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Full Paper

Composting of tobacco plant waste by manual turning and forced aeration system

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Abstract: The efficiency of tobacco plant waste composting, by the manual turning and the forced aeration system, was compared. Tobacco plant waste, cow manure, urea fertiliser, and a compost inoculum mixture at a 100:10:0.2:0.01 ratio respectively, with 60% (w/v) moisture content, were set up in piling forms. The piles of the manual turning system were provided with turning aeration by hand at intervals of 7 days during the composting process. For the forced aeration system, each pile was aerated by a 3-HP air pump with a flow rate of 19 litres min^{-1} for 15 minutes every morning and evening. The completely randomised design of turned and force-aerated piles was performed in triplicate. The composting activity of both systems during the composting period was measured by several parameters: temperature, pH, moisture content, C/N ratio, growth of microorganisms, cellulase activity, and nicotine degradation in the set-up piles. Both systems had similar temperature, pH, and moisture content conditions in the piles during the composting process. However, the forced aeration system was more advantageous for the growth of mesophilic and thermophilic microorganisms, for cellulase activity from cellulase-producing microorganisms, and for nicotine degradation, when compared to the manual turning system. In conclusion, the forced aeration system was more efficient than the manual turning system in composting and is a viable alternative method for the composting process.

Keywords: composting, tobacco waste, manual turning, force-aerated system

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Introduction

It is estimated that roughly 300,275 tons of nicotine waste are produced globally every year [1]. In Thailand, approximately 500-650 tons of tobacco plant waste is generated annually at various stages during post-harvest processing and manufacturing of tobacco products. Tobacco plant waste has no immediate use, and cigarette companies are required to pay for its disposal. The majority of tobacco plant waste is burned, although this has a high cost of operation because of air pollution problems. On the other hand, several soil microorganisms, such as *Arthrobacter nicotianae*, *A. nicotinovorans*, *A. globiformis*, *Enterobacter cloacae*, *Psedomonas putida*, *Cellulomonas* sp., and *Alcaligenes paradoxus* are able to degrade toxic nicotine [2-7]. Therefore composting, which utilises several types of microorganisms, may be used as an alternative method for organic waste treatment, similar to that of sewage sludge and animal manure. Composting toxic tobacco plant waste from the agricultural industry would minimise waste and be useful for agricultural purposes. Currently, composting is used in the agro-industrial process to obtain products that can be applied to soil in order to increase the organic matter content as well as to enhance the soil structure and cation exchange capacity [8]. Utilisation of organic waste composts is particularly important for unfruitful soils that have low organic matter content. Likewise, many Asian agricultural regions have defective soils since farmers have used inorganic fertilisers for many years without regard to their long-term effect on soil structure and thus greatly need this type of treatment.

In order for composting to be accepted, development of the operating strategy, followed by the success of the composting process, is necessary. Therefore, proper evaluation of composting systems is required if an acceptable product is to be generated, and the efficiency of the system must be maximised [9-10]. Manual turning is often labour intensive, creates air pollution (e.g. dust), and requires additional space for the pile. Therefore, other operating strategies that can reduce manpower and space are worth exploring. In addition, the composting time can be shortened by other composting strategies. In one report, force-aerated composting, which maintains temperatures in the upper thermophilic range and provides an effective inactivation of pathogens, was a more efficient composting method [11]. Force-aerated windrow composting uses a ventilation unit (centrifugal blower) to force air into the perforated pipe system, located underneath the compost pile, to induce air convection movement into the material and to deliver oxygen to the microorganisms [11,12-14]. Bulking agents, such as wood chips, straw, peat, or sawdust, are often mixed with the compost material to give the required open structure and to ensure adequate aeration [12,15-16]. This composting system is also a non-turning method and therefore saves space compared to the conventional composting method.

The present study compares the composting efficiency of conventional composting systems to force-aerated composting systems. In this study, chemical and biological parameters were determined to assess the maturity of the compost from tobacco plant waste. Meanwhile, the reduction of nicotine was determined for both composting systems.

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Materials and Methods

Raw materials

The tobacco plant waste was collected from the storehouse of the Chiang Mai Tobacco Office, Thailand Tobacco Monopoly. The tobacco waste was air dried for 4 weeks prior to use.

Cow manure was obtained from the cow farm, located at the Faculty of Agriculture at Chiang Mai University, Chiang Mai, Thailand. Sub-samples of cow manure were air dried at 80°C for 24 h and ground and pressed through a 0.2 mm sieve. The samples were then stored prior to usage.

Compost inoculum (CMU) was produced in the Department of Biology at Chiang Mai University. The CMU consists of 4 strains of bacteria in the genus *Bacillus*, 1 strain of actinomycetes in the genus *Streptomyces*, and 2 strains of fungi in the genus *Aspergillus ,* and one unknown strain.

Experimental establishment

The tobacco plant waste, cow manure, urea fertiliser, and compost inoculum, in a 100:10:0.2:0.01 ratio respectively, were homogeneously mixed, and the moisture content was adjusted to 60% (w/v) with tap water before piling. The completely randomised design (CRD) with two treatments, i.e. manual turning and forced aeration, were performed in triplicate. Each pile was triangular in shape, and approximately 2.5×3.5 m at the base and 1.5 m in height [17]. For the turned pile, aeration was performed manually by turning the piles every 7 days. During the composting process, the ambient temperature and temperature at a depth of 60 cm was monitored every 2 days in the first week and every 10 days thereafter. For the force-aerated pile, 10-cm diameter polyvinyl chloride (PVC) pipes perforated in lengths of 2.5 m, were laid at the base of the piles. Wood chips were used to cover the perforated sections of the pipes to prevent blockage of the holes. Aeration for the piles was supplied by a 3-HP air pump, with an average flow rate of 19 litres min^{-1} and a maximum output of 24 litres min^{-1}. The air pump was operated 15 minutes in the morning and 15 minutes in the evening during the entire period of composting. Every two days in the first week and then every 10 days thereafter, the temperature of each pile was measured in the following locations: at the top, 130 cm from the base of the pile; in the middle, 75 cm from the base of the pile; at the bottom of the pile, 30 cm from the base of the pile; and at the surface of the pile, 5 cm from the surface of the pile.

Analytical sample

Composite samples were taken at five symmetrical locations in each pile at the starting time and then every 10 days until the end of the composting process (50 days).

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Chemical analysis

1) Moisture content

The moisture content in the composting piles was determined by the weight loss of 10 g samples, which were dried for 48 h at 105°C in an incubator, or until the weight of the compost mixture was constant. The percentage of moisture content was then calculated.

2) pH value

To obtain the pH value in the compost, 10 g of solid sample was extracted with deionised water, at a sample-to-water ratio of 1:5 (v/v). After an equilibration time of 30 min, with occasional stirring, the pH was measured with a pH metre.

3) C/N ratio

The total organic carbon content of the 10 g of compost sample was determined by oxidation with potassium dichromate in an acid medium. The excess of the dichromate was measured using Mohr's salt, according to the method previously described [18].

The total nitrogen content of the 10 g of compost sample was determined using the regular-Kjeldahl method [19]. Kjeldahl nitrogen was quantified by mineralisation within a strong acid medium, containing 98% sulfuric acid, followed by steam distillation and titrimetric determination of NH^+_4/NH_3. Organic nitrogen was obtained by subtraction of NH^+_4/NH_3 nitrogen from Kjeldahl nitrogen. The C/N ratio was then calculated by dividing the total organic carbon content by the total nitrogen content.

4) Cellulase activity

The compost sample (5 g) was mixed with 25 ml distilled water for 30 min, using an ultrasonic bath, and was then centrifuged for 15 min at 6000 RPM. The cellulase activity was assayed by measuring the reduced sugars, as represented by glucose, using the dinitrosalicylic acid method [20]. The reaction mixture contained 0.5 ml of the above supernatant and 0.5 ml of 1% carboxymethylcellulose (Sigma Co.) in a 0.05 M potassium phosphate buffer at pH 7.0. After incubation at 50°C for 30 min, the reaction was terminated by adding 3 ml of dinitrosalicylic acid. The colour of the reaction mixture was developed in a boiling water bath in 15 min, and the absorbance was read at 540 nm. Sugar values were read from a glucose calibration curve. One unit of enzyme activity was defined as the amount of enzyme which released 1 μg of reducing sugar per min.

5) Microbiological analysis

The growth of both mesophilic and thermophilic bacteria and fungi were determined. In the preliminary experiments, various media for bacteria and fungi were tested, and the media in which the largest number of isolates appeared was adopted as the plate count media. The media utilised in this work was Trypticase soy agar (BBL Microbiology Systems) media for bacteria (trypticase peptone, 17 g; phytone peptone, 3 g; NaCl, 5 g; K_2HPO_4, 2.5 g; glucose, 2.5 g; agar, 20 g; distilled water, 1 litre [pH 7.3]), and Rose Bengal agar media for fungi (peptone, 5 g; dextrose, 10 g; KH_2PO_4, 1 g; $MgSO_4$, 0.5 g; agar, 15 g; Rose Bengal 35 mg; distilled water, 1 litre [pH 5.2]). In order to determine the total counts of microorganisms, 10 g of sample was subjected to 10-fold serial dilutions using a sterile

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phosphate buffer solution at pH 7. From each dilution, a 0.1 ml aliquot was spread onto plates containing media, as described above, in duplicate. The incubation temperatures were 30°C for mesophile growth and 50°C for thermophile growth. The incubation time was 24 h for mesophilic bacteria, 48 h for thermophilic bacteria, and 72 h for mesophilic and thermophilic fungi. The cultures were prepared from the plates, and those that corresponded to the same dilution (from duplicates) and showed between 30-300 colonies were selected, and their microbial numbers, as CFU g^{-1}, were calculated.

6) Nicotine analysis

In order to extract nicotine, 5 g of compost sample was added to a distillation flask with 50 ml of alkali-salt solution and then distilled with a current of steam. The distillate was diluted, and the spectrophotometric method was used to measure the percentage of nicotine residue using the AOAC official method 960.07 [21].

Results and Discussion

Temperature profile

The initial temperature of the turned pile was 29°C and rapidly rose to a peak of 62°C after a 4-day composting period. The high temperature continued until 20 days of composting, after which the temperature dropped to 36°C by the 30th day of composting. Thereafter the temperature varied within a narrow range (36-32°C) (Figure l). The temperature change of the force-aerated pile and turned pile were similar. The pattern of these temperatures, i.e. first an increase to a high temperature, persistence of a high temperature, and then a decrease to a low temperature, is a typical temperature profile of the composting process, especially for cow manure and wheat straw composts [22], citrus waste compost [23], spent pig manure and sawdust litter composts [24], and filter cake and bagasse composts [25]. The temperature levels in the compost piles tended to increase and reach 50-60°C due to the energy released from the biochemical reactions of the microorganisms in the compost piles, while the temperature levels in the compost piles tended to decrease after the thermophilic phase due to a loss of the substrate and a decrease in microbial activity [26]. The temperature in the compost piles can be used as an indicator of the compost maturity [19,27]. It was previously reported that the compost material can be considered mature when an ambient temperature of 28°C is reached, and that the compost in a force-aerated pile, after 30 days of the composting process, is closer to maturity than the one in a turned pile [19,27].

pH Change

During the composting process, the pH of the turned piles and the force-aerated piles were similar (Figure 2). At first, the pH of both piles increased from an initial pH of 9.54 and 9.10 respectively, to a pH of 11.78 and 10.97 after 10 days. This increase in pH during the composting process could be due to the production of ammonium as a result of the ammonification process [24]. Subsequently, the pH of both piles decreased from 11.78 and 10.97, to 7.78 and 7.80 respectively.

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This may be due to a high organic carbon content in these piles that was subsequently degraded to organic acids by the acid-forming bacteria that exist in the compost piles [28]. In addition, the pH decrease may also be caused by the mineralisation of organic acids and the large quantity of carbon dioxide released during the composting process [9,19,22,24,25,28]. Our results show that the pH of both composts decreased to a final, mature pH of approximately 8.0, based on the compost regulations of pH 5.0-8.0 for the US, and pH 5.5-8.0 for the Council of European Communities (CEC) [47].

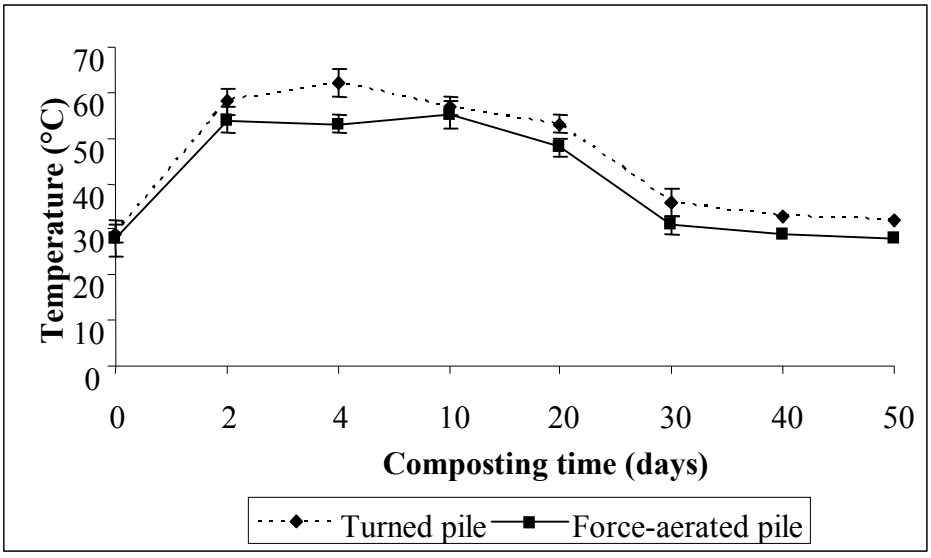

Figure 1. Temperature profiles during composting of tobacco plant waste

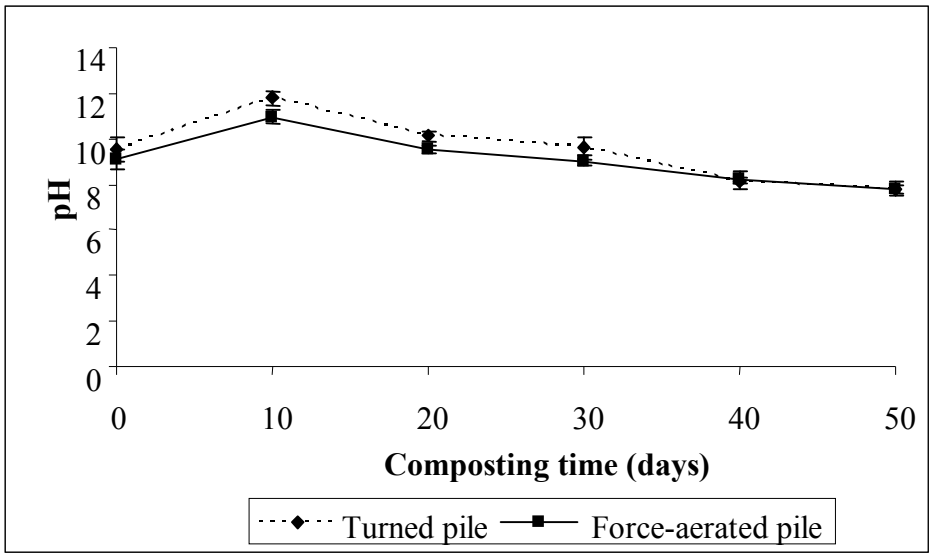

Figure 2. Change of pH during composting of tobacco plant waste

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Moisture content

At the initial stage of composting, the moisture content of the turned and force-aerated piles were approximately 60-63% and then dropped gradually throughout the composting time (Figure 3). The moisture content of both piles slowly decreased to a final concentration of approximately 42-46% at the end. An optimum level of moisture content was previously reported to have a strong effect on the oxygen consumption rate of aerobic heterotrophic microorganisms and was efficient between 50-60% for composting spent litter, and therefore, during the entire composting process. All of the compost piles were adjusted to have a moisture content of 60% [9]. Water evaporation occurred as an effect from the heat generated from the microbial reactions during composting, which resulted in a decrease in moisture content in the compost pile [30]. When an ambient temperature was reached, the addition of water was stopped, although the composting process continued. The force-aerated pile, after a 30 day composting period, was thought to have achieved an acceptable level of quality for mature compost, with a less than 50% moisture content [31].

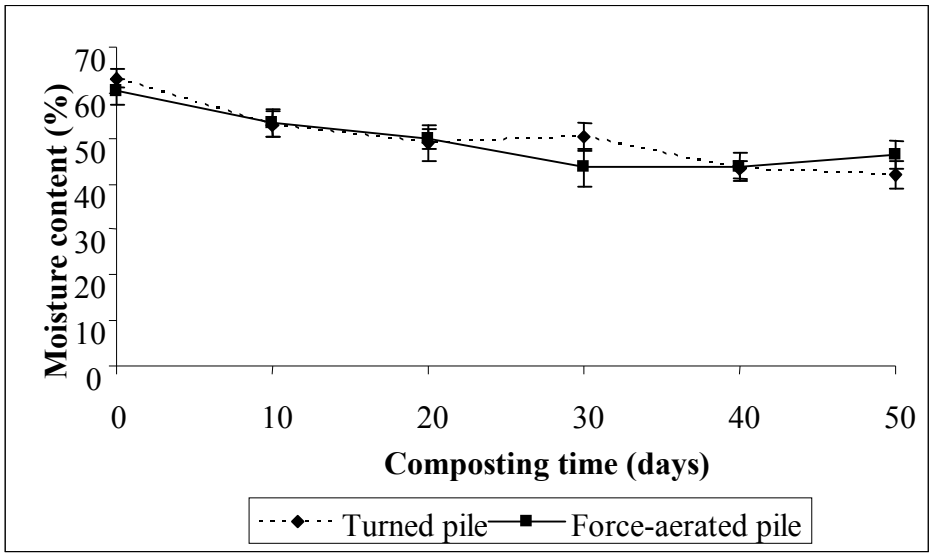


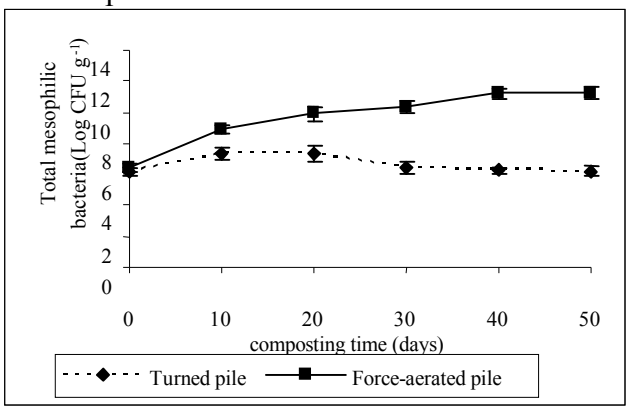
Figure 3. Change of moisture content during composting of tobacco plant waste

Growth of microorganisms

The growth of both the mesophilic and thermophilic bacteria and fungi, at various stages of the composting process, was determined (Figures 4-5). The growth of the mesophilic bacteria in a force-aerated pile regularly increased with time and reached a maximal growth of approximately 10^{11} CFU g^{-1} after 40 days of composting, while the growth of the mesophilic bacteria in the turned pile was retarded and was only 10^{6}-10^{7} CFU g^{-1} (Figure 4a). Thermophilic bacteria in the force-aerated pile first grew rapidly, reaching CFU of approximately 10^{7}-10^{9} CFU g^{-1} within 20 days of decomposition and then slowly declined to 10^{8} CFU g^{-1} at the end of composting time. The thermophilic bacteria in the turned pile was reduced to 10^{6}-10^{7} CFU g^{-1} (Figure 4b). The growth of the mesophilic and

thermophilic fungi in the force-aerated pile increased with time and reached a maximum titre of 10^9-10^{10} CFU g^{-1} after 30 and 40 days of composting time respectively (Figures 5a-5b). Their titres declined thereafter. The growth rates of the mesophilic and thermophilic fungi in the turned pile were mostly parallel to those in the force-aerated pile but resulted in overall lower cell counts. These results indicate that composting through force-aerated pile allowed the mesophilic and thermophilic bacteria and fungi to grow better in comparison with the turned pile composting. During the decomposition, the temperature of the material was raised, which favours the growth of bacteria and fungi during that particular stage of composting. The proliferation of the mesophilic and thermophilic microorganisms during composting is related to the mesophilic and thermophilic stages of the composting system [32-35]. Microbial succession plays a key role in the composting process and in the appearance of certain microorganisms that reflect the quality of the maturing compost [34,36].

a. Mesophilic bacteria



b. Thermophilic bacteria

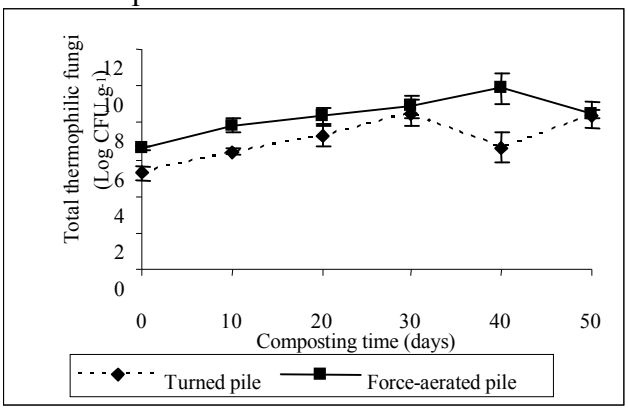
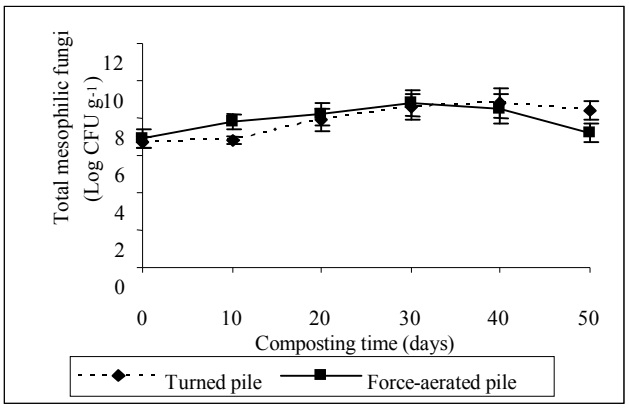


Figure 4. Change of mesophilic (a) and thermophilic (b) bacterial growth during the composting of tobacco plant waste

a. Mesophilic fungi



b. Thermophilic fungi

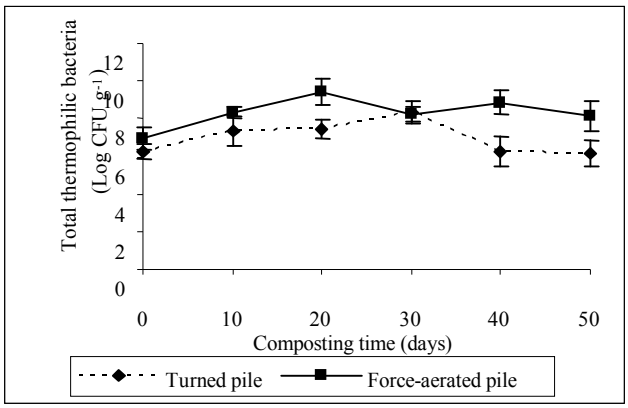


Figure 5. Change of mesophilic (a) and thermophilic (b) fungi growth during the composting of tobacco plant waste

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Change in C/N ratio

Both the turned and force-aerated piles decreased in C/N ratio with the composting time and reached maturation with a C/N ratio of approximately 13 after roughly 50 days of composting (Table 1). The initial C/N ratio was the main factor that affected the time required for compost maturity [27]. Based on the nutritional requirements of the microbes that are active in composting, the C/N ratio of the organic matter should be on the order of 20-25 parts carbon to 1 part nitrogen; a departure from this ratio leads to slow composting. On the other hand, the 20/1 ratio is critical in terms of crop production. If the C/N ratio is higher than 20/1, there is a strong possibility of nitrogen shortage for the crop plants [37-38]. Composts with a C/N ratio of 20, but not higher, are required for proper maturation [39]. Several previous studies have concluded that a C/N ratio of 20-25 indicates the maturity of the final compost product [40-41]. However, although the C/N ratio is a factor used for indicating compost maturation, it cannot be used in this study as an absolute indicator of compost maturation since the initial C/N ratio was below 20. For instance, the C/N ratio of compost at maturity was 13-27 for the co-composting of chestnut burr and leaves with solid poultry manure [42] and 11-17 for the composting of bagasse with sewage sludge [43].

Table 1. Change of C/N ratio during composting of tobacco plant waste

	C/N ratio	
Treatment	day 0	day 50
Turned pile	17.76	12.27
Force-aerated pile	19.04	13.75

Cellulase activity

The cellulase activity of both the turned and force-aerated piles is presented in Figure 6. The results show that the force-aerated pile had a rapid increase in cellulase activity during the first 30 days of the composting period followed by a prompt decline in cellulase activity, which continued until the end of the composting period. In the turned pile, the cellulase activity was similar to that of the force-aerated pile, but with an overall lower activity level. These results are consistent with a previous report that also stated that the cellulase activity increased to a maximum level at 30 days in all treatments, followed by a decline in cellulase activity until 60 and 90 days [44]. In general, cellulose decomposition limits the rapid production of compost more than any other substrate [45]. Cellulase activity, involved in the degradation of cellulose, depends on the type of cellulolytic microorganisms that develop in organic waste [45]. For the most part, fungi are involved in the decomposition of cellulose, hemicellulose, and lignin that are present in the organic matter. The force-aerated system can provide aerobic conditions for microorganisms, and this is favourable for cellulase-producing fungi in

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order to decompose the cellulose of the organic matter and allow for higher availability of nitrogen [46].

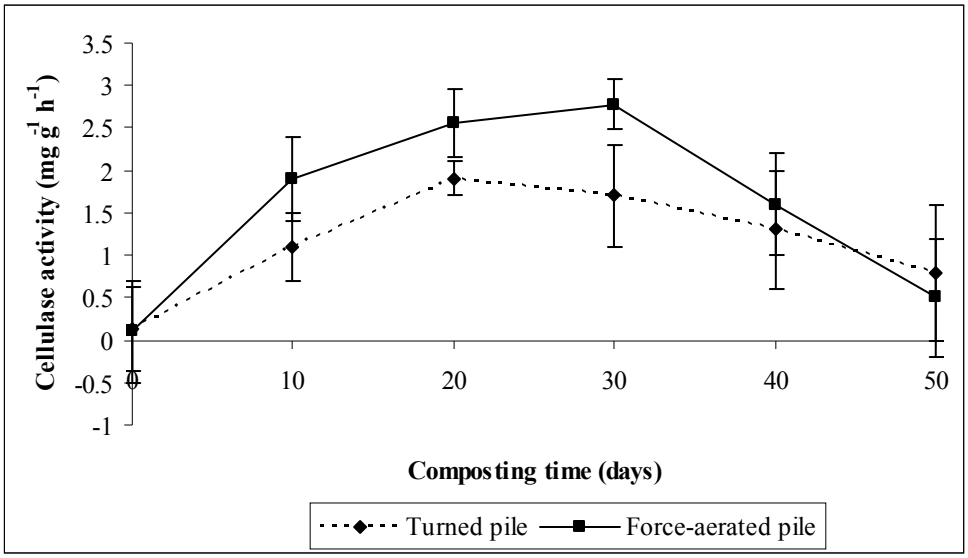

Figure 6. Change of cellulase activity during composting of tobacco plant waste

Nicotine

As shown in Table 2, the nicotine content dropped at the end of the composting process from 1.75 to 1.51% in the turned pile and from 1.75 to 1.18% in the force-aerated pile. The compost obtained by the forced aeration system had significantly ($p<0.05$) less nicotine residue (5700 mg kg^{-1} loss) than that by the manual turning system (2400 mg kg^{-1} loss). A similar result was obtained previously, and it was reported that at the end of composting the tobacco waste, the nicotine content dropped from 2000 to 450 mg kg^{-1} [47]. These results are supported by an additional report that stated that composting with tobacco waste could accelerate the breakdown of nicotine and result in the production of a less toxic and more useful organic improvement [48]. The forced aeration system, with its higher aeration, may encourage microbial growth, which may decompose the nicotine residue more quickly [1-7].

Table 2. Percentage and loss of nicotine residue in turned piles and force-aerated piles

	Nicotine (%)		Loss of nicotine (mg kg^{-1})
Treatment	day 0	day 50	After 50 days
Turned pile	1.75 b	1.51 ab	2400
Force-aerated pile	1.75 b	1.18 a	5700

Note: Different letters in the table indicate significant differences at $p<0.05$.

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Conclusions

This investigation has revealed that the composting activities of both the turning and forced aeration systems are similar in temperature, pH, and moisture content. However, the forced aeration system had higher titres of both the mesophilic and thermophilic bacteria and fungi and a higher cellulase activity from cellulose-degrading organisms compared to the turning system. This indicates that the forced aeration system may supply more aerobic conditions for both microorganism growth and cellulase activity, which are vital to the composting process. As a result of this study, we have concluded that the forced aeration system of composting enhances the growth of the related microorganisms, cellulase activity, and degradation of nicotine content in tobacco waste compared to composting by the manual turning system. Therefore, the forced aeration system is an alternative method for the composting process; however, the proper duration and interval times of the forced aeration system should be established in order to improve the composting process. Nevertheless, compost from both turned and force-aerated piles apparently reached maturity faster (within 30-40 days, as indicated by a decrease to ambient temperatures) than previous studies.

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