Clean Techn Environ Policy (2009) 11:323–327
DOI 10.1007/s10098-008-0190-3

ORIGINAL PAPER

An eco-biotechnological process for the treatment of residual lemon pulp

A. R. Navarro · L. Dorado · M. C. Maldonado

Received: 2 July 2008 / Accepted: 7 November 2008 / Published online: 3 December 2008

Abstract A biological treatment was designed for lemon pulp waste, a residual product from the lemon processing industry that causes many economic and environmental problems. The technique was carried out in two stages. In the first one, the pulp was treated with microorganisms until reaching partial digestion and increasing pH from 3.8 to neutrality. In the second, the predigested pulp of the first stage was treated with earthworms of the *Eisenia foetida* variety for 90 days, thus transforming the residue into humus and propagating the earthworms with an individual mean growth of 2.34 mg/day. The process is simple and transforms a highly contaminant solid residue into commercially valuable products like humus and earthworms. Different germination and phytotoxicity assays were performed with assorted seeds to establish humus properties.

Introduction

The lemon industry generates an important amount of liquid and solid effluents when manufacturing juice concentrate, essential oil and lemon peel. Lemon pulp, the solid residue with 93% humidity, is 9% of the weight of fresh lemon; it causes many economic and environmental problems because of its fermentability (Tripodo et al. 2004). Pulp residue (called rug) consists of the fraction screened from the pulp, wall segments or membranes and seeds. A plant that processes 80,000 tn lemon/year generates 7,200 tn of residual pulp with a dry weight of 504 tn. A small percentage is used for animal feed and the rest must be sent to a waste disposal plant. This involves substantial transportation costs (Tripodo et al. 2004).

The treatment of waste is complicated because of its low pH (3.5–4.0) and high water content. Its physical characteristics make it difficult to dry in common industrial devices and disposal of its high organic matter content is not easy (Crupi et al. 2001).

Composting is one of the most common biological processes used in the treatment of agroindustrial residues. This technology requires adjusting pH (6.5–8.0), 60% maximum humidity and solid granulometry (Rynk 1992).

Lemon residual pulp has 85–90% humidity and low pH which makes composting difficult. Another alternative currently employed is vermiculture, but it cannot be adopted with pulp because of its low pH. In this study, a microbial process was applied to increase pH and partially degrade more complex organic matter. On a second stage earthworms were used to complete pulp transformation.

No precedents of lemon residual pulp treatment with earthworms were found in the literature.

Vermicomposting is an eco-biotechnological process that transforms complex organic substances into a stabilized humus-like product (Benitez et al. 2000), rich in chelating and phytohormonal elements (Tomati et al. 1995).

The purpose of this work was to develop a low cost biotechnology process to treat residues transforming them into a commercially valuable product such as humus which could be used as fertilizer in lemon plantations. Thus, part of the nutrients would be returned to the soil from which they were taken.

A. R. Navarro (✉) · L. Dorado · M. C. Maldonado
Instituto de Biotecnología, Facultad de Bioquímica, Universidad Nacional de Tucumán, Ayacucho 465, 4000 Tucumán, Argentina
e-mail: biotec@fbqf.unt.edu.ar

Materials and methods

Chemical analysis

Pulp and vermicompost were analyzed by standard methods. Organic carbon was determined by the partial-oxidation method (Walkley and Black 1934).

Total nitrogen was measured by using the micro-Kjeldhal method.

To determine pH, 10 g of vermicompost was weighed, 25 ml distilled water was added and the mixture was stirred for 10 min. It was left to settle for 30 min and pH was measured in an Orion model 420A pHmeter.

Ash was determined by muffle furnace calcination at 560°C and organic matter (OM) was calculated with the formula %OM = 100 − %Ash.

Potassium determination was carried out by calcination of 1 g of the sample at 560°C for 8 h, ash was dissolved in HCl 2 N and a Metrolab 315 flame photometer was used in the measurements.

Pulp microbiological treatment

Different pulp concentrations (in %dw) 15.0; 17.5; 20.0; 22.5 and 25.0, suspended in water, with the addition of 0.15 g/l urea and 0.20 g/l diamonic phosphate, were tested to ascertain the maximum pulp concentration that could be treated in the fermentor. A Bio Flow C fermentor from New Brunswick Scientific, USA, was used in batch systems with an 8 l work volume for each pulp concentration and it was inoculated with 5.0 g (dw/l) of mixed culture of microorganisms. It was aerated with 1.5 l air/l culture medium × hours and stirred at 300 rpm at 37°C.

The mixed culture was obtained by microorganism propagation in a Bio Flow fermentor for 24 h at 30°C. We used microorganisms from the lemon industry wastewater because they are adapted to growth on lemon wastes; they were separated by centrifugation.

Predigested pulp treatment with earthworms

Earthworms were fed with a mixture of predigested pulp (filtered with Whatman paper No. 11) and vermicompost in a 1:1 ratio by mass to improve anelid acceptance of this substrate. Humidity, N content and C/N ratio were considered. Horse manure vermicompost was produced in the laboratory with the same earthworms and conditions used in the assays. The mixture was left in a 40 × 60 × 25 cm plastic container for 5 days and earthworms *Eisenia foetida* were added. A stocking density of 1.60 kg-worms/m^2 and a feeding rate of 0.75 kg-feed day/kg worm (Ndegwa et al. 1999) were used in this study. Individual earthworm biomass and cocoon number were monitored every 30 days and total mortality was calculated at the end of the experiment.

All containers were incubated in a humid and dark place at 30 ± 0.5°C. Moisture was maintained at about 65% spraying distilled water throughout the study period. The experiments were terminated at the end of the third month after all the substrate had been consumed by the worms.

Phytotoxicity test

Since the organic matter nonhumified in organic waste, would produce phytotoxicity in the plant, a phytotoxicity test (Warman 1999) was performed.

The vermicompost aqueous extract effect on the germination and root development of lettuce and chicory seeds was determined. The aqueous extract was obtained by adding 50 ml distilled water to 10 g of the sample, stirring for 30 min and centrifugating for 15 min at 12,000 rpm. A Whatman No. 3 cellulose paper filter was placed on a Petri dish. It was saturated with 5 ml aqueous extract and 25 lettuce seeds were placed on it, wrapped in paper and incubated in darkness at 25°C. The seeds were superficially sterilized with sodium hypochlorite (Somasegaran and Hoben 1994) to eliminate bacterial or fungal contamination that might interfere with the assay.

The same procedure was performed in triplicate for each seed and aqueous extract assay. On the fourth day of incubation germination percentage, root and hypocotyle length were calculated. Distilled water was used as control and a value of 100% was assigned to the germinated seeds. Percentage germination (%G) was determined according to it. With these data the germination index was calculated with the formula:

$$I_g = \%G \times L_m / L_c$$

where L_m is the root mean length + hypocotyle mean length of the samples and L_c the root mean length + control hypocotyle mean length.

Germination assay

A germination test, conducted under controlled temperature (24°C), and 2/3 to moisture equivalent, was performed to demonstrate the capacity of the vermicompost to germinate different seeds (Gariglio et al. 2002).

This assay was carried out to calculate germination the index (I_g) of each plantule grown in the pulp and horse manure vermicompost using stem and leave length; soil was used as control. The samples, 25 corn, tomato and pumpkin seeds, were placed in 30 × 20 × 10 cm separate plastic containers. The plantule length was measured on the fifth day and the germination index was calculated. The soil was assigned the value 100.

Table 1 Residual lemon pulp analysis

pH	3.9
%K (mg/g)	19.0
%N	1.82
%C	49.89
C/N	27.52

Statistical analysis

All determinations were performed in triplicate and standard deviation was calculated by Microsoft Excel.

Results and discussion

Pulp fermentation

The results of residual lemon pulp analysis are shown in Table 1.

As a first stage, pulp microbiological digestion is necessary to modify their physicochemical characteristics so that it may be accepted by the earthworms that will complete the degradation process at a second stage. Out of all pulp concentrations tested, the 20% (dw) one yielded the best results and it was selected for all assays. Concentrations of 22.5 and 25% (dw) were difficult to aerate and microorganism growth became slow.

The parameter used to stop fermentation was pH; at 32 h, pH was 7.40, and fermentation was interrupted because values above 8 are not convenient for earthworms. The increment in pH occurs because microorganisms consume organic acids.

In Table 2, pH, %C and %N variations are observed according to fermentation time. The 34% N increase at the end of fermentation is due to nitrogen incorporation into their cellular mass by pulp associated microorganisms at the expense of N added to the fermentation medium.

Table 2 Lemon pulp fermentation: values of pH, %C, %N and C/N ratio versus time

Time (h)	pH	%C	%N	C/N
0	3.8	39.9	1.87	21.06
5	4.3	36.7	1.94	18.91
10	5.7	33.1	2.04	16.22
15	6.5	28.6	2.20	13.00
20	6.7	29.1	2.31	15.60
25	6.8	29.6	2.38	12.44
30	7.0	29.8	2.48	12.02
32	7.4	29.8	2.5	11.92

The %C showed a different behavior; at the beginning it diminished from 39.9 to 28.6% in 15 h and after that it remained almost constant. This reduction is due to CO_2 release because of microbial metabolism.

As a result of N and C variations, the C/N ratio decreased 43.4% during the fermentative process until reaching 11.92 at 32 h of incubation. This value is too low and noxious for earthworms because, when there is too much nitrogen, bacterial flora generates ammonia that kills them (Ferruzi 1994). They can consume substrates with a C/N ratio close to 10, but with nitrogen values of 1 or 2%. The way nitrogen is present is also important; as ammonia, it is noxious for earthworms, but it is not toxic as nitrate, the form naturally found in the soil.

Substrate acceptance test

To determine earthworm pulp acceptance, a batch of 50 earthworms was placed in 20 cm deep plastic containers with 900 g of fermented pulp. At the beginning they behaved normally, eating and gaining weight. However, on the sixth day they began to leave their substrate and died on the following days probably because of ammonia increase. Hence, it was thought that vermicompost, a product with a low nitrogen concentration, should be mixed with the fermented pulp to increase the C/N ratio. According to Ndegwa and Thompson (2000), feedstock with an initial C-to-N ratio of 25 would be optimal for vermicomposting using *Eisenia foetida*.

Substrate mix transformation by earthworms

During the first 10 days, pH increased up to 8.8 because of the ammonia produced by protein and nitrogen metabolization. This result agrees with those obtained by Cegarra (1998). After the tenth day, the pH began to decrease because nitrifying bacteria oxide ammonia into nitrate, the most common form of occurrence of nitrogen in the vermicompost. According to Soto and Muñoz (2002), acidification is also due to phenol condensation with NH_4^+ during humus formation.

Carbon percentage decreases during vermicomposting due to CO_2 release in the oxidative process of organic matter by earthworms and microorganisms (Table 3).

Fermentation and vermicomposting both resulted in a carbon loss because of mineralization.

Nitrogen content decrease during vermicomposting may have been due to ammonification, NH_3 volatilization and denitrification (Martins and Dewes 1992; Bernal et al. 1996). Similar results were reported by Benitez et al. (1999), who observed a 36% nitrogen decrease during sewage sludge vermicomposting.

Atiyeh et al. (2000) reported earthworms have a considerable impact on nitrogen transformation in manures by

Table 3 Vermicomposting of fermented pulp: values of pH, %C, %N and C/N ratio versus time

Time (days)	pH	%C	%N	C/N
0	7.0	37.8	1.80	21.00
10	8.8	36.8	1.73	21.29
20	8.3	36.0	1.68	21.43
30	8.1	35.2	1.61	21.86
40	7.9	34.3	1.58	21.71
50	7.7	33.3	1.53	21.76
60	7.6	32.7	1.44	22.71
70	7.5	31.6	1.40	22.57
80	7.4	30.7	1.37	22.41
90	7.4	30.5	1.32	23.11

enhancing it, so that mineral nitrogen was retained in the nitrate form.

The C/N ratio was kept almost constant up to day 50, but then it began to grow since carbon decrease was lower than that of nitrogen. The vermicompost thus reached normal values with this increase. Organic matter content decreased 15% as a consequence of mineralization, which together with humus formation by microorganisms and earthworms is responsible for the main transformations of organic matter. Ash concentration increased 9% during the first 45 days of vermicomposting.

Mineralization is a very beneficial process for plants and soil since it supplies nutrients like K and P which are easy to assimilate. When it is used as biofertilizer (Grant and Long 1989), humus will provide physical support to prevent nutrients from being washed away by irrigation or rain water.

The average weight of the earthworms at the beginning of the experiment was 280 mg, with an individual mean growth of 2.34 mg/day, about 9.3% more than the value obtained in this work with horse manure beddings. The mortality of the earthworms after 60 days was 5.43%.

Test results from lemon pulp and horse manure vermicomposts are shown in Table 4 together with bibliographical data.

Vermicompost results from lemon pulp and those from horse manure are quite similar and agree with bibliographical data. Pulp vermicompost K% is high because of the high K content of the pulp (Sinclair 1984). This is an important advantage since its use as fertilizer for lemon trees would return K to the soil which would recover part of the minerals the plants need. Microorganism amount (MO) in the vermicompost is within the range of those obtained from the literature.

Germination bioassays

A germination assay and a phytotoxicity test were performed to confirm that the vermicompost lacked toxic products and that it was suitable as a fertilizer. Germination index results are shown in Table 5.

Table 4 Analysis of vermicomposts and bibliographic data

Vermicompost			
Parameters	Pulp	H. manure	Bibliography[a]
%N	1.32	1.19	1.0–3.2
%C	30.50	21.86	14.0–33.8
%Ash	71.20	72.70	74.4–78.0
%OM	28.8	27.30	20–30
No. MO	1.2×10^9	1.9×10^9	1×10^9 to 1×10^{12}
%P	1.70	1.82	1.00–1.94
%K	1.59	0.98	0.78–1.73
pH	7.5	7.8	6.8–7.5

[a] Range of values taken from the bibliography: Cegarra (1998), Ferruzi (1994), and Suthar (2006)

Table 5 Corn, tomato, and pumpkin seeds germination index with soil, pulp vermicompost and horse manure

Germination index (I_g)			
Seeds	Control	Pulp vermicompost	Horse manure vermicompost
Corn	100 (±3.1)	154.2 (±2.5)	151.3 (±3.9)
Tomato	100 (±4.2)	185.9 (±3.6)	162.4 (±4.5)
Pumpkin	100 (±3.4)	132.5 (±3.7)	146.8 (±3.6)

Standard deviation values are indicated in parentheses

Table 6 Phytotoxicity test for chicory and lettuce seeds in pulp and horse manure extract

Seeds	Germination index (I_g)		
	Distilled water control	Pulp vermicompost extract	Horse manure vermicompost extract
Chicory	100 (±3.6)	122.1 (±4.4)	123.2 (±3.8)
Lettuce	100 (±3.1)	119.5 (±3.2)	118.9 (±4.3)

Standard deviation values are indicated in parentheses

Germination index values are higher for the pulp vermicompost than for soil or horse manure. Hence, the vermicompost would be confirmed as a fertilizer or soil enricher.

Phytotoxicity test

Table 6 summarizes the results obtained in the germination assay with pulp and horse manure vermicompost extracts. Distilled water was used as control. In all the cases the values were higher than control. These results show that the pulp vermicompost is not toxic.

Conclusions

The two-stage process, microbial digestion and vermicomposting, successfully transforms a contaminant like lemon residual pulp into valuable commercial products like humus and earthworms.

The growth rate and mortality values of earthworms confirm that partially digested lemon pulp by microorganisms is an appropriate earthworm substrate.

The chemical and microbiological analysis of lemon pulp humus proved it had similar characteristics to those of horse manure humus.

Germination assays and the phytotoxicity test ratified that it is possible to use this humus as a fertilizer that would partially return soil nutrients extracted by lemon trees and that it could improve their yields.

This study revealed that the proposed process could be used efficiently to solve the problems of agricultural waste management on a low input basis.

References

Atiyeh RM, Domínguez J, Edwards C (2000) Changes in biochemical properties of cow manure during processing by earthworms and the effects on seeding growth. Pedobiol 44:709–724

Benitez E, Nogales R, Elvira C, Masciandro G, Ceccanti B (1999) Enzyme activities as indicators of the estabilization of sewage sludge composting with *Eisenia foetida*. Bioresour Technol 67:293–303

Benitez E, Nogales R, Masciandro G, Ceccanti B (2000) Isolation by isoelectric focusing of humic–urease complexes from earthworm (*Eisenia foetida*) processed sewage sludge. Biol Fertil Soils 31:489–493

Bernal M, Navarro AF, Roig A, Cegarra J, Garcia D (1996) Carbon and nitrogen transformation during composting of sweet soghum bagasse. Biol Fertil Soils 22:141–148

Cegarra J (1998) Compostaje y lombricompostaje, características de los compost. Residuos orgánicos 2:39–52

Crupi F, Gugo P, Di Giacomo G, Rispoli G, Di Giacomo A (2001) Sulla produzione del succo di arancia in Italia Part I. Ezzenze derivati agrumari 71:121–132

Ferruzi C (1994) Manual de lombricultura. Cap. 1 y 2. Ed. Mundi-Prensa, Madrid, España

Gariglio N, Buyatti M, Pilatti R, Gonzalez Rossia D, Acosta M (2002) Use of germination bioassay to test compost maturity of willow sawdust. New Zealand J Crop Hortic Sci 30:135–139

Grant WD,Long PE (1989) Microbiologia Ambiental. Cap. 1 y 6, 21–28 y 131–141. Ed. Acribia S.A. Zaragoza, España

Martins O, Dewes T (1992) Loss of nitrogenous compounds during composting of animal wastes. Bioresour Technol 42:103–111

Ndegwa P, Thompson SA (2000) Effects of C-to-N ratio on vermicomposting of biosolids. Bioresour Technol 75(1):7–12

Ndegwa P, Thompson SA, Das KC (1999) Effect of stocking density and feeding rate on vermicomposting of biosolids. Bioresour Technol 71:5–12

Rynk R (1992) On farm composting handbook. Northeast Regional Agricultural Engineering Service, New York, p 186

Sinclair W (1984) The lemon, Chap 3, pp 100–105. University of California, Div Agr Sci, USA

Somasegaran P, Hoben HJ (1994) Handbook for rhizobia: methods in legume-rhizobium technology, pp 366–367, USA

Soto G, Muñoz C (2002) Consideraciones teóricas y prácticas sobre el compost y su empleo en la agricultura orgánica. Manejo integrado de plagas y agroecología 65:123–129

Suthar S (2006) Vermicomposting potential of *Perionyx sansibaricus* (Perrier) in different waste material. Bioresour Technol 98:1231–1237

Tomati U, Galli E, Pasetti LD, Volterra E (1995) Bioremediation of olive mill waste water by composting. Waste Manage Res 13:505–518

Tripodo MM, Lanuzza F, Micali G, Coppolino R, Nucita F (2004) Citrus waste recovery: a new environmentally friendly procedure to obtain animal feed. Bioresour Technol 91(2):111–115

Walkley A, Black I (1934) An examination of the Degtjareff method for determining soil organic matter and prepared modification of the chromic acid titration method. Soil Sci 34:29–38

Warman P (1999) Evaluation of seed germination and growth test for assessing compost maturity. Compost Sci Util 7:33–37