World Journal of Microbiology & Biotechnology (2005) 21:747–752
DOI 10.1007/s11274-004-5407-y

Bio-production of compost with low pH and high soluble phosphorus from sugar cane bagasse enriched with rock phosphate

Gaber Zayed* and Heba Abdel-Motaal
Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Minia, Egypt
**Author for correspondence: Tel.: 002-086-344030, Fax: 002-086-362182, E-mail: zayed000@yahoo.com*

Keywords: Alkaline soil, *Aspergillus niger,* bagasse, broad bean, phosphorus, *Trichoderma viride*

Summary

Aspergillus niger and *Trichoderma viride* strains were used together as a fungal activator in the presence or absence of farmyard manure (FM) for composting of bagasse enriched with rock phosphate. Quality of the composts produced was compared with that obtained from non-inoculated bagasse. The composts were evaluated as organic phosphatic fertilizers, for broad bean plants. The results showed that composting of bagasse without microbial inoculation or FM addition was not complete after 105 days of fermentation. An excellent decomposition in a relatively short time however was obtained with the use of *A. niger* and *T. viride* as inoculant agents with or without FM. The inoculation with *A. niger* + *T. viride* with or without FM, also represented the most suitable conditions for phosphate solubilization. Acidic conditions (pH 4–5) at the end of the experiment were obtained in all piles receiving *Aspergillus niger* and there was a correlation between the amounts of soluble phosphorus and the reduction in pH values in the compost piles. There were no phosphate-dissolving fungi present in any composted piles except those treated with *Aspergillus niger* and *Trichoderma viride*. The number of phosphate-dissolving bacteria increased only in the treatments that were treated with FM. The non-fertilized sandy soil and the non-inoculated bagasse compost did not provide broad bean plants with phosphorus while the composts produced by inoculation with *A. niger* + *T. viride* provided the plants with the highest amounts of phosphorus.

Introduction

One of the largest agro-industrial byproducts in Egypt is sugarcane bagasse, a fibrous residue of cane stalks left over after the crushing and extraction of the juice from the sugar cane. This byproduct is commonly burnt or used by the sugar factories as a fuel for the boilers. Some reports have described the conversion of bagasse into value-added compost that has the potential to improve productivity of crops and reduce the problem of environmental pollution. However, some bagasse characteristics offer unique challenges to processors. For example, this material is broken down slowly by the native microorganisms originally present in it, even with all environmental conditions maintained at an optimum level, because of the unfavourable C:N ratio and the high content of lignocellulose (Li-Xin *et al.* 2002). Bagasse may be used in composting if it is first shredded (Nandi *et al.* 1996), enriched with other substances (Tengerdy & Szakacs 2003) and/or inoculated with certain cellulose degrading microorganisms (Dimalanta & Latiza 1990; Li-Xin *et al.* 2002). These workers however produced composts with a final pH of 7–8, which would not be useful in providing the growing plants with soluble phosphorus in the alkaline soils of Egypt. Use of a phosphate-dissolving fungal strain in addition to a cellulose-degrading one for the production of compost from bagasse offers a solution to the waning interest of farmers in the use of organic phosphatic fertilizers in the alkaline soils.

Therefore, experiments were planned in this work to use cellulose degrading *Trichoderma* and phosphate-dissolving *Aspergillus* strains with or without farmyard manure (FM) for production of compost from bagasse enriched with rock phosphate. The effect of these treatments on the microbial community structure of the produced composts was studied. The composts produced were evaluated as organic phosphatic fertilizers in pots cultivated with broad bean (*Vicia faba*) plants.

Materials and methods

Bagasse

Bagasse used in this study was obtained from a sugar factory in Abu-Korkas City, Egypt. The bagasse was air-dried and crushed into about 3–6 cm pieces using a shredding machine.

Microorganisms

Cellulose-degrading *Trichoderma viride* 104 and phosphate-dissolving *Aspergillus niger* 111 strains were obtained from the culture store of the Department of Agricultural Microbiology, Faculty of Agriculture, Minia University. The cultures were grown and maintained on potato dextrose agar slants.

Inocula preparation

A spore suspension was prepared by adding 3 ml of sterile distilled water to each slant and shaking vigorously for 1 min. The number of the spores in this suspension was about 10^8 c.f.u./ml. Cloth bags each contained a mixture of 1 kg of corn meal and 10 kg of sand were autoclaved for 30 min. Each autoclaved wet corn meal–sand mixture bag was inoculated under aseptic condition with 6 ml spore suspension of either *Aspergillus niger* or *Trichoderma viride*. The inoculated corn meal–sand mixture was incubated at 30 °C for 5 days until the green spores of *Trichoderma* or the black ones of *Aspergillus* arose on the corn meal-sand mixture. These mixtures were then used as inocula for compost preparations.

Preparation of piles

A plastic sheet was placed on the soil surface to control leaching from the piles. Dry shredded bagasse was enriched with rock phosphate (100 g/kg, w/w). To activate the microbial action, a small dose (20 g/kg, w/w) of ammonium sulphate was added to the dry shredded bagasse. Windrow piles each of 150 cm length, 80 cm width and 60 cm height were set up on the plastic sheet. Three replicates of the following treatments were included:

(1) Piles without microbial inoculation or FM addition (reference experiments).
(2) Piles treated with FM at a rate of 100 g/kg.
(3) Piles inoculated with 2.5% (w:w) corn meal–sand mixture *T. viride* inoculum and 2.5% (w:w) corn meal-sand mixture *A. niger* inoculum.
(4) Piles inoculated with FM (100 g/kg), 2.5% (w:w) corn meal–sand mixture *T. viride* inoculum and 2.5% (w:w) corn meal–sand mixture *A. niger* inoculum.

Moisture was maintained at 60% (the moistening was considered satisfactory when a hand-full of composted materials would wet the hand but not drip). The composting was allowed to continue for 105 days. The piles were turned for aeration once a week and three replicate samples were taken from different spots in each pile at 15 days intervals for subsequent analysis.

Pot experiments

Three seeds of broad bean were planted in each fired clay pot contained 10 kg of sandy soil. Three replicate-planted pots for each of the following treatments were carried out:

(1) Pots without any fertilizer.
(2) Pots fertilized each with 300 g of compost as follows:
(a) Pots fertilized with the composted bagasse without any treatment.
(b) Pots fertilized with the composted bagasse treated with FM.
(c) Pots fertilized with the composted bagasse treated with *T. viride* and *A. niger*.
(d) Pots fertilized with the composted bagasse treated with FM, *T. viride* and *A. niger*.

Microbial determinations

The total number of bacteria and fungi were determined using the standard pour plate method on agar-soil extract (Mahmoud *et al.* 1969) and Martins (1950) media, respectively. The Bunt & Rovira (1955) medium modified by Abdel-Hafez (1966) was used for counting the phosphate-dissolving bacteria. To each flask, containing 90 ml of the melted medium free from phosphate, 5 ml of a 10% K_2HPO_4 sterile solution followed by 10 ml of a 10% $CaCl_2$ sterile solution were added and thoroughly mixed with the agar. This was carried out immediately before pouring into the Petri dishes. This method was found to form a fine precipitate of insoluble calcium phosphate in the medium. The pH was readjusted to 6.8 by sterile standard 4% NaOH solution. The standard pour plate method was used for counting phosphate-dissolving bacteria, which were readily detected by clear zones around the colonies after incubation at 30 °C for 48 h.

For the determination of phosphate-dissolving fungi, we modified the Martins medium by replacing K_2HPO_4 with KCl to make it free from soluble phosphate. A fine precipitate of insoluble calcium phosphate was formed in the medium as described above and the same procedure used for the determination of phosphate-dissolving bacteria was used.

Chemical analysis

Organic carbon in the composts produced was determined by a procedure described by FAO (1977). The Micro-Kjeldahl method was used for determination of the total nitrogen in 0.1 g of milled homogenized compost samples. Phosphorus was determined colorimetrically at a wave length of 660 μm by the reduction of the phosphomolybdate complex with the stannous chloride in sulphuric acid system. In this method 10 g of compost sample was collected from different spots in each well-turned pile. Each sample was thoroughly mixed with 100 ml distilled water at room temperature. The suspension was agitated for 1 h, filtered using Whatman filter paper No. 15 and 1 ml of the supernatant was used for phosphorus determination. The

quantity of soluble phosphorus was calculated with reference to a standard curve prepared according to the method mentioned above.

For the determination of phosphorus uptake, the whole dried plant material from each pot was pulverized. A representative sample of exactly 0.1 g was digested using sulphuric acid and hydrogen peroxide. The digest was quantitatively transferred into a 100-ml volumetric flask and made to volume by distilled water. One ml was used for the colorimetric determination of phosphorus and phosphorus uptake was calculated as mg/kg of plant dry weight.

Results and discussion

C/N ratio of composted bagasse

In the reference experiment, the results showed that decomposition of bagasse without microbial inoculation or FM addition was not complete, since the value of C/N ratio was high (40:1) after 105 days of composting. This is because the initial C/N ratio of the non-treated bagasse was very high (70:1), which made the conditions not suitable for the natural microflora to start their activity. Furthermore, the high content of bagasse lignin (Dimalanta & Latiza 1990) increased the adverse conditions for these microflora. The results showed that addition of FM led to an increase in the rate of bagasse decomposition with a final C/N ratio of 25:1 after 90 days of fermentation, because FM is a good source of bioavailable carbon and other nutrient and heterotrophic microorganisms. An excellent bagasse composting was obtained with the use of *A. niger* + *T. viride* as inoculation agents. The C/N ratio gradually decreased during composting till reaching a static figure (constant value) of 19:1 after 60 days indicating a complete bagasse digestion at the end of the process. The results showed that there was no difference regarding C/N ratio values between using *A. niger* + *T. viride* with or without FM for bagasse composting. Similar results were reported by Biswas & Narayanasamy (2002) and Tengerdy & Szakacs (2003).

Phosphorus release from composted bagasse

Soluble phosphorus (15–20 mg/kg) was initially present in the different treatments. Results of the reference experiment showed that composting of the bagasse without any inoculation resulted in a low release of soluble phosphorus. The maximum amount of released phosphorus was 180 mg/kg after 75 days of composting (Table 1) indicating the need of an additional source of organic matter, active cellulolytic strains, and/or active phosphate dissolvers. The results showed that addition of FM increased the amounts of soluble phosphorus to 570 mg/kg after the same period of composting. It may be that more active microorganisms were introduced with FM. However the inoculation with *A. niger* + *T. viride* with or without FM released the highest amounts of soluble phosphorus. For example the amount of soluble phosphorus reached 810 mg/kg after 75 days when the piles were inoculated with *A. niger* + *T. viride*. This could be explained by rapid decomposition of cellulose as a result of *T. viride* inoculation and active phosphate-solubilizing process as a result of *A. niger* inoculation (Zayed & Abdel-Motaal 2004). These results also indicated that in the case of using certain microbial strains (*Aspergillus* and *Trichoderma*) for composting of a bagasse-rock phosphate mixture, there is no need to add farmyard manure.

Oxidation of the organic acids produced or the formation of neutral substances on the expense of the organic acids previously formed may explain the decrease of soluble phosphorus at the end of composting periods as shown in Table 1.

pH values of composted bagasse

The results showed that the tested bagasse was alkaline raw material with an initial pH around 8. During the composting process there was a correlation between the amounts of soluble phosphorus released and the reduction in pH values. For example the amounts of phosphorus released after inoculation with *A. niger* and *T. viride* were 47, 60, 330 and 810 ppm after bagasse composting for 30, 45, 60 and 75 days respectively. These amounts correlated to reduction in the pH values from initial 8 to 7.26, 6.11, 5.88 and 4.8, respectively. The pH of all piles receiving an *Aspergillus niger*, decreased to a high acidic condition (pH 4–5) till the end of the experiment. These results are, however, in contrast with those of Rajbanshi *et al.* (1998) and Lei *et al.* (2000) who reported that after 2–4 days of aerobic composting, the pH usually tended to

Table 1. Phosphorus released from bagasse enriched with rock phosphate and composted for 105 days with different treatments.

Treatments of bagasse enriched with rock phosphate	Released phosphorus ± SD[a] (mg/kg) during different composting times					
	30 days	45 days	60 days	75 days	90 days	105 days
Without any treatment	31 ± 1.2	47 ± 1	160 ± 2.6	180 ± 3	150 ± 2.6	130 ± 2.6
Treated with FM	40 ± 0	60 ± 3.6	271 ± 2.6	572 ± 2	572 ± 3.5	603 ± 2.6
Treated with *A. niger* and *T. viride*	40 ± 1	90 ± 2	331 ± 1.7	810 ± 3	573 ± 3.8	421 ± 2.6
Treated with *A. niger*, *T. viride* and FM	40 ± 1	57 ± 3.6	662 ± 3.5	790 ± 4	782 ± 1.8	573 ± 3.6

[a] Standard deviation.

rise up to 8–9 towards the end of the process. These workers aimed to keep the pH of their compost around the neutral value by using some additives such as calcium carbonate and by maintaining the aerobic condition. However, we aimed to produce composts with low pH, which is the favourable condition for release of soluble phosphorus from the added rock phosphate. We have maintained the acidic conditions during the composting time by inoculation with sufficient amounts of a selected aerobic acid-producing fungal strain (*A. niger*). The above results showed that we were successfully able to decrease the pH values during the composting time and consequently increase the amounts of released phosphorus.

Number of total and phosphate-dissolving fungi in composted bagasse

To determine whether the released phosphorus was directly related to the inoculation with the fungal strains, the total number of fungi as well as the number of phosphate-dissolving fungi were followed during the composting process. The results showed that the numbers of total fungi in the non-treated bagasse experiment were low (11–16 $\times 10^6$ c.f.u./g of dry weigh) throughout the fermentation period (Table 2). Inoculation with FM did not increase the total number of fungi in any treatment. This may be explained by the very low fungal content of the FM used (0 to 1×10^3 c.f.u./g of dry FM). The inoculation with *Aspergillus niger* and *Trichoderma viride* greatly increased the total number of fungi compared to the non-treated bagasse after the same periods of fermentation. These results are in harmony with the above C/N ratio, pH and phosphorus solubilization results, which means that the beneficial effects are related to the added microorganisms. The results in Table 3 made this conclusion clearer, since nearly there were no phosphate-dissolving fungi present in composted bagasse except for those treated with *Aspergillus niger* and *Trichoderma viride*. The numbers of phosphate-dissolving fungi in bagasse treated with *Aspergillus niger* and *Trichoderma viride* were of course lower than the total number of fungi. This is because not both of the added fungi are phosphate dissolvers although they are both cellulolytic fungi.

Number of total and phosphate-dissolving bacteria in composted bagasse

As cleared above the addition of FM alone increased the soluble phosphorus (Table 1) although it did not increase either the total number of fungi or the number of phosphate-dissolving fungi (Tables 2 and 3). To explain the increase of soluble phosphorus in case of FM addition, it has been decided to measure the total number of bacteria as well as the number of phosphate-dissolving bacteria during composting of bagasse. The results in Table 4 showed that addition of FM in all treatments greatly increased the total number of bacteria, comparing to the non treated compost. The presence of a very high total number of bacteria in the added FM (400–500 $\times 10^6$ c.f.u./g of dry weight FM) may explain the role of FM in increasing the total number of bacteria in composted materials. No reports in the literature about the effect of FM addition on the structure of microbial community in composted bagasse are available. Results in Table 5 confirmed the idea of phosphate solubilization through the activity of bacteria (and not through the activity of fungi) in case of FM addition. This is because the number of phosphate-dissolving

Table 2. Total number of fungi in bagasse enriched with rock phosphate and composted for 105 days with different treatments.

Treatments of bagasse enriched with rock phosphate	Total number of fungi ± SD[a] ($\times 10^6$ c.f.u.)/g of dry weight during different composting times					
	30 days	45 days	60 days	75 days	90 days	105 days
Without any treatment	12 ± 0	12 ± 1	16 ± 0	11 ± 0	11 ± 0	11 ± 0
Treated with FM	10 ± 0	26 ± 1	16 ± 1	10 ± 0	13 ± 0	10 ± 0
Treated with *A. niger* and *T. viride*	20 ± 1	66 ± 2	160 ± 3	130 ± 2	178 ± 4	20 ± 0
Treated with *A. niger*, *T. viride* and FM	33 ± 1	50 ± 2	167 ± 3	116 ± 3	153 ± 3	42 ± 2

[a] Standard deviation.

Table 3. Number of phosphate-solubilizing fungi in bagasse enriched with rock phosphate and composted for 105 days with different treatments.

Treatments of bagasse enriched with rock phosphate	Number of phosphate solubilizing fungi ± SD[a] ($\times 10^5$ c.f.u.)/g of dry weight during different composting times					
	30 days	45 days	60 days	75 days	90 days	105 days
Without any treatment	1 ± 0	1 ± 0	2 ± 0	9 ± 0	1 ± 0	1 ± 0
Treated with FM	0	5 ± 0	3 ± 0	1 ± 0	0	2 ± 0
Treated with *A. niger* and *T. viride*	100 ± 2	160 ± 2	220 ± 3	184 ± 3	168 ± 2	142 ± 2
Treated with *A. niger*, *T. viride* and FM	100 ± 2	125 ± 2	225 ± 3	193 ± 2	153 ± 3	124 ± 2

[a] Standard deviation.

Table 4. Total number of bacteria in bagasse enriched with rock phosphate and composted for 105 days with different treatments.

Treatments of bagasse enriched with rock phosphate	Total number of bacteria ± SD[a] (× 10^6 c.f.u.)/g of dry weight during different composting times					
	30 days	45 days	60 days	75 days	90 days	105 days
Without any treatment	14 ± 1	36 ± 2	42 ± 2	40 ± 2	26 ± 1	15 ± 1
Treated with FM	100 ± 2	330 ± 4	330 ± 4	330 ± 3	310 ± 4	90 ± 2
Treated with *A. niger* and *T. viride*.	100 ± 2	150 ± 3	200 ± 3	250 ± 3	110 ± 3	110 ± 2
Treated with *A. niger*, *T. viride* and FM	150 ± 3	180 ± 3	240 ± 4	480 ± 5	170 ± 3	120 ± 2

[a] Standard deviation.

Table 5. Number of phosphate-solubilizing bacteria in bagasse enriched with rock phosphate and composted for 105 days with different treatments.

Treatments of bagasse enriched with rock phosphate	Number of phosphate solubilizing bacteria ± SD[a] (× 10^5 c.f.u.)/g of dry weight during different composting times					
	30 days	45 days	60 days	75 days	90 days	105 days
Without any treatment	19 ± 1	17 ± 1	10 ± 0	31 ± 2	14 ± 0	12 ± 0
Treated with FM	12 ± 0	30 ± 1	116 ± 3	259 ± 3	110 ± 2	40 ± 2
Treated with *A. niger* and *T. viride*.	18 ± 1	13 ± 0	13 ± 0	38 ± 2	32 ± 2	18 ± 1
Treated with *A. niger*, *T. viride* and FM	60 ± 2	110 ± 3	119 ± 3	331 ± 4	320 ± 5	70 ± 2

[a] Standard deviation.

bacteria increased only in the treatments including inoculation with FM.

Greenhouse experiments

To evaluate the resulted composts as organic phosphatic fertilizer, broad beans were planted in pots and fertilized with different types of the compost obtained. The experiments were conducted in sandy soil and control non-fertilized pots were included. A factor taken in consideration to judge the quality of compost was the determination of plant phosphorus uptake by end of the growth period. The results in Table 6 show that the non-fertilized sandy soil did not provide broad bean plants with enough phosphorus. It is well known that available phosphorus is deficient generally in non-amended alkaline soils of Egypt (Zayed 1997). Table 6 also shows that broad beans fertilized with the compost of uninoculated bagasse did not absorb higher amount of phosphorus as compared to those grown in non-fertilized control pots. A higher amount of phosphorus uptake was recorded when the plants were fertilized with the bagasse compost treated with FM alone. However, the composts resulting from inoculation with *A. niger* + *T. viride* with or without FM were much better as a source of soluble phosphorus compared to inoculation with FM alone. Chattopadhyay *et al.* (1993) found that plant phosphorus uptake rates were significantly enhanced by the application of compost inoculated with *T. viride* compared to non-inoculated compost, FM or inorganic fertilizer. El-Din *et al.* (2000) reported that different types of compost receiving fungal activators induced a significant increase in phosphorus uptake of tomato plants relative to the FM and mineral fertilizer treatments.

Table 6. Phosphorus uptake of broad bean plants fertilized with bagasse enriched with rock phosphate and composted for 105 days with different treatments.

Broad bean fertilization	Phosphorus uptake ± SD[a] (mg/kg)
Non-fertilized	40 ± 1
Fertilized with non-treated bagasse	45 ± 1.7
Fertilized with bagasse treated with FM	141 ± 2.6
Fertilized with bagasse treated with *A. niger* + *T. viride*	210 ± 2
Fertilized with bagasse treated with FM + *A. niger* + *T. viride*	221 ± 3.4

[a] Standard deviation.

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