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Evolution of the pathogen content during co-composting of winery and distillery wastes

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Abstract

The aim of this study was to monitor some microbial indicators and pathogen contents (sulphite reducers clostridia, total enterobacteriaceae, total coliforms, faecal coliforms (*Escherichia coli*), enterococci, *Staphylococcus aureus* and *Salmonella* spp.) throughout the co-composting of wastes from the winery and distillery industry with other organic residues, as well as the effect of the composting system used. Seven different piles using mixtures of winery–distillery wastes with other organic materials were prepared. P1 and P2 were made using grape stalk (GS), grape marc (GM), exhausted grape marc (EGM) and sewage sludge (SS), whereas in P3 and P4 were also used exhausted grape marc with cow manure (CW) and poultry manure (PM), respectively, using the Rutgers system. Additionally, P2 was watered with vinasse (V). The rest of piles (P5, P6 and P7) were prepared with grape marc, exhausted grape marc, cow manure and poultry manure, using the turning system. The effectiveness of the composting process to reduce the pathogen content was higher in the static aerated piles than in those elaborated with the turning. The relatively high temperatures (50–60 °C) reached in some of the piles produced a notable decrease in some microbial groups, such as total and faecal coliforms (*E. coli*), but the characteristics of the raw materials used notably influenced the pathogen contents of the end-product.

Keywords: Winery wastes; Distillery wastes; Compost; Pathogens

1. Introduction

The wine production of the Mediterranean countries represents about 65% of the entire worldwide production, being Spain, together with France and Italy, the greatest producers (Food and Agriculture Organisation, 2004). As a consequence of this powerful wine-producing activity, several wastes of great contaminating potential, mainly on account of their seasonal character (the peak production being during August–October) and some polluting properties, are generated. Grape stalk, grape marc and wine lee are the main solid wastes obtained during wine-making, while exhausted grape marc is the final solid waste from alcohol distilleries. On the other hand, winery wastewater and vinasse are the effluents produced in the different steps of wine production and after the distillation process, respectively.

In general, the winery and distillery solid wastes are characterised by an acidic pH, high organic matter, polyphenol and potassium contents, as well as significant levels of nitrogen, and phosphorus, being the last ones important factors in soil fertility. Winery wastewater and vinasse also show an acidic pH, a high organic load and notable polyphenol content, as well as significant heavy metal contents, especially in Pb (Bustamante et al., 2005).

Research studies on an agricultural use of the winery and distillery wastes are, nowadays, extremely limited and have

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been carried out using, especially, vinasse and grape marc. The direct incorporation of grape marc without any pretreatment into agricultural soils, a common practice, may cause an adverse effect derived from the release of several degradation products which can inhibit root growth (Inbar et al., 1991). On the other hand, the production of urban wastes, such as sewage sludge, as well as that of residues with an animal origin (cattle manure, pig slurries, poultry manure, etc.) is also increasing, with the associated problems that imply their management. Among different strategies suggested for using and valuating these wastes, composting is one of the most promising since it allows taking advantage of the nutritional and energy properties of these residues without causing environmental damage. Composting is defined as the aerobic biological decomposition and stabilisation of organic substrates, under conditions that allow development of thermophilic temperatures as a result of biologically produced heat. The co-composting of winery and distillery wastes with other organic residues has been studied by different authors. Ferrer et al. (2001) studied the co-composting process of grape waste and hen droppings and the effect of the application of the compost obtained as soil conditioner for corn seed germination in greenhouse, and Bertrán et al. (2004) reported the evolution of different parameters throughout the composting process of mixtures elaborated with grape stalk and sewage sludge in different proportions. The final product, compost, is a stable material which provides valuable benefits for plant growing by increasing soil fertility (Golueke, 1982; Haug, 1993). However, depending on the characteristics of the raw material and the management of the process, composts may also contain substances harmful to the environment such as pathogens, bioaerosols, heavy metals and toxic organics (Déportes et al., 1995). Moreover, a composting process not properly managed can induce the proliferation and dispersion of potentially pathogenic and/or allergenic thermotolerant/thermophilic fungi and bacteria, such as *Salmonella*, *Shigella*, *Escherichia coli*, *Enterobacter*, *Yersinia*, *Streptococci* and *Klebsiella*, among the bacteria, and *Aspergillus fumigatus*, among the fungi, which can emerge and cause infections for compost handlers and agricultural users (Hassen et al., 2001). It is necessary, therefore, to guarantee the sanitary safety of any compost previously to its application. Traditional indicator organisms, such as faecal coliforms, faecal *streptococci* and *E. coli*, are generally monitored to ensure compost quality (Sidhu et al., 1999). On the other hand, the composting system used can also influence the efficiency of the process and, thus, the quality of the end-product obtained. The turning system in windrows (conventional composting method) is often labor intensive, creates air pollution (e.g. dust) and requires additional space for the pile, though allows a better homogenization of the mixture. In contrast, forced-aerated composting is a more efficient composting method, which ensures temperature in the upper thermophilic range and its control, ensures adequate aeration and saves space compared to the turning system (Tiquia and Tam, 1998).

This paper is focused on the study of the sanitation capacity of two different composting systems, Rutgers and turning, in relation to different human pathogens and microbial groups used as contamination indicators, such as sulphite reducers clostridia, *E. coli*, *Staphylococcus aureus* and *Salmonella* spp. and total enterobacteriaceae and total coliforms, respectively.

2. Methods

2.1. Composting procedure

Seven different piles were prepared using in all of them wastes from the winery and distillery industry. The Rutgers static pile composting system was used in four piles and the turning system was used in the rest.

2.1.1. Piles using Rutgers system

Four different piles (P1, P2, P3 and P4) were prepared with mixtures of grape stalk (GS), grape marc (GM), exhausted grape marc (EGM), sewage sludge (SS), cow manure (CM) and poultry manure (PM) (Table 1). Fresh collected vinasse (V) was used in P2 for watering. SS came from a treatment plant of municipal wastewater placed in Torrevieja (Alicante). CM was collected from a cattle farm placed in Santomera (Murcia) with a productivity of 35.000 heads per year. PM was collected from a poultry farm with 30.000–40.000 laying hens located in Orihuela (Alicante). GS and GM used were collected from a winery placed in Jumilla (Murcia) and EGM and V from an alcohol distillery placed in Villarrobledo (Albacete). The analyses of the raw materials are shown in Table 2.

The mixtures (about 1800 kg each) were composted in a pilot plant, in trapezoidal piles (1.5 m high with a 2×3 m base), supplied with forced aeration conducted through three basal PVC tubes (3 m length and 12 cm diameter). Aeration system imposed was 30 s every 30 min, with 55 °C as ceiling temperature for continuous ventilation. Turning treatments for improving both homogeneity and fermentation process were applied when necessary (Table 1).

2.1.2. Piles using turning system

Three different piles (P5, P6 and P7) were prepared with winery and distillery wastes: GM and EGM. To these wastes were added two organic wastes rich in nitrogen: CM and PM. GM was collected from an alcohol distillery placed in Villarrobledo (Albacete), whereas the origin for the rest of organic wastes was the same as that of the wastes used in the piles using Rutgers system.

The mixtures (about 140 kg each) were composted in domestic thermo-composter (85 cm high with a 70×70 cm base and a 350 L volume). Turning times are detailed in Table 1.

In all the cases, both static and turned piles, the bio-oxidative phase of composting was considered finished when the temperature of the pile was stable and near to that of

Table 1
Characteristics of composting heaps

Waste[b]	P1		P2		P3	P4	P5	P6	P7
	Initially	After 17 days	Initially	After 17 days					
Composition[a]									
GS	63 (56)	44 (51)	63 (54)	44 (44)	–	–	–	–	–
EGM	25 (28)	18 (25)	25 (27)	18 (22)	70 (80)	70 (79)	70 (76)	–	70 (71)
GM	12 (16)	9 (15)	12 (16)	9 (13)		–	–	70 (72)	–
CM	–	–	–	–	30 (20)	–	30 (24)	30 (28)	–
PM	–	–	–	–	–	30 (21)	–	–	30 (29)
SS	–	29 (9)	–	29 (13)	–	–	–	–	–
V	–	–	0.4* (3)	0.8* (8)	–	–	–	–	–
Composting system	Rutgers		Rutgers		Rutgers	Rutgers	Turning	Turning	Turning
Turnings (days)	18–53–86		17–39–87		92	144	14–25–28	14–25–28	10–16–26–37
							40–53–67	40–53–67	49–63–84

* Data expressed as L/Kg.
[a] Data expressed as percentage on a fresh weight basis (dry weight basis in brackets).
[b] GS: grape stalk; EGM: exhausted grape marc; GM: grape marc; CM: cow manure; PM: poultry manure; SS: sewage sludge; V: vinasse.

Table 2
Microbiological characteristics of composted wastes

Waste[a]	SRC	TE	TC	FC	Enterococci	*S. aureus*	*Salmonella*
SS	$>1.00 \times 10^4$	1.02×10^8	$>2.40 \times 10^3$	1.10×10^3	$>2.40 \times 10^3$	$<3.00 \times 10^0$	D
CM	6.00×10^3	3.33×10^2	9.80×10^1	9.80×10^1	$>2.40 \times 10^3$	$<3.00 \times 10^0$	D
PM	$>1.00 \times 10^4$	3.60×10^4	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	3.20×10^2	D

SRC: sulphite reducers clostridia; TE: total enterobacteriaceae; TC: total coliforms; FC: faecal coliforms (*E. coli*).
[a] SS: sewage sludge; CM: cow manure; PM: poultry manure.

the surrounding atmosphere. Then, the piles were allowed to mature for two months. The moisture of the piles was controlled weekly by adding the necessary amount of water to obtain a moisture content not less than 40%. Excess water leached from the piles was collected and added again to the piles. Samples were obtained by mixing subsamples coming from seven different zones of the piles and they were extracted at the beginning of the process (I), at the thermophilic phase (T), at the end of the bio-oxidative phase (E) and at the mature phase (M) in order to analyse biologic, physical and chemical parameters. Additionally, samples from P1 and P2 were extracted when sewage sludge was added (S). Each sample was immediately frozen and kept for microbiological analysis.

2.2. Analytical methods

2.2.1. Microbiological assays

Samples were investigated in relation to the presence of different microbial pathogen groups according to methods described by association of official analytical chemists (AOAC). Microorganisms investigated were sulphite reducers clostridia (SPS Agar, 24–48 h at 42 °C), Total Enterobacteriaceae (McConkey Agar, 24 h at 37 °C), Total Coliforms (MPN with Lactose Broth and confirmation in Levine EMB Agar, 24–48 h at 37 °C), Enterococci (Rothe Broth, 24 h at 37 °C), *Staphylococcus aureus* (MPN with Giolitti–Cantoni, isolation in Mannitol Salt Phenol Red Agar and confirmation in DNAse Agar, 24 h at 37 °C) and *Salmonella* (pre-enrichment in Buffered Peptone Water, enrichment in Selenite–Cystine Broth, isolation in Hektoen Agar and confirmation in Kligler Medium, 18–24 h at 37 °C). *E. coli* was investigated from Total Coliforms confirmative EMB plates by isolating in McConkey Agar plates and confirmed by the Indole Test.

All the analyses were made in triplicate and the results were expressed as the number of colony-forming units per gram of compost or waste.

3. Results and discussion

3.1. Temperature evolution of the compost piles

The temperature is one of the main parameters to evaluate the composting process, since its value determines the rate at which many of the biological reactions take place as well as the sanitation capacity of the process. From a biological point of view, three are the intervals that govern the different aspects: temperatures above 55 °C to maximise sanitisation, between 45 and 55 °C to improve the degradation rate and between 35 and 40 °C to increase microbial diversity (Stentiford, 1996).

Fig. 1 shows the temperature curves of each pile together with the atmospheric temperature. In relation to the piles with the Rutgers system, P3 and P4 showed a longer thermophilic phase than P1 and P2, moreover the temperature within these piles reached higher values (>50 °C). The presence of grape stalk, a material that is

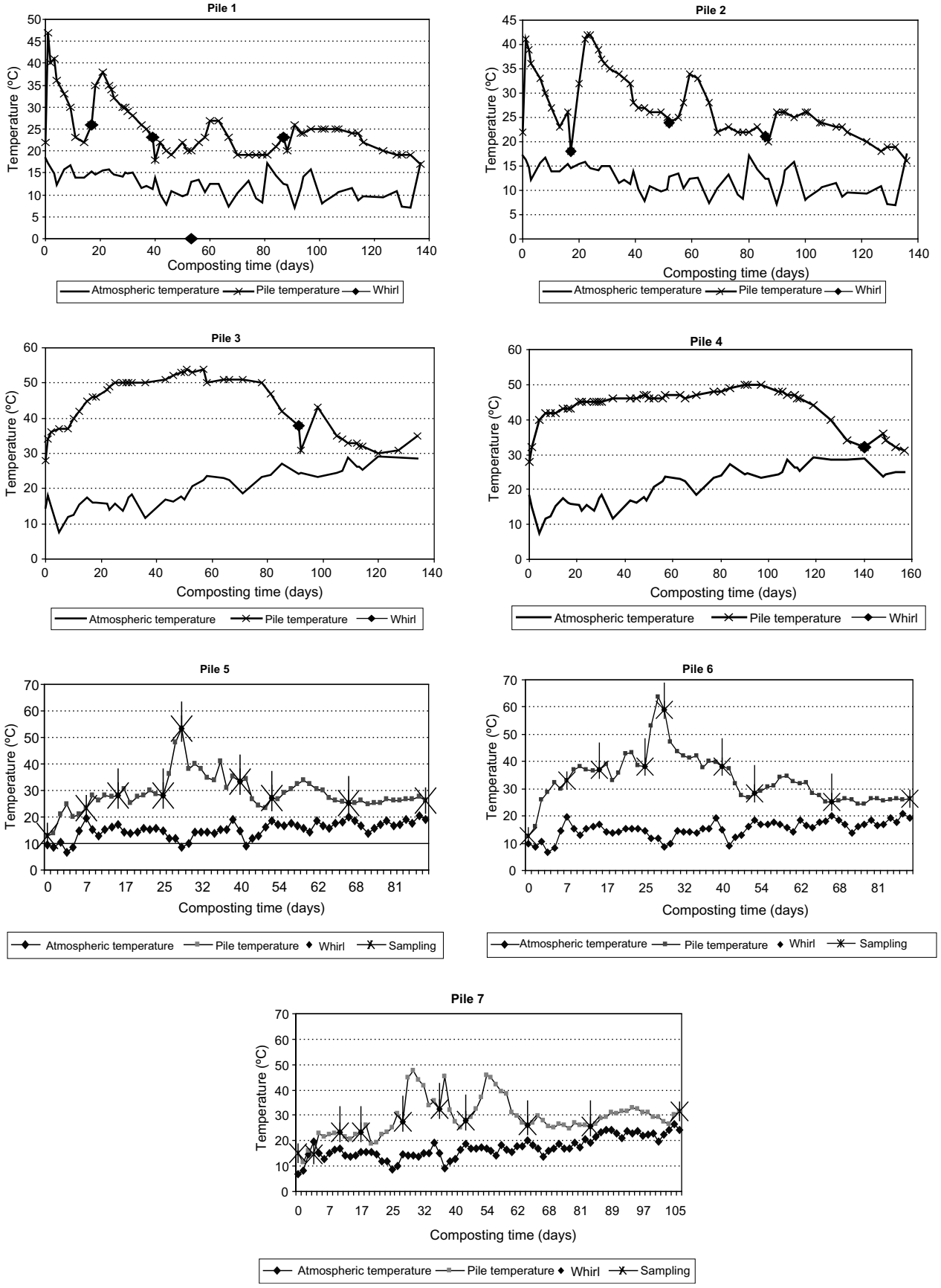

Fig. 1. Development of ambient temperature and temperature within the different piles during the composting process.

characterized by high polyphenolic content, in P1 and P2, probably was responsible of these differences. Polyphenolic compounds, especially tannins, could influence the composting process by means of their antimicrobial effect (Scalbert, 1991). Nevertheless, a rapid increase in the temperature was recorded for P1 and P2, so that on the first 20 days the maximum temperature values were reached. This fact was also observed in an experiment composting grape stalk with sludge (Bertrán et al., 2004).

On the other hand, the piles elaborated using the turning system (P5, P6 and P7), showed concrete temperature values similar to those observed for P3 and P4, but with a shorter thermophilic phase and a faster fell to environmental thermal values.

The piles P1, P2, P5, P6 and P7 were elaborated during winter. This fact could also explain that the temperature values reached in these piles were lower and the thermophilic phase shorter than in P3 and P4, which were performed during summer. In these conditions, the great difference of temperature between the piles and the environment favours an energetic flux from those to the surrounding atmosphere (Tiquia et al., 1997), which makes difficult to reach high thermal values.

3.2. Evolution of the microbiological parameters

3.2.1. Sulphite reducers clostridia (SRC)

The evolution of each microbial group in the composting piles throughout the different stages of the composting process is showed in Table 3. The presence of SRC (*Clostridium perfringens*) not only is interesting on account its pathogenic character but because of it can be a suitable indicator for other pathogens of faecal origin (Araujo et al., 2004).

None of the composting systems showed enough sanitation capacity to promote the total elimination of SCR. This is not a surprising result since microorganisms belonging to the genus *Clostridium* are among the most resistance to several adverse conditions, such as temperature or molecules with anti-microbial activity (Juneja and Marmer, 1998; Payment, 1999). Misterlich and Marth (1984) reported that clostridial spores survive a temperature of 100 °C at pH 6.0

Table 3
Evolution of microbial groups in different composting piles

Sample[a]	SRC	TE	TC	FC	Enterococci	*Staphylococcus aureus*	*Salmonella*
P1							
I	ND	ND	$<3.00 \times 100$	$<3.00 \times 10^0$	$>2.40 \times 10^3$	$<3.00 \times 10^0$	ND
T	ND	2.35×10^6	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	4.00×10^0	ND
S	4.03×10^3	8.47×10^6	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	6.00×10^0	ND
E	1.20×10^3	3.15×10^5	$>2.40 \times 10^3$	1.66×10^2	$>2.40 \times 10^3$	1.37×10^1	ND
M	1.00×10^3	2.70×10^6	$>2.40 \times 10^3$	$>2.40 \times 10^3$	3.36×10^1	$<3.00 \times 10^0$	ND
P2							
I	ND	8.60×10^2	$>2.40 \times 10^3$	5.15×10^1	1.75×10^2	$<3.00 \times 10^0$	ND
S	1.00×10^3	1.90×10^7	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	3.50×10^0	ND
T	6.00×10^2	8.93×10^5	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	7.00×10^0	ND
E	3.00×10^3	5.77×10^5	1.18×10^3	1.18×10^3	4.29×10^2	7.66×10^0	ND
M	4.87×10^3	9.73×10^5	9.11×10^2	9.11×10^2	4.17×10^1	$<3.00 \times 10^0$	ND
P3							
I	9.00×10^2	1.70×10^4	9.20×10^1	8.87×10^1	$>2.40 \times 10^3$	3.33×10^0	ND
T	ND	1.95×10^6	$>2.40 \times 10^3$	5.70×10^1	$>2.40 \times 10^3$	1.07×10^1	D
E	1.00×10^2	6.33×10^6	3.00×10^0	$<3.00 \times 10^0$	$>2.40 \times 10^3$	$<3.00 \times 10^0$	ND
M	1.00×10^1	1.75×10^7	9.00×10^0	9.00×10^0	$>2.40 \times 10^3$	$<3.00 \times 10^0$	ND
P4							
I	6.67×10^0	2.89×10^6	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	1.03×10^1	D
T	5.66×10^2	3.97×10^5	8.87×10^1	8.87×10^1	1.33×10^3	3.00×10^0	D
E	1.00×10^2	7.13×10^6	4.05×10^2	1.40×10^1	1.91×10^2	4.03×10^0	D
M	2.33×10^2	5.70×10^6	1.51×10^2	2.53×10^1	1.61×10^2	1.20×10^1	D
P5							
I	2.00×10^3	7.90×10^6	4.05×10^2	1.74×10^2	$>2.40 \times 10^3$	$<3.00 \times 10^0$	ND
M	3.67×10^1	2.93×10^5	$<3.00 \times 10^0$	$<3.00 \times 10^0$	8.08×10^2	$<3.00 \times 10^0$	ND
P6							
I	1.73×10^3	1.80×10^4	1.83×10^1	1.13×10^1	$>2.40 \times 10^3$	$<3.00 \times 10^0$	ND
M	$>1.00 \times 10^4$	7.13×10^6	4.66×10^1	1.77×10^1	$>2.40 \times 10^3$	1.83×10^0	D
P7							
I	$>1.00 \times 10^4$	2.27×10^6	$>2.40 \times 10^3$	$>2.40 \times 10^3$	$>2.40 \times 10^3$	1.66×10^2	D
M	$>1.00 \times 10^4$	1.46×10^6	2.03×10^1	1.77×10^1	$>2.40 \times 10^3$	$<3.00 \times 10^0$	D

SRC: sulphite reducers clostridia; TE: total *enterobacteriaceae*; TC: total coliforms; FC: faecal coliforms (*E. coli*).
[a] I: beginning of the process; S: sewage sludge addition; T: thermophilic phase: E: end of bio-oxidative phase; M: mature phase.

to 7.4 for more than 110 min, even though the vegetative cell shows similar resistance to other bacteria. Otherwise, the ubiquitous distribution of this group (Juneja et al., 2003) difficult the existence of free *Clostridium* environments, even in aerobic environments such as composting piles. In one hand, the spores can survive at those conditions (Böhnel and Lube, 2000; Jones and Martin, 2003) and, on the other hand, some anaerobic zones can persist that makes the growth of anaerobic bacteria possible (Pourcher et al., 2005). Probably, the greater level of SCR in the turning piles, generally above 10^4 cfu/g, in relation to Rutgers piles, below 10^3 cfu/g in almost all cases (Table 3), may be on account a lesser efficacy of the aeration system.

In the piles operated with the Rutgers system, the incorporation of SS (P1 and P2) favoured higher concentration of SCR. Nevertheless, a decrease in SCR counts was observed in P1, in contrast to P2, where the watering of the pile with V probably limited the efficiency of the process. In P3 and P4, the evolution in the SRC contents was also different. In P3, the number of SRC decreased from 9.00×10^2 cfu/g compost to 1.00×10^1 cfu/g compost, while in P4 increased from 6.67×10^0 cfu/g compost to 2.33×10^2 cfu/g compost. In P3, during the thermophilic phase, higher temperature values than in P4 were reached, thus a more effective elimination of SRC could have been produced.

3.2.2. Total enterobacteriaceae (TE)

The Enterobacteriaceae group includes microorganisms capable of fermenting glucose, such as those belonging to the genus *Salmonella*, *Escherichia* or *Enterobacter*. Although many of their members are enough important to be investigated on their own, as a group, this family is used as faecal contamination indicator. The contents of TE were high in all piles. In case of the piles elaborated with the Rutgers system, in P1 and P2, the incorporation of SS into the composting biomass produced a considerably increase in the number of TE. This was an expected result since enteric bacteria are the most prevalent group in sludge (Hay, 1996). Only in P2 a decrease in the TE contents was observed at the end of the composting process, from 1.90×10^7 to 9.73×10^5 cfu/g compost. In relation to the turning piles, P5 and P7 showed a slight decrease in the TE contents during the composting process, while P6 showed an increase.

3.2.3. Total coliforms

Since they are easily determinable and their presence is usual in wastes (Kelley and Walker, 1999) the total coliforms are also used as indicators of faecal pollution in environments related to soil and water (Hassen et al., 2001; Tallon et al., 2005). In the static aerated piles (Rutgers system), the evolution of total coliforms was very different. The highest contents of total coliforms were observed throughout the composting process in P1. However, in P2, P3 and P4, the number of total coliforms decreased at the end of composting. P3 and P4 showed the lowest values of total coliforms, probably due to the relatively high thermal values maintained for longer time than in the other piles. In general, the turning piles also showed lower contents of total coliforms in the mature samples, except in case of P6, which showed a slight increase from 1.83×10^1 to 4.66×10^1 cfu/g compost. This regrowth of coliforms has been also described by other authors (Hachicha et al., 1993; Hassen et al., 2001) and is usually attributed to recontamination phenomena.

3.2.4. Faecal coliforms (E. coli)

As the most representative member of the faecal coliforms (Déportes et al., 1998), and on account of its character as emerging pathogen (Hess et al., 2004), *E. coli* is one the microorganisms usually investigated in processes in which faecal related materials are integrated. The evolution of FC in all the piles was similar to those described for total coliforms. In P1 and P2, the addition of SS increased the number of *E. coli*. However, in P2, at the end of composting a decrease was observed in comparison with the *E. coli* values during the S phase ($>2.40 \times 10^3$ cfu/g compost to 9.11×10^2 cfu/g compost), although a secondary growth appeared in the maturation phase in P1. In case of P3 and P4, a decrease in the number of *E. coli* was observed, especially in P4, from $>2.40 \times 10^3$ to 2.53×10^1 cfu/g compost. This decrease was presumably the result of the high temperatures (50–55 °C), although these values did not promote a complete elimination of *E. coli*. Similar results have been registered by Hess et al (2004), who described a decrease and a consequently regrowth of *E. coli* at 50 °C. Pourcher et al. (2005) showed even temperature as high as 66 °C did not inactive completely this microorganism.

In the turning piles, the number of faecal coliforms decreased considerably in P5 and P7, being the values reached less than 2.00×10^1 cfu/g compost, even though the possible effect of redistribution during the turnings of the windrows formerly commented.

3.2.5. Enterococci

The enterococci (*Streptococcus faecalis*) are commonly considered as the best indicators of faecal pollution and they are characterised for being more resistant to different environmental factors than coliforms (Pourcher et al, 2005; Tallon et al., 2005). In P1 and P2 a considerably reduction in the number of enterococci was observed at the maturation phase, from $>2.40 \times 10^3$ to 3.36×10^1 and 4.17×10^1 cfu/g compost, respectively. P4 also showed a decrease in the enterococci contents throughout the composting process, especially due to the temperature values reached in this pile, because temperature is an important factor in the inactivation of these organisms (Jones and Martin, 2003; Hassen et al., 2001). However, the number of enterococci was maintained at the end of the composting in P3. The number of enterococci in the turning piles did not change in relation to the values at the beginning of the pro-

cess, except in P5, whose content was reduced from $>2.40 \times 10^3$ to 8.08×10^2 cfu/g compost. These counts at the maturation phase were higher than those observed for coliforms, which confirms the greater capacity of enterococci to persist throughout composting processes.

3.2.6. Staphylococcus aureus

Staphylococcus aureus is one of the main causes of collective toxic infections of food (Vernozy-Rozand et al., 2004). This microorganism also generates cutaneous infections that represent a risk for compost handlers and agriculturists during farm compost spreading (Tallon et al., 2005). Generally, levels of *S. aureus* detected throughout the different composting phases were low and modifications were scarce. The number of *S. aureus* increased during the bio-oxidative phase in P1 and P2, and at the thermophilic phase in P3, but decreased at the maturation phase in all piles, being the value less than 3.00×10^0 cfu/g compost. Only P4 showed a regrowth of *S. aureus*. In the turning piles, the number of *S. aureus* also decreased at the maturation phase, except in P5, where the number of *S. aureus* was maintained.

3.2.7. Salmonella

Salmonella is considered as the major and specific problem of the hygienic quality of compost (Brinton and Droffner, 1994; Hay, 1996; Yanko, 1995). Salmonellae come from food wastes, essentially from poultry, meats, milk and its derivatives, as well as some wastes, especially poultry waste. *Salmonella* was detected in all raw materials with an animal origin used. Among the piles elaborated with the Rutgers system, P1 and P2 did not show *Salmonella* in any stage of the composting process, and just one of the samples was positive in the pile P3. On the contrary, this microorganism was detected in all the analysis performed in the pile P4, the only one with poultry manure as ingredient of the composting raw material. Although *Salmonella* is not among more resistant microorganisms, some strains have been described to persist at temperature higher than 50 °C (Brinton and Droffner, 1995), maximal value registered for P4. In the turning piles, *Salmonella* was detected in P6 and P7. In P6 was only detected in the mature phase, probably due to a recontamination and in P7 was detected at the beginning and at the end of the process, possibly because this pile was also elaborated using as a raw material poultry manure.

4. Conclusions

In conclusion, in the static aerated piles (Rutgers system), in general, the contents of sulphite reducers clostridia, enterococci and *Salmonella* were reduced in a more effective way than in the turning piles. On the other hand, the occurrence of relatively high thermal values during the composting process not always ensures a complete sanitisation of the end-product. Other factors, among them humidity, nutrient availability, or competitive microbiota, can influence the ability for growing of some pathogens, such as *E. coli* or *Salmonella*. Therefore, in order to guarantee the effectiveness of the composting process to produce a sanitised compost material is necessary to monitor the process and especially the cooling phase, because in this phase a microbial regrowth or reactivation is also possible.

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