International Journal of Scientific Research in Science and Technology
Available online at : www.ijsrst.com
OPEN ACCESS
Print ISSN: 2395-6011 | Online ISSN: 2395-602X
doi : https://doi.org/10.32628/IJSRST24112118

Isolation and Characterization of Microorganisms Involved in Biogas Production from Agricultural Waste

Siddharth, Shesh Vijay Sharma, V. K. Pandey

Department of Environmental Science, Veer Bahadur Singh Purvanchal University Jaunpur, Uttar Pradesh., India

Corresponding author Email id: drvivekpandey4@gmail.com

ARTICLEINFO

Article History:

Accepted: 12 April 2024

Published: 22 April 2024

Publication Issue :

Volume 11, Issue 2

March-April-2024

Page Number :

718-730

ABSTRACT

The study aimed to investigate the microbial composition involved in the biogas production process using a diverse range of substrates, including *Spinacia oleracea* (vegetable), banana peel, plant extract, watermelon residue, wheat straw and paddy straw, sourced from multiple locations in Jaunpur. Over a period of five weeks (30 days), the research employed established microbiological methodologies and customized anaerobic bio-digesters for the comprehensive analysis of the isolates and substrates to assess biogas generation. The evaluation revealed dynamic fluctuations in the digester temperature within the range of 30°C to 36°C, accompanied by initial pH levels ranging from 4.2 to 8.3, which subsequently decreased to pH 5-6 during and after the anaerobic digestion process. The identified anaerobic bacterial species encompassed *Staphylococcus sp., Micrococcus, Enterobacter, Escherichia, Citrobacter, Bacillus sp.*, and *Pseudomonas aeruginosa*. Furthermore, the findings demonstrated a hierarchy in the percentage of biogas yield from the substrates, with the following ranking: synergistic mixture > plant extract > banana > wheat straw > spinach > watermelon. Notable disparity in the volume of biogas produced was observed across different substrate treatments and digestion periods. The research underscored the pivotal role of methanogens and other auxiliary bacteria in the overall biogas production process. Additionally, the average pH levels were determined to range between 6.3 - 7.2 before and 5.0 - 6.2 during and after anaerobic digestion. The observed decline in pH during the anaerobic digestion process was associated with the production of metabolites such as acetate, hydrogen gas, carbon dioxide and other volatile fatty acids, exerting significant influence on the substrates within the digesters.

Keywords : Agricultural Wastes, Biogas Digester, Methanogenic bacteria. Biogas production, Anaerobic digestion, pH range, Isolation and characterization

I. INTRODUCTION

Anaerobic digestion is a biological process in which microorganisms decompose biodegradable organic material in the absence of oxygen. This process takes place in a sealed system known as an anaerobic bioreactor. Throughout anaerobic digestion, diverse types of microorganisms operate sequentially to break down intricate organic compounds, leading to the generation of biogas and a nutrient-rich digestate. The process usually entails four phases: breakdown, acidogenesis, acetogenesis and methanogenesis. During breakdown, complex organic molecules are broken down into simpler compounds by hydrolytic bacteria. In the acidogenesis stage, acidogenic bacteria convert these simpler compounds into volatile fatty acids, carbon dioxide, hydrogen, and ammonia. Following this, acetogenic bacteria further decompose the volatile fatty acids into acetate and hydrogen. Finally, methanogenic archaea transform the acetate, hydrogen and carbon dioxide into biogas, mainly consisting of methane and carbon dioxide. The biogas yielded through anaerobic digestion can be harnessed as a sustainable energy source for purposes such as electricity generation, heating and vehicle fuel. Furthermore, the nutrient-rich digestate left after the digestion process can function as a valuable organic fertilizer for agricultural uses. In anaerobic digestion offers a sustainable and eco-friendly approach to managing organic waste while generating renewable energy and nutrient-rich outputs.

Biogas stands out as a highly effective alternative energy source for several compelling reasons. Firstly, the process of biogas production actively contributes to the reduction of greenhouse gas emissions by capturing methane, a particularly potent greenhouse gas, which would otherwise be released during the decomposition of organic waste. This sustainable approach plays a crucial role in environmental preservation and climate change mitigation efforts. Moreover, biogas provides a sustainable and environmentally responsible method for managing organic waste. By utilizing organic waste as the primary feedstock for biogas production, it significantly reduces the dependence on landfills and incineration, offering a more eco-friendly waste management solution. The versatility of biogas further enhances its appeal as an alternative energy source. Its application spans across various energy requirements, including electricity generation, heating, and cooking, making it a versatile and adaptable option for diverse energy needs. Additionally, the abundant availability of input feedstocks such as agricultural waste, animal manure, sewage and food residuals underscore its potential as a decentralized energy solution. In conclusion, the effective utilization of biogas presents a renewable, environmentally friendly energy option. Its potential to reduce reliance on traditional fossil fuels and foster sustainable energy practices positions it as a significant player in the global transition towards cleaner, more sustainable energy sources. The escalating cost and scarcity of conventional petroleum products utilized for industrial, agricultural, and domestic purposes pose significant challenges, particularly in developing nations like Nigeria. This reality has fueled an intensified exploration for renewable and sustainable alternatives to fossil fuels

(Asikong et al., 2016). Biogas, a flammable gas generated through the anaerobic fermentation of organic materials, has emerged as a promising alternative energy source with potential applications in electricity generation, vehicle fueling and domestic cooking (Madu and Sodeinde, 2001). The anaerobic digestion process comprises four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Bitton, 2005; Gerardi, 2003; Nwuche and Ugoji, 2008, Nwuche, and Ugoji, 2010). These stages are facilitated by synergistic of microorganisms participating in syntrophic interrelations (Angelidaki, 1993).The microorganisms involved in each stage are categorized as hydrolyzers, acidogens, acetogens and methanogens, responsible for the breakdown of complex organic compounds, conversion of sugars and amino acids into carbon dioxide, hydrogen and organic acids, conversion of volatile fatty acids into acetate and hydrogen and the production of methane from acetate or hydrogen and carbon dioxide, respectively (Zieminski et al., 2012; Schink, 1997). The complex composition of these microbial synergistic is significantly influenced by various factors such as substrate composition, temperature, pH, and the design of the anaerobic digester (Roland et al., 2012). This study was initiated to isolate and characterize methanogenic microorganisms from vegetable (Spinacia oleracea), banana, plant extract and watermelon substrates, as well as from synergistic substrates sourced from different locations within Jaunpur. The focus lies in understanding the microbial dynamics and their role in the production of biogas from diverse organic feedstocks, laying the groundwork for the development of sustainable biogas production systems.

II. MATERIALS AND METHODS

Samples Collection

The substances utilized in this investigation comprised agricultural and vegetable remnants procured from various sites in Jaunpur. Furthermore, husks and manure were obtained from rural regions, Samples were gathered from various sites in Tanda, Uttar Pradesh, India. The collected specimens encompassed animal feces (gobar), agricultural remnants (such as sugarcane bagasse, corn stover, wheat straw, and rice straw), as well as by-products of food processing (including fruit pomace, grain processing remnants, and vegetable trimmings), while vegetable leftovers were acquired from households in native dwellings in both Prayagraj and Jaunpur. The resources utilized in this research included agricultural and vegetable waste collected from a variety of sites. Alongside this, husks and excrement were sourced from rural areas, and vegetable scraps were obtained from homes in local residences in Jaunpur.

Media

The study employed a diverse set of media to support its investigative processes. These included starch agar, which is commonly used for the cultivation of microorganisms due to its ability to support the growth of a wide range of organisms. Carboxymethyl cellulose agar, another vital medium utilized, is well-known for its role in determining the cellulase activity of microorganisms. Additionally, egg yolk agar, renowned for its use in the detection of lecithinase and lipase activities, was a crucial component of the investigative toolkit. Moreover, the utilization of nutrient-gelatin agar, prized for its capacity to promote the growth of microorganisms while also facilitating the liquefaction of gelatin, contributed significantly to the breadth of the study. Triple Sugar Iron Acquisition of notable mention is the inclusion of triple sugar Iron, which was sourced from the esteemed Hi Media Lab Pvt. Ltd, a renowned provider of high-quality laboratory solutions located in Mumbai, Maharashtra. This particular medium, characterized by its ability to differentiate enteric bacteria based on their ability to ferment lactose, sucrose, and glucose, played a pivotal role in the comprehensive investigative approach adopted in the study.

The judicious selection and procurement of these media from a reputable source underline the meticulous methodology underpinning the study, ensuring the reliability and accuracy of the experimental outcomes.

Formulation of Media and Reagents

The media and reagents utilized in the study were meticulously prepared and preserved instrict accordance with the specifications outlined by the manufacturer.

Preparation of the Raw Substrates for Microbial Screening

(i) Wheat and Paddy straw, banana peel, and watermelon residue: 20 g of the substrates were individually weighed and aseptically crushed into powder. It was transferred into 80ml of sterile distilled water contained in a 100 ml volumetric flask. The mixture was agitated and allowed to settle.

(ii) Spinach Leaves and roots (*Spinacia oleracea*) waste: The stalks of the ground vegetable were mixed with 20 milli liter aseptically transferred in a 80 ml of sterile distilled water contained in a 100 ml volumetric flask. The mixture was agitated and allowed to settle.

(iii) Substrate mixture: Equal quantity of all substrate was thoroughly grounded after which 20 milliliter was aseptically transferred into a 80 ml sterile distilled water in a100 ml volumetric flask. The mixture was agitated and allowed to settle.

Bacteria Isolation and Evaluation

The methodology proposed by Aderonke et al., 2017 was employed with minor adjustments. The prepared substrates were calibrated to a 10^{-4} dilution. Subsequently a 10-milliliter aliquot from the 10^{-6} dilution was measured into another volumetric flask containing 90 milliliters of sterile distilled water, resulting in a 10^{-2} dilution. Sequential dilutions were then conducted until reaching a dilution level of 10^{-9}. Sampling occurred every three days to ascertain the total heterotrophic counts.

For bacterial screening, dilutions ranging from 10^{-4} to 10^{-6} of the samples (following serial dilution) were plated on starch agar, carboxymethyl cellulose agar, egg yolk agar and nutrient-gelatin agar (media conducive to hydrolytic bacteria). The plates were incubated for 36 hours at 36°C. Enumeration of colony forming units per gram (CFU g-1) of bacterial growth within the range of 50 to 350 colonies was carried out. Subsequently, the formed colonies underwent subculture and identification utilizing cultural, morphological and biochemical methods.

Bacterial Isolate Characterization

Pure colonies were subjected to preliminary characterization based on their cultural and morphological attributes, following the guidelines established by Holt et al., 1994. The cell shape and arrangement characteristics were observed under a compound microscope subsequent to standard staining procedures. The determination of the isolates' gram characteristics was conducted using the 3% (w/v) KOH test as described by Gregersen, 1978. Additionally, biochemical tests including catalase, coagulase, lactose, glucose, sucrose, citrate, indole, H2S production, urease, gas, methyl red, Voges-proskauer, spore formation, oxidase, and motility were performed to further characterize the isolates.

Design of biodigester

In this current investigation, 425-liter rigid plastic vessels with transparent walls, featuring a diameter of 40cm and 30cm, were employed as bio-digesters. These containers were meticulously perforated with a hot iron rod at two specific locations, enabling the introduction of substrates through one inlet and the release of the produced biogas through the other outlet. The outlet of the biogas was linked to a 10-liter receptacle containing water, serving as an integral component of the system. This 10-liter container was

further interconnected with an additional 10-liter container, serving the purpose of accumulating the displaced water.

Preparation of Slurry and Loading of Digesters

Enhanced Experimentation and Methodology for Biogas Production

In the pursuit of optimizing biogas production efficiency, a systematic approach was undertaken to meticulously configure four distinct bio-digesters. Commencing with a precisely formulated composite boasting a 10% solid content, each bio-digester was meticulously loaded with slurry, reaching a volumetric capacity of 70%. Bio-digester- A, enriched with 2.0 kg of animal manure expertly amalgamated with 17 liters of water. Similarly, Bio-digester B, was enriched with 2.0 kg of crop residues, harmoniously blended with 17 liters of water. Bio-digester C, was the infusion of 2.0 kg of food processing residues intricately combined with 17 liters of water. Noteworthy is the amalgamation in Bio-digester D, comprising a 2.0 kg fusion of all previously mentioned samples blended with 17 liters of water.

An essential phase of inoculation involved the introduction of 0.30 kg of freshly cut cattle rumen into each bio-digester, serving as a catalyst to initiate the fermentation process. Over an observation period spanning 30 days, the fermentation process unfolded under meticulously maintained mesophilic conditions. The pivotal control of pH within each bio-digester - meticulously regulated within the critical range of 4.8-8 - played a crucial role in promoting the optimal biogas yield. The strategic precaution of shielding each bio-digester from sunlight, accomplished through the meticulous application of black polythene coverings, aimed to prevent undesirable external influences on the fermentation process. Integral to the operational methodology was the recurrent agitation of the content within each bio-digester, orchestrated twice daily during morning and evening intervals. This deliberate practice was designed to ensure the comprehensive and uniform fermentation of the medium, aligning with the established practices articulated by Aremu and Aggaray (2013), thus enhancing the robustness and efficiency of the biogas production process.

Assessment of Gas Generation

For precision in quantifying the biogas output from each bio-digester, the water displacement method served as the cornerstone of this meticulous evaluation process. This method facilitates the measurement of the biogas volume by gauging the displacement of water from one vessel to another. Consequently, the quantification encompassed meticulous tracking of the amount of biogas generated, coupled with regular measurements of temperature and pH at three-day intervals, aligning with the established methodologies advocated by Aremu and Aggaray (2013). This strategic approach ensured the systematic assessment of biogas production, with a keen eye on the evolution of temperature and pH dynamics within each bio-digester. Such comprehensive monitoring not only facilitated the accurate quantification of biogas yield but also provided invaluable insights into the variabilities and trends evolving throughout the biogas production process.

III.STATISTICAL ANALYSIS

Analysis Utilizing 2-Way ANOVA for Data Examination

The thorough scrutiny of data arising from the diverse treatments entailed the application of a potent 2-way analysis of variance (ANOVA). This resilient statistical approach facilitated the exploration of relationships between two distinct factors, delivering invaluable insights into the impacts of each treatment. Subsequent to the ANOVA analysis, the Fisher's Least Significant Difference (LSD) was utilized to discern the disparities among the averages derived from the

treatments. This method not only ensures the precision and accuracy of the findings but also provides a comprehensive comprehension of the discrepancies between the treatment means at a 5% significance level. To ensure the precise presentation of results, meticulous representation was upheld, expressing all data as means ± standard deviation. This methodology affords a comprehensive overview of the variability within the dataset, further reinforced by the fact that this representation was based on triplicate trials, thereby affirming the robustness and dependability of the statistical conclusions. The significance level conventionally applied for the Fisher's Least Significant Difference (LSD) method is 5%. This significance level is regularly selected to ascertain the statistical significance of differences observed between treatment means.

IV. RESULTS AND DISCUSSION

The fluctuations in hydrogen ion concentration of the distinct substrates treatments before, during, and after digestion

pH Variations of the Substrates

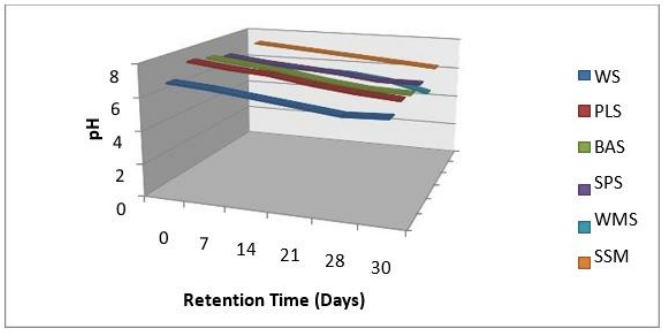


Figure 1: Changes in pH the digester content before, during and after anaerobic digestion

Key: WS = Wheat Straw, PLS = Plant substrate, BAS = Banana substrate, SPS =Spinach substrate, WMS = Watermelon substrate, SSM = Synergistic substrate mixture

The variation in the pH levels before, during, and after the anaerobic digestion process provides crucial insights into the complex biochemical transformations occurring within the substrates. The average pH was observed to span from 4.2 to 8.3 before digestion and from 5-6 during and after the anaerobic digestion process, indicating dynamic changes in hydrogen ion concentration throughout the stages of digestion.

This notable decrease in the hydrogen ion concentration during the anaerobic digestion is a significant finding, and its implications are multifaceted. It is linked to the formation of sulphide in the slurries due to the breakdown of biodegradable sulphur-containing organic and inorganic compounds. Additionally, the generation of fatty acids by acetogenic methanogens during the digestion process contributes to this reduction (Bagudo, 2007). Remarkably, the observed pH aligns with previous studies by Yerima et al., 2001, which highlight the optimal pH range (between 5 and 8) for methanogen growth during biogas production. Similarly, Garba and Sambo (1992) have reported that the optimum pH range for biogas production lies between 6 and 7. These findings reinforce the significance of pH regulation in biogas production processes. Furthermore, the decline in pH observed in this study can be attributed to the accumulation of metabolites such as acetate, hydrogen gas, carbon dioxide, and several volatile fatty acids like propionic, all of which have a substantial impact on the substrates in the digesters (Tsunatu et al., 2004). This underscores the intricate biochemical dynamics at play during anaerobic digestion processes. Moreover, research conducted by Anuputtikul and Rodtong (2004) supports the observed variations in pH, highlighting the meaningful correlation between the acidogenic and methanogenic phases when the pH remains within the range of 6.2 to 7.6 during the anaerobic digestion of substrates. These collective findings underscore the importance of pH management and

regulation in optimizing the efficiency and efficacy of anaerobic digestion processes, offering valuable insights for the advancement of biogas production technologies. The fluctuating pH levels observed during digestion can be ascribed to a network of interconnected elements:

1. Degradation of Organic and Inorganic Compounds: The decomposition of biodegradable organic and inorganic substances during digestion results in the release of diverse chemical by-products, exerting an impact on hydrogen ion concentration and subsequently shaping the pH milieu.

2. Microbial Activity: The metabolic activity of microorganisms, such as methanogens and acetogenic bacteria, yields by-products like fatty acids and gases including hydrogen and carbon dioxide. Subsequently, these by-products influence the chemical composition of the digestion environment, contributing to the oscillations in pH levels.

3. Generation of Sulphide: The genesis of sulphide stemming from the breakdown of sulphur-containing compounds can also contribute to pH dynamics during digestion. The production of sulphide is linked to the degradation of biodegradable sulphur-containing organic substances, inducing shifts in chemical equilibrium and affecting pH levels.

4. Accumulation of Metabolic By-products: The accrual of metabolic by-products, such as acetate and volatile fatty acids, directly impacts the acid-base equilibrium within the digestion process, precipitating pH fluctuations.

5. Microbial Growth Conditions: The growth and metabolic activity of specific microbial populations, such as methanogens, are responsive to pH levels. Consequently, the metabolic processes of these microorganisms can instigate pH variations within the digestion environment.

By comprehending and effectively addressing these factors, it becomes feasible to optimize the parameters for anaerobic digestion processes, ensuring the efficient and productive generation of biogas.

Temperature Variations of Substrates

Results of Temperature Variations in Anaerobic Digestion Process

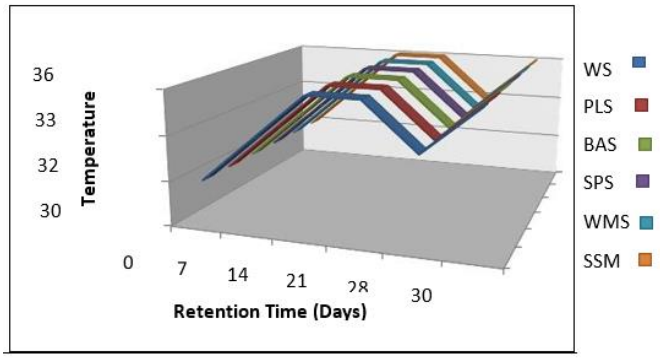


Figure 2: Temperature changes in the digester content before, during and after anaerobic digestion.

Key: WS = Wheat Straw : PLS = Plantain substrate: BAS = Banana substrate, SPS = Spinachsubstrate: WMS = Watermelon substrate: SSM = Synergistic substrate mixture

Variations in temperature wield significant influence over biogas production by directly impacting microbial activity, enzymatic reactions, and metabolic processes within the anaerobic digestion system. Here's a comprehensive delineation of how temperature fluctuations can affect biogas production:

1. Microbial Activity: The temperature sensitivity of microorganisms involved in biogas production, such as methanogenic archaea and acidogenic bacteria, is paramount. Different temperature ranges favor the growth and activity of specific microbial populations. For instance, mesophilic bacteria thrive at moderate temperatures (approximately 20°C to 45°C), while thermophilic bacteria prefer elevated temperatures (around 50°C to 60°C). Fluctuations in temperature can provoke shifts in microbial communities, directly impacting the efficiency of biogas production.

2. Enzymatic Reactions: Temperature significantly influences the enzymatic activities of microorganisms engaged in anaerobic digestion. Enzymes responsible for decomposing organic materials into volatile fatty acids and eventually biogas exhibit varying levels of activity at different temperatures. Consequently, temperature oscillations can alter the rates of these enzymatic reactions, thereby affecting the overall biogas production process.

3. Biogas Composition: Temperature variations can influentially mold the composition of biogas. Higher temperatures can favor the production of biogas with a higher methane content, while lower temperatures may lead to a decrease in methane production. Given that methane is the primary component of biogas and a significant energy source, this influence is particularly noteworthy.

4. Rate of Digestion: The rate at which organic matter is broken down and transformed into biogas, known as the digestion rate, is directly impacted by temperature. Elevated temperatures generally result in faster digestion rates, leading to more rapid biogas production.

5. Process Stability: Fluctuations in temperature can profoundly influence the overall stability of the anaerobic digestion process. Sudden temperature changes can disrupt the microbial ecosystem within the digester, potentially resulting in process instability, longer lag phases, and reduced biogas yields.

By comprehending the intricate relationship between temperature and biogas production, it becomes feasible to optimize the temperature conditions within the anaerobic digester to enhance biogas yield and quality. This may entail implementing temperature control measures, such as insulation, heating, or cooling, to uphold the ideal temperature range for microbial activity and biogas production efficiency. Substantial variations in temperature were observed across different substrate treatments both before and during the anaerobic digestion process. Average Temperatures throughout the anaerobic digestion process, the mean temperatures within the digester ranged between 30°C and 36°C, showcasing a significant deviation from the ambient temperature registered prior to the commencement of digestion. Research Consistency The findings align with studies by Surnaso et al. (2010), Aremu and Agarry (2012), and El-Mashed et al. (2003) focusing on biogas production using anaerobic biodigesters with cassava starch effluent, reinforcing the reliability of the observed temperature trends. Optimal Temperature Range Adelekan and Bamgboye (2009) reported that digestion temperatures within the range of 28°C to 39°C are favorable for hemophilic bacterial populations and are well-tolerated by anaerobic bacteria, indicating an ideal temperature range for maximizing biogas production efficiency implications The controlled temperature conditions play a crucial role in the biogas production process by influencing the microbial activity and metabolic processes, underscoring the importance of maintaining suitable temperatures for efficient anaerobic digestion.

Morphological and Biochemical Characterization of the Isolated Microbial Isolates

Table 1. Morphological characteristics of bacteria isolates obtained from the substrates

Substrate	Isolate	Colony Morphology	Cell Morphology
Banana	BA1	White to Yellow	Oval
	BA2	Golden-brown	Rod

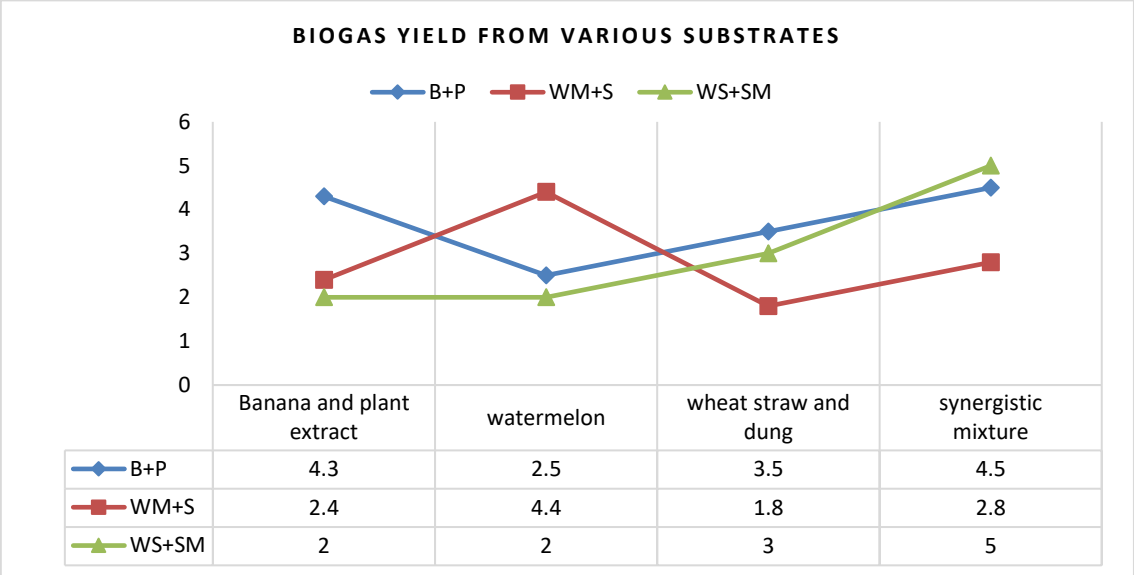
Wheat , paddy straw	WSI	Grey	Rod
	PS2	Off-white	Rod
Watermelon Residues	WM1	Rainbow	Rod
	WM2	Golden-brown	Rod
Spinach leaves	SP1	Semi-transparent	Rod
	SP2	Grey	Rod
Synergistic	SM1	Blue-green	Rod
	SM2	Golden-brown	Rod

Table 2. Biochemical characterization of the bacteria isolates obtained from the substrates

Substrate/Test	Banana Rind		Wheat, Paddy straw		Watermelon residues		Spinach Leaves and stems		Synergistic Mixture	
	BA^1	BA^2	WU^1	PU^2	WM^1	WM^2	SP^1	SP^2	SM^1	SM^2
Catalase	+	-	-	-	-	+	-	-	+	-
Coagulase	-	-	+	-	-	-	+	-	+	-
Lactose	-	-	+	-	-	-	+	-	-	-
Glucose	-	-	+	-	-	-	+	-	-	-
Sucrose	-	-	+	-	-	-	+	-	-	-
Citrate	-	-	+	-	+	-	-	+	-	-
Indole	-	-	+	-	+	-	-	+	-	-
H2S	-	-	+	-	+	-	-	+	-	-
Urease	-	-	+	-	-	-	-	-	-	-
Gas	-	-	-	-	-	-	+	-	-	-
MR	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-
Spore	-	-	-	-	+	-	-	+	+	-
Oxidase	-	-	+	+	-	-	-	-	-	-
Bacterial Isolated	S	M	E	E	C	M	BF	ES	PA	MS

Key: (+) = Positive, (-) = Negative, S = *Staphylococcus sp*, M = *Micrococcus spp*, ES = *Enterobacter spp*, Ec = *Escherichia,* C = *Citrobacter sp.* , B = *Bacillus spp.* , Ps = *Pseudomonas aeruginosa*, B = Banana, WS = Wheat straw , WM = Watermelon, SP = Spinach, , CS- Synergistic spp.

Biogas Yield from Substrates :



	Banana and plant extract	watermelon	wheat straw and dung	synergistic mixture
B+P	4.3	2.5	3.5	4.5
WM+S	2.4	4.4	1.8	2.8
WS+SM	2	2	3	5

Figure 3: Volume of biogas produced from each substrate

The biogas yield varied significantly between the substrate treatments and the digestion intervals (days). Maximum biogas yield was obtained within 21 days (3rd week) of digestion for the banana, watermelon, wheat and Paddy straw and the synergistic substrate mixtures.

The biogas production varied considerably across the substrate treatments and digestion intervals. The maximum biogas yield was attained within 21 days (3rd week) of digestion for the banana, watermelon, wheat straw, and the combined substrate mixtures. Conversely, the plant extract and spinach substrates exhibited their peak yield at 28 days (4th week) over the 30-day digestion period. As per Jaenicke et al. (2011), methanogens commonly dominate methanogenic sub-communities in diverse anaerobic digester systems, and this study has revealed the critical role of different methanogenic sub-communities in the anaerobic degradation process for methane synthesis (Dhevagi et al., 1992). These findings align with Demirel and Scherer (2008), who affirmed that the anaerobic transformation of organic wastes involves various bacterial groups such as hydrolyzing, acidifying, acetogenic, and methanogenic bacteria, ultimately leading to the production of CO2 and methane as the primary products of the digestion process. The biogas volume peaks between 21 – 28 days result from the acclimatization of biogas-producing microorganisms following the hydrolysis of substrates by hydrolyzing organisms. Subsequently, the decline in biogas volume is attributed to reduced activities, primarily of methanogens, as well as other factors such as pH decrease and temperature increase, deposition of microbial metabolites, gradual depletion of available nutrients from the substrates, and the proliferation of organisms utilizing the by-products of their activities (Asikong et al., 2016). The highest percentage yield of biogas was observed in the co-digestion of all substrates, possibly due to the diverse proximate composition of the substrates, positive synergistic effects of co-digestion providing balanced nutrients, increased buffering capacity, and reduced impact of toxic compounds (Mata-Alvarez et al., 2000; Li et al., 2009; Jianzheng et al., 2011). Synergistic Effects at Certain substrate combinations can elicit synergistic interactions, where the presence of multiple substrates enhances the overall methane production capacity of the anaerobic digestion system. This can

result in increased biogas yield compared to individual substrates. By combining different substrates, the system's buffering capacity may be improved, leading to better pH regulation within the digester.

This can create more favorable conditions for the microbial communities responsible for biogas production, ultimately contributing to higher biogas yields. Mitigation of Inhibitory Compounds Some substrate mixtures have the potential to mitigate the presence of inhibitory compounds that can hamper microbial activity and biogas production. This mitigation effect can lead to improved overall biogas yield. Improved Microbial diversity combining diverse substrates can support a wider array of microbial species with complementary metabolic capabilities, potentially enhancing the efficiency of the anaerobic digestion process and promoting higher biogas yields, substrate mixtures can influence biogas yield by optimizing nutrient availability, inducing synergistic effects, enhancing buffering capacity, mitigating inhibitory compounds, and fostering microbial diversity.

V. CONCLUSION

.

The research work highlighted the complex dynamics of anaerobic digestion processes and their vital role in biogas production. The diverse microbial composition and fluctuating pH levels underscored the intricate biochemical transformations occurring within the substrates. This emphasizes the significance of precise pH regulation and comprehensive research validates the viability of employing organic waste materials including wheat straw, paddy straw, banana peels, plant substrates, watermelon residues and spinach stalks for biogas production, utilizing cellulolytic, lipolytic, proteolytic, and amylolectic microorganisms. These specific microorganisms play a crucial role in enabling the conversion of organic waste into biogas, highlighting the potential for utilizing these substrates in sustainable biogas generation practices. This study underscores the significant potential of organic waste materials as valuable resources for biogas production through the metabolic activities of specific microorganisms. By identifying the role of cellulolytic, lipolytic, proteolytic, and amylolectic microorganisms in facilitating biogas generation from diverse organic substrates, this research contributes to the advancement of sustainable practices in biogas production. The findings advocate for the efficient utilization of these organic waste sources to promote eco friendly and renewable biogas production processes which is understanding of the interconnected elements involved in anaerobic digestion. The findings provide valuable insights for optimizing biogas production technologies and advancing sustainable energy production methods.

VI. REFERENCES

[1]. Adelekan, B. and Bamgboye, A. (2009). Comparison of the biogas productivity of cassava peels in selected ratios with major livestock waste types. African Journal of Agricultural Research, 4, 571-577.

[2]. Aderonke, A., Wasiu, A. and Moses, O. (2017). Isolation and Characterization of Hydrolytically Proficient Microorganisms during Anaerobic Digestion and Biogas Production from Cow Dung and Rice Husk. Journal of Natural Sciences Research, 7(5), 20-30.

[3]. Angelidaki, I., Ellegaard, L. and Ahring, B.K.A. (1993). Mathematical model for dynamic simulation of anaerobic digestion of complex substrates with a focus on ammonia inhibition. Biotechnology and Bioengineering, 42, 159–166.

[4]. Anuputtikul, W. and Rodtong, S. (2004). Investigation of the potential production of biogas from cassava tubers. 70, 12-23.

[5]. Aremu, M. and Agarry, S. (2012). Comparative study of biogas production from cow dung and pig dung under mesophilic conditions.

International Referred Journal of Engineering and Science, 4,16-21.

[6]. Asikong, B.E., Idire, S.O. and Tiku, D.R. (2016). Microbial Consortia Associated with Biogas Production Using Vegetable Wastes (Telfairia occidentalis), Banana Peels, and Pig Dung as Substrates. British Microbiology Research Journal. 16(3): 1-12.

[7]. Baba, S., Shedu, U., Abubakar, I. and Nasir, I. (2012). Anaerobic digestion of cow dung for biogas production. ARPN Journal of Engineering and Applied Sciences, 7, 169-172.

[8]. Bagudo, B.U. (2007). "Studies on Biogas Production from Selected Agricultural Waste Materials" A Ph.D Thesis submitted to Postgraduate School Usmanu Danfodiyo University Sokoto.

[9]. Bitton, G. (2005). Wastewater Microbiology. 3rd ed. Hoboken, New Jersey: John Wiley & Sons Inc., 345–369.

[10]. Demirel, B. and Scherer, P. (2008). Roles of Acetotrophic and Hydrogenotrophic Methanogens during Anaerobic Conversion of Biomass to Methane. Review of Environmental Science and Technology, 7, 73-901.

[11]. Dhevagi, P., Ramasamy, R. and Oblisami, G. (1992). Biological Nitrogen Fixation and Biogas Technology. Bioresource Technology, 6, 14-23.

[12]. El-Mashed, H., Zeeman, G., Loon, W., Bot, G. and Lettinga G. (2003). Effect of Temperature Fluctuation on Thermophilic Anaerobic Digestion of Cattle Manure. Bioresource Technology, 95, 213-221.

[13]. Ezeohu, S., Dioha, I. and Eboatu, N. (2005). Daily Biogas Production from Different Wastes and Identification of Methanogenic Bacteria Involved. Nigerian Journal of Solar Energy, 15, 80-85.

[14]. Garba, B. and Sambo, A.S. (1992). "Effect of Operating Parameters on Biogas Production Rate". Nigerian Journal of Renewable Energy, 3(1 and 2), 36-44.

[15]. Gerardi, M.H. (2003). Wastewater Microbiology series: The Microbiology of Anaerobic Digesters. New York: John Wiley and Sons Inc.

[16]. Gopinath, L.R., Merlin C.P., Mahesh, K., Bhuvaneswari, R. and Divya, D. (2014). Identification and Evaluation of Effective Bacterial Synergistics for Efficient Biogas Production. IOSR Journal of Environmental Science, Toxicology and Food Technology, 8(3), 80-86.

[17]. Gregersen, T. (1978). Rapid Method for Distinction of Gram-negative from Gram-positive Bacteria. Applied Micobiol. Biotechnol, 5(2), 123-127.

[18]. Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T., Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology, 9th Edition. (H. W. R, Ed.).

[19]. Williams & Wilkins Jaenicke, S., Zakzewski, M., Ander, C. and Bekel, T. (2011). Comparative and Joint Analysis of Two Metagenomic Data Sets from a Biogas Fermenter by 454-pyrosequencing. Plus One, 6(1), 14-19.

[20]. Jianzheng, L., Ajay, K., Junguo, H., Qiaoying, B., Sheng, C. and Peng, W. (2011). Assessment of the Effects of Dry Anaerobic Co-digestion of Cow Dung with Wastewater Sludge on Biogas Yield and Biodegradability. Int. J. Phys. Sci. 6(15), 3679-3688.

[21]. Li, X., Li, L., Zheng, M., Fu, G. and Lar, J. (2009). Anaerobic Co-digestion of Cattle Manure with Corn Stover Pretreated by Sodium Hydroxide for Efficient Biogas Production. Energ. Fuel, 23, 4635-4639.

[22]. Madu, C. and Sodeinde, O. (2001). Relevance of biomass in the sustainable energy development in Nigeria. Proceedings of the national engineering conference and annual general meeting of the Nigerian Society of Engineers. pp. 220-227

[23]. Mata-Alvarez, J., Mace, S. and Labres, P. (2000). Anaerobic digestion of organic solid wastes. An

overview of research achievements and perspectives. Rev. Paper Bio-resource Technol. 74:3-16.

[24]. Nwuche, C. O. and Ugoji E. O. (2008). Effects of heavy metal pollution on the soil microbial activity. International Journal of Environmental Science and Technology, 5(3): 409-414.

[25]. Nwuche, C. O. and Ugoji E. O. (2010). Effect of co-existing plant species on soil microbial activity under heavy metal stress. International Journal of Environmental Science and Technology. 7(4): 697-704.

[26]. Roland, W., Etelka, K., Gergely, M., Zoltan, B., Gabor, R. and Kornel, L.K. (2012). Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. Biotechnology for Biofuels. 5(41): 231 – 241.

[27]. Schink, B. (1997). Energetics of syntrophic cooperation in methanogenic degradation.

[28]. Microbiology and Molecular Biology Reviews. 61(2): 262–280.

[29]. Surnaso, S., Siswo, S. and Budiyono, Y. (2010). Biogas production using anaerobic biodigester from cassava starch effluent. International Journal of Science and Engineering. 1(2):33-37.

[30]. Tsunatu, D., Yavini, U., Usman, H., Taura, N. and James, M. (2014). Comparative study of mesophilic biogas production potentials of selected agro-wastes. International Journal of Engineering and Science. 3(2):1-6.

[31]. Yerima, M.B, Rahman A.T.M.F. and Ekwenchi, M.M. (2001).”Effect of Buffering on Biogas Fermentation of Chicken Droppings” Nigerian Journal of Renewable Energy, 9(1 and 2): 47- 49

[32]. Ziemiński, K. and Magdalena, F. (2012). Methane fermentation process as anaerobic digestion of biomass: Transformations, stages and microorganisms. African Journal of Biotechnology. 11(18): 4127-4139.