Biovalorisation of Two-Phase Olive Mill Waste by Composting Process

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Abstract: ***The two -phase olive mill waste resulting from the extraction of olive oil a major problem for the olive industry because of their polluting power which originates from a high loading of phenolic compounds. The treatment of Two-phase olive mill (TPOM) was performed by the composting process with horse manure and spent coffee ground for 24 weeks. During composting, the intense microbial activity has translated by an increase in temperature (61.78°C -67.98 °C) during a week. The final composts show an increase in pH, electrical conductivity, total nitrogen, and a decrease in organic carbon. These final composts have a C/N ratio close to 10,and abundance of the agrochemical element (P, K, Na, Ca). The humic substrates of the composts were evaluated in order to monitor the composting progress. The polyphenol reduction was about 93.06%. The phytotoxicity, determined by the effect of water-soluble extracts on the germination of cress seeds, after 24 weeks of composting.***

Keywords: Two-phase olive-mill waste **,**Composting, Horse manure, Polyphenol.

1. Introduction

Among the Mediterranean countries, Morocco results to be the sixth largest producer of olive oil. The average annual of olive production is about 1.5 million tons, 65% are reserved for trituration 25% for table olives and the 10% represents the consumption and losses[1]. Olive oil extraction is based on a mechanical process. Three different systems can be used industrially: the traditional press-cake system; the three phase decanter system; and the two-phase centrifugation system. In the three-phase process, water is added to improve the separation, and two residues are generated: olive-mill wastewater (OMWW) composed of vegetation water and added water, and the olive kernel[2]. The two-phase centrifugation system is considered the most ecologically friendly techniques, because it saves >80% of the water (the process requires only a small amount of water) and >20% of the energy required by the three-phase system. Then, using this two-phase technology, only a semi-solid waste composed of olive pomace and vegetation water is generated, called two-phase olive mill waste (TPOMW)[3]. These wet olive pomace is a dense sludge, with lower moisture content than OMWW, but higher than that of olive kernel from the three-phase system. Also, the TPOMW is slightly acidic and presents high concentrations of organic matter (containing lignin, hemicelluloses and cellulose), and also proteins, lipids. It is also highly polluting to soil and water because of its high content of phenolic compounds[2], [4].

The biotransformation of olive mill waste by composting has proved to be an efficient valorization method as its final product is characterized as an excellent soil amendment. During the composting process, the organic matter from the raw materials is degraded by native microbial population due to its metabolic activity[5], [6]. This biotransformation is carried out by a complex temporal succession of a large number of microorganisms included bacteria, actinobacteria and fungi. Nevertheless, the olive mill wastes have certain properties inadequate for composting, such as excessive moisture content (>80% in TPOMW) and the presence of non-easily biodegradable compounds (lipids, polyphenols) that exhibit antimicrobial action[6]. The composting of olive mill waste have been developed with several bulking agents (grape stalks, wheat straw, cotton gin waste, agricultural by-products, animal manure, , municipal solid waste, etc.)[5]. Therefore, using bulking agents increase the compost porosity and the oxygen availability, optimize microbial activities and hence temperature increase[5], [6]. Several critical factors in the composting process have examined, including bulk density, porosity, particle size, nutrient content, C/N ratio, temperature, pH, moisture and aeration, since they determine the optimal conditions for microbial development and the biodegradation of organic matter.

However, the feasibility of using composting to stabilize TPOMW has provide [7]. In Morocco, the agriculture sector has a great importance, composted this two-phase olive mill waste (TPOMW) can be an excellent solutions for farmers, while simultaneously providing significant environmental benefit.

The aim of this paper is to study the viability of recycling TPOMW by means of composting with horse manures and spent coffee ground , as well as to evaluate the quality of the composts obtained.

2. Materials and Methods

2.1 Composting Performance

The TPOMW was taken from olive-mill located in province of Meknes during the olive campaign season 2013–2014. Two mixtures were made by adding the selected bulking agents (horse manure HM, spent coffee ground SCG) to the TPOMW (the main characteristics of the raw materials shown in table 1). and placed in plastic bins (30cm diameter ×50cm depth) were filled 10 kg of feed mixed. The mixtures were prepared in the following proportions, on a fresh-weight basis:

- Bin 1: 60%TPOMW+40% HM.
- Bin 2: 60%TPOMW+30%HM+10%SCG.

After bins were formed, manual turnings were performed twice a week. The moisture of the bins was controlled weekly by adding the necessary amount of water to obtain a moisture content of not less than 60%. The temperature of the bins was measured daily at different levels using a thermometer. Homogeneous samples of each mixture were taken at T0 and T24 week.

Table 1: Main characteristics of the raw materials used in the composting experiments (dry weight)

Parameters	*TPOMW*	*HM*	*SCG*
Moisture %	64.0 0	52.56	64.36
pH	5.50	8	6.83
EC (dS/m)	5.33	2.1	2.59
COT g/kg	205.1	211.6	201.68
MOT g/kg	353.6	364.9	347.70
TN g/kg	5.61	6.7	7.7
C/N ratio	36.56	31.6	26.19
TPC g/kg	26.82	0.001	24.98

2.2 Evaluation of chemical and physicochemical parameters

The compost samples were analyzed for EC and pH in a 1:2.5 and 1:5 (w/v) water-soluble extract respectively [8]. The dry matter of the samples was determined after 12 h at 105°C. Total organic carbon (C) and the organic matter (OM) were assessed using the Walkley and Black method and total nitrogen (TN) was determined in 0.5 g samples by the Kjeldahl method[9]. After HNO3/HClO4 digestion, P was measured colorimetrically as molybdovanadate phosphoric acid, K , Ca and Na by flame photometry[10]. Carbone content of the humic acid (HA) and fulvic acid (FA) fractions was determined by a previously described method of quantitative extraction and fractioning of humic substances[11]. The total phenolic compounds (TPC) were determined according the method described by Ait Baddi et al., 2009. The phytotoxicity of compost extracts was determined according to the method by Zucconi et al., using seed germination technique Cress seeds (lepidum sativum)[12]. The chemical and physicochemical parameters were carried out in triplicate and the standard deviation (SD) was calculated.

2.3 Statistical Analyses

Statistical analyses of data were made using the SPSS 22.0 Programme for Windows. The t-test was calculated for the results of the composting samples to determine changes in the parameters with time.

3. Results and Discussion

3.1 Temperature

The temperature is an important factor for composting process, as it governs the biochemical process and the microbial activities of compost. It is usually used as a parameter to indicate the end of the composting[13]. Figure 1, presents time series of bins and ambient temperature during the composting process. The thermophilic phase established after 3-4 weeks for about 1 week. In bin 1 the maximum temperature (67.98°C) was observed at week 3, while in bin 2 the maximum temperature (61.78°C) was observed at week 4. The range of 52–60°C is the most favorable for decomposition and the temperatures above 55 °C are required to kill pathogenic microorganisms[13] .The addition of water at week 6 caused a decrease in temperature. Temperature then gradually decreased from 30°C to 10°C ambient temperature by 14 weeks . It appears that activity of mesophilic microorganism dominated this period.

A review of OMW composting experiments and applications in the literature reveals that successfully reached the thermophilic phase (48°C-72°C)[6]. However , the duration of the thermophilic phase presents substantial variations (33-365 days)[5], [6]. These differences in temperature values and thermophilic phase durations can most probably be attributed to several factor (moisture, bulking agents, aeration system, the volume of the compost, organic matter content)[5].

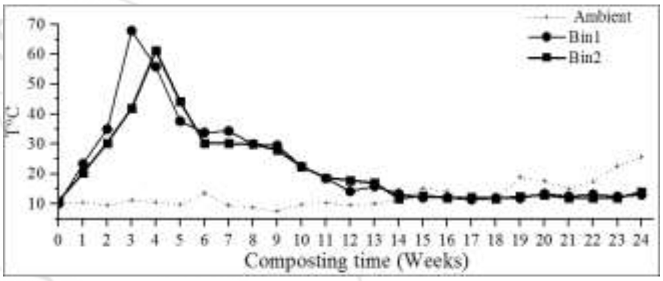

Figure 1: Temperature profile during composting process.

3.2 Compost quality obtained

Table 2. shows a substantial increase in both the pH and EC during the composting process. The increase in pH could be attributed to the degradation and consumption of organic acids by micro-organism[14]. Also , this may occur due to the mineralization of carbon and the subsequent production of OH- ions[13]. The EC increase could be caused by the reduction of organic matter and release of different minerals. The EC values should be lower than 4000 mS/cm to be safe for agricultural purposes[5] .

During the process, organic matter OM content and TOC decrease in both bins. Bacteria are active during the mesophilic phase, fungi are active throughout the entire process, and Actinomycetes sp. dominate the maturation phase. Losses in carbon content in the course of composting due to the degradation of the cellulose and hemicellulose components[6], [13]. The concentration of nitrogen in bins had increased significantly ($p<0.05$), with composting time as carbon is utilized by microorganisms. Moreover, the N increase may be a consequence of a substrate's lower lignin and cellulose content [15].

As shown in Table 2, a decrease in C/N ratio was observed in both bins, at the end of maturation phase the values were very close to 11. This decrease could be attributed to release of organic matter content. The compost can be characterized as mature only when the C/N ratio is below 20 and nitrogen content is above 3% [16].

Table 2: Changes in some parameters during composting of the TPOMW mixture (dry weight)

Parameters	*Bin 1*		*Bin 2*	
	Initial	*Final*	*Initial*	*Final*
pH	6.30	8.01	6.48	8.02
EC (dS/m)	2.51	3.71	2.64	4.21
COT g/kg	222.92	198.42	228.94	198.51
MOT g/kg	383.40	342.12	394.65	342.28
TN g/kg	7.25	17.82	7.66	18.52
C/N ratio	30.7	11.13	30	10.71

Table 3 shows that he final composts contain a higher concentration of micronutrient (P, K, Na, Ca). This micronutrient are important to improve the quality of composts intended for agricultural usage[5]. Several experiments have been reported that composts based on OMW have high mineral nutrient contents (P:0.1–3%; K: 0.12–4.4%; Na:0.05–4.1%)[6]. In our results reveal that bin 1 and bin 2 was specially rich in K (bin1:17.93 g/kg;bin2: 17.83g/kg), which is a common characteristic in olive-mill by-products.

Table 3: The agrochemical of compost after 24 weeks (dry weight)

Margin	*Bin 1*	*Bin2*
Phosphor (P) g/kg	4.81	4.95
Potassium (K) g/kg	17.93	17.83
Sodium (Na) g/kg	1.84	1.63
Calcium (Ca) g/kg	13.18	12.00

3.3 Humification fraction

Composting is a biotechnological process where microorganisms are used to mineralize organic matter and improve the humification level of recalcitrant compounds [16]. The chemical fractionation of humic acids (HA) and fulvic constitute the main fraction of organic matter due to their effect on soil ecology, structure, fertility, and plant growth[16]. According to Table 4 , a significant increase in humic acid was observed at the end of this composting process. The level of humic acid in both bins was 10.1% (bin1) and 10.7% (bin2). Several studies had shown that the increase in HA is an indicator of the degree of humification of organic matter and the maturity of composts[13], [16]. Subsequently, a trend of decrease in the FA was also observed from (bin 1) and (bin 2) at the end of the process. Huang et al., (2006) also explained that the humification of organic matter occurs mainly through the HA fraction and little through the fraction FA. The majority of OMW composting research presents final humic acid and fulvic acid values of 18.6% and 12.8%, respectively, while composts with contents above 7% are characterized as high quality soil amendments [17]. In addition, humification reactions are associated to the degradation processes, as humic substrates are formed by the condensation and polymerization of aromatic units such as phenolic compounds with cellular debris such as sugars and amino-acids.[6]. However, the HA/FA ratio is an indicator of the maturity of compost, the circulation of residue polymerization[18] .We noted a significant increase in the HA/FA ratio for all bins at the end of composting. Also, the compost with a HA/FA ratio greater than 1.9 is considered as mature compost[19], [20]. Our results reveal that the final composts are mature.

Table 4: Changes in humic fraction (humic acid HA, fluvic acid FA) during composting of the TPOMW mixture (dry weight)

Parameters	*Bin 1*		*Bin 2*	
	Initial	*Final*	*Initial*	*Final*
HA %	2.34	10.10	2.23	10.7
FA %	5.22	2.86	6.39	3.94
HA/FA ratio	0.45	3.56>1	0.35	2.56>1

3.4 Polyphenol degradation and phytotoxicity

The toxic compounds in the TPOMW is the high concentration of phenolic compounds. This have been seen to contribute to the phytotoxicity of TPOMW as also to their antimicrobial properties[5]. Therefore, before applying the compost to agricultural crops, its potential phytotoxicity should be assessed[6]. This is very important for composts of OMW as their high content of phenolic compounds could reduce the quality of compost[5], [6].

In our composting substrates, the decrease in TPC was evident clearly at the end of the process, bin 1, bin2. The total polyphenol degradation (TDP) was about 93.06% in both bins . Chowdhury et al., have reported that the phenolic content is reduced by 93% after the maturation phase during the OMW composting[16]. (Table 4)

Table 2: The total phenolic compounds of compost at the beginning (Initial) and after 3 month (Final) and germination index (GI) at end of composting

Margin	*Bin 1*		*Bin 2*	
	I	*F*	*I*	*F*
TPC g/kg	21.75	1.51	22.39	1.55
TDP %	93.06		93.06	
GI %	86.45		85.12	

The phytotoxicity test is one of the key indicators of compost maturity[16]. GI value of 80 % has been used as an indicator of disappearance of phytotoxicity in composts [16].The GI values recorded were bin1 and bin2. The phytotoxicity screening (GI) indicate that the mature compost of this study was not phytotoxic and therefore it could be applied as a high quality organic soil amender.

4. Conclusion

The difficulty of composting TPOMW is mainly related to its high concentration of organic substances (phenols compounds), which make degradation a difficult and expensive task. The presence of a bulking agent also increases compost porosity, and hence, oxygen availability facilitating the microbial activities. In our work, horse manure used like bulking agent have facilitated the degradation of organic substances (phenols compounds), by microorganisms. At the end of composting of TPOMW, the nutrient content was high thus benefiting plant growth. Also, the increase of humic acid contents indicate a high quality soil amendment. Finally, the germination index revealed that the mature compost presented germination indices of approximately 80%, indicating that the current compost had excellent characteristics.

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