Chapter 4

Microbial diversity and nutrient mobilization in lignocellulosic waste (spent mushroom straw) based vermicomposting systems

Introduction

Commercial cultivation of mushrooms generates a substantial amount of employment in many countries [1,2]. About 200 species of oysters are cultivated over the world [3]. Mushroom industry is growing at a good rate and approximately 25 million tons of mushrooms are annually produced worldwide [4]. Rice or cereal straws are mostly used as a substrate for cultivation of mushrooms. They have a significant impact on the growth and functional properties of the fungus (i.e mushrooms). It has been estimated that about 5kg of SMS is generated for production of 1kg of mushrooms [5]. Generally the used-up substrates, hereinafter spent mushroom substrates (SMS), are mostly disposed of in landfills or partly by composting after harvesting of the mushrooms [4,6]. In many countries open-burning is also one of the major disposal pathways of SMS that eventually creates considerable air pollution hazards[5].

Presently, the environmental legislations and vigilance activities have compelled the mushroom growers to search for sustainable disposal pathways of SMS in India[7]. As such, apart from the cereal straws, wood saw dust and cotton waste are also frequently evidenced in SMS [8]. Occurrence of such cellulose rich materials in large proportion gives rise to the recalcitrant nature of the material. Consequently, establishment of sustainable avenue for SMS disposal is a challenging task. As a result, the land filled SMS causes large scale eutrophication of surface water bodies through leaching of nutreints (N and P) [6].

Recycling such spent substrate through composting approaches has been proposed by few workers [9–11].Although vermicomposting is one of the most efficient solid waste stabilizing routes, its application for SMS recycling has been rarely evidenced in the literature. The decaying process of SMS under traditional composting systems is very slow owing to the strong crystalline structure of the polymer; which greatly retards microbial activity. In recent past, X-ray diffractometry (XRD) based assessment of crystallinity in cellulosic biomass has been identified as a dependable technique [12,13].To the best of available knowledge, so far, no studies have applied such

technique for understanding the lignocellulosic biomass conversion efficiency of earthworm species commonly used in vermitechnology. Moreover, how the interactions between earthworms and microbial communities vary in SMS mediated vermibeds would be an interesting area to study for the first time. In fact, the synergy between earthworm and microorganisms is largely responsible for the benefit achieved during vermicomposting with respect to nutrient availability and enzyme activity [14–17].

The phospholipid fatty acid (PLFA) profiling is a dependable biological index to study the microbial diversity in different environmental situations [18]. The PLFAs are produced by the microorganisms for sustaining the cell membrane stability and cellular functions under varied environmental conditions [19,20]. However, this tool has seldom been applied for studying the microbial community in vermicomposting systems. Hence, the identified research questions for the current study were: a)How different earthworm species respond to SMS based feedstock? ; b) Does the presence of earthworms influences the microbial community structure in such feedstock?; and c) Does the change in biomass crystallinity varies depending on the earthworm species used for vermicomposting?. The third question was important because the crystalline arrangement in lignocellulosic materials largely inhibits the enzymatic degradation process [21]. Therefore, the changes in SMS feedstock during vermicomposting with three earthworm species (*Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*) were assessed on the basis of XRD derived crystallinity index (CI), Fourier transforms infrared spectroscopy (FTIR), nutrient availability (C, N, and P), microbial growth, metabolism, and microbial community structure through PLFA assay.

Finally, seed germination assay was conducted to evaluate the impact of the vermicomposted SMS on plant growth.

Materials and methods

Experiment design, Collection , treatments

Phase 1

The SMS samples were procured from the mushroom production unit of Defence Research & Development Organization (DRDO), Defence Research Laboratory (DRL), Tezpur, Assam, India. The SMS was the byproduct of the standardized oyster mushroom (*Pleurotusostreatus*) cultivation system. Clitellated specimens of three epigeic and well

documented earthworm species (*Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*), weighing about 300-400mg were collected from designated stock maintained in the vermiculture unit of Department of Environmental science, Tezpur University. 2-3 days old urinefree cowdung samples were used in this experiment.

Phase 2:

Three parallel series of earthen vermireactors were arranged for three different species. The vermireactors were designed according to the standardized size and dimensions [size: 3 l; dimensions: 45 cm (height) × 15 cm (base radius) × 30 cm (top radius)] with one leachate hole at the base as reported earlier[22]. The vermireactors were placed on concrete floored vermi-yard having a corrugated sheet made roof with open sides. The collected SMS materials were thoroughly mixed with cowdung (CD) in3:1 ratio after enumerating the inherent physico-chemical characteristics **(table 4.1)**. Subsequently, 3 kg of the homogenized mixtures of SMS and CD were poured in the vermireactors assigned for the experiments. Then the specimens of the selected earthworm species were separately introduced @ 10 worm kg-1in the respective series of reactors as mentioned above. A series of aerobic composting reactor with identical feedstock was maintained under same condition for comparison.

Phase 3:

The details of treatment combinations are given below:
Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control)
VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida*
VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae*
VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*

The experiment was conducted for 60 days within a range of 27-31 ºC and 40-50% moisture contents. Such conditions were maintained by sprinkling water and turning up the pile twice daily at 9 am and at 4 pm. Therate of leachate generation was negligible owing to adequate aeration ensured by the daily mixing operation. Despite, the generated leachates were collected at the bottom in earthen trays and eventually recycled for watering. The moisture and temperature were regularly recorded with the help of a wet bulb thermometer. At the end of the incubation period (60 days), earthworms and their cocoons were carefully sieved out from the vermicomposted materials. Then, the

prepared vermicompost were kept under shade for drying for 6-7 days till a stable weight was achieved. The average temperature in the drying shade was about 32 ± 2 ºC. Afterwards, the samples were grinded into smaller pieces, passed through 2 mm mesh sieve, and partly used for analysis. The other parts of the processed samples were stored in plastic containers in freezer

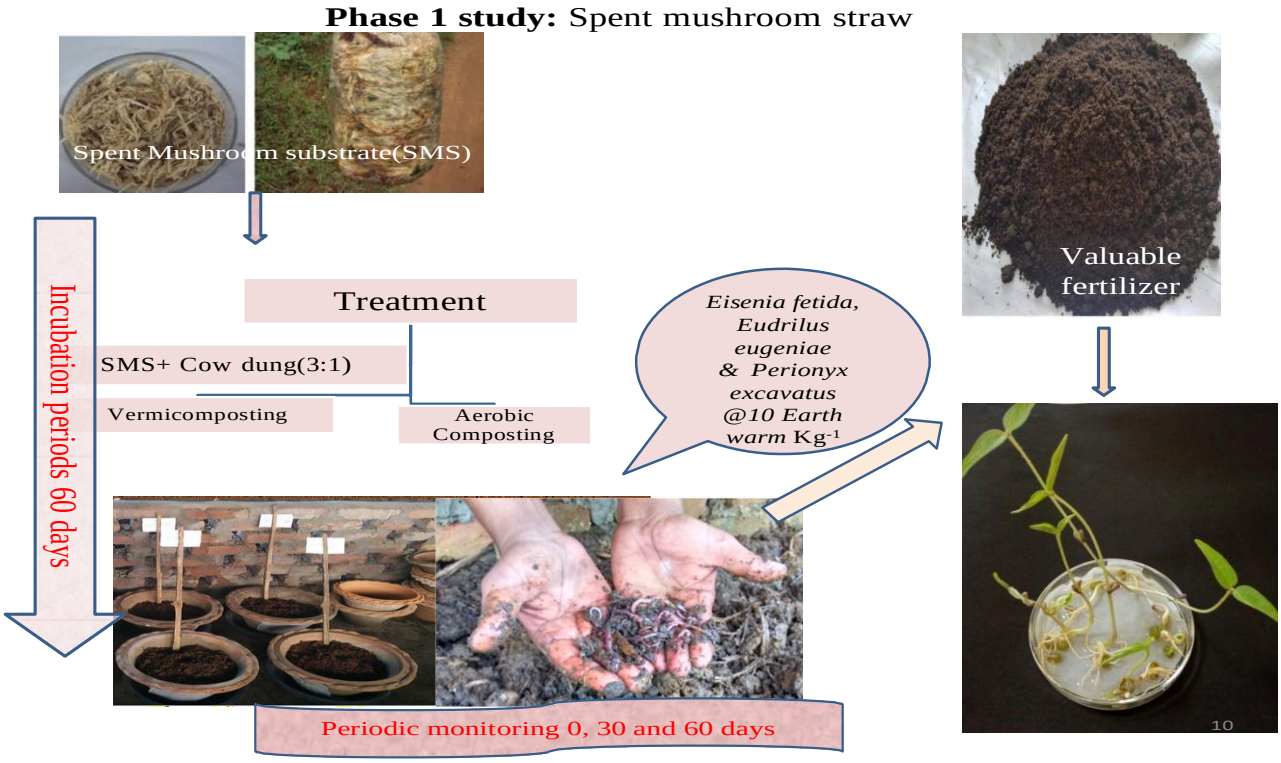

Fig 4.1 Schematic representation of the work done under

Chemical analysis

The temporal changes in chemical properties of the bioprocessed SMS were analyzed on the basis of pH, Total organic carbon (TOC), Total Kjeldahl nitrogen (TKN), and available Phosphorous following standard protocols [23]. Suprapure chemical and deionized water were used for preparation of reagents and other analytical solutions. All glass wares were cleaned with deionized water before use to ensure the removal of adherents and eventually the glass wares were dried at 420C [24].

pH

Requisite

1. pH meter
2. Distilled water

Procedure

1 Taken 10 g air-dry soil (< 2-mm) into a conical flask.

2 Then 25 mL distilled water was added using a graduated cylinder or 50-mL volumetric flask in a 1:2.5 soil suspension.

3 Mixed well with a glass rod, and allow to stand about 1 hour.

4 Calibrate the EuTech pH meter (pH 700) and recorded the pH.

Total Organic Carbon (%) (Modified Walkley and Black method, 1934

Requisite

APPARATUS

1. Beaker
2. Pipette
3. Measuring cylinder
4. Burette
5. Volumetric flask
6. Conical flask
7. Morter and Paster
8. Waste sample.

REAGENTS

1. 1N Potassium dichromate solution (K2Cr2O7): Dissolve 49.04g of Potassium dichromate in one liter of distilled water.

2. 0.5N Ferrous ammonium sulphate solution {FeSO4(NH4)2SO4.6H2O}: Dissolve 196.1g of Ferrous iron solution in one liter of distilled water followed by 20ml of conc. H2SO4.

3. Diphenylamine indicator: 0.5g of diphenylamine indicator dissolve in 20ml of distilled water by the addition of 100ml of conc. H2SO4.

4. 85% Orthophosphoric acid (H3PO4)

5. Conc. Sulphuric acid (H2SO4).

Procedure:

1. Weighed 1.0 g of the soil sample was taken in 500 ml conical flask.
2. Added 10 ml of K2Cr2O7 solution and 20 ml concentrated H2SO4and heated until a few bubble comes.
3. Kept it some time for cooling.
4. Diluted the reaction mixture with 200 ml water.

5. After that added 10 ml of orthophosphoric acid, 1.5ml of diphenylamine indicator.
6. Titrated the solution with standard 0.5M FeSO solution to a brilliant green colour.
7. A blank without sample was run simultaneously.

Calculation: Total organic carbon (%) = $\frac{Vk(1-\frac{Vs}{Vb})}{W}$ ×Sk× 0.3

Where,

Vk: Volume of K2Cr2O7 solution

Vs: Titrant reading

Vb: Blank reading

Sk: Strength of K2Cr2O7 solution

W: Weight of soil sample

0.3 = 3 × 10-3 × 100, where 3 is the equivalent weight of C.

Note:

0.3 = weight of C (1 000 ml 0.1667M K2 Cr2 O7 = 3 g C. and 1 ml 0.1667M K2 Cr2 O7 = 0.003 g C. Thus 100 ml 0.1667M K2 Cr2 O7 = 0. 3 g C).

Total kjeldahl nitrogen (TKN

REAGENTS:

(i) Concentrated Sulphuric acid(H2SO4)
(ii) Copper Sulphate(CuSO4)
(iii) Potassium Sulphate(K2SO4)
(iv) 0.1 N H2SO4: 2.8 ml H2SO4add to 1000 ml distilled water.
(v) 0.1N NaOH: 4 g NaOH dissolve in 1000 ml distilled water.
(vi) Mixed indicator: 0.5 g bromocrescol green + 0.1 g methyl red add to 100 ml alcohol.

Procedure:

1. Taken 1 g soil sample, add 0.8 g CuSO4 and7 g K2SO4.
2. Then added 12- 15ml conc. H2SO4
3. Digested the contents at 420 0 C for 1 hr , keep it to cool and transfer the contents to distillation flask and add 80ml distilled water followed by 50 ml 40% NaOH until the appearance of black colour.

4.Then, started distillation and the distillate was collected in a conical flask containing 20 ml 0.1 N H2SO4 and 3-4 drops of mixed indicator.

5. Finally titrated with 0.1 N NaOH.

Calculation:

Total Nitrogen (%) = (Volume of H2SO4× strength – Volume of NaOH consumed ×strength) ×0.014×100) ÷ Weight of soil

Available Phosphorus (Olsen's method)

REAGENTS:

1. 0.5 M NaHCO3 (pH 8.5) solution (Olsen extretant): Dissolve 42g NaHCO3 in distilled water and make the volume up to 1000ml.
2. Charcoal
3. Ammonium molybdate solution (1.5 %): Dissolve 15g ammonium molybdate [(NH4)6Mo7O.4H2O] in 301ml con HCL and make up the volume with distilled water upto 1000ml in volumetric flask .
4. Stannous chloride solution (5 %): Dissolve 2.5g Stannous cholrid (SnCl2.2H2O) in 5ml con HCl and make the volume upto 50 ml with warm distilled water.
5. 4*N NH*4OH: Dissolve 27ml Ammonia hydroxide (NH4OH) in distilled water and make the volume upto 100ml.
6. 4 N HCl: Dissolve exactly 34.5 ml con HCl in distilled water and make the volume upto 100 ml.
7. 2, 4 dinitophenol indicator : Dissolve 2, 4 dinitophenol in distilled water and make the volume upto 250 ml, filter and use the solution .
8. Standard P solution: 50 ppm stock solution was prepared by dissolving 0.2196 g of potassium orthophosphate (KH2PO4) in 400 ml distilled water. Added 25 ml of 7N H2SO4 to the solution and diluted up to 1 L. From the stock solution, taken 20 ml in a 500 ml volumetric flask and diluted with distilled water up to the mark to prepare a 2 ppm standard P solution.

Preparation of standard curve: Pipetteting out 1, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 20.0 ml 2ppm standard P solution in individual 50 ml volumetric flasks. Added 3 drops of dinitophenol to each flask. To each solution, drop wise added 4 N NH4OH till yellow color appears. Then, add 4 NHCl drop by drop until yellow color disappears and adjust the, pH of each solution to 3. To each flask, add 10 ml of (NH4)6Mo7O.4H2O solution and 2, 3 drops of SnCl2 solution. Making the volume up to the mark.Recorded the

reading against blank solution at 660nm in Chemito 2600 UV-Visible spectrophotometer. Plot the Optical density reading against standard concentration and draw a curve.

PROCEDURE:

1. 2g sample had been taken in 250 ml conical flask.
2. Added 20ml of extracting 0.5 *M* NaHCO3 (pH 8.5) solution and 1g of P free charcoal in the sample.
3. Shaken the flask with sample in the mechanical shaker for 30 minute. After 30-minute filter the sample solution with Whatman no. 42 filter paper.
4. Mixed 5ml of filtrate with 4 to 5 drops of 2, 4 dinitophenol, 4*N*NH4OH, 4 *N* HCl, (5 %) Stannous chloride and taken 5 ml ammonium molybdate and maked the volume up to 50 ml with distilled water.
5. Stired the solution to mix and the reading is taken in the spectrophotometer at 660 nm.

CALCULATION: Total P = P concentration × 10(dilution factor)

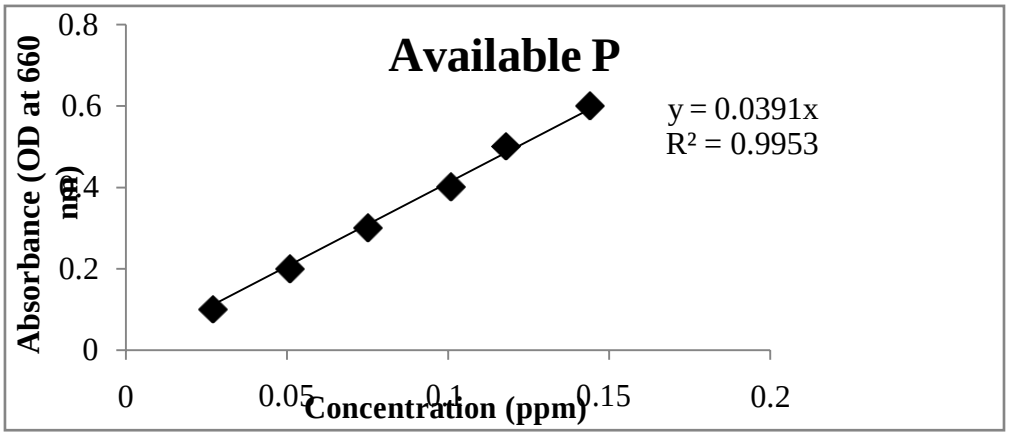

Fig:4.2

FIG: Standard curve of available phosphorus

X-ray diffraction (XRD) and Fourier transforms infrared spectroscopy (FTIR) analysis: Crystallinity and functional of the vermicomposted SMS

X-ray diffraction (XRD)

The air dried samples were kept in glass sample holder and analyzed in plateau conditions in an X-ray diffractometer. Subsequently, the crystallinity index (CI) was computed from the XRD intensity recorded at~ 180 of 2θ (amorphous plane) and at~ 22.80 of 2θ(crystalline plane) using a formula detailed below [23]:

$$\mathrm{CI} = \frac{(I_{002} - \mathrm{I_{amor}})}{I_{002}} \times 100$$

Where,

CI = Crystallinity Index

I002(crystalline) = Intensity at 22.80

Iamor = Intensity at 180

The CI was used to assess the process of degradation of the SMS under different vermicomposting systems.

Fourier transforms infrared spectroscopy (FTIR) analysis

Fourier transforms infrared spectroscopy (FTIR) was performed for determination of the abundance of various functional groupsin vermicomposted samples. The analysis also helps to know the chemical composition of studied materials[13]. The samples were dried at 50 0C for 24 h; subsequently 2 mg of each powdered sample was dispersed on IR grade potassium bromide (200 mg); and the formed pellets were used for analysis. The FTIR spectra were derived at a resolution of 1 cm-1 in a NICOLET spectrophotometer (Model IMPACT 410).

Microbial growth, Microbial biomass C (MBC), compost respiration (CR), microbial quotient (Mq), microbial metabolic quotient (qCO2), and Phospholipid fatty acid (PLFA) analysis

Microbial growth

Total bacterial and fungal counts in vermicomposted samples were assessed after Pramer and Schmidt (1964)[26]. Bacterial and fungal colonies in each vermicomposted SMS samples were grown in petriplates following serial dilution technique and eventually the population were counted in a colony counter and expressed as colony forming units per gram of sample (CFU g-1). Bacterial and fungal colonies were grown in nutrient agar and potato dextrose agar respectively.

Microbial biomass C (MBC)

Reagents and equipment's:

a) Ninhydrin reagent: Dissolved 0.8 g ninhydrin and 0.12 g hydrindantin in 30ml dimethyl sulfoxide. To the above add10ml of lithium acetate buffer. Used always fresh reagents.

b) Lithium-acetate buffer:Add 168g lithium hydroxide (LiOH.H2O) to about 500ml ofwater.By stirring dissolved about half of the ingredient and add 293ml of glacial acetic and make the volume up to 1L. Adjust the pH to 5.2 ± 0.05 either with acetic acid or lithium hydroxide. Allow the solution to cool overnight and then make up to 1L.

c) Ethanol-water: Diluted 165ml ethanol (95%) with water to 300 ml.

d) Potassium chloride solution (2M KCl): Dissolved 149g potassium chloride in water and make volume upto 1L.

e) Chloroform: Used HPLC grade chloroform stabilized with 0.006%. 2-methyl -2-butane.

f) Require Glass apparatus.

g) Spectrophotometer.

h) Water bath at 100°C

i) Nitrogen standards: Dissolved 47mg of Leucine in 2 M KCl and make up to 50 ml. This contained 100 (µg Nml-1). This solution was serially diluted to 0, 2, 4, 8, 16 µg Nml-1 with 2 M KCl solution.

Procedure: Taken sieved field moist soil adjust to 40% WHC and incubate in dark for 3 days at 25°C. Take two portions 10g each of the moist soil and weight accurately. Take one portion in a 250 ml conical flask fitted with a stopper. Immediately extracted this with 40ml 2M KCl solution for 30 min in an oscillating shaker at 200 rpm. After filtration take the filtered solution and stored at -15°C. Carry out fumigation using a 50ml beaker containing the weighed soil. Place the beaker in a vacuum desiccator lined with a wet tissue paper and vial containing 10 g soda lime. Place the beaker containing a few boiling chips and 25ml chloroform inside a desiccator, which is then evacuated until chloroform boiled for 2 min. After fumigation remove the beaker containing chloroform by repeated evacuations (6 times for 2 min with a gap of 3 min between two consecutive evacuations). Transfer the soil to 250ml conical flask for extraction with 40 ml 2 M KCl as mentioned for the unfumigated sample. Used the extract for ninhydrin – N estimation.

Determination of ninhydrin-reactive N

Calibration: Into 50 ml test tubes,pipette 1 ml of each leucine standard and then added slowly 0.5 ml of ninhydrin reagent. After mixing the contents thoroughly heat all the standard solutions in duplicate in a boiling water bath. The test tubes were cool at room temperature and added then 9.5 ml of ethanol-water to each of the test tubes and mixed

thoroughly. Measured the absorbance value in a spectrophotometer at a wavelength of 570nm with KCl solutions as the blank. Draw Calibration curve with the absorbance against N- concentration. In the similar fashion colors of the samples were developed. Thus determined the concentration of the extract with the standard curve.

Calculation:

Initial calculation:

MBC (µg g-1) = [(Fumigated concentration – Unfumigated concentration) x DF]

Oven dry soil wt. difference

Oven dry soil wt difference means, if moist wt was 10 g and oven dry wt = 9.23 g, hence the difference would be 10 – 9.23 = 0.77

Microbial Biomass C (µg g-1 oven dry soil) = 31 × ninhydrin N

Microbial Biomass N (µgg-1 oven dry soil) = 4.6 × ninhydrin N

Compost respiration (CR), microbial quotient (Mq), microbial metabolic quotient (qCO2)

The microbial quotient (Mq) was derived from the ratio between MBC (Cmic) and TOC (Corg) [27]; while microbial metabolic quotient (qCO2)was measured from the ratio of MBC and CR [28].

Phospholipid fatty acid (PLFA) analysis

The phospholipid fatty acid (PLFA) analysis was performed to study microbial community structure in selected vermicomposts samples; such selection was done on the basis of the results obtained for microbial count, Mq, and qCO2. Air dried vermicomposted samples were primarily extracted with Bligh-Dyer extractant, eventually lipids were separated by solid phase extraction technique, and finally samples were prepared for gas chromatographic analysis as detailed in our previous paper [22].

Seed germination assay

The efficacy of the various vermicomposted SMS as plant growth promoting agents was assessed on the basis of their effects on seed germination index, relative root and shoot growth potentials. The assay was performed using fresh, robust, and disease free seeds of green gram (*Vignaradiata*)following standard methods [29]. 10 g of composted and vermicomposted SMS samples were dissolved in Milli-Q water at 1:10 ratio in sealed

plastic containers and undergone shaking at 140rpm in mechanical shaker for one hour. Then, the containers were kept in rest for some time till the stabilization of the precipitates and the supernatants were collected. The supernatants of respective treatments were used for moistening the green gram seeds (10 in number) kept in filter papers placed in series of sterilized glass petriplates. Eventually, the petriplates were kept under incubation at 25oC under dark. The counts of germinated seeds, sprouted roots and shoots were accordingly recorded. The equations used for enumeration of relative seed germination (RSG), relative root growth (RRG), relative shoot growth (RShG), and germination index (GI) are given as below:

$$\text{RSG \%} = \frac{\text{Number of seed germinated with the extracts of treated vermicompost samples}}{\text{Number of seeds germinated in distilled water}} \times 100$$

$$\text{RRG \%} = \frac{\text{Mean root length of seeds treated with vermicompost samples}}{\text{Mean root length of seeds in distilled water}} \times 100$$

$$\text{RShG \%} = \frac{\text{Mean shoot length of seeds treated with vermicompost samples}}{\text{Mean shoot length of seeds in distilled water}} \times 100$$

$$\text{GI \%} = \frac{\text{RSG}}{\text{RRG}} \times 100$$

Statistical analysis

All obtained data of the vermicomposting experiment were for two-way ANOVA, considering different vermicomposting systems and duration as two governing factors. Moreover, Least Significant Difference (LSD) test was performed to find out the significant variations among the various treatments.

Results and Discussion

Characteristics of the raw materials (SMS and CD)

The inherent properties of the SMS and CD have been presented in Table 1. Rice straw was the main ingredient of the SMS under study. In fact, rice straw is one of the most widely used materials for edible mushroom cultivation [30,31].

The physical nature of the SMS and CD material were assessed on the basis of crystallinity index (CI) as discussed earlier. The CI of the SMS was about three times greater than CD indicating stronger crystalline arrangement owing to the lignocellulosic

composition of the material [32]. The SMS material was alkaline in nature owing to high salt content with slightly lower TOC content as compared to the CD[23]. Edible mushrooms are generally cultivated on various plant litters; therefore, their physicochemical characteristics considerably fluctuate from place to place[31]. The N and P contents have been found to be high in the SMS by previous workers (Gupta et al., 2004; Nik Nor Izyan et al., 2009). Contrarily, N level was low(<1%) but P availability was satisfactory in the SMS material sued in this study. This may be due to recalcitrant nature of the SMS that greatly retards the organic end to inorganic end transformation. Interestingly, MBC was lower in the SMS material with higher respiration level as compared to the CD. This indicated that wasteful expenditure of microbial energy was greater in SMS than CD [34].

Table 1:Physico-chemical characteristics of the spent mushroom substrate(SMS) and cow dung (CD) used for the study. Values represent mean ±standard deviation

	SMS	**CD**
pH	6.27±0.02	6.84±0.01
Total Organic Carbon (%)	2.73±0.35	3.90±0.00
Total Nitrogen (%)	0.66±0.04	1.23±0.251
Available Phosphorus (mg kg-1)	110.5±0.02	100.38±0.04
Microbial Biomass Carbon (µg g-1)	1128.4±2.98	1470.4±0.47
Compost Respiration (µg g-1 h-1)	3423.61±1310.74	2760.42±2604.17
Crystallinity index (%)	18.15±0.01	6.8±0.06

4.7.2 X-ray diffraction and Fourier Transform Infrared Spectroscopy (FTIR) based physico-chemical analysis

The cellulose rich SMS material is largely resistant to enzymatic hydrolysis owing to their crystalline structure [21]. The hydrogen bonds linking the glucose molecules render crystalline characteristics in cellulosic substances [12]. However, the crystalline arrangements of biomolecules in SMS material (Cellulose, hemicelluloses,lignin etc) were hypothesized to degrade due to vermicomposting. Therefore, the crystallinity index (CI) was enumerated using XRD spectrum and the data is presented in **Fig. 1** The CI value substantially reduced due to vermicomposting in the SMS feedstocks. Such reduction was greatest in *Perionyx* mediated vermibeds (1.37 folds) followed by *Eudrilus* (1.34 folds) and *Eisenia* (1.31 folds) at 60 days ($p < 0.01$; LSD=0.28). Conceptually, lower CI values indicate lesser abundance of crystalline complexes vis-à-vis greater predominance of amorphous components in the materials [13,35]. Hence, the results signify that the matrix of lignocellulosic compounds in the feedstock have been efficiently degraded by the earthworm and/or the microbial enzymes contributed by the earthworm intestines.

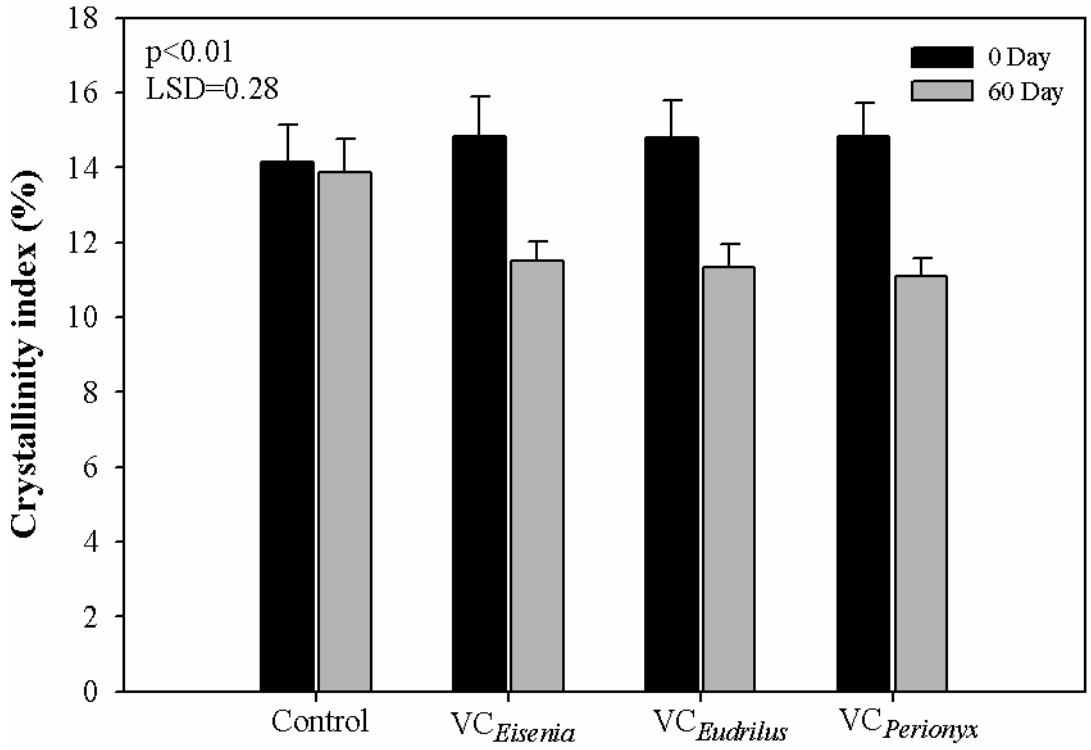

Fig.4.1. Structural deformation in the spent mushroom substrate (SMS) under the vermicomposting and composting treatments as verified from the crystallinity index. [Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control);

VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida;* VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae;* VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation; LSD means Least Significant Difference.

The data on FTIR spectral analysis are presented in **table 4.2**.The FTIR spectroscopy was also used to assess the degradation status of SMS due to vermicomposting with three earthworm species. Generally, the typical stretching frequencies of vital functional groups (C=O, NH, OH etc.) occur between 4000-1300 cm-1 spectral regions [36]. The frequency in the range 3400-3500 cm-1 indicated the occurrence of O-H groups of alcohols, phenols, and aldehydes in all the samples collected at 0 and 60 days. The evidences of C-H and C=C stretching vibrations of hydrocarbons, alkanes, and alkenes (spectral region: 2850-300 cm-1 and 1640-1650 cm-1 respectively) were also found in both composted and vermicomposted samples. Similarly, N compounds in forms of amines (NH2 and N-H) and esters (S-OR) were omnipresent in the feedstocks **(table 4.2**). In addition, the typical bending vibrations of Si-O-Si and O-Si-O in the spectral region of 465-470 cm-1 was commonly evidenced in all the vermibeds as well as the compost beds. Signatures of sulfur compounds such as sulfates (S=O) were initially detected in all the feedstocks in IR spectral region of 1350-1390 cm-1; which were however absent in vermicomposted feedstocks after 60 days. Interestingly, in spite of overall similarity in spectral distributions in the vermicomposted SMS; spectral region of 1420-1425 cm-1 assigning to aromatic ring structures was only detected in VC*Eudrilus* and VC*Perionyx*samples after 60 days of incubation.These variations indicate that the earthworm mediated biodegradation process encouraged the formation of stable humified substances with destruction of sulfur compounds.

Anoticeable reduction in the transmittance energy (%T) was recorded after 60 days in the composted and vermicomposted (VC*Eudrilus* andVC*Perionyx*)SMS. Generally, lowering of peak intensity vis-à-vis transmittance at any frequency in the FTIR spectra signifies the predominance of bonds that have same vibrational energies to that of the incident light, which prevents the light to pass on to the other side of the sample [37]. As such, this is an eventual outcome of breakdown of the lignocellulosic crystals [38]. This is interesting because these findings strongly substantiated the results of the XRD based crystallinity analysis.

Table 4.2:Transmittance values (T%) and the main absorbancebands in FTIR spectra of thecomposted and vermicomposted samples along with theirassignments

		Control				**VC*Eisenia***				**VC*Eudrilus***				**VC*Perionyx***			
Wave number (cm-1)	**Functional Groups**	**0 d**	**T%**	**60 d**	**T%**	**0 d**	**T%**	**60 d**	**T%**	**0 d**	**T%**	**60 d**	**T%**	**0 d**	**T%**	**60 d**	**T%**
3400 - 3500	O-H groups of alcohols, phenols, aldehydes	Yes	53	Yes	41	Yes	54	Yes	52	Yes	53	Yes	50	Yes	50	Yes	43
2850-3000	C-H stretching of hydrocarbon alkanes	Yes	60	Yes	57	Yes	58	Yes	57	Yes	55	Yes	59	Yes	62	Yes	53
1640-1650	C=C of hydrocarbon alkene; C=O of amides	Yes	59	Yes	59	Yes	58	Yes	56	Yes	54	Yes	53	Yes	55	Yes	48
1420-1425	Aromatic ring structure	No	-	No	-	No	-	No	-	No	-	Yes	58	No	-	Yes	58
1350-1390	S=O of sufates of other S compounds	Yes	49	Yes	47	Yes	48	No	-	No	-	No	-	Yes	51	No	-
1090-1100	Silicon compounds e.gSilane (S-OR)	Yes	40	Yes	30	Yes	43	No	40	Yes	46	No	43	Yes	40	No	38
660-850	Amines (NH2 and N-H), Esters (S-OR)	Yes	62	Yes	56	Yes	63	Yes	63	Yes	62	Yes	65	Yes	63	Yes	57
500-540	S-S disulfide bonds	Yes	59	Yes	57	Yes	58	Yes	58	Yes	62	Yes	60	Yes	60	Yes	59
465-470	Bending vibration of Si-O-Si and O-Si-O, Si-aliphatic, alkyl, and alkenes	Yes	56	Yes	46	Yes	58	Yes	55	Yes	55	Yes	55	Yes	40	Yes	38

Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida*; VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae*; VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*

4.7.3. Changes in pH, TOC, N, and P levels under three different conditions

The data on changes in pH, TOC, total N and available P during vermicomposting of SMS feedstock by three different earthworm species presented in **Fig. 4.2**. In general, the substrate pH reduced over time in *Eisenia* and *Eudrilus* mediated vermicomposting systems (VC*Eisenia* and VC*Eudrilus*)as compared to the initial values. On the other hand the pH slightly increased in *Perionyx* mediated vermibeds (i.e VC*Perionyx*). Contrarily, *Perionyx excavatus* has been reported to reduce pH in different feedstocks by some earlier workers (Pattnaik and Reddy, 2010; Tran, 2016). Theincrement in pH of the VC*Perionyx* substrates may be attributed to the activity of the calciferous glands in the esophageal epithelium of the earthworms contain carbonic anhydrase; which fixes CO2 as Calcium Carbonate and prevent reduction in pH [40]. Nevertheless, the substrate characteristics play a vital role in governing the pH during vermicomposting and composting [41]. On the other hand, the shift in pH towards acidity under *Eisenia* and *Eudrilus* system has been reported earlier by many workers [42–45]. Such reduction in pH is contributed by the organic acid, CO2, Nitrate (NO3-) produced during the process of organic matter mineralization.

The TOC of the vermibeds significantly reduced over times by 1.4 to 2.5 folds (**Fig. 4.2**); whereas TOC reduction was nominal in the feedstock under aerobic composting. However, the reduction of TOC was most prominent in VC*Eisenia* system followed by VC*Perionyx* and VC*Eudrilus* systems. In general, the reduction in TOC signifies the efficiency of vermitechnology in regard to nutrient mineralization owing to accelerated microbial activity [22,46].Correspondingly, TKN level significantly increased due to vermicomposting as compared to the composting series (Fig. 2). At 60 days TKN level was slightly higher in VC*Eudrilus* than VC*Perionyx* and VC*Eisenia*. However, such variation was not statistically significant (Fig. 2). The nitrogen in the vermicomposts is generally contributed by the N-fixing microorganisms released via Earthworm excreta and abundance of small to medium polysaccharides [47].

Overall, bioavailability of Phosphorus was significantly high in vermicomposting than composting after 60 days of incubation. The P availability was significantly high in VC*Eudrilus* vermibeds followed by VC*Eisenia* and VC*Perionyx* (P value=0.000; LSD= 10.30). The efficiency of *Eudrilus eugeniae* in solubilizing higher amount of Phosphorus recently been reported by Paul et al. (2018). Generally, bioavailability of P is greatest

within neutral pH range (6.5-7.1) [48]. Interestingly, the pH at 60 Days of *Eisenia* and *Eudrilus* mediated vermibeds were nearly neutral range, while the *Perionyx* feedstock was slightly alkaline. Hence the alkalinity might have been restricted the P solubility despite of considerable proliferation of P-solubilizing microorganism in such feedstocks.

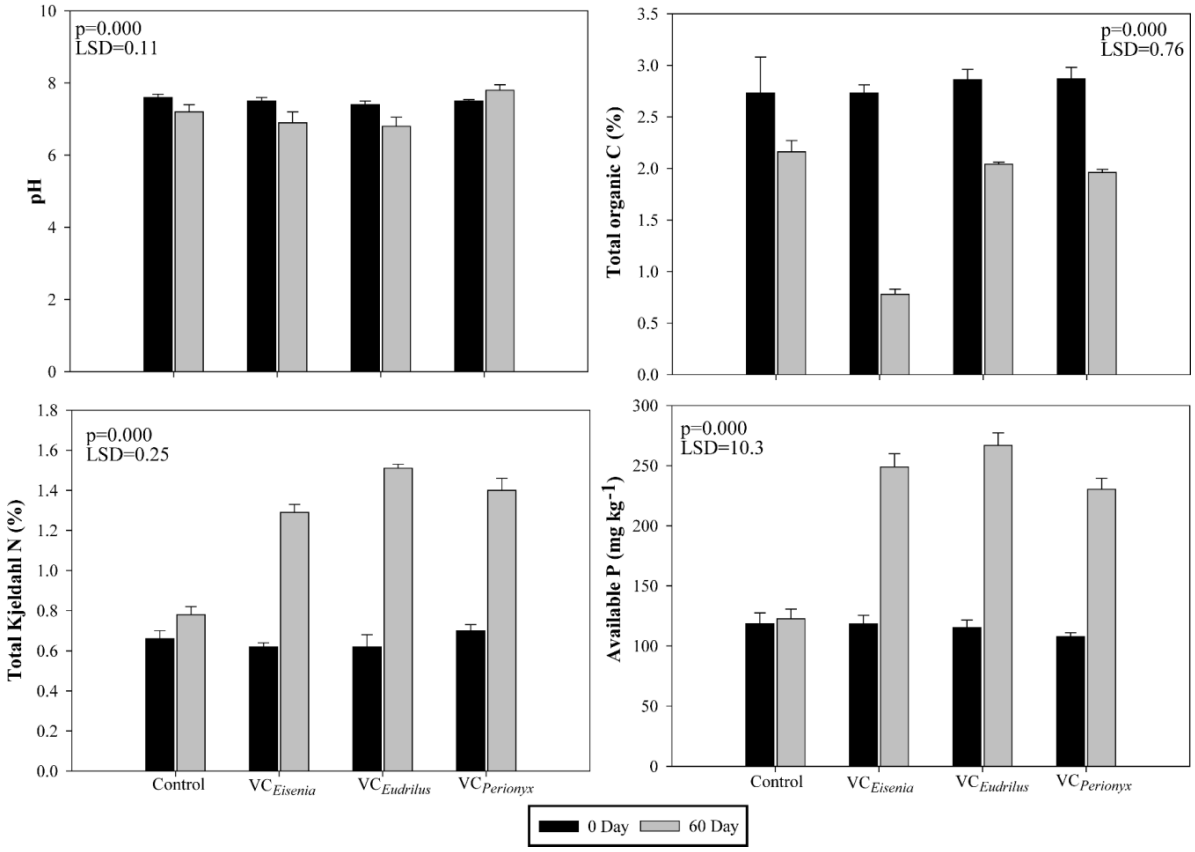

Fig.4.2. Temporal variation in pH, total organic C, total Kjeldahl N, and available P of spent mushroom straw based feedstocks under the composting and vermicomposting system [Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida;* VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae;* VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation; LSD means Least Significant Difference.

Growth, metabolism, and diversity of microbial communities: Assessment based on MBC, CR. Mq, qCO2, colony count, and PLFA structure

The data on changes in MBC, CR, Mq, and qCO2 are presented in **Fig.4.3**. The MBC and CR increased by 1.6-4.8 folds after 60 days in all the feedstocks. Such increment was greatest in *Perionyx excavatus* treated feedstock followed by *Eudrilus eugeniae* (P value: MBC & CR = 0.000; LSD: MBC = 73.1, CR = 392.06). The rise in MBC implies improvement in microbial health and proliferation due to vermicomposting. Earthworm intestines harbor diversified microbial genera by providing favorable growing conditions [16,49]. All such microorganisms are introduced in the vermibeds along with the digested feedstocks, enzymes, and other plant growth stimulators through the earthworm excreta [50]. However, microbial respiration (i.e CR) was lowest in VC*Eisenia*despite of significant rise in MBC after 60 days. The C in the microbial biomass greatly contributes to the total organic C and thus the enhancement in MBC greatly resulted in high Mq (i.e the ratio of MBC and TOC) in VC*Perionyx* reactors followed by VC*Eisenia* and VC*Eudrilus* (Fig. 3). While, the qCO2 (i.e ratio of CR and MBC) in the feedstocks was in the order: C > VC*Eisenia* = VC*Eudrilus*> VC*Perionyx*(P = 0.000; LSD = 0.80) (Fig. 3). The CR is a measure of CO2 evolution contributed by the metabolic activity of microorganisms in the feedstock; which often considered as wasteful expenditure of acquired energy for maintenance rather the growth in challenged environments [51]. Therefore, low qCO2 in thevermicomposted feedstock signifies quicker decline in the microbial respiration thereby stabilization of biodegradation process as compared to aerobic composting [22,34].

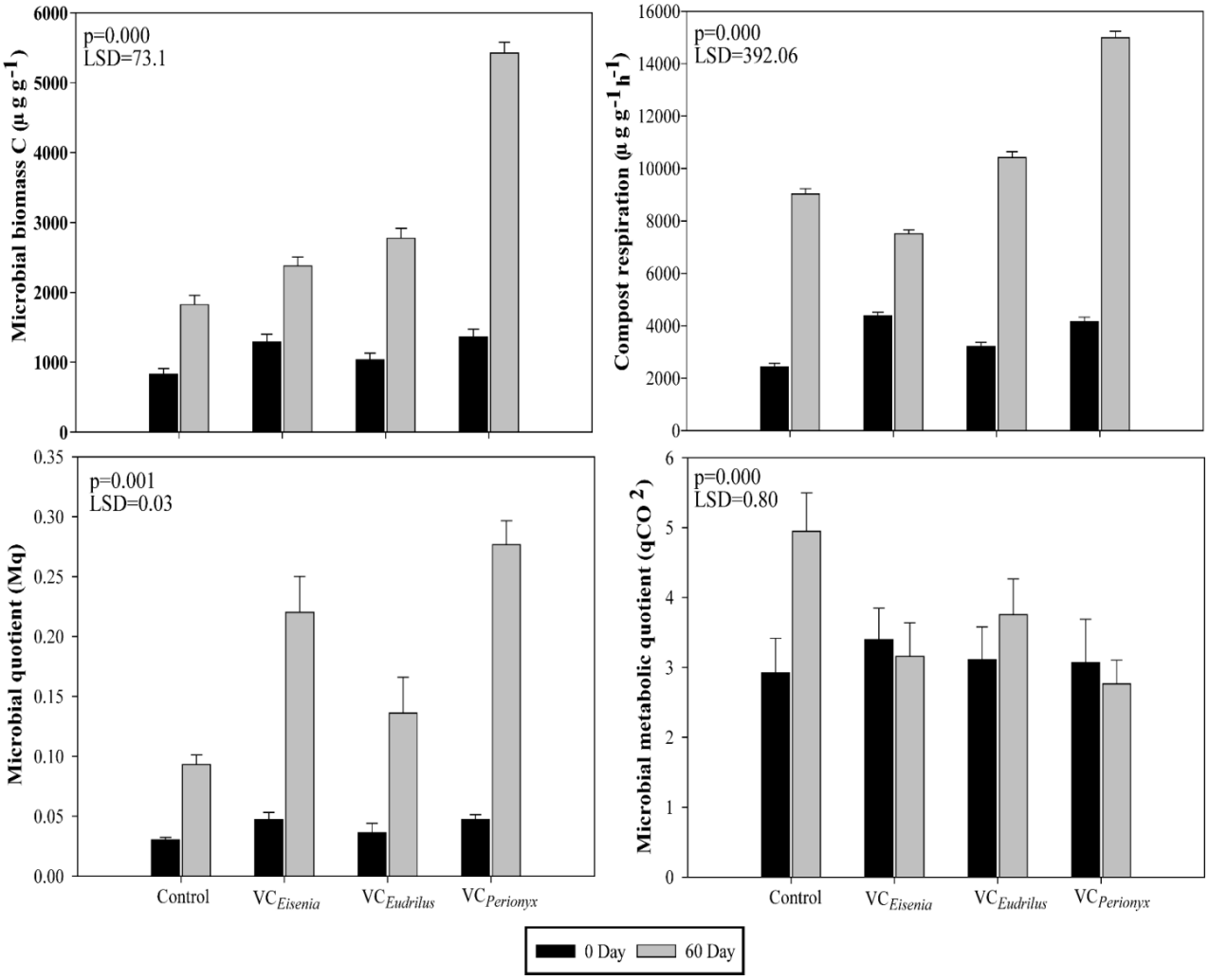

Fig. 4.3. Changes in microbial biomass C, compost respiration, microbial quotient, and microbial metabolic quotient under various bio-composting treatments. [Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida;* VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae;* VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation; LSD means Least Significant Difference.

Correspondingly, the microbial counts were considerably greater in the vermicomposted SMS than the composted ones (**Table 4.3**). The total bacterial count was significantly higher in VC*Perionyx*than the other systems; whereas fungal growth was highest in VC*Eudrilus* followed by VC*Perionyx* and VC*Eisenia* (P = 0.000; LSD =10.6). Moreover, proliferation of P-solubilizing and N-fixing bacteria was remarkably greater in VC*Perionyx* as compared to VC*Eisenia* and VC*Eudrilus* (P value = 0.000; LSD =108.02). Earthworms efficiently pulverize the feed materials into minute pieces with the help of their gizzard thereby expose larger surfaces for microbial action [41]. However, the variability among the vermicomposting systems in regard to microbial growth was clearly evidenced. In fact, the levels of compatibilitybetween earthworm species and feed composition greatly dictate the microbial enrichment in the processed materials [34]. The results are in good agreement with some previous findings [16,43].

Table 4.3: Count of total bacteria (TBC), total fungi (TFC), P solubilizing bacteria (PSB),and N fixing bacteria (NFB) in the composted and vermicomposted SMS at 60 days of incubation.Values represent mean ± standard deviation

	TBC (x105 CFU g-1)	NFB (x104CFU g-1)	PSB (x105CFU g-1)	TFC (x102CFU g-1)
Control	230±5	73±6	18±2	80±4
VC*Eisenia*	1000±9	400±17	280±7	120±9
VC*Eudrilus*	900±16	210±6	350±11	1900±17
VC*Perionyx*	3600±11	2000±19	1440±9	1300±14
p	0.001	0.000	0.000	0.000
LSD	116.7	83.97	108.02	10.6

Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida*; VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae*; VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus; CFU= Colony forming unit; LSD= Least Significant Difference*

The PLFA assay was performed to understand the structural adjustments in microbial communities in response to SMS dominated vermibeds. The long and short chain phospholipid fatty acids provide strength and stability to the microbial cell membrane [20]. The structural compositions of PLFAs greatly vary among microbial communities depending on their immediate environments[18]. In this study, the total microbial biomass was remarkably high in VC*Perionyx* as compared to VC*Eisenia* and VC*Eudrilus* **(Fig. 4.4**). The Gram negative bacterial PLFA was greater in VC*Eudrilus* than the other two systems; whereas Gram positive bacterial PLFAs were highly significant in VC*Perionyx* followed by VC*Eisenia*. Occurrence of actionmycetes communities were also significantly high in VC*Perionyx* followed by VC*Eudrilus* and VC*Eisenia* (**Fig. 4.4**). As such, abundance of Gram positive bacterial communities in VC*Perionyx* and VC*Eisenia* signifies favorable earthworm-feedstock compatibility in these systems. Whereas, the dominance of Gram negative bacterial communities in VC*Eudrilus*indicates that the SMS feedstocks were probably more stressful for the *Eudrilus* mediated system because Gram negative organisms are known to exhibit high tolerance to aberrant environments[52]. Although the fungal population was almost of same size in all the three vermireactors; arbuscular mycorrhizal fungi (AMF) were detected in VC*Perionyx* and VC*Eisenia*. AMFs are natural biofertilizer who normally resides in the root zone soil in agricultural or forest lands [53]. Hence, occurrence of AMFs in the SMS vermicomposts would certainly add greater value for sustainable recycling of the waste in agriculture.

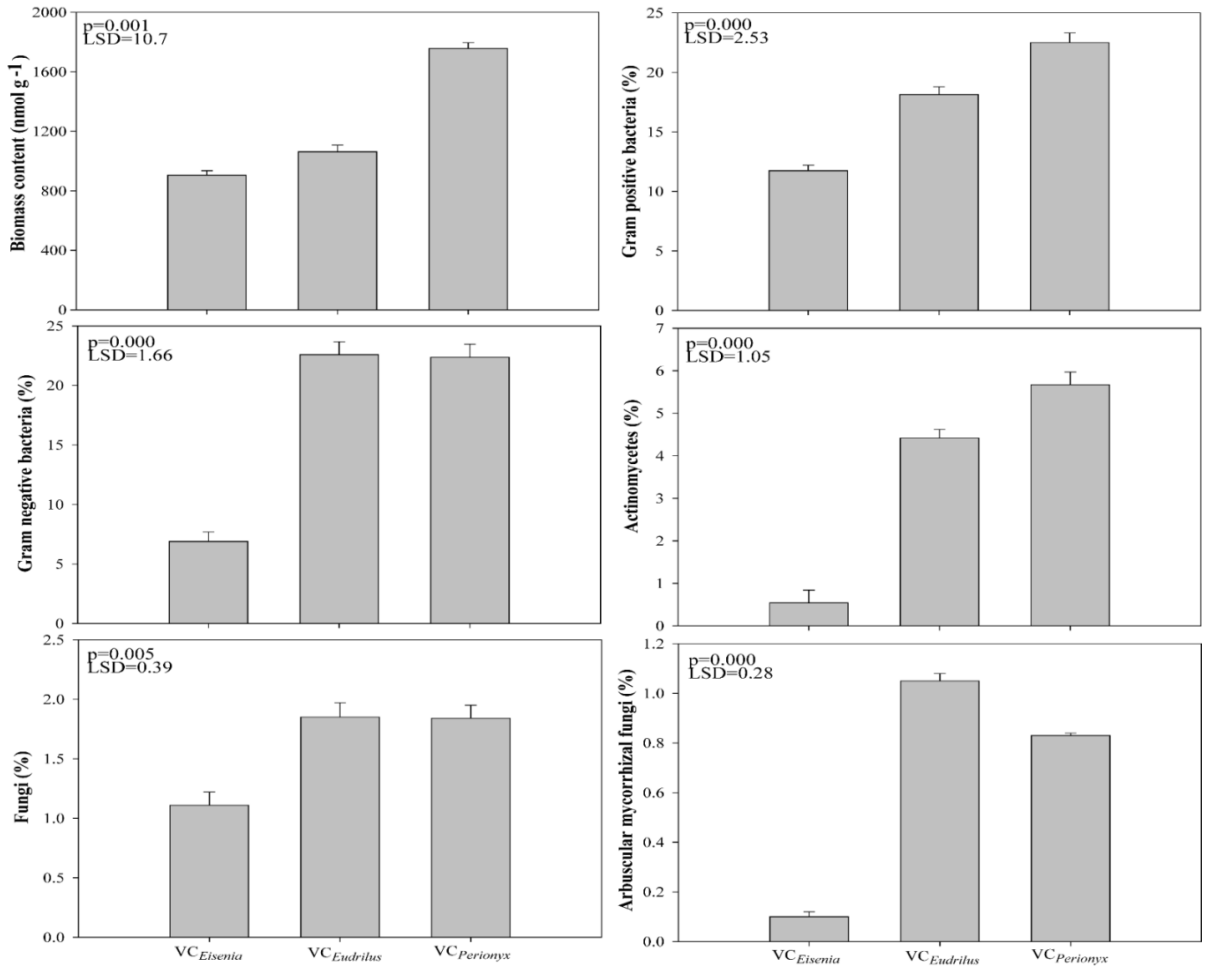

Fig.4.4. Phospholipid fatty acid (PLFA) identified microbial groups in the composted and vermicomposted SMS-based feedstocksat 60 days of incubation. [Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida;* VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae;* VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation; LSD means Least Significant Difference.

Interestingly, the proportions of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) in the total PLFA was highest in VC*Perionyx* and VC*Eisenia* respectively (**Fig. 4.5**). As such, both MUFA and PUFA are known to maintain membrane fluidity in many bacterial species, thereby ensure membrane stability during unfavorable conditions [54]. Moreover, the signatures of some typical PUFA (18:1ω9c and 18:2ω6,9c) in the PLFA profiles of the vermicomposted SMS samples revealed presence of eukaryotes and ectomycorrhizal fungal communities in the processed material [55].

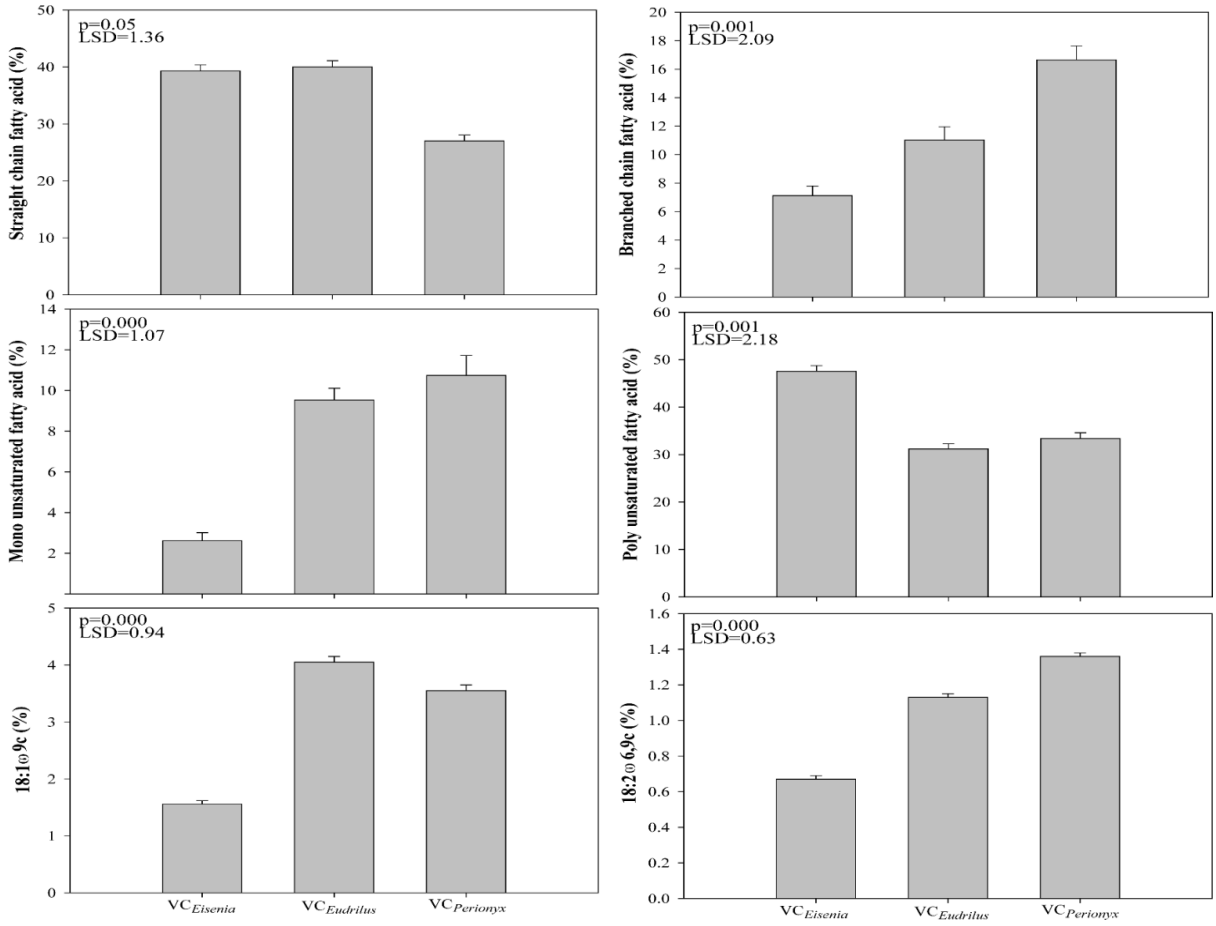

Fig.4.5. Percentage composition of different types of fatty acids in the vermicomposted and composted feedstocks detected through Phospholipid fatty acid (PLFA) analysis. [Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida;* VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae;* VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus*]; Error bars represent the standard deviation; LSD means Least Significant Difference.

Effects of composted and vermicomposted SMS on seed vigor: germination assay
The results of the germination assay are presented in **table 4.4**.This assay is a reliableparameter for testing the response of plant species to any growth stimulant [22,56]. The germination index and the relative seed germination were significantly higher in VC*Perionyx* treated seeds followed by VC*Eisenia* and VC*Eudrilus* (P = 0.000; LSD =3.18). Concurrently, the relative root and shoot growth (RRG and RShG) were in the order: RRG - VC*Perionyx*> VC*Eisenia*> VC*Eudrilus*> Control (P = 0.000; LSD = 6.85); RShG - VC*Perionyx*> VC*Eisenia* = VC*Eudrilus*> Control (P = 0.000; LSD = 5.76). This result was fascinating because it strongly substantiatedsome results of this experiment. In general, vermicomposts are known to energize hormonal and enzymatic activity in the growing medium, which in turn remarkably induce viability of crop seeds [29,57,58].In the present study, the profuse microbial enrichment in VC*Perionyx* system probably resulted in qualitativeimprovement of SMS vermicompostthat induced significant seed vigor.

Table 4.4: Comparison between the composted and vermicomposted SMS extracts on relative root (RRG), shoot growth (RShG), relative seed germination (RSG), andgermination index (GI) ofgreen gram (*Vigna radiata*)

	RSG	RRG	RShG	GI
Control	70.0±8.97	67.3±6.50	64.7±6.09	47.1±3.22
VC*Eisenia*	90.0±10.0	96.5±10.3	69.8±4.99	107.4±10.2
VC*Eudrilus*	80.0±9.22	88.2±12.9	66.3±5.49	86.8±5.87
VC*Perionyx*	121.7±16.1	141.4±11.8	96.5±5.67	113.1±11.9
p	0.000	0.000	0.000	0.000
LSD	3.18	6.85	5.76	8.08

Control – Aerobic composting with SMS+CD (3:1) feedstock (as composting control); VC*Eisenia* – Vermicomposting of SMS+CD (3:1) with *Eisenia fetida*; VC*Eudrilus* - Vermicomposting of SMS+CD (3:1) with *Eudrilus eugeniae*; VC*Perionyx* - Vermicomposting of SMS+CD (3:1) with *Perionyx excavatus; LSD= Least Significant Difference*

Conclusions

The XRD and FTIR spectroscopy suggested that cellulosic crystallinity of SMS was more efficiently broken in VC*Perionyx*than VC*Eudrilus* and VC*Eisenia* vermireactors. Correspondingly, Microbial proliferation (bacteria, fungi, N-fixers, and P-solubilizers) and metabolic activitywere greatest in VC*Perionyx*.The fatty acid profiles noticeably varied among the vermibeds. However, the N and P availability was greater in VC*Eisenia* compared to others. Correspondingly, significant vigor and growth of green-gram seeds were evidenced due to VC*Perionyx* treatment. Thus, SMS was highly palatable for *P. excavatus*and *E. fetida*; and microbial augmentation was the major key to produce high quality SMS-vermicompost.

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