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Exploration of Microbial Diversity and Enzyme Activity for Effective Biogas Production from Organic Waste

Dr. Shilanjali Bhalerao[1]*, Dr. Dayanand Agsar[2], Dr. Gangagni Rao Anupoju[3], Dr. Kumaraguru Thenkrishan[4]

[1]Project Assistant, Department of Microbiology, Gulbarga University, Sedam Road, Jnana Ganga, Kalaburagi, Karnataka 585106, India
[2]Professor, Department of Microbiology, Gulbarga University, Sedam Road, Jnana Ganga, Kalaburagi, Karnataka 585106, India
[3]Chief Scientist and head, Department of energy and Environmental engineering (DEEE), CSIR-Indian Institute of Chemical Technology (IICT), Tarnaka , Hyderabad 500007, Telangana, India
[4]Senior scientist, Organic synthesis & process chemistry, CSIR-Indian Institute of Chemical Technology (IICT), Tanaka, Hyderabad 500007, Telangana, India

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***Corresponding Author**
Dr. Shilanjali Bhalerao

Project Assistant, Department of Microbiology, Gulbarga University, Sedam Road, Jnana Ganga, Kalaburagi, Karnataka 585106, India

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A B S T R A C T

This study investigates the microbial ecology of an Anaerobic Gas-Lift Reactor (AGR) to enhance biogas (methane) production from organic waste streams. ***Methods*:** Samples of vegetable waste, seed inoculum and food waste were collected from various locations in Hyderabad. Microbial isolation, characterization, and enzyme screening were conducted to identify key microbial species and their enzymatic capabilities. Growth dynamics under varying temperature and pH conditions were analyzed, and a microbial consortium was tested in a lab-scale biogas production system. ***Results*:** A diverse microbial community was identified, with extracellular enzyme screening revealing species with multifunctional enzymatic potential. Temperature and pH significantly influenced microbial growth and biogas production efficiency. ***Conclusion*:** The study provides valuable insights into optimizing microbial consortia for enhanced methane production, highlighting the critical roles of temperature and pH in regulating microbial activity. These findings contribute to developing efficient AGR systems for sustainable biogas generation from organic waste.

Keywords: Anaerobic Gas-Lift Reactor (AGR), Microbial Ecology, Biogas Production, Organic Waste Management, Extracellular Enzymes.

INTRODUCTION

The generation of biogas from organic waste offers an effective solution for waste management and renewable energy production, making it a critical area of research and application globally [1]. Anaerobic digestion (AD) is a biological process that breaks down organic waste, including food scraps, agricultural residues, cattle manure, and the organic fraction of municipal solid waste (OFMSW), into biogas, primarily composed of methane (CH_4) and carbon dioxide (CO_2). This methane-rich biogas is a valuable energy source that can be used for combined heat and power (CHP) generation or upgraded for use as a fuel [2]. AD also produces digestate, a nutrient-rich byproduct used as a biofertilizer, further enhancing its environmental benefits.

The AD process consists of four key stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each stage is mediated by specific microbial communities, including bacteria and archaea, that work together to break down organic material and produce methane. The efficiency of this process is influenced by several critical parameters such as temperature, pH, and the availability of micronutrients (MNs) or trace elements. Maintaining optimal conditions for microbial growth and activity is essential for maximizing biogas yield. For instance, the optimal temperature for AD ranges

between 35°C (mesophilic conditions) and 55°C (thermophilic conditions), while a pH range of 6.5 to 7.5 is ideal for methanogenic activity [3].

The presence of MNs such as cobalt (Co), nickel (Ni), iron (Fe), and molybdenum (Mo) is also crucial, as these elements act as cofactors for enzymes involved in methane production. Supplementation with these MNs can stimulate methanogenic archaea and enhance biogas production [4]. However, excessive amounts of MNs can inhibit microbial growth and reduce reactor performance [5].

In recent years, research has explored the potential of using nanoparticles (NPs) of MNs to further enhance AD performance. NPs have unique properties, including a high surface area-to-volume ratio, which increases their reactivity and bioavailability. Studies have shown that the addition of MN NPs, such as Fe_3O_4 and NiO, can significantly improve biogas production by facilitating microbial interactions and enzyme activity [6]. For example, it found that nickel NPs increased biogas production by over 70% in cattle manure-based AD systems.

Despite these promising findings, the effects of NPs on microbial communities and reactor stability are not fully understood. There is a need for further research to establish the optimal concentrations and combinations of NPs for improving AD efficiency without causing toxicity to methanogens [7].

This study aims to design a substrate-specific microbial consortium for biogas production and compare its efficiency with a natural consortium. By isolating and screening key microbial species involved in AD, this research seeks to develop a synthetic microbial consortium optimized for specific substrates. The performance of this consortium will be tested in terms of biogas yield and reactor stability, contributing to the broader goal of enhancing biogas production for sustainable energy generation.

Anaerobic digestion (AD) is intrinsically a sequential complex chemical and biochemical process, and many factors (microbiological, operational, and chemical) can affect its performance [8].

A major part of this increase is expected to come from new centralized biogas plants. The annual potential for biogas production from biomass resources available in Denmark is estimated to be approx. 30 Peta Joule (PJ) [9].

The effect of bioaugmentation by anaerobic hydrolytic bacteria on biogas production was determined by the biochemical methane potential assay. Microbial biomass from full scale up flow anaerobic sludge blanket reactor treating brewery wastewater was a source of active microorganisms and brewery spent grain a model lignocellulosic substrate [10]. The biomethane potential of four different substrates (molasses, bio-refinery waste, liquid manure and high-rate activated sludge) was determined by means of four different inoculate from full-scale anaerobic digestion plants [11].

A group consisting of *Bacteroidales, Pseudomonadales,* and *Enterobacterales* was identified to be putatively responsible for the hydrolysis of microalgal biomass [12].

Materials and Methods

1. Collection of Samples

The bacterial consortium for this study was developed using organic waste collected from various sources. The samples included vegetable waste, food waste, and seed inoculum. The collection sites included households, local markets, and industrial biogas plants. These materials were chosen because they are rich in microbial diversity, essential for effective anaerobic digestion.

2. Isolation of Microorganisms

Bacterial strains were isolated from collected organic waste samples through serial dilution and plating on nutrient agar. The plates were incubated at 37°C for 24-48 hours. After incubation, distinct bacterial colonies were selected based on morphology and were sub cultured to obtain pure isolates. The isolated strains were stored at 4°C in glycerol stocks for further analysis.

3. Screening for Extracellular Enzyme Activities

To assess the ability of bacterial strains to degrade organic matter, they were screened for the production of key extracellular enzymes involved in the degradation of carbohydrates, proteins, and lipids. The following tests were conducted:

- **Amylase Activity:** Tested using starch agar plates, which were flooded with iodine after incubation to detect starch hydrolysis zones.
- **Protease Activity:** Assessed using skim milk agar plates to identify clear zones indicating protein degradation.

- **Cellulase Activity:** Evaluated on carboxymethyl cellulose (CMC) agar plates by staining with Congo red to identify cellulose-degrading strains.
- **Pectinase Activity:** Detected on pectin agar plates, where clear zones around colonies indicated pectin degradation.
- **Lipase Activity:** Assessed using spirit blue agar to detect lipid hydrolysis.

3. RESULTS AND DISCUSSION

1.1. Sampling of the site:

Vegetable wastes are collected from Bowenpally market at Hyderabad (Fig 1), Seed inoculum Samples are collected from Erragada place from biogas plant at Hyderabad (Fig 2),food waste (Fig 3) and Monitored pH 4.5, 5.5 and 7.0 sample of Seed Inoculum is collected from CSIR-IICT institute.

1.2. Microbial Isolation and Characterization from Organic Waste Samples for Biogas Production

This study investigated the **microbial diversity** associated with various organic waste streams relevant to biogas production in India. Samples were obtained from three distinct sources:

a) Grinded vegetable waste: Sourced from the Bowenpally Biogas Plant, this sample represents the typical organic substrate used for biogas generation.

Fig1: Bowenpally market at Hyderabad

Fig 2: Erragada place from biogas plant at Hyderabad

Fig3: CSIR-IICT institute at Hyderabad

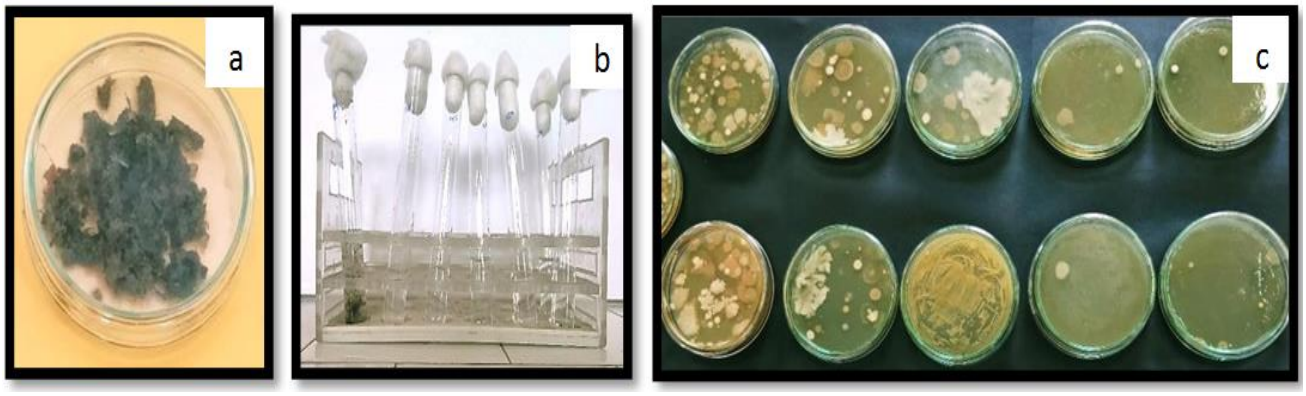

Fig 4:a)Vegetable waste b)Serial dilution tubes c) Colony plates

b) Seed inoculum: Obtained from the Erragada Biogas Plant, this sample representsTheactivemicrobialpopulationalreadyestablishedinafunctioningbiogasdigester.

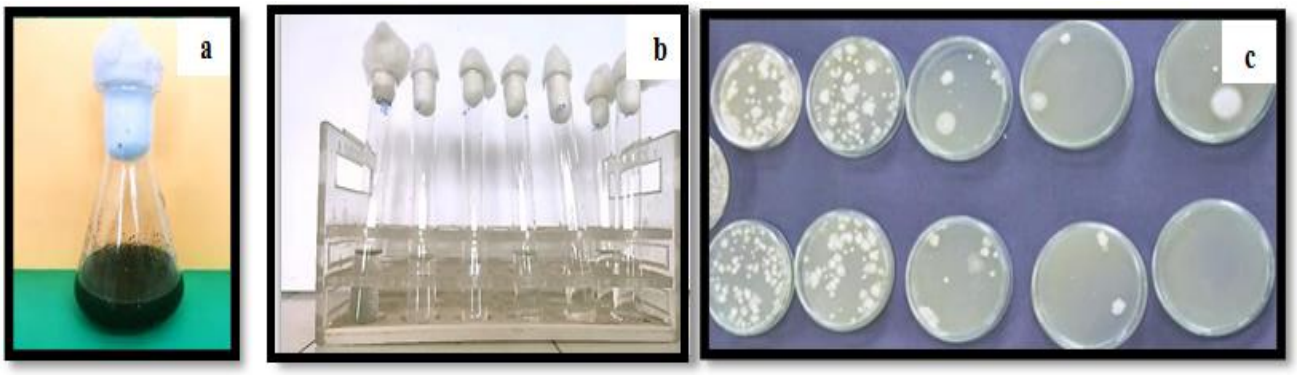

Fig 5: a) Seed inoculum b) Serial dilution tubes c) Colony plates

c) Cooked, grinded food waste: Collected from the IICT Canteen, this sample explores the potential of utilizing food waste for biogas production.

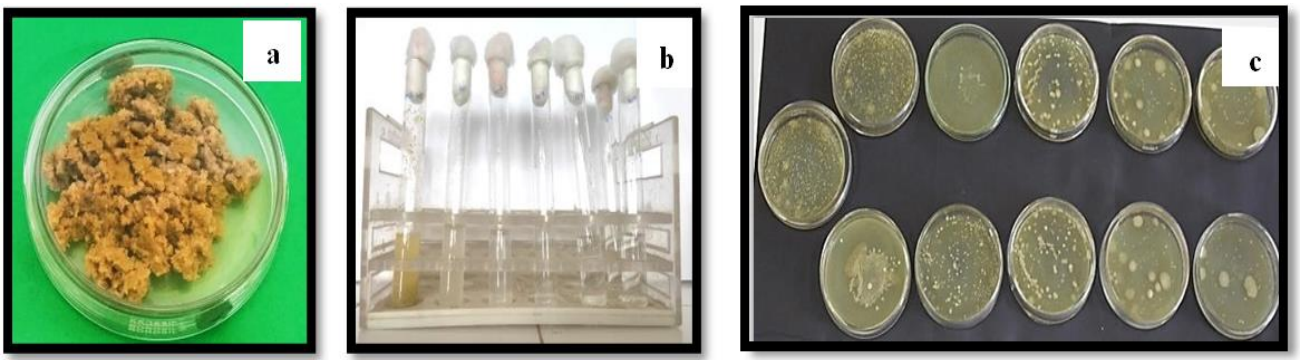

Fig6: a)Food wastes b)Serial dilution tubes c) Colony plates

To further understand the influence of pH on microbial composition, monitored pH samples were prepared from the seed inoculum at three different pH levels: 4.5, 5.5, and 7.0.

d) Monitored pH4.5 (0hrs)

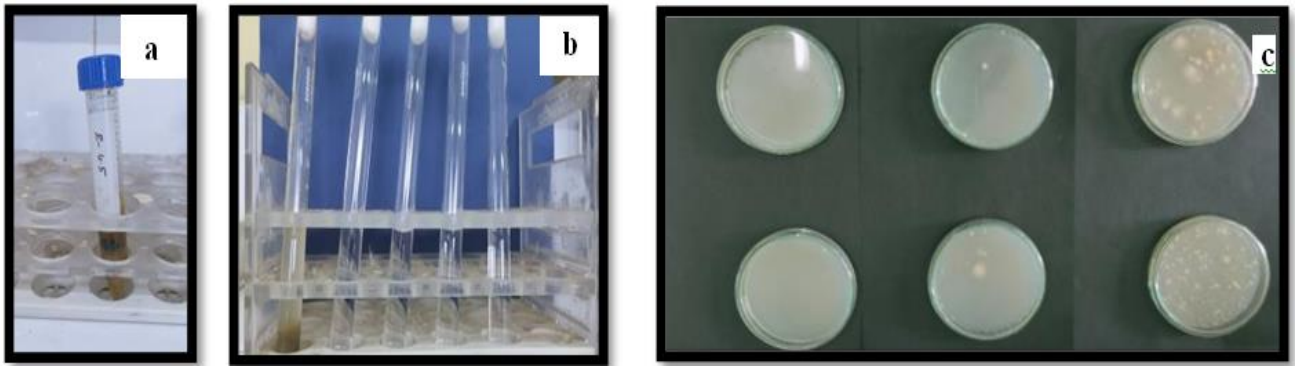

Fig7:a)Monitored pH 4.5(0hrs) b)Serial dilution tubes c) Colony plates

e) Monitored 4.5(24hrs)

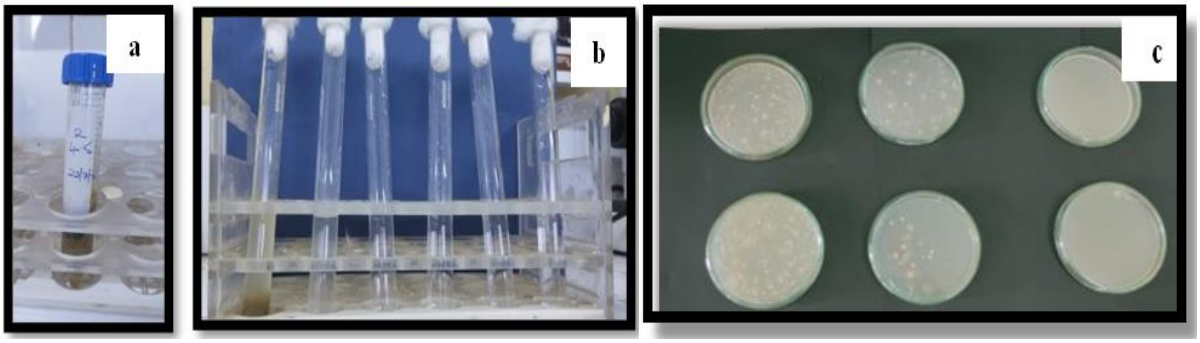

Fig8:a)Monitored pH 4.5 (24hrs) b)Serial dilution tubes c) Colony plates

The experiment commenced with the collection of seed inoculum samples from the IICT Bees Lab, and their initial pH was meticulously recorded at 4.5. The experimental setup involved visually inspecting tubes containing the samples at 0 hours (0 HRS) and 24 hours(24 HRS).

f) MonitoredpH5.5(0hrs)

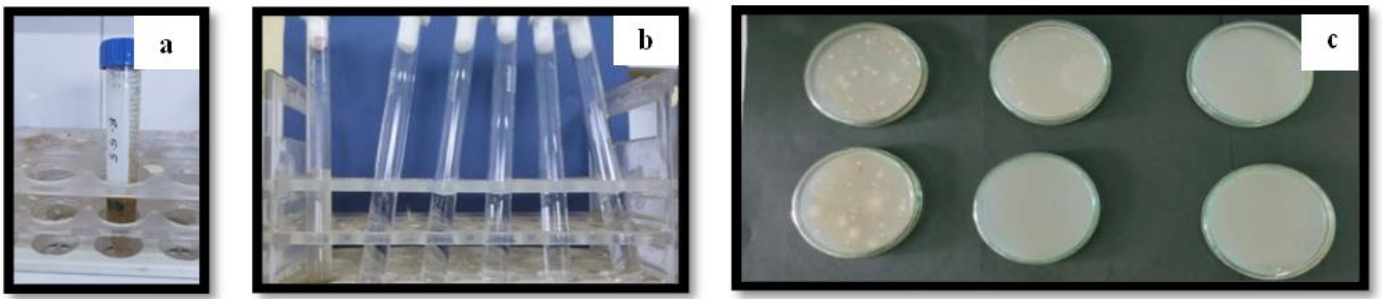

Fig9:a)Monitored pH 5.5 (0hrs) b)Serial dilution tubes c) Colony plates

g) MonitoredpH5.5 (24hrs)

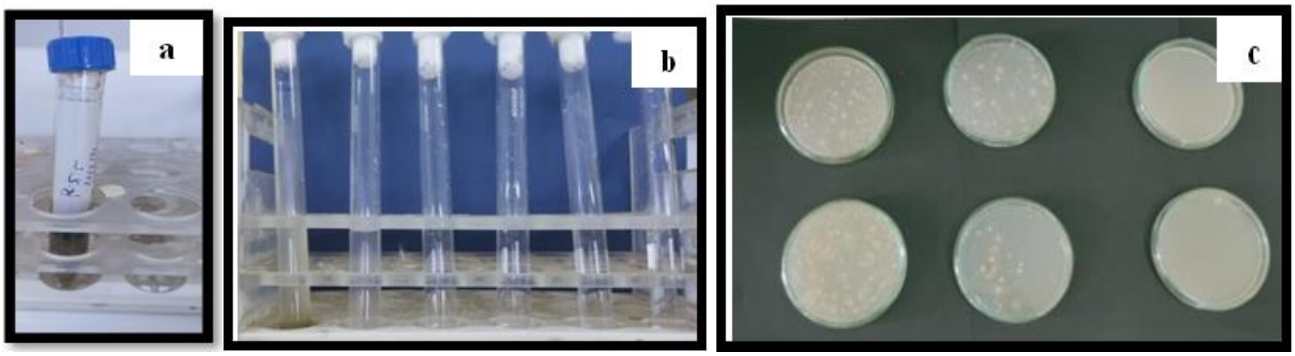

Fig10:a)Monitored pH 5.5(24hrs) b)Serial dilution tubes c) Colony plates

Following the baseline pH monitoring, a pH adjustment was implemented to set the conditions at 5.5. The experiment continued with the same visual inspection at 0 hours (0HRS) and 24 hours (24 HRS), capturing the dynamic changes in acidity.

h) MonitoredpH7.0 (0hrs)

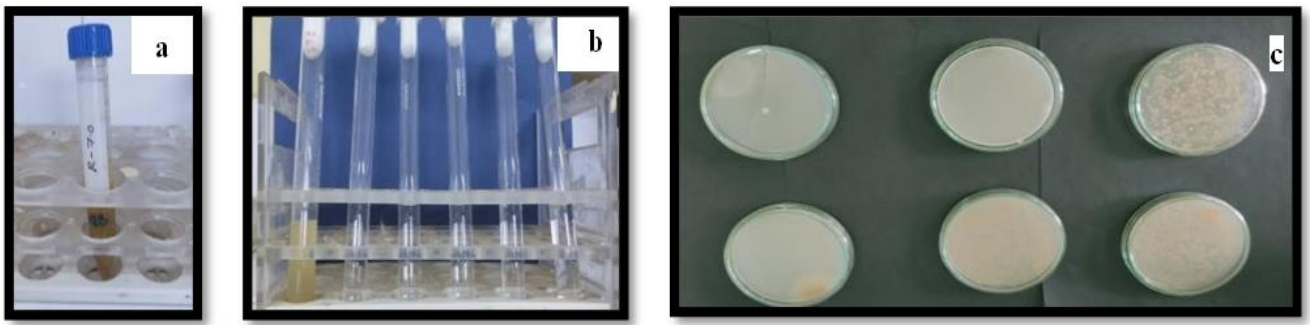

Fig11: a)Monitored pH 7.0 (0hrs) b)Serial dilution tubes c) Colony plates

i) MonitoredpH7.0(24hrs)

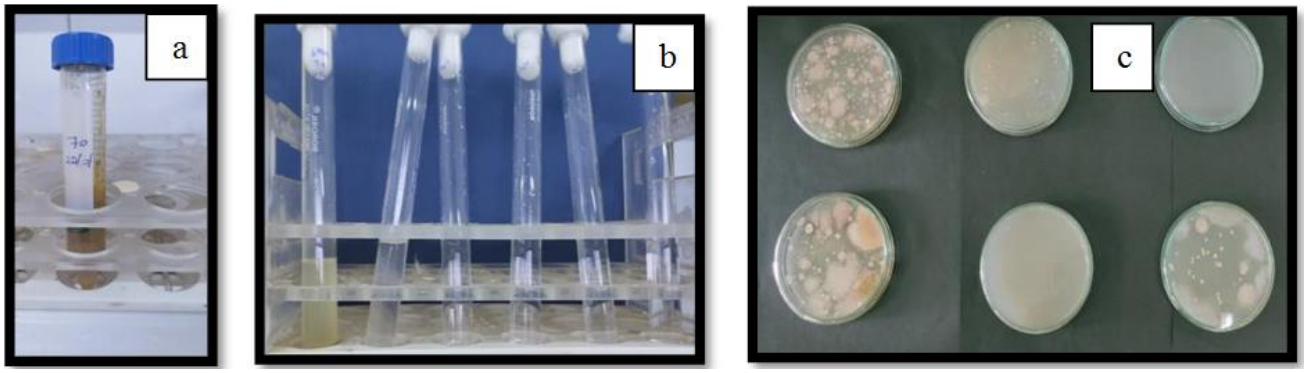

Fig12: a) Monitored pH 7.0 (24hrs) b) Serial dilution tubes c) Colony plates

Further modifications were introduced to establish a neutral pH condition at 7.0 for the seed inoculum samples. Visual inspection of tubes at 0 hours (0 HRS) and 24 hours (24 HRS) was carried out to monitor any changes in acidity. The accompanying image showcases the tubes with the seed inoculum at the neutral pH, providing a visual record of the experimental progression.

Serial dilution and subsequent microbial plating were crucial steps in the experimental process, contributing to a more comprehensive understanding of the seed inoculum's characteristics and its potential applications.

Important stages in the experiment included serial dilution and subsequent microbial plating, which helped to provide a deeper knowledge of the properties of the seed inoculums and its possible uses. The use of microbial plates highlights the impact of pH on microbial growth and adds to the extensive discourse on the behaviour of the seed inoculum under various environmental circumstances.

Microbial isolation from the heterogeneous organic waste samples sourced from Erragada Biogas Plant (seed inoculum), Bowenpally Biogas Plant (grinded vegetable waste), IICT-Canteen (cooked, grinded food waste) and Monitored pH sample of Seed Inoculum, involved the application of specialized culture media and techniques optimized for promoting the growth of a diverse microbial consortia. A total of 300 isolates were successfully obtained, constituting a comprehensive representation of the microbial community associated with the distinct organic substrates. Subsequent taxonomic analysis revealed a substantial predominance of bacteria, encompassing 190 isolates, and a discernible presence of actinobacteria, with 17 isolates identified. This nuanced microbial profile c contributes to a deeper understanding of the taxonomic diversity and functional potential of microorganisms in the biogas production milieu, offering valuable insights for further ecological and biotechnological investigations in the context of organic waste management.

1. Identification of the dominating microbial community at various phases of substrate digestion (Table 1)

Table 1:

SL.NO	SAMPLES	MICROBES	NUMBER
1	VEGETABLEWASTE	BACTERIA	20
		ACTINOBACTERIA	1
2	FOODWASTE	BACTERIA	45
		ACTINOBACTERIA	4
3	SEEDINOCULUM	BACTERIA	41
		ACTINOBACTERIA	7

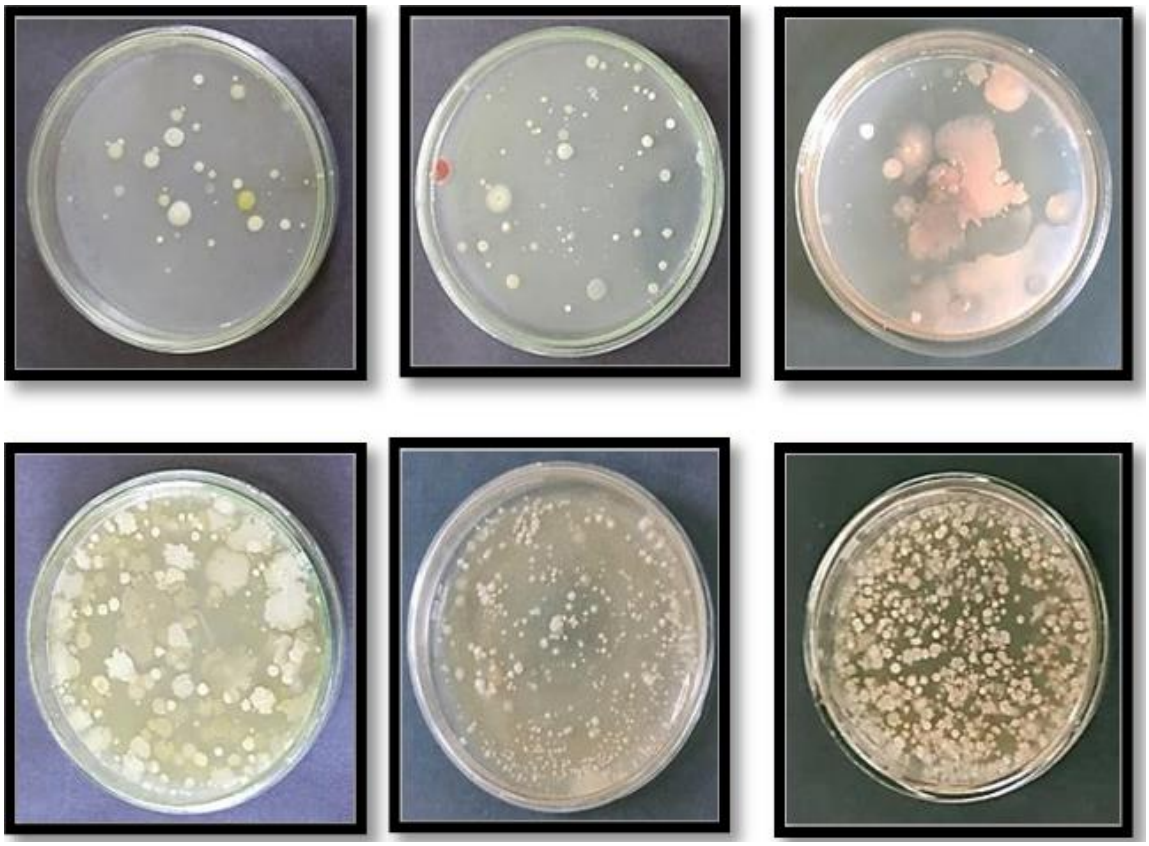

Fig 13: Organic substrates samples: Serial Dilution Diversity Plate

2. Identification of the dominating microbial community at various phases of substrate digestion (Table 2)

Table 2:

SL. NO	SAMPLE	PERIOD	MICROBES	NUMBER
1	pH4.5	0HRS	BACTERIA	34
			ACTINOBACTERIA	2
		24HRS	BACTERIA	42
			ACTINOBACTERIA	1
2	pH5.5	0HRS	BACTERIA	38
			ACTINOBACTERIA	14
		24HRS	BACTERIA	48
			ACTINOBACTERIA	-
3	pH7.0	0HRS	BACTERIA	36
			ACTINOBACTERIA	1
		24HRS	BACTERIA	52
			ACTINOBACTERIA	-

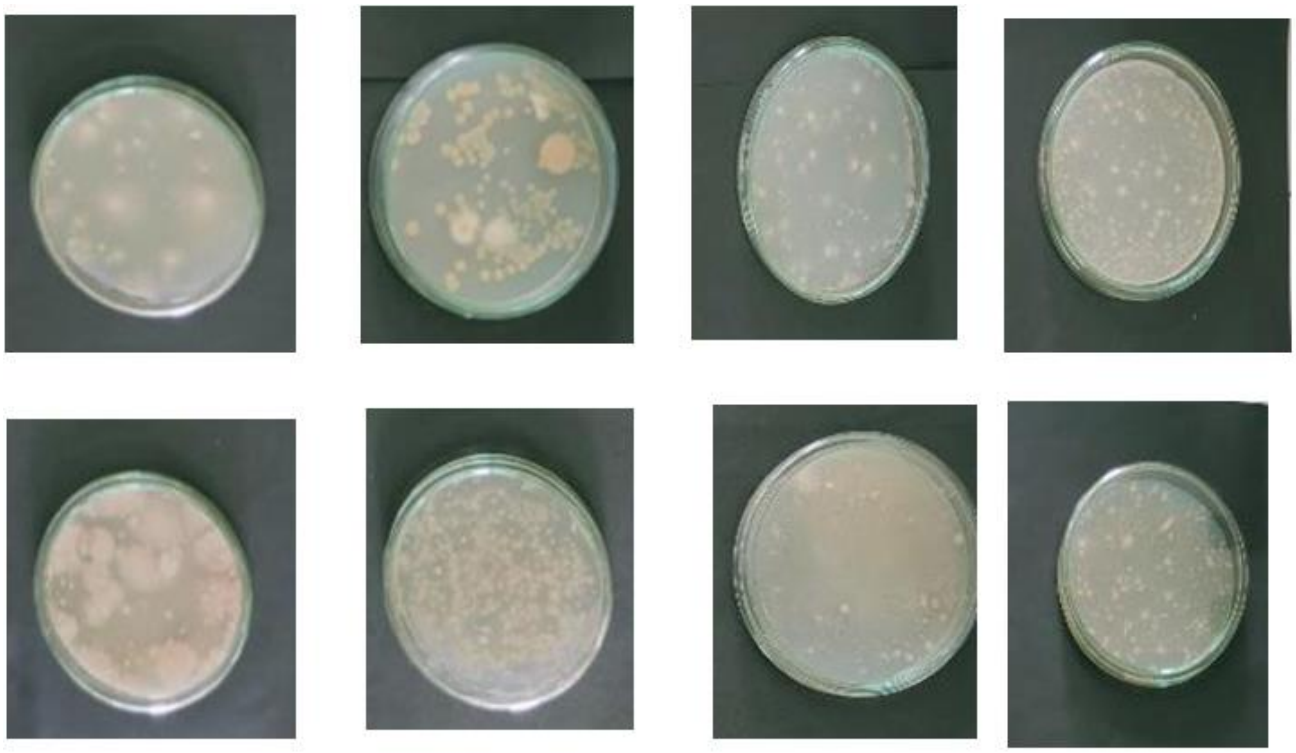

Fig14: pH samples: Serial Dilution Diversity Plate

3. Screening of the isolate for extracellular enzymes (Table3)

Table3:

Sl. No	ISOLATES	AMYLASE	CELLULASE	PECTINASE	PROTEASE	LIPASE
1.	VHNP-8	3mm	2mm	-	-	-
2.	S2EVW8	2mm	-	3mm	-	-
3.	S2EVW14	4mm	2mm	-	-	-
4.	pH4NS10	3mm	5mm	-	-	-
5.	pH4NS14	2mm	3mm	-	-	-
6.	pH4NS16	4mm	2mm	-	-	-
7.	pH4NS17		2mm	-	-	1mm
8.	pH5NS3	3mm	1mm	-	-	-
9.	pH5NS4	2mm	3mm	-	-	-
10.	pH5NS10	5mm	1mm	-	-	-
11.	pH5NS11	4mm	4mm	-	-	-
12.	pH5NS12	6mm	3mm	-	-	-
13.	pH5SC 3	2mm	5mm	-	-	-
14.	pH5SC 25	5mm	3mm	-	-	-
15.	pH5SC26	4mm		-	2mm	-
16.	pH5SC 28	3mm	6mm	-	-	-
17.	pH7NS 1	22mm	18mm	28mm	-	-
18.	pH7NS4	5mm	3mm	-	-	-
19.	pH7NS5	2mm	5mm	-	-	-
20.	pH7NS6	4mm	1mm	-	-	-
21.	pH7SC 1	-	3mm	4mm	-	-
22.	pH7SC 2	2mm		6mm	-	-
23.	pH7SC 18	-	4mm	7mm	-	-
24.	pH7SC 26	3mm	-	8mm	-	-
25.	pH4AS5	5mm	3mm	-	-	-
26.	pH4AS12	2mm	5mm	-	-	-
27.	pH4AS14	3mm	6mm	-	-	-
28.	pH4AS19	5mm	-	-	-	2mm

29.	pH4AS21	4mm	1mm	-	-	-
30.	pH4BS7	3mm	4mm	-	-	-
31.	pH4BS11	5mm	2mm	-	-	-
32.	pH4BS12	2mm	-	-	1mm	-
33.	pH5BS6	6mm	3mm	-	-	-
34.	pH5BS7	1mm	4mm	-	-	-
35.	pH5BS8	3mm	5mm	-	-	-
36.	pH5AS3	5mm	2mm	-	-	-
37.	pH7BS1	2mm	4mm	-	-	-
38.	pH7BS7	4mm	1mm	-	-	-
39.	pH7BS10	3mm	2mm	-	-	-
40.	pH7BS15	5mm	-	-	-	2mm
41.	pH7BS23	6mm	1mm	-	-	-
42.	pH7AS13	4mm	3mm	-	-	-
43.	pH4BS4	2mm	3mm	5mm	-	-
44.	pH4BS2	-	3mm	5mm	-	-
13.	pH 7NS4	3mm	12mm	-	10mm	-
45.	pH 7NS5	15mm	11mm	2mm	-	-
46.	pH 7NS6	14mm	11mm	25mm	-	-
47.	pH 7AS1	-	13mm	9mm	3mm	-
48.	pH 7AS3	11mm	10mm	11mm		-
49.	pH 7AS4	5mm	3mm	22mm		-
50.	pH 7NS3	-	13mm	10mm	2mm	-

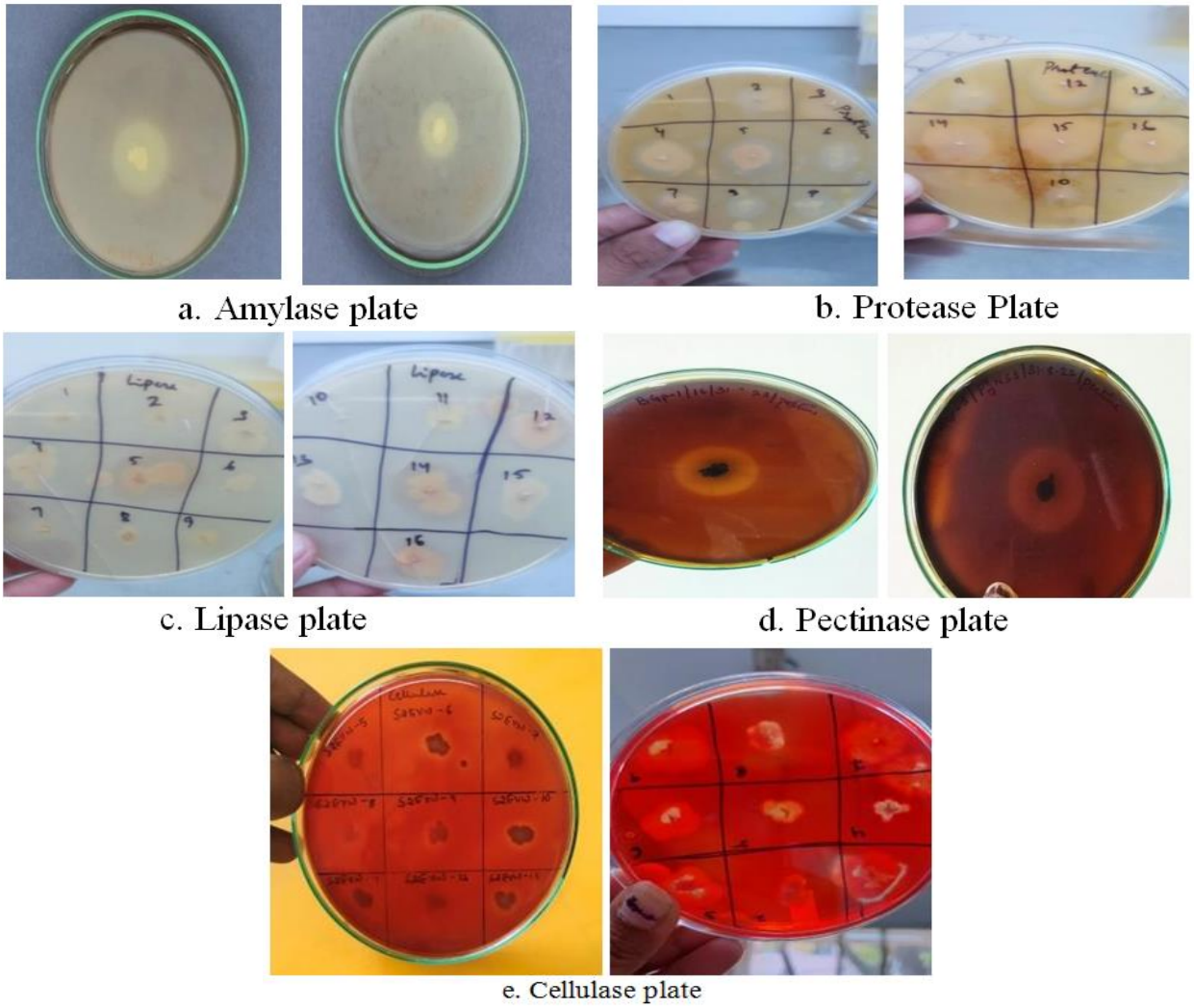

Fig15: Hydrolytic plate of Enzymes

The screening of microbial isolates for extracellular enzyme activity is a crucial step in understanding their potential applications. Among the isolates labeled as pH7NS1, pH7NS4,pH7NS5, pH7NS6, pH7AS1, pH7AS3, pH7AS4, and pH7NS3, eight isolates demonstrated positive results for three significant hydrolytic enzymes: amylase, protease, and pectinase. This multi-enzyme positive profile indicates the versatile enzymatic capabilities of these isolates.

The presence of amylase signifies the ability to hydrolyse starch into simpler sugars, showcasing the isolates' potential in various industrial applications, such as biofuel production and food processing. Protease activity suggests the capability to break down proteins, indicating potential applications in biotechnology, detergent formulation, and leather processing. Pectinase activity is essential for the breakdown of pectin, making these isolates promising candidates for applications in fruit and vegetable processing industries.

Moreover, the simultaneous detection of lipase and cellulase activities further amplifies the significance of these isolates. Lipases play a crucial role in lipid hydrolysis and are essential for various biotechnological applications, including biodiesel production and the food industry. The presence of cellulase activity suggests the ability to break down cellulose, a complex carbohydrate found in plant cell walls, making these isolates valuable for biomass conversion and biofuel production.

The positive screening results for multiple hydrolytic enzymes in these isolates, underlining their potential for diverse enzymatic activities, highlight their significance in biotechnological and industrial processes. Further characterization and exploration of these isolates may uncover additional enzymatic capabilities, paving the way for their utilization in various sectors of biotechnology and industrial production.

DISCUSSION

This study emphasizes the critical importance of microbial diversity and enzymatic activity in enhancing biogas production from organic waste. The isolated microbial consortia demonstrated a rich diversity, which is vital for the various stages of anaerobic digestion, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each of these stages is crucial for breaking down complex organic materials into biogas, with different microbial species specializing in different processes. For example, during hydrolysis, specific bacteria break down polysaccharides into simple sugars, which are then converted into organic acids during acidogenesis. The multifunctional enzymatic capabilities of these microbial isolates enhance the degradation of complex substrates like carbohydrates, proteins, and lipids, showcasing the adaptability of these consortia in handling diverse organic waste streams.

Identifying optimal conditions specifically a temperature of 35°C and a pH range of 6.5 to 7.5—was essential for maximizing microbial growth and biogas yield. Maintaining these conditions ensures that metabolic activities are efficient and consistent, which is crucial for stable anaerobic digestion processes. Moreover, the development of substrate-specific microbial communities tailored to the characteristics of different organic wastes can significantly boost production efficiency. By selecting and enriching microbial consortia that excel in degrading particular substrates, operators can optimize microbial interactions and metabolic pathways, leading to enhanced biogas production.

These findings present exciting prospects for industrial-scale anaerobic digestion systems, promoting sustainable waste management and renewable energy generation. The ability to effectively convert organic waste into biogas not only contributes to energy recovery but also reduces the environmental impact of waste disposal. By harnessing these specialized microbial communities, industries can improve the reliability and efficiency of biogas production, potentially reducing greenhouse gas emissions associated with waste management. Ultimately, this research lays the groundwork for innovative strategies to leverage microbial consortia for enhanced biogas production, providing a sustainable pathway for energy generation from organic waste.

CONCLUSION

In conclusion, this study provides valuable insights into the importance of microbial diversity and enzymatic potential in enhancing biogas production from organic waste. The identification of key microbial species and their multifunctional enzymatic activities underscores the necessity of developing substrate-specific microbial consortia to optimize anaerobic digestion processes. By establishing optimal conditions for microbial growth, this research paves the way for improved biogas yield and reactor stability, contributing to sustainable energy generation. Future research should aim to scale these findings to practical applications, exploring the potential of these microbial isolates in real-world anaerobic digestion systems, thereby enhancing organic waste management and renewable energy solutions.

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