{c}	0.25^{c}	0.25^{b}
T_5	0.24^{c}	0.48^{b}	0.96^{b}	1.68^{a}	2.88^{b}	4.8^{b}	5.67^{b}	3.36^{c}	1.92^{d}	0.96^{c}	0.48^{d}	0.24^{e}	0.24^{d}	0.24^{c}	-

(Means with different superscript are significantly different)

T_1- cow dung alone

T_2- cow dung + azolla (1: 0.25)

T_3- cow dung + azolla (1: 0.5)

T_4- cow dung + azolla (1: 0.75)

T_5- cow dung + azolla (1: 1)

4.1.6 Physico-chemical properties of enriched slurry

The influence of various treatments on physico-chemical properties of enriched slurry are given in Table 14.

Table 14. The physico-chemical properties of enriched slurry (Oven dry basis)

Treatment	Total Solids (%)	Bulk density (gm cc^{-1})	OC (%)	pH	N (%)	P (%)	K (%)
T_1	5.40^{a}	0.98^{a}	26.1^{a}	7.1^{c}	1.72^{e}	0.73^{b}	2.43^{c}
T_2	4.32^{b}	0.97^{a}	23.0^{b}	7.3^{bc}	2.34^{d}	0.74^{ab}	2.67^{b}
T_3	3.80^{c}	0.96^{ab}	21.4^{c}	7.5^{b}	2.76^{c}	0.74^{ab}	2.90^{a}
T_4	3.49^{d}	0.95^{b}	21.6^{c}	7.8^{a}	3.23^{b}	0.77^{a}	2.91^{a}
T_5	2.68^{e}	0.95^{b}	22.2^{bc}	7.8^{a}	3.44^{a}	0.74^{ab}	2.93^{a}

(Means with different superscript are significantly different)

T_1- cow dung alone
T_2- cow dung + azolla (1: 0.25)
T_3- cow dung + azolla (1: 0.5)
T_4- cow dung + azolla (1: 0.75)
T_5- cow dung + azolla (1: 1)

The total solids varied significantly in different treatments with treatment T_1 (cow dung alone) registering the highest value of 5.40 per cent whereas the lowest total solids (2.68 per cent) was obtained in T_5 (cow dung+azolla, 1: 1). The total solids in T_2 (cow dung+azolla, 1: 0.25), T_3 (cow dung+azolla, 1: 0.5) and T_4 (cow dung+azolla, 1: 0.75) was 4.32, 3.8 and 3.49 per cent respectively.

The bulk density of enriched slurry was highest (0.98 gm cc^{-1}) in the treatment T_1 (cow dung alone) and lowest (0.95 gm cc^{-1}) in the treatment T_5 (cow dung+azolla, 1: 1) and T_4 (cow dung+azolla, 1: 0.75).

In general, the pH of the enriched slurry was in alkaline range. It was 7.8 in the treatments T_4 (cow dung+azolla, 1: 0.75) and T_5 (cow dung+azolla, 1: 1) and 7.1 in T_1 (cow dung alone). It was noted that the pH value increased by increasing the quantity of azolla. The treatment T_2 (cow dung+azolla, 1: 0.25) and T_3 (cow dung+azolla, 1: 0.5) recorded the pH value of 7.3 and 7.5 respectively.

With respect to the organic carbon, maximum content (26.1 per cent) was recorded in T_1. The lowest content (21.4 per cent) was associated with the treatment T_3 (cow dung+azolla, 1: 0.5) followed by T_4 (21.6 per cent), T_5 (22.2 per cent) and T_2 (23 per cent).

The highest nitrogen content of 3.44 per cent was recorded in the treatment T_5 (cow dung+azolla, 1: 1) and the lowest in T_1 (cow dung alone) with 1.72 per cent. The nitrogen content of enriched slurry was significantly increased by increasing the proportion of azolla with cow dung. The treatments T_2 (cow dung+azolla, 1: 0.25), T_3 (cow dung+azolla, 1: 0.5) and T_4 (cow dung+azolla, 1: 0.75) recorded 2.34, 2.76 and 3.23 per cent nitrogen respectively.

The maximum content of P was recorded in T_4 (0.77 per cent) and minimum in T_1 (0.73 per cent). The treatments T_2, T_3 and T_5 were on par with same content of P (0.74 per cent).

With respect to potassium, the maximum content (2.93 per cent) was recorded in T_5 and the minimum (2.43 per cent) in T_1. The other treatments T_2, T_3 and T_4 were found to contain 2.67, 2.9 and 2.91 per cent of potassium respectively.

4.2 Preparation of enriched manure

4.2.1 Selection of enriched slurry I and enriched slurry II

The slurry obtained from different treatments were categorized to select the best and the second best for persuing further on preparing a quality manure. It was observed that cow dung and azolla in the ratio of 1: 1 could enrich slurry with 3.44 per cent N, 0.74 per cent P and 2.93 per cent K over cow dung alone and this treatment was selected as enriched slurry I. The treatment T_4 with cow dung and azolla in the ratio of 1: 0.75 which recorded 3.23 per cent N, 0.77 per cent P and 2.91 per cent K and was selected as enriched slurry II.

4.2.2 Physico-chemical properties of solid materials

The physico-chemical properties of solid materials used to prepare enriched manure are given in Table 15.

Table 15. Nutrient content of solid materials (Oven dry basis)

Materials	Total carbon (%)	pH	N (%)	P (%)	K (%)	C/N ratio
Saw dust	46.30	5.1	0.10	0.18	0.20	463
Coir dust	44.40	5.3	0.51	0.33	0.32	87
Powdered coconut leaves	48.80	5.6	0.80	0.42	0.93	61

The highest content of total carbon was obtained in powdered coconut leaves (48.80 per cent) whereas the lowest in coir dust (44.40 per cent). Maximum nitrogen content was also obtained in powdered coconut leaves with a value of 0.80 per cent while saw dust contained only 0.10 per cent N. Powdered coconut leaves contained more P (0.42 per cent) than saw dust (0.18 per cent) and coir dust (0.33

per cent). The C/N ratio was 61, 87 and 463 for powdered coconut leaves, coir dust and saw dust respectively.

4.2.3 Quantity of materials required to prepare enriched manure

The quantity of different materials viz. saw dust, coir dust and powdered coconut leaves required to prepare enriched manure is given in table 16.

Table 16. Quantity of solid materials required for preparing enriched manure

Treatment	Saw dust ($g\ l^{-1}$)	Coir dust ($g\ l^{-1}$)	Powdered coconut leaves ($g\ l^{-1}$)
T_1	343^{c}	463^{d}	595^{d}
T_2	347^{bc}	468^{c}	601^{c}
T_3	349^{ab}	470^{bc}	605^{b}
T_4	350^{ab}	472^{ab}	607^{b}
T_5	352^{a}	475^{a}	612^{a}

(Means with different superscript are significantly different)

T_1- cow dung alone
T_2- cow dung + azolla (1: 0.25)
T_3- cow dung + azolla (1: 0.5)
T_4- cow dung + azolla (1: 0.75)
T_5- cow dung + azolla (1: 1)

Among the materials tried tried for preparing enriched manure, the quantity required to bring down the moisture to 70 per cent per litre was highest for powdered coconut leaves and the lowest for saw dust (Table 16). In the case of powdered coconut leaves, the quantity varied from 595 to 612 $g\ l^{-1}$ of enriched slurry obtained from treatments whereas for coir dust, it ranged from 463 $g\ l^{-1}$ to 475 $g\ l^{-1}$. The values when saw dust was used as the material for preparing enriched manure ranged from 343 $g\ l^{-1}$ to 352 $g\ l^{-1}$.

4.2.4 Physico-chemical properties of enriched manure I and II as influenced by different materials

Effect of powdered coconut leaves

The physico-chemical properties of enriched manures mixed with powdered coconut leaves are given in Table 17a.

Table 17a. Physico-chemical properties of enriched manures added with powdered coconut leaves

Treatment		TS (%)	pH	OC (%)	N (%)	P (%)	K (%)	C:N
Enriched manure I	Before	2.68	7.8	22.20	3.44	0.74	2.93	6.45
	After	29.60	6.7	35.50	4.10	1.14	3.89	8.6
	Per cent increase	1004	-14.1	59.90	19.18	54.05	32.76	33.33
Enriched manure II	Before	3.49	7.8	21.60	3.23	0.76	2.91	6.68
	After	29.80	6.7	35.20	3.91	1.17	3.85	9.00
	Per cent increase	753	-14.1	62.96	21.05	53.94	32.30	34.73

The data revealed that mixing with powdered coconut leaves had a positive effect on total solids and nutrient content of enriched manure I and II. In both types, pH showed a decrease from 7.8 to 6.7.

Enriched manure I- Total solids increased from 2.68 to 29.6 per cent. Carbon content registered 59.9 per cent increase. In case of N, the increase was 19.18 per cent only whereas for P, it was 54.05 per cent. The K content of manure on mixing with coconut leaves increased from 2.93 to 3.89 per cent registering an increase of 32.2 per cent.

Enriched manure II- The total solids increased from 3.49 to 29.8 per cent. Carbon content registered 62.96 per cent increase. In case of N, the increase was 21.05 per cent only whereas for P, it was 53.94 per cent. The K content of manure on mixing with coconut leaves increased from 2.91 to 3.85 per cent registering an increase of 32.3 per cent.

Effect of coir dust

The physico-chemical properties of enriched manures mixed with coir dust are given in Table 17b.

Table 17b. Physico-chemical properties of enriched manures added with coir dust

Treatment		TS (%)	pH	OC (%)	N (%)	P (%)	K (%)	C:N
Enriched manure I	Before	2.68	7.8	22.2	3.44	0.74	2.93	6.45
	After	28.5	6.5	33.1	3.74	1.01	3.23	10.2
	Per cent increase	963.4	- 16.6	49.0	8.72	36.4	10.23	58.1
Enriched manure II	Before	3.49	7.8	21.6	3.23	0.76	2.91	6.68
	After	29.3	6.6	32.3	3.52	1.03	3.20	11.1
	Per cent increase	739.5	- 15.3	49.5	8.97	35.5	9.96	66.6

The data revealed that mixing with coir dust had a positive effect on total solids and nutrient content of enriched manure I and II.

Enriched manure I- The total solids increased from 2.68 to 28.5 per cent. Carbon content registered 49 per cent increase. In case of N, the increase was 8.72 per cent only whereas for P, it was 36.4 per cent. The K content of manure on mixing with coir dust increased from 2.93 to 3.23 per cent registering an increase of 10.23 per cent.

Enriched manure II- The pH showed decrease from 7.8 to 6.6. The total solids increased from 3.49 to 29.3 per cent. Carbon content registered 49.5 per cent increase. In case of N, the increase was 8.97 per cent only whereas for P, it was 35.5 per cent. The K content of manure on mixing with coir dust increased from 2.91 to 3.2 per cent registering an increase of 9.96 per cent.

Effect of saw dust

The physico-chemical properties of enriched manures mixed with saw dust are given in Table 17c.

Table 17c. Physico-chemical properties of enriched manures added with saw dust

Treatment		TS (%)	pH	OC (%)	N (%)	P (%)	K (%)	C:N
Enriched manure I	Before	2.68	7.8	22.2	3.44	0.74	2.93	6.45
	After	29.5	6.4	32.7	3.53	0.88	3.12	9.8
	Per cent increase	1000	-17.9	47.2	2.61	18.9	6.48	51.6
Enriched manure II	Before	3.49	7.8	21.6	3.23	0.76	2.91	6.68
	After	29.8	6.4	33.1	3.32	0.94	3.09	10.6
	Per cent increase	753.8	-17.9	53.2	2.78	23.6	6.18	58.6

Enriched manure I- The pH showed decrease from 7.8 to 6.4. The total solids increased from 2.68 to 29.5 per cent. Carbon content registered 47.2 per cent increase. Compared to other solid materials, N content did not increase on mixing since saw dust contained less N (Table 15) and the increase was 2.61 per cent only whereas for P, it was 18.9 per cent. The K content of manure on mixing with saw dust increased from 2.93 to 3.12 per cent registering an increase of 6.48 per cent.

Enriched manure II- The pH showed decrease from 7.8 to 6.4. The total solids increased from 3.49 to 29.8 per cent. Carbon content registered 53.2 per cent increase. In case of N, the increase was 2.78 per cent only whereas for P, it was 23.6 per cent. The K content of manure on mixing with saw dust increased from 2.91 to 3.09 per cent registering an increase of 6.18 per cent.

4.3 Crop response to enriched manure

4.3.1 Physico-chemical properties of soil

Before the experiment

The physico-chemical properties of soil before the experiment are given in Table 18.

Table 18. The physico-chemical properties of soil before the experiment

S. N.	Properties	Value
1.	pH	4.7
2.	Organic carbon, %	1.01
3.	Available Nitrogen, kg ha^{-1}	232.08
4.	Available Phosphorus, kg ha^{-1}	6.80
5.	Available Potassium, kg ha^{-1}	72.04

The basic properties of soils were studied before the conduct of the pot experiment. Soil samples were collected from 0-15 cm depth, processed and analyzed for pH, organic carbon and available N, P_2O_5 and K_2O content employing standard procedures. The soil was acidic in reaction with a pH of 4.7. The organic carbon content of soil was 1.01 per cent. Among the major nutrients, the content of available N, P_2O_5 and K_2O were 232.08, 6.8 and 72.04 kg ha^{-1} respectively.

After the experiment

The effect of different treatments on physico-chemical properties of soil after pot culture experiment is presented in Table 19.

Table 19. The physico-chemical properties of soil after crop harvest

Treatments	pH	Total carbon (%)	N (mg kg^{-1})	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})
T_1- control	4.6^{c}	1.01^{d}	1049^{c}	31.3^{f}	328^{d}
T_2- NPK+FYM	4.8^{c}	1.45^{a}	1479^{a}	48.8^{d}	370^{c}
T_3- FYM alone	4.8^{c}	1.43^{a}	1142^{b}	41.8^{e}	354^{c}
T_4- Enriched manure I	5.4^{a}	1.23^{b}	1217^{b}	52.3^{ab}	435^{b}
T_5- Enriched manure II	5.3^{ab}	1.21^{bc}	1236^{b}	52.7^{a}	428^{b}
T_6- Enriched manure I + NPK	5.1^{b}	1.2^{bc}	1517^{a}	51.7^{c}	466^{a}
T_7- Enriched manure II + NPK	5.1^{b}	1.17^{c}	1536^{a}	52.1^{bc}	471^{a}

(Means with different superscript are significantly different)

The pH increased to 5.4 in the treatment T_4 (enriched manure I) from an initial value of 4.7 followed by 5.3 in T_5 (enriched manure II). The treatments T_6, T_7, T_3 and T_2 registered a pH of 5.1, 5.1, 4.9 and 4.8 respectively whereas in treatment T_1 pH decreased to 4.6 from an initial value of 4.7 (Table 19).

The highest content of total carbon was recorded in the treatment T_2 (1.45 per cent) followed by T_3 (1.43 per cent) and they were on par.

The highest available N content of 1536 mg kg^{-1} of soil was recorded in the treatment T_7 followed by the treatment T_6 with 1517 mg kg^{-1}. The treatments T_3, T_4

and T_5 recorded 1142, 1217 and 1236 mg kg^{-1} of soil and were on par. The treatment T_2 recorded 1479 mg kg^{-1} whereas control (T_1) recorded 1049 mg N kg^{-1} of soil.

Maximum content of phosphorus was obtained in the treatment T_5 with 52.7 mg kg^{-1} of soil which was on par with the treatment T_4. The treatments T_6 and T_7 registered 51.7 and 52.1 mg kg^{-1} of soil respectively. With respect to the available potassium, T_7 recorded maximum content of 471 mg K_2O kg^{-1} of soil followed by T_6 with 466 mg kg^{-1}. The lowest K_2O content of 328 mg kg^{-1} of soil was recorded in T_1 (absolute control).

4.3.2 Biometric observations

The effect of treatment on plant height, number of tillers per pot, panicle length and number of grains per panicle at crop harvest stage are furnished in Table 20.

Table 20. Effect of treatments on biometric characters of crop at harvest stage

Treatments	Plant height (cm)	No. of tillers	Panicle length (cm)	No. of grains per panicle
T_1- control	55.66^{c}	8.6^{c}	15.2^{f}	104^{d}
T_2- NPK+FYM	60.33^{b}	12.3^{a}	20.1^{b}	114^{bc}
T_3- FYM alone	56.00^{c}	10.0^{b}	16.4^{e}	110^{c}
T_4- EM I*	56.33^{c}	10.6^{b}	17.2^{d}	112^{c}
T_5- EM II**	57.66^{c}	10.6^{b}	17.8^{c}	113^{bc}
T_6- EM I* + NPK	62.33^{a}	12.6^{a}	20.7^{a}	118^{b}
T_7- EM II**+ NPK	63.00^{a}	13.6^{a}	21.1^{a}	123^{a}

(Means with different superscript are significantly different)

*EM I – Enriched manure I   **EM II- Enriched manure II

Effect of treatments on plant height

The height of the plant as influenced by different treatments is given in Table 21. There was significant difference in plant height among the treatments at harvest stage. The maximum plant height (63 cm) was observed in T_7 (enriched manure II+ NPK) followed by T_6 (62.33 cm) and T_2 (60.33 cm). The treatments T_1, T_3, T_4 and T_5 recorded 55.66, 56.00, 56.33 and 57.66 cm respectively and were on par.

Effect of treatments on number of tillers per plant

The number of tillers per pot at harvest stage as influenced by different treatments is furnished in Table 21. The maximum number of tillers was observed in treatment T_7 (13.6) followed by T_6 (12.6) and T_2 (12.3). The treatments T_3, T_4 and T_5 registered 10, 10.6 and 10.6 respectively and were on par whereas treatment T_1 recorded only 8.6 numbers of tillers per pot.

Effect of treatments on panicle length

The average panicle length as recorded by the plant in different treatments is given in Table 21. The maximum panicle length of 21.1 cm was measured in the treatment T_7 followed by T_6 (20.7 cm) and T_2 (20.1 cm). The treatments T_3, T_4 and T_5 registered 16.4, 17.2 and 17.8 cm respectively. The minimum panicle length (15.2 cm) was obtained in the treatment T_1.

Effect of treatments on number of grains per panicle

The number of grains per panicle as affected by different treatments is given in Table 21. It was observed that there was significant difference in number of grains per panicle among the treatments at harvest stage. The maximum number of grains per panicle was observed in T_7 (123 grains) followed by T_6 (118 grains) and

T_2 (114 grains). The treatments T_3, T_4 and T_5 recorded 110, 112 and 113 grains respectively and were on par. The minimum number of 104 grains per panicle was observed in treatment T_1.

4.4 Grain and straw yield

The grain and straw yield as influenced by different treatments are reported in Table 21.

Table 21. Grain and straw yield of rice as influenced by different treatments

Treatments	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})
T_1- control	25.96^{e}	45.14^{d}
T_2- NPK+FYM	40.41^{b}	65.26^{b}
T_3- FYM alone	29.25^{d}	54.95^{c}
T_4- Enriched manure I	32.80^{c}	58.90^{c}
T_5- Enriched manure II	30.22^{d}	52.72^{c}
T_6- Enriched manure I+ NPK	42.56^{b}	69.38^{ab}
T_7- Enriched manure II+ NPK	44.97^{a}	72.73^{a}

(Means with different superscript are significantly different)

There was significant difference among the treatments on grain yield. The maximum grain yield was recorded in T_7 with 44.97 g pot^{-1} followed by T_6 (42.56 g pot^{-1}) and T_2 (40.41 g pot^{-1}). The treatments T_3 and T_5 recorded 29.25 and 30.22 g pot^{-1} respectively and were on par. The treatment T_4 recorded 32.8 g pot^{-}1 whereas the minimum yield of 25.96 g pot^{-1} was observed in treatment T_1.

The maximum straw yield was obtained in T_7 (72.73 g pot^{-1}) and minimum in T_1 (45.15 g pot^{-1}). There was significant difference among the treatments. The treatments T_3 (54.95 g pot^{-1}), T_4 (58.9 g pot^{-1}) and T_5 (52.72 g pot^{-1}) were on par. The treatments T_2 (65.26 g pot^{-1}) and T_6 (69.38 g pot^{-1}) were also on par.

4.5 Nutrient uptake

The effect of treatments on nutrient uptake by crop (Rice var. Jyothy) is furnished in Table 22.

Table 22. Effect of treatments on nutrient uptake by rice crop (mg pot^{-1})

Treatments	N (mg pot^{-1})	P (mg pot^{-1})	K (mg pot^{-1})
T_1- control	2430^{f}	500^{e}	2510^{d}
T_2- NPK+FYM	3840^{c}	830^{b}	4154^{b}
T_3- FYM alone	2660^{e}	590^{d}	2722^{d}
T_4- Enriched manure I	2860^{d}	700^{c}	2930^{c}
T_5- Enriched manure II	2950^{d}	690^{c}	3152^{c}
T_6- Enriched manure I+ NPK	4130^{b}	938^{a}	4211^{a}
T_7- Enriched manure II+ NPK	4260^{a}	930^{a}	4263^{a}

(Means with different superscript are significantly different)

Among the major nutrients, total N content (mg pot^{-1}) ranged from 2430 (T_1) to 4260 mg pot^{-1} in T_7 followed by T_6 (4130 mg pot^{-1}). The treatments T_4 (2860 mg pot^{-1}) and T_5 (2950 mg pot^{-1}) were on par whereas T_2 and T_3 recorded 3840 and 2660 mg pot^{-1} respectively.

The total P content ranged from 500 (T_1) to 938 mg pot^{-1} (T_6) followed by T_7 (930 mg pot^{-1}). The treatments T_4 (700 mg pot^{-1}) and T_5 (690 mg pot^{-1}) were on par whereas T_2 and T_3 recorded 830 and 590 mg pot^{-1} respectively.

The maximum total K content was associated with T_7 (4263 mg pot^{-1}) followed by T_6 (4211 mg pot^{-1}) and they were on par. The minimum content (2510 mg pot^{-1}) was recorded in T_1. The treatments T_4 and T_5 were on par.

Discussion

5. DISCUSSION

The results obtained from present investigation are discussed in this chapter in the light of available literature in this line.

5.1 Anaerobic digestion of substrates

5.1.1 Temperature inside the digester of biogas plant

The data in section 4.1.2 and Fig. 1 depicts the mean temperature inside digester at 5 days interval of 30 days anaerobic digestion period. The highest mean temperature was recorded in T_3 (cow dung+azolla, 1: 0.5) on 15-20th days of digestion period and the lowest in T_1 (cow dung alone) on 25-30th days. This variation clearly shows an optimum C/N ratio in substrates for methanogenic bacteria and hence higher microbial activity. On the basis of experimental results, it may be inferred that addition of azolla in T_3 (cow dung+azolla, 1: 0.5) in the ratio of 1: 0.5 brought down the C/N ratio of substrates to optimum for higher microbial growth and maximum temperature than other treatments.

5.1.2 Hydraulic Retention Time (HRT)

As cited in section 4.1.3, the shortest HRT was observed in T_3 (cow dung+azolla, 1: 0.5) and the longest on T_1 (cow dung alone). This difference in HRT might be due to higher temperature and microbial activity in T_3 (cow dung+azolla, 1: 0.5) than T_1 (cow dung alone) so that substrates digested in short period of HRT. The increased pH (Fig. 4) inside digester in T_4 and T_5 might be toxic to methanogenic bacteria and hence responsible for slow digestion of substrates.

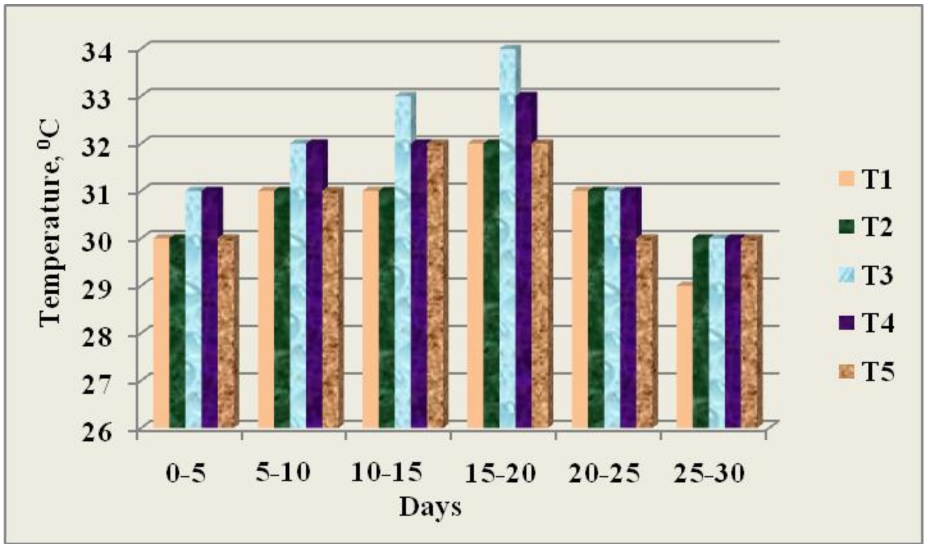


Fig 1. Effect of treatments on temperature inside the digester of biogas plant (^{0}C)

T_1- cow dung alone

T_2- cow dung+azolla (1: 0.25)

T_3- cow dung+azolla (1: 0.5)

T_4- cow dung+azolla (1: 0.75)

T_5- cow dung+azolla (1: 1)

5.1.3 Volume of gas produced

Fig. 2 illustrates the effect of different treatments on total biogas production during anaerobic digestion of specific substrates used for the study. The results are presented under section 4.1.4 and 4.1.5. The highest gas volume was recorded by T_3 (cow dung+azolla, 1: 0.5) with 0.29 $m^3\ kg^{-1}$ of total solids. Similar results have been reported by Das *et al*. (1994) and Dipu *et al*. (2011). The rate of gas production also registered the peak value in the same treatment. The temperature inside the digester of biogas plant was found to be maximum under this treatment which indicates temperature as the main factor responsible for maximum gas production. The low C/N ratio as recorded by the treatments T_1 and T_2 and less acetate production in cow dung (Singh *et al.*, 1983) might have inhibited the rate as well as total gas production under these treatments. The increased pH inside the digester as recorded by treatments T_1, T_2 and T_3 can be very well compared with T_4 and T_5 (Table 14). This might have caused adverse influence on methanogenic bacteria (Shilpakar and Shilpakar, 2009).

The data in Table 14 revealed that the total solids decreased after increasing the ratio of azolla in T_4 and T_5. This might also be the reason for low gas production in these treatments as solid particles were too diluted (Budiyono *et al*., 2011).

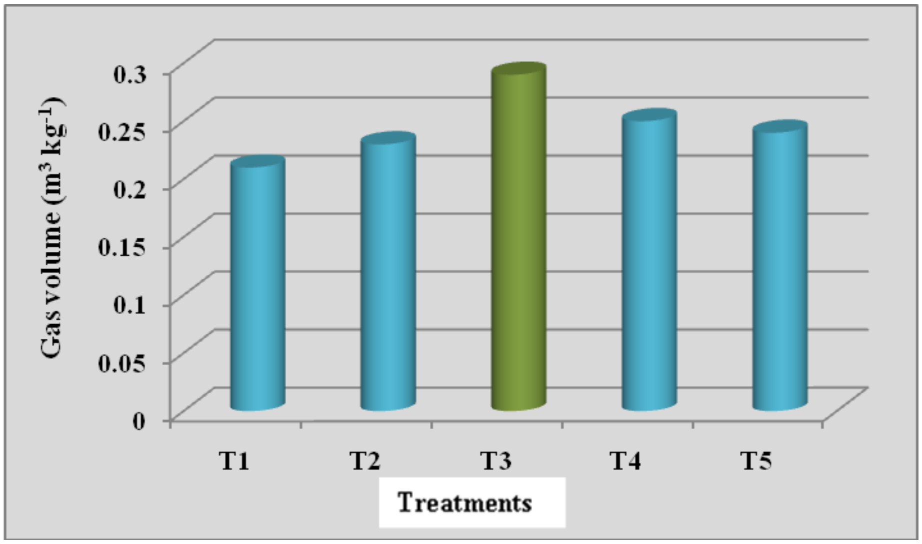


Fig 2. Effect of treatments on total gas production (m^3 kg^{-1} of TS)

T_1- cow dung alone

T_2- cow dung+azolla (1: 0.25)

T_3- cow dung+azolla (1: 0.5)

T_4- cow dung+azolla (1: 0.75)

T_5- cow dung+azolla (1: 1)

5.1.4 Rate of gas production

The gas production in each treatment was recorded daily and value was calculated at two days interval until the digestion completed within 30 days (Table 13).

The biogas production obeyed sigmoid function (Fig 3). It was slow at the beginning and also towards the end of digestion. In all the treatments, anaerobic digestion started only after two days. The production of Volatile Fatty Acids (VFA) by acid forming bacteria declined the pH and in turn the growth of methanogenic bacteria leading to reduced gas production. This is in conformity with the findings of Cuzin *et al*., (1992) and Deshpande *et al.* (1979) who also reported similar effect of pH at the initial stage. The temperature inside the digester was another major factor deciding methanogenesis. In all the treatments, temperature was maximum at 15-20 days. There might be higher methanogenic activity when temperature increased. It can be concluded that microbial activity, temperature and gas production are interrelated in anaerobic digestion process.

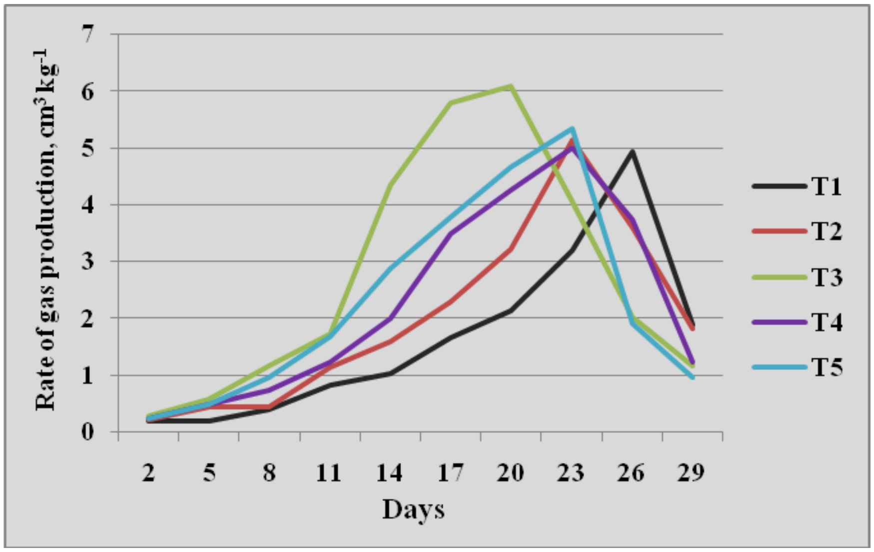


Fig 3. Effect of treatments on rate of gas production (cm^3 kg^{-1} of TS)

T_1- cow dung alone

T_2- cow dung+azolla (1: 0.25)

T_3- cow dung+azolla (1: 0.5)

T_4- cow dung+azolla (1: 0.75)

T_5- cow dung+azolla (1: 1)

5.1.5 Manurial value of slurry

The data as presented in section 4.1.6 and Fig. 4 revealed an inverse relationship of carbon content with gas volume production. The higher carbon content was determined in T_1 and the lowest in T_3. The higher gas volume production in treatment T_3 could decrease carbon content due to the formation of methane (CH_4) and carbon dioxide (CO_2) during digestion process. Similar findings have been reported by Zennaki *et al*. (1996).

The data in section 4.1.6 and Fig. 5 showed the total solids content of enriched slurry. The reduction in total solid content was observed in all the treatments. This is in line with the observations made by Malte *et al*. (1991). The data revealed the significant differences among the treatments. The increasing quantity of azolla in treatment T_2, T_3, T_4 and T_5 could decrease TS content in enriched slurry since azolla contained low dry matter (6 per cent) than cow dung (21 per cent) as presented in section 4.1.1.

The pH of the enriched slurry was in alkaline range (Hobson *et al.*, 1981) for all treatments (Fig. 6). It was observed that increasing ratio of azolla could increase pH as azolla is rich in protein. The pH was increased due to liberation of more ammonia with an increase in quantity of azolla in respective treatments. This is in line with the observations made by Das *et al*. (1994).

The data in section 4.1.6 depicts the N content of digested slurry significantly differed among treatments under study. Similar findings have been reported by Das *et al*. (1994). The N content in slurry increased with increasing ratio of azolla in T_2, T_3, T_4 and T_5 respectively which was nitrogen rich and could narrow C/N ratio (Fig 7 and 8).

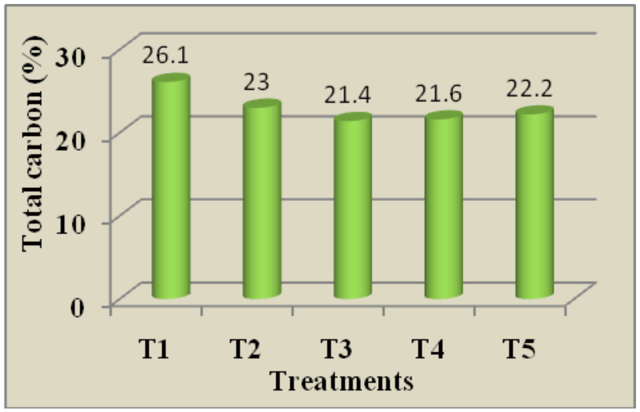


Fig 4. Effect of treatments on total carbon of slurry

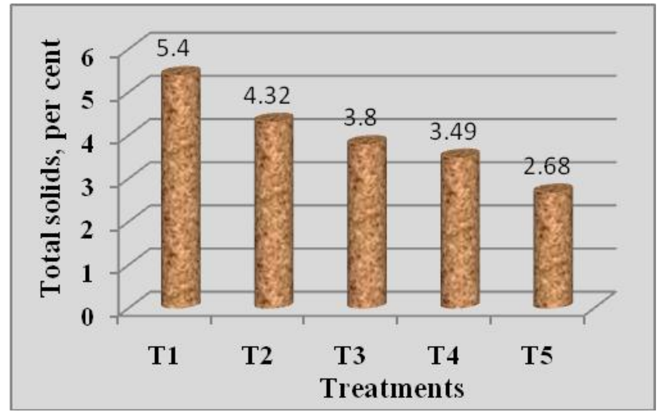


Fig 5. Effect of treatments on total solids of slurry

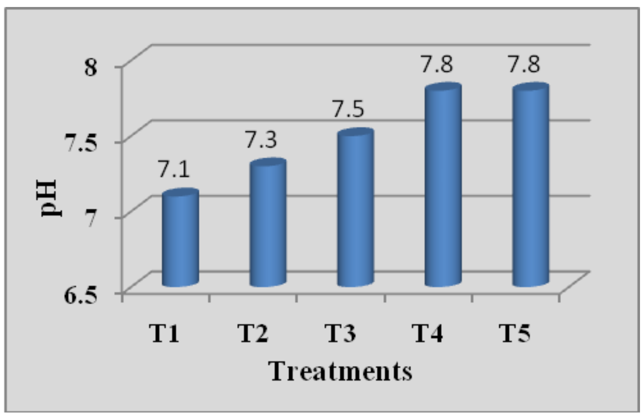


Fig 6. Effect of treatments on pH of slurry

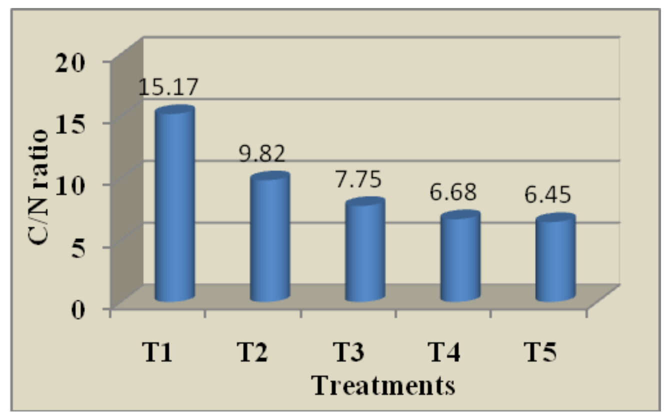


Fig 7. Effect of treatments on C/N ratio of slurry

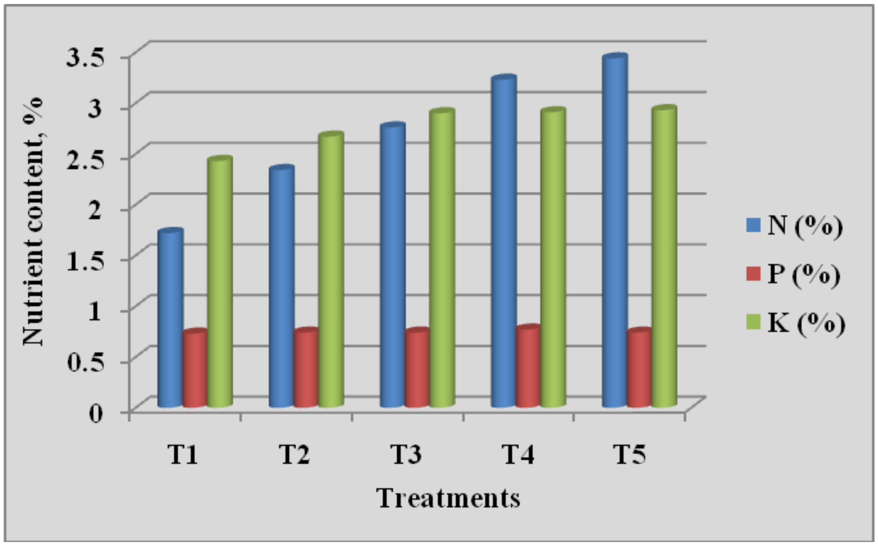


Fig 8. Effect of treatments on N, P and K content of enriched slurry

T_1- cow dung alone
T_2- cow dung+azolla (1: 0.25)
T_3- cow dung+azolla (1: 0.5)
T_4- cow dung+azolla (1: 0.75)
T_5- cow dung+azolla (1: 1)

The data presented in section 4.1.6 further revealed that there were no significant differences in P content among treatments with the addition of azolla but K content was found to be higher in all the treatments. It was also observed that P content could not increase in digested slurry as N and K content. Addition of azolla could increase K content in slurry (Fig 8) since azolla was inherently rich in K. This is in line with the observations made by Das *et al*. (1994).

5.2 Preparation of Enriched manure

The second objective of the study was to mix and to prepare enriched manure to increase handling property and ease for immediate application. It is reported by several workers that possible phytotoxicity of fresh slurry (Poggi-Varaldo *et al*., 1999 and Wang, 1991), viscosity and odor (Smet *et al*., 1998), handling difficulty and expensive soil application approaches (Tchobanoglous *et al*., 2002) and up to 90 per cent NH_4^+ that might be lost as NH_3^+ during sun drying (Rivard *et al.*, 1995), so further treatment of slurry by mixing with solid materials was tried. The treatments T_5 (cow dung+ azolla, 1: 1) and T_4 (cow dung+ azolla, 1: 0.75) were selected as enriched slurry I and enriched slurry II respectively on the basis of high N, K content and narrow C/N ratio (Section 4.1.6).

5.2.1 Quantity of solid materials

Among the materials tried to prepare enriched manure, the quantity required for bringing down the moisture to 70 per cent per litre of the slurry was highest for powdered coconut leaves and the lowest for saw dust (Section 4.2.3 and Fig. 9). This variation among solid materials was due to density of materials on weight by volume basis. Powdered coconut leaves had higher density and low moisture absorbing capacity than saw dust and coir dust.

5.2.2 Suitability of solid materials

The data in section 4.2.4 illustrates the total solids content after mixing of enriched slurry with solid materials. The data revealed that total solids increased significantly in both enriched manures I and II. The dry matter content was highest in solid materials than enriched slurry I and II. This was the reason for this increment. The pH of both enriched manure I and II decreased (Section 4.2.4) after mixing with solid materials thereby reducing the chances of NH_3^+ -N volatilization as against in enriched slurry in which pH was high (Section 4.1.6). This reduction in pH was due to low pH value of these materials than enriched slurry. The data presented in section 4.2.4 depicts the organic carbon content after mixing of enriched slurry with solid materials. The carbon content increased significantly due to high carbon content in solid materials (Section 4.2.2) than enriched slurry I and enriched slurry II.

The N content of enriched manure I and enriched manure II increased after mixing with solid materials (Section 4.2.4 and Fig. 10) based on the N content in these materials. The highest N content was obtained when mixed with powdered coconut leaves due to high N content in powdered coconut leaves (Section 4.2.2) among solid materials. The P and K content of the manure were also higher than enriched slurry due to its addition from the solid materials.

Enriched manure I and II recorded maximum N and K content after mixing with powdered coconut leaves due to higher N and K content in coconut leaves and these two enriched manures were selected for crop response study.

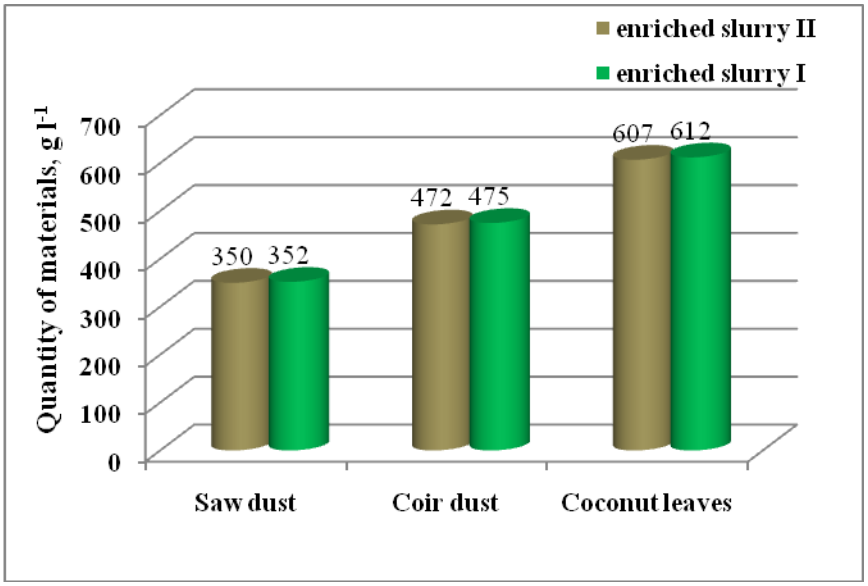


Fig 9. Quantity of solid materials required to bring down moisture of slurry to 70 per cent

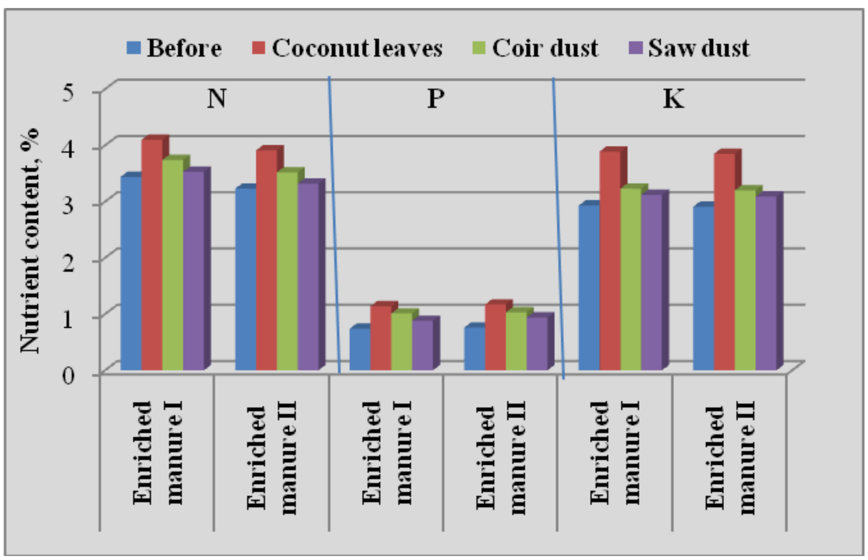


Fig 10. Effect of solid materials on N, P and K content of enriched slurry before and after mixing

5.3 Crop response studies

5.3.1 Studies on soil properties

The objective of crop response study was to observe the response of crop to enriched manure by improving the nutritional status of the soil.

Soil pH and organic carbon

Section 4.3.1 illustrates the soil pH after crop harvest. The pH was higher under treatment T_4 (enriched manure I) and T_5 (enriched manure II). This can be ascribed to addition of basic cations from the organic materials (Aitken, 1992) used in enriching. Both enriched manure I and II had higher pH than initial soil pH value . The NH_4^+ rich enriched manure temporarily reduced the pool of H^+ in the soil.

The data in section 4.3.1 on organic carbon content in soil revealed the application of FYM and enriched manure could increase total carbon status of soil in comparison with control. The organic carbon content of the soil increased from an initial value of 1.01 per cent (Section 4.3.1) to as high as 1.45 per cent under T_2 (FYM+NPK). This might be due to higher organic carbon content in FYM and its slow decomposition. Similar observations were made by Rajshree *et al.* (2005). The low organic carbon content under chemical fertilizer is mostly due to rapid mineralization of the organo-mineral complex (Yoshida and Padre, 1975). Due to reduction in carbon content in enriched slurry during anaerobic digestion, enriched slurry application could not increase carbon content in soil.

Available N, P_2O_5 and K_2O

The significant increase in available N, P_2O_5 and K_2O was observed in all the treatments than control (Section 4.3.1 and Fig. 11). The slow mineralization of N in FYM and higher quantity of N in enriched manure could add N in soil. The enhanced soil pH due to addition of enriched manure was favorable for N fixation. Since at low pH, N fixation would be initiated by high H^+ concentration as well as high Fe or Al in laterites (Mohan *et al*., 1987).

The P_2O_5 content of soil at crop harvest could increase from its initial value. The available P_2O_5 content in lateritic soil is very less (Section 4.3.1). Largest portion of the added P_2O_5 is fixed as aluminium compounds followed by iron compounds in acid soils (Sanyal and De Datta, 1991). Application of slurry stimulated beneficial microbiological activities in respect to phosphorous solubilizers and nitrogen fixing azotobactor (Karki, 2001) and enhanced the conversion of insoluble P_2O_5.

Fig. 11 depicts the K_2O content in soil after crop harvest. The data revealed the significant increase of available K_2O after application of manures and fertilizers. The enriched manure II along with N, P_2O_5 and K_2O application recorded the maximum quantity of available K_2O in soil. This might be due to the high K content in enriched manure (Section 4.2.2) and chemical fertilizers (Appendix III) in respective treatments.

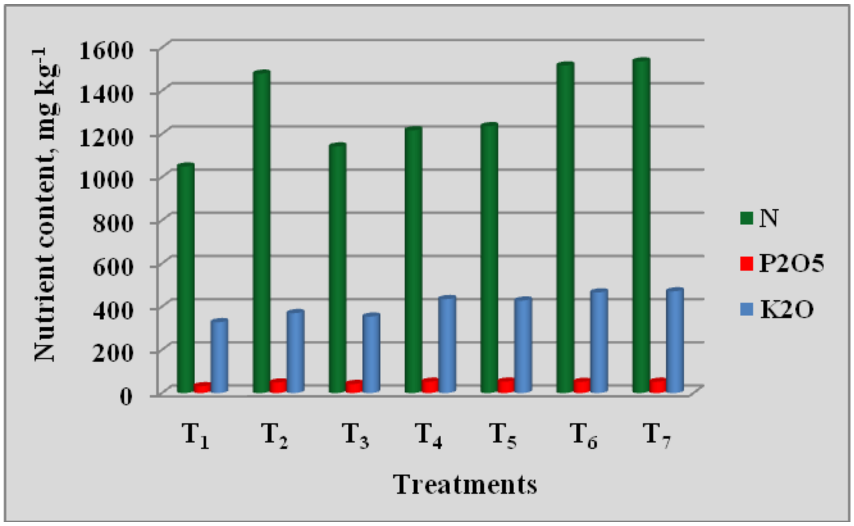


Fig 11. Effect of treatments on N, P_2O_5 and K_2O content of soil at harvest

T_1- Control
T_2- FYM+NPK
T_3- FYM alone
T_4- Enriched manure I
T_5- Enriched manure II
T_6- Enriched manure I+ NPK
T_7- Enriched manure II+ NPK

5.3.2 Biometrical parameters and yield

The effect of treatments on biometrical parameters at crop harvest stage is depicted in Section 4.3.2. The plant height and number of tillers were significantly different in case of enriched manure I and II along with N, P_2O_5 and K_2O application. Application of adequate nutrients from organic manures and chemical fertilizers resulted in increased plant height and number of tillers per hill (Singh and Mandal, 1997). The longest panicle length and maximum number of grains per panicle was recorded by T_7 (Enriched manure II+NPK). This is due to high N and K content in enriched manure I and II and chemical fertilizers which were readily available to rice crop.

The data on grain yield at harvest stage (Section 4.2 and Fig. 12) showed that grain yield increased significantly with the application of enriched manure II+ N, P_2O_5 and K_2O (44.97 g pot^{-1}). Since enriched manure II was prepared from the treatment T_4 (cow dung+azolla, 1: 0.75) of the first experiment which was more digested (based on gas volume produced) than the treatment T_5 (cow dung+azolla, 1: 1) (Section 4.1.4 and Fig 2). Nutrient availability to the crop might be higher due to reduction in C/N ratio and increased content of NH_4^+-N which is dependent upon the degree of digestion of biomaterials (Goldstein, 2000). However, the total N content (Section 4.1.6 and Fig. 8) was higher in enriched manure I, the NH_4^+ -N might be higher in enriched manure II than enriched manure I which was readily available to rice crop. Similar findings on grain yield of rice with the application of slurry were observed by Zhang *et al*. (2009).

The data in section 4.2 and Fig. 12 depicts the straw yield at harvest stage. The data revealed that the application of enriched manure II+ NPK (T_7) increased straw yield (Fig. 12). The nutrients content provided by chemical fertilizers and enriched manure II were responsible for higher straw yield in treatment T_7.

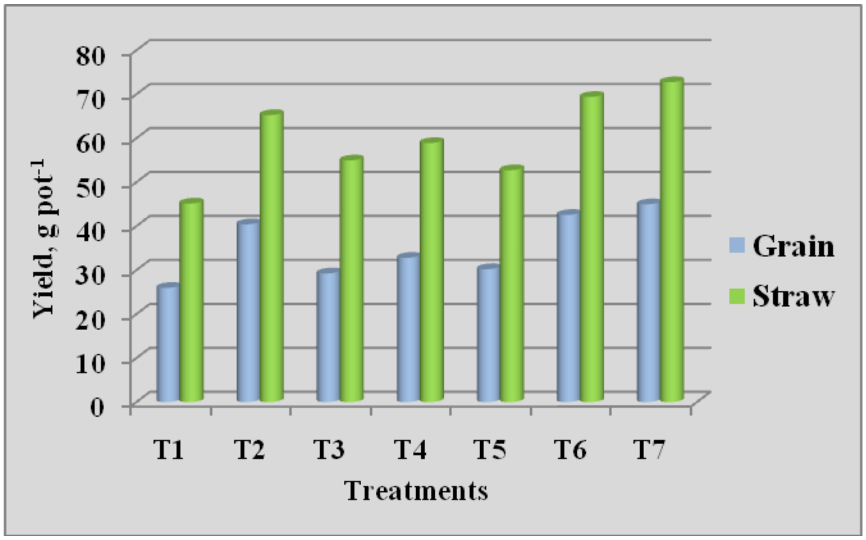


Fig 12. Effect of treatments on grain and straw yield (g pot^{-1})

T_1- Control
T_2- FYM+NPK
T_3- FYM alone
T_4- Enriched manure I
T_5- Enriched manure II
T_6- Enriched manure I+ NPK
T_7- Enriched manure II+ NPK

5.4 Uptake of major nutrients by rice crop

It is apparent from section 4.5 that combined application of Enriched manure II and NPK results in higher uptake of N, P and K than other treatments. James *et al*. (1967) reported that higher rate of metabolic activity with rapid cell division brought by slurry application, can be resulted in high uptake of nutrients and thus it might have resulted in increased utilization of nitrogen. The higher nutrient uptake under these treatments was responsible for the higher yield of rice (Section 4.2). Similar findings have been reported by Sajwan (1995) and Sarangi *et al*. (1997). Organic matter (as fresh slurry was mixed with powdered coconut leaves) also contributed nutrients in addition to N, P_2O_5 and K_2O. Improved soil reaction due to alkaline nature of enriched slurry and supply of substrates and nutrients through enriched slurry and chemical fertilizer might have stimulated microbial activity (Sarangi *et. al*, 1997 and Rautaray, 2003) leading to higher nutrient release and availability to rice plants. Anon (2000) reported that 50 per cent organic form of N is converted into NH_4-N during anaerobic digestion of biomaterials. This might be the reason for higher uptake of N which was readily available to rice crop. Xianjun *et al.* (2011) also reported that slurry application increased soil microflora and amounts of phosphobacteria, silicate bacteria and ammonifying bacteria as compared with the control and those treated by chemical fertilizers only.

The higher uptake of P in enriched manure along with chemical fertilizers application was assessed in T_6 and T_7. The pH of soil increased after enriched manure application due to high pH than initial value which favored P uptake by crop. Moreover, the enriched manure stimulated phosphobacteria which enhanced release of bound P and made available to the crop.

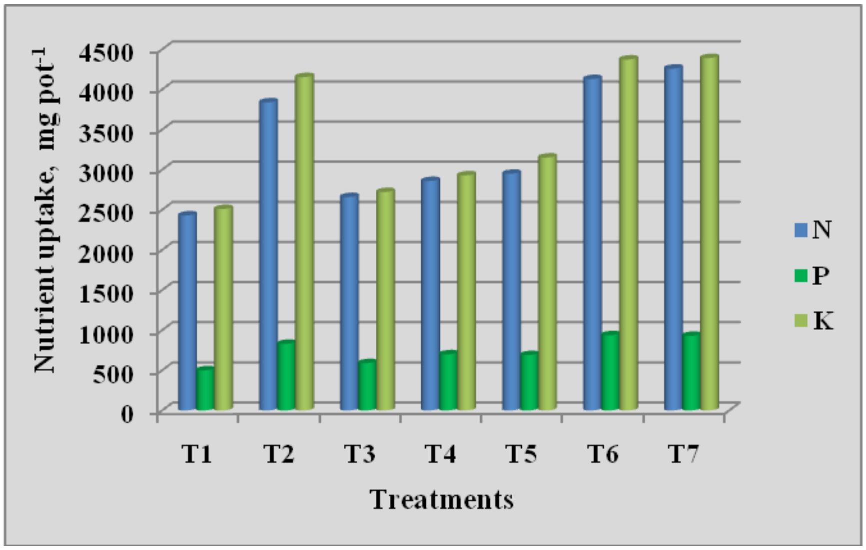


Fig 13. Effect of treatments on N, P and K uptake by crop

T_1- Control
T_2- FYM+NPK
T_3- FYM alone
T_4- Enriched manure I
T_5- Enriched manure II
T_6- Enriched manure I+ NPK
T_7- Enriched manure II+ NPK

The highest K uptake was determined in T_7 followed by T_6 since the enriched slurry was rich in K content which was readily available to the crop. The increase in K uptake due to increased K availability consequent to shifting of relatively unavailable forms to more available forms in soil.

Summary

6. SUMMARY

An investigation on “Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment” was conducted during 2010-11 at College of Horticulture, Vellanikkara. The major objectives of the study were to evaluate the optimum ratio of cow dung and azolla for biogas slurry enrichment, to identify the best material to prepare quality organic manure from the enriched slurry and also to evaluate the crop response to the enriched organic manure.

- For studying the optimum ratio of cow dung and azolla for biogas slurry enrichment, different proportions viz. 1: 0.25, 1: 0.5, 1: 0.75 and 1: 1 of the substrates were tried and tested against cow dung alone using floating drum biogas digester of 0.5 m^3 capacity. Temperature inside the digester was recorded daily. It recorded a peak of 34 ^{0}C in the treatment cow dung+azolla (1: 0.5) during 15-20 days of digestion and was found to be greatly influenced by the substrates. The minimum temperature of 29 ^{0}C was recorded in the absence of azolla.

- Volume of gas produced was measured on a daily basis. Combination of cow dung and azolla in 1: 0.5 ratio excelled (0.29 m^3 kg^{-1} TS) all other treatments in terms of volume of gas produced registering an increase of 27.58 per cent over cow dung alone. The Hydraulic Retention Time (HRT) which indicates the completion of digestion could be reduced to 20 days from 26 days, as registered in cow dung alone treatment, by adding azolla along with cow dung.

- The digested slurry obtained from different combinations of cow dung and azolla was analysed. Based on its nutrient status, it was designated as enriched slurry I (from the treatment cow dung+azolla in 1: 1) and enriched slurry II (from the treatment cow dung+azolla in 1: 0.75). For improving the handling property of enriched slurry, it was mixed with saw dust, coir dust and powdered

coconut leaves. Powdered coconut leaves were identified as the best material for modifying the liquid nature of the enriched slurry. The requirement was 612 g of powdered coconut leaves per litre in enriched slurry I and 607 g of powdered coconut leaves per litre in enriched slurry II for preparing organic manure with better handling property attributing to its increased acceptability.

- The effectiveness of organic manure prepared was tested in rice crop (variety- Jyothy). Grain and straw yield was maximum (44.97 g pot^{-1} and 72.73 g pot^{-1} respectively) when the crop was nourished with enriched manure II along with recommended dose of chemical fertilizers. Uptake of N and K was also the highest in this treatment.

- The data on soil physico-chemical properties also revealed a positive trend consequent to manure application. Soil pH increased to 5.4 from the initial value of 4.7 with the addition of enriched manure I. Available N and K_2O status was the highest when the enriched manure II was applied along with recommended dose of chemical fertilizer whereas P_2O_5 content was maximum when chemical fertilizers was excluded.

Based on the present investigations, the following inferences can be drawn:

- The ideal ratio of cow dung and azolla for maximum gas production was 1: 0.5 registering an increase of 27.58 per cent over cow dung alone.

- Nitrogen content of slurry increased by 50 per cent when cow dung and azolla was mixed in the ratio of 1: 1 than cow dung alone.

- Powdered coconut leaves were selected as the best material to obtain enriched manure from the slurry.

- Application of Enriched manure II along with the recommended dose of chemical fertilizers produced highest grain and straw yield.

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References

REFERENCES

Abdullahi, Y.A., Akunna, J.C., White, N.A., Hallett, P.D. and Wheatley, R. 2008. Investigating the effects of anaerobic and aerobic post-treatment on quality and stability of organic fraction of municipal solid waste as soil amendment. *Bioresour. Technol.* 99: 8631-8636.

Alexander, D., Rajan, S., Rajamony, L., Ushakumari, K. and Kurien, S. 2009. *Package of Practices Recommendations (Adhoc) for Organic Farming: Crops*, Directorate of Extension, Kerala Agricultural University, Thrissur, 14p.

Aitken, R.L. 1992. Relationships between extractable Al, selected soil properties, pH buffer capacity and lime requirement in some acidic Queensland soils. *Aust. J. Soil. Res.* 30: 119-130.

Anon, 2000. Farm Scale Biogas and Composting to improve Bathing Waters – a report for the Scottish Executive by Enviros/Greenfinch report.
Available via the link:
http://www.scotland.gov.uk/Resource/Doc/1057/0048383.pdf

Bajpai. R.K., Chitale. S., Upadhyay. S.K. and Urkurkar, J.S. 2006. Long-term studies on soil Physico-chemical properties and productivity of Rice-Wheat system as influenced by Integrated Nutrient Management in Inceptisol of Chattisgarh. *Indian Soc. Soil Sci.* 54: 24-29

Bastian, R.K. and Ryan, J.A. 1986. *Design and management of successful land application system, Utilization Treatment and Disposal of Waste on Land*. Soil Science Society of America, Madison, WI, USA, pp. 217–229.

Bhattarai, S. and Maskey, S.L. 1988. Effect of Azotobacter Inoculation in combination with different sources of organic manure. *Proceeding of National Conference on Science and Technology*; 24-29 April, 1988; Khumaltar, Lalitpur, Nepal, pp. 81-85.

Bray, R.H. and Kurtz, L.T. 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil sci*. 59: 39-45.

Budiyono, Widiasa, I. N., Johari, S. and Sunarso. 2011. Increasing Biogas Production Rate from Cattle Manure Using Rumen Fluid as Inoculums. *International Journal of Basic & Applied Sciences*. 10(1): 68-75.

Burton, C.H. and Turner, C. (2003). Manure management. Treatment strategies for sustainable agriculture. 2nd Edition. Silsoe Research Institute, Bedford, UK, pp. 55-67.

Chowdhary, S.D.R., Gupta, S.K., Banergy, S.K., and Chowdhry, S.D. 1994. Evaluation of the potentiality of tree leaves for biogas production. *Indian Forester*. 120(8): 720–728.

Chawla, O.P. 1986. Methane fermentation technology. In: Bali, J.B. and Sarma, P.S.N. (eds.), *Advances in Biogas Technology*. Gowarsons Publishers, New Delhi, pp. 31-54.

Cuzin, N., Farinet, J.L., Segretain, C. and Labat, M. 1992. Methanogenic fermentation of cassava peel using a pilot plug flow digester. *Bioresource Technol*. 41: 259-264.

Das, D., Sikadar, K., and Chatterjee, A.K. 1994. Potential of *Azolla pinnata* as biogas generator and as a fish feed. *Indian J. Enviorn*. 36: 186-191.

De la Rubia, M.A., Pérez, M., Romero, L.I. and Sales, D. 2006. Effects of Solids Retention Time (SRT) on pilot scale anaerobic thermophilic sludge digestion. *Proc. Biochem.* 41: 79-86.

Desai, M. and Madamwar, D. 1994. Anaerobic digestion of a mixture of cheese whey, poultry waste and cattle dung: a study of the use of adsorbents to improve digester performance. *Environ .Pollut*. 86 (3): 337–340.

Deshpande, P., Sarnaik, S., Godbole, S.H. and Wagle, P.M. 1979. Use of water hyacinth as an additive in bio-gas production. *Curr. Sci.* 48(11): 490-492.

Dipu, S., Kumar, A.A. and Thanga, V.S.G. 2011. Potential application of macrophytes used in phytoremediation. *World Applied Sci. J*. 13 (3): 482-486.

Eghball, B., B.J. Wienhold, J.E. Gilley, and R.A. Eigenberg. 2002. Mineralization of manure nutrients. *J. Soil Water Conserv*. 57(6): 470-472.

Eghball, B. and Power, J.F. 1994. Beef cattle feedlot manure management. *J. Soil Water Conserv*. 49: 113–122.

FAO [Food & Agriculture Organization]. 2007. Biogas processes for sustainable development Israel, www.fao.org/docrep, retrieved on12/15/2007.

Freed, R. 2006. MSTAT-C version 7. Department of Crop and Soil Science. Michigan State University.

Forster-Carneiro, T., Pérez García, M. and Romero García, L.I. 2007. Composting potential of different inoculum sources on modified SEBAC system treatment of municipal solid wastes. *Bioresour.Technol.* 98: 3354-3366.

Gallert, C., Henning, A. and Winter, J. 2003. Scale-up of anaerobic digestion of the biowaste fraction from domestic wastes. *Water Res.* 37:1433-1441.

Goldstein, J. 2000. Anaerobic digestion advances. *BioCycle.* 41: 30–32.

Goutam, D.N., Jain, M.C., Kumar S., Sarkar, K. and Sinha, S.K. 1996. Methane emissions from rice fields amended with biogas slurry and farm yard manure. *Indian J. Environ*. 33(1): 97-109.

Gurung, J.B. 1996. Review of literature on effects of slurry use on crop production, The Biogas Support Program, Kathmandu, Nepal, pp. 12-23.

Herzalla, A. Nagat, Amer, A.A., Mahmoud H.M., and El-Banna, A.A.M. 2001. .*Azolla* carbohydrate contents and/or its photosynthesis activity under senescence conditions due to *Azollza* fronds crowding. *Al-Azhar J. Agric. Res*. 34: 349-361.

Hesse, P.R. 1971. *A Textbook of Soil Chemical Analysis*. John Murray Publishers Ltd., London, 520p.

Hobson, P.N., Bousfield, S. and Summers, R. 1981. *Methane Production from Agricultural and Domestic waste*. Applied Science Publishers, England, 269p.

Ituen, E.E., John, N.M. and Bassey, B.E. 2007. Biogas production from organic waste in Akwa Ibom State of Nigeria. Appropriate Technologies for Environmental Protection in the Developing World. Selected Papers from ERTEP 2007, July 17-19, Ghana.

Jackson, M.L. 1958. Soil Chemical Analysis (Indian Reprint 1967). Prentice Hall of India Pvt. Ltd., New Delhi, 498p.

Janotti, E.L., Porter, J.H., Fischer, J.R. and Sievers, D.M., 1986. Changes in swine manure during anaerobic digestion. *Dev. Ind. Microbiol*. 20: 519–529.

James, P.T., Harwin, D. and Heilman. 1967. Influence of moisture and fertilizer on growth and nitrogen and phosphorus uptake by sweet pepper. *Agron. J.* 59(1): 27-30.

Kanaiyan, S. and Somporn, C. 1987. Studies on sporulation, biomass production and nitrogen fixing potential of eleven cultures of aquatic fern *Azolla*. *Indian J. Microbiol*. 27: 22-25.

Karki, A.B. and Dixit K. 1984. *Biogas Field Book*. Sahayogi Press, Nepal, 365p.

Karki, K. B. 2001. *Response to Bio-Slurry Application on Maize and Cabbage in Lalitpur District*, Ministry of Science and Technology Alternative Energy Promotion Centre, His Majesty's Government of Nepal, Krishna Galli, Pulchok, Lalitpur, p10.

KAU [Kerala Agricultural University]. 2007. *Package of Practices Recommendations*: *Crops* (12^{th} Ed.). Kerala Agricultural University, Thrissur, 334p.

Kirchmann, H and Lundvall, A. 1993. Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry. *Biol. Fertil. Soils.* 15: 161-164.

KVIC.1983. Khadi and V I Commission and its Non-conventional Energy Programmes, KVIC, Bombay, India, pp. 34-56.

Laxamanan, A.R., G. Kuppuswamy and A. Jeyabal, 1993. Use of Biogas Effluents as Seed Coating Media for Successful Crop Production/ In Biogas Slurry Utilsation. New Delhi.

Laxminarayana, K. 2006. Effect of Integrated use of inorganic and organic manures on soil properties, yield and nutrient uptake of rice in ultisols of Mizoram. *J. Indian Soc. Soil Sci.* 54: 120-123.

Lumpkin, T.A. and Plucknett, D.L. 1980. *Azolla*: Botany, physiology and use as a green manure. *Econ. Bot.* 34(2): 111-153.

Liu, C.C. 1979. *Use of Azolla in rice production in China*. In: Nitrogen and Rice. The International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines. pp. 375-394.

Malte, D.B., Mahajan, S.J. and Chaphale, S.D. 1991. Manurial value of effluent slurry from biogas plants. *Journal of Soils and Crops*. 1(1): 93-95.

Maurya, M.S., Singh, L., Sairam, M. and Alam, S.I. 1994. Production of biogas from night soil: Effect of temperature and volatile solids. *Indian J. Microbiol*. 34(3): 223–228.

Mclnerny, M.J. and Bryant, M.P. 1981. Review of methane fermentation fundamentals. In: Wise, D.L. (ed.), *Fuel Gas Production from Biomass* (1st Ed.). CRC Press, USA, 480p.

Möller, K., Stinner, W., Deuker, A. and Leithold, G. 2008. Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. *Nutr. Cycl. Agroecosyst.* 82: 209-232.

Mohan, S., Jayaraj, Purushothaman, S. and Rangarajan, A.V. 1987. Can the use of Azospirillum biofertilizer control sorghum shooty fly? *Curr. Sci.* 56: 723-725.

Monnet, F. 2003. Digested biomass as fertiliser. Available online: http://www.landbrugsraadet.dk/view.asp?ID=2281.

Nina, C.F. 1999. Production potential of aquatic plants in systems mixing floating and submerged macrophytes. *Freshwater Biol.* 41: 183-191.

Opperman, M.H., Wood, M.H. and Hriss, P.J. 1989. Changes in inorganic N following the application of cattle slurry to soil at two temperatures. *Soil Biol. Biochem*. 21: 319-321.

Panse,V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers* (4^{th} ed.). Indian Council of Agricultural Research, New Delhi, India, 347p.

Paul, J.W. and Beauchamp, E.G. 1995. Availability of manure slurry ammonium for corn using ^{15}N-labelled $(NH_4)_2SO_4$. *Can. J. Soil Sci*. 75: 35-42.

Perez, M., Romero, L.I. and Sales, D. 1997. Steady state anaerobic thermophilic degradation of distillery wastewater in fluidized bed bioreactors. *Biotechnol. Prog.* 13: 33-38.

Poggi-Varaldo, H.M., Trejo-Espino, J., Fernandez-Villagomez, G., Esparza-Garcia, F., Caffarel- Mendez, S. and Rinderknecht-Seijas, N. 1999. Quality of anaerobic compost from paper mill and municipal solid wastes for soil amendment. *Water Sci. Technol.* 40: 179-186.

Prasad, R. 2007. *Crop Nutrient - Principles and Practices*. New Vishal Publications, New Delhi, 272p.

Prasad, R. and Power, J.F. 1997. *Soil Fertilizer Management for Sustainable Agriculture*. CRC-Lewis, Boca Raton, FI., USA, 347p.

Rajshree, M., Wandile, Maya, M., Raul, Swati, V., Washimakar and Bharti, S.B. 2005. Residual effect of long-term application of N, P, Zn and FYM on soil properties of Vertisols, yield, protein and oil content of soybean. *J. Soils and Crops*. **15**(1): 155- 159.

Rautaray, S.K., Ghose, B.C. and Mittra, B.N. 2003. Effect of fly ash, organic wastes and chemical fertilizers on yield, nutrient uptake, heavy metal content and residual fertility in a rice-mustard cropping sequence under acid lateritic soils. *Bioresour. Technol.* 90:275-283.

Rivard, C.J., Rodriguez, J.B., Nagle, N.J., Self, J.R., Kay, B.D., Soltanpour, P.N. and Nieves, R.A. 1995. Anaerobic digestion of municipal solid waste. Utility of process residues as a soil amendment. *Appl. Biochem. Biotech.* 51-52: 125-135.

Safley, L.M., Westernman, P.W., Barker, J.C., King, L.D. and Bowman, D.T. 1986. Slurry dairy manure as a corn nutrient source. *Agric. Wastes*. 18: 123-136.

Sajwan, K.S. 1995. The effect of fly ash/sewage sludge mixtures and application rates on biomass production. *J. Environ. Sci. Health A.*30(6): 1327-1337.

Sanyal, S.K. and De Datta, S.K. 1991. Chemistry of phosphorus transformation in soil. In: Advances in Soil Science (B.A. Stewart, Ed.), Vol. 16, pp. 1-120, Springer-Verlag, New York, Berlin, London.,pp.1-120

Sarangi, P.K., Mishra, T.K. and Mishra, P.C. 1997. Soil metabolism, growth and yield of *Oryza sativa* L. in fly ash amended soil. *Indian J. Environ. Sci.*1(1):17-24.

Savant, M.K., De Datta, S.K. 1982. Nitrogen transformations in wetland rice soils. *Adv. Agron*. 35: 241-302.

Schollenberger, C.J. 1945. Determination of soil organic matter. *Soil Sci*. 59:53-56.

Shilpakar, P. and Shilpakar, D. 2009. *Handbook of Biogas Technology*. Agrotech Publishing Academy, Udaipur, 400p.

Singh, Y.V. and Mandal, B.K. 1997. Nutrition of rice (*Oryza sativa*) through Azolla, organic materials and urea. *Indian J. Agron.* 42 (4):626-633.

Singh, P.K. 1979. Use of *Azolla* in rice production in India. In: Nitrogen and Rice. International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines, pp. 407-418.

Singh, R., Jain, M.K. and Tauro, P. 1983. Pre-digestion to improve production of biogas from cattle waste. *Agric.Wastes.* 6: 167–174.

Smet, E., Van-Langenhore, H. and De-Bo, I.Z. 1998. The emission of volatile compounds during the aerobic and the combine anaerobic/aerobic composting of biowaste. *Atmos. Environ.* 33: 1295-1303.

Subbaiah, B.V. and Asija, C.L. 1956. A rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci*. 25: 259-260.

Suthar, S. 2010. Potential of domestic biogas digester slurry in vermitechnology. *Bioresour. Technol*. 101(14): 5419-5425.

Swift, M.J. and Woomer, P.L. 1993. Organic matter and the sustainability of agricultural system: Definition and measurement. In: K. Mulongoy and R. Merckx (eds.), *Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture.* Proceedings of an international symposium, Leuven, John, Wiley & Sons, Belgium, pp. 3-18.

Tchobanoglous, G., Kreith, F. and Williams, M.E. 2002. Introduction. In: Tchobanoglous, G. and Kreith, F (eds.), *Handbook of solid waste management* (2^{nd} Ed.)., cGraw-Hill Professional, New York, NY, U.S.A, pp. 13-18.

Thimmaiah, S.K. 1989. A Manual for research methods for analysis of agricultural products. University of Agricultural Sciences, Dharwad, 263p.

Thomas, C.G. 2008. *Forage Crop Production in the Tropics* (2^{nd} Ed.). Kalyani Publishers, Ludhiana, 239p.

Tolanur, S.I. and Badanur, V.P. 2003. Changes in organic carbon, available N, P and K under integrated use of organic manure, green manure and fertilizer on sustainable productivity of pearl millet-pigeonpea system and fertility of an Inceptisol. *J. Indian Soc. Soil Sci.* 51: 37-41.

Tomar, S.S. 1995. *Energy, Agriculture & Enviornm*ent. Mittal Publications, New Delhi, 213p.

Tuomela, M., Vikman, M., Hatakka, A. and Itävaara, M. 2000. Biodegradation of lignin in a compost environment: a review. *Bioresour. Technol.* 72: 169-183.

Van Hove, C., Diara, H.F. and Godard, P. 1983. Azolla in west Africa. Azolla project of the west Africa Rice Development Association and Belgium Administrations for Development and cooperation Brussels, 56p.

Van Hove, C. 1989. Azolla and it's multiple uses with emphasis on Africa. Food and Agriculture Organization, Rome, 52p.

Voca, N., Kricka, T., Cosic, T., Rupic, V., Jukic, Z. and Kalambura, S. 2005. Digested residue as a fertilizer after the mesophilic process of anaerobic digestion. *Plant Soil Environ.* 51: 262-266.

Walkley, A.J. and Black, I.A. 1934. Estimation of soil organic carbon by chromic acid titration method. *Soil Sci*. 31: 29-34.

Wang, W. 1991. Ammonia toxicity to macrophytes (common duckweed and rice) using stating and renewal methods. *Environ. Tox. Chem.*, 10: 1173-1177.

Wilkie, A.C. 2000. Reducing Dairy Manure Odor and Producing Energy. *BioCycle*. 41(9): 48-50.

Xianjun, H., Jianping, H. and Zhiwei, Q. 2011. Effect of Biogas Slurry on Biological Properties of Cabbage Continuous Cropping Soil. *Chinese J. Appl. Environ. Biol*. 17(3): 384-387.

Yadava, L.S. and Hesse, P.R. 1981. The Development and Use of Biogas Technology in Rural Areas of Asia. *Improving Soil Fertility through Organic Recycling*, FAO/UNDP Regional Project RAS/75/004, Project Field Document No. 10.

Yadvika, S.T. (2004), Enhancement of biogas production from solid substrates using different techniques-a review. *Bioresour. Technol*. 95: 1-10.

Yoshida, T. and Padre Jr., B.C. 1975. Effect of organic matter application and water regimes on the transformation of fertilizer N in a Philippine soil. *Soil Sci. Plant Nutr.* 21:281-292.

Zennaki, B.Z., Zadi, A., Lamini, H., Aubinear M. and Boulif, M. 1996. Methane Fermentation of cattle manure: Effects of HRT, temperature and substrate concentration. *Tropicul. Tural*. 14: 134-140.

Zhang, J., Zhang, M., Shan, S., Luo, L and Wang, M. 2009. Effect of biogas slurry application on growth, grain yield and heavy metals content of rice. *J. Agro-environ. Sci.* 28(10): 2005-2009.

Appendices

Appendix I

Details of substrates used for anaerobic digestion

T. N.	substrates loaded
1	cow dung alone 50 kg cow dung and 50 L water
2	cow dung + azolla (1: 0.25) 40 kg cow dung + 10 kg azolla and 50 L water
3	cow dung + azolla (1: 0.5) 33.32 kg cow dung + 16.68 kg azolla and 50 L water
4	cow dung + azolla (1: 0.75) 28.57 kg cow dung + 21.42 kg azolla and 50 L water
5	cow dung + azolla (1: 1) 25 kg cow dung + 25 kg azolla and 50 L water

Appendix II

Atmospheric temperature during anaerobic digestion period

Date	Temperature (^{0}C)	Date	Temperature (^{0}C)
01/08/2011	29.6	16/08/2011	29.6
02/08/2011	27.0	17/08/2011	32.0
03/08/2011	27.6	18/08/2011	28.8
04/08/2011	29.7	19/08/2011	29.9
05/08/2011	27.6	20/08/2011	29.0
06/08/2011	28.7	21/08/2011	29.1
07/08/2011	28.0	22/08/2011	30.5
08/08/2011	29.2	23/08/2011	30.2
09/08/2011	30.1	24/08/2011	30.0
10/08/2011	30.6	25/08/2011	28.6
11/08/2011	30.6	26/08/2011	30.0
12/08/2011	27.6	27/08/2011	29.6
13/08/2011	29.0	28/08/2011	30.1
14/08/2011	30.8	29/08/2011	29.2
15/08/2011	31.8	30/08/2011	29.4

(Source: Department of Agricultural Meteorology, College of Horticulture, KAU)

Appendix III

Nutrient content of fertilizers and organic manure

Sl. No.	Fertilizers and Manures	Nutrient content (%)		
		N	P_2O_5	K_2O
1	Urea	46.00	0.00	0.00
2	Rajphos	0.00	18.00	0.00
3	Muriate of Potash	0.00	0.00	60.00
4	Farmyard manure	0.76	0.43	0.56
5	Enriched manure I	4.10	1.14	3.89
6	Enriched manure II	3.91	1.17	3.85

Appendix IV

Nutrient management of rice (var. Jyothy) (As per package of practices recommendations, KAU)

Treatment	Fertilizer/manure dosage per pot (10 kg soil)
T_1	Absolute control- crop without manuring
T_2	NPK+FYM 70 N: 35 P_2O_5: 35 K_2O kg ha^{-1} Urea - 0.34 g Rajphos - 0.39 g MOP – 0.13 g FYM – 11.2 g
T_3	FYM alone FYM – 11.2 g
T_4	Enriched manure I alone (@ 5 ton ha^{-1}) Enriched manure I – 11.2 g
T_5	Enriched manure II alone (@ 5 ton ha^{-1}) Enriched manure II – 11.2 g
T_6	Enriched manure I+NPK 70 N: 35 P_2O_5: 35 K_2O kg ha^{-1} Urea – 0.34 g Rajphos – 0.39 g MOP – 0.13 g Enriched manure I – 11.2 g (@ 5 ton ha^{-1})
T_7	Enriched manure II+NPK 70 N: 35 P_2O_5: 35 K_2O kg ha^{-1} Urea – 0.34 g Rajphos – 0.39 g MOP – 0.13 g Enriched manure II – 11.2 g (@ 5 ton ha^{-1})

SUITABILITY OF AZOLLA (*Azolla pinnata*) FOR BIOGAS SLURRY ENRICHMENT

By

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ABSTRACT OF THE THESIS

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ABSTRACT

A study on "Suitability of azolla (*Azolla pinnata*) for biogas slurry enrichment" was conducted during 2010-11 at College of Horticulture, Vellanikkara to evaluate the optimum ratio of cow dung and azolla for biogas slurry enrichment, to identify the best material to prepare quality organic manure from the enriched slurry and also to adjudge the crop response to enriched organic manure. To attain the objectives, three separate experiments were conducted in a phased manner.

In order to determine the optimum ratio of cow dung and azolla for biogas slurry enrichment, the floating drum biogas digesters of 0.5 m^3 capacity were used. The different proportion of the substrates tried were cow dung alone and cow dung and azolla in 1: 0.25, 1: 0.5, 1: 0.75 and 1: 1 ratio. The results indicated that mixing of cow dung and azolla in a proportion of 1: 0.5 produced the highest volume of gas (0.29 m^3 kg^{-1} TS) in 20 days of Hydraulic Retention Time (HRT). But the proportion of 1: 1 favored in terms of N (3.44 per cent) content in slurry followed by 3.23 per cent in 1: 0.75 ratio. These treatments were designated as enriched slurry I (3.44 per cent N, 0.74 per cent P and 2.93 per cent K) and enriched slurry II (3.23 per cent N, 0.77 per cent P and 2.91 per cent K). Addition of azolla increased pH from 7.1 to 7.8 but decreased the total solids of slurry from 5.40 per cent in cow dung alone to 2.68 per cent in the ratio of 1: 1.

For identifying the best material for preparing quality manure from the enriched slurry, it was mixed with saw dust, coir dust and powdered coconut leaves which varied in the nutrient content and C/N ratio. Based on the nutrient content, powdered coconut leaves were identified as the best material and the requirement was 612 g per litre in enriched slurry I and 607 g per litre for enriched slurry II.

Crop response to the enriched manure I and II was assessed in pot culture with rice (variety- Jyothy) as the test crop. The treatments were control (T_1), NPK+FYM (T_2), FYM alone (T_3), enriched manure I (T_4), enriched manure II (T_5), enriched manure I+NPK (T_6) and enriched manure II+ NPK (T_7). The grain (44.97 g pot^{-1}) and straw (72.73 g pot^{-1}) yield was maximum when the crop was nourished with enriched manure II in association with the recommended dose of chemical fertilizers. Crop uptake of N (4260 mg pot^{-1}) and K (4263 mg pot^{-1}) was also the highest in this treatment.

Soil physico-chemical properties were also favorably influenced by the manure application. A shift in pH from 4.7 to 5.4 was obtained consequent to applying enriched manure I. Soil status of available N (1536 mg kg^{-1}) and available K_2O (471 mg kg^{-1}) was the highest when enriched manure II was applied along with the recommended dose of chemical fertilizers. The content of available P_2O_5 in soil was the maximum (52.7 mg kg^{-1}) when chemical fertilizers were excluded from this treatment.