Grape Winery Waste as Feedstock for Bioconversions: Applying the Biorefinery Concept

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Received: 20 May 2016 / Accepted: 22 August 2016 / Published online: 27 August 2016
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Abstract Grape wine is among the most important alcoholic beverages in the globe, with a continuously rising world demand, currently sizing at 25 billion litres. Such a large and heavily industrialised market calls for the maintenance of a steady production of raw materials to end products. Consequently, intensive cultivation of land, harvesting of the goods and manufacturing for the production of commercially available products are being implemented. Wine making is a timed, multistage process producing a large amount of organic and inorganic waste. It has been calculated that during cultivation and harvesting about 5 tonnes of solid waste are generated per hectare per year, while the winery wastewater varies according to the production size from 650,000 m³ (Greece) to over 18,000,000 m³ (Spain) per year. Conventional treatments of winery waste are becoming increasingly expensive, demanding significant amounts of effort, resources and energy for safe waste discharge. Therefore, the need to recycle, reuse and recover energy and valuable chemicals from winery waste and wastewater becomes apparent. Valorisation of winery waste is possible when introducing the concept of biorefinery, i.e. the use of winery waste as bioconversions feedstock in order to produce platform chemicals, biofuels, heat and energy.

Keywords Biorefinery · Winery waste · Feedstock · Bioconversion · Biofuels · Platform chemicals · Waste valorisation

Introduction

Grape wine represents one of the most important alcoholic beverages in the world, with a continuously growing demand. While traditionally wine production and consumption was concentrated in the European continent, currently over 67 nations produce, export, import and consume wine including Australia, New Zealand, Latin America (Chile, Argentina) and South Africa, all competing for a share of above 25 billion litres world market [1].

The industry continuous to be dominated by the “Big Three”: Italy, France and Spain; however the US and Australia are becoming producers of significant size [2]. France is the first wine producing country in the world with 41.4 million of hectolitres or 16.4 % of the global production each year, followed by Italy with 40.1 million of hectolitres or 15.9 % and Spain with 30.4 million of hectolitres or 12.1 % [3, 4]. China, on the other hand, is the largest producer of grapes contributing 13 % of the world’s production, but limited information is available on wine production in the country and the majority of grapes are exported elsewhere [2].

Such a large and heavily industrialised market calls for the maintenance of a steady production of products. Therefore, intensive cultivation of land, harvesting of the goods and manufacturing is needed and is implemented. Wine making is a timed, multistage process producing a large amount of organic and inorganic waste. During cultivation and harvesting, waste has been calculated at about 5 tonnes per hectare of land per year [5, 6] while the winery wastewater varies according to the production size from 650,000 m³ (Greece) to over 18,000,000 m³ (Spain) per year [7].

The winemaking industry has been majorly positively portrayed, due to the socioeconomic and cultural benefits
attributed to it [8, 9]. Regardless of the vast amounts of waste generated, the great use of water resources and the exhaustive land usage, the industry has not been viewed negatively by the general public. This, in turn, has encouraged its development and consequent generation of higher amounts of waste.

Waste can be seen as a virtually inexhaustible resource, being utilized in industrial markets to generate combined heat and power (CHP) and fertilizers, in the affluent developed world [10, 11]. Within the coming decade, these markets will develop further, as well as shifting into recovering chemicals and generating energy, synthetic materials, feeds and food from the waste, in an effort to reduce the carbon footprint of their production, as a result of legislative, environmental, economic and social drivers [12]. Utilizing natural resources will place limitations on manufacturing, but will also achieve environmental sustainability and will constitute non-solid waste safe for environmental discharge, in the form of particle, nutrient free and sterile effluents [13]. Therefore the utilization of waste as a valuable commodity and platform chemicals “mine” is an important step for the development and deployment of alternative sources of energy production [14].

Conventional treatment of waste is becoming increasingly expensive, demanding significant amounts of effort, resources and energy for safe waste discharge into the environment [15]. Tightening legislations regarding waste disposal call for alternative solutions to methods such as landfilling, landspreading or disposal in water streams such as rivers. In the current knowledge-driven economy that aims for low carbon use, and with the growing awareness of environmental protection—due to climate change and natural resources exhaustion—, the need to recycle, reuse and recover energy and valuable chemicals from waste and wastewater becomes apparent [16].

Therefore, the overall aim of this review is to explore schemes that could be applied at an industrial scale to valorise winery waste, introducing the concept of biorefinery, i.e. the use of winery waste as source of platform chemicals, fuels, heat and energy.

Energy and Commodities Formation from Alternate Origins: The Biorefinery Idea

Using agricultural goods for the production of other products is barely a novelty. However, the use of plant biomass as a raw material for the production of numerous products using complex physicochemical processing methods, a concept similar to petroleum refinery, is a rather new idea, first initiated in the 1980s [8, 17]. This approach though successful to an extent has several drawbacks. Plant based biomass is a rich source of lignin, carbohydrates, proteins and fats, also containing in smaller amounts vitamins, dyes and flavours [18, 19]. Its utilisation as bioconversion substrate requires extensive, often costly, pre-treatment in order to be processed successfully by the microorganisms. It has to be intensively cultivated and grown to produce considerable amounts of fuels, chemicals and power. This leads to land competition for crops development, potential shortage of feedstock, environmental constraints, due to excessive use of fertilisers, human food and export market, as well as possible water shortage [20].

Therefore, in recent years there is a shift from the whole crop concept—where an entire crop of wheat, rye, barley, corn or triticale is used as feedstock—to the waste based concept mainly in lignocellulose feedstock, where hard fibrous plant materials generated from agricultural or forestry activities are used [21]. This approach, albeit beneficial, has been hard to apply due to the extensive demand in pre-treatment (enzymatic hydrolysis or chemical digestion) for the production of cellulosic and hemicellulose material [22].

Moreover, several researchers [23, 24] have highlighted the importance of recycling waste, municipal, agricultural, domestic, and industrial, through bioconversion, i.e. applying a biorefinery (Fig. 1) concept, but with waste as the main feedstock.

This approach has been voiced by numerous governmental and non-governmental bodies and most importantly by the European Union [25–29] which has called for the increase of the recycling and preparing for re-use of municipal waste to 70% by 2030, and has stipulated phasing out landfilling recyclable waste (including plastics, paper, metals, glass and bio-waste) in non-hazardous waste landfills, reducing landfilling to a maximum of 25% by 2025.

Waste, depending on its origin, contains various high-value chemical substances and elements, including carbon sources in the form of carboxylic and other acids, carbohydrates, proteins, nitrogen (N) as ammonia, phosphorus (P) and metals. The use of recovered materials from waste would be highly beneficial for the environment and the economy. For example; phosphate rock is a non-renewable natural resource, of critical importance because of its numerous applications including drinking water softening, feed and food additives, and fertilisers. Although its production is carbon neutral, mining P is gradually becoming more costly and supply risks, related to environmental and socio-political issues, have risen. It has been reported that by 2035 the demand for P will outpace the supply as the finite resource becomes increasingly expensive (800% rise between 2006 ($50) and 2008 ($400), current value of over $500/tonne) On the other hand, P removal from wastewater has to improve as water discharge standards become more stringent, raising the costs of wastewater treatment [30]. Substantial value also exists in the high content of metal ions in numerous agricultural and industrial wastes.
Ammonia, another resource, has a market value of $800/tonne and its global consumption exceeds 150 million tonnes. As well as being used heavily in fertilisers, it is also an important component of various commercial and industrial products. These include fuels, antimicrobial agents, woodworking agents and cleaners. It has a large production carbon footprint (best practice being 2.2 tonnes of CO₂ per tonne of ammonia), as during its synthesis methane is reformed to produce H₂ and CO₂. In addition, the disposal and return of ammonia to the atmosphere through nitrification and denitrification adds additional costs to wastewater treatment [30].

Therefore, reclaiming these valuable chemicals into formulated feedstock suitable for biochemical conversion to industrially relevant products, is a crucial step in improving sustainability and reducing environmental impact. Multiple benefits lie in this approach including: recycled materials will substitute newly synthesized or mined materials; the reduction in the volume and concentration of waste will reduce demand and costs in waste treatment plants and methane emissions in the landfills; recovery of ferrous and non-ferrous metals from the waste streams for recycling is more energy efficient than mining for virgin resources; electricity generated by methane generation through anaerobic digestion offsets electricity generated from fossil fuels; valuable streams, such as formulated of nutrient streams, are created for application in agriculture and bioprocessing [31, 32].

Waste: A Sustainable Point of Supply of Resources and Energy

In the context of a current high energy demand economy, with growing awareness of environmental protection and the strengthening of water resource and wastewater related legislation; the need to recover and produce energy and chemicals from wastes becomes apparent [33]. The continuously rising human population results in rising demand for food, energy and water. This growing global urbanization coupled with elevated environmental awareness, expressed by various steep legislative frameworks over waste disposal as well as public pressure, are pushing private and public waste treatment providers to review and reengineer their waste management strategies [29, 34].

The development of novel, cost-effective waste management methodologies is of great interest to various groups such as contractors, engineering consultants, equipment providers, policy regulators (agencies, politicians, and think tanks), and the general public and depends on the needs of the community in a microscale but also on the general good in a macroscale (Fig. 2) [23, 35, 36]. Waste can be divided in numerous categories (Fig. 3) according to type, governing legislation origin or state of matter [37].

Probably not all waste types are suitable to use as biorefinery feedstock, since several complications due to their complex physicochemical nature might occur. Implications relevant to transportation or the need of extensive costly pre-treatment might hinder the use, for instance, of construction waste. Construction waste may include lignocellulosic material but due to its heavily mixed nature and current ways of collection is unsuitable for such an approach [37].

Waste generated by the beverage, food, feed, and agricultural industry is certainly the best candidate for the biorefinery approach, satisfying criteria such as size, continuity of supply and nutritive content. Beverage and food production has become heavily industrialised and therefore regulated generating tons of waste per annum [38, 39]. The food industry is shifting towards the intensive production of ready to eat foods (RTE) that are consumed in venues...
that have fewer conventional methods of stabilizing food, therefore resulting in even larger amounts of waste [40]. In addition to the directly occurring waste due to food processing (slaughterhouse, dairy, wheat and corn milling, confectionary, sugar and starch processing, vegetative processing, fish and poultry processing, alcoholic and non-alcoholic beverages and soft drinks manufacturing and processing), the food industry is linked to agricultural waste (organic waste and agricultural residues) produced by intensive animal and crop farming to satisfy food demand, reaching a 264,854 tonnes per annum [41] in United Kingdom alone. Agricultural waste is third in terms of waste industry size, comparable only to municipal solid waste [42, 43] and it imposes environmental threats, since conventional treatments—such as landfilling or land-spreading—may cause eutrophication and land and water toxicity, due to freely available nutrients and metals spread in water and soil. There are also human health concerns due to land related pathogenicity contained in the raw materials [44].

Industrial wastewaters from food processing industries, wineries, breweries and agricultural wastewater from animal confinements are ideal candidates for biotechnological production of high value substances and platform chemicals [45, 46] however their effective formulation remains a desideratum. These effluents, if used as nutrient media, are potentially highly profitable, especially when compared to the traditional synthetic media or that derived from food sources such as crops. For example, the cost per kilo of Man de Rogosa broth, a well-known nutrient medium used in research and development of starter cultures used in dairy industry can reach $1311 per kilo, while a formulated waste deriving nutritive effluent can cost as little as $2.4 per kilo of nutrients (acids, ammonia, phosphate) recovered [47].

Previous research [48–50] has shown the strong potential of discharged waste effluents to be used as feedstock
for the production of various biobased chemicals (Fig. 4). Consequently, waste can represent an ideal feedstock, since the main focus of a biorefinery is to produce low-value, high-volume (LVHV) products to meet the global energy demand simultaneously with the production of high-value, low-volume (HVLV) products that enhance profitability, while the production of CHP can be used to reduce the costs of processing procedures.

Among the several kinds of food industry related waste, wine industry waste is of major interest for such an approach.

The Winery Waste as Biorefinery Substrate

Grape Wine Production Process

Wine is produced by the botanical genus *Vitis* (grapes), while most of the European wines are produced from the species *Vitis vinifera*.

Wine production is an important part of agriculture and beverage industry worldwide. According to the latest evidence, in 2012 only, 253,000,000 hectolitres of wine where produced worldwide [51]. An average winery is capable of crushing 100 tons of grapes per season, since wine making is a seasonal task occurring in the south hemisphere from January to April and in the north hemisphere between August and October. Grape wine has three main genres, still, sparkling and fortified, with still wine production gaining the major part of the market. Still wine is produced via fermentation through three different routes (skins, peeled and smashed grapes) resulting in different types, white, rose and red. In brief, wine making follows a multiple step process including destemming, crushing, and fermentation, pumping over and pressing (Fig. 5).

The grapes are normally delivered to the winery during autumn (August–October). Destemming, the process of partial or total removal of stems from the grapes, is applied for white or rose wines. Then the grapes are separated depending on whether they can or cannot be crashed, so pulp and juice are released. Crushing is done mechanically, since former manual process may split the skin or simply crack it. The grapes come through a pneumatic press and produce must and solid residues. The produced amount of must is about 80 L per 100 kg of grapes [52–54]. The fermentation stage for red wine is done on solid parts; the fermenting must is in contact with the seeds, skins, and sometimes even stems, while for white wine the solid parts are not that much involved and the decanting stages might be different. The conversion of grapes sugar into alcohol and carbon dioxide by yeasts takes place in a stainless steel, cement or wooden fermentation tanks after pressing, since the solids part should be in contact with the must to impart colour, odour and texture. During fermentation, continuous mixing is required, as grapes’ solid parts have the tendency to surface. Continuous mixing ensures the homogenous distribution of physicochemical conditions and yeasts.

After fermentation, decanting takes place. During this process, the supernatant wine is separated from the produced wine lees and is fed by pumps to empty tanks that are filled completely for further stabilization. The wine lees are at a concentration of 5 % v/v, and are used for to alcohol production [55]. The next stage is maturation,
where decanted wine is kept in maximum capacity filled vessels. After maturation and stabilisation, wine is clarified using chemical agents (fining) (Fig. 5) for quality improvement and then is decanted into empty tanks. After the desired timed period for settling has elapsed, wine is bottled on transportation tanks and distributed to the contact points.

**Origins of Grape Winery Waste**

Winery waste can be divided into two main categories, solid and liquid waste. Solid waste is generated during the collection of grapes and liquid waste is generated during the wine making process (Fig. 5). Solid winery waste, namely grape stalks, grape pomace and grape seeds, varies in chemical composition and texture. In terms of percentage it is composed of up to 7.5% grape stalks, up to 45% grape pomace, up to 6% grape seeds and various other waste sources [56]. Grape stalks are the major by-product of vineyards with an average production of 5 tonnes per hectare per year [57]. They are rich in lignin, cellulose, N and potassium (K), having a high agronomic value and are used for composting [58]. Grape stalks have been found to be highly effective for soils, as they have low organic matter content [59].

Grape pomace contains up to 15% sugars, 0.9% pigments and phenolics, especially in the case of red grape pomace, up to 1% tartrate acid and up to 40% fibre. Grape pomace is being used as a feed additive due to its high fibre content. Grape seeds are very rich in linoleic acid and omega-6 fatty acids, with up to 17 and up to 6% phenolics [60].

Winery waste, however, is not limited to waste generated at the first stages of grape harvesting and initial stages of wine formulation. Waste known as lees, composed of solid and liquid fractions, is generated during the fermentation and maturation stages [61, 62]. The solid part is comprised of the remains precipitated at the bottom of the tanks, mainly consisting of bacterial biomass, undissolved carbohydrates of hemi- or cellulosic nature, phenolic compounds, lignin, proteins, metals, inorganic salts, organic acid salts (mainly tartrates, in the case of wine lees) and other materials such as pips (tannins sustaining grape seeds), fruit skins, grains and seeds. The liquid phase is represented mainly by the spent fermentation broth, often rich in organic acids and ethanol. Vinasses, a by-product of the wine lees, are defined as liquid fraction waste deriving from the distillation process of the wine lees, which is carried out to recover ethanol and elaborate distilled beverages [13, 62].

A vast amount of waste, in the form of wastewater is generated during the further stages of processing, including fermentation (vessels pre- and postwashing), storage and maturation (pre- and post-washing of storage tanks, pre- and postwashing of fermentation vessels, spillages), clarification (wastewater generated from filtration) decanting and bottling (spillages and cleaning of vessels and bottles). Cleaning is not only done with water (cold or lukewarm) but also with solvents, detergents and chemical agents, such as sodium hydroxide. Each wine production step generates a varying amount of wastewater, with qualitative characteristics relevant to the process stage (Table 1) [63].

Winery wastewater overall is produced in high amounts; it has been calculated as 0.5–14 litres per litre of wine produced [64], is mostly acidic, phytotoxic, with high biochemical oxygen demand (BOD) and bactericidal phenols. As can be easily assumed, the generation of wine demands vast amounts of water that have been estimated between 1 and 4 litres per litre of wine produced resulting into 26,000,000–100,500,000 hectolitres of water consumption globally per year [63], while less conservative calculations raise the number to 1,000,000,000 hectolitres per annum in worldwide wine production [63, 65].

The unregulated, unmonitored release of winery wastewater to the soil and water streams can change their chemical and physical characteristics such as pH, conductivity and colour, as well as having several other detrimental effects to the ecosystem. The high organic matter, indicated by BOD, chemical oxygen demand...
COD) and total organic carbon (TOC), results in reduction of oxygen levels in the aquatic environment causing death of several aquatic organisms and generating odours due to the anaerobic decomposition [63]. High alkalinity or extreme acidity, indicated by the pH, affects the solubility of ions and heavy metal content, thus making water toxic and influencing detrimentally both crops and marine organisms. Sodicity of soil—the high sodium content of soil-, indicated by sodium adsorption ratio (SAR), can cause disintegration of soil structure, resulting in surface crusting, which in turn causes low infiltration and hydraulic conductivity. On the other hand, high nutrient content such as N, K and P leads to eutrophication and algal blooms, while the drinking water if containing nitrite and nitrate can be highly toxic to humans. High ionic content or salinity indicated by electrical conductivity (EC) and total dissolved solids (TDS) influences the palatability of water, its uptake by the crops, the flora as well as the wellbeing of fauna. High content in solids, indicated by total solids and total suspended solids (TS and TSS), can reduce light transmission, endangering the ecosystem’s health and smothering its habitants [63].

The high organic and salts content and acidity of winery waste may cause plant growth inhibition, while alterations in conductivity result in retardation of germination, hindering the water uptake by the seeds [66]. Typical composition of winery wastewater is summarised in Table 2 and the elemental composition of solid winery waste grape marc is given in the Table 3.

On the other hand, winery waste is generally biodegradable with a high BOD and COD (Table 5), due to carbohydrate and alcohol content and therefore constitutes a good candidate for fermentation feedstock, provided the use of acid tolerant microorganisms. Commonly the COD concentration of winery waste streams varies from 320 to 49,105 mg L\(^{-1}\) with a mean value of 11,886 mg L\(^{-1}\), while the BOD ranges between 203 and 22,418 mg L\(^{-1}\) with a mean value of 6570 mg L\(^{-1}\) [19].

### Applying the Biorefinery Idea Using Grape Winery Waste as Substrate

The biorefinery concept was introduced to tackle the global energy crisis and climate change, attributed to the intensive industrialisation across the globe. Energy production is among the most polluting processes, based majorly on non-renewable sources such as coal, oil and natural gas. On the other hand, the biorefinery concept was and still is majorly applied to cereals (crops such as wheat and corn) causing implications such as land competition, food shortages, and depletion of natural resources such as water and soil nutrients.

Nowadays, the concept has been extended to the formulation of a biobased economy that has been estimated to grow globally by 2020 to $250 billion in value ($77 bn at 2005, $125 bn at 2010) generating up to 380,000 jobs (120,000 at 2005, 190,000 at 2010) However, currently biobased goods replace just 0.2 % of petroleum-based goods, but alternatives exist for over 90 % of them [67, 68]. The prospect for scaling up has enlivened both supporters and critics of the technology [49, 50].

**Table 1** Wine production stages in relation to generation of wastewater [63, 113, 114]

| Period          | Season            | Wine production process                                                                 | Effect on wastewater characteristics                                    | Effect of wastewater volume |
|-----------------|-------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------|
| Pre-harvest     | Winter–Spring     | Cleaning processes                                                                    | Increase (↑) of K, COD, pH                                                | Up by 33 %                 |
| Early harvest   | (Jan–May)         | Alkali washing and neutralisation                                                      | Increase (↑) of P, Cl, COD                                                | Up by 43 %                 |
| Peak harvest    |                   | Rinsing (tanks, floors, bottling, barrels transfer lines, pipes etc.)                  |                                                                            |                            |
| Late harvest    |                   | Clarification–maturation processes                                                    |                                                                            |                            |
| Post-harvest    | Summer–Winter     | Filtration                                                                            | Increase (↑) of various contaminants, COD, EC                            | Up by 15 %                 |
| Non harvest     | (Jun–Dec)         | Stabilisation–acidification                                                            | Increase (↑) of chemicals such SO₄, NaCl, COD, EC, pH                     | Up by 3 %                  |
|                 |                   | Cooling                                                                               | Increase (↑) of various salts, COD, EC                                    | Up by 6 %                  |
|                 |                   | Other stages                                                                           |                                                                            |                            |
|                 |                   | Winery practices                                                                       | Increase (↑) various salts, pH, COD                                       | Up by 10 %                 |
Economically, implementation of biorefinery into large scale has not always proven feasible, due to the high cost of feedstock production and processing [69, 70]. Several factors should be taken into account while estimating the feasibility of such a process (Fig. 6) Several attempts have been made to reduce the dependence on energy crops, involving the use of lignocellulosic material; however several complications regarding the cost of processing have arisen [71].

During the last decade the need for sophisticated treatment strategy of waste has emerged, due to the rise of environmental awareness, the continuously stringent rules applied on waste disposal and the elevated cost of the conventional waste treatments.

Waste can be seen as an inexhaustible resource due to its rich content in valuable nutrients, with agriculture waste (crops, plant and vegetation) related to food, feed and beverage production becoming a strong nominee as biorefinery feedstock. Agricultural waste complex physicochemical nature might require pre-treatment, however in the case of winery waste due to its generation process this need is minimised [72].

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Both the solid and the liquid winery waste can be used successfully as feedstock for the production of high value chemicals either in a format of conventional biorefinery (lees, vinasses, marc) (Fig. 7) or as green (leafs, pomace) (Fig. 8) or a lignocellulosic (LCF) (Fig. 9) (stalks, peels, seeds, trimming vine shots, pips, pomace) biorefinery, where the effluent winery waste can be used as bioconversion feedstock. In the case of winery wastewater, the high content is organic matter expressed by the COD.

In a LCF biorefinery (Fig. 9) the hard fibrous plant parts (for example pomace, seeds, stalks or seeds) are fractionated, by enzymatic or chemical hydrolysis in three basic chemical parts namely (a) hemicellulose, pentoses, 5-C polymers, (b) cellulose, hexoses, 6-C polymers and (c) lignin, phenols. These fractions will be further converted to useful chemicals such as ethanol, carboxylic acids (acetic, butyric acid acetic acid), butanol, acetone and others [48, 73]. A biorefinery requires, nevertheless, a demanding capital investment and, if based in one major conversion technology, the cost of outputs for the consumers is increased. Therefore several conversion technologies (thermochemical, biochemical/biotechnological chemical, biological) can be integrated (Fig. 8), so that the biorefinery will not only be limited to the production of chemicals but also include production of heat and electricity.

### Bioconversion of Grape Winery Waste to High Value Products and Energy

#### Case Studies

The concept of biological treatment of winery waste has been applied extensively in wastewater treatment plants proving their biodegradability. Taking a step further, several case studies have been conducted over the past 15 years to apply bioconversions and biotransformations of winery waste and wastewater to high value products. These attempts, mostly practised in laboratory scale, have had varying success rates, however they have gone far beyond proving the concept and most of them have shown highly promising results. In these studies, wine lees, grape marc, vinasses, and winery wastewater have been used as feedstock to produce platform chemicals such as lactic acid, biofuels including ethanol, enzymes, chemical intermediates and energy through pyrolysis and anaerobic digestion.

| Parameters                          | Unit      | Min   | Mean  | Max   |
|-------------------------------------|-----------|-------|-------|-------|
| pH                                  | mg L⁻¹    | 2.5   | 5.3   | 12.9  |
| Total solids (TS)                   |           | 190   | 8660  | 18,332|
| Total suspended solids (TSS)        |           | 66    | 1700  | 8600  |
| Total volatile solids (TVS)         |           | 661   | 5625  | 12,385|
| Chemical oxygen demand (COD)        |           | 320   | 11,886| 49,105|
| Biochemical oxygen demand (BOD₅)    |           | 181   | 6750  | 22,418|
| Total organic carbon (TOC)          |           | 41    | 1876  | 7363  |
| Total phosphorous (TP)              |           | 2.1   | 53    | 280   |
| Total nitrogen (TN)                 |           | 10    | 118   | 415   |
| Total phenolic compounds (TPh)      |           | 0.51  | 205   | 1450  |
| Electrical conductivity (EC)        | mS cm⁻¹   | 1.1   | 3.46  | 7.2   |

| Elemental composition (%)           |          |
|-------------------------------------|----------|
| C        | H        | O   | N       | S      | Ash    |
| White grape marc         | 52.97    | 5.94 | 34.22   | 0.54   | 4.16   | 2.18   |
| Red grape marc           | 41.21    | 5.93 | 45.50   | 0.66   | 3.24   | 3.46   |
Fig. 6 Factors affecting the biorefinery concept applied on the winery waste

Fig. 7 The chemical/biochemical biorefinery assortment applied to winery waste [58, 59, 67, 69, 73, 117]

Fig. 8 The green biorefinery assortment applied to winery waste [67, 69, 73, 117]
Utilisation of Grape Marc and Vine Shoots as Substrate

Grape marc is rich in hemicellulosic sugars that, if hydrolysed, will produce mixtures of xylose and glucose that could be under the presence of microorganisms converted to lactic acid. Numerous researchers [74–77] have used effectively *L. pentosus* and *L. rhamnosus* and it has been found that the production of biosurfactants is induced simultaneously with the production of lactic acid. The produced biosurfactants have been proven effective when tested on several non-hydrophilic plant based substrates [20, 78]. Other studies include the use of grape seed oil for the production of rhamnolipid, a biosurfactant generated successfully from the propagation of *Pseudomonas aeruginosa* J4, while grape marc was used as substrate for lactobacilli spawned anti-allergic substances [74]. Grape marc has been also successfully used in solid state fermentations for the production of hydrolytic enzymes such as exo-polygalacturonase, xylanase, b-glucosidase, pectinase and cellulase [79–82], substances effective against allergies or bioethanol [83]. Efforts to recover phenols from grape marc, using ultrasound [84] and solvents (ethanol, methanol) [85] and supercritical fluid consecutive [86] extraction method, have been made with a high success rate.

Grape marc on its own or as a part of an agroindustrial substrate mixture has also been used effectively as an anaerobic digestion substrate for the generation of biogas and methane [87–89] and it is estimated that a small–medium fully operational winery could produce 7800 kW h year\(^{-1}\) electrical and 8900 kW h year\(^{-1}\) thermal energy. Further studies using grape marc for biotechnological production of goods are summarised in Table 4.

To address the numerous difficulties (reduced financial resources, seasonal productivity, transportation costs, complex procedures) researchers [74] have suggested the use of grape marc as compost, even though the phytotoxic attributes of the waste demand extensive pre-treatment. A promising and possibly financially viable process which has been proposed entails a mixture of grape marc, grape stalks and vine shoots to be used as a substrate for growth of edible mushrooms *Agaricus bisporus*.

Other uses of grape marc include its use as a feed additive for livestock (pigs, goats, ewes). The global market value of feed additives has been estimated to reach by 2017 $27.6 billion, due to the expansion of meat and livestock production especially in developing counties. Grape marc has been found to improve sensory abilities and enhance the metabolism of livestock. Trials have been made by treating grape marc with fungi (*Aspergillus*, *Rhizopus* and *Trichoderma* spp.) to enhance protein content in order to provide a nutritious animal feed (protein contents increase between 5 and 26 % and digestibility increased from 25 to 50 %) [90].

A similar approach to the treatment of grape marc has been applied to vine shoots which, when pre-treated (hydrolysis), can be converted by fermentative
microorganisms into chemicals such as xylitol, ethanol, lactic acid and biosurfactants [91–93]. *Bacillus tequilensis* has been grown successfully on pre-treated vine shoots (enzymatic, alkaline hydrolysis) generating approximately 1.52 g L\(^{-1}\) of biosurfactants [94].

Debaryomyces hansenii NRRL Y-7426 and *L. rhamnosus* co-cultures, propagated on vine trimming wastes, have been used to generate biosurfactants and xylitol at 27.5 g L\(^{-1}\) [95].

*Lactobacillus pentosus* [96] and other microorganisms have been successfully used to produce lactic acid from vine shoot samples treated with water and acid to an amount of 24.5 g L\(^{-1}\), as well as to produce a mixture of xylooligosaccharides and single sugars [97, 98]. Co-cultures of *L. pentosus* and *L. plantarum* have been utilised to produce 43.0 g L\(^{-1}\) of lactic acid, 1.58 mM of polylactic acid and 2.6 mg L\(^{-1}\) of biosurfactants from trimming vine shoot hydrolysates [99].

Although not a direct use in the concept of a biorefinery, vine shoots can be effectively used as crude material for pulp paper production, in sites of abundant vineyards such as Spain [2, 100]. The main products produced of trimming vine shoots are summarized in Table 4; most of the cited studies involve the production of lactic acid, biosurfactants, cellulose, pulp and phenolic compounds (Table 5).

### Table 4  Biobased treatments of winery waste [5, 6, 74]

| Winery waste                          | Treatment                                                                 | Product                                                                 |
|---------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------|
| Vinification lees                     | –                                                                         | Nutritional supplement for lactobacilli                                 |
|                                      | Extraction of tartaric acid                                               | Nutritional supplement for *Debaryomyces Hansenii*                      |
| Vinasses                              | Alkali treatment, microwave, fermentation                                 | Lactic acid                                                            |
|                                      | Solubilisation and precipitation                                          | Tartaric acid                                                          |
|                                      | Fermentation                                                              | Protein rich fungal biomass                                             |
| Lees, grape marc                      | Yeast induced fermentation                                                | Protein                                                                |
| Vinasses and grape marc               | Fermentation with *Trichoderma Viride*                                    | Biocontrol agent                                                       |
| Trimming vine shoots                  | Hydrolysis, fermentation of hemicellulosic sugars by *L. Pentosus*        | Lactic acid, biosurfactants                                             |
|                                      | Hydrolysis, delignification, simultaneous saccharification and fermentation of cellulose fraction | Lactic acid                                                             |
|                                      | Hydrolysis and fermentation of hemicellulosic sugars with *Lactobacillus and Debaryomyces Hansenii* | Lactic acid; xylitol; biosurfactants                                   |
|                                      | Solid state fermentation with *Pleurotus*                                 | Source of microbial and human food                                      |
|                                      | Hydrolysis, fermentation of hemicellulosic sugars by *L. Pentosus*        | Lactic acid, biosurfactants                                             |
| Grape marc                            | Hydrolysis, fermentation *L. Pentosus*                                    | Lactic acid, biosurfactants                                             |
|                                      | Extraction                                                                | Tannins as wood adhesives, polyphenols                                 |
|                                      | Solid state fermentation with lactobacilli                                | Hydrolytic enzymes                                                     |
|                                      | Solid state fermentation                                                  | Anti-allergens                                                         |
|                                      | Yeast-induced fermentation                                                | Hydrolytic enzymes, bioethanol                                         |
| Grape marc, lees                      | Fermentation with lactobacilli                                            | Protein                                                                |
| Grape seed oil                        | Fermentation with *Pseudomonas aeruginosa*                                | Biosurfactants                                                         |
| Grape marc seed                       | Extraction                                                                | Oil                                                                    |

### Table 5  Indicative composition of COD in winery wastewater [115, 116]

| Component                     | Concentration (mg L\(^{-1}\)) | Composition (%) |
|-------------------------------|-------------------------------|-----------------|
| COD (dissolved)               | 12,700                        | 100             |
| Ethanol                       | 4900                          | 80.3            |
| Carbohydrates (glucose–fructose) | 870                  | 7.3             |
| Glycerol                      | 320                           | 3.1             |
| Tartaric acid                 | 1260                          | 5.3             |
| Malic acid                    | 70                            | 0.4             |
| Lactic acid                   | 160                           | 1.2             |
| Acetic acid                   | 300                           | 2.6             |
Utilisation of Vinasse as Substrate

The products obtained from vinasse, in most of the cited studies (Table 4), involve the production of nutritional microbial media, tartaric acid, protein rich biomass and plant growth substrates [101, 102]. It has been reported that non treated vinification lees may be used either alone or combined with other cheap waste products, such as corn steep liquor, to formulate inexpensive nutrient media to be used for fermentative production of lactic acid or xylitol [103]. The production of xylitol, when using the liquid fraction of white wine lees, reached 31.9 g L⁻¹, while on the solid fraction 22.5 g L⁻¹ were produced [104].

Vinasse, pre-treated with alkali solutions and microwaving prior to fermentation, has been used as feedstock for the successful production of lactic acid. Lactic acid production was achieved at approximately 17.5 g L⁻¹. The pre-treatment step is enhancing the rate of conversion of pre-processed vinasse to lactic acid and the utilization rate of cellulose and hemicellulose can reach values around 23.8 and 71 %, respectively [105].

Other uses of vinasse include the production of protein rich fungal biomass, as an aquaculture feed ingredient [74], and the production of single cell protein (SCP) A combination of vinasses and trimming vine shoots has also been used successfully for the production of lactic acid and surfacing, a biosurfactant at a final total concentration (hemicellulosic and liquid fraction) of 25.1 g L⁻¹ and 3.2 mg L⁻¹ respectively [106].

From vinasses, tartaric acid can be effectively recovered. Tartaric acid is widely applied in the food and beverage industry as natural acid preservative and an alternative to the citric and phosphoric acids. Grape distilled lees, from which tartaric acid has been recovered using chemical extraction, have been freeze dried and used as a nutrient medium for Lactobacillus pentosus growth [107], achieving a lactic acid production of 18.9 g L⁻¹ [61, 62]. Although researchers [108] have identified this, they have used methods based on chemical extraction (treatment with HCl and precipitation with CaCl₂ generating 1:2 ratio) that may be ineffective in terms of waste generation.

Utilisation of Grape Winery Wastewater as Substrate

Winery wastewater, i.e. the post cleaning operation (crushing, pressing etc.) wastewater, has not been widely used as biotechnological conversion feedstock [109, 119]. Limited studies have been conducted, with winery wastewater being used as substrate for Gluconacetobacter xylinus for the production of cellulose at a 6.26 g L⁻¹ [110, 111]. Other studies involve the use of fungi, Trichoderma viride, Aspergillus niger and Aspergillus oryzae for the production of SCP at a 5 g L⁻¹ and a simultaneous reduction of COD to 90 % [112].

The vast majority of waste distilleries have been treated using traditional wastewater treatment processes, such as land spreading or anaerobic digestion, with the focus being the treatment on BOD and COD, rather than the production of energy or platform chemicals.

Conclusions

Winery waste can be successfully used as feedstock in the biorefinery concept. The seasonal availability of the waste, however, demands judicious handling and treatment to achieve economic feasibility and efficiency. Further research and practical experimentation is necessary since, in the case of winery waste, limited studies have been conducted and life cycle analysis regarding full economic costing of the use wine waste as a resource is needed. The currently available results on the biotechnological use of winery waste are a promising alternative to the current treatment techniques that are focusing on the waste remediation and treatment, rather than resource recovery.

Acknowledgments This work has benefited from the helpful comments of Dr Anna Charalambidou, Lecturer in English Language at Middlesex University London.

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