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Investigation of Compost Production from Cane Organic Wastes With the Different Treatment of Urea and pH by Using Trichoderma Fungi

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Abstract: This research investigates compost production from sugar cane organic wastes by using Trichoderma fungi. A factorial completely randomized design was done included 12 treatments and they were replicated three times. Treatments included 1. Adjusting pH of water used for moistening organic matters in three levels (pH equal 5.5, 4.5 and 3.5) and 2. Four levels of urea (0.5, 1, 1.5 and 2% fertilizer/total weight of organic matters). Each treatment contained 50, 50, 20 and 12 kg of bagasse, filter cake, manure and fresh alfalfa, respectively, in pits (1×1×1 m). The microorganism's suspension was sprayed on the raw materials amounted 2.5 mL kg^{-1} dry organic matters. Organic wastes were manually mixed at a week intervals throughout the composting period to provide aeration, also, adjusting moisture to 60% by weight. A representative sample was taken from composite sub-samples collected from each composting pile every after mixing. In sub-samples after 5th and 10th weeks, total nitrogen and phosphorus, organic carbon and potassium were measured. The pH and EC were determined on a water extract from compost using compost to water ratio of 1:5 by weight. Results indicated that the trend of temperature variations was same for different treatments and more influence by the microorganism's activity in compared with treatment kind. C/N ratio reduced below 30 in 5 weeks and below 20 in 10 weeks after composting. The best treatment pay attention to carbon, nitrogen, C/N ratio, decrease in compost weight and costs is 0.5% urea treatment moistened with pH = 5.5 water.

Key words: Cane bagasse, filter cake, compost, Trichoderma fungi

INTRODUCTION

Global sugar production was nearly 100 million tons in 2002 from sugarcane in over 130 countries (Meuchang *et al*., 2005). There is several sugar mills located in the sugar cane cultivation areas of Iran. On an annual basis, the sugar production process releases filter cake and bagasse by-products amounted 30000 and 20000 ton, respectively, in haft tappeh sugar factory. Sugar extraction from the cane stalks provides a cellulosic residue (bagasse) as a by-product of the plant fresh weight.

The utilization of this residue is important for the economy of the global use of the crop. One of the options for bagasse utilization is as organic soil amendment. There is a market for bagasse as a fuel or matter for paper production and domesticated meal, but no market for filter cake. Currently, the only option for filter cake utilization is as an organic soil amendment or as landfill (Meunchang *et al*., 2005). However, the direct incorporation into the soil of raw wastes such as the bagasse is not usually suitable because they may cause undesirable effects such as phytotoxicity and soil nitrogen immobilization (Negro *et al*., 1999). It is a well known fact that composting is one of the most suitable ways of converting organic wastes into more stable products which are safe and beneficial to plant growth, as well as an environmentally friendly and economical alternative method for treating solid waste (Huang *et al*., 2006). With regards to the great C/N ratio in bagasse, it is important that how this material can rapidly be converted to a stable product which may be useful in crop production. Over the past decades, effective inoculation has been reported by Shin *et al*. (1999), Makaly Biey *et al*. (2000), Baheri and Meysami (2002), Xi *et al*. (2005) and Barrena *et al*. (2006). Various specialized inocula have been applied in practice. Hatakka (1994) studied lignin-modifying enzymes from selected white-rot fungi and found that white-rot fungi played an important role in lignin degradation.

Whit-rot fungi such as *Phanerochate chrysosporium* and *Coriolus versicolor* are the most efficient ligninolytic

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organisms described to date. Their ability to degrade lignin and a wide variety of aromatic compounds is due to a non-specific extracellular enzyme system, which involves lignin peroxidases, laccases and manganese-dependent peroxidases as well as hydrogen-producing oxidases (Kirk and Farrell, 1987; Reddy, 1995). Several reports have demonstrated the contribution of these enzymes to humification, e.g., Ruttimann-Johnson and Lamar (1996) and Chefetz *et al.* (1998). Production of ligninolytic enzymes is influenced by culture condition and the composition of the medium (Ikehata *et al.*, 2004) and optimum levels for these factors vary according to microorganism and substrate (Zhao *et al.*, 1996). Lignin-degrading microorganisms accelerate the compositing process and increase the compost quality (Lopez *et al.*, 2002; Elorrieta *et al.*, 2002).

Excluding C/N ratio, temperature, aeration, mixing and turning, other factors such as pH and materials added to organic mass can influence the decomposition of organic wastes by microorganisms and thus derived compost quality. Therefore, the aim of this study in addition to investigation of compost formatting from sugar cane mill wastes by two lignocelluloytic fungi is focuses on the effects that different treatments of nitrogen and pH of water used for moistening organic wastes have on compost quality.

MATERIALS AND METHODS

This study was conducted during 2005-2006 years in haft tappeh sugar mill. Filter cake and bagasse were collected from above factory after sugar extraction in Khuzestan province, Iran. Both by-products were air dried to a moisture content of 10% and had characteristics as shown in Table 1. The C/N ratio needed for effective composting is between 25 and 40, depending on the particular organic substance cited by Golueke (1991) and Meunchang *et al.* (2005). In this research, therefore, fresh alfalfa and manure were added to bagasse and filter cake for reduction of C/N ration to about 35. At the first stage, 36 mass were provided that each contained 50, 50, 20 and 12 kg, respectively of bagasse, filter cake, manure and fresh alfalfa, i.e., 12 treatments and each in three replicates. The ligno-cellulolytic microorganisms were composed of *Trichoderma harzianum* and *T. koningii* provided by the Zhongjia biological Technique Company limited (China).

These microorganisms were cultivated by potato dextrose agar (potato extract 200 g L^{-1} and agar 20 g L^{-1}). During cultivating process, the microbial colonies were counted using a standard dilution-planting procedure until to reach the desired concentration of 1×10^9 cfu mL^{-1} for composting inoculation. The experimental design was factorial completely randomized included 12 treatments and they were replicated three times with two factors (treatment and time). Treatment included three level of pH water used in moistening organic matter (pH equal 3.5, 4.5 and 5.5) and four level of urea fertilizer (0.5, 1, 1.5 and 2% w/w fertilizer/organic matter). At the initial stage of composting, the microorganisms' suspension was sprayed on the raw material amounted 2.5 mg kg^{-1} dry organic matters. Treatment were done in pits (1×1×1 m) and for maintaining temperature and moisture, the pits surface have been covered by plastic. During compositing, materials were manually mixed at a week intervals throughout the composting period to provide aeration. The compost temperature was monitored using glass thermometers.

Table 1: Some properties of bagasse and filter cake used

Analysis	Bagasse	Filter cake
pH in 1:5 extract	4.50	7.80
EC in 1:5 extract (dS m^{-1})	0.03	0.62
Total nitrogen (%)	0.48	1.64
Carbon/nitrogen ratio	110.00	15.00
Total phosphorus (%)	0.05	0.84
Total potassium (%)	0.16	0.33

One thermometer was placed in the central part of each pile of compost and read daily. The moisture level of the mixtures were measured gravimetrically every a week and an appropriate amount of water was sprinkled onto the pile to increase the moisture content to 60% by weight. The pile was mixed to uniformly distribute the water. A representative sample was taken from composite sub-samples collected from each compositing pile every week after mixing. In the end of composting process (after 70 days), when the piles temperature reached below 35°C, *Azotobacter* inoculation as modified molasses mixture amounted 2 mL kg^{-1} dry organic matters was added to the piles (20 g molasses/1 L water with pH = 7). Final samples were taken from treatments at the end of experiment.

The samples were air dried and ground to pass through a 1 mm sieve. Total Kjeldahl Nitrogen (TKN) and Total Organic Carbon (TOC) of samples were estimated by using a micro-Kjeldahl method (Singh and Pradhan, 1981) and Walkey and Blacks (1934) Rapid titration method. Total Phosphorus (TP) was determined spectrophotometrically while Total Potassium (TK) was detected by flame emission technique. Microelements were also determined on by Atomic Absorption technique. The pH and EC were determined on a water extract from compost using compost to water ratio of 1:5 by weight.

All the results reported are the means of three replicates. One way Analysis of Variance (ANOVA) was done using the SAS and MSTATC programs. The objective of statistical analysis was to determine any significant differences among the parameters analyzed for different treatment.

RESULTS AND DISCUSSION

The thermophylic phase started near the beginning of the composting process. In this phase, the temperature is more than 60°C for approximately 14 days and then decreased slowly to reach about 52°C for 42-45 days and continued to decreased rapidly to ambient at 70 days (Fig. 1).

Effect of time: Figure 2, 3 and 4 shows the mean C and N percent and C/N ratio during time, respectively. In initial 14 days, decomposition was very slow and only physical from of organic matter had changed, although alfalfa had approximately been decomposed. Loss of CO_2 in this stage was 3.76% of total carbon. This is due to the thermophylic bacteria activity that this temperature (59-65°C) is an activator for their activity. Overall, at the first stage, composting has been done by bacteria, but at next stages by fungi. In first phase, reduction of carbon is sharp to denote on decomposition of the carbonic compounds with low molecular weight such as soluble sugar. At the second phase, i.e., next 28 days, temperature slowly reduced from 59 to 51.5°C. When easily decomposable organic matters were decomposed, consequently the great C/N organic compounds remained; and when temperature reduced below 60°C, this can to prepare a suitable environment for fungi activity (Tang *et al*., 2007). Although a high C/N ratio is usually required for lignin degradation by fungi, the nitrogen source greatly affects this activity (Lopez *et al*., 2006). Capability of *Trichoderma* fungi in digesting cellulitic materials have been proved by Hataka (1994), Meunchang *et al*. (2005) and Singh and Sharma (2002). Total C has only declined 1.2% that is due to the high resistance of remind compounds to decomposition.

At third stage (28 days), loss of carbon was 7.12%. The great part of cellulose and lignin decomposition is done at the end of composting process by fungi, when temperature reduces. Temperatures 34-55°C and pH = 7.5 to 7.9 are suitable conditions for these fungi.

Total N of second and third phases was greater than first phase. There is a distinct increase in total N of compost due to decrease in C than N during composting. Total N increased 0.36% at the second stage and 0.41% at the third stage. In these stages, temperature has fallen below 55°C and carbohydrates to be made available for N fixation by *Azotobacters*. During the maturation phase of composting, N was stabilized because of decrease in NH_3 volatilization, pH and temperature and increase in nitrification (Meunchang *et al*., 2005). The maturing of compost is a very important parameter for both compost production process and its application. In mature compost, NH_4^+/NO_3^- and C/N rations have reduced in comparison to the original material (Bernal *et al*., 1998; Zucconi *et al*., 1981).

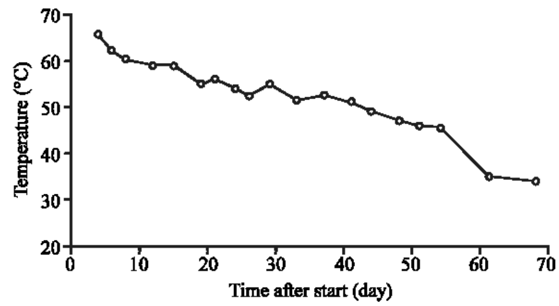

Fig. 1: Mean temperature during the time of composting

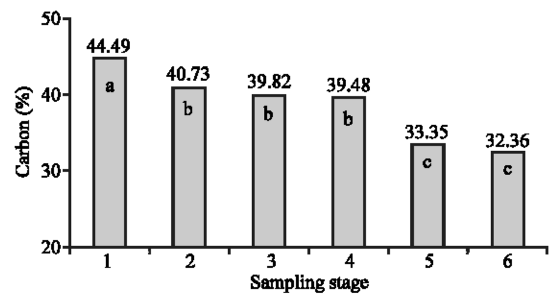

Fig. 2: Mean carbon (%) during the time of composting

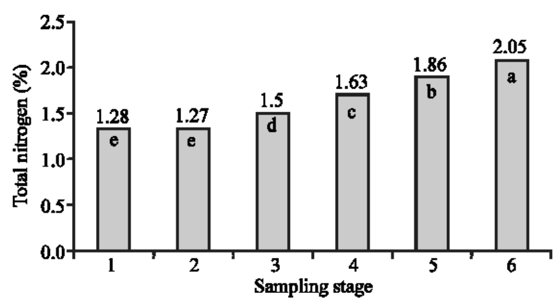

Fig. 3: Mean total nitrogen (%) during the time of composting

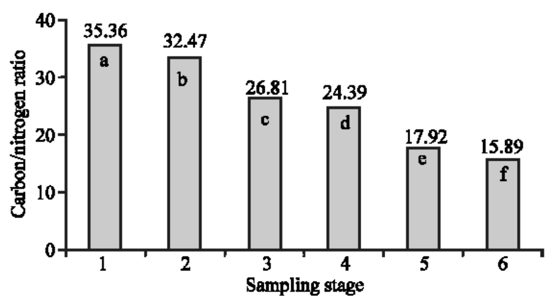

Fig. 4: Mean carbon/nitrogen ratio during the time of composting

Table 2: Carbon, nitrogen and carbon/nitrogen ratio in treatment after 5 weeks from start of process

Treatment No.	Treatments	Carbon (%)	Nitrogen (%)	Carbon/ nitrogen ratio
1	pH = 3.5 plus 0.5% urea	41.40ab	1.51cd	29.01a
2	pH = 3.5 plus 1% urea	42.53a	1.59abcd	29.28a
3	pH = 3.5 plus 1.5% urea	41.72ab	1.57bcd	28.72a
4	pH = 3.5 plus 2% urea	40.12bcd	1.63ab	26.22bc
5	pH = 4.5 plus 0.5% urea	38.53cde	1.66ab	24.61bc
6	pH = 4.5 plus 1% urea	37.41ef	1.62abc	24.11bc
7	pH = 4.5 plus 1.5% urea	39.81bcd	1.64ab	24.94bc
8	pH = 4.5 plus 2% urea	40.49abc	1.65ab	26.28b
9	pH = 5.5 plus 0.5% urea	39.37bcd	1.69a	25.56bc
10	pH = 5.5 plus 1% urea	38.14de	1.60abc	25.67bc
11	pH = 5.5 plus 1.5% urea	40.01bcd	1.48d	29.83a
12	pH = 5.5 plus 2% urea	39.37bcd	1.58bcd	28.78a

Different letter(s) means significant differences at $p<0.05$

Table 3: Carbon, Nitrogen and carbon/nitrogen ratio in treatment after 10 weeks from start of process

Treatment No.	Treatments	Carbon (%)	Nitrogen (%)	Carbon/ nitrogen ratio
1	pH = 3.5 plus 0.5% urea	32.6b	2.0cd	16.3cd
2	pH = 3.5 plus 1% urea	32.5b	2.1abcd	17.3bc
3	pH = 3.5 plus 1.5% urea	37.6a	2.1bcd	17.6b
4	pH = 3.5 plus 2% urea	32.5b	2.0ab	16.0de
5	pH = 4.5 plus 0.5% urea	36.7a	2.2ab	16.6bcd
6	pH = 4.5 plus 1% urea	30.2b	2.0ab	15.0ef
7	pH = 4.5 plus 1.5% urea	32.8b	2.1ab	15.6de
8	pH = 4.5 plus 2% urea	33.6b	2.2ab	15.6de
9	pH = 5.5 plus 0.5% urea	33.0b	2.1a	15.6de
10	pH = 5.5 plus 1% urea	30.0c	2.1abc	14.3f
11	pH = 5.5 plus 1.5% urea	33.5b	1.7d	19.5a
12	pH = 5.5 plus 2% urea	34.1b	1.8bcd	18.8a

Different letter(s) means significant differences at $p<0.05$

The C/N ratio of compost was stabilized after approximately 70 day. The final C/N ratio of approximately 15.89 indicated that it would be good source of N. C/N ratio reduced approximately 3, 8 and 8.5% at phases 1, 2 and 3, respectively. This reduction at the first phase is due to decrease of C, at the second phase due to increase of N.

The final compost materials appear to be suitable for agronomic use. It contain suitable characteristics of pH, EC are likely of pathogens because of the high temperatures (Haug, 1993). Compost appears to be good source of plant nutrients such as N, P, K and Ca.

Treatment effect

Short time period (5 weeks): Table 2 shows that the remind carbon in treatments pH = 3.5 is more than other treatments that is because of less composting. In these treatments, different amounts of urea satisfactory were not caused to increase in organic wastes decomposition. It seems that bacteria activities have excessively decreased in this pH. Above pH do not create problem for fungi activity, but the growth and activity of bacteria for fungi instigation is an important factor in composting process. In the group of treatments with pH = 3.5 remind carbon has only decreased in 2% urea treatment that is due to dissolving urea and creating alkaline reaction. Increase in pH tend to the promotion of ecological conditions in organic wastes, consequently, carbon has more lost in pH = 4.5. This denote on the compatibility of microbial groups led to decompose organic wastes until conditions be suitable for *Trichoderma* activity. With regards to Table 2, carbon variations is depends on urea level in pH = 4.5 and 5.5 treatments. Carbon percent is less at the low level of urea (0.5 and 1%); therefore, treatments 5, 6, 9 and 10 are better because of the less carbon. It should be regarded that urea to create a more limitation for biological activity than pH because of NH_3 production. Yang *et al*. (1980) reported that the addition of nitrogen in small quantities to woody materials can increase lignin degradation rates.

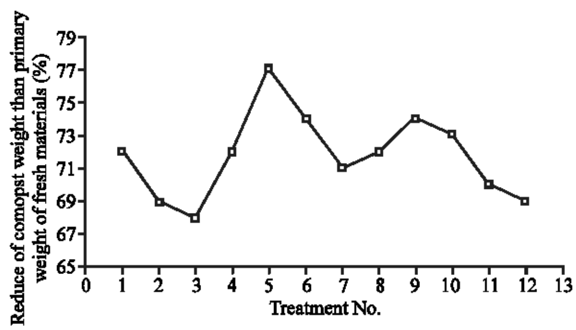

Fig. 5: Reduce of compost weight than primary weight of fresh materials (%)

In pH = 5.5 as compared with pH = 4.5, wastes nitrogen level is more by using 0.5 and 1% urea, whereas this is reverse in 1.5 and 2% urea treatment. It seems the increase in urea level has changed ecological equilibrium and has established limitations for microbial activity which is led to increase in nitrogen and decrease in carbon.

Since C/N ratio relate to the carbon and nitrogen amount, every factor to affect on carbon and nitrogen led to change in C/N ration. In general, treatment 1, 2, 3 and 4 that have been moistened by water with pH = 3.5 have a less importance in composting sugar cane wastes.

Long time period (10 weeks): Table 3 shows the final sampling results of organic wastes. Base on results, C/N ration has decreased below 20 in all treatments at the end of composting process. It should be regarded; of course, they have considerable differences with the view of carbon and nitrogen variations and compost quality. The lowest C/N is related to treatment 6, 7, 8, 9 and 10.

Results relate to decrease in compost weight than primary fresh weight of organic wastes is shown in Fig. 5. Organic wastes have approximately lost 2/3 of its weight in composting process that this is important with the view of better transportation, store keeping and distribution of compost. The highest loss of weight is related to treatment 5, 6, 9 and 10 that have the greatest loss in carbon, greatest nitrogen, consequently, they have the greatest C/N. Mature compost can be of high value for crop nutrition, in contrast to immature compost which may result in net immobilization of soil N into the microbial biomass and may induce N deficiency in crops (Inbar *et al.*, 1993).

CONCLUSION

Regarding derived results, we can to decrease C/N ratio below 30 in 5 weeks period and below 20 in 10 weeks period by using *Trichoderma* fungi inoculation, urea and adjusting pH of water used in moistening organic wastes. Using derived compost after 5 weeks can be suitable, but it is better to use it 10 weeks after composting because to promote its quality. Treatment 5, 6, 9 and 10 were suitable pay attention to carbon, nitrogen, C/N ratio and decrease in compost weight, but the best treatment from this view and decrease in costs, moreover, less usage of urea in abundant production of compost is treatment 9.

It is proposed to investigate the effect that adding new compounds to bagasse and filter cake have on compost quality, for example using molasses that is enrichment with the view of potassium; or using ammonium sulfate instead of urea.

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