Article

Composting agroindustrial waste inoculated with lignocellulosic fungi and modifying the C/N ratio

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Abstract

The current disposal of waste involves a strong environmental impact that can be minimized by recycling these materials. Composting is one of the best options to achieve this purpose. The objective of this work is to evaluate the effect of the inoculation of *Trichoderma harzianum* and *Aspergillus* sp. in the composting process of bagasse of maguey mezcalero (BM-*Agave angustifolia* Haw.) and bagasse of sugar cane (BC-*Saccharum officinarum* L.), both with reduced C/N ratio. During 2012, in Santa Cruz Xoxocotlan (Oaxaca, Mexico), an experiment was developed under a completely randomized design. The composting process of BM and BC, inoculated with the fungi *T. harzianum* and *Aspergillus* sp., including an uninoculated control, was monitored. For both residues, the C/N ratio was modified with the addition of bovine manure. The duration of the experiment was 133 days. The temperature of the masses in fermentation was recorded every week. Samples were taken in triplicate for analysis on days 0, 37, 70, 103 and 133 after the start of the process. The addition of bovine manure was enough to reduce the value of the C/N ratio and reach the thermophilic phase in a few days. The inoculation with *T. harzianum* and *Aspergillus* sp., reduced the degradation time of BM but not BC. *Aspergillus* sp., Generated greater degradation in both residues. BM reached C/N values that qualify it as a mature compost as of day 103 of composting, this was not the case with BC.

Keywords: maguey bagasse, cane bagasse, composting, lignocellulolytic fungi.

Reception date: January 2018
Acceptance date: February 2018

Introduction

With the current scenario of waste generation from very diverse origins, and its inadequate final disposal, several methods are being studied to turn them into useful products in the shortest time possible (Raj, 2011; Shafawati and Siddiquee, 2013). Various options for the use and recycling of lignocellulosic waste generated in agroindustries have been described. Waste from the wine industry is used to obtain tartrates (Carmona *et al*., 2012). From the bagasse of maguey tequila, processes have been developed for the production of seedlings (Crespo-Gonzáles *et al*., 2013), biopolymers, enzymes and other metabolites (González-García, 2005).

Sugarcane bagasse has been bioprocessed to obtain livestock feed (Valiño *et al*., 2003). Other lignocellulosic residues are used as raw material to produce ethanol, for the manufacture of paper and the obtaining of organic acids, amino acids, vitamins, among others (Sánchez, 2009).

In the agricultural sector, these materials cannot be used directly due to the high content of phenolic components, which cause phytotoxic effects, causing a decrease in the growth or even death of plants (González-García, 2005; Aranda *et al*., 2008). In order for these residues to be used, they must be subjected to a degradation process whereby the compounds that cause such toxicity are reduced or even eliminated (Aranda, 2008). Composting is a key tool in the conversion of lignocellulosic waste to useful products. It is an aerobic process in which microorganisms (mainly fungi and bacteria) are responsible for transforming organic matter into a stable and pathogen-free product, due to the high temperatures that are generated. Also during this process the recalcitrant organic compounds are slowly degraded and with this the phytotoxicity is eliminated (Bernal, 2009, Fornes *et al*., 2012).

During the process of degradation of lignocellulosic residues, the availability of nutrients for fungi is linked to the C/N ratio, the range considered as optimum for composting is 25-30 (Bernal *et al*., 2009). A C/N ratio greater than 30 translates into a slow process with low availability of N, which is necessary for the fungi to develop and remain active (Heredia-Abarca *et al*., 2008). On the other hand, a C/N ratio of less than 25 indicates higher N content, which causes the excess production of inorganic N, which can be lost by volatilization in the form of ammonium or by leaching in the form of nitrate (Bernal *et al*., 2009). Agroindustrial waste has high values of C/N ratio, and that is why its natural degradation is slow. If it is desired to accelerate the mineralization process, the addition of materials with a high nitrogen content is recommended (Flores, 2009).

Inoculation of lignocellulosic residues with fungi is a viable option both to reduce the time of composting and to improve the characteristics of the final product obtained. Many fungi have been identified as lignocellulolytic organisms, in the group of the basidiomycetes *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus* and the group of the ascomycetes *Aspergillus niger*, *Trichoderma harzianum*, *T. reesei*, *T. pseudokoningii*, *Fusarium oxysporum*, among others (Valenzuela and Pinochet, 2008; Sánchez, 2009; Haddadin *et al*., 2009; Charitha, 2012).

Fungi are the main responsible for the degradation of lignin and cellulose, and this degrading capacity is associated with the habit of mycelial growth that allows the fungus to transport scarce nutrients, such as nitrogen and iron, at considerable distances within the lignocellulosic substrate (poor in nutrients) that constitutes its carbon source (Sánchez, 2009; Haddadin *et al*., 2009). They also require N in large quantities, not only to synthesize cellular structural compounds such as proteins, nucleic acids and chitin, but also for the synthesis of enzymes that are necessary to extract the nutrients from the environment (Heredia-Abarca *et al*., 2008).

The objective of this work is to evaluate the effect of the inoculation of *T. harzianum* and *Aspergillus* sp. in the composting process of bagasse of maguey mezcalero (*Agave angustifolia* Haw.) and bagasse of sugarcane (*Saccharum officinarum* L.), both with reduced C/N ratio.

Materials and methods

The composting of bagasse of maguey mezcalero and bagasse of sugarcane, both mixed with bovine manure (4:1 and 5:1 v/v respectively) was monitored for the reduction of the C/N ratio (Bernal, 2009), and inoculated with lignocellulosic fungi.

The experiment was carried out in the composting module of the CIIDIR-Oaxaca Unit in Santa Cruz Xoxocotlan, Oaxaca. The bagasse, both recently generated, were collected in the community of San Baltazar Yatzachi the Alto, district of Villa Alta, Oaxaca. Cattle manure was acquired in the livestock production module of the Technological Institute of the Valley of Oaxaca. The composting piles have a capacity of 1.8 m^3 (3 × 1 × 0.6 m).

The fungi used were *T. harzianum* and *Aspergillus* sp. The first was obtained from a commercial product (Michoderma®) and was cultivated using the plating technique, using the 10-4 dilution. The fungus *Aspergillus* sp. It was obtained from compost in process and isolated in agarized culture medium. Both fungi were isolated in PDA culture medium (Holguin and Mora-Delgado, 2009) and multiplied using the technique of infected rice (Posada-Floréz, 2008) for subsequent inoculation to composting piles. To the bagasse of agave, 4.95 kg of rice infected with the fungus *T. harzianum* and 4.87 kg with *Aspergillus* sp. to the bagasse of cane, 5.38 kg of rice infected with *T. harzianum* and 5.25 kg with *Aspergillus* sp.

The experiment was developed under a completely randomized design, with six treatments, bagasse of maguey mezcalero inoculated with, *T. harzianum* (BM-Th), with *Aspergillus* sp. (BM-A) and without inoculation (BM-C). The same three treatments were applied to sugarcane bagasse (BC-Th, BC-A, BC-C). Each compost pile was an experimental unit. The temperature was measured weekly as an average of 10 measurements in the pile, at a depth of 10 cm. Samples were collected in triplicate at 0, 37, 70, 103 and 133 days after the start of the process. They measured the pH and electrical conductivity (CE) (Altieri and Esposito, 2010), the Ash Content (CEN) and organic matter (MO) (Ansorena, 1994), the total organic carbon (TOC) by the formula proposed by Golueke (1977), total nitrogen (NT) (Bremner, 1965) and the C/N ratio (R C/N). The data were subjected to variance analysis followed by a means separation test (Tukey $p \leq 0.05$). The IBM SPSS 20 software was used.

Results and discussion

The composition and properties of the original materials are reported in Table 1. The properties of the BM used are similar to those reported by Iñiguez *et al*. (2011) for the maguey tequila bagasse. The values of pH, COT, NT and R C/N of the BC used are similar to those reported by Chandler *et al*. (2008) and the properties of manure coincide with the values presented by Bernal *et al*. (2009).

Table 1. Initial composition of bagasse of mescalero maguey (*Agave angustifolia*-BM), bagasse of sugar cane (*Saccharum officnarum*-BC) and bovine manure (E), raw materials used in the evaluation of composting of the first materials.

Parámetro	Unidades	BA	BC	E
CEN	(%)	6.15 b	1.99 b	35.7 a
MO	(%)	93.9 a	98 a	64.3 b
COT	(%)	52.1 a	54.5 a	35.7 b
NT	(%)	0.35 b	0.21 b	1.43 a
R C/N		150.6 b	261.9 a	25.1 c
pH		4.97 c	5.13 b	8.47 a
CE	(dS m^{-1})	1.43 b	0.84 c	3.97 a

CEN= ashes; MO= organic material; COT= total organic carbon; NT= total nitrogen; R C/N= carbon/nitrogen ratio; CE= electrical conductivity. Values with the same letter, in each row, are statistically equal to each other (Tukey, $p \leq 0.05$).

The temperature in the piles increased rapidly until reaching maximum values in the first 21 days in the mixtures of maguey bagasse (Figure 1), reaching values higher than 40 °C. In BM-Th and BM-A treatments, the highest values were recorded. In BC, treatment C recorded the highest temperature value on day three of composting; the BC-Th treatment registered lower values than the control, in the BC-A treatment a maximum temperature of 31.6 °C was reached at day 45.

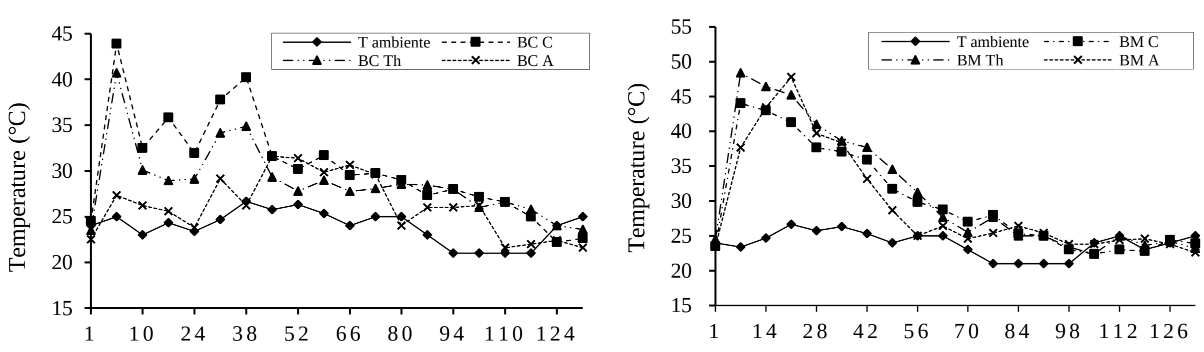

Figure 1. Temperature dynamics in the composting process (133 days) of maguey bagasse (*Agave angustifolia*-BM) and sugar cane (*Saccharum officinarum*-BC) inoculated with the fungi *Aspergillus* sp. (A) *Trichoderma harzianum* (Th) or without inoculation (C). reference, ambient temperature (TA).

Temperature is an indicator of microbial activity in the composting process. In this work, the temperatures rose immediately propitiating the thermophilic phase in the first week after the start of composting, this is attributed to the fact that the particle size of the bagasse allowed simultaneous diffusion of oxygen and moisture retention at an adequate level to promote microbial activity (Flores, 2009). Tortarolo *et al*. (2008) point out that the temperature factor contributes to the decomposition of the waste and to the health of the compost, affirming that the ranges of 45 to 55 °C maximize biodegradation.

In the batteries with BM temperatures were reached within this range, in those of BC the maximum temperature was less than 45 °C. Haddadin *et al*. (2009), during the composting of olive residues, indicates that the maximum degradation of lignocellulosic compounds increases with increasing temperature, although this factor may also be a limitation for inoculated organisms, since at certain levels of temperature can decrease their activity enzymatic (Tuomela *et al*., 2000).

In Table 2 it is observed that there are significant differences between the treatments for each type of bagasse in the values of CEN, MO and COT. In CEN, significant differences were recorded at days 37 and 133 in BM, the highest values are for treatments C and A. In BC there are significant differences from day 103, with the highest value for treatment A. Iñiguez *et al*. (2011) report final values of 11.8-18.6% ash in maguey tequila compost with addition of urea and vinasse. The high values obtained in this work are due to the addition of manure with high ash content and to the reduction of organic matter due to its mineralization (Haddadin *et al*., 2009). The content of MO and COT of BM decrease between 22 to 29% in the 133 days of experiment.

Table 2. Ash content (CEN), organic matter (MO) and total organic carbon (COT) during the 133-day composting process of maguey bagasse (*Agave angustifolia* - BM) and sugar cane bagasse (*Saccharum officinarum* - BC) inoculated with the fungi *Trichoderma harzianum* (Th), *Aspergillus* sp. (A) or without inoculation (C).

	CEN (%)			MO (%)			COT (%)		
Día	Th	A	C	Th	A	C	Th	A	C
Bagasse of maguey mezcalero (BM)									
0	17.2a	17.2a	17.2a	82.8a	82.8a	82.8a	46a	46a	46a
37	26.9b	33.1a	25.2b	73.1a	66.9b	74.8a	40.6a	37.2b	41.6a
70	29.9a	36.5a	33.6a	70a	63.5a	66.4a	38.9a	35.3a	36.9a
103	33.2a	36.5a	36.6a	66.8a	63.5a	63.4a	37.1a	35.3a	35.2a
133	35.8b	41.3a	39.6a	64.2a	58.7b	60.4b	35.7a	32.6b	33.6b
Bagasse of sugar cane (BC)									
0	9.8a	9.8a	9.8a	90.2a	90.2a	90.2a	50.1a	50.1a	50.1a
37	19.5a	20.9a	17.6a	80.5a	79.1a	82.4a	44.7a	43.9a	45.8a
70	22.1b	37.1a	24.6b	77.9a	62.9a	75.4a	43.3a	34.9a	41.9a
103	22.4b	38.8a	25.5b	77.6a	61.2b	74.5a	43.1a	34b	41.4a
133	28.6b	38.2a	35.3ab	71.4a	61.8b	64.7ab	39.7a	34.3b	36ab

Values with the same letter, in each row, are statistically equal to each other (Tukey, $p \leq 0.05$).

With the treatments C and Th the highest final values of MO were recorded, while with A the lowest values of both parameters were obtained. In BC there are no significant differences in the content of OM and TOC at day 133, although the inoculation with the mushroom A has a higher rate of mineralization, by reducing the content of both of them by 31.5%. Charitha and Kumar (2012) mention that the enzymatic activity of cellulose is high in strains of Aspergillus sp. and Trichoderma sp. Tuomela *et al*. (2000); Sánchez (2009) mention that bagasse has a high percentage of cellulose (32-44%), which may explain that with Aspergillus sp. a greater percentage of degradation is registered in sugar cane bagasse.

Fungi of the genus *Aspergillus* sp. they are thermotolerant, being able to resist 52-55 °C (Tuomela *et al*., 2000), which leads to suppose that, when the temperature of the thermophilic phase decreases, this is the dominant fungus during the rest of the composting process (Nusbaumer *et al*., 1996 cited by Tuomela *et al*., 2000). Conversely, Trichoderma has an optimum temperature for the degrading activity of 30 °C (Haddadin *et al*., 2009), a factor that would explain the lower *Trichoderma* mineralization activity.

In the NT content, significant differences were recorded in all sampling dates in both materials (Figure 2). There is a constant increase in the content of NT from the beginning until day 103 in the treatments in BM, with a final decrease in the treatments C and Th. In BC fluctuations in the content of NT are recorded but the final value is greater than initial value. Sharma *et al*. (2012), when inoculating crop residues with different species of *Trichoderma*, report increases in the N content, which attributes to the release of this element due to the death and degradation of the microorganisms that assimilated it.

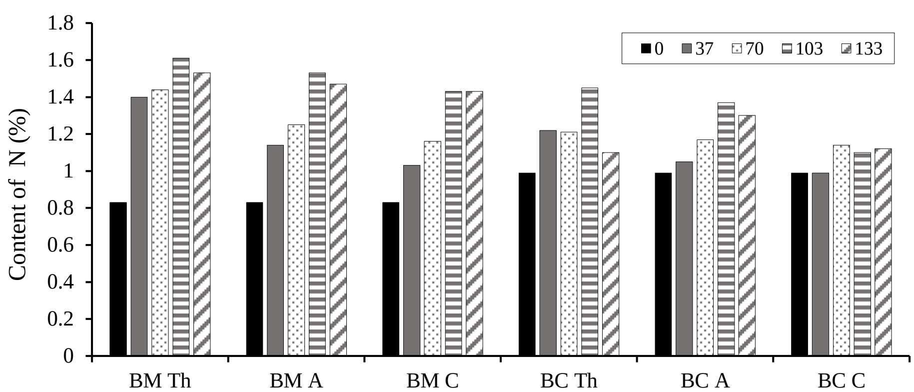

Figure 2. Behavior of the content of N in the composting process (133 days) of bagasse of maguey (*Agave angustifolia*-BM) and of sugar cane (*Saccharum officinarum*-BC) inoculated with the fungi *Aspergillus* sp. (A), *Trichoderma harzianum* (Th) or without inoculation (C).

Raj and Antil (2011) mention that at the beginning of composting there is an increase of ammoniacal N, and after 60 days there is a rapid conversion to nitric N. This content increases as the thermophilic phase passes and the composting process advances. They also point out that in the composting of agroindustrial waste the processes of ammonification and nitrification are accelerated due to the fact that higher aeration conditions are generated, favoring the activity of the microorganisms in the immobilization of NH_3, which avoids their loss due to volatilization. The results obtained in this investigation show a relationship with the previously described, being lignocellulosic residues with low N content.

In the electrical conductivity in BM, significant differences were recorded from day 70, with a constant decrease, treatment C with the highest CE. In BC there are significant differences until day 133, with treatment A with the highest value (Table 3). Flores (2009) reports increases in CE throughout the composting process of maguey tequila bagasse, with final values of 11.9 to 14.4 dS m^{-1}. Mazuela and Urrestarazu (2005) report values of 22.9 to 34.3 dS m^{-1} in compost generated from horticultural waste. The behavior observed in BC coincides with Gordillo *et al*. (2011), who report an increase in the initial phase due to the mineralization of the organic matter, followed by a decrease caused by the leaching of metabolites and residues, finally a maturation phase with a decrease in the CE, indicating the end of the process.

Table 3. Behavior of pH, Electric Conductivity (CE) and the C/N ratio (R C/N) during the 133 days composting process, of maguey bagasse (*Agave angustifolia* - BM) and cane bagasse sugar (*Saccharum officinarum* - BC) inoculated with the fungi *Trichoderma harzianum* (Th), *Aspergillus* sp. (A) or without inoculation (C).

	Bagasse of maguey mezcalero			Bagasse of sugar cane		
Day	Th	A	C	Th	A	C
			pH			
0	6.89a	6.89a	6.89a	7.92a	7.92a	7.92a
37	9.45b	9.78a	9.57ab	9.04a	8.01b	9.01a
70	8.54b	9.46a	9.62a	9.22a	8.62b	8.52b
103	9.29a	9.03b	9.21ab	9.02a	8.84a	8.94a
133	9.24a	9.13a	9.11a	9a	8.52b	8.66b
			CE (dS m^{-1})			
Day	Th	A	C	Th	A	C
0	4.19a	4.19a	4.19a	2.49a	2.49a	2.49a
37	2.78ab	1.79a	2.88a	2.86a	2.6a	3.41a
70	2.14b	1.62c	2.88a	2.51a	1.81a	2.49a
103	1.93b	1.44a	2.57a	2.68a	1.8b	2.37ab
133	1.31b	1.31b	2.22a	1.45b	2.42a	1.39b
			R C/N			
Day	Th	A	C	Th	A	C
0	55.4a	55.4a	55.4a	50.6a	50.6a	50.6a
37	35.6a	36.1a	29.7b	42.7a	44.4a	37.4a
70	31.1a	30.4ab	25.6b	37a	30.6a	34.7a
103	24.3a	24.7a	21.9a	31.5a	30.9a	28.6a
133	24.3a	22.8b	22b	30.5a	30.7a	32.6a

Values with the same letter, in each row, are statistically equal to each other (Tukey, $p \leq 0.05$).

The addition of manure increased the pH value of the residues (Table 3). In BM, significant differences were recorded only on day 70, while in BC there are significant differences on dates 37, 70 and 133. All treatments show an increase at the beginning of the process. The Th treatment has the highest numerical value of pH in both residues at the end of the evaluation. Boulter-Bitser *et al*. (2006) report increases in pH, reaching values of 8.4. They attribute this increase to the mineralization of organic compounds and degradation of organic acids.

The management of aeration is one of the factors that modifies the pH values, the higher the concentration of oxygen, the action of the microorganisms in the degradation of organic acids is favored, which increases the pH (Iñiguez *et al*., 2011). An explanation of the behavior recorded by treatments A, with a higher level of mineralization compared with Th, is that the optimum pH range for the enzymatic activity of *T. harzianum* is 4 to 4.5 (Haddadin *et al*., 2009). The results obtained in this work contrast with those reported by Zayed and Abdel-Motal (2005), who when composting bagasse of sugarcane inoculated with *T. viride* and *A. niger* observed reductions in pH, which can be attributed to the fact that *A. niger* is a highly acidifying organism of the medium for its ability to synthesize organic acids.

The values of R C/N at the beginning of the process are 55.4 and 50.6 in BM and BC, respectively, due to the addition of bovine manure (Table 3). There are significant effects of the treatments in BM, but not in BC. With treatment C in BM, the greatest total reduction was registered. In the inoculated treatments, A promoted a greater reduction of R C/N than Th (58.9 and 56.2%). In BC, a greater reduction of R C/N was observed with the treatments with inoculation (39.7% for *T. harzianum* and 39.3% for *Aspergillus* sp.). Zayed and Abdel-Motal (2005) report a R C/N value of 40 after 105 days of cane bagasse composting with inoculation of *A. niger* + *T. viride*. With the addition of farm waste, a value of 25 is reported after 90 days of processing.

In BC, values are recorded above the range considered optimal (18-20) by Iñiguez *et al*. (2011). Raj y Antil (2011) mention that in the composting of agroindustrial waste the decrease in R C/N is faster than in farm waste. Iñiguez *et al*. (2011) report final values of R C/N of 14.5 and 16.2, which indicate a high degree of stability. The R C/N is used as an indicator of maturity and stability in compost, and the values used as a reference range from 10 to 25 (Iñiguez *et al*., 2011; Raj and Antil, 2011).

In this BM work, with any of their treatments, they have values of 21.9 to 24.3, which can already be considered stable. The values in BC are greater than the indicated range, due to the low contents of initial N in this residue. Flores (2009) mentions that high values of R C/N can affect the immobilization of N because, due to the high contents of C in the compost, the action of the microorganisms continues and they require N for their development.

Conclusions

The addition of bovine manure in the proportions used in this research, are sufficient to reduce the value of the C/N ratio to an appropriate level to reach in a few days the thermophilic phase of composting. The use of the lignocellulolytic fungi *T. harzianum* and *Aspergillus* sp. The time of degradation of the bagasse of mezcal, but not of the cane bagasse, was reduced. *Aspergillus* sp. It showed a higher degree of degradation in both evaluated bagasse, which was reflected in the lower values of organic matter and total organic carbon. The bagasse of maguey mezcalero, with addition of bovine manure and inoculated with any of the two fungi evaluated, reached values of the C/N ratio that value it as a mature compost from day 103 of composting. After 133 days the same thing does not happen for the bagasse of sugarcane, for which reason this product is valued as an immature compost.

Gratefulness

The work was financed by the Research and Postgraduate Secretariat of the National Polytechnic Institute. The first author thanks CONACYT for the scholarship for Master's studies.

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