बायोगैस अवशिष्ट तथा उर्वरक के एकीकृत उपयोग द्वारा गेहूँ में नाइट्रस ऑक्साइड का उत्सर्जन

N_2O EMISSION IN WHEAT WITH INTEGRATED USE OF BIOGAS SLURRY AND FERTILIZERS

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2016

N_2O emission in wheat with integrated use of biogas slurry and fertilizers

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A Thesis

Submitted to the Faculty of the Post-Graduate School,

Indian Agricultural Research Institute, New Delhi

In partial fulfillment of the requirements

For the award of the degree of

MASTER OF SCIENCE

In

ENVIRONMENTAL SCIENCES

2016

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CERTIFICATE

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It is further certified that all the assistance and help availed during the course ofinvestigation as well as all sources of information have been duly acknowledged by him.

Date: July, 2016
Place: New Delhi-110012

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Chairman

ACKNOWLEDGEMENT

I acknowledge the great power, who has paved the way on which I have walked so far. As a prelude to my thanks giving, at first I wish to thank and bow my head before Lord Almighty, for blessing me with all his grace to successfully complete my studies and research endeavor in IARI.

It is my great privilege to express deep sense of gratitude to ***Dr. Shakeel A. Khan****, Sr. Scientist, Centre for Environmental Science and Climate Resilient Agriculture, Indian Agricultural Research Institute, New Delhi and the Chairman of my advisory committee, who guided me as a teacher, helped me like a friend and cared me as guardian for providing me an opportunity to work under his guidance. I am also thankful for his valuable guidance, personal attention, constructive criticism, immense patience, untiring help, wholehearted support and encouragement to me throughout the period of my study and preparation of this manuscript.*

I express my sincere thanks and gratefulness to Dr.H. Pathak, Principal Scientist and Professor,Centre for Environmental Science and Climate Resilient Agriculture, IARI, New Delhi and Co-Chairman of my advisory committee for encouraging me to undertake thisstudy and providing me with his precious advice, necessary guidance and help rendered during my course of study.

I convey my respectful sincere thanks to themember of my Advisory Committee,Dr.Namita D. Saha, for their concrete suggestions and commendable help throughout the experimental period.

I would like to extend my heartfelt gratitude to Dr.Ranjan Bhattacharya, Senior Scientist, CESCRA, IARI, New Delhi and Dr.JoydeepMukharjee, Senior Scientist , Division of Agricultural Physics, IARI, New Delhi, the members of my Advisory Committee for their most valuable help rendered in relation to planning, execution and analysis of this work.

It gives me immense pleasure to thank Dr. S.D. Singh, Head, all my teachers and scientists of the Division, for their valuable suggestions and constant encouragement during my course of research.

My sincere thanks go to Dr.Bhawana Joshi, Dr.Preeti, Dr. RituTomer, Dr. Amit Kumar and all staff members of CESCRA for their timely help.

No words can describe the unending love, moral support and help by my dearest friends Pawan, Ranjeet, Soumen, Jayanta and Chandrashekharduring my course of study. I would like to thank especially to my senior LalchandMalav who helped me a lot during writing of manuscript and data analysis.

I affectionately cherish the blessings and good wishes of my beloved parents, my loving siblings, and all my relatives who were the constant sources of inspiration to me from miles apart.

I wish to express my heart full thanks to my seniorsNasir Ahmad Haqbeen,Khalil Nawab,ReazaJoudaki,Amir Jan Dawlatzaifor supporting me.

I wish to express my heart full thanks to my good friends Ahmad Fayez WalidSiawashHabibi, NaqibullahAzizi, Fardinsadri, KhujaSafiullah, Usmani,EmalShegiwal,AliMohammadDawlatza,NaqibullahShenwari,Jawid Safi and SayedHazart Alawi.

I will ever remain indebted for the facilities and knowledge, IARI provided to me.

Date : July, 2016 **SHAHABUDEN KHWAHANY**

Place: New Delhi-110012

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1. INTRODUCTION

We required environmentally safe energy technologies for the sustainable production and use. Therefore, they should contribute to decreased emissions of greenhouse gasses to the atmosphere and strengthen the ecosystem services and food production. Agricultural biogas plants have become increasingly attractive (Tiwari *et al.*, 2000; Thran and Kaltschmitt, 2007). This has led to the increasing use of biogas slurry, the remains of anaerobic degradation, as a fertilizer in arable crop production (Loria *et al.*, 2007). Bio-slurry is the byproduct of biogas technology and help in (i) mitigate green house gas emissions, (ii) better substitution of chemical fertilizer,(iii) source of many essential micronutrients and (iv) improve the economics condition of farmers. In addition, the nutritional value of biogas slurry can also be improved by combining with other sources of nutrients; e.g. ashes (Bougnom *et al.*, 2012). Tightening of the nutrient cycle, recycling and local production of renewable energy are examples of key principles in organic agriculture (IFOAM, 2011).

Carbon dioxide, methane, and nitrous oxide are the three major greenhouse gases (GHG) contributing altogether 87% of the total radiative forcing (IPCC, 2014). The atmospheric concentrations of these gases have substantially exceeded the pre-industrial levels by about 40, 150, and 20%, respectively (IPCC, 2014), which has resulted in global warming and may adversely affect natural and agro-ecosystems. Globally agriculture, forestry and other land use sector accounts for 24% of total global anthropogenic emissions of GHGs mainly methane and nitrous oxide (IPCC, 2014). Of global anthropogenic emissions in 2010, agriculture accounted for about 43% increase in N_2O and about 47% increase in CH_4 emissions as compared to 1970 (IPCC, 2014).

Nitrogen (N) fertilizer input into agricultural systems is considered the major source of N_2O emissions from agricultural soils (Mosier and Kroeze, 2000). N_2O is produced in soils mainly by the bacterial processes of nitrification and denitrification after fertilizer application. Nitrous oxide with its current concentration of 326 ppbv in the atmosphere; besides being an important greenhouse gas is responsible for destruction of stratospheric ozone (Zaman *et al.*, 2012). Atmospheric concentration of nitrous oxide is of great concern because of its long life time of 121 years (IPCC, 2014). Global annual emission of nitrous oxide is 6.7 Tg yr^{-1} which includes direct

emission from agricultural soils through loss of N to aquatic system and atmosphere (IPCC, 2007). Globally agricultural N_2O emission has increased by nearly 17% from 1990 to 2005 (IPCC, 2007). Soil is considered to be one of the major sources of nitrous oxide emission contributing 65% to the total global emission (Ehhalt *et al.,* 2001). Soils receiving chemical fertilizer and biologically fixed N contribute to nitrous oxide emission during the process of nitrification and denitrification. Contribution of different sources to nitrous oxide emission from Indian agricultural soils like fertilizer, manure (animal and green), soil mineralization, and crop residues, was 77, 13, 9 and 1%, respectively, to the total N input in the year 2010 (Pathak *et al.,* 2014).

The microbial processes of nitrification and denitrification are the natural phenomenon for N_2O production. The soil physico-chemical properties play important role in N_2O emission because the interactions among the physical, chemical and biological variables that control N_2O production are complex. N_2O fluxes from agricultural systems are highly variable in both time and space (Smith 1990; Mc Taggart *et al.* 1994; Smith *et al*. 1999; Mosier and Kroeze 1999). As a result, measured N_2O emissions associated with a unit of N application show a wide variation, with a range from the order of 0.1% to the order of 10% (IPCC 2000). Over 250 estimates of direct N_2O emission from agricultural fields, (Bouwman,1996) reported, agricultural systems emit large amounts of N_2O, but was unable to establish systematic patterns of N_2O flux with soil texture, soil pH, soil drainage, crop type, or residue management.

Agricultural soils system exhibit a high degree of spatial variability in terms of physicochemical and biological properties. Soil microbes play a crucial role in transformation of organic matter that regulates several essential ecosystem functions like decomposition, mineralization of nutrients to be available to plants. Any organic production system relies on such microbially regulated ecosystem functions and thus important to investigate the effects of organic practices on such microbially derived activities. As opposed to the conventional and synthetic fertilizer based agricultural practices, organic cultivation system differ significantly in different aspects like, it reduces nutrient losses, improve soil fertility (Burger and Jackson, 2003; Gattinger *et al.*, 2012), and reduce global warming potential (Burger *et al.,* 2005; Cavigelli *et al.*, 2013) while supporting similar crop yields in certain contexts (Seufer *et al.*, 2012).

The soil microbial biomass and their activity is generally controlled by its existing SOM and carbon and nitrogen inputs (Fierer *et al*., 2009; Kallenbach and Grandy, 2011). Hence, the quality and the quantity of the organic amendments are also distinctly stimulating microbial biomass through increases in labile organic matter (Marriott and Wander, 2006; Smukler *et al*., 2008; Kallenbach and Grandy, 2011) and/or total soil C on time frames from months to decades (Drinkwater and Wagoner, 1998; Kong *et al*., 2005). The enzymatic activity of soil, derived from active microorganisms and the stabilized pool in clay- humus complexes (Tabatabai, 1994; Burns *et al*., 2013), plays a major role in the depolymerization of structurally diverse polymeric macromolecules, which is considered the rate-limiting step in decomposition and nutrient mineralization potential of soil (Schimel and Bennett, 2004).

Soil management practices are directly affected by the microbiological attributes such as biomass and their activities, (Elliot, 1997). The soil physico-chemical properties are the indicator of the changes in biological properties and detect an early signal of improvement or a warning of degradation (Pankhurst et al., 1995).) To differentiate microbial communities from several habitats, Community level physiological profiles (CLPPs) methodology is used which is based on the ability of microorganisms to oxidize different carbon substrates (Garland, 1997; Gomez et al., 2004). CLPPs are a sensitive approach to estimate the microbial functional diversity and detect modifications due to soil management (Stenberg, 1999; Gomez *et al*., 2004). Biolog EcoPlate^{TM} assay is most significant methodology and reported in many literatures, allows testing a more reduced number of ecologically relevant substrates and the possibility of larger number of replicates. However, there is less information available as regards the use of Eco Plate assay to detect changes in microbial functional diversity due to soil management practices.

Other than CLPPs, it has been reported that organic management practices increase overall enzyme activity (Mäder *et al*., 2002; García-Ruiz *et al*., 2008; Moeskops *et al*., 2010), but the composition of the organic amendments, the relative availability of nutrients and soil physico-chemical properties affect the activities of specific enzymes (Stursová and Baldrian, 2010).

The overall objective of this study was to examine how different doses of biogas slurry (which is a byproduct from Biogas plant and enriched with different nutrient components) application and nutrient management practices affect N_2O

emission, soil microbial activity and microbial community composition in organic agricultural systems, using an on-farm approach under wheat crop.

Thus the proposed research work was carried out with the following objectives:

1. To examine the greenhouse gas (N_2O) mitigation potential of biogas slurry in substitution of N fertilizer.
2. To examine the effect of slurry application on microbial biomass community composition and its functional diversity

2. REVIEW OF LITERATURE

Worldwide large amounts of biogas slurry are produced every day from different sources such as livestock, industries, agriculture and households. These wastes need to be managed and utilized in an appropriate way. One way to make waste management sustainable is to use the organic wastes as sources in renewable energy production, to produce e.g. biogas. However, the use of organic wastes in bioenergy production also leads to generation of 'secondary' residues, because not all organic material fed into a bioprocess can be utilized by the microbes. Such residues are known to be rich in plant nutrients and can be used as fertilizers, thereby recycling the nutrients back to agricultural soils (Odlare *et al.,* 2011; Svensson *et al.,* 2004).

The application of organic residues to agricultural soils has become an important approach to increase soil fertility (Pérez-Piqueres *et al.,* 2006; Ayuso *et al.,* 1996), but also presents challenges, as the beneficial effects of these materials may be accompanied by risks to both the soil and the environment due to contaminants (Bationo *et al.,* 2007; Albihn, 2002). Most types of organic fertilizers such as conventional animal manures and crop residues have been extensively studied from different perspectives, e.g. crop yield, soil microbiology and environmental effect (Zhong *et al.,* 2010; Möller *et al.,* 2008;Goyal *et al.,* 2006; Grandy *et al.,* 2006;Ghosh *et al.,* 2004; Goyal *et al.,* 1992),whereas organic residues generated from renewable energy sources, such as biogas residues (BRs), are poorly documented.

The number of biogas plant reactors is increasing and will continue to increase rapidly in many countries over the coming years to meet the demand for energy (Rutz, 2010). Within the European Union, biogas production increased six-fold from 1990 to 2005 (EUROSTAT, 2007). In early 2010, about 5,900 biogas plants were installed in Eastern Europe (Zuber, 2010). In Germany, the biogas market is booming and currently there are about 4,500biogas plants (Rutz, 2010). Because of this great increase in number of anaerobic digestion plants, large amounts of BRs are expected to be produced (Angelidaki *et al.,* 2003). For instance, Swedish Waste Management reported that in 2011 more than 555,000 tons biodegradable wastes were digested in biogas processes, resulting in 594,000 tons of slurry BRs (Swedish Waste Management, 2012).

The biogas process, also called anaerobic digestion, consists of the stages hydrolysis, fermentation, acetogenesis and methanogenesis (Angelidaki *et al.*,2011), all of which are performed by diverse groups of microorganisms, leading to the overall

degradation of complex organic compounds. During anaerobic digestion, a large proportion of the energy contained in the organic waste is retained in the methane molecules (Neves *et al.*, 2006). At the same time, the nitrogen (N) and most other nutrients are preserved in the residues (Massé *et al.*, 2007). For example, organically bound N in manure and crop residues is mineralised to ammonium (NH_4^+), which is a soluble form of N that plant roots can easily absorb (Möller & Stinner, 2009).

Overall, anaerobic digestion in a biogas plant results in a residue that differs profoundly from the 'raw materials' fed to the process in having e.g. a higher pH, lower contents of dry matter and total carbon (C), a higher proportion of NH_4^+-N to total N and a lower carbon to nitrogen (C/N) ratio. However there is generally no alteration in total N, K and P (Field *et al.*, 1984; Kirchmann & Witter, 1992). Therefore, application of BRs can be expected to lead to different effects on the soil ecosystem and the environment compared with the use of regular organic fertilizers (Levén & Schnurer, 2005; Engwall &S chnürer, 2002; Angelidaki *et al.*, 2000; Grossi *et al.*, 1998).Thus, BRs can be generated using different operating parameters and therefore the recycling of residues to arable soils might lead to different effects.

2.1 Fertilization and greenhouse gases emissions

Recycling organic residues to arable soils can carry the risk of increased emissions of greenhouse gases (GHG) such as N_2O, CH_4 and CO_2 (Flessa *et al.*, 2002), all of which contribute to global warming. N_2O is produced in the soil by microbiological activity through nitrification and denitrification (Bremner, 1997). N_2O has a global warming potential 310 times higher than that of CO_2 due to its absorptive capacity and long persistence in the atmosphere, *i.e.* 114 years (Forster *et al.*, 2007).

Furthermore, emissions of N_2O have received great attention due to the potential of this gas to destroy the ozone layer protecting the earth from ultraviolet radiation from the sun (Ravishankara *et al.*, 2009). In addition, emissions of N_2O result in losses of N from the soil, thereby withdrawing availability of this nutrient from arable land (Whalen, 2000). The agricultural sector accounts for 65-80% of the total global emissions of N_2O, which means that agriculture represents the largest anthropogenic source of this gas (IPCC, 2007). A number of factors have been identified as affecting N_2O emissions from soils either directly or indirectly, of which soil moisture (Blackmer *et al.*, 1982), oxygen concentration (Hwang & Hanaki, 2000), mineral N (Peng *et al.*, 2011), available C (Velthof *et al.*, 2003), soil texture

(Maag & Vinther, 1996), pH (Šimek & Cooper, 2002) and temperature (Goodroad & Keeney, 1984) are thought to be the most important.

The overall soil moisture is probably the most important factor regulating N_2O production by directly affecting the cellular activities of nitrifying and denitrifying bacteria and influencing the solubility and diffusion rate of organic C, mineral N and N_2O in the soil system. In addition, soil moisture affects the reduction of N_2O to N_2, thereby influencing the gaseous composition of the gaseous emissions (Blackmer *et al.*, 1982). Soil moisture also indirectly affects N_2O emissions through dissolution and diffusion of organic C, making it available to aerobically respiring organisms and contributing to lowering the partial pressure of oxygen in the system (FAO, 2001).

2.1.1 Nitrous oxide

Nitrogen (N) fertilizers contribute significantly to anthropogenic nitrous oxide (N_2O) emissions (Davidson 2009). Nitrous oxide is a greenhouse gas with a global warming potential 310 times that of carbon dioxide over a 100-year timeframe (IPCC 2014). The prerequisite of developing management practices to minimize N_2O emissions from managed ecosystems is an understanding of the source and factors controlling N_2O emissions. Atmospheric N_2O concentration has increased, particularly after 1960 because of the increasing use of N fertilizers (Davidson 2009). A major amount of N_2O production following the application of N fertilizers occurs because of biotic processes, namely nitrification and denitrification (Wrage *et al.*, 2001). Biological processes (denitrification, nitrification, dissimilatory nitrate reduction and assimilatory nitrate reduction) as well as chemical reactions (chemo denitrification) are the possible mechanisms of N_2O emissions from soil. Production of N_2O from nitrification and denitrification occurs simultaneously, but the dominant process depends on the availability and type of substrates and the soil oxygen and moisture levels (Khalil *et al.*, 2002, 2004).

2.1.2 Nitrification as a source of N_2O

Nitrifying bacteria are a well-known source of N_2O in soils (Davidson, 1992). Nitrifier is mainly chemo lithoautotrophic bacteria obtaining their energy from oxidation of NH_3 (Hofman & Lees, 1952). Nitrification is performed by two different groups of bacteria belonging to the β and γ subgroups of proteo-bacteria: AOB

(ammonia oxidizing bacteria) gaining energy from oxidation of NH_3 to NO_2 - and NOB (Nitrite oxidizing bacteria) oxidizing NO_2^- to NO_3^- (Fig. 1).

Common AOB in the soil belong to the genera *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus*and *Nitrosovibrio,* whereas NOB are commonly represented by *Nitrobacter* and *Nitrospira* (Myrold, 1999). AOB are widely distributed in soils, rocks, freshwater and seawater, and in wastewater treatment systems (Koops *et al.,* 2006; Teske *et al.,* 1994). Ammonia oxidation is catalysed by the enzyme ammonia monooxygenase, which oxidises NH_3 to hydroxylamine (NH_2OH). The intermediate NH_2OH is further oxidised to NO_2^- by another enzyme called NH_2OH oxido reductase. The NO_2^- produced is further oxidised by NOB in a one-step reaction to NO_3^- using the enzyme nitrite oxido reductase. It has been shown that N_2O can be produced through chemical decomposition of the intermediate NH_2OH (Fig. 1) (Poth & Focht, 1985; Hooper & Terry, 1979), especially under oxygen-limited conditions (Bremner, 1997).

In addition, using $14NH_4^+$ and $15NO_2^-$ it has been shown that N_2O, at low oxygen tension, can also be produced by NO_2^- reduction (Poth & Focht, 1985). Recently, high numbers of putative ammonia monoxygenase genes in archaeans belonging to the 45 phylum Crenarchaeota have been detected in soil, indicating high ammonia oxidising potential of this prokaryotic group (Kelly *et al.*, 2011), also suggesting contribution to N_2O emission (Cabello *et al.,* 2004). However, nitrogen metabolism is much less known in archaea than in bacteria.

2.1.3 Denitrification as a source of N_2O

Denitrification is a biological process responsible for gaseous losses of N_2O and N_2 from soil. Anaerobic conditions prompt the denitrifiers to use nitrogen oxides as an electron acceptor when oxygen is limited. The main enzymes involved in the denitrification process are NO_3^- reductase, NO_2^- reductase, NO reductase and N_2O reductase, catalysing the chain of reductions from NO_3^- to NO_2^- and then further to NO and N_2O and finally, in most cases to N_2 (Knowles, 1982). Under certain conditions, such as those in acidic soil, N_2O reductase is inhibited, so the N_2O/N_2 ratio in the gaseous product will increase (Šimek *et al.*, 2002), while in conditions of high soil moisture and low oxygen the N_2O/N_2 ratio will be low (Ciarlo *et al.*, 2007). Denitrification can lead to emissions of NO, which even though it has very short lifetime can still be emitted from the soil (Jeffrey Peirce & Aneja, 2000; Remde &

Conrad, 1991). Denitrification is performed by a wide range of bacterial species (Knowles, 1982), and potent denitrifiers have been identified as belonging to the genera *Achromobacter, Alcaligenes, Bacillus, Burkholderia-Ralstonia, Clostridium Moraxella, Pseudomonas, Streptomyces* and *Xanthomonas-Frateuria*(Chènebyet *al.*, 2000; Myrold, 1999; Zumft, 1997). Furthermore, N_2O can be produced by other groups of microorganisms such as archaeans (Cabello *et al.*, 2004) and fungi (Ma *et al.*, 2008). However, it has been shown that N_2O emissions are driven by bacteria rather than archaeans in N-rich grassland soils (Di *et al.*, 2010).The main soil microbial processes leading to emissions of N_2O are illustrated in Fig. 1.

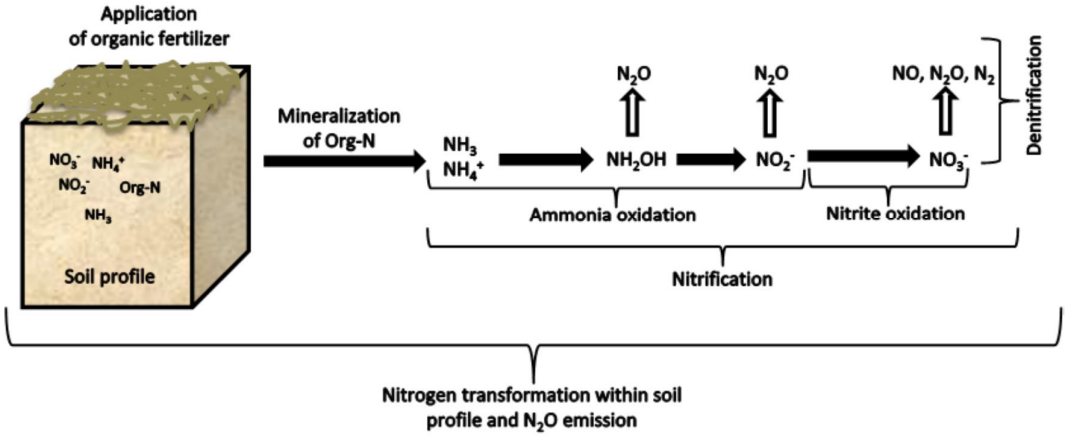


Figure 1. Nitrous oxide emissions by nitrification and denitrification during biological transformation of N.

2.1.4 Chemical formation of N_2O

Nitrous oxide can also be formed by chemical reactions when NO_2 or NH_2OH are decomposed in acid soils, producing N_2O.

$$\mathbf{NH_2OH + HNO_2 \rightarrow N_2O + 2H_2O}$$

However, the formation of N_2O by chemical reaction of NO_2 and hydroxylamine does not seem to be important since there was no significant increase of the rate of N_2O production by the addition of NO_2^- or NH_2OH in soils (Pathak , 1999). It is generally assumed that the majority of N_2O production occurs in the proximity to the surface.

2.1.5 Factors affecting the emissions of nitrous oxide

Factors controlling seasonal N_2O emissions may include management practices, particularly fertilization, irrigation, selected crop, and temporal climatic variations (Lee *et al.,* 2009).The emissions of N_2O and N_2 and their ratios are affected by various soil and management factors, including mineral N concentration, available C, soil pH, soil aeration status, soil temperature and their interactions. Some of the important factors and their impacts are given in Table 1.

Table 2.1 Factors affecting N_2O and N_2 emissions and their ratios.

Factors	Management	Impact	Management practices
Soil NH_4^+ & NO_3^- concentration	Decrease	Reduce nitrification &denitrification and lower N_2O:N_2	Use urease and nitrification inhibitors to enhance fertilizer use efficiency; split N fertilizer application to synchronize plant N demand and to minimize N losses; avoid over grazing; manipulation of animal diet; use of constructed or natural riparian wetland, improving water use efficiency to avoid anaerobicity.
Soil organic C	Increase	Improve soil health, facilitate denitrification and thus lower N_2O:N_2	Sequester more C by adopting management practices including zero or minimum tillage, retention of crop residues, mulching, application of organic and farm wastes, biochar, and applying chemical fertilizers with organic amendments.
Soil pH	Increase	Facilitate nitrification and denitrification and thus lower N_2O:N_2	Regular liming each year or if possible with every N fertilizer application.
Soil aeration and water status	Improve	Facilitate nitrification and denitrification and thus lower N_2O:N_2	Improving soil structure via C sequestration, avoiding soil compaction; improving soil drainage condition and also water use efficiency.

Source: Zaman *et al.,* (2012)

3. Relation between recycling of organic residues and N_2O emissions

The use of N-fertilizers influences directly the amount of NH_4^+ or NO_3^- available in the soil. The greater the amount of $N\text{-}NH_4^+$ in the fertilizer, the greater will be the nitrification process (Mosier 2001, Khalil *et al.,* 2004, Liu *et al.,* 2005). Global emission of N_2O from cultivated land is estimated to be 3.5 Tg N per year out of which synthetic nitrogenous fertilizer contributes 1.5 Tg annually (Hirsch *et al.,* 2006). Plants grown on normally aerated soil can release significant amounts of N_2O into the atmosphere. Blackmer *et al.,* (1980) reported that the amount of N_2O evolved from plots treated with $(NH_4)_2SO_4$ or urea markedly exceeded those from plots receiving the same amount of N as $Ca(NO_3)_2$. Emissions of N_2O will also be greater when NO_3^- in the soil is high (Carmo *et al.,* 2006, Ruser*et al.,* 2006, Zanatta *et al.,* 2010). When the NO_3^- availability decreases, N_2O emissions will also decrease, because denitrification is reduced (Hellebrand *et al.,* 2008). On the other hand, N-fertilization implies a higher plant biomass production, and then more crop residues (and carbon sources) would be available in the soil, which could increase N_2O emissions for a long period, after the N-fertilizer application (Hellebrand *et al.,* 2008).

3.1 Amendment with organic manure

With increasing interest in the use of organic amendments for remediating soils, it is important to investigate their interactive effects on soil biogeochemical processes including greenhouse gas emissions, particularly nitrous oxide (N_2O) and carbon dioxide (CO_2). Denitrifiers use organic C compounds as electron donors for energy and synthesis of cellular constituents. Plant residues, green manure and farmyard manure have been reported to increase rates of N_2O emmisions (Bhatia *et al,* 2005, Pathak *et al.*, 2010).

3.1.1 Effect of biogas residues on N_2O emissions

Application of mineral fertilizers and animal manures to soils has frequently been shown to increase N_2O emissions (van Groenigen *et al.*, 2004; Akiyama & Tsuruta, 2003; Petersen, 1999; Olivier *et al.,* 1998; Beauchamp, 1997). Therefore, fertilization with BRs can also be expected to increase N_2O emissions. However, total emissions and flux patterns of N_2O after fertilization with BRs may differ to those from mineral fertilizers and conventional animal manures due to the fact that BRs originate from technical anaerobic processes. In a biogas reactor the raw feedstock is biochemically

altered in a different way to digestion in the gastro-intestinal system of animals (Field *et al.*, 1985).

Moreover, the raw material fed to the biogas process is more variable than the feed given to animals. Anaerobic digestion increases the NH_4^+concentration and pH in the processed material, but also decreases the organic dry matter and total organic C content. Therefore, the C/N ratio in BRs is lower than that in the raw organic substrate (Field *et al.*, 1984). During the biogas production process the most easily degradable organic C is consumed and other organic components are partly degraded, resulting in digested residues with more recalcitrant material. Such material when applied to soil might be more limiting for microbial activities than conventional organic fertilizers (Ernst *et al.*, 2008; Clemens *et al.*, 2006; Kirchmann& Witter, 1992).

To get a full picture of the effects of different BR types on N_2O emissions, studies under both controlled conditions and field conditions are needed. However, in the field, climate factors are unstable, which makes the results difficult to interpret. In addition, field experiments are expensive, time-consuming and labour intensive to conduct because of the high spatial and temporal variability of N_2O fluxes from soil (Ambus & Christensen, 1994). Nevertheless, field studies are important in order to quantify N_2O emissions on regional and global scales.

N_2O emissions were studied in two laboratory incubation experiments performed by using a repacking soil core technique, which is a common method for studying GHG emissions under controlled conditions (Scheer *et al.*, 2009; Ball *et al.*, 2008; Dannenmann *et al.*, 2008; Jan Dick, 2006; Butterbach-Bahl *et al.*, 2002). The experiments were performed by amending three soil types (sandy, clay and organic) with two types of BRs (BR-C and BR-D) and CS. The amendments were added at a fertiliser rate corresponding to 70 kg NH_4^+-N ha^{-1}. In the first experiment N_2O fluxes were measured 12 times during 24 days, with the first gas sampling made 24 hours after amendment and thereafter on days 2, 4, 6, 8, 10, 11, 13, 15, 17, 20 and 24. The second experiment aimed to quantify the N_2O fluxes at times when peaks were expected as inferred from the first incubation experiment. Furthermore, PAO and PDA, NH4 + and NO_3^-as well as total C (Tot-C) and N (Tot-N) were analysed at the times when N_2O was expected to peak.

N_2O emissions were studied in a clay and organic soil after amendment with two types of BRs: (1) a conventional biogas residue (BR-F) from a large-scale biogas plant treating ley crop silage and source-separated household waste, and (2) a solid-fraction residue (BR-G) from a pilot-scale biogas reactor treating the same material as above, but with the residues 47 processed in an ultra-filtration membrane unit to separate the solid fraction from the liquid fraction of the slurry. This mechanical solid-liquid separation of the digested residue was done to reduce the dry matter content of the recirculated process water and thereby increase the capacity to load and treat more material in the reactor. This allows more biogas to be produced and, hence, more electricity (Bauer *et al.*, 2009). Another benefit with solid-liquid separation is that it yields a BR with a lower water content, which makes the product easy to store and handle and lowers the costs of transportation and spreading. In the experiment the flux of N_2O was measured 24 hours and 7 days after amending the two soils with non-separated slurry and the solid fraction of the BR.

BRs (BR-C and BR-D) and CS yielded substantially different flux patterns of N_2O and also differed in total N_2O-N losses. Amending the sandy soil with BR-D and CS led to higher total N_2O-N losses (0.32 and 0.18 mg N_2O-N m^2, respectively) than amending it with BR-C (0.02 mg N_2O-N m^2). This can probably be attributed to the higher levels of organic N and organic C in BR-D and CS than in BR-C *per se*. In fact, the actual organic N and Tot-C in the doses, corresponding to 70 kg NH4 +-N ha-1, were higher in BR-D and CS and lower in BR-C. In contrast, addition of BR-C to clay soil increased the N losses significantly compared with BR-D and CS (0.25, 0.15 and 0.02 mg N_2O-N m^2 for BR-A, BR-B and CS, respectively).

The organic C content in BR-C was obviously not high enough to provide the denitrifying bacteria with the energy required to further reduce N_2O to N_2, as apparently was the case for BR-D and CS. It has been shown that applying rich material to fine-textured soil at relatively high soil moisture contents decrease the N_2O/N_2 ratio in the gas emitted (Weier *et al.*, 1993; Cady & Bartholomew, 1960). Therefore, we believe that the different content and quality of organic C were responsible for the higher emissions observed for the BRs. Organic soil amended with BR-C and BR-D showed lower total N_2O-N losses compared with soil amended with CS (0.09, 0.08 and 0.31 mg N_2O-N m^2 for BR-A, BR-B and CS, respectively). Furthermore, the residue type showed a clear effect on the peak size, with amendment with CS generating alarger peak than amendment with BR-C and BR-D.

This may be related to both the quantity and quality of the C in CS (Florinsky *et al.*, 2004; Velthof *et al.*, 2003). The organic C in BRs may not be of the same quality as that in CS (Ernst *et al.*, 2008) because during anaerobic digestion most of the organic C will be degraded and converted to CH_4 and CO_2 (Venkata Mohan *et al.*, 2005; Angenent*et al.*, 2004).

Amending clay and organic soils with BR-G increased the N_2O flux significantly compared with the unfiltered BR-F at days 1 and 7. The high N_2O flux after application of BR-G could probably be explained by stimulated overall denitrification due its high dry matter content contain inorganic C, as described by Bauer *et al.* (2009). The filtration process resulted in a doubling of the dry matter content of the solid fraction compared with the unfiltered residue. Another interpretation could be that despite its high quantity of organic matter, the solid fraction did not contain sufficient amounts of easy available C to support the denitrifiers in completing the full denitrification pathway to N_2, thus leaving N_2O as a dominant end product. It has been reported previously that the liquid fraction of anaerobic digested residues contains most of the soluble C, such as volatile fatty acids (Lee *et al.*, 2000).Yet another factor to consider is that the pH of the residues was considerably higher in BR-G (pH 10) than in the unfiltered BR-F (pH 7.5), which should have led to increased soil pH after application and lower N_2O emissions, especially in the organic soil. However, BR-G still generated higher N_2Oemissions than BR-F, and thus it seems that short-term application may not affect the soil pH and N_2O emission. Therefore, the long-term effect of fertilisation with the solid fraction of BRs needs to be investigated to determineits impact on soil pH and N_2O emissions.

4. Soil biological properties

Soil biological properties are defined as various activities performed by living microorganisms, soil fauna and roots. Examples of activities within the N cycle are mineralization, nitrification and denitrification. Based on the results from the incubation experiments reported, PAO and PDA in the three soils at the times when N_2O generally peaked, *i.e.* day 1 and 11, could not explain the individual peaks in the soils, but were more useful in explaining the different flux patterns between soil types and total losses. PAO was low in the sandy and organic soils and higher in the clay soil, while PDA was higher in the clay and organic soils and lower in the sandy

soil. This indicates that most of the N_2O flux during the first week in the sandy soil should have originated from nitrification. This was probably not the case in the clay and organic soils, where PDA was higher. However, it should be kept in mind that measuring51PAO and PDA is only indicative and in order to determine whether N_2Ooriginates from nitrification or denitrification an alternative approach is needed, such as determining the 15N-isotope abundance (Koster *et al.*, 2011; Yoshida, 1988). Using such a method, Koster *et al.* (2011) observed a rapid shift from denitrification to nitrification in soil after application of BR to soil cores. They attributed this shift to depletion of organic C, the driving force for bacterial denitrification, as indicated by decreasing CO_2 release and the presence of increasingly abundant nitrate due to ongoing nitrification. At this stage nitrification had probably become the main source of N_2O production relative to denitrification.

The quantity and quality of existing SOM and carbon (C) and nitrogen (N) inputs crucially controls on soil microbial biomass and activity (Fierer *et al.*, 2009; Kallenbach and Grandy, 2011). Thus, the quality (e.g. manure, leguminous cover crops, and composted materials) and the quantity (doses) of the organic amendments are also distinctly stimulating microbial biomass through increases in labile organic matter (Marriott and Wander, 2006; Smukler *et al.*, 2008; Kallenbach and Grandy, 2011) and/or total soil C on time frames from months to decades (Drinkwater and Wagoner, 1998; Kong *et al.*, 2005).

Biological, especially microbiological attributes such as biomass and activities, are sensitive to soil management practices (Elliot, 1997). Modifications in biological properties may precede detectable changes in soil physical and chemical properties and thus provide an early signal of improvement or a warning of degradation (Pankhurst *et al.*, 1995). Community level physiological profiles (CLPPs) based on the ability of microorganisms to oxidize different carbon substrates have been successfully used to differentiate microbial communities from several habitats (Garland, 1997; Gomez *et al.*, 2004). The estimation of microbial functional diversity by carbon source use profiles has been reported to be a sensitive approach to detect modifications due to soil management (Stenberg, 1999; Gomez *et al.*, 2004). Most of the literature published on CLPPs used the Biolog GN™ assay, which tests the metabolization of 95 carbon sources. The more recently developed Biolog EcoPlate™ assay allows testing a more reduced number of ecologically relevant substrates and the possibility of larger number of replicates. However, till

date there is less information available as regards the use of Eco Plate assay to detect changes in microbial functional diversity due to management practices.

Other than CLPPs, organic management increases overall enzyme activity (Mäder *et al*., 2002; García-Ruiz *et al*., 2008; Moeskops *et al*., 2010), but activities of specific enzymes may differ depending on the composition of the amendments and the relative availability of nutrients, as well as other soil physic- chemical factors, such as soil type and its unique characteristics, e.g. pH and texture (Stursová and Baldrian, 2010).

3. MATERIALS AND METHODS

A field experiment was conducted under irrigated condition during Rabi season of 2015-16 to study "N_2O emission in wheat with integrated use of biogas slurry and fertilizers". The details of the materials used and techniques followed during the course of investigation are described below.

3.1Study area

3.1.1Location

The NCT of Delhi with an area of 1483 km^2 is situated between the Himalaya and Aravali range in the heart of the Indian sub-continent and lies between north latitude 28° 24′ 17′′ and 28° 50′ 00′′ and east longitude 76° 50′ 24′′ and 77° 20′ 37′′. It is located on the banks of the Yamuna and major part of territory lies on the western side of the river. Its greatest length is around 33 miles and greatest breadth is 30 miles. Altitude ranges between 213 to 305 meters above sea level.

3.1.2Climate and soil

Delhi state falls under fourth agro-climatic region compromising hot arid climate of northern plain and central high lands including Aravali (Mahapatra *et al.*,2000). Its climate is mainly influenced by its inland position and prevalence of continental type of air during the major part of year. Main characteristics of climate is high variation between summer and winter temperatures and precipitation with an intense hot summer and cold winter as compared to other parts of country.

Temperature

The average annual temperatures are around 29°C, although they can vary around 25°C on rainy days to 32°C during dry spells. Summers start in early April and peak in May and June, with mean maximum and minimum temperatures 39.6°C and 27.2°C respectively, although occasional heat waves can result in high close to 45°C on some days. January is the coldest month in this region with mean maximum and minimum temperature 20.8°C and 7.8°C respectively.

Rainfall

The monsoon starts in late June and lasts until mid-September, with mean annual rainfall of about 716.2 mm. About 80% of annual rainfall is received during monsoon with maximum about 206.5 mm in the month of August, rest is received in winter. The monsoons recede in late September, and post monsoon season continues till late October, with average temperatures sliding from 29°C to 21°C.

Humidity

The driest months (April-may) have average relative humidity of 20% to 30% whereas in the monsoon season (July-august) it is maximum 63% to 75% during morning hours.

Soil

The soil of Delhi is grouped under order Inceptisols (81.3%) and Andisol (8.7%) as reported by Mahapatra*et al.,* (2000). The soils are alluvial in origin (sandy loam) and influenced by annual rainfall and flooding of Yamuna River due to rain during June to September.

3.2 Previous crop on the experimental site

An irrigated Baby corn crop was grown in sequence during 2013-14 and 2014-15 on the experimental field.

Table3.1:Physical and Chemical properties of soil in the experimental site

Sr. No.	Properties	Methods Employed
1.	**Physical properties**	
a.	Bulk Density (g/cc)	Core sampler method (Dastane, 1967)
b.	Porosity	
2.	**Chemical properties**	
a.	Available Nitrogen (kg/ha)	Alkaline Permanganate method (Subbiah and Asija, 1956)
b.	Available P_2O_5 (kg/ha)	Olsen's method (Jackson, 1973)
c.	Available K_2O (kg/ha)	Flame photometry (Jackson, 1973)
d.	Organic carbon (%)	Walkley and Black's wet oxidation method (Jackson, 1973)
e.	Soil pH (1:2.5 soil: water)	Potentiometry (Piper, 1966)
f.	Electrical conductivity (dS/m)	Conductivity Bridge (Jackson, 1967)
3.	**DTPA Extractable micronutrients**	
a.	Zn (mg/kg)	DTPA Extractable method (Lindsay and Norvell, 1978)
b.	Cu (mg/kg)	DTPA Extractable method (Lindsay and Norvell, 1978)
c.	Mn (mg/kg)	DTPA Extractable method (Lindsay and Norvell, 1978)
d.	Fe (mg/kg)	DTPA Extractable method (Lindsay and Norvell, 1978)

Table 3.2:Physicochemical properties of Biogas slurry

Sr. No.	Properties	Methods Employed
1.	Total N (%)	Micro kjeldahl's method,
2.	Total P (%)	Vanadomolybdate phosphoric yellow colour method (Jackson, 1973)
3.	Total K (%)	Flame photometer method, (Jackson, 1973)
4.	pH (1:2.5 soil: water)	Potentiometry (Piper, 1966)
5.	Electrical conductivity (dS/m)	Conductivity Bridge (Jackson,1967)
6.	DTPA Extractable micronutrients	DTPA Extractable method (Lindsay and Norvell, 1978)

3.3 Experimental details

The details of the experiment with regard to treatments evaluated, the design adopted and plot size are given below.

3.3.1 Design and layout

To fulfill the objective of this study the experiment was conducted a field of IARI New Delhi. The field was divided into 24 plots; every plot represented one experimental unit. The field experiment conducted in a randomized block design (RBD) comprising 6 treatment of different combination of biogas slurry and chemical fertilizer with 4replication each.

3.3.2 Treatments

Wheat will be cultivated as a :

1. T0 – Control (where no urea/biogas slurry applied),
2. T1 – 100% N by recommended dose of fertilizer (Urea),
3. T2 – 75% N by recommended dose of fertilizer + 25% N by BGS
4. T3 – 50% N by recommended dose of fertilizer + 50% N by BGS
5. T4 – 25% N by recommended dose of fertilizer +75% N by BGS,
6. T5 – 100% N by BGS

3.3.3 Plot size: 20 m^2

3.3.4Spacing: Row to row spacing should be 22.5 cm

3.3.5Design: Randomized block design

3.3.6 Date of slurry application: 1 week before the sowing

3.3.7 Date of sowing: Rabi – 23rd Nov. 2015

3.3.8 Date of harvesting: Rabi- 24th April, 2016


T2
T3
T4


T2
T3
T4

Plan of Layout of Experiment in RDB

Replication		Treatments					
REPLICATION	R1	T0	T1	T2	T3	T4	T5
	R2	T5	T4	T3	T2	T1	T0
	R3	T2	T3	T4	T5	T0	T1
	R4	T1	T2	T0	T4	T5	T3

3.4 Cultural operations

3.4.1 Land Preparation

After harvest of previous crop land was ploughed once with mould board plough and soil was brought to fine tilth by crushing the clods with cultivator and harrowing twice.

3.4.2 Manures and Fertilizer Application

The required quantity of liquid biogas slurry as per treatment was applied for each plot 1 weeks before the sowing. Recommended dose of fertilizer (150:60:60 kg/ha) was applied in the field. Nitrogen, phosphorus and potassium were applied in the form of urea, diammoniumphosphate (DAP) and muriate of potash (MOP) respectively to the representative treatment of wheat.

3.4.3 Seeds and sowing

The seeds of wheat HD 2967 variety were used for experimentation. Sowing was done at 5-6 cm deep below the soil.

3.4.4 Harvesting

The crop was harvested when the grains become hard and the straw becomes yellow, dry and brittle.

3.5 Collection of gas samples

Collection of gas samples was carried out by closed chamber technique (Hutchinson and Mosier, 1981; Bhatia *et al.*, 2005). Gas samples were collected on 2, 9, 19, 34, 41, 56, 65, 73, 86, 87, 93, 103, 118, 126, 145 and 153 DAS for nitrous oxide emission from different treatments. The aluminum channels were used as plate form for the acrylic box, were inserted 10 cm inside the soil and filled with water to make the system air tight. Generally a thermometer was placed inside to monitor the temperature. The chamber was flushed 3-4 times with a 50 ml syringe before taking the samples. Gas samples were drawn with the help of needle. Head space volume inside the box was noted, which was used to calculate flux of N_2O. Samples of 3 replications of each treatment were taken and the average was taken as representative value for the treatment. Gas samples at 0, 30 minute and 1 hr were collected from the acrylic box.

3.6 Gas sample analysis

Nitrous oxide concentration in the gas samples collected from the fields was estimated by Gas Chromatograph (Hewlett Packard 5890 Series II) fitted with an electron capture detector (ECD) and 6' x 1/8" stainless steel column (Porapak N). Column, injector, and detector temperatures were 50, 120 and 350°C, respectively. The carrier gas was N_2 with a flow rate of 14 ml min^{-1}. Hewlett Packard integrator was used to plot and measure the peak area. A GC-computer interface was used to plot and measure the peak area. N_2O standards of 500 ppbv and 1 ppm obtained from Spectra gases (NIST standards) were used for calibration.

Estimation of total N_2O emissions during the crop season was done by successive linear interpolation of average emission on the sampling days assuming that emission followed a linear trend during the periods when no sample was taken (Pathak et al., 2002).

3.6.1Calculation of N_2O flux

Cross sectional area of the chamber = A m^2

Headspace = H m


T2
T3
T4


T2
T3
T4

Volume of headspace = AHm^3 = 1000 x AH l

N_2O concentration at 0 time = Co

N_2O concentration after time t = Ct

Change in concentration in time t = $(C_t - C_o)$ ppbv

= $(C_t - C_o)$ nl l^{-1}

Volume of N_2O evolved in time t = $(C_t - C_o)$ nl l^{-1} x 1000 AH l

= $(C_t - C_o)$ x AH µl

When t is in hours, then flux is

$F = [(C_t - C_o) \times AH] / (A \times t)$ µlm^{-2} h^{-1}

= Y µl m^{-2} h^{-1}

Now 22.4µl of N_2O is 44 µg at STP

So, Y µl of N_2O is (44xY/22.4) µg at STP

Therefore, Flux = Y x (44/22.4) µg $m^{-2}h^{-1}$

Hence, Flux = $[(C_t-C_o)/t] \times H \times (44/22.4)$ µg $m^{-2}h^{-1}$

So, for one hectare /day N_2O

$$\text{Flux} = \frac{[(Ct - Co)/t] \times H \times 44/22.4 \times 10000 \times 24 \text{ mg}}{1000}$$

$$N_2O\text{-N flux mg ha}^{-1}\text{ d}^{-1} = \frac{[(Ct - Co)/t] \times H \times 44 \times 240 \times 28 \text{ mg ha}^{-1}\text{ d}^{-1}}{22.4 \times 44}$$

$= [(Ct - Co)/t] \times 300$ mg ha^{-1} d^{-1}

3.7 Soil Sample analysis for different microbial properties

3.7.1 Microbial densities

The standard dilution plating procedures methods were used for the estimation of densities of cultivable actin omycetes, fungi and bacteria. One gram of soil sample from each treatment were weighed and blended in 9 ml of sterile water and keep on shaker for 20 min. We diluted it 10 fold for further analysis. The quantification of bacteria were done from 10^{-5} dilution on Yeast-Peptone-Glucose-Agar (yeast extract 5 g /l, peptone 5 g/l, glucose g/l, agar 15 g/l) supplied with cycloheximide (100 mg/l) and fungi were quantified in melting malt extract agar (malt 15 g/l, agar 10 g/l) supplied with antibiotics (citric acid 250 mg/l, chlortetracycline 50 mg/l and streptomycin 100 mg/l). The quantification of actin omycete was done in actin

omycete isolation readymade agar. For counting the similar process followed as earlier mentioned but, incubated at 35°C for 72 hours. For total microbial count, Nutrient Agar media was used and incubated at 30^{o}C.

3.7.2 Community level physiological profiles of bacteria:

Community level physiological profiles (CLPPs) profile of bacteria were assessed by the Biolog Eco Plate^{TM} system (Biolog Inc., CA, USA). Each Biolog Eco Plate have 96-well with three replicates, each one comprising 31 sole carbon sources and water blank. Soil suspensions (soil 10 g, distilled water 100 ml) were taken and kept at shaker for 1 h (Dick et al., 1996). Biolog Eco Plate^{TM} was incubated at 25 ^{o}C, and color development in each well was recorded at optical density (OD) at 590 nm with a plate reader at regular intervals up to 264 hours. Microbial activity in each Biolog Eco Plate^{TM}, expressed as average well-color development (AWCD) and was determined as follows:

$AWCD = \sum ODi/31$

Where, ODi is the optical density value from each well. Richness (R), as the number of oxidized C substrates, and the Shannon–Weaver index (H) (i.e., the richness and evenness of response) were calculated using an OD of 0.25 as threshold for positive response (Garland, 1997). Shannon–Weaver index was calculated as follows:

$H = - \sum pi \, (\ln pi)$

Where, *pi* is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates $\sum$ODi. Plate readings at 96 h of incubation were used to calculate AWCD, R and H, since it was the shortest incubation time that allowed the best resolution among treatments. Average well-color development (AWCD), richness and Shannon–Weaver index and SOC were analyzed by ANOVA and multiple comparisons of means by the Duncan test. The SOC was related with R and H by a regression function. Principal component analysis was performed on OD data from the 31 carbon sources at 96 h of incubation. The data were standardized by the average well-color development in each microplate to remove inoculum density effects (Garland, 1997).

3.7.3 Enzyme assays

The potential activities of three hydrolytic enzymes (α-Glucosidase, β-Glucosidase, N-Acetyl-Glucosaminidase) for each sample using fluorometric

techniques modified following Steinweg *et al.* (2012). We measured the activities of two enzymes hydrolyzing C-rich substrates (β-Glucosidase and α-Glucosidase), one for N-rich substrates (N-Acetyl-Glucosaminidase). Soil samples from three different growth phase (i.e. vegetative, reproductive and harvesting) (2.75 g) were homogenized with 91 mL of 50 mMTris buffer (pH 8.2) using a vortex. Plates with 96 deep-wells (2 ml capacity) were used for the enzyme assay as well as reference standards, in which samples were arranged in columns and different enzymes and standards in rows. Aliquots (800 µL) of each homogenized sample were pipetted into seven wells in one of the 12 columns of a plate using an 8-channel pipetter. When the plate was filled with homogenized samples (up to 12 samples for a plate), 200 µL of each substrate dissolved in DI water was added to each aliquot of sample. Each of the three substrates (4-MUB-α-D-glucopyranoside for AG, 4-MUB-β-D-glucopyranoside for BG and 4-MUB-N-acetyl- β -D-Glucosaminide for NAG) was pipetted into wells in a given row (up to 12 wells). The concentration of each substrate was determined based on tests prior to the experiment. We employed 200 $\mu mol \cdot L^{-1}$ for the rest of the substrates so that 200 µL of substrate would not be completely degraded by enzymes during an incubation period (Arrhenius et al., 1889). After a lid was firmly placed on the plate after substrate addition, the plate was inverted several times to mix soil samples and substrates well, and immediately placed in an incubator at 35^{o}C for 1.5 hours. Reference standards were prepared in a similar manner as the soil samples described above using the same apparatus. In the standard plates, we added fluorescent standards, instead of the substrates, in seven concentrations ranging from zero to up to 100 µM. We used 4-methylumbelliferone (MUB) as fluorescent standard. For a set of 12 samples, we set up four plates, each of which was incubated at 35^{o}C. Four additional plates were prepared as reference standards. The standard set incubated at 35°C was used to calculate potential enzyme activities.

After incubation, the plates were centrifuged at 350 g for three minutes, the supernatant was removed from each well and pipetted into a corresponding well of a 96-well black plate with transparent base. Fluorescent activities were immediately measured using Glomaxmicroplate reader with a set of wavelength at 365 and 450 nm for excitation and emission, respectively. Readings of the fluorescent activities from standards were used to calculate potential enzyme activities for each sample.

3.8 Statistical analysis and interpretation of data

The data collected from the field and laboratory work were computed and tabulated properly. The data relating to each character were analyzed as per the procedure of analysis of variance and significance of a Completely Randomized Design (CRD) was tested by "F" test (Gomez and Gomez, 1984). Standard Error of Means (SEm±) and Critical Difference (CD) at 5% level of significance were worked out for each parameter. The CD values were used to draw conclusions from treatment comparison. The software of Microsoft Excel 2003 (Microsoft Corporation, Pullman WA) and SPSS 13.0 (SPSS Inc., Chicago IL) were used for statistical analysis.

4. RESULTS

Biogas slurry have a number of specific properties that are relevant in relation to the yield and N_2O mitigation in wheat, maintenance of soil productivity and environment quality. The aim of this was that how biogas slurry combination with chemical fertilizer affects the growth and yield of wheat crop and mitigation of N_2O.Further, soil microbial community and different enzymes activities would be vary from field to field and these variations may be due to quantity and composition of SOM as well as other environmental factors related to the type of organic amendments. The composition of microbial community would be influenced by different source of nutrient.

Analysis of various physico-chemical properties of soil and biogas slurry was done, which is given in Table 4.1 and 4.2. It was sandy loam, alkaline in nature with normal electrical conductivity and low in organic carbon. Results regarding biogas slurry and soil analysis and their impact on yield parameters of wheat and mitigation of nitrous oxide are being discussed here.

4.1 Physico-chemical characteristics of biogas slurry

The physico-chemical parameters, which decide suitability of biogas slurry as a manure for crop production are pH, total nitrogen, total phosphorous, total potassium and micronutrients (Fe, Cu, Zn, Mn) etc. The physic-chemical characteristics of biogas slurry analysis are given in Table 4.1.It can be concluded from the initial analysis that biogas slurry contains many rich and nutritive elements including nitrogen, phosphorous, Potassium and trace elements (Zn, Fe Ni, Cu,) and biogas slurry has higher manorial value then other organic fertilizer like compost, FYM etc. The processing of manure in an anaerobic digester is only a partial manure degradation process therefore material remaining after digestion must be utilized in some manner, most logically land-applied as a crop nutrient resource. In addition, biogas slurry is free of weed seeds because anaerobic digestion kills more seeds than any manure processing system. The nitrogen in animal manure is normally available in an organic form but after passing through the fermentation process in a biogas digester it is changed (by bacteria) to inorganic form mostly ammonia nitrogen (NH_4^+) which is easily soluble and utilized by crop plants.

Table 4.1:Physico-chemical composition of biogas slurry taken from IARI biogas plant

S. N.	Parameters	Range	Average ± Stand. dev.
1	pH	7.7 -8.2	7.95± **0.16**
2	Total nitrogen (%)	1.95-2.27	2.11± **0.16**
3	Total phosphorus (%)	0.97-1.13	1.05± **0.07**
4	Potassium (%)	0.87-1.12	0.99± **0.13**
5	Fe (ppm)	0.32-0.34	0.33± **0.03**
6	Cu (ppm)	0.004-0.007	0.005± **0.01**
7	Mn (ppm)	0.085-0.093	0.089± **0.01**
8	Zn (ppm)	0.021-0.023	0.022± **0.00**

Before sowing of wheat (HD 2967), liquid biogas slurry samples were collected. Samples were brought to the laboratory of CESCRA. After analysis of sample results shown that the pH, total nitrogen, total phosphorus, potassium, Fe, Cu, Mn, and Zn value of the biogas slurry were 7.95, 2.11%, 1..05%, 0.99, %0.33 ppm, 0.005 ppm, 0.089 ppm and 0.022 ppm respectively.

4.2 Physico-chemical properties of soil

The physico-chemical parameters which decide suitability of crop for particular type of soil are electrical conductivity (EC), pH, organic carbon, available N, P, K and micronutrients (Cu, Fe, Mn, Zn) etc. The results of physico-chemicalanalysis of soil are given in Table 4.2.

Table 4.2: Physico-chemical composition of soil before sowing

S. No.	Parameters	Range	Average ± stand. Dev.
1.	**Physical properties**		
a.	Bulk Density (g/cc) **0-15 cm** **15-30 cm**	 1.44-1.48 1.55-1.60	 **1.46 ± 0.023** **1.58 ± 0.019**
2.	**Chemical properties**		
a.	Available Nitrogen (kg/ha)	214.25-272.78	**243.52 ± 27.42**
b.	Available P2O5 (kg/ha)	38.86-47.65	**43.26 ± 4.45**
c.	Available K_2O (kg/ha)	248.72-253.42	**251.07 ± 5.45**
d.	Organic carbon (%)	0.42-0.47	**0.44 ± 0.038**
e.	Soil pH (1:2.5 soil: water)	7.8-8.0	**7.9 ± 0.139**
f.	Electrical conductivity (dS/m)	0.49-0.58	**0.53 ± 0.037**
3.	DTPA Extractable micronutrients		
a.	Zn (mg/kg)	1.97-3.22	**2.59 ± 0.442**
b.	Cu (mg/kg)	1.31-1.45	**1.38 ± 1.16**
c.	Mn (mg/kg)	17.9-18.6	**18.25 ± 0.280**
d.	Fe (mg/kg)	5.04-7.16	**6.10 ± 0.739**

Before sowing of wheat (HD 2967), soil samples were collected. Samples were brought to the laboratory of CESCRA. After analysis of sample results shown that the pH, EC,bulk density of soil at 0-15 cm and 15-30 cm depth, organic carbon, available nitrogen, phosphorus, potassium, Fe, Cu, Mn, and Zn value of soil were7.9,0.53 dSm^{-1}1.46gcm^{-3}, 1.58 gcm^{-3},0.44%, 243.52, 43.26, 251.07 kg ha^{-1},6.10 ppm, 1.38 ppm, 18.25 ppm and 2.59 pp mrespectively.

4.3 Effect of BGS along chemical fertilizer on wheat yield

Effect of biogas slurry (BGS) along with combination of chemical fertilizer on wheat yield is shown in Figure 4.1. The results showed that the application of BGS with chemical fertilizer significantly affected the wheat yield. Control (T0) and (T5) treatments showed minimum wheat yield where no nutrient source and only bio gas

slurry applied respectively. All treatments with biogas slurry application showed an increase in yield ranging from 25 to 56 % over control. Application of 50% biogas slurry with combination of 50 % N through urea (T3) showed maximum yield,which was 56 % higher than that of control (T0) and 11% higher than that of recommended dose of urea (T3).Growth of cereals is generally affected by source of nitrogen in any form (Rahman*et al*., 2008).

The data also showed that other combinations of BGS along with inorganic nitrogen containing treatments caused almost same yield as in the recommended dose of nitrogen. The reason is that nutrients release through biogas slurry is generally slower than chemical source of nitrogen. Biogas slurry increases the water and nutrient use efficiency of soil. That's why, 50% BGS application and 50% inorganic nitrogen showed better results than inorganic chemical nitrogen.

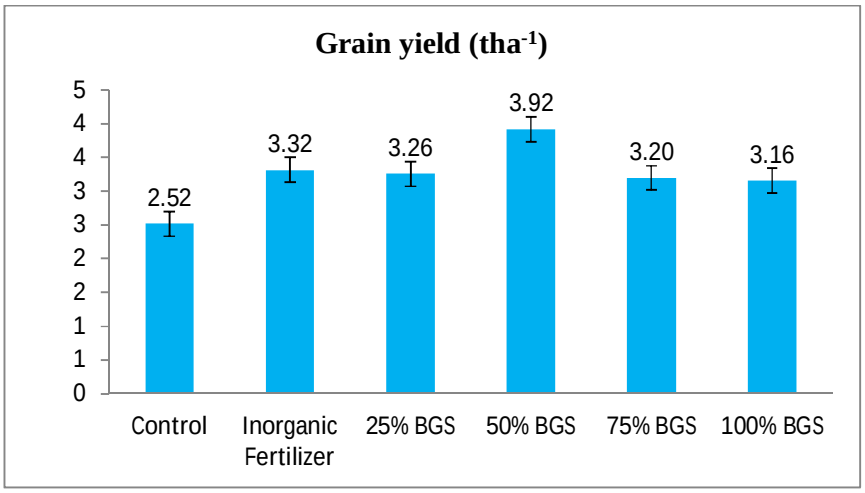


Figure 4.1: Effectiveness of biogas slurry on wheat (HD 2967) yield

4.4 Effect of biogas slurry on Biomass yield

Effect of biogas slurry (BGS) along with combination of chemical fertilizer on wheat yield is shown in Figure 4.2. The results showed that the application of BGS with chemical fertilizer significantly affected the wheat yield. Control treatment showed minimum wheat yield(7.472 t ha^{-1}),where no nutrient source applied respectively. All treatments with biogas slurry application showed an increase in yield. Application of 50% biogas slurry with combination of 50 % N through urea (T3) showed maximum yield, which was 11% higher than that of recommended dose of urea (T3).

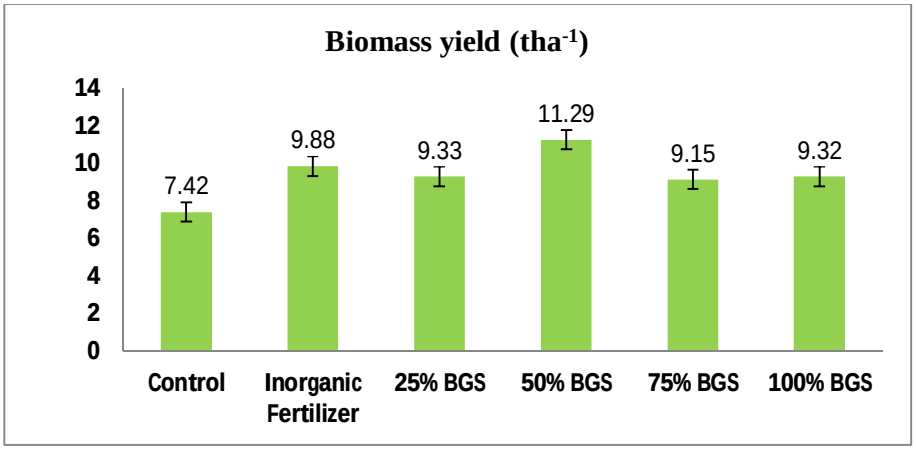


Figure 4.2: Effectiveness of biogas slurry on biomass yield

4.5 Effect of biogas slurry on harvest index

Response of biogas slurry (BGS) with different rates of inorganic nitrogen on harvest index is shown in figure 4.3.Significantly higher harvest index (39 %) was noticed in T3 treatments. Among other treatments did not differ significantly with respect to harvest index. The minimum harvest index was found in T0 (30%).

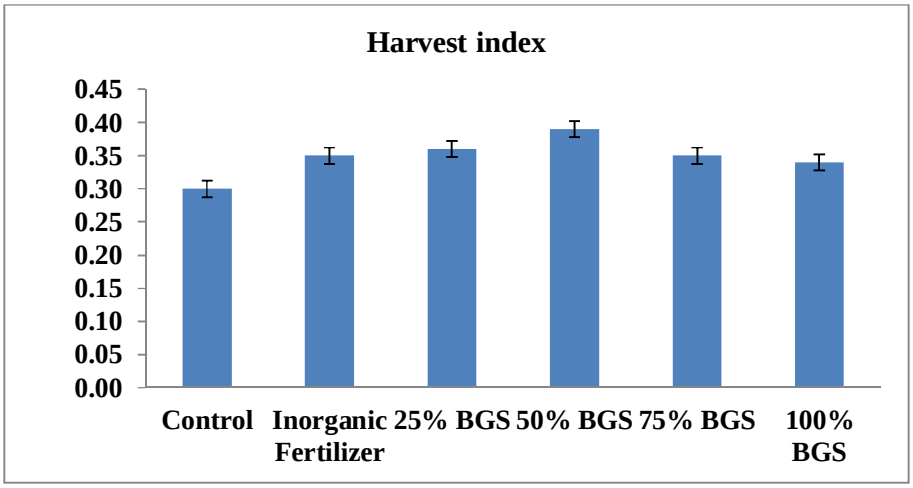


Figure 4.3: Effectiveness of biogas slurry on harvest index

4.6 Effect of biogas slurry on nitrous oxide emission

N_2O flux from the treatments showed more or less similar temporal trends with appearance of a peak of N_2O emission three days after urea applications, however, the magnitude of flux differed (Fig.4.4, 4.5, 4.6). The observed trend of N_2O flux in wheat was in agreement with Malla*et al.* (2005). Urea takes two to three days for

hydrolysis into NH_4-N under optimum moisture condition (Lloyd and Sheaffe, 1973), which undergoes further nitrification to NO_3-N resulting in a peak of N_2O flux generally three to four days after urea application.

The cumulative emission of N_2O-N from wheat treatments varied from 0.59 to 0.92 kg ha^{-1}. The emission was significantly higher (11-54 %) under recommended dose of fertilizer treatment (T1) as compared to control and BGS treatments (T0, T2, T3, T4, T5), however, application of higher BGS with urea fertilizer led to significant reduction in N_2O emission.Highest N_2O emission reduction was found in control (54%) which was devoid of both urea and BGS application but N_2O emission was significantly reduced with the application of BGS. After control, 100 % BGS treatment (T5) showed maximum N_2Oemission reduction (25%) over there commended dose of fertilizer treatment (T1).

The cumulative N_2O emission from different treatments were in the order of T0<T5<T4<T3<T2<T1 in wheat crop during 2015-16.The lower N_2O emission in higher BGS treatments was observed due to slow release of N in the soil with high soil moisture contents that reduces rate of diffusion of oxygen into soil promoting anaerobic condition.

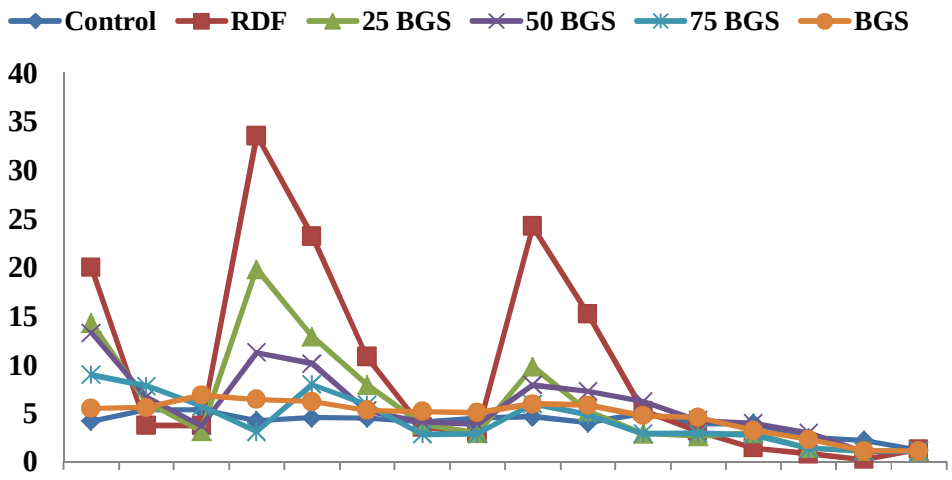


Figure 4.4: Effectiveness of biogas slurry on nitrous oxide emission

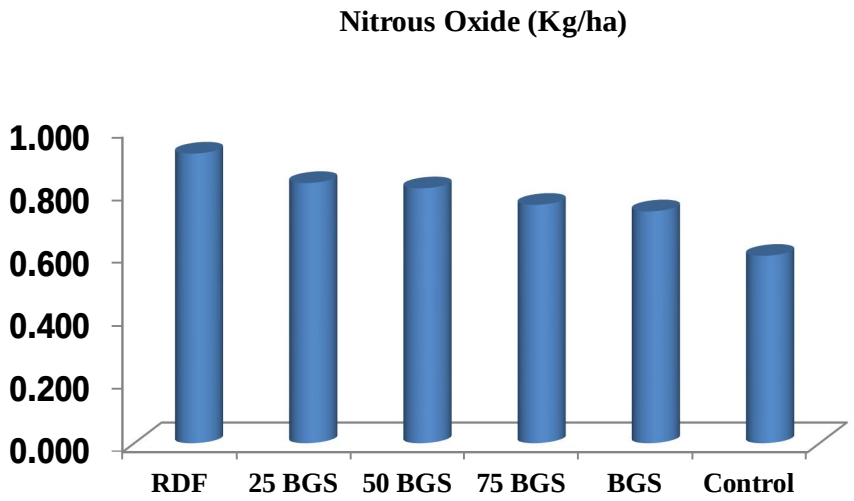


Figure 4.5: Effectiveness of biogas slurry on nitrous oxide emission

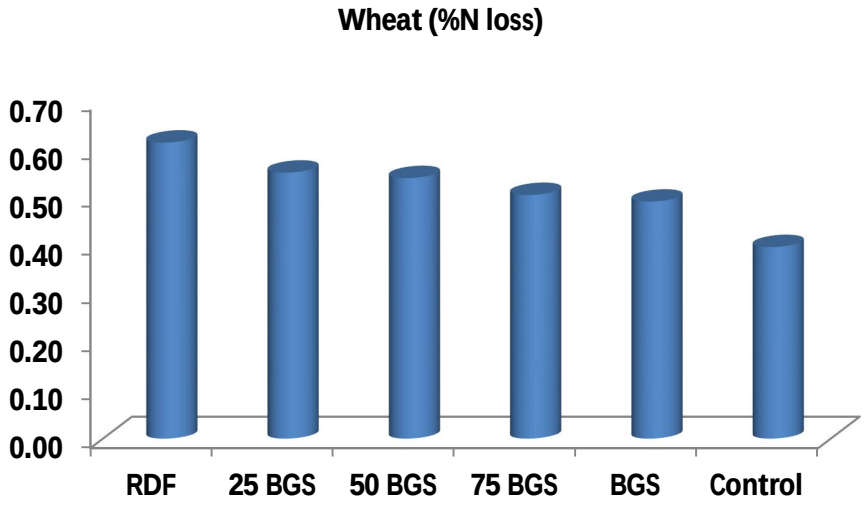


Figure 4.6: Effectiveness of biogas slurry on nitrous oxide emission

Table 4.3: Nitrous oxide emission from wheat field

Treatments	Kg/ha	%N loss
RDF	0.921	0.61
25 BGS	0.827	0.55
50 BGS	0.810	0.54
75 BGS	0.758	0.51
BGS	0.737	0.49
Control	0.596	0.40

4.7 Culturable microbial densities:

The enumeration of culturable total microbial count and count of bacteria and fungi was undertaken at distinct plant developmental stages of wheat. We assessed developmental stage-dependent community fluctuations of culturable microbial populations under different doses of biogas slurry application and 100% inorganic fertilizer application. Rhizosphere community densities were compared with baseline population densities enumerated in control soil at three time points throughout the plant growing season: Vegetative phase, Reproductive phase and Harvesting phase. There was significant temporal variation in total viable counts in three different growth phases in all the six treatments. In all treatments (irrespective of Inorganic and organic treatments), community densities of culturable heterotrophs were significantly more ($p<0.05$) during reproductive growth phase. Although the densities of fungal populations were significantly less ($p<0.05$) in vegetative phase but, remained almost consistent in both reproductive and harvesting stage (statistically not significant). The numbers of culturable heterotrophs and fungi in wheat rhizospheres were maximum in T4 and T5 (statistically at par) [Figure. 4.7, 4.8, 4.9].

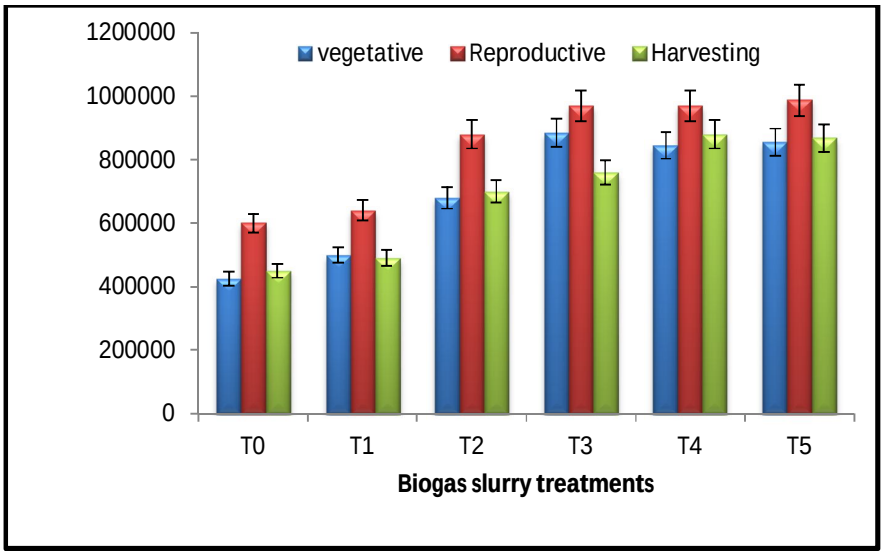


Figure 4.7: Total culturable microbial densities at three different growth phases (Vegetative, Reproductive and Harvesting) of wheat under different doses of biogas slurry treatments.

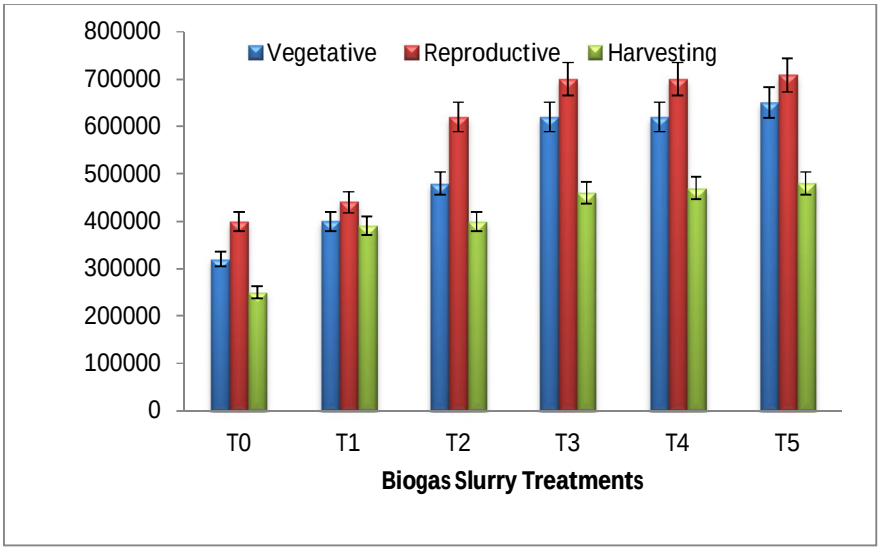


Figure 4.8: Total culturable bacteria at three different growth phases (Vegetative, Reproductive and Harvesting) of wheat under different doses of biogas slurry treatments.

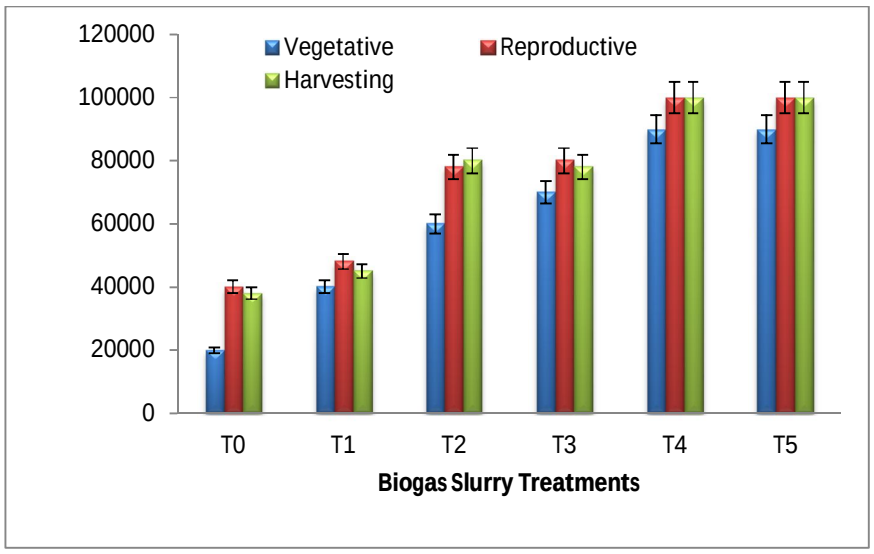


Figure 4.9: Total culturable fungi at three different growth phases (Vegetative, Reproductive and Harvesting) of wheat under different doses of biogas slurry treatments.

4.8 Community Level Physiological Profiling (CLPP) of rhizospheric soils of wheat under different treatments:

Significant differences ($p<0.01$) were found in Average Well Color Development (AWCD), Richness (R) and Shannon-Weaver Index (H) in control (T0) and different doses of slurry treatments (T2-T5) (Figure. 2-4)**.** We have assessed AWCD, R and H for reproductive phase of soil collected from rhizozpheric soils of wheat crop with the hypothesis in mind that, during reproductive phase, crops secretes more through roots and thus treatment effect (if any) could be well differentiated at this stage. The maximum AWCD (0.64) was measured at 96 hours of incubation in T3 treatment with a dose of 50% biogas slurry and 50% of recommended dose of inorganic fertilizer. There was no significant difference in AWCD in case of 100% inorganic fertilizer treatment (T1) and 25% Biogas slurry +75% inorganic fertilizer application (Figure. 4.10).

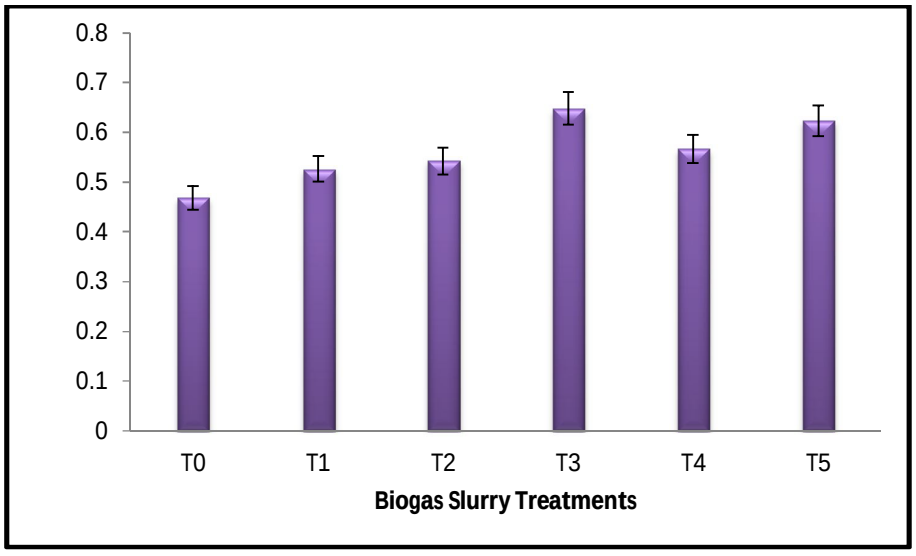


Figure 4.10: Average Well Color Development [AWCD] of metabolized substrates in Biolog EcoPlate™ at 96 hours of incubation as at this point treatment differences were well distinguishable. Error bars indicate ±SE (standard error) of the three replications within each plate (n=3).

Richness (R) was highest (27.66) in T3 followed by T5 (Figure. 4.11). Control treatment (T0) with no fertilizer and no slurry application had shown lowest Richness of diversity with a value of 23.66. Shannon-Weaver index (H) was higher in all the amended plots (including 100% inorganic fertilizer application) compared

to control one. However, Maximum *H* was measured in T5 followed by T3 but both were statistically at per (Figure.4.12).

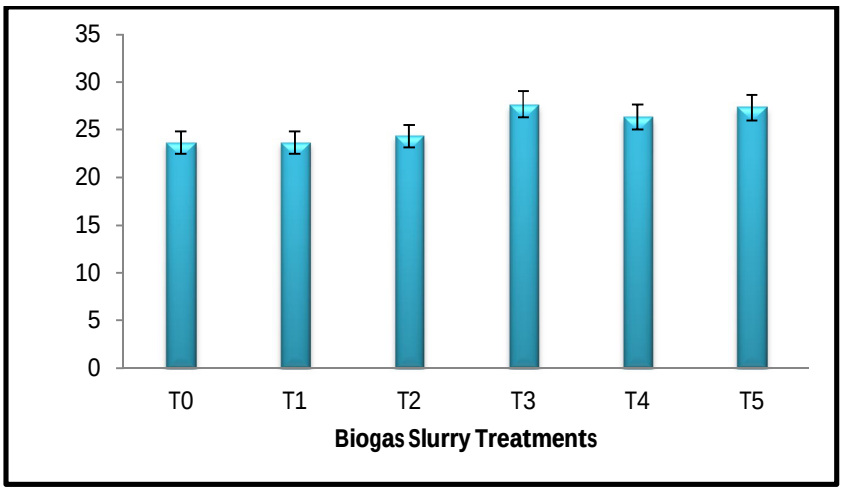


Figure 4.11: Richness (R) of metabolized substrates in BiologEcoPlate™. Richness was calculated at 96 hours after incubation as at this point treatment differences were well distinguishable. Error bars indicate ±SE (standard error) of the three replications within each plate (n=3).

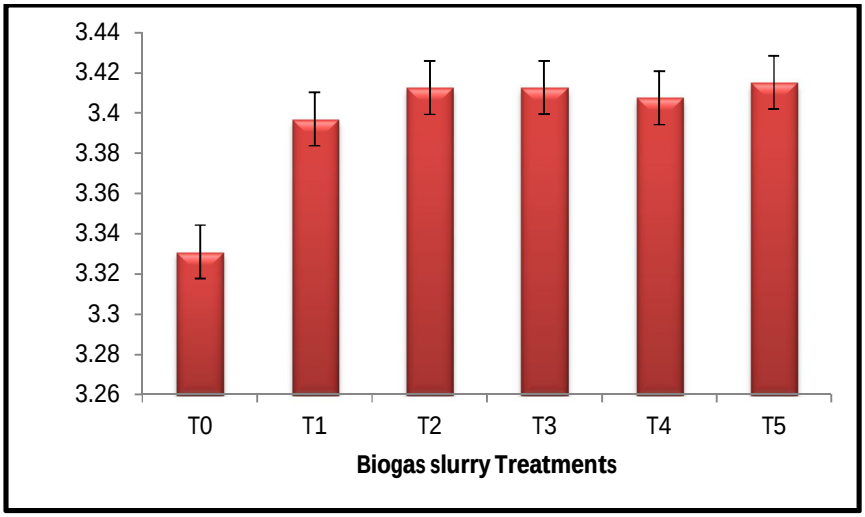


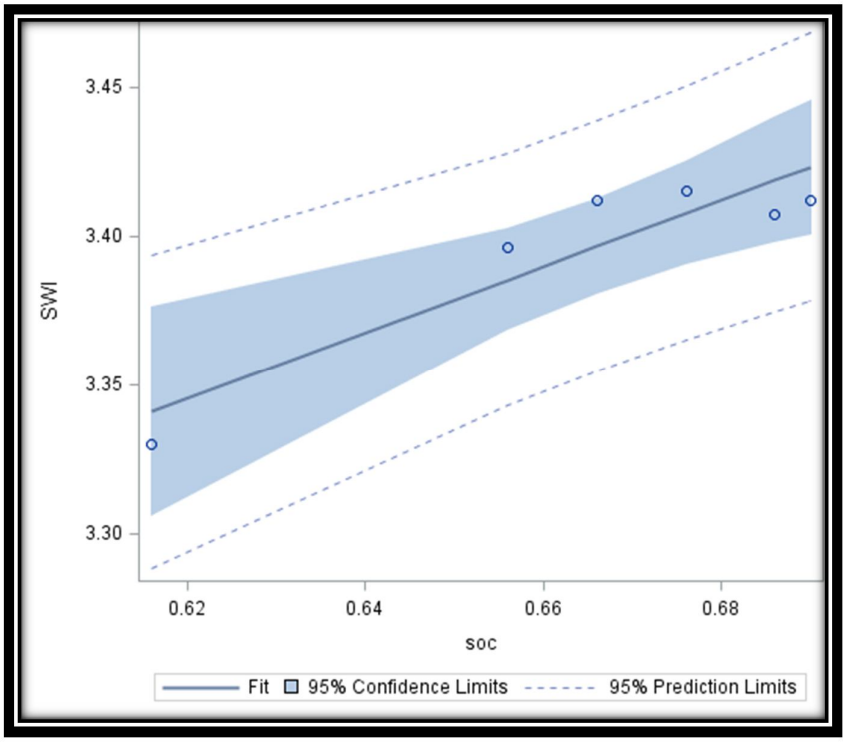
Figure 4.12: Shannon–Weaver index (H) of metabolized substrates in BiologEcoPlate™. This index represents diversity index of rhizospheric soils of reproductive phase of wheat. Error bars indicate ±SE (standard error) of the three replication within each plate (n=3).

The regression functions showed significantly positive linear relationships when Shannon weaver index and AWCD were related to SOC, with R^2 = 0.85 and 0.76 respectively (Figure. 4.13 and 4.14). In order to determine the extent of differentiation of treatments from control and different doses of Biogas slurry treatments with regard to carbon source metabolization, each of the treatments were subjected to principal component analysis. The first and second principal component (PC1 and PC2) explained 64.2% and 17.26% of data variance, respectively (Figure.4.15). The treatments T2, T3, T4 and T5 clustered together and were differentiated from the control (T0) and 100% inorganic fertilizer (T1) treatment. Further to this, T0 and T1 are also located in different quadrant which implies huge differences in terms of catabolic potential and other community level physiological attributes (Figure. 4.15).

Parameter estimates for SWI and SOC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Intercept	1	2.65	0.15	17.20	**<.0001****
soc	1	1.11	0.23	4.81	**0.0086****
Error	4	0.0007	0.0001		

** significant at 1% level of significance



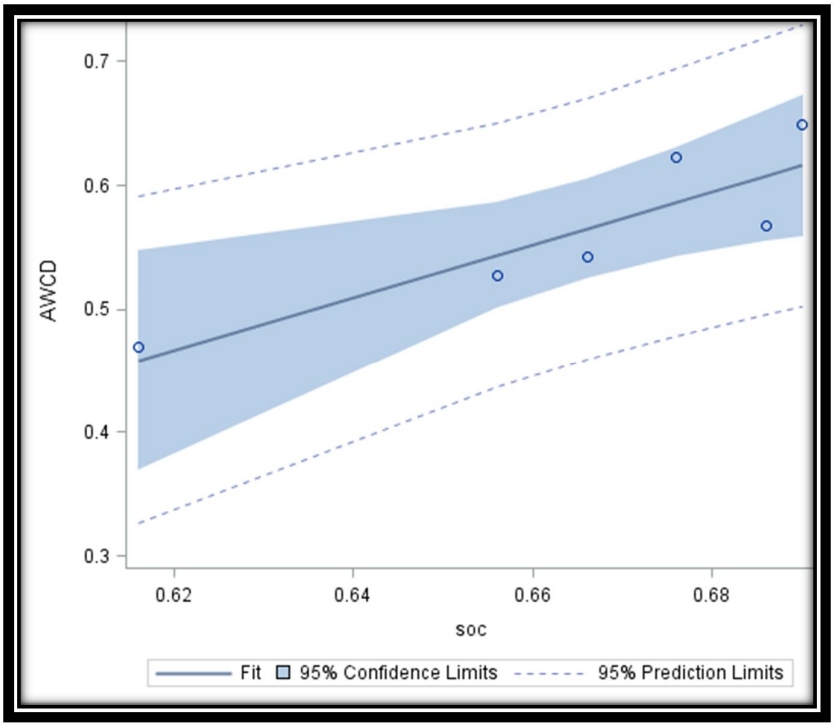
SWI = 2.65 + 1.11*soc

R^2 = 0.8524

Figure4.13: Regression function between Shannon Weaver Index (H) from metabolized substrates in Biolog Ecoplate and Soil Organic Carbon (SOC) during reproductive phase where, (n=6).

Parameter estimates for AWCD and SOC					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Intercept	1	-0.85	0.38815	-2.19	0.0934
soc	1	2.13	0.58328	3.65	**0.0219***
Error	4	0.005	0.00125		

* significant at 5% level of significance



AWCD = -0.85 + 2.13*soc

R^2 = 0.7686

Figure 4.14: Regression function between Average Well Color Development (AWCD) from metabolized substrates in Biolog Ecoplate and Soil Organic Carbon (SOC) during reproductive phase where, (n=6).

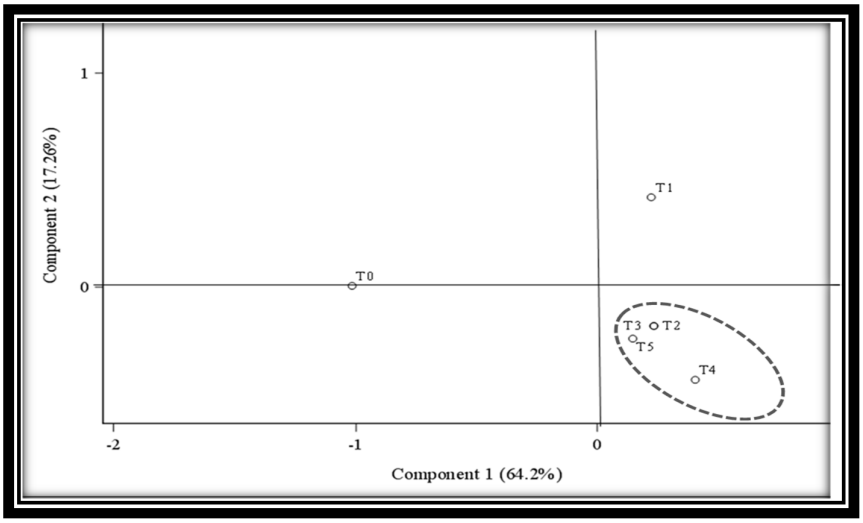


Figure. 4.15: Principal components on data of activity on carbon substrates in Biolog Ecoplates.

The carbon sources those are significantly correlated with PC1 and PC2 (R>.60) are mentioned in **Table. 4.4.** Substrates such as D-Cellobiose, I-erithritol, D-Xylose, Phenyl ethyl Amine were intensively (OD>2.1 at 264 hours of incubation) metabolized by microbial community of control (T0) treatment. Among these carbon substrates, all belong to carbohydrate groups except Phenyl ethyl amine which belongs to Amines group of carbon substrates. Microbial communities from all other treatments of biogas slurry irrespective of different doses of slurry application, intensively used Tween-40 (Polymers), I-erithritol (Carbohydrates), L-Phenyl Alanine (amino acid) and Putrescine (Amines). Interestingly, this indicates that functional diversity of microbes (in terms of different group of carbon substrates utilization capacity) was enhanced in case of biogas slurry application (irrespective of doses). The treatment with full dose of inorganic fertilizer (T1), had also shown different pattern. Soil microbial community of T1 had shown intensive utilization of D-Cellobiose, I-erithritol, and L-Asparagine. Out of these, first two belong to carbohydrate group and L-Asparagine belongs to Amino acid group which are comparatively easily utilizable. This trend of findings is a good indication about catabolic potential of soil microbial communities, amended with different inputs. Optical density (<.25) was recorded for 2-hydroxy benzoic acid and 4-Hydroxy Benzoic acid in all treatments (T0 to T5). This result clearly depict that, functional diversity in terms of utilization of easily to recalcitrant types of carbon substrates are enhanced in case of biogas slurry amended soils.

Table 4.4: Carbon substrates utilized by microorganisms in Biolog Ecoplate™, significantly correlated with to PC1 and PC2 (R>60).

PC1	PC2
Pyruvic Acid Methyl Ester	N-Acetyl D Glucosamine
D-Mannitol	Phenyl ethyl Amine
Tween- 40	4-hydroxy benzoic acid
Tween-80	L-Serine
α-cyclodextrin	L-Threonine
I-erithritol	Glycil-L-Glutamic acid
D-Cellobiose	
α-D Lactose	
L-Arginine	
L-Asperginine	
D-Xylose	
D-Malic Acid	

4.9 Microbial metabolic potential:

The metabolic capacity of soil communities collected from six different treatments, were assessed temporally over 264 hours of incubation period. Maximum initial AWCD was noticed in case of T1 (100% recommended dose of inorganic fertilizer). This may be because of easily available nutrients were more in case of T1 in comparison to other treatments. The temporal response of metabolic potential varied from treatment to treatment significantly upto 144 hours of incubation period and was statistically at per from 144 hours onwards to 264 hours of incubation time. However, a significant ($P < 0.05$) stimulation of microbial C utilization capacity was noticed in case of T5 at 96 hours with a maximum AWCD even at later stages also. (Figure4.16).

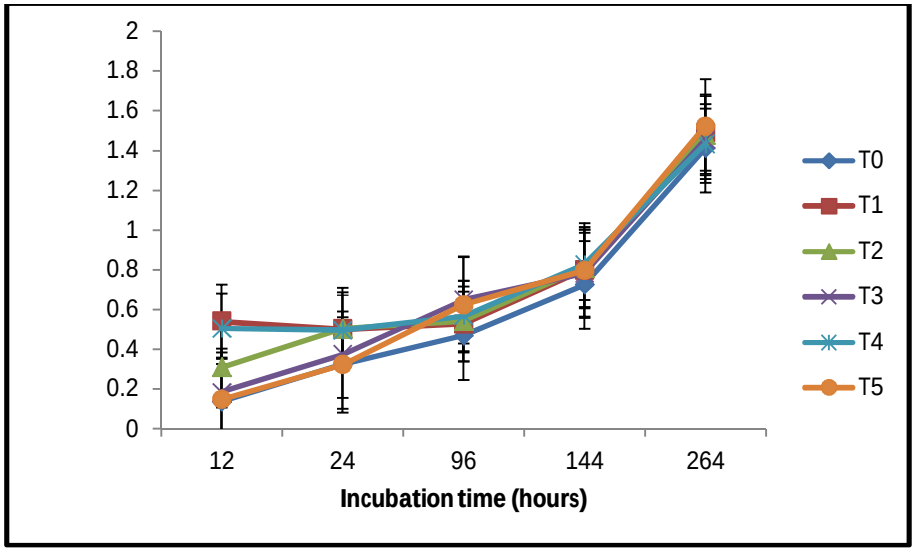


Figure 4.16. Average well color development (AWCD) trend [over incubation time period] of six different biogas slurry treatments on wheat. Error bars indicate ±SE (standard error) of the three replication within each plate (n=3).

4.10 Patterns of soil enzyme activities:

The activities of three soil enzymes (AG, BG and NAG) showed different trends across the six field treatments (Figure. 4.17, 4.18, 4.19). Maximum AG activity (594.99 nmoles/g dry soil/hour) was noticed in the reproductive phase of T3 treatment with a balanced dose of Slurry and inorganic fertilizer application. However, the response of T0, T1 and T2 for AG was statistically at per (Figure.

4.17). Although at T5, higher doses of slurry application were done, but the response was significantly less in comparison to T3 and T4. This may be because; immediate availability of inorganic nitrogen was quite less in case of T5. Generally availability of nitrogen boosts up the secretion of carbon degrading enzymes. Similar trend is also noticed in case of BG. Maximum BG activity (226.1 moles/g dry soil/hour) was noticed in case of T3 in reproductive phase (Figure. 4.18). However, in contrary to the earlier findings of AG, comparatively higher BG activity was noticed in T1 with complete inorganic fertilizer application. This might happened because of residual organic matters present in soil from earlier crop, as this particular field is cultivated for three consecutive years with biogas slurry application. Overall, a trend is noticed that, C-cycling enzyme potential activities increased with inorganic N availability while those of N-cycling enzymes increased with C availability.

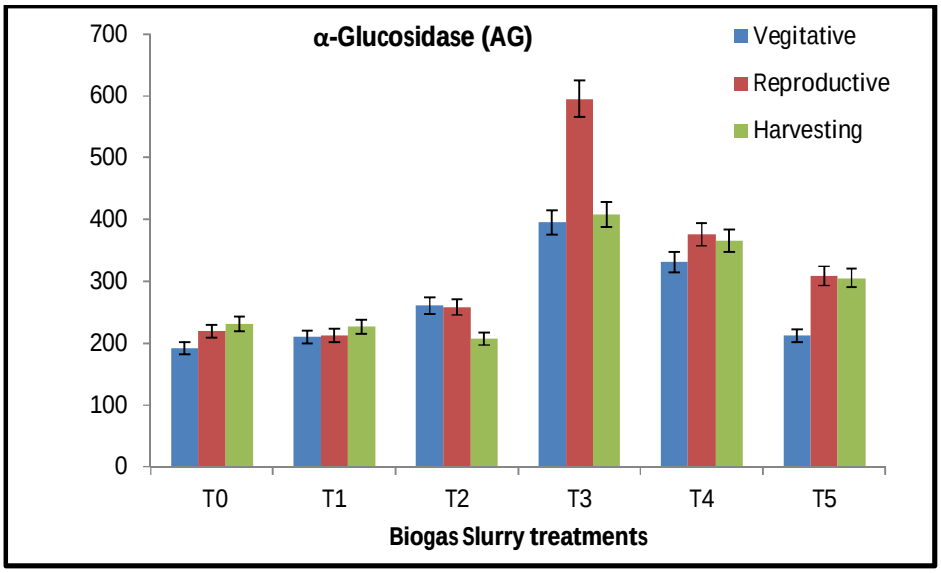


Figure 4.17. Temporal response of α-glucosidase (AG) activity due in different treatments. Vertical bars indicate average confidence intervals at 5% probability (n=3).

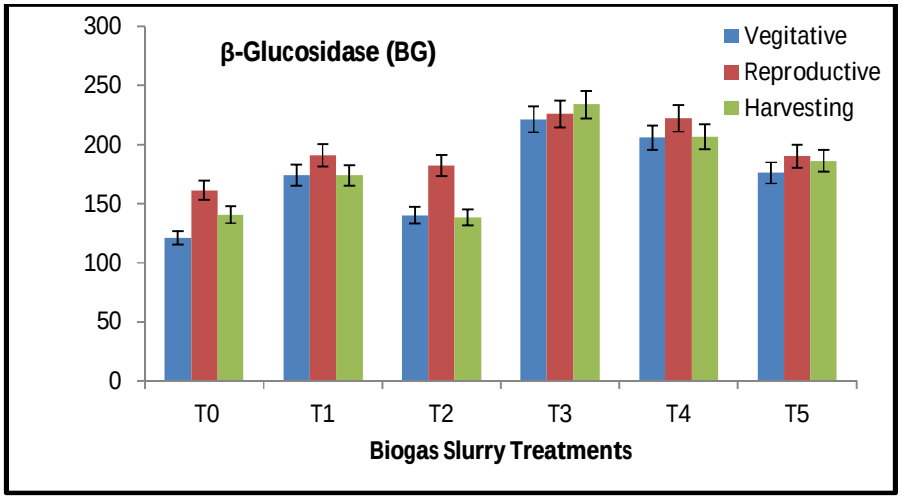


Figure 4.18. Temporal response of β-glucosidase (BG) activity due in different treatments. Vertical bars indicate average confidence intervals at 5% probability (n=3).

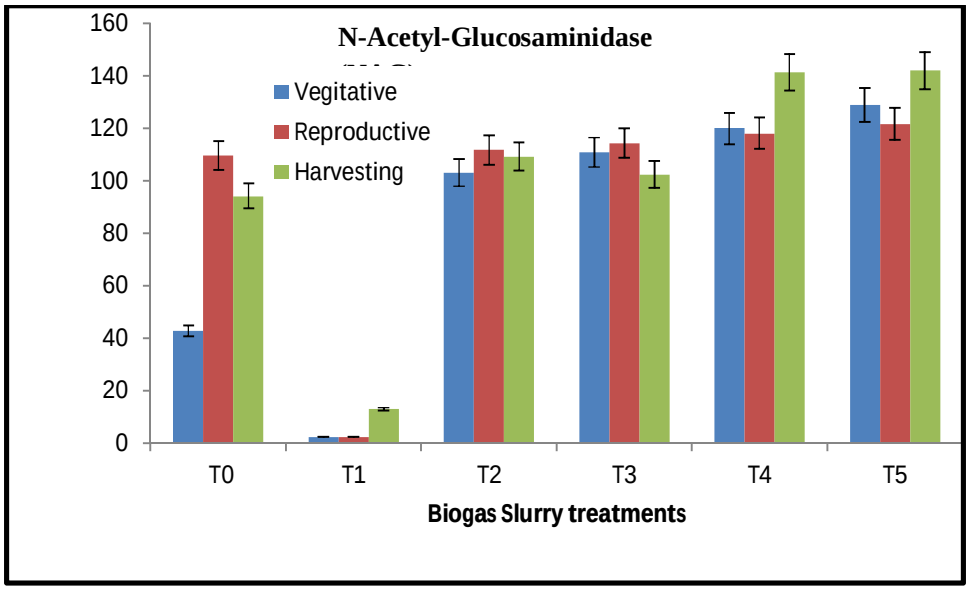


Figure 4.19. Temporal response of N-Acetyl-Glucosaminidase (NAG) activity due in different treatments. Vertical bars indicate average confidence intervals at 5% probability (n=3).

5. DISCUSSION

The amount of residues generated by biogas production has increased dramatically due to worldwide interest in using renewable energy. Biogas slurry (BGS) produced from anaerobic degradation of organic wastes has high content of N, P, K and other valuable nutrients so it can be used as organic fertilizers (Odlare *et al*., 2011). Recycling biogas residues to arable soils as fertilizer has become a more common approach not only to meet the increased demand for food but also to mitigate the rising costs for mineral fertilizers. Most studies on biogas residues have focused on their value as a fertilizer and less on environmental effects (Svensson *et al*., 2004; Odlare *et al*., 2011; Abubaker *et al*., 2012). To maintain ecological safety, the treated materials should be evaluated for their effects on the environmental system. Thus, for the wider application of BGS on crop production and soil ecosystem, its effects need to be investigated. Therefore, on-farm method was used to examine the impacts of different doses of biogas slurry application to compare with conventional and recommended doses of fertilizer application, in wheat growing field because of their (1) ability to contribute better yield by providing nutrients in plants, (2) effects on greenhouse gas emissions such as nitrous oxide (N_2O) and (3) impact on the soil microbial ecosystem.

One environmental problem that has received considerable attention is the emission of greenhouse gases such as nitrous oxide (N_2O) from agricultural ecosystems (Beauchamp, 1997). Emissions of N_2O contribute to the greenhouse effect and lead to destruction of the ozone layer (Crutzen, 1972). N_2O molecule has 310 times global warming potential than that of CO_2 because of their high absorptive capacity of radiation and atmosphere stability (114 yr) (Forster et al., 2007). Emissions of N2O from the agricultural sector account for 60% of total global anthropogenic emissions (IPCC, 2007). Nitrous oxide is produced in the soil mainly by denitrification and nitrification under oxygen-limiting conditions (Bremner, 1997). Denitrification is an anaerobic respiration process whereby NO^3 and NO_2 in particular are used as alternative terminal electron acceptors to oxygen, leading to their reduction to NO, N_2O, and N_2. Nitrification, on the other hand, is the energy-yielding oxidation of NH_4^+ to NO_3^-, which is performed in two steps–ammonium

oxidation followed by nitrite oxidation–both of which produce N_2O under low oxygen conditions (Davidson, 1992).

N_2O flux from the treatments showed more or less similar temporal trends with appearance of a peak of N_2O emission three days after urea applications, however, the magnitude of flux differed (Fig.4.4, 4.5, 4.6). The observed trend of N_2O flux in wheat was in agreement with Malla *et al.* (2005). Urea takes two to three days for hydrolysis into NH_4-N under optimum moisture condition (Lloyd and Sheaffe, 1973), which undergoes further nitrification to NO_3-N resulting in a peak of N_2O flux generally three to four days after urea application.

The cumulative emission of N_2O-N from wheat treatments varied from 0.59 to 0.92 kg ha^{-1}. The emission was significantly higher (11-54 %) under recommended dose of fertilizer treatment (T1) as compared to control and BGS treatments (T0, T2, T3, T4, T5), however, application of higher BGS with urea fertilizer led to significant reduction in N_2O emission. Highest N_2O emission reduction was found in control (54%) which was devoid of both urea and BGS application but N_2O emission was significantly reduced with the application of BGS. After control, 100 % BGS treatment (T5) showed maximum N_2Oemission reduction (25%) over the recommended dose of fertilizer treatment (T1). The cumulative N_2O emission from different treatments were in the order of T0<T5<T4<T3<T2<T1 in wheat crop during 2015-16.The lower N_2O emission in higher BGS treatments was observed due to slow release of N in the soil with high soil moisture contents that reduces rate of diffusion of oxygen into soil promoting anaerobic condition.

Our results showed that the use of biogas slurry as a nitrogen fertilizer increased the yield as well as biomass of the wheat. Optimization of application rate was done to get the maximum growth. It was concluded that highest production was obtained when 50% BGS and 50% chemical fertilizer was used. More studies has to be done for the use of different combination of organic and inorganic fertilizers in such a way that it is cost effective, efficient and a sustainable way for the production of crops which will provide a wider scope in the future.

The quantity and quality of rhizodeposition, moisture content of the soil and the root architecture (surface area, depth of penetration, etc.) was affected by the change in population densities in the rhizosphere and also different dose of biogas slurry was affected by the different input. Certain groups of bacteria have the ability to respond to different quantity and quality of input and in part be selected from the

soil community may explain why a relatively high proportion of bacteria present can be grown in the laboratory when compared with bulk soil, where estimates of the proportion that are recalcitrant to isolation are close to 99.99%. The community densities present in biogas slurry treatments showed remarkable difference from those recorded with control treatments and inorganic treatments.

Differences in soil microbial functional diversity from the control site and from different dose treatments of biogas slurry application indicated community level physiological profiles. The control site showed a lesser average well color development, richness and Shannon weaver index. These findings suggest that, biogas slurry application had enriched soil microbial functional diversity of native soil. In agreement to this, many researchers reported that different amendments of organic matter in soil increased microbial populations (Weon., 1999, Lee *et al.,* 2004). In contrast to our result, previous report demonstrated about high functional diversity and more catabolic potential to utilize carbon substrates by microbial community of native soils (which were not amended with different organic manures) (Staddon*et al.,* 1998, Goodfriend, 1998, Gomez *et al.,* 2004). We assessed catabolic potential of whole microbial community of soil through BIOLOG. The AWCD reflects the oxidative capacity or metabolization capacity of soil microbial community and is a robust indicator to indicate about microbial activity and the employ of BIOLOGTM plates along with environmental samples for CLPP has been recognized as an indicator of metabolic potential, ecosystem function and functional diversity and also showed limitations (Garland & Mills, 1994). A major limitation is that the culturable members of the microbial community which is a small proportion of total populations present in the soil is targeted. Inspite of this, it can provide useful insights when these data are used to match data from nonculturebased techniques. Such as by DGGE analysis of 16S rRNA genes will give community diversity profiling of the rhizosphere that will provide more true picture of actual diversity present in soil. The diversity of carbon substrate utilization profiles (intentisty of utilization of different carbon substrates) and relative abundance (culturable density) confirmed that the developmental stages of crop along with different slurry treatments were a significant driver of community structure.

The transformation of nutrients and organic manures added in soil is directly affected by the microbial activity because it plays a great role in biological and biochemical soil processes. Not only this, their association of extracellular hydrolytic

enzymes qualitatively and quantitatively is found to be important in the process of decomposition and mineralization of organic matter (Kiss *et al.,* 1975; Nakas*et al.,* 1987; Elliott *et al.,* 1993). The assessment of biological and biochemical soil properties and β− glucosidase, α- Glucosidase, N-Acetyl-Glucosaminidase activity have been recommended because of their connection to the soil C cycle, Nitrogen mineralization and the sensitivity of these indicators to detect changes resulting from agricultural management practices (Nannipieri*et al.,* 1990; Dick &Tabatabai, 1993; Gil-Sotres*et al.,* 2005; Lagomarsino *et al.,* 2009). Microbial communities in the soil are enhanced and stimulated by the addition of organic waste, especially due to the presence of readily available nutrients and C compounds. In general, organic waste has high levels of macronutrients such as N, P, K, Ca (Aita*et al.,* 2007; Giacomini*et al.,* 2009), and micronutrients such as B, Zn and Mn and thus increases secretion of different hydrolytic enzymes in soil.

In our experiment, Maximum AG activity (594.99 nmoles/g dry soil/hour) was noticed in the reproductive phase of T3 treatment with a balanced dose of slurry and inorganic fertilizer application. However, the response of T0, T1 and T2 for AG was statistically at per (Figure. 11). Although at T5, higher doses of slurry application were done, but the response was significantly less in comparison to T3 and T4. This may be because; immediate availability of inorganic nitrogen was quite less in case of T5. Generally availability of nitrogen boosts up the secretion of carbon degrading enzymes. Similar trend is also noticed in case of BG. Maximum BG activity (226.1 moles/g dry soil/hour) was noticed in case of T3 in reproductive phase (Figure. 12). However, in contrary to the earlier findings of AG, comparatively higher BG activity was noticed in T1 with complete inorganic fertilizer application. This might happened because of residual organic matters present in soil from earlier crop, as this particular field is cultivated for three consecutive years with biogas slurry application. Overall, a trend is noticed that, C-cycling enzyme potential activities increased with inorganic N availability while those of N-cycling enzymes increased with C availability.

Increases in β−glucosidase activity after compost application consisting of municipal solid residues were also reported by Marcote*et al.,* 2001 and Ros*et al.* (2006), indicating the effect of the substrate. An increased enzyme activity is also an indicator of microbial activity related to the cycling of chemical elements, and significant increases in biogas slurry amended fields, compared to the control

(Figures. 11, 12 and 13) may be due to increased microbial biomass, which may have produced these enzymes (Liang *et al.,* 2005; Tejada*et al.,* 2006; Bastida*et al.,* 2008). The detection of the β-glucosidase enzyme is related to cellulose decomposition which is synthesized by fungi, bacteria and other soil organisms. However, biogas slurry is not as stable as compost (compared to uncomposted residues or sewer sludge) which provides C compounds that are more resistant (Pascual*et al.,* 1997) and more slowly hydrolyzed by enzymes. Thus, addition of biogas slurry had induced stimulated production and secretion of extra cellular enzymes (C and N clycling one). In case of NAG, a nice trend was noticed. In presence of more available nitrogen in T1, detection of Nitrogen cycling enzyme was significantly less in comparison to other biogas slurry treatments. That means N-cycling enzymes increased with C availability. This finding also, corroborates with findings of Bowels *et al.* (2014). Overall, the microbial activity was probably increased because of the contribution of organic C and nutrients available to soil microorganisms and this kind of findings also reported by Emmerling*et al.,* 2000.

The use of organic matters and manures of different kind, to restore soils with low organic C content has been very successful. Because organic suppliments provide high levels of organic C and a facet of nutrients, it effectively improves soil microbial activity, resulting in better soil quality.

Producing wheat with biogas slurry is an agricultural practice commonly used to improve soil quality, reduce green house gas emission (from traditional fertilizer application) and also to manage organic wastes to produce organic food for human consumption. As opposed to the conventional and synthetic fertilizer based agricultural practices, organic cultivation system differ significantly in different aspects like, it reduces nutrient losses, improve soil fertility, and reduce global warming potential while supporting similar crop yields in certain contexts.

6. SUMMARY AND CONCLUSIONS

Organic matter which is the important source of plant nutrients, enhancing the CEC, improves soil structure, increasing water and nutrient holding capacity of soils. Biogas slurry is a good source of plant nutrients and replacing chemical fertilizers with biogas slurry can not only achieve resource utilization of slurry, but also reduce the amount of fertilizer. Moreover, Biogas slurry is not only rich in macronutrients and micronutrients, but also rich in amino acids, vitamins B complex, a variety of hydrolysis enzymes, some plant hormones, materials or factors inhibited the pests and diseases. Combination of organic with chemical fertilizers improves crop growth through modification soil physico-chemical properties. But there is hardly ever study on the effects of biogas slurry application on the yield and N_2O mitigation potential in wheat. The main challenge however was finding the optimal combinations of organic and inorganic fertilizers input rates to enhance the soil microbiology and N_2O mitigations. The aim of this was that how biogas slurry combination with chemical fertilizer affects the growth and yield of wheat crop, mitigation of $N_2O$and its impact on soil functional diversity. With this background, a field trial was carried out to study the "N_2O emission in wheat with integrated use of biogas slurry and fertilizers" at Indian Agricultural Research Institute, New Delhi during *rabi*– 2015-16, with the following objectives.

1. **To examine the green house gas (N_2O) mitigation potential of biogas slurry in substitution of N fertilizer.**
2. **To examine the effect of slurry application on microbial biomass community composition and its functional diversity**

A field experiment was conducted to quantify the N2O loading to the atmosphere from the N- fertilized soil and the efficacy of different levels of biogas slurry in mitigating N2O emission as well as examine the microbial functional diversity of the soil. Wheat, variety HD 2967 was used as a test crop. There were 24 treatment combinations consisting of five levels of organic and inorganic manures, one liquid organic manure sources and one control. The experiment was laid out in a random block design (RBD) with three replications. In field experiment biogas slurry applied @ 0, 25, 50, 75 and 100% along with 100, 75 and 50, 25 and 0 % levels of recommended nitrogen. A field experiment was conducted at Top Block 2 B, farm research Area, Indian Agricultural Research Institute, New Delhi. After harvest of

previous crop (baby corn) land was ploughed once with mould board plough and soil was brought to fine tilth by crushing the clods with cultivator and harrowing twice.

Before sowing of wheat (HD 2967), liquid biogas slurry samples were collected. Samples were brought to the laboratory of CESCRA. After analysis of sample results shown that the pH, total nitrogen, total phosphorus, potassium, Fe, Cu, Mn, and Zn value of the biogas slurry were 7.95, 2.11%, 1..05%, 0.99, %0.33 ppm, 0.005 ppm, 0.089 ppm and 0.022 ppm respectively.BGS consists of 90% water and 10% dry matter of which 4.5% was organic and 2.5% inorganic matter. The required quantity of liquid biogas slurry as per treatment was applied for each plot 1 week days before sowing.

Collection of gas samples was carried out by closed chamber technique (Hutchinson and Mosier, 1981; Bhatia et al., 2005). Gas samples were collected on 2, 9, 19, 34, 41, 56, 65, 73, 86, 87, 93, 103, 118, 126, 145 and 153 DAS for nitrous oxide emission from different treatments. At the top of chamber three-way stopcock was fitted to collect the gas samples. The chamber was thoroughly flushed several times with a 50 ml syringe. Gas samples were drawn with the help of hypodermic needle (24 gauge). After drawing the sample, syringes were made air tight with a three-way stopcock. Head space volume inside the box was recorded, which was used to calculate flux of N_2O. Samples of four replications of each treatment were taken from the plots and the average was taken as representative value for the treatment. Gas samples at 0, 30 minute and 1 hr were collected from the chamber.

Outcome of this study showed that the biogas slurry as a source of nitrogen along with chemical fertilizer stimulated the growth and yield of wheat and. N_2O flux from the treatments showed more or less similar temporal trends with appearance of a peak of N_2O emission three days after urea applications, however, the magnitude of flux differed. Different source of nutrient, soil moisture, structure of root and amount of rhizosphere deposits would shows variations in population densities in the rhizosphere. It was showed that biogas slurry treatment along with chemical fertilizer affects the community densities significantly. The results of the experiments are summarized as follows:

1. The results showed that the application of BGS with chemical fertilizer significantly affected the wheat yield. Control (T0) and (T5) treatments

showed minimum wheat yield where no nutrient source and only bio gas slurry applied respectively.

2. The data reflected that all treatments with biogas slurry application showed an increase in yield ranging from 25 to 56 % over control. Application of 50% biogas slurry with combination of 50 % N through urea (T3) showed maximum yield, which was 56 % higher than that of control (T0) and 11% higher than that of recommended dose of urea (T3).
3. The results showed that the application of BGS with chemical fertilizer significantly affected the wheat yield. Control treatment showed minimum wheat yield (7.472 t ha^{-1}), where no nutrient source applied respectively. All treatments with biogas slurry application showed an increase in yield. Application of 50% biogas slurry with combination of 50 % N through urea (T3) showed maximum yield, which was 11% higher than that of recommended dose of urea (T3).
4. The maximum yield per plot was observed on the application of 50% BGS along with 50 % N of recommended inorganic N (T3) fertilizers which was 56 % more than that of control and 11% more than that of recommended dose of urea.
5. There were significant difference ($P < 0.01$) in biomass yield between the control and T3 (50% BGS + 50% inorganic nitrogen). Control (T0) had the lowest biomass yield and the highest yield was observed in T3 as the BGS and inorganic nitrogen enhance the N mineralization.
6. The cumulative emission of N_2O-N from wheat treatments varied from 0.59 to 0.92 kg ha^{-1}. The emission was significantly higher (11-54 %) under recommended dose of fertilizer treatment (T1) as compared to control and BGS treatments (T0, T2, T3, T4, T5).
7. Highest N_2O emission reduction was found in control (54%) which was devoid of both urea and BGS application but N_2O emission was significantly reduced with the application of BGS. After control, 100 % BGS treatment (T5) showed maximum N_2Oemission reduction (25%) over the recommended dose of fertilizer treatment (T1).
8. The cumulative N_2O emission from different treatments were in the order of T0<T5<T4<T3<T2<T1 in wheat crop during 2015-16.

9. Community level physiological profiles indicated differences between soil microbial functional diversity from the control site and as well from different dose treatments of biogas slurry application. The control site showed a lower average well color development, richness and Shannon weaver index. These findings suggest that, biogas slurry application had enriched soil microbial functional diversity of native soil. The residual effect of BGS on various parameters of soil has been evaluated.
10. Although it was a short term study but, it can be concluded from this experiment that the soil physical properties would improved for the benefit of crops if we apply the biogas slurry.
11. The use of organic matters and manures of different kind, to restore soils with low organic C content has been very successful. Because organic supplements provide high levels of organic C and a facet of nutrients, it effectively improves soil microbial activity, resulting in better soil quality.
12. The Biolog EcoPlate assay is a robust tool and was sensitive to changes in the short term due to management practices. Overall, a trend is noticed that, C-cycling enzyme potential activities increased with inorganic N availability while those of N-cycling enzymes increased with C availability. The soil microbial biomass, populations of bacteria, fungi and total count as well as enzyme activity increased significantly in the biogas slurry treated soil.

Producing wheat with biogas slurry is an agricultural practice commonly used to improve soil quality, reduce green house gas emission (from traditional fertilizer application) and also to manage organic wastes to produce organic food for human consumption. As opposed to the conventional and synthetic fertilizer based agricultural practices, organic cultivation system differ significantly in different aspects like, it reduces nutrient losses, improve soil fertility, and reduce global warming potential while supporting similar crop yields in certain contexts. This study has proven it although it was the result of the single year. More research work is needed to develop cost-effective and efficient use of different combination of organic and inorganic fertilizers for green house mitigation and improvement of soil organic matter for sustainable crop production in near future.

N_2O emission in wheat with integrated use of biogas slurry and fertilizers

Abstract

The amount of residues generated by biogas production has increased dramatically due to worldwide interest in using renewable energy. Biogas slurry (BGS) originate from anaerobic degradation of organic wastes has been proposed as organic fertilisers because of their high content of N, P, K and other valuable macro- and micro-nutrients. The effects of BGS on crop production and on the soil ecosystem and environment urgently need to be investigated before their wider use. Therefore, an on-farm approach was used to investigate impacts of different doses of biogas slurry application to compare with conventional and recommended doses of fertilizer application, in wheat growing field with respect to their (1) ability to provide plants with necessary nutrients for better yield, (2) effects on emissions of the greenhouse gas nitrous oxide (N_2O) and (3) impact on the soil microbial ecosystem. Results from the present study showed that the application of biogas slurry as a nitrogen fertilizer stimulated the growth of wheat yield and biomass. N_2O flux from the treatments showed more or less similar temporal trends with appearance of a peak of N_2O emission three days after urea applications, however, the magnitude of flux differed. The Biolog EcoPlate assay is a robust tool and was sensitive to changes in the short term due to management practices. Overall, a trend is noticed that, C-cycling enzyme potential activities increased with inorganic N availability while those of N-cycling enzymes increased with C availability. The soil microbial biomass, populations of bacteria, fungi and total count as well as enzyme activity increased significantly in the biogas slurry treated soil.

In conclusion, as opposed to the conventional and synthetic fertilizer based agricultural practices, BGS cultivation system differ significantly in different aspects like, it reduces nutrient losses, improve soil fertility, and reduce global warming potential while supporting better crop yields in certain contexts.

बायोगैस अवशिष्ट तथा उर्वरक के एकीकृत उपयोग द्वारा गेहूँ में नाइट्रस ऑक्साइड का उत्सर्जन

सारांश

विश्वभर में नवीनीकरणीय ऊर्जा के बढ़ते उपयोग के कारण बायोगैस उत्पादन के द्वारा उत्पन्न अवशेषों की मात्रा में नाटकीय रूप से वृद्धि हुई है। जैविक अवशेषों के अवायवीय निम्नीकरण द्वारा उत्पन्न बायोगैस अवशिष्ट में N,P,K एवं अन्य मूल्यवान स्थूल तथा सूक्ष्म पोषक तत्वों की प्रचुरता के कारण इसका प्रयोग जैविक खाद के रूप में किया जाता है।बायोगैस अवशिष्ट काव्यापक उपयोग करने से पहले फसल उत्पादन, मृदा पारिस्थितिकी तंत्र तथा पर्यावरण पर इसके प्रभाव की तत्काल जाँच करने की आवश्यकता है। अतः बायोगैस अवशिष्ट के विभिन्न खुराकों के साथ अलग अलग पारंपरिक तथा संस्तुत विधियों के प्रभावों का (1) अधिक उत्पादन के लिए पौधों को आवश्यक पोषक तत्वों को प्रदान करने की क्षमता (2) नाइट्रस ऑक्साइड (N_2O) के उत्सर्जन पर प्रभाव तथा (3) मृदा सूक्ष्मजीवीय पारिस्थितिकी तंत्र पर प्रभावकी जाँच करने के लिए किया गया। वर्तमान अध्ययन के परिणाम से पता चला कि बायोगैस अवशिष्ट का एक नाइट्रोजन उर्वरक के रूप में प्रयोग करने से गेहूँ की उपज तथा जैव भार में वृद्धि हुई।N_2O का प्रवाह यूरिया प्रयोग के तीन दिन बाद अधिकतम पायी गयी तथा अन्य समयों पर प्रवाह लगभग एक समान ही थी। बायोलोग ईको प्लेटपरख एक मजबूत उपकरण है तथा प्रबंधन तरीकों के कारण कम समय में हुए बदलावों के प्रति काफी संवेदनशील है। कुल मिलाकर, एक प्रवृत्ति देखी गयी, जिसमें यह पाया गया कि अकार्बनिक N की उपलब्धता में वृद्धि के साथ C आवर्तीय एंजाईम की शशक्त क्षमता में वृद्धि हुई जबकि N आवर्तीय एंजाईमकी शशक्त क्षमता में वृद्धिC की उपलब्धता में वृद्धिके साथ हुई। बायोगैस अवशिष्ट से उपचरित मृदा में सुक्ष्मजीवीय जैव भार, जीवाणुओं तथा कवकों की जनसंख्या तथा एंजाईमगतिविधिमें विशेष रूप से वृद्धि हुई।

अंत में, पारंपरिक और सिंथेटिक उर्वरक आधारित कृषि पद्धतियों की तुलना में बायोगैस अवशिष्ट आधारित कृषि कई मायनों में अलग है। यह पोषक तत्वों की क्षति को कम करने के साथ साथ मिट्टी की उर्वरता में सुधार तथा हरित गृह प्रभाव की क्षमता को भी कम करता है तथा फसलों की बेहतर पैदावार में मदद भी करता है।

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