Waste Management 155 (2023) 320–328

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Potato peels waste as a sustainable source for biotechnological production of biofuels: Process optimization

Beatriz Rodríguez-Martínez [a], Eduardo Coelho [b,c], Beatriz Gullón [a], Remedios Yáñez [d,e,*], Lucília Domingues [b,c]

[a] Universidade de Vigo, Departamento de Enxeñaría Química, Facultade de Ciencias, As Lagoas, 32004 Ourense, Spain
[b] CEB–Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal
[c] LABBELS - Associate Laboratory, Braga/Guimarães, Portugal
[d] Universidade de Vigo, Departamento de Enxeñaría Química, Escola de Enxeñaría Industrial, Campus Lagoas-Marcosende 9, Vigo 36310, Spain
[e] CINBIO, Universidade de Vigo, 36310 Vigo, Spain

ARTICLE INFO

Keywords:
Potato peel waste
Bioethanol
Liquefaction
Enzymatic hydrolysis
PSSF
VHGF

ABSTRACT

Potato peel waste (PPW) is a starchy by-product generated in great amounts during the industrial processing of potatoes. It can be used as a low cost alternative, and renewable feedstock for the production of second generation bioethanol. In order to intensify this process, *Saccharomyces cerevisiae* Ethanol Red®, a robust and thermotolerant yeast strain, was selected and two experimental designs and response surfaces assessment were conducted to enable very high gravity fermentations (VHGF) using PPW as feedstock. The first one focused on the optimization of the liquefaction and enzymatic hydrolysis stages, enabling a maximum ethanol concentration of 116.5 g/L and a yield of 80.4 % at 72 h of fermentation; whereas, the second one, focus on the optimization of the pre-saccharification and fermentation stages, which further increased process productivity, leading to a maximum ethanol concentration of 108.8 g/L and a yield of 75.1 % after 54 h of fermentation.

These results allowed the definition of an intensified pre-saccharification and simultaneous saccharification and fermentation (PSSF) process for ethanol production from PPW, resorting to short liquefaction and pre-saccharification times, 2 h and 10 h respectively, at an enzyme loading of 80 U/g PPW of Viscozyme and 5 UE/g PPW of SAN Super and a higher fermentation temperature of 34 °C due to the use of a thermotolerant yeast. Overall, with these conditions and solely from PPW without any supplementation, the outlined PSSF process allowed reaching a high ethanol concentration and yield (104.1 g/L and 71.9 %, respectively) standing at high productivities with only 54 h of fermentation.

1. Introduction

Nowadays, about 80 % of global energy is produced from fossil fuels such as oil, coal, natural gas, etc. Therefore, they remain the main source of energy in the world (Mazaheri and Pirouzi, 2020; Sheikh et al., 2016; Barampouti et al., 2021). However, these non-renewable fuels are considered an important source of environmental pollution due to the greenhouse gas emissions, which contribute to global warming (Mazaheri and Pirouzi, 2020; Sheikh et al., 2016; Soltaninejad et al., 2022; Barampouti et al., 2021).

In the coming decades, the increase in energy demand will be associated with the world population growth and with the industrialization of developing countries (Sheikh et al., 2016; Ude et al., 2020; Barampouti et al., 2021). On the other hand, the expected significant decline in oil production by the year 2050 (Ude et al., 2020), together with the limitation and availability of fossil energy sources and the energy price increase, stress the need of research efforts in this field to develop renewable and sustainable energy sources (Mazaheri and

Abbreviations: FAO, Food and Agriculture Organization of the United Nations; HPLC, high performance liquid chromatography; LSR, liquid-solid ratio; PE, preliminary experiments; PPW, potato peel waste; PSSF, pre-saccharification and simultaneous saccharification and fermentation; RI, refractive index; RSM, response surface methodology; VHG, very high gravity; VHGF, very high gravity fermentation; VHGF SSF, very high gravity simultaneous saccharification and fermentation.

* Corresponding author at: Universidade de Vigo, Departamento de Enxeñaría Química, Escola de Enxeñaría Industrial, Campus Lagoas-Marcosende 9, Vigo 36310, Spain.
E-mail address: reme@uvigo.es (R. Yáñez).

https://doi.org/10.1016/j.wasman.2022.11.007
Received 22 August 2022; Received in revised form 21 October 2022; Accepted 5 November 2022
Available online 19 November 2022

Pirouzi, 2020; Ude et al., 2020; Barampouti et al., 2021).

Under this premise, biomass-based fuels or biofuels arise as a renewable and sustainable source from zero cost raw materials (Ben Atitallah et al., 2019; Soltaninejad et al., 2022). Among them, bioethanol is by far the most widely used biofuel in the transport sector worldwide. Its use as fuel causes the reduction of carbon emissions up to 80 % and the elimination of acid rain derived from sulphur dioxide (Ben Atitallah et al., 2019; Soltaninejad et al., 2022; Ude et al., 2020). Moreover, bioethanol is considered a starting material for the synthesis of several chemicals such as ethylene, propylene, isobutene, gasoline, acetaldehyde, diethyl ether, acetic acid, acetone o ethyl acetate (Xiang et al., 2022; Zhang et al., 2010). Among the advantages of this biofuel are: high octane rating (thus avoiding the need to use methyl tertiary butyl ether or lead), low carbon monoxide production (reduced thanks to its high oxygen content), easy storage and facility for mixing with gasoline (Ude et al., 2020; Barampouti et al., 2021; Mithra et al., 2018). On the other hand, second generation bioethanol has high operating costs in addition to other production challenges such as the need for biomass pretreatment or the high enzyme costs (Cunha et al., 2020b; Gomes et al., 2021b; Mithra et al., 2018).

An important factor in the sustainable production of second generation bioethanol by the fermentative route is the biomass selection; since it must be zero cost and not compromise food safety. In this context, agricultural residues or industrial by-products are interesting due to their high availability and low cost (Ben Atitallah et al., 2019). Specifically, starchy by-products have gained attention recently as alternative raw materials in fermentative processes because they present high polysaccharide and nutrient contents, in addition of high biodegradability (Chohan et al., 2020).

Potato is the third most important food crop in the world. According to data provided by Food and Agriculture Organization of the United Nations (FAO), its production is continuously growing, reaching almost 390 million tons in 2021 (Barampouti et al., 2021). Its industrial processing, boosted in recent years by the growing demand for fast food, generates large volumes of residues (20–50 % of the raw product) (Ben Atitallah et al., 2019; Chohan et al., 2020; Ude et al., 2020). Potato peel waste (PPW), the main waste generated in their processing, contain large amounts of starch, as well as cellulose, hemicelluloses and lignin (Ben Atitallah et al., 2019).

Due to their availability and their high content of fermentable carbohydrates, they are considered as potential raw materials in biotechnological processes. In a circular economy context, the production of second generation bioethanol, could open new alternatives for the valorisation of this by-product, which is normally disposed of in landfills (Ben Atitallah et al., 2019; Chohan et al., 2020; Ude et al., 2020; Barampouti et al., 2021). However, the bioethanol production from starchy feedstock, such as PPW, requires previous stages of liquefaction and saccharification. For attaining this purpose, several pretreatment stages have been reported, including thermal, acid, basic or enzymatic ones (Ben Atitallah et al., 2019; Zhang et al., 2010) but the ethanol titers reported so far from this substrate are modest impairing the feasibility of this valorisation route (Ben Atitallah et al., 2019; Chohan et al., 2020; Barampouti et al., 2021).

On the other hand, in last years very high gravity fermentation (VHGF) has been proposed to increase bioethanol production (Gomes et al., 2021a; Zhang et al., 2010). VHGF is defined as the preparation and fermentation of a mash containing at least 270 g/L of dissolved solids (Chao et al., 2017). This technology allows for reaching high ethanol concentrations, as well as the reduction of energy consumption in the distillation processes and increasing the ethanol yield (Chao et al., 2017; Zhang et al., 2010). Although VHGF has been successfully employed for the production of bioethanol from cereals (Zhang et al., 2010), corn (Li et al., 2017) and cassava (Nguyen et al., 2014), to the best of our knowledge, its application in tubers such as the potato, which present high viscosity, has rarely been reported (Zhang et al., 2010). In this context, this study deals with the optimization of the liquefaction, enzymatic hydrolysis and VHG SSF (very high gravity simultaneous saccharification fermentation) of PPW for bioethanol production.

In this work, the use of Very High Gravity (VHG) methodology to obtain high bioethanol titers from PPW was evaluated. Two experimental designs were proposed in order to optimize the liquefaction and hydrolysis enzymatic stages as well as the fermentative process. In the first experimental design, the parameters tested were the liquefaction time, SAN Super and Viscozyme concentrations; while the presaccharification time, the fermentation temperature and the load of fresh yeast were evaluated in the second one.

2. Materials and methods

2.1. Raw material

PPW from the Kennebec variety were washed with water and oven-dried at 50 °C for 48 h. Afterwards, they were grinded to a particle size of 0.5 mm, before being frozen at −18 °C until use.

2.2. Liquefaction and enzymatic hydrolysis stages

PPW liquefaction experiments were carried out in 50 mL Erlenmeyer flasks in a water bath at 90 °C. In this stage, the loading of the commercial enzyme Termamyl® SC 4X was fixed at 75 UE/g PPW, the liquid–solid ratio (LSR) in 2 g/g and the pH at 6 (sodium citrate buffer 1 N); whereas the effects of the time (1–3 h) and of adding the PPW in once or twice loads on liquefaction were evaluated.

After the liquefaction stage, the Erlenmeyer flasks were cooled to the desired temperature before adding the selected commercial enzyme preparations: a blend of glucoamylase, acid amylase and cellulase (Saczyme® Yield) or an amyloglucosidase with a balanced content of acid alpha-amylase and proteinase (SAN Super® 360 L) and a mixture of beta-glucanases, pectinases, hemicellulases and xylanases (Viscozyme ® L from *Aspergillus* sp.). All enzymatic blends were kindly provided by Novozymes (Denmark). In this stage, the agitation of the orbital shaker was maintained at 200 rpm and the temperature at 50 °C. The amylolytic activities of Termamyl® SC 4X, Saczyme® Yield and SAN Super® 360 L (78405, 12,507 and 4079 UE/mL, respectively) were determined following the protocol reported by Murado et al. (1993). The activity of Viscozyme (4348 U/mL) was measured as the amount of enzyme releasing 1 μmol of galacturonic acid per min from polygalacturonic acid 0.5 % under specific assay conditions (37 °C and pH = 5) (Martínez et al., 2009). Samples were withdrawn at the required times and centrifuged before analysis by high performance liquid chromatography (HPLC).

After some preliminary assay, in order to optimize the liquefaction and enzymatic hydrolysis stages, a Box–Behnken design of three variables at three levels was proposed (see Table 1). In this set of experiments, the selected independent variables were the liquefaction time or x_1 (1–3 h) and the SAN Super and Viscozyme loadings, denoted as x_2 and x_3 (5–15 UE/g PPW, and 30–90 U/g PPW, respectively).

2.3. Microorganism and inoculum preparation

In fermentation assays, two yeast with improved thermotolerance were selected, the *Saccharomyces cerevisiae* Ethanol Red® strain, which has been recently characterized regarding thermotolerance traits (García-Ríos et al., 2022; Lip et al., 2020; Pinheiro et al., 2020) and *Kluyveromyces marxianus* S9 isolated from cocoa fermentation with thermotolerance widely recognized (Baptista et al., 2021). Both yeasts were maintained on yeast peptone dextrose (2 % of glucose, 2 % of peptone and 1 % of yeast extract) agar medium at 4 °C. For the preinocula yeast were grown overnight at 30 °C and 200 rpm in a sterilized medium containing 20 g peptone/L, 10 g yeast extract/L and 20 g glucose/L. To recover the biomass, cells were centrifuged 10 min at 4000 rpm (ScanSpeed 416) and re-suspended in 0.9 % NaCl to obtain

Table 1
Experimental design expressed in terms of the dimensional variables liquefaction time, SAN Super and Viscozyme concentrations and dimensionless variables x_1, x_2 and x_3.

Experiment	Dimensionless, normalized, independent variables			Dimensional independent variables		
	x_1	x_2	x_3	Liquefaction time (h)	SAN Super concentration (UE/g)	Viscozyme concentration (U/g)
1A	−1	−1	0	1	5	60
2A	−1	0	−1	1	10	30
3A	−1	0	1	1	10	90
4A	−1	1	0	1	15	60
5A	0	−1	−1	2	5	30
6A	0	−1	1	2	5	90
7A	0	0	0	2	10	60
8A	0	0	0	2	10	60
9A	0	0	0	2	10	60
10A	0	1	−1	2	15	30
11A	0	1	1	2	15	90
12A	1	−1	0	3	5	60
13A	1	0	−1	3	10	30
14A	1	0	1	3	10	90
15A	1	1	0	3	15	60

solutions of 200–600 g of fresh yeast/L, which were employed for inoculation.

2.4. Pre-saccharification and simultaneous saccharification and fermentation (PSSF)

Preliminary PSSF evaluation was performed testing *S. cerevisiae* and *K. marxianus* at 37 and 42 °C, with an inoculum concentration of 5 mg of fresh yeast/g total solution, orbital agitation of 200 rpm and without the addition of external nutrients. Liquefaction and enzymatic hydrolysis conditions were fixed: 3 h of liquefaction at 90 °C, 75 U/g PPW of Termamyl, LSR of 2 g/g and pH = 6 (sodium citrate buffer 1 N), 21 h of pre-saccharification at 50 °C, Saczyme or SAN Super loads of 17.5 and 7.5 UE/g PPW, respectively and 42 U/g PPW of Viscozyme.

Under the selected liquefaction and enzymatic hydrolysis conditions, the PSSF stage using *S. cerevisiae* as yeast was optimized following a Box–Behnken design of three variables at three levels as shown in Table 2. In this second set of experiments, the selected independent variables were the pre-saccharification time or x_1 (10–20 h), the fermentation temperature or x_2 (30–42 °C) and the inoculum concentration or x_3 (5–15 mg of fresh yeast/g total solution). In this stage, the agitation of the orbital shaker was also maintained at 200 rpm and no external nutrients were supplied.

PSSF experiments were performed in Erlenmeyer flasks fitted with air-locks filled with glycerol, allowing CO_2 exhaustion while preventing entrance of oxygen into the flask. PSSF kinetics were followed by measuring weight difference of Erlenmeyer flasks (associated with the release of carbon dioxide) as previously described (Cunha et al., 2019, 2020a, 2021). In the end, samples were withdrawn from the media, centrifuged, and the liquid phase was analysed by HPLC for the quantification of glucose, ethanol and other minor metabolites. The ethanol kinetics were calculated taking into account the reaction stoichiometry (1 g of glucose generates 0.51 g of ethanol and 0.49 g of carbon dioxide) and final ethanol concentration was determined by HPLC. Ethanol yield and volumetric productivity (Q_p) were calculated following Eqs. (1) and (2):

$$\text{Ethanol yield } (\%) = \frac{([\text{EtOH}]_t - [\text{EtOH}]_0)}{0.51 \cdot \text{Gn}/\text{C}_{\text{Est}} \cdot \text{S}} \quad (1)$$

$$Q_p(g/L \cdot h) = \frac{[\text{EtOH}]_t}{t} \quad (2)$$

where $[\text{EtOH}]_t$ and $[\text{EtOH}]_0$ are ethanol concentrations at times t and 0, 0.51 is the stoichiometric factor for glucose to ethanol conversion, Gn is the content of glucose polysaccharides of the PPW (sum of glucan and starch), CEst is the stoichiometric conversion factor of glucose polysaccharides to glucose (162/180), S is the concentration of solid at the beginning of the fermentation and t the time.

Table 2
Experimental design expressed in terms of the dimensional variables pre-saccharification time, fermentation temperature and yeast load and dimensionless variables x_1, x_2 and x_3.

Experiment	Dimensionless, normalized, independent variables			Dimensional independent variables		
	x_1	x_2	x_3	Pre-saccharification time (h)	Fermentation temperature (°C)	Fresh yeast load (mg/ g total solution)
1B	−1	−1	0	10	30	10
2B	−1	0	−1	10	36	5
3B	−1	0	1	10	36	15
4B	−1	1	0	10	42	10
5B	0	−1	−1	15	30	5
6B	0	−1	1	15	30	15
7B	0	0	0	15	36	10
8B	0	0	0	15	36	10
9B	0	0	0	15	36	10
10B	0	1	−1	15	42	5
11B	0	1	1	15	42	15
12B	1	−1	0	20	30	10
13B	1	0	−1	20	36	5
14B	1	0	1	20	36	15
15B	1	1	0	20	42	10

2.5. Analytical methods

2.5.1. Chemical composition of the raw material

Samples of PPW were subjected to moisture (TAPPI T-264-om-88 method), ash (T-244-om-93 method) and extracts content (TAPPI T-264-om-88 method) determination. The content of minerals was evaluated by atomic absorption spectrometry in a Varian Spectra AA 220/FS. 5 mL of HNO_3 65 % (w/w), 1 mL of H_2O_2 30 % (w/v) and 0.5 mL of HF 40 % (w/w) were employed for the microwave assisted acid digestion. The nitrogen content was determined in an elemental analyser (Thermo Finnigan EA 1112), and the protein content was calculated assuming 6.25 g protein/g nitrogen. The Megazyme kit (Total Starch Assay Procedure (Amyloglucosidase/A-amylase method) K-TSTA-50A/K-TSTA-100A 11/20, AOAC Method 996.11, AACC Method 76–13.01) was employed for the starch determination. Finally, the content of hemicelluloses, acetyl groups, glucose polymers and Klason lignin was determined by quantitative acid hydrolysis (TAPPI T13 m method). The glucan content was obtained by difference between the glucose polymers and the total starch contents. Klason lignin (TAPPI T13 m assay) was gravimetrically determined from the oven-dried solid residue from hydrolysis. The liquid phase was filtered through 0.22 μm membranes and analysed by HPLC using an Agilent 1200 equipped with a refractive index (RI) detector and an Aminex HPX-87H column (BioRad, Life Science Group, Hercules, CA). Other conditions in HPLC analysis were the following: 3 mM H_2SO_4 as the mobile phase, flow rate of 0.6 mL/min, oven temperature 50 °C, detector temperature 35 °C. An aliquot of the liquid phase was also subjected to uronic acid determination by spectrophotometry, with galacturonic acid as standard (Blumenkrantz and Asboe-Hansen, 1973). Analysis were carried out in triplicate.

2.5.2. Chemical characterization of liquid fractions

Samples from the liquefaction and enzymatic hydrolysis and PSSF experiments were filtered through 0.22 μm membranes and analysed for glucose, ethanol, glycerol and acetic, succinic and lactic acids by the HPLC method described in the previous section.

2.6. Data analysis

The data obtained were fitted using the commercial software Microsoft Excel (Microsoft, USA).

3. Results

3.1. Chemical composition of PPW

Table 3 shows the chemical composition of the PPW (expressed in g/100 g oven-dry weight ± standard deviation). It should be noted the high content of starch (close to 46 %), followed by 10.88 % of protein. Several polysaccharides were determined in smaller amounts: xylan (5.75 %), glucan (4.69 %) and arabinan (0.88 %). Other compounds of minor interest in this study were: ashes (7.06 %) and lignin (3.14 %). Finally, the extracts were quantified (6.67 %), which contained mostly monomeric sugars and organic acids with low antioxidant activity. These results are similar to those published by Khawla et al. (2014) for the same raw material. Concerning the minerals quantification, also included in Table 3, potassium is the mineral present in higher amount, followed by magnesium and calcium. Taking into account its high content in polysaccharides (57 %) and that these mainly derive from starch, an easily accessible sugar polymer, PPW is a promising feedstock for biofuels production when compared for example with lignocellulosic substrates that pose an additional challenge due to their recalcitrant structure and the difficult conversion of cellulose to fermentable sugars. Also, its significant content in other nutrients, as for example protein or salts, ensures the required nutritional needs of yeast for proper fermentation metabolism, sparing the need for supplementation. Hence, PPW is a good candidate for bioethanol production, providing a potentially profitable alternative to mitigate the environmental impact of fossil fuels and add value to this industrial by-product, while avoiding consumption of other substrates that can find value in the food chain.

Table 3
Chemical composition of PPW.

Component	Content (g/100 g dry PPW)
Glucan	4.42 ± 1.32*
Starch	46.18 ± 0.44
Xylan	5.72 ± 0.10
Arabinan	0.87 ± 0.10
Acetyl groups	0.33 ± 0.02
Lignin	3.12 ± 0.45
Uronic acids	2.21 ± 0.23
Protein	10.88 ± 0.16
Ash	7.02 ± 0.09
Extracts	6.67 ± 0.24
Moisture	1.14 ± 0.31
Magnesium	0.15 ± 4.34E^{-03}
Calcium	0.15 ± 5.98E^{-03}
Sodium	0.06 ± 1.28E^{-03}
Potassium	3.24 ± 0.09
Zinc	1.86E^{-03} ± 2.01E^{-05}
Copper	9.84E^{-02} ± 4.50E^{-03}
Iron	0.05 ± 3.53E^{-03}
Manganese	2.16E^{-03} ± 5.57E^{-05}
ND	7.80

*Calculated by difference.

3.2. Preliminary assays

In the first part of this study, several preliminary assays were carried out in order to select and evaluate the influence of the more relevant variables in the liquefaction, saccharification and fermentative stages. The optimization of operational parameters is necessary from an economically point of view, since it could result in important cost reduction in industrial processes. In a study carried out by Nieder-Heitmann et al. (2020), it is concluded that the continuous optimization of different pretreatments could increase the profitability of biorefineries for the valorisation of lignocellulosic biomass. In this work, the optimization of enzyme and yeast loads allowed significant economic savings since these are expensive consumables. On the other hand, the optimization of liquefaction and pre saccharification times and fermentation temperature can also allow energy savings, since the utilities cost is considered one of the most important economic factor in the production cost (Dávila et al., 2017; Gomes et al., 2021c). Finally, the production of high ethanol concentrations from zero cost raw materials is also a relevant factor to take into account when designing proposals to be implemented at an industrial level.

3.2.1. Liquefaction and saccharification stage

A set of eight experiments was carried out (denoted as PE-1 to PE-8, see Table S1) with the purpose of addressing: 1) the influence of the addition of the solid on the liquefaction stage in one or two loads (half at time zero and the other half after one hour), 2) the effect of two commercial glucoamylases (SAN Super and Saczyme) and 3) the effect of Viscozyme loading. All the operational variables were selected according to previous experiences and to studies performed with several starchy raw materials (Coelho et al., 2020; Khawla et al., 2014; Nkomba et al., 2016).

Taking into account the results provided in Table S1 from this set of experiments, it can be observed that the combination of Saczyme + Viscozyme yielded a higher final concentration and yield of glucose (see experiment PE-7). However, as can be seen in Fig. 1A, when normalizing to initial enzyme loading, calculating the ratio of concentration of glucose produced (after liquefaction + saccharification stages) per enzyme unit, the experiments with SAN Super resulted in values between 0.5 and 1.2, significantly higher than the ones attained with

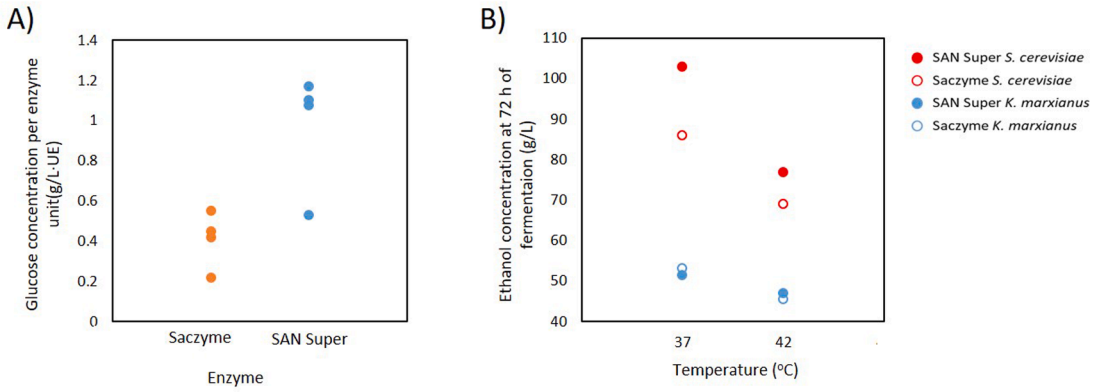

Fig. 1. Concentration of glucose produced per enzyme unit in the preliminary experiments of liquefaction and saccharification performed with Saczyme and SAN Super, respectively (A) and ethanol concentration achieved for the preliminary fermentation stage experiments (B).

Saczyme (0.2–0.55). In fact, if we do a head to head comparison of experiments PE-1 and PE-6, it can be clearly seen that similar concentrations and yields were obtained with lower enzyme loadings using SAN Super, leading to its selection moving forward in process optimization. Nevertheless, the higher yield obtained in experiment PE-7 provided a preliminary proof of the importance for the optimization of enzyme loading to attain satisfactory yields, pursued in the first design of experiments.

3.2.2. *Fermentation stage*

Since the optimal operating temperatures of the enzymes (between 40 and 50 °C for Viscozyme and 55–60 °C for SAN Super) are higher than that of the selected yeasts (between 30 and 40 °C), it is intended to identify compromise operating conditions where effective enzymatic hydrolysis stage is performed and at the same time the fermentative stage is viable. While for *S. cerevisiae* Ethanol Red® strain the optimum growth temperature was shown to be 35 °C (Lip et al., 2020), for lignocellulosic fermentation this commercial yeast strain has been applied at 35 °C in SSF of *Eucalyptus globulus* hydrolysate supplemented with cheese whey (Cunha et al., 2018) and at 40 °C for the consolidated bioprocessing of corn con liquor (Cunha et al., 2020a) and corn cob supplemented with cheese whey (Cunha et al., 2021). The thermotolerant yeast *K. marxianus* is typically used at 37 °C (Baptista et al., 2021; Palacios et al., 2021) and ethanol production from lignocellulosic substrates has been carried out at 30–43 °C (reviewed in Baptista and Domingues (2022)). Under the selected liquefaction and saccharification operational conditions (75 UE/g PPW of Termamyl, one solid load, LSR = 2 g/g, 3 h of liquefaction at 90 °C, 17.5 UE/g PPW of Saczyme or 7.5 UE/g PPW of SAN Super, 42 U/g PPW of Viscozyme and 21 h of pre-saccharification at 50 °C), a new set of 8 PSSF experiments was conducted (see PE-9 to PE-16 in Table S2 of Supplementary information and Fig. 1B). The first four allowed comparing the performance of *S. cerevisiae* and *K. marxianus* operating at 37 °C, both with SAN Super and Saczyme; whereas, the other four evaluated the effect of increasing the fermentation temperature to 42 °C, maintaining the other parameters as previously described.

As can be observed in Fig. 1B, the best results were obtained with the combination SAN Super and *S. cerevisiae* (filled red symbols) when the fermentation process was performed at 37 °C. Comparing similar runs, *S. cerevisiae* (represented by red symbols) led consistently to higher ethanol concentration and titters than *K. marxianus* (blue symbols). If VHG conditions are imposed, the lower stress tolerance to high sugar concentration and to ethanol of *K. marxianus* when compared with *S. cerevisiae* most probably provokes the catabolic suppression, hence affecting negatively fermentation yield (Baptista and Domingues, 2022). The same behaviour was previously reported by Palacios et al. (2021) when they studied the potential of banana peels for the ethanol production in PSSF process at high solid loading.

Regarding enzyme selection, SAN Super (see filled symbols in Fig. 1B) led to overall higher ethanol yields than Saczyme (empty symbols), corroborating the previously observed in the liquefaction and saccharification trials. These differences are even greater if we consider that such higher ethanol yields where achieved at significantly lower loading of SAN Super (7.5 UE/g PPW) than Saczyme (17.5 UE/g PPW). Also, differences between SAN Super and Saczyme were even greater in the SSF runs than on the previous hydrolysis trials, hinting that SAN Super advantage over Saczyme can be even greater when lower process temperatures are imposed. These differences between Saczyme and SAN Super are faded in the runs conducted with *K. marxianus*, due to the lower efficiency of this yeast previously discussed.

Taking into account the preliminary results obtained, the combination of SAN Super and *S. cerevisiae* commercial strain was selected to continue the study.

3.3. *Experimental design proposed for the optimization of liquefaction and enzymatic saccharification stages*

In order to optimize the liquefaction and enzymatic hydrolysis stages, a Box–Behnken design of three variables at three levels was proposed (see Table 1). In this case, the selected independent variables were the liquefaction time or x_1 (1–3 h) and the SAN Super and Viscozyme loadings, denoted as x_2 and x_3 (5–15 UE/g PPW, and 30–90 U/g PPW, respectively).

Fig. 2A shows the kinetics of 5 experiments selected from design 1. From the experimental results, it can be stated that one of the most influential independent variables on ethanol concentration was the Viscozyme load. This hypothesis is corroborated by the ethanol time courses of assays 2A and 3A (see Fig. 2A), carried out with the same liquefaction time (1 h) and SAN Super load (10 UE/g PPW), but with the minimum and maximum Viscozyme loads (30 and 90 U/g PPW, respectively). Moreover, this assumption would also be confirmed by experiment 11A, since in this case with the highest loads of Viscozyme and SAN Super, the highest concentration of ethanol was achieved (values close to 117 g/L). The need for the addition of enzymes to reduce the viscosity of culture media formulated with starchy materials has been previously reported (Zhang et al., 2010). This step will enable the full release of sugars, enabling to reach high ethanol concentrations in the fermentation stage and thus increasing the economic feasibility of the distillation process (Gomes et al., 2021c). In previous studies, lignocellulosic substrates have been mixed with cheese whey, in a multi-waste valorisation approach, to raise the sugar concentration and thus enable the economic feasibility of ethanol production (Cunha et al., 2021, 2018). PPW was in here shown to enable high ethanol concentrations by itself.

On the other hand, the favorable kinetics obtained both in experiment 11A and in the central point of the design (experiments 7A-9A),

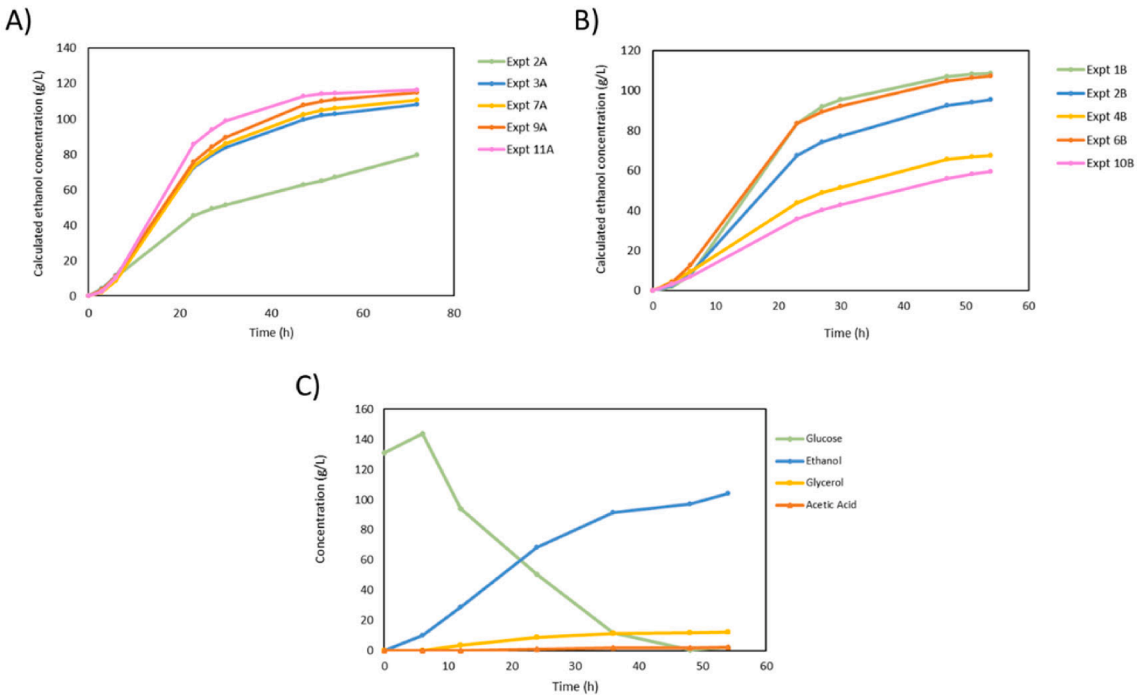

Fig. 2. Ethanol kinetics of some selected experiments of the design 1 (A), of selected experiments of the design 2 (B) and ethanol, glucose, glycerol and acetic acid time courses obtained in the PSSF experiment performed under the selected conditions (C).

indicate that intermediate liquefaction times could be sufficient to reach high concentrations of ethanol.

In all experiments, the maximum Q_p were reached at 23 h, with values in the range 3.73–1.97 g/(L·h). As can been expected, the highest and the lowest ones were obtained in experiments 11A and 2A, respectively.

Response surface methodology (RSM) was used for a straightforward interpretation of the ethanol concentrations and yields obtained in the two proposed designs. Table 4 shows the dependent variables (y_1 to y_4) which are correlated with the independent variables (liquefaction time, SAN Super and Viscozyme loads for design 1; and pre-saccharification time, fermentation temperature and yeast load for design 2) using empirical models according to the following formula:

$$y_j = b_0 + \sum_{i=1}^{3} b_i x_i + \sum \sum_{i<j=1}^{3} b_{ij} x_i x_j + \sum_{i=j}^{3} b_{ii} x_i^2 \quad (3)$$

where y_j ($j = 1$ to 4) corresponds to dependent variables; x_i or x_j (i or j: 1 to 3, $j \geq i$) corresponds to the normalized independent variables that are described in the Table 1 for the first design and in the Table 2 for the second design; and $b_{0j} \ldots b_{ijj}$ are the regression coefficients calculated from the experimental data by multiple regression employing the least-squares method.

Table 4 shows the fitting parameters of the two designs carried out experimentally. Statistical parameters such as regression coefficients, their statistical significance and the statistical significance of the models are also included.

The correlation coefficients obtained in the first design for the

Table 4
Regression coefficients and statistical parameters measuring the correlation and significance of models obtained for ethanol concentration and yield for both designs.

Coefficient	Design 1		Design 2	
	Ethanol concentration at 72 h or y_1 (g/L)	Ethanol yield at 72 h or y_2 (%)	Ethanol concentration at 54 h or y_3 (g/L)	Ethanol yield at 54 h or y_4 (%)
b_{0j}	110.62[a]	76.50[a]	104.60[a]	72.26[a]
b_{1j}	8.68[a]	6.01[a]	−0.06	−0.04
b_{2j}	−0.64	−0.47	−18.85[a]	−13.07[a]
b_{3j}	7.75[a]	5.21[a]	4.22[b]	2.87b
b_{12j}	4.47[c]	3.09[c]	2.27	1.57
b_{13j}	−5.68[b]	−3.94[b]	−1.66	−1.15
b_{23j}	−0.33	−0.19	1.21	0.93
b_{11j}	−8.03[a]	−5.58[a]	−0.10	−0.12
b_{22j}	−1.57	−1.07	−19.34a	−13.32a
b_{33j}	−0.25	−0.16	−0.79	−0.50
Statistical parameters				
R^2	0.873	0.872	0.958	0.958
F experimental	11.73	11.60	36.61	36.40
Significance level %	99.277	99.259	99.951	99.950

[a] Significant coefficients at the 99% confidence level.
[b] Significant coefficients at the 95% confidence level.
[c] Significant coefficients at the 90% confidence level.

variables y_1 and y_2 (R^2 of 0.872 and 0.873, respectively) and the high values of experimental F (11.73 for y_1 and 11.60 for y_2) indicate that the proposed equation had a good fit.

Concerning the regression coefficients collected in Table 4, it is observed that the most influential variable on ethanol concentration at 72 h (y_1) was the liquefaction time, followed by its quadratic term and by the Viscozyme load. With the exception of the interaction of liquefaction time and SAN Super load, all the quadratic terms had a negative effect on the ethanol concentration. However, the linear terms of liquefaction time and Viscozyme load exerted a positive effect. On the other hand, the SAN Super load and its quadratic term were not significant variables. However, previous studies performed with other starchy materials reported a higher influence of the SAN Super load. For instance, in the enzymatic hydrolysis of chestnut puree, a higher SAN Super load led to higher sugar concentrations (López et al., 2004). As can been expected, in our work, a similar behaviour pattern has been observed for the ethanol yield at 72 h (y_2), with values for the coefficients slightly lower.

The calculated response surfaces for the ethanol concentration (y_1) for SAN Super loadings of 5, 10 and 15 UE/g PPW are displayed in Fig. 3. As can be seen, the surfaces increase with the concentration of Viscozyme, this increase being more pronounced at low liquefaction times. Concerning the SAN Super load, at low liquefaction times, low loads of SAN Super resulted in higher ethanol concentrations and yields. At intermediate liquefaction times (in the range 1.7–2.5 h) the best results were predicted for intermediate SAN Super loads. However, at liquefaction times higher than 2.5 h, SAN Super loads of 15 UE/g PPW resulted in the higher ethanol concentrations and yields. Similar liquefaction times (2.5 h) were previously selected by Nkomba et al. (2016), in a study performed with white sorghum grain.

Therefore, taking into account everything previously described, the conditions selected to continue with the establishment and optimization of the PSSF process were the following: 2 h liquefaction time, Viscozyme load of 80 U/g PPW and SAN Super load of 5 UE/g PPW, since no important improvements were predicted at higher Viscozyme and SAN Super loads.

3.4. Experimental design proposed for the optimization of the pre-saccharification and SSF stages

In the second Box–Behnken design (see Table 2), the selected independent variables were the pre-saccharification time or x_1 (10–20 h), the fermentation temperature or x_2 (30–42 °C) and the inoculum concentration or x_3 (5–15 mg of fresh yeast/g total solution).

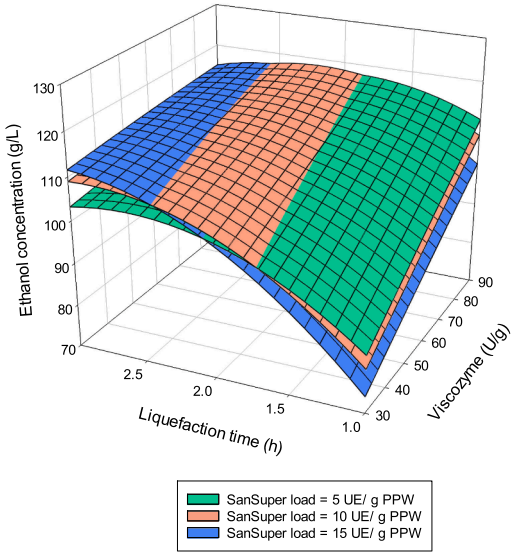

Fig. 3. Response surfaces obtained in experimental design 1 for the ethanol concentration after 72 h at SAN Super loads of 5, 10, and 15 UE/g PPW.

As a representative example, Fig. 2B shows the bioethanol production profiles obtained for the experiments 1B, 2B, 4B, 6B, and 10B of the second experimental design. As can be seen in Table S3, the experimental ethanol concentrations obtained at 54 h of fermentation ranged between 66.3 and 108.5 g/L. The minimum and maximum values corresponded to the experiments 15B and 14B, respectively. As can be observed in Fig. 2B, the ethanol concentration increased sharply (about 83 g/L) after 23 h of fermentation (runs 1B and 6B), when the temperature of fermentation was maintained at their lowest value. Afterwards, this increase in ethanol concentration occurred in a less pronounced way. However, in the experiments performed at the highest temperature tested (42 °C, run 4B and 10B), the increase in ethanol concentration was lesser pronounced (about 46.7 and 35.7 g/L, respectively). This behaviour highlights the known effect of supra-optimal temperatures on yeast physiology and the necessity for selecting robust yeast with thermotolerant traits (Lip et al., 2020).

In all experiments the maximum values of Qp were reached at 23 h, the results at this time were: 3.63 (run 1B), 2.93 (run 2B), 1.89 (run 4B), 3.63 (run 6B) and 1.55 (run 10B) g of ethanol/(L·h), respectively. In general, these volumetric productivities can be compared favourably with those reported for other cheap raw materials such as distillers' dried grains (1.61–1.67 g of ethanol/(L·h)) (Nkomba et al., 2016) or PPW (0.939 g of ethanol/(L·h)) (Chohan et al., 2020) and (1.18–2.39 g of ethanol/(L·h)) (Aruwajoye et al., 2020).

RSM was also applied to assess the effect of the selected independent variables on the concentration and yield of ethanol at 54 h (dependent variables denoted as y_3 and y_4). Regression coefficients are also presented in Table 4. The favorable results determined for R^2 (0.958) and F (over 36) indicate a good fitting of both models.

In accordance with the regression coefficients, it can be inferred that the linear and quadratic terms of the temperature of fermentation were the more influential variables and exerted a negative influence on the concentration and yield of ethanol. On the contrary, the linear factor of yeast load had a lower but positive effect on both responses and the pre-saccharification time and its quadratic term had practically no influence on the dependent variables analysed.

Aruwajoye et al. (2020) studied the effect of the fermentation temperature on the bioethanol concentration from cassava peels and found that an increase in fermentation temperature leads to a decrease in ethanol production. This behaviour was also noted by Chohan et al. (2020) when they optimized bioethanol production from PPW. In fact, the negative effect of temperature on fermentation is more pronounced under VHG conditions which is explained by the consequent increase of ethanol-related stresses (Gomes et al., 2021a). When developing cell recycling systems for VHG processes, Pereira et al. (2012) observed a significant improvement on yeast cell performance when the temperature was reduced from 30 to 27 °C. For 400 g/L initial glucose, the yeast cells were able to produce 18.2 % (v/v) ethanol with a residual glucose of 80 g/L at 30 °C while at 27 °C, produced 20.1 % (v/v) and the glucose residual decreased to 60 g/L.

Fig. 4 displays the response surfaces for ethanol concentration (at 54 h in function of pre-saccharification time and fermentation temperature for fixed yeast load values (5, 10, and 15 mg fresh yeast/g total solution). As can be seen in the calculated surfaces, the highest ethanol concentration (114. 8 g/L) and ethanol yield (79.2 %) were attained at 34 °C, 10 h of pre-saccharification time and 15 mg fresh yeast/g total solution.

These results are in line with those obtained by Nkomba et al. (2016) who reported an ethanol concentration of 130.4 g/L at 30 °C, corresponding to an ethanol yield of 89.7 % when they fermented whole sorghum grains using *S. cerevisiae* yeast. In another study, Khawla et al. (2014) managed to reach 71.4 % of ethanol yield when PPW was subjected to a stage of chemical treatment followed by enzymatic hydrolysis (performed by adding an amylolytic and a cellulolytic enzyme) and using *S. cerevisiae*.

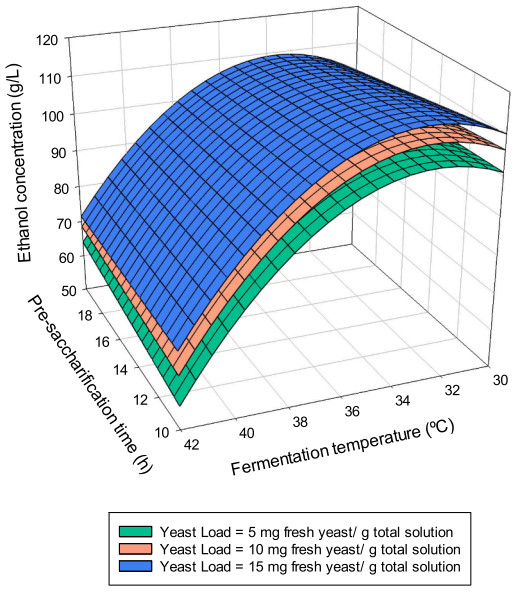

Fig. 4. Response surfaces obtained in experimental design 2 for the ethanol concentration at 54 h at yeast loads of 5, 10, and 15 mg of fresh yeast /g total solution.

3.5. Validation of the second model

According to the model predictions and taking into account the low influence of the yeast and SAN Super load, the operational conditions selected for the validation of the model were the following: 2 h of liquefaction, 80 U/g PPW of Viscozyme, 5 UE/g PPW of SAN Super, pre-saccharification time of 10 h, fermentation temperature of 34 °C and the lowest *S. cerevisiae* yeast load of 5 mg fresh yeast/g total solution. As can be seen in Fig. 2C, the high experimental ethanol concentration at 54 h was 104.1 ± 2.7 g/L, corresponding to a yield of 71.9 ± 1.8 %. These values match the ones predicted by the empirical models (103.2 g/L and 71.4 %, respectively). In this experiment, the highest productivity (2.86 g/(L·h)) was obtained at 24 h, and was higher than the previously cited for other starchy feedstock.

On the other hand, as can be also observed in Fig. 2C, other fermentative co-products were detected, mainly glycerol and acetic acid (reaching values of 12.1 ± 0.8 g/L corresponding to a yield of 4.2 ± 0.3 % and 2.05 ± 0.01 g/L with a yield of 0.7 ± 0.0 % at 54 h, respectively).

These results compare favourably with other previously reported in studies using PPW: 21.2 g/L of ethanol with an acid pretreatment (Ben Atitallah et al., 2019), 23.8 g/L with a basic pretreatment (Barampouti et al., 2021) or 22.5 g/L with enzymatic liquefaction (Chohan et al., 2020). Moreover, ethanol concentration achieved in this work is comparable to those obtained by VHGF with other raw materials, such as white sorghum grain, 125 g/L of ethanol at 120 h (Nkomba et al., 2016).

4. Conclusions

PPW were subjected to PSSF for bioethanol production by VHG strategies with *S. cerevisiae* commercial strain. With this purpose, the conditions for the liquefaction, enzymatic hydrolysis and fermentation stages were optimized by RSM. The experiment performed under the selected conditions resulted in a high ethanol concentration and yield, higher than the obtained in previous works with this raw material. The proposed models showed a high degree of fitting. Therefore, this study opens new valorisation opportunities for this feedstock in line with the circular economy and zero residues industries in a food by-products biorefinery context.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, by the "Xunta de Galicia" (GRC ED431C 2022/08 and GRC ED431C 2021/46), Centro Singular de Investigación Biomédica "CINBIO", Project ED431F 2020/03, partially funded by the FEDER Program of the European Union ("Unha maneira de facer Europa") and by MINECO (project PID2020-116717RB-I00 and project PID2019-110031RB-I00). B. Gullón would like to express her gratitude to the Spanish Ministry of Economy and Competitiveness for her postdoctoral grant (Reference RYC2018-026177-I). R. Yáñez and B. Rodríguez to the Iacobus Program for the mobility fellowships. The authors would like to thank the Centro de Apoio Científico-Tecnolóxico á Investigación (C.A.C.T.I.) for performing the nitrogen and mineral content determinations. Funding for open access charge: Universidade de Vigo/CISUG.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2022.11.007.

References

Aruwajoye, G.S., Sewsynker-Sukai, Y., Kana, E.B.G., 2020. Valorisation of cassava peels through simultaneous saccharification and ethanol production: Effect of prehydrolysis time, kinetic assessment and preliminary scale up. Fuel 278, 118351. https://doi.org/10.1016/j.fuel.2020.118351.

Baptista, M., Cunha, J.T., Domingues, L., 2021. Establishment of *Kluyveromyces marxianus* as a microbial cell factory for lignocellulosic processes: Production of high value furan derivatives. J. Fungi 7 (12), 1047. https://doi.org/10.3390/jof7121047.

Baptista, M., Domingues, L., 2022. *Kluyveromyces marxianus* as a microbial cell factory for lignocellulosic biomass valorisation. Biotechnol. Adv. 60, 108027 https://doi.org/10.1016/j.biotechadv.2022.108027.

Barampouti, E.M., Christofi, A., Malamis, D., Mai, S., 2021. A sustainable approach to valorize potato peel waste towards biofuel production. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-021-01811-4.

Ben Atitallah, I., Antonopoulou, G., Ntaikou, I., Alexandropoulou, M., Nasri, M., Mechichi, T., Lyberatos, G., 2019. On the evaluation of different saccharification schemes for enhanced bioethanol production from potato peels waste via a newly isolated yeast strain of *Wickerhamomyces anomalus*. Bioresour. Technol. 289, 121614 https://doi.org/10.1016/j.biortech.2019.121614.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New Method for Quantitative Determination of Uronic Acids. Anal. Biochem. 54 (2), 484–489.

Chao, B., Liu, R., Zhang, X., Zhang, X.u., Tan, T., 2017. Tannin extraction pretreatment and very high gravity fermentation of acorn starch for bioethanol production. Bioresour. Technol. 241, 900–907. https://doi.org/10.1016/j.biortech.2017.06.026.

Chohan, N.A., Aruwajoye, G.S., Sewsynker-Sukai, Y., Gueguim Kana, E.B., 2020. Valorisation of potato peel wastes for bioethanol production using simultaneous saccharification and fermentation: Process optimization and kinetic assessment. Renew. Energy 146, 1031–1040. https://doi.org/10.1016/j.renene.2019.07.042.

Coelho, E., Ballesteros, L.F., Domingues, L., Vilanova, M., Teixeira, J.A., 2020. Production of a distilled spirit using cassava flour as raw material: Chemical characterization and sensory profile. Molecules 25, 1–13. https://doi.org/10.3390/molecules25143228.

Cunha, J.T., Soares, P.O., Romaní, A., Thevelein, J.M., Domingues, L., 2019. Xylose fermentation efficiency of industrial *Saccharomyces cerevisiae* yeast with separate or combined xylose reductase/xylitol dehydrogenase and xylose isomerase pathways. Biotechnol. Biofuels 12, 1–14. https://doi.org/10.1186/s13068-019-1360-8.

Cunha, J.T., Romaní, A., Inokuma, K., Johansson, B., Hasunuma, T., Kondo, A., Domingues, L., 2020a. Consolidated bioprocessing of corn cob-derived hemicellulose: Engineered industrial *Saccharomyces cerevisiae* as efficient whole cell biocatalysts. Biotechnol. Biofuels 13, 1–15. https://doi.org/10.1186/s13068-020-01780-2.

Cunha, J.T., Soares, P.O., Baptista, S.L., Costa, C.E., Domingues, L., 2020b. Engineered *Saccharomyces cerevisiae* for lignocellulosic valorization: a review and perspectives

on bioethanol production. Bioengineered 11, 883–903. https://doi.org/10.1080/21655979.2020.1801178.
Cunha, J.T., Gomes, D.G., Romaní, A., Inokuma, K., Hasunuma, T., Kondo, A., Domingues, L., 2021. Cell surface engineering of *Saccharomyces cerevisiae* for simultaneous valorization of corn cob and cheese whey via ethanol production. Energy Convers. Manag. 243, 114359 https://doi.org/10.1016/j.enconman.2021.114359.
Cunha, M., Romaní, A., Carvalho, M., Domingues, L., 2018. Boosting bioethanol production from Eucalyptus wood by whey incorporation. Bioresour. Technol. 250, 256–264. https://doi.org/10.1016/j.biortech.2017.11.023.
Dávila, J.A., Rosenberg, M., Castro, E., Cardona, C.A., 2017. A model biorefinery for avocado (*Persea americana mill.*) processing. Bioresour. Technol. 243, 17–29. https://doi.org/10.1016/j.biortech.2017.06.063.
García-Ríos, E., Alonso-del-Real, J., Lip, K.Y.F., Pinheiro, T., Teixeira, J., van Gulik, W., Domingues, L., Querol, A., Guillamón, J.M., 2022. Genome-wide effect of non-optimal temperatures under anaerobic conditions on gene expression in *Saccharomyces cerevisiae*. Genomics 114 (4), 110386. https://doi.org/10.1016/j.ygeno.2022.110386.
Gomes, D., Cruz, M., de Resende, M., Ribeiro, E., Teixeira, J., Domingues, L., 2021a. Very high gravity bioethanol revisited: Main challenges and advances. Fermentation 7, 1–18. https://doi.org/10.3390/fermentation7010038.
Gomes, D., Cunha, J., Zanuso, E., Teixeira, J., Domingues, L., 2021b. Strategies towards Reduction of Cellulases Consumption: Debottlenecking the Economics of Lignocellulosics Valorization Processes. Polysaccharides 2, 287–310. https://doi.org/10.3390/polysaccharides2020020.
Gomes, D.G., Teixeira, J.A., Domingues, L., 2021c. Economic determinants on the implementation of a Eucalyptus wood biorefinery producing biofuels, energy and high added-value compounds. Appl. Energy 303, 117662. https://doi.org/10.1016/j.apenergy.2021.117662.
Khawla, B.J., Sameh, M., Imen, G., Donyes, F., Dhouha, G., Raoudha, E.G., Oumèma, N. E., 2014. Potato peel as feedstock for bioethanol production: A comparison of acidic and enzymatic hydrolysis. Ind. Crops Prod. 52, 144–149. https://doi.org/10.1016/j.indcrop.2013.10.025.
Li, Z., Wang, D., Shi, Y.C., 2017. Effects of nitrogen source on ethanol production in very high gravity fermentation of corn starch. J. Taiwan Inst. Chem. Eng. 70, 229–235. https://doi.org/10.1016/j.jtice.2016.10.055.
Lip, K.Y.F., García-Ríos, E., Costa, C.E., Guillamón, J.M., Domingues, L., Teixeira, J., van Gulik, W.M., 2020. Selection and subsequent physiological characterization of industrial *Saccharomyces cerevisiae* strains during continuous growth at sub- and-supra optimal temperatures. Biotechnol. Reports 26, e00462. https://doi.org/10.1016/j.btre.2020.e00462.
López, C., Torrado, A., Fuciños, P., Guerra, N.P., Pastrana, L., 2004. Enzymatic Hydrolysis of Chestnut Purée: Process Optimization Using Mixtures of α-Amylase and Glucoamylase. J. Agric. Food Chem. 52, 2907–2914. https://doi.org/10.1021/jf035164o.
Martínez, M., Gullón, B., Yáñez, R., Alonso, J.L., Parajó, J.C., 2009. Direct enzymatic production of oligosaccharide mixtures from sugar beet pulp: Experimental evaluation and mathematical modeling. J. Agric. Food Chem. 57, 5510–5517. https://doi.org/10.1021/jf900654g.
Mazaheri, D., Pirouzi, A., 2020. Valorization of *Zymomonas mobilis* for bioethanol production from potato peel: fermentation process optimization. Biomass Conv. Bioref. 12 (8), 3389–3398. https://doi.org/10.1007/s13399-020-00834-7.
Mithra, M.G., Jeeva, M.L., Sajeev, M.S., Padmaja, G., 2018. Comparison of ethanol yield from pretreated lignocellulo-starch biomass under fed-batch SHF or SSF modes. Heliyon 4, e00885. https://doi.org/10.1016/j.heliyon.2018.e00885.
Murado, M.A., Pastrana, L., Miron, J., 1993. Enhancement of the bioproduction potential of an amylaceous effluent 44, 155–163.
Nguyen, C.N., Le, T.M., Chu-Ky, S., 2014. Pilot scale simultaneous saccharification and fermentation at very high gravity of cassava flour for ethanol production. Ind. Crops Prod. 56, 160–165. https://doi.org/10.1016/j.indcrop.2014.02.004.
Nieder-Heitmann, M., Haigh, K., Louw, J., Görgens, J.F., 2020. Economic evaluation and comparison of succinic acid and electricity co-production from sugarcane bagasse and trash lignocelluloses in a biorefinery, using different pretreatment methods: dilute acid (H2SO4), alkaline (NaOH), organosolv, ammonia fibre expa. Biofuels Bioprod. Biorefining 14, 55–77. https://doi.org/10.1002/bbb.2020.
Nkomba, E.Y., van Rensburg, E., Chimphango, A.F.A., Görgens, J.F., 2016. The influence of sorghum grain decortication on bioethanol production and quality of the distillers' dried grains with solubles using cold and conventional warm starch processing. Bioresour. Technol. 203, 181–189. https://doi.org/10.1016/j.biortech.2015.12.045.
Palacios, A.S., Ilyina, A., Ramos-González, R., Aguilar, C.N., Martínez-Hernández, J.L., Segura-Ceniceros, E.P., González, M.L.C., Aguilar, M., Ballesteros, M., Oliva, J.M., Ruiz, H.A., 2021. Ethanol production from banana peels at high pretreated substrate loading: comparison of two operational strategies. Biomass Convers. Biorefinery 11, 1587–1596. https://doi.org/10.1007/s13399-019-00562-7.
Pereira, F.B., Gomes, D.G., Guimarães, P.M.R., Teixeira, J.A., Domingues, L., 2012. Cell recycling during repeated very high gravity bio-ethanol fermentations using the industrial *Saccharomyces cerevisiae* strain PE-2. Biotechnol. Lett. 34, 45–53. https://doi.org/10.1007/s10529-011-0735-0.
Pinheiro, T., Lip, K.Y.F., García-Ríos, E., Querol, A., Teixeira, J., van Gulik, W., Guillamón, J.M., Domingues, L., 2020. Differential proteomic analysis by SWATH-MS unravels the most dominant mechanisms underlying yeast adaptation to non-optimal temperatures under anaerobic conditions. Sci. Rep. 10, 1–18. https://doi.org/10.1038/s41598-020-77846-w.
Sheikh, R.A., Al-Bar, O.A., Soliman, Y.M.A., 2016. Biochemical studies on the production of biofuel (bioethanol) from potato peels wastes by *Saccharomyces cerevisiae*: effects of fermentation periods and nitrogen source concentration. Biotechnol. Biotechnol. Equip. 30, 497–505. https://doi.org/10.1080/13102818.2016.1159527.
Soltaninejad, A., Jazini, M., Karimi, K., 2022. Sustainable bioconversion of potato peel wastes into ethanol and biogas using organosolv pretreatment. Chemosphere 291, 133003. https://doi.org/10.1016/j.chemosphere.2021.133003.
Ude, M.U., Oluka, I., Eze, P.C., 2020. Optimization and kinetics of glucose production via enzymatic hydrolysis of mixed peels. J. Bioresour. Bioprod. 5, 283–290. https://doi.org/10.1016/j.jobab.2020.10.007.
Xiang, H., Xin, R., Prasongthum, N., Natewong, P., Sooknoi, T., Wang, J., Reubroycharoen, P., Fan, X., 2022. Catalytic conversion of bioethanol to value-added chemicals and fuels: A review. Resour. Chem. Mater. 1, 47–68. https://doi.org/10.1016/j.recm.2021.12.002.
Zhang, L., Chen, Q., Jin, Y., Xue, H., Guan, J., Wang, Z., Zhao, H., 2010. Energy-saving direct ethanol production from viscosity reduction mash of sweet potato at very high gravity (VHG). Fuel Process. Technol. 91, 1845–1850. https://doi.org/10.1016/j.fuproc.2010.08.009.