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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 pretreatment with autohydrolysis and enzymatic hydrolysis, a broth containing 44.1 g/L total sugars was obtained. Twelve *Clostridium* strains were compared to find an appropriate microorganism for the transformation of tomato pomace hydrolysate. *C. acetobutylicum* DSM 792 produced 6.20 ± 0.63 g/L butanol and 8.28 ± 0.85 g/L isopropanol, whereas *C. beijerinckii* DSM 6423 produced 6.52 ± 0.17 g/L butanol and 7.30 ± 0.23 g/L isopropanol. In addition, another hydrolysate with a higher total sugar content (85.1 g/L) was prepared and 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. According to these results, tomato pomace could be an interesting feedstock for ABEI biorefineries.

Keywords: tomato waste; lignocellulosic biomass; butanol; ethanol; isopropanol

1. Introduction

Nowadays, in an environmentally challenging context, it is necessary to explore and develop research that permits 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 (Otles and Kartal, 2018). 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 (Hassan et al., 2019).

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 (FAO, 2019). That same year, about 5.16 Mt tomato were harvested in Spain, of which 58.13% were destined to the processing industry (Eurostat, 2019). According to Shrestha et al. (2016), a kilogram of processed tomatoes generates 20 g of discarded tomatoes and 20 g of tomato pomace, whereas del Valle et al. (2006) indicated that tomato pomace could represent at most 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 (Lenucci et al., 2013). The wet pomace contains 33% seed, 27% skin and 40% pulp while the dried pomace contains 44% seed and 56% pulp and skin (Kaur et al., 2008). 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 (del Valle et al., 2006; Hegde et al., 2018; Scoma et al., 2016; Singh et al., 2012). 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) (Scoma et al., 2016).

Tomato pomace has been used mostly as animal feed (Hegde et al., 2018), but recently it has been proposed as a source of bioproducts, like polysaccharides (Tommonaro et al., 2008), lycopene, β -carotene, phenolic compounds, flavonoids, levulinic acid, phytosterols, vegetal oil, cutin (Fritsch et al., 2017; Ravindran and Jaiswal, 2016; Scoma et al., 2016) and as plant fertiliser or microbial growth medium

(Fritsch et al., 2017). The generation of biofuels like ethanol and butanol from a substantial number of lignocellulosic and food processing wastes has been extensively assessed (Diaz et al., 2018; Hijosa-Valsero et al., 2018a, 2018b; Huzir et al., 2018). However, the application of tomato waste in biofuel production by fermentation is not well studied (Hegde et al., 2018), in spite of the recent methods described to release simple sugars from tomato by-products (Díaz et al., 2017). So far, bioethanol production from tomato wastes has yielded poor results (Aramrueang et al., 2017; Kasavi et al., 2012; Lenucci et al., 2013; Patle and Lal, 2007). 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 autohydrolysis 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.

2. Material and Methods

2.1. 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 six 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.

The chemical characterisation of dry tomato waste was performed as described elsewhere (Hijosa-Valsero et al., 2017). It was composed of 30.6% total carbohydrates (1.46% soluble carbohydrates, 16.05% glucan and 11.27% hemicellulose), 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).

2.2. Hydrolysis of tomato waste

In order to obtain sugar-rich fermentable hydrolysates, tomato waste was pretreated by autohydrolysis (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. 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), by addition of 36 µl Cellic CTec2 (activity 100 FPU/mL; Novozymes, Tianjin, China) and 10 µl Viscozyme L (activity 41 CMC/mL; Novozymes, Bagsvaerd, Denmark) per 1 g of initial solid biomass. The hydrolysis time was set at 48 h or 120 h, depending on the biomass load (20% or 30%, respectively).

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.

2.3. 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, *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 (Yerushalmi and Volesky, 1987) as described previously (Paniagua-García et al., 2018). 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 NH₄Cl, 1 g/L KH₂PO₄ and 5 g/L CaCO₃ were added. The bottles were sterilised at 121°C for 15 min and, after adding 0.01 g/L FeSO₄·7H₂O, 0.2 g/L MgSO₄·7H₂O 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 N₂ 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.

2.4. Production of ethanol

2.4.1. 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, *K. marxianus* DSM 5418, *K. marxianus* DSM 7239, *K. thermotolerans* DSM 3434 (currently classified as *Lachancea thermotolerans*), *Saccharomyces cerevisiae* DSM 70449, *Scheffersomyces stipitis* DSM 3651, *S. stipitis* 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 7-24 h depending of 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 (Silbir et al., 2014) 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).

2.4.2. Alcoholic fermentation

Tomato waste hydrolysate was not sterilised and it was not supplemented with any nutrients. Its pH 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 waste, where gaseous N₂ 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 KH₂PO₄ for yeasts; and 7 g/L yeast extract, 2.5 g/L K₂HPO₄, 1.6 g/L (NH₄)₂SO₄ and 1 g/L MgSO₄·7H₂O for bacteria). All samples and controls were fermented in triplicate in an orbital shaker at 30 °C and 150 rpm during 72 h.

2.5. Chemical analyses

Samples of hydrolysates and fermented broths were centrifuged, filtered and analyzed according to Hijosa-Valsero et al. (2017) 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 ($W_{B/S}$, $W_{E/S}$) were calculated as

reported by Hijosa-Valsero et al. (2017), based on total sugar consumption.

2.6. Statistical analyses

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

3. Results and discussion

3.1. 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).

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

Compound	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

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 the release of 58.5% 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 carbohydrate release efficiency of 66.6%. Other tomato pomace hydrolysates reported in

literature and destined for ethanol fermentation contained 36-57 g/L total sugar (Lenucci et al., 2013; Patle and Lal, 2007). Therefore, the applied pretreatment in the present work was efficient for sugar release. In fact, autohydrolysis, followed by enzymatic hydrolysis, has been proven as an efficient pretreatment for tomato wastes (del Campo et al., 2006; Díaz et al., 2017).

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.

3.2. Biobutanol production from tomato waste

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 (Chen et al., 2013). 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) (Table 2). 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 (Table 2). On the other hand, some strains, such as *C. beijerinckii* CECT 508, DSM 51, DSM 552, DSM 791, *C. pasteurianum* DSM 526, *C. saccharoperbutylacetonicum* DSM 2152 and DSM 14923, were unable to properly ferment tomato pomace (Table 2). 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. 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 A1). 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 (Bahl et al., 1986). In addition, the ratios of isopropanol:acetone are affected by the concentrations of acetate, butyrate and vitamin B3 (Xin et al., 2017).

Table 2. Parameters of ABEI fermentation for tomato pomace hydrolysates. *Note:* Superscripts (a, b, c, etc.) represent statistical differences among strains ($p < 0.05$). If two strains share at least one letter for a given column, they do not differ significantly.

Species	Strain	A (g/L)	B (g/L)	E (g/L)	I (g/L)	Acetate (g/L)	Butyrate (g/L)	Sum ABEI (g/L)	Total sugar consumption (%)	Y _{B/S} (g/g)	W _B [g/(L·h)]
<i>C. acetobutylicum</i>	DSM 792	0.22 ± 0.11 ^{bde}	6.20 ± 0.63 ^{ab}	0.36 ± 0.09 ^{ac}	8.28 ± 0.85 ^b	2.92 ± 0.72	0.13 ± 0.04 ^b	15.07 ± 1.33 ^b	70 ± 8 ^b	0.203 ± 0.009 ^b	0.065 ± 0.007 ^b
<i>C. acetobutylicum</i>	DSM 6228	3.75 ± 0.12 ^c	5.06 ± 0.16 ^a	0.83 ± 0.05 ^a	< 0.05 ^a	n.q.	2.02 ± 0.58 ^{ac}	9.64 ± 0.33 ^e	79 ± 1 ^{ab}	0.155 ± 0.005 ^{ab}	0.053 ± 0.006 ^a
<i>C. beijerinckii</i>	DSM 6423	0.27 ± 0.03 ^{bde}	6.52 ± 0.17 ^{ab}	0.39 ± 0.02 ^c	7.30 ± 0.23 ^c	4.72 ± 0.22	0.45 ± 0.06 ^b	14.48 ± 0.07 ^b	81 ± 3 ^a	0.184 ± 0.005 ^{ab}	0.068 ± 0.006 ^b
<i>C. beijerinckii</i>	CECT 508	0.42 ± 0.22 ^{bde}	0.48 ± 0.33 ^d	0.07 ± 0.00 ^{bd}	< 0.05 ^a	n.q.	3.66 ± 0.18 ^d	0.97 ± 0.55 ^d	18 ± 4 ^{de}	0.066 ± 0.046 ^{ef}	0.005 ± 0.006 ^d
<i>C. beijerinckii</i>	DSM 51	0.09 ± 0.01 ^{bde}	2.35 ± 0.04 ^c	0.08 ± 0.01 ^{bd}	0.16 ± 0.00 ^a	5.46 ± 0.06	4.95 ± 0.19 ^e	2.67 ± 0.02 ^{cd}	59 ± 2 ^c	0.097 ± 0.003 ^{cd}	0.024 ± 0.006 ^c
<i>C. beijerinckii</i>	DSM 552	0.08 ± 0.00 ^d	0.51 ± 0.03 ^d	0.07 ± 0.01 ^{bd}	0.14 ± 0.01 ^a	n.q.	4.09 ± 0.63 ^{de}	0.80 ± 0.05 ^d	32 ± 0 ^f	0.038 ± 0.001 ^f	0.005 ± 0.006 ^d
<i>C. beijerinckii</i>	DSM 791	0.67 ± 0.27 ^e	1.02 ± 0.45 ^d	0.06 ± 0.01 ^{bd}	< 0.05 ^a	n.q.	1.61 ± 0.14 ^{ac}	1.75 ± 0.73 ^d	21 ± 7 ^{de}	0.115 ± 0.030 ^{cd}	0.011 ± 0.006 ^d
<i>C. beijerinckii</i>	DSM 1820	3.87 ± 0.45 ^c	7.00 ± 0.05 ^b	0.17 ± 0.01 ^{bd}	< 0.05 ^a	4.36 ± 0.08	1.90 ± 0.33 ^{ac}	11.03 ± 0.51 ^{ae}	81 ± 13 ^{ab}	0.209 ± 0.011 ^b	0.073 ± 0.006 ^b
<i>C. pasteurianum</i>	DSM 526	0.14 ± 0.05 ^{bde}	2.60 ± 0.26 ^c	0.13 ± 0.01 ^{bf}	1.52 ± 0.21 ^d	n.q.	2.38 ± 0.48 ^c	4.38 ± 0.48 ^c	54 ± 2 ^c	0.111 ± 0.017 ^{cd}	0.027 ± 0.006 ^c
<i>C. saccharobutylicum</i>	DSM 13864	5.95 ± 0.36 ^a	5.82 ± 0.26 ^{ae}	0.22 ± 0.02 ^{af}	< 0.05 ^a	n.q.	1.07 ± 0.16 ^{ab}	11.99 ± 0.63 ^a	86 ± 0 ^a	0.144 ± 0.006 ^{ac}	0.061 ± 0.006 ^{ab}
<i>C. saccharoperbutylacetonicum</i>	DSM 2152	0.62 ± 0.06 ^{bde}	1.21 ± 0.14 ^d	0.13 ± 0.01 ^{bf}	< 0.05 ^a	n.q.	2.10 ± 0.41 ^c	1.96 ± 0.21 ^d	24 ± 1 ^{def}	0.116 ± 0.003 ^{cd}	0.013 ± 0.006 ^d
<i>C. saccharoperbutylacetonicum</i>	DSM 14923	0.45 ± 0.03 ^{bde}	0.61 ± 0.10 ^d	0.09 ± 0.01 ^d	< 0.05 ^a	n.q.	1.98 ± 0.04 ^{ac}	1.15 ± 0.14 ^d	22 ± 3 ^e	0.065 ± 0.005 ^{ef}	0.006 ± 0.006 ^d

n.q. Not quantified due to chromatographic interferences.

Total sugar consumption varied between 18 and 86% depending on the strain (Table 2). 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. The highest butanol yields ($Y_{B/S}$) attained were about 0.20 g/g (Table 2). 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 (Hijosa-Valsero et al., 2017, 2018a), but similar to that of potato peel (Hijosa-Valsero et al., 2018b).

3.3. Ethanol production from tomato waste

The yeast and bacterial strains employed in this study for ethanol production differ in their abilities to ferment or assimilate various carbon sources (Table 3). 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 A2). However, not all of them were able to cope with tomato pomace hydrolysate. 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 (Table 4). 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 3), 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* (Olofsson et al., 2008). Bellido et al. (2011) 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, a 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 (Table 4). 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 3).

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) (Pentjuss et al., 2017). 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 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 (Rodrussamee et al., 2011). 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 4).

Table 3. Carbohydrate sources fermented (+) or assimilated (A) by the strains tested for alcoholic fermentation.

Species	Glu	Man	Fru	Suc	Mal	Gal	Lac	Raf	Tre	Xyl	Inu	Cel	Rha	Ara	Sta	References
<i>Lachancea thermotolerans</i>	+			+	+	+		+	+		+					Lachance and Kurtzman, 2011
<i>Kluyveromyces lactis</i> var. <i>lactis</i>	+			+	+	+	+	+	+	A	A	A				Lachance, 2011; Lane et al., 2011
<i>Kluyveromyces marxianus</i>	+	+	A	+		+	+	+		+	+	A		A		Lachance, 2011; Lane et al., 2011; Pentjuss et al., 2017; Rodrussamee et al., 2011
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+		+	A				A			van Maris et al., 2006; Vaughan-Martini and Martini, 2011
<i>Scheffersomyces stipitis</i>	+	+		A	+	+	A		+	+		+	A	A	A	du Preez et al., 1986; Kurtzman, 2011
<i>Zymomonas mobilis</i>	+		+	A												de Araújo et al., 2018; Silveira and Jonas, 2002; Sprenger, 1996

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.

Table 4. Alcoholic fermentation of tomato pomace hydrolysate. *Note:* Superscripts (a, b, c, etc.) represent statistical differences among strains ($p < 0.05$). If two strains share at least one letter for a given column, they do not differ significantly.

Species	Strain	Ethanol (g/L)	Acetic acid (g/L)	Lactic acid (g/L)	Total sugar consumption (%)	Glucose consumption (%)	$Y_{E/S}$ (g/g)	W_E [g/(L·h)]
<i>Lachancea thermotolerans</i>	DSM 3434	20.56 ± 0.30 ^{cef}	2.98 ± 0.11 ^a	0.29 ± 0.31	78 ± 1 ^c	100 ± 0 ^b	0.318 ± 0.010 ^b	0.286 ± 0.004 ^{cef}
<i>Kluyveromyces marxianus</i>	DSM 5422	21.74 ± 0.89 ^c	2.63 ± 0.17 ^a	0.20 ± 0.08	80 ± 2 ^c	100 ± 0 ^b	0.322 ± 0.009 ^b	0.302 ± 0.012 ^c
<i>Kluyveromyces marxianus</i>	DSM 5418	18.40 ± 1.43 ^{be}	3.96 ± 0.01 ^a	0.10 ± 0.01	82 ± 1 ^c	100 ± 0 ^b	0.261 ± 0.022 ^{ab}	0.256 ± 0.020 ^{be}
<i>Kluyveromyces marxianus</i>	DSM 7239	18.39 ± 0.65 ^{be}	3.41 ± 0.29 ^a	5.09 ± 0	84 ± 2 ^c	100 ± 0 ^b	0.253 ± 0.007 ^{ab}	0.255 ± 0.009 ^{be}
<i>Kluyveromyces lactis</i>	DSM 70799	17.90 ± 0.69 ^{bf}	2.27 ± 0.53 ^a	< 0.05	80 ± 1 ^c	100 ± 0 ^b	0.260 ± 0.009 ^{ab}	0.248 ± 0.009 ^{bf}
<i>Saccharomyces cerevisiae</i>	CECT 1383	16.96 ± 1.51 ^b	2.96 ± 0.36 ^a	< 0.05	81 ± 2 ^c	100 ± 0 ^b	0.248 ± 0.015 ^{ab}	0.236 ± 0.021 ^b
<i>Saccharomyces cerevisiae</i>	DSM 70449	6.61 ± 2.12 ^d	3.85 ± 2.76 ^a	4.08 ± 5.65	34 ± 14 ^b	53 ± 16 ^c	0.240 ± 0.030 ^{ab}	0.092 ± 0.029 ^d
<i>Saccharomyces cerevisiae</i>	Ethanol Red®	21.61 ± 1.21 ^{ce}	3.10 ± 0.11 ^a	0.11 ± 0.03	90 ± 0 ^c	100 ± 0 ^b	0.284 ± 0.015 ^{ab}	0.300 ± 0.017 ^{ce}
<i>Saccharomyces cerevisiae</i>	Hércules	20.11 ± 1.35 ^{bc}	2.27 ± 0.63 ^a	< 0.05	90 ± 2 ^c	100 ± 0 ^b	0.266 ± 0.017 ^{ab}	0.279 ± 0.019 ^{bc}
<i>Scheffersomyces stipitis</i>	DSM 3651	0.20 ± 0.02 ^a	2.81 ± 0.06 ^a	0.11 ± 0.02	8 ± 8 ^a	8 ± 7 ^a	0.204 ± 0.310 ^{ab}	0.003 ± 0.003 ^a
<i>Scheffersomyces stipitis</i>	DSM 3652	0.19 ± 0.09 ^a	3.48 ± 0.69 ^a	3.85 ± 3.40	20 ± 4 ^{ab}	18 ± 1 ^a	0.011 ± 0.003 ^a	0.003 ± 0.001 ^a
<i>Zymomonas mobilis</i>	DSM 3580	16.63 ± 0.39 ^b	4.61 ± 0.01 ^a	13.01 ± 0.34	53 ± 4 ^d	75 ± 2 ^d	0.384 ± 0.043 ^b	0.231 ± 0.005 ^b

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 (2007) 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. (2012) 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. (2013) subjected tomato pomace to enzymatic hydrolysis and obtained a broth with 28.6 g/L total sugars, which was fermented by *S. cerevisiae* strain Cisca 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. In order to obtain profitable concentrations of ethanol, it is necessary to start the fermentation with a sufficient sugar concentration (Díez-Antolínez et al., 2016, 2018; Olsson and Hahn-Hägerdal, 1996). Because of that, the biomass-to-solvent ratio during the pretreatment has to be increased. The minimum solids concentration for economic ethanol production

is about 15% (Parawira and Tekere, 2011). As mentioned in section 3.1, 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.

4. Conclusions

Autohydrolysis was successful to pretreat tomato pomace. 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 (~20 g/L) are the highest reported, this value is insufficient for industrial exploitation. However, our results indicate that *K. marxianus*, *K. lactis* or *L. thermotolerans* are potential biocatalysts for lignocellulosic biomass hydrolysates containing inhibitory substances.

In contrast, tomato pomace was a suitable feedstock for ABEI fermentation. Before considering the use of this biomass in industrial biorefineries, it would be necessary to optimise fermentation and nutrient conditions to reduce costs.

Appendix A

The results of fermentation controls are shown in Table A1 (ABEI) and Table A2 (ethanol).

Declaration of interests: None.

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APPENDIX

Table A1. Acetone-butanol-ethanol-isopropanol (ABEI) fermentation of control solutions containing glucose, xylose and nutrients.

Species	Strain	A (g/L)	B (g/L)	E (g/L)	I (g/L)	Acetate (g/L)	Butyrate (g/L)	Sum ABEI (g/L)	Glucose consump. (%)	Xylose consump. (%)	Total sugar consump. (%)	Y _{B/S} (g/g)	W _B [g/(L·h)]
<i>C. acetobutylicum</i>	DSM 792	0.15 ± 0.01	10.75 ± 0.75	0.19 ± 0.01	3.73 ± 0.19	0.79 ± 0.86	0.62 ± 0.36	14.82 ± 0.94	98 ± 0	82 ± 4	94 ± 1	0.235 ± 0.014	0.112 ± 0.008
<i>C. acetobutylicum</i>	DSM 6228	3.78 ± 0.06	7.63 ± 0.23	0.62 ± 0.06	<0.05	2.98 ± 0.08	2.93 ± 0.04	12.02 ± 0.31	99 ± 0	92 ± 11	97 ± 3	0.165 ± 0.001	0.079 ± 0.002
<i>C. beijerinckii</i>	DSM 6423	0.15 ± 0.03	10.39 ± 0.11	0.14 ± 0.03	3.75 ± 0.19	1.35 ± 0.07	0.79 ± 0.11	14.43 ± 0.26	99 ± 1	71 ± 1	92 ± 0	0.234 ± 0.003	0.108 ± 0.001
<i>C. beijerinckii</i>	CECT 508	1.34 ± 0.14	7.34 ± 0.25	0.09 ± 0.14	< 0.05	2.49 ± 0.24	1.89 ± 0.45	8.77 ± 0.39	70 ± 4	73 ± 3	71 ± 3	0.219 ± 0.006	0.076 ± 0.003
<i>C. beijerinckii</i>	DSM 51	0.08 ± 0.02	3.24 ± 0.30	0.07 ± 0.02	0.48 ± 0.16	3.43 ± 0.09	3.37 ± 0.11	3.87 ± 0.41	53 ± 2	62 ± 3	55 ± 2	0.123 ± 0.011	0.034 ± 0.003
<i>C. beijerinckii</i>	DSM 552	0.25 ± 0.04	5.10 ± 0.20	0.09 ± 0.04	0.39 ± 0.06	3.03 ± 0.61	2.84 ± 0.24	5.83 ± 0.28	71 ± 4	58 ± 2	67 ± 3	0.159 ± 0.003	0.053 ± 0.002
<i>C. beijerinckii</i>	DSM 791	0.43 ± 0.06	4.30 ± 0.38	0.07 ± 0.06	< 0.05	2.81 ± 0.11	1.74 ± 0.54	4.80 ± 0.44	29 ± 5	100 ± 0	48 ± 3	0.186 ± 0.004	0.045 ± 0.004
<i>C. beijerinckii</i>	DSM 1820	1.62 ± 0.03	5.97 ± 0.05	0.10 ± 0.03	< 0.05	2.78 ± 0.10	1.13 ± 0.21	7.68 ± 0.06	60 ± 1	52 ± 1	58 ± 1	0.215 ± 0.004	0.062 ± 0.001
<i>C. pasteurianum</i>	DSM 526	0.14 ± 0.00	7.46 ± 0.45	0.12 ± 0.00	1.64 ± 0.26	2.49 ± 0.30	1.92 ± 0.44	9.36 ± 0.71	78 ± 2	65 ± 2	74 ± 2	0.206 ± 0.008	0.078 ± 0.005
<i>C. saccharobutylicum</i>	DSM 13864	3.78 ± 0.28	5.75 ± 0.29	0.22 ± 0.28	< 0.05	2.60 ± 0.03	0.69 ± 0.08	9.75 ± 0.59	97 ± 1	46 ± 2	84 ± 0	0.147 ± 0.007	0.060 ± 0.003
<i>C. saccharoperbutylacetonicum</i>	DSM 2152	3.59 ± 0.05	11.06 ± 0.09	0.77 ± 0.05	< 0.05	0.88 ± 0.12	0.23 ± 0.05	15.42 ± 0.12	99 ± 0	66 ± 7	91 ± 2	0.251 ± 0.005	0.115 ± 0.001
<i>C. saccharoperbutylacetonicum</i>	DSM 14923	6.18 ± 0.05	8.58 ± 0.07	0.44 ± 0.05	< 0.05	1.10 ± 0.08	0.25 ± 0.02	15.20 ± 0.09	98 ± 0	93 ± 1	97 ± 0	0.183 ± 0.001	0.089 ± 0.001

Table A2. Alcoholic fermentation of control solutions containing glucose, xylose and nutrients.

Species	Strain	Ethanol (g/L)	Acetic acid (g/L)	Lactic acid (g/L)	Glucose consumption (%)	Xylose consumption (%)	Total sugar consumption (%)	Y _{E/S} (g/g)	W _E [g/(L·h)]
<i>Lachancea thermotolerans</i>	DSM 3434	19.91 ± 0.20	0.00 ± 0.00	< 0.05	100 ± 0	57 ± 4	85 ± 1	0.303 ± 0.004	0.276 ± 0.003
<i>Kluyveromyces marxianus</i>	DSM 5422	17.40 ± 0.09	3.25 ± 0.11	< 0.05	100 ± 0	38 ± 1	82 ± 0	0.269 ± 0.001	0.242 ± 0.001
<i>Kluyveromyces marxianus</i>	DSM 5418	15.25 ± 0.27	4.56 ± 0.22	< 0.05	100 ± 0	47 ± 0	81 ± 0	0.242 ± 0.005	0.212 ± 0.004
<i>Kluyveromyces marxianus</i>	DSM 7239	15.69 ± 0.50	4.39 ± 0.34	< 0.05	100 ± 0	49 ± 0	82 ± 0	0.247 ± 0.008	0.218 ± 0.007
<i>Kluyveromyces lactis</i>	DSM 70799	13.15 ± 0.56	0.00 ± 0.00	< 0.05	90 ± 2	32 ± 0	70 ± 2	0.243 ± 0.008	0.183 ± 0.008
<i>Saccharomyces cerevisiae</i>	CECT 1383	17.42 ± 0.56	1.40 ± 0.03	< 0.05	100 ± 0	26 ± 0	79 ± 0	0.281 ± 0.009	0.242 ± 0.008
<i>Saccharomyces cerevisiae</i>	DSM 70449	14.31 ± 1.64	0.74 ± 0.09	< 0.05	88 ± 6	30 ± 3	68 ± 5	0.273 ± 0.023	0.199 ± 0.023
<i>Saccharomyces cerevisiae</i>	Ethanol Red®	16.08 ± 0.16	5.49 ± 0.22	< 0.05	100 ± 0	26 ± 0	79 ± 0	0.260 ± 0.002	0.223 ± 0.002
<i>Saccharomyces cerevisiae</i>	Hércules	17.45 ± 0.34	0.13 ± 0.06	< 0.05	100 ± 0	37 ± 0	82 ± 0	0.271 ± 0.005	0.242 ± 0.005
<i>Scheffersomyces stipitis</i>	DSM 3651	18.36 ± 0.37	0.79 ± 0.02	< 0.05	98 ± 2	11 ± 2	73 ± 2	0.321 ± 0.002	0.255 ± 0.005
<i>Scheffersomyces stipitis</i>	DSM 3652	18.02 ± 0.21	0.67 ± 0.01	< 0.05	100 ± 0	36 ± 2	82 ± 1	0.281 ± 0.002	0.250 ± 0.003
<i>Zymomonas mobilis</i>	DSM 3580	27.48 ± 0.20	0.45 ± 0.04	0.23 ± 0.07	100 ± 0	30 ± 0	76 ± 0	0.459 ± 0.009	0.382 ± 0.003