ABSTRACT

Nearly 50% of the solid waste generated in the world is biogenic and contains lignocellulosic substances. According to a recent report, the annual global generation of lignocellulosic biomass is more than 200 billion tons, with paddy straw and food waste as the dominant components. The food waste generated worldwide has a similar predominance of lignocellulosic substances, which are integral components of plant cell walls, complex in structure, and inherently hydrophobic. Complex networks of these polymeric compounds not only resist microbial intervention but also deactivate some vital enzymes. The recalcitrance of lignocellulosic materials thus limits their efficient utilization, and appropriate treatment is required to successfully recover nutrients from these substances. Composting and vermicomposting are key technologies for converting numerous types of solid waste into valuable organic fertilizers. Composting is an aerobic biological system that promotes the inherent microorganisms to decompose organic biowastes. Vermicomposting, on the other hand, takes advantage of synergy among earthworm activity, earthworm gut microorganisms, and microbial communities inherent in feedstocks. In this context, epigeic earthworms like *Eisenia fetida* are most effective due to their wide adaptability, unique defense mechanisms, and voracious feeding habit.

Nevertheless, the true value of earthworm-mediated lignocellulosic waste transformation can only be assessed by studying the microbial community structure during the biocomposting process. This can only be achieved through high-level next generation sequencing approaches. As such, little is known about the functional and taxonomic diversity of *Eisenia fetida* mediated lignocellulosic waste-based vermicomposting systems. So far, microbial metagenomic analyses are used to interpret microbial diversity and community shifts in response to change in the immediate environment. Such knowledge is rarely applied to generate microbial resources.

Previous 16S rRNA gene-based studies of bacterial and fungal communities provided only partial knowledge because numerous eukaryotic and prokaryotic organisms can arise during bio-composting. A next-generation sequencing (NGS)-based whole metagenomic approach can properly and comprehensively assess the organismal diversity and functionality found in vermicomposting systems.

Considering those research gaps, I was interested to investigate the vermicomposting and composting systems for optimization of the waste valorization process to successfully recover nutrients from these substances, to study the variation in microbial diversity of lignocellulosic waste-based feedstocks under composting and vermicomposting, and to identify how earthworms (*Eisenia fetida*) regulate microbial community structures in such feedstocks. Also, I was interested to isolate and identify efficient lignocellulosic waste (LCW) degrading microorganisms from vermibeds and earthworm intestines and asses their performance regarding plant growth promoting traits and bioethanol production from LCW. Finally, I wanted to form and optimize microbial consortiums for rapid conversion of agricultural field stubbles.

The study was initiated through critical analysis of different feedstock combinations for vermicomposting and aerobic composting. Temporal changes in various physicochemical and microbial attributes were assessed for two months (60 days) from May to June 2019. Eventually, one of the most enriched feedstocks from each condition (composting and vermicomposting) was selected for whole metagenome analyses on the Illumina NGS platform. This assessment provided strong scientific basis of the standardized vermitechnology for large scale application. The study provided some vital information and knowledge for standardization of the vermitechnology. The knowledge acquired from NGS-derived taxonomic and functional information was utilized for the first time to isolate and characterize a few bacterial strains with promising plant-growth-promotional traits. Eventually, industrially suitable ethanologenic bacteria were isolated from lignocellulosic waste-based vermicomposting systems. Additionally, these strains were used for consortium building and some of the most effective consortiums were applied in the field for determination of efficient field stubble degradation.

The first chapter of the thesis is comprised of a general introduction addressing the background, severity of the identified problem, and research gaps; based on which the research objectives were derived. The second chapter comprised of an extensive review of literature focusing on most recent and relevant research information in the subject domain. The third chapter describes the methodology and broad research plan in brief. The fourth chapter deals with the study on characterization and LCW mineralization potential of vermitechnology using selected earthworm species (*Eisenia fetida*), which is followed by the fifth chapter describing the isolation, characterization, and applications of efficient

LCW degrading bacterial strains from vermibeds and earthworm intestines as consortium candidates and ethanol production. The sixth chapter deals with the invitro and field-based assessment with crop refuge using bacterial consortiums. The last chapter (Chapter 7) summarizes the whole study featuring the major findings of the research.

Biocomposting experiments and their outcomes

The first objective (i.e., to assess the efficacy of vermitechnology for rapid degradation of lignocellulosic wastes (LCW) with respect to time, product quality, and microbial community structure) has been addressed in the Chapter 4 of the thesis. Food waste (FW) samples were collected from the waste disposal yard of Tezpur University, Assam, India and hostel kitchen. At the same time, paddy straw (PS) and cow dung (CD) samples were collected from the agricultural field of a local farmer in Tezpur. The lignocellulosic wastes (paddy straw and food waste) have been mixed with and without cow dung in different ratios and vermicomposted with *Eisenia fetida*, while using a series of aerobic composting as a control. 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) along with a concurrent increment of nutrients (NPK) (∼2–3.5 fold). Significant augmentation in microbial activity (biomass and respiration) and growth (bacteria and fungus) is observed under vermicomposting. A considerable shift in taxonomic diversity, accompanied by differential functional diversity of the microbial communities, is detected between paddy straw-food waste (1:1) vermicompost and compost after 60 days of incubation. The overall gene volume is greater in the vermibeds than in the compost, and genes of a few well-known microbial communities with good plant growth promoting traits (e.g., *Beijerinckiaceae* and *Propionibacteriaceae*) are exclusive to the vermicompost. Additionally, genes associated with beneficial microbial activities, such as amino acid transport, nuclear structure development, and lipid transport, are found to be more abundant in vermicompost than in compost. These data are helpful in identifying suitable feedstock for isolating scalable microbial species with beneficial traits. Subsequently, six multi-dimensional plant-growth-promoting endophytic bacterial species are isolated from both the vermibeds and earthworm guts. Interestingly, close genetic resemblances are found for a few of these isolates with the metagenomically detected genes. In conclusion, this is the first study to identify the practical utility of next-generation sequencing-based metagenomic analyses

for the meaningful isolation of economically viable microbial species from vermicomposting systems that might replace a sizeable portion of the chemical fertilizers used in agriculture.

Insight on isolation of novel and industrially suitable ethanologenic bacteria from lignocellulosic waste-based vermicomposting systems

In Chapter 5 the details of the biocomposting experiments followed by isolation of ethanologenic bacteria and their applicability assessment studies are presented. This experiment accomplished the second and third objective (i.e., to isolate and identify efficient LCW degrading microorganisms from vermibeds and earthworm intestines; and performance assessment of the identified microbes regarding bioethanol production from LCW and plant growth promoting traits). All the strains that were isolated from the vermibeds and earthworm gut could exhibit efficient sugar and cellulose solubilizing potential along with high ethanol production ability without pre-treatment. Additionally, these strains were strongly tolerant to inhibitory factors. We postulated through enzyme assay that appropriate activation of enzymes like alcohol dehydrogenase was one of the critical attributes that imparted high ethanol-producing capability in *Bacillus alcalophilus* C5. The study with flow cytometry and confocal microscopy revealed that reverse/delayed apoptosis and strong mem-brane integrity could be the defence strategies in bacteria that facilitate their growth and maintain ethanol production capacity in sugar-enriched conditions. High waste-to-wealth conversion efficiency with a significant benefit–cost ratio strongly substantiates the practical applicability of the identified organisms for bioethanol generation from recalcitrant biowastes.

Performance assessment on formation and optimization of microbial consortiums for rapid conversion of agricultural field stubbles

The Chapter 6 deals with the fourth objective of the thesis (i.e., the development of bacterial consortiums, for assessing the efficacy of the developed consortiums to decompose agriculture field stubbles and crop residues). A total of 12 bacterial consortiums was developed using eight different strains, out of which five consortiums showed significant results (weight reduction, total organic carbon and total nitrogen) in-Vitro. Correspondingly, the performance of the developed consortiums was assessed in comparison with other treatments through field experiments with rice straw as the test

material. It was conducted in the mid-winter season for 20 days in the year 2024. The results showed that the consortium S7 comprising of five bacterial strains (*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8) was most efficient to degrade rice straw and field stubbles along with enhancement in microbial biomass carbon.

Concluding summary

The salient outcomes of the study are:

- 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.

- Next-generation sequencing (NGS) revealed distinct microbial community profiles between compost and vermicompost. *Proteobacteria* were dominant in both, but *Actinobacteria*, *Acidobacteria*, and *Bacteroidetes* were more abundant in vermicompost (LCWv), indicating a richer microbial diversity.

- Vermicompost samples (LCWv) exhibited higher numbers of DNA reads and N50 scaffolds, suggesting more extensive microbial activity and diversity facilitated by earthworms.

- 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.

- *Aeromonas hydrophila* T3 vs. *Erwinia tasmaniensis* PB4 shows the highest aggressivity value (2.757 mm^2), 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(*Aeromonas hydrophila* T3 + *Aeromonas Sp.* OS2 + *Bacillus cereus* B3 + *Erwinia tasmaniensis* PB4 + *Serratia marcescens* PB1 + *Bacillus aerius* B6 + *Citrobacter freundii* OS8 + *Bacillus halotolerans* B8), S2(*Aeromonas hydrophila* T3 + *Bacillus cereus* B3 + *Bacillus aerius* B6 + *Bacillus halotolerans* B8 + *Citrobacter freundii* OS8), S7(*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8), S10(*Serratia marcescens* PB1 + *Erwinia tasmaniensis* PB4 + *Bacillus aerius* B6) and S11(*Aeromonas hydrophila* T3 + *Serratia marcescens* PB1 + *Bacillus halotolerans* B8 + *Bacillus aerius* 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 (*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8) (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.