BACTERIAL AND FUNGAL COMMUNITY STRUCTURE OF AN ABATTOIR RUMEN-CONTENT SUBSTRATE USED FOR BIOGAS PRODUCTION IN A FLEXIBLE-MATERIAL BIODIGESTER IN NIGERIA

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ABSTRACT

Anaerobic digestion is driven by a coordinated microbial consortium of hydrolytic and fermentative bacteria, fungi, and methanogenic archaea, yet the microbial ecology of digesters fed with abattoir-derived substrates in Nigeria remains largely uncharacterized. This study combined selective culture-based isolation with molecular metagenomic profiling to characterize the bacterial and fungal community structure of an abattoir rumen-content substrate used to feed a locally fabricated flexible-material biodigester (NFB 1000). Selective agar media and Gram staining were used to isolate anaerobic bacterial morphotypes displaying superficial colony and cell characteristics resembling published methanogen reference profiles; because methanogenic archaea cannot grow on nutrient or MacConkey agar, and Gram staining alone cannot distinguish Archaea from Bacteria, these isolates are more accurately interpreted as presumptive facultative anaerobic bacterial co-isolates rather than confirmed methanogens. Full-length 16S rRNA gene amplicon sequencing (PacBio Revio) classified 41.36% of reads to the bacterial kingdom, with the community overwhelmingly dominated by Pseudomonadota (72.78%), particularly Gammaproteobacteria (72.62%), and the genera Azonexus (43.05%), Acinetobacter (19.22%), and Aliarcobacter (17.04%). Internal Transcribed Spacer (ITS1) amplicon sequencing (PacBio Sequel IIe) fully resolved the fungal community (100% Fungi), which was dominated by Ascomycota (82.25%), with Aspergillus (31.61%), Fusarium (22.89%), and Penicillium (18.58%) as the leading genera. The bacterial profile indicates a substrate rich in fast-growing, metabolically versatile organisms associated with hydrolysis and acidogenesis, while the fungal profile indicates strong cellulolytic and hemicellulolytic potential supporting breakdown of fibrous rumen material. Neither dataset directly captured archaeal methanogens, and a substantial fraction of bacterial reads (58.64%) remained taxonomically unassigned. These findings indicate that the substrate used in the NFB 1000 was biologically well suited to the early and intermediate stages of anaerobic digestion, while confirming that targeted archaeal profiling is required to fully link microbial community structure to methane yield.

Keywords: anaerobic digestion; microbial community; metagenomics; 16S rRNA gene sequencing; ITS sequencing; abattoir waste; biogas

1 INTRODUCTION

Anaerobic digestion (AD) is a multi-stage biological process in which hydrolytic and fermentative bacteria, syntrophic organisms, and methanogenic archaea act in coordination to convert complex organic matter into biogas [1, 2]. Fungi also participate in the early hydrolytic stage of digestion, particularly in substrates containing lignocellulosic or fibrous material, by secreting extracellular enzymes that solubilise complex polymers ahead of bacterial fermentation [3]. Because methane is ultimately produced only by methanogenic archaea, the overall efficiency of a digester depends on how effectively

bacterial, fungal, and archaeal populations are coupled within a stable microbial consortium [4, 5] Microbial community characterization is therefore a necessary complement to the process-performance evaluation of any biodigester, yet it is frequently omitted from studies of low-cost, locally fabricated systems in sub-Saharan Africa, where evaluation has typically focused on biogas yield and economic viability rather than the underlying microbial ecology. Abattoir rumen content, increasingly investigated as a digester feedstock in Nigeria, carries a distinctive gut- and feedstock-associated microbial signature that differs from municipal solid waste or livestock manure typically used in comparable studies [6, 7], making substrate-specific characterization valuable rather than merely confirmatory. This study characterized the microbial community of an abattoir rumen-content substrate used to feed a locally fabricated flexible-material biodigester (the NFB 1000, described previously). Two complementary approaches were used: selective culture-based isolation with Gram staining, to presumptively identify methanogenic morphotypes; and molecular metagenomic sequencing, to obtain a comprehensive, culture-independent profile of the bacterial and fungal community at multiple taxonomic levels. The specific objectives were to (i) presumptively identify methanogen-associated morphotypes using selective agar media and microscopic examination; (ii) characterize the full bacterial community structure using 16S rRNA gene amplicon metagenomics; (iii) characterize the fungal community structure using Internal Transcribed Spacer (ITS) amplicon metagenomics; and (iv) interpret the resulting community profiles in terms of their functional implications for the anaerobic digestion process.

2 MATERIALS AND METHODS

2.1 Digester Substrate and Sampling

The substrate characterized in this study was abattoir rumen-content waste used to feed the NFB 1000 biodigester. For culture-based analysis, a representative sample of biodigester slurry was collected in a sterile, airtight container from the day-1 substrate loading and transported to the laboratory in an ice box to minimize disturbance of the microbial community; subsequent culture-based samples were collected at 3-day intervals over the monitoring period to support the isolation work described in Section 2.2. The core standards according to [8], are rapid post-mortem sampling (≤15 min), anaerobic handling, sterile tools, controlled temperature (39 °C for culture; chilled for DNA), and sealed transport in pre-warmed thermic bottles up to 300 min without loss of microbial viability while following [9] which provides the general framework for microbiological examinations and reporting, [10] which provides the broader laboratory competence and quality-assurance framework and [11] which is relevant to the preparation, production, storage and performance testing of the culture media. Laboratory procedures should be consistent with the selected standard and the laboratory's biosafety requirements. Finally, [12] provides for the appropriate sample preparation and dilution procedures that was followed. Molecular metagenomic analysis, by contrast, was performed on a single substrate sample taken from the day-1 loading only (Inqaba Biotec reference 1DCC-16S); no longitudinal time-series metagenomic data were generated, and the bacterial and fungal community profiles reported in Section 3 should accordingly be read as characterizing the day-1 baseline feedstock rather than tracking community change over the digestion period. This sample was transported to the Soil Microbiology Laboratories of the International Institute of Tropical Agriculture (IITA), Ibadan, for DNA extraction ahead of sequencing.

2.2 Feedstock Integrity and History

The substrate was collected as rumen content already discharged at the abattoir’s designated collection point. (Figure 1(a). The precise interval between slaughter and collection was not known, and any intermediate holding conditions at the abattoir prior to transport, were not systematically recorded [13]. This is a relevant gap given that the microbiome findings reported in Section 3.6 are more consistent with post-slaughter handling and holding-pit exposure than with freshly drawn, intact rumen fluid, and standardized recording of post-slaughter age and handling history is recommended for future substrate characterization work [14].



Figure 1. Waste collection, substrate feeding and sample collection (a)Abattoir rumen content waste collection (b) Digester substrate feeding (c) Substrate sampling for laboratory analysis

2.3 Culture-Based Isolation and Presumptive Screening of Anaerobic Bacterial Morphotypes

Bacterial isolation followed the protocols of [15, 16]. The homogenized sample was serially diluted (10^1, 10^2, 10^3) in distilled water, and 1 mL aliquots from the 10^2 and 10^3 dilutions were spread-plated onto Nutrient Agar and Tryptic Soy Agar (for total heterotrophic bacterial counts), MacConkey Agar (selective for Gram-negative organisms), and three basal mineral agar formulations (Agar A, supplemented with sodium formate; Agar B, supplemented with sodium formate and sodium nitrate; and Agar C, supplemented with methanol), each designed to selectively support the growth of specific methanogen genera. All plates were incubated at 37°C for 48 hours in an anaerobic jar. Representative colonies of distinct morphology were sub-cultured to purity, Gram-stained, and examined under oil-immersion microscopy (1000×) to determine Gram reaction, cell morphology, and arrangement, which were compared against the reference morphotypes reported by [15, 16] for presumptive genus-level naming. Because methanogenic archaea cannot grow on nutrient, Tryptic Soy, or MacConkey agar, and Gram staining alone cannot distinguish Archaea from Bacteria, these isolates cannot be confirmed as methanogens by this method; they are more accurately described as presumptive anaerobic bacterial morphotypes displaying superficial colony and cell characteristics resembling published methanogen reference profiles. Confirmation of true methanogenic identity would require anaerobic roll-tube or Hungate culturing, F_{420} coenzyme epifluorescence microscopy, or targeted molecular screening using archaeal-specific markers such as mcrA PCR, none of which was performed on these isolates.

2.4 DNA Extraction

Total genomic DNA was extracted from the biodigester substrate at the IITA Ibadan laboratories using the ZymoBIOMICS™ DNA Miniprep Kit, following the manufacturer's protocol with minor modifications for environmental, inhibitor-rich samples [17, 18]. Briefly, 50–100 mg of substrate was subjected to combined mechanical (bead-beating) and chemical lysis, and the resulting lysate was clarified by centrifugation, filtered, and bound to a silica spin column. The column was washed sequentially with proprietary pre-wash and wash buffers to remove PCR inhibitors such as humic and fulvic acids, and DNA was eluted in DNase/RNase-free water. DNA concentration and purity were assessed spectrophotometrically (A260/A280 and A260/A230 ratios), consistent with performance benchmarks reported for this kit on inhibitor-rich environmental samples [19].

2.5 Bacterial and Fungal Metagenomic Sequencing

Metagenomic sequencing and taxonomic analysis were performed by Inqaba Biotechnical Industries Inqaba Biotec™ (Pretoria, South Africa) following their PacBio full-length 16S rRNA sequencing protocol following the IITA, Ibadan Nigeria, DNA extraction with the ZymoBiomics kit, amplification with primers 27F/1492R, SMRTbell library preparation, and sequencing on the PacBio Revio platform using SMRT Cell 25M" [20]. For the bacterial community, full-length 16S rRNA gene amplicons were sequenced on the PacBio Revio platform. Raw sub-reads were processed through the SMRTlink (v25.1.0.257715) Circular Consensus Sequencing (CCS) algorithm to generate high accuracy reads (>QV40), which were subsequently processed through DADA2 for quality filtering and denoising and QIIME2 for taxonomic classification. For

the fungal community, Internal Transcribed Spacer (ITS1F) amplicons were sequenced on the PacBio Sequel IIe platform, processed through SMRTlink (v13.1) CCS to generate high accuracy reads, and taxonomically classified using vsearch with report generation through the NextITS pipeline. Full-length 16S amplification is typically performed with the universal bacterial primer pair 27F/1492R, and ITS1 amplification with ITS1F paired with a suitable reverse primer (e.g., ITS2 or ITS4), consistent with standard PacBio full-length amplicon protocols.

2.6 Data Analysis

Relative abundance was calculated at the phylum, class, order, family, genus and species levels for both the bacterial and fungal datasets. The relative abundance of each taxon was expressed as the percentage of classified sequencing reads assigned to that taxon relative to the total number of reads classified at the corresponding taxonomic level. This approach follows the standard practice of generating taxonomic abundance profiles from sequencing-derived microbial community data [21, 22]. For the bacterial dataset, the proportion of unassigned reads was also recorded separately to indicate the extent of taxonomic classification achieved and the proportion of sequences for which taxonomic identities could not be confidently established.

3 RESULTS AND DISCUSSION

3.1 Presumptive Screening of Anaerobic Bacterial Morphotypes by Selective Culture and Gram Staining

Table 1 summarizes the Gram reaction, cell morphology, and presumptive identification of isolates recovered from the day-1 substrate sample across the three selective media systems. Nutrient and Trypticase Soy agar predominantly yielded Gram-positive cocci, morphologically consistent with Methanococcus spp., alongside Gram-positive rods resembling Methanobacterium spp. [23]. MacConkey agar, which selectively favours Gram-negative bacteria through the inhibitory action of bile salts and crystal violet, yielded Gram-negative rod-shaped organisms [24], initially associated with Methanoplanus spp. The basal mineral agars, formulated with different nutritional substrates to favour specific methanogenic groups, yielded morphotypes presumptively consistent with Methanobacterium spp. (Agar A), Methanococcus spp. (Agar B) and Methanosarcina spp. (Agar C). Such substrate-dependent cultivation approaches reflect the varying nutritional and metabolic requirements of methanogenic archaea [25, 26]. It should be noted however, that the genus-level identifications should be regarded as presumptive because Gram reaction and cell morphology alone cannot conclusively identify methanogenic archaea.

Table 1. Gram reaction, cell morphology, and presumptive anaerobic morphotype naming of isolates recovered from selective culture media. Genus-level names are working labels based on morphological resemblance to published methanogen reference profiles and do not represent confirmed archaeal identification

Selective medium	Isolate code	Gram reaction	Cell morphology	Arrangement	Presumptive organism
Nutrient/Tryptic Soy agar	A	+ve	Cocci	Clustered	Methanococcus sp.
Nutrient/Tryptic Soy agar	B	+ve	Cocci	Clustered	Methanococcus sp.
Nutrient/Tryptic Soy agar	C	+ve	Rods	Clustered	Methanobacterium sp.
Nutrient/Tryptic Soy agar	D	+ve	Cocci	Clustered	Methanococcus sp.
Nutrient/Tryptic Soy agar	E	+ve	Cocci	Clustered	Methanococcus sp.
MacConkey agar	A	−ve	Rods	Clustered	Methanoplanus sp.
MacConkey agar	B	−ve	Rods	Clustered	Methanoplanus sp.
Basal agar A	A	+ve	Rods	Clustered	Methanobacterium sp.
Basal agar B	B	+ve	Cocci	Clustered	Methanococcus sp.
Basal agar C	C	−ve	Cocci	Clustered	Methanosarcina sp.

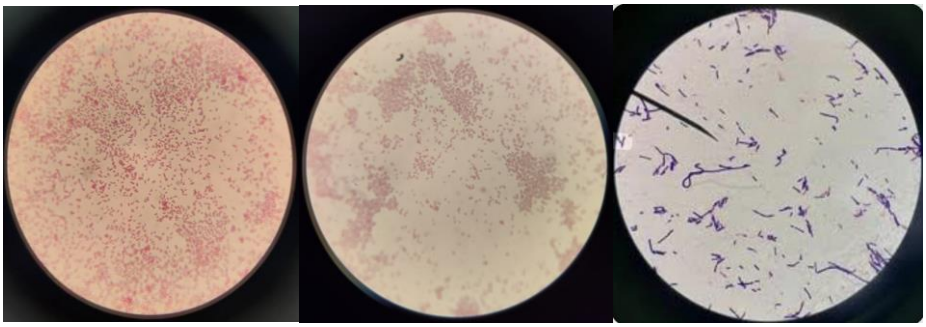


Figure 2. Representative Gram-stained micrographs of isolates recovered from selective culture media: (a) Methanoplanus sp., pink Gram-negative clustered rods (MacConkey agar); (b) Methanosarcina sp., pink Gram-negative clustered cocci (Basal agar C); (c) Methanobacterium sp., purple Gram-positive rods (Basal agar A)

These culture-based results should be regarded as presumptive, as identification was based on colony and cellular morphology compared with published descriptions, without confirmation through sequencing, F_{420} autofluorescence or archaeal-specific molecular markers. Morphological characteristics alone are insufficient for definitive identification of methanogens, for which complementary approaches such as F_{420} epifluorescence microscopy and molecular detection targeting archaeal 16S rRNA and mcrA genes are established methods [27, 28, 29]. Methanogenic archaea are strictly anaerobic and require specialized cultivation conditions, including oxygen-free media and appropriate reducing environments [25, 30]. Consequently, growth observed on the conventional Nutrient and MacConkey agar formulations used in this study should not be interpreted as evidence of methanogen isolation, particularly where strict anaerobic cultivation conditions were not maintained. MacConkey agar is primarily selective for Gram-negative bacteria [24]. The recovered organisms are therefore more appropriately interpreted as bacterial co-isolates, potentially including facultative and aerotolerant anaerobes, displaying morphological similarities to published methanogen descriptions. Accordingly, these findings indicate the presence of a diverse culturable bacterial population in the substrate but do not independently establish the presence or taxonomic diversity of methanogenic archaea.

3.2 Overview of the Bacterial Metagenomic Classification

Metagenomic analysis of the substrate sample (Inqaba Biotec reference 1DCC-16S) classified 41.36% of the total sequencing reads as bacterial, while 58.64% remained taxonomically unassigned. This substantial unassigned fraction may reflect several factors commonly encountered in complex environmental and host-associated microbiome studies. These include incomplete representation of uncultured and insufficiently characterised microbial lineages in reference databases, particularly those associated with specialised ecosystems such as the rumen [31]. Furthermore, taxonomic assignments can vary considerably depending on the reference database and classification algorithm employed; therefore, differences between SILVA and GTDB should be interpreted in the context of their respective sequence coverage, taxonomic curation and classification performance rather than assuming that one database consistently provides superior coverage [32, 33]. The presence of residual plant material in rumen contents may also introduce chloroplast and mitochondrial DNA that can be co-amplified by universal 16S rRNA primers, potentially contributing reads that are not assigned to bacterial taxa [34]. Additionally, PCR amplification of complex microbial templates can generate chimeric sequences, which may remain in sequencing datasets unless specifically identified and removed during bioinformatic processing [35, 36]. Although PacBio circular consensus sequencing improves sequence accuracy, additional quality-control procedures remain necessary to identify sequencing artefacts and chimeric amplicons [37].

3.3 Bacterial Taxonomic Composition

At the phylum level, the bacterial community was overwhelmingly dominated by Pseudomonadota (72.78%), followed by Campylobacterota (17.93%), with minor contributions from Spirochaetota (2.42%), Synergistota (2.26%), Verrucomicrobiota (1.53%), Bacteroidota (1.13%), and Bacillota-related groups

collectively below 2% (Figure 3). The occurrence of these bacterial groups is consistent with the taxonomic diversity previously reported in rumen-associated microbial communities, although their relative abundances vary with host, diet and sampling location [38, 39]. At finer resolution, Gammaproteobacteria constituted the dominant class (72.62%), with Burkholderiales (51.94%) and Pseudomonadales (19.79%) as the leading orders. The dominant families were Rhodocyclaceae (43.05%), Moraxellaceae (19.22%), and Arcobacteraceae (17.04%), corresponding at genus level to Azonexus (43.05%), Acinetobacter (19.22%), and Aliarcobacter (17.04%) (Figure 4). The occurrence of Azonexus in cattle rumen-associated bacterial communities has previously been documented by [40], while Acinetobacter has been identified among the Gammaproteobacteria of the cattle rumen [39]. Similarly, the detection of Aliarcobacter is consistent with reports of Arcobacter diversity and persistence in abattoir-associated microbial communities [41]. At species level, Azonexus sp026261605 (34.57%), Acinetobacter sp002135245 (13.68%), and Aliarcobacter cibarius (10.04%) were the most prevalent taxa. Table 2 summarises the dominant taxon at each classification level.

Table 2. Summary of dominant bacterial taxa by classification level

Taxonomic level	Dominant taxon	Relative abundance	Second taxon	Relative abundance
Phylum	Pseudomonadota	72.78%	Campylobacterota	17.93%
Class	Gammaproteobacteria	72.62%	Campylobacteria	17.93%
Order	Burkholderiales	51.94%	Pseudomonadales	19.79%
Family	Rhodocyclaceae	43.05%	Moraxellaceae	19.22%
Genus	Azonexus	43.05%	Acinetobacter	19.22%
Species	Azonexus sp026261605	34.57%	Acinetobacter sp002135245	13.68%

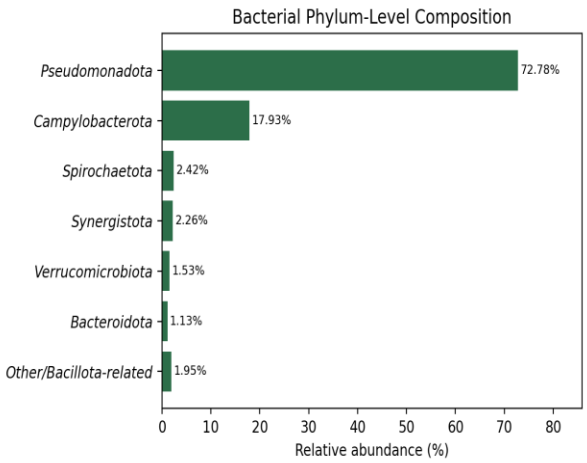


Figure 3. Bacterial phylum-level community composition of the digester substrate

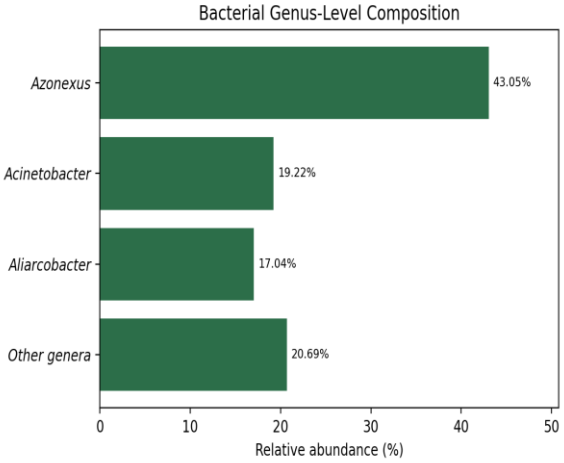


Figure 4. Bacterial genus-level community composition of the digester substrate

3.4 Overview of the Fungal Metagenomic Classification

In contrast to the bacterial dataset, all 11,194 reads retained for taxonomic classification in the ITS dataset were assigned to Kingdom Fungi (100% kingdom-level assignment). This result is consistent with the use of ITS1F, a fungal-targeted primer designed to preferentially amplify fungal ribosomal DNA [42]. However, this high kingdom-level assignment should not, by itself, be interpreted as evidence of superior sequencing or overall data quality relative to the bacterial 16S dataset. The two assays target different genetic markers and taxonomic groups, and their classification outcomes are influenced by primer specificity, marker characteristics, and the coverage and curation of the relevant reference databases [43, 44]. Consequently, differences in the proportions of classified reads may reflect differences in assay design and reference-database representation rather than differences in the underlying quality of the sequencing data.

3.5 Fungal Taxonomic Composition

At the phylum level, the fungal community was dominated by Ascomycota (82.25%), followed by Basidiomycota (14.75%) and Rozellomycota (2.51%), with trace proportions of Zoopagomycota and Neocallimastigomycota (Figure 5). Ascomycota and Basidiomycota are among the major fungal groups reported in rumen-associated fungal communities, although their relative representation varies with host species, diet, sample type and analytical approach [45, 47]. At the class level, Eurotiomycetes (50.22%) and Sordariomycetes (24.22%) predominated, corresponding principally to Eurotiales (50.19%) and Hypocreales (23.66%), and the families Aspergillaceae (50.19%) and Nectriaceae (22.89%). The dominance of Aspergillus, Fusarium and Penicillium at genus level (31.61%, 22.89% and 18.58%, respectively; Figure 6) is consistent with previous reports of these genera in cattle rumen-associated fungal communities [46, 45]. Lower-abundance yeast and yeast-like genera included Vishniacozyma (6.29%), Diutina (3.12%), Meyerozyma (1.46%), Candida (0.85%) and Trichosporon (0.75%), reflecting the diversity of yeast taxa previously reported in bovine rumen samples [46[. At species level, Aspergillus subversicolor (31.20%), Fusarium tonkinense (22.82%) and Penicillium citrinum (18.54%) were the most abundant assigned taxa. Table 3 summarises the dominant fungal taxa at each classification level. Notably, Neocallimastigomycota, which comprises obligately anaerobic gut fungi that contribute substantially to the degradation of lignocellulosic plant material in the rumen, accounted for only 0.01% of the classified reads [47]. This low relative abundance is noteworthy given the rumen origin of the substrate; however, it should not be interpreted as evidence that anaerobic fungi were genuinely scarce in the original rumen community. Previous studies have demonstrated that primer selection, ITS sequence variability, amplification efficiency and taxonomic assignment can influence the detection and estimated abundance of Neocallimastigomycota in amplicon-sequencing studies [48].

Table 3. Summary of dominant fungal taxa by classification level

Taxonomic level	Dominant taxon	Relative abundance	Second taxon	Relative abundance
Phylum	Ascomycota	82.25%	Basidiomycota	14.75%
Class	Eurotiomycetes	50.22%	Sordariomycetes	24.22%
Order	Eurotiales	50.19%	Hypocreales	23.66%
Family	Aspergillaceae	50.19%	Nectriaceae	22.89%
Genus	Aspergillus	31.61%	Fusarium	22.89%
Species	Aspergillus subversicolor	31.20%	Fusarium tonkinense	22.82%

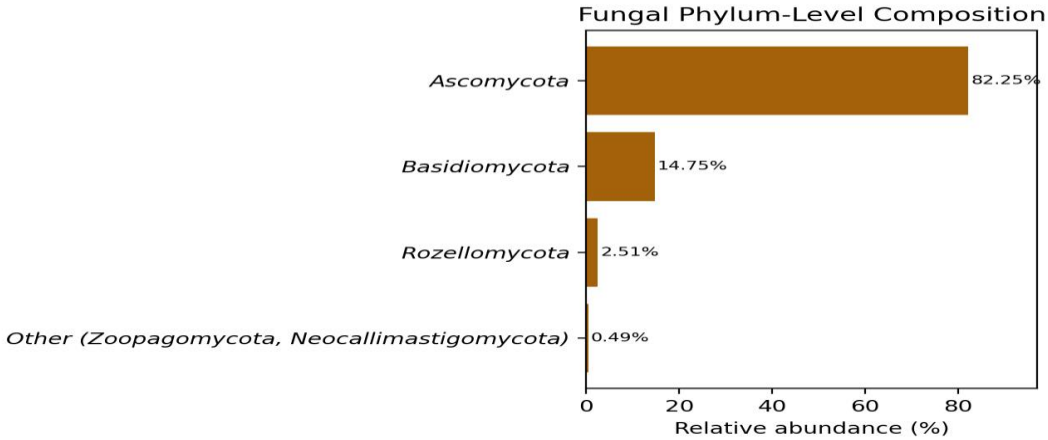


Figure 5. Fungal phylum-level community composition of the digester substrate

Together, the culture-based and metagenomic datasets indicate a substrate microbiologically rich in both bacteria and fungi, with the bacterial dataset only partially resolved (41.36% assigned) and the fungal dataset fully resolved (100% assigned).

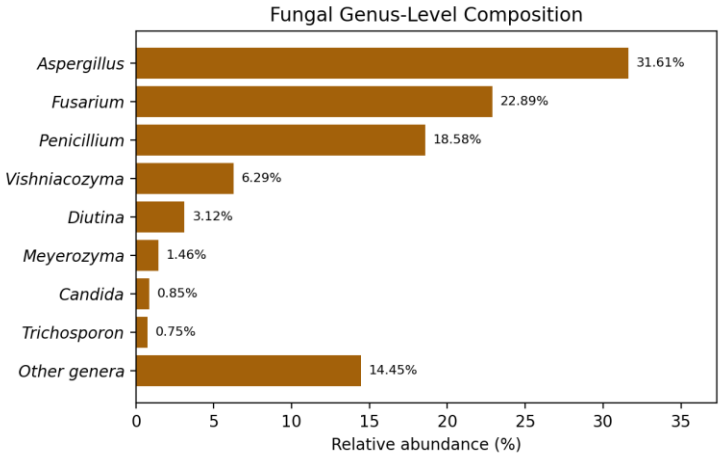


Figure 6: Fungal genus-level community composition of the digester substrate

3.6 Functional Implications of the Bacterial Community for Anaerobic Digestion

The overwhelming dominance of Pseudomonadota, and specifically Gammaproteobacteria, indicates a substrate microbiome skewed toward fast-growing, metabolically versatile bacteria associated with substrate breakdown, intermediary fermentation, and redox transitions [49]. In anaerobic digesters, Proteobacteria are commonly linked to active hydrolysis and early acidogenesis rather than to the terminal methanogenic stage of digestion; more stable, strongly methanogenic systems typically depend on a broader syntrophic network involving Bacillota/Firmicutes, Bacteroidota, and methanogenic archaea acting in concert [5]. The comparatively low abundance of Bacteroidota (1.13%) and Bacillota-related groups (<2%) observed here, both of which are typically dominant hydrolytic and fermentative taxa in anaerobic digesters, suggests that classical fibre- and protein-degrading bacteria were under-represented relative to many previously characterised digester systems [50]. The dominance of Azonexus (43.05%), a genus of chemoorganoheterotrophic denitrifying bacteria within the family Rhodocyclaceae typically associated with wastewater and nutrient-transforming environments [51], points to active degradation of soluble organic matter and nitrogen-transforming activity that may support pre-methanogenic conversion, although denitrification pathways can also compete with methanogenesis for available electron donors. The substantial presence of Acinetobacter (19.22%), a metabolically adaptable genus known to persist in manure and digestate environments [52], suggests a resilient bacterial population capable of tolerating the variable field conditions typical of a locally fabricated digester, though this genus is not itself a signature organism of high-rate methanogenesis [1]. The high relative abundance of Aliarcobacter (17.04%), including A. cibarius, is consistent with the intestinal, abattoir origin of the substrate, as members of this genus are well documented as gut- and faeces-associated organisms in livestock and slaughterhouse environments, several of which are considered emerging zoonotic or food-chain-associated pathogens [6, 53]. Taken together, the co-dominance of a denitrifying wastewater-associated genus (Azonexus), an environmentally persistent opportunist (Acinetobacter), and a gut- and faeces-associated zoonotic genus (Aliarcobacter) is not the profile expected of intact bovine rumen fluid, in which Bacteroidota and Bacillota (notably Prevotella, Ruminococcus, and Fibrobacter) typically predominate; it instead more closely resembles the microbial signature of substrate that has passed through post-slaughter handling and holding-pit conditions at the abattoir, consistent with the feedstock-history gap noted in Section 2.1. This confirms the biodegradability of the substrate while also indicating that digestate hygienisation should be considered before agricultural reuse. Although present at low abundance, Spirochaetota (2.42%, represented mainly by Sphaerochaeta) and Synergistota (2.26%) are microbiologically significant, as both groups are frequently implicated in

syntrophic acetate oxidation and amino-acid fermentation in anaerobic systems [54, 55]. Their presence indicates that, despite the numerical dominance of facultative and denitrifying groups, the substrate retained bacterial members capable of channelling fermentation intermediates toward methanogenic partners, provided a compatible archaeal population was also active [56].

3.7 Functional Implications of the Fungal Community for Anaerobic Digestion

The strong dominance of Ascomycota, and specifically Eurotiomycetes (Aspergillus, Penicillium) and Sordariomycetes (Fusarium), indicates a fungal community well equipped for extracellular enzymatic degradation of lignocellulosic and fibrous material, since these genera are widely reported for cellulase, hemicellulase, xylanase and protease production [57, 58, 3]. This is functionally relevant to the abattoir rumen-content substrate, which contains partially digested fibrous and proteinaceous material requiring hydrolysis before downstream fermentation and methanogenesis can proceed efficiently, hydrolysis being frequently the rate-limiting step for particulate substrates [59] The detection of yeast and yeast-like genera, including Vishniacozyma, Diutina, and Meyerozyma, further indicates active fermentation of soluble sugars into alcohols and organic acids that serve as substrates for downstream acetogenic and methanogenic activity, even though fungi do not themselves produce methane [60]. The near absence of Neocallimastigomycota (0.01%), the phylum containing obligately anaerobic gut fungi specialized in degrading fibrous plant material within herbivore digestive systems [61], is a notable finding given the rumen origin of the substrate. This may reflect primer bias or short-term oxygen exposure during sample handling immediately prior to DNA extraction, but the more parsimonious explanation, given the co-occurring bacterial signature reported in Section 3.6, is that the substrate had already undergone substantial aerobic exposure, aging, or partial drying at the abattoir prior to collection (Section 2.1), which would be expected to disfavour the obligately anaerobic Neocallimastigomycota while favouring the terrestrial and airborne Ascomycota that dominate the fungal profile reported here. Under this interpretation, the fungal hydrolytic contribution captured in this dataset was carried primarily by aerobic and facultative Ascomycota that colonised the substrate after slaughter, rather than by the specialised anaerobic gut fungi native to the intact bovine rumen.

3.8 Cross-Validation Between Culture-Based and Metagenomic Findings

The culture-based and metagenomic datasets provide partially complementary rather than directly overlapping evidence. Selective culture and Gram staining recovered anaerobic and facultative bacterial morphotypes bearing superficial resemblance to published methanogen reference profiles (Section 3.1) across all three media systems, but, as noted above, none of these media supports methanogen growth and Gram staining cannot itself distinguish Archaea from Bacteria; these results therefore do not constitute evidence that methanogenic archaea were cultured. The metagenomic datasets independently failed to detect methanogens for a distinct and expected methodological reason: the bacterial community was profiled using full-length 16S rRNA gene amplicon sequencing with primers designed for broad bacterial coverage (Section 2.5), and universal bacteria-targeted primers such as the 27F/1492R family have well-documented mismatches against the corresponding archaeal 16S rRNA gene regions, so that Archaea are intentionally under-amplified or excluded by this primer chemistry regardless of their true abundance in the sample. The absence of methanogen sequences in the metagenomic dataset is accordingly an expected consequence of the bacteria-specific primer choice rather than an anomaly of the substrate or evidence against the presence of methanogens; neither the culture-based nor the metagenomic approach used here can confirm or quantify the archaeal community, and targeted archaeal 16S primers (e.g., Arch349F/Arch806R) or archaeal-specific functional markers such as mcrA [62, 63] are required to do so.

3.9 Process-Level Implications for the NFB 1000 and Biogas Production

Taken together, the bacterial and fungal community profiles indicate that the abattoir rumen-content substrate loaded into the NFB 1000 was microbiologically active and well suited to the early and intermediate stages of anaerobic digestion, particularly hydrolysis, fermentation, and nitrogen-associated metabolism. However, neither dataset directly demonstrates a methanogen-rich terminal community, since methane is produced by archaea rather than by the bacteria or fungi profiled here [4]. The results therefore

support the interpretation that methane productivity in the NFB 1000 depended on effective coupling of this hydrolytic and fermentative consortium with methanogenic archaea under favorable operating conditions of pH, retention time, and organic loading, consistent with the digestion kinetics and pH dynamics reported for this system elsewhere [64]. Comparatively low abundance of classical hydrolytic bacteria (Bacteroidota, Bacillota) alongside strong fungal hydrolytic representation suggests that, in this particular substrate, fungi may have played a proportionally larger role in initial substrate breakdown than is typical in digesters dominated by bacterial hydrolysis alone, an interpretation that would benefit from further substrate-specific investigation.

5 CONCLUSIONS

This study combined selective culture-based isolation with 16S rRNA gene and ITS amplicon metagenomics to characterize the bacterial and fungal community structure of an abattoir rumen-content substrate used in a locally fabricated flexible-material biodigester in Nigeria. Culture-based screening recovered four presumptive anaerobic bacterial morphotypes bearing morphological resemblance to published methanogen reference profiles across selective media, though confirmation of true archaeal identity was not possible using the media and staining methods employed. Metagenomic profiling classified 41.36% of reads to Bacteria, dominated by Pseudomonadota and the genera Azonexus, Acinetobacter, and Aliarcobacter, and fully resolved the fungal community (100% Fungi), dominated by Ascomycota and the genera Aspergillus, Fusarium, and Penicillium. These profiles indicate a substrate biologically well suited to the hydrolytic and fermentative stages of anaerobic digestion, while the absence of archaeal-specific data identifies a clear direction for future work: targeted archaeal profiling to directly link microbial community structure to methane yield in locally fabricated Nigerian biodigesters.

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