



Project
funded by the
EUROPEAN UNION



Mediterranean Cooperation in the Treatment and Valorisation of Olive Mill Wastewater (OMW)

MEDOLICO

Deliverable 5

Activity 7: Feasibility and evaluation of OMW by-products recovery

- Activity 7.1 - By-products recovery and evaluation



University
of Cyprus



BGU

July, 2015



EXECUTIVE SUMMARY

Activity 7: Feasibility and evaluation of OMW by-products recovery

Activity 7.1 By-products recovery and evaluation

Simultaneously with the evaluation of the performance for the different olive mill wastewater (OMW) treatment processes tested at pilot scale and carried out under the activity 6 in the framework of MEDOLICO project (Deliverables 3 and 4), activity 7 will evaluate the feasible methods for recovering by-products from olive mill wastewater, in an attempt to offset the initial costs incurred by the purchase of the OMW treatment equipment. An additional advantage to recovering by-products is to eliminate or reduce to absolute minimum any waste stream.

A summary of the Activity 7 objectives are:

1. Polyphenols recovery from OMW process streams:
 - ▶ to obtain bioactive concentrates enriched in phenolic compounds from fresh OMW using multiple membrane steps (UF and NF);
 - ▶ to generate a cleaned aqueous stream that can be treated in a municipal wastewater plant;
 - ▶ to recover biophenols from this concentrate using a Membrane Aromatic Recovery System (MARS) based on a newly developed thin film composite membranes (TFCM);
 - ▶ to analyze MARS permeate for phenols and other products to determine component/quality characteristics of different MARS membranes;
 - ▶ to determine the factors affecting the membrane recovery process, to evaluate the feasibility of biophenols recovery by the developed method and further development optimization.
2. Valorisation of OMW for enzyme production
 - ▶ to assess the suitability of OMW as a fermentation medium for the production of lipase.

1. Polyphenols recovery from OMW process streams

During the MEDOLICO project, BGU developed a hybrid membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from olive mill wastewater (OMW) was developed. A membrane technology was investigated for recovering in a cost effective way a concentrated hydroxytyrosol product from the OMW effluent. Selective permeability of hydroxytyrosol and tyrosol with very low passage of other OMW components such as acids, carboxylate, and non-aromatic carbon chains, was achieved by the use of specific modification of the selective barriers by formation of specific channels. Improved membrane permeability and selectivity were achieved by selective layer thickness 1-3 μm and by incorporating tyrosol into the selective films matrix. The crosslinking of the fluorinated silicone polymers provided several advantages to the obtained membranes: it imparted mechanical stability of the membrane, it also conferred chemical stability of the membrane so it doesn't get washed off during the application process and it also reduced the pore size to increase selectivity and prevent transfer of salts from the feed solution, preventing thus transfer of base from the strip solution to the feed solution. Treating waste stream concentrates from nanofiltration (NF), treated OMW allows efficiently increase TOC concentration from $\sim 5\%$ to $>15\%$. Mobile OMW Membrane Treatment System (MARS) with treating capacity of 133 m^3 per day was proposed. The presented data earnestly demonstrated the payback of the proposed system.

The OMW was acidified to stabilize the OMW and promote hydrolysis to release the hydroxytyrosol and tyrosol from oleuropein. Then, there were three sequential membrane stages:

- 1) After removal of top and bottom phases that separate, submerged ultrafiltration (UF) treatment (0.04 micron) of the acidified OMW is carried out to remove fine suspended solids and large molecules.
- 2) After this NF or reverse osmosis (RO) treatment of the UF permeate on a pilot plant is carried out.
- 3) Third stage is Polyphenols recovery from NF or RO concentrate with use of a membrane contactor, which strips polyphenols from an acidified feed with a high pH strip solution on the other side. This process is denoted in the literature as a Membrane Aromatic Recovery System (MARS) [1-3].

- 4) The NF or RO retentate, containing enriched and purified low molecular weight polyphenols, has potential applications in the food, pharmaceutical or cosmetic industries, while UF retentate can be used as fertilizer. Because of the high value of tyrosol and hydroxytyrosol (14000-20000 EUR per pure kg), the extract can reach 80-520 EUR/kg of extract depending on the extent of its purification from other components in the OMW. Further concentration and recovery of such valuable components by the MARS would be a significant valorisation of olive oil waste. An additional potential of the developed membrane system (UF-NF-MARS) is that further developments may lead to the recovery of other high value OMW by-products.

UNIGE proposed an integrated membrane process for OMW treatment and simultaneous recovery of polyphenols [4]. Proposed OMW treatment process consist of two consecutive membrane processes: microfiltration (MF) followed by the reverse osmosis (RO). The MF process uses a porous multichannel ceramic membrane to retain suspended materials and to produce a clarified permeate to feed the RO process. The RO step provides separation of dissolved substances from water, thus produces purified water of a very high quality and minor volume of polyphenols rich concentrate (total phenols up to 7 g/L). Further separation of polyphenols from other substances present in the RO concentrate and their purification, for instance in MARS system, as proposed by BGU, were not investigated by UNIGE.

2. Valorisation of OMW for enzyme production

The composition of OMW includes polysaccharides, sugars, phenolic compounds, tannins, polyalcohols, proteins, organic acids and lipids. Due to its oil residual content, OMW is a potential source of lipase-producing microorganisms and a complex medium potentially suitable for lipase production. Lipases, triacylglycerol hydrolases, are an important group of enzymes with several applications in food, dairy, detergent and pharmaceutical industries. Additionally, lipases have an important application in the field of bioenergy. The lipase-catalyzed esterification of vegetable oils has been studied as an environmentally friendly process to produce fatty acid methyl ester (FAME), which is called biodiesel fuel.

The purpose of this study was to evaluate the suitability of OMW as growth medium for lipase production by a non-conventional lipase-producing yeast strain JOR5 which was previously isolated and identified as *Magnusiomyces capitatus* by DNA sequencing. This species had not yet been

described in literature as having lipolytic properties and was tested in this study. A statistical design following the Doehlert distribution was used to evaluate the influence of nitrogen (NH_4Cl) concentration and oxygen availability (k_{La}) on *M. capitatus* JOR5 growth and to improve lipase production. The experimental design allowed reaching 1.44 U mL^{-1} for lipase activity, and concluding that lipase production can be increased with increasing of NH_4Cl concentration. The influence of the oxygen availability was less significant in lipase production. There was a positive interaction of the two factors on yeast growth and for intact membrane cells but the effect of oxygen availability was higher than nitrogen concentration. Biomass growth was stable as indicated through flow cell cytometry analysis in which membrane cells presented an average of 99% integrity, under the tested conditions.

This work demonstrated that the yeast strain *M. capitatus* JOR5 is a lipase-producing microorganism. Valorisation of OMW is possible as fermentation growth medium for the yeast production of lipase under controlled conditions.

References

1. Han, S., Ferreira, F.C. and Livingston, A. (2001). J. Membr. Sci. 188, 219.
2. Han, S., Ferreira, F.C. and Livingston, A. (2002). Desalination 148, 267-273.
3. Han, S., Ferreira, F.C. and Livingston, A. (2005). J. Membr. Sci. 257, 120-133
4. EUROMED 2015 - Desalination for Clean Water and Energy, Cooperation among Mediterranean Countries of Europe and the MENA Region, 10–14 May 2015, Palermo, Italy: “Treatment of olive mill wastewater through integrated pressure driven membrane processes”, A. Bottino, G. Capannelli, A. Comite, R. Firpo, A. Jezowska.

Abbreviations

<i>MARS</i>	<i>Membrane Aromatic Recovery System</i>
<i>MF</i>	<i>Microfiltration</i>
<i>NF</i>	<i>Nanofiltration</i>
<i>OMW</i>	<i>Olive Mill Wastewater</i>
<i>RO</i>	<i>Reverse osmosis</i>
<i>TOC</i>	<i>Total Organic Carbon</i>
<i>UF</i>	<i>Ultrafiltration</i>

Contributions by:

BGU - Ben-Gurion University of the Negev

Zeev Wiesman

Jack Gilron

Charles Linden

Janna Abramovich

Leonid Kogan

Galina Neimark

LNEG – National Laboratory of Energy and Geology

Vera Salgado

Céu Penedo

Belina Ribeiro

Ana Eusébio