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Evaluation of Mesophilic Biodegraded Grape Marc as Soil Fertilizer

Ana B. Moldes[1,3], Manuel Vázquez[2,*], José M. Domínguez[3], Francisco Díaz-Fierros,[1] and María T. Barral[1]

[1]Departamento de Edafología y Química Agrícola. Facultad de Farmacia. Universidad de Santiago de Compostela, España;
[2]Área de Tecnología de los Alimentos, Departamento Química Analítica, Escuela Politécnica Superior, Universidad de Santiago de Compostela-Campus de Lugo, 27002-Lugo, España, E-mail: vazquezm@lugo.usc.es;
[3]Departamento de Ingeniería Química. Facultad de Ciencias de Orense. Universidad de Vigo. Orense, España.

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Abstract

The wine industry generates a large amount of wastes, including grape marc and vinification lees. These substances can be used to produce enzymes or other food additives. Composting could be a successful strategy for the sustainable and complete recycling of grape marc. In this work, the mesophilic biodegradation of grape marc during 60 d under microaerobic conditions using several ratios of seeds, skin, and stem were studied. The presence of *Penicillium* spp. was detected at the beginning of the composting. Changes in chemical and biological parameters were evaluated. Biodegraded grape marc with stem showed the best organic matter properties (C/N ratio of 14 and N content of 37 g/kg) and a germination index of 155% for the growth of ray grass seeds. The results suggest that the biodegraded of grape marc could be used as fertilizer, especially for ray grass crops.

Index Entries: Biodegradation; compost; winery; grape marc; seeds; germination index.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

The wine industry produces large volumes of waste products *(1–5)*. These residues are an environmental problem and it is needed to recycling them. Composting is the biological decomposition and stabilisation of biodegradable components, which under controlled conditions can be used as soil conditioners and/or organic fertilizers.

The wastes from the wine industry include grape marc, vinification lees and even the water that is used to carry off the waste materials. Grape marc is composed mainly by cellulose, hemicelluloses and lignin. Cellulose is a long chain of glucose molecules, linked to one another primarily with β(1–4) glycosidic bonds. During composting, enzymes are required for cellulose degradation. Some microorganisms like *Aspergillus, Trichoderma* and *Penicillium* produce these enzymes. *Trichoderma resei* produces an enzymatic complex constituted by endoglucanases, exoglucanases and celobiase *(6–8)*. Cellulase and xylanase enzymes produced by *Penicillium janthinellum* NCIM 1171 hydrolyzed pretreated sugar cane bagasse *(9)* and *Penicillium brasilianum* IBT 20888 cultivated on three different carbon sources produced cellulolitic enzymes *(10)*. These microorganisms convert cellulose to microbial biomass and reduce the composting period.

Hemicelluloses are branched polymers of xylose, arabinose, galactose, mannose and glucose. Hemicelluloses bind bundles of cellulose fibrils to form microfibrils, which enhance the stability of the cell wall. They also cross-link with lignin, creating a complex web of bonds which provide structural strength, but also challenge microbial degradation *(11)*.

Lignocellulosic materials can be used as substrates for fermentation processes or additives extraction. The enzyme laccase was produced by solid state fermentation using seeds from grape bagasse as an inert support *(12)*. Vinification lees were used as nutritional medium for lactic acid bacteria *(3)* and trimming vine shoots as carbon source for lactic acid production *(4–5)*. Other food additives like antioxidants can also be extracted, for example from the grape bagasse *(13)*. However, the competition of other substrates sometimes delays the immediate use of grape marc for food additives production. In any case, some fractions always remain unconverted.

In order to reduce the environmental impact generated by the winery industry, it would be interesting the whole use of grape marc. Mesophilic biodegradation can be considered as an incomplete compost that could be a successful strategy for the sustainable complete recycling of grape bagasse and an alternative to the traditional disposal of residues. Some authors have reported that grape marc can be used as raw material for composting. Inbar et al. *(1)* composted grape marc, grape skin and seeds in windrows for 378 d without additives, obtaining C/N ratio of 19.9. Composted beet vinasse and grape marc in plastic bins at 55 ºC for 43 d followed by a second state at room temperature for 40 d gave C/N ratios in the range 21.9–32.8 *(2,14)*. In order to improve these results will be inter-

esting to reduce the C/N ratio and the composting period, being especially important the grape marc components.

With the aim of evaluating the influence of the grape marc composition in the mesophilic biodegradation process, different combinations of seeds, skins and stems were tested as substrates for biological decomposition under microaerobic conditions and the final products obtained were characterized by chemical analysis and biological tests to evaluate their effect as fertilizer.

Materials and Methods

Materials

Grape marc, from white wine-making technology, was supplied by Cooperativa Vitivinícola do Ribeiro (Ourense, Spain). At the beginning of the vinification process, the must was separated from the grape marc, which is considered a residue.

Experimental Procedure

Biodegradation processes were carried out at laboratory scale under micro-aerobiosis conditions by turning to keep the mass microbially active. Reactors of 12 cm diameter and 7 cm height were loosely filled with different substrates. Substrates consisted in grape marc with stem (13.5% w/w stem, 50.3% w/w skin and 36.2% w/w seeds); grape marc without stem (58% w/w skin and 42% w/w seeds); skin of grape marc and mashed seeds. Experiments were carried by triplicate during 2 mo at room temperature (19–21°C).

Analytical Methods

For all analyses, substrates were sieved to <20 mm and homogenized. The substrates were milled prior to carbon, nitrogen and elemental analysis. Electrical conductivity (EC) and pH were determined in aqueous extract (substrate/extractant ratio: 1/10 v/v) by adding 100 mL of water to 10 g of fresh sample. Following after 1 h of shaking the pH value and the EC in the suspension was determined using a pH meter Crison and a conductivity meter (HANNA HI 9033), respectively.

Moisture (M) was determined by drying at 105°C until constant weight. Total organic matter content (TOMC) was determined by weight loss on ignition at 450°C for 16 h.

Total nitrogen content was evaluated by Kjeldahl digestion and steam distillation. The soluble content of nitrate, ammonia and magnesium was determined after extraction of the substrate with 0.0125 M $CaCl_2$ solution (ratio 1:10) using the methods proposed by the German Federal Compost Quality Assurance Organization *(15)*. After extraction, nitrate and ammonia were measured by steam distillation, and magnesium by flame photometry.

The soluble content of phosphorous and potassium was determined after the extraction of fresh compost with a solution of calcium acetate, calcium lactate and acetic acid buffered to pH 4.1 (ratio 1:10). In the extract, phosphorous was measured with a spectrophotometer at 840 nm and potassium by flame photometry.

Analyses of the main fractions (cellulose, hemicelluloses and Klason lignin) of initial grape marc and composted grape marc were carried out by performing a quantitative acid hydrolysis under standard conditions *(16)*.

Penicillium was isolated employing potato dextrose agar as solid culture medium for the detection and enumeration of yeast and moulds in foods. *Penicillium* was identified on the base of vegetative and general characteristics.

The maturity of biodegraded grape marc was evaluated by measuring the phytotoxicity level of water extracts of compost samples, and calculating the germination index *(17)*. Three species, namely garden cress (*Lepidium sativum L.*), barley (*Hordeum vulgare L.*) and ray grass (*Lolium perenne L.*) with three replicates were used for the test. After 5 d of incubation in the dark, the seed germination percentage and root length of seedlings immersed in the compost extracts as well as in deionized water were determined. The values obtained for the deionized water were used as the control. The germination index (GI) was calculated as follows: $GI = G \times (La)/Lc$, where G is the number of germinated seeds expressed as percentage of control values, La is the average value of root length in the compost extracts and Lc is the average value of root length in the control.

Statistical Analysis

Chemical analyses and biological tests were carried out by triplicate and means are given. Data were analyzed by analysis of variance using SPSS statistical software package and significant differences were assessed by Tukey's multiple range test ($p < 0.05$).

Results and Discussion

The substrates evaluated for biodegradation were: (1) grape marc with stem; (2) grape marc without stem; (3) skins of grape marc and (4) mashed seeds. After 5 d of composting, the massive presence of *Penicillium spp.* was identified by microscopy in all the substrates.

Table 1 shows the composition of the main fractions of grape marc at the beginning and at the end of the mesophilic biodegradation process after 60 d. During composting, it is speculated that extracellular enzymes from *Penicillium spp.* broke the cellulose and hemicellulose chains to cellulosic and hemicellulosic mono or disaccharides such as glucose, cellobiose, manose, xylose, arabinose, or rhamnose. Usually, during the process the degradation of cellulose is very slow because all the microorganisms present in organic wastes have not the ability to hydrolyze the long chains of cellulose. In this work, the cellulose content decreased down to 49% after

Table 1
Change in Composition of Grape Marc (in % of dry weight) at the Beginning and the End of the Biodegradation Process[a]

	Initial grape marc	Biodegraded grape marc with stem	Biodegraded grape marc without stem
Cellulose (%)	18.2b	9.3a	9.7a
Lignin (%)	56.7a	63.3b	66.0c
Hemicellulose (%)	8.0b	2.6a	3.0a

[a]Values are the means of three determinations and different letters in each row indicate significant differences ($p < 0.05$).

Table 2
Physicochemical Characterisation and Change in Nitrogen and Carbon in Initial Grape Marc (Control) and Biodegraded Materials[a]

	Grape marc	Composted skin	Composted mashed seeds	Composted grape marc without stem	Composted grape marc with stem
Moisture (%)	72.2b	73.3b	26.6a	72.7b	73.5b
pH	3.8a	8.2c	8.0c	7.5b	7.5b
EC (dS/m)	0.04a	0.06b	0.08b	0.03a	0.04a
TOMC (g/kg)	945c	896a	938c	917b	902a
C (g/kg)	548c	520a	544c	532b	523a
N (g/kg)	22a	31b	20a	38c	37c
C/N ratio	24.5	16.7	27.2	14.0	14.1
$N\text{-}NO_3$ (mg/kg)	0a	0a	18.9b	21.0b	98.0c
$N\text{-}NH_4^+$ (mg/kg)	192c	11.2a	385d	44.8b	17.0a

[a]Values are means of three determinations and different letters in each row indicate significant differences ($p < 0.05$).

two months of composting, whereas the decrease in hemicelluloses was down to 65%. There was not statistic difference between the cellulose and hemicellulose content of grape marc composted with or without stem. Lignin showed a slow increase up to 16%. The lignin increase is attributed to the effect of concentration by the decomposition of the other compounds and not due to the generation of lignin.

Table 2 shows some physicochemical parameters of grape marc that are important to evaluate their quality as soil fertilizer. Grape marc showed an acid pH (3.8). This is compatible with the biodegradation because it is known that composting is possible in the pH range of 3.0 to 11.0. After mesophilic biodegradation, the four treatments studied showed an increase in the pH up to values of 7.5–8.2. The alkaline pH at the end of the process has also been reported in composting processes using different raw materials *(18–21)*.

The EC value of the initial grape marc was low (0.04 dS/m). EC showed no change during the biodegradation of grape marc with or without stem. This is probably due to the no-release of soluble salts through organic decomposition *(22)*. In composted skins or composted mashed seeds, the measured EC values were lightly higher (0.06–0.08 dS/m), but the tolerable level (2 dS/m) was not exceeded *(23)*.

The TOMC decreased in the biodegraded grape marc with or without stem. For raw grape marc, some risk exists of *N* immobilization in soil after the addition of waste, as a result of the absorption of available *N* by microorganisms. However, the mesophilic biodegradation stabilizes the organic matter and decreases the C/N ratio, thus reducing the risk of *N* immobilization. The *N* content increased in the treated materials. The highest increase corresponded to the biodegraded grape marc without stem (38 g/kg). The C/N ratio of the grape marc was 24.5. The usual recommended range for C/N ratios at the beginning of composting process is about 30, but this ideal ratio may vary depending on the carbon source. As carbon is converted to CO_2 (and assuming minimal nitrogen losses), the C/N ratio decreases during the process, with the ratio of finished compost typically close to 10 or 15, although composted grape marc at 55°C obtaining a final C/N ratio of 33 *(2)*. The final C/N ratio showed that the mesophilic biodegraded grape marc was mature (C/N ratio of 16.7–14.1), except for the biodegraded mashed seeds (C/N ratio of 27). This possibility should be confirmed by biological tests that confirm the higher phytotoxic effects of biodegraded mashed seeds (discussed later).

Concerning the inorganic *N* content, biodegraded mashed seeds showed the highest content of $N\text{-}NH_4^+$, whereas biodegraded grape marc with stems (13.5% stem, 50.3% skin and 36.2% seeds) showed the highest $N\text{-}NO_3$ concentrations.

In our experiments, no changes in temperature were observed in the reactors. This fact can be attributed to the low quantities of waste materials composted at a laboratory scale, and to the fact that grape marc has low concentrations of easily biodegradable components which otherwise would be quickly decomposed by microorganisms, thus increasing temperature.

Table 3 shows the available phosphorus, potassium and magnesium. Mashed seeds showed the highest contents of available phosphorus (617 mg/kg) indicating a mineralization of the samples. On the other hand, Composted grape marc with steam showed a lower value (335 mg/kg) than the original grape marc (420 mg/kg) suggesting a fixation, precipitation or microbial immobilization.

All biodegraded materials showed a decrease in available potassium, being the lowest value (2586 mg/kg) for the biodegraded grape marc with stem. Biodegraded skins showed the highest potassium concentration (5233 mg/kg), that it is similar to the potassium content of the raw grape marc (5480 mg/kg).

Table 3
Mineral Characterization in Raw Material (Control) and Biodegraded Materials[a]

	Initial grape marc	Biodegraded skin	Biodegraded mashed seeds	Biodegraded grape marc without stem	Biodegraded grape marc with stem
P (mg/kg)	420b	420b	617c	567c	335a
K (mg/kg)	5480c	5233c	4424b	4548b	2586a
Mg (mg/kg)	177b	193c	212d	188bc	53a

[a]Values are the means of three determinations and different letters in each row indicate significant differences ($p < 0.05$).

Mashed seeds showed the highest contents of available magnesium (212 mg/kg). The finished biodegraded grape marc showed magnesium, phosphorus and potassium content above the minimum acceptable, according to the specifications recommended by the European Union for these kinds of materials *(17)*.

The GIs combine measurements of relative seed germination and relative root elongation. In our study garden cress, barley and ray grass were used. A soil fertilizer is mature when the GI is over 80% *(24)*. GI for the initial grape marc was determined and a value of 1.9% was obtained for barley, 0% for garden cress and 0% for ray grass. The restriction in germination can be attributed to the low pH (3.8) and/or to phytotoxic compounds, such as ethanol, acetic acid, or lactic acid coming from the degradation of single sugars by microorganisms. This phytotoxic effect demonstrates that the direct disposal of this waste in the fields could be harmful for crops.

Figure 1 shows the GI of garden cress seeds grown on the biodegraded materials. Biodegraded grape marc with or without stem showed values over 80%, hence they can be considered mature fertilizers. From the statistical point of view, biodegraded grape marc with stem showed the best behaviour for growing cress seeds and biodegraded skin and biodegraded mashed seeds gave the worse results.

Figure 2 shows the GI of barley seeds grown on the biodegraded materials. Values in the range 62–89% were obtained for the four materials studied. The statistical analysis indicated that there is not difference in the GI for the four composted materials. This results are in accordance with the strict requirements of barley seeds.

Figure 3 shows the GI of ray grass seeds grown on the composted materials. biodegraded skin and biodegraded mashed seeds showed low values (51–64%). However, biodegraded grape marc, with our without steam, showed very high GI, 125% and 169%, respectively. In this range, a GI of 155% has also been reported for the same seeds grown on the co-composting

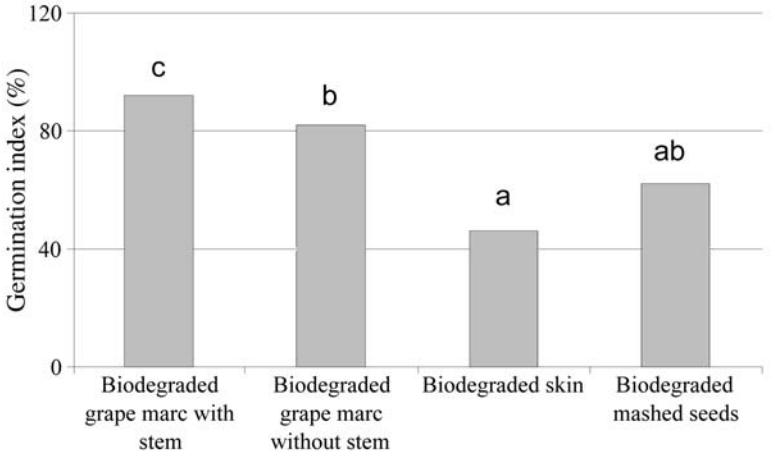

Fig. 1. Germination index of garden cress (*Lepidium sativum*) seeds grown on the biodegraded materials. Different letters indicate differences ($p < 0.05$) between treatments.

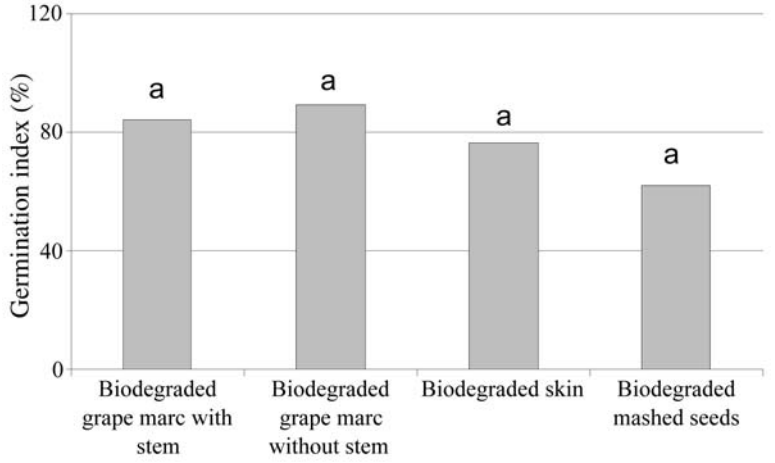

Fig. 2. Germination index of barley (*Hordeum vulgare*) seeds grown on the biodegraded materials. Different letters indicate differences ($p < 0.05$) between treatments.

of chestnut burr/leaf litter with solid poultry manure *(20)*. GI values above 100% indicate the positive influence of some compounds present in the water extract of the biodegraded grape marc.

Among the three seeds employed in the biological methods, ray grass seeds gave similar behaviour than cress seeds. Statistically, it can be said that biodegraded grape marc with stem produced the best GI for ray grass seeds whereas biodegraded mashed seeds and biodegraded skin produced the worse GI.

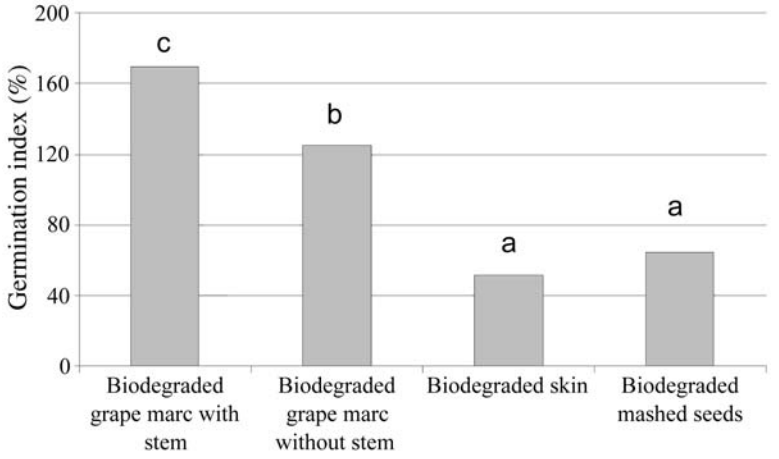

Fig. 3. Germination index of ray grass (*Lolium perenne*) seeds grown on the biodegraded materials. Different letters indicate differences ($p < 0.05$) between treatments.

During biological assays it was observed that grape marc composted with stem gave the best GI. This fact can be due to that grape marc with stem is less compacted than skin or mashed seeds, therefore it can be supposed that during biodegradation this material present higher air content in comparison with skin or mashed seeds.

The statistical analysis also indicated that there is not difference in the GI among composted skin and composted mashed seeds. Therefore the results suggest that composted grape marc with stem can be used as fertiliser in ray grass crops.

Conclusions

Mesophilic biodegradation of grape marc during 60 d decreased the C/N ratio while maintaining nutrient concentrations. On the basis of biological test, biodegraded grape marc with stem could be used as fertilizer, especially for ray grass crops.

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References

1. Inbar, Y., Chen, Y., and Hadar, Y. (1991) *Soil Sci.* **152,** 272–282.
2. Díaz, M. J., Madejón, E., López, F., López, R., and Cabrera, F. (2002) *Process Biochem.* **37,** 1143–1150.
3. Bustos, G., Moldes, A. B., Cruz, J. M., and Domínguez, J. M. (2004) *J. Agric. Food Chem.* 52, 5233–5239.

4. Bustos, G., Moldes, A. B., Cruz, J. M., and Domínguez, J. M. (2005) *J. Sci. Food Agric.* **84,** 2105–2112.
5. Bustos, G., Moldes, A. B., Cruz, J. M., and Domínguez, J. M. (2004) *J. Sci. Food Agric.* **85,** 466–472.
6. Phillips, J. A. and Humprey, A. E. (1982) *Liquid Fuel Dev.* **00,** 65–95.
7. Schurz, J., Billiani, J., Honel, A., et al. (1985) *Acta Polymerica* **36,** 76–80.
8. Camacho, R., González, T., Jurado, A., and Páez, D. (1986) *Ing. Quím.* **18,** 117–123.
9. Adsul, M. G., Ghule, J. E., Shaikh, H., et al. (2005) *Carbohydrate Polymers* **62,** 6–10.
10. Jorgensen, H. and Olsson, L. (2006) *Enz. Microbial. Technol.* **38,** 381–490.
11. Ladisch, M. R., Lin, K. W., Voloch, M., and Tsao, G. T. (1983) *Enz. Microbial. Technol.* **5,** 82–102.
12. Rodriguez-Couto, S., Lopez, E., and Sanrroman, M. A. (2006) *J. Food Eng.* **74,** 263–267.
13. Cruz, J. M., Domínguez, H., and Parajó, J. C. (2004) *J. Agric. Food Chem.* **52,** 5612–5620.
14. Madejón, E., Díaz, M., López, R., and Cabrera, F. (2001) *Biores. Technol.* **76,** 275–278.
15. FCQAO: Federal Compost Quality Assurance Organization (1994), Methods book for the analysis of compost. Bundesgütegemeinschaft Kompost e.V. Germany
16. Browing, B. L. (1967) *Methods of Wood Chemistry*. John Wiley & Sons, New York.
17. Zucconi, F., Monaco, A., Forte, M., and De Bertoldi, M. (1985), in *Composting of Agricultural and Other Wastes* (Gasser, J. K. R., ed.). Commission European Communities, Elsevier Applied Sciences, London: pp. 73–86.
18. Bangar, K. C., Kapoor, K. K., and Mishra, M. M. (1988) *Biol. Wastes* **25,** 227–231.
19. Guerra-Rodríguez, E., Díaz-Raviña, M., Vázquez, M. (2001) *Biores. Technol.* **78,** 107–109.
20. Guerra-Rodríguez, E., Díaz-Raviña, M., and Vázquez, M. (2001) *J. Sci. Food Agric.* **81,** 648–652.
21. Guerra-Rodríguez, E., Vázquez, M., and Díaz-Raviña, M. (2000) *Biores. Technol.* **75,** 223–225.
22. Wong, J. W. C., Fang, M., Li, G. X., and Wong, M. H. (1997) *Environ. Technol.* **18,** 563–568.
23. Tiquia, S. M. and Tam, N. F. Y. (2000) *Biores. Technol.* **72,** 1–7.
24. Saviozzi, A., Levi-Minzi, R., Riffaldi, R., and Benetti, A. (1992) *Biocycle* **Feb,** 72–75.