Definition of biochemical parameters in composting in a Chilean Pisco industry

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Abstract
Pisco industry in Chile generates liquid and solid wastes, whose disposal and recycling constitute an environmental problem. Wastes, including grape pomace, bunch stems and other material like chipped pruning material (only produced during April- June). Composting was proposed to treat these two organic wastes and goat manure for recycling its organic matter content to the vineyard crops. In this work, the thermophilic biodegradation of organic solid wastes during 120 d using several ratios of seeds- skin, and manure were studied. Temperature and EC (electrical conductivity) were determine weekly and microbial functional groups including celulolitic, amililitic, total fungi and yeast were determined, and enzymatic activities including urease, acid and alkaline phosphodiesterase, B glucosidase three times during the process. At the same time, chemical properties were determined to characterize compost quality: %Carbon, %Nitrogen, C/N ratio, pH and some humification index like %humic and fulvic acids and E_4/E_6 ratio. Biodegraded product showed good organic matter properties (pH 6.97-8.66; %HA 3.62- 19.1; %FA 1.54- 6.38 and statistical differences in ureases, phosphatase and B glucosidase activity) The results suggest that biodegraded grape marc could be used as fertilizer.

Introduction
Main factors influencing the composting process are temperature, water content, oxygen concentration in the composting matrix, porosity and free air space (FAS). Temperature is both a consequence of the composting process (microbial metabolism) and a control parameter. According to Haug, 1993 and Bernal *et al.* 2009 temperatures providing the maximum degradation velocity are in the range of 40-70 ^{o}C. The optimization of the composting process in wine and pisco industry is possible using goat manure, and stalk appears to be an ideal bulking agent for composting, providing C and physical properties such as porosity (provided by its branch- type structure) and resistance to biodgradation of the hard-wood fraction (Tuomela *et al.* 2000). Its chemical properties are also optimal. Stalk C/N ratio is high (around 39) and equilibrates the low C/N ratio of sludge (around 5). Cocomposting goal manure and pisco solid wastes as bulking agent would generate a stabilized fertilizer suitable for its application to the vineyard crops. Chemical and microbiological changes have been studied during manure composting under field conditions in windrows and heaps (Godden *et al.* 1983, 1986; N'Dayegamiye and Isfan, 1991; Martins and Dewes, 1992; Atallah *et al.* 1995) and there are different criteria for compost quality and maturity (Bernal *et al.* 2009). The aim of this study was to investigate the evolution of some physical, chemical and microbiological parameters in solid pisco industry wastes and goat manure in a composting system.

Material and methods
Description of the experiment
The compost is based on the raw materials available within Empresas Bauzá, located in the Coquimbo Region of Chile; these are pruning material, residues from the pisco industry, fruit remains, and goat manure. The total amount of each material is currently being surveyed. Compost will be manufactural and controlled using wireless temperature and moisture sensors. Before composting, raw materials will be analyzed for total C, N, lignin, and some other evaluations that can be of interest. After composting, some compost-derived products will be obtained. Each product will be analyzed in terms of its chemical composition, physical characteristics as well as microbial activity. Stalk, wheat straw and goat manure (in ratio 10:1; a practical ratio for the farm system used in the experiment), mixed at different ratios (Table 1). Automated thermocouple-sensors were placed along the length of between the windrows to determine the temperature daily. Weekly temperature was also defined using a thermometer inserted 50 cm and pH and electrical conductivity defined. Aeration was provided by mechanical turning weekly and the process was carried out for 14 weeks.

Table 1. Treatments evaluated- Proportion of different materials.

Treatment	horse manure	goat manure	grape residues	cane, oat residues	Total %
1	1		89	10	100
2	9	7	82	2	100
3	4		91	5	100
4		50	50		100
5		63	33	4	100
6	22	25	25	28	100
7	22	24	48	6	100
8	42	20	33	5	100
9		66	34		100

Samples for microbiological and chemical analysis were collected during composting from the infeed and from the material in the middle of the pile. The samples from the heaps were collected when the compost was turned and at the end of the experiment, after the whole heap was mixed thoroughly by hand.

Analytical determinations

Immediately after sampling, the samples (101) were transported to the laboratory and homogenized by hand. Subsamples were taken for immediate analysis of microbiological parameters, moisture, pH, conductivity and water-soluble nutrients, and for drying at 40°C. The rest of the sample was frozen and stored for later use. Total carbon was determined by Walkley & Black (1934). Ammonium was determined pH (in water and in 0.01 M $CaCl_2$, 1:3 fresh compost/liquid ratio, w/w), dry matter content (% fw, 105°C) were all measured in triplicate.

Microbiological analysis

The heterotrophic microbial populations were determined using the micro drop method. Fresh compost samples (4 g) and sterile saline solution 0,85 % (36 ml) were homogenized with a vortex, after which additional dilutions were made up to 10^{-6}. A 20µL aliquot of the dilutions 10^{-6}, 10^{-5} and 10^{-4} were cultivated in nutritive agar (Merck); when the drop was dry, the plates were incubated at 30°C for 48 hours to count the number of colonies in each drop and establish the UFC/g. From the same dilutions, count was made of cullulolytic, amilolytic, proteolytic, phosphate solubilizer microorganisms and total fungi and yeast by surface plate count method in specific media: cellulose agar, starch agar, milk agar, SMRS1 agar and potato dextrose agar (PDA Merck media) respectively. The plates were incubated at 30°C for 8 days except the fungi and yeast cultures which were incubated at 25° for the same time. For the count of the functional of enzymatic and solubilizer activity groups, it only the colonies who presented halo were counted; for the cases of the amilolytic and cellulolytic count, it was necessary reveal the activity by the addition of lugol (Merck) and congo red 1% (m/v) respectively.

Enzymatic activity

Urease activity

5 g of compost were moistened with 2.5 mL of urea 0.08 M and incubated for 2 hours at 37°C and 100 rpm; then 50 mL of KCl 1 N acidified in HCL 0.01 N was added and re incubated for 30 minutes at 100 rpm and room temperature. The suspension was filler with Whatman filter paper number 2 and 1 mL of the extract was used to determine the NH_4 produced with the colorimetrical method using indophenol blue (Mulvaney, 1996). The urease activity was expressed as µmol of NH_4 /g*h.

Phosphatase activity

This activity was determined by the methodology established by Dick *et al.* (1996), using 4 mL of Buffer MUB (pH 6,5 for acid phosphatase or pH 11 for alkaline phosphatase) and 1 mL of p-nitrophenil phosphate 0,05 M. The product p-nitrophenol was determined spectrophotometrically at 410 nm using a calibration curve with standar solutions of p-nitrophenol (0, 2, 4, 6, 8 and 10 µg/mL). The phosphatase units (UP) were expressed as µmol of p-nitrophenol /g*h.

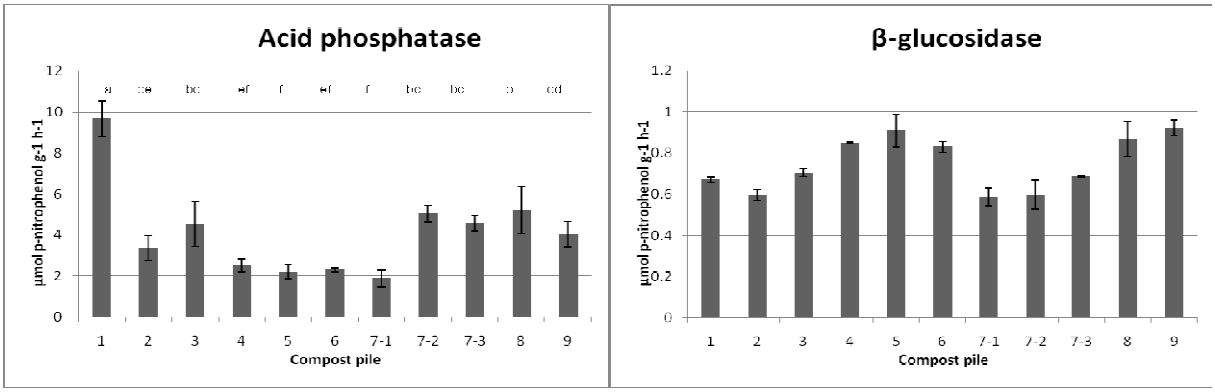
β Glucosidase activity

For the determination of this enzyme, the method established by Dick *et al.* (1996) was used, where 1 g of the compost was mixed with 0.2 mL of toluene, 4 mL of Buffer MUB (pH 6,0) 1 mL of p-nitrophenil β-D-glucopiranoside 0.05 M. The product p-nitrophenol was determined spectrophotometrically at 410 nm using a calibration curve with standard solutions of p-nitrophenol (0, 2, 4, 6, 8 and 10 µg/mL). The β glucosidase units (UBG) were expressed as µmol of p-nitrophenol /g*h.

Results

Compost piles showed a normal increase in temperature in week 1, reaching temperatures approaching 50 ° C (data not shown), which was maintained for 8 weeks. After this time the temperature dropped to reach environment temperature. The process was carried out in winter (12-4°C). Regarding the biochemical data obtained, there is a behavior associated with maturation of the material in the mixture for the treatments 4, 5 6 and 7 without statistical differences between them. Treatment No. 5 presents a lower concentration of B-glucosidase enzymes, and phosphatases, as well as a C / N of 12, meeting all the parameters in relation to food safety (absence of Salmonella sp., - Data not shown) and concentration heavy metals, as defined in rule NCh. 2880

Treatment	C/N	C	Cr	Cu	Ni	Pb	Cd	Zn
		%	(----------mg/kg----------)					
1	16	27	14.5	33	7.2	4.5	<0.01	36
2	13.5	29.8	7.3	31	6.4	4	<0.01	40
3	15.1	29.4	13.3	30	6.5	4.3	<0.01	35
4	13	26.4	6.2	31	8.5	5.8	<0.01	55
5	12.2	24.5	10.3	32	8.8	4.6	<0.01	57
6	15.1	31.1	12.5	33	7.2	5	<0.01	44.5
7	12.5	28.2	12	33	8.3	4.7	<0.01	43.5
8	11	24.1	9.3	36	9.1	5.5	<0.01	50
9	12	27.5	13	31	9.7	5.7	<0.01	58



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