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REVIEW ARTICLE

EFFECT OF HIGH BIOWASTE MOISTURE CONTENT ON PATHOGENS HYGIENIZATION POTENTIAL OF IN-VESSEL COMPOSTING TECHNOLOGY DURING COMPOST PRODUCTION

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ABSTRACT

Nutrient recovery through composting of biowaste stands for many years as promising strategy for replenishing nutrients to barren lands and soil conditioner. Unfortunate, microbiological safety of the resulting compost has been of great concern owing to higher pathogens contamination levels in the biowaste. This study aimed at investigating performance of In-vessel composting technology (ICT) at providing complete inactivation of pathogens from biowaste with higher moisture content. *E. coli* and *S. senftenberg* were spiked on Potato peels waste (PPW) having 77.1% moisture to simulate contamination in biowaste. Five sample formulations: 1:0, 1:1, 3:2, 7:3 and 0:1 (PPW: Compost soil) were aerobically composted at laboratory scale based on ICT. Except for moisture, other physicochemical attributes in PPW were within acceptable composting ranges. The moisture content in the peels turned composting process anaerobic, hampered the composting process and discharged more leachate in composting vessels which affected both temperature-time patterns and pathogens hygienization in the finished compost. Air drying of PPW for 36hrs reduced moisture to 67.7%. This improved temperature-time pattern from 48˚C for 2hrs to 59.2˚C for 4days in 3:2 formulations thus confirming moisture as critical factor.

INTRODUCTION

Land application of compost prepared from biowaste (biodegradable organic fraction of municipal solid waste) has been a practice to many countries for so many years (Day and Shaw, 2001). Even though the presence of microbial pathogens in biowaste is well researched and documented (Bagge *et al.*, 2005; Aderemi *et al.*, 2012; Lemunier *et al.*, 2005; Ceustermans *et al.*, 2007; Sahlström, 2006), in many developing countries for instance where open dumping of commingled municipal solid waste is still the most predominant method of waste disposal (United Nations, 2011), and that segregation of waste is not practiced either because of lack of public motivation or negative attitudes towards waste management (Mbuligwe *et al.*, 2002), yet urban-agriculture practitioners willingly collect the rotten-garbage direct from the dumps and use as compost for their horticultural cropping systems. Acceptable compost for land application according to DG ENV.A.2 (2001) is a stable, sanitized and humus-like material rich in organic matter and free from offensive odours resulting from the composting process of separately collected biowaste and complies with environmental classes of quality.

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Uncontrolled dumping of waste has many disadvantages, among others; it does not allow for resource recovery (Read *et al.*, 2001), contributes in accelerating onset of climate change through emission of greenhouse gases (Adila and Nawaz, 2009) and poses risks to human, animal and environment through spreading of diseases (Thomas and Young, 2013; Sahlström, 2006).

The concern on survival and spread of potentially microbial pathogens as results of compost use has become one of the key research activities in environmental science and technology. Among the existing biowaste composting technologies, In-vessel composting technology (ICT) promise a more stabilized and consistent finished compost compared to static pile or windrow technologies (Franke-Whittle and Insam, 2013). Additionally, ICT requires relatively small area and is said to provide better containment of odour (Franke-Whittle and Insam, 2013). If well monitored and managed, this technology is reported to enhance activities of aerobic bacteria on breakdown of organic matter in biowaste leading to a raise in vessel temperature as higher as 55-70˚C and maintain for longer time (Schultz, 2009) which is enough assurance of safe resulting final compost. One disadvantage of using ICT is that, apart from temperature monitoring devices, the system has no provision for mixing or turning of the materials during composting process. Existing of cool zones and blockage of air circulation porosity probably as results of compact feeding or draining leachate from biodegrading organic fractions aggravate the chance of pathogens to survive thus induces proliferation of potentially pathogens in the final compost. In this laboratory scale based study, the performance of aerated ICT at providing complete inactivation of pathogens in biowaste with higher moisture content was investigated. By studying both physicochemical characteristics of fresh and partially air dried potato peels waste (PPW) (laboratory simulated biowaste) and temperature –time pattern of PPW during composting, and assessment of sensory attributes that include texture, colour and odour of the resulted finished compost, we now have a better understanding on the basic factors that dictates inactivation of microbial pathogens in ICT.

MATERIALS AND METHODS

Potato peels for simulation of biowaste with high moisture content were collected from Potatoes restaurant (Kartoffelkiste, Spremberger), Straße 37 Cottbus, Germany and in case of shortfall; additional PPW were obtained through manual peeling of round potatoes bought from tradition open markets within Cottbus City. Two microbial pathogens, *Escherichia coli* from Germany culture collection (DSMZ– Deutsche Sammlung von Mikroorganismen und Zellkulturen, Liebniz Institut, Germany) and *Salmonella senftenberg* from IFB (Institut für Bioanalytik, Umwelttoxikologie und Biotechnologie, Halle, Germany) were selected because they are potentially pathogens of highly abundance in poorly managed biowaste (Deportes, 1998) and indicator of faecal contamination. In the course of assessing efficiency of in-vessel composting process at inactivation of the spiked pathogens, Brilliant green agar selective media for salmonella and Endo Agar selective media for *E.coli* were the two culture media used in spread plate count. Other media such as nutrient broth and sterilized water were prepared in the laboratory and used as received.

Preparation of study samples

The study samples were prepared as described in Figure 1. In this case, the peels were firstly blended using Multipro compact blender of 1.5 Liter capacity to reduce the size to about 10-12mm. Then the two microbial pathogens were propagated in nutrient broth (Carl Roth GmbH), filtered under 2.1mm Ø (Isopore Membrane Filters) and their viability status confirmed using Fluorescent Microscope (NiKON Eclipse LV 100) using 2 phases contrast at 20 fold magnification. Bacteria cells on Syto9 and propedium iodide (live/dead) were counted using NIS Element BR 3.1 software. Based on spatial distribution of live bacteria cells, the final total bacteria cells liable for spiking was calculated according to the following mathematic expression:

$$\text{BacteriaCells/ mL} = \frac{\sum Cellcounts}{\text{Segment/ Area}(mm^2)} X \frac{FilterArea(mm^2)}{SampleVol(\text{mL})} XDilution \qquad (1)$$

The blended peels were divided into two portions. To one portion, the pathogens were artificially spiked at a ratio of 100:1 (PPW: Pathogens solution) in order to simulate the microbial contaminated biowaste. This ratio was established from *Enterobacteria* contamination levels observed from the real municipal solid waste samples (results not provided in this paper) and was used as benchmark for setting microbial contamination in the simulated biowaste.

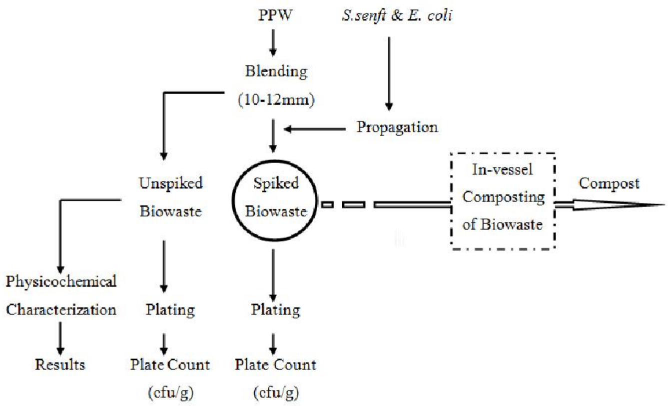

Figure 1. Experimental layout

Physicochemical and microbiological characterization of biowaste for composting

Physicochemical attributes of PPW as baseline for microbial activity and reflection of quality of finished compost were analyzed according to *Bundersgutegemeinschaft Kompost* of 1994 described in Burkhardt, (2013). In this experiment, moisture content, pH measured in 0.01M $CaCl_2$, total solids, loss on ignition, ash and mineral nutrients of PPW were determined before and after composting process. Similarly, the ratio of carbon (analyzed as total organic carbon using Eltra high temperature furnace and multi N/C 2100S (Analytik jena) to nitrogen (analyzed as TKN using Kjeldahl apparatus) was determined only in the compost according to Christensen, *et al*., (2002) and Burkhardt (2013). Determination of macro and essential nutrients in the PPW which reflect the quality of composting material was done using inductively coupled plasma atomic emission spectrometry (ICP-AES) (iCAP 6000, Thermo Scientific). Some of the macronutrients analyzed included phosphorous and potassium. Other determined nutrients essential for microorganism activity were calcium, magnesium and sodium. Microbiological analysis based on spread plate count as described elsewhere (Hurst *et al*., 2007; Benson, 2002) was used for bacteria culturing while enumeration of resulting colonies as colony forming units (cfu/g) was calculated according to the following equation:

$$cfu / g = \frac{\sum colonies}{SampleVol(mL)\,\text{x}(\text{n}_1 + 0.1\text{n}_2)} XDilution \qquad (2)$$

Set-up of In-vessel Composting process

Composting of PPW was carried out in an air forced flasks (Figure 2). Five samples categorized by ratio of PPW: Compost soil 0:1, 1:0, 7:3, 3:2 and 1:1 were formulated for composting process. In this study compost soil was used as inoculum/starter. The compost soil was sieved through 6.0mm sieve to remove debris, un-composted plant parts and stones before use. Each formulated sample was thoroughly mixed and 2000g was transferred into composting reactor having 28cm height, 11cm diameter and 2100mL volume. Air was supplied in the system using external pump (Model JUN Aquarium ACO-5502) in a rate of 1air bubble per second.

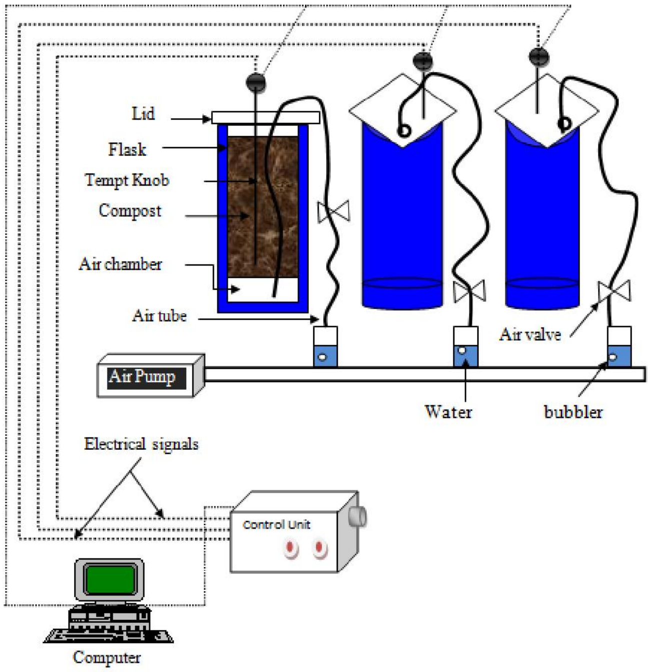

Figure 2. Laboratory scale air forced In-vessel composting technology

Temperature and time were the two parameters monitored during composting. The knobs for temperature reading were calibrated (LAUDA eco Re.104 equipment) to 45.0±0.2°C. Six knobs were used for temperature recording out of which five knobs were directly inserted into each composting flask with samples while the remained knob was left hanging to record room temperature. During the whole composting process, temperature and time were automatically recoded in every two hour using computer with T-Mess Me-2600 V37 vee software.

RESULTS AND DISCUSSION

Size reduction of biowaste firstly increases the surface area and exposes the peels to microbes for easy decomposition and secondly it enhances air circulation by increasing porosity of the composting material mixture within In-vessel composting system. Sample pH determines the effectiveness of composting process by providing conducive environment for decomposing microbes.

For the purpose of biowaste decomposition, an optimum pH-value of 6.0-8.0 has to be maintained. It was worth noting that all sample formulation (Table 1) exhibited the required pH value before and after biowaste composting except for the formulation (1:0) i.e PPW without compost soil for which the final pH was 5.7 before composting and the value dropped even more to pH 5.0 after composting, an indication that peels alone are not to be treated as feed material for aerobic composting. The pH determination using $CaCl_2$ was preferred. This is because 0.01mol/L $CaCl_2$ solution is the perfect choice of neutral salt extractant and has an ionic strength closer to the expected value in soil solution upon which the finished compost is to be applied (Element and Element, 2007).

The physicochemical analysis of PPW prior to and after composting process in Table 1 further confirms that the peels had moisture content higher enough than the recommended ranges of 50-60%. Zhu (2006) and Richard (2007) pointed out that too much moisture >65% in biowaste hamper biowaste decomposition, accelerate onset of anaerobic decomposition in the system, and result into production of off-odour and nutrient leaching. The slower the decomposition the poorer microbial activities in the system thus lead to lower and irregular time-temperature patterns (Figure 3). Compost heat being a by-product of microbial breakdown of organic fraction of biowaste can be optimally reached only if the biowaste moisture content, C/N ratio and aeration in the system are properly regulated. Although the C/N ratio prior to composting was not analyzed, research recommends 20-35:1 range (Jimenez and Garcia 1992; Cooperband 2002). The C/N ratio of the final-product ranged between 8-19/1 confirmed high nitrogen content of the input material. Excess mineral salts and nutrients content in final compost have been reported to cause negatively affect on plants by changing electrical conductivity (EC) as results of loading the soil with un-standardized nutrients. The results of composition of essential nutrient in the peels waste (Table 2) were found within acceptable range for land application and conform to the findings reported in Hargreaves *et al*., (2008).

Pathogens inactivation in PPW

Microbial composting of biowaste passes through three phases (Day and Shaw, 2001). Phase-I normally dominated by mesophillous microorganisms lasting between 1-4days in which temperature raises at logarithmic rate ranging 31-45˚C, then phase-II dominated by thermophilic microorganisms from day 5-13 with temperature between 50-65˚C and last

Table 1. Physicochemical analysis of formulated sample before and after In-vessel composting

Sample	Attributes before composting					Attributes after composting					
	Mc	TS	LOI	Ash	pH-$CaCl_2$	Mc	TS	LOI	Ash	C/N	pH- $CaCl_2$
	Values given as % w/w					Values given as % w/w					
PPW:C (0:1)	38.2	61.8	16.6	83.4	6.8	38.5	61.5	16.4	83.6	n/a	6.6
PPW:C (1:0)	77.1	22.9	94.2	5.8	5.7	69.5	30.8	94.8	5.2	16:1	5.0
PPW:C (7:3)	60.1	39.4	51.5	48.5	6.2	61.7	38.6	57.7	42.3	19:1	6.0
PPW:C (3:2)	57.4	42.4	47.0	53.0	6.1	58.8	41.1	50.5	49.5	17:1	6.0
PPW:C (1:1)	54.2	45.6	32.6	67.4	6.4	59.6	40.5	40.5	59.2	8:1	6.1

Key: PPW:C = Ratio of Potato peels to Compost soil; Mc = Moisture content
C/N = Ratio of Carbon to Nitrogen; LOI=Loss on ignition
TS= Total solids; n/a = not analyzed

Table 2. Composition of essential nutrients in PPW

Parameter	Results	Units
Ca	574.9	mg/kg
Na	494.9	mg/kg
K	23270	mg/kg
Mg	1105	mg/kg
P	2023	mg/kg

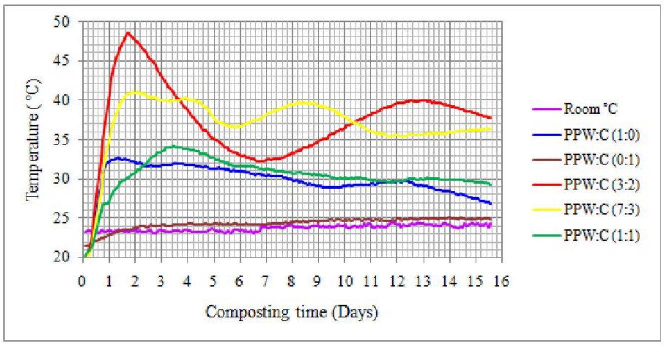

Figure 3. Temperature patterns for In-vessel composting of fresh PPW

phase-III from day 14 normally characterized by self-inhibition of microorganism in which actinomycetes, algae and fungi are dominant (Mbuligwe *et al*., 2002). In this phase, cooling of the compost and degassing of plant-harming compounds of ammonia and H_2S, and maturation or curing of the compost (Cooperband, 2002; Ceustermans *et al*., (2007). The maximum attained temperature 48˚C for 2hrs was observed in sample formulation 3:2 (PPW: C) (Figure 3), the temperature was lower than the minimum suggested of 55˚C in 4hrs for inactivation of *E.coli* (Bijlsm *et al*., 2013) and 60˚C in 4hrs for inactivation of *S. senftenberg* (Ceustermans *et al*., 2007; Richard, 2007) in the compost. The irregular pattern was an indication of blocked porosity within sample due to high moisture content caused by leachate formation. Except for formulation (7:3) which attained a maximum of 41˚C, the other sample formulations could not even reach 35˚C. At the end of 16days of composting, the results showed 9.9% reduction in moisture from the PPW. The temperature-time patterns indicate that composting ended around mesophillic range. No thermophilic microbes managed to colonize the composting process.

(i)Peels-to-Compost ratio (1:0)

(ii)Peels-to-Compost ratio (3:2)

(iii)Peels-to-Compost ratio (7:3)

(iv)Peels-to-Compost ratio (1:1)

Figure 4. Unhygienized finished compost formulation

This could be due to the fact that leachate drained from the compost blocked air circulation and therefore inhibited thermophilic bacteria within the system. Although at the end of day 16, compost were obtained (Figure 4) these compost were unsafe for land application due to unattained temperature capable of effecting pathogens hygienization in the final product. As pointed out in Cooperband (2002) and Zhu (2006), when composting sample is too wet, aerobic bacteria can't get enough oxygen leading to the onset of anaerobic decomposition. Bonhotal *et al*., (2011) revealed that, anaerobic decomposition of biowaste causes slower decomposition, slurry compost accompanied by off-odour and leachate. Similar phenomenon was observed to all sample formulations in this study and even more at the end of the process. Following these distasteful results, it was of the essence to adjust PPW moisture. PPW were air dried for about 36hrs to 67.7% moisture. Moisture adjustment brought improvements in temperature-time pattern (Figure 5), increased composting time to 30days and sensory indicators of finished compost (Figure 6). Results revealed that in sample with formulation 1:1 for instance, although irregular temperature pattern still existed; there was an improved temperature to 55.2˚C which was maintained for 5hrs. This temperature-time regime was enough to completely inactivate *E. coli* from its initial level 8.8$\log_{10}$cfu/g but not sufficient for *S. senftenberg* and therefore managed to reduce microbial load from its spiked level 9.0$\log_{10}$ to 6.2$\log_{10}$cfu/g.

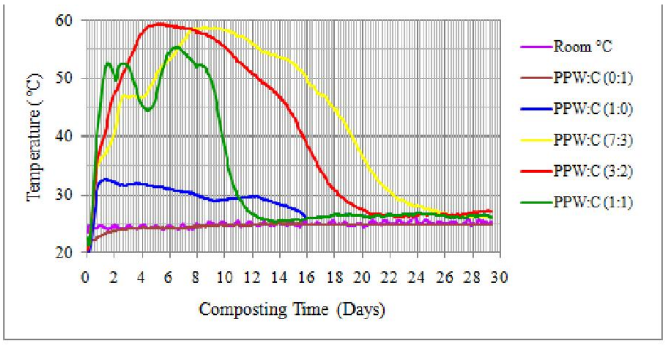

Figure 5. Temperature patterns for In-vessel composting of open air dried PPW

The irregularity of the temperature pattern could be accounted by moisture content of the sample since the moisture content of 67.7% attained after drying was still above 50-60% recommended range (Di Maria *et al*., 2008; Koufodimos and Samaras 2002; Read *et al*. 2001) for biowaste composting. Specification of compost to be used for land application according to regulation EC no.208/2006 requires zero cfu/25g of *Salmonella spp* in the compost and a maximum of 10^3cfu/g for *E. coli*. Inactivation of *E. coli* and substantial decrease in *S. senftenberg* contamination up to 5.6$\log_{10}$cfu/g was observed in the sample with ratio 7:3 which attained 58.6˚C and maintained for 2days.

(i) Peels-to-Compost ratio (1:0)

(ii) Peels-to-Compost ratio (3:2)

(iii) Peels-to-Compost ratio (7:3)

(iv) Peels-to-Compost ratio (1:1)

Figure 6. Finished compost following air dying of PPW

Complete inactivation of pathogens was obtained in sample with ratio 3:2 which attained temperature of 59.2˚C which was maintained for 4 days. Due to the absence of inoculum in sample with 1:0 (peels: soil compost), there was no improvement in both sensory characteristics and temperature-time pattern, and therefore no microbial inactivation was expected.

Conclusion and Recommendation

The study focused on the treatment by composting of simulated biowaste and studies the hygienization of the substrate. Moisture content of the feed for composting stands a prime parameter for consideration if and only if microbiological hygienization of the finished compost is to be realized. Temperature in the reactor depends on the activity of the microbes under the influenced by conducive environment like optimum moisture, aeration, pH and mineral salts. Imbalance in these conditions ends up disturbing microbes and in most cases turning aerobic process into anaerobic, the later being associated with formation of offensive off-odours and lower temperature in the reactor. Therefore even when compost is about to be prepared, the composting plant workers (also called producers) should focus on hygienic potential of the process which determine safety in the final product. The introduced pathogens in the reactor through the use of contaminated biowaste should be inactivated by the inherent temperature so as to hamper health and environmental threatening condition provided the un-hygienized compost are to applied on land. The findings from this study in a nutshell recommends that since high moisture content of the feed hinders pathogens hygienization potential of the ICT, therefore prior to composting, laboratory assessment of biowaste based on physicochemical composition is inevitable. Drying of biowaste for moisture adjustment and/ or intentionally mixing of biowaste with other more dry materials like agriculture waste and yard waste before composting helps in optimizing the moisture content of the feed for better composting process. In addition, thorough investigation of potentially pathogens of great abundance should be done on finished compost for microbiological safety thus avoids land application of contaminated compost.

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