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RESEARCH ARTICLE

MICROBIAL SUCCESSION PATTERN, ISOLATION AND CHARACTERIZATION IN *BRASSICA OLERACEA* VAR. *CAPITATA* AT DIFFERENT DECOMPOSITION STAGES

Stephanie Okeyo, Methuselah Mang'erere Nyamwange*, Ezekiel Mugendi Njeru

Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya.
****Corresponding author Email: nmethu1992@gmail.com***

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ABSTRACT

Decomposition is a microbial driven process and man has maximized this natural process in composting which is the biological decomposition of biodegradable solid waste under controlled conditions. In Kenya, waste from *Brassica olerecea* var. *capitata,* which is the main vegetable crop accounts for the largest proportion of solid waste generated in food markets. Majority of the population use landfill as a disposal method, although this is neither sustainable nor environmentally friendly. Moreover, cabbage wastes release glucosinolates during the initial stages of decomposition which is deleterious to beneficial biodegradation microbial communities. This study focused on isolating and characterizing microorganisms involved in decomposition, determining microbial succession patterns during decomposing stages, and assessing changes in temperature during decomposition. Leaves of different *Brassica* cultivars were collected from Githurai food market, Nairobi at four different points. The samples were then piled in sterile bags, three replicates per sample and maintained in a greenhouse for ninety days. Temperature readings were taken after every two days while samples for microbial isolation and characterization were taken after every two weeks. All microorganisms were characterized both morphologically and biochemically. Interestingly, bacterial load CFU/ml differed significantly ($P < 0.001$) based on the composting stage, where those at stage zero (0) recorded the lowest bacterial load in week 2. Remarkably, 17 bacteria isolates were detected where only three isolates could grow at 55 ºC.

KEYWORDS

Decomposition*, Brassica olerecea var*. capitata, microbial load, temperature, microbial characterization

1. INTRODUCTION

Decomposition is the process whereby organic substances are broken down to simpler matter (Pinero, 2009). Dead organic substances contain useful compounds that are often in short supply in ecosystems; carbon tied up in large carbohydrate molecules, calcium and other minerals, organic nitrogen bound up in proteins (Ryckeboer et al., 2003). Without the help of decomposers, these elements would be leached from the food chain and gradually become so rare that the ecosystem would reduce productivity. Man has maximized this natural process in processes such as composting which is the controlled aerobic biological decomposition of organic matter into stable, humus like product called compost (Veas et al., 2003).

Biotic decomposition processes also known as biodegradation, are mediated by microorganisms that are extremely important for environment maintenance, nutrients and organic matter cycling, changing organic matter into inorganic matter and providing nutrients which propitiates energetic balance in ecosystems (Nicholson et al., 2000). The world's urban population increased to more than 50 per cent of the world's total population in 2015 and will rise further to approximately 70 per cent of the total by 2050 (Ryckeboer et al., 2003). Increase in population has led to high food demand resulting to large deposition of organic wastage from food markets. In Kenya, waste from *Brassica olerecea* var. *capitata* accounts for the largest proportion of solid waste generated in food markets especially in Nairobi. The waste from the market places always pose challenge in handling due to slow rate of decomposition (Veas et al., 2003).

Landfills have been the only ultimate method of disposing *Brassica olerecea* var. *capitata* food waste. However, the disposal method is neither sustainable nor environmentally friendly and also there is no enough space to accommodate large volumes of waste from the market (Nicholson et al., 2000). In addition, low decomposition of waste from landfills enhances harboring pathogenic microbes which are threat to surrounding community (Ryckeboer et al., 2003). Bioremediation of *Brassica olerecea* var. *capitata* food waste using microorganisms can significantly accelerate the rate of decomposition and clean the environment within short duration. Bioremediation using microorganism is environmentally friendly and the waste will be converted into organic fertilizers vital for agricultural production (EPA, 2009).

Composting food waste and other organic waste produces an effective soil amendment that improves soil quality and is environmentally sustainable (Pinero, 2009). The organic fertilizer from *Brassica olerecea* var. *capitata* produces compounds that suppress plant disease and improve soil water retention (Nicholson et al., 2000). There is also limited information on the availability and characteristics of microorganism responsible for decomposition of *Brassica olerecea* var. *capitata* and their characteristics. It is therefore, vital to isolate and determine the characteristics and microbial succession patterns of *Brassica olerecea* var. *capitata* at different decomposition stages.

In this study, we tested the hypothesis that; (1) microorganisms involved

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in the decomposition of *Brassica olerecea var. capitata* are biochemically and morphologically diverse, (2) there is variation of microbial population in the decomposing *Brassica olerecea* var. *capitata,* and (3) variation in decomposition temperature affects microbial load in *Brassica olerecea* var. *capitata*. The specific objectives of the study were; (1) to isolate and characterize biochemically and morphologically microorganisms involved in the decomposition of *Brassica olerecea* var. *capitata* at different decomposing stages, (2) to determine changes in the microbial population during *Brassica olerecea* var. *capitata* decomposition, and (3) to determine changes in temperature in the decomposing *Brassica olerecea* var. *capitata.*

2. Materials and Methods

2.1 Study area

The study was carried out at Kenyatta University Microbiology Laboratory, Kiambu County in Kenya. Samples were collected from Githurai 45 open air market (S 1° 11' 6.1296", E 36° 55' 50.3652") which serves as a main grocery collection point for people living in Nairobi and Kiambu County.

2.2 Sample collection

Five samples of *Brassica olerecea* var. *capitata* (common cabbage) were selectively collected, shredded and put in sterilized open zipbags. The criteria used for differentiating the samples was levels of decomposing stages based on visually decay.

2.3 Experimental Design

The experiment was established on 7th April 2021 at Kenyatta University Microbiology Laboratory, Kiambu County in Kenya. The open zip bags were kept in a secluded area in the laboratory for 56 days. Bacterial enumeration and inoculation were done after every two weeks. Sample 0, 1, 2 and 3 were decomposed in ascending order with sample 3 being the most decomposed. Sample 5 was a mixture of sample 0-3. The purpose of this study was; (1) to isolate and characterize biochemically and morphologically microorganisms involved in the decomposition of *Brassica olerecea* var. *capitata* at different decomposing stages, (2) to determine changes in the microbial population during *Brassica olerecea* var. *capitata* decomposition, and (3) to determine changes in temperature in the decomposing *Brassica olerecea* var. *capitata*.

2.4 Microbial isolation

Isolation and enumeration of bacteria was done using spread plate method. Ten grams of the sample collected was suspended in 90 ml of sterile water blanks in 250 ml conical flasks and diluted serially to yield three dilutions. Using a sterile pipette, 0.1 ml of the the first dilution (10^{-1}) was plated onto three petri dishes containing Potato Dextrose agar (PDA), 0.1 ml of 10^{-2} dilution was plated onto three petri dishes containing M1 media while 0.1 ml of 10^{-3} dilution was plated onto three petri dishes containing nutrient agar (NA). The developed colonies were isolated and sub cultured repeatedly to obtain pure isolated colonies. Presumptive identification of bacteria was carried out by colony morphology and use of the Gram-positive and Gram-negative bacteria biochemical tests such as triple sugar-iron (TSI) test, Methyl Red-Voges Proskauer (MR-VP) test, Simmons citrate test, sulfide, indole and motility (SIM) test, nitrate reduction test, urease test, starch test, cellulase test and catalase test.

2.5 Data analysis

Data was tested for homogeneity of variance by Bartlett test. Data on microbial load were analyzed by ANOVA and wherever feasible means were separated by Tukey's Honest Significance Difference (HSD) at $p < 0.05$ using SAS (version 9) software.

3. Results

3.1 Morphological characteristics of microbial isolates

During the study, 13 groups of bacterial isolates based on morphological characteristics were obtained from the decomposition of Brassica olerecea var. capitata obtained from Githurai market (Table 1). Sample 3 recorded highest of the isolates obtained during the study followed by sample 2. Majority of the isolates had characteristic cream color and others translucent. However, some isolates were very distinct with orange color and some yellow. Eight of the isolate groups had their diameter size greater than 2 mm while 5 of the groups had their diameter size less than 2 mm. A part from two isolates that had flat elevation, the other 11 isolates groups had raised elevation. The margins of the isolates comprised of entire, irregular, serrated and filamentous (Table 1).

Table 1: Bacterial cultural Characteristics on nutrient agar (NA)

Isolate	Colour	Size (mm)	Shape	Elevation	Margin
1	Translucent	0.8	Round	Raised	Entire
2	Cream	0.7	Irregular	Raised	Irregular
3	Cream	2.3	Round	Raised	Irregular
4	White	2.4	Irregular	Flat	Irregular
5	Orange	2.7	Round	Raised	Entire
6	Yellow	1.2	Round	Raised	Entire
7	Cream	2.4	Irregular	Raised	Serrated
8	Translucent	2.5	Irregular	Raised	Irregular
9	White	1.3	Round	Raised	Filamentous
10	Cream	1.1	Round	Raised	Entire
11	Translucent	2.4	Round	Raised	Irregular
12	White	2.7	Round	Flat	Irregular
13	Translucent	2.1	Round	Raised	Entire

The actinomycetes were isolated during the study using M1 agar media. Four isolates were obtained based on their morphological characteristics (Table 2).

Table 2: Morphological characteristics of actinomycetes on M1 media

Isolate	Morphological characteristic
1	Creamish, yellow, mucoid and chained
2	Tan, pigment, flat, dry appearance
3	White, smooth, flat, feathery appearance
4	White, irregular, flat, dry appearance

3.2 Biochemical characteristics of the microbial isolates

The study showed that majority (13) of the isolates were Gram-positive (Table 3). The Gram-positive bacteria isolates ranged from filamentous, rods and bacilli. The other two isolates (7 and 15) were Gram negative where isolate 7 was short streptobacilli and isolate 15 was short coccibacilli (Table 3). Thirteen (13) isolates were glucose fermenters, isolate 8 was a lactose and sucrose fermenter and isolate 4,12,9 and 3 did not ferment sucrose, lactose or glucose. Five isolates were motile 3, 6, 14, 15 and 17. All isolates were catalase positive. Nine isolates could hydrolyze starch while eight isolates could hydrolyze cellulase. Isolate 1 and 15 had tryptophanase enzyme. Fourteen isolates were able to metabolize glucose to pyruvic acid (Table 3).

3.3 Microbial population and succession

During isolation, there was significant ($p<0.001$) variation on microbial population in the first two weeks of decomposition. Sample 3 recorded the highest CFUs with an average of 292.33 CFUs whereas sample 0 had the least CFUs with an average of 122.00 CFUs (Table 4). After decomposition period was increased to 4 weeks, there was significant ($p<0.001$) variation in CFUs recorded. However, the microbial population increased with increase in the duration of decomposition which resulted to use dilutions of 10^5 and 10^6. Similarly, sample 3 recorded the highest CFUs while sample 0 recorded the least CFUs (Table 4).

Table 3: Biochemical characteristics of bacteria isolates

Isolate	**Gram stain**	**TSI**				**SIM**			**Urease**	**Cit**	**MR**	**VP**	**Nit**	**Sta**	**Celu**	**Cata**
		Sl	B	Su	G	S	I	M								
1	Gram + rods	P	Y	+	+	+	+	-	+	-	+	-	+	-	+	+
2	Gram + rods	P	Y	-	-	-	-	-	+	-	+	+	+	+	+	+
3	Gram + rods	P	Y	-	-	-	-	+	+	-	+	+	+	+	+	+
4	Gram + rods	P	P	-	-	-	-	-	+	+	-	+	-	+	-	+
5	Gram + rods	P	Y	-	-	-	-	-	-	-	+	-	+	+	-	+
6	Gram + rods	P	Y	-	-	-	-	+	+	-	+	+	+	+	+	+
7	Gram - rods	P	Y	-	-	-	-	-	-	-	+	+	+	+	+	+
8	Gram + rods	Y	Y	-	-	-	-	-	-	-	-	-	+	-	-	+
9	Gram + rods	P	P	-	-	-	-	-	+	-	+	-	+	-	+	+
10	Gram + rods	P	Y	-	-	-	-	-	+	-	+	+	-	+	-	+
11	Gram + rods	P	Y	-	+	-	-	-	-	-	-	-	-	-	-	+
12	Gram + rods	P	P	-	-	-	-	-	-	-	+	-	+	-	+	+
13	Gram + rods	P	Y	-	-	-	-	-	-	-	+	+	+	-	-	+
14	Gram + rods	P	Y	-	-	-	-	+	-	-	+	-	+	+	-	+
15	Gram - cocci	P	Y	-	+	-	+	+	-	-	+	-	+	-	-	+
16	Gram + rods	P	Y	-	-	-	-	-	-	-	+	-	+	+	+	+
17	Gram + rods	P	Y	-	-	-	-	+	-	+	+	-	+	-	-	+

Key: Sl-Slant, B-Butt, Su-Sulphide, G-Gas, I-Indole Production, M-Motility, TSI-Triple Sugar- Iron test, SIM- Sulphide Indole and Motility test, Cit-Simmons citrate test, MR-Methyl Red, VP-Vogues Proskauer, Nit-Nitrate test, Sta-Starch, Celu-Cellulase, Cata-Catalase, P-Pink, Y-Yellow

Table 4: Mean CFUs of microbial load at various stages of decomposition

	Week 2		**Week4**		**Week 6**	
Dilution	**10^2**	**10^3**	**10^5**	**10^6**	**10^7**	**10^8**
Sample 0	122.00 ±1.09d	36.33± 0.54b	38.35± 0.52c	50.67± 2.08c	122.00 ±1.80c	251.67 ±1.92b
Sample 1	162.00 ±2.39c	49.00± 0.57b	295.00 ±3.61a	256.33 ±1.81b	153.67 ±2.04b	263.33 ±2.12ab
Sample 2	277.00 ±3.64b	125.33 ±3.12a	120.67 ±2.51b	51.67± 2.51c	172.33 ±2.11b	277.00 ±2.42a
Sample 3	292.33 ±4.93a	163.67 ±3.95a	293.67 ±3.06a	280.33 ±2.52a	263.67 ±3.08a	297.00 ±2.60a
P Values	0.001	0.001	0.001	0.001	0.001	0.001

The values of average mean CFUs ± standard error after one-way ANOVA followed by Tukey's HSD test. Values followed by the same letter in the same column are not significantly different at P < 0.05 (Tukey's HSD test).

There was gradual significant (p = 0.001) difference in microbial population from sample 5 with increase in duration of decomposition (Table 5).

Table 5: Mean CFUs of microbial load of sample 5 at various stages of decomposition

Week	Week 4		Week 6		Week 8	
Dilution	10^5	10^6	10^5	10^6	10^5	10^6
Sample 5	226.33 ± 3.11b	77.33 ± 1.69d	292.33 ± 3.51a	99.33 ± 2.21c	51.33 ± 0.86f	66.33 ± 1.22e
P Values	0.001	0.001	0.001	0.001	0.001	0.001

The values of average mean CFUs ± standard error after one-way ANOVA followed by Tukey's HSD test. Values followed by the same letter in the same column are not significantly different at P < 0.05 (Tukey's HSD test).

3.4 Growth of isolates at different temperatures

There was presence of actinomycetes, mesophilic and thermophilic bacteria with mesophilic bacteria being the dominant group. There was reduction on the microbial population with increase in temperature for both mesophilic and thermophilic bacteria. However, some bacterial isolates were resistant to change in temperature and persisted up to after 24 hours (Figure 1).

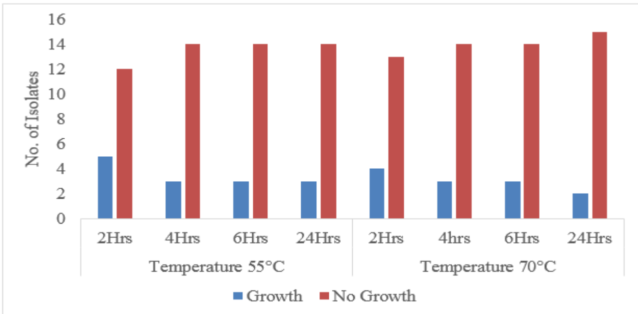

Figure 1: Effect of temperature on growth of the isolates

3.5 Temperature variation during composting

Temperature increased gradually during the first 42 days in sample 0, 1, 2 and 3. Sample 5 recorded highest temperature change for the first two weeks, a slight increase for two days followed by decreasing temperatures (Figure 2).

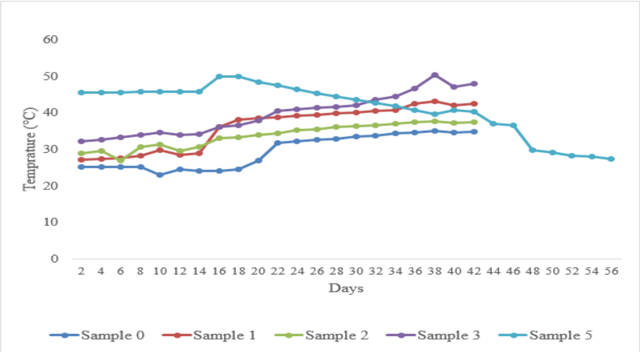

Figure 2: Temperature change during decomposition within different samples

4. Discusion

4.1 Morphological and biochemical characteristics of the microbial isolates

In this study, there was diverse biochemical and morphological characteristics of the isolates from decomposition of Brassica olerecea var. capitata. Based on morphological characteristics, the isolates were grouped into 13 distinct groups. The high diversity of isolates demonstrates that the decomposition of Brassica olerecea var. capitata harbors diverse microbial population due to rich sources of carbon in the compost (Ryckeboer et al., 2003). The results of this study are in agreement with during a study on microbiological aspects of biowaste during composting in a monitored compost bin concluded that the microbial abundance, composition and activity changed substantially during composting and compost maturity was correlated with high microbial diversity and low activity of microbes (Nicholson et al., 2000).

Majority of bacterial morphological and biochemical characteristics resembled those of Staphylococcus species and Bacillus species. Gram positive bacteria and actinomycetes were more in numbers compared to Gram negative bacteria. Similar findings have been reported during the assessment of bacterial diversity when composting agricultural by-products (Chandna et al., 2013). Further, a group researcher reported that majority of bacterial genera found in the compost were gram positive such as Staphylococcus, Serratia, Klebsiella, Enterobacter, Bacillus, Microbactrium, Kocuria, Acidovorax and Comamonas (Ryckeboer et al., 2003).

An isolate which resembled Bacillus species in both biochemical and morphological characteristics was dominant throughout the decomposition process. This is because Bacillus species are capable of surviving in thermophilic temperatures by producing endospores (Nicholson et al., 2000). These findings were in agreement with those by who reported that Bacillus species were found in the thermophilic phase of composting garbage (Ishii et al., (2000). Additionally, some surveyed on bacteria and fungi occurring during composting and self-heating processes and reported that Bacillus species was the most dominant bacterial genera recovered from compost feedstock (Veas et al., 2003).

Some isolates produced morphological characteristics which resembled Aspergillus species, indicating that fungi were also involved in the composting process. Similarly, reported presence of Thermomyces species and Aspergillus species while studying dynamic changes of the dominant functioning microbial community in the compost of a 90 m3 aerobic solid state fermentor where hemicellulose was degraded (Zhang et al., 2016).

4.2 Effect of temperature on decomposition of *Brassica olerecea* var. *capitata*

The study showed that the increase in temperature increased the rate of decomposition since change in temperature affect the functionality of microorganisms involved (Chandna et al., 2013). The difference in assimilation of the decaying substrate gives rise to entrance of different microbial communities which in turn accelerates the decomposition process (Kell et al., 1998). Changes in bacterial population were analyzed by cultivation-based method which reveals variations in the number of bacteria during the composting process. In this study, there is increase in bacterial population as dilution factor used increases with time. This could be ascribed to availability of nutrients from the decaying *Brassica olerecea* var. *capitata*. A decrease in bacterial population in sample 5 could be because of the cooling stage of decomposition hence limitation of nutrients (Chandna et al., 2013).

In this study disappearance of fungal population from the fourth week was observed and can be attributed due to rise in temperature as decomposition continued. Different studies have also reported similar findings whereby fungi and yeasts disappeared almost totally during peak heating during the whole thermophilic phase (Fujio and Kume, 1991; Herrmann and Shann, 1997; Choi and Park, 1998). Temperature is a significant factor in determining the relative advantage of some population over another due to variation in temperature during decomposition (Ishii et al., 2000). This in turn determines the type of microbes present thus affecting microbial load and bioremediation.

During composting, temperature phases are divided into mesophilic, thermophilic, cooling and maturation phase. The study showed an increase in microbial load between weeks 2-8 in sample 0,1,2,3 whereas sample 5 had the highest microbial load up to week 6 before a decrease was recorded. This could be due to population shift in mesophilic and thermophilic bacteria which occurs during the composting process (Corominas et al., 1987; Falcon et al., 1987). At higher temperatures bacteria species diversity decreases (Finstein and Morris, 1975; Fogarty and Tuovinen, 1991; Stenbro-Olsen, 1998).

High temperatures favour cellulose degradation hence cellulolytic organisms appear mainly at the end of the thermophilic stage (Shilesky and Maniotis, 1969; Gray et al., 1971). At the end of the composting process, the cellulose is inaccessible to enzymatic attack because of low water content or association with protective substances such as lignin, resulting in a decrease of the number of cellulolytic organisms (Stutzenberger et al., 1970). It is also indicated that in the material with a high cellulose content, thermotolerant microorganisms with the ability to degrade cellulose will dominate at the end of the composting process (Stenbro-Olsen, 1998). Decomposition slows down as time progresses in week 8 because efficiency of the composting process drops at the higher thermophilic levels (Golueke, 1986). Similar findings have been reported (Ryckeboer et al., 2003).

5. Conclusions

Morphological and biochemical characteristics from our experimental findings revealed that bacteria, fungi and actinomycetes present in decomposition of *Brassica olerecea* var. *capitata*, where of the mesophilic group. Therefore, indicating that decomposition was carried out by mesophilic microorganisms. Further, our experimental findings indicated that there was microbial population variation during decomposition since microbial load increase was observed during the initial stages followed by a decrease as the process continues. This was a clear indication that at the end of the composting process, the cellulose is inaccessible to enzymatic attack because of low water content or association with protective substances such as lignin, thus decrease in microbial load. Moreover, temperature increase led to increase in decomposition process, thus high temperatures favored cellulose degradation.

References

Berg, B., Laskowski, R., 2006. Litter decomposition: a guide to carbon and nutrient turnover. San Diego: Elsevier Academic Press.

Bot, A., Benites, J., 2005. The importance of soil organic matter: key to drought-resistant soil and sustained food and production. Rome: Food and Agriculture Organization of the United Nations.

Chandna, P., Nain, L., Singh, S., Kuhad, R.C., 2013. Assessment of bacterial diversity during composting of agricultural byproducts. BMC Microbiology, 13 (1), Pp. 99.

Choi, M.H., Park, Y.H., 1998. The influence of yeast on thermophilic composting of food waste. Letters in Applied Microbiology, 26, Pp. 175–178.

Chynoweth, D.P., Owens, J.M., Legrand, R., 2000. Renewable methane from anaerobic digestion of biomass. Renewable Energy, 22 (1–3), Pp. 1–8.

Chynoweth, D.P., Owens, J.M. and Legrand, R., 2000. Renewable methane from anaerobic digestion of biomass. Renewable Energy, 22 (1–3), Pp. 1–8.

Chynoweth, D.P., Owens, J.M. and Legrand, R., 2000. Renewable methane from anaerobic digestion of biomass. Renewable Energy, 22 (1–3), Pp. 1–8.

Das, S., Bhattacharyya, B.K., 2017. Selective organic fraction of municipal solid waste degradation under controlled composting conditions. Journal of Environment and Waste Management, 4 (1), Pp. 156-163.

De Deyn, G.B., Cornelissen, J., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters, 11, Pp. 516–531.

Deqiang, Z., Dafeng, H., Yiqi, L., and Guoyi, Z., 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. J Plant Ecol., 1 (2), Pp. 85-93.

Fujio, Y., and Kume, S., 1991. Isolation and identification of thermophilic bacteria from sewage sludge compost. Journal of Fermentation and Bioengineering, 72, Pp. 334–337.

Golueke, C.G., 1986. Compost research accomplishments and needs. Biocycle 27, 40–43.

Hearn, E.T., 2017. Method Development for the GC In-Vessel Composting System Using Post-Consumer Food Waste from the MAX. Appropriate, N., & On, M. A. (n.d.). Nationally appropriate mitigation action on a circular economy solid waste management approach.

Herrmann, R.F., and Shann, J.F., 1997. Microbial community changes during the composting of municipal solid waste. Microbial Ecology, 33, Pp. 78–85.

Insam H., de Bertoldi, M., 2003. Microbiology of the composting process. In: Golueke C., Bidlingmaier W., de Bertoldi M., Diaz L., Eds, Compost Science and Technology, Elsevier Science Ltd (in press).

Intergovernmental Panel on Climate Change – IPCC. 2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands. Switzerland: IPCC, 2014.

Ishii, K., Fukui, M., Takii, S., 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J. Appl Microbiol., 89 (5), 768-777, 2000.

Kumar, A., Prakash, A., Johri, B.N., 2011. Bacillus as PGPR in Crop Ecosystem. Bacteria in Agrobiology: Crop Ecosystems. Berlin Heidelberg: Springer-Verlag, (10), Pp. 642-649.

Liesack, W., Schnell, S. and Revsbech, N.P., 2000. Microbiology of flooded rice paddies. FEMS Microbiology Reviews, 24 (5), Pp. 625-645.

Metcalfe, D.B., Asner, G.P., Martin, R.E., Silva Espejo, J.E., Huasco, W.H., and Malhi, Y., 2014. Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests. Ecology Letters, 17 (3), Pp. 324–332.

Moorhead, D.L., Sinsabaugh, R.L., Linkins, A.E., Reynolds, J.F., 1996. Decomposition processes: modelling approaches and applications. Sci.Total Environ., 183, Pp. 137-149.

Nakasaki, K., Shoda, M. and Kubota, H., 1985. Effect of temperature on composting of sewage sludge. Applied and Environmental Microbiology, 50, Pp. 1526–1530.

Nicholson, W.L., 2002. Roles of Bacillus endospores in the environment. Cellular and Molecular Life Sciences, 59 (3), Pp. 410-416.

Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J., Setlow, P., 2000. Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiology and molecular biology reviews, 64 (3), Pp. 548-572.

Nurhidayati, N., Ali, U., Murwani, I., 2017. Chemical Composition of Vermicompost Made from Organic Wastes through the Vermicomposting and Composting with the Addition of Fish Meal and Egg Shells Flour. The Journal of Pure and Applied Chemistry Research, 6 (2), Pp. 127-136.

Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., Swings, J., 2003. Microbiological aspects of biowaste during composting in a monitored compost bin. Journal of Applied Microbiology, 94 (1), Pp. 127–137.

Sharma, M., Dangi, P., Choudhary, M., 2014. Actinomycetes: Source, Identification, and Their Applications. International Journal of Current Microbiology and Applied Sciences, 3 (2), Pp. 801–832.

Shilesky, D.M., Maniotis, J., 1969. Mycology of composting. Compost Science, 9, Pp. 20–23.

Stenbro-Olsen, P.W., 1998. Studies on the microbial ecology of open windrow composting. PhD Dissertation. 212 pp. Dundee: University of Abertay.

Tuomela, M., Vikman, M., Hatakka, A., Itävaara, M., 2000. Biodegradation of lignin in a compost environment: A review. Bioresour. Technol., 72 (2), Pp. 169-183.

Wang et al., 2013. Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses, 23, Ecological Applications, Pp. 255.