CHAPTER 5
GENERAL DISCUSSION

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Recycling of organic waste irrespective of its origins is highly relevant as far as sustainability of natural resource bases such as soil, water and biological and physical environment are concerned. Recycling of organic waste residue as organic fertilizers continues despite of several competitive uses of organic waste residue of agri and horticultural origin emerging in recent time. Use of organic waste of cities for conversion into compost has also received attention and scientific interventions are also required to reduce nutrient loss during composting for which population load of pathogenic microorganisms and to enhance its manurial value by increasing population load of beneficial bacteria in the final compost.

This research estimated organic portion of waste generated in Imphal city, characterized the waste in terms of level of human pathogenic bacteria and agriculturally important beneficial microorganisms and nutrient content in the waste. Efficient method to convert the city waste by mixing with agricultural (mainly rice straw and cow dung) and aquatic biomass was developed through a series of in-vitro and in-vivo experiments. In these experiments, inocula of an efficient strain of CDM and one efficient earthworm species, Eisenia fetida were used. Compost as well as enriched compost quality parameters were evaluated by both physico-chemical analysis in laboratory and bioassay in pots using french bean as test crop.

1. Quantification and characterisation of city waste

A total of 24.84 MT of green biodegradable waste is generated daily in Imphal city. The total compostable waste represents 35.62 MT/day including cellulose material rich cartoon and papers which is 52% of the total waste generated in the KRYPSA waste disposal site. The quantity of waste generated by household of the Imphal city is 125.5 g/person/day only and thus the quantity of city waste generated in Imphal city is less compared to thickly populated city in countries like China generating 640 g waste/person/day (Daniel et al., 2013; Yu Dawei, 2012) and USA generating 730 g waste/person/day (Eurostatistics, 2009). Yet, this is a significant quantity of waste in Imphal city in terms of potential biodegradable materials available locally for production of organic manure. Currently, most of this waste undergoes natural decomposition in the dump site or individual home without any effort for realising their
value through compost production. However, during our waste quantification study in municipality area of Imphal, we learnt that farmers, who have small scale commercial rearing of different domestic animals in the outskirt of the city, collect the vegetable market waste for use as feeds for their animals. In our calculation of total city waste, the waste generation in household waste are not added as the waste disposal vehicles of different NGOs collect them and dump in the KRYPSA disposal site. In order to determine the manurial potential of the waste, it was essential to characterise the biodegradable portion of the municipality waste in terms of level of harmful and beneficial microorganisms, nutrient content and other parameters.

2. Population of microorganisms in the municipality biowaste due to different treatments

A very important factor considered in the compost production from the municipality biowaste is the level of pathogenic microorganisms (PM). If the level of PM in the final compost produced from the municipality biowaste is found to be very high, this limits in practical use. A high level of PM in the raw MW may also lead to their high level in the final compost. The level of pathogenic bacteria (PB) in the genera *Salmonella* spp., *Shigella* spp., *Enterobacter* spp. and *Micrococcus* spp. were found to be 0.69 - 9.44, 1.16 - 9.54, 0.92 - 9.05 and 1.26 - 9.15 log cfu/g dry biomass respectively. In general, a level of $2.0 \times 10^{3}$ to $2.9 \times 10^{7}$ cfu/g dry wt. of different PM had been reported in the city waste generated elsewhere (Tsado, 2013; Zikrullah et al., 2011; Anela et al., 2010; Anela et al., 2010; Bhila et al., 2010; Adegunloye D.V., 2007). We also found that depending upon time of the year, the level of PM varies. In addition to PB, the municipality biowaste also contain agriculturally beneficial bacteria (BB) although the level of BB in municipality biowaste based on their isolation and counting on selective media has not been reported previously. We found population of *Azospirillum*, PSB (fluorescent pseudomonas) and cellulose degrader in the range of 9.09 – 9.23 log cfu/g dry biomass after plating. However, result based on use of such selective media for reporting occurrence and level of BB and PB bacteria may not be very conclusive, as BB of one selective medium can grow in another selective medium (Thakuria et al., 2005 and Elizabeth et al., 2013). To be confident on the demarcation of PB and BB, both BB and PB colonies derived based on selective medium were cross streaked in the selective media. We found that the BB and PB colonies grew on cross
streaked plates but their colony morphologies were found to be distinctly different on cross streaked plates. This was confirmed by using the reference standards of the BB and PB (Fig. 10).

The MW was mostly alkaline in nature and depending upon season of collection, pH varied. In general, pH of Imphal municipality waste ranged from 7.41 – 9.05 and EC 374 – 497 µS/cm. The variation could be the result of mainly variation in types of vegetable waste found in different seasons. Khwairakpam et al., 2011 and Suthar, 2009a reported variation in pH and EC of different vegetable waste. Similarly, the nutrient content in the city waste also varied depending upon season.

3. Effect of different treatments on level of PBs and BBs in the municipality waste

Depending upon the method of pre-treatment of the MW, the population of both PBs and BBs were either slightly increased or decreased significantly. For example, spreading the MW (83.4 % moisture) in plastic chamber and in net house resulted in different level of PBs. The plastic house temperature was higher during day due to green house effect with maximum temperature reaching 30 - 50°C during night and day inside the net house. It was interesting to observe increase in population of the PBs during the first two days after spreading the waste in the net house. During first two days, the PBs owing to higher temperature might have undergone multiplication. The waste was in ambient temperature of 24 – 30°C prior to placing in the plastic house. However, reduction in population from 3rd to 5th day may be due to loss of moisture as a result of evaporation caused by higher temperature in the plastic house. Reduced moisture and high temperature might have caused death of PBs. A comparison of the population of the MW kept at 24 – 34°C (net house) and those at 30 – 50°C for 5 days could support this result as there was no decline in the population of the MW in the net house. The moisture content of the MW kept in net house at 24 – 34°C was also not reduced to great extent. However, determination of PBs in the green house at daily interval would have been ideal. The population data of Enterobacter spp. suggest that 50°C day time temperature and 30°C during night in the plastic house is not favourable for its growth. Earlier worker (Iversen et al., 2004) reported that Enterobacter spp. grow at 30°C ambient temperature. Thus, this experimental result showed that there is no advantage of pre-treatment of MW in either plastic chamber or net house prior to its placing in compost heap, specifically when it is desired to reduce the level of PBs.
In another experiment, heating of the MW at different temperature and time phases inside oven was carried out and found to be efficient in reducing the level of PBs significantly. *Salmonella* spp. and *Enterobacter* spp. were reduced/eliminated but *Micrococcus* spp. could survive in reasonable number even after 100°C 12 hr. and 30°C 38 hr. heating. The resistance of *Micrococcus* spp. to heating and survival for longer duration due to their ability to develop cyst like structure was reported earlier (Ansu *et al*., 2002). The population of four groups of beneficial bacteria due to heat treatment followed a similar trend. Detection of P. solubiliser and cellulose degrader even after heating at 30 – 100°C for 6 hr. suggest that they may belong to *Bacillus* genus. Several *Bacillus* spp. possess P. solubilising and cellulose degradation ability (Ken-Jer *et al*., 2006; Pankaj *et al*., 2012).

Exposure of the layer of municipality waste in thickness of about 10 cm to UV radiation for a period of 7 hrs. followed by 5 hrs. of normal light had no effect on population of the PBs. There are previous reports of reduction of PBs in manure applied to soil by UV rays of natural light (Gayan *et al*., 2010; Kim *et al*., 2002; Bernal *et al*., 2009; Nicholson *et al*., 2005). Based on the previous report, we exposed the MW to UV radiation but contrary to our expectation, there was no consistent effect observed. The thickness of the layer of MW may have been more for the UV light to penetrate, although we mixed the materials before exposure to UV rays for 7 hrs. daily, for a duration of 16 days. Thus, among the different treatments to reduce PBs, heating in oven only was found to be effective in removing/reducing PBs, except the *Micrococcus* spp. However, in actual decomposition experiment, decomposition of the MW by different treatments combination including decomposer agents such as CDMs and earthworm, we did not use the pretreated municipality waste. But the population of PBs in final compost of all experimental treatment were determined and it was found that decomposition treatment reduced PBs to varying level from their initial level which is discussed latter.

Although decomposition of different biomass takes place in nature by action of microorganisms naturally occurring in the biowaste, complexity of the lignin and cellulose in different materials vary (Sudip *et al*., 2013; Yuan *et al*., 2011; Allinson *et al*., 2009; Stephen K. R., 2008; Keto *et al*., 2006; Haita *et al*., 2002; Benner *et al*., 1984; Kirk *et al*., 1977) and can reduce overall rate of decomposition of the biowaste. The
population of the naturally occurring degraders may be either less or the efficient strains of degraders may be missing. In efficient management of biowaste through controlled strategy, short duration of decomposition is desirable. Therefore, isolation, screening and development of inocula of efficient strains of CDMs were important component of this research. Forty six CDM isolates with distinct colony morphology were obtained from different types of materials including excreta of several herbivores, soil and cellulose rich materials such as sugarcane trash. It was found that excreta of herbivores contained CDMs population in the range of 7.48 – 8.54 log cfu/g dry materials with highest population in elephant and horse dung. Among different cellulose rich organic waste materials, sugarcane trash was found to contain CDM population of 8.35 log cfu/g dry material. Previous research showed occurrence of CDM in herbivores in the range of $10^{10}$ to $10^{11}$ bacteria/ml of ruminal content (Mahesh S., 2012; Qi et al., 2006) and in the range of $6.7 \times 10^4$–$7.2 \times 10^4$ cfu/g in poultry dropping, (Akpomie et al., 2013).

The forty six isolates showed cellulolytic activity in qualitative CMC agar plate assay. CMC agar plate assay is used by other workers to carry out initial screening of CDM for cellulolytic activity (Ponting, 1999; Maki, 2011). In quantitative assay (DNS method), we found that some isolates did not show any activity. Overall cellulase activity of the isolates was found in the range of 0 to 1.26 µM/min. The cellulase activity of the efficient CDMs of our study is comparable with those reported by other workers (Gautam et al., 2012; Gautam et al., 2010; Sadaf et al., 2005). Although we could not keep reference standard in this experiments, the cellulase activity of the reference standard, *Cellulomonas cellulans* MTCC 23, was found to be 0.036 – 0.068 µM/min in similar condition (30°C) of determination by DNS method (Miller, 1956).

Different parameters influence result of quantitative assay of cellulase activity of different isolates. It was initially difficult to obtain uniform result adding similar number of colonies for each isolates in cellulose supplemented broth as cell size or colony size of different isolates were different. However, uniform standard dose of inocula from the colony growth, temperature and standard solution storage condition were all worked out for maintaining uniform conditions all throughout different experiments which involved determination of cellulase activity. We found that for getting similar level of inocula, different no. of colonies of the isolates were needed. Despite of equal inoculum level, some isolates showed very low cellulase activity.
indicating these isolates were inherently inferior in cellulose degradation. Variation in cellulase activity of bacteria, actinomycetes and fungi is reported by many workers (Athanasios et al., 2007; Roshan et al., 2013; Akpomie et al., 2013). Ten isolates with larger diameter of clearing zones were selected for determination of their cellulase activity by quantitative method (Miller, 1956; Ghose T. K., 1987).

Out of the 46 isolates, CDM9 showed maximum diameter of clearing zone in qualitative assay and cellulase activity in quantitative assay (DNS method). For other cultures, there was statistical correlation between cellulase activities determined by the two methods. Cellulase is a complex enzyme with three subtypes such as β – glucosidase, exoglucanases and endoglucanases (Patricia et al., 2012; Leonid et al., 2012; Zverlov et al., 2005; Sen Bok Lee., 2004; Rabinovich et al., 2002; Bayer et al., 2000) and the interaction of these enzymes with cellulose substrate as component in solid and liquid medium may be different. Since CDM9 showed highest activity in both the methods, it was an obvious choice for production of inoculum for use in the subsequent decomposition experiments. But yet, we evaluated the effect of inoculation of CDM9 and the other six isolates on their cellulase activity and CO₂ evolution in cellulose powder added to the other composition of the Omeliansky medium. On 7th and 14th day after inoculation, the relationships between two parameters were positive and only for 14th day data, it was statistically significant. This result implies that cellulase enzyme has released glucose for the CDMs to utilise in meeting carbon and energy requirement. A CDM with low cellulase activity may also release less glucose and perhaps due to less biomass generation by it. By 21st day, the + ve correlation was not visible and in absence of fresh nutrient supply or for other reason like possible production of toxic metabolite by old cultures, the growth and glucose metabolism might have been different (Bames et al., 2003; Gellert et al., 2002). In this study also, CO₂ evolution and cellulase activity were highest in case of the isolate, CDM9. The inoculum of isolate, CDM9, was consistently used in all experiments carried out subsequently and to determine suitable treatment combinations inclusive of decomposer agent for production of compost with fewer loads of pathogenic bacteria, high nutrient content, finer particulate materials and overall superior attribute in bioassay using test crop.
4. Characterisation of the CDM isolates by classical and molecular techniques

Out of the 46 isolates, ten superior isolates which were screened for their cellulase activity by different methods, were also first subjected to morphological, physiological and biochemical test using methods described in Bergy’s manual of systematic bacteriology. Isolates were classified as CDM1 (*Klebsiella pneumonia*), CDM2 (*Sphingobacterium* spp.), CDM3 (*Cellulomonas* spp.), CDM4 (*Cellulomonas biozotea*), CDM5 (*Streptomyces* spp.) CDM6 (*Bacillus cereus*), CDM7 (*Renibacterium* spp.), CDM8 (*Aeromonas* spp.), CDM9 (*Streptomyces* spp.), CDM10 (*Listeria* spp.).

In recent times, molecular methods have been increasingly accepted for identification of microorganisms with higher level of confidence (Athanasios et al., 2013) and based on 16S rRNA gene partial sequence, the ten isolates were identified to have close resemblance (% similar) with different reported strains (Fig. 23).

The CDM5 was a fungal isolate and based on its ITS gene partial sequence, it was found to have close resemblance (% similarity) with several earlier reported strains of *Aspergillus versicolor* (Fig. 24). The most efficient CDM was an actinomycetes in the genus, *Streptomyces*. It is to be noted that the isolate nomenclature based on the classical taxonomy and molecular analysis are different. However, the strains are maintained in laboratory by both accession nos. i.e. 16S rRNA and ITS gene partial sequence based accession and classical method based accession for each isolates. CDM2 and CDM9 were sequenced (1500 bp) full length and identified as *Sphingobacterium multivorum* and *Streptomyces xanthochromogenes*.

5. Decomposition of MW in different sets of experiments

Decomposition of MW in mixture with other biowaste was carried out in a series of experiments. The treatment combinations of biowaste and experimental conditions were designed in each successive experiments to generate scientific data which could finally help to select the best treatment combinations for (1) better quality compost in terms of nutrient compositions, (2) less load of harmful microorganisms and more load of beneficial microorganisms, (3) finer compost materials with high surface area, (4) short duration composting and (5) less loss of volatilizable nutrients such as N in NH₃ form or other in leachate. The experiments were conducted under ambient temperature and humidity which varied season to season. Similarly, municipality biowaste was a mixture of a diverse type of vegetable materials and depending upon the season, there
was slight variation in their nutrient composition for which the comparison of results of two experiments may be different but taken all the experimental results together, practically useful techniques of composting have emerged from results of different experiments. The details of the municipality biowaste mixture used in each experiment have been presented in appropriate chapter. The calculation of N loss of all the experiments was done on the basis of results obtained using CHNSO analyser.

In the first experiment, in which mixture of MW, RS and CD were used in coarse size fraction for decomposition, presence of earthworm resulted in better compost as reflected in C:N ratio of 9.72 as compared to C:N ratio of 11.67 and 13.6 in MW alone and MW + RS + CD mix composting, respectively over a period of 106 days. N content also followed similar trend. Initial N content of the three treatments were somewhat similar and after 106 days of composting, MW + RS + CD in presence of earthworm, the content of N in the final compost was found to be higher. Higher level of N content in compost produced in presence of earthworm is reported earlier (Dvidson et al., 2006; Depak et al., 2012). There was less N content in MW alone treatment and its mixture with RS and CD without EW (Table 39). Thus, the total amount of N in the three composts was less than that in the initial mix and total content in composts was maximum where earthworm was used as decomposer agent. This also suggests loss of N during decomposition and importance of decomposer agent, earthworm, in reducing N loss during decomposition.

In the second experiment, the municipality waste mix contained more N than the N content in MW used in first expt. Its mixture with low N containing RS and CD reduced the N content of the initial composting mix. However, the N content in compost from MW alone was reduced by 0.53% suggesting loss of large quantity of N during decomposition. Loss of N during decomposition of municipality waste was observed by previous workers (Stefano et al., 2010; Yulian et al., 2012). Mixture of MW with RS and CD in presence of decomposer agent, CDM and EW in this experiment, resulted in higher content of N in the final composts. Both the result of first and second experiment on N suggest that mixing low N containing biowaste and using decomposer agent, N loss can be prevented to a greater extent. The result of the 2nd experiment also clearly showed that decomposition of MW with high N content can increase loss of N substantially. For example, in MW alone treatment, 24.3% N was lost and N loss was
higher (30.9%) in presence of EW compared to N loss (18.2%) in presence of CDM. It was also interesting to observe that N content in compost produced from MW in presence of EW was higher (2.19%) although the N loss was 30.9% whereas in presence of CDM inoculum, N content in the final compost was 2.03% and N loss was 18.2%. When the quantity of N loss during composting and N present in the final compost were added, the total N content was found to be higher than N content in the initial composting mix. This raises the question as where this N additional N might have originated from. It is possible that passage of the microflora of the composting mix through earthworm gut provide them the congenial environment during the transit through earthworm gut and might stimulated N fixing bacteria to fix more atmospheric nitrogen. Occurrence of fixation of nitrogen by N fixing microorganisms during their passage through earthworm gut has been reported by previous workers (Atiyeh et al., 2000; Sarma S., 2002; Chitrapriya et al., 2013). In fact, in all our experiment, we have clearly shown presence of nitrogen fixer population such as *Azotobacter* spp. and *Azospirillum* spp. in the composting materials and final compost. In EW treated MW decomposition treatment, the level of *Azotobacter* spp. varied from 7.60 to 9.15 log cfu/gdry wt. and *Azospirillum* spp. from 8.20 to 9.15 log cfu/gdry matter. The presence of both the decomposer agents resulted in retention of less N (44.4% of initial content) in the compost compared to that in the only earthworm treatment perhaps due to faster rate of decomposition. A faster rate of decomposition may release more N than the organisms assimilate and thus leaving more N in the system for loss through volatilization. This was also evident from the date of the 4th experiment in which N loss increased to 42.0% in the decomposing mix of MW + RS + CD in presence of earthworm and CDM. The N loss was 34.8% in the decomposing mix without the two decomposer agents. Interestingly, in this experiment also, N content was more in the compost of decomposer agent treatment despite of greater loss of N. This result also suggests fixation of N by nitrogen fixing bacteria such as *Azospirillum* spp. whose population was enhanced by action of earthworm. The role of increased action of earthworm during composting in enhancing the population of N fixers has been reported by Kunwar et al., 2010.

The loss in N during decomposition may occur by different ways (Steiner et al., 2010). The loss in N content in our study could occur both through drainage of liquid
produced during decomposition or through volatilization loss from heap during the entire period of decomposition (Rana et al., 2010; Ashish et al., 2013). This conclusion is based on the observation that N content in the drained out liquid did not account for the total loss of N estimated by subtracting total N content in the final compost from the value of N content in the initial feed mix at the time of start of the experiment.

In third experiment, we determined the factors which might be associated with loss of large quantity of N from the composting mix. Maintaining the moisture in MW at 70% resulted in reduction of loss of N (35.9%) compared to those (38.5%) from MW maintained at 90% moisture. In this experiment, the different wastes were completely dried in shade to which moisture was added to maintain the level of % moisture at 70% and 90%. In the final compost of the experiment also, %N content was higher than that in the initial mix. This was expected as during decomposition, large amount of biomass C and other constituent elements were lost. However, the % N in the final compost could be much higher if the loss was less. Similarly, when MW was mixed with CD and RS and decomposed with or without the decomposer agent at 90% moisture, there was significant reduction in either %N content or N loss in the drained out liquid compared to the first two experimental results. In the 1st and 2nd experiments, the decomposing mix was 100% water saturated and perhaps under this condition, more N was coming out in the drained out liquid. Alternatively, higher level of moisture may result inefficient decomposition and immobilization. However, ineffectiveness of the two agents in increasing the C/N ratio in the final compost obtained from MW + RS +CD mixture at 90% moisture content is difficult to explain.

Overall, the results of three experiments could throw light on few possible factors for substantial N loss from the MW composting system. In the 3rd experiment, we determined the pH of the drained out liquid collected at 0 – 20, 21 – 30 and 31 – 107 intervals. It was found that pH increased gradually i.e. 8.58 – 9.63, 9.64 – 9.88 and 9.96 – 10.05 in liquid collected progressively with time. Earlier Rana et al., 2010 observed gradual increase in pH compost wash produced during decomposition. Similar trend was also observed in case of electrical conductivity. The collected liquids were stored in capped bottle to prevent evaporation of H₂O or volatilization loss of N and all samples were analysed at one time after storing drained out liquid collected at successive samplings. The pH of the composting mix was higher (10.23 -10.28) in case of the
treatment MW and MW + RS + CD mixture. Particularly, at 10th day after composting of Loktak *phumdhi* alone had low pH but mixing of MW with LP resulted in increase of pH.

A portion of the drained out liquid of MW treatment alone was subjected to analysis for N content and pH at 4 different dates by keeping either in opened and close container amended with NH$_4$Cl or HCl. NH$_4$Cl was added to increase NH$_4$ content and HCl to reduce pH. In open container, % N decreases during successive determination despite of loss of water due to evaporation. If there was no N volatilisation, the concentration of N should have been increased due to reduction in volume of liquid. The concentration of N in the close container where volume of the liquid remained the same did not change. Thus, it can be indicated that the high pH of the composting mix caused in loss of substantial amount of N from the liquid kept in opened container over a period of 15 days (Table 61). Neutralisation of the drained out liquid by HCl in open container produced expected result i.e. there was no vaporisation loss of N and % N increased as the volume of liquid was reduced due to evaporation. Similarly, addition of NH$_4$Cl to the drained out liquid of high pH resulted in increase in loss of N from open container compare to those in close container, which maintained %N content (Table 61) in liquid at different dates of determination. By subtracting from the initial N content, the total N in the compost and in the drained out liquid and the sediment, we find that 0 to 7.12 g N can be lost from 10 kg fresh MW during composting.

To reduce volatilisation loss of N from alkaline composting mix, such as MW or its mixture with RS and CD, we have shown that the floating biomass (Loktak *phumdhi*, LP) of Loktak lake which is acidic in nature, can be mixed with alkaline MW mix. The compost produced from *phumdhi* of Loktak lake are in general of inferior quality despite of its high N content. This is mainly due to low pH of the compost derived from LP. The floating biomass is a mixture of root mass with sediment below water surface and green vegetation at surface. Because of less O$_2$ environment, organic acids such as low molecular wt. fatty acids are produced for which the pH of biomass is around 4.0 – 5.0. Mixing MW with LP resulted in a congenial pH for its decomposition by microorganisms. The rate of decomposition of LP alone was very low as reflected in the production of very low quantity of liquid and also varies less reduction of biomass during composting. Mixing MW and LP together enhanced rate of decomposition,
reduced N loss and resulted in higher N content in the final compost. The decomposer agents further reduced N loss and increased N content of compost from MW + LP mix.

In 4th experiment, we could reduce loss of N further during composting. For example, there was only 23.9% loss of N against 32.8% N loss observed in 3rd experiment. Presence of decomposer agent reduced the loss further and the N content in the compost was also much higher. The pH of the final compost of this treatment was 7.7 whereas pH of the compost from MW + RS + CD + EW + CDM was 9.2 which again suggest N loss by volatilization during composting. In the 4th experiment, composting duration was only 53 days due to finer size of materials for faster decomposition. It is likely that quicker decomposition release N in quantity which is more than that utilised by the decomposing microorganisms. This might subsequently be lost by volatilization. The observed N loss (37.3%) from MW + RS + CD + EW + CDM treatment of 3rd expt. compared to N loss (42.0%) from the same treatment of 4th expt. suggests such possibility. In relatively longer duration of decomposition taken by relatively bigger size waste materials, the rate of formation of simpler forms of easily utilisable organic N or mineral N might be somewhat similar to rate at which the decomposer population assimilate them whereas in situation of faster decomposition presented by fine size particle, the N mineralisation may be more than what the decomposer agent can utilize. As a result, more amount of N left in the system either to assimilate by decomposer agent by faster rate or to be drained out. This needs to be firmly established experimentally. Coarse materials of 3rd experiment had less surface area and took longer (112 days) for decomposition whereas in 4th experiment, it took only 56 days for decomposition.

The population of PB and PB in the final compost obtained under different composting experiments did not show any consistent trend. However, it was apparent that population of PB generally declined from its population present in the composting mix at the time of start of composting. The population level was also different depending upon types of the PBs. The initial population of the 4 PBs in MW ranged from 6.39 to 9.54 log cfu/g dry materials and under different treatments, the level of all the 4 PBs was in the range of 3.55 to 8.87 in the first experiment. In the 2nd expt., the PB population ranged from 5.56 to 8.87 log cfu/g dry materials. MW + RS + CD + EW + CDM treatment’s final compost carried lowest level of the 4 types of PBs. In the
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compost of 3rd experiment, only Salmonella spp., and Micrococcus spp. population were
determined. LP contained lowest level of Salmonella spp. population, Micrococcus spp.
population was highest in compost produced from MW and different treatments reduced
the population slightly. Salmonella spp. and Micrococcus spp. population in compost
produced from MW + LP was slightly lower than in the compost produced from MW +
RS + CD mix. Although presence of EW and CDM tended to reduce level of PBs in
different experiments, it was not consistent in compost of all experiments. In general,
the population of BBs in the compost was increased in treatments of different
experiments containing the decomposer agents except in few cases. The population of
BBs increased due to inoculation of decomposer agent, earthworm, was reported by
Aira et al., 2006; Aira et al., 2008 and Dominguez J., 2011.

Quality of the compost is also determined by composition of different size
fractions in it. It was consistently observed that presence of either EW or both EW and
CDM inocula resulted in highest % of materials in finer size fraction (< 2.00 mm) and
lowest % of materials in coarse size fraction (> 4.0 mm) in the final compost. This
result was consistent across all experiments. When the MW was decomposed either
alone or in combination with RS + CD or LP. LP cannot be decomposed easily and
most of the compost produced from it is present in coarse fraction. Although, treatments
of composting experiments in which MW was composted with CD and RS using the
inocula of decomposer agents, CDM and EW, were found to be superior in terms of
both macro and micronutrients content, higher load of BBs and smaller size fractions,
the real test of superiority was its ability to stimulate crop growth and improve soil
quality in bioassay. Two bioassay, one with 8 treatments comprised of 7 composts of 3rd
decomposition experiment and a non-compost added control were tested on green gram
as test crop to evaluate the best compost treatment for maximum plant growth
stimulation. The other bioassay was to test the effect of superior BB strains enriched
compost obtained from the selected treatments of composting experiment sets 2 and 3.

In the bioassay, growth of green gram was found to be highest due to application
of compost produced by composting MW along with cowdung and rice straw using
inocula of the two decomposer agents, CDM9 and earthworm. The increase in total
biomass (root and shoot) was 86.3% over the control. The compost of this treatment had
pH 9.99, 36.6% finer (< 2.0 mm) fraction and lowest quality (45.7%) of coarse fraction
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(> 4.0 mm), 1.92% N, 0.24% P, and 0.5% K. In fact, among all treatments, the content of P and K in compost under MW + RS + CD + EW + CDM treatment was the highest. Although, compost from mixture of MW and LP contained highest %N, the other nutrient contents was low and thus for good growth of green gram, the nutrient content in compost of this treatment may not have been adequate nutrients. Furthermore, load of naturally occurring BB i.e. P. solubilisers and *Azospirillum* group was highest in this treatment. Thus, an ideal composting treatment combination for MW which produced best compost in terms of physical, chemical and biological parameters has been confirmed by result of the bioassay with green gram. The biomass of the Loktak *phumdhi* alone is not good for ideal compost is reflected in the result of the bioassay expt. As discussed earlier, the pH of Loktak *phumdhi* compost is lowest and the coarse fraction is highest although it contained higher level of N. It is to note that the mixture of MW and LP in presence of the decomposer agent produced very high quality compost in terms of different physical, chemical and microbiological parameters and it would have been good if a bioassay could be carried out to test its effect on crop. In this research work, it was not possible to conduct a bioassay experiment using the compost generated in 4th experiment. However, based on the result of the bioassay on french been and the observed match between the quality parameters of compost and plant growth, we predict that combination of LP and MW of Imphal city for composting using the two decomposer agents, will be ideal to provide better quality compost for use in crop production. This may be tested in future field experiments using different test crops.

To determine that application of compost affect soil properties, we analysed the C and N content of the soils which were adhered to root surface. Cotxarrera *et al.* (2002) reported that the analysis of root adhered soil provide a better idea on effect of different soil amendment treatments. In our study, it was found that the total N content in root adhered soil was highest due to application of compost produced under MW + RS + CW + EW + CDM treatment. Perhaps this compost modify the soil environment near roots or increase load of higher population of N$_2$ fixer for increasing N content of soil in addition to higher uptake of N by the crop. This type of analysis of soil of root region should be extended to many other parameters in future experiments.
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GENERAL DISCUSSION

6. Beneficial bacterial strain inoculation into MW compost and crop growth studies in bioassay

Bacteria inocula or more popularly known as bacterial biofertiliser of superior strains are known to enhance plant growth and improve soil quality. It is also accepted that superior strains of beneficial bacteria are potential agent for improving quality of organic manure through their inoculation in the manure. The general notion is that the inoculated beneficial bacterial strains may multiply in the manure and increase their population. However, experimental evidences are limited. We observed that the population of phosphate solubilizers in six final composts obtained from 2nd composting experiment did not change significantly after application of the inocula of the BB strain, *Bacillus megaterium* MTCC 4126. Similarly, the population of the BB strain, *Azospirillum amazonase* MTCC 4128, also did not change significantly. Previous research results on enrichment of manure are inconsistent. Tantawy *et al.*, 2009 reported the beneficial effect of inoculation of manure with bacterial strains on plants but conclusive data on extent of population increase of the beneficial bacteria is lacking. Based on the results of our experiments, we speculate that it is unlikely that a culture of beneficial bacteria grown in culture medium will be able to use the solid compost nutrients to either maintain or increase its population. However, sterilised compost has been found to be a good carrier nutrient of beneficial bacterial strain (Thakuria *et al.*, 2005). In this study also, we found that inoculation of sterilised compost of 2nd composting expt. (Table 75) resulted in cfu of 9.7 and 9.2/g of dry compost of the two strains after 5 days of their inoculation. The population of the two strains did not decrease significantly even upto 10th day after inoculation. It is known that sterilised compost can maintain inoculated microbial population for more than six months (Kostov *et al.*, 1998; Talukdar *et al.*, 2004).

That inoculation with the BB did not cause any significant change on the population of P. solubilizers and *Azospirillum* spp. in the compost was also reflected in the result of expt. to study their effect on the growth of okra. Although growth of okra, in terms of shoot length and biomass was conspicuous due to application of BB inoculated sterilised compost and the inoculated unsterilized compost, the difference was not statistically significant from the other BB inoculated compost (Table 77). As such compost of FT6 treatments had higher nutrient content (Fig. 30) and load of
natural population of beneficial bacteria (Table 53) which could be attributed to its superior effect on growth of okra. The sterilised compost of FT2 treatment contained population of two efficient strains in substantial number and also higher N content (Table 75). However, it is difficult to attribute whether the better effect on vegetative growth is due to higher N content resulting from death and lysis of the native microflora of the compost or higher population of the efficient BB strain in the compost. A treatment of sterilised compost without BB strain could have been ideal to confirm this best we could not include such a treatment. However, in the 2nd enrichment experiment conducted using the compost generated from the 3rd composting experiment could throw some light on this. The compost produced from MW + RS + CD (DT3) and MW + RS + CD + EW + CDM (DT4) contained 1.99 and 1.92% 2.25% of N, respectively (Table 56). The cfu of P- solubiliser in the two treatments was 8.09 and 8.29/g dry compost and of _Azospirillum_ 6.72 – 6.92/g dry compost, respectively (Table 65). On sterilisation, the N content increased which may be due to release of N from dead cell lysis and also release of N from complex humus N. Correspondingly, dry shoot and root biomass of the green gram were found to be different (Table 79). For example, the total biomass of the 45 days old green gram was higher in DT3 treatment compost than that of the DT4 treatment compost. However, comparision of the effect of the BB added unsterilized compost with that of BB added sterilised compost suggest that sterilisation of the compost produced better effect which may be due to higher N content. Because at the time of application of the different compost to the soil green gram seeded pot soil, there was no conspicuous difference in the population of the two groups of beneficial bacteria (Table 78). This result also shows that mixing both cultures of superior strain of BB with MW derived compost can produce more plant biomass than the uninoculated compost.

This research also generated data on diversity of bacteria in the compost produced by mixing MW with other waste materials. In the different composting experiments, we have shown that population of the beneficial bacteria were higher in the treatments where the two decomposer agents were used either alone or together in the 3 out of 4 composting experiments. However, by the cultural techniques alone, it is not possible to know about total bacterial diversity. By using DGGE profiling of the PCR product of the 16S rRNA gene V3 region primers, interesting result has been
obtained. Presence of decomposer agents, CDM and EW, resulted in somewhat uniform composition of the bacteria which is evident from clustering of GT2 and GT4 together in the dendrogram (Fig. 48). The composting mix in GT1 and GT2 treatments is different from that of GT3 and GT4 and the physico-chemical environment is also different in the two types of mixtures. It appears the decomposer agents create a uniform environment supportive to uniform proliferation of selective bacteria during decomposition of diverse materials.

Furthermore, application of the mother composts of DT3 (MW + RS + CD) and DT4 (MW + RS + CD + EW + CDM) treatments of 3rd composting along with sterilised and unsterilized BB added composts of the two treatments with diverse bacterial composition affected the bacterial diversity in the green gram rhizosphere soil. This is evident from clustering pattern of the DGGE profile of the PCR products of metagenomic DNA of rhizosphere soils obtained from different compost treatment pots. Bacterial community of the control soil with no added nutrient broth was part of the same cluster but yet was different than those of nutrient broth added and DT3 treatment’s compost added rhizosphere soil. This suggests that addition of the nutrient broth stimulated soil bacteria near root zone as it appears in the same line of cluster with DT3 compost added soil. DT3 contained a different bacterial community and therefore was expected to change the rhizosphere soil bacteria. Similarly, the two superior BB strains and the superior attribute of manures of DT4 (sterilised but BB inoculated) resulted in a different bacterial community as it formed a completely different cluster in the dendrogram (fig. 49). The BB strain inoculated unsterilized compost of DT3 treatments and BB inoculated sterilised compost of DT3 resulted in a single cluster in the dendrogram suggesting these treatments affected rhizosphere soil community similarly. In case of BB inoculated unsterilized and sterilised compost of DT4 also resulted in a single cluster showing similar rhizosphere community. Thus, the DGGE profile data provide a better picture of 16S rRNA gene based diversity in compost of different treatments and in green gram rhizosphere soils to which different composts were added.