MINI-REVIEW

Bioremediation and biovalorisation of olive-mill wastes

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Abstract Olive-mill wastes are produced by the industry of
olive oil production, which is a very important economic
activity, particularly for Spain, Italy and Greece, leading to
a large environmental problem of current concern in the
Mediterranean basin. There is as yet no accepted treatment
method for all the wastes generated during olive oil
production, mainly due to technical and economical
limitations but also the scattered nature of olive mills
across the Mediterranean basin. The production of virgin
olive oil is expanding worldwide, which will lead to even
larger amounts of olive-mill waste, unless new treatment
and valorisation technologies are devised. These are
encouraged by the trend of current environmental policies,
which favour protocols that include valorisation of the
waste. This makes biological treatments of particular
interest. Thus, research into different biodegradation
options for olive-mill wastes and the development of
new bioremediation technologies and/or strategies, as well
as the valorisation of microbial biotechnology, are all
currently needed. This review, whilst presenting a general
overview, focusses critically on the most significant recent
advances in the various types of biological treatments, the
bioremediation technology most commonly applied and
the valorisation options, which together will form the
pillar for future developments within this field.

Keywords Olive-mill wastes · Microbial treatments ·
Bioremediation · Valorisation

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Introduction

The olive oil industry generates large amounts of by-
products that are harmful to the environment. According to
the Food and Agriculture Organisation of the United
Nations (FAOSTAT 2006), 2.7 millions of tonnes of olive
oil are produced annually worldwide, 76% of which are
produced in Europe, with Spain (35.2%), Italy (23.1%) and
Greece (16.1%) being the highest olive oil producers. Other
olive oil producers are Africa (12.5%), Asia (10.5%) and
America (0.9%). Olive oil production is a very important
economic activity, particularly for Spain, Italy and Greece
(where combined exports are valued at more than 5,500
million US$); worldwide, there has been an increase in
production of about 30% in the last 15 years (FAOSTAT
2006). Moreover, olive oil production is no longer
restricted to the Mediterranean basin, and new producers
such as Australia, USA and South America will also have

to face the environmental problems posed by olive-mill wastes (OMWs).

The chemical composition of olives, which is the raw material for olive oil extraction, is very variable and depends on factors such as the olive variety, soil type and climatic conditions, but in general it consists of 18–28% oil, 40–50% vegetation water and stone (pit) and 30–35% of olive pulp (Niaounakis and Halvadakis 2004). Following olive oil extraction, mainly by mechanical procedures in olive mills, a large quantity of liquid and solid residues is produced, with a high organic load, the nature of which depends on the extraction system employed. Three systems are used worldwide for the industrial-scale extraction of oil from olives, *viz*. the traditional press-cake system, the three-phase decanter system and the modern two-phase centrifugation system (Fig. 1). Nowadays, two-phase and three-phase centrifugation systems are most commonly used.

The three-phase system, introduced in the 1970s to improve extraction yield, produces three streams: pure olive oil, olive-mill wastewater (OMWW) and a solid cake-like by-product called olive cake or *orujo*. From an environmental point of view, OMWW is considered the most critical waste emitted by olive mills in terms of both quantity and quality (Niaounakis and Halvadakis 2004). The olive cake, which is composed of a mixture of olive pulp and olive stones, is transferred to central seed oil extraction plants where the residual olive oil can be extracted. The two-phase centrifugation system was introduced in the 1990s in Spain as an ecological approach for olive oil production since it drastically reduces the water

consumption during the process. This system generates olive oil plus a semi-solid waste, known as the two-phase olive-mill waste (TPOMW) or *alpeorujo*. This review will focus on the microbial treatment of OMWW and TPOMW due to their high production (Table 1) and thus high environmental impact.

The problems arising from OMWW are derived from its high organic load and its chemical composition (Table 2), which renders it resistant to degradation. The OMWW contains a majority of the water-soluble chemical species present in the olive fruit, a high organic load and high C/N ratio (chemical oxygen demand (COD) values up to 200 g l^{-1}) and has an acidic pH of between 4 and 6. The organic fraction contains large amounts of proteins, lipids and polysaccharides, but unfortunately OMWW also contains phytotoxic components that inhibit microbial growth (Capasso et al. 1995; Ramos-Cormenzana et al. 1996), as well as the germination and vegetative growth of plants (Linares et al. 2003). Olive oil phenolic compounds are the main determinants of antimicrobial and phytotoxic actions of olive-mill wastes. These compounds are either originally synthesised by the olive plants as a defence against a remarkable variety of pathogens (Bianco et al. 1999) or formed during the olive oil extraction process (Pannelli et al. 1991). Once in the olive oil, olive oil phenols show a range of antioxidant, functional, nutritional and sensory properties (Saija and Uccella 2000). Because olive oil phenols are amphiphilic, only a fraction of the phenolics enters the oil phase, and a large proportion (>98%) is lost with the waste stream during processing (Rodis et al. 2002). It is estimated that the toxic load of OMWW in

Fig. 1 Simplified flow chart of industrial-scale olive oil extraction processes: traditional press-cake system, three-phase decanter system and two-phase centrifugation system
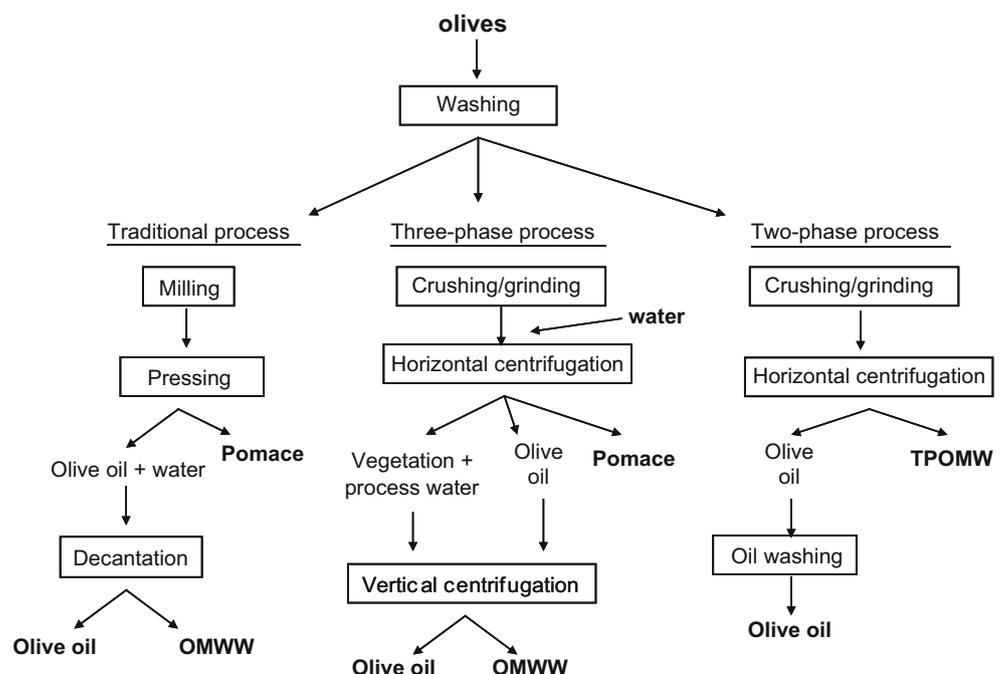
Table 1 Input–output data for the three olive oil production processes

Production process	Input	Amount of input	Output	Amount of output (kg)
Traditional press process	Olives	1 ton	Oil	~200
	Wash water	0.1–0.12 m^3	Solid waste	~400
	Energy	40–63 kWh	Wastewater	~600
Three-phase process	Olives	1 ton	Oil	200
	Wash water	0.1–0.12 m^3	Solid waste	500–600
	Fresh water for decanter	0.5–1 m^3	Wastewater	1,000–1,200
	Energy	90–117 kWh		
Two-phase process	Olives	1 ton	Oil	200
	Wash water	0.1–0.12 m^3	Solid + water waste	800–950
	Energy	<90–117 kWh		

Adapted from Azbar et al. (2004)

terms of phenolic compounds is up to a thousand times larger than that of domestic sewage (Niaounakis and Halvadakis 2004). Due to their instability, OMWW phenols tend to polymerise during storage into condensed high-molecular-weight polymers that are particularly difficult to degrade (Ayed et al. 2005; Crognale et al. 2006). For these reasons, the uncontrolled disposal of OMWW has traditionally become a great problem in Mediterranean countries because of their polluting effects on soil and water (Sierra et al. 2001; Piotrowska et al. 2006).

The waste stream of the two-phase system, TPOMW, comprises about 800 kg per 1,000 kg of the processed olives, and its production may exceed four million tons annually in Spain alone (Alburquerque et al. 2004). The TPOMW consists of a thick sludge that contains water and pieces of stone plus the pulp of the olive fruit. This semi-solid effluent has a water content of about 65%, a slightly acidic pH and a very high content of organic matter, mainly composed of lignin, hemicellulose and cellulose (Table 3). It has also a considerable proportion of fats, proteins, water-soluble carbohydrates and a small but active fraction of hydrosoluble phenolic compounds (Alburquerque et al. 2004).

The phenolic profile of OMWs is complex and variable. The occurrence of specific phenolic compounds depends on the fruit (e.g. maturity, cultivar), climatic conditions and

Table 2 Chemical composition of OMWW

Parameter	Mean	Range
Dry matter (%)	6.72	6.33–7.19
pH	4.84	4.2–5.17
EC (dS/m)	8.36	5.5–12
Organic Matter (g/l)	55.80	46.5–62.1
TOC (g/l)	37.00	34.2–39.8
TN (g/l)	0.96	0.62–2.1
C/N	53.32	52.3–54.3
P$_2$O$_5$ (g/l)	0.57	0.31–0.7
K$_2$O (g/l)	4.81	2.37–10.8
Na (g/l)	0.26	0.11–0.42
Ca (g/l)	0.35	0.2–0.64
Mg (mg/l)	121.25	44–220
Fe (mg/l)	81.70	18.3–120
Cu (mg/l)	3.15	1.5–6
Mn (mg/l)	5.15	1.1–12
Zn (mg/l)	6.13	2.4–12
Density (g/cm^3)	1.04	1.02–1.048
Lipids (g/l)	6.39	1.64–12.2
Phenols (g/l)	4.98	0.98–10.7
Carbohydrates (g/l)	7.16	1.4–16.1
COD (g/l)	124.67	67–178
BOD5 (g/l)	65.00	46–94

Data were calculated from eight independent studies reported in Roig et al. (2006)

Table 3 Chemical composition of TPOMW

Parameter	Mean	Range
Humidity (%)	62.16	49.6–71.4
pH (H$_2$O)	5.48	4.9–6.8
EC (dS/m)	2.99	1.2–5.24
Organic matter (%)	90.66	60.3–98.5
C/N	44.99	29.3–59.7
TN (g/kg)	11.99	9.7–18.5
P (g/kg)	0.97	0.3–1.5
K (g/kg)	18.73	6.3–29
Ca (g/kg)	5.08	2.3–12
Mg (g/kg)	1.03	0.5–1.7
Na (g/kg)	0.67	0.2–1
Fe (mg/kg)	1,107.80	526–2,600
Cu (mg/kg)	41.20	13–138
Mn (mg/kg)	25.80	13–67
Zn (mg/kg)	19.60	10.01–27
Lignin (%)[a]	38.82	19.8–47.5
Hemicellulose (%)[a]	29.70	15.3–38.7
Cellulose (%)[a]	23.47	17.3–33.7
Lipids (%)[a]	11.01	3.76–18
Protein (%)[a]	6.95	6.7–7.2
Carbohydrates (%)[a]	12.32	9.6–19.3
Phenols (%)[a]	1.36	0.5–2.4

Data were calculated from eight independent studies reported in Roig et al. (2006)

[a] (% w/w) of total organic matter

storage time, in addition to the processing technique. Besides, analytical factors such as the solvent of choice to extract the phenolic compounds from the residues (e.g. methanol, ethanol, hydroalcoholic mixtures, ethyl acetate), the different methods used to store/prepare the samples (e.g. direct extraction, chemical hydrolysis, steam treatments) and the diversity of analytical techniques employed to conduct qualitative or quantitative analysis (e.g. high-performance liquid chromatography (HPLC), HPLC–mass spectrometry (MS), gas chromatography–MS, capillary electrochromatography) add more complexity to the intrinsic variability of the phenolic profiles of OMWs. In fact, the complexity of the phenolic fraction has been highlighted in the literature, and 20 phenolic substances have been identified in OMWW using HPLC–MS–MS chemical analysis (Bianco et al. 2003). Using this approach in three different varieties of olives, the main phenolic compounds detected were tyrosol, hydroxytyrosol, oleoside methyl ester, oleoside dimethyl ester and oleuropein. Lesage-Messen et al. (2001) studied the phenolic composition of OMWs as a function of the extraction system (three-phase and two-phase mills), and they reported that the phenolic profiles identified by HPLC (after acid extraction with ethyl acetate) were similar in the residues from the two extraction systems (OMWW and TPOMW), with hydroxytyrosol (approximately 1% dry residue) and tyrosol being the major compounds detected. Nevertheless, the contents of individual compounds (hydroxytyrosol, tyrosol, caffeic acid, ferulic acid and *p*-coumaric acid), with the exception of vanillic acid, were higher for the two-phase system. Research on the bioactivity and chemical analysis of these substances has focussed on their antioxidant and antimicrobial activities (reviewed by Obied et al. 2005).

Besides traditional decantation, several disposal methods have been proposed for olive-mill wastes, such as thermal processes (combustion and pyrolysis), physico-chemical treatments (e.g. precipitation/flocculation, ultrafiltration and reverse osmosis, adsorption, chemical oxidation processes and ion exchange), extraction of valuable compounds (e.g. antioxidants, residual oil, sugars), agronomic applications (e.g. land spreading), animal-breeding methods (e.g. direct utilisation as animal feed or following protein enrichment) and biological treatments (Niaounakis and Halvadakis 2004). Among the different options, biological treatments are considered the most environmentally compatible and the least expensive of wastewater treatments methods (Mantzavinos and Kalogerakis 2005). These processes use microorganisms to break down the chemicals present in olive-mill wastes and/or to valorise the residues by the production of added-value compounds such as a diverse range of microbial-derived substances including biopolymers and biofuels. The actual type of microorganism that is involved depends on the conditions under which the

olive-mill waste is treated, particularly whether it is aerobic or anaerobic. Aerobic processes are applied to waste streams with low organic loads and/or concentration of nutrients or as a polishing step to further remove residual organic matter and nutrients from olive-mill wastewaters, whereas anaerobic processes are applied to waste streams with high organic loads (Niaounakis and Halvadakis 2004). In any case, high organic loads, presence of some classes of antimicrobial or biostatic compounds such phenols and lipids, low pH values, low water activity in the case of TPOMW and unbalanced composition of nutrients all represent barriers that should be overcome to achieve an optimal biological process.

Aerobic biodegradation

Aerobic biological treatments, such as activated sludge and trickling filters, are usually exploited to remove pollutants from wastewaters. A plethora of aerobic biological processes, technologies and microorganisms have been tested for the treatment of OMWs, aimed at reducing organic load, dark colour and toxicity of the effluents. Early studies focussed on the use of specific bacterial species, primarily to reduce the toxicity of OMWW (Ramos-Cormenzana et al. 1996; Ehaliotis et al 1999). In general, aerobic bacteria appeared to be very effective against some low-molecular-mass phenolic compounds but are relatively ineffective against the more complex polyphenolics responsible for the dark colouration of OMWs (McNamara et al. 2008).

Ramos-Cormenzana et al. (1996) evaluated the reduction of the phenolic content of OMWW by *Bacillus pumilis*, obtaining a biodegradation up to 50% of these compounds. Using a similar approach, Ehaliotis et al. (1999) demonstrated that the N_2-fixing bacterium *Azotobacter vinelandii* was able to reduce the phytotoxicity of OMWW by approximately 90% at the end of two 5-day period cycles in an aerobic biowheel-type reactor. The treated effluent was thus suitable for use as fertiliser (Ehaliotis et al. 1999; Piperidou et al. 2000). Nevertheless, it should be noticed that the conditions used by Ehaliotis et al. (1999) and Piperidou et al. (2000) were not sterile, and thus other microorganisms could, at least partially, have contributed to the chemical changes reported in their studies.

Aerobic bacterial consortia from different sources have also been utilised for bioremediation of OMWW (Benitez et al 1997; Zouari and Ellouz 1996a). Several recent studies have focussed on this subject, but available information about the indigenous microbiota of OMWs is still scarce. Reported studies have used different approaches, such as the direct isolation of phenolic-degrading bacteria from OMWW (Di Gioia et al. 2002) or the determination of the ecophysiology and diversity of bacterial isolates obtained

from TPOMW (Jones et al. 2000; Ntougias et al. 2006). Interestingly, olive-mill wastes, due to their particular characteristics, provide a source of new microorganisms with biotechnological potential, like the exopolysaccharide-producing bacterium *Paenibacillus jamilae* (Aguilera et al. 2001) and the obligate alkaliphilic *Alkalibacterium olivoapovliticus* (Ntougias and Russell 2001).

In general, available scientific information shows that fungi are more effective than bacteria at degrading both simple phenols and the more complex phenolic compounds present in olive-mill wastes. The reason for this lies in the structure of the aromatic compounds present in OMWs; they are analogous to that of many lignin monomers, and only a few microorganisms, mainly wood-rotting fungi, are able to efficiently degrade lignin by producing ligninolytic

enzymes such as lignin peroxidases, manganese peroxidases and laccases (Hattaka 1994).

Consequently, the bioremediation of OMWs using specific strains of fungi (some of them isolated directly from olive-mill wastes), primarily filamentous fungi, white rot fungi and yeasts, has been extensively investigated. There are a considerable number of reports on the application of fungi to reduce the organic load and phenolic content of OMWs (Table 4). For instance, several species of the genus *Pleurotus* were found to be very effective in the degradation of the phenolic substances present in OMWs. It should be highlighted that in general a close relationship has been found between the decrease of phenolic content and the decrease of phytotoxicity. In fact, several studies have reported a reduction of phytotoxicity following the

Table 4 Aerobic treatment of OMWs by fungi

Culture	Residue	Method	OM reduction	Phenol reduction (%)	Reference
Aspergillus niger	OMWW	Flasks	73% as COD	76	García García et al. 2000
Aspergillus spp.	OMWW	Flasks	52.5% as COD	44.30	Fadil et al. 2003
Aspergillus terreus	OMWW	Bioreactor	66% as COD	n.a.	Garrido Hoyos et al. 2002
Aspergillus terreus	OMWW	Flasks	63% as COD	64	García García et al. 2000
Candida boidinii	TPOMW	Fed-batch microcosm	n.a.	57.7	Giannoutsou et al. 2004
Candida tropicalis	OMWW	Flasks	62.8% as COD	51.70	Fadil et al. 2003
Coriolus versicolor	OMWW	Flasks	65% as COD	90.00	Yesilada et al. 1997
Coriolopsis rigida	TPOMW	Flasks	9% as TOC	89	Sampedro et al. 2007
Geotrichum spp.	OMWW	Flasks	55% as COD	46.60	Fadil et al. 2003
Geotrichum candidum	OMWW	Bubble column pilot scale station	70% as COD	n.a.	Assas et al. 2000
Geotrichum candidum	TPOMW	Fed-batch microcosm	n.a.	57	Giannoutsou et al. 2004
Funalia trogii	OMWW	Flasks	70% as COD	93.00	Yesilada et al. 1997
Lentinula edodes	OMWW	Immobilized mycelium	73–80% as TOC	88.50	D'Annibale et al. 1998
Lentinula edodes	OMWW	Flasks	65% as COD	88.00	D'Annibale et al. 2004a
Penicillium spp.	OMWW	Flasks	25–38 as COD	32–45	Robles et al. 2000
Phanerochaete flavido-alba	OMWW	Bioreactor	n.a.	52.00	Blánquez et al. 2002
Phanerochaete flavido-alba	TPOMW	Solid-state cultures	n.a.	70.00	Linares et al. 2003
Phanerochaete chrysosporium	OMWW	Bioreactor	75% as COD	92	García García et al. 2000
Phanerochaete chrysosporium	TPOMW	Flasks	9.2% as TOC	14.50	Sampedro et al. 2007
Phlebia radiata	TPOMW	Flasks	13% as TOC	95.70	Sampedro et al. 2007
Pleurotus ostreatus	OMWW	Solid cultures and flasks	n.a.	64–67	Fountoulakis et al. 2002
Pleurotus ostreatus	OMWW	Flasks	n.a.	90.00	Martirani et al 1996
Pleurotus ostreatus	OMWW	Bioreactor	n.a.	Nearly complete	Aggelis et al. 2003
Pleurotus spp.	OMWW	Solid cultures and flasks	n.a.	69–76	Tsioulpas et al. 2002
Pleurotus ostreatus	OMWW	Flasks	n.a.	>90	Sanjust et al. 1991
Pleurotus ostreatus	TPOMW	Plastic bag	22% as TOC	90	Saavedra et al. 2006
Pleurotus floridae	OMWW	Flasks	n.a.	>90	Sanjust et al. 1991
Pleurotus pulmonarius	TPOMW	Flasks	9.7% as TOC	66.2	Sampedro et al. 2007
Poria subvermispora	TPOMW	Flasks	13.2% as TOC	72.3	Sampedro et al. 2007
Pycnoporus cinnabarinus	TPOMW	Flasks	7.6% as TOC	88.7	Sampedro et al. 2007
Saccharomyces spp.	TPOMW	Fed-batch microcosm	n.a.	61	Giannoutsou et al. 2004
Yarrowia lipolytica	OMWW	Flasks	20–40% as COD	<30	Lanciotti et al. 2005

n.a. not available

Table 5 Phytotoxicity reduction by aerobic treatment of OMWs with fungi

Culture	Residue	Method	Phenol reduction (%)	Toxicity analysis	Toxicity reduction	Reference
Coriolopsis rigida	TPOMW	Flasks	89	Growth inhibition of tomato plants	Growth inhibition decreased 57.4% after treatment	Sampedro et al. 2007
Lentinula edodes	OMWW	Flasks	88.00	Durum wheat germinability	>50% in twofold diluted OMW treated with *Lentinula edodes*	D'Annibale et al. 2004a
Phanerochaete flavido-alba	TPOMW	Solid-state cultures	70.00	Tomato plant germinability	Improvement of >40% in germination rates	Linares et al. 2003
Coriolopsis rigida	TPOMW	Flasks	73	Tomato plant germinability	Decreased phytotoxicity	Aranda et al. 2006
Candida holstii	OMWW	Flasks	39	Barley plant germinability	Improvement of 80% in germination rates	Ben Sassi et al. 2008

treatment of the OMWs with fungi (Table 5). However, the use of filamentous fungi (compared with bacteria) for OMWW treatment in large-scale processes is considered problematic due to the difficulty of achieving a continuous culture because of the formation of fungal pellets and other aggregations (Niaounakis and Halvadakis 2004). To overcome this limitation, the use of yeast in bioreactors could be a way forward. Some yeasts able to reduce COD and phenolic content of OMWs include *Geotrichum candidum* (Assas et al. 2000; Giannoutsou et al. 2004), *Candida tropicalis* (Fadil et al. 2003), *Candida boidinii* and *Saccharomyces* sp. (Giannoutsou et al. 2004; Table 4). In contradiction to the general relationship between phenolic content reduction and phytotoxicity reduction, Tsioulpas et al. (2002) reported that different strains of *Pleurotus* spp. were able to remove phenolics from OMWW but suggested that the remaining phenolics and/or some of the oxidation products of the laccase reaction were more toxic than the original phenolic mixture. In this study, phytotoxicity was quantified by using the phenol toxicity index (a function of the germination index of *Lepidium sativum* seeds and the phenolic concentration), which was suggested to quantitatively express the toxicity of phenolic content. Two possible explanations for these contradictory findings are, first, an increase of the toxicity of the phenolics after oxidation (Field and Lettinga 1989) and, second, a selective accumulation of a toxic fraction of phenolics not oxidised by the fungal laccase. Thus, it is suggested that further research is still required to clarify the effect of treating OMW with fungi on the phytotoxicity of the residue. Moreover, most of the aforementioned studies have been conducted under strictly controlled laboratory conditions, low waste volumes, axenic conditions and treated waste (e.g. sterile, filtrated, lyophilised, diluted), conditions which are far from real case scenarios. Thus, in spite of the considerable research effort to find single species to achieve optimal mineralisation or detoxification of OMWs, this has not led to environmentally useful strategies at industrial scale.

The application of culture-independent (molecular) techniques to study microbial communities involved in the biodegradation of olive-mill wastes provides a valuable source of additional information. By using laboratory-scale bioreactors, it was shown that the genetic potential of the indigenous microbiota was able to metabolise polyphenolic compounds present in TPOMW under aerobic conditions, through the stimulation of the fungal fraction by nutrient supplementation (N and P), and it was also observed that predominant fungi identified by polymerase chain reaction (PCR)–temperature time gradient electrophoresis included members of the genera *Penicillium*, *Candida*, *Geotrichum*, *Pichia*, *Cladosporium* and *Ascochyta* (Morillo et al. 2008a). Moreover, it was demonstrated that, compared to the inoculation of a single-strain (or consortium) approach, indigenous microorganisms could have a broader range of different biodegrading activities and thus sterilisation of the substrate is not necessary. The amendment with nutrients to alter the C/N ratio allowed the microbial activity and the phenolic content reduction to be significantly improved during aerobic biodegradation of either OMWW (El Hajjouji et al. 2008) or TPOMW (Morillo et al. 2008a). Apart from changing the structure of the microbial communities involved in the bioremediation of OMWs, the addition of nutrients can also modify the pattern of degrading enzymes production by specific microorganisms. In fact, applying nitrogen supplementation on some axenic white root fungi cultures has resulted in a significant decrease in OMW toxicity (de la Rubia et al. 2008).

Anaerobic biodegradation

Biodegradation of OMWs using anaerobic biodegradation approaches has been widely investigated. This technique presents a number of advantages in comparison to the classical aerobic processes: (a) quite a high degree of

purification with high-organic-load feeds can be achieved; (b) low nutrient requirements are necessary; (c) small quantities of excess sludge are usually produced; and (d) a combustible biogas is generated (Borja et al. 2006; Wheatley 1990). However, the nutrient imbalance of OMWW, due mainly to its high C/N ratios (~50), low pH (~5), low alkalinity (~0.6 g CaCO$_3$ per litre) and the presence of biostatic and inhibitory substances, represents a problem for the anaerobic degradation of these wastewaters (Boubaker and Cheikh Ridha 2007). An additional problem of TPOMW is its doughy consistency, which makes its transport, storage and handling difficult.

To overcome problems of the nutrient imbalance, toxicity and other difficulties derived from the composition of OMWW, the wastewaters can be subjected to pre-treatments before anaerobic digestion. Apart from dilution with water, other pre-treatments include (a) aerobic biological treatment (Borja et al. 1998; Hamdi 1996), (b) pre-treatment with specific aerobic organisms like the fungi Phanerochaete chrysosporium (Gharsallah et al. 1999) and (c) the addition of a source of nitrogen (Boari et al. 1984; Demirer et al. 2000). Another approach adopted by researchers to minimise the difficulties of OMWW anaerobic digestion is the co-digestion of OMWW with other substrates to compensate for its low alkalinity and nitrogen. For example, co-digestion with nitrogen-rich substrates such as animal manure has been explored (Angelidaki and Ahring 1997; Angelidaki et al. 2002).

In spite of these limitations, OMWW and TPOMW may be metabolised using anaerobic digestion once the process parameters have been optimised. A reduction of more than 80% in COD and yields of methane production of ~0.1–0.3 m^3 CH$_4$ per kilogramme of COD removed have been widely reported for anaerobic process at mesophilic temperatures (Table 6). Although most of these studies utilised microbial consortia derived from sewage treatment plants, other more "exotic" microbes have also been tested, including the use of a bacterial consortium isolated from termites (Hamdi et al. 1992).

Knowledge of the microbial communities involved in the anaerobic biodegradation of OMWs would be useful in order to better understand and monitor these processes. In a recent study, molecular identification of the microbial species (Bacteria and Archaea) involved in a process of anaerobic treatment of diluted TPOMW showed that the composition of the microbial communities changed with the operational conditions (Rincón et al. 2008). Firmicutes, mostly represented by the genus Clostridium, were the predominant bacteria at low organic loading rate (OLR), whereas other bacterial communities containing Gammaproteobacteria, Actinobacteria, Bacteroidetes and Deferribacteres were the most abundant at high OLR. The Archaea were mainly represented by four phylotypes belonging to the genus Methanosaeta independent of the OLR.

Comparing anaerobic to aerobic biodegradation, the former process requires generally higher capital investment (e.g. reactors), expert labour and transport of waste from generation point to treatment point resulting in higher fuel costs and higher emissions. Furthermore, it has been traditional practice to use composting of these wastes as a preferred aerobic biodegradation treatment, due partially to the reasons mentioned above but also due to the seasonal production of these wastes. Nevertheless, recent efforts have been focussed on anaerobic treatments because of their potential for production of biofuels (Antizar-Ladislao and Turrion-Gomez 2008).

Table 6 Anaerobic biodegradation of OMWs

Residue	Method	COD reduction (%)	Methane yield (cubic metre CH$_4$ per kilogramme COD removed)	Reference
OMWW	Anaerobic sludge bed reactors UASB	70	0.35	Ubay and Öztürk 1997
OMWW	Anaerobic sequencing batch reactor	80	n.a.	Ammary 2005
OMWW	Stirred batch reactor with sepiolite	90	0.345	Borja et al. 1998
OMWW	Anaerobic reactors packed with GAC	78.4	0.08	Bertin et al. 2004
OMWW	Anaerobic batch reactor	85.4–93.4	n.a.	Ergüder et al. 2000
OMWW	Co-digestion with solid wastes in tubular digesters	89	n.a.	Boubaker and Cheikh Ridha 2007
OMWW	Co-digestion with sewage and sewage sludge	75-85	0.32	Boukchina et al. 2007
OMWW	Two-stage up-flow and fixed-bed bioreactors	83	n.a.	Dalis et al. 1996
OMWW	Co-digestion with piggery effluent in an up-flow filter type	70–80	n.a.	Marques 2001
TPOMW	Laboratory-scale stirred tank reactor	88	~0.30	Borja et al 2002
TPOMW	Laboratory-scale stirred tank reactor	77–97	0.244	Rincón et al. 2008

n.a. not available, UASB up-flow anaerobic sludge blanket, GAC granular activated carbon

Bioremediation of olive-mill waste by composting

Among many other treatment technologies, composting is one of the most popular technologies aimed at utilising OMWs and producing a fertiliser from such wastes. Composting typically removes the phytotoxicity of the residues within a few weeks and allows the subsequent enrichment of croplands with compost nutrients that were originally taken up by olive tree cultivation (Arvanitoyannis and Kassaveti 2007). Such a tight circulation and recycling of nutrients has a certain aesthetic and practical appeal, particularly in local situations where the small-scale production of oil by individual mills can re-utilise the wastes to improve subsequent cropping, be it olives or other produce such as tomatoes. The local/rural economy might also benefit from the sale of surplus soil conditioner (Vlysides et al. 1989).

The effectiveness of compost recycling in agriculture depends mostly on the quality of the compost; therefore, characterisation of the process plus evaluation of the quality of the mature compost are crucial (Cayuela et al. 2008a, b). In this respect, fulvic acid levels could constitute a tool in order to follow the maturity of the product during composting of OMWs (Ait Baddi et al. 2004). Due to the characteristics of these residues, it is also important to follow the changes occurring in phenols and biotoxicity during composting. In an experimental composting process of OMWW plus barley straw, Zenjari et al. (2006) found that degradation of the phenols reached 95% after the maturation phase and the toxicity disappeared after only 2 months of composting. In this regard, much active research is oriented towards the adaptation of composting technologies to the specific requirements of OMWs.

Composting of OMWs requires the proper adjustment of pH, temperature, moisture, oxygenation and nutrients, thereby allowing the adequate development of the microbial populations (Arvanitoyannis and Kassaveti 2007). In general, ideal conditions for an optimal composting process are a carbon-to-nitrogen ratio of the composting material between 20 and 40, moisture content between 50% and 65%, an adequate oxygen supply, a small particle size and enough void space through which air can flow (Chang et al. 2006). In order to equilibrate the nutrient imbalance of OMWs, the preferred approach in the majority of cases has been the application of co-composting with other residues, such as those derived from cattle and poultry farming (Paredes et al. 2001; Hachicha et al. 2008), arable farming (Alburquerque et al. 2007; Paredes et al. 2002) or industry (Sánchez-Arias et al. 2008). Direct amendment with nutrients such as urea has also been proved to be a possibility (Tomati et al. 1995), although it is less environmentally desirable. The OMWW has to be pre-absorbed in a solid substrate in order to proceed with the composting process. To obtain good results, a broad variety of bulking agents, including wheat straw (Galli et al. 1997; Tomati et al. 1995), maize straw (Paredes et al. 2000) and solid olive and olive tree waste (Vlyssides et al. 1999; Filippi et al. 2002), have been found acceptable.

Among the possible technologies for recycling the TPOMW, composting is gaining interest as a sustainable strategy to recycle this residue for agricultural purposes (Alburquerque et al. 2006; Filippi et al. 2002; Cayuela et al. 2008a, b). The TPOMW needs also to be mixed with bulking agents such as grape stalk (Alburquerque et al. 2006) or straw (Madejón et al. 1998), due to its semi-solid consistency and low porosity. Compost, rich in organic matter and free of phytotoxicity, can thus be obtained (Alburquerque et al. 2006). Composting of TPOMW is a valid process from technical and economical standpoints, as demonstrated by its application at industrial scale (Kobek 2004). Vermicomposting of TPOMW amended with manure has been also suggested as a suitable alternative (Plaza et al. 2008).

A characteristic feature during the composting of TPOMW is the high pH (>9) reached (Cayuela et al. 2004; Alburquerque et al. 2006). This fact was explained by Cayuela et al. (2008a, b) as being a consequence of the decarboxylation of organic anions during the aerobic decomposition of TPOMW and could represent a limitation for its soil application. Roig et al. (2004) suggested the addition of elemental sulphur during the composting process as a suitable strategy to control this pH increase. Composting of TPOMW is also characterised by a prolonged thermophilic period, which can be optimised and even reduced in length of time by the use of appropriate bulking agents (Manios et al. 2006). Hydrolytic enzymes involved (Cayuela et al. 2008b) and the pattern of organic matter transformation and humic substances produced during composting have been also characterised (Ait Baddi et al. 2004).

Monitoring of microbial diversity is one of the most fundamental tasks to understand the composting process. Although its importance is claimed, there are only few studies in the published literature that focus on the analysis of the microbial communities during the composting process (Antizar-Ladislao et al. 2008). In a recent study, olive-mill wastewater sludge obtained by a physico-chemical treatment of OMWW (electro-Fenton oxidation) was composted in a bench-scale reactor and the evolution of microbial species within the composter was followed using a respirometric test and by means of both cultivation-dependent and cultivation-independent approaches (PCR–single strand conformation polymorphism (SSCP); Abid et al. 2007). It was reported that during the period of high respiration rates (7–24 days), the cultivation method showed that thermophilic bacteria, as well as actinomycetes,

dominated over eumycetes, whilst, during the composting process, the PCR–SSCP method showed a higher diversity of the bacterial community than the eukaryotic one. Finally, after 60 days of composting, the compost exhibited a microbial stability and a clear absence of phytotoxicity.

The main problem of the application of composting from olive by-products is odour emission and the drainage water that has to be treated. Biofilters are applied to treat the released gas from composting piles, which then increases the total costs of this technology (Kobek 2004).

Bioconversion of olive-mill wastes to high added-value products

Many applications have been attributed to OMWs, with or without further treatment to obtain added-value products (Fig. 2). For example, OMWs have been used as substrate for the culture of certain microorganisms in order to obtain a potentially useful microbial biomass and/or to induce a partial bioremediation of the residues. Such applications have a long history, and already 50 years ago Fiestas Ros de Ursinos (1961) reported the production of yeast biomass using OMWW in a chemostat for use in industrial

applications. A few edible fungi, especially species of *Pleurotus*, can also be grown using OMWs as the source of nutrients by the application of different strategies (Kalmis et al. 2008; Sanjust et al. 1991; Zervakis et al. 1996). These authors also reported biochemical changes and detoxification of the substrates due to the active excretion of ligninolytic enzymes and partial consumption/adsorption of the organic fraction by fungi. Kalmis et al. (2008) recently suggested the cultivation of the oyster mushroom (*Pleurotus ostreatus*) on wheat straw substrate containing a mixture of tap water and OMWW (25% OMWW, v/v) as an environmentally friendly solution for the purpose of commercial mushroom production. Similar approaches have been exploited by several small companies for more than a decade (Ramos-Cormenzana et al. 1995). Mention should also be made of the possibility of using the microbial biomass produced from OMW fermentations either as an additive to animal feed or to improve its agronomic use. For example, Laconi et al. (2007) achieved an intense degradation of most polluting substances of OMWW and the production of biomass that could be used as an animal feed integrator using a chemical–biological method (alkaline-oxidative treatment to decrease the polyphenolic content followed by fermentation with a fungal

Fig. 2 Potential uses and microbial valorisation of olive-mill wastes
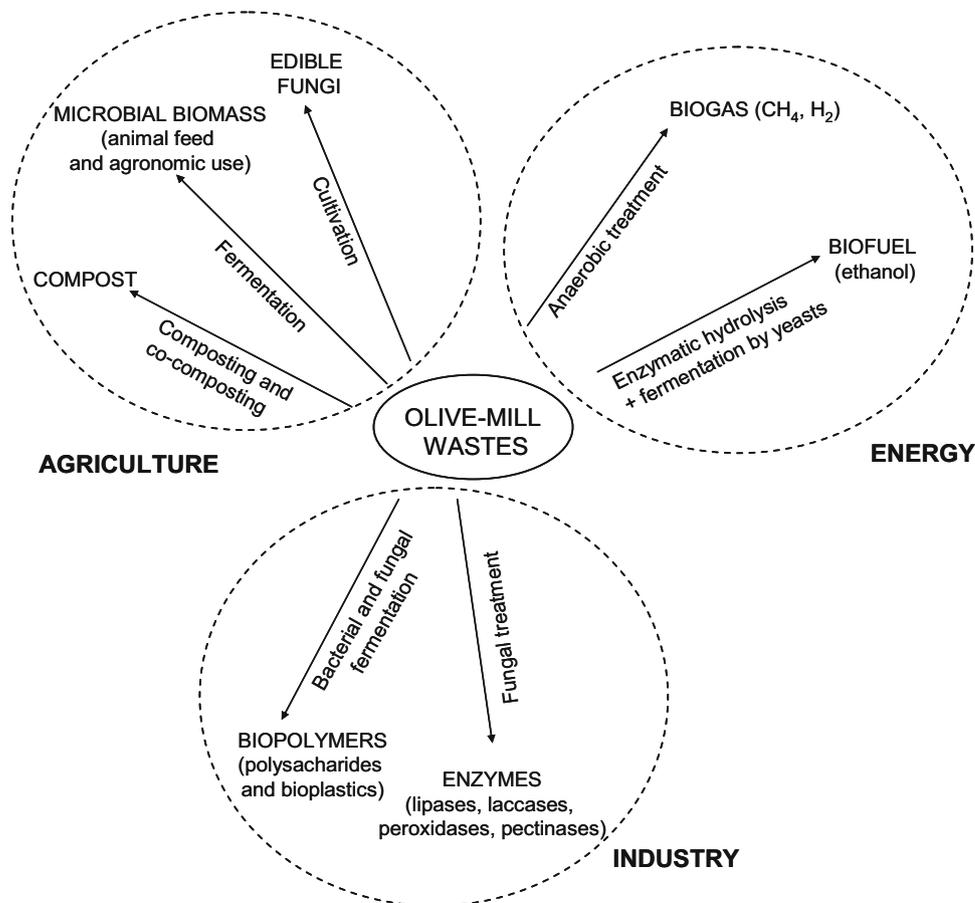
mixture). As far as agronomic use of the waste is concerned, the idea of re-using microbially treated OMWW as fertiliser has been also proposed (Vassilev et al. 1997). To this end, a strain of the acidogenic fungus *Aspergillus niger* was grown in either free or immobilised form on OMWW with rock phosphate added in order to solubilise it. After fermentation, the phosphorus-enriched OMWW was tested as fertiliser on soil/trifolium (*Trifolium repens* L.) and soil/wheat (*Triticum durum*) systems, resulting in enhancement of plant growth (Cereti et al. 2004). These authors highlighted the production of biopolymers, enzymes and biofuels as added-value products of such fermentations.

Biopolymers and enzymes

The production of microbial biopolymers using OMWs as a low-cost fermentation substrate has been proposed, focussing on polysaccharides and biodegradable plastics in a process that has the additional benefit of being environmentally beneficial (Ramos-Cormenzana et al. 1995). This microbial biotechnological valorisation is supported by the fact that olive-mill wastes present certain similarities with the standard media for microbial polymer production, mainly with respect to the high carbon-to-nitrogen ratio. This approach could reduce the cost of polymer production because it is the market prices of the substrate that is often the first limiting factor and so might improve the process economics.

Microbial exopolysaccharides (EPSs) often show clearly identified properties that form the basis for a wide range of applications in food, pharmaceuticals, petroleum and other industries (Sutherland 1990). Xanthan gum, an EPS produced by the bacterium *Xanthomonas campestris* (the most commercially accepted microbial polysaccharide), has been obtained from OMWW (Lopez and Ramos-Cormenzana 1996). An improved EPS yield could be obtained with a selection of the proper *X. campestris* strain and an adequate balance between waste concentration and nutrient supplementation (López et al. 2001). In these studies, it was reported that a dilution of OMWW (below 60%) in order to reduce the inhibitory effect of phenols and the addition of nitrogen and/or salts led to a significant increase of xanthan yields, with a maximum of 7.7 g l^{-1}. A similar approach has successfully been used to obtain the metal-binding EPS produced by *P. jamilae* from OMWs. In these studies, maximal EPS production (5.1 g l^{-1}) was reached in batch-culture experiments with a concentration of 80% of OMWW as fermentation substrate (Morillo et al. 2007). In the case of the use of TPOMW as substrate, maximal EPS yield (2 g l^{-1}) was obtained in cultures prepared with an aqueous extract of 20% TPOMW (*w/v*). An inhibitory effect was observed on growth and EPS production when TPOMW concentration

was increased (Morillo et al. 2006). This EPS produced by *P. jamilae* through fermentation of OMWs has been investigated in relation to its potential application as a biofilter of heavy-metal-contaminated water (Morillo et al. 2008b).

The fungus *Botryosphaeria rhodina* has also been used for the production of the polysaccharide β-glucan from OMWW with a satisfactory yield of 17.2 g l^{-1} and a partial dephenolisation of the substrate, which was attributed to an adsorption phenomenon of the mycelial biomass due to the absence of a phenol-degrading activity (Crognale et al. 2003). It has been reported that OMWW can also be used as a fermentation substrate to obtain other types of microbial polymers, such as homo- and co-polymers of polyhydroxyalkanoates (PHAs; Martinez-Toledo et al. 1995; Gonzalez-Lopez et al. 1996; Pozo et al. 2002). These substances are accumulated as intracellular granules in a variety of bacteria and are a source of new biodegradable plastics. The production of PHA by *Azotobacter chroococcum* strain H23 in a medium prepared with OMWW (diluted up to 60% with water) as the only source of carbon was increased by aeration of the cultures and the addition of nitrogen (0.12% ammonium acetate *w/v*), with a maximal yield of 6.2 g PHA per litre culture medium (Pozo et al. 2002).

The production of enzymes by fungi using OMWs as substrate with commercial interest offers another interesting opportunity for the biotechnological valorisation of the residues. The microbial enzymes obtained by fungal treatment of OMWs include different families of lipases, laccases, Mn-dependent peroxidases and pectinases (Crognale et al. 2006).

Microbial lipases employed in the dairy, pharmaceutical, detergent and other industries can be obtained from the fermentation of OMWW based on the (variable) amount of residual oil present in these wastes (Cordova et al. 1999). Amongst a series of fungal strains belonging to the known lipolytic species, D'Annibale et al. (2006) found a promising potential for lipase production by *Candida cylindracea* NRRL Y-17506. The aromatic-degrading ability of white rot fungi is associated with the production of extracellular oxidases, enzymes with low substrate specificity and good stability against various potentially denaturing agents and thus with a possible use in a wide range of industrial applications (Crognale et al. 2006). Within this context, the production of laccases and Mn-dependent peroxidases by OMWW fermentation by *Panus tigrinus* CBS 577.79, a strain able to cope with high organic loads, has been proposed (D'Annibale et al. 2004b). The development of effective methods to purify/isolate the enzymes and biopolymers from the bulk fermentation is an important point in order to scale the processes to the industrial scale. The required methodologies are specific of the enzyme/technology considered and generally involves

further steps of concentration, precipitation and chromatography (Morillo et al. 2006; D'Annibale et al. 2004b).

All the aforementioned studies are very promising, but at present it is unlikely that biopolymers and enzymes obtained from these wastes will be produced at an economic industrial scale, mainly due to their low demand, which will render their production unprofitable, and to the high competition with other established technologies. Therefore, these biopolymers and enzymes are still mainly produced at the laboratory scale.

Bioconversion of olive-mill waste to biofuels

In recent years, considerable attention has been directed towards the production of energy from lignocellulosic wastes. As mentioned above, anaerobic digestion is a practical alternative for the treatment of TPOMW, which produces biogas (Borja et al. 2006; Antizar-Ladislao and Turrion-Gomez 2008). As it has been reported for other agroindustrial residues, such as potato pulp (Zhu et al. 2008) or cattle manure (Güngör-Demirci and Demirer 2004), anaerobic processes applied to OMWs, whether in one or two stages, must be selected according to the C/N ratio of the residues in order to obtain a satisfactory anaerobic degradation. The TPOMW is biodegradable by anaerobic digestion at mesophilic temperatures in stirred tank reactors, with COD removal efficiencies in the range of 72–89% and an average methane yield coefficient of 0.31 dm^3 CH_4 per gramme COD removed (Borja et al. 2006). Similar production of hydrogen and methane has been reported using thermophilic reactors at 55°C (Gavala et al. 2005) and in mesophilic anaerobic treatment of TPOMW in continuous and batch experiments, in which approximately 0.28 dm^3 CH_4 per gramme COD was removed, and hydrogen production was coupled with a subsequent step for methane production, giving the potential for production of 1.6 mmol H_2 per gramme of TPOMW (dry matter; Borja et al. 2006).

Additionally, the high content of organic matter makes OMWs an interesting alternative resource to produce ethanol as a biofuel (Li et al. 2007). Even if the content of free reducing sugar in these wastes is low, different kinds of polysaccharides can be converted to ethanol via different reactions that occur in two separate steps: first an enzymatic hydrolysis using commercial enzymes followed by the conversion of reducing sugars to ethanol performed by yeasts (alcoholic fermentation; Zanichelli et al. 2007). Amongst the many parameters that can affect the process of alcoholic fermentation, the presence of inhibiting compounds in OMWs is critical. Bambalov et al. (1989), using a collection of several yeast strains of different species, confirmed that fresh OMWW was unfavourable to yeast growth and ethanol production. The removal of the phenolic fraction using an adsorption/desorption technique seems to be a necessary procedure for efficient ethanol production from OMWW (Zanichelli et al. 2007).

The utilisation of TPOMW as a potential substrate for production of ethanol has also been proposed. Ballesteros et al. (2001) reported the production of ethanol by a simultaneous saccharification (by the addition of cellulases) and fermentation process, using the two main components of TPOMW (stones and olive pulp) as substrates. Although a pre-treatment was not necessary to bioconvert a fraction of the olive pulp into ethanol, pre-treatment of fragmented olive stones by sulphuric-acid-catalysed steam explosion increased the enzymatic digestibility. The yield of the enzymatic hydrolysis (expressed as glucose obtained in the enzymatic hydrolysis divided by potential glucose in the raw material) was in the range 38–49%, and concentrations of >10 g l^{-1} of glucose after the hydrolysis could be obtained (Ballesteros et al. 2001). Pre-treatment of TPOMW with hot water (200–250°C) combined with the use of feed-batch procedure is another option to improve the production of ethanol (Ballesteros et al. 2002). In another recent study (Georgieva and Ahring 2007), an enzymatic hydrolysis and subsequent glucose fermentation by baker's yeast were evaluated for ethanol production using dry matter of TPOMW. The enzymatic hydrolysis resulted in an increase in glucose concentration by 75%. The results showed that yeasts could effectively ferment TPOMW without nutrient addition, resulting in a maximum ethanol production of 11.2 g l^{-1} and revealing the tolerance of yeast to TPOMW toxicity. In this study, pre-treatment of the residue was not performed prior to being subjected to enzymatic hydrolysis and ethanol fermentation, in order to avoid sugar degradation in the substrate (Georgieva and Ahring 2007).

It is widely recognised that clean and sustainable technologies, e.g. biofuels, are only part of the solution to the impending energy crisis. Comparing the heating value of biohydrogen (121 MJ kg^{-1}), methane (50.2 MJ kg^{-1}) and bioethanol (23.4 MJ kg^{-1}), the production of hydrogen will be more attractive (Nazaroff and Alvarez-Cohen 2002). Nevertheless, at present, the use of biohydrogen is still not practical (Duerr et al. 2007), and thus there is a higher demand for methane and bioethanol because they can be used directly as biofuels with the existing technology (Antizar-Ladislao and Turrion-Gomez 2008).

Summary

Although much olive oil is produced in modern cooperatives and its consumption has become globalised, overall, in the Mediterranean region, its production remains essentially a local activity. Thus, solutions for dealing with the wastes generated the need to be effective at both small- and

medium-scale levels. A corollary is that treatment regimes should be relatively simple to operate and preferably low cost, not simply towards removing the toxic waste but by converting it to environment-enhancing products.

Given this background, it is perhaps surprising that no particular OMW-remediation technology has been more universally adopted. No single method has proven superior enough to become adopted by an industry that retains a diversity of practices. We would argue that a multifactorial approach is needed, combining a biological, i.e. bioremediation, stage (this might integrate more than one type of biotreatment) with innovative process engineering to handle the wastes and derived products. For instance, there might be sequential anaerobic and aerobic biological treatments, which delivered fractions that were subjected to specific biological (or chemical) treatments. These would give a variety of specific added-value products, ranging from bulk fertilisers and other soil amendment products (e.g. for germination enhancement or selective pathogen suppression) to specialised products such as antioxidants, enzymes, biofuels and bioplastics. It is the development of industries producing useful value-added products that will alter the mindset for dealing with OMWW from one that is focussed on its deleterious (toxic) properties to one that emphasises its beneficial qualities and realises its economic benefit.

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