

## **Wastewaters from the olive-oil extraction process: disposal or valorization?**

Otpadne vode iz procesa ekstrahiranja maslinovog ulja:  
uklanjanje ili valoriziranje?

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### ABSTRACT

Olive vegetation waters (OVW) represent a serious environmental problem for the solution of which several disposal methods have been proposed, such as, e.g., physico-chemical treatments (decantation with lime and/or total oxidation; concentration, drying and incineration; ultrafiltration and reverse osmosis), agronomic (aquaculture; land-spreading) and animal-breeding methods (direct utilization as animal feed or after protein enrichment by, e.g. yeast or fungal fermentation) and of the "biotechnological" type (fermentation; biological treatments). The OVW might also be a possible resource due to the presence of value products (e.g., antioxidants) and of simple and complex sugars which make the waste a possible basis medium for fermentation processes. This work presents a brief survey of studies conducted at the Dipartimento di Agrobiologia e Agrochimica, University of Tuscia, on the possible chemical and biotechnological valorization of the OVW. Technical feasibility of various fermentative processes either to obtain products of high added value (polysaccharides and enzymes such as lipase, laccase, Mn-peroxidase and pectinase) or to improve the waste's agronomic use has been assessed.

Main residues of the olive-oil extraction process are vegetation waters (OVW) and husks. Extraction system, pressure and centrifugation play an important role in the amount and characteristics of both olive oil and residues (Amirante et al., 1993).

In the traditional centrifugation system, 50-100 l of water are added to 100kg of olive paste to reduce viscosity and to improve oil separation: as a consequence, large amounts of OVW are produced. During the last ten/twenty years, new centrifugation systems have been developed that require either less (10-20 l) or no water at all added during oil separation (Fig. 1). In either case, however, residues of the olive-oil extraction may represent a serious environmental problem.

After recovery of residual oil by solvent extraction, husks from traditional processes can find valid utilization as animal feed or as alternative fuel and in compost preparation. OVW, with washing waters, make the oil-mill waste waters (black waters) generally characterized by large volume and high polluting load. Moreover, compounds with biostatic activity (e.g., polyphenols) are largely present.

Key words: wastewaters, olive-oil, extraction, disposal, valorization

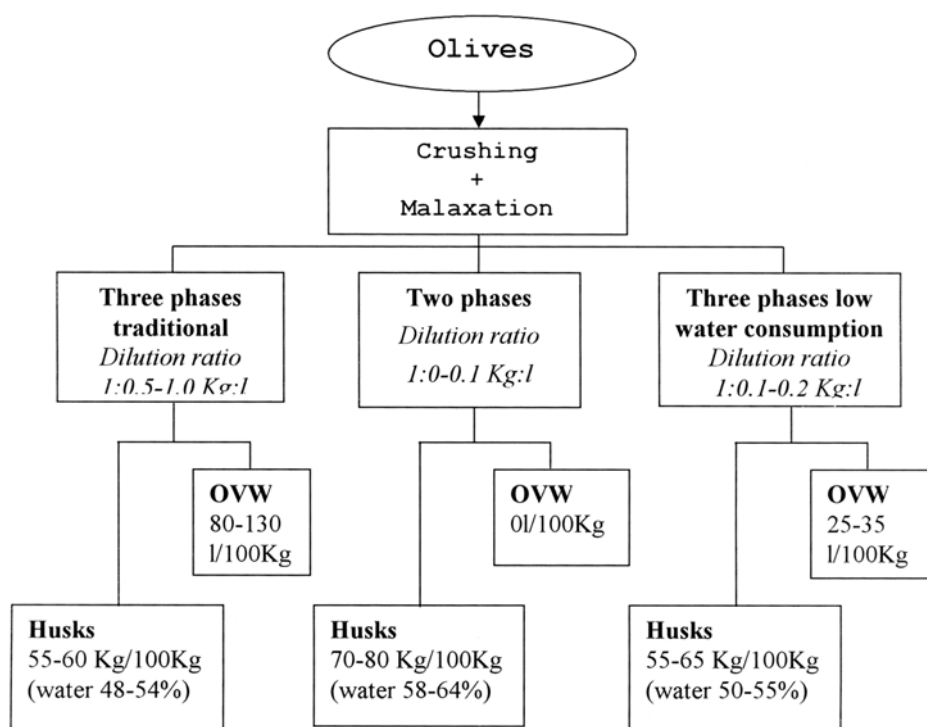
## SAŽETAK

Vegetacijske vode masline (OVW) predstavljaju ozbiljan okolišni problem za čije se rješenje predlaže nekoliko metoda uklanjanja kao što su npr. fizikalno-kemijski postupci (uklanjanje vapnom i/ili potpuna oksidacija, koncentriranje, sušenje i spaljivanje, ultra filtriranje i obrnuta osmoza), agronomske (akvakultura, rasprostranjenje po zemlji) te metode u uzgoju životinja (direktna uporaba kao krmiva za životinje ili nakon obogaćenja npr. kvascem ili gljivičnom fermentacijom) i biotehnološki oblik (fermentacija, biološko tretiranje). OVW je također mogući resurs zbog dragocjenih sastojaka (npr. antioksidanata) te jednostavnih i složenih šećera koji čine ovaj otpad mogućim osnovnim sredstvom za procese fermentacije. Ovaj rad daje kratak pregled istraživanja na Dipartimento di Agrobiologia e Agrochimica Sveučilišta Tuscia moguće kemijske i biotehnološke valorizacije OVW-a. Procjenjuje se tehnička izvedivost raznih fermentacijskih procesa za dobivanje proizvoda visoke dodane vrijednosti (polisaharidi i enzimi kao što su lipaza, lakaza, Mn-peroksidaza i pektinaza) ili za poboljšanje agronomske iskoristivosti otpada.

Glavni talozi u procesu ekstrahiranja maslinovog ulja su vegetativne vode (OVW) i ljuska. Sustav ekstrahiranja, pritisak i centrifugiranje igraju važnu ulogu u količini i značajkama maslinovog ulja i taloga (Amirante et al., 1993).

U tradicionalnom sustavu centrifugiranja dodaje se 50 do 100 l vode na 100 kg pulpe maslina da se smanji viskoznost i poboljša separacija ulja pa se kao posljedica stvaraju velike količine OVW. U zadnjih deset/dvadeset godina razvijeni su novi sustavi centrifugiranja koji zahtijevaju manje vode (10 - 20 l) ili uopće ne trebaju vodu za vrijeme separacije ulja (Sl. 1). U oba slučaja, međutim, ekstrakcije maslinovog ulja mogu predstavljati ozbiljan okolišni problem. Nakon vađenja preostalog ulja izlučivanjem pomoću otapala, ljuske se u tradicionalnom procesu mogu korisno upotrijebiti kao hrana za životinje ili kao alternativno gorivo, te u spremanju komposta. OVW, što za ispiranja čine otpadne vode u mlinu za masline /crna voda/ općenito obilježavaju veliki volumen i visoki teret onečišćenja. Osim toga, velikim su dijelom prisutni spojevi biostatične aktivnosti (npr. polifenoli).

Ključne riječi: Vegetacijska voda, maslina, ekstrahiranje, uklanjanje, valorizacija



*Fig. 1 - Flow chart of continuous olive oil extraction process*

*Slika 1. Dijagram neprekidnog procesa ekstrahiranja maslinovog ulja*

Besides the traditional decantation, several disposal methods have been proposed for this waste, such as, e.g., physico-chemical treatments (decantation with lime and/or total oxidation; concentration, drying and incineration; ultrafiltration and reverse osmosis), agronomic (aquaculture; land-spreading) and animal-breeding methods (direct utilization as animal feed or after protein enrichment by, e.g. yeast or fungal fermentation) and of the "biotechnological" type (fermentation; biological treatments).

## 1. CHEMICAL TREATMENTS

Though generally considered as the best approach to the treatment of OVW, total oxidation appears to be an impracticable solution due to the waste's high content of organic substance. It could represent, however, the

final step of a process which, in a preliminary phase, would eliminate most of the polluting load. Of particular interest is the oxidative approach based on the combined treatment with enzyme complexes (peroxidases) and H<sub>2</sub>O<sub>2</sub>. Neutralization with lime represents a cheap and effective pre-treatment often proposed to make flocculation of suspended solids easier and to accelerate the natural but slow microbial degradation so to avoid (or to limit) the development of bad smell during storage.

## 2. PHYSICAL TREATMENTS

Concentration by thermal evaporation has also been proposed; in this case, however, overheads are definitely not neglectable though subject to reduction by the use of 'multiple-effects' evaporators and/or utilizing the resulting residual solids as alternative fuel. Concentration can also be achieved by ultrafiltration and reverse osmosis: availability of suitable membranes, however, is of fundamental importance to ensure the success of this technology because of possible rapid membrane obstruction. Suitable pre-treatments such as flocculation of the proteic fraction, microbial fermentation and/or raw pre-filtration, however, can greatly reduce, though not completely eliminate, this problem.

## 3. DISPOSAL ON AGRICULTURAL LAND

Most likely, disposal on agricultural land by field spreading is the best of all solutions so far proposed to solve the problem of OVW disposal. Field spreading is now allowed by the Italian legislation while simply accepted in Spain.

In general, all experiments carried out so far have shown that OVW can be applied to several types of cultures without causing any apparent phytotoxic effects (Bonari et al., 2001). The appearance and the degree of such possible harmful effects seem to be dependent upon the amount of waste waters supplied to the soil and upon the period of time elapsing between the spreading of the waste and the sowing of crops. With a 60-day period allowed between treatment and planting no harmful effects upon the seedling germinative characteristics and early stages of growth should be observed, provided that the doses do not exceed 60-80 m<sup>3</sup> ha<sup>-1</sup> (Bonari et al., 2001).

#### 4. MICROBIAL TREATMENTS

This kind of treatments, largely applied in the case of many other biological wastes, in the case of OVW has encountered several contraindications due, in particular, to the antimicrobial action of phenols and, with certain microorganisms, to the unbalanced composition of nutrients. For this reason, convey of OVW to the sowing system or to already existing plants for the disposal of other organic liquid wastes is possible only after suitable dilution. Aerobic as well as anaerobic fermentations have sometimes been considered as integrative processes of other depurative technologies.

Each of the above approaches has its own essential validity for the reduction of the polluting load and, consequently, for the waste eventual disposal. With probably the only exception of field spreading, however, none appears to be a suitable and definitive solution to the problem, according to the present legislation.

**Table 1.** Average composition of olive vegetation waters

**Tablica 1.** Prosječan sastav vegetacijskih tekućina masline

Density	1.023-1.054
pH	4.6-6.7
Turbidity	11,000-65,000
Water (%)	82.4-96.0
Dry extract (%)	3.0-18.0
Suspended solids (%)	0.04-1.04
Mineral compounds (%)	0.4-7.2
Organic compounds (%)	3.9-16.5
Total sugars (%)	1.0-8.0
Total pectins (%)	0.05-0.15
Total polyphenols (%)	0.15-1.75
Total nitrogen (%)	0.1-7.2
BOD (mg/l)	9,600-110,000
COD (mg/l)	30,000-195,000

Besides being a serious environmental problem, however, OVW can also represent a possible resource of chemical substances (Figure 2) potentially useful as such, after either direct recovery or chemical transformation (Fki et al., 2005), and of simple and complex sugars (Table 1) that could be of use as a basis for fermentation processes (D'Annibale et al., 2003).

### Chemical valorization

In the OMW, several low-molecular-weight phenolic compounds are present such as, for instance, the phenolic derivatives of hydroxycinnamic, ferulic and caffeic acids and, in larger amounts, tyrosol (4-hydroxyphenetil alcohol) and hydroxytyrosol (3,4-dihydroxyphenetil alcohol) (Figure 2). Although to a different extent, all these compounds are characterized by high antioxidant activity and are, therefore, of great interest in the cosmetic and pharmaceutical industries and in food processing and food products conservation. After filtration to eliminate the suspended solids, all compounds of potential interest can be recovered by physico-chemical processes such as ultrafiltration, nanofiltration and reverse osmosis.

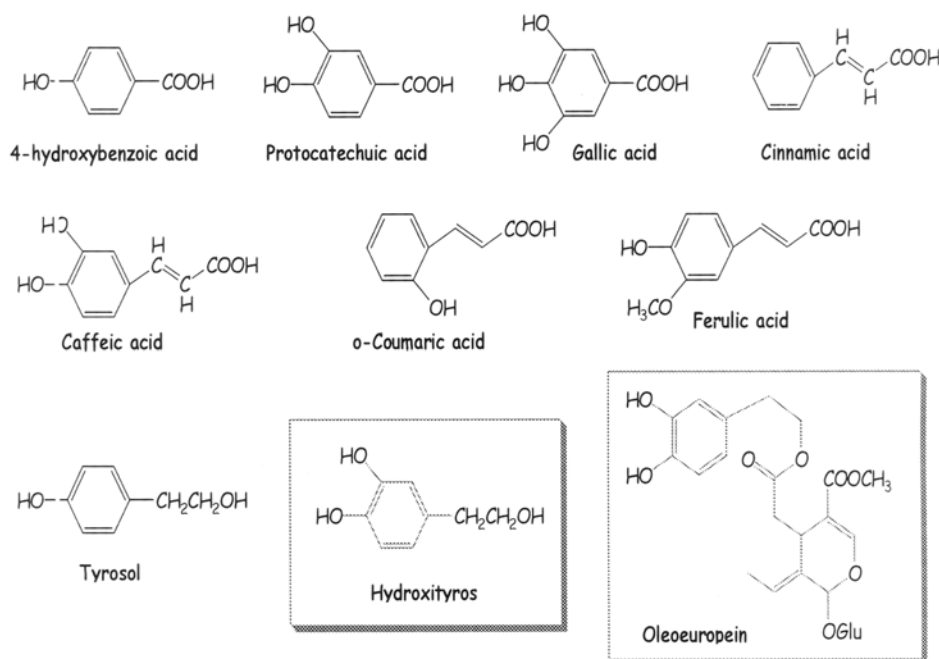


Fig. 2. Phenols and phenolic acids in olive mill wastewaters.

Slika 2. Fenoli i fenolne kiseline u otpadnim tekućinama u mlinu za masline

Hydroxytyrosol is, among all compounds present in the OMW, of greatest importance being characterized by antioxidant activity quite similar to that of

BHT (2,6-ditert-butyl-*p*-hydroxytoluene) as well as other synthetic antioxidants; at present it is obtained by total synthesis and commercialized at very high prices.

The large presence of hydroxytyrosol in the OMW makes, therefore, this waste of great potential importance for its direct recovery and, of even greater commercial importance, for the possibility of developing catalytic methods, environmentally friendly and economically sustainable, to convert other compounds (tyrosol, above all), present in the OMW, into hydroxytyrosol. In this context, research has been conducted in our Department to develop and to experiment effective catalysts (MTO, for instance) (Saladino et al., 2001).

### *Biotechnological valorization*

We report here on some different biotechnological approaches tested by our research group at the University of Tuscia, for the possible, eventual industrial utilization of OMW (Crognale et al., 2005).

## 1. ENZYME PRODUCTION

### *1.1 Lipase*

Microbial lipases catalyze the hydrolysis of ester linkages in lipids and are, therefore, employed in food technology (mainly, in the dairy industry), and in the detergent, pharmaceutical, cosmetic and leather industries. The use of cheap growth substrates, such as agro-industrial wastes can lead to the reduction of their production costs. OMW contains, among others, residual lipids as a consequence of incomplete olive oil extraction, that can stimulate, and sometimes induce, microbial production of lipases.

We have, therefore, tested several fungal strains for the ability to grow on the waste producing lipase activity (Table 2) (D'Annibale et al., 2005). The highest enzyme activities were obtained with *Geothricum. candidum* NRRL 553 (0.52 U ml<sup>-1</sup>) and *Candida cylindracea* (0.46 U ml<sup>-1</sup>), organisms already known for their ability to produce lipases on defined media but, so far, never tested on wastewaters. Optimisation of culture medium, in terms of possible integrations (salts, inducers, etc.) to OMW, and fermentation conditions significantly improved the enzyme production.

**Table 2. Lipase activity production and productivity (LP) by fungal strains grown on olive-mill wastewater****Tablica 2. Proizvodnja aktivnosti lipaza i proizvodnost (LP) pomoću gljivičnih sojeva (gljivica uzgojenih na otpadnim tekućinama u mlinu za masline)**

Strain	Lipase activity (U ml <sup>-1</sup> )	LP (U l <sup>-1</sup> h <sup>-1</sup> )
<i>Penicillium citrinum</i> NRRL 1841	0.37±0.02 <sup>a</sup>	2.92±0.16 <sup>a</sup>
<i>Penicillium citrinum</i> NRRL 3754	0.34±0.04 <sup>ac</sup>	4.58±0.54 <sup>b</sup>
<i>Penicillium citrinum</i> ISRIM 118	0.38±0.07 <sup>a</sup>	5.42±1.00 <sup>c</sup>
<i>Aspergillus niger</i> NRRL 334	0.33±0.01 <sup>ac</sup>	2.08±0.06 <sup>de</sup>
<i>Aspergillus oryzae</i> NRRL 485	0.38±0.02 <sup>a</sup>	2.08±0.11 <sup>de</sup>
<i>Aspergillus oryzae</i> NRRL 1988	0.34±0.03 <sup>ac</sup>	2.08±0.18 <sup>de</sup>
<i>Candida cylindracea</i> NRRL Y-17506	0.46±0.01 <sup>b</sup>	2.92±0.06 <sup>a</sup>
<i>Geotrichum candidum</i> NRRL Y-552	0.36±0.07 <sup>ac</sup>	3.75±0.73 <sup>f</sup>
<i>Geotrichum candidum</i> NRRL Y-553	0.52±0.01 <sup>b</sup>	2.92±0.06 <sup>a</sup>
<i>Rhizopus arrhizus</i> NRRL 2286	0.30±0.02 <sup>c</sup>	1.67±0.11 <sup>d</sup>
<i>Rhizopus oryzae</i> NRRL 6431	0.36±0.05 <sup>ac</sup>	2.50±0.35 <sup>ae</sup>
<i>Rhizopus</i> sp. ISRIM 383	0.35±0.03 <sup>ac</sup>	2.92±0.25 <sup>a</sup>

Column means followed by the same superscript letter were not significantly different ( $P < 0.05$ ) as determined by the Tukey test.

### 1.2. Laccase and Mn-dependent peroxidase

The aromatic degrading ability of white-rot fungi is due to the release of extracellular oxidases, such as laccase and Mn-dependent peroxidase, characterized by a low substrate specificity and good intrinsic stability towards several potentially denaturing agents. As a consequence, the use of these biocatalysts in several commercial applications including textile and lignocellulosic fibers processing, wine stabilization and wastewater treatment has been suggested.

Production of these enzymes by the white-rot basidiomycete *Panus tigrinus* CBS 577.79 was investigated using OMW as a low-cost growth medium both in liquid submerged (LSF) and solid state (SSF) fermentation, where straw was added (Table 3) (Fenice et al., 2003). Fermentation in the rotary drum reactor (RDR) gave the highest values for total enzyme activity ( $EA_{tot}$ ) and in shorter time than in STR, thus suggesting its use with solid state materials such as, for instance, humid olive husks. Nevertheless, both submerged fermentation



systems (air-lift in particular) showed unitary volumetric activity ( $EA_{vol}$ ) and average volumetric productivity significantly higher than those of RDR.

**Table 3.** Process performances of laccase production by *Panus tigrinus* CBS 577.79 in a 25-l rotary drum reactor (RDR), 3-l stirred tank reactor (STR) and 3-l air-lift reactor using an OMW-based medium (OM)

**Tablica 3.** Proces lakaze pomoću *Panus tigrinus* CBS 577.79 u 25l reaktoru s kružnim bubnjem (RDR), 3l reaktoru bazenu za miješanje (STR) i 3l reaktoru na zračno dizanje korištenjem medija na bazi OMV(OM).

	RDR*	STR	Air lift
$EA_{max}$ (U l <sup>-1</sup> )	1309 ± 20 <sup>a</sup>	4603 ± 98 <sup>b</sup>	4300 ± 23 <sup>c</sup>
$EA_{tot}$ (U)	22514 ± 208 <sup>a</sup>	8990 ± 280 <sup>b</sup>	10468 ± 104 <sup>c</sup>
$EA_{vol}$ (U l <sup>-1</sup> )	1125 ± 46 <sup>a</sup>	2997 ± 94 <sup>b</sup>	3489 ± 34 <sup>c</sup>
$Y_{a/s}$ (U g <sup>-1</sup> )	32.2 ± 0.2	N.D.	N.D
Biomass (g l <sup>-1</sup> ) §	69 ± 3.5 <sup>a</sup>	2.4 ± 0.26 <sup>b</sup>	3.8 ± 0.45 <sup>c</sup>
$EA_{sp}$ (U l <sup>-1</sup> )	466 ± 28	1917 ± 40	1131 ± 6
$Y_{p/s}$ (U l <sup>-1</sup> )	-	9206 ± 196 <sup>a</sup>	8604 ± 46 <sup>b</sup>
$\check{R}_t$ (U l <sup>-1</sup> h <sup>-1</sup> )	5.2 ± 0.03 <sup>a</sup>	9.6 ± 0.31 <sup>b</sup>	20.8 ± 0.13 <sup>c</sup>
OMW <sub>tr</sub> (l)	0.0325	0.33	0.42
V <sub>bio</sub> (l)	20	3	3
T (h)	216	312	168

Legend:  $EA_{max}$  = maximal enzyme activity;  $EA_{tot}$  = total enzyme activity from a single fermentation batch;  $EA_{vol}$  = unitary volumetric activity: activity per unit of reactor volume;  $Y_{a/s}$  = yield of activity per g of substrate;  $EA_{sp}$  = specific activity: activity referred to weight unit of the mycelial biomass; T = time to reach the maximal activity;  $Y_{p/s}$  = production yield: enzyme production per unit of OMW;  $\check{R}_t$  = mean volumetric productivity calculated at the time of maximal production; OMW<sub>tr</sub> = amount of omw treated in a single fermentation batch per unit of reactor volume; V<sub>bio</sub> = total bioreactor volume.

\*OMW was used as a moistening agent (65%) for maize stalks; § data are expressed in g mycelium (kg solid substrate)<sup>-1</sup>.

Results are mean of three replicates ± standard deviation. Row means followed by the same superscript letter were not significantly different (P < 0.05) as determined by the tukey test.

## 2. PRODUCTION OF CHEMICALS

### 2.1 Exopolysaccharides

Microbial exopolysaccharides (EPS) might be a valid alternative to plant and algal products for their unusual molecular structures and peculiar conformations

that make them unique and potentially interesting properties in view of possible industrial uses. Commercial usage of fungal exopolysaccharides such as, *e.g.*, scleroglucan and schizophyllan might be encouraged by the development of fermentative processes possibly using cheap substrates such as agro-industrial residues and/or wastes.

To this end, three fungal strains have been tested on OMW: *Sclerotium glucanicum* NRRL 3006 and *Sclerotium rolfsii* ATCC 15206, already well known as good producers of beta-glucans, and *Botryosphaeria rhodina* DABAC-P82.

On undiluted OMW (COD, 74.0 g l<sup>-1</sup>), *S. glucanicum* and *S. rolfsii* did not grow while *B. rhodina* DABAC-P82 grew well (approximately, 13.0 g/l of fungal biomass) and produced high levels of EPS (more than 10 g/l after only 96 h of fermentation) (Figure 3) (Crognale et al., 2003).

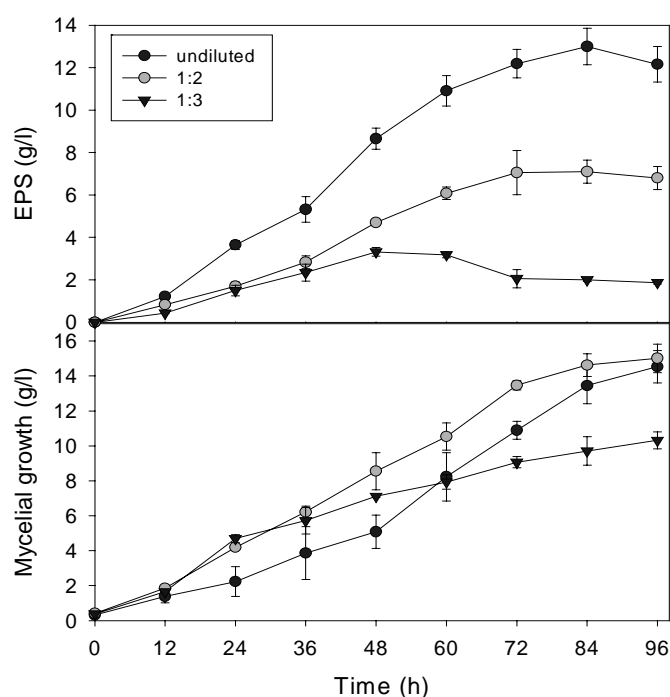


Fig. 3. EPS production by *Botryosphaeria rhodina* DABAC-P82 grown on undiluted and diluted OMW

Slika 3. Proizvodnja EPS pomoću *Botryosphaeria rhodina* DABAC-P82 uzgojenog na nerazrijeđenom i razrijeđenom OMW

The potential of this fungus to use OMW as a substrate for the production of EPS appears to be noticeable. However, further studies aimed at assessing the process technical feasibility with different OMW typologies in terms of origin, polluting load and time and way of storage are yet needed.

### 3. IMPROVEMENT OF OMW IN VIEW OF AGRONOMIC USE:

#### 3.1 Microbial enrichment of OMW with soluble phosphate

This work was aimed at performing partial removal of pollutants from OMW with concomitant enrichment in soluble phosphorus in order to use the treated effluent for field spreading as a low cost P fertilizer. To this end, OMW supplemented with  $3 \text{ g l}^{-1}$  of rock phosphate (RP) were treated with *Aspergillus niger* in repeated batch process: maximum soluble P reached  $0.75 \text{ g l}^{-1}$  at the 3<sup>rd</sup> batch (Figure 4).

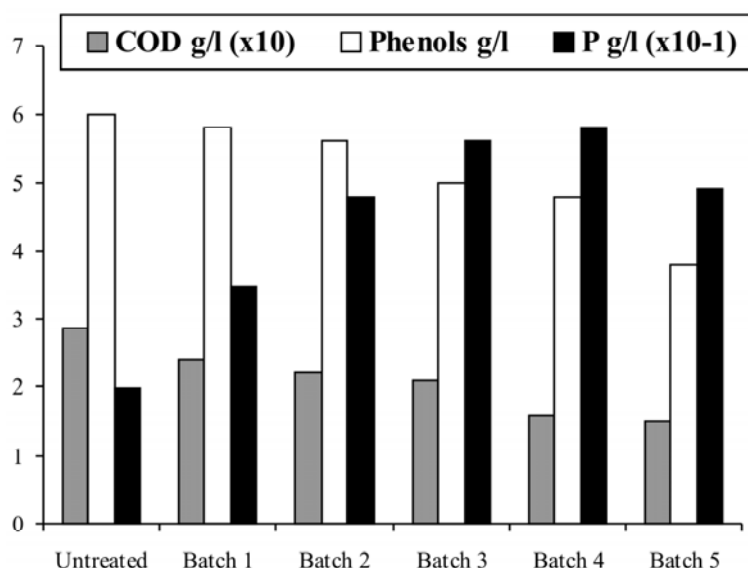


Figure 4. Repeated batch fermentation using immobilised *Aspergillus niger* on OMW + RP, shaken flasks

Slika 4. Ponovljena fermentacija mase korištenjem imobiliziranog *Aspergillus niger* na OMW + HP

Several types of OMW  $\pm$  RP, microbially-treated or not, were tested for their fertilizing ability on a soil-wheat (*Triticum durum*) model system in green house in order to simulate the effects of possible land spread of the effluent (Cereti et al., 2003). Plants grown on soil watered with OMW treated as above showed an increase in seed biomass, spike number and kernel weight (Tab. 4); Harvest Index was also highest (0.53).

**Table 4. Preliminary agricultural tests on wheat in greenhouse using treated and untreated OMW**

**Tablica 4. Preliminarni poljoprivredni testovi na pšenici u plasteniku korištenjem tretirane i netretirane OMW**

	Spikes/plant (N°)	Kernel weight (mg)	Grain yield (g/pot)	Harvest Index
Control (soil)	1.4 $\pm$ 0.7	27.3 $\pm$ 0.06	0.49 $\pm$ 0.18	0.20 $\pm$ 0.06
Soil + P fertilizer	2.0 $\pm$ 0.6	30.2 $\pm$ 0.03	0.98 $\pm$ 0.34	0.25 $\pm$ 0.01
Soil + Untreated OMW	1.0 $\pm$ 0.0	30.1 $\pm$ 0.25	0.26 $\pm$ 0.03	0.22 $\pm$ 0.04
Soil + Treated OMW	3.8 $\pm$ 0.2	48.3 $\pm$ 0.02	3.05 $\pm$ 0.57	0.53 $\pm$ 0.02

## CONCLUSIONS

The results obtained in the research summarized above suggest that OMW can be regarded as a useful residue for the recovery of fine chemicals and for different biotechnological applications such as the production of important metabolites, and biotreatment to improve its characteristics as a fertilizer. Nevertheless, it should be taken into account that there are several obstacles and difficulties in OMW upgrading at an industrial scale. These technical constraints include the seasonality of the olive oil production, the highly variable chemical composition of OMW as well as the need for an accurate storage.

Therefore, it seems that fermentative biotechnologies can be applied successfully only if limited amounts of OMW are to be treated, while in the case of large amounts controlled compost production might probably be more indicated.

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