AGRICULTURAL REUSE OF OLIVE MILL EFFLUENTS AFTER ENERGY RECOVERY

A. Nastri, N. A. Ramieri, R. Abdayem, P. Gioacchini, C. Marzadori, C. Ciavatta
Dipartimento di Scienze e Tecnologie Agroambientali, Alma Mater Studiorum Università di Bologna, Viale G. Fanin n. 40, I-40127 Bologna, Italy. claudio.ciavatta@unibo.it

ABSTRACT

Aim of this research, within the UE BIOTROLL Project, was the physical and chemical characterization of the olive pulp (OP) and of the effluents arising from the biological treatment for the production of hydrogen (EH_2) and methane (ECH_4) and the agricultural reuse of these products for soil fertilization. Preliminary results showed that they contain respectively 28.4%, 7.8% and 4.6% of dry matter; around 60% of organic carbon; appreciable amount of macro- (especially organic N and K_2O) and micronutrients and negligible amount of heavy metals. pH and electrical conductivity are in a normal range. Their addition to a sandy soil showed different intensity of mineralization processes, any phytotoxicity phenomenon and a significant bio-stimulant effect on wheat seed germination.
Keywords: *Olive oil pulp, BIOTROLL, Organic wastes, Energy production.*

INTRODUCTION

The technology for olive oil extraction has significantly progressed since the beginning of the seventies, when the three-phase centrifugation system appeared. The main problem of this technology is the generation, in a short time, of large quantities of wastewaters, characterized by a high pollutant load due to their high content of organic compounds, in particular, carbohydrates, polyphenols and lipids (crude fats).

Recently the olive oil industry has adopted a new continuous two-phase centrifugation system, that strongly reduces the water needed for the extraction and separates the oil from the olive pulp, a semi-solid and humid by-product. The olive pulp is rich in carbohydrates and so it can be an ideal substrate for the production of energy in the form of ethanol, hydrogen and biogas (methane) through aerobic and anaerobic microbial digestion. At the end of the processes, however, it is necessary to get rid of the remaining by-products, and their use in agriculture as soil improving agents or fertilizers might be a possible solution. In order to make this reliable and profitable from an economic point of view, these substances must be physically and chemically characterized and their effects on the dynamics of soil nutrients and on the plant growth must be tested.

The aim of this study, within the UE BIOTROLL[1] Project, is: i) the physico-chemical characterization of the olive pulp (OP) and of the effluents arising from the biological treatment for the production of hydrogen (EH_2) and methane (ECH_4); ii) the evaluation of their phytotoxic effects on seed germination and plant growth; iii) the evaluation of the effects on soil nitrogen processes.

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MATERIALS AND METHODS

Chemical and Physical Characterization

In order to characterize chemically and physically the olive pulp (OP) and the effluents from its fermentation for the production of hydrogen (EH_2) and methane (ECH_4), the following parameters were determined: dry matter, pH and electric conductivity (EC), total lipids (crude fats), phenolic compounds, organic carbon (TOC), extracted organic C (TEC), humic-like substances (HLS), nitrogen, macro- and micronutrients.

The dry matter was determined by drying the products at 105 °C for 24 hours; pH and EC in a water extract, 3:50 (w/v) and 1:10 (w/v) respectively. The total lipids content was determined using the Soxhlet method and extraction with petroleum ether. Phenolic substances, expressed as gallic acid, were analyzed by the Folin-Ciocalteau method after extraction with water+methanol (1:1) in a ratio 1:20 (w/v) and colorimetric determination (Box, 1983; Ryan et al., 2001). The TEC and the HLS were extracted in $NaOH+Na_4P_4O_7$ 0.1M and a polyvinylpyrrolidone column was used to separate HLS from the non-humic substances (Ciavatta and Govi, 1993). The TOC was determined with the Springer-Klee method (Ciavatta et al., 1989). The total N was determined using an elemental analyzer and the total P colorimetrically using the Murphy and Riley method. Micronutrients and heavy metals were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) after digestion with nitric acid 65% (w/v).

Phytotoxicity Tests

Two different phytotoxicity tests were carried out to evaluate the effects of the three materials on the germination of seeds of durum wheat (*Triticum durum* L., cv. Duilio) and on the early stages of plant development: a filter paper test and a soil test. The soil used was a sandy soil (*Typic Ustifluvent*), slightly alkaline with a low content in N and TOC, sampled in an area of the South Italy characterized by a high olive oil production. The experiments were held in a growth chamber, in controlled conditions for light and temperature.

The **Filter Paper Test** was carried out on four layers of filter paper in 100 mm x 10 mm Petri dishes. Six different doses of materials were used: 0, 60, 120 and 180 kg N ha^{-1} and 70 t ha^{-1} and 80 m^3 ha^{-1}; the last two doses correspond to the Italian law limits for the sludges from agro-industries and for olive mill wastewaters, respectively. The amount of N added using 70 t ha^{-1} and 80 m^3 ha^{-1} corresponds to about 200 kg N ha^{-1} for the two effluents and 450 kg N ha^{-1} for OP, respectively. A complete randomized design with three replicates was used. Germination percentage was measured daily and at the end of this process the number of abnormal seeds was measured.. The seedlings were grown for 10 days, then the final shoot height and the root and shoot dry biomass (at 105 °C for 24h) were recorded.

The **Soil Test** was performed like the previous experiment, but 50 g of soil in each Petri dish was used as growth medium. The three products were mixed to the soil at the doses of 0-60-120-180 kg N ha^{-1}.. Four different sowing times (0, 15, 30 and 60 days after soil treatment with the three materials) were compared as well, in order to evaluate if there were differences of phytotoxicity in time.

Gross N mineralization

The gross mineralization of N was measured with the ^{15}N pool dilution technique at different time intervals from the incubation of the three matrices in soil: immediately after their addition to the soil (0-day) and after 7, 20 and 40 days. During all the period the samples were kept in controlled conditions. In each sample, the amount of product added to 100 g of soil (dry basis) was equivalent to a N supply of 150 kg ha^{-1}. The gross mineralization was measured by adding 3 mL of $(NH_4)_2SO_4$ solution containing 167 µg $N\text{-}NH_4^+$ mL^{-1} to the soil. The solution was ^{15}N-labelled at 10% ^{15}N abundance. The extraction of mineral N with $CaCl_2$ was done two hours after the ^{15}N injection and then after 96 and 168 hours. The amount of $N\text{-}NH_4^+$ in the extract was measured colorimetrically (Keeney and Nelson, 1982) whereas for the measure of the $N\text{-}NH_4^+$ isotope ratio we carried out the diffusion procedure of Khan et al. (1998). The isotope ratio of NH_4 was measured by CF-IRMS (Finnigan-Mat mod. Delta Plus). The gross mineralization was calculated according to the equation of Barraclough (1991).

RESULTS AND DISCUSSION

OP has the highest dry matter content, whereas ECH_4, which is the by-product subjected to the longest biological process with the production of ethanol and methane, has the lowest, since part of the organic matter was used by the fermentation agents as substrate (Table 1). The pH values were acidic in OP and EH_2 and moderately alkaline in ECH_4. The EC is higher in OP and ECH_4, whereas EH_2 shows a lower value; even though ECH_4 has the highest moisture content the EC is high, probably as a consequence of the salts added during its extraction. The lipid content is high in OP and EH_2, much lower in ECH_4; lipids, as well as carbohydrates, are a readily available substrate for the microbial activity, so the double biological extraction of ethanol and methane in ECH_4 might explain its low value. A very important parameter for the agricultural evaluation is the content of phenol compounds, which together with the lipids is related to the phytotoxic and antimicrobial effects; their content tends to increase in the dry matter, whereas in the fresh material decreases from 3.9 (OP) to 1.5 (ECH_4) g kg^{-1} with the intensifying of the biological processes for the energy production. The TOC is similar in the three materials, whereas the TEC and the HLS content show an increase in the digested materials; this trend was confirmed by the humification rate (HR) which shows a significant increase after energy production. The total N increases with the OP digestions. The concentration of phosphorus (expressed as P_2O_5) and potassium (expressed as K_2O) increases in digested samples, as a consequence of the addition of P and K salts as nutrients for micro-organisms during the anaerobic digestion. Among the micronutrients, Fe shows a content of a interest, because it is reasonable to suppose it in an available form (organic matter Fe-complex) to crop needs. The amount of heavy

***Table 1**. Physical and chemical properties of the three samples studied.*

Parameters		OP	EH_2	ECH_4
Dry matter (%)	(*)	28.4	7.8	4.6
pH	(*)	5.39	5.12	8.05
EC (dS m^{-1})	(*)	1.27	0.58	1.13
Total lipids (crude fats) (%)		19.4	24.9	8.1
Phenolic compounds (g kg^{-1})		13.7	25.7	32.6
TOC (%)		58.4	61.8	56.3
TEC (%)		20.6	37.5	39.9
HA+FA (%)		13.4	22.3	29.8
HR (%)		23	37	52
N (%)		2.2	4.0	5.4
P_2O_5 (%)		3.4	10.7	25.8
K_2O (%)		2.0	4.1	6.0
Fe (mg kg^{-1})		433	677	1317

(*) data referred to the fresh materials; all the other data are referred to the dry matter.

metals, including the micronutrients Cu and Zn, was complessively negligible (data not shown).

In the Filter Paper Test no phytotoxic effects on germination and plant growth were found as a consequence of the addition of the three products, with the exception of OP at the two law limit doses (not used for crop wheat) that determined, compared to the control, a decrease in shoot (-54%) and root (-37%) development and an important increase in the percentage of abnormal seeds (+1150%). In the Soil Test the effects of the three products varied in function of the different sowing times. An increase of the germinative energy (+30%) was observed with the application of the three materials when the wheat was sown soon after the soil treatment (0 days); with the sowing at 15 and 30 days there were no differences among treatments; whereas with the sowing at 60 days a decrease can be observed with the application of ECH_4 (-31%). Plant growth was never negatively affected by the application of the three products.

The rate of N mineralization was affected by both materials and incubation time. Soon after the addition of the products to soil, the highest mineralization rate was observed with OP and ECH_4, with an increase of about 150% compared to the control and EH_2. Then at 7 days from the beginning of the incubation their mineralization rate decreased, nevertheless it was still about 50% higher than the control. In the OP treatment the same rate of mineralization was measured also at 20 and 40 days from the incubation, whereas in the ECH_4 treatment the rate furtherly decreased to the control level. The EH_2 treatment, on the contrary, caused an increase in the N mineralization, double compared to the control, only after 7 days from the incubation.

CONCLUSIONS

The possibility of using the three products seems to be very promising. All three samples have shown physical and chemical characteristics compatible with their use in agriculture. EH_2 and ECH_4 on one hand had no negative effect upon the germination and plant growth, on the other hand had a positive effect on seedling growth, this might be due to an action of bio-stimulating effect on the mechanism of seed development. On the contrary OP at the two law-limit doses decreased the germinative energy and the plant growth; these effects might be attributed to a higher presence of phytotoxic components such as phenol compounds and lipids. Mineralization was not negatively affected by the application of the three products.

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