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Composting of sugar cane bagasse by *Bacillus* strains

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Composting of sugar cane bagasse with *Bacillus sp.* CMAGI2 and *Bacillus subtilis* JCM 1465^T strains was carried out during five months at horticultural center. Chemical, biochemical and microbial parameters were followed during this process. There was a difference between inoculated composts and non-inoculated compost. These bacterial additives allowed greater biodegradation compared to control compost. The inoculated composts were more degraded than the control compost with compost3 which presented the highest OM loss with 91.37%, compost1 with 90.15% and compost2 had 89.47% of OM loss. Control compost showed the lowest C/N ratio, however compost3 had the highest C/N ratio compared to compost1 and compost2. Microbiologically, *Bacillus* strains in compost1 and compost2 had probably inhibitory effect on microflora statistically if they were inoculated alone when the mixture of two strains (compost3) had no inhibitory effect on microflora during the composting process. The inoculated composts presented higher enzymatic activities than control compost, probably due to the presence of *Bacillus* strains.

Key words: Sugar cane bagasse, composting, *Bacillus sp.*CMAGI2, *Bacillus subtilis* JCM 1465^T.

INTRODUCTION

In these last years, a decrease in humus soil has been observed in most agricultural fields. This issue is a result of number of human activities and environmental phenomena such as: large use of chemical fertilizer in intensive agriculture, bad management of agricultural wastes, soil erosion, and various environmental pollutions. This problem has significantly impacted on agricultural productivity, and finding solutions for this issue has become a vital and dynamic research focus.

The use of compost has been thought to be an option, for enhancing soil quality. Composting is a biological process which converts heterogeneous organic wastes into humus like substances by mixed microbial population under controlled optimum conditions of moisture, temperature and aeration (Ryckeboer et al., 2003b; Ahmad et al., 2007; Insam and de Bertoldi, 2007;

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Jurado et al., 2015). The resulting product is called compost and can be used as soil conditioner and or organic fertilizer. It is well known that soil microbiome plays important role in compost biodegradation to specific and precise stages, however it is very difficult to count the number of microorganisms involved in this bioprocess (Ryckeboer et al., 2003b). Among the composting microorganisms, bacteria, actinomycetes and fungi constitute the major active groups. Bacteria are also the most diverse group of compost organisms, using a broad range of enzymes to chemically degrade a variety of organic matters (Ryckeboer et al., 2003b). The high surface/volume ratio of bacteria allows a rapid transfer of soluble substrates into the cell. They are usually far more dominant than larger microorganisms such as fungi (Tuomela et al., 2000; Ryckeboer et al., 2003b; Insam and de Bertoldi, 2007; Mehta et al., 2014). The ubiquitous genus *Bacillus* is often found in environment mostly in composts at any stage (Ryckeboer et al., 2003, b; Insam and de Bertoldi, 2007; Franke-Whittle et al., 2014). Their capacity to produce spores allowing to survive in unfavorable environmental condition is an advantage over other bacteria. They can produce extracellular polysaccharide hydrolysing enzymes (Priest, 1977).

The inoculation with specific microorganisms can be a useful method for enhancing the properties of compost and decreasing the composting time (Adebayo et al., 2011; de Figueirêdo et al., 2013; Jurado et al., 2015) but the use of inoculants remains controversial due to contradictory results presented by many authors (Adebayo et al., 2011; de Figueirêdo et al., 2013). Indeed, environmental and nutritional conditions are not the only parameters that can affect microbial growth; the presence of other microorganisms can influence the activity of the tested microorganism, either positively or negatively (Franke-Whittle et al., 2014). The aim of this study was to investigate the evolution of physico-chemical parameters and changes in microbial population during the sugar cane bagasse composting.

MATERIALS AND METHODS

In this study, we used bagasse as substrate and *Bacillus sp.* CMAGI2 and *Bacillus subtilis* JCM 1465^T strains as inocula. The sugar cane bagasse was taken from the Senegalese Sugar Company (Compagnie Sucrière Sénégalaise C.S.S.) which is located to the north of Senegal (Richard-Toll, St-Louis). This substrate was chosen for its low microbial activity, which may allow an assessment of the behavior of inoculated *Bacillus* strains during the process. The composting of sugar cane bagasse was carried out at horticultural center UPPF of Senegalese Institute of Agricultural Research "Institut Sénégalais de Recherches Agricoles" (ISRA), located at Sangalkam, Dakar, Senegal.

Preparation of bacterial inoculants

B. subtilis JCM 1465^T strain spores were kindly provided by Belgian partners from Gembloux University. It was reactivated on nutrient agar (*Bio-Rad*) then preserved at 4°C and stored too in 20% glycerol at -80°C. *B. sp.*CMAGI2 was isolated from mature sugarcane bagasse compost in laboratory of Applied Microbiology and Industrial Engineering (Microbiologie Appliquée et Génie Industriel, MAGI) (Diallo et al., 2015). The bacterial biomass of inoculum (*B. sp.* CMAGI2 and *B. subtilis* JCM 1465^T) was prepared with their specific culture media growth.

B. sp CMAGI2 was cultured in medium containing: 5.0 g of glucose, 5.0 g casamino-acids, 3.0 g beef extract, 5.0 g peptone in 1 L distilled water at pH 8. *B. subtilis* was cultured in a medium containing: peptone 5.0 g, beef extracts 3.0 g in 1 L distilled water at pH 7.

The inocula were prepared by growing young colonies of the two strains in fresh sterilized culture media in 5 L flasks and incubated for two days at 40°C. The resulting bacterial cultures were diluted in 20 L of sterile water before inoculation in the process.

Composting process

Windrows composting were carried out in this project. We built four cemented composters with 5 m length, 1 m width and 60 cm depth, and filled an amount of 2 m^3 bagasse with 60% humidity. They were inoculated with 10% (20 L) bacterial culture with a final bacterial concentration of 10^9 CFU/ml and a bacterial population between 10^7 and 10^8 CFU/g of fresh compost was obtained at the beginning of composting (de Figueirêdo et al., 2013). One composter was the control compost and was non inoculated, that is, was also designed by replacing the bacterial inocula by an equal volume of sterilized distilled water, Compost1 was inoculated with *B. subtilis* JCM 1465^T, Compost2 was inoculated with *Bacillus* sp. CMAGI2 and Compost3 with the mixed culture of the two strains (*B.* sp.CMAGI2 and *B. subtilis* JCM 1465^T. At the beginning of composting, the bacterial suspensions were sprayed on the moistened bagasse and the material was turned upside down after inoculation to spread the bacteria. The organic matter was humidified every two days, and the inoculation was repeated every two months.

During the first month, the return was done manually and weekly, then from the second month bimonthly and monthly until the end. At the third month, heated cow manure taken from local family farmhouse at Sangalkam was added in order to increase the pH and decrease the C/N ratio. 2.5 volumes of bagasse and 1 volume of cow manure were mixed. The composting was carried out for 5 months.

Microbial parameters

The changes of composting microflora at different stages were determined by enumeration. Microbial samples were collected at different times (days) and were taken from different points of the windrow, and then mixed before analysis: 0, 7th, 14th, 21th, 28th, 35th, 42th, 49th, 63th, 84th, 105th, 112th, 126th and 147th day. The targeted microbial groups were: mesophilic fungi and yeasts, total mesophilic bacteria, spore forming bacteria, faecal coliforms, faecal *Streptococci*, *Clostridia*, *Salmonella* and mesophilic actinomycetes. The analysis for *Staphylococci*, *Escherichia coli*, *Vibrio*, *Listeria* and *Bacillus cereus* were carried out only in the final compost (147th day) samples.

About 25 g of fresh compost were suspended in 225 ml sterile buffered peptone water (*Scharlau, Spain)* and shaken at 150 rpm for 30 min at room temperature (22°C) to allow the microorganisms to migrate into the solution. Then the resulting suspension was diluted (ten-fold serial dilutions) using sterile buffered peptone water in test tube (9 ml) and was used for microbial counts. One milliliter

from each dilution was spread on sterile Petri dishes containing the required culture media. After incubation, the number of colony forming units (CFU) was counted and the microbial number of each sample expressed in terms of CFU/g of fresh weight.

Fungi and yeasts were counted on chloramphenicol glucose agar (Scharlau, Spain) and plates were incubated at 25 to 30°C for 48 to 72 h according to ISO 7954 and FIL-IDF 94B standards. Total bacteria were determined by the plate pouring technique using plate count agar (Liofilchem, Italy) containing sterile cycloheximide at a concentration of 250 µg/ml. They were incubated at 30°C for 3 days. The number of spore-forming bacteria was determined as above, only; diluted suspensions were first incubated at 80°C for 20 min, then plated in nutrient agar (Liofilchem, Italy) supplemented with sterile cycloheximide (250 µg/ml) for 24 h at 30°C. *Clostridia* were detected on trypticase sulfite neomycin agar (*Bio-Rad, USA*) and incubated at 44°C for 24 h. Violet red bile lactose agar (Liofilchem, Italy) was used as selective differential medium to enumerate faecal coliforms after incubation at 44°C for 24 h. Bile esculin agar (Scharlau, Spain) was used for detecting faecal *Streptococci* after incubation at 44°C for 24 h. Actinomycetes were cultured in specific medium (Wink, 2004) composed of 1 g/l L-Asparagine, 10 g/l Glycerol, 1g/l K_2HPO_4, 20 g/l agar and 1 ml salt solution (1 g $FeSO_4$ $7H_2O$; 1 g MnCl2 $4H_2O$; 1 g $ZnSO_4$ $7H_2O$ in 100 ml distilled water) adjusted at pH 7.2 and supplemented with sterile cycloheximide (250 µg/ml). The incubation was done at 30°C for 7 to 15 days.

We monitored *Salmonella* during the process as recommended by standard methods of ISO 6579. The compost suspensions (25 g in 225 ml of buffered peptone water) were used for this analysis. On the samples of final compost (day 147), we used Baird Parker medium (Bio-Rad, USA) for *Staphylococci* and plates were incubated at 37°C for 24 h; the Mossel medium (Bio-Rad, USA) was used for *B. cereus* incubated at 30°C for 24 h. *E. coli* was counted on Rose Gal 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) agar (Biokar, France) after incubation at 44°C for 24 h. *Vibrio* were cultured in thiosulfate-citrate-bile-saccharose medium (Biokar, France) at 37°C for 24 h. *Listeria* was detected as recommended by standard methods of ISO 11290.

Analytical methods

The temperature was determined with electronic temperature probe *Checktemp1 (Hanna instruments*) and it was measured at 20 cm depth (the middle of pile). The measurements were taken at three points along the windrows. The pH was determined with an electrode pH-meter Cyberscan *(*laboratoires Humeau*)* on a water extract from compost using a ratio of 10:100 (w/v) compost/distilled water. Before reading the pH values, the mixture was shaken at room temperature for 30 min. The humidity content was determined by oven-drying at 105°C until constant weight (16 h). For these parameters, the samplings were done weekly for a better monitoring of the process. Ash content (A) was determined by loss on ignition of samples at 900°C with furnace (*carbolitte*) until a constant weight (24 h). Total organic carbon (TOC) content was determined by oxidation with potassium dichromate according to Walkley and Black (1934) modified method. The total nitrogen (TN) was analyzed by the method of Kjeldahl.

Loss of organic matter (OM) during composting was determined and we considered OM decreased when ash content increased. According to Paredes et al. (1996), we calculated the following formula:

$$\text{OM loss } \% = 100 - 100\left(\frac{\text{Ai}(100 - \text{Af})}{\text{Af}(100 - \text{Ai})}\right)$$

Where, Ai is the initial ash level and Af the final ash level.

The cellulase and xylanase activities were quantified by the methods of Miller (1959) and Wood and Bhat (1988) which are colorimetric methods using carboxymethylcellulose and xylan respectively as substrates. The alkaline and acid phosphatases activities were estimated by using ρ-nitrophenylphosphate prepared in acid and alkaline buffer respectively as substrate according to Tabatabai and Bremner (1969). These analyses for enzymatic activities were performed in triplicate. They were done on days 0, 28, 56, 84, 112 and 147 and values were read with an UV/Vis spectrophotometer *Analytik Jena* (*Specord 200 Plus*) equipped with basic *WinASPECT PLUS*® software. Results were expressed in unit enzyme corresponding to 1 µmole of product liberated per gram dry weight enzyme per minute.

Statistical analysis

Data obtained in the study were the mean values of three replicates. Data were subjected to statistical evaluation using one-way ANOVA ($p < 0.05$) with XLSTAT (v2008.1.01) software and Tukey (HSD) test for multiple comparisons.

RESULTS AND DISCUSSION

Humidity, temperature and pH during composting

Humidity is a very important parameter for composting and it may become a limiting factor if not well monitored (Ahmad et al., 2007). That is the reason why, the moisture content was determined every week for a better monitoring of the composting process. The best range of moisture is 40 to 60% (FAO, 2005; Ahmad et al., 2007). An initial humidity of 60% is acceptable in the beginning of the composting process; however that should decrease up to 30% to prevent further biological activity in the final product. In this process, the four designs of composting were in the ideal range of humidity (Figure 1). With 60% humidity at the beginning of process, the four composts reached around 30% of moisture at the final stage with highest values in control compost.

Temperature is an important factor for tracking the composting process evolution. It was weekly monitored during all the process. The four composts presented the same temperature profile (Figure 2). After 2 months, the highest temperature (45.07°C) was recorded in compost3, and lowest temperature was noted after the seventh week with weak value of temperature 37.53°C in control compost confirmed statistically (p=0,013) with significant difference between control compost and compost3. These low temperature values can be attributed to the decrease in pH, which can affect microbial growth and activity.

According to Sundberg et al. (2004), low pH is an inhibiting factor in the transition from mesophilic to thermophilic phase, which explained low values of composting temperatures. But in control compost, there is no low pH value, so the recorded heat in other

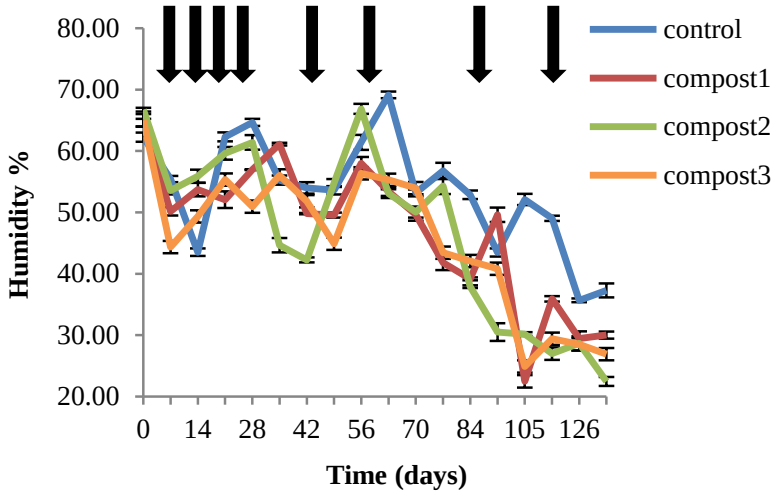

Figure 1. Humidity evolution in the four windrows composting. Arrows indicate turnings. The vertical bars represent the standard deviation of the mean calculated for triplicates.

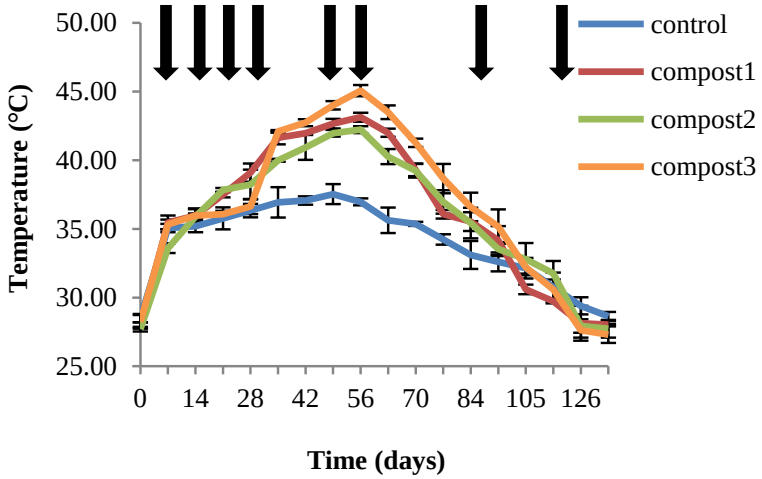

Figure 2. Temperature profile of the four composts. Arrows indicate turnings.

windrows may be caused by the activity of inoculated bacteria. At initial stage of composting, low pH was due to the low pH of the bagasse which was 3.56 (Table 1) and increased during the first month.

One month after, we observed a decrease in pH and the condition was found to be acidic in inoculated windrows which may be attributed to the presence of short chain organic acids, mainly lactic and acetic acids. These products resulted from the activity of acid-forming bacteria that break down complex carbonaceous material to organic acids as intermediate products (Tuomela et al., 2000; Beck-Friis et al., 2001).

In control compost, any decrease in pH was not observed (Figure 3) and confirmed statistically with significant difference between control compost and inoculated composts ($p < 0.05$). At the third month, after incorporation of the cow manure, the pH increased up to 7.0 in all composts corresponding to the ideal neutral final pH with a high natural buffering capacity (FAO, 2005). Evolution of composts was monitored during the experimental period of 5 months and characteristics of final composts are presented in Table 2.

According to the formula of Paredes et al. (1996); post-hoc comparisons using HSD test indicated in terms of

Table 1. Physico-chemical characterization of bagasse and cow manure before composting.

Parameter	Bagasse	Cow manure
Humidity (%)	9.58(0.83)	8.35(0.34)
pH	3.56(0.03)	8.62(0.01)
Organic carbon (%)	66.38(0.49)	33.54(1.50)
Nitrogen (%)	0.34(0.00)	2.66(0.00)
C/N ratio	195.23(1.43)	12.61(0.56)
Ash (%)	20.50(1.13)	51.55(1.32)
Organic matter (%)	79.50(1.13)	48.45(1.32)

Values in parenthesis are deviation standard of the triplicates.

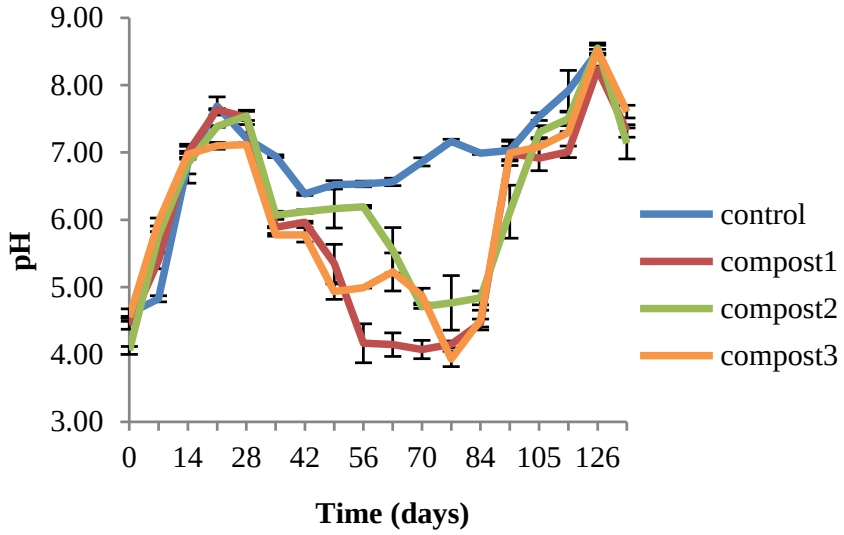

Figure 3. Changes in pH for the four types of composting.

OM loss that compost3 presented the highest value (91.37% OM loss), followed by compost1 and compost 2 with 90.15 and 89.47% OM loss, respectively. The control compost which showed the lowest OM loss (85.55%) presented a significant difference ($p < 0.05$) compared to the inoculated ones (Figure 4). Thereby, control compost was less degraded compared to others. This result is in agreement with that of C/N ratio, which indicated that control compost was less degraded (Figure 5). These two parameters (MO and the C/N ratio) are considered as maturity parameters.

Microbial evolution

This process involves complex microbial community which plays key role during composting (Insam and de Bertoldi, 2007). Many factors determine microbial evolution during composting like temperature, pH, humidity and aeration.

Fungi and yeasts

Mesophilic fungal populations were low at the beginning of composting process in all windrows. A rapid growth of this microbial group was observed in the first four weeks of the experiment, and the highest fungal population was obtained at the 28th day. The control presented the highest fungal population (1.26×10^{13}CFU/g), followed by compost3 (1.91×10^{12} CFU/g) and compost2 (3.25×10^{10} CFU/g) (Figure 6). Compost1 presented the lowest fungal population with 1.0×10^{9} CFU/g. After the 28th day, a drastic decrease in fungal population was observed, which may be due to the increasing temperature, since fungi are very sensitive to the high temperature (Tuomela et al., 2000; Mehta et al., 2014). Surprisingly, compost3 in which the highest temperature was recorded presented

Table 2. Physical and chemical characterization of the four different final composts.

Parameter	Control	Compost1	Compost2	Compost3
Humidity%	37.29(1.14)	30.01(0.57)	22.44(0.72)	26.89(0.20)
pH	7.48(0.07)	7.32(0.09)	7.13(0.23)	7.61(0.09)
Nitrogen%	0.82(0.03)	1.12(0.00)	1.24(0.14)	1.09(0.09)
C/N ratio	37.56(3.03)	19.12(0.92)	16.41(1.49)	21.05(1.74)
Organic matter	36.17(0.80)	22.38(0.58)	26.72(0.12)	24.51(0.59)
Na%	0.22(0.01)	0.14(0.01)	0.24(0.01)	0.20(0.00)
Mg%	0.35(0.00)	0.27(0.00)	0.30(0.01)	0.40(0.00)
K%	0.68(0.00)	0.62(0.01)	0.70(0.02)	0.80(0.01)
Ca%	1.19(0.02)	0.74(0.02)	0.93(0.02)	1.16(0.00)
P(ppm)	551(0.00)	240.34(0.00)	251.27(0.00)	419.86(0.00)
Color	Brown	Brown	Brown	Brown
Odor	Damp earth	Damp earth	Damp earth	Damp earth

values in parenthesis are deviation standard of the triplicates.

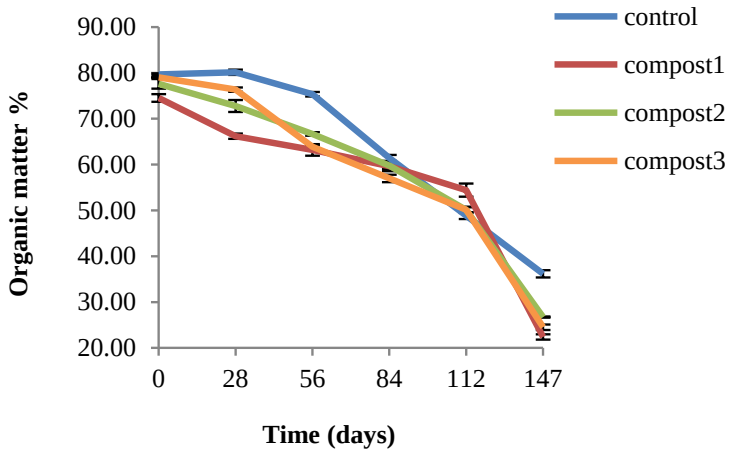

Figure 4. Evolution of organic matter during composting.

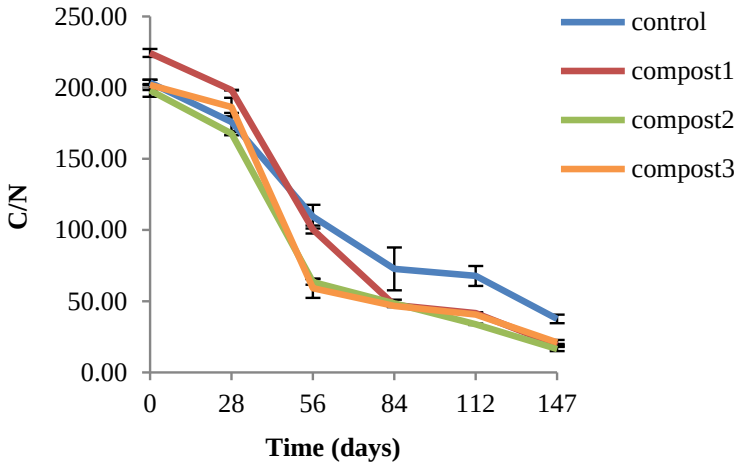

Figure 5. C/N ratio during composting.

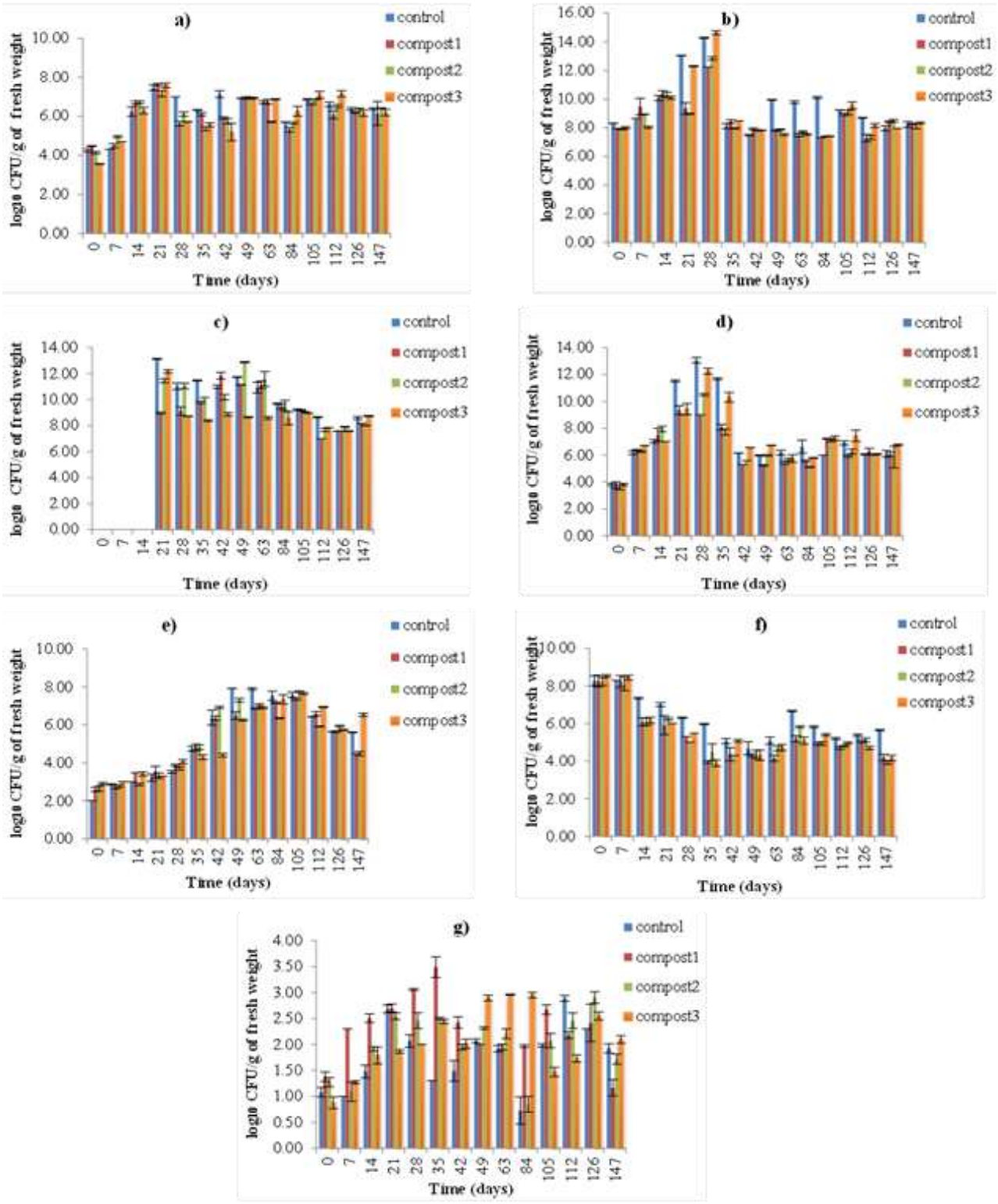

Figure 6. Evolution of different microbial groups during different composting. (a) spore-forming bacteria. (b) Bacteria. (c) Actinomycetes. (d) fungi and yeasts. (e) Faecal coliforms. (f) Faecal Streptococci. (g) Clostridia. The vertical bars represent the standard deviation of the mean calculated for triplicates.

the highest fungal population compared to other inoculated composts. When the pH increased and the temperature decreased, mesophilic fungal population increased and recolonized again the substrate. The number of fungal population presented significant difference ($p < 0.05$) across different composts (Table 4).

Mesophilic bacteria

During composting, bacteria are mainly responsible for substrate decomposition and heat generation (Tuomela et al., 2000; Insam and de Bertoldi, 2007; Mehta et al., 2014). Initial bacterial population was near 10^7 to 10^8 CFU/g in the different windrows. Bacterial growth occurred in the first four weeks with significant difference between control compost, compost3 and inoculated composts with single strain ($p < 0.05$). After 28^{th} day a decrease in bacterial population was observed. The highest bacterial population was found in compost3 (4.52×10^{14} CFU/g) followed by control compost (1.93×10^{14} CFU/g), and then compost2 and compost1 had no difference statistically. From the fourth week, the mesophilic bacterial population began to decrease in all windrows probably due to the decreasing pH and elevation of temperature. Among the four designed composting, control compost had the most important number of bacteria, this may be explained by the fact that, its pH had never fallen to acidity level throughout the composting process (Table 4).

Spore-forming bacteria

During the first month, the density of spore-forming bacteria increased from 10^4 to 10^7 CFU/g in all windrows. A progressive increase of spore-forming bacteria was observed because of the increasing temperature and the decreasing of pH in all windrows. However, the population of sporulated bacteria of control compost was higher than the others ($p < 0.05$) (Table 4). Probably, this was due to nutrient competition phenomenon available for bacteria or these bacteria could not use the readily degradable cellulosic substrate. When the temperature decreased and the pH increased meaning favorable environment, there was a germination of spores and a decrease of spore-forming bacteria.

Actinomycetes

Actinomycetes play key role in degradation of organic compounds such as cellulose, lignin, chitin and proteins (Epstein, 1997). In this process, they appeared from the third week and reached 1.37×10^{13} CFU/g in control compost and the lowest population was noted in compost1 with 9.59×10^8 CFU/g. Their non-appearance during the initial stage might be due to their slow growth rate compared to bacteria or fungi. This result is consistent with many reports in which actinomycetes appeared during the thermophilic phase as well as the cooling and maturation phases of composting (Tuomela et al., 2000). During this composting in all windrows, actinomycetes population presented significant difference in all composts ($p < 0.05$).

Faecal coliforms

The count of faecal coliforms is a good indicator of the sanitary quality of soil, food and environment. A low faecal coliform population was noted at initial stages, then an increase up to 10^8 CFU/g was noted probably due to recontamination, redistribution by equipment used for windrows turnings or water used for humidification. When the temperature cooled down in last month, coliforms decreased to 4.05×10^5 in control compost, 3.0×10^4 in compost1, 3.14×10^4 in compost2 and 3.43×10^6 in compost3 ($p < 0.05$) (Table 3).

Faecal Streptococci

Faecal *Streptococci* are ubiquitous and are the best indicators of faecal pollution. The number of *Streptococci* decreased considerably from 2.09×10^8 in control compost, 2.16×10^8 in compost1, 1.83×10^8 in compost2 and 3.42×10^8 in compost3 ($p < 0.05$) at initial stages to 4.58×10^5 in control compost, 1.65×10^4 in compost3, 1.52×10^4 in compost1 and 8.52×10^3 in compost2 ($p < 0.05$) at the end. Similar results were reported by Hassen et al. (2001).

Clostridia

Clostridia are telluric bacteria and involved in biodegradation of soil. *Clostridium* converts organic compounds to sugar, acids and alcohol and play important role in compost maturation (Ryckeboer et al., 2003b; Franke-Whittle, 2014), that is, anaerobic microorganisms decompose organic matter after aerobic bacteria had consumed oxygen in composts or when gas exchange is very slow. During the composting process, the count in compost1 was higher than the others, compost2 and compost3 had the same number and control compost presented the lowest number ($p < 0.05$) (Table 4).

Table 3. Microbial parameters of the final composts.

Parameter	Control compost	Compost 1	Compost 2	Compost 3
Spore-forming bacteria (CFU/g)	9.0×10^5	6.5×10^5	8.0×10^5	5.0×10^4
Fungi and Yeasts (CFU/g)	1.40×10^6	1.22×10^6	2.17×10^6	5.74×10^6
Bacteria(CFU/g)	1.8×10^8	1.85×10^8	1.35×10^8	2.15×10^8
Actinomycetes (CFU/g)	4.03×10^8	1.50×10^8	1.04×10^8	5.37×10^8
Faecal coliforms (CFU/g)	4.05×10^5	3.0×10^4	3.14×10^4	3.43×10^6
Faecal *Streptococci* (CFU/g)	4.58×10^5	1.65×10^4	8.52×10^3	1.52×10^4
Clostridium (CFU/g)	85	15	53	126
Salmonella (25g)	absence	absence	absence	absence
Staphylococci (CFU/g)	0	0	0	0
E.coli (CFU/g)	0	0	0	0
B. cereus (CFU/g)	0	0	0	0
*Listeria (*25 g)	Absence	Absence	Absence	Absence
Vibrio (CFU/g)	0	0	0	0

Table 4. Microbial number of different composts during the composting process.

Log_{10} CFU/g of fresh weight	Control compost	Compost 1	Compost 2	Compost 3
Fungi and yeasts	103.380^a	91.137^b	92.663^b	101.880^a
Bacteria	133.957^a	121.237^c	120.763^c	125.803^b
Spore-forming bacteria	88.343^a	84.653^b	84.707^b	85.293^b
Actinomycetes	113.027^a	103.477^c	109.533^b	97.063^d
faecal coliforms	74.350^a	71.953^b	71.850^b	73.633^a
faecal streptococci	86.797^a	74.643^c	76.103^{bc}	76.967^b
Clostridium	24.920^c	32.217^a	28.250^b	28.900^b

^{a,b,c,d}represent significant differences ($p<0.05$) in the same line.

***Salmonella* were not detected at any stage during the whole composting process**

Except *Clostridium*, microbial population was found to be higher in control compost probably due to low temperature which was recorded in control compost. It was followed by compost3. The compost1 and compost2 had the lowest count and often equal statistically for most microbial groups with ($p < 0.05$). Probably, *Bacillus sp.* CMAGI2 and *Bacillus subtilis* JCM 1465^T had an inhibitory effect on microbial flora if inoculated alone. For instance in compost3, where a mixed culture of the two strains was inoculated, microbial population was higher than that of compost1 and compost2 inoculated with a single strain. When these two *Bacillus* strains were inoculated together, they probably had no inhibitory effect on microflora (Table 4).

Enzymes are biomolecules involved in specific catalyzing biological reactions (Alef and Nanniperi, 1995). During the composting, secretions and changes in enzymatic activities were caused by the action of many microorganisms. These enzymes participate in return in complex microbial successions. Enzymes are the main mediators of various biodegradation processes (Goyal et al., 2005). In this study, three important enzymes involved in organic matter biodegradation were targeted: cellulases, xylanases, acid and alkaline phosphatases.

The initial cellulases activities were very low in all designed composts. On day 56, these activities were equal in compost1 and compost3 and started to increase ($p < 0.05$) compared to cellulase activity of control compost and compost2. Highest values were significantly different in windrows and noted on 112^{th} day. Indeed, compost3 scored a cellulase activity of 3.1369 $U.g^{-1}.mn^{-1}$ while compost2 and compost1 scored 2.5691 and 2.1532 $U.g^{-1}.mn^{-1}$ respectively (Table 5). The lowest value was recorded in control compost with 1.7734 $U.g^{-1}.mn^{-1}$. That may be explained by low level of nitrogen reported to be a limiting factor in cellulose degradation elsewhere (Tuomela et al., 2000). Increase in cellulase activity

Table 5. Evolution of cellulase, xylanase, acid phosphatase, alkaline phosphatase activities during composting.

Time (days)	Cellulase (U.g^{-1}dry matter)						Xylanase (U.g^{-1}dry matter)						Acid phosphatase (mU.g^{-1}dry matter)						Alkaline phosphatase (mU.g^{-1}dry matter)					
	0	28	56	84	112	147	0	28	56	84	112	147	0	28	56	84	112	147	0	28	56	84	112	147
Control compost	0.08	0.07	0.15	0.12	1.77	0.47	0.21	0.26	0.12	0.2	1.29	0.46	20.96	33.11	49.23	45.88	42.14	47.31	0.02	3.94	0.44	0.59	1.89	1.01
Compost1	0.11	0.15	0.25	0.51	2.15	0.37	0.10	0.15	0.30	0.61	12.18	1.01	29.18	65.86	72.83	47.27	21.15	15.94	0.07	1.95	0.38	1.60	1.26	1.02
Compost2	0.13	0.09	0.16	0.43	2.57	0.60	0.30	0.14	0.24	0.52	11.46	0.81	26.72	69.47	45.93	24.47	13.35	27.07	0.03	3.22	2.41	3.07	1.63	1.34
Compost3	0.09	0.15	0.28	0.58	3.14	0.68	0.17	0.22	0.43	0.39	7.24	1.33	26.53	53.10	72.62	35.31	33.72	23.48	0.07	2.48	2.47	1.92	1.93	1.13

observed on the 112th day is attributable to the incorporation of cow manure which increased the compost microflora. Since sugar cane bagasse contained about 50% cellulose, 25% hemicellulose and 25% lignin (Tuomela et al., 2000; Insam and de Bertoldi, 2007), we can understand why these low values were obtained.

Xylanases activities presented the same trend as cellulases activities, that is, the highest activity was remarked on day 112. This similar trend between cellulases activities and xylanases activities were also observed by Zeng et al. (2010).

Compost1 and compost2 had no significant difference and scored the highest values with 12.181 and 11.455U.g^{-1}.mn^{-1}, but they presented difference compared to control compost and compost3 with xylanases values 1.291 and 7.237 U.g^{-1}.mn^{-1} respectively.

The slow biodegradation kinetics of cellulose and hemicellulose can be explained by the environmental condition under which the experiment was carried out. In fact, the optimun degradation activity of these enzymes are obtained in low pH and high temperature (around 50°C) (Schinner and von Mersi, 1990). Also, like any enzymatic reaction, concentration, location and mobility of the enzymes in the compost impact in enzymatic activity (Hayano, 1986). Presence of more easily-decomposable substrates lead to the suppression of components rich with xylan and cellulose decomposition. Hence, we can assume that the strong activity on day 112 was caused by high microbial diversity noted during the cooling and maturation phases of composting (Ryckeboer et al., 2003b; Insam and de Bertoldi, 2007).

Phosphatases catalyse the hydrolysis of phosphate esters and are enzymes with relatively broad specificity, capable of acting on a number of different structurally related substrates, but at widely different rates (Alef and Nanniperi, 1995).

In the phosphatase assay, phosphomonoesterases were searched and were classified as acid, neutral and alkaline phosphatases; the first two enzymes have been detected in animal, microbial and plant cells and the other has been found only in microorganisms and animals (Alef and Nanniperi, 1995)..

In this process, we noted high activities of acid phosphatase during the first month. Statistically, all windrows presented significant values with 69.47 mU.g^{-1} for compost2, 66.59 mU.g^{-1} for compost1, 53.10 mU.g^{-1} for compost3 and 33.11 mU.g^{-1} for the control compost for the second month for the others and then declined. This phenomenon is similar to the studies of Albrecht et al. (2010). The highest value was observed in compost1 with 72.83 mU.g^{-1}.mn^{-1} to the second month.

Initial alkaline phosphatases were very low and its activity increased in the first month with significant difference in all windrows when the pH increased. The highest alkaline phosphatase activity was recorded in control compost with 3.94 mU.g^{-1}.mn^{-1} and the lowest value of enzyme was 1.95 mU.g^{-1}.mn^{-1} in compost1. The activity of this enzyme decreased in the second month, increased again in the third month and decreased again at the end in all composts.

Compost1, compost2 and compost3 showed higher enzymatic activities than control compost. These results are consistent with the evolution of organic matter and C/N ratio and confirmed by the study of Albrecht et al. (2010). These three inoculated composts were found to be better degraded than the non inoculated control compost, implying that the the use of the two *Bacillus* strains would enhance organic matter biodegradation rate, and shorten the composting process.

Conclusion

The aim of this study was to use two *Bacillus* strains (*Bacillus* sp. and *B. subtilis*) as inocula during a composting with bagasse as substrate. These strains were tested as to whether they could enhance the biodegradation rate of sugar cane bagasse. Physicochemical and microbiological parameters were monitored to follow maturation of composts. Interestingly, *Bacillus* strains presented a high metabolic activity, and could reduce the composting time of sugarcane bagasse. Besides, these strains decreased the microbial microflora count when they were inoculated alone with significant difference compared to control and the other with mixed bacterial culture windrows. The obtained products would contribute and increase the fertilization of soils.

Conflict of Interests

The authors have not declared any conflict of interests.

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