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Current status and future challenges of table olive processing wastewater valorization

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ABSTRACT

Table olive production is expanding worldwide. This production is associated with the generation of enormous amounts of polluting and difficult to handle wastewater streams. Thus, research on different technologies to decompose their organic load is currently needed. The economic benefit of the potential detoxification routes is expected to increase if processes for upgrading Table olive processing wastewaters are designed. This makes biological treatment of particular interest. The current review analyzes a relevant number of scientific studies dealing with promising technologies for biological treatment of table olive processing wastewaters from laboratory to pilot-scale systems. Concise details of the various technologies involved such as anaerobic digestion, lactic acid fermentation and fungal fermentation are determined. The most significant advances in the manufacturing of value-added products (e.g. biogas, platform chemicals, natural antioxidants) from table olive processing wastewaters through different microorganisms, bioreactor design modifications, and operational conditions are critically discussed. Future prospects of valorizing table olive wastewaters are presented.

1. Introduction

Table olives are considered predominant constituents of the Mediterranean diet. Their nutritional properties are related to the presence of high content of unsaturated fatty acids, biophenols, minerals, fibers and vitamins [1,2]. According to the International Olive Council (IOC), the world table olive production for the 2013/2014 season accounted for approximately 2.7 million tonnes. The European Union (EU) contributed 30% of the total production. The EU's largest producers are Spain, Greece and Italy (72, 16 and 9% of the total EU production, respectively). Other main producing countries are Egypt (15%) and Turkey (16%) [3]. This situation is clearly illustrated in Figs. 1 and 2. Also, in Fig. 2 the international trade and consumption of table olives for the same period is presented (based on IOC statistical data). Noticeably, low- or even non-producing countries such as USA, Russia and Brazil consume significant amounts of the product.

The main commercial types of table olives are the Spanish-style green olives (~50% of total production), Californian-style black ripe olives (~25% of total production), and naturally black olives in brine (~25% of total production) [4], the manufacturing of which generates considerable quantities of different types of wastes [4–6]. The fact that table olive production is expanding worldwide results in the continuous increase of table olive processing wastewater (TOPW) generation. On the other hand, table olive industry is forced to follow environmental regulations towards the elimination of organic pollutants in TOPWs. The prospect of the sustainable valorization of TOPWs for the production of high value-added products is an economically and environmentally attractive alternative. This approach is in line with the circular economy model promoted by the EU [7].

The present review is focused on the current knowledge about bioremediation and biovalorization of TOPWs. Particular attention is given to the most significant advances in the manufacturing of value-added products (e.g. biogas, chemical feedstocks, natural antioxidants) from TOPWs through different biological treatments. While excellent reviews dealing with the bio-based valorization of olive mill wastes are available [e.g. 8–10], no detailed review is available up to now on this issue. Moreover, future prospects of valorizing TOPWs are presented.

To develop innovative and economically feasible valorization strategies for TOPWs, it is important to identify the critical processing steps that contribute mostly in the generation of these streams and to determine the volume/composition of the latter. Thus, in the next paragraphs a careful review of the different

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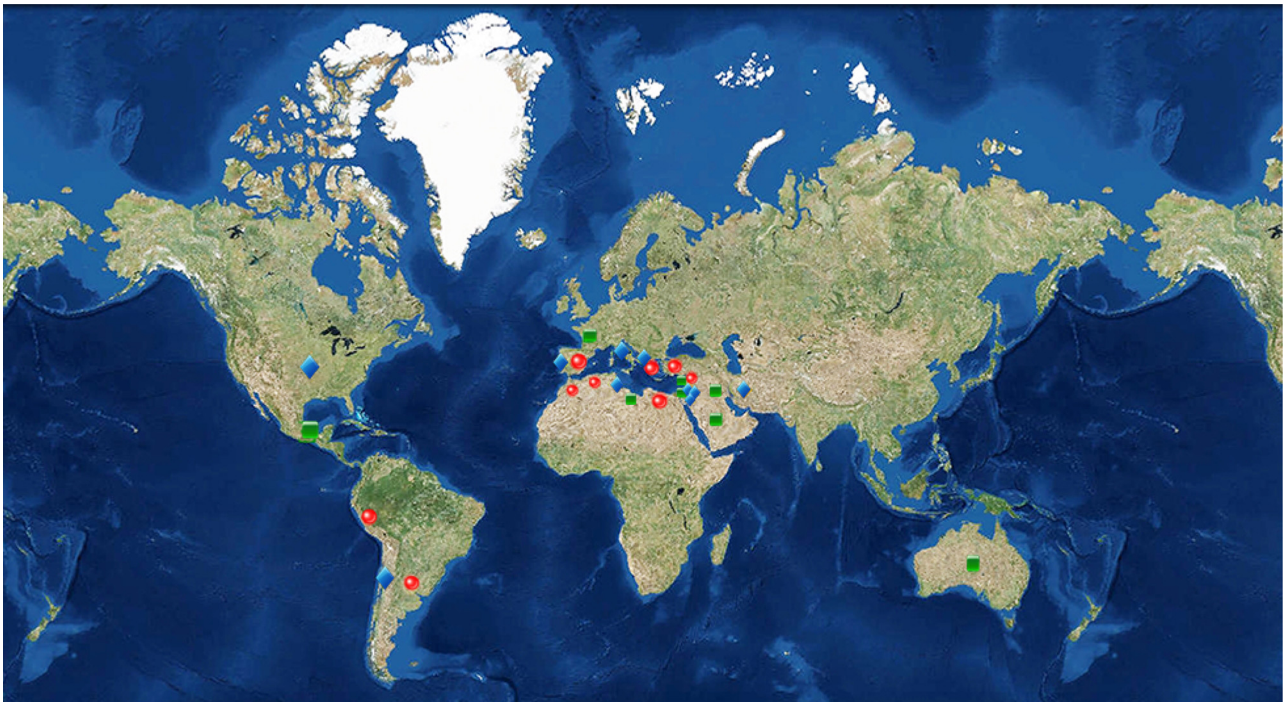

Fig. 1. Table olive producing countries in 2013/2014. 85,000–572,000 (red bullets), 15,000–85,000 (blue rhombus), 1000–15,000 (green squares) tonnes of olives [3]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

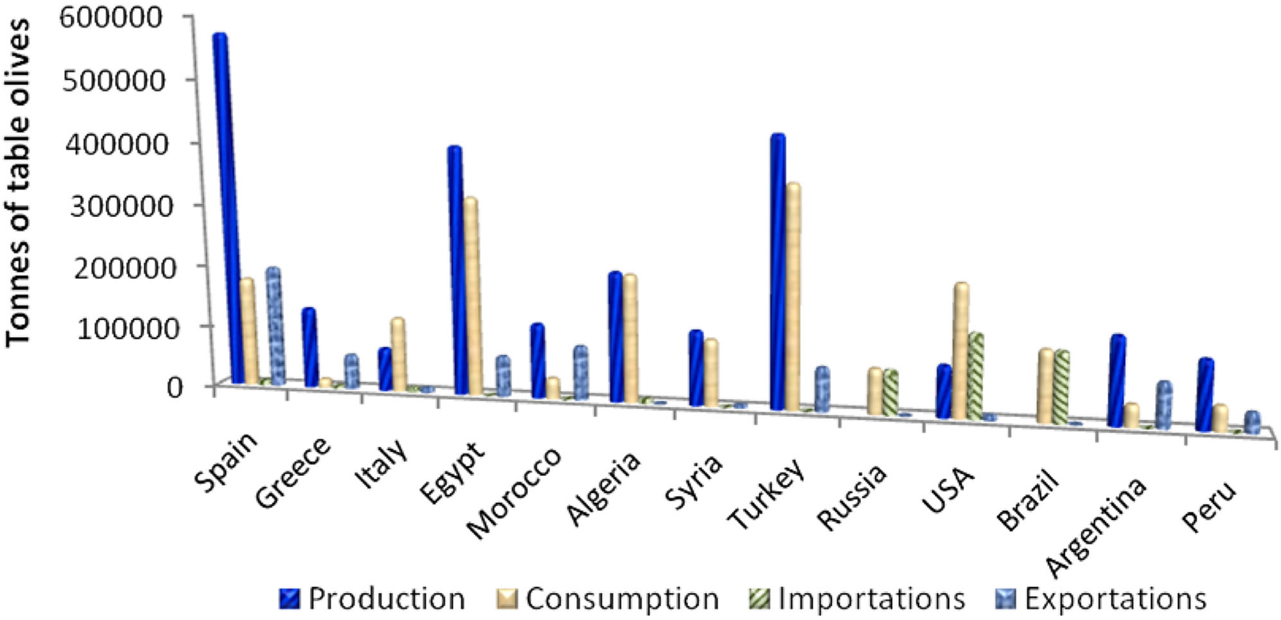

Fig. 2. Table olive production, consumption and trade in different countries in 2013/2014 [3].

manufacturing processes of table olives and the waste streams derived is presented.

2. Table olive processing

The processing stages that take place during the production of the main commercial types of table olives are shown in Fig. 3 [11–13].

For the Spanish-style and Californian-style processes, olive bitterness is removed by means of lye treatment using 1–2% w/v NaOH aqueous solution (debittering stage) [5,13]. In this step, the hydrolysis of oleuropein takes place with the concomitant formation of elenolic acid glucoside and hydroxytyrosol (Fig. 4) [14]. After this step, fruits are washed with water several times for the removal of the alkali from the flesh [4,12,15].

Upon the Californian-style process, olives are treated with lye immediately or stored before treatment. During storage, olives are immersed into acidified brine (4–6% NaCl) or acidified water (2.4% acetic acid) under aerobic and anaerobic conditions, respectively [16]. A mild fermentation may take place for the period of storage [17]. In this process, debittering is exhaustive by applying sequentially three to five lye treatments [12]. Between the treatments,

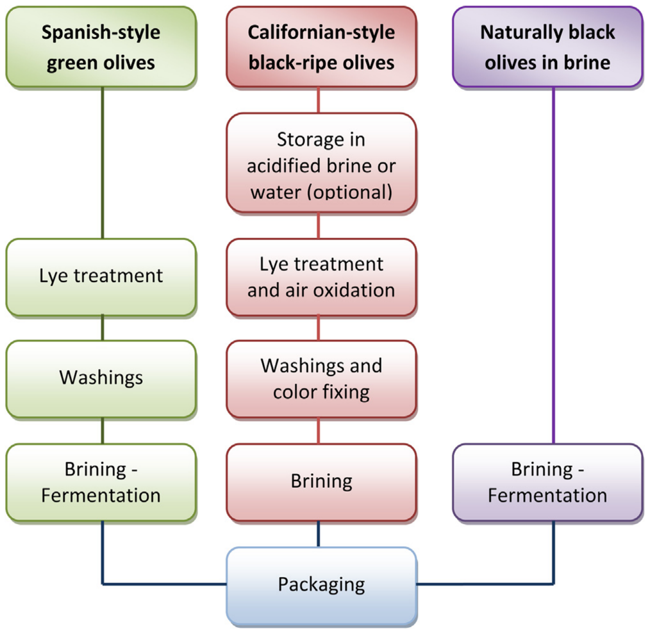

Fig. 3. Production process flow charts for Spanish-style green olives, Californian-style black-ripe olives and naturally black olives in brine [11–13].

fruits are placed in water and oxidation of polyphenols takes place by injecting air under pressure to achieve blackening of the fruit skin. Also, addition of 0.1% w/v ferrous gluconate or 0.06% ferrous lactate in the final washing treatment ensures the stabilization of the black color [17].

After the washing step, processed olives are transferred in tanks filled with brine (aqueous solution of NaCl). The concentration of NaCl in brine is 2–3% in the Californian-style [15] and 4–8% in the Spanish-style [4,18] processes. In the latter case, spontaneous fermentation takes place during the storage in brine. For the production of naturally black olives, the debittering step is not applied before fermentation (in 8–10% NaCl solution) [19] and removal of oleuropein in the olives is incomplete (untreated olives) [12].

Other table olive commercial types, with low impact to international market, are the Californian-style green-ripe olives (treated) and the naturally green-ripe and turning color olives (untreated) [11,12]. In contrast to the Californian-style black-ripe olives, air oxidation and treatment with gluconate solution are not applied for the production of the Californian-style green-ripe olives [17]. Untreated green and turning color olives are placed directly in brine and preserved by spontaneous fermentation via indigenous lactic acid bacteria (LAB) and yeast metabolic activities [11,12].

3. TOPWs volumes and composition

During the various stages of table olive processing, large amounts of water and chemicals (e.g. NaOH, NaCl) are required that result in the generation of polluting and difficult to handle wastewater streams. The quantities of these streams and their composition vary widely according to the treatment applied in each production process.

3.1. The volumes of TOPWs

Fig. 5 gives a representative graph of the average volumes of wastewaters generated from the different treatment schemes based on published data [6,12,20]. The most polluting ones are those that involve lye treatment and exhaustive washings for the removal of the alkali (i.e. Californian-style black-ripe, Spanish-style green, Californian-style green-ripe olives). Among them, the production of Californian-style black-ripe olives has the highest pollutant potential (maximum total volume of ~6 L/kg olives produced). On the other hand, processing that is based on untreated olives (i.e. naturally black, green or turning color olives) produces lower volumes of wastewaters (~1 L/kg olives produced) generated from the fermentation brine. Complete information on the volumes of wastewaters produced from table olive processing should also include those derived from post-treatment (e.g. packaging).

3.2. Composition of TOPWs

Based on literature data, the physicochemical characteristics of the wastewater streams derived from the three main types of table olive processing are shown in Tables 1–3. The organic fraction of TOPWs consists mainly of sugars, acids, phenolic compounds and nitrogenous compounds that contribute to the chemical oxygen demand (COD) value. For the Spanish-style processing wastewaters, this value has been estimated to be on average between 16 and 19 g O_2/L depending on the treatment applied in each step (lye treatment, washings, brining) [4–6,18,25–32] (Table 1). Moreover, the lye/washing water effluents are characterized by high pH values of 10–12 and alkalinity, in contrast to the acidified brine effluent (average value of pH 4.0) [4–6,18,21–33]. This dramatic difference is attributed to variation in the composition of the lye and brine effluents, which consist of high amount of NaOH (up to 9 g/L) and lactic acid (8 g/L), respectively [4,21–28,34]. The enormous amount of NaCl in the brine effluent (near 70 g/L) reflects the elevated value of electrical conductivity as well as the high content of chloride ions and inorganic dissolved solids [4–6,18,21,24–30,32,34]. In the Californian-style process, the average values of COD and total phenol content of the generated effluents (lyes plus washing waters) range between 2.0–4.0 g O_2/L and 0.3–0.4 g/L, respectively [12,35–37] (Table 2). These values are noticeably lower in comparison with those from Spanish-style processing [4–6,18,22–32,34]

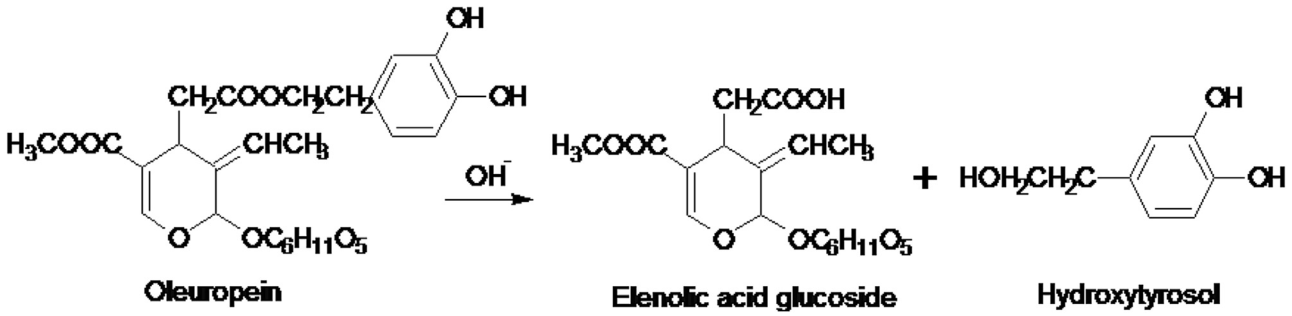

Fig. 4. Alkaline hydrolysis of oleuropein during the olive debittering stage [14].

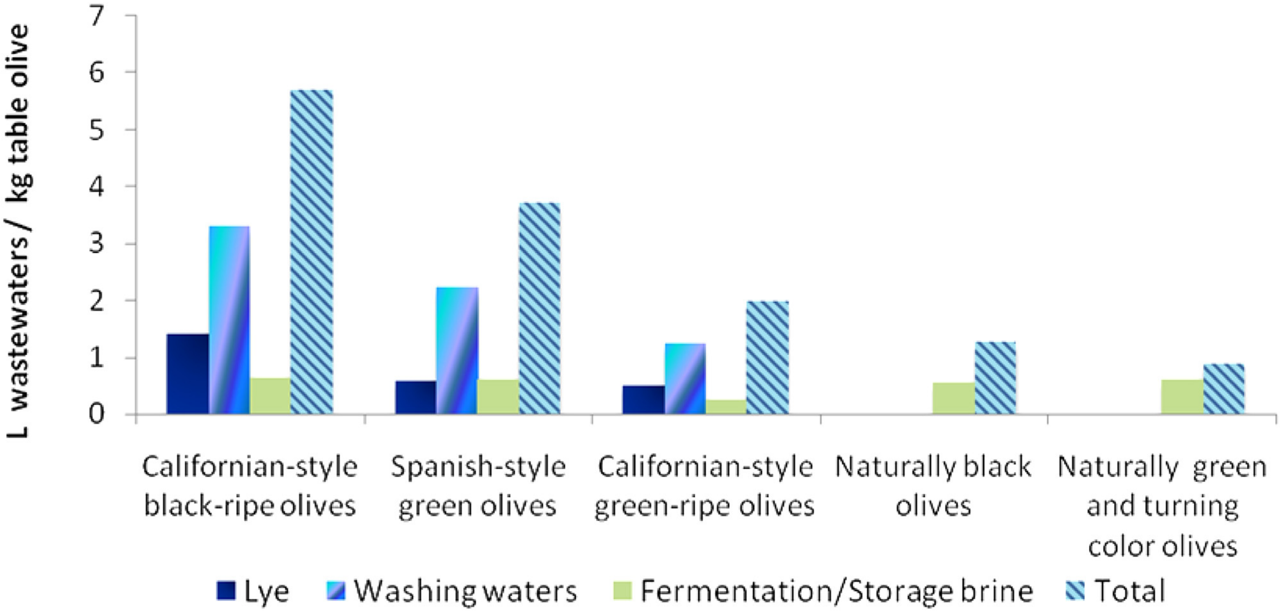

Fig. 5. Average volumes of wastewaters generated from the various stages of different types of table olive processing [6,12,20].

Table 1
Average composition of Spanish-style green olive wastewaters.

Parameters	Lye		Washing waters		Brine		Refs.
	Average	Range	Average	Range	Average	Range	
pH	12.1	9.5–13.2	10.0	7.2–11.5	4.0	3.6–4.6	[4–6,18,21-33]
Acidity (g of lactic acid/L)	–	–	–	–	8.0	3.5–15.0	[4,21–28,34]
Electrical conductivity (mS/cm)	12.1	11.1–13.0	10.2	–	81.4	53.1–94.2	[5,18,29,30]
COD (g O2/L)	18.8	9.4–35.0	16.1	0.3–35.0	15.9	6.8–26.0	[4–6,18,25-32]
BOD (g O2/L)	9.5	3.1–20.0	11.0	0.1–21.0	10.6	2.2–20.0	[4–6,25,26,34]
Dissolved organic solids (g/L)	21.9	13.1–30.0	24.2	19.1–30.0	18.8	13.6–25.2	[4,26,28,32,34]
Dissolved inorganic solids (g/L)	21.9	15.4–35.0	20.0	6.8–54.3	73.9	20.9–110.0	[4,26–28,32,34]
Total suspended solids (g/L)	2.03	0.10–3.42	0.07	0.03–0.10	0.81	0.08–2.00	[6,18,25,29–31]
Total phenolic compounds (g/L)	1.78	0.21–4.00	2.32	0.45–4.00	2.78	0.18–6.00	[4,5,18,22–31,34]
Sugars (g/L)	6.6	4.9–9.0	6.4	4.7–9.0	–	–	[4,34]
Total Kjeldahl nitrogen (g/L)	0.58	0.50–0.75	–	–	0.32	0.27–0.36	[18,30,31]
NaOH (g/L)	9.0	6.9–11.0	1.5	0.9–2.0	–	–	[4,34]
NaCl (g/L)	0.0005	0–0.0010	0.0005	0–0.0010	67.8	52.0–90.0	[6,21,24,25,27–28,34]
Cl^- (g/L)	0.32	0.00–0.60	0.30	0.00–0.60	49.1	36.4–62.7	[6,18,29,30]

Table 2
Average composition of Californian-style black-ripe olive wastewaters.

Parameters	Lyes		Washing waters		Gluconate Solution	Refs.
	Average	Range	Average	Range	Average	
pH	11.89	11.70–12.11	9.93	6.90–13.60	3.7	[12,35,36]
COD (g O2/L)	2.4	2.0–2.6	4.1	3.5–6.7	1.5	[12,35–37]
BOD (g O2/L)	–	–	3.1	1.4–5.0	–	[35–37]
Total solids (g/L)	–	–	12.3	5.7–22.9	–	[35–37]
Dissolved organic solids (g/L)	14.5	12.1–19.3	24.0	3.1–36.6	43.4	[12]
Dissolved inorganic solids (g/L)	4.5	2.4–6.9	6.2	3.2–8.1	7.3	[12]
Volatile suspended solids (g/L)	–	–	2.5	0.3–4.6	–	[35,36]
Total Kjeldahl nitrogen (mg/L)	–	–	25.0	–	–	[37]
Total phenolic compounds (g/L)	0.42	0.18–0.59	0.29	0.12–0.64	0.80	[12,35–37]
NaOH (g/L)	5.9	4.3–8.9	–	–	–	[12]

(Table 1). This might be related with the high COD values estimated for the storage brine effluent (reaching 35 g/L) [12] (Fig. 3). In the case of naturally black olives processing, the fermentation brine is the main waste stream of the process. This stream is considered more polluting than the respective one derived from the Spanish-style processing because of its higher organic load (average value of COD equal to 32.3 g O_2/L) and elevated content of phytotoxic phenolic compounds (4.3 g/L) [6,38–40] (Table 3). This difference is attributed to the absence of the debittering and washing stages during the natural processing of olives (Fig. 3).

These data along with the fact that large volumes of wastewaters are produced within a limited period of time (2–4 months) in restricted geographic ranges by small-sized enterprises reflect the magnitude of the disposal problems. When considered in absolute terms, Spanish-style processing appears to generate the largest volume of TOPWs (in liters of waste generated). Also, taking into

Table 3
Average composition of naturally black olive wastewaters (brine).

Parameters	Average	Range	Refs.
pH	4.31	3.60–5.00	[6,38–40]
Electrical conductivity (mS/cm)	111.5	–	[38,39]
COD (g O2/L)	32.3	11.0–60.0	[6,38,39]
BOD (g O2/L)	24.0	3.0–38.3	[6,40]
Dissolved organic solids (g/L)	101.3	95.3–118.8	[40]
Total suspended solids (g/L)	0.13	0.10–0.15	[6]
Total phenolic compounds (g/L)	4.3	3.2–5.2	[38–40]
NaCl (g/L)	66.4	56.0–77.0	[6,40]
Cl^- (g/L)	39.4	33.3–45.5	[6]

account their high COD mean values (Table 1) priority needs to be given to these streams.

4. TOPW biological treatment options

In Mediterranean countries, TOPWs are disposed untreated in land, rivers or the sea. The most common treatment is just to store TOPWs in open evaporation ponds [5,41]. This may lead to the production of malodorous gases by putrefactive or methanogenic bacteria under uncontrolled environmental conditions [42,43]. High risk of contamination attributable to waste leakage and migration into groundwater and deep soil due to the improper pond construction can occur [31,41,43].

The prospect of converting TOPWs into a by-product is challenging and the related processes may reduce the cost of the main product. Up to now, the main focus has been on the development of various physical (e.g. membrane filtration), physico-chemical (e.g. flocculation) and thermal methods (e.g. evaporation), as reviewed by Niaounakis and Halvadakis [44]. Moreover, advanced oxidation methods have been applied for the remediation of TOPWs such as the combined use of ozone and hydrogen peroxide or UV radiation [45], photocatalysis using titanium dioxide and UV radiation [39], wet air oxidation [46] and electrochemical oxidation [38]. However, many of these technologies have the main drawback of generating side streams with concentrated pollutants that need further treatment and/or relatively high energy/cost disadvantage [4,47].

Biological treatment technologies (bioremediation and biovalorization) are considered economical and efficient processes compared to other competing ones [47]. These processes employ microbial cells able to degrade or convert the pollutants into value-added compounds via specific metabolic pathways. Still, in the case of TOPW treatment, adverse environmental conditions such as extreme pH values, high salinity, unbalanced composition of nutrients and the presence of antimicrobial compounds may inhibit microbial growth and metabolism. This fact reflects the complexity underlying these bioprocesses. Below, the available knowledge about the bio-based remediation and valorization strategies examined for TOPWs is presented and critically discussed as these will support future plans and further developments within this field.

4.1. Anaerobic digestion

In general, anaerobic digestion (AD) is an established agro-industrial waste valorization strategy resulting in the combined waste detoxification and the production of value-added products, such as biogas. During AD organic compounds are converted into biogas (mainly methane and carbon dioxide) through the enzymatic activities of naturally occurring microorganisms under conditions of oxygen depletion [48,49]. Apart from biogas production, other advantages of the process are its applicability to high organic load waste streams and the low production of surplus activated sludge [31,49]. Despite these advantages, the high content of phenolic compounds in TOPWs has a marked inhibitory effect on methanogenesis. This is verified by the findings of a kinetic study of the AD of green table olive wastewaters conducted by Beltran et al. [50]. A modified Monod model successfully simulated the batch kinetic of waste digestion and methane production in a spherical magnetically stirred anaerobic digester (internal diameter: 17 cm) containing activated sludge from a municipal wastewater treatment plant. The kinetic constant decreased with an increase of the initial waste load and the total phenol content due to inhibition effects. The application of the Levenspiel model to the experimental results led to the determination of the critical inhibition concentration of phenolic compounds (0.34 g/L) [50]. This value is 6-fold lower than the average value of total phenolic content reported for the Spanish-style green olive wastewaters [4,5,18,22–31,34] (Table 1) and 10-fold higher than that for maximum methane yield (295 mL/g $COD_{degraded}$) [50]. The points above highlight the strong inhibitory effect of this type of waste in process performance.

To overcome problems of TOPW toxicity, the wastewaters can be subjected to pretreatments for polyphenol removal before anaerobic digestion. Biotreatment with white-rot basidiomycete could be efficient for polyphenols removal as suggested by Aggelis et al. [31]. Also, Benitez et al. [35] discussed the efficiency of chemical pretreatment of black olive washing waters by applying UV radiation and Fenton's reagent oxidation on methane production by AD of this waste stream. The results achieved by this approach were an enhancement in the methane yield from 250 to 282 mL/g $COD_{degraded}$ and an improvement in the rate of AD process, as indicated by an increase of the rate constant from 0.015 up to 0.034 min^{-1} [35]. Methane yield was comparable to that achieved by AD of other wastewaters such as olive mill wastewaters (150–260 mL CH_4/g $COD_{degraded}$) [51,52] and cassava wastewaters (183 mL CH_4/g $COD_{degraded}$) [53].

Another approach adopted by researchers to minimize the difficulties of AD of TOPW is its co-digestion with other substrates to compensate for its toxicity and nutrition unbalance and to enhance synergetic phenomena between the microorganisms. For example, co-digestion of green table olive debittering and washing effluents together with cattle and pig manures resulted in more than 50% increase on methane production compared to single cattle manure fed system. Up to 40% addition of TOPW in the wastewater mixture is considered feasible for the AD process. However, the addition of more than 25% of this waste stream in the mixture is expected to result in lower methane production due to sub-optional growth especially of mesophilic flora (which is usually preferred due to low cost), as pointed out by the researchers [41]. The selection of proper bioreactor type is also very important to achieve high product yield [31].

4.2. Lactic acid fermentation

Another potentially interesting approach to advanced valorization of TOPWs is the application of lactic acid fermentation to produce lactic acid and hydroxytyrosol [33,42]. Castro and Brenes [33] pointed out on the importance of the initial pH of the green

Table 4
Bio-based routes for remediation and valorization of table olive processing wastewaters (TOPWs).

TOPW streams	Microorganism(s)	Optimal process operation	Removal efficiency	Valorized product	Remarks	Refs.
Washing waters (Californian-style processing)	Activated sludge (municipal WWs)	UV radiation or Fenton oxidation pretreatment (1 L reactor, 0.055 M H_2O_2, 2.4×10^{-3} M Fe^{2+}) and AD (1 L magnetically stirred batch digester, 35 °C).	63–78% COD	Methane (277–282 mL/g COD_{deg})	Methane yield and rate constant of digestion step were improved when a chemical pretreatment was applied.	[35]
Lye (Spanish-style processing)	Activated sludge (1: industrial WWs; 2: municipal WWs)	AD pretreatment (10 L draw-and-fill digester, pH ~7, 35 °C) and aerobic biological treatment (1 L stirred draw-and-fill reactor, COD:N:P 100:5:1, pH <8.5, 25 °C).	83% COD, 28% TP	Methane-rich biogas (93 mL/L/d)	Influents' pH control was not required; Low biogas production was attributed to the presence of methanogenic inhibitors.	[31]
WWs (Spanish-style processing)	Activated sludge (municipal WWs)	AD (20 L magnetically stirred batch digester, 35 °C).	81–94% COD	Methane (295 mL/g COD_{deg})	The critical inhibition concentration of polyphenols was determined to be 0.34 g/L.	[50]
Lye/Washing waters (Spanish-style processing)	Activated sludge	Anaerobic co-digestion of TOPWs (25–40%), cattle and pig manure (50 L stainless steel complete mix anaerobic digester, continuous operation, feed rate 1.66 L/d, agitation 60 rpm for 6 min/h, 55 °C).	81% TOC, 17% TP	Methane (~250–300 mL/g VS_{added})	50–61% higher methane yield than that from single cattle manure; Higher than 25% TOPW may inhibit microbial growth; Thermophilic digestion (55 °C) was more stable than mesophilic (35 °C) one.	[41]
Washing waters (Californian-style processing)	Activated sludge (municipal WWs)	Aerobic biological pretreatment (2 L CMBR, COD:N:P 100:5:1, pH control at 7, air flow rate 125 L/h, 28 °C) and ozonation (1.2 L CMBR, gas flow rate 25 L/h).	99% COD, 98% TP	–	The 2-step process improved the kinetic parameters of ozonation; Ozone oxidized the majority of phenolic compounds.	[36]
Mixture of WWs from various processings	Activated sludge (municipal WWs)	Aerobic biological treatment (20 L submerged membrane bioreactor, pore size 0.04 µm, continuous operation, 10 L/m^2 h, air flow rate 8 N dm^3/min).	92% TOC, 83% TP	–	Stable performance at moderate biomass concentration (<10 g/L).	[47]
Fermentation brine (Spanish-style processing)	Activated sludge (bioreactor landfill)	Aerobic biological treatment (sequencing stirred batch reactor, air flow rate 550 L/h, feed rate 150 mL/d, 18–20 °C).	75–88% COD, 97–98% TP	–	Simultaneous adaptation of biomass to salinity and the presence of phenols enhanced the efficiency of the process.	[18]
Washing waters (Spanish-style processing)	Indigenous microflora	Spontaneous fermentation under anaerobic conditions (initial pH 4, 20–25 °C).	–	Lactic acid (13.9 g/L), HT (4.7 g/L)	Correction of initial pH value to 4 enhanced lactic acid and HT yields.	[33]
Washing waters (Spanish-style processing)	*Lactobacillus pentosus*	Lactic acid fermentation (500 L batch fermenter, initial pH 5, 25 °C) and vacuum evaporation (65 °C).	–	Lactic acid (123.7 g/L), HT (36.4 g/L)	pH correction of the fermentation effluent to 8.3 before evaporation enhanced the yield of the valorized products.	[42]
Lye (Spanish-style processing)	*Pleurotus ostreatus*	Aerobic biological treatment (50% dilution with water, initial pH 6, 28 °C).	52–76% TP, 49% color	–	MnP activity was related to decolorization; Laccase activity was related to reduction of phenolic content but not phytotoxicity.	[30]
Lye/Washing waters (Spanish-style processing)	*Aspergillus niger*	Aerobic biological treatment (9000 L external loop air-lift bioreactor, semi-batch operation, air flow rate 0.1 v/v/min, initial pH 4.5–5.0) followed by EO (iron plate electrodes, 12 V, 1.6% H_2O_2) and coagulation (0.4% CaO).	98% COD	–	Laboratory results were replicated successfully at pilot scale plant treating about 4000 L/d TOPWs.	[66]
Lye/Washing waters (Spanish-style processing)	*Aspergillus niger*	Aerobic biological treatment (250 mL shake flasks, agitation 150 rpm, addition of 3 g/L glucose, initial pH 5, 30 °C).	65% COD, 48% TP, 62% Color	–	Tannase activity was related to the reduction of phenolic content.	[67]

WWs: wastewaters; AD: anaerobic digestion; COD: chemical oxygen demand; COD_{deg}: COD degraded; TP: total phenols; TOC: total organic carbon; VS: volatile solids; CMBR: completely mixed batch reactor; HT: hydroxytyrosol; MnP: manganese peroxidase; EO: electrochemical oxidation.

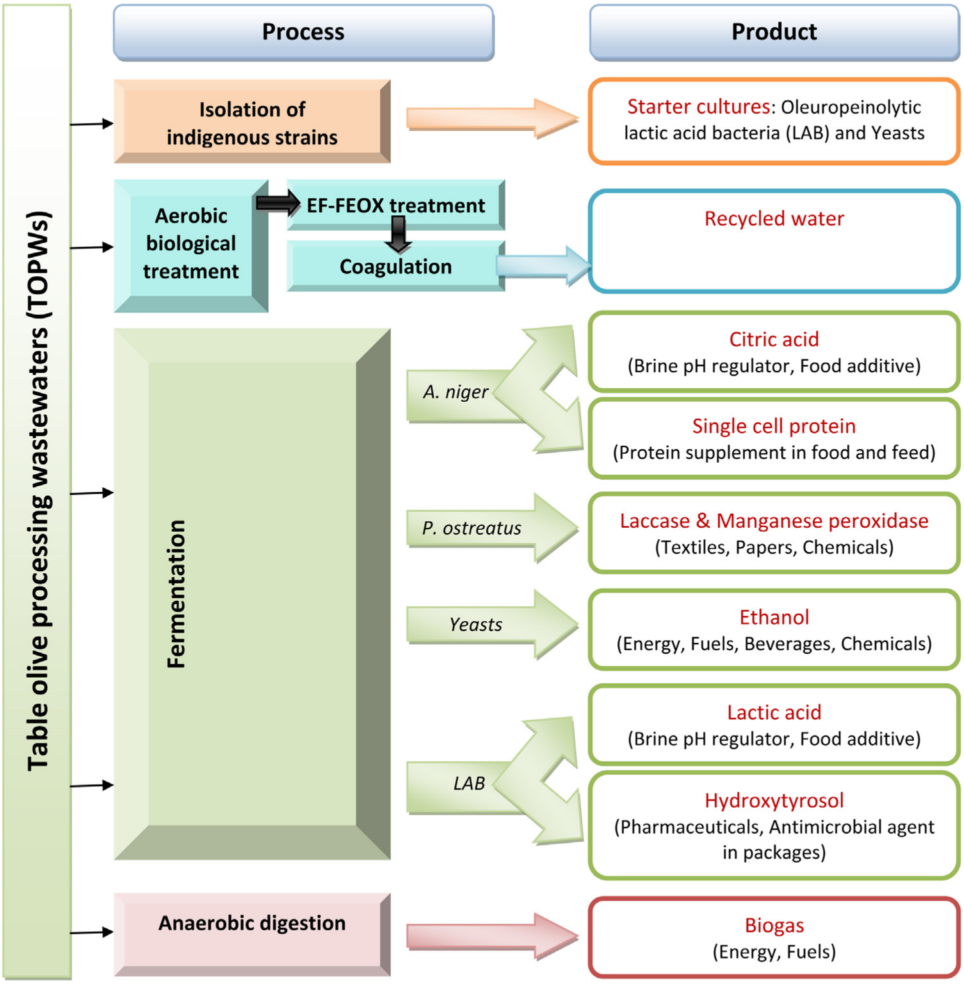

Fig. 6. Scenarios for TOPWs valorization.

table olive washing water effluent that can influence the final products of the process enormously. Correction of initial pH to 4 gave rise to lactic acid fermentation by indigenous microflora of this waste stream. Lactic acid yield was considered satisfactory (14 g/L) [33]. LAB grew well despite the presence of the antibacterial agent hydroxytyrosol. This can be attributed to the fact that this compound was found in lower level (4.8 g/L) than the minimum inhibition concentration reported for hydroxytyrosol against *Lactobacillus plantarum* (7.7 g/L) [54]. Moreover, proteins and amino acids present in the washing waters may bind to the hydroxytyrosol molecule resulting in the neutralization of its inhibitory effect. This suggestion is in line with the mechanism proposed for the growth of LAB in olive brine [55].

Brenes et al. [42] standardized the above process through the inoculation of indigenous starter cultures (*Lactobacillus pentosus* and *Enterococcus casseliflavus*) in a semi-industrial scale (500 L fiberglass reactor) in terms of yield and selectivity of the target compounds. Acidification of the waste (pH value of 5) before inoculation ensured the stability of the process due to the inhibition of spoilage putrefactive bacteria. Under more acidic conditions (when initial pH was set to 3.4) only indigenous yeasts could survive resulting in the formation of ethanol as the main fermentation product with the concomitant reduction of hydroxytyrosol level. Upon the semi-industrial scale fermentation process following by evaporation of the fermented waste stream, the optimum lactic acid content of the concentrate solution was 124 g/L [42]. This value is comparable or even higher than those reported in literature for other waste streams (100 and 162 g/L for wheat flour and barley starch hydrolysates, respectively, and 95.7 g/L from sugar molasses) [56–58]. The factor that is expected to expand the economics of the proposed process is the selective recovery of the bioactive solute hydroxytyrosol at the expense of elenolic acid glucoside due to its hydrolysis under acidic conditions. Noticeable, the yield of hydroxytyrosol achieved is dramatically higher than those recovered from the three-phase olive mill wastewaters (36 g/L vs 0.25–1.2 g/L) [42,59,60].

The innovative recovery and transformation of hydroxytyrosol into more lipophilic derivatives that will promote their use in stabilizing fats and oils triggered developments towards suitable enzymatic transformations processes [4].

4.3. Fungal fermentation

Aspergillus niger, a microorganism that has been proposed for the bioremediation of olive oil wastewaters [61–63], was also found capable of degrading organic load of TOPWs [64–67]. The use of *A. niger* is reported for the first time in treatment of wastewaters from the debittering process of green table olives and the subsequent washings in the patent of Lasaridi et al. [64]. In that patent, as expected, no details are available with regards to bioremediation engineering aspects. However, in the extended research paper of the same group [65] the quantitative data on the reduction of COD (70%) as well as the phenolic load (41% and 85% total and simple phenolic reduction, respectively) are presented. Experiments were conducted in a bubble column bioreactor (central tube of a Pyrex glass, 140 cm height and 12 L total volume) operated in a batch mode for 3 days followed by continuous operation with a hydraulic retention time of 2 days and a biomass recycling ratio of 80%. The threshold value of dissolved oxygen concentration was 3 ppm (air flow rate of 12 L/min) [65]. The above findings offered a solid basis for a scale of the proposed method up to the pilot plant of an air-lift reactor of 4 m^3/day capacity successfully, achieving a COD removal of 71% [66].

Taking into account the acidophilic character of the microorganism and the high alkalinity of this type of waste, pH correction near to 5 as a pretreatment step ensures the optimal performance of the microorganism [65–67]. Among other process parameters tested (fermentation time, dilution of the effluent, glucose addition level, $(NH_4)_2SO_4$, dose of inoculum and agitation), supplementation of TOPWs with glucose is expected to enhance significantly the removal of COD and polyphenols, and decolorization. From the biochemical point of view, oxidation of phenolic compounds via the action of tannase and lignin peroxidase is responsible for the bioremediation of TOPWs. Absorption of phenolic compounds onto the fungal mycelium should not be precluded [67].

There are several pros and cons of the TOPW fermentation by *A. niger* that need to be considered. Acclimatization of biomass and sterile conditions is not required offering significant advantages to the bioprocess, especially when compared with the AD [50,65,66]. However, in view of the seasonal production of large quantities of wastewaters, an obvious disadvantage of the bioprocess is the fact that the efficiency of the process decreases when TOPWs need to be stored before treatment. This has been attributed to the autoxidation and subsequent polymerization of phenols that take place during storage resulting in the formation of less biodegradable compounds [66]. Also, despite the significant decrease of the organic load in the effluent of the biological treatment, chemical treatment of the latter by applying advanced oxidation processes (Fenton's oxidation or electrolysis in the presence of hydrogen peroxide) seems to be mandatory to eliminate the pollutants of the waste and meet the various regulatory restrictions [65,66]. Nevertheless, the use of expensive chemicals will cause the cost of the overall process to increase.

Almost in parallel to the above mentioned efforts for the bioremediation of Spanish-style TOPWs using a selected strain of *A. niger*, the work of Aggelis et al. [30] stressed on the mechanism of detoxification and decolorization of the debittering wastewaters by white-rot fungi. To overcome toxicity problems, the wastewaters can be subjected to 50% dilution with water and pH control near to 6. Among eight strains, the major reduction in phenolic compounds was achieved by *Pleurotus ostreatus* (51.5%), *Abortiporus biennis* (54.5%), *Panellus stipticus* (42.2%) and *Dichomitus squalens* (36.4%). Only *P. ostreatus* could effectively decolorize the wastewaters (48.9% reduction) [30]. This ligninolytic fungus has been proposed as efficient biocatalysts for aerobic olive mill wastewater remediation [68]. The reduction of phenolic compounds and decolorization has been correlated with the activity of lignolytic enzymes, laccase and manganese peroxidase. Aggelis et al. [30] proposed laccase as an early indicator to select white fungi for the degradation of phenolic compounds. However, the importance of the formation of precipitates of higher oligomers and polymers of low solubility after laccase treatment in the efficient detoxification of TOPWs was not underestimated as these products can be removed easily by sedimentation or filtration [69]. Otherwise, the remaining phenolics and/or the oxidation products may be more toxic than the original, in line with observations made for olive mill wastewaters [9].

5. Valorized products from TOPWs and future challenges

The depletion of natural resources and the insistent demand to reduce the environmental impact of food production and consumption stimulate EU's transition towards a bio-based economy [70]. Noticeably, investment of €1 in EU-funds supporting the bioeconomy research and innovation will return approximately €10 of added-value to bioeconomy sectors by 2025 [71]. Future bioeconomy will need the development of sustainable biorefineries from specific feedstocks.

The problem of wastewater generation from table olive processing has attracted the interest of researchers, regulatory bodies and the industry. Table 4 provides a list of the most effective conditions to treat TOPWs using microorganisms. Given this background, it can be argued that bioprocessing may be combined with advanced chemical treatment (e.g. ozonation, Fenton's oxidation, UV radiation) to remove chemical pollutants from TOPWs. For example, pilot scale (4000 L/d TOPWs) testing of the combined biological treatment with *A. niger* and electrochemical oxidation with $Ca(OH)_2$ coagulation resulted in an effluent accepted in the public sewage system. Thus, this process can be considered as an industrial scenario for TOPW treatment and recycling [66]. The efficiency and sustainability of TOPW treatment can be enhanced by applying research into the biorefinery concept to develop an optimal mix of processes for the production of biofuels and bio-based industrially important chemicals.

In this direction, promising valorized products that can be obtained from TOPWs are illustrated in Table 4 and Fig. 6. For example, methane-rich biogas, which can be produced in considerable amounts by AD of TOPWs [50], is considered a clean renewable fuel that can replace fossil fuels and reduce some of the negative aspects of the latter, such as environmental impact [48]. In 2010 Europe's biogas production reached 10.9 millions of tonnes, while by the end of the same year China's total biogas output was 13.90 billion m^3 [72,73]. Despite its high market volume, biogas has a low value that limits the economic benefits that arise from the investment in biofuel-only operations.

High value, lower volume bio-based chemicals are expected to provide the economic advantage to support the sustainable development of the biorefining industry. This is the case of lactic acid that offers an interesting opportunity for the biovalorisation of TOPWs. The worldwide production of lactic acid is about 400,000 t annually [74]. This chemical has a primary role as an acidulant, flavor enhancer and antimicrobial preservative in food, pharmaceutical and other chemical industries [49,75]. Importantly, lactic acid is regarded as a top value platform chemical [74] for the production of poly-lactic acid (PLA), an economically interesting biodegradable polymer with many applications in medicine and plastics [75,76]. Prices of L-(+)-lactic acid of high purity (≥98%) range between 200 to 500 euros/10 g [77]. Lactic acid is mainly produced by microbial fermentation. Among the pros are the high purity and the perspective of a cost-effective, environmentally friendly investment due to low production temperature and low energy requirements. The main drawback is the high raw materials cost that contributes more than 34% of the total cost of the fermentative production of lactic acid [75]. Therefore, the production of lactic acid from low or even no-cost resources such as TOPWs will improve the process economics. The economic benefit of the proposed route for TOPW biorefining is expected to increase if production of more than one value-added compounds is designed (multi-product biorefineries). The fermentation scheme proposed by Brenes et al. [42] offers a solid basis to develop a cost effective plant for lactic acid and hydroxytyrosol recovery from TOPWs. Hydroxytyrosol is one of the main phenolic compounds in olive fruits with high value due to its antimicrobial and antioxidant properties and other health protective benefits (e.g. anticancer, anti-inflammatory, antidiabetic, neuroprotective activity) [78,79]. These benefits reflect its high price; hydroxytyrosol of high purity (≥98%) sold as reagent costs ~250 euros/25 mg [80]. Development of effective methods to recover these compounds from the bulk fermentation is an important point. This view is supported by recent studies on the development of sustainable processes for the recovery of lactic acid produced in fermentation media [81] and hydroxytyrosol from TOPWs [82,83].

Mention should also be made of the possibilities for TOPW valorization based on scenarios that exist for the exploitation of wastes from olive oil production [69,84–86], similar in nature with TOPWs. For example, the ability of fungi such as *A. niger* and white-rot fungi to grow in TOPWs [30,65–67] makes this waste an interesting alternative resource to produce high value fungal-based products. Among potential candidates are single-cell protein and citric acid from *A. niger* [87–89] and phenol-degrading enzymes (e.g. laccase, lignin peroxidase, manganese peroxidase) from white-rot fungi [69,90]. Citric acid is a food additive used as an acidifying agent mainly in the pharmaceutical and food industries. The great global demand/consumption of this chemical is due to its low toxicity when compared with other acidulants [88,89]. Also, phenol-degrading enzymes in free or immobilized form can be used under extreme conditions of pH, temperature, and salinity to degrade high and low contaminant concentrations without acclimation and in a more controllable process than that of whole cell systems [69]. Last but not least, TOPWs indigenous microflora, mainly LAB and yeasts, offers starter cultures in a cost-effective way. Predominant LAB species, due to their acid tolerance and oleuropeinolytic properties [4], provide a powerful tool to table olive industry for lactic acid fermentation control and elimination of the debittering stage.

All the aforementioned studies are very promising and are expected to create an additional source of input with a concomitant decrease in disposal expense. The discrepancy between sustainable bioprocess techniques developed by R&D teams and their adoption for practical application and use by industries is because of the uncertainty factors involved in the scaling-up process as well as the long lag time that is customary for new materials to be commercialized and readily available according to safety measures and market rules. Adopting a fully-evolved system – even integrating the facilities necessary to produce and recover indispensable products – alongside the co-operation between industries, will convert what is currently poorly valorized as waste stream into a greatly profitable by-product.

6. Conclusion

The problem of wastewater generation from table olive processing has attracted the interest of researchers, regulatory bodies and industry. The prospect of utilizing TOPW as raw materials in a biorefinery is challenging. This approach will support the circular economical model that produces virtually zero waste and is urged by EU. To this direction, in this study scientific findings that provide opportunities for effective valorization of TOPW towards the sustainable production of chemicals of high economic importance as well as water and energy conservation are presented. These findings demonstrated that bioprocessing may be combined with green chemical treatment in order to develop TOPW-based successful biorefinery strategies. Process scaling-up and optimization in terms of economic and technical feasibility should take place in order to recognize the highly profitable processes. Greater collaboration between researchers and stakeholders will help the interested parties to develop dynamic technology transfer and reduce the time required for commercialization of each new ingredient due to safety precautions and market rules.

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