Using a Second-Order Polynomial Model to Determine The Optimum Vinasse/Grape Marc Ratio For In-Vessel Composting

M.J. Díaz¹, E. Madejón², F. Cabrera², L. Jiménez³ and M. De Bertoldi⁴

1. Departamento de Ingeniería Química, Facultad de Ciencias Experimentales Campus del Carmen, Universidad de Huelva, Huelva, Spain

2. Instituto de Recursos Naturales y Agrobiología de Sevilla (CSIC), Sevilla, Spain

3. Departamento de Ingeniería Química, Campus de Rabanales, Universidad de Córdoba, España

4. Dept. of Industrial Microbiology, University of Udine, Udine, Italy

This paper evaluates the influence of operating conditions (pH, Kjeldahl-N losses, NO_3^- -N, NO_4^- -N, C/N ratio and biodegradation) used in the vinasse/grape marc incubation on the properties of products obtained to determine the best incubation conditions (time and amount of vinasse proportion) to aid in the design of an in-vessel composting system. A second-order polynomial model consisting of two independent process variables was found to accurately describe, with <10% differences between experimental values and model prediction, the vinasse-grape marc incubation. The dependent variables measured were pH, Kjeldahl-N losses, NO_3^- -N, NO_4^- -N, C/N ratio and biodegradation and the independent process variables were operation time and amount of vinasse. Results determined products with acceptable chemical properties, high biodegradation and germination index and minimum Kjeldahl-N losses entails operating at medium-to-short operation time (20-35 days) and medium-to-low vinasse percentages (0-20%).

Introduction

Beet vinasses are beet molasses almost completely biochemically desugarized, distilled and sometimes concentrated. Vinasse (V) presents two main environmental problems: high organic load (61000-70000 mg $\text{O}_2 \text{ l}^{-1}$) and high salinity (EC 250-300 dS m^{-1}) (López *et al.* 1992). The high levels of N (30 g kg^{-1}), K (30 g kg^{-1}) and organic matter (350 g kg^{-1}) of the concentrated vinasse can be beneficial factors in recycling this waste for agricultural purposes. However, the direct application of vinasse to land has some drawbacks because of its high salinity, P content (P 0.06 g kg^{-1}) and dense liquid character (1.3 g cm^{-1}) (López *et al.* 1992).

Grape marc, a primary waste of wine production, could be recycled as a soil conditioner due to its organic matter (850 g kg^{-1}) and nutrient contents (N 15 g kg^{-1} , P 5 g kg^{-1} , K 10 g kg^{-1}). The direct incorporation of grape marc into agricultural land, a common practice, has proved to be a serious problem since the degradation products can inhibit root growth due to presence of phenols, tannins, etc. (Inbar, *et al.* 1991).

An alternative to overcome problems found in the direct application of vinasse and grape marc to soil and to recycle both products is cocomposting. The success of the composting process depends on several

basic conditions including: the moisture content (50-60%) (Crawford 1983, Jeris and Regan 1973, a,b), effectiveness of the aeration (Haug 1993, Choi 1999), the C/N ratio of the initial mixture (20-30) (Kipp 1992), and reaching a temperature of 50-60°C (Shulze 1962; McGregor *et al.* 1981; McKinley *et al.* 1985; Stentiford 1996; Smärs *et al.* 1999). Another important parameter is the correct proportion of each material.

This work aims to elucidate the optimum vinasse/grape marc ratio in order to determine the best composting conditions (operation time and vinasse) to achieve a correct composting design (adequate physico-chemical characteristics and minimum costs).

Materials and Methods

Relevant characteristics of the raw materials and initial mixtures are reported in Table 1. The experiments were carried out in plastic bins (35 x 20 x 30 cm). The walls and bottom of each bin were perforated with 100 cm^2 holes. The mixtures (ca. 5 kg) were placed in the bins and incubated in thermostatic chambers at 55°C provided by a blower (70 l h^{-1}) that delivered air continuously in the thermostatic chamber during the entire period of composting. A randomized complete block design with four replicates per mixture was used. Mois-

TABLE 1.
Chemical characterisation^a of the raw materials, the initial mixtures and the final composts (day 50).

		V ^f		GM ^g		Mixtures With 20% Of Vinasse		Mixtures With 40% Of Vinasse	
		AV ^d	SD ^e	AV	SD	AV	SD	AV	SD
Day 1									
pH		4.7	0.4	6.8	0.6	5.2	0.5	5.0	0.7
OM ^b	g kg ⁻¹	270	14.1	846	43.7	798	41.3	776	40.2
Nc	g kg ⁻¹	25.0	1.3	12.5	0.8	18.0	1.0	20.1	0.9
NO ₃ ⁻ -N	mg kg ⁻¹	350	17.7	410	21.1	720	37.1	1120	58.0
NH ₄ ⁺ -N	mg kg ⁻¹	50	2.6	10	0.4	20	1.1	35	1.9
C/N		6	0.6	37.5	2.0	23.4	1.1	21.4	1.2
P	g kg ⁻¹	0.3	0.1	3.1	0.4	2.0	0.5	1.9	0.4
K	g kg ⁻¹	30	1.4	10.5	0.7	14.5	0.9	16.8	0.9
Ca	g kg ⁻¹	3.4	0.5	27.3	1.1	26.3	1.3	28.5	1.3
Mg	g kg ⁻¹	5.0	0.6	4.2	0.4	5.4	0.4	6.9	0.6
Day 50									
pH				8.2	0.5	8.0	0.4	8.1	0.7
OM	g kg ⁻¹			695.0	36.1	667.0	34.6	666.0	34.3
N	g kg ⁻¹			11.7	0.8	16.3	0.7	14.9	0.8
NO ₃ ⁻ -N	mg kg ⁻¹			23.6	1.3	39.2	1.9	97.4	4.7
NH ₄ ⁺ -N	mg kg ⁻¹			3.0	0.5	4.0	0.7	5.0	0.5
C/N				36.5	1.7	22.3	1.3	25.7	1.2
P	g kg ⁻¹			3.4	0.5	3.3	0.5	3.1	0.3
K	g kg ⁻¹			13.8	0.9	18.8	1.0	21.9	1.2
Ca	g kg ⁻¹			28.7	1.5	27.4	1.5	30.7	1.5
Mg	g kg ⁻¹			4.9	0.5	5.9	0.6	7.7	0.7

^aEach value is the average of four samples, dry weight basis; ^bOM=Organic matter; ^cN=Kjeldahl-N; ^dAV=Average; ^eSD=Standard deviation; ^fV=Vinasse; ^gGM=grape marc

ture content was established at 55%, for the initial materials during the thermophilic phase, moisture losses were compensated by daily addition of water and manual mixing during incubation. By mass balance, after moisture characterization, the necessary water to maintain 55% of moisture was calculated. Particle size of grape marc (< 2.36 cm) was the same for all experiments. Vinasse absorbed in the grape marc did not modify the initial particle size. The vinasse and the operation time of the different experiments of the factorial design were 0%, 20% and 40% and 20, 35 and 50 days respectively.

Samples were collected and separated in two fractions (wet and dry analyses). Moisture was determined by drying at 105°C to a constant weight. For dry sample analyses, subsamples were dried (60°C) and ground (0-25 mm). The mixtures were analyzed, and results expressed on a dry matter basis, for the following parameters: pH (1:5 w/v) using a pH electrode, total organic matter (OM) by loss on ignition (550°C for 5h) (Allison 1965), total P (acid digest) using the ascorbic acid method (APHA 1995), total K, Ca and Mg (acid digest) by atomic absorption spectrophotometry (APHA 1995), Kjeldahl-N (Bremnen 1996) and NH₄⁺-N, NO₃⁻-N using the KCl extraction method (Mulvaney 1996)

and germination index (Gi) was determined using seeds of *Lepidium sativum* L. The number of germinated seeds and the primary root length were measured and expressed as a percentage of the control. The Gi was obtained by multiplying the germination percentage by the root length percentage, divided by 100 (Zucchini *et al.* 1985). Biodegradation, a parameter that relates initial and final content of compost organic matter during composting, based on ash conservation (Haug 1993, Tchobanogolus *et al.* 1984), was also calculated for each mixture. Biodegradation, K_b, was calculated using the equation (Haug 1993).

$$K_b = \frac{[(OM_i - OM_f)100]}{[OM_i (100 - OM_f)]} \dots \dots \dots (1)$$

where:

OM_f is the organic matter content (%) at the end of the process (dry matter basis) and

OM_i is the organic matter content (%) at the beginning of the process (dry matter basis).

The Kjeldahl-N losses during the process have been calculated as percentages, based on the initial content of N-Kjeldahl, and the evolution of the organic matter, assuming the mineral amount in each mixture is constant.

The main factors controlling cocomposting systems include: environmental parameters (temperature, moisture content, pH, aeration, operation time) and substrate nature parameters (particle size, and nutrient contents).

The process parameters were fixed at the beginning of the experiment, excepting pH, operation time and the vinasse (i.e. vinasse/grape marc ratio). Operation time and vinasse were selected as the independent variables. Time was selected due to its influence on the volume of the composting reactor. Vinasse proportion was chosen because of the importance of the amount of vinasse in the quality of the resulting compost. Kjeldahl-N losses, NO_3^- -N, NH_4^+ -N, C/N ratio, biodegradation and GI were the dependent variables, which were observed in relation to the independent variables. GI was used as an indication of maturity because of its sensitivity to toxic substances and speed of germination.

Experimental Design for the Incubation Process

In order to relate the dependent (pH, Kjeldahl-N losses, NO_3^- -N, NH_4^+ -N, C/N ratio, biodegradation and germination index) and independent (time and vinasse) variables with the minimum possible number of experiments, a 2^n central composite factor design for two factors was used that enabled the construction of second-order polynomials in the independent variables and the identification of statistical significance in the variables (Montgomery 1991, Aknazarova and Kafarov 1982). This design meets the general requirement that every parameter in the mathematical model can be estimated from a fairly small number of experiments. The total number of observations required (N) for two independent variables (time and vinasse) was calculated using the following equation:

$$N = 2^K + 2 \cdot K + 1 \dots \dots \dots (2)$$

where K is the number of independent variables, 2 in this design and therefore, N is 9. The central combination for the experimental design was as follows: $t = 35$ days and vinasse (V) = 20%. The results were subjected to multiple linear regression as implemented in SAS system package to discard non-significant variables, i.e. those variables with Snedecor's F coefficients values less than 4 (Aknazarova and Kafarov 1982). The independent variables values were normalized (X_n) from -1 to +1 using equation 3 to improve the comparison of the coefficients and the visualization of the effects of the individual independent variables on the response surface. This normalization also resulted in more accurate estimation of the regression coefficients as it reduced interrelationships between linear and quadratic terms (Montgomery 1991).

$$X_n = \frac{X - \bar{X}}{(X_{\max} - X_{\min})/2} \dots \dots \dots (3)$$

where X is the real value of a independent variable, $\bar{X}$ is its average value and $X_{\max}$ and $X_{\min}$ are its maximum and minimum values, respectively.

The independent variables used in the equations relating to both types of variables were those having a statistically significant coefficient (viz. those not exceeding a significance level of 0.05 in Student's t-test and having a 95% confidence interval excluding zero).

The amount of vinasse and operation time used in the different experiments of the central composite experimental design were 0%, 20% and 40% and 20, 35 and 50, days respectively. Thus, normalized values of both the independent variable, " X_v " (vinasse) and " X_t " (time) were -1, 0 and +1.

Results and Discussion

Table 2 shows the normalized values of independent variables and its subsequent dependent variables of the products obtained in the incubation process, using the proposed experimental designs. Each value in experimental results is an average of four samples. The deviations for these parameters from their respective means were all less than 5%.

Multiple linear regression analysis yields the equations showed in Table 3 relating dependent variables as functions of independent variables. Plots in Figures 1-5 show the response surfaces for most representative dependent variables.

Figure 1 shows that the amount of vinasse has greater influence on pH than time. As this figure shows, pH slowly increases during the thermophilic phase at low levels of vinasse. However, at high vinasse levels, a different development was observed. The pH values

FIGURE 1. pH variation as a function of operation time and vinasse.

TABLE 2.
Values of the independent variables and the chemical properties of the mixtures
obtained in the incubation process using the proposed experimental design

Normalized And Real Values Of Time And Vinasse				pH		^a B		Kjeldahl-N Losses (%)		C/N		NH ₄ ⁺ -N (mg kg ⁻¹)		NO ₃ ⁻ -N (mg kg ⁻¹)		^b Gi	
^c X _t	^d X _v	^e t	^f V	AV ^g	SD ^h	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
-1,	-1	20	0	8.56	0.50	51.0	5.53	17.1	1.53	35.8	5	440	48.16	70	5.34	36	2.29
0,	-1	35	0	8.45	0.68	57.3	4.46	18.7	1.90	35.5	3.79	2	0.13	39	2.69	59	4.58
+1,	-1	50	0	8.25	0.86	57.7	5.38	20.9	1.86	36.5	3.65	8	0.42	31	2.02	80	7.22
-1,	0	20	20	8.89	0.68	43.9	5.22	11.6	0.91	21.0	2.69	450	47.71	100	9.01	25	2.45
0,	0	35	20	8.55	0.84	48.4	4.84	15.0	1.07	21.7	1.33	113	6.74	65	6.37	44	3.40
+1,	0	50	20	8.01	0.75	48.8	2.86	18.5	1.36	22.3	1.27	3	0.32	53	4.26	65	6.18
-1,	+1	20	40	9.04	0.60	30.7	2.07	26.1	2.25	24.0	1.85	285	19.90	158	11.16	16	1.59
0,	+1	35	40	8.55	0.92	41.7	4.82	26.5	1.59	23.6	0.77	50	3.82	97	5.60	35	1.46
+1,	+1	50	40	8.07	0.44	41.7	1.11	34.0	2.50	25.7	2.15	6	0.45	98	10.39	51	2.22

^aBiodegradability; ^bGi Germination Index; ^cX_t, normalized value of the operation time; ^dX_v normalized value of the vinasse; ^et, real value of the operation time (days); ^fV real value of the vinasse (%); ^gAV Average; ^hSD Standard deviation

TABLE 3.
Equations yielded for each dependent variable

Eq.		r ²	F
4	PH = 8.48 - 0.37 X _t - 0.16 X _t X _v	0.93	40.0
5	BIOD = 49.1 + 3.76 X _t - 8.69 X _v - 3.47 X _t ²	0.98	65.4
6	NL = 5.01 + 3.11 X _t + 4.98 X _v + 8.88 X _v ²	0.97	48.5
7	CN = 21.05 + 0.61 X _t - 5.77 X _v + 8.52 X _v ²	0.99	367.4
8	NO ₃ = 67 - 24.33 X _t + 35.5 X _v + 18.0 X _t ²	0.92	34.3
9	NH ₄ = 54.83 - 193 X _t + 143 X _t ²	0.96	42.5
10	GI = 45.55 + 20.01 X _t - 12.5 X _v - 2.00 X _t X _v	0.99	205.3

Where PH denotes pH, NL the nitrogen losses (%), CN the C/N ratio, NH₄ the NH₄⁺-N content (mg/kg), NO₃ the NO₃⁻-N content (mg/kg), BIOD the biodegradability and GI the germination index. X_t and X_v the normalized value of the operation time and vinasse, respectively. The differences between the experimental values and those estimated by using the previous equations never exceeded 10%.

FIGURE 2. Biodegradability variation as a function of operation time and vinasse.

decreased continually. Both evolutions could be due to loss volatilization of N as ammonia (Figure 3).

Biodegradation was similarly affected by both operation time and vinasse. In Figure 2 it can be observed that to obtain a high biodegradation, medium to long operation time (35-50 days) and low vinasse (0-20%) are recommended. The observed biodegradation values of the current mixtures was similar to those reported by other authors in similar residues (22-71% for several residues, Kayhanian and Tchobanogolus 1992; (60-70% for coffee and agricultural wastes, Nogueira *et al.* 1999).

Figure 3 demonstrates the large influence of the quadratic term in the vinasse factor in equation 7 (Table 3). The statistical influence of vinasse is

FIGURE 3. Kjeldahl-N loss variation as a function of operation time and vinasse.

greater than that of the time (Figure 3). The initial values of Kjeldahl-N were significantly greater in the mixtures with higher proportion of vinasse, due to the relative high N content of the vinasse (Table 1). However, the final contents of Kjeldahl-N do not follow this same behavior. As it can be seen in Figure 3, lower losses of N took place in medium vinasse (20%). The losses of N can be related with the pH evolution (Figure 1). Therefore, medium-to-low vinasse (0-20%) could be used to reduce Kjeldahl-N losses. Although the initial C/N ratio in the mixtures was higher than 20, important N losses were recorded during the process. This fact could be explained assuming that the available C for the microorganisms ($C_{\text{biodegradable}}$) is lower than the total (C_{total}), and therefore the $C_{\text{biodegradable}}/N$ ratio, is lower than the C_{total}/N ratio (Díaz, *et al.* 2002). The Kjeldahl-N losses in the present mixtures were similar to those reported by other authors in similar residues (9-21% for wheat straw compost, Bannick and Joergensen 1993; 20-30% for sorghum bagasse composts, Negro *et al.* 1999; 12-28% for cattle deep litter composts, Sommer 2001).

The statistical influence of vinasse in the C/N ratio is higher than that of the time (Eq 7 in Table 3). The optimum C/N ratio of the initial mixtures is between 25-35 (Poincelot 1974, Haug 1993, Nakasaki *et al.* 1993, Choi 1999). Therefore, medium-to-low vinasse (0-20%) was recommended. The C/N ratio decreased during the composting process to reach of 10-15 - typical values of finished compost (Poincelot 1974, Rao *et al.* 1995). Due to positive and negative statistical influence of the vinasse term in C/N evolution (Eq. 7 in Table 3), to obtain low C/N ratios for final mixtures medium-to-low vinasse (0-20%)

should be used. The operation time could be medium-to-short (20-35 days) due to its scarce statistical influence (Eq. 7 in Table 3). Long operation time could give rise to greater investment and maintenance costs.

The statistical influence of vinasse on NO_3^-N final values was higher than that of the operation time (Figure 4). Final values NO_3^-N increased as vinasse increased and decreased as operation time increased. However, equation (Table 3) shows no statistical influence of the vinasse on NH_4^+N final values. At the same time, Eq. 9 shows that to obtain suitable NH_4^+N values, long operation time (50 days) should be recommended. A high NO_3^-N content of the mixtures at the beginning of incubation was observed (Table 1). This fact could be attributed to initial nitrogen mineralization of the substrates during their storage. Figure 4 shows that the NO_3^-N decreased in the initial phases of the thermophilic incubation and then, slightly increased during the final phase of the process. Therefore, to obtain high NO_3^-N contents in the final product, high vinasse (40%) should be used. Other authors reported similar NO_3^-N evolution during composting (Bishop and Godfrey 1983, De Bertoldi *et al.* 1985, Mahimairaja *et al.* 1994). The content of NH_4^+N in the final products was lower than the value advised for mature compost (40 mg/kg, Zucconi and De Bertoldi 1987, Mathur *et al.* 1993). The decreased of NH_4^+N during the process did not correspond with an increase of NO_3^-N , suggesting that N was lost during incubation. Probably, most of the NH_4^+ was lost through NH_3 volatilization due to the high temperature (55°C) and pH values (>7).

Figure 5 shows the variation of the germination

FIGURE 4. NO_3^-N variation as a function of operation time and vinasse.

FIGURE 5. Germination Index (Gi) variation as a function of operation time and vinasse.

index (Gi) as a function of time and vinasse. Phytotoxicity decreased, or Gi increased, in all cases during the incubation. From the Equation 11 (Table 3), it can be deduced that the positive statistical influence of the operation time is higher than the negative statistical influence of the vinasse proportion. If a high (Gi) is desired, a medium-to high operation time (35-50 days) and medium-to-low vinasse (0-20%) should be used. For low vinasse mixtures, intermediate operation time (35 days) was enough to ensure a suitable maturity (Gi >60) (Zucconi *et al.* 1985).

The initial (day 0) and final (day 50) nutrients content in mixtures are shown in Table 1. As the amount of vinasse increases in the initial mixtures, concentrations of K, Ca and Mg increased and concentration of P decreased. Increases of nutrient contents were observed at the end of composting, probably because the decrease of organic matter concentration.

For economic reasons, medium operating time (35 days) is recommended. At an operating time of 35 days, 20% of vinasse represents the point in the experimental design at which the ratio of biodegradation to nitrogen loss (i.e. maximum degradation with minimum nitrogen loss) is highest (3.22). Hence, this could be a suitable combination for in-vessel composting.

Conclusions

The results of the experiments indicated that composting of vinasse and grape marc can be considered as an ecological way of recycling these wastes. Results of the incubation experiment show that a final product (compost) with acceptable properties (maturity and chemical) entails operating at medium operation time (20-35 days) and medium-to-low vinasse (0-20%). Moderate amount of vinasse (10-20%) would be the best compromise to compost this waste with grape marc.

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