Biogas effluent as a sustainable biofertilizer: Biochemical, microbiological and agronomic Insights

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Abstract:

Biogas is produced through anaerobic digestion of kitchen waste. A liquid effluent (bioslurry) is also produced in the process. The effluent is often discarded and its disposal poses a concern for large-scale biogas systems. This study presents a preliminary assessment of bioslurry through physical, chemical, microbiological, and agronomic analyses. Nutrient profiling revealed nitrogen (1790 ppm), phosphorus (21ppm), and potassium (110 ppm) indicating macronutrient availability. In addition, micronutrients like calcium, magnesium, sulphur, iron, manganese, boron, zinc, copper and sodium were also detected. The pH of the bioslurry was in the alkaline range. The BOD and COD levels were found to be 32.5 mg/L and 279 mg/L respectively and were within standard limits as prescribed by the World Health Organisation; the standards being BOD < 100 mg/L, COD < 300 mg/L for untreated or anaerobic biogas effluent or bioslurry. Microbial isolates demonstrated degradative capacities due to the presence of enzymes such as amylase, cellulase, gelatinase, lipase, pectinase and protease. Root tray assays on *Zea mays* and *Vigna radiata* demonstrated improved growth parameters at 0.1% bioslurry dilution compared to 1% bioslurry dilution. The findings highlight the potential of biogas effluent as an eco-friendly biofertilizer, reducing dependence on chemical alternatives.

Key words: biogas, biofertilizer, bioslurry, sustainability, soil fertility, macronutrients, micronutrients

Introduction:

The extensive use of inorganic fertilizers in agriculture to ensure food security has led to innumerable health issues and irrecoverable damage to the environment, reduction in soil fertility being the major one. Soil carries out essential ecological services that sustain life by supporting plant growth, regulating water and nutrient cycles, sustaining biodiversity and filtering contaminants. Hence, soil health management is of utmost importance. Chemical fertilizers like nitrogenous, phosphate and potassium fertilizers are added to increase crop productivity and enhance soil fertility. But the consistent use of these fertilizers affects the soil's organic content resulting in its decline. It is coupled with a decline in the quality of soil health, soil fertility, soil

microbial activity in addition to hardening of soil and alteration in soil pH. Nutrient availability and microbiological activities of the soil depend on soil organic carbon. Soil organic carbon is found to increase with continuous application of organic manure (Kumari *et al.*,2024). Nearly all plants have diverse, interactive and complex communities of microorganisms colonized on them. This colonization can be endodontic, rhizospheric and epiphytic on plants. Plant microbiome plays an important role in promoting the growth and yield of crop plants by producing phytohormones, degrading organic components, and enhancing the availability of nutrients. The plant microbiome can thus be used as biofertilizers. Biofertilizers consist of living cells (like bacteria, fungi or algae) which are applied to the soil directly or to seedlings or to seeds. By solubilising zinc, phosphate and potassium from the soil, the biofertilizers help in improving availability of nutrients and their ready uptake from the soil. Thus, microorganisms have become important components for better functioning of the soil ecosystem (Kumar *et al.*,2023). Awareness of the long-term damages to soil and crops caused by chemical fertilizers has led to the increase in the use of organic fertilisers by farmers. Some popular organic fertilisers are vermiculture and cow dung. Vermiculture converts organic waste viz., municipal, agriculture, animal and sewage into fertilizer (Gupta *et al.*,2008; Suthar *et al.*,2009; Singh *et al.*,2012; Yadav *et al.*,2013). It is rich in micro- and macro-plant nutrients which are in available forms like nitrate, phosphate, sulphate, etc. to the plants. They help in plant growth and productivity (Singh *et al.*,2012). Cow dung is also considered as a good biofertilizer. The application of cow dung manure and vermicompost increases the soil's organic matter content, and this leads to improved water infiltration and water holding capacity as well as an increased cation exchange capacity (Raj *et al.*,2014). Biogas is a sustainable and renewable source of energy produced by anaerobic digestion of organic components like food waste, cow dung waste and poultry waste. Biogas slurry is a by-product of biogas production. It is a useful organic fertilizer because of its substantial nutrient content like nitrogen, phosphorus, potassium and other trace elements like iron, manganese and zinc. In recent studies, it was observed that sun dried biogas slurry approximately has carbon (41.6 %), nitrogen 0.72 %, phosphorus (0.91 %) (Kumar *et al.*,2023). It supplies more nitrogen to plants than common chemical fertilizers (You *et al.*,2019). The microbial diversity in biogas slurry such as nitrogen fixing and phosphate solubilizing bacteria helps in faster nutrient recycling, more robust soil health and promotes a balanced ecosystem in agriculture fields. Biogas slurry constitutes about 93 % of water, 7 % is organic (4.5 %) and inorganic (2.5 %), pH of slurry is in the neutral range . It is also a rich source of slow-release minerals if used in compost form. The organic elements in biogas slurry reduces soil erosion by improving physical properties of soil, permeability of air, along with increase in organic carbon content in soil. An increase in photosynthesis is observed due to greater availability of macro and micronutrients (Zhang *et al.*,2025). The present study attempts to evaluate various physico-chemical, nutrient, biochemical and agronomic parameters of biogas slurry (obtained from

anaerobic digestion of cooked food waste) with the aim of assessing its potential as a sustainable biofertilizer.

Materials and Methods:

PHYSICAL AND CHEMICAL ANALYSIS: Parameters such as electrical conductivity, pH, nitrogen, phosphorous and potassium content, total organic carbon, total dissolved solids, total alkalinity, chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids, total suspended solids, sodium absorption ratio and boron content were determined using standards methods.

MICROBIAL AND BIOCHEMICAL ANALYSIS: Isolation of microorganisms from the bioslurry was done by serially diluting it and using spread plate and pour plate techniques. Nutrient agar and Sabouraud Dextrose agar were used for the isolation of bacteria and fungi respectively. Each morphologically different colony was checked for its ability to produce a variety of enzymes such as amylase, cellulase, gelatinase, lipase, pectinase and protease using the grid plate technique. For determination of each enzyme, different media were used such as starch agar, carboxymethylcellulose agar, gelatin broth, tributyrin agar, pectin agar, and milk agar, respectively.

ROOT TRAY ASSAY: Root tray assay was conducted by planting monocotyledonous seeds ie. *Zea mays* (maize) and dicotyledonous seeds ie. *Vigna radiata* (moong) in a root tray. The tray chambers were moistened with sterile tap water, 0.1% and 1% dilutions of bioslurry. Sterile tap water was used as control and was added daily while 0.1% and 1% bioslurry dilutions were added on the first day and on the fifth day of the assay. The assay was conducted in triplicates for ten days.

AGRONOMIC ANALYSIS: The germination of seeds, number of leaves, length of stem of *Zea mays* and *Vigna radiata* were recorded after every two days and the average values were noted. At the end of the assay, i.e. after ten days, the average wet weight of the plantlets and average length of the roots were also noted.

Statistical analysis: The t-test was applied to the results.

Result and Discussion:

PHYSICO-CHEMICAL ANALYSIS: The pH of the bioslurry was found to be in the alkaline range similar to that of soil pH thereby supporting soil health and soil micro flora. The nitrogen content was found to be much more than the required concentration which is 150-200 ppm. (table 1). The phosphorus and potassium content were within the optimum range. These results are in agreement with earlier studies wherein a bioslurry was applied to maize fodder. (Zhang *et al.*,2025; Islam *et al.*,2010). Biogas produced after anaerobic digestion exhibited pH of 7.13 and high nutrient content (Wentzel *et al.*,2015). Analysis of trace elements also revealed that they were present in appropriate amounts essential for the growth of the plants. Similar results were obtained for biogas slurry in earlier studies (Gupta *et al*, 2007; Yu *et al*, 2010; Xu *et al*, 2021;) BOD and COD values are important to evaluate organic pollution, assess waste water treatment efficiency, protect aquatic

ecosystems and ensure compliance with environmental standards (World Health Organisation, 2006). The values obtained were 32.5 mg/L and 279 mg/L respectively indicating that they are within the limits of WHO standards (WHO, 2006). For agricultural reuse, the standards are BOD < 100 mg/L, COD < 300 mg/L for untreated or anaerobic biogas effluent or bioslurry.

Table 1: Physicochemical analysis of the bioslurry

Sr. No.	Parameter	Test value
1	pH	9
2	Nitrogen (N)	1789.7 ppm
3	Phosphorus (P)	21.4 ppm
4	Potassium (K)	110 ppm
5	Calcium	21 ppm
6	Magnesium	3.12 ppm
7	Sulphur	1.78 ppm
8	Iron	7.8 ppm
9	Manganese	0.32 ppm
10	Zinc	0.19 ppm
11	Copper	0.08 ppm
12	Sodium	1200 ppm
12	Boron	1 ppm
13	Total organic carbon	3058 ppm
14	Chemical oxygen demand	279 ppm
15	Biological oxygen demand	32.5 ppm
16	Total dissolved solids	1000 ppm
17	Total suspended solids	47.1 ppm

DETECTION OF VARIOUS ENZYMES BY COLONIES OBTAINED IN THE BIOSLURRY: Twenty-one different isolates which were visually distinct were analysed for their ability to produce enzymes such as amylase, cellulase, gelatinase, lipase, pectinase and protease that would aid in the degradation of kitchen waste. It has been reported by Goyal et al. (2016) and Kaur *et al.*, (2017) that bioslurry obtained from biogas produced from poultry waste and cow dung can be used as a substrate for protease production. Zhao *et al.* (2013) identified 18 operational taxonomic units (OTUs) in biogas slurry compost. In 2022, Wang *et al*. showed that biogas slurry flooding treatment around *Panax notoginseng*, an important herbal plant used in Chinese medicine altered the physicochemical properties and reshaped the bacterial and fungal communities, reduced the relative abundance of potential fungal pathogens like (*Fusarium*, *Cylindrocarpon*, *Alternaria*, and *Phoma*), and suppressed culturable fungi and *Fusarium.* The paper by Wentzel *et al.*, (2016) showed that application of bioslurry boosted grass growth but led to lower fungal biomass and

reduced mycorrhizal colonisation. This was due to high nutrient availability in the bioslurry, highlighting bioslurry's potential to increase fertility of the soil.

Table no.2: Biochemical analysis

Sr. No.	Enzymes	No. of colonies showing enzyme activity
1.	Amylase	13
2.	Cellulase	04
3.	Lipase	21
4.	Gelatinase	09
5.	Pectinase	-
6.	Protease	18

AGRONOMICAL ANALYSIS: Agronomical analysis was carried out using two dilutions of the bioslurry (1% and 0.1%). Tap water was used as control. Significant changes in the parameters such as % germination, root and shoot length, wet weight was observed for both *Zea mays* and *Vigna radiata* (Tables 3 & 4, Fig.1 to Fig.8) as compared to the control values. Similar reports on a study of the effect of bioslurry on maize plants were also obtained by Islam *et al*, 2010.

a) *Zea mays* (Maize):

Table no.3

Sr.No.	Parameters tested	Control (Average values)	Treatment 1 (Average values)	Treatment 2 (Average values)
1.	Percentage of seed germination	58.5	77.8	75.0
2.	Height of stem (in cm)	7.1	7.8	7.4
3.	Number of leaves	2	3	2
4.	Length of root (in cm)	9.2	12.6	10.5
5.	Wet weight of the plantlet (in grams)	5.90	7.57	6.07

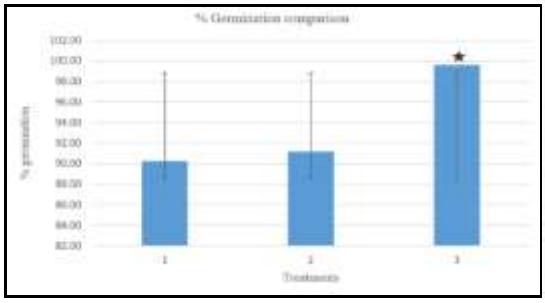


Fig.1: Percentage of germination comparison

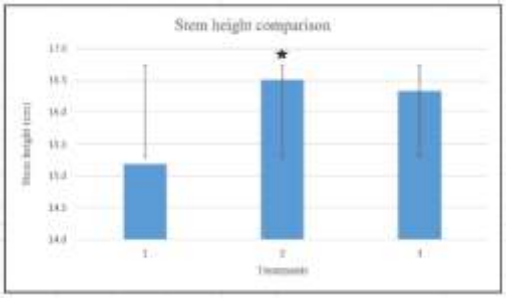
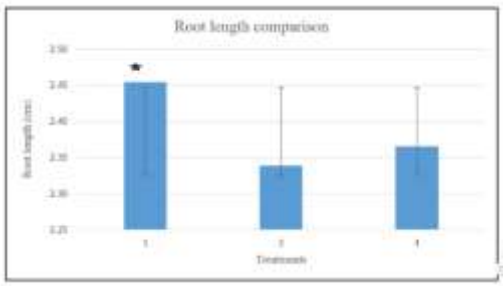


Fig.2: Stem-height comparison



Fig,3 Root length comparison

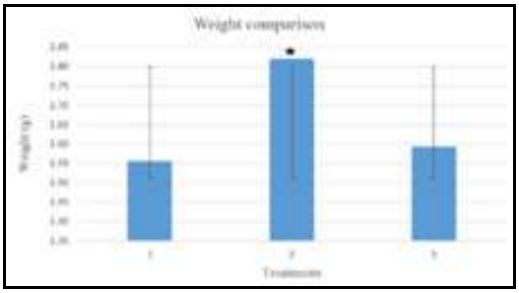


Fig.4 Weight comparison

★ indicates effective treatment.Treatment 1: Control, Treatment 2: 0.1 % slurry; Treatment 3: 1.0 % slurry

b) *Vigna radiata* (moong):

Table no. 4

Sr.No.	Parameters tested	Control (Average values)	Treatment 1 (Average values)	Treatment 2 (Average values)
1.	Percentage of seed germination	90.27	91.20	99.61
2.	Height of stem (in cm)	*15.2*	*16.5*	*16.3*
3.	Number of leaves	2	2	2
4.	Length of root (in cm)	15.2	16.5	16.3
5.	Wet weight of the plantlet (in grams)	2.56	2.82	2.59

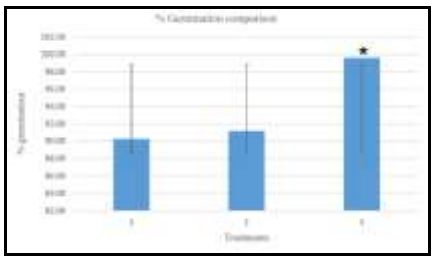


Fig 5: Percentage of germination of seeds

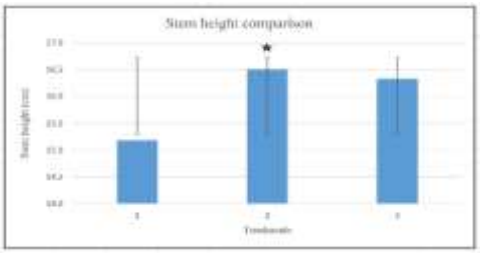


Fig 6: Stem height comparison

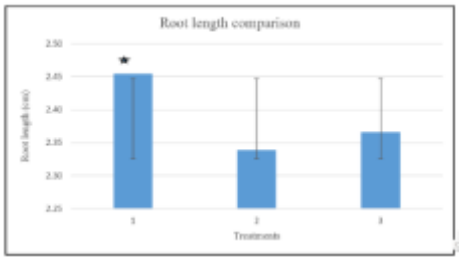


Fig 7: Root length comparison

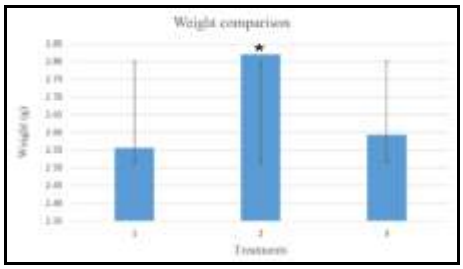


Fig 8: Weight comparison

★ indicates effective treatment.Treatment 1: Control, Treatment 2: 0.1 % slurry; Treatment 3: 1.0 % slurry

Conclusion:

The spent liquid or effluent of a biogas system or bioslurry is usually discarded. Its disposal is a matter of concern to large biogas producing systems. Physical, chemical, biochemical, microbiological, agronomical and statistical analyses of the effluent or bioslurry were studied that revealed that the bioslurry could be used as a biofertilizer effectively, thereby, providing an alternative to chemical fertilizers.

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