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Tomato Waste from Processing Industries as a Feedstock for Biofuel Production

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Abstract

Tomato pomace, a solid by-product of tomato processing industries, was assessed for the first time as a feedstock for acetone-butanol-ethanol-isopropanol (ABEI) fermentation. After the pretreatment with hydrothermal process and enzymatic hydrolysis of tomato pomace with a 20% solid-to-solvent ratio, a broth containing 44.1 g/L total sugars was obtained (61.0% saccharification yield). Twelve *Clostridium* strains were compared to find an appropriate microorganism for the transformation of tomato pomace hydrolysate. Four of these strains produced 0.22–5.95 g/L acetone, 5.82–7.00 g/L butanol, 0.17–0.39 g/L ethanol, 0.05–8.28 g/L isopropanol and 11.03–15.07 g/L ABEI. The strain *C. beijerinckii* DSM 6423 obtained 2.67 g butanol, 2.99 g isopropanol and 5.92 g ABEI from 100 g dry tomato pomace (containing 30.6 g carbohydrates). In addition, another pretreatment was performed with a 30% solid-to-solvent ratio in order to obtain higher sugar concentrations, obtaining a hydrolysate with 85.1 g/L total sugars (66.8% saccharification efficiency), which was employed for bioethanol production by comparing twelve different yeast and bacterial strains. *Kluyveromyces marxianus* DSM 5422, *Saccharomyces cerevisiae* Ethanol Red®, *S. cerevisiae* Hércules and *Lachancea thermotolerans* DSM 3434 produced 20.1–21.7 g/L ethanol (about 5.21 g ethanol per 100 g dry tomato pomace). According to these results, tomato pomace could be an interesting feedstock for ABEI biorefineries.

Keywords Tomato waste · Lignocellulosic biomass · Butanol · Ethanol · Isopropanol

Introduction

Nowadays, in an environmentally challenging context, it is necessary to tackle certain problems, such as greenhouse emissions, management of landfill wastes and the assurance of the food supply to a growing human population. Within this situation, the improvement in waste treatment during the food-chain production is mandatory in order to reduce its environmental repercussion [1]. Over recent years, the idea of a biorefinery concept has emerged as an approach to deal with waste management problems by converting agricultural and food processing wastes into high-valued compounds such as alcohols (butanol, ethanol and isopropanol) that can be utilised by bioenergetic or chemical industries [2]. In this sense, lignocellulosic agri-food wastes could become an interesting feedstock for both butanol and ethanol fermentation processes.

Between 1910 and 1960, butanol and acetone were produced worldwide at industrial scale by the acetone-butanol-ethanol (ABE) fermentation [3, 4]. In this process, sugar- or starch-rich feedstocks, such as maize, molasses, grain or potatoes, were transformed by solventogenic *Clostridium* strains through a two-phase fermentation route whose main products are acetone (or alternatively isopropanol), n-butanol, ethanol, CO_2, H_2, acetate and butyrate [3, 5, 6]. The elevated price of those feedstocks and the rapid development of petrochemical industry caused the decline of ABE fermentation plants [3, 6, 7]. Currently, butanol is mainly produced via synthesis from petroleum-derived propylene [3].

Regarding alcoholic fermentation, 96% of the ethanol produced in the European Union in 2017 was obtained from corn, cereals and sugars, whereas only 4% came from lignocellulosic biomass [8]. Most industrial fermentations use the yeast

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Saccharomyces cerevisiae due to its high ethanol yield, osmotolerance and resistance to inhibitors. However, *S. cerevisiae* wild strains can only metabolise hexose sugars (such as those present in traditional sucrose-rich and starch-rich feedstocks) [9], but they are unable to ferment pentoses, which are common in lignocellulosic biomass. Therefore, it is necessary to unearth new microorganisms that are capable of transforming lignocellulosic-derived sugars into ethanol and thus avoid the use of expensive feedstocks that could be devoted to human or animal nutrition.

Upon completion of the alcoholic or ABE fermentation in conventional industrial plants, the solvents were generally recovered and purified by distillation [4]. However, distillation implies high economic and energetic costs, which makes this process financially unfeasible in biorefineries, where tight profit margins and hard competition with petrochemical industries are expected. Recently, other recovery methods for solvents, such as gas stripping, two-stage gas stripping, pervaporation, liquid-liquid extraction, adsorption, non-ionic surfactants, pervaporation, perstraction and reverse osmosis, have been reviewed and proposed as alternatives to distillation [6, 10–14].

Lignocellulosic agri-food by-products are composed mainly of cellulose, hemicellulose and lignin. Conventional fermentation microorganisms are not able to directly metabolise these substrates and a pretreatment is necessary to degrade the complex lignocellulosic structure and release the sugars contained in cellulose and hemicellulose [10]. These pretreatments involve physical, chemical, physicochemical and/or biological procedures, among which the most common are milling, acid or alkaline pretreatment at high temperatures, ammonia fibre explosion (AFEX), organosolv pretreatment, hydrothermal processes, steam explosion and enzymatic hydrolysis [5, 9, 12, 15–17]. However, lignin- and sugar-degradation products, such as acetic acid, formic acid, levulinic acid, furfural or 5-hydroxymethylfurfural (5-HMF), can also be released during the pretreatment and they are known to inhibit fermentative microorganisms [14, 18]. Therefore, in some cases, depending on the pretreatment type, on the nature of the substrate and on the sensitivity of the microbial strain, detoxification methods (e.g. extraction, evaporation, adsorption, electrodialysis, neutralisation, overliming, steam stripping or enzymatic treatment) are recommended to remove inhibitors before fermentation [19], which raises the global price of the production process.

In the context of tomato agriculture, worldwide production reached 182 Mt in 2017, with a total surface of $4.84 \cdot 10^6$ ha devoted to this crop [20]. That same year, about 5.16 Mt tomato were harvested in Spain, of which 58.13% were destined to the processing industry [21]. According to Shrestha et al. [22], a kilogramme of processed tomatoes generates 20 g of discarded tomatoes and 20 g of tomato pomace, whereas del Valle et al. [23] indicated that tomato pomace could represent up to 4% of the total fruit weight processed. Tomato pomace is the solid waste generated during the processing of tomatoes to obtain tomato juice, paste, sauce, puree or ketchup [24]. The wet pomace contains 33% seed, 27% skin and 40% pulp while the dried pomace contains 44% seed and 56% pulp and skin [25]. The chemical composition of tomato pomace is variable depending on the sample analysed, but it is normally in the range of 10–18% starch, 27–32% cellulose, 5–18% hemicellulose, 11–26% simple sugars, 7.6% pectin, 31% lignin, 12–23% protein, 5–20% fat and 4–6% ash [23, 26–28]. Tomato waste contains important amounts of Ca, K (~ 7–11 g/kg), Mg, Na and P (~ 2–3 g/kg), and lower amounts of Fe, Mn and Cu (15–30 mg/kg) [27].

Tomato pomace has been used mostly as animal feed [26] or it has been left on the soil causing environmental problems [29]. However, recently tomato pomace has been proposed as a source of bioproducts, like polysaccharides [30], lycopene, β-carotene, phenolic compounds, flavonoids, levulinic acid, phytosterols, vegetal oil, cutin, polyhydroxyalkanoates and vitamin B12 [27, 29, 31, 32], and as plant fertiliser or microbial growth medium [31]. The generation of biofuels like ethanol and butanol from a substantial number of lignocellulosic and food processing wastes has been extensively assessed [15, 33–35]. However, the application of tomato waste in biofuel production by fermentation is not well studied [26], in spite of the recent methods described to release simple sugars from tomato by-products [36]. So far, bioethanol production from tomato wastes has yielded poor results [24, 37–39]. On the other hand, to the best of our knowledge, no information about biobutanol production from tomato pomace has been published yet.

In this work, tomato pomace was subjected to a soft pretreatment including hydrothermal process and enzymatic hydrolysis to release its fermentable sugars and obtain a liquid hydrolysate. The suitability of this hydrolysate as a feedstock for butanol production by acetone-butanol-ethanol-isopropanol (ABEI) fermentation and for ethanol production by alcoholic fermentation was assessed with twenty-four different bacterial and yeast strains (Fig. 1).

Material and Methods

Tomato Waste Description

Wet tomato wastes from a processing industry were provided by Conservas Vegetales de Extremadura S.A. (Badajoz, Spain) in August 2018. The raw biomass was dried in the open air during 6 days and then dried further in an oven at 45 °C during 48 h. The dried biomass was ground in a rotary mill SM100 Comfort (Retsch GmbH, Haan, Germany) and kept in an airtight container until use.

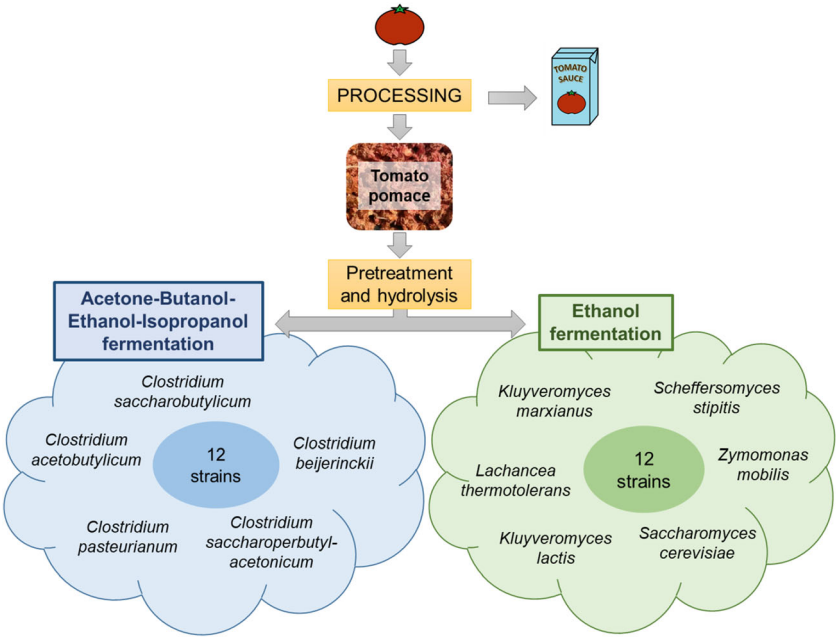

Fig. 1 Schematic view of the proposed process for tomato pomace fermentation to obtain biofuels

The chemical characterisation of dry tomato waste was performed as described elsewhere [40]. It was composed of 1.46% soluble carbohydrates, 16.05% glucan (sum of cellulose and starch) and 11.27% hemicellulose (i.e. 30.6% total carbohydrates), 5.27% galacturonic acid, 24.62% Klason lignin, 17.88% protein, 4.42% fat, 5.13% ash and 6.25% moisture. Its content of total phenolic compounds was 20.5 mg/g, expressed as gallic acid equivalents (GAE).

Hydrolysis of Biomass

In order to obtain sugar-rich fermentable hydrolysates, tomato waste was pretreated by hydrothermal process (i.e. using water as the only solvent and reagent). For the experiments of ABEI fermentation, the biomass was pretreated in a high-pressure 2-L reactor made of alloy Carpenter 20 (Parr Instrument Company, Moline, IL, USA) at 121 °C during 20 min, with a solid-to-solvent ratio of 20% (*w*/*w*). The total mass introduced in the reactor vessel for each batch was 400 g. The reactor temperature was increased from 22 to 121 °C in 21.5 min; it was kept at 121 °C during 20 min, and then the reactor was cooled down again in 5 min with a water refrigeration system. For the experiments of ethanol fermentation, the biomass was pretreated in an autoclave at 121 °C during 20 min, with a solid-to-solvent ratio of 30% (*w*/*w*). It was necessary to employ a greater biomass load in the case of ethanol experiments (30%) in order to guarantee a hydrolysate with a higher initial concentration of simple sugars, which is essential for a profitable ethanol fermentation process.

The samples were cooled down and then the solid/liquid mixture was subjected to enzymatic hydrolysis at pH 5.0 (citrate buffer 50 mM), 50 °C and 180 rpm in an orbital shaker (HT Minitron, Infors AG, Bottmingen, Switzerland). The cellulase cocktail Cellic CTec 2 (activity 100 FPU/mL) was provided by Novozymes (Tianjin, China) and the endo-β-1,4-glucanase enzyme Viscozyme L (activity 41 CMC/mL) was obtained from Novozymes (Bagsvaerd, Denmark). Both of them were applied to the sample at a dose of 3.6 FPU/g biomass and 0.41 CMC/g biomass, respectively. These doses were chosen based on previous works in the case of Cellic CTec2 [41] and on experimental data in the case of Viscozyme L. The hydrolysis time was set at 48 h or 120 h, depending on the biomass load (20% or 30%, respectively). Saccharification efficiency was calculated as the ratio between the mass of dissolved sugars present in the hydrolysate and the initial mass of carbohydrates from tomato pomace present in the sample just before the hydrothermal pretreatment, expressed as a percentage (Eq. 1):

$$\text{Saccharification}\ (\%) = \frac{m_{\text{RS}}}{m_{\text{C}}} \times 100 \qquad (1)$$

where m_{RS} is the amount of released sugars after the enzymatic hydrolysis (expressed in g), calculated taking into account sugar concentrations and the recovered total liquid volume; and m_{C} is the amount of total carbohydrates present in the dry tomato pomace sample before any pretreatment (expressed in g).

The hydrolysates of the different batches were mixed and homogenised to guarantee the same initial composition of all the samples. These hydrolysates were directly employed (without filtration) in fermentation experiments.

Biobutanol Production

Twelve bacterial strains were compared for their performance in tomato waste fermentation. The strains *Clostridium*

acetobutylicum DSM 792, DSM 6228; *C. beijerinckii* DSM 51, DSM 552, DSM 791, DSM 1820, DSM 6423; *C. pasteurianum* DSM 526; *C. saccharobutylicum* DSM 13864; and *C. saccharoperbutylacetonicum* DSM 2152 and DSM 14923 were purchased from DSMZ (Braunschweig, Germany), whereas the strain *C. beijerinckii* CECT 508 was supplied by CECT (Paterna, Spain). Seed cultures for fermentation were prepared from stocks of pure spore solutions of each strain maintained at 4 °C in sterile distilled water. These spores were heat activated in reinforced clostridial medium (RCM) broth or in the case of strains DSM 2152 and DSM 792 in a potato-based medium [42] as described previously [43]. Inocula from the non-spore forming strain DSM 6228 were performed by using a single bacterial colony from a 48-h RCM plate culture (2% agar) obtained from a bacterial stock maintained at − 80 °C in glycerol (80% *v*/*v*). Liquid inocula were maintained at 35 °C until reaching a cell density of $5{\cdot}10^8$ cells/mL (24–48 h) as determined by counting in a Bürker chamber (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany).

For fermentation experiments, 50 mL of tomato hydrolysates were placed in 100-mL glass bottles, where 5 g/L yeast extract, 2.1 g/L NH_4Cl, 1 g/L KH_2PO_4 and 5 g/L $CaCO_3$ were added. The bottles were sterilised at 121 °C for 15 min to eliminate any possible bacterial contamination typical of ABEI processes [44] and, after adding 0.01 g/L $FeSO_4{\cdot}7H_2O$, 0.2 g/L $MgSO_4{\cdot}7H_2O$ and 0.5 g/L cysteine, the pH was adjusted to 6.0 with a solution of NaOH 40%. The bottles were closed in an airtight manner with rubber caps and they were inoculated with 3% (*v*/*v*) of the corresponding bacterial strain. Gaseous N_2 was bubbled into the bottom of the sample for 5 min to guarantee anaerobic conditions. Then, the bottles were incubated at 35 °C and 100 rpm during 96 h in an orbital shaker (Infors HT Minitron, Infors AG, Bottmingen, Switzerland). Control fermentations were carried out for all the strains, with an aqueous solution containing glucose and xylose mixtures at similar concentrations to those of tomato hydrolysates (37 g/L glucose and 12 g/L xylose) and the abovementioned nutrients. All experiments were performed in triplicate.

Bioethanol Production

Strain Cultivation and Inocula Preparation

Twelve different bacterial and yeast strains were compared for ethanol production. *Kluyveromyces lactis* var. *lactis* DSM 70799, *K. marxianus* DSM 5422, DSM 5418, DSM 7239, *K. thermotolerans* DSM 3434 (currently classified as *Lachancea thermotolerans*), *Saccharomyces cerevisiae* DSM 70449, *Scheffersomyces stipitis* DSM 3651, DSM 3652 and *Zymomonas mobilis* DSM 3580 were provided by DSMZ (Braunschweig, Germany); *S. cerevisiae* Ethanol Red® was obtained from Lesaffre Advanced Fermentations (Marcq-en-Baroeul, France); *S. cerevisiae* Hércules-green was provided by Lesaffre Ibérica S.A. (Valladolid, Spain); and *S. cerevisiae* CECT 1383 was purchased from CECT (Paterna, Spain).

Yeast strains, from a cryopreserved solution (glycerol 80% *v*/*v*), were inoculated on culture plates (10 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract, 5 g/L soy peptone, 20 g/L agar) and maintained at 20 °C until obtaining colonies of 1–2 mm. Subsequently, seed cultures for fermentation were prepared in liquid medium from a single yeast colony and kept at 30 °C and 150 rpm during the time necessary to reach a concentration of $1{\cdot}10^8$ cells/mL (incubation time varied from 7 to 24 h depending on the strain used, with the exception of strain DSM 3434 which required 48 h). In the case of *Z. mobilis* DSM 3580, cryopreserved stock solutions were cultured as described previously [45] during 24 h. Then, a single colony was inoculated in 50 mL of liquid medium in a 100-mL glass bottle capped with a rubber stopper in anaerobic conditions by injecting gaseous nitrogen in the headspace during 5 min. The inoculum was kept 24 h at 30 °C (cell density $1{\cdot}10^8$ cells/mL). For all strains, cell density was determined by using a Bürker counting chamber (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany).

Alcoholic Fermentation

Because of the lower nutritional requirements of yeasts in comparison with *Clostridium*, tomato waste hydrolysate was not supplemented with any nutrients for alcoholic fermentation. In addition, due to the competitive capacity of yeasts, the broth was not sterilised before fermentation, which implies energy savings. Alcoholic fermentations with the bacterium *Z. mobilis* DSM 3580 were also performed without nutrient supplementation and without sterilisation for comparison purposes. The pH of tomato waste hydrolysate was adjusted to 5.0 with NaOH and it was inoculated with 3% (*v*/*v*) of liquid inoculum containing yeasts or bacteria. All yeast fermentations were performed in 100-mL Erlenmeyer flasks containing 50 mL tomato hydrolysate, plugged with foam stoppers, under aerobic conditions. Fermentations with *Z. mobilis* DSM 3580 were carried out in 100-mL rubber-capped bottles containing 50 mL tomato hydrolysate, where gaseous N_2 was bubbled during 5 min to guarantee anaerobic conditions. Fermentation controls were prepared with aqueous solutions at pH 5.0 containing glucose and xylose mixtures at similar concentrations to those of tomato hydrolysates (60 g/L glucose, 22 g/L xylose), and supplemented with nutrients and salts (10.4 g/L yeast extract and 1.47 g/L KH_2PO_4 for yeasts; and 7 g/L yeast extract, 2.5 g/L K_2HPO_4, 1.6 g/L $(NH_4)_2SO_4$ and 1 g/L $MgSO_4{\cdot}7H_2O$ for bacteria). All samples and controls were fermented in triplicate in an orbital shaker at 30 °C and 150 rpm during 72 h.

Chemical Analyses

Samples of hydrolysates and fermented broths were centrifuged, filtered and analysed according to Hijosa-Valsero et al. [40] for the quantification of sugars (cellobiose, maltose, glucose, xylose, galactose, mannose, rhamnose and arabinose), potential fermentation inhibitors [formic acid, acetic acid, levulinic acid, 5-hydroxymethylfurfural (5-HMF) and furfural] and fermentation products (acetone, butanol, ethanol, isopropanol, acetic acid and butyric acid). Butanol and ethanol fermentation yields ($Y_{B/S}$, $Y_{E/S}$) and productivities (W_B, W_E) were calculated as reported elsewhere [40], based on total sugar consumption (Eqs. 2 and 3, respectively):

$$Y_{i/S} = \frac{i_t}{S_0 - S_t} \quad (2)$$

$$W_i = \frac{C_{it}}{t} \quad (3)$$

where $Y_{i/S}$ is the fermentation yield of metabolite i (expressed in g/g); i_t is the mass (g) of metabolite i at the end of the fermentation; S_0 is the mass (g) of total sugars at the beginning of the fermentation, S_t is the mass (g) of total sugars at the end of the fermentation; W_i is the fermentation productivity of metabolite i [expressed in g/(L·h)], C_{it} is the concentration (g/L) of metabolite i at the end of the fermentation and t is the fermentation time (h).

In addition, the liquid and solid fractions of the hydrolysates were separated and measured after the enzymatic pretreatment and after the fermentation in order to perform a mass balance.

Statistical Analyses

Fermentation samples were compared with a one-way ANOVA and Tukey's HSD test using the software Statistica 7 (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Hydrolysis of Tomato Waste

High solvent-to-solid ratios were employed during the pretreatment (20–30% biomass, *w*/*w*) in order to guarantee a sufficient sugar concentration in the hydrolysate for fermentations, since the total carbohydrate content of dry tomato waste was relatively low (30.6% carbohydrates). It was observed that the enzymatic hydrolysis step was slowed down and prolonged from 48 to 120 h when increasing the solid biomass load from 20 to 30% *w*/*w*. Therefore, the hydrolysis of the samples destined for ABEI fermentation (20% solid load) was faster than that of the samples prepared for ethanol fermentation (30% solid load).

The chemical composition of tomato waste hydrolysates is shown in Table 1. The broth used for ABEI fermentation contained 44.1 g/L total sugars, which implies a saccharification efficiency of 61.0% of the carbohydrates present in tomato waste biomass. On the other hand, the broth prepared for ethanol fermentation was composed of 85.1 g/L total sugars, entailing a saccharification efficiency of 66.8%. Other tomato pomace hydrolysates reported in literature and destined for ethanol fermentation contained 36–57 g/L total sugar [24, 39]. Therefore, the applied hydrothermal pretreatment in the present work (121 °C, 20 min) was efficient for sugar release. In fact, previous works have evidenced that the use of reagents, such as acids, is not necessary for the pretreatment of tomato wastes and that the hydrothermal process, followed by enzymatic hydrolysis, is an efficient pretreatment for tomato wastes [36, 46]. Working temperatures for the physicochemical pretreatment of tomato pomace have been reported between 110 and 135 °C [36, 46]. Treatment times for hydrothermal processes of tomato and other vegetable wastes are in the range of 5–60 min [35, 36, 40, 46].

Regarding the generation of fermentation inhibitors, only formic acid (0.23–0.36 g/L) and acetic acid (1.64–2.79 g/L) exhibited relevant values, especially in the case of the hydrolysate allocated to ethanol fermentation (Table 1). Levulinic acid, 5-HMF and furfural were present at very low concentrations.

Biobutanol Production from Tomato Waste

The *Clostridium* species selected to perform the fermentation screening corresponded to typical solventogenic Clostridia widely mentioned in the scientific literature [6, 10]. Some strains, such as DSM 51, DSM 552, DSM 791, DSM 1820, DSM 6423, DSM 13864, DSM 2152, DSM 792, DSM 6228,

Table 1 Chemical composition (g/L) of tomato waste hydrolysates

	Biomass 20% (for ABEI fermentation)	Biomass 30% (for ethanol fermentation)
Cellobiose + maltose	< 0.05	< 0.05
Glucose	31.4 ± 1.0	56.0 ± 1.3
Xylose + mannose + galactose	9.3 ± 1.6	22.0 ± 1.0
Rhamnose	3.2 ± 0.3	5.7 ± 0.3
Arabinose	0.3 ± 0.1	0.94 ± 0.14
Total sugars	44.1 ± 3.0	84.6 ± 2.5
Formic acid	0.23 ± 0.08	0.36 ± 0.04
Acetic acid	1.64 ± 0.06	2.79 ± 0.06
Levulinic acid	< 0.02	0.11 ± 0.01
5-HMF	< 0.02	< 0.02
Furfural	< 0.02	0.06 ± 0.01

DSM 14923 or CECT 508, have been previously evaluated for the fermentation of glucose/xylose mixtures and lignocellulosic materials [34, 35, 43, 47, 48]. In the present work, some of the tested strains produced acetone (A), butanol (B) and ethanol (E) as main solvents, whereas other strains transformed part of the acetone into isopropanol (I), according to their typical metabolic pathways [3]. The highest acetone production was achieved by *C. saccharobutylicum* DSM 13864 (5.95 ± 0.36 g/L acetone), the best butanol concentration was obtained by *C. beijerinckii* DSM 1820 (7.00 ± 0.05 g/L butanol), whereas the greatest isopropanol value was provided by *C. acetobutylicum* DSM 792 (8.28 ± 0.85 g/L isopropanol) (Fig. 2, Table S1). Ethanol concentrations were below 1 g/L for all the tested strains. Regarding the total production of solvents (sum of concentrations, ABEI), the strains *C. acetobutylicum* DSM 792 and *C. beijerinckii* DSM 6423 were significantly superior to the rest of strains ($p < 0.05$), attaining concentrations of 14.5–15.1 g/L ABEI (Fig. 2,Table S1). On the other hand, some strains, such as *C. beijerinckii* CECT 508, DSM 51, DSM 552, DSM 791; *C. pasteurianum* DSM 526; and *C. saccharoperbutylacetonicum* DSM 2152 and DSM 14923, were unable to properly ferment tomato pomace (Fig. 2, Table S1). Although isopropanol concentration was slightly higher for *C. acetobutylicum* DSM 792 than for *C. beijerinckii* DSM 6423 ($p < 0.05$), the fermentation broths of the former were difficult to handle during the posterior analytical procedures due to their gelatinous nature and high viscosity. This fact should be taken into account for potential industrial applications.

It must be noted that the isopropanol:butanol ratio of the two abovementioned strains (DSM 792 and DSM 6423) was remarkably high for the fermentation of tomato wastes (A:B:E:I was approximately 1:28:1.6:38 for DSM 792, and 1:24:1.4:27 for DSM 6423), a fact which could be related to the chemical composition of tomato pomace hydrolysate. In fact, isopropanol:butanol ratios were clearly lower in the case of control fermentations of simple synthetic media (Table S2). It has been observed that the acetone:butanol ratio in *C. acetobutylicum* can vary depending on the nutrient composition of the fermentation medium (mainly iron and phosphorus) and on fermentation conditions like pH [49]. In addition, the ratios of isopropanol:acetone are affected by the concentrations of acetate, butyrate and vitamin B3 [50].

Total sugar consumption varied between 18 and 86% depending on the strain (Fig. 2, Table S1). Sugar consumption was related to a successful ABEI fermentation. Actually, all the strains with a sugar consumption above 70% (DSM 6228, DSM 6423, DSM 1820, DSM 13864, DSM 792) were able to produce at least 5 g/L butanol. It was not possible to measure cell density in tomato pomace hydrolysates and fermented broths due to the presence of microscopic biological debris. The highest butanol yields ($Y_{B/S}$) attained were about 0.20 g/g (Table S1). This value is lower than the yields of 0.27 g/g reported in literature for the fermentation of other food wastes like apple pomace or coffee silverskin [34, 40], but similar to that of potato peel [35].

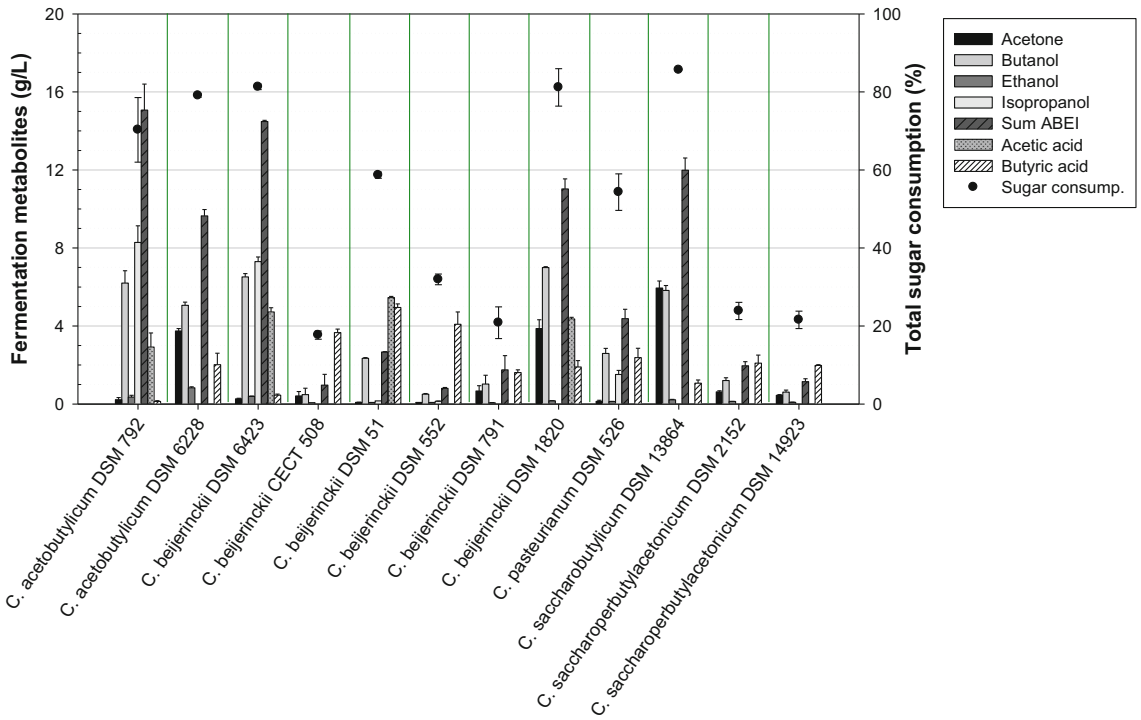

Fig. 2 Parameters of ABEI fermentation for tomato pomace hydrolysates using twelve *Clostridium* strains. Statistical differences among strains are shown in Table S1. *Note*: For strains DSM 6228, CECT 508, DSM 552, DSM 791, DSM 526, DSM 13864, DSM 2152 and DSM 14923, acetic acid was not quantified due to chromatographic interferences

Taking the strain *C. beijerinckii* DSM 6423 as a model, a mass balance for ABEI fermentation was proposed (Fig. 3). From 100 g dry tomato pomace (containing 30.6 g carbohydrates), it is possible to obtain 2.67 g butanol, 2.99 g isopropanol and 5.92 g ABEI.

The butanol (6.2–7.0 g/L) and isopropanol (7.3–8.3 g/L) concentrations obtained in the best cases represent a promising starting point for future research focused on process optimisation. In addition, it must be highlighted that tomato pomace hydrolysate was directly fermentable without the need of any detoxification step to remove potential inhibitors, which simplifies the process. The possibility of using tomato wastes for ABEI fermentation could enlarge the variety of potential lignocellulosic feedstocks and contribute to the engineering development of biorefineries. According to Jiang et al. [7], feedstock costs would represent 30% of the total process costs in an ABE biorefinery based on cellulosic biomass, whereas in the traditional corn-based ABE fermentation this cost attained 66%. The cost of the petrochemical process to produce n-butanol is about 1.23 US$/L, while that of traditional ABE fermentation from corn is about 1.51–1.90 $/L, and 0.80–1.07 $/L from a lignocellulosic by-product like corn stover [7]. In order to sustain an economically viable biorefinery, minimum selling prices for lignocellulosic biobutanol have been calculated to be in the range of 0.40–1.38 $/L [51–53]. However, technoeconomic drawbacks still have to be overcome before the establishment of commercial ABEI biorefineries based on lignocellulosic by-products [10, 14].

Ethanol Production from Tomato Waste

The selection of the yeast and bacterial strains for alcoholic fermentation was based on previous studies in the case of *K. lactis* DSM 70799, *K. marxianus* DSM 5422, DSM 5418, DSM 7239, *S. cerevisiae* Ethanol Red, Hércules-green, CECT 1383 [54], *S. stipitis* DSM 3651 [55] and *Z. mobilis* DSM 3580 [56]. On the contrary, the available information about fermentation of agri-food by-products with *L. thermotolerans* is scarce [57]. The strains employed in this study for ethanol production differ in their abilities to ferment or assimilate various carbon sources (Table 2). The species *S. stipitis*, *K. marxianus* and *K. lactis* var. *lactis* have the potential to grow on a broad variety of carbohydrate substrates.

According to the results obtained in control fermentations, all the tested strains were capable of fermenting glucose/xylose mixtures (Table S3). However, not all of them were able to cope with tomato pomace hydrolysate, although cell densities at the end of the fermentation were similar to those of

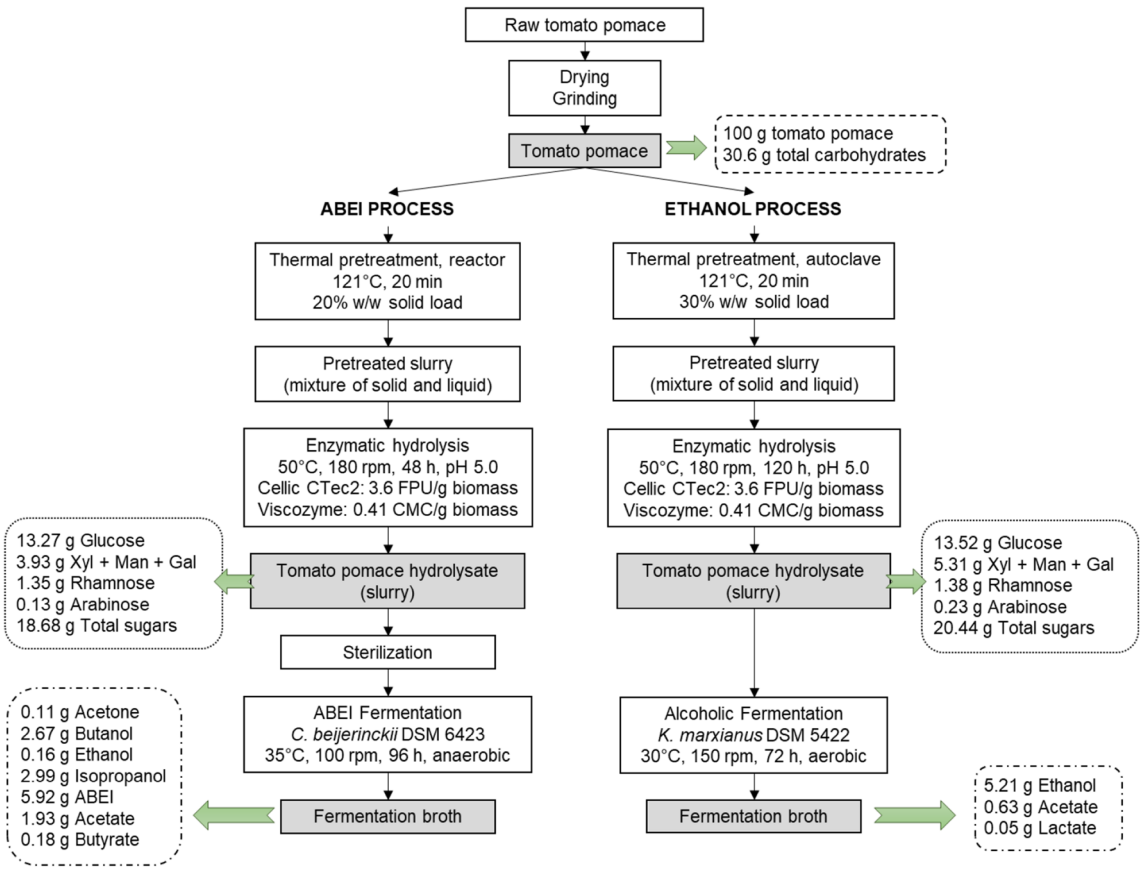

Fig. 3 Mass balance of ABEI and alcoholic fermentation (normalised to an initial amount of 100 g dried tomato pomace) for two model microorganisms. Mass losses due to evaporation have been considered for the calculation. *Note*: Xyl, xylose; Man, mannose; Gal, galactose

Table 2 Carbohydrate metabolism of the strains tested for alcoholic fermentation

Species	Glu	Man	Fru	Suc	Mal	Gal	Lac	Raf	Tre	Xyl	Inu	Cel	Rha	Ara	Sta	References
Lachancea thermotolerans	+			+	+	+		+	+		+					[58]
Kluyveromyces lactis var. *lactis*	+			+	+	+	+	+	+	A	A	A				[59, 60]
Kluyveromyces marxianus	+	+	A	+		+	+	+		+	+	A		A		[59–62]
Saccharomyces cerevisiae	+	+	+	+	+	+		+	A				A			[63, 64]
Scheffersomyces stipitis	+	+		A	+	+	A		+	+		+	A	A	A	[65, 66]
Zymomonas mobilis	+		+	A												[67–69]

+ fermented, *A* assimilated, *Glu* glucose, *Man* mannose, *Fru* fructose, *Suc* sucrose, *Mal* maltose, *Gal* galactose, *Lac* lactose, *Raf* raffinose, *Tre* trehalose, *Xyl* xylose, *Inu* inulin, *Cel* cellobiose, *Rha* rhamnose, *Ara* L-arabinose, *Sta* starch

control samples in most cases (Table S4). In this regard, the four best-performing strains for tomato samples were *K. marxianus* DSM 5422, *S. cerevisiae* Ethanol Red®, *L. thermotolerans* DSM 3434 and *S. cerevisiae* Hércules, with ethanol values of 20.1–21.7 g/L (Fig. 4, Table S4). Other strains like *K. marxianus* DSM 5418, *K. marxianus* 7239, *K. lactis* DSM 70799 and *Z. mobilis* DSM 3580 offered ethanol values above 16 g/L. On the contrary, the two strains of *S. stipitis* (DSM 3651, DSM 3652) were unable to ferment tomato pomace hydrolysate, in spite of their ability to assimilate and ferment numerous sugar sources (Table 2), a fact which could indicate their sensitivity to inhibitory compounds present in tomato hydrolysate. It has been reported that *S. stipitis* is less tolerant to inhibitors than *S. cerevisiae* [70]. Bellido et al. [55] observed that a concentration of 2.5 g/L acetic acid caused an inhibition of 60% in growth and ethanol production in *S. stipitis* DSM 3651, an acetic acid value similar to that of the tomato pomace hydrolysate (Table 1).

Total sugar consumption was 78–90% for all the yeasts that fermented tomato pomace successfully (Fig. 4, Table S4). Nevertheless, the bacterial strain *Z. mobilis* DSM 3580 had a clearly lower ($p < 0.05$) sugar consumption, which could be related to its limited carbohydrate utilisation (Table 2).

According to metabolic stoichiometry, 1 mol of glucose produces 2 mol of ethanol, and 1 mol of xylose produces 1.6 mol of ethanol (or even 0.96 mol ethanol) [61]. This implies theoretical ethanol yields (Y_E) of 0.511 g/g for glucose and 0.294–0.491 g/g for xylose. Under experimental

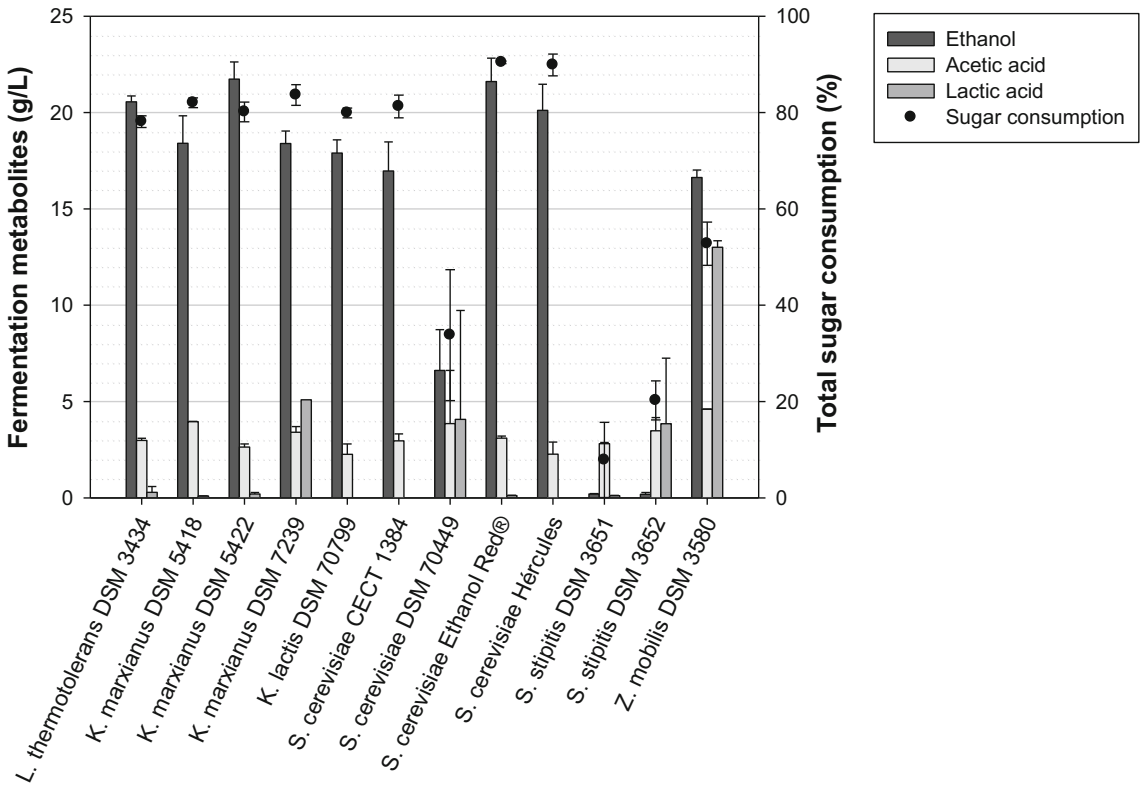

Fig. 4 Parameters of alcoholic fermentation for tomato pomace hydrolysates using twelve yeast and bacterial strains. Statistical differences among strains are shown in Table S4

conditions, the observed ethanol yields $Y_{E/S}$ for glucose:xylose mixtures are temperature dependent and lie in the range of 0.37–0.49 g/g [62]. The ethanol yields based on total sugars ($Y_{E/S}$) calculated in the present study are slightly lower than the abovementioned results; for instance, the four best-performing yeasts for tomato pomace hydrolysate obtained yields of 0.266–0.323 g/g (Table S4).

The best ethanol concentration of 21.7 g/L (obtained by *K. marxianus* DSM 5422) is clearly higher than those reported in literature for the fermentation of similar tomato wastes. Patle and Lal [39] employed a co-culture of *Z. mobilis* MTCC 92 and *Candida tropicalis* TERI SH 110 to ferment a tomato waste hydrolysate containing 36 g/L sugar, and obtained 14 g/L ethanol. Kasavi et al. [38] fermented untreated tomato peels with *S. cerevisiae* strains BC187, L-1374, L-1528, K11 and Y9, but with an extremely low sugar initial concentration (1 g/L carbon), which resulted in an ethanol concentration of 0.16–0.27 g/L and a sugar consumption of 42.3–54.8%. Lenucci et al. [24] subjected tomato pomace to enzymatic hydrolysis and obtained a broth with 28.6 g/L total sugars, which was fermented by *S. cerevisiae* strain Cispa 161 to about 10 g/L ethanol in 12 h; in addition, they prepared a more concentrated broth with 57 g/L total sugars, which yielded 15 g/L ethanol in 72 h.

The strain *K. marxianus* DSM 5422 was selected to calculate the mass balance of the alcoholic fermentation (Fig. 3). It was estimated that 5.21 g ethanol could be obtained from 100 g dry tomato pomace.

In order to obtain profitable concentrations of ethanol, it is necessary to start the fermentation with a sufficient sugar concentration [54, 71, 72]. Because of that, the biomass-to-solvent ratio during the pretreatment has to be increased. The minimum solids concentration for economic ethanol production is estimated at about 15% [73]. As mentioned in section Hydrolysis of Tomato Waste, the biomass ratio of 30% resulted in a slow enzymatic hydrolysis. Taking into account this problem and the low ethanol titres obtained, tomato pomace does not seem a good feedstock for bioethanol production under the conditions tested in this study.

Conclusions

Tomato pomace can be successfully pretreated with a hydrothermal process followed by enzymatic hydrolysis. Saccharification efficiencies of 60–67% can be attained for biomass loads of 20–30% (*w*/*w*). However, to avoid long treatment times during the enzymatic hydrolysis, solid-to-solvent ratios of up to 20% (*w*/*w*) are recommended.

Although the ethanol concentrations obtained for tomato pomace fermentation (~20 g/L) are the highest hitherto reported, this value is insufficient for industrial exploitation. The fact that the ethanol values reached in control solutions were similar to those of tomato pomace hydrolysates could indicate that the glucose/xylose composition of the sample was not very favourable for yeast fermentation. In any case, the good performance demonstrated by some strains of *K. marxianus*, *K. lactis* or *L. thermotolerans* suggests that they could be interesting alternatives to *S. cerevisiae* as potential biocatalysts for other lignocellulosic biomass hydrolysates containing a different sugar composition.

In contrast, tomato pomace was a suitable feedstock for ABEI fermentation. The butanol (6.2–7.0 g/L) and isopropanol (7.3–8.3 g/L) concentrations attained by some strains are only slightly below industrial values for conventional ABEI processes. Another remarkable fact is the possibility of directly fermenting tomato pomace hydrolysates without the need of any detoxification technique by selecting the most appropriate microbial strains. However, before considering the use of this biomass in commercial biorefineries, further research would be necessary to optimise fermentation and nutrient conditions to reduce costs.

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Data Availability All data generated or analysed during this study are included in this article [and its supplementary information files].

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Otles S, Kartal C (2018) Food waste valorization. In: Galanakis CM (ed) Sustainable food systems from agriculture to industry. Academic Press, London, pp 371–399. https://doi.org/10.1016/B978-0-12-811935-8.00011-1
2. Hassan SS, Williams GA, Jaiswal AK (2019) Lignocellulosic biorefineries in Europe: current state and prospects. Trends Biotechnol 37:231–234. https://doi.org/10.1016/j.tibtech.2018.07.002
3. Chen J, Zidwick M, Rogers P (2013) Organic acid and solvent production: butanol, acetone, and isopropanol; 1,3- and 1,2-propanediol production; and 2,3-butanediol production. In: Thompson F (ed) Rosenberg E, DeLong EF, Lory S, Stackebrandt

E. The Prokaryotes. Springer-Verlag, Berlin Heidelberg, pp 77–134. https://doi.org/10.1007/978-3-642-31331-8_386
4. Jones DT, Woods DR (1986) Acetone-butanol fermentation revisited. Microbiol Rev 50(4):484–524 PMCID: PMC373084
5. Vivek N, Nair LM, Mohan B, Nair SC, Sindhu R, Pandey A, Shurpali N, Binod P (2019) Bio-butanol production from rice straw – recent trends, possibilities, and challenges. Bioresource Technol Rep 7:100224. https://doi.org/10.1016/j.biteb.2019.100224
6. Pugazhendhi A, Mathimani T, Varjani S, Rene ER, Kumar G, Kim S-H, Ponnusamy VK, Yoon J-J (2019) Biobutanol as a promising liquid fuel for the future - recent updates and perspectives. Fuel 253: 637–646. https://doi.org/10.1016/j.fuel.2019.04.139
7. Jiang Y, Liu J, Jiang W, Yang Y, Yang S (2015) Current status and prospects of industrial bio-production of n-butanol in China. Biotechnol Adv 33:1493–1501. https://doi.org/10.1016/j.biotechadv.2014.10.007
8. ePURE (2018) European renewable ethanol – key figures 2017. https://www.epure.org/resources/statistics/. Accessed 28 June 2019
9. Mupondwa E, Li X, Tabil L, Sokhansanj S, Adapa P (2017) Status of Canada's lignocellulosic ethanol: part II: hydrolysis and fermentation technologies. Renew Sust Energ Rev 79:1535–1555. https://doi.org/10.1016/j.rser.2016.11.037
10. Gottumukkala LD, Haigh K, Görgens J (2017) Trends and advances in conversion of lignocellulosic biomass to biobutanol: microbes, bioprocesses and industrial viability. Renew Sust Energ Rev 76:963–973. https://doi.org/10.1016/j.rser.2017.03.030
11. Outram V, Lalander C-A, Lee JGM, Davis ET, Harvey AP (2016) A comparison of the energy use of in situ product recovery techniques for the acetone butanol ethanol fermentation. Bioresour Technol 220:590–600. https://doi.org/10.1016/j.biortech.2016.09.002
12. Bharathiraja B, Jayamuthunagai J, Sudharsanaa T, Bharghavi A, Praveenkumar R, Chakravarthy M, Yuvaraj D (2017) Biobutanol – an impending biofuel for future: a review on upstream and downstream processing tecniques. Renew Sust Energ Rev 68:788–807. https://doi.org/10.1016/j.rser.2016.10.017
13. Xue C, Zhao J, Chen L, Yang S-T, Bai F (2017) Recent advances and state-of-the-art strategies in strain and process engineering for biobutanol production by *Clostridium acetobutylicum*. Biotechnol Adv 35(2):310–322. https://doi.org/10.1016/j.biotechadv.2017.01.007
14. Abo BO, Gao M, Wang Y, Wu C, Wang Q, Ma H (2019) Production of butanol from biomass: recent advances and future prospects. Environ Sci Pollut R 26:20164–20182. https://doi.org/10.1007/s11356-019-05437-y
15. Huzir NM, Aziz MMA, Ismail SB, Abdullah B, Mahmood NAN, Umor NA, Muhammad SAFS (2018) Agro-industrial waste to biobutanol production: eco-friendly biofuels for next generation. Renew Sust Energ Rev 94:476–485. https://doi.org/10.1016/j.rser.2018.06.036
16. Mupondwa E, Li X, Tabil L, Sokhansanj S, Adapa P (2017) Status of Canada's lignocellulosic ethanol: part I: pretreatment technologies. Renew Sust Energ Rev 72:178–190. https://doi.org/10.1016/j.rser.2017.01.039
17. Ruiz HA, Thomsen MH, Trajano HL (eds) (2017) Hydrothermal processing in biorefineries: production of bioethanol and high added-value compounds of second and third generation biomass. Springer International Publishing AG, Basel. https://doi.org/10.1007/978-3-319-56457-9
18. Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33. https://doi.org/10.1016/S0960-8524(99)00161-3
19. Grisales-Díaz VH, Olivar-Tost G (2018) Economic optimization of in situ extraction of inhibitors in acetone-ethanol-butanol (ABE) fermentation from lignocellulose. Process Biochem 70:1–8. https://doi.org/10.1016/j.procbio.2018.04.014
20. FAO (2019) FAOSTAT, Production, Crops. http://www.fao.org/faostat/en/#data. Accessed 16 January 2019
21. Eurostat (2019) Crop production in EU standard humidity. https://ec.europa.eu/eurostat/data/database. Accessed 16 January 2019
22. Shrestha N, Fogg A, Wilder J, Franco D, Komisar S, Gadhamshetty V (2016) Electricity generation from defective tomatoes. Bioelectrochemistry 112:67–76. https://doi.org/10.1016/j.bioelechem.2016.07.005
23. del Valle M, Cámara M, Torija ME (2006) Chemical characterization of tomato pomace. J Sci Food Agric 86:1232–1236. https://doi.org/10.1002/jsfa.2474
24. Lenucci MS, Durante M, Montefusco A, Dalessandro G, Piro G (2013) Possible use of the carbohydrates present in tomato pomace and in byproducts of the supercritical carbon dioxide lycopene extraction process as biomass for bioethanol production. J Agric Food Chem 61:3683–3692. https://doi.org/10.1021/jf4005059
25. Kaur D, Wani AA, Oberoi DPS, Sogi DS (2008) Effect of extraction conditions on lycopene extractions from tomato processing waste skin using response surface methodology. Food Chem 108: 711–718. https://doi.org/10.1016/j.foodchem.2007.11.002
26. Hegde S, Lodge J, Trabold T (2018) Characteristics of food processing wastes and their use in sustainable alcohol production. Renew Sust Energ Rev 81:510–523. https://doi.org/10.1016/j.rser.2017.07.012
27. Scoma A, Rebecchi S, Bertin L, Fava F (2016) High impact biowastes from south European agro-industries as feedstock for second-generation biorefineries. Crit Rev Biotechnol 36:175–189. https://doi.org/10.3109/07388551.2014.947238
28. Singh A, Kuila A, Adak S, Bishai M, Banerjee R (2012) Utilization of vegetable wastes for bioenergy generation. Agric Res 1:213–222. https://doi.org/10.1007/s40003-012-0030-x
29. Boccia F, Di Donato P, Covino D, Poli A (2019) Food waste and bio-economy: a scenario for the Italian tomato market. J Clean Prod 227:424–433. https://doi.org/10.1016/j.jclepro.2019.04.180
30. Tommonaro G, Poli A, De Rosa S, Nicolaus B (2008) Tomato derived polysaccharides for biotechnological applications: chemical and biological approaches. Molecules 13:1384–1398. https://doi.org/10.3390/molecules13061384
31. Fritsch C, Staebler A, Happel A, Cubero-Márquez MA, Aguiló-Aguayo I, Abadias M, Gallur M, Cigognini IM, Montanari A, López MJ, Suárez-Estrella F, Brunton N, Luengo E, Sisti L, Ferri M, Belotti G (2017) Processing, valorization and application of bio-waste derived compounds from potato, tomato, olive and cereals: a review. Sustainability 9:1492. https://doi.org/10.3390/su9081492
32. Ravindran R, Jaiswal AK (2016) Exploitation of food industry waste for high-value products. Trends Biotechnol 34:58–69. https://doi.org/10.1016/j.tibtech.2015.10.008
33. Diaz AB, Blandino A, Caro I (2018) Value added products from fermentation of sugars derived from agro-food residues. Trends Food Sci Tech 71:52–64. https://doi.org/10.1016/j.tifs.2017.10.016
34. Hijosa-Valsero M, Garita-Cambronero J, Paniagua-García AI, Díez-Antolínez R (2018) Biobutanol production from coffee silverskin. Microb Cell Factories 17:154. https://doi.org/10.1186/s12934-018-1002-z
35. Hijosa-Valsero M, Paniagua-García AI, Díez-Antolínez R (2018) Industrial potato peel as a feedstock for biobutanol production. New Biotechnol 46:54–60. https://doi.org/10.1016/j.nbt.2018.07.002
36. Díaz AI, Laca A, Laca A, Díaz M (2017) Treatment of supermarket vegetable wastes to be used as alternative substrates in bioprocesses. Waste Manag 67:59–66. https://doi.org/10.1016/j.wasman.2017.05.018
37. Aramrueang N, Zicari SM, Zhang R (2017) Characterization and compositional analysis of agricultural crops and residues for ethanol production in California. Biomass Bioenergy 105:288–297. https://doi.org/10.1016/j.biombioe.2017.07.013

38. Kasavi C, Finore I, Lama L, Nicolaus B, Oliver SG, Oner ET, Kirdar B (2012) Evaluation of industrial *Saccharomyces cerevisiae* strains for ethanol production from biomass. Biomass Bioenergy 45:230–238. https://doi.org/10.1016/j.biombioe.2012.06.013
39. Patle S, Lal B (2007) Ethanol production from hydrolysed agricultural wastes using mixed culture of *Zymomonas mobilis* and *Candida tropicalis*. Biotechnol Lett 29:1839–1843. https://doi.org/10.1007/s10529-007-9493-4
40. Hijosa-Valsero M, Paniagua-García AI, Díez-Antolínez R (2017) Biobutanol production from apple pomace: the importance of pretreatment methods on the fermentability of lignocellulosic agro-food wastes. Appl Microbiol Biotechnol 101:8041–8052. https://doi.org/10.1007/s00253-017-8522-z
41. Giacobbe S, Balan V, Montella S, Fagnano M, Mori M, Faraco V (2016) Assessment of bacterial and fungal (hemi)cellulose-degrading enzymes in saccharification of ammonia fibre expansion-pretreated *Arundo donax*. Appl Microbiol Biotechnol 100:2213–2224. https://doi.org/10.1007/s00253-015-7066-3
42. Yerushalmi L, Volesky B (1987) Culture conditions for growth and solvent biosynthesis by a modified *Clostridium acetobutylicum*. Appl Microbiol Biotechnol 25:513–520. https://doi.org/10.1007/BF00252009
43. Paniagua-García AI, Hijosa-Valsero M, Díez-Antolínez R, Sánchez ME, Coca M (2018) Enzymatic hydrolysis and detoxification of lignocellulosic biomass are not always necessary for ABE fermentation: the case of *Panicum virgatum*. Biomass Bioenergy 116:131–139. https://doi.org/10.1016/j.biombioe.2018.06.006
44. Zverlov VV, Berezina O, Velikodvorskaya GA, Schwarz WH (2006) Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery. Appl Microbiol Biotechnol 71:587–597. https://doi.org/10.1007/s00253-006-0445-z
45. Silbir S, Dagbagli S, Yegin S, Baysal T, Goksungur Y (2014) Levan production by *Zymomonas mobilis* in batch and continuous fermentation systems. Carbohydr Polym 99:454–461. https://doi.org/10.1016/j.carbpol.2013.08.031
46. del Campo I, Alegría I, Zazpe M, Echeverría M, Echeverría I (2006) Diluted acid hydrolysis pretreatment of Agri-food wastes for bioethanol production. Ind Crop Prod 24:214–221. https://doi.org/10.1016/j.indcrop.2006.06.014
47. Niglio S, Procentese A, Russo ME, Sannia G, Marzocchella A (2019) Investigation of enzymatic hydrolysis of coffee silverskin aimed at the production of butanol and succinic acid by fermentative processes. Bioenerg Res 12(2):312–324. https://doi.org/10.1007/s12155-019-09969-6
48. Magalhães BL, Grassi MCB, Pereira GAG, Brocchia M (2018) Improved n-butanol production from lignocellulosic hydrolysate by *Clostridium* strain screening and culture-medium optimization. Biomass Bioenergy 108:157–166. https://doi.org/10.1016/j.biombioe.2017.10.044
49. Bahl H, Gottwald M, Kuhn A, Rale V, Andersch W, Gottschalk G (1986) Nutritional factors affecting the ratio of solvents produced by *Clostridium acetobutylicum*. Appl Environ Microbiol 52:169–172 PMCID: PMC203431
50. Xin F, Chen T, Jiang Y, Dong W, Zhang W, Zhang M, Wu H, Ma J, Jiang M (2017) Strategies for improved isopropanol–butanol production by a *Clostridium* strain from glucose and hemicellulose through consolidated bioprocessing. Biotechnol Biofuels 10:118. https://doi.org/10.1186/s13068-017-0805-1
51. Malmierca S, Díez-Antolínez R, Paniagua AI, Martín M (2017) Technoeconomic study of biobutanol AB production. 2. Process design. Ind Eng Chem Res 56:1525–1533. https://doi.org/10.1021/acs.iecr.6b02944
52. Tao L, He X, Tan ECT, Zhang M, Aden A (2014) Comparative techno-economic analysis and reviews of n-butanol production from corn grain and corn stover. Biofuels Bioprod Biorefin 8:342–361. https://doi.org/10.1002/bbb.1462
53. Kumar M, Goyal Y, Sarkar A, Gayen K (2012) Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks. Appl Energ 93:193–204. https://doi.org/10.1016/j.apenergy.2011.12.079
54. Díez-Antolínez R, Hijosa-Valsero M, Paniagua-García AI, Garita-Cambronero J, Gómez X (2018) Yeast screening and cell immobilization on inert supports for ethanol production from cheese whey permeate with high lactose loads. PLoS One 13(12):e0210002. https://doi.org/10.1371/journal.pone.0210002
55. Bellido C, Bolado S, Coca M, Lucas S, González-Benito G, García-Cubero MT (2011) Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by *Pichia stipitis*. Bioresour Technol 102:10868–10874. https://doi.org/10.1016/j.biortech.2011.08.128
56. Pinilla L, Torres R, Ortiz C (2011) Bioethanol production in batch mode by a native strain of *Zymomonas mobilis*. World J Microb Biot 27:2521–2528. https://doi.org/10.1007/s11274-011-0721-7
57. Bollók M, Réczey K (2000) Screening of different *Kluyveromyces* strains for simultaneous saccharification and fermentation. Accred Qual Assur 5:59–70. https://doi.org/10.1556/AAlim.29.2000.1.6
58. Lachance MA, Kurtzman CP (2011) *Lachancea* Kurtzman (2003). In: Kurtzman C, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier science, Amsterdam, pp 511–519. https://doi.org/10.1016/B978-0-444-52149-1.00061-6
59. Lachance MA (2011) *Kluyveromyces* van der Walt (1971). In: Kurtzman C, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier science, Amsterdam, pp 733–746. https://doi.org/10.1016/B978-0-444-52149-1.00061-6
60. Lane MM, Burke N, Karreman R, Wolfe KH, O'Byrne CP, Morrissey JP (2011) Physiological and metabolic diversity in the yeast *Kluyveromyces marxianus*. Antonie Van Leeuwenhoek 100:507–519. https://doi.org/10.1007/s10482-011-9606-x
61. Pentjuss A, Stalidzans E, Liepins J, Kokina A, Martynova J, Zikmanis P, Mozga I, Scherbaka R, Hartman H, Poolman MG, Fell DA, Vigants A (2017) Model based biotechnological potential analysis of *Kluyveromyces marxianus* central metabolism. J Ind Microbiol Biotechnol 44:1177–1190. https://doi.org/10.1007/s10295-017-1946-8
62. Rodrussamee N, Lertwattanasakul N, Hirata K, Suprayogi S, Kosaka T, Yamada M (2011) Growth and ethanol fermentation ability on hexose and pentose sugars and glucose effect under various conditions in thermotolerant yeast *Kluyveromyces marxianus*. Appl Microbiol Biotechnol 90:1573–1586. https://doi.org/10.1007/s00253-011-3218-2
63. van Maris AJA, Abbott DA, Bellissimi E, van den Brink J, Kuyper M, Luttik MAH, Wisselink HW, Scheffers WA, van Dijken JP, Pronk JT (2006) Alcoholic fermentation of carbon sources in biomass hydrolysates by *Saccharomyces cerevisiae*: current status. Antonie Van Leeuwenhoek 90:391–418. https://doi.org/10.1007/s10482-006-9085-7
64. Vaughan-Martini A, Martini A (2011) *Saccharomyces* Meyen ex Reess (1870). In: Kurtzman C, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier science, Amsterdam, pp 733–746. https://doi.org/10.1016/B978-0-444-52149-1.00061-6
65. du Preez JC, Bosch M, Prior BA (1986) The fermentation of hexose and pentose sugars by *Candida shehatae* and *Pichia stipitis*. Appl Microbiol Biotechnol 23:228–233. https://doi.org/10.1007/BF00261920
66. Kurtzman CP (2011) *Scheffersomyces* Kurtzman & M. Suzuki (2010). In: Kurtzman C, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier science, Amsterdam, pp 773–777. https://doi.org/10.1016/B978-0-444-52149-1.00061-6
67. de Araújo LCA, de Cássia Dias Mendes T, dos Santos BS, da Mota Silveira Filho V, de Souza Lima GM, de Araújo JM, dos Santos

Correia MT, de Oliveira MBM, Morais Júnior MA, da Silva MV (2018) Molecular identification and physiological characterization of *Zymomonas mobilis* strains from fuel-ethanol production plants in north-east Brazil. Lett Appl Microbiol 67:54–63. https://doi.org/10.1111/lam.12888

68. Silveira M, Jonas R (2002) The biotechnological production of sorbitol. Appl Microbiol Biotechnol 59:400–408. https://doi.org/10.1007/s00253-002-1046-0
69. Sprenger GA (1996) Carbohydrate metabolism in *Zymomonas mobilis*: a catabolic highway with some scenic routes. FEMS Microbiol Lett 145:301–307. https://doi.org/10.1016/S0378-1097(96)00396-5
70. Olofsson K, Bertilsson M, Lidén G (2008) A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels 1:7. https://doi.org/10.1186/1754-6834-1-7
71. Díez-Antolínez R, Hijosa-Valsero M, Paniagua-García AI, Gómez X (2016) Very-high-gravity fermentation of non-supplemented cheese whey permeate by immobilized *Kluyveromyces marxianus*. Chem Engineer Trans 49:529–534. https://doi.org/10.3303/CET1649089
72. Olsson L, Hahn-Hägerdal B (1996) Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb Tech 18:312–331
73. Parawira W, Tekere M (2011) Biotechnological strategies to overcome inhibitors in lignocellulose hydrolysates for ethanol production: review. Crit Rev Biotechnol 31:20–31. https://doi.org/10.3109/07388551003757816