ORIGINAL ARTICLE - OPEN ACCESS

World Journal of Multidisciplinary Research and Reports DOI: http://dx.doi.org/10.51521/WJMRR.2025.1101

Contents lists available at bostonsciencepublishing.us

World Journal of Multidisciplinary Research and Reports

A Metagenomic Approach to Microbiota Occurrence in Mixed Waste and Their Methanogenic, Lignolytic and Indutrial Pottensials

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ARTICLE INFO

Article history:
Received 28 December 2024
Revised 17 January 2025
Accepted 22 January 2025
Published 29 January 2025

KEYWORDS:
Mixed-waste,
Methanogens,
Metagenomic analysis,
Biogas,
Molecular characterization,
and Potentials.

ABSTRACT

Background: This work demonstrated the requisite necessity of microorganisms derived from waste feed-stock in environmental, industrial and metabolic processes by confirming their degradative and gas producing capacity. Methanogens and other logninolytic microorganisms were isolated and identified. This study aimed at isolation and identification using molecular characterization and metagenomic approach from waste derived microbiome and their potentials. The organisms of interest are the ligninolytic and gas producing microbes (methanogens). Samples (Fruit and vegetable residuez+wood waste+animal waste) were collected from AMAC Relocation market, Lugbe, F.C.T. and analysed at the International Institute for Tropical Agriculture ((IITA) Oyo state, the 16SrNA and mcrA encoding the alpha ά unit of methyl co-enzyme reductase genes as markers was used for the detection of phylogenic analysis of anaerobic bacteria community, a metagenomic approach was also carried out for identification. Mineral solution containing $CuSO_4$ was used to identify cellulose degraders. Their degradative ability was confirmed by the formation of a clear zone. Twenty-four anaeroboic bacteria were isolated out of which six bacteria species were identified as *Methanothermobacter thermautotrophicus* NPK, *Candidatus methanoperedenaceae* GB37, *Methanothermobacter thermautotrophicus* CCSD, *Methanobacteriaceae archaeon* KR-H07 A06, *Methanobacteriaceae archaeon* RK-H07, and *Candidatus methanoperedenaceae* GB50. Biogas yield increased from 3.0 to 8.0mL in the non-inoculated sample while the inoculated ranged from 5.0 - 24.0mL on day 30 with higher gas yield in the inoculated compared to the non-inoculated sample, gas yield increased from 0mL - 5mL on day 60 in the inoculated as compared to the non-inoculated. All the methanogens identified produce gas in their metabolic processes of breaking down the complex polymeric substances from the waste derived feedstock to smaller monomeric units. A total of 180 bacterial species were found in the non-inoculated sample of which *Bacteroidota* (26.91%), *Fermicutes* (25.87%) and *Proteobacteria* (7.24%) predominated. Seventy (70.0%) in the non-inoculated were anaerobes while 30% were aerobes. *Clostridium* were the highest having (28.8%) amongst the anaerobic bacterial community while *Acidobacteriota* was the least (0.01%). *Eschericia coli* was the highest aerobic species having (14.5%) while *Streptomyces* was the least (0.01%). The ability to produce ligninolytic enzyme makes some microorganisms suitable for application in many industries, including the production of biofuels, antibiotics, bioremediation, biomedical applications as biosensors.

Introduction

Globally, agriculture produces an average of 23.7 million tons of food every day while also producing 21% of the world's greenhouse gas emissions (Duque-Acevedo *et al.,* 2020). Field waste are present after harvesting crops and include stems, leaves, stalks, and waste after processing crops includes seeds, peels, husks. (Rao P *et al*., 2019). Microorganisms play an important role in reducing this waste to value added products; methanogens play integral role in such functions. Microorganisms are of great benefits to human, as they have a wide range of potentials in the human body as probiotics. They are also used in industrial processes and waste management strategies. Ruminant animals such as cows, sheep, and deer feed primarily on grass and leaves as they have special digestive system. This system rely on microbes to help them break down cellulose from plant matter and other cellulolytic foods, since vertebrates don not have the necessary enzymes to do that themselves, methanogens breakdown this cellulose and release methane gas into the environment (Jayasekara and Ratnayake, 2019). Methanogens can be used to digest organic matter and thus produce biogas. In sewage treatment plant for example, anaerobic digestion of solid waste is carried out by methanogens (Lei *et al*., 2018). The gasses produced by this process mainly methane and carbon dioxide are harmful to the environment (Augelletti, and Annesini, 2017). Biogas can be used as fuel for cooking or to power lighting and other devices. This use is seen especially in rural areas that have a lot of cattle that produce plenty raw materials as cowdung is one of them. Enzymes produced by microorganisms have more demand in the recent years due to its numerous applications in biotechnology. Some lignolytic microorganisms be it bacteria, fungi or algae produce enzymes

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such as Manganese peroxidase which has potential biotechnological as well as bioremediation application such as production of natural aromatic flavor, decolouration of various industrial waste water (Bilal *et al.,* 2017). Biodegrading microorganisms have demonstrated great potentials in the degradation of natural rubber, oxidizing phenolic and non-phenolic compounds, dye decolourization, Cosmetic industry, lignin degradation, bioethanol production and diagnostic kits. (Bilal *et al.,* 2017, Asgher *et al*., 2014).

MATERIALS AND METHODS

Description of Study Location

Samples were collected from two sampling points;

1. Cow Dung, Mixed fruit and vegetable residue and Saw Dust from AMAC Market and Cattle Ranch, Lugbe, Federal Capital Territory.
2. Mixed fruit and Vegetable residue from Odi and Yenagoa environs, Bayelsa state.
3. Cow dung from slaughter house, Ibadan, Oyo state.

Description of study location 1 One (1).

The Federal Capital Territory is the capital of Nigeria, Abuja. It is within the middle belt region of the country with a total land area of 7, 315 KM^2 (2,824 sq mi), a total population of 2, 238,800 and a density of 190/KM^2 (500/sq mi). It has a coordinate of 9^0 4'0"N $7^0$29'0"E. The AMAC, Lugbe Relocation Market is located along airport road in the upcoming Lugbe Area of the F.C.T. The Ultra-Modern Market boast over 900 types of shops including Warehouses, Cold Rooms, and Meat shops, Slaughter House, Wood Shade and Cattle Ranch from where the samples were collected. Plate 3.1 and 3.2 shows the description of the two sampling points.

Description of sampling point one (1)

Samples were collected at the Cattle Ranch and the AMAC Lugbe relocation Market. Animal dung was collected from the cattle ranch (Cow droppings) in the early hours of the day from the cattle. Fruit and vegetable residue was also collected from house refuse at life camp, Abuja.

Description of Study location (2).

Yenagoa is a Local Government in Bayelsa State, Nigeria 'and is the Capital of Bayelsa State in the Niger Delta regions of Nigeria. Its headquarters are in the town of Yenagoa (the State capital) in the South of the area at a latitude of 40 55'29"N60 15'15'51"E and it has a coordinate; 50 02'N60 20'E. It has a total area of 1,698 Km2 (656 sq mi). Tombia Market is located at Tombia round about Yenagoa, Bayela State.

Description of sampling point 2.

Fruits and vegetable residue was collected from their vendors at their selling tent in the Market. And also, sawdust was collected at the saw mill while Cowdung was also collected from the residential area of cattle rearers in Odi community of Bayelsa State.

STUDY DESIGN AND SCOPE

Scope of Study

This study focuses on the microbiota in mixed-waste (fruits and vegetable waste, saw-dust and cow dung waste) and the effect of seeding using these microbiotas on biogas yield.

Study Design.

In this study, five reactors were used (Anaerobic digesters) for the three set up which include

1. Control
2. Mixed substrate with cow dung, mixed fruit and vegetable residue and Saw dust.
3. Single substrate

The process efficiency, microbial structure,digest morphology and biogas yield was checked for each of the set up and comparisons were made.

Sampling process and sample collection

Sampling Process

Cow dung sample was collected at Tipa garage airport road, Ahmadu Bello Way, Lugbe, Abuja. Other non-wood substrate as mixed-fruit and vegetable

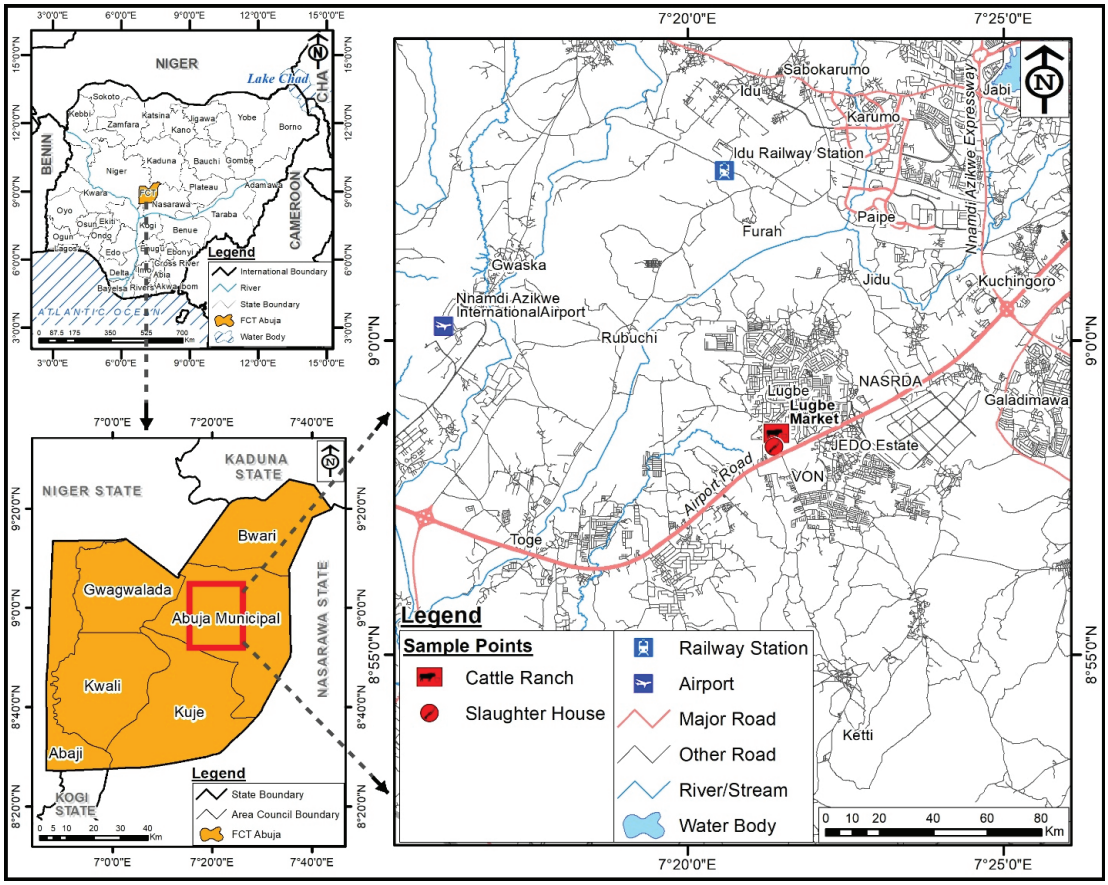

Figure 1: Map of Abuja Municipal Area Council showing Sample Points (Cattle Ranch and Slaughter House, Lugbe Market), Abuja.

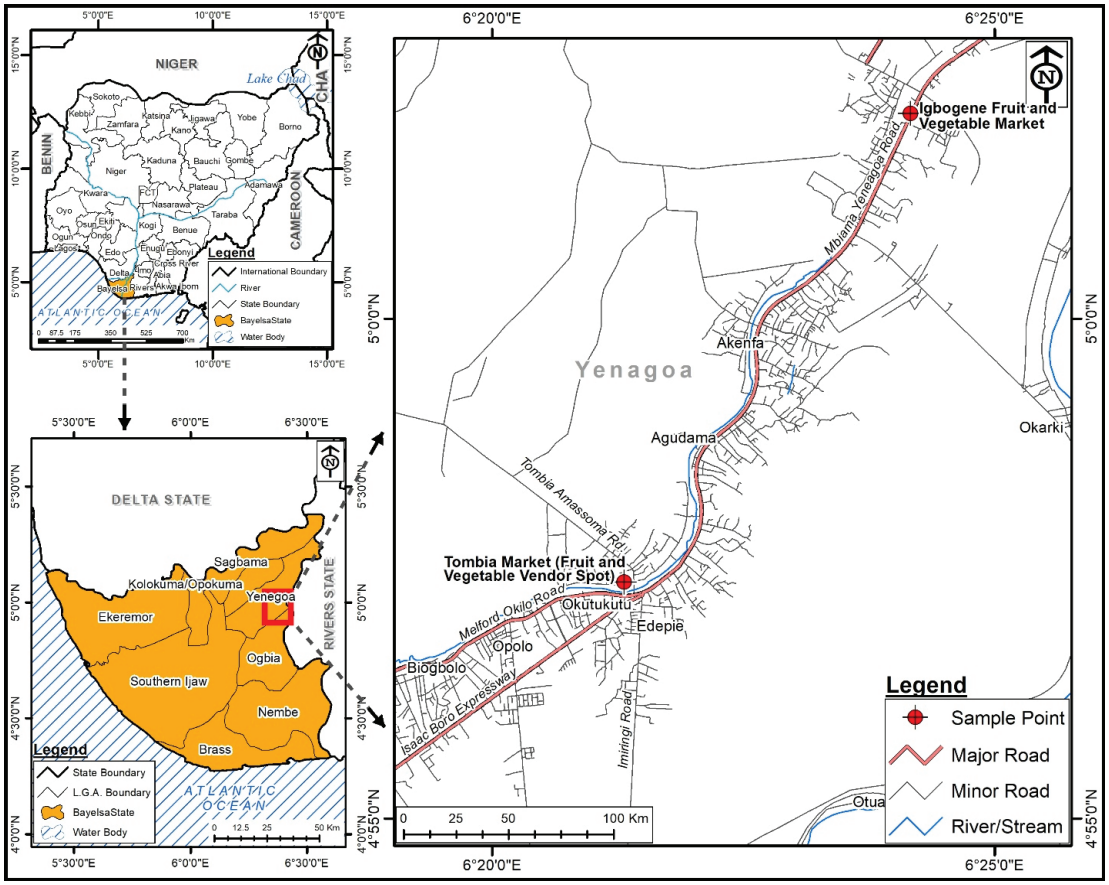

Figure 2: Map of Yenagoa showing Sample Points (Fruit Markets).

waste were collected from homes in life camp areas of F.C.T. At least 100kg of each of the samples was collected.

Determination of microbiological parameters

Media Used for Microbiological Analysis

The media that were used in the study are, Nutrients agar, Minimal Salt Media (MSML) PDA (Potatoe Dextrose Agar) and MSA (Mannitol Salt Agar) using the method of Akintokun and Wasiu Abibu 2017.

For bacterial screening

Dilutions 10^{-1}, 10^{3}, 10^{5} and 10^{-7} of the samples (upon serial dilution) were plated on Nutrient agar and nutrient-gelatin agar (hydrolytic bacteria media). The plates were incubated for 48hrs and colonies were observed, there was no significant number of colonies after 48hrs, therefore the plates were further incubated for 48 more hrs (96 hrs in all) which showed a high number of colonies with distinct clear zones. (Khan *et al*., 2013). Colonies on plates were counted, colony forming units per gram (CFU g-1) of bacterial growth between 30 - 300 colonies were enumerated. The colonies formed were further sub cultured on nutrient agar and minimal salt medium, colonies were observed after gram staining using cultural, morphological and biochemical methods. Scanning electron microscope (Hitachi S 5200) was used for microscopy.

Anaerobic Count (TVC)

The colonies formed were sub cultured and identified using a microscope as described (Ellis *et al*., 2007). Dilutions 3, 5 and 6 were plated on the Msml media using spread plate method. Plates were incubated at 28^{0}C for 48hrs. Colonies on plate were counted, colonies with clear zones around them were selected and sub cultured on Msml media for purification.

Media Preparation

Nutrient agar used for primary isolation was prepared according to manufacturer's instruction and sterilized by autoclaving at 121^{0}C for 15 minutes. Media was plated on Petri-dishes and allowed to solidify before use.

Minimal salt media (Mslm) was prepared for purification of isolates (Methanogens) using the following components with their concentrations; KH_2PO_4 (3.09), K_2HPO_4 (1.26), $MgCl_2.6H_2O$ (0.19), $CaCl_2.2H_2O$ (0.21), NH_4Cl (13.08), NaCl (10.27), Sodium acetate (20.0), resazurin (0.003), Na_2CO_3 (1.51) and $Na_2S.9H_2O$ (1.78); 10mL/litre each of the trace element solution.

Laboratory Sample Preparation

1g of each of the sample was dissolved using a votex mixer in 100mls of distilled water. Serial dilution was carried out by taking 1ml into the first tube containing 9mls of distilled water coupled with mixing using a vortex mixer to homogenize the suspension. Dilutions 10^{1}, 10^{3}, 10^{5} and 10^{7} was inoculated on the plates containing nutrient agar media.

Biochemical Test for the Identification of Biodegrading Bacteria (ligninolytic)

Oxidase Test

Tested bacteria colonies were smeared in the filter paper previously saturated with freshly prepared oxidase reagent. Positive oxidase test were recorded as the development of a blue purple color within 10 seconds.

Catalase Test

Hydrogen peroxide was added to the microscopic slide containing the isolates, this was done to induce catalase activity. The appearance of bubbles on the slide indicate catalase activity and suggest the presence of catalase positive-bacteria

Identification of Methanogens

As methanogenic bacteria are extremely sensitive towards oxygen, they require reducing conditions, and pre reduced media conditions, these are essential for their growth and development as described by Derza *et al*., 2015 where microbial were grown on serum vials or Basal medium (35.5mL) stoppered with aluminum seal**.** In this work, anaerobic jar was used to grow methanogenic cells.

Methanogenic Sample Enrichment

Samples were inoculated into sodium bicarbonate media incubated at 37^{0} C for 15days. The growth of methanogens was observed using morphological and Biochemical test**.**

Preparatory set up of digesters with the thermostat regulator for gas production carried out at Microbiology department, University of Ibadan.

Screening of isolate for lignolytic ability

Isolates were screened for lignolytic property (mineral solution, 2.5g\L, KH2PO41 g\L, $Nacl_2$, 2.5g\L.MgS0.7H20 0.2g\L, $CaCl_2$ 0.1 g\L (clear zone target). Javasecara *et al*., (2019).

Molecular characterization

This was carried out using the method of Jennifer Ogunnusi *et al*., (2022).

Metagenomics

Samples were sequenced on the Sequel IIe system by PacBio (www.pacb.com). Raw sub-reads were processed through the SMRTlink (v11.0). Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These highly accurate reads were then processed through vsearch (https://github.com/torognes/vsearch) and taxonomic information was determined basedonQIMME2. Reportgenerationcommandused:$create_vsearch_single_sample_pdf_report_pacbio.pycreate_vsearch_single_sample_pdf_report_pacbio.py MJ-Control-16S_M13_bc1001_F--M13_bc1067_R.hifi_reads-filtered-feature-table-asv.tsvM13_bc1001_F--M13_bc1067_R MJ-Control-16S 20240203_Cell2 16S.

RESULTS AND DISCUSSION

Tentative identification of isolates with lignolytic and methanogenic properties based on performance using biochemical test

Biochemical test and microscopy result presented in table I was interpreted as shown in the table II which revealed the presence of isolates positive for Citrase, sucrose and glucose with rods observed under the microscope at messophilic and thermophilic temperature (35-45^{0}C). This is typical of the genus *methanocella*. Microscopy of some isolates revealed the presence of cocci and were positive for mannitol and mannose at

Type		Glucose	Sucrose	Citrase	Mannose	Mannitol	Gram-stain	Morphology
1	Isolate	Positive/gas	Negative	Negative	Positive	Negative		
2	*Isolate*	Negative	Negative	Positive	Negative	Negative	**I**	**Rods**
3	Isolate	Negative	Positive	Negative	Positive/gas	Positive	-	Cocci
4	Isolate	Positive/gas	Positive/gas	Positive	Positive/gas	Positive		
5	Isolate	Positive	Positive/gas	Positive	Positive/gas	Negative	-	Short rods
6	Isolate	Negative	Positive/gas	Positive	Positive/gas	Negative	-	Tiny short rods
7	Isolate	Positive/gas	Positive/gas	Negative	Positive/gas	Positive		
8	Isolate	Positive	Positive/gas	Negative	Negative	Positive	-	Cocci
9	Isolate	Positive/gas	Positive	Negative	Negative	Positive/gas		
10	Isolate	Negative	Negative	Negative	Positive/gas	Negative	**I**	Mixed
11	Isolate	Negative	Negative	Positive/gas	Negative	Negative		Short Rods
12	Isolate	Negative	Positive/gas	Negative	Positive	Positive		Cocci
13	Isolate	Positive/gas	Positive/gas	Negative	Negative	Positive		Cocci
14	Isolate	Positive/gas	Positive/gas	Negative	Positive/gas	Positive		Short rods & short rods
15	Isolate	Negative	Positive	Negative	Positive/gas	Positive		Cocci
16	Isolate	Positive	Positive	Negative	Positive/gas	Negative		Cocci
17	Isolate	Positive	Positive/gas	Negative	Positive/gas	Negative		
18	Isolate	Negative	Negative	Positive	Positive/gas	Negative		
19	Isolate	Positive	Negative	Negative	Positive/gas	Negative		Short rods
20	Isolate	Negative	Negative	Negative	Negative	Negative		Cocci
21	Isolate	Negative	Negative	Negative	Negative	Positive/gas		Rods
22	Isolate	Negative	Negative	Positive	Positive/gas	Positive/gas		Cocci
23	Isolate	Negative	Positive/gas	Positive	Negative	Positive/gas		Cocci
24	Isolate	Negative	Positive/gas	Negative	Positive/gas	Positive/gas		Rods
25	Isolate	Positive	Positive/gas	Negative	Negative	Negative		Cocci

Methanolinear
Methanococcus
Methanomicrobium
Methanoregukar
Methanomicrobacterium
Methanolinear and lignolytic
Lignolytic *Thermophiles*
Lignolytic
Li Lignolytic

Table 1: Biochemical test results.

thermophillic and slightly mesophilic temperatures, this is typical of the genus *Methanococcus* as shown on table I. *Methanomicrobium* was also identified as microscopy revealed short rods with the isolates positive to mannose, citrase and sucrose. *Methanoregular* was identified as microscopy revealed the presence of rods, cocci, short and tiny rods and isolates were positive to mannose test which is typical of the genus *methanoregular.* Isolates with short rods under the microscope and positive for citrase were observed which is typical of the genus *Methanomicrobacterium.* Short rods isolates positive for Glucose, mannose, sucrose and mannitol were also identified as *methanolinear* which is typical of its genus.

Ligninolytic microorganisms were found present as they have utilized the glucose and produced enzymes during the biochemical test. Formation of clear zone was used to screen lignolytic microrganisms using selective aforementioned mineral solution.

Table II: **Results obtained for isolates tested for ligninolytic and methanogenic potentials from Saw Dust, Cow Dung, Mixed fruit and Vegetable Substrate**.

Genus	Biochemical test Substrate(+)	Morphology	Temperature	Lignin degradation (clear zone)
Methanocella	Citrase, sucrose and glucose	Rods	35-45^{0}C	-
Methanococcus	Mannitol and mannose	Cocci	20-35^{0}C	-
Methanomicrobium	Mannose,citrase, sucrose	Shortrods	50^{0}C	-
Methanoregular	Mannose	Rods, cocci, short and tiny rods	30-35^{0}C	-
Methanomicrobacterium	Citrase	Short rods	40^{0}C	-
Methanolinea	Glucose, mannose, sucrose, mannitol	Short rods	37-50^{0}C	-
Ligninolytic Methanolinea.	Glucose, sucrose and mannose	Short rods	22^0-40^0	+
Ligninolytic thermophiles	Mannitol and mannose	Rods	35^0-055 20^0-60^0	+
Ligninolytic	Mannitol and mannose	Cocci	20^0-60^0	+
Ligninolytic	Mannitol sucrose	Cocci	^{0}20-60^0	+

Six bacteria species were identified as *Methanothermobacter thermautotrophicus* NPK, *Candidatus methanoperedenaceae* GB37, *Methanothermobacter thermautotrophicus* CCSD, *Methanobacteriaceae archaeon* KR-H07 A06, *Methanobacteriaceae archaeon* RK-H07, and *Candidatus methanoperedenaceae* GB50. Figure one shows the percentage similarities of the microrganisms identified.

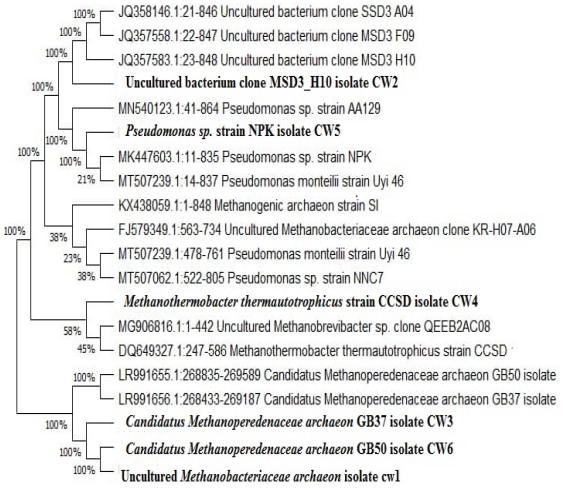

Figure 3: Phylogenetic tree for methanogens used in biogas production.

Isolate Code	Name	Percentage identity	Accession no.
CW 1	Uncultured *Methanobacteriaceae archaeon* clone KR-H07-A06	94%	FJ579349.1
CW 2	Uncultured bacterium clone MSD3_H10	97%	*JQ357583.1*
CW 3	*Candidatus Methanoperedenaceae archaeon* GB37	97.48%	*LR991656.1*
CW 4	*Methanothermobacter thermautotrophicus* strain CCSD	97.0%	DQ649327.1
CW 5	*Pseudomonas sp.* strain NPK	96.09%	*MK447603.1*
CW 6	*Candidatus Methanoperedenaceae archaeon* GB50	98.9%	LR991655.1

Table 3: Molecular identification of species.

Results obtained from metagenomic analysis

A total of 180 bacterial species were found in the NSFCS of which *Bacteroidota* (26.91%), *Fermicutes* (25.87%) and *Proteobacteria* (7.24%) predominated. Seventy (70.0%) of the NSFCS were anaerobes. *Clostridium* was the highest (28.8%) amongst the anaerobic bacterial community, while *Acidobacteriota* was the least (0.01%). *Escherichia coli* was the highest aerobic species (14.5%), while *Streptomyces* was the least (0.01%).

Gas producing ability of the methagenomic isolates in consortium

Figure 4 shows results obtained from the inoculation (seeding) of cow dung substrate using the identified methagenomic species during gas production.

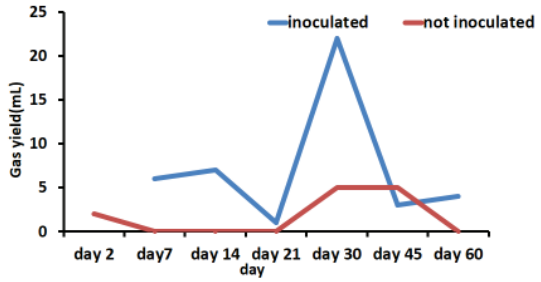

Figure 4: Effects of Microbial seeding on gas yield from cow dung substance

Applications

Ligninolytic microorganisms and methanogens (gas producing microorganisms) are largely essential in;

Medicine

Enzymes produced by these microorganisms during their metabolic activities (biodegradation) are used in producing diagnostic kits.

Industry

Food industry: Bioethanol production, probiotics

Textile industry: degradation of natural rubber, dye decolourization.

Pharmaceutical:

Oxidizing phenolic and non-phenolic compounds

Probiotics

Antibiotics

Conclusion and Recommendation

Most of the ligninolytic and gas producing (methanogens) microorganisms (*Methanocella, Methanococcus, Methanomicrobium, Methanoregular, Methanomicrobacterium, Methanolinea, and Ligninolytic Methanolinea)* isolated in this work produce enzymes. Some of these enzymes such as manganese per-oxide are key contributors in ligninolytic system. They also act as oxidizing agents, which breaks phenolic lignin and produce free radicals that have a tendency to disintegrate involuntarily, this has a great application potential and ample opportunities in diverse areas such

as Alcohol, pulp and paper, biofuel, agriculture, cosmetic, textile, and food industries. Nigerian Government should prioritize and promote research and funding in the areas of bioremediation, biodegradation (probiotics) and other enzyme related-industrial processes which will reduce cost and enhance a sustainable environment if technically applied especially in countries like Nigeria where oil spillage and other indiscriminate waste disposal has become the order of the day.

Declaration of Interest

There is a high interest in this work as it processes are consequential in industry, environment, probiotics and pharmaceutical applications. All these areas have shown high interest in lignolytic and gas producing microorganisms. Our interest in this work is basically to harness the potentials of these microbes in different fields of endeavor in science.

Acknowledgment

To commence with, we appreciate the entire management of the National Biotechnology Development Agency for providing such a ground for us to extend the frontiers and bridge the gap of research and scientific set back in our country. We greatly acknowledge the efforts of my Chief Executive officer and Director General, Prof. Mustapha Abdullahi and my co-authors for their immense effort in this, am so much grateful for this opportunity. I anticipate for more of its kind.

REFERENCES

1. Asgher M, Bashir F, & Iqbal H. M. N (2014) A comprehensive ligninolytic pre-treatment approach from lignocellulose green Biotechnology to produce bioethanol. Chem Eng Res Des 92(8):1571-1578.
2. Augelletti, R., Conti, M., & Annesini, M. C. (2017). Pressure swing adsorption for biogas upgrading. A new process configuration for the separation of biomethane and carbon dioxide. *Journal of Cleaner Production*, *140*, 1390-1398.
3. Bilal M, Asgher M, Iqbal HMN, HuH, Wang., & W, Zhang X (2017) Bio catalytic performance, lland dye based industrial pollutants degradation potential of agar rose immobilized MnP using a packed bed reactor system. Int J Biol Macromol 102:582-590.
4. Derya Ozuolmez, Hyunsoo Na, Mark A. Lever, Kasper U. Kjeldsen, Bo B,Jorgensen ., & Caroline M. 2015. Plugge. Methanogenic Archaea and sulfate reducing bacteria co-cultured on acetate:team work. Front. Microbial 6:492. doi: 10.3389/fmicb.00492.
5. Jayasekara, S., & Ratnayake, R. (2019). Microbial cellulases: an overview and applications. *Cellulose*, *22*.
6. Lei, Y., Wei, L., Liu, T., Xiao, Y., Dang, Y., Sun, D., & Holmes, D. E. (2018). Magnetite enhances anaerobic digestion and methanogenesis of fresh leachate from a municipal solid waste incineration plant. *Chemical Engineering Journal*, *348*, 992-999.
7. Ogunnusi, T.A., & Salaudeen, H. (2022). Occurrence of diversity of bacteria during anaerobic fermentation of cow dung and corn cob. ABUAD International Journal of Natural and Applied Science, 2(2),49-58. http://doi.org:/10.53982/aijnas.2022.0202.01-j.