NEW OLIVE MILL WASTEWATER PROCESS TO ELIMINATE THE ENVIRONMENTAL LOAD AND TO RECOVER SEVERAL FINE CHEMICALS AS POLIPHENOLS AND PURIFIED WATER

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SUMMARY: A new Olive Mills Wastewater (OMW) treatment process is presented, in a new sustainable approach, in order to eliminate the pollution load and simultaneously to recover and re-use the organic matter content, such as nitrogen compounds, sugars, fibres, purified water, and polyphenolic compounds, in particular verbascoside and hydroxytyrosol polyphenols, which have important antioxidant properties.

The process flow sheet is reported in figure 1. Five treatment steps are necessary. Chemical and enzymatic pre-treatment of the raw OMW matrix, followed by four tangential flow membrane filtration steps: micro-filtration (MF), ultra-filtration (UF), nano-filtration (NF) and reverse osmosis (RO). The MF section is equipped with ceramic tubular membrane with a molecular weight cut-off (MWCO) of 0.14 mm. All the other membrane sections are equipped with spiral wound polymeric membranes with MWCO ranging between 6,000- 200 Daltons.

The filtration processes allow the recovery of five main liquid fractions, in different volumetric percentages, all of which are suitable for commercial use in food, nutraceutical and cosmetic industries. All the concentrate fractions, including the UF concentrate, are requested in the food industries and in the nutraceutical sector. The purified water, RP permeate, representing the highest volume fraction, has a COD level of 100 ppm O_2, and has the characteristics of potable water.

This water can represent a new potable water class (vegetable water) with hypotonic and antioxidant properties due to the presence of hydroxytyrosol traces and the typical olive flavours.

A preliminary technical and economic evaluation of the entire new process, covered by an international patent WOO2005123604, is reported, in order to promote the application of our technologies in the Mediterranean area.

1. INTRODUCTION

In the Mediterranean countries, Spain, Italy Greece and Morocco, etc. , several million tons of waste are produced in three months by Olive Mills Wastewater (OMW), that represent one of the greatest environmental problems of the olive agro industry.
In table 1, an estimation of large volume of OMW produced in Europe is reported.

Table 1. Estimation of Olive Mill Wastewater produced in Europe

Countries	Ton/Year
Spain	8,0
Italy	9,0
Greece	2,0
Total	15-20

OMW has been a pollution source, for thousands of years, but now its effect on the environment is more noticeable, because the sensitivity of people to environmental problems is much higher than in the past.
In Italy the total organic load per year produced from OMW is equivalent to that produced by 25 million of people, causing dangerous effects on soil fertility and on water stratum contamination.
In fact, where laws allow it, as do the Italian regulations, OMW can be spread out on agricultural fields due to the difficulties and the cost of conventional treatment methods.
Negative consequences on the soil properties are object of several studies (1-2).
Although the chemical composition of OMW changes from one soil area to another, from olive cultivar, olive ripening, and olive crashing procedure, chemical compounds are well known, but the high COD (Chemical Oxygen Demand) of OMW ranging between 80 to160 g/L of O_2 represents a serious disposal problem. The typical chemical composition of OMW is reported in table 2.

Table 2. Physical and chemical properties of OMW

Parameters	OMW
pH	4,5-6,0
Electric conductivity (mS/cm)	5-10
Minerals (g/L)	5-10
BOD_5 (g/L di O_2)	50-150
COD (g/L di O_2)	80-210
Total solids (%)	5,5-17,6
Organic substances (g/L)	30-160
Oil (g/L)	0,3-23
Sugars (g/L)	10-80
Nitrogen substances (g/L)	5-20
Total polyphenols (g/L)	3-11

The COD load is due to high organic content, including nitrogen compounds, sugars, organic acids, oils, cellulose and polyphenolic compounds, with concentration ranging between 3 to 11 g/L.
These chemical compounds represent the main obstacle to the OMW disposal, because they have antibacterial and phyto-toxic properties, they inhibit both aerobic and anaerobic treatment processes (3-5), due to which the development of a specific treatment process is required .
The treatment of OMW is extremely difficult, due to the chemical composition

(polysaccharides, sugars, polyalcohols, proteins, polyphenols, oil), a considerable amount of suspended solid, that may reach 190 g/L (4), and because of the large volume produced each year.

Several treatments, based on chemical, physico-chemical, and biological processes, have been studied in order to reduce the pollution load, but up to now the practical results are very poor.

A large number of OMW treatment processes consist of destroying the polyphenols trough the oxidation way, performed by air-oxygen, ozone etc. and a few of them are based on the application of microorganisms, as selected yeasts, able to metabolize the poliphenols as hydroxityrosol, verbascoside, etc.

Recently new studies concerning the more interesting biological properties of the polyphenols present in the OMW have focused on its recovery and reutilization, rather than its destruction.

Although polyphenols can be considered an environmental problem, they belong to a very important category of antioxidant, phytochemical compounds that are useful for the pharmaceutical and cosmetic industry.

The concentration of polyphenols in olive oil ranges from 100 to 800 mg/kg of oil, depending on the olive variety, fruit ripening, extraction system, etc. This amount of antioxidants in the olive oil is only 1-2 % of the available pool of antioxidants present in the olive fruits, the rest is lost in the wastewater (approximately 50%) and in the pomace (about 50 %).

More than 30 biophenols compounds are identified in OMW , the majority of which exhibit antioxidant activity, cardio protective action and cancer-preventing activities in humans, as reviewed by Obied and al(6-7).

In this context a new treatment process of OMW, completely based on the application of membrane technologies, is studied and developed..

2. MATERIALS AND METHOD

Experiments were carried out in the laboratories of the Casaccia Research Centre of ENEA in Rome.

OMW used was supplied from an olive mill operating in a continuous extraction process, located at north of Rome. After the collection, OMW were acidified at pH 3,5 using HCl 35% supplied by J.T. Baker.

The nitrogen compounds were determined through colorimetric titration following the kjeldhal method, using digestion B-426 and distiller B-323 units Buchi.

Polyphenolic concentration was determined in HPLC using a high pressure gradient pump Gynkotec with an UV detector Varian 9050 operating at 280 nm. Chromatographic separation was obtained using the Lichrosorb 5 RP18 250x4,6 mm column, working in gradient of pure water acidified at pH 3,2 with H_3PO_4 and acetonitrile. All the polyphenolic standards were from SIGMA with the exception of oleuropein obtained from Genay. Hydroxytyrosol standard was obtained from quantitative hydrolysis of oleuropein in a 1% solution of citric acid.

The enzyme Natuzym Olimax, supplied by Addfood Company, was used at 0,02 % for 60 minutes, in a stirred batch reactor at 37°C. Natuzym Olimax is a combination of pectinase and cellulase, produced from selected strains of *Aspergillus niger.* This enzyme was developed especially for olive oil extraction in the milling procedure.

Membrane filtration experiments were realized with MF, UF, NF and RO ENEA pilot plants.

MF pilot plant, was equipped with a centrifugal pump Grundfos, with a total head of 91,8 m, a maximum flow rate of 10 m^3/h and a nominal power of 3 kW; two housing* containing one ceramic tubular membrane; two flow meters on the retentate and permeate lines able to measure fluxes of 4 m^3/h and 320 L/h; three manometers, before and after the ceramic membrane and in the permeate line, with a measuring range of 0-4 bars; a cooling system controlled by an electro valve regulated with a thermocouple with range of ± 1°C; feed and permeate tanks of about 120 L; a back pulse apparatus for membrane continuous cleaning with permeate stream at prefixed interval time.

UF and NF experiments were carried out with a pilot plant,, employing polymeric spiral-wound membranes. The plant consists of a centrifugal pump Grundfos with a total head of 140 m, a maximum flow rate of 6 m^3/h; a vessel containing one polymeric membrane 4’’x40’’; two flow meters in the retentate and permeate lines able to measure 6 m^3/h and 1 m^3/h; two manometers, before and after the vessel, with a range of 0-25 bars; a cooling system controlled by an electro valve regulated with a thermocouple with range of ± 1°C; a tank of 500 L. RO experiments were carried out with a pilot plant made by Osmo Sistemi, consisting of a pre-alimentation pump Lowara with a total head of 36 m, a maximum flow rate of 4 m^3/h and a nominal power of 0,45 kW; a high pressure pump with installed power of 1,85 kW, able to reach 60 bar of pressure; a vessel containing a spiral wound membrane 4’’x40’’; two flow meters in the pre-alimentation and in the concentrate lines able to measure 1,6 m^3/h; two manometers at the exit of the vessel in the concentrate line and in the pre-alimentation line respectively with ranges of 0-100 bars and 0-6 bars; a water cooling system; a tank of 500 L; in line sensors and a display to show pressures, feed flow, permeate flux, permeate conductivity, amount of produced permeate.

The specifics of the MF, UF, NF and RO membranes employed are reported in table 3.

Table 3. Membrane technical features.

Membrane	Process	Material	Conformation	Cut-off and area
Ceramic, Tami	MF	ZrO_2	Tubular 23 canals (3,6 mm F)	0.14 m (0,35 m^2)
Polymeric Osmonics	UF	PES	Spiral-wound 4’’x40’’ inches 28 mils spacer	6 Kd (8,36 m^2)
Polymeric,Nadir	NF	Polyamide TFM	Spiral-wound 4’’x40’’ inches 20 mils spacer	150-300 Da (8,36 m^2)
Polymeric Hydronautics	RO	Composite polyamide	Spiral-wound 4’’x40’’ inches	99,5% salts rejection (7 m^2)

ZrO_2: Zirconium oxide
PES: Polyethersulfone
TFM: Thin Film Composite

3.RESULTS AND DISCUSSION

The treatment of OMW is based on the application of membrane technology (MT) with the aim of extracting and recovering the maximum amount of polyphenols in order to facilitate the disposal of these wastes (8-9) .

It is known that membrane technologies employ special filters (membrane) that are operated in a special fluido-dynamic condition (tangential flow) that reduce the filter fouling and consequently assures a high permeate flux as a function of time (10). These technologies are defined BAT (Best Available Technology) from the EPA and are also recognized by the European Union (UE).

The MT is largely applied in the world not only for wastewater treatment but especially for dispersed solutes recovery, often pollutant, and to generate purified water.

All these technical characteristics are perfectly achieved in the new OMW treatment process, in which the raw matrix is completely separate in five liquid fractions, of different chemical

compositions.
Membrane technologies, microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), are used in sequence. The permeate stream of each section, feeds the successive filtration step. Four liquid fractions, respectively constituted of concentrated organic matter, depleted of polyphenolic content (MF and UF retentate), and concentrated high molecular weight (OMW) polyphenols.
The freshly collected OMW was acidified with HCl or H_2SO_4 until pH = 3,5 in order to inhibit the endogenous polyphenol-oxidase and subsequently treated with a pectolytic enzyme (Natuzym) in order to hydrolyse the cellulose and hemi-cellulose structure of the OMW responsible for entrapping the polyphenols compound. The enzymatic reaction takes place in a steered batch reactor at 37°C for 120-150 minutes. This enzymatic treatment enhances the concentration of soluble polyphenol and subsequently permits, a phase separation, in a relatively clear red suspension, on the bottom and a dense paste on the top. The suspension is collected from the bottom of the reactor and pumped in the first membrane step, the microfiltration one.
The dense and colloid phase, in the form of micro-emulsion due to the presence of oil micro-drops should be retreated with the enzyme, but in our experiment it is simply discharged.
The hydraulic scheme of OMW treatment with membrane is reported in the figure 1.

Figure 1. The new process scheme of OMW treatment

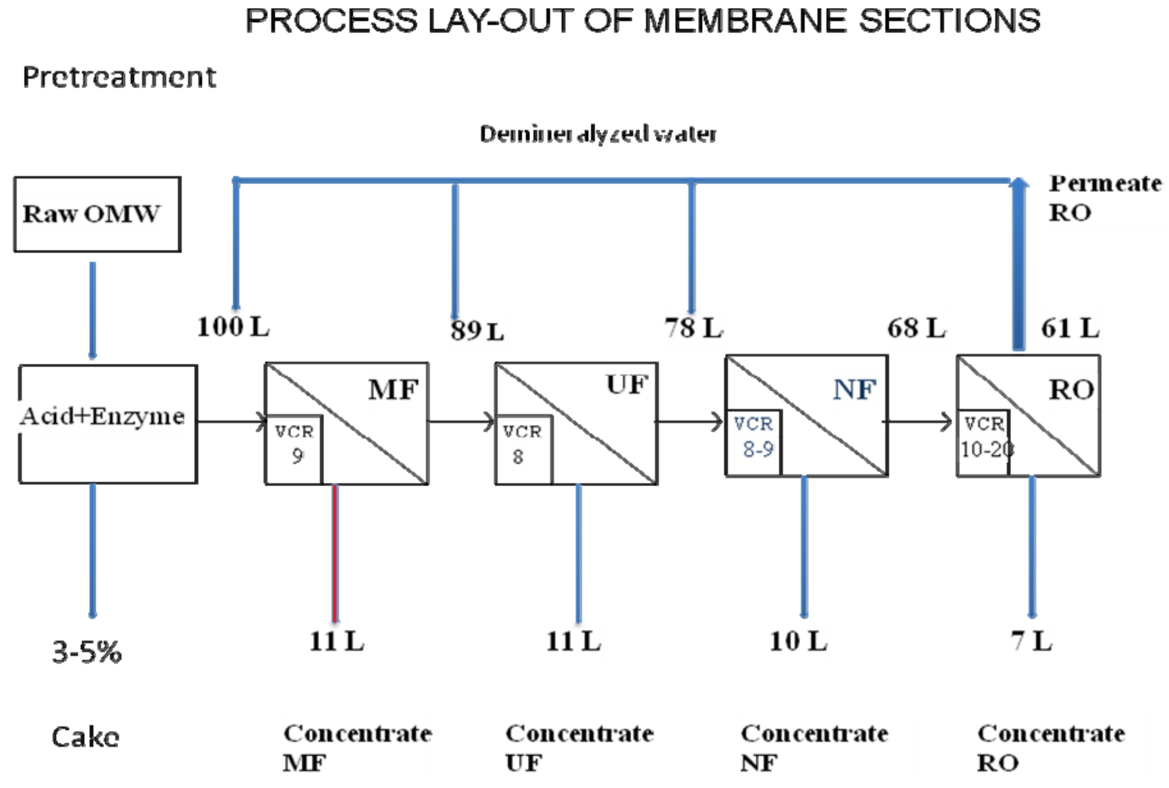

Figure 1 shows the treatment pathway of raw OMW fractionation as previously discussed.

Considering 100 L of the volume of OMW red suspension, as the feed of MF, the respective volumes of permeate and concentrate of each membrane section are reported. The volume concentration ratio (VCR) between the initial feed volume/final retentate volume of each membrane section is reported in figure 1. All four retentate fractions of membrane, respectively 11 % of MF, 11 % of UF, 10 % of NF and 7 % of RO constitute the new refined products with the addition of the 60-70 % of RO permeate, constitute a purified water that has a COD of about 100 ppm of 0_2.

The volume retentate percentage must be varied in the process, as a function of the chemical composition of the raw OMW (more o less diluted) and as a function of the specification required from the market of products rich in poliphenols, especially the NF and the RO retentate.

In order to increase the polyphenols extraction yield, both in the NF and RO concentrate, a suitable quantity of RO permeate solution is added to each retentate fraction, as indicated in the figure 1. Nevertheless, the maximum polyphenols extraction in the NF and RO retentate fractions remained under 85 % of the total present in the feed solution.

The total polyphenols distribution in the four fractions exit from the membrane are reported in table 4.

Table 4. Polyphenols concentrations (g/L) in each membrane fraction

Total polyph.ls	Row OMW	MF		UF		NF		RO	
		R.	P.	R.	P.	R.	P.	R .	P.
	4,66	5,09	4,50	2,15	4,8	9,10	4,01	28,3	0,012

R= R=retentate; P=permeate

The permeate flux as a function of time, for the MF, UF, NF and RO sections are reported in the figure 2, as a function of a continuous process run of 450 minutes.

The permeate flux reduction, as shown in figure 2, represents a classical trend for the membrane technologies, but the slow decreasing productivity profiles of the four filtration sections is a demonstration of a satisfactory process parameter regulation.

Table 4 summarize the process conditions of each membrane section and the permeate flux average value measured in about 7.5 hours of continuous process run .

Figure 2. Permeate flux vs. time of MF,UF,NF and RO membrane sections.

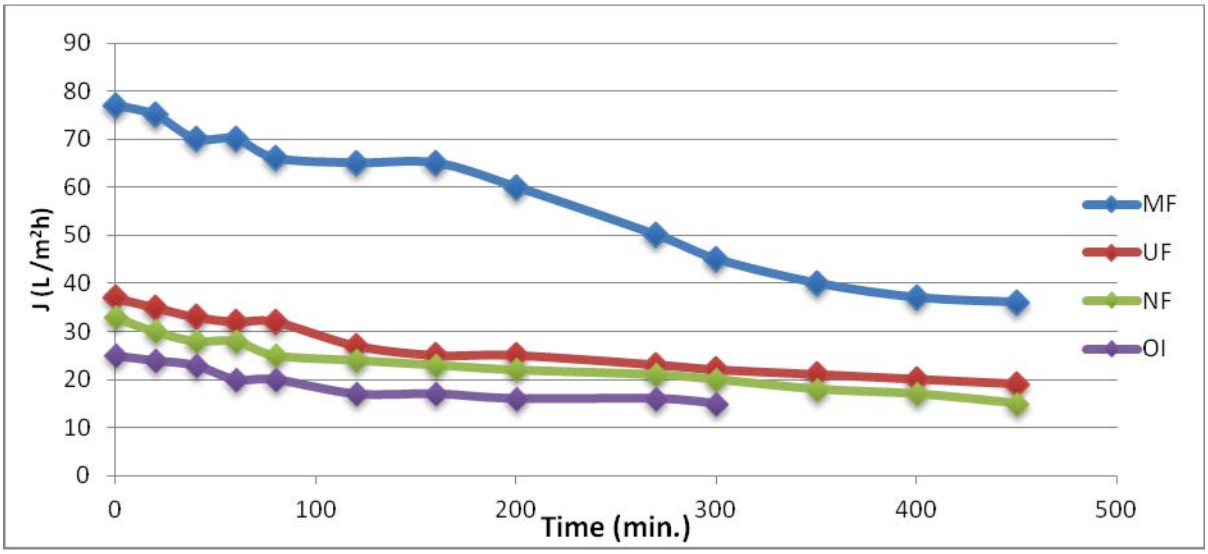

Table 4. Membrane process conditions, average of the productivities and COD of permeate streams.

Membrane sections	DP (bar)	Temperature (°C)	Permeate flux (l/m^2h)	Permeate COD (mg/L)
MF	3-4	40-45	62	130,000
UF	2-3	18-20	23	20,000
NF	16-20	15-20	25	3,000
OI	30-35	15-20	20	80-100

4.CONCLUSIONS

The new membrane filtration processes allow the recovery of five main liquid fractions (figure 1) in different volumetric percentages, all of which are suitable for commercial use in food, nutraceutical and cosmetic industries.

The MF and UF retentate can be used in the food industry as an additive for bread, pizza, chokers, etc., but is also more interesting as an anaerobic substrate for methane production.

The NF concentrate contains purified polyphenols (verbascoside) used as a food integrator; RO concentrate contains 52 to 70% of the hydroxytyrosol present in the row OMW.

All the concentrate fractions, including the UF concentrate, are requested in the food industries and in the nutraceutical sector. The purified water, representing the highest volume fraction, shows a COD level of 100 ppm O_2 , having all the characteristics of potable water .

Membrane performances, in terms of productivities requires a continuous monitoring and control of process parameters as pressure, temperature, feed capacity and permeation flux.

In this condition the membrane fauling, that represent a critical point of this technology, must be kept under control.

In order to avoid the lengthy membrane cleaning and regeneration procedures it is necessary to respect all the operating parameters and the VCR of each membrane section (table 3).

The new process represents a realistic solution for solving the environmental problem of OMW disposal , in fact about 60-70% of initial OMW volume is transformed in a purified water having a COD of about 100 ppm of O_2, with the propertied of drinkable water. In addition, the new process allows the production of purified and concentrated polyphenols from a natural matrix which allows a pay back of the investment costs in little time.

The process is now developed in an industrial plant scale of 10 ton/day of OMW, demonstrating not only the feasibility of the elimination of the environmental impact, but also the economical convenience.

5.REFERENCES

1) Fiorentino A., Gentili A., Isidori, M., Monaco P.; Nardelli A., Parrella A., Temussi F. (2003) Environmental effects caused by olive mill wastewaters: toxicity comparison of low-molecular-weight phenol components. *In Journal. Agric. Food Chem.* 51,pp 1005-1009
2) Sierra J., Martì E., Montserrat G., Cruanas R., Garau M.A. (2001) Characterisation and evolution of a soil affected by olive oil mill wastewater disposal. *In Sci. Total Environ.* 214 pp 279, 207
3) Ranalli, A.; Lucera, L.; Contento S. (2003) Antioxidizing potency of phenol compounds in olive mill wastewater. *Journal. Agric. Food Chem.,* 51, 7636-7641.
4) Zouari N., (1998) Decolorization of olive oil mill effluent by physical and chemical treatment

prior to anaerobic digestion. *Journal. Chem. Technol. Biotechnol.* 73 pp 297-303

5) Rozzi A., Malpei F, (1996) Treatment and disposal of olive mill effluent; *In International Biodeterioration & Biodegradation*, pp 135-144
6) Obied H.K., Allen M.S., Bedgood D.R., Prenzler P.D., Robards K., Stockmann R., (2005) Bioactivity and analysis of biophenols recovered from olive mill waste. *In Journal. Agric. Food Chem.*, 53 pp 823-837
7) Visioli, F., Romani A., Mulinacci N., Zarini S., Conte D., Vincieri F., Galli C. (1999) Antioxidant and other biological activities of olive mill wastewaters. *In Journal. Agric. Food Chem.* 47 pp 3397-3401
8) Pizzichini M., Russo C., (2005) Process for recovering the components of olive mill wastewater with membrane technologies. *International. Patent WO2005123603*.
9) Russo C., (2007) A new membrane process for the selective fractionation and total recovery of polyphenols, water and organic substances from vegetation waters . ., 288 pp 239-246
10) Cheryan M. (1986) Ultrafiltration handbook, *Ed. Technomic Publishing Comp. Inc.*