CHAPTER-7

7.1. Summary

The annual global generation of LCW exceeds 200 billion tons, with paddy straw and food waste being the primary components. The resistance of lignocellulosic materials to microbial intervention due to their complex structures necessitates effective treatment methods for nutrient recovery. Vermicomposting act as an effective technology for converting solid waste into organic fertilizers. It relies on the synergistic activity of earthworms, earthworm gut microorganisms, and microbial communities in the feedstocks. The study notes that vermicomposting's efficiency is influenced by the nature of the feedstocks, the compatibility of the earthworms, and microbial growth. Previous studies using 16S rRNA gene-based analyses provided limited insights into the microbial communities in vermicomposting systems. This study identifies a gap in the use of next-generation sequencing (NGS) techniques to assess microbial diversity in systems based on lignocellulosic waste, such as paddy straw and food waste.

7.1.1. Key Findings

- Significant decreases in pH, organic C (~3 fold), and XRD-derived crystallinity are seen most evidently in the paddy straw-food waste (1:1) mixtures upon vermicomposting (compared to composting).
- Concurrent increment of nutrients (NPK) (~2–3.5 fold). Vermicomposting significantly increased the bioavailability of phosphorus (P) and potassium (K) compared to composting. After 60 days, K-availability was notably higher in vermibeds PS+FW(1:1)v (V4) with lignocellulosic feedstocks like Paddy straw (PS) and Food waste (FW).
- Vermicomposting enhanced microbial biomass carbon (MBC) and microbial respiration (MR). The highest MBC was observed in PS+FW(1:1)v (V4) followed by PS+FW+CD(3:3:2)v V3 and PS+FW+CD(2:2:1)v (V2), indicating substantial microbial proliferation. Microbial respiration (MR) also increased significantly over time, particularly in vermibeds PS+FW+CD(3:3:2)v (V3) and PS+FW(1:1)v (V4).

- The total bacterial and fungal counts were significantly higher in vermicomposting treatments. For instance, bacterial count peaked in PS+FW (1:1) vermibeds (V4), and fungal populations followed a similar trend.
- The NGS approach revealed significant differences in microbial communities and functional genes between vermicompost and traditional compost. Vermicomposting systems showed an enhanced presence of bacterial families like *Beijerinckiaceae* and *Propionibacteriaceae*, which improve soil quality. *Proteobacteria* were dominant in both, but *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes* were more abundant in vermicompost (LCWv), indicating a richer microbial diversity.
- Vermicompost samples (LCWv) had higher concentrations of genes related to amino acid transport, RNA processing, and nuclear structure development compared to compost samples, which had more genes associated with carbohydrate transport and defense mechanisms.
- Vermicompost samples (LCWv) exhibited higher numbers of DNA reads and N50 scaffolds, suggesting more extensive microbial activity and diversity facilitated by earthworms.
- Vermicomposting showed higher waste conversion efficiency (WCE) and benefit-cost ratio (BCR) compared to aerobic composting.

The experiment revealed the superiority of vermicomposting, mediated by *Eisenia* species, in handling recalcitrant biomass compared to aerobic composting. The advanced metagenomic analysis provided deeper insights into the microbial dynamics, which could guide the selection of suitable feedstocks for enhanced vermicomposting efficiency. The study concludes by suggesting that this approach can lead to the isolation of economically viable microorganisms for broader applications.

In the phase 2 of this study, the main aim was to study comprehensively the capabilities of novel bacterial strains derived from earthworm gut and vermicomposting systems to contribute to the development of sustainable and efficient bioethanol production processes. The study is conducted in two stages, with the first focusing on isolating and characterizing bacteria with plant-growth-promotional traits including nitrogen-fixing, phosphate and potassium-solubilizing, siderophore-producing, and indole-3-acetic acid and the second on isolating ethanologenic bacteria from lignocellulosic biosolids. The isolation process involves collecting and preparing earthworm specimens and vermicompost samples, followed by microbial colony

development and screening for cellulose-degrading and carbohydrate-utilizing bacterial strains. Conventional bioethanol production involves feedstock pretreatment and enzymatic hydrolysis to convert cellulosic materials into sugars. Here, we aimed to optimize bioethanol production by reducing process steps. Cellulolytic microorganisms are crucial for breaking down cellulose in LCW, accelerating composting, and improving compost quality. Despite the promise of yeast (*Saccharomyces cerevisiae*) in ethanol production, its limitations in utilizing cellulose and hemicelluloses prompt the search for alternative microorganisms.

Gas chromatographic estimation of ethanol production is conducted using sugar solutions and banana peel substrates. The ethanol production potential of selected bacterial isolates is compared with yeast. Additionally, sedimentation rate, ethanol, and sugar tolerance of microbial cultures are assessed to determine their industrial applicability. The identification of microorganisms is carried out through Gram staining, 16S rRNA sequencing, and fatty acid methyl ester (FAME) analysis. The study concluded with the exploration of the osmo-adaptation mechanism of selected bacteria compared to yeast, assessing the activities of key enzymes involved in bioethanol production.

7.1.2. Key Findings

- Selected bacterial strains demonstrated significant potential for ethanol production. This was compared to the yeast Saccharomyces cerevisiae, commonly used in industrial bioethanol production.
- The sedimentation rate, ethanol, and sugar tolerance of these bacterial strains were measured. These factors are crucial for assessing the industrial applicability of the strains. The activities of key enzymes involved in bioethanol production were analyzed, offering insights into the microbial mechanisms at play.
- Gram staining and 16S rRNA sequencing were used to identify the bacterial strains. Some strains were also identified through fatty acid methyl ester (FAME) analysis.
- Out of total 9 strains isolated, 6 bacterial species (*Citrobacter freundii* OS8, *Serratia marcescens* OS6, *Bacillus albus* T24, *Serratia marcescens* PB1, *Bacillus cereus* B3, and *Bacillus halotolerans* B8) showed plant-growth-promoting traits and cellulose degrading efficiency.
- The remaining three strains (*Kosakonia sacchari* C1, *Enterobacter cloacae* C3 and *Bacillus alcalophilus* C5) showed ethanol production capabilities without the need for pre-treatment or externally supplemented enzymes. Specifically, *Bacillus alcalophilus*

C5 produced significantly more ethanol (~ 5–15 g L^{-1}) than yeast, and were tolerant to ethanol and sugar shocks, highlighting their industrial applicability.

- The IAA production capabilities varied among the strains, with *Citrobacter freundii* OS8 showing the highest production.
- The phylogenetic analysis based on 16S rRNA sequences revealed the evolutionary relationships among the isolated strains. The strains showed affiliations with specific taxonomic groups, indicating a significant modification of the microbial community profiles in the LCW feedstocks during vermicomposting by earthworm activities.
- Comparative bioinformatics analyses provided insights into the microbial diversity and the overall abundance of taxonomic groups at the phylum, class, and order levels in the vermicomposting systems. This included the dominance of *Proteobacteria* and the presence of beneficial microbial species in the LCWv samples.
- The study also characterized the metabolic and biochemical properties of the isolated strains. For example, *Bacillus albus* T24 isolated from *Eisenia fetida* guts exhibited multiple plant-growth-promoting traits, such as nitrogen fixation and cellulose degradation.

In the third phase, the major aim here was to visualize the environmental and economic challenges posed by managing lignocellulosic waste (LCW), particularly agricultural residues like field stubbles. Traditional disposal methods such as open burning lead to air pollution and the loss of valuable biomass. An innovative solution explored in this work is the development of bacterial consortia to enhance the biodegradation of LCW. These consortia, consisting of multiple efficient bacterial strains, isolated from previous phase of work demonstrate superior efficiency in breaking down complex organic compounds compared to single strains, thereby benefiting soil health and reducing environmental impact. Bacterial strains were selected and combined based on their cellulose-degrading capabilities and synergistic relationships. Nutrient agar plates and broths were used to grow and measure the synergy between the strains. Various economically viable and efficient media, including Rice washed water (RWW), Rice starch (RS), Rice washed water (RWW) + Rice starch (RS) (50:50) ratio, and Nutrient broth (NB) (Positive control), were tested to determine optimal growth conditions of the strains. Twelve consortia were developed and tested in vitro by mixing them with LCW and measuring changes in carbon and nitrogen content, as well as weight changes over 20 days. The five most effective consortia were then selected for large-scale field trials. In these trials, the consortia were applied to plots with straw, and their effects on LCW degradation and soil attributes were

monitored during the initial days of the experiment and the final days of maturity through volume reduction measurements, changes in pH, total organic carbon, humic acid, fulvic acid, total nitrogen and microbial biomass carbon.

7.1.3. Key Findings

- The in vitro experiments showed that specific bacterial consortia significantly enhanced the degradation of LCW. The combination of S7 (PB4 + T3 + B6 + B3 + OS8), for instance, exhibited strong synergistic interactions, leading to efficient LCW breakdown.
- Field trials demonstrated that the consortia-treated plots had increased levels of total organic carbon and total nitrogen, indicating enhanced soil fertility.
- The pH levels in the consortia-treated plots increased from acidic to neutral in all the treatments except for treatment S1 (T3 + OS2 + B3 + PB4 + PB1 + B6 + OS8 + B8) (8.07±0.04), which is slightly higher basic then all the other treatments
- The bacterial consortia effectively accelerated the decomposition of LCW in the field, reducing residue accumulation and promoting better nutrient cycling in the soil providing an eco-friendly alternative to traditional disposal methods, mitigating the adverse environmental impacts of agricultural residue burning.
- *Aeromonas hydrophila* T3 vs. *Erwinia tasmaniensis* PB4 shows the highest aggressivity value, indicating a strong competitive interaction between these strains. The lowest aggressivity was found in *Erwinia tasmaniensis* PB4 vs *Citrobacter freundii* OS8 (-0.013 mm^2).

- Through the development of five effective microbial consortia S1 (T3 + OS2 + B3 + PB4 + PB1 + B6 + OS8 + B8), S2 (T3 + B3 + B6 + B8 + OS8), S7 (PB4 + T3 + B6 + B3 + OS8), S10 (PB1 + PB4 + B6) and S11 (T3 + PB1 + B8 + B6), our study illustrates their superior capability in breaking down lignocellulosic waste compared to individual microorganisms without any pretreatment.
- The data suggests that selecting strain combinations with lower aggressivity could lead to more effective and cooperative consortia.
- The volume of the lignocellulosic biomass greatly reduced in most of the treatments. The highest reduction in volume was found in S7 (7.85±0.18) approximately 70.84% reduction followed by S10 (9.68±0.13) approximately 63.99% reduction and S2 (10.82±0.46) approximately 59.74% reduction.

- During the in-vitro and on-field experiments, consortium S7 (*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8) stood out as the most efficient consortia.