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Abstract: This study is aimed at assessing the potential of apple pomace (AP) as a substrate for biofuel production following a biorefinery approach. Two different APs, from juice and cider production were evaluated. First, bioethanol generation was performed and its fermentation residues, together with available biobutanol fermentation residues, were studied for biogas production. Moreover, co-digestion of AP and swine manure was investigated following a factorial design. Twelve different bacterial and yeast strains were compared for bioethanol production, obtaining bioethanol concentrations about 50 g L-1 by different strains of Kluyveromyces marxianus, K. lactis, Lachancea thermotolerans and Saccharomyces cerevisiae, with yields of 0.371 - 0.444 g g-1. Specific methane yields of the fermentation residues of bioethanol and biobutanol production were 463 and 290 mL CH4 g -1 VS added, respectively. Methane yield for the co-digestion of AP and swine manure was 596 mL CH4 g-1 VS added, with an AP percentage of 14.6 % and a substrate concentration of 9.38 g VS L-1.

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Dear Editor:

Will you please consider the enclosed manuscript **"**Valorization of apple pomaces for biofuel production: a biorefinery approach" for publication in Biomass and Bioenergy.

(1) Authors: Beatriz Molinuevo-Salces, Berta Riaño, María Hijosa-Valsero, Isabel González- García, Ana I. Paniagua-García, David Hernández, Jerson Garita-Cambronero, Rebeca Díez-Antolínez and María Cruz García-González, mutually agree that the article should be submitted to Biosystems Engineering.

(3) Authors state that the present article is an original work.

(4) Authors state that the present manuscript has not been previously submitted to Biomass and Bioenergy.

(5) Novelty in results/findings, or significance of results.

The production of apples in the world generates large quantities of apple pomace (AP). Most of this AP is currently incinerated or used for compost production, resulting in a potential source of GHG emissions. Biofuel production from AP could be a sustainable alternative for the valorization of this by-product. In this way, the present work evaluates two valorization ways for AP, namely a biorefinery approach for biofuel production and the co-digestion of AP and swine manure.

First, bioethanol production from AP was successfully produced. Bioethanol concentrations about 50 g L^{-1} were obtained by different strains of *K. marxianus, K. lactis, L. thermotolerants* and *S. cerevisiae* with yields of 0.371-0.444 g g^{-1}. Then, specific methane yields of the exhausted broths after bioethanol and biobutanol production were 463 and 290 mL CH_4 g $^{-1}$ VS added, respectively. Finally, co-digestion of AP and swine manure was investigated following a factorial design and the highest methane yield (596 mL CH_4 g $^{-1}$ VS added) was obtained with a substrate concentration of 9 g L^{-1} SV and an AP content in the mixture of 15%.

Yours sincerely,

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***Highlights (for review)**

- Apple pomaces are valorized for biofuel production
- Bioethanol yield was up to 0.371-0.444 g g-1 from twelve different strains
- Methane yield of 463 mL CH_4 $g^{-1}_{VS\ added}$ of the bioethanol fermentation residue
- Methane yield of 290 mL CH_4 $g^{-1}_{VS\ added}$ of the biobutanol fermentation residue
- Co-digestion of AP and manure produced up to 596 mL CH_4 $g^{-1}_{VS\ added}$

Valorization of apple pomaces for biofuel production: a biorefinery approach.

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Abstract

This study is aimed at assessing the potential of apple pomace (AP) as a substrate for biofuel production following a biorefinery approach. Two different APs, from juice and cider production were evaluated. First, bioethanol generation was performed and its fermentation residues, together with available biobutanol fermentation residues, were studied for biogas production. Moreover, co-digestion of AP and swine manure was investigated following a factorial design. Twelve different bacterial and yeast strains were compared for bioethanol production, obtaining bioethanol concentrations about 50 g L^{-1} by different strains of *Kluyveromyces marxianus*, *K. lactis*, *Lachancea thermotolerans* and *Saccharomyces cerevisiae*, with yields of 0.371 - 0.444 g g^{-1}. Specific methane yields of the fermentation residues of bioethanol and biobutanol production were 463 and 290 mL CH_4 $g^{-1}_{\text{VS added}}$, respectively. Methane yield for the co-digestion of AP and swine manure was 596 mL CH_4 $g^{-1}_{\text{VS added}}$, with an AP percentage of 14.6 % and a substrate concentration of 9.38 g VS L^{-1}.

Keywords: apple pomace, biobutanol, bioethanol, biogas

1. Introduction

The production of apples in the world is approximately 54.2 million tons per year. Around 26% of this production is processed in the apple industry for obtaining different products as juice, jelly or cider [1]. The solid waste produced after generating the different apple products is called apple pomace (AP) and it accounts for approximately 25% of the total processed biomass [2]. Regarding Spain, half a million ton of apples is produced every year and around 9 metric tons of apple pomace is generated from cider production in Asturias, the main cider producer region in Spain [3, 4]. Apple pomaces obtained from juice and cider production are similar in composition, containing 20-30% solids, with a high amount of lignocellulosic material. Currently, a fifth of the produced AP is used as animal or human feed. The rest is incinerated or either used for compost production, resulting in a potential source of GHG emissions [2]. Alternative uses such as production of biofuels, extraction of antioxidants and nutraceuticals, production of pectin or production of materials for the development of scaffolds for cell growth have been proposed in the last years for alleviating waste disposal [4, 5, 6].

In the last decades, many efforts have been made for biofuel production from food by-products. AP can be potentially converted into biobutanol [6] and bioethanol [7] in biorefineries by fermentation. In order to ferment AP, either by alcoholic or acetone-butanol-ethanol (ABE) fermentation, a pretreatment is necessary to release the simple sugars contained in cellulose and hemicellulose, thus obtaining a hydrolysate rich in hexose and pentose sugars. However, this pretreatment also generates toxic compounds that can inhibit fermentation. Not all microbial species are able to ferment the wide variety of sugars present in lignocellulosic hydrolysates and their tolerance to inhibitors is very variable. Therefore, it is essential to select an adequate strain to deal with

lignocellulosic hydrolysates. In the case of alcoholic fermentation, *Saccharomyces cerevisiae* has been preferentially employed for traditional substrates due to its bioethanol production capacity and tolerance, but other microorganisms are emerging as interesting alternatives for lignocellulosic biomasses like AP, such as *Kluyveromyces* sp., *Scheffersomyces stipitis* or *Zymomonas mobilis* [8, 9]. In the case of ABE fermentation from AP, the species *Clostridium beijerinckii* has been successfully employed [6, 10].

The most common residual streams generated during ethanol or ABE fermentation are exhausted fermentation broths, also known as stillage or spent wash (i.e. broths from which solvents have been recovered by distillation or similar techniques). They consist of aqueous suspensions with low concentrations of free sugars, containing AP solids, microorganisms and microbial debris. Currently, stillage from ethanol distilleries is used as soil fertiliser, although different authors have demonstrated it can cause pollution problems [11, 12]. It has been suggested that these ethanol distillation by-products could also be employed as feedstock in various bioprocesses to obtain microbial biomass, proteins, ethanol, surfactants, bioplastics, fatty acids, edible fungi, enzymes or biogas by anaerobic digestion [11, 12, 13, 14].

Anaerobic digestion (AD) is a biological process by which organic matter is transformed into renewable energy. AD contributes to greenhouse gas mitigation, odor and pathogen reduction, and organic nitrogen mineralization into available nitrogen for plant growth [15]. Previous authors have investigated methane potential of AP, being in the range of 137-231 mL CH_4 g volatile solids $(VS)^{-1}$ [16, 17]. However, anaerobic digestion of the exhausted broths after biofuel production has been scarcely evaluated

and it would be interesting since AD could be a sustainable alternative to the current uses of this by-product. Moreover, it would result in a biorefinery approach, where the by-product obtained after biofuel production would be the substrate for AD. Anaerobic co-digestion of AP and livestock wastes has been also evaluated, resulting in positive synergetic effects on biogas production between both substrates, if compared to animal manure alone [18]. According to The European Biogas Association, the number of agricultural biogas plants in Spain was 139 at the end of 2015. This number is quite low, if compared to the number of biogas plants in other EU countries such as Germany or Italy. However, from 2014 to 2015 it has exponentially grown from 39 to 139 (EBA 2014, EBA 2015). These plants are co-digestion plants where the main substrate is manure and different carbon-rich co-substrates are fed during the year. Since AP is seasonally produced, there is a special interest in investigating the potential of AP as co-substrate for AD plants to valorize this substrate during the months where it is produced.

This work aims to study two valorization ways for AP, namely a biorefinery approach for biofuel production and the co-digestion of AP and swine manure. First, bioethanol production from AP was studied, paying special attention to the microbial strain selection. Then, the biochemical methane potential of different AP fermentation residues (exhausted broths from alcoholic and ABE processes) was determined. Finally, co-digestion of AP and swine manure was investigated following a factorial design in order to determine the optimal ratio AP/manure that would ensure a stable AD process for the existing biogas plants.

2. Material and Methods

2.1. **Origin of the substrates (apple pomaces and exhausted fermentation broths), swine manure and inoculum.**

Two apple pomaces were studied. The first one (AP1) was provided by Muns Agroindustrial S.L., located in Lleida, Spain. It was a dry AP obtained after juice extraction and drying for preservation. AP1 was ground and sieved. The final size ranged between 0.5 and 1.0 mm. Two fermentation by-products from AP1 (i.e. exhausted fermentation broths) were obtained, from bioethanol production (AP1-E) and biobutanol production (AP1-B), respectively. The stream AP1-E was obtained after removing the bioethanol from the broth corresponding to a 72-h fermentation of AP1 hydrolysate by *S. cerevisiae* Ethanol Red® (see section 2.2 for more details). Bioethanol was removed by gas stripping, with T_{feed} = 70 °C, $T_{refrigeration}$ = 0 °C and gas flow 1.34 L min^{-1} during 4 h. The stream AP1-B was prepared from a fermented hydrolysate of AP1 containing 1.42 g L^{-1} acetone, 5.45 g L^{-1} butanol, 0.16 g L^{-1} ethanol, 4.28 g L^{-1} acetic acid and 4.98 g L^{-1} butyric acid, previously pretreated by autohydrolysis and fermented, with *C. beijerinckii* CECT 508, according to Hijosa-Valsero et al. [6]. Subsequently, the broth of the ABE fermentation was subjected to gas stripping according to the conditions described by Díez-Antolínez et al. [19] in order to remove ABE solvents. These exhausted broths were stored at 4 ºC for further use.

The second AP (AP2) was provided by the Regional Research and Development Service of Asturias (SERIDA), Asturias, Spain. In this case, the AP was a fresh product obtained after apple pressing for cider production (AP2-fresh). The biomass was dried at 60ºC, obtaining AP2-dried. This biomass was then ground (AP2- dried powder) in a SM100 Comfort rotary mill (Retsch GmbH, Haan, Germany) and sieved, with a size range of 0.5–1.0 mm.

The chemical composition of both apple pomace samples (AP1 and AP2) is shown in Table 1.

Swine manure (SM) was obtained from a pig farm located in Guardo, Palencia, Spain. The inoculum used for AD (AD inoculum) was a mesophilic anaerobic sludge that was obtained from the municipal wastewater treatment plant (WWTP) in Valladolid, Spain and subsequently stored at 4 ºC for further use.

2.2. **Bioethanol production**

2.2.1. Hydrolysis of apple pomace

In order to perform bioethanol fermentation, it is necessary to release the simple sugars (glucose, xylose, galactose, etc.) that are contained within AP polysaccharides (cellulose and hemicellulose). Hence, AP was subjected to a physicochemical pretreatment and an enzymatic hydrolysis. In the first place, AP was autoclaved in an aqueous solution at 121 °C during 20 min, with a solid-to-solvent ratio of 30% (w/w). Then, the samples were cooled down, and citric acid and NaOH were added to obtain a citrate buffer of 50 mM and pH 5.0. Afterwards, 36 µL of the enzyme Cellic CTec 2 (activity 100 FPU mL^{-1}; Novozymes, Tianjin, China) and 10 µL of the enzyme Viscozyme L (activity 41 CMC mL^{-1}; Novozymes, Bagsvaerd, Denmark) were added per each 1 g of dry AP. The enzymatic hydrolysis was performed in an orbital shaker (HT Minitron, Infors AG, Bottmingen, Switzerland) at 50 °C and 180 rpm during 48 h. This pretreatment was applied to AP1 and AP2. However, probably due to the high pectin content (quantified as galacturonic acid) or to other intrinsic characteristics of AP2 (Table 1), it was not possible to hydrolyse it. Therefore, bioethanol fermentation was only carried out with

hydrolysate of AP1. Sugar composition of these hydrolysates was analysed by HPLC according to section 2.5.

2.2.2. Strain cultivation and inocula preparation

Twelve different bacterial and yeast strains were compared for bioethanol production. *K. 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*), *S. cerevisiae* DSM 70449, *S. stipitis* DSM 3651, *S. stipitis* DSM 3652 and *Z. 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). Yeasts were cultivated in Petri dishes (10 g L^{-1} glucose, 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract, 5 g L^{-1} soy peptone, 20 g L^{-1} agar) at 20 °C under aerobic conditions until the formation of colonies of 1-2 mm. Then, a colony was transferred to an Erlenmeyer flask with 50 mL of liquid medium (10 g L^{-1} glucose, 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract, 5 g L^{-1} soy peptone). The flask was capped with a foam stopper and incubated at 30 °C and 150 rpm in an orbital shaker until cell density reached $1 \cdot 10^8$ cells mL^{-1} (approximately 7-24 h). *Z. mobilis* was grown in Petri dishes (50 g L^{-1} sucrose, 7 g L^{-1} yeast extract, 2.5 g L^{-1} K_2HPO_4, 1.6 g L^{-1} $(NH_4)_2SO_4$, 1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 20 g L^{-1} agar) at 30 °C under anaerobic conditions until the formation of colonies of 1-2 mm. Then, a colony was transferred to a bottle with 50 mL of liquid medium (50 g L^{-1} sucrose, 7 g L^{-1} yeast extract, 2.5 g L^{-1} K_2HPO_4, 1.6 g L^{-1} $(NH_4)_2SO_4$, 1 g L^{-1} $MgSO_4 \cdot 7H_2O$). The bottle was capped with a rubber stopper and gaseous N_2 was injected in the headspace for 5 min. The bottles were

incubated at 30 °C in an oven until cell density reached $1 \cdot 10^8$ cells mL^{-1} (approximately 24 h). Cell density was determined with a Bürker counting chamber (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany).

2.2.3. Alcoholic fermentation

The hydrolysate of AP1 was used directly for alcoholic fermentation, without filtration, centrifugation, sterilization or nutrient addition. Its pH was adjusted to 5.0 with a concentrated NaOH aqueous solution 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 of hydrolysate of AP1 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 of hydrolysate of AP1 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 hydrolysate of AP1 (82 g L^{-1} glucose, 70 g L^{-1} xylose), and supplemented with nutrients and salts (20 g L^{-1} yeast extract and 2.69 g L^{-1} KH_2PO_4 for yeasts; and 7 g L^{-1} yeast extract, 2.5 g L^{-1} K_2HPO_4, 1.6 g L^{-1} $(NH_4)_2SO_4$ and 1 g L^{-1} $MgSO_4 \cdot 7H_2O$ for bacteria). All samples and controls were fermented in triplicate in an orbital shaker at 30 °C and 150 rpm during 72 h. Bioethanol fermentation yields ($Y_{E/S}$) and productivities ($W_{E/S}$) were calculated as reported by Hijosa-Valsero et al. [6], based on total sugar consumption.

2.3. **Biochemical methane potential (BMP) experiments**

The biochemical methane potential (BMP) of the different substrates was carried out in bottles with a total volume of 0.57 L. For substrate AP1, BMPs of AP1, AP1-B and AP1-E were determined. In the case of AP2, the substrate was evaluated as a fresh

substrate (AP2-fresh), a dry substrate (AP2-dried), and a dry and ground substrate (AP2-dried powder). The ratio substrate (So) to inoculum (Xo) was 1, expressed as g VS g^{-1} VS. This ratio 1 was chosen to study the maximum biochemical methane potential that can be obtained from the substrates, while ensuring a stable process [20]. Anaerobic sludge was used as AD inoculum and it presented TS and VS concentrations of 21.1 ± 0.0 and 11.1 ± 0.4 g L^{-1}, respectively. Chemical characteristics of the substrates are shown in Tables 1 and 2. In every bottle, water up to a final amount of 0.30 L of liquid mixture was added, thus allowing a headspace for the gas of approximately 0.27 L. For the determination of endogenous methane production, blanks containing only AD inoculum were run. The BMP assays were run in triplicate using the method described by Molinuevo-Salces et al. [21]. After the set-up of each bottle, the headspace was flushed with N_2 in order to ensure anaerobic conditions. Then, the bottles were placed in an incubator at 36 ± 1 ºC and continuous agitation was provided by a shaker. The incubation time was 37 days. The volume of biogas produced by the different substrates was calculated by measuring the pressure of the bottle's headspace. Biogas composition was analyzed once per week. Specific methane yield, expressed as mL of CH_4 per gram of VS added, was calculated.

2.4. **Anaerobic co-digestion of AP and swine manure: central composite design and data analyses**

A central composite design (CCD) was carried out to study the anaerobic co-digestion of AP2 and swine manure (SM). In order to facilitate the performance of the different mixtures, apple pomace corresponding to the sample AP2-dried powder was chosen. Two factors, namely the substrate concentration (SC), based on VS, and the proportion of AP (% AP) in the co-digestion mixture, based on VS, were selected for the

experimental design. The selected range for factor 1 (SC) was from 2.5 to 49.5 g L^{-1} VS. The selected range for factor 2 (% AP) was between 0 and 100%. All the treatments were carried out in triplicates except for the central point (T9) which was repeated 6 times in order to estimate the experimental error. Batch bottles were prepared as previously explained in Section 2.3. In this case, the experiments lasted for 92 days.

Response surface methodology was used to fit the experimental data into a second-order polynomial equation. Two experimental responses were selected, namely VS removal and specific methane yield. The following equation describes the influence of the two selected factors over the responses:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{12} X_1 X_2$$

where Y is the predicted response value, namely VS removal or specific methane yield. β_0, β_1, β_2, β_{11}, β_{22} y β_{12} are the regression coefficients. X_1 and X_2 are the evaluated factors (SC and % AP).

Excel was used to obtain the regression coefficients from the data set. The determination coefficient (R^2) was calculated to assess the quality of the fit of the polynomial model equation. The impact of the regression coefficients on the predicted response was determined by *p*-values and significant model terms were indicated by *p*-values lower than 0.05.

2.5. **Chemical analyses**

Glucan (sum of cellulose and starch), hemicellulose, Klason lignin, proteins, fats and total phenolic compounds in dry AP samples were analyzed as described by Hijosa-Valsero et al. [6]. To determine galacturonic acid content, dry AP samples were submitted to a two-stage sulfuric acid hydrolysis procedure and were analyzed using an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with an Aminex HPX-87H (Biorad, Hercules, CA, USA) and a Refractive Index Detector (RID) G1362A (Agilent Technologies), according to NREL [22]. Regarding ethanol and ABE hydrolysis and fermentation, aqueous samples of hydrolysates and fermented broths were centrifuged, filtered and analyzed according to Hijosa-Valsero et al. [6] 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), furfural and total phenolic compounds) and fermentation products (acetone, butanol, ethanol, acetic acid and butyric acid).

Analyses of moisture, total solids (TS), VS, ash, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total ammonia nitrogen (TAN) and total Kjeldahl nitrogen (TKN) were performed in duplicate in accordance with APHA [23]. In the case of the AP1-E, AP1-B and AP2-fresh samples and to avoid overestimation of the specific methane yield, TS and VS measurements were corrected by adding the dry-oven losses of volatile organic compounds to the standard dry matter determination. In this way, 89.2% of the total volatile fatty acids (TVFA), 37.5% of the lactic acid, and 100% of the ethanol present in those samples were added to the experimentally obtained TS-VS concentrations [24]. Duplicate samples of 30 g of

sample were added to 150 mL of water in a closed flask. The resultant liquids after 19 h at 4 ºC were used for TVFA, lactic acid and ethanol determination.

Biogas composition was analyzed using a gas chromatograph (Agilent 7890A, USA) with a thermal conductivity detector, provided by a HP-Plot column (30 m 0.53 mm 40 µm) followed by a HP-Molesieve column (30 m 0.53 mm 50 µm). Helium (7 mL min^{-1}) was used as the carrier gas. The injection port temperature was set at 250 ºC and the detector temperature was 200 ºC. The temperature of the oven was set at 40 ºC for 4 min and thereafter increased to 115 ºC. Methane values were expressed at normal conditions (i.e. 0 ºC and 1 atm). The concentrations of acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate and caproate were determined by using a gas chromatograph (Agilent 7890A, USA) equipped with a Teknokroma TRB-FFAP column of 30 m length and 0.25 mm i.d. followed by a flame ionization detector (FID). The carrier gas was helium (1 mL min^{-1}). The temperature of the detector and the injector was 280 ºC. The temperature of the oven was set at 100 ºC for 4 min, then increased to 155 ºC for 2 min and thereafter increased to 210 ºC. TVFA were calculated as the sum of those acids. Ethanol and lactic acid concentrations were determined by a HPLC (Agilent 1200, USA) equipped with an AMINEX HPX-87H column of 300 mm length, 7.8 mm i.d. followed by a refractive index detector (RID) and a diode-array detector (DAD). The mobile phase was sulfuric acid 4 mM (0.6 mL min^{-1}). The temperature of the column and the RID were 65 and 30 ºC, respectively.

3. **Results and Discussion**

3.1. **Bioethanol production**

The hydrolysate of AP1 employed in alcoholic fermentations contained 153 ± 4 g L^{-1} total sugars (3.7 ± 2 g L^{-1} cellobiose-maltose, 81.5 ± 1.8 g L^{-1} glucose, 53 ± 1.8 g L^{-1} xylose-mannose-galactose, 3.3 ± 0.1 g L^{-1} rhamnose, 12 ± 0.4 g L^{-1} arabinose), 0.3 ± 0.0 g L^{-1} formic acid, 2.9 ± 0.1 g L^{-1} acetic acid and 0.35 ± 0.0 g L^{-1} 5-HMF. Bioethanol production from the hydrolysate of AP1 by the different strains tested is shown in Table 3. In general, all *Kluyveromyces* and *Lachancea* strains obtained bioethanol concentrations between 49.9 and 51.5 g L^{-1}. The performance of *S. cerevisiae* strains was more variable, with ethanol values ranging from 25.7 to 51.0 g L^{-1}. On the other hand, the strains of *Z. mobilis* and *S. stipitis* were unsuccessful for the fermentation of hydrolysate of AP1 (Table 3). There were no significant differences ($p < 0.05$) for bioethanol concentrations among the six best-performing strains (DSM 70799, DSM 5418, DSM 5422, DSM 7239, DSM 3434 and Ethanol Red®). Total sugar consumption was 74-84% for all the strains that produced more than 40 g L^{-1} bioethanol, with bioethanol yields of 0.371-0.444 g g^{-1}. The strain *S. cerevisiae* Ethanol Red® exhibited the highest sugar consumption among all strains ($p < 0.05$). The performance of control fermentations (with synthetic solutions) was remarkably lower, with bioethanol concentrations ranging from 2.7 g L^{-1} (*Z. mobilis* DSM 3580) to 29.9 g L^{-1} (*S. stipitis* DSM 3651).

The highest bioethanol concentrations obtained in the present work (~50 g L^{-1}) are similar to those reported for *S. cerevisiae* ATCC 4124 (Magyar et al., 2016) and for a mixed culture of *Z. mobilis* MTCC 92 and *Candida tropicalis* TERI SH 110 (Patle and Lal, 2007) with apple pomaces containing 116-122 g L^{-1} total sugars. Although nutrient supplementation is not necessary for AP fermentation [7, 25], it is important to have high initial sugar concentrations in the broth in order to guarantee an economically

efficient fermentation, which is usually achieved by increasing the solid-to-solvent ratio during the pretreatment and enzymatic hydrolysis above 15% [26]. Therefore, low initial sugar concentrations imply low bioethanol production. For instance, Nogueira et al. [27] fermented an extract of apple pomace containing 76.2 g L^{-1} total sugars and obtained ~34 g L^{-1} bioethanol with *S. cerevisiae* Uvaferm CK. For an initial sugar concentration of 10-20 g L^{-1} in an AP acid hydrolysate, Ucuncu et al. [28] obtained only 1.67 g L^{-1} bioethanol with *Trichoderma harzianum* NRRL 31396.

According to literature, all the tested strains are able to ferment glucose and galactose into bioethanol; except *Z. mobilis*, which cannot ferment galactose. *S. cerevisiae*, *K. marxianus* and *S. stipitis* can ferment mannose. Only *K. marxianus* and *S. stipitis* can ferment xylose, and *K. lactis* var. *lactis* has been reported to assimilate xylose. *S. cerevisiae* and *S. stipitis* can assimilate rhamnose, whereas *K. marxianus* and *S. stipitis* can assimilate L-arabinose. Regarding disaccharides, *S. stipitis* can ferment cellobiose and maltose, whereas *S. cerevisiae, K. lactis* var. *lactis* and *L. thermotolerans* can ferment maltose. *K. lactis* var. *lactis* and *K. marxianus* can also ferment lactose, but this sugar is not present in the hydrolysate of AP1 [8, 29, 30, 31, 32, 33, 34]. In the present work, differences were observed in sugar consumption between control samples and AP1 hydrolysate samples, which could be associated with the different composition of both media. In fact, fermentation results indicate that *K. marxianus, L. thermotolerans, K. lactis* var. *lactis* and certain strains of *S. cerevisiae* were the most adequate for AP fermentation (Table 3). This could be related to their tolerance to fermentation inhibitors. Actually, the initial concentration of acetic acid in AP1 hydrolysates was 2.93 ± 0.12 g L^{-1}. This may have affected *S. stipitis*, whose bioethanol production was among the highest ones in control fermentations. Bellido et al. [35] reported that growth

and bioethanol production were totally inhibited in *S. stipitis* DSM 3651 when acetic acid concentration was 3.5 g L^{-1}, whereas a concentration of 2.5 g L^{-1} caused an inhibition of 60%. Regarding the poor performance of *Z. mobilis* for the fermentation of hydrolysate of AP1, it might be related to its narrow sugar utilisation (it only assimilates glucose, fructose and sucrose), because this species is known for its high tolerance to fermentation inhibitors [36].

3.2. **Biochemical methane potential**

Six different triplicate experiments were carried out. Experiments 1 to 3 corresponded to BMPs for apple pomace from juice extraction (AP1) and the correspondent exhausted broths from bioethanol (AP1-E) and biobutanol (AP1-B) productions (Fig. 1). Specific methane yield of the AP1 sample was 258 mL CH_4 g^{-1} VS added . Specific methane yields for AP1-E and AP1-B were 463 and 290 mL CH_4 g^{-1} VS added , respectively. Specific methane yield from AP1-E was 1.8-fold higher than that obtained from the original substrate (AP1), indicating that the process carried out for bioethanol production worked as a pretreatment for the original biomass, enhancing methane potential of the original substrate. The exhausted fermentation broth after bioethanol production (AP1-E) was characterized by a high COD and simple sugars (i.e. xylose, galactose, arabinose, etc.) concentrations (Table 2). Previous studies have reported COD values in the range of 30 to 154 g L^{-1} for a variety of stillages from the fermentation of different biomasses [37]. Methane yields of 249 and 401-458 mL CH_4 g^{-1} VS added have been reported for anaerobic digestion of the exhausted broths after cassava and corn fermentations, respectively [38, 39]. The specific methane potential obtained from AP1-E was in the same range, probably due to the similar composition between those biomasses. The exhausted fermentation broth after biobutanol production (AP1-B)

resulted in a specific methane yield of 290 mL CH_4 g^{-1} VS added, which was in the range of the original AP1. To the best of our knowledge, this is the first time that ABE distillation wastes are used as feedstock for anaerobic digestion.

Experiments 4 to 6 corresponded to BMPs for apple pomace obtained after apples pressing for cider production (AP2) (Fig. 2). Final methane yields in the range of 204-254 mL CH_4 g^{-1} VS added were obtained for AP2. These values are in accordance to those reported by Kafle and Kim. [18] for the batch AD of apple pomace. No significant difference in specific methane yield was found between the dry (AP2-dried) and the dried and ground (AP2-dried powder) samples. On the contrary, significant differences in the specific methane yield between these two samples and the fresh one (AP2-fresh) were obtained ($p > 0.05$). It is noteworthy that a correction in the VS content of AP2-fresh was performed to avoid overestimations on the specific methane yield [24]. In spite of the VS correction, specific methane yield for AP-fresh was 1.25 and 1.15 times higher than for AP2-dried and AP2-dried powder, respectively. This finding indicates that to obtain the maximum methane yield of this by-product, preservation methods must minimize the loss of volatile organic compounds. The dried and ground sample was utilized for investigating co-digestion of AP and swine manure.

3.3. **Co-digestion of AP and swine manure (SM)**

Anaerobic co-digestion of AP2-dried powder and SM was investigated following a central composite design (CCD). The coded and actual values corresponding to the studied factors (concentration of substrate (SC) and proportion of AP (% AP)) and responses (i.e. VS removal and specific methane yield) from the batch tests are presented in Table 4.

3.3.1. VS Removal

The regression analysis for the co-digestion of AP and SM resulted in Eq. (1) for the response VS removal:

$$Y_{VS} = 57.3 - 5.3\,(SC) + 4.8\,(\%\,AP) - 8.2\,(SC)^2 - 3.9\,(\%\,AP)^2 + 2.8\,(SC)\,(\%\,AP) \quad \text{Eq. (1)}$$

The response model presented an adjusted R^2 coefficient of 0.8790, which means that the assessed factors and their interactions are able to explain 88% of the data variability found in the response VS removal. Regression results show a statistically significant model, since the actual F-value (25.69) is higher than the calculated one (5.1×10^{-6}). As it can be observed in Table 5, both factors presented a significant effect on VS removal. Moreover, the quadratic term of both factors (*b11*, *b22*) and the interaction term between the two studied factors (*b12*) also presented a significant effect on VS removal. VS removal percentages were in the range of 29.7 to 57.3%. The resulting surface plot indicates that VS removal increases with a substrate concentration increase (Fig. 3A). The percentage of AP contributes to VS removal. These results fit well with those obtained by Molinuevo-Salces et al. [40], who found that VS removal increased with the increase in the proportion of vegetable by-products to AD of swine manure. The higher biodegradability of vegetable by-products in comparison to swine manure is the responsible of this increase in VS removal. However, VS removals in this study were lower than those obtained by Molinuevo-Salces et al. [40], who obtained values in the range of 82% VS removal.

3.3.2. *Specific methane yield*

The regression analysis for the co-digestion of AP and SM resulted in Eq. (2) for the specific methane yield:

$$Y_{CH4} = 433.1 - 20.1\,(SC) - 123.6\,(\%\,AP) - 16.2\,(SC)^2 - 15.8\,(\%\,AP)^2 + 20.8\,(SC)\,(\%\,AP) \quad \text{Eq. (2)}$$

The response model presented an adjusted R^2 coefficient of 0.9408, which means that the assessed factors and their interactions are able to explain 94% of the data variability found in the response specific methane yield. Regression results show a statistically significant model, since the actual F-value (67.79) is higher than the calculated one (3.4×10^{-10}). P-values were lower than 0.05 for both studied factors (Table 5), indicating that both of them have a significant influence on specific methane production. The highest specific methane yield was 596 mL CH_4 g^{-1} VS, with a SC of 9.38 g VS L^{-1} and an AP percentage of 14.6 % (T2).

Fig. 4 shows accumulated methane yield for T1 to T9. T1 and T3 contained 85.36 % of AP while T2 and T4 contained 14.64 % of AP (Table 4) but in both pairs, the higher SC, the longer lag-phase for methane production. More specifically, methane production stops after 10 days for treatments with low SC; these are T1, T2 and T7 with 9.4, 9.4 and 2.5 g VS L^{-1}, respectively. In T9 (SC of 26 g VS L^{-1}) the production stops after 15 days. The other treatments present a lag-phase between 15-35 days to start producing methane. T3 presents the longest lag-phase of about 35 days. The mixture in T3 contained a high SC (42.62 g SV L^{-1}) and a high AP percentage (85.36 %), which

could have led to TVFA accumulation. Once TVFA were consumed, methane production started.

On the other hand, if treatments with a constant value for SC (i.e. T5, T6 and T9) are compared, it is seen that an increase in AP content resulted in a decrease in specific methane yield (Fig. 4). In this vein, specific methane yield for T5 (100 % AP) is 215 mL CH_4 g^{-1} VS while specific methane yield for swine manure (T6) is 567 mL CH_4 g^{-1} VS. Kafle and Kim [18] obtained similar values when anaerobically digesting apple by-products (i.e. 267 mL CH_4 g^{-1} VS).

3.3.3. AD stability

pH values at the end of the experiment were between 7.0 and 7.8. TVFA concentration at the end of the experiment were low, indicating that anaerobic microorganisms successfully converted organic matter into methane. Moreover, high ammonium concentrations could lead to process failure due to ammonia-mediated inhibition of the AD microorganisms activity. The inhibitory concentrations are between 4 and 10 g TAN L^{-1}. Ammonium concentrations at the end of the experiments were below those inhibitory levels, so that no ammonia-mediated inhibition was expected.

4. **Conclusions**

Bioethanol and methane can be successfully produced from apple pomaces (AP) following a biorefinery approach. As a first step, alcoholic fermentation could be used as a method to obtain bioethanol as well as to pretreat AP, enhancing methane production by anaerobic digestion. Bioethanol concentrations about 50 g L^{-1} were obtained by different strains of *K. marxianus*, *K. lactis*, *L. thermotolerants* and *S. cerevisiae* with yields of 0.371-0.444 g g^{-1}. Specific methane yield of the exhausted

broths after bioethanol and biobutanol production were 463 and 290 mL CH_4 g^{-1} VS added, respectively. Specific methane yield of the exhausted broth after bioethanol production was 1.8 higher than that of the original AP (i.e. 258 mL CH_4 g^{-1} VS added). Anaerobic co-digestion of manure and AP is favoured at low substrate concentrations and low AP content in the mixtures. The highest methane yield (596 mL CH_4 g^{-1} VS added) was obtained with a substrate concentration of 9 g L^{-1} SV and an AP content in the mixture of 15%.

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Figure captions

Figure 1. Accumulated methane yields for AP1, AP1-E and AP-B. Data are means of triplicate experiments.

Figure 2. Accumulated methane yields for AP2 samples. Data are means of triplicate experiments.

Figure 3. Surface response plot for VS removal response (A) and specific methane yield response (B).

Figure 4. Accumulated specific methane yields for T1-T9. Data are means of duplicate experiments, exception made for T6, with six replicate experiments.

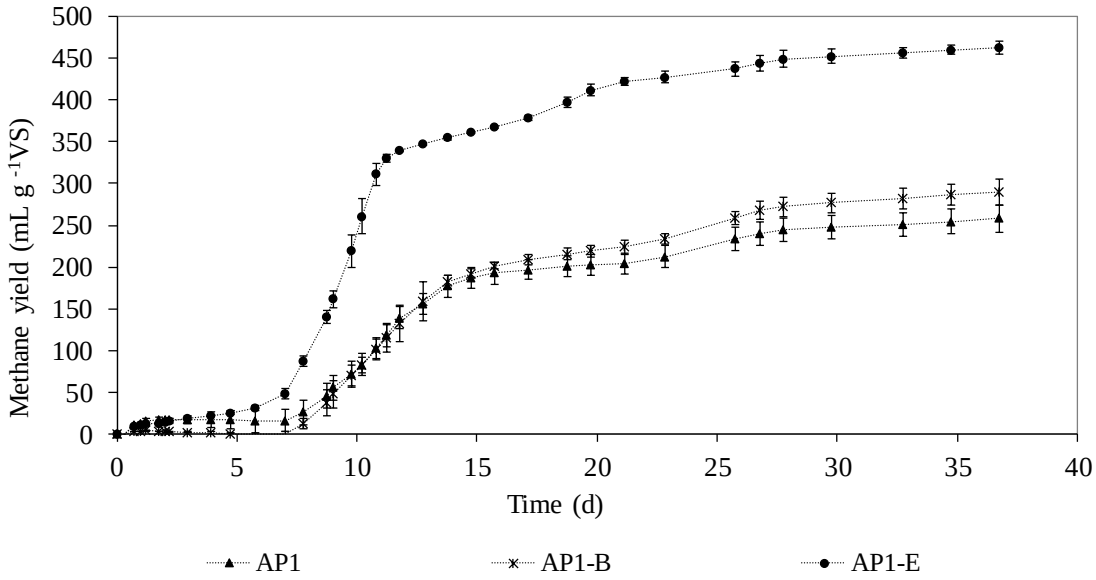
Methane yield (mL g -1VS)
500
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
Time (d)
AP1
AP1-B
AP1-E

Figure 1.

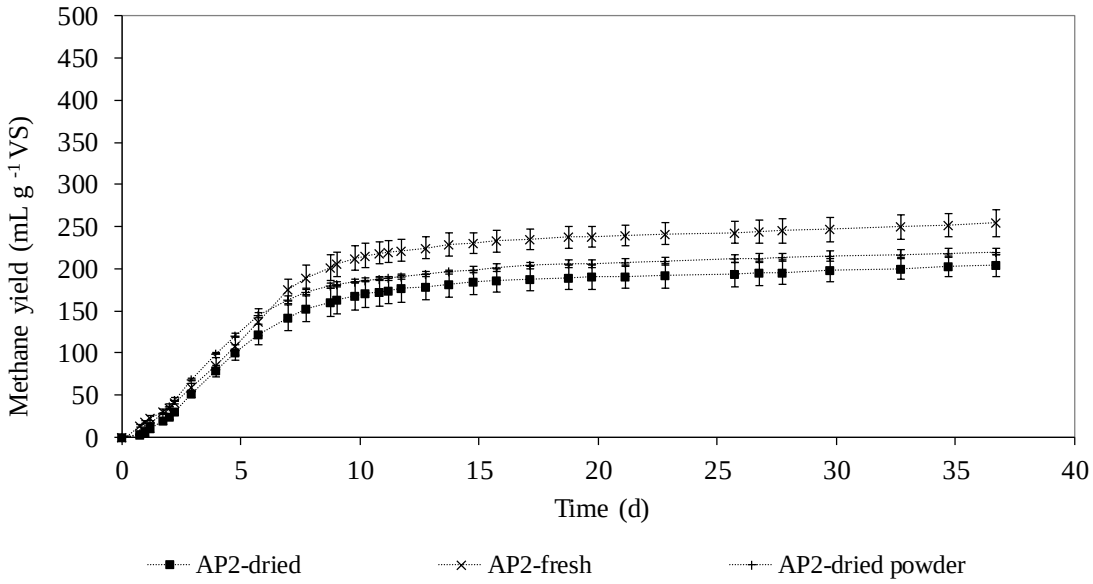
Methane yield (mL g -1 VS)
500
450
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
Time (d)
AP2-dried
AP2-fresh
AP2-dried powder

Figure 2.

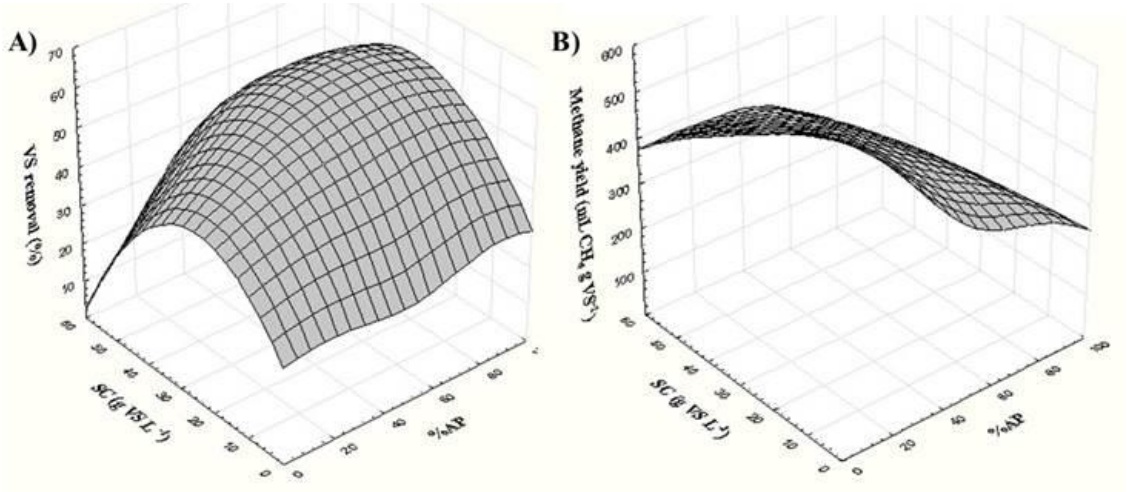
A)
VS removal (%)
SC (g VS L-1)
%AP
B)
Methane yield (mL CH4 g VS-1)
SC (g VS L-1)
%AP

Figure 3.

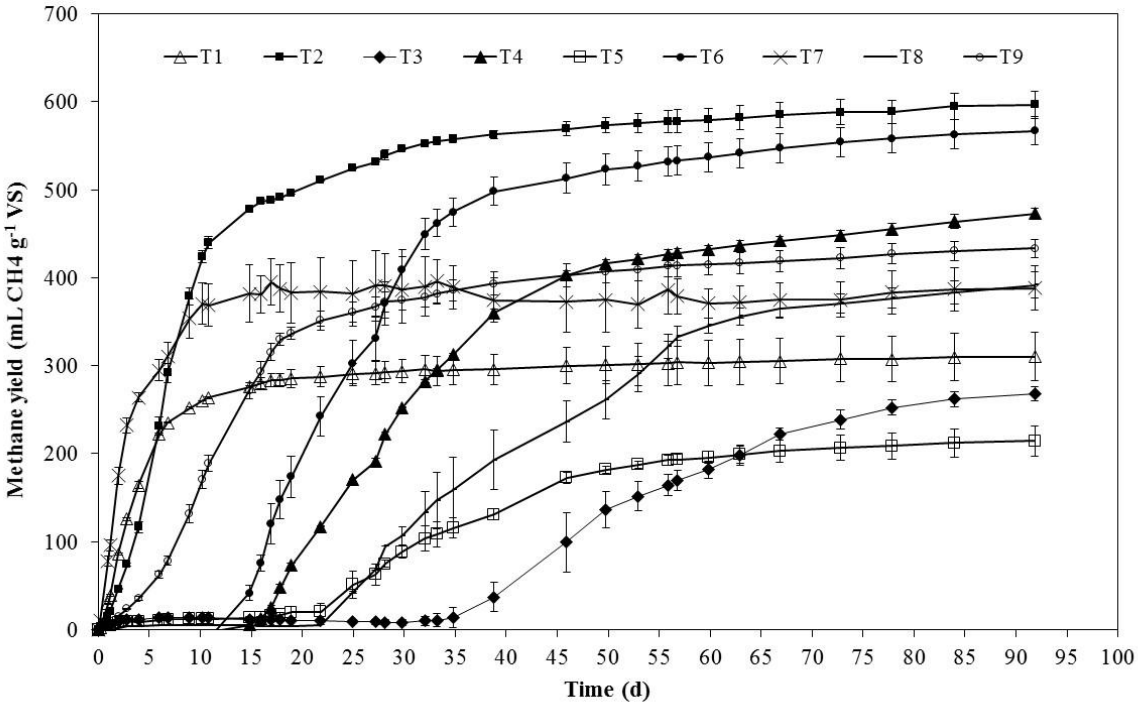
T1
T2
T3
T4
T5
T6
T7
T8
T9
Methane yield (mL CH4 g-1 VS)
Time (d)
0
100
200
300
400
500
600
700
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

Figure 4.

Table 1. Composition of the apple pomaces (AP1 and AP2). Average and standard deviations are shown for TS and VS.

	Units	AP1	AP2
TS	g kg^{-1}	865.6 ± 51.3	923.1 ± 2.4
VS	g kg^{-1}	720.2 ± 56.6	910.3 ± 2.2
Total carbohydrates	% TS	59.78	50.54
Soluble carbohydrates	% TS	16.64	0.16
Glucan	% TS	22.71	29.02
Hemicellulose	% TS	15.79	16.14
Galacturonic acid	% TS	5.47	8.00
Klason lignin	% TS	19.80	21.47
Protein	% TS	5.21	5.71
Fat	% TS	1.52	2.49
Total phenolic compounds	mg g^{-1}	3.5	2.2

Table 2. Composition of the exhausted fermentation broths after solvent recovery by gas stripping (AP1-E and AP1-B), which were used for AD. n.d. stands for not determined. Average and standard deviations are shown.

	Units	AP1-E	AP1-B
TS	g L^{-1}	214.7 ± 5.9	88.8 ± 4.0
VS	g L^{-1}	133.2 ± 5.1	52.5 ± 2.3
CODt	g L^{-1}	296.2 ± 25.7	95.4 ± 0.9
CODs	g L^{-1}	167.5 ± 4.5	85.1 ± 1.7
TVFA	g L^{-1}	6.2 ± 0.2	11.4 ± 0.3
Cellobiose + Maltose	g L^{-1}	<0.05	<0.05
Glucose	g L^{-1}	0.05	6.41
Xylose + Galactose + Mannose	g L^{-1}	12.37	0.53
Rhamnose	g L^{-1}	7.02	1.86
Arabinose	g L^{-1}	15.62	0.71
Acetone	g L^{-1}	n. d.	0.63
Butanol	g L^{-1}	n. d.	1.5
Ethanol	g L^{-1}	3.6	0.12
Acetic acid	g L^{-1}	n. d.	3.75
Butyric acid	g L^{-1}	n. d.	3.91

Table 3. Bioethanol fermentation parameters for the different strains with hydrolysate of AP1. Averages and standard deviations are shown.

Species	Strain	Bioethanol (g L^{-1})	Acetic acid (g L^{-1})	Total sugar consumption (%)	$Y_{E/S}$ (g g^{-1})	W_E (g L^{-1} h^{-1})
Kluyveromyces lactis	DSM 70799	49.9 ± 0.5	3.77 ± 0.10	80.0 ± 0.5	0.402 ± 0.006	0.694 ± 0.007
Kluyveromyces marxianus	DSM 5418	50.5 ± 0.6	6.73 ± 0.09	78.8 ± 0.4	0.412 ± 0.003	0.701 ± 0.008
Kluyveromyces marxianus	DSM 5422	50.1 ± 0.8	4.79 ± 0.21	78.6 ± 0.2	0.410 ± 0.005	0.695 ± 0.011
Kluyveromyces marxianus	DSM 7239	49.9 ± 0.1	5.40 ± 0.15	78.7 ± 0.5	0.408 ± 0.003	0.693 ± 0.002
Lachancea thermotolerans	DSM 3434	51.5 ± 0.4	3.78 ± 0.23	74.5 ± 0.9	0.444 ± 0.009	0.715 ± 0.005
Saccharomyces cerevisiae	CECT 1383	46.5 ± 0.5	3.51 ± 0.04	79.9 ± 1.4	0.374 ± 0.003	0.646 ± 0.007
Saccharomyces cerevisiae	DSM 70449	25.7 ± 10.5	2.10 ± 0.75	37.9 ± 13.5	0.463 ± 0.025	0.357 ± 0.146
Saccharomyces cerevisiae	Ethanol Red®	51.0 ± 1.0	4.05 ± 0.11	84.0 ± 0.3	0.398 ± 0.009	0.708 ± 0.014
Saccharomyces cerevisiae	Hércules-green	44.5 ± 0.8	3.60 ± 0.05	77.3 ± 3.6	0.371 ± 0.012	0.619 ± 0.012
Scheffersomyces stipitis	DSM 3651	0.0 ± 0.0	3.03 ± 0.16	0.0 ± 0.0	0.000 ± 0.000	0.000 ± 0.000
Scheffersomyces stipitis	DSM 3652	0.2 ± 0.05	3.13 ± 0.17	0.4 ± 1.2	0.391 ± 0.776	0.003 ± 0.001
Zymomonas mobilis	DSM 3580	7.8 ± 4.7	2.60 ± 1.47	37.3 ± 35.8	0.342 ± 0.370	0.108 ± 0.065

Table 4. Coded values, actual values and response values for the co-digestion of AP and SM. Averages and standard deviations are shown.

	Coded values		Actual values		Responses	
	SC (g VS L^{-1})	% AP	SC (g VS L^{-1})	% AP	VS removal (%)	Specific methane yield (mL CH_4 g^{-1} VS added)
Treatments						
T1	-1	1	9.38	85.36	45.3 ± 0.7	310.3 ± 27.5
T2	-1	-1	9.38	14.64	39.7 ± 0.5	596.4 ± 15.4
T3	1	1	42.62	85.36	57.0 ± 0.5	268.0 ± 8.5
T4	1	-1	42.62	14.64	40.1 ± 1.5	472.8 ± 5.8
T5	0	1.4142	26.00	100.00	54.7 ± 2.2	214.6 ± 17.2
T6	0	-1.4142	26.00	0.00	43.6 ± 0.5	566.8 ± 16.1
T7	-1.4142	0	2.50	50.00	29.7 ± 0.1	388.2 ± 25.1
T8	1.4142	0	49.50	50.00	51.3 ± 3.6	391.6 ± 15.3
T9	0	0	26.00	50.00	57.3 ± 0.9	433.1 ± 10.4

Table 5. Regression results for the two studied responses in the co-digestion of AP and SM.

	VS removal		Specific methane yield	
	Coefficient	Prob	Coefficient	Prob
β0	57.3	<0.001	433.1	<0.001
β1	- 5.3	<0.001	-20.1	0.01
β2	4.8	<0.001	-123.6	<0.001
β11	-8.2	<0.001	-16.2	0.066
β22	-3.9	0.011	-15.8	0.072
β12	2.8	0.027	20.8	0.054
	R^2= 0.9146, Adj. R^2= 0.8790, r= 0.9563 F value= 25.69 Prob>F= $5.1x10^{-6}$		R^2= 0.9549, Adj. R^2= 0.9408, r= 0.9772 F value= 67.79, Prob>F= $3.4x10^{-10}$	

R^2, determination coefficient; Adj. R^2, adjusted determination coefficient; r, regression coefficient; F value.

Supplementary material

Table S. 1. Bioethanol fermentation parameters for the different strains with control solutions (82 g L^{-1} glucose, 70 g L^{-1} xylose). Averages and standard deviations are shown.

Species	Strain	Ethanol (g L^{-1})	Acetic acid (g L^{-1})	Total sugar consumption (%)	$Y_{E/S}$ (g g^{-1})	W_E (g L^{-1} h^{-1})
Kluyveromyces lactis	DSM 70799	27.7 ± 0.2	0.92 ± 0.06	66.8 ± 0.4	0.313 ± 0.004	0.384 ± 0.003
Kluyveromyces marxianus	DSM 5418	23.9 ± 0.2	5.69 ± 0.09	67.8 ± 0.4	0.267 ± 0.001	0.332 ± 0.003
Kluyveromyces marxianus	DSM 5422	26.5 ± 0.0	2.86 ± 0.03	66.6 ± 0.0	0.301 ± 0.000	0.368 ± 0.000
Kluyveromyces marxianus	DSM 7239	24.1 ± 0.0	5.63 ± 0.16	67.7 ± 0.2	0.269 ± 0.000	0.334 ± 0.000
Kluyveromyces thermotolerans	DSM 3434	29.7 ± 0.1	0.82 ± 0.10	65.4 ± 0.2	0.343 ± 0.000	0.412 ± 0.001
Saccharomyces cerevisiae	CECT 1383	23.0 ± 0.1	5.49 ± 0.11	66.4 ± 0.2	0.262 ± 0.000	0.319 ± 0.001
Saccharomyces cerevisiae	DSM 70449	27.4 ± 0.4	1.33 ± 0.04	98.9 ± 0.1	0.193 ± 0.003	0.380 ± 0.005
Saccharomyces cerevisiae	Ethanol Red ®	24.0 ± 0.6	2.20 ± 0.15	62.7 ± 0.4	0.309 ± 0.008	0.333 ± 0.008
Saccharomyces cerevisiae	Hércules-green	25.2 ± 0.0	5.45 ± 0.14	65.3 ± 0.1	0.293 ± 0.000	0.351 ± 0.000
Scheffersomyces stipitis	DSM 3651	29.9 ± 0.2	0.50*	54.2 ± 0.2	0.402 ± 0.002	0.415 ± 0.002
Scheffersomyces stipitis	DSM 3652	27.7 ± 0.2	0.57*	56.9 ± 0.2	0.355 ± 0.002	0.385 ± 0.002
Zymomonas mobilis	DSM 3580	2.7 ± 0.1	0.27 ± 0.00	9.1 ± 0.4	0.197 ± 0.003	0.038 ± 0.001

*Acetic acid values correspond to a single measure.

Table S. 2. Sugar consumption (%) during bioethanol fermentation for the different strains. Note: In the case of AP1 samples, the low initial concentrations of cellobiose-maltose, rhamnose and arabinose may have provoked some incertitude in consumption values. Average and standard deviations are shown.

		Control		Apple pomace hydrolysate of AP1				
Species	Strain	Glucose	Xylose	Cellobiose + Maltose	Glucose	Xylose + Mannose + Galactose	Rhamnose	Arabinose
Kluyveromyces lactis	DSM 70799	100 ± 0	25.4 ± 0.8	88.1 ± 1.1	99.8 ± 0.1	78.5 ± 0.9	0 ± 0	0 ± 0
Kluyveromyces marxianus	DSM 5418	100 ± 0	27.6 ± 0.8	86.7 ± 0.7	99.9 ± 0.0	79.6 ± 0.4	0 ± 0	0 ± 0
Kluyveromyces marxianus	DSM 5422	100 ± 0	25.0 ± 0.0	89.0 ± 0.4	100 ± 0	79.1 ± 0.2	0 ± 0	0 ± 0
Kluyveromyces marxianus	DSM 7239	99.9 ± 0.1	27.8 ± 0.9	88.9 ± 1.2	99.9 ± 0.0	79.3 ± 0.5	0 ± 0	0 ± 0
Lachancea thermotolerans	DSM 3434	99.4 ± 0.0	26.0 ± 0.8	88.6 ± 1.2	99.7 ± 0.2	74.1 ± 1.1	0 ± 0	0 ± 0
Saccharomyces cerevisiae	CECT 1383	99.7 ± 0.0	24.8 ± 0.4	88.5 ± 0.6	100 ± 0	80.2 ± 1.6	0 ± 0	0 ± 0
Saccharomyces cerevisiae	DSM 70449	97.9 ± 0.2	20.9 ± 0.2	0 ± 0	67.0 ± 17.7	20.3 ± 12.6	0 ± 0	0 ± 0
Saccharomyces cerevisiae	Ethanol Red ®	99.9 ± 0.1	16.0 ± 1.0	59.2 ± 2.5	100 ± 0	95.9 ± 0.3	0 ± 0	0 ± 0

Saccharomyces cerevisiae	Hércules-green	100 ± 0	22.0 ± 0.2	76.2 ± 18.9	99.8 ± 0.2	75.0 ± 5.8	0 ± 0	0 ± 0
Scheffersomyces stipitis	DSM 3651	100 ± 0	50.6 ± 69.9	8.0 ± 5.3	0 ± 0	0 ± 0	100 ± 0	0 ± 0
Scheffersomyces stipitis	DSM 3652	100 ± 0	54.6 ± 64.2	12.7 ± 5.4	0 ± 0	0 ± 0	100 ± 0	0 ± 0
Zymomonas mobilis	DSM 3580	12.3 ± 0.6	5.3 ± 0.2	0 ± 0	42.9 ± 32.4	36.7 ± 36.3	0 ± 0	31.6 ± 39.3

Table S. 3. pH, TKN, TAN, TVFA at the end of the AD experiments. Average and standard deviations are shown.

Treatment	pH	TKN (mg L^{-1})	TAN (mg L^{-1})	TVFA (mg L^{-1})
AP1	7.16 ± 0.04	1108 ± 76	531 ± 4	42 ± 2
AP1-E	7.45 ± 0.02	1249 ± 40	621 ± 9	117 ± 7
AP1-B	7.26 ± 0.01	1449 ± 6	812 ± 8	111 ± 21
AP2 - fresh	7.01 ± 0.01	1054 ± 0	517 ± 13	33 ± 6
AP2 - dried	7.04 ± 0.02	1059 ± 21	538 ± 0	70 ± 3
AP2 –dried and ground	7.08 ± 0.02	1067 ± 23	544 ± 9	150 ± 17

Table S. 4. pH, TS, VS, TKN, TAN, TVFA at the end of T1-T9. Average and standard deviations are shown.

Treatment	pH	TS (g L^{-1})	VS (g L^{-1})	TKN (mg L^{-1})	TAN (mg L^{-1})	TVFA (mg L^{-1})
T1	7.12 ± 0.01	11.0 ± 0.1	6.9 ± 0.1	1051 ± 10	636 ± 4	64 ± 28
T2	7.41 ± 0.01	12.5 ± 0.2	7.5 ± 0.1	1634 ± 57	1073 ± 32	58 ± 2
T3	7.29 ± 0.04	16.9 ± 0.3	14.5 ± 0.2	1603 ± 43	944 ± 14	124 ± 14
T4	7.76 ± 0.00	28.3 ± 0.5	17.8 ± 0.5	4017 ± 1	3128 ± 13	93 ± 24
T5	6.98 ± 0.03	13.5 ± 0.7	9.6 ± 0.5	1031 ± 4	429 ± 4	38 ± 2
T6	7.64 ± 0.01	21.3 ± 0.2	12.4 ± 0.1	3136 ± 24	2475 ± 14	62 ± 13
T7	7.29 ± 0.00	9.6 ± 0.1	5.6 ± 0.0	961 ± 90	656 ± 16	14 ± 20
T8	7.67 ± 0.06	26.2 ± 1.7	17.7 ± 1.3	3153 ± 88	2274 ± 28	42 ± 13
T9	7.48 ± 0.00	17.3 ± 0.3	10.6 ± 0.2	2067 ± 81	1522 ± 31	21 ± 11