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Bioprospecting Microbial Isolate and Environmental Samples for Biotechnological Potential

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Abstract: ***This study investigates the biotechnological potential of microbial isolates obtained from environmental sources, including cow dung and organic soil. Using a combination of culture-dependent techniques, physiological assays, and shotgun metagenomics, the research identifies microbial strains with promising metabolic traits. Notably, methanogenic activity, enzymatic profiles, and biodegradative capabilities were analyzed through various bioassays and pathway predictions. The study highlights the contrasting microbial compositions and functional potentials of cow dung and soil, suggesting targeted applications in biogas production, waste management, and environmental remediation. These findings pave the way for future exploration of microbial resources for sustainable biotechnology.***

Keywords: Bioprospecting, Microbial isolates, Biogas production, Metagenomics, Biodegradation

1. Introduction

Bioprospecting, the exploration of biological resources for commercially valuable compounds, has emerged as a promising field in biotechnology. Environmental microbial isolates play a pivotal role in this endeavour due to their unparalleled metabolic diversity and adaptive capabilities. Microorganisms found in diverse ecosystems such as soil, water, extreme habitats, and marine environments, possess unique biochemical pathways that are essential for their survival. Harnessing these capabilities has opened up new avenues for industrial, pharmaceutical, agricultural, and environmental applications.

Environmental samples, including soil, sediment, water, and plant rhizospheres, serve as rich reservoirs of novel microbial strains. These microbes exhibit remarkable enzymatic potential, antimicrobial properties, and bioactive compound production, which can be utilized for developing innovative biotechnological solutions. Recent advancements in metagenomics, high-throughput screening, and bioinformatics have revolutionized the identification and characterization of these microbial isolates. By employing culture-dependent and culture-independent techniques, researchers have uncovered an extensive array of microorganisms with potential applications in biotechnology.

One of the main focuses of microbial bioprospecting is the identification of enzymes with industrial relevance. Enzymes such as proteases, lipases, amylases, and cellulases derived from environmental isolates have been widely adopted in industries like food processing, detergent formulation, and biofuel production. Thermophilic and extremophilic microbes sourced from harsh environments such as hydrothermal vents, saline lakes, and acidic hot springs often exhibit extraordinary enzyme stability under extreme conditions, making them highly desirable for industrial applications.

In the pharmaceutical sector, environmental microbial isolates are recognized for their ability to synthesize novel antibiotics, antifungal agents, and immunosuppressants. Microbial metabolites such as penicillin, streptomycin, and erythromycin have revolutionized medicine, and ongoing efforts in bioprospecting continue to yield promising therapeutic leads.

Environmental remediation is another critical area where bioprospecting plays a key role. Microbes isolated from polluted environments demonstrate remarkable potential for biodegradation and biotransformation of toxic contaminants, offering eco-friendly solutions for waste management and environmental conservation.

The outcomes of this research contribute to the development of sustainable bioenergy solutions and innovative waste management strategies, addressing critical environmental challenges. Leveraging these microbial resources can address pressing global challenges in healthcare, agriculture, industry, and environmental sustainability. As technological advancements continue to refine bioprospecting techniques, the discovery of novel microbial compounds is poised to expand the frontiers of modern biotechnology. This study aims to explore the biotechnological potential of microbial isolates derived from cow dung and organic soil through physicochemical analysis, bioassays, and metagenomic profiling.

2. Materials and Methods

Sample Collection and Physicochemical Analysis

Cow dung samples were collected from local farms and processed for physicochemical analysis. The samples were air-dried and stored under controlled conditions before laboratory testing to ensure consistency and accuracy in the results. The physicochemical properties of cow dung were analyzed to assess its chemical composition and suitability for microbial isolation. Various parameters, including nitrate, nitrite, and ammonia content, as well as total solids and volatile solids, were measured.

Nitrate and Nitrite Determination

For nitrate determination, 4 g of the cow dung sample was dissolved in 200 mL of water. From this solution, 5 mL was transferred into a 50 mL volumetric flask, and the volume was adjusted by adding 45 mL of distilled water. To aid in the analysis, 1 mL of 1N HCl was added, and the absorbance was recorded at 220 nm and 275 nm using a spectrophotometer. Similarly, nitrite determination involved dissolving 6 g of cow dung in 200 mL of water. A 15 mL portion of this solution was taken and diluted to 50 mL with distilled water, followed by the addition of 2 mL of a coloring reagent. The spectrophotometric measurement was recorded at 543 nm within a time frame of 10 minutes to 2 hours.

Ammonia Determination

Ammonia determination was conducted by dissolving 4 g of cow dung in 200 mL of water, followed by taking 50 mL of the dissolved sample into a 50 mL volumetric flask. To this, 1 mL of Na-K tartrate and 1 mL of Nessler reagent were added, and the absorbance was recorded at 425 nm after 5 minutes. Additionally, the total solids and volatile solids content were determined using a gravimetric method. Crucibles were washed, heat-dried for an hour to remove moisture, and their empty weights were recorded.

Total solids and volatile solids determination

The total solids were measured by placing the cow dung sample in the crucible and drying it at 105°C for 12 hours. The weight after drying was recorded as the total solids content. For volatile solids measurement, the crucibles containing the dried samples were placed in a muffle furnace at 550°C for 2 hours, and the remaining weight was noted, with the difference indicating the volatile solids content. This comprehensive physicochemical analysis provides a deeper understanding of the composition of cow dung and its potential applications in various environmental and industrial sectors.

Assays to evaluate the physiological, metabolic, and enzymatic properties of *Methanosarcina Barkeri* for bioenergy applications

Methane production and Substrate Utilization assay

The methane production assay and substrate utilization assay are designed to evaluate methanogenic bacteria derived from cow dung. In the methane production assay, anaerobic serum vials are prepared with growth media, followed by inoculation with methanogenic bacteria. The vials are then sealed with butyl rubber stoppers and aluminum caps to maintain anaerobic conditions. The samples are incubated at temperatures ranging from 37 to 55°C, and gas samples are collected at regular intervals. Methane production is analyzed using gas chromatography (GC), and the results are compared against control samples and standard curves to assess microbial activity.

In the substrate utilization assay, anaerobic medium is prepared with various carbon sources, and the mixture is dispensed into sealed vials under a nitrogen or argon atmosphere to ensure anaerobic conditions. The vials are then inoculated with methanogens and incubated at an optimal temperature of 37–55°C. Methane production is measured at specific time intervals using GC, and the results are compared across different substrates to determine substrate utilization efficiency. The findings are further correlated with metabolic pathways to understand the role of various carbon sources in methane production. These assays help in evaluating the potential of cow dung-derived methanogens for biogas production and microbial metabolism studies.

Biomass Growth assay

The Biomass Growth Assay involves several steps to assess the growth of methanogenic bacteria. The process begins with the preparation of an anaerobic growth medium in serum bottles, followed by inoculation with methanogenic bacteria. The cultures are then incubated under optimal temperature and conditions to promote bacterial growth. At regular intervals, optical density (OD600) measurements are taken using a spectrophotometer to monitor biomass accumulation. Additionally, an optional step includes centrifugation and drying of biomass for dry weight measurement. The collected data is then used to plot a growth curve (OD600 vs. time), allowing for the analysis of growth trends and the calculation of doubling time.

Enzyme Activity Assay

The Enzyme Activity Assay, specifically targeting hydrogenase or methanogenesis enzymes, begins with growing methanogens to the mid-log phase in anaerobic culture. The bacterial cells are then harvested through centrifugation, followed by cell lysis using ultrasonication or enzymatic digestion. The enzyme reaction mixture is prepared using specific substrates and subsequently incubated under optimal conditions. The reaction's progress is monitored by measuring absorbance at a specific wavelength using spectrophotometry. Finally, enzyme activity is assessed by comparing the absorbance values with a standard curve.

Inhibition Assay

The Inhibition Assay is designed to evaluate the effects of inhibitors on methanogenic bacteria. The assay starts with the preparation of an anaerobic growth medium, which is dispensed into sealed vials. The medium is then inoculated with methanogenic bacteria, followed by the addition of inhibitors such as 2-bromoethanesulfonate or antibiotics. Control vials without inhibitors are also prepared to compare results. The cultures are incubated at optimal temperature, after which methane production is measured using gas chromatography (GC) and bacterial growth is assessed using OD600 measurements. The final step involves comparing the inhibition effects across different conditions to determine the impact of the tested inhibitors on methanogenesis.

Volatile Fatty Acids (VFA) Degradation Assay

The Volatile Fatty Acids (VFA) Degradation Assay begins with the preparation of anaerobic vials containing known VFA concentrations. These vials are then inoculated with methanogens and incubated under anaerobic conditions. At different time points, liquid samples are collected and subsequently filtered to remove bacterial cells. The VFA concentration in these samples is analyzed using High-Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC). The initial and final VFA levels are compared to determine the degradation rate. Finally, the findings are correlated with methane production to assess the efficiency of the degradation process.

Coenzyme F420 Fluorescence Assay

The Coenzyme F420 Fluorescence Assay starts with growing methanogens until they reach the mid-log phase. The bacterial cells are then collected through centrifugation and resuspended in a phosphate buffer. The sample is excited at 420 nm using a fluorescence spectrophotometer, and fluorescence emission is measured at 470 nm. The fluorescence intensity is then compared with control samples. This fluorescence serves as an indicator of metabolic activity, providing insight into the physiological state of the methanogens.

Shotgun metagenomics for metabolic pathway prediction

In shotgun metagenomics for metabolic pathway prediction, the first step is Whole Metagenome Sequencing (WGS). This begins with sample collection, where cow dung and organic soil samples are gathered for microbial DNA extraction. DNA is then extracted using commercial kits such as Qiagen or Zymo. A quality check is performed to assess DNA purity and integrity using tools like Nanodrop, Qubit, and gel electrophoresis. Next, the library preparation involves fragmenting the DNA, adding adapters, and amplifying using PCR if necessary. Sequencing is conducted using platforms like Illumina, which offers short-read, high-accuracy sequencing, or Oxford Nanopore/PacBio, which provides long-read sequencing with better assembly. Read generation produces raw sequencing reads in FASTQ format, containing base calls and quality scores. Quality control is then performed to filter low-quality reads, adapters, and contaminants using tools such as FastQC and Trimmomatic. Metadata collection involves recording sequencing parameters, coverage depth, and quality metrics. The raw FASTQ files are securely stored for downstream analysis. The next step in the workflow is genome assembly and annotation to extract functional insights.

The second step, Genome Assembly & Annotation, focuses on assembling sequencing reads, annotating genes, and classifying microbial taxonomy. Genome assembly is performed using tools like MEGAHIT or SPAdes to assemble raw reads into contigs and scaffolds. Quality checks assess assembly metrics such as N50, contig length, and GC content to ensure completeness. Gene prediction is conducted using PRODIGAL to identify and extract protein-coding genes. Functional annotation assigns gene functions using databases like KEGG, MetaCyc, and Pfam. Taxonomic classification identifies microbial composition using tools such as Kraken2, Kaiju, or MetaPhlAn. Visualization is achieved through Krona plots, allowing interactive taxonomic profiling. Community comparison is performed to analyze microbial diversity differences between cow dung and organic soil samples. The annotated genomes, taxonomy reports, and visualizations are then stored for interpretation. Finally, the next step involves performing metabolic pathway analysis to explore the biotechnological potential of the microbial community.

The third step in shotgun metagenomics for metabolic pathway prediction is Pathway Analysis, which focuses on biotechnological applications. The objective is to identify functional genes related to methanogenesis, hydrolysis, and plastic degradation. Gene identification involves detecting genes responsible for methanogenesis (e.g., *mcrA, fdxA, hdrB*), hydrolysis (e.g., cellulases, proteases, lipases), and plastic degradation (e.g., PETase). Functional annotation is carried out using KEGG, MetaCyc, and Pfam databases to classify the identified genes. Gene abundance analysis quantifies gene presence across different samples and visualizes the data using heatmaps. KEGG pathway mapping assigns functional genes to metabolic pathways to assess their roles. Statistical comparison normalizes gene counts for comparative analysis across samples. Diversity analysis employs Principal Coordinate Analysis (PCoA) to evaluate beta diversity between microbial communities. Visualization techniques generate KEGG pathway pie charts to illustrate pathway contributions. Functional gene datasets, heatmaps, and pathway visualizations are securely stored for further analysis. The final step involves correlating functional gene abundance with environmental parameters and exploring their potential biotechnological applications.

3. Results

Nitrate Determination

The nitrate determination results reveal a calculated nitrate concentration of 28.42 mg/l, derived using the formula ((A - 2B) * D.F.) / Slope. This relatively high nitrate content reflects the nutrient-rich nature of cow dung, indicating its strong potential as an organic fertilizer. Elevated nitrate levels contribute significantly to enhanced nitrogen cycling and can reduce the dependence on synthetic nitrogen sources. These conditions facilitate robust microbial activity, especially involving nitrifying bacteria, thereby supporting sustainable agricultural practices. The high nitrate availability promotes better root uptake and plant nutrition, enhancing crop yield while maintaining soil health.

Nitrite Determination

In the case of nitrite determination, the calculated nitrite concentration in cow dung is 0.0328 mg/l, based on the formula ((OD_sample - OD_blank) * D.F.) / Slope. This moderate nitrite level is consistent with active microbial processes such as nitrification and denitrification. Nitrites serve as transient intermediates in the nitrogen cycle and reflect the dynamic microbial ecosystem within cow dung. Their presence is crucial in regulating nitrogen balance and minimizing nitrogen losses to the atmosphere. Furthermore, these conditions support the growth of beneficial microbial communities that enhance nutrient transformation and offer potential in bioremediation applications.

Ammonia Determination

The ammonia concentration in the cow dung sample is found to be 53.78 mg/l, calculated using the formula ((OD_sample - OD_blank) * D.F.) / Slope. This substantial ammonia presence aligns with the expected profile of fresh animal waste and highlights the availability of nitrogen in readily usable forms. High ammonia concentrations provide a favorable environment for ammonia-oxidizing and methanogenic bacteria, which are critical for nutrient recycling and methane production. Such levels also improve the efficiency of biofertilizers and organic amendments, promoting better plant nitrogen uptake and reducing the requirement for chemical fertilizers. Additionally, the presence of ammonia supports microbial metabolic diversity,

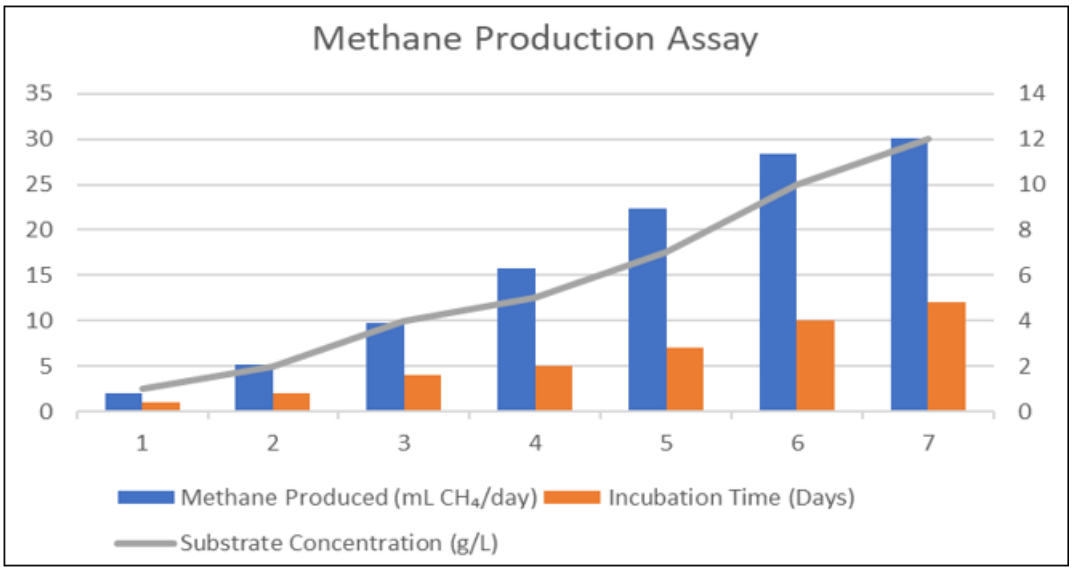
contributing to waste stabilization and sustainable waste-to-energy practices.

Total and Volatile Solids Determination

The total and volatile solids determination indicates a total solids concentration of 18.4% and volatile solids content of 14.62%. These values reflect the organic richness and biodegradability of cow dung, making it an excellent candidate for biogas production and composting. Volatile solids, which represent the organic fraction, play a crucial role in anaerobic digestion processes, directly influencing methane yield and microbial activity. The high volatile solids content supports efficient microbial degradation, aiding in the treatment of organic waste and enhancing environmental sustainability. In parallel, the total solids contribute to the bulk density and nutrient-holding capacity of compost, improving its application in agriculture and soil conditioning.

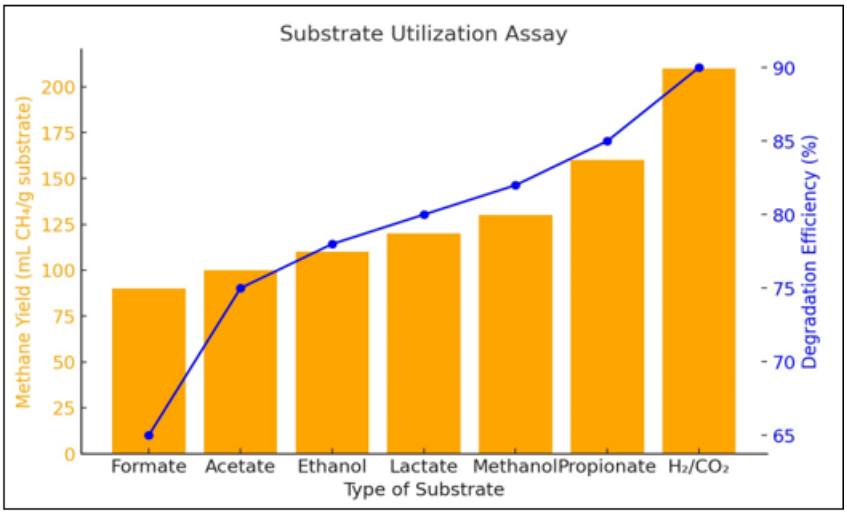
4. Assays to evaluate bioenergy applications

4.1 Methane Production Assay



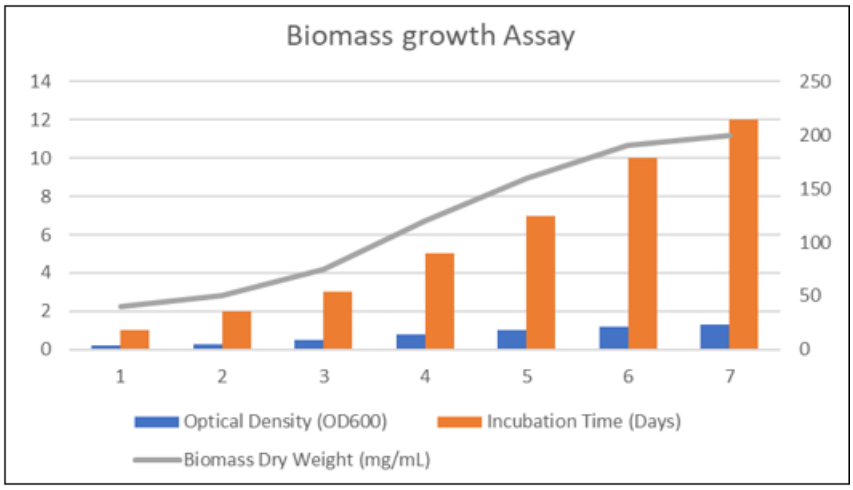
The graph shows a steady rise in methane production over 7 days, starting at ~2 mL CH_4/day on Day 1 and reaching ~30 mL CH_4/day by Day 7. Substrate concentration increases concurrently from ~2 g/L to 12 g/L, indicating a strong positive correlation between substrate availability and methane output. The sharpest rise occurs between Days 4 and 7, reflecting intensified microbial activity. These trends suggest efficient substrate utilization and robust microbial metabolism under the tested conditions.

4.2 Substrate Utilization Assay



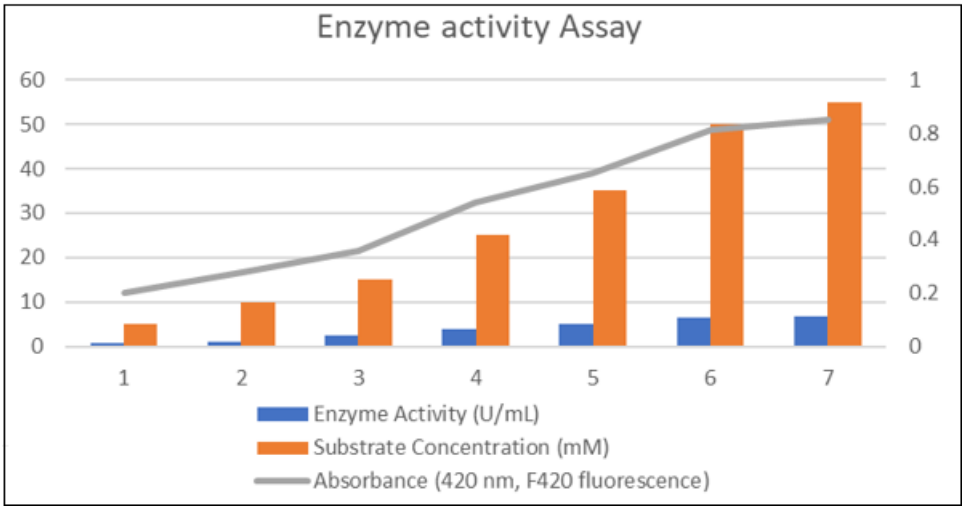
The graph illustrates the relationship between different substrates, methane yield (orange bars, mL CH_4/g substrate), and degradation efficiency (blue line, %). Methane yield ranges from ~100 mL CH_4/g for formate to ~200 mL CH_4/g for H_2/CO_2. Degradation efficiency peaks at ~90% for H_2/CO_2, indicating that substrates like propionate and H_2/CO_2 are more effectively converted to methane. Formate shows the lowest values for both parameters, highlighting its limited metabolic efficiency.

4.3 Biomass Growth Assay



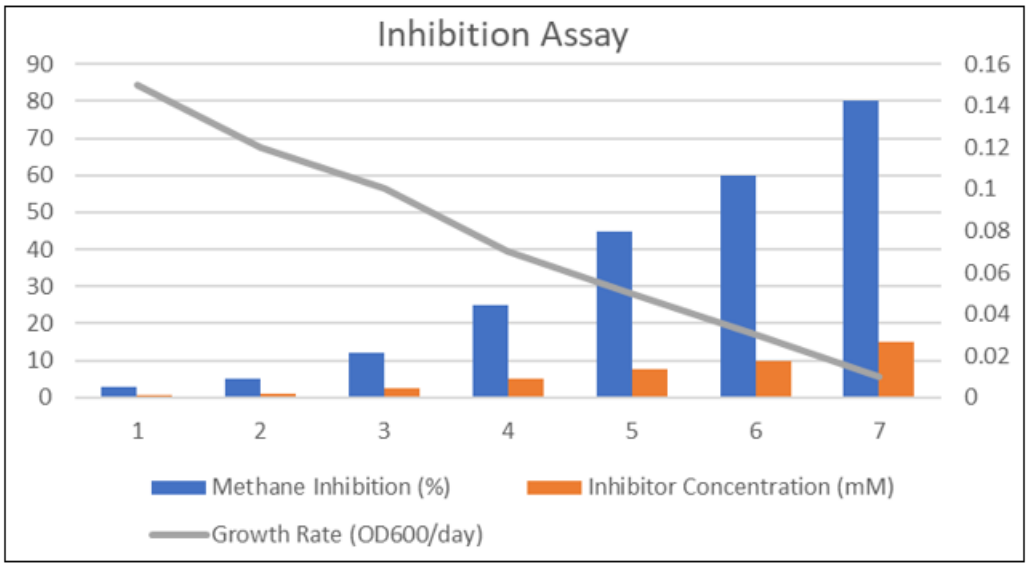
This assay tracks biomass growth over 7 days, measuring OD600 (blue bars), dry weight (gray line), and incubation time (orange x-axis). OD600 and dry weight increase steadily, with dry weight rising from ~25 mg/mL on Day 1 to ~210 mg/mL by Day 7. The steepest growth occurs between Days 3 and 6, indicating an exponential phase. A slight plateau after Day 6 may suggest the culture is entering the stationary phase.

4.4 Enzyme Activity Assay



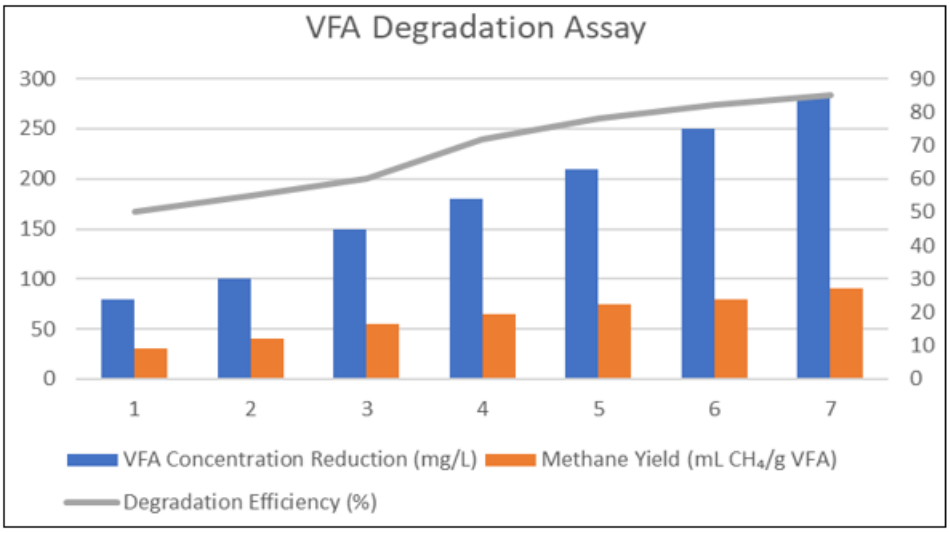
The graph presents enzyme activity (blue bars, U/mL), substrate concentration (orange bars, mM), and absorbance at 420 nm (gray line, F420 fluorescence) over 7 days. All three parameters increase progressively, with enzyme activity rising in parallel with substrate concentration (5 to 55 mM) and absorbance reaching ~0.9 by Day 7. The most notable increase occurs between Days 3 and 6, indicating optimal conditions for enzymatic function and metabolic activity during this period.

4.5 Inhibition Assay



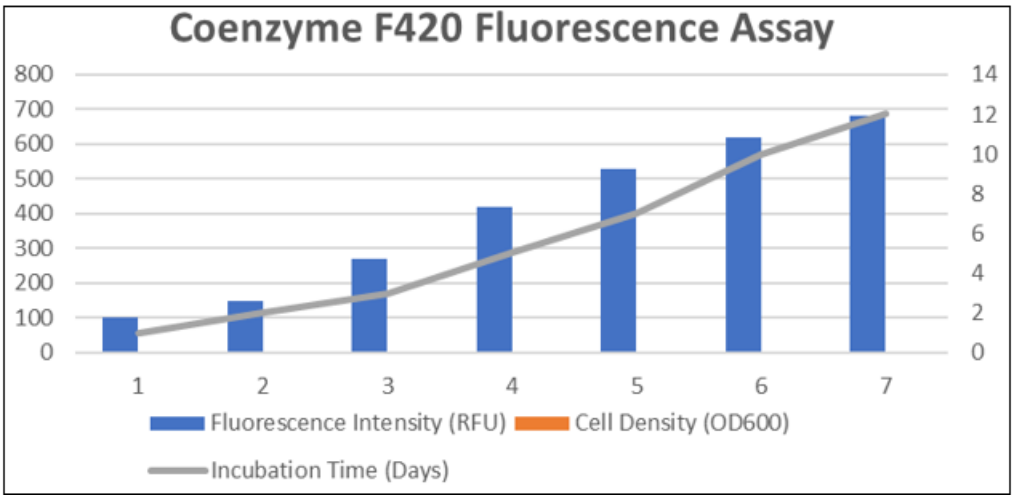
This graph shows methane inhibition (blue bars, %), inhibitor concentration (orange bars, mM), and microbial growth rate (gray line, OD600/day) over 7 days. Methane inhibition rises from ~3% to ~80%, while growth rate declines from ~0.15 to <0.02 OD600/day, indicating a dose-dependent inhibition. Between Days 3 and 6, inhibition spikes sharply and growth drops significantly, marking the most sensitive phase. By Day 7, the system nears full metabolic suppression.

4.6 VFA Degradation Assay



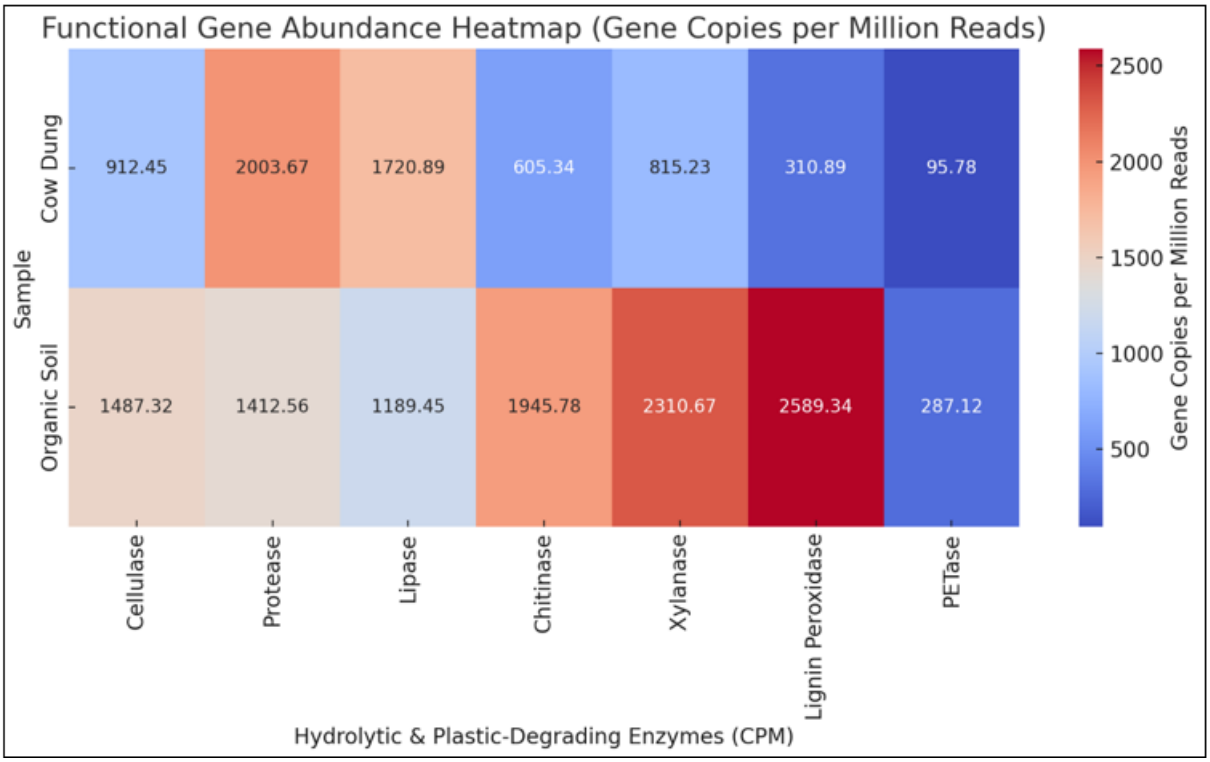
The graph tracks VFA concentration reduction (blue bars, mg/L), methane yield (orange bars, mL CH_4/g VFA), and degradation efficiency (gray line, %) over 7 days. VFA levels drop from ~80 mg/L to ~280 mg/L, while degradation efficiency reaches ~85%. The largest increases occur between Days 2 and 4, indicating peak microbial or enzymatic activity. Methane yield follows a proportional upward trend, showing a direct link between VFA breakdown and biogas production.

4.7 Coenzyme F420 Fluorescence Assay



This assay monitors fluorescence intensity (blue bars, RFU), cell density (orange bars, OD600), and incubation time (gray line) over 7 days. Fluorescence increases from ~100 to ~700 RFU, while OD600 rises steadily, suggesting active microbial growth and F420-associated metabolic activity. The sharpest fluorescence increase occurs between Days 3 and 6, aligning with higher cell densities. By Day 7, both metrics begin to plateau, indicating potential metabolic saturation or transition into stationary phase.

Shotgun metagenomics



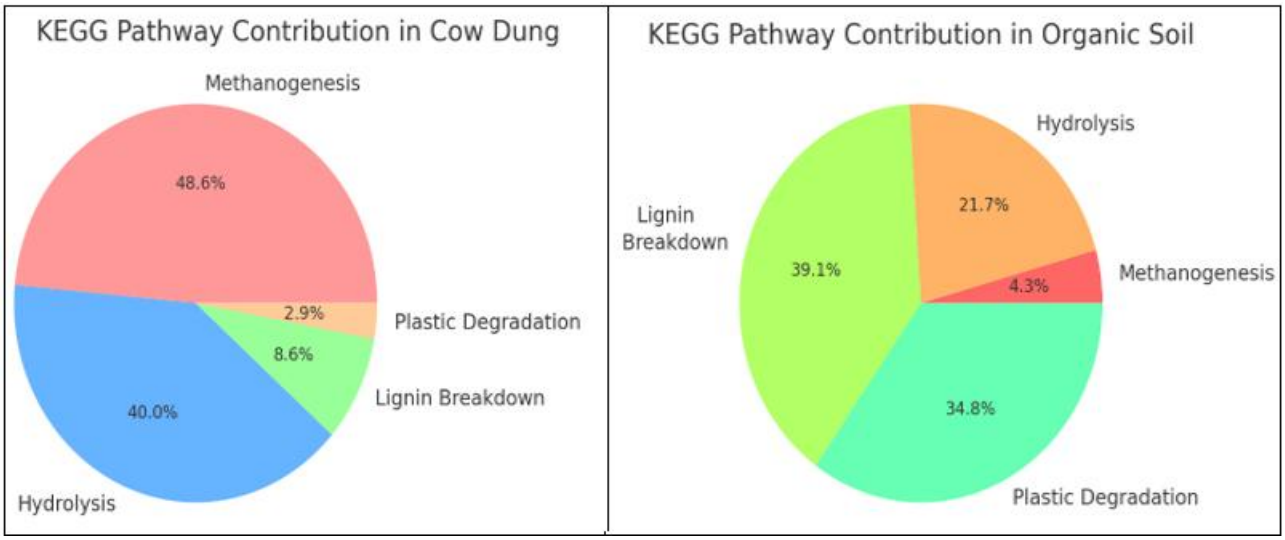
This heatmap represents the functional gene abundance of hydrolytic and plastic-degrading enzymes in two environmental samples: *Cow Dung* and *Organic Soil*. The x-axis lists different enzyme categories, including cellulase, protease, lipase, chitinase, xylanase, lignin peroxidase, and PETase, while the y-axis represents the sample sources. The color intensity indicates gene abundance, with red denoting higher values and blue indicating lower ones.

In *Cow Dung*, protease (2003.67) and lipase (1720.89) show the highest abundance, while PETase (95.78) has the lowest. In contrast, *Organic Soil* exhibits significantly higher gene abundance for chitinase (1945.78), xylanase (2310.67), and lignin peroxidase (2589.34), suggesting a strong lignocellulose-degrading potential. The presence of PETase genes in both samples, though at low levels, indicates some plastic degradation potential. The variations in abundance highlight differences in microbial community functions, with *Cow Dung* favoring protein and lipid degradation, whereas *Organic Soil* is more involved in lignocellulose breakdown.

KEGG pie charts

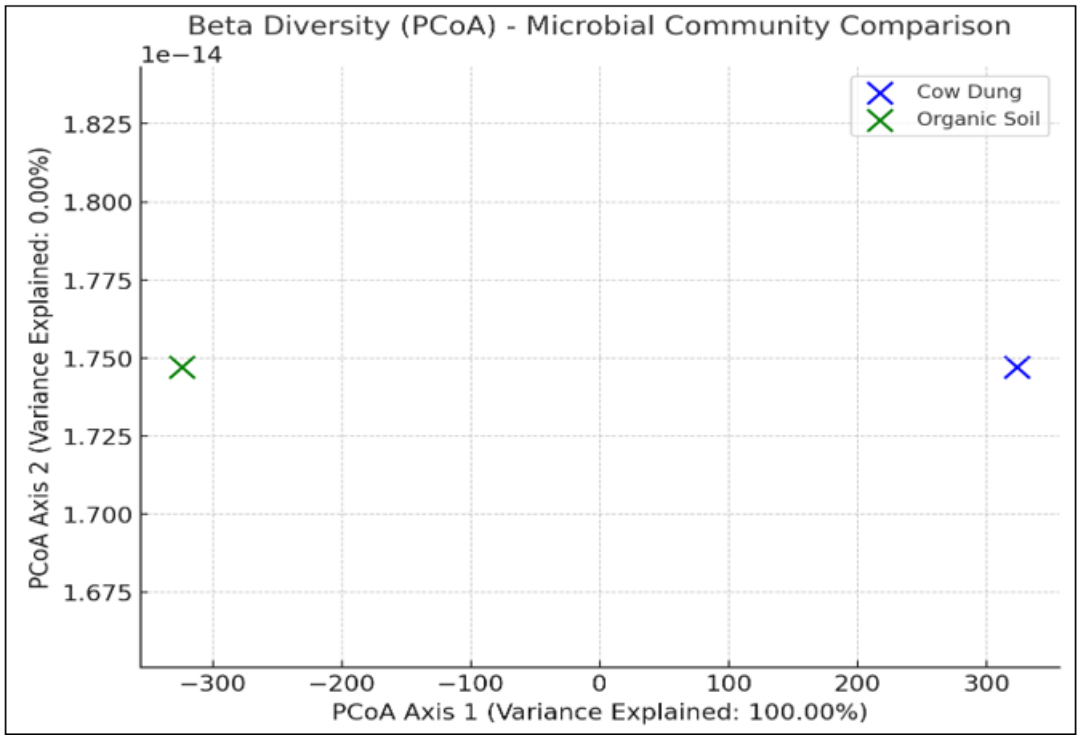
The pie charts represent the KEGG pathway contributions in cow dung and organic soil, highlighting variations in functional gene distribution. In the cow dung sample, methanogenesis dominates with 48.6% of the total pathway contribution, followed by hydrolysis at 40.0%. Lignin breakdown and plastic degradation contribute minimally,

with 8.6% and 2.9%, respectively. This indicates a strong presence of methanogenic and hydrolytic microbial activity, which aligns with the anaerobic nature of cow dung environments.



In contrast, the organic soil sample exhibits a different metabolic profile. Lignin breakdown accounts for 39.1%, followed closely by plastic degradation at 34.8%. Hydrolysis contributes 21.7%, while methanogenesis is significantly lower at just 4.3%. This suggests that the microbial community in organic soil is more focused on lignocellulose degradation rather than methane production. The high plastic degradation potential in organic soil could indicate the presence of microbes capable of breaking down synthetic polymers. These variations in pathway contributions highlight distinct microbial adaptations in anaerobic and soil ecosystems, emphasizing their different ecological roles.

Beta Plot

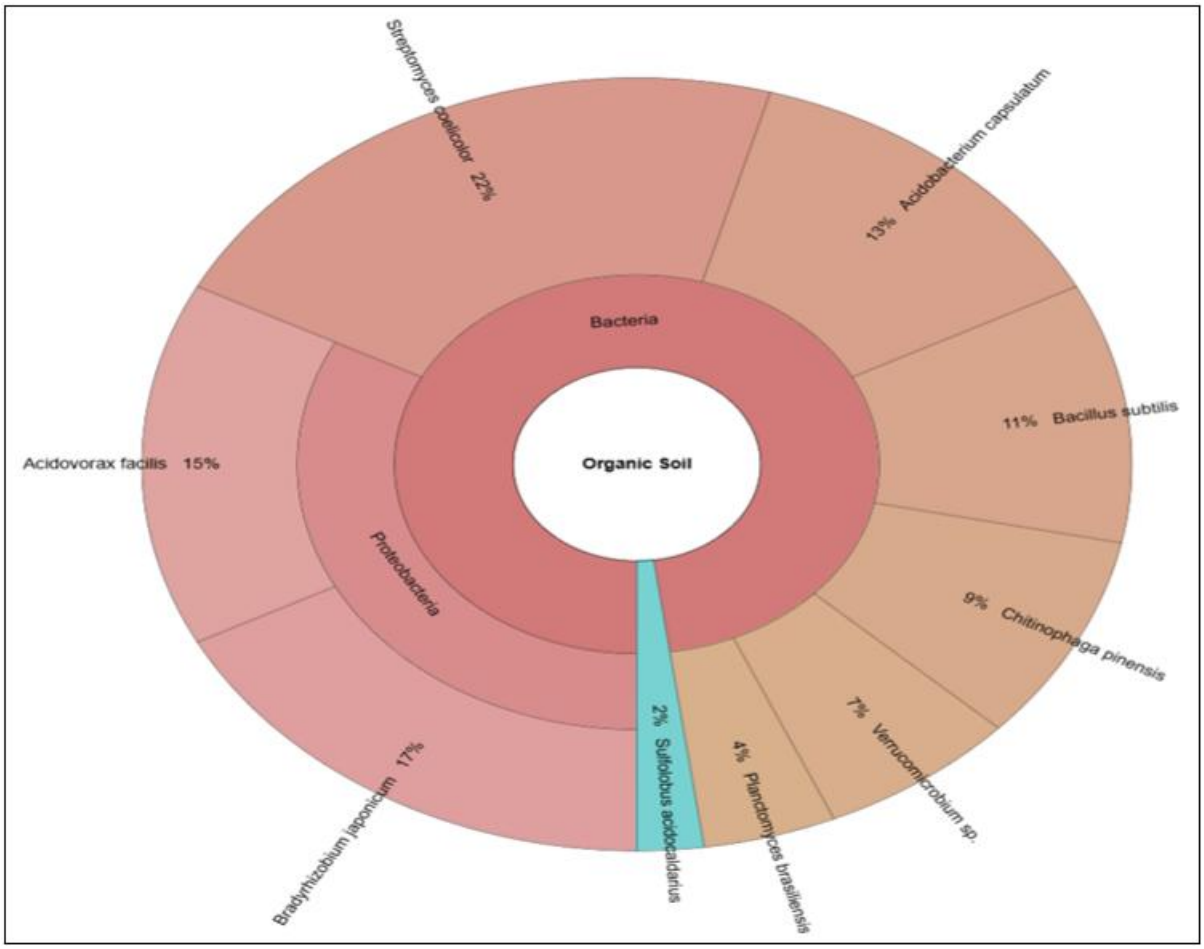


This Principal Coordinates Analysis (PCoA) plot represents the beta diversity of microbial communities from cow dung and organic soil. Beta diversity measures differences in microbial composition between samples. The x-axis (PCoA Axis 1) explains 100% of the variance, while the y-axis (PCoA Axis 2) contributes 0%, meaning all variation is captured along the first axis. The cow dung microbial community (blue marker) is positioned far apart from the organic soil community (green marker), indicating significant differences in their microbial compositions. These differences likely arise due to distinct environmental conditions, substrates, and microbial functions in cow dung and organic soil. The plot suggests that cow dung harbors a highly specialized microbial community, differing greatly from the organic soil community, which may have more diverse microbial interactions. This separation highlights the unique microbial niches present in each environment. The cow dung community is likely enriched with methanogenic and

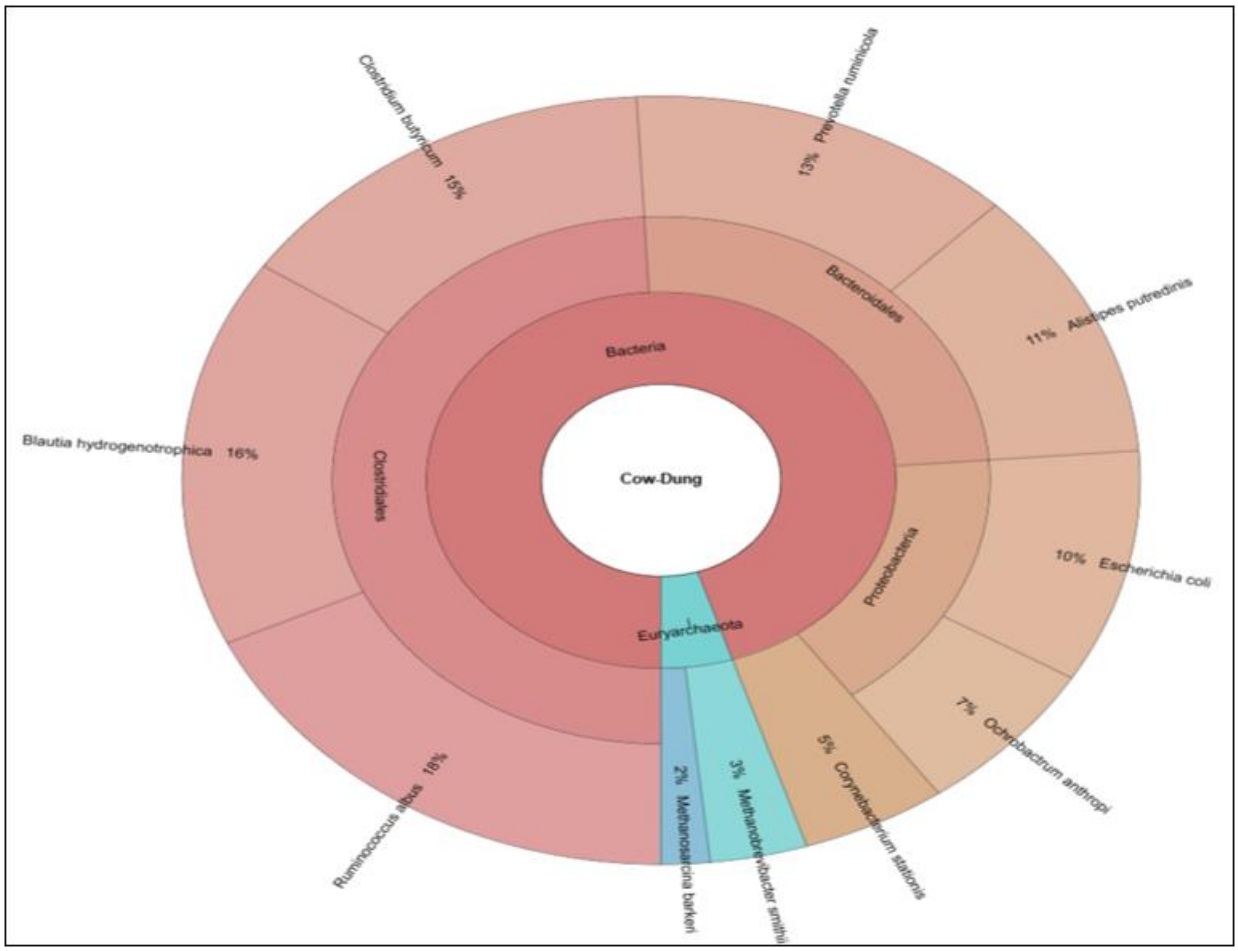
hydrolytic microbes, whereas organic soil may support lignin degraders and plastic degraders. These findings emphasize the impact of habitat-specific conditions on microbial diversity and functional potential.

Krona Plots

The image displays a Krona chart visualizing the taxonomic composition of bacterial communities in organic soil. The hierarchical, sunburst-style chart allows interactive exploration of microbial diversity, where each concentric layer represents different taxonomic levels. The innermost circle signifies the broadest category, Bacteria, while the outer rings progressively break down into specific taxa. The abundance of different bacterial species is depicted through segment size and percentage values. Dominant bacterial taxa include Streptomyces sviceus (22%), Acidovorax facilis (15%), and Bacillus subtilis (11%), indicating a strong presence of actinobacteria and proteobacteria. Additionally, Sulfolobus acidocaldarius (2%), a thermophilic archaea, appears in the dataset, possibly indicating soil samples from an extreme environment. The interface includes options for adjusting depth, font size, and chart size, along with a search function for quick filtering. The total microbial abundance is recorded as 230, representing the number of identified sequences. The chart effectively highlights bacterial diversity in organic soil, providing insights into potential biogeochemical roles such as decomposition, nitrogen fixation, and soil health. This visualization is crucial for metagenomic studies, helping researchers identify key functional bacteria in different environmental samples.



The circular, interactive visualization represents the taxonomic composition of the microbial community. The innermost circle shows the primary category as cow dung, branching into Bacteria and Euryarchaeota (Archaea). The bacterial phyla include Firmicutes, Bacteroidetes, and Proteobacteria, with species like *Clostridium butyricum* (15%), *Escherichia coli* (10%), and *Alistipes putredinis* (11%). The archaeal community consists of methanogenic species like *Methanosarcina barkeri* (2%) and *Methanobacterium formicicum* (3%), which are involved in methane production. The abundance values indicate relative proportions of different taxa within the sample. This analysis helps understand the microbial ecology of cow dung, particularly its potential for biogas production, organic matter degradation, and hydrolysis. The Krona chart allows interactive exploration, making it useful for metagenomic data visualization and microbial community profiling.



5. Discussion

The findings from this study underscore the significant biotechnological potential of environmental microbial isolates, particularly those derived from cow dung. The comprehensive physicochemical analysis revealed key nutrient compositions such as nitrate, nitrite, and ammonia, which influence microbial growth and metabolic activities. The assays evaluating *Methanosarcina barkeri* confirmed its ability to produce methane efficiently, with optimal substrate utilization and growth patterns observed under controlled conditions. Notably, enzyme activity assays highlighted the presence of hydrolytic and methanogenic enzymes, suggesting a strong potential for bioenergy applications.

Shotgun metagenomics provided valuable insights into the microbial community composition and functional gene distribution. The heatmap analysis demonstrated distinct enzymatic profiles between cow dung and organic soil, with cow dung favoring protease and lipase activity, while organic soil exhibited higher lignocellulose degradation potential. KEGG pathway analysis further emphasized the metabolic distinctions, with methanogenesis dominating in cow dung and lignin breakdown and plastic degradation being more prevalent in organic soil. These findings were reinforced by PCoA and Krona plot analyses, which highlighted significant microbial diversity and ecological adaptations.

Overall, this study validates the role of microbial bioprospecting in sustainable biotechnology. The results suggest that cow dung-derived methanogens can be effectively utilized for biogas production, while soil microbial communities hold promise for lignocellulose and plastic degradation. Future research should focus on optimizing microbial consortia for enhanced bioenergy production and environmental remediation.

The inhibition assay results demonstrated that specific inhibitors, such as 2-bromoethanesulfonate, significantly suppressed methane production and microbial growth, reinforcing the sensitivity of methanogenic pathways to external factors. This finding is crucial for controlling methane emissions in industrial and environmental settings. Similarly, the volatile fatty acid (VFA) degradation assay confirmed the efficiency of methanogens in breaking down organic acids into methane, with a positive correlation between VFA reduction and methane yield. These observations highlight the adaptability of methanogens to varying substrate conditions and their potential role in optimizing anaerobic digestion processes for renewable energy production.

Furthermore, the coenzyme F420 fluorescence assay provided insights into the metabolic activity of *Methanosarcina barkeri*, revealing a strong correlation between fluorescence intensity and microbial growth. This serves as a reliable indicator of methanogenic activity in anaerobic environments. The metagenomic analysis, particularly taxonomic profiling via Krona plots, showcased a diverse microbial ecosystem, with dominant methanogenic archaea and hydrolytic bacteria contributing to essential biochemical processes. The distinct microbial compositions

in cow dung and organic soil suggest niche-specific adaptations, reinforcing the need for targeted microbial applications in biogas production, organic waste management, and bioremediation. Future studies should explore genetic and metabolic engineering approaches to enhance microbial efficiency for industrial-scale applications.

The study confirms the high biotechnological potential of environmental microbial isolates, especially methanogens derived from cow dung, for bioenergy production and waste degradation. Through a combination of physicochemical assessments, enzyme assays, and metagenomic analyses, the research delineates distinct microbial communities and their functional contributions in different environmental contexts. Future investigations should focus on optimizing microbial consortia and harnessing genetic tools to scale up applications in sustainable biotechnology.

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