CHAPTER 3

Methodology and planning of the experiments

The aim of this chapter is to present a summary of the methods employed in conducting the experiments. The work plan of the study was divided into four phases.

3.1 Phase I: Efficiency evaluation of *Eisenia fetida* mediated vermicomposting system with respect to aerobic composting.

In the first phase, two types of lignocellulosic wastes (LCWs), food waste (hereafter, FW) and paddy straw (hereafter, PS), were undergone vermicomposting and composting in combination with cow dung to accomplish the first objective. The FW samples were collected from the waste disposal yard, hostel kitchen of Tezpur University, Assam, India. At the same time, the PS and cow dung (hereafter, CD) samples were collected from the agricultural field of a local farmer in Tezpur. The location of the study area is presented in Fig 3.1.

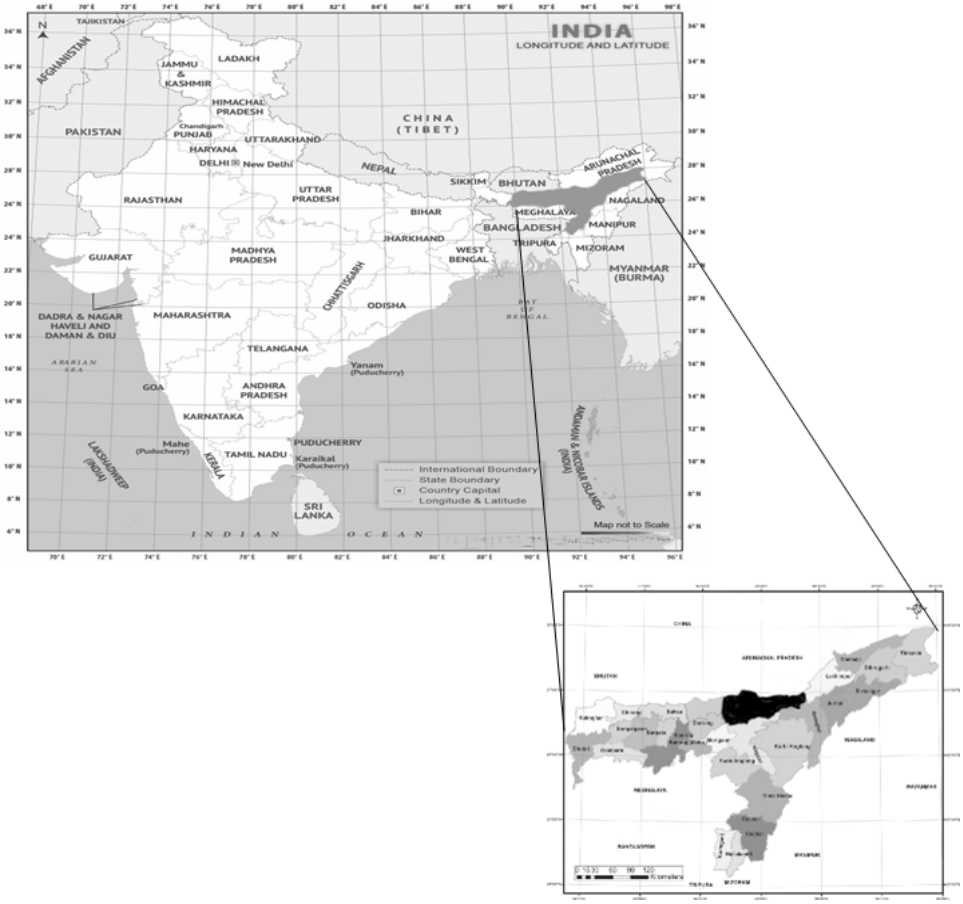

Fig. 3.1: Location map of the waste sample collection area

Subsequently, the physicochemical properties of the waste and CD samples were comprehensively analyzed and the vermicomposting and composting experiment was conducted with eight combinations of FW, PS, and CD. More details of the experimental set up are provided in Chapter 4 of the thesis. The efficiency of the vermicomposting technology was compared with aerobic composting in regard to nutrient enrichment, end product maturity for optimization of vermitechnology for rapid mineralization of lignocellulosic wastes (LCW), with respect to time, nutrient dynamics (e.g. C/N ratio, available P, available K, microbial biomass C, microbial metagenomic assessment, etc.) by employing established methodologies [1].

3.2 Phase II: Isolation and characterization of potent plant growth promoting and biomass degrading microorganisms from earthworm gut and vermicompost samples

In the second phase, an effort was made to isolate and identify LCW degrading microorganisms from vermibeds and earthworm intestines (*Eisenia fetida*), based on the results of the first phase experiment using standard protocol [2,3]. The detail results of this study can be found in the Chapters 4 and 5 of the thesis. The performance efficacy of the isolated microbial strains was assessed in regard to a few useful traits (cellulose degradation, ethanol production, N-fixation, P-solubilization, siderophore production, and other plant growth promoting activity) [4]. Among these beneficial traits, major emphasis was given on cellulose degradation and bioethanol production. As the results of bioethanol production potential study was highly encouraging, the efficacy of presently identified strains was evaluated in comparison with yeast (*Saccharomyces cerevisiae*) and a few earthworm-gut isolated previously reported strains and the underlying mechanism of bacteria-mediated bioethanol production from LCW was examined using confocal microscopy and flow cytometry techniques [5]. The previously isolated strains were reported for their N-fixing and P-solubilizing potential of one of my seniors of my lab [3]. This effort was made to develop comprehensively effective microbial consortia for treating LCWs. These results can be found the Chapter 6 of this thesis. The 2nd phase experiments could accomplish the 2nd and 3rd objectives of the study, respectively.

3.3 Phase III: Formation of microbial consortiums for rapid conversion of agricultural field stubbles

Finally, 5 prolific consortiums were developed from the previous study and upscaled for rapid conversion and degradation of agricultural field stubbles during the 4th phase of the research. The efficacy of the developed consortiums was initially assessed in the laboratory and based on the lab-scale evaluation; five potent consortiums were scaled-up for the field experiment. Rice starch solutions were used as media for field application due to its profuse availability and excellent ability to sustain the growth of microbial colonies. The field experiment was conducted in a farmer's field after the harvest of the monsoon rice. For addressing the 4th objective, the farmer was directed not to remove the field stubbles and crop residues. Moreover, the crop residues were uniformly spread over the experimental plots and the efficacy of the bacterial consortiums were assessed in comparison with ashes of burnt crop residue, as positive control. Burning of crop residue and mixing the burnt ash as soil conditioner is a conventional practice in India. Therefore, this treatment was considered as positive control of the experiment.

3.4 Quality assurance and quality control

The experimental analyses were performed following the general quality control (QC) guidelines published by Tezpur University. Quality assurance and Quality control are defined below.

Quality Assurance is defined as "a set of coordinated actions such as plans, specifications, and policies used to assure that a measurement program can be quantifiable and produce data of known quality".

Quality control is defined as "the routine use of procedures designed to achieve and maintain a specified level of quality for a measurement system". A monitoring system without adequate QA/QC runs the risk of not being able to control the quality of data, and not being able to assure accuracy and precision.

3.4.1 Sample storage and preservation

Upon collection, samples from different treatments underwent air-drying, grinding, and sieving as per AOAC guidelines. These air-dried samples were then stored in labeled

plastic zipper bags, with dates corresponding to their collection. Most samples were analyzed within 2-3 days of collection. If any samples needed to be stored, they were kept at 4°C for up to 28 days. Before initiating any measurements or analyses, it's essential to verify that all equipment, materials, and reagents are free of contaminants. Moreover, it's important to keep blank values to a minimum to ensure the accuracy and reliability of the results.

3.4.2 Purity of chemicals, reagents and labwares

All chemicals utilized were of GR grade (guaranteed reagent) and certified to have a purity between 90-98%. Ultrapure water sourced from a water purification system (Sartorius Stedim, Germany) was consistently used for reagent preparation. Reagents were freshly prepared on the day of each analysis to maintain their integrity. Before each use, glassware and plasticware were cleaned using reagent water, followed by rinsing with double-distilled water, and then oven-dried.

3.4.3 Calibration procedures

Prior to use, all analytical instruments were calibrated and adjusted as necessary. The calibration was then validated one day before commencing the analysis of new samples. Each calibration procedure included at least one blank and multiple standards. For instruments like the pH meter that do not require blanks, regular standard measurements were conducted. Throughout the experiments, standard reference materials (SRMs) with certified analyte values were employed to ensure the accuracy of our analyses. We maintained the highest purity standards for all reagents, including those used for the pH meter, electrical conductivity meter and UV-Vis spectrophotometers.

3.4.4 Initial demonstration of performance

The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges) and laboratory performance i.e. determination of method detection limits prior to performing analyses.

3.4.5 Linear calibration range (LCR)

The LCR for major instruments like UV Vis spectrophotometer 5and Kjeltec N analyzer, was initially determined and verified every 6 months or whenever a significant change in instrument response was observed. Generally, the verification of linearity was verified by using 1 blank and 3 standards. In general, the linearity was re-established in case when verification data exceeded the initial values by ± 10%.

3.4.6 Instruments and equipments

Operations and maintenance

All instruments and equipments were maintained in optimized condition with all records of correct operation, calibration and trouble- shooting most often. Guidelines followed for daily operations of the following laboratory equipments are as follows:

Equipments: UV-VIS Spectrophotometers, pH Meter & probes Conductivity Meter, Balances and Automated Pipettes.

Calibrations

UV-VIS Spectrophotometers: After initial warm-up of the instrument. Verification of calibration was done after every 40 samples analyzed. Verification of calibration was done after every 40 samples analyzed.

Maintenance: Maintenance yearly once by professionals.

pH Meter & probes: Every 90 samples, calibrating the instrument at pH 4, 7 and 9 with standard solutions.

Maintenance: Checking of probes regularly and ensuring that electrodes are filled.

Conductivity Meter: The conductivity probes were cleaned with double distilled water before and after every use. The surfaces of the probes were usually wiped with high absorbent paper towels to ensure its dryness. The instrument was frequently calibrated with known solutions supplied with the instrument.

Maintenance: Checking of probes regularly as specified by the manufacturer.

Balances: The balances were checked daily before use. The balances were kept in air tight cabinets to nullify the errors in measurement. The precision of the balances was routinely checked by weighing authentic weighs.

Maintenance: After every use necessary cleaning was performed with ethanol. The dust and fallen chemicals during weighing was vacuum cleared. The instrument was serviced and calibrated by certified company engineers once in a year.

Automated pipettes: Used to be calibrated in every 15 days.

Maintenance: Adequate cleaning after every use.

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