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Effect of aeration rate on composting of penicillin mycelial dreg

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ABSTRACT

Pilot scale experiments with forced aeration were conducted to estimate effects of aeration rates on the performance of composting penicillin mycelial dreg using sewage sludge as inoculation. Three aeration rates of 0.15, 0.50 and 0.90 L/(min·kg) organic matter (OM) were examined. The principal physicochemical parameters were monitored during the 32 day composting period. Results showed that the higher aeration rate of 0.90 L/(min·kg) did not corresponded to a longer thermophilic duration and higher rates of OM degradation; but the lower aeration rate of 0.15 L/(min·kg) did induce an accumulation of NH_4^+-N contents due to the inhibition of nitrification. On the other hand, aeration rate has little effect on degradation of penicillin. The results show that the longest phase of thermophilic temperatures ≥ 55°C, the maximum NO_3^--N content and seed germination, and the minimum C/N ratio were obtained with 0.50 L/(min·kg) OM. Therefore, aeration rates of 0.50 L/(min·kg) OM can be recommended for composting penicillin mycelial dreg.

Introduction

China has been regarded as one of the largest producers and exporters of antibiotics, and the annual yield of antibiotics from manufacturers reached approximately 147 thousand tons in 2009 (Li et al., 2012b). Among these antibiotics, penicillin production accounted for about 100 thousand tons (Fang, 2014). Penicillin mycelial dreg (PMD) is a type of bio-waste from the penicillin production process, mainly containing the organic medium designed for *penicillium* growth and reproduction, and the remaining penicillin. Therefore, PMD is characterized by a high proportion of nutritional materials such as starch, maize slurry, glucose, peptone and nutrient salts as well as the high levels of penicillin residue. In order to utilize its nutritional value and medical effect, PMD had been applied as a feed additive for livestock and poultry to promote growth and reduce pathogenic bacterial infection (Zhang, 2000, 2002). However, the poor absorption by animals results in 70%–90% of the antibiotic residue being excreted in manure (Kumar et al., 2005; Phillips et al., 2004). It is likely that if these manures were used for land application without pretreatment, the antibiotics contained in them would be transported into surface water and ground water, posing a potential risk to the environment, namely promoting the generation and spread of antibiotic resistant bacteria (Jiang et al., 2013).

Composting is one of the most effective ways of reducing the danger of solid wastes (Ding et al., 2014; Hu et al., 2011) and transforming them into a resource that can be applied to soil systems (Seymour et al., 2001; Veeken et al., 2002). Composting is also a controlled-microbiological aerobic process to degrade organic matters through the actions of enzymes, microorganisms and oxygen during the entire process. The main products of biological metabolism are carbon dioxide, water and heat under aerobic conditions (Bari and Koenig, 2001). Thus, a suitable level of aeration is

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essential for maintaining a relatively high activity of microbes, being directly responsible for the substrate degradation rate and temperature variation during the composting process; and aeration rate is believed to be one of the most important parameters influencing the composting process and compost quality (Gao et al., 2010; Kuter et al., 1985). Too little aeration can induce an anaerobic environment, while too much aeration can induce premature cooling, impairing the thermophilic conditions giving the optimum decomposition rates (Ahn et al., 2007). Therefore, it is important to obtain an appropriate aeration rate to make the composting process more efficient.

Various aeration rates were used in some previous studies. Gao et al. (2010) investigated composting of chicken manure and sawdust at aeration rates from 0.3 to 0.7 L/(min·kg) organic matter (OM) and found that an aeration rate of 0.5 L/(min·kg) OM is more efficient than others. Guo et al. (2012) reported that the aeration rate of 0.48 L/(min·kg) dry matter was suitable for co-composting of pig feces and corn stalks. Kulcu and Yaldiz (2004) found that the highest OM degradation and temperature value were obtained at the aeration rate of 0.4 L/(min·kg) OM in composting of agriculture wastes. Li et al. (2008) suggested an aeration rate of 0.25 L/(min·kg) OM in the composting of dairy manure with rice straw. Rasapoor et al. (2009) recommended a rate of 0.4–0.6 L/(min·kg) OM for composting of active municipal solid waste. These variations in the recommended aeration rate show that there might be a close association between the optimal aeration rate and the resource material composition in composting. Although a vast amount of research has been conducted on the aeration rate for effective composting, there is lack of information on the effect of aeration rate on the composting of PMD, rice straw and sawdust (as bulking agent) with sewage sludge (as inoculation). The main objectives of this work were to investigate the evolution of various physical and physicochemical properties in composting at different aeration rates to obtain an optimal aeration rate, with a view to saving operational cost for practical application.

1. Methods

1.1. Source materials

The PMD was provided by a local biological pharmaceutical industry (Harbin, China). Dewatered sewage sludge, consisting of primary and secondary biosolids, was collected from a local municipal wastewater treatment plant (Harbin, China). Both PMD and sewage sludge were stored in a freezer at −20°C prior to use. Sawdust (SD) and rice straw (RS) were collected from a local wood processing facility in Harbin. SD is characterized by powdery particles of sawn wood, the average diameter of which was about 0.55 mm. RS was manually cut into a length of 1.0–2.0 cm. They were used as bulking agents in the experiments. The physical and chemical properties of the source materials are presented in Table 1.

1.2. Composting experiment

The composting reactor has a volume of 390 L (100 cm height, 65 cm length and 60 cm width) and was covered with 2 cm thickness styrene cystosepiment (foam board) for thermal insulation. To ensure a uniform gas distribution, the composting material was supported by a stainless steel grid installed in the reactor about 7 cm above the bottom. Three sampling ports (5 cm inter diameter) on the side of the reactor were set at equal intervals (30 cm). The central positions of three sampling ports were located at the heights of 17 cm, 47 cm and 77 cm from the bottom of the reactor, respectively (Fig. 1). The ports were sealed by rubber stoppers during the reaction.

The rectangular reactor had a removable lid. A Pt100 temperature sensor (WRPX-12, Changjiang Temperature Meter Factory, Shanghai city, China) located at 50 cm height from the bottom was connected to a digital thermometer (XMT, Changjiang Temperature Meter Factory, Yuyao city, Zhejiang, China) to automatically record the data. A hole on the side near the reactor bottom was used for aeration.

In our previous study, a series of experiments on the effect of bulking agents on the composting performance were conducted (data not shown here). Results revealed that the mixture of PMD, sewage sludge, SD and RS added with the wet weight ratio of 2.0:1.6: 1.0:1.0 was optimal. Thus, three composting treatments (T-1, T-2 and T-3) using the aforementioned ratio were conducted to investigate the effect of three aeration rates, namely 0.15, 0.50, and 0.90 L/(min·kg) OM. Each treatment weighed about 98 kg and was carried out in triplicate. The homogeneity of each composting treatment was ensured before composting. The characteristics of the raw materials are presented in Table 2.

Each composting process lasted 32 days. The initial water content and C/N was adjusted to about 65% and 23, respectively. The C/N ratio adjustment focuses on C due to the relatively excess N in the matrix. According to Li et al. (2013), the C source supplement depended partly on glucose. The treatments were turned on the 9th day, and water was added

Table 1 – Physicochemical parameters of penicillin mycelial dreg (PMD), sewage sludge, sawdust (SD) and rice straw (RS).

Parameters	PMD	Sewage sludge	RS	SD
pH	7.3 ± 0.4	6.8 ± 0.7	7.0 ± 0.4	6.4 ± 0.3
Moisture content (%)	76.5 ± 1.5	78.3 ± 1.7	11.1 ± 0.5	13.5 ± 0.7
OM [a] (g/kg)	765.7 ± 16.8	414.5 ± 11.1	801.4 ± 10.4	862.4 ± 12.2
TOC [a] (g/kg)	446.4 ± 8.3	205.6 ± 6.2	483.3 ± 8.8	471.7 ± 8.3
TKN [a] (g/kg)	75.6 ± 1.9	26.6 ± 0.7	5.1 ± 0.4	2.7 ± 0.6
C/N	5.6	7.7	87.2	182.3

[a] Dry weight base; data are presented as mean ± standard deviation of 3 replicates; OM: organic matter; TOC: total organic carbon; TKN: total Kjeldahl nitrogen; C/N: carbon/nitrogen ratio.

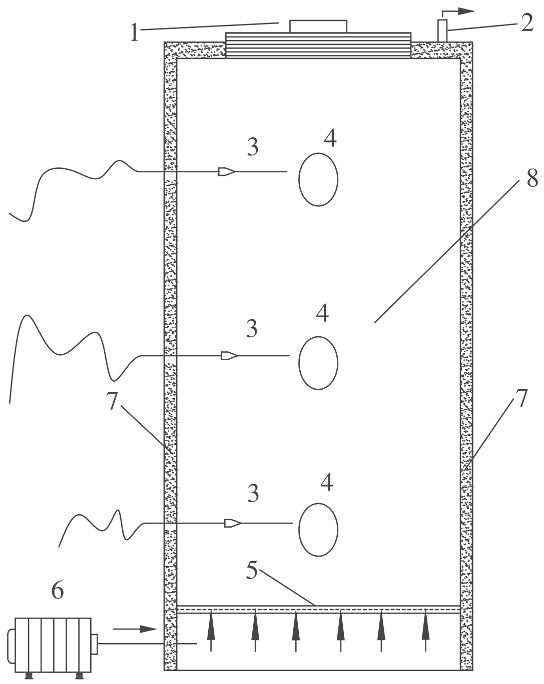

Fig. 1 – Schematic drawing of the composting reactor: (1) removable lid; (2) gas exit; (3) temperature sensor; (4) sampling port; (5) stainless steel grid; (6) aeration pump; (7) styrene cystosepiment; (8) reactor.

to maintain about 65% moisture. The air pump ran intermittently, turned on for 10 min and off for 20 min during the first 7 days and turned off for 40 min during the next 25 days. The air flow rate was measured by a volumetric air-flow meter. Temperature in the center of treatments was recorded daily.

A representative sample weighing 2 kg was collected by mixing three sub-samples from three locations corresponding to sampling ports every day in the first 7 days and then once every 3 days until the end. The sample was divided into two parts, of which one was immediately analyzed and the other was air-dried, ground and then passed through a 1-mm sieve to analyze.

1.3. Compost analysis

The moisture was determined by drying the fresh sample at 105°C in an oven for a period of 24 hr. The pH and electrical conductivity (EC) were measured in the water extract from the sample according to the procedures described by Hu et al. (2009). OM was determined by sample burning (previously dried at 105°C) at 550°C for 4 hr in a muffle furnace. Total organic carbon (TOC) was determined by a TOC analyzer (SSM-5000A, Shimadzu, Tokyo, Japan). NH_4^+-N and NO_3^--N were determined according to the reference (Li et al., 2012a). The seed germination (GI) test was used to evaluate the phytotoxicity of the compost, and its measurement procedures were carried out according to the description of Gao et al. (2010).

1.4. Determination of penicillin

Penicillin was analyzed by an improved method described by Kakimoto et al. (2007). Air dried samples were crushed and sifted by a 50 mesh sieve. A 5 g sifted sample was accurately weighed into a 50 mL centrifuge tube and mixed with 20 mL extraction buffer solution composed of acetonitrile and 0.1% formic acid at 3:1 ratio (*V*/*V*). After being left to stand for 25 min, the mixture was vortex mixed for 5.0 min. The mixture was centrifuged for 10.0 min at 6000 r/min, and then 10 mL of the supernatant was accurately withdrawn. The same extraction procedure was repeated twice.

The purification and pre-concentration of the extract liquid collected were processed with solid phase extraction by a solid phase extraction column (Waters Oasis HLB, 3 cm^3/60 mg) conditioned with 5 mL methanol and 5 mL ultrapure water previously. The extract liquid went through the solid phase extraction cartridge (Supelco Visiprep™, USA) with a flow rate of 1.0 mL/min, and then the cartridge was rinsed with 5 mL of 5% methanol/water solution. The analyte of interest was eluted with 5 mL of the extraction buffer solution. The eluent collected was concentrated to near dryness under a gentle stream of N_2 gas at 25°C, and then reconstituted with 1 mL injection solution (50% acetonitrile in ultrapure water) followed by filtration with a 0.45 μm nylon syringe filter before analysis.

Penicillin was analyzed by high-performance liquid chromatography (Waters 2487, Milford, MA, USA) at 216 nm with the Eclipse XDB-C18 column. The binary mobile phase was composed of A: 0.1% formic acid in ultrapure water; B: acetonitrile. Isocratic elution in the ratio of 60/40 (A/B) was used at the flow rate of 1.0 mL/min. The 30°C column temperature and injection volume of 10 μL were selected. All experiments were carried out at least in duplicate and the standard deviations of experimental data were usually within 5% unless otherwise noted.

1.5. Statistical analysis

Three measurements were carried for each characterization and analysis. Data were calculated using Excel (Microsoft 2003), and the standard deviation is reported along with the average value.

Table 2 – Characteristics of the raw composting materials for T-1, T-2 and T-3.

Treatments	OM (g/kg)	pH	C/N	EC (mS/cm)	NO_3^--N (mg/kg)	NH_4^+-N (g/kg)
T-1	785.4 ± 14.7	6.78 ± 0.24	24.2 ± 0.7	1.3 ± 0.02	426 ± 8	1398 ± 17
T-2	771.5 ± 13.3	6.68 ± 0.46	23.6 ± 0.6	1.2 ± 0.03	475 ± 6	1504 ± 23
T-3	783.6 ± 14.1	6.73 ± 0.34	24.5 ± 0.7	1.2 ± 0.03	412 ± 8	1634 ± 19

Data are presented as mean ± standard deviation of 3 measurements.
EC: electrical conductivity.

2. Results and discussion

2.1. Temperature profile

Temperature has been widely recognized as an important parameter to monitor the composting process and is correlated with microbial activities, affecting not only the biological reaction rates and the population dynamics of microbes, but also the physicochemical characteristics of composts (Antizar-Ladislao et al., 2005; Hu et al., 2009). According to temperature evolution, the composting process was divided into four phases, namely mesophilic, thermophilic, cooling, and maturation phases (marked for T-2) (Fig. 2). The temperature increased rapidly in the three treatments due to the microbial proliferation, and reached above 55°C in the initial 5 days. The highest temperature in each treatment was 60, 65 and 67°C for the T-1, T-2 and T-3, respectively, appearing on the 5th, 4th and 2nd day. The T-1 temperature slowly increased before reaching the peak, while that of T-3 increased rapidly. The longest thermophilic phase was observed in T-2, which sustained the temperature above 55°C 4 days, while 3 and 2 days were observed for T-1 and T-3, respectively. According to the compost sanitation requirements specified in the Chinese national standard (GB7989-87), temperature must be maintained above 55°C for 3 days. Obviously, the T-3 performance did not satisfy this requirement. This may be because that its aeration rate was excessive and caused heat loss. On the other hand, the temperature in the T-1 treatment during the maturation phase was relatively higher than that of T-2, suggesting that the degradation extent of organic matter in T-1 was inadequate. The possible reason may be attributed the relatively low aeration rate required for microbe metabolism (Gao et al., 2010; Guo et al., 2012). Therefore, the T-2 aeration rate might be considered the most desirable in terms of temperature change pattern.

2.2. Changes in chemical parameters

2.2.1. Organic matter and C/N ratio

OM content dropped during composting for all treatments due to the microbial activity. In general, the OM content reached a relatively stable status at the later period, meaning that composting had reached the stable phase (Bustamante et al., 2008). The C/N ratio has also been used to assess compost maturity (Nolan et al., 2011).

The OM contents of the three treatments decreased during composting (Fig. 3a). It is evident that a higher rate of OM degradation was observed for the thermophilic phase than the cooling and maturation phases. Finally, the T-1 and T-3 OM degradation rates were 36.6% and 40.8%, respectively. The maximum rate of OM degradation rate occurred in the T-2 treatment, corresponding to 44.2% with a moderate aeration rate, suggesting that too little or much aeration adversely affected OM decomposition during composting. This may be because the T-2 aeration rate not only provided adequate oxygen concentration for microorganisms, but also avoided evaporating water too rapidly, resulting in the inhibition of microbial activity.

For all three treatments, the final C/N ratio was lower than the initial C/N ratio. The T-1, T-2, and T-3 C/N ratios decreased from the initial values of 24.2, 24.5, and 23.6 to 15.7, 13.1, and 14.5, respectively (Fig. 3b). The low C/N ratio of the final compost was mainly a result of the degradation of the carbon fraction in the pile (Zhu, 2007). During the composting process, carbonaceous materials such as carbohydrates, fats and amino acids (degraded quickly in the first stage of compost), and also cellulose, hemicelluloses and lignin (partially degraded at a later stage) are partially mineralized, leading to carbon losses, mainly as carbon dioxide, throughout the process (Bernal et al., 2009). Obviously, the C/N ratio appeared stable in the maturation stage during composting. Moreover, the T-2 and T-3 (but not T-1) C/N ratios decreased below 15.0, suggesting that their composts had satisfied an acceptable standard of maturation, as reported by other authors (Bernal et al., 2009). The relatively low C/N ratio of the T-2 treatment may be due to the suitable aeration rate, favoring the further degradation rate of OM during composting.

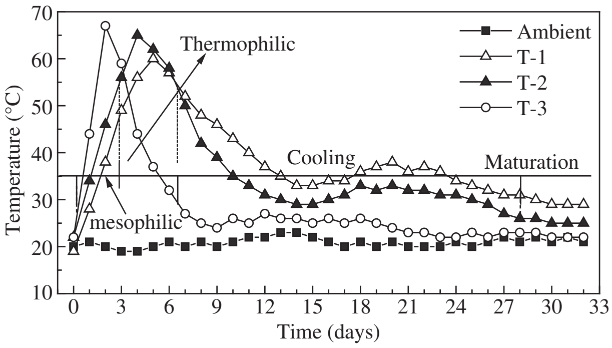

Fig. 2 – Temperature profiles of composting at three aeration rates for T-1, T-2 and T-3.

2.2.2. Changes in NH_4^+-N and NO_3^--N

NH_4^+-N was generated due to the decomposition of organic matter during composting; high NH_4^+-N concentration also contributes to ammonia emission due to the conversion of NH_4^+-N into NH_3 by its volatilization under high pH and temperature conditions; high NH_4^+-N content in compost indicates instability (Sánchez-Monedero et al., 2001).

From Fig. 4a, the NH_4^+-N content of T-1, T-2 and T-3 reached its peak value of 7883, 7717 and 7017 mg/kg on the 6th, 5th and 3rd day, respectively, and finally was 532, 371 and 327 mg/kg in T-1, T-2 and T-3, respectively. The initial increase in NH_4^+-N content is due to the hydrolysis of the nitrogenous material in the biodegradable fractions of the pile (De Gioannis and Muntoni, 2007). The final NH_4^+-N content in the T-2 and T-3 treatments did not exceed the stabilization limit of 400 mg/kg (Cáceres et al., 2006). However, the NH_4^+-N content of the T-1 treatment remained higher than the others, not meeting the stabilization standard. This may be because NH_4^+-N content during composting is strongly affected by temperature and air flow rate. In particular, during the later period of composting, the nitrification process in the T-1 pile was inhibited to some extent due to the relatively high temperature (Grunditz and Dalhammar, 2001). Moreover, the low aeration rate for the T-1 treatment restricted ammonia gas volatilization.

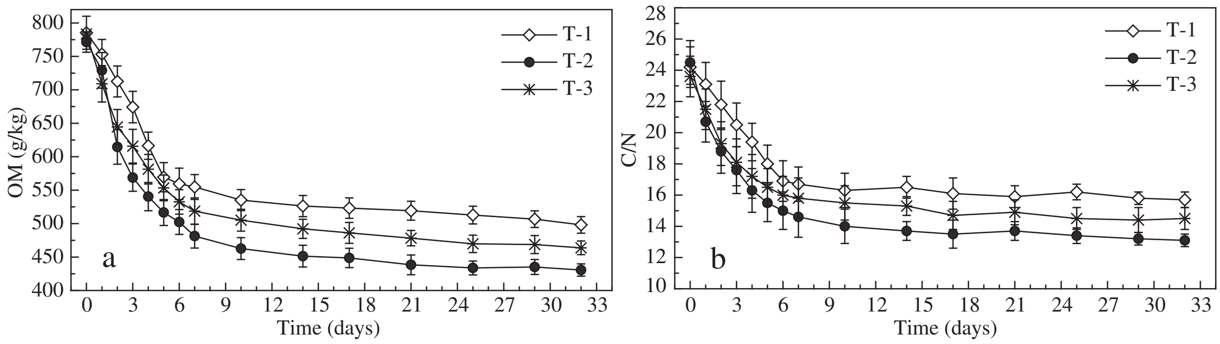

Fig. 3 – Changes in organic matter (OM) (a) and C/N ratio (b) at three aeration rates for T-1, T-2 and T-3.

Nitrification was inhibited in the three treatments during the initial 6 days (Fig. 4b). NO_3^--N then showed a rising trend until the end. Finally, the NO_3^--N content was 2557, 3262 and 2934 mg/kg in T-1, T-2 and T-3, respectively. After the thermophilic phase, an obvious increase in NO_3^--N content occurred due to the weakening of the inhibition of nitrifying bacteria growth. The increase in NO_3^--N contents coincided with the decrease in NH_4^+-N contents, which resulted from the nitrification by microorganisms during composting. Nitrification can make it possible to obtain a product with a high concentration of available N (Cáceres et al., 2006). Therefore, the T-2 aeration rate was preferable to others, considering that its NO_3^--N contents were the highest finally.

2.2.3. Changes in pH and EC

The pH level is one of the important characteristics of the composting process. The optimum pH values for composting are between 5.5 and 8.0 (Venglovsky et al., 2005). EC reflects the degree of salinity and the amount of ions during composting, which indicates its possible phytotoxic/phyto-inhibitory effects on the growth of plants (Gao et al., 2010).

As Fig. 5a shows, the pH values of the three treatments showed a similar pattern. The increase in pH during the composting coincided with the temperature change. The pH for the T-1, T-2 and T-3 treatments first increased to 8.56, 8.78 and 8.72, respectively, and decreased due to the increased production of organic acids or increased nitrification as a result of H^+ released during microbial nitrification. Finally the pH decreased in the three treatments to below 8.0 (7.48 in T-1; 7.04 in T-2 and 7.24 in T-3), indicating that all composts had reached acceptable values between 5.5 and 8.0, as reported by Venglovsky et al. (2005).

Fig. 5b shows the patterns of change in EC of the T-1, T-2 and T-3, which increased to the maximum 4.8, 4.5 and 4.1 mS/cm, respectively, and then dropped till the end. The initial increase in EC could be due to the release of mineral salts such as sulfur, phosphates, magnesium and ammonium ions from the decomposition of organic substances. With the development of composting, the volatilization of ammonia and the precipitation of mineral salts resulted in the decrease of EC in the cooling and maturation stage (Gao et al., 2010). Finally, the compost EC of T-1, T-2 and T-3 were 2.7, 2.4 and 2.1 mS/cm, respectively, which did not exceed the stability limit of 3.0 mS/cm (Cáceres et al., 2006).

2.3. Phytotoxicity and penicillin residue

GI is an integrated biological indicator and has been used to evaluate variation in compost phytotoxicity during the composting process (Gao et al., 2010; Tiquia and Tam, 1998). Additionally, the land application of the compost containing penicillin residue may induce antibiotic resistance genes and antibiotic resistant bacteria in the natural environment. Therefore, it is most desirable that the penicillin level in compost be as low as possible.

The changes in GI of the three treatments are shown in Fig. 6a. Low GI values were recorded in the early composting stage (<2.5%), suggesting severe phytotoxicity in the substrates, and then the GI increased to 50%, indicating that the severe toxicity was greatly weakened. The compost GI was

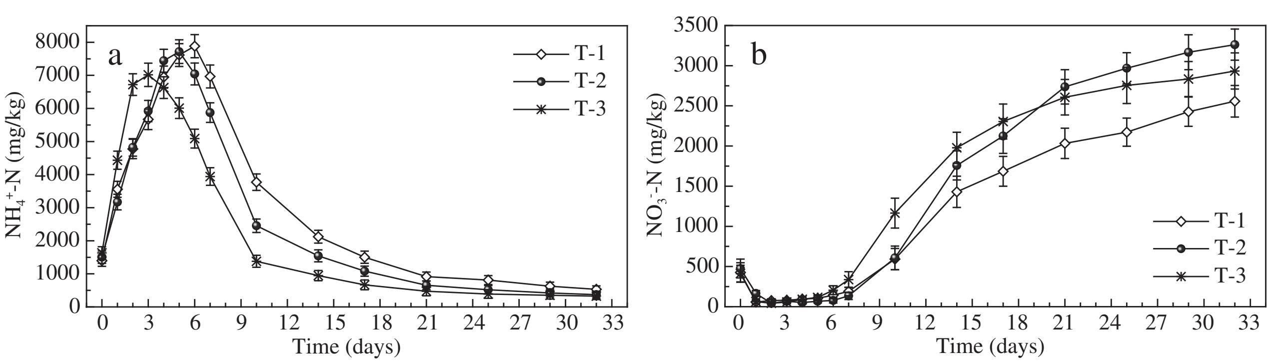

Fig. 4 – Changes in NH_4^+-N (a) and NO_3^--N (b) at three aeration rates for T-1, T-2 and T-3.

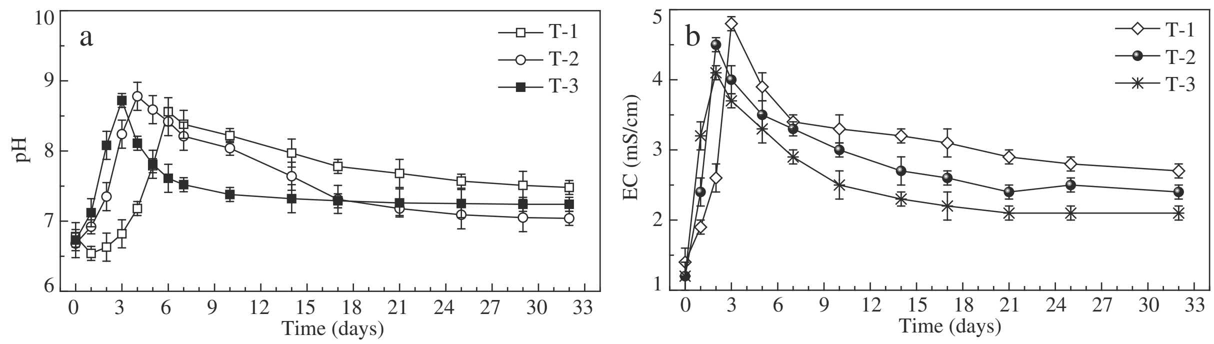

Fig. 5 – Changes in pH (a) and EC (electrical conductivity) (b) at three aeration rates for T-1, T-2 and T-3.

more than 80%, except for T-1, whose GI was 79.2%. This showed that T-1 contained relatively high phytotoxicity, which is also related to excessive salinity (Tiquia, 2010). This fact also accorded with the T-1 EC value (4.8 mS/cm) in Section 2.2.3. The T-1 EC reached 4.8 mS/cm, higher than the others. The final GI from the T-2 and T-3 treatments was respectively 96.6% and 85.7%. The difference in GI between T-2 and T-3 may have been the result of differences in microbial activity due to the different aeration rates. Usually, microbial activity affects its ability to metabolize low molecular weight substances such as NH_3 and organic acids, whose content is negatively correlated with GI. The highest GI was obtained for the T-2 treatment, with a moderate aeration rate, meaning the disappearance of the compost phytotoxicity, according to Tiquia and Tam (1998).

From Fig. 6b, a notable decrease in the penicillin contents was detected during composting, and similar trends were observed for the three treatments. The maximum penicillin degradation mainly occurred in the initial 4 days. The initial contents of penicillin in the treatments were in the range between 1065.2 and 1101.6 mg/kg, and on the 4th day those of T-1, T-2 and T-3 were 282.4, 97.7 and 164.7 mg/kg, corresponding to degradation rates of 74.3%, 90.9% and 85.1%, respectively. It is worthy to note that there were no significant differences in the penicillin contents finally. The penicillin contents of T-1, T-2 and T-3 were finally 15.5, 7.2 and 6.4 mg/kg, the remove rate of which reached 98.5%, 99.3% and 99.4%, respectively. The most likely explanation is that penicillin has a very sensitive ring structure (beta-lactam ring) and the ring is easily cleaved when the environmental temperature rapidly increases. Besides temperature, other factors such as phosphate, ammonia, and pH also impact on penicillin degradation (Kakimoto et al., 2007). As a similar compound containing the same beta-lactam ring, amoxicillin degradation in composting has been reported. Kakimoto and Funamizu (2007) found that phosphate, ammonia, and hydroxyl ion in the matrix can contribute to amoxicillin degradation during composting.

3. Conclusions

The composting results showed that a higher aeration rate did not correspond to a longer thermophilic duration and higher rate of OM degradation; but the lower aeration rate did induce an accumulation of NH_4^+-N content due to the inhibition of nitrification. On the other hand, aeration rate has little effect on the degradation of penicillin. The aeration rate of 0.50 L/(min·kg) OM is recommended for composting PMD, mainly depending on the fact that the longest phase of thermophilic temperatures ≥55°C, the maximum NO_3^--N contents and GI, and the minimum C/N ratio were obtained under this aeration rate.

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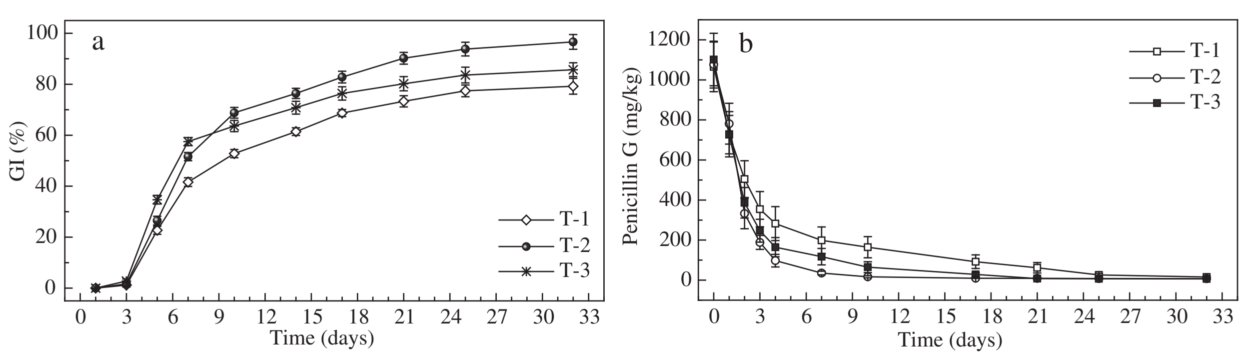

Fig. 6 – Changes in germination index (GI) (a) and penicillin residue (b) at three aeration rates for T-1, T-2 and T-3.

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