

Bioethanol production from grass pea and wild oat hydrolysates using *S. cerevisiae* ETP53, *K. marxianus* ETP87, and *P. fermentans* ETP22

Asmamaw Tesfaw¹ · Deniz Kosebent² · Ebru Toksoy Oner³ · Fassil Assefa⁴

Received: 13 July 2021 / Accepted: 8 October 2021

Published online: 23 October 2021

© The Author(s) 2021 [OPEN](#)

Abstract

Conversion of lignocellulosic biomass into bioethanol is essential to reduce dependency on fossil fuels. After the grass pea and wild oat straws were characterized, they were hydrolyzed by live fungi (in situ), crude fungal enzymes, and chemical methods (sodium hydroxide and sulphuric acids at 0.5, 1, 2, and 3%). The fungi used for in situ degradation and crude lignocellulosic enzymes were *Aspergillus niger* JMC22344, *Trichoderma reesei* JMC22, *Pleurotus ostreatus* M2191, and *Pleurotus sajor-caju* M2145. Furfural content of hydrolysates was analyzed by GC–MS. The potential of activated charcoal, overliming, and sequential activated charcoal-overliming potential to reduce furfural in the acid hydrolysates were evaluated. The hydrolysates were fermented by *S. cerevisiae* ETP53, *K. marxianus* ETP87, and *P. fermentans* ETP22. Sulphuric acid hydrolyzed common wild oat (8.91 g/L) yielded higher sugars than NaOH (3.88 g/L) and NaOH treated wild oat (8.14 g/L) was superior in terms of sugars released than grass pea (4.21 g/L). *P. ostreatus* M2191 liberated the highest sugar (18.63 g/L) extracts than *T. reesei* JMC22676, *A. niger* ETP22344 and *P. sajor-caju* M2145. The 23–77% of the furfural in acid hydrolysates was reduced by overliming, activated charcoal, and the sequential activated charcoal-overliming. Yeasts produced optimal ethanol from straws hydrolyzed by crude enzymes from *A. niger* JMC22344. Generally, the ethanol titer was directly proportional to the amount of sugars released.

Keywords Bioethanol · Cellulase · Simultaneous saccharification fermentation · Separate saccharification fermentation · Lignocellulose · Hydrolysates

1 Introduction

The ever-increasing demand for energy and the problem of environmental pollution necessitate the search for renewable energy from various alternatives [1]. The biofuels obtained from agricultural residues, industrial wastes, and other lignocellulose sources are alternative cheap and sustainable sources of energy supply.

In 2016, 2.01 billion tons of solid waste was generated worldwide and it is expected to raise to 3.40 billion tons in 2050 [2]. Among the solid wastes, agricultural residues are important sources of biomass for ethanol production in Africa and the estimated total ethanol from agricultural residues and municipal solid waste (excluding paper) is 20.2 and 1.8 Billion liters per year respectively [3]. The highest available biomass was estimated to be 136 million tons per year in Western

✉ Asmamaw Tesfaw, asmamawtesfaw@dbu.edu.et; asmamawtesfawbelay2004@gmail.com; Deniz Kosebent, denizkosebent@gmail.com; Ebru Toksoy Oner, ebru.toksoy@marmara.edu.tr; Fassil Assefa, asefafasil2013@gmail.com; fasil.asefa@aaau.edu.et | ¹Department of Biology, Debre Berhan University, P.O. Box 445, Debre Berhan, Ethiopia. ²Department of Bioengineering, Marmara University, Goztepe Campus, P.O. Box 34722, Istanbul, Turkey. ³IBSB, Department of Bioengineering, Marmara University, Goztepe Campus, P.O. Box 34722, Istanbul, Turkey. ⁴Department of Microbial Cellular and Molecular Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia.



Africa followed by 123 million tons per year in Eastern Africa [3]. According to a World Bank report in 2012, Ethiopia has the potential to generate up to 14 million liter of ethanol that can be produced from its lignocellulosic agricultural and municipal residues.

Lignocellulosic materials are rigid structures consisting of mainly cellulose and hemicelluloses which are cross-linked and strongly bound to lignin [4]. This structural complexity makes them recalcitrant and they must be saccharified physically, chemically or/and biologically in order to release simple sugars to enhance ethanol fermentation. The biological saccharification includes enzymatic treatment and in situ degradation by cellulosic microorganisms whereas the chemical methods include acid, alkali, salt, solvent, ozone, and hydrogen peroxide treatment of which acid and alkali treatments are relatively cheap and efficient in hydrolyzing lignocelluloses [5].

The degradation of lignocellulosic residues releases phenolic compounds and furan derivatives as by-products that are inhibitors of microbial growth and ethanol fermentation [6]. Therefore, the detoxification of the lignocellulosic hydrolysates using physical, chemical and biological methods is important for successful hydrolysate fermentation process [7, 8]. A variety of methods are developed to remove the inhibitory substances of which the most inexpensive ones are over-liming [8–10] and activated charcoal treatment [11, 12].

Apart from physical and chemical treatments, co-culturing and enzyme treatment of hydrolysates can enhance ethanol production. Accordingly, Jang et al. [13] cocultured amylase producing fungus *Rhizopus oryzae* and *S. cerevisiae* in the presence of 5 mM NaCl to produce ethanol and coculture produced the highest amount of ethanol compared to monoculture control. Tanti paibulvut et al. [14] produced economically viable ethanol for fuel by direct fermentation of starch (from desizing waste) by *Bacillus subtilis* and *S. cerevisiae*. A coculture of *Saccharomyces diastaticus* (amylolytic yeasts) and *S. cerevisiae* 21 produced higher (48%) ethanol than the yield obtained with the monoculture [15].

Effective coculturing of *Aspergillus niger* and *K. marxianus* resulted in the maximum ethanol yield of 23.10 g/L [16]. In some studies, separate reactors for amylolytic (*A. niger*) and ethanologenic (*S. cerevisiae*) microorganisms were designed in one pool reactor system to solve different growing requirements for the two organisms [17]. *K. marxianus* MTCC1389 was able to produce 22.9 g/L ethanol from hydrothermal pretreated palm wood after hydrolyzing using *Trichoderma reesei* MTCC4876 [18].

All taken together, in order to produce ethanol from lignocellulosic residues, there is a need to select yeasts that can ferment both hexose and pentose sugars, tolerate inhibitors in the hydrolysate and give high ethanol yield [19].

In the first hand, even if grass pea (*Lathyrus sativus* L.) and common wild oat (*Avena fatua* L.) is economically important in developing nation; their biomass is considered as neglected agricultural residue [20, 21]. In some counties, the two crops might be categorized under neglected crops. On the other hand, the biomass productivity of the two crops is very high compared to other similar gain crops. All these make the biomass of grass pea and wild oat cheap and ideal to use them as substrate for bioethanol production.

The aim of this study was to evaluate the potential of using *S. cerevisiae* ETP53, *K. marxianus* ETP87, and *P. fermentans* ETP22 to ferment lime, activated carbon and cellulase enzyme treated acid/alkali hydrolysates of grass pea and wild oat to ethanol production.

2 Materials and methods

2.1 Sources and characterization of lignocelluloses

Two lignocellulosic substrates (wild oat and grass pea) were collected from different sources. Wild oat, also called common wild oat, (*Avena fatua* L.) straw was obtained from North Shoa Agricultural Research Center, Amhara Regional State and grass pea (*Lathyrus sativus* L.) straw was collected from Enemay Woreda, East Gojjam, Amhara Regional State.

Acid soluble lignin, alkali soluble lignin, moisture content and total solid of wild oat and grass pea were characterized using the National Renewable Energy Laboratory (NREL) method [22] and the result is presented in Table 1.

2.2 Sources of microorganisms

Pichia fermentans ETP22, *S. cerevisiae* ETP53, and *K. marxianus* ETP87 which were isolated locally from different sources were used for ethanol production [23]. *Pleurotus ostreatus* M2191 and *Pleurotus sajor-caju* M2345 were obtained from MYCELIA, Belgium whereas *Aspergillus niger* JCM22344 and *Trichoderma reesei* JCM22676 were obtained from Japan Collection of Microorganisms, Japan were used as source for cellulase enzyme.

Table 1 Wild oat and grass pea straws characterization

Variable	Wild oat	Grass pea
Total solid (%)	87.77 ± 0.96	86.89 ± 0.72
Moisture content (%)	12.23 ± 0.96	13.11 ± 0.72
Acid (1% v/v H ₂ SO ₄) soluble lignin (%)	3.87 ± 0.49	5.38 ± 0.41
Alkali (1% w/v NaOH) soluble lignin (%)	4.99 ± 0.35	7.17 ± 0.37
Protein (%)	0.91 ± 0.075	3.74 ± 0.062

2.3 Treatment of straws

Wild oat (3.13 g) and 3.11 g grass pea straws were transferred to screwed 250 mL bottles containing 100 mL of 0.5%, 1% and 3% sulfuric acid or sodium hydroxide [11]. The screw was closed very well but not tightly. The biomass was autoclaved at 121 °C for 90 min. After cooling, the pretreated biomass was filtered with filter paper to analyze the liquid portion for total reducing sugars and acid soluble lignin using a spectrophotometer whereas glucose, cellobiose, xylose, arabinose, and galactose were analyzed by high pressure liquid chromatography (HPLC). The furfural was quantified by GC–MS. The oven dried solid portion of previously treated straws was retreated with the same condition to evaluate for further possible saccharification.

2.4 Crude protein of grass pea and wild oat

Pre-dried grass pea (1 g) and wild oat (1 g) were put in 500 mL Kjeldahl digestion flask. A mixture of copper sulfate and potassium sulphate (10 g) and 25 mL of 0.1 N sulfuric acid was added. The flask was placed in an inclined position and heated gently. It was boiled for 30 min after the solution cleared. After cooling for 15 min, 250 mL of distilled water and then 90 mL of 40% NaOH solution were added. The distillation apparatus was connected with loops and 300 mL Erlenmeyer flask containing 30 mL boric acid indicator solution (methyl red plus methyl blue). Then it was distilled and titrated with 0.1 N HCl until colorless endpoint was reached.

$$\%N = \frac{1.4 \times (\text{ml sample titrant} - \text{ml blank titrant}) \times N \text{ of Sulfuric acid}}{\text{weight of sample}} \quad (1)$$

$$\%CP = N \times 8.25 \quad (2)$$

2.5 Furfural reduction in acid hydrolysates

The detoxification of acid hydrolysate was run by employing the method of Kuhad et al. [24]. First, the acid hydrolysates (0.5 and 1% H₂SO₄) of grass pea and wild oat were overlimed by mixing with calcium hydroxide until pH reached 10 [24]. Second, activated charcoal (1.5%, w/v) was added to acid hydrolysate according to Kuhad et al. [24]. The two mixtures were stirred with magnetic stirrer at higher speed for 30 min and 60 min to see also the effects of retention time on inhibitor reduction. Third, the overlimed hydrolysate was further mixed with activated charcoal (1.5%, w/v) and continuously stirred to investigate the synergetic effect of overliming and activated charcoal. Then, overlimed, activated charcoal and overlimed-activated charcoal treated acid hydrolysates were centrifuged at 8000 revolutions per minute (Eppendorf, Centrifuge 5418 R) to separate the precipitated calcium sulphate and activated charcoal from hydrolysates. The furfural was measured before and after treatment using GC–MS. The percentage of furfural reduction due to overliming and adsorbing with activated charcoal was calculated as

$$FR\% = \left[1 - \frac{FAT}{FBT} \right] \times 100 \quad (3)$$

where, FR=Percentage of furfural reduction, FAT=Furfural (g/L) after treatment with overliming and activated charcoal, FBT=Furfural (g/L) before treatment with overliming and activated charcoal.

Then, yeasts extract (0.1 g/L) and peptone (0.5 g/L), which were separately sterilized, was added and yeasts were inoculated and incubated at 30 °C.

2.6 Solid state fermentation (SSF) of cellulase producing fungi

Grass pea and wild oat straws were used as a substrate for solid state fermentation of *P. ostreatus* M2191, *P. sajor-caju* M2345, *A. niger* JCM22344 and *T. reesei* JCM22676. The fungi were grown for 8 days on PDA agar from which 1 mm × mm cube was transferred into 250-mL Erlenmeyer flasks containing the medium and 10 g of treated straw (at 3% (w/v) loading rate with 1% sulfuric acid for 1 h at 121 °C) and moistened with 15 mL of modified mineral salt solution in SSF [25]. The modified mineral salt solution contained (g/L): (NH₄)₂SO₄, 1.86; KH₂PO₄, 2.0; urea, 0.3; CaCl₂, 0.03; MgSO₄·7H₂O, 0.3; yeast extract, 2.0; FeSO₄·7H₂O, 0.005; MnSO₄·H₂O, 0.0016; ZnSO₄·7H₂O, 0.0014; CoCl₂, 0.002; (w/v) and peptone, 3.0. Flasks with untreated grass pea and wild oat straws were used as control. They were incubated at 25 °C for 8 days. After 8 days growth, 100 mL distilled water was added and shaken over night at 100 rpm and room temperature to extract the sugar and enzymes. The solid particles were filtered first with cheese cloth and then by suction through Whatman No. 1 filter paper. The reducing sugars released to the substrates were quantified by DNS [26].

2.7 Crude cellulase enzyme preparation and hydrolysis

One mL filtrate from above was centrifuged at 13,000g× for 10 min to remove cell debris and fungal spores and 3 mL cold acetone which was put at – 18 °C over-night was added to supernatant in acetone compatible tube. After vortexing, the acetone-supernatant mixture was incubated at – 18 °C for 2 h and centrifuged at 13,000g× (Eppendorf, Centrifuge 5418 R) for 10 min to separate crude enzymes from the mixture. The supernatant was carefully decanted. Finally, 0.5 mL acetate buffer at pH 5.0 was added to dissolve the enzyme pellet. Crude enzymes obtained from 10 mL of each culture filtrate were used to hydrolyze 1% (v/v) sulfuric acid pretreated straws (6% w/v on dry weight bases). As a positive control and comparison, carboxymethylcellulose (CMC) was hydrolyzed by crude enzymes from *A. niger* JMC22344. The mixture was incubated at 50 °C for 12 h. The sugar released was quantified by DNS [26]. The absorbance was measured at 540 nm (Jenway 6405 UV/Vis).

2.8 Yeast fermentation of acid or alkali hydrolysate

Bioethanol production from straw hydrolysate was carried out using three local yeast isolates; *P. fermentans* ETP22, *S. cerevisiae* ETP53, and *K. marxianus* ETP87 were isolated from compost, tella, and traditional yoghurt, respectively. The procedure was according to Jung et al. [11] and Martins et al. [27] with a little modification. The pH of acid and alkali treated hydrolysate were 0.9 and 13.2 respectively. The acid hydrolysate pH was adjusted near to 5 using first solid sodium hydroxide and then to 5.5 employing 3 N sodium hydroxide dropwise whereas the alkali hydrolysate pH was adjusted near to 5 using concentrated hydrochloric acid (45%) then to 5.5 by adding 3 N HCl dropwise. The solid NaOH and concentrated HCl were employed to avoid sugar dilution [27].

The hydrolysates were centrifuged at 10,000g× for 15 min and the filtrates were separated from solid particles by decanting. All these were done aseptically to prevent contaminations. Then 50 mL of the hydrolysates were supplemented with sterilized peptone (0.5 g/L) and yeast extract (0.1 g/L) and transferred into 250-mL flask, and inoculated with (approximately 7.5×10^6) *P. fermentans* ETP22, *S. cerevisiae* ETP53, and *K. marxianus* ETP87. They were incubated in shaker incubator at 30 °C and 130 rpm. Samples were taken at 8 and 24 h for yeast growth, sugar consumption, furfural content, butanol, isoamyl alcohol and ethanol analysis [11].

2.9 Ethanol fermentation of enzymatically degraded straws

The hydrolysates from enzymatic hydrolysis were boiled to reduce the volume by half and increase the sugar concentration two times. The hydrolysate (100 mL) was transferred to 250 Erlenmeyer flask and supplemented with yeast extract 1% and peptone 2% for ethanol production. Then it was inoculated with 7.5×10^6 cells of each yeast and incubated at 30 °C for 24 h.

2.10 Analytical methods

2.10.1 High pressure liquid chromatography

The analysis of sugar contents of hydrolysates (such as cellobiose, glucose, galactose, xylose, and arabinose) was performed by high pressure liquid chromatography (HPLC) (Agilent 1100Series). The hydrolysates were centrifuged at $14,500g\times$ (Eppendorf, Centrifuge 5418 R) two times for 10 min to remove any unsolublized materials or debris. The HPLC (Agilent 1100Series) was equipped with refractive index (RI) detector (G1362A) and quaternary pump (G1311A). MetaCarb Pb Plus (300 mm \times 7.8 mm, Agilent A5241) column was operated at 80 °C and the flow rate was 0.1 mL/min. The injection volume was adjusted at 20 μ L. The mobile phase consisted of 100% HPLC grade water.

2.10.2 Ethanol and furfural determination using gas chromatography

In order to determine the ethanol and furfural, the fermented products and hydrolysates were centrifuged at $14,000g\times$ for 5 min. The supernatant was analyzed by Gas Chromatography (Agilent 6890N) coupled with a mass spectrometer (MS) with headspace auto-sampler (Agilent 7694E). The GC–MS was equipped with Mass Selective Detector (Agilent-5973Network) and a polar polyethylene glycol (PEG) phase DB-wax122-7032 capillary column. Helium was used as a carrier gas. The flow rate for the column was 1 mL/min. The column temperature was held at 40 °C for 4 min, and then raised to 240 °C at 40 °C/min; the inlet temperature was 220 °C. The GC was operated with 20:1 split injection of the headspace. The mass-to-charge ratios (m/z) for ethanol was 31–45 m/z range with the retention time of 3.36 min and for furfural 32–95 m/z range with the retention time of 7.64 min. The MS Quadrapole, MS source and transfer line temperature were 150 °C, 230 °C, and 250 °C respectively. The conditions of the headspace autosampler were 25 min for the GC cycle time, 10 min for the vial equilibration time, 0.5 min for the pressurization time, 1 min for the injection time and a constant vial pressure of 14.0 psi. The temperatures are at 110 °C for the transfer line to the column and at 90 °C for the loop. The equilibration temperature was 80 °C for 10 min. Fermentation samples (200 μ L) were put in 10 mL headspace vials.

2.10.3 Yeast production

Yeast production was determined as a dry weight (g/L). One mL of yeast broth grown for 24 h was transferred into 1.5 mL eppendroff tube and centrifuged at $13,000g\times$ (Eppendorf centrifuge 5418 R, Germany). The pellets were carefully transferred and dried in dry oven at 70 °C for 3 days and weighed using precision balance (Radwag WAS220/C/2, Poland).

3 Results and discussion

3.1 Straw hydrolysis by dilute acid and alkali

The amount of reducing sugars released from acid- and alkali-treated lignocellulosic residues is shown in Table 2. The sugar yield of acid treated was between 5.3–8.9 g/L and 7.3–13.9 g/L from wild oat and grass pea, respectively compared with 3.4–4.2 g/L and 6.5–8 g/L obtained from the alkali treated wild oat and grass pea respectively, indicating that the sugar released from acid hydrolysis was 1.5 times higher than alkali hydrolysis. The sugar contents were drastically decreased when more than 2% concentrations of acid/alkali was used for the treatment. Jung et al. [11] attained optimal sugars (14.5 g per 100 g straw) from Thai Mission grass (*Pennisetum polystachyon*) which was hydrolyzed by 1% H_2SO_4 (w/v). In the first hand, Juarez et al. [28] reported the liberating of higher reducing sugar from spent coffee at 5% (v/v) sulfuric acid and a hydrolysis time of 3 h. On the other hand, more reducing sugars were obtained when the algal (*Chlorella vulgaris*) biomass was hydrolyzed by 3% (v/v) sulfuric acid [29]. Similarly, 3% (v/v) sulfuric acid treatment of palm wood liberated higher reducing sugars [18].

The sugar yield of 4.26–8.19 g/L obtained from 2% alkali treated substrates in this study is much lower than 6.6 g/100 g straw released at 3% (w/v) NaOH hydrolyzed mission grass pretreated with microwave Tatijarern et al. [30], and the

Table 2 Reducing sugars released by dilute acid and alkali hydrolysis at 121 °C for 90 min

Straw	Hydrolyzing chemical	Treating chemical concentration (% v/v)	Released Reducing sugars (g/L)	
Wild oat (<i>Avena fatua</i> L.)	H ₂ SO ₄	0.5	8.36	
		1	8.91	
		2	5.32	
	NaOH	3	0.42	
		0.5	3.42	
		1	3.88	
	Grass pea (<i>Lathyrus sativus</i> L.)	H ₂ SO ₄	2	4.26
			3	1.53
			0.5	13.03
NaOH		1	13.9	
		2	7.48	
		3	0.73	
		0.5	6.45	
		1	7.64	
		2	8.19	
		3	1.42	

application of 4–7% NaOH resulted in the degradation of monomeric sugars released from switch grass [31]. Therefore, the lower sugar at higher alkali and acid treating chemical might be the reason.

3.2 Hydrolysate sugar composition

The composition of the different types of sugars in the acid and alkali treated hydrolysates is shown in Table 3. In all cases, the concentration of xylose was higher than glucose, except from 1% NaOH hydrolyzed grass pea and this suggests that the acid treatment might attack the hemicellulose portion of lignocellulose. This shows that most of the xylose (75–90%) present in hemicellulose could be extracted by dilute acid pretreatment technology [32]. Similarly, de Cássia et al. [33] reported that D-xylose (19.19 g/L) was the major product in hydrolysates released from acid treated sugarcane bagasse hemicellulose whereas the concentrations of D-glucose (0.981 g/L) and L-arabinose (1.82 g/L) were very low. The authors showed that the mild hydrolysis condition was not enough to release D-glucose from the cellulose fraction. However, glucose was found to be dominant in dilute acid hydrolysis of algal biomass [29] and this might be higher starch present in the algae. Like de Farias Silva and Bertucco [29] studies and unlike this study, glucose concentration was higher than xylose in dilute acid hydrolysates of globe artichoke crop residues [34].

3.3 Ethanol production from acid and alkali hydrolysates

It is interesting to note that, although the acid-treated hydrolysates contained higher sugar contents than the alkali-treated ones, more ethanol was produced from the alkali treated ones (Table 4). Accordingly, the ethanol content from alkali-treated wild oat and grass pea hydrolysates was in the range of 0.8–2.6 g/L and 0.3–3.3 g/L, respectively. The higher ethanol was produced by *S. cerevisiae* ETP53 (2.6 g/L ethanol) (8 h) and *P. fermentans* ETP22 (4.2 g/L ethanol) (24 h) and 3.3 g/L (8 h). The lowest ethanol (1.8 g/L) was detected from *K. marxianus* ETP87 (24 h). *P. fermentans* ETP22 was able to grow on xylose, the dominant sugar in hydrolysates and it might use xylose for growth and leave the glucose for more

Table 3 Chemical components of acid and alkali hydrolysates (g/L)

Substrate	Treatment	Cellobiose	Glucose	Xylose	Arabinose	Galactose
Wild oat	1% H ₂ SO ₄	1	1.48	2.68	1.16	0.26
Wild oat	1% NaOH	0.28	0.46	0.84	0.9	0.16
Grass pea	1% H ₂ SO ₄	0.98	2.24	5.34	1.44	0.32
Grass pea	1% NaOH	0.2	1.22	0.24	0.38	0.22

Table 4 Ethanol production (g/L) from alkali and acid hydrolysate incubated at 30 °C in bath shaker

Straw	Treatment	<i>K. marxianus</i> ETP87		<i>S. cerevisiae</i> ETP53		<i>P. fermentans</i> ETP22	
		8 h	24 h	8 h	24 h	8 h	24 h
Wild oat	H ₂ SO ₄ (1%)	0.8 ^a	0.2 ^a	1.7	0.4	0.8	0.2
Wild oat	NaOH (2%)	1.3	1.4	2.6	0.8	1.3	4.2
Grass pea	H ₂ SO ₄ (1%)	0.4	0.6 ^a	0.9	0.4	0.4	0.5
Grass pea	NaOH (2%)	1.1	1.8	1.4	0.3	3.3	0.7

^aIsoamyl alcohol was detected even if its amount was very small

ethanol production. Huang et al. [35] suggested that xylose fermenting yeasts are highly needed to produce ethanol from acid hydrolysates that contains higher percentage of xylose than glucose. *Candida tropicalis* JH030 which was grown in non-detoxified rice straw acid hydrolysates containing 4.5 g/L glucose, 35.9 g/L xylose, and 4.3 g/L arabinose resulted 3.3 g/L ethanol [35]. The ethanol produced by the *C. tropicalis* JH030 was higher (3.3 g/L) than half of the glucose ($1/2 \times 4.5 = 2.25$) and this shows that *C. tropicalis* JH030 utilize xylose to produce ethanol.

In general, the yeast species showed variations in ethanol production upon different incubation periods. Thus *S. cerevisiae* ETP53 attained the highest concentration of ethanol (4.6 g/L) in 8 h; whereas *P. fermentans* ETP22 achieved optimum production of 4.2 g/L and 3.3 g/L in 24 h and 8 h on alkali treated hydrolysates. On the contrary, *K. marxianus* ETP87 did not show a significant pattern in ethanol production at different incubation times. Different studies have demonstrated variation on *K. marxianus* and *S. cerevisiae* potential to tolerate inhibitors and produce ethanol depending upon treatments, types of substrates and strains [36].

In this study, however, *K. marxianus* ETP87 was able to grow and ferment sugars both in acid and alkali hydrolysates but ethanol amount was lower than *S. cerevisiae* ETP53 (Table 4). *K. marxianus* is the efficient ethanol producer from non-Saccharomyces yeast and it is competent enough to produce ethanol from lignocellulosic hydrolysates [18]. Isoamyl alcohol was produced by only *K. marxianus* ETP87 that grew in acid treated wild oat and grass pea straws.

3.4 Detoxification of hydrolysates

The effect of treatment with liming and active carbon and their combination on the possible removal of the inhibitory by-products (furfurals) was tested on the acid treated hydrolysates (Table 5). The data showed that the combination of overliming and activated charcoal removed 77% of the furfurals from wild oat and 70% from grass pea, followed by treatment with overliming with 53% removal efficiency of furfural (wild oat) and 40% (grass pea) in almost 30 min. However, treatment of the hydrolysates with activated carbon removed furfural by 35% and 43% from grass pea and 23% and 30% from wild oat upon 30 and 60 min, respectively, indicating that raising the adsorption time from 30 to 60 min slightly, but not significantly, decreased the furfural removed by activated charcoal and overliming but raising time had no effect on the synergetic application of overliming and activated charcoal (Table 5). Activated charcoal reduced not only furfural but also other inhibitors organic acid (acetic and formic) and 5-hydroxymethylfurfural and this enhanced ethanol production by *S. cerevisiae* [37].

Table 5 Ethanol production before and after hydrolysate detoxification by activated charcoal and overliming (Incubation time was 24 h)

Substrate	H ₂ SO ₄	Detoxification method	Furfural reduction (%)		<i>K. marxianus</i> ETP87 (g/L)		<i>S. cerevisiae</i> ETP53 (g/L)		<i>P. fermentans</i> ETP22 (g/L)	
			30 min	60 min	Before	After ^a	Before	After ^a	Before	After ^a
Grass pea	0.5%	Overliming	52.94	53.22	0.91	1.46	1.11	1.69	0.84	0.92
Grass pea	0.5%	Activated carbon	35.29	42.67	0.91	1.23	1.11	1.41	0.84	0.91
Grass pea	0.5%	Overliming-activated charcoal	70.59	68.62	0.91	1.57	1.11	1.73	0.84	1.22
Wild oat	1%	Overliming	40.00	38.89	0.53	1.12	1.67	2.86	0.46	0.86
Wild oat	1%	Activated carbon	23.08	31.53	0.53	1.08	1.67	2.52	0.46	0.51
Wild oat	1%	Overliming-activated charcoal	76.92	77.45	0.53	1.18	1.67	2.73	0.46	0.83

^aAfter 30 min detoxification time

The removal efficiency of furfural by overliming, therefore, was higher than the 41.75% of furfural removal from acid hydrolysate (1.5% H₂SO₄) of *S. spontaneum* [38] and 38% removal from mission grass hydrolysates [10]. Kuhad et al. [24] reported that overliming reduced furfurals (by 34.28%) present in dilute acid hydrolysates of red sage (*Lantana camara*) while subsequent detoxification with activated charcoal resulted in further reduction of furfural (65.0%).

Combined detoxification through overliming followed by activated charcoal strongly reduced furfural content by 77.45% and 68.62% for grass pea and wild oat hydrolysate respectively whereas overliming and activated charcoal separately detoxified the furfural to a less extent (Table 5). In the first hand, Keshav et al. [39] reported that activated charcoal treatment and ammonia neutralization of acid hydrolyzed oil palm empty fruit bunches removed 90% of the furfural [11].

The potential of the yeasts to produce ethanol from detoxified and non-detoxified hydrolysates is shown in Table 5. All the three yeasts produced higher ethanol in detoxified hydrolysates than non-detoxified counter-parts. The yeast *S. cerevisiae* ETP53 and *K. marxianus* ETP87 produced more ethanol from wild oat hydrolysate than the grass pea. On the contrary, *P. fermentans* ETP22 produced relatively high ethanol on grass pea hydrolysate than wild oat. Likewise, *S. cerevisiae* ETP53 produced the highest ethanol (2.86 g/L) in wild oat hydrolysate overlimed with Ca(OH)₂ whereas *P. fermentans* ETP22 produced the lowest (0.51 g/L) ethanol in wild oat hydrolysates treated with activated charcoal (Table 5).

Fermentation of dilute acid hydrolysates of red sage by *Pichia stipitis* resulted in 5.16 g/L ethanol [24], which was higher than the present study. The maximum ethanol concentration produced by *P. stipitis* grown in over-limed acid hydrolysates of rice straw was 9.29 g/L ethanol which may be associated with xylose utilization [32]. In this study, however, even if *P. fermentans* ETP22 metabolized xylose, it did not produce enough ethanol. In general, the production of more ethanol in detoxified hydrolysates is due to increased cell growth and sugar consumption when inhibitors were reduced by different methods [12, 36, 37, 40].

3.5 Ethanol production by employing sugars extracted from solid state grown fungi

The effect of enzymatically treated hydrolysates from *T. reesei* JMC22676, *A. niger* IMC22344, *P. ostreatus* M2191, and *P. sajor-caju* M2345 in wild oat and grass pea straw is indicated in Table 6.

The data indicated that the amount of sugar produced in the hydrolysates of the two treated and untreated substrates was in the range of 7.25–18.63 g/L. The highest reducing sugar was derived from oat straw ranging from 12.72 to 18.63 mg/L (average 15.42) compared to the 7.25–10.41 mg/L (average = 8.91) released from grass pea straw indicating

Table 6 Ethanol production from sugars released by fungi in solid state fermentation of treated and untreated straws

Straws	Acid treatment condition	Cellulose degrading fungi	Reducing Sugar (g/L)	Ethanol (g/L) produced by		
				<i>K. marxianus</i> ETP87	<i>S. cerevisiae</i> ETP53	<i>P. fermentans</i> ETP22
Wild oat	Treated	<i>T. reesei</i> JMC22676	16.14 ± 0.34	6.32 ± 0.20	6.05 ± 0.13	5.23 ± 0.08
Wild oat	Untreated	<i>T. reesei</i> JMC22676	14.25 ± 0.26	4.79 ± 0.17	5.20 ± 0.04	3.50 ± 0.06
Wild oat	Treated	<i>A. niger</i> JMC22344	15.37 ± 0.25	5.17 ± 0.21	4.69 ± 0.15	4.75 ± 0.05
Wild oat	Untreated	<i>A. niger</i> JMC22344	14.62 ± 0.36	3.49 ± 0.36	3.32 ± 0.18	2.69 ± 0.02
Wild oat	Treated	<i>P. ostreatus</i> M2191	15.34 ± 0.14	6.47 ± 0.35	6.16 ± 0.05	5.71 ± 0.05
Wild oat	Untreated	<i>P. ostreatus</i> M2191	18.63 ± 0.49	8.25 ± 0.25	7.76 ± 0.1	6.95 ± 0.06
Wild oat	Treated	<i>P. sajor-caju</i> M2345	12.72 ± 0.40	6.20 ± 0.20	6.11 ± 0.02	5.29 ± 0.05
Wild oat	Untreated	<i>P. sajor-caju</i> M2345	16.35 ± 0.50	7.45 ± 0.35	7.45 ± 0.15	6.79 ± 0.13
Average			<u>15.4</u>	<u>6.01</u>	<u>5.84</u>	<u>5.11</u>
Grass pea	Treated	<i>T. reesei</i> JMC22676	9.38 ± 0.22	3.58 ± 0.35	3.30 ± 0.17	3.56 ± 0.06
grass pea	Untreated	<i>T. reesei</i> JMC22676	8.77 ± 0.35	2.85 ± 0.17	2.40 ± 0.10	2.08 ± 0.07
grass pea	Treated	<i>A. niger</i> JMC22344	8.79 ± 0.12	2.62 ± 0.20	2.54 ± 0.05	1.86 ± 0.03
grass pea	Untreated	<i>A. niger</i> JMC22344	8.56 ± 0.28	2.00 ± 0.20	1.86 ± 0.04	1.42 ± 0.08
grass pea	Treated	<i>P. ostreatus</i> M2191	8.69 ± 0.17	2.34 ± 0.05	2.36 ± 0.05	1.78 ± 0.03
grass pea	Untreated	<i>P. ostreatus</i> M2191	10.41 ± 0.34	3.53 ± 0.08	3.43 ± 0.05	2.45 ± 0.11
grass pea	Treated	<i>P. sajor-caju</i> M2345	7.25 ± 0.26	2.14 ± 0.15	1.91 ± 0.11	1.28 ± 0.01
grass pea	Untreated	<i>P. sajor-caju</i> M2345	9.39 ± 0.25	3.40 ± 0.10	2.97 ± 0.15	2.86 ± 0.06
Average			<u>8.9</u>	<u>2.81</u>	<u>2.6</u>	<u>2.16</u>

twice as much as reducing sugars released from oat. The quantity of reducing sugar showed a pattern in that untreated substrates contained more sugars in their hydrolysates than the treated ones. The lower sugar in treated straw might be associated with loss of sugars by treatment before the fungus was inoculated. The hydrolysate derived from *P. ostreatus* on oat straw was the highest (18.63 g/L) followed by *T. reesei* and *P. sajor-caju*.

The amount of ethanol produced from the hydrolysate also showed variations amongst the fermenting yeast (Table 6). Accordingly, *K. marxianus* ETP87 produced the highest amount of ethanol from oat hydrolysate (3.49–8.25 g/L) with average ethanol of 6.02 g/L, followed by *S. cerevisiae* ETP53 with 3.32–7.76 g/L with average ethanol production of 5.84; and *P. fermentans* ETP22, with a range of 2.69–6.95 g/L and mean of 5.11 g/L. However, Raja Sathendra et al. [18] were able to produce higher ethanol than this study by mixing, *K. marxianus* and *T. reesei* in acid pretreated palm wood.

The efficiency of ethanol production by all the yeasts corresponded with the amount of sugar released in acid treated/untreated but enzymatically treated wild oat with *P. ostreatus* M2191 (Fig. 1); followed by the same substrate treated with by *P. sajor-caju*. The data showed that all the yeast strains produced the highest concentration of ethanol derived from the hydrolysates of *P. ostreatus* grown on wild oat (6.95–8.25 g/L) which was almost twice higher than the ones treated with *A. niger* although the difference in the amount of sugar released by the latter was only 21%.

Singhania et al. [1] reported that 10.5 g/L ethanol was generated by *K. marxianus* MTCC 4136 from 27 g/L glucose released by crude enzymatic activity from acid pre-treated and crude cellulases from *Penicillium janthinellum*.

In this study, the ethanol concentration in sugars released by crude enzymes obtained from treated substrates was lower than acid or alkali hydrolysates and fungi hydrolysate. This is due to 10% of enzymes extracted from 15 g of solid state fermented fungi were used to degrade 12 g of pretreated straws. Cunha et al. [41] suggested that due to complex structure and recalcitrant nature of lignocellulose, high crude enzyme loadings are needed in the production of ethanol from biomass.

3.6 Yeast production from acid and alkali hydrolysates

The yeast productivity in acid hydrolyzed straws was higher than alkali hydrolyzed straws (Fig. 2). The yeast dry weight obtained from *P. fermentans* ETP22 was higher on 1% acid treated grass pea with 7.0 g/L (biomass) followed by 6 g/L

Fig. 1 Ethanol production from acid pre-treated wild oat (A) and grass pea (B) straws hydrolysed by fungi crude enzymes

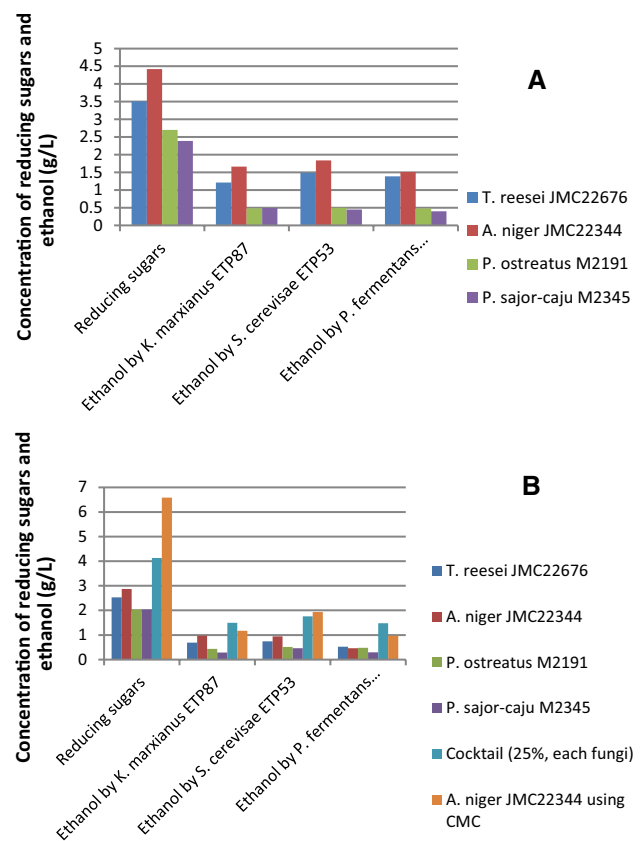


Fig. 2 Yeast production in acid and alkali hydrolysates incubated at 30 °C for 24 h

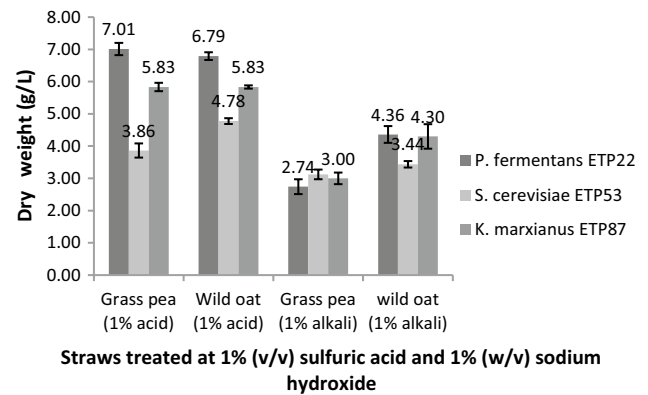
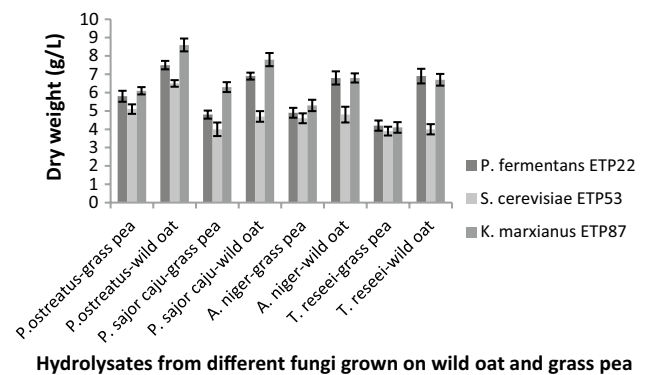


Fig. 3 Yeast dry weights from straws degraded by in situ growing fungi



from *K. marxianus* and 4–5 g/L with *S. cerevisiae* on the same substrate, respectively. The biomass recovered from acid-hydrolysates was higher than alkali-hydrolysates (Fig. 2). Minimum yeast dry biomass was obtained in all yeasts grown on grass pea treated with 1% (w/v) sodium hydroxide. Generally, yeast growth and ethanol production are inversely related since most of the sugars are utilized for cell growth and functions during yeast growth.

3.7 Biomass production from in situ degraded straws

The effect of fungal treated hydrolysates on biomass production by the three test yeasts is shown in Fig. 3. Accordingly, *K. marxianus* ETP87 and *P. fermentans* ETP22 gave higher biomass than the *S. cerevisiae* ETP53; except on *P. sajor-caju* and *T. reesei* treated grass pea, the biomass production ranged from 5.0 to 8.0 g/L. The pattern showed that more biomass was recovered from wild oat than grass pea. This was much higher than 2 g/L biomass obtained from *S. cerevisiae* grown on the hydrolysates of Chrysanthemum waste by *P. ostreatus* degradation [42].

4 Conclusion and recommendations

The ethanol concentration produced from straws varies depending on the amount of sugars and inhibitors released during chemical hydrolysis. Alkali hydrolysis released less furfural and solubilized more lignin than acid hydrolysis. Both alkali and acid hydrolysis of grass pea resulted in higher sugar and ethanol production than wild oat. The pentose sugars, which were not fermented by *S. cerevisiae* ETP53, *P. fermentans* ETP22, *K. marxianus* ETP87, were dominant in chemical hydrolysates of wild oat and grass pea. This further reduces the ethanol concentration.

All the detoxifying methods such as overliming, activated carbon, and overliming followed by activated charcoal reduced the furfural content of acid hydrolysates. Prolonged time (greater than 30 min) for overliming and overliming-activated charcoal did not further reduce the furfural content of acid hydrolysate but activated carbon increased the reduction when the incubation time was increased up to 60 min.

Authors' contributions AT: conceptualization, investigation, methodology, data analysis and writing first draft. DK: conceptualization, methodology, and data analysis. ETO: funding acquisition, resources, supervision and validation. FA: resources, supervision, validation, review and editing. All authors read and approved the final manuscript.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Singhania RR, Saini JK, Saini R, Adsul M, Mathur A, Gupta R, et al. Bioethanol production from wheat straw via enzymatic route employing *Penicillium janthinellum* cellulases. *Biores Technol.* 2014;169:490–5.
2. The World Bank. Solid waste management. Washington, DC: The World Bank; 2019.
3. Bensah EC, Kemausuor F, Miezah K, Kádár Z, Mensah M. African perspective on cellulosic ethanol production. *Renew Sustain Energy Rev.* 2015;49:1–11.
4. Pu Y, Hu F, Huang F, Davison BH, Ragauskas AJ. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. *Biotechnol Biofuels.* 2013;6(1):15. <https://doi.org/10.1186/1754-6834-6-15>.
5. Bensah EC, Mensah M. Chemical pretreatment methods for the production of cellulosic ethanol: technologies and innovations. *Int J Chem Eng.* 2013. <https://doi.org/10.1155/2013/719607>.
6. Sarawan C, Suinyuy T, Sewsynker-Sukai Y, Kana EG. Optimized activated charcoal detoxification of acid-pretreated lignocellulosic substrate and assessment for bioethanol production. *Bioresource Technol.* 2019;286: 121403.
7. Tesfaw A, Assefa F. Current trends in bioethanol production by *Saccharomyces cerevisiae*: substrate, inhibitor reduction, growth variables, coculture, and immobilization. *Int Sch Res Notices.* 2014. <https://doi.org/10.1155/2014/532852>.
8. Nandal P, Sharma S, Arora A. Bioprospecting non-conventional yeasts for ethanol production from rice straw hydrolysate and their inhibitor tolerance. *Renew Energy.* 2020;147:1694–703. <https://doi.org/10.1016/j.renene.2019.09.067>.
9. De Bari I, Cuna D, Di Matteo V, Liuzzi F. Bioethanol production from steam-pretreated corn stover through an isomerase mediated process. *New Biotechnol.* 2014;31(2):185–95.
10. Prasertwasu S, Khumsupan D, Komolwanich T, Chaisuwan T, Luengnaruemitchai A, Wongkasemjit S. Efficient process for ethanol production from Thai Mission grass (*Pennisetum polystachion*). *Biores Technol.* 2014;163:152–9.
11. Jung YH, Kim IJ, Kim HK, Kim KH. Dilute acid pretreatment of lignocellulose for whole slurry ethanol fermentation. *Biores Technol.* 2013;132:109–14.
12. Kim S-K, Park D-H, Song SH, Wee Y-J, Jeong G-T. Effect of fermentation inhibitors in the presence and absence of activated charcoal on the growth of *Saccharomyces cerevisiae*. *Bioprocess Biosyst Eng.* 2013;36(6):659–66.
13. Jang S-W, Kim J-S, Park J-B, Jung J-H, Park C-S, Shin WC, et al. Characterization of the starch degradation activity from newly isolated *Rhizopus oryzae* WCS-1 and mixed cultures with *Saccharomyces cerevisiae* for efficient ethanol production from starch. *Food Sci Biotechnol.* 2015;24(5):1805–10. <https://doi.org/10.1007/s10068-015-0235-4>.
14. Tantipaibulvut S, Pinisakul A, Rattanachaisit P, Klatin K, Onsriprai B, Boonyaratsiri K. Ethanol production from desizing wastewater using co-culture of *Bacillus subtilis* and *Saccharomyces cerevisiae*. *Energy Procedia.* 2015;79:1001–7.
15. Verma G, Nigam P, Singh D, Chaudhary K. Bioconversion of starch to ethanol in a single-step process by coculture of amylolytic yeasts and *Saccharomyces cerevisiae* 21. *Biores Technol.* 2000;72(3):261–6.
16. Manikandan K, Viruthagiri T. Simultaneous saccharification and fermentation of wheat bran flour into ethanol using coculture of amylolytic *Aspergillus niger* and thermotolerant *Kluyveromyces marxianus*. *Front Chem Eng China.* 2009;3(3):240–9. <https://doi.org/10.1007/s11705-009-0205-6>.
17. Jeon BY, Kim DH, Na BK, Ahn DH, Park DH. Production of ethanol directly from potato starch by mixed culture of *Saccharomyces cerevisiae* and *Aspergillus niger* using electrochemical bioreactor. *J Microbiol Biotechnol.* 2008;18(3):545–51.
18. Raja Sathendra E, Baskar G, Praveenkumar R, Gnansounou E. Bioethanol production from palm wood using *Trichoderma reesei* and *Kluyveromyces marxianus*. *Biores Technol.* 2019;271:345–52. <https://doi.org/10.1016/j.biortech.2018.09.134>.
19. Chandel AK, da Silva SS, Singh OV. Detoxification of lignocellulose hydrolysates: biochemical and metabolic engineering toward white biotechnology. *BioEnergy Res.* 2013;6(1):388–401. <https://doi.org/10.1007/s12155-012-9241-z>.
20. Dixit GP, Parihar AK, Bohra A, Singh NP. Achievements and prospects of grass pea (*Lathyrus sativus* L.) improvement for sustainable food production. *Crop J.* 2016;4(5):407–16. <https://doi.org/10.1016/j.cj.2016.06.008>.
21. Esfeld K, Uauy C, Tadele Z. Application of TILLING for orphan crop improvement. In: Jain SM, Dutta Gupta S, editors. *Biotechnology of neglected and underutilized crops*. Dordrecht: Springer; 2013. p. 83–113.

22. Scarlata C, Sluiter J, Templeton D, Crocker D. Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory-NREL/TP-510-42618 Laboratory Analytical Procedure (LAP) Golden, CO. 2011.
23. Tesfaw A, Oner ET, Assefa F. Optimization of ethanol production using newly isolated ethanogenic yeasts. *Biochem Biophys Rep.* 2021;25:100886.
24. Kuhad RC, Gupta R, Khasa YP, Singh A. Bioethanol production from lantanacamura (red sage): pretreatment, saccharification and fermentation. *Biores Technol.* 2010;101(21):8348–54.
25. Trivedi N, Reddy C, Radulovich R, Jha B. Solid state fermentation (SSF)-derived cellulase for saccharification of the green seaweed *Ulva* for bioethanol production. *Algal Res.* 2015;9:48–54.
26. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem.* 1959;31(3):426–8.
27. Martins LB, Soares J, da Silveira WB, Sousa RdCS, Martins MA. Dilute sulfuric acid hydrolysis of *Chlorella vulgaris* biomass improves the multistage liquid-liquid extraction of lipids. *Biomass Conv Bioref.* 2020. <https://doi.org/10.1007/s13399-020-00661-w>.
28. Juarez GFY, Pabiloña KBC, Manlangit KBL, Go AW. Direct dilute acid hydrolysis of spent coffee grounds: a new approach in sugar and lipid recovery. *Waste Biomass Valor.* 2018;9(2):235–46. <https://doi.org/10.1007/s12649-016-9813-9>.
29. de Farias Silva CE, Bertuccio A. Dilute acid hydrolysis of microalgal biomass for bioethanol production: an accurate kinetic model of biomass solubilization, sugars hydrolysis and nitrogen/ash balance. *React Kinet Mech Catal.* 2017;122(2):1095–114. <https://doi.org/10.1007/s11144-017-1271-2>.
30. Tatijarern P, Prasertwasu S, Komalwanich T, Chaisuwan T, Luengnaruemitchai A, Wongkasemjit S. Capability of Thai Mission grass (*Penisetum polystachyon*) as a new weedy lignocellulosic feedstock for production of monomeric sugar. *Biores Technol.* 2013;143:423–30.
31. Hu Z, Wen Z. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. *Biochem Eng J.* 2008;38(3):369–78.
32. Huang C-F, Lin T-H, Guo G-L, Hwang W-S. Enhanced ethanol production by fermentation of rice straw hydrolysate without detoxification using a newly adapted strain of *Pichia stipitis*. *Biores Technol.* 2009;100(17):3914–20.
33. de Cássia LR, Rocha GJ, Rodrigues D, Filho JH, de Graças AFM, Pessoa A. Scale-up of diluted sulfuric acid hydrolysis for producing sugarcane bagasse hemicellulosic hydrolysate (SBHH). *Bioresource Technol.* 2010;101(4):1247–53.
34. Pesce GR, Fernandes MC, Mauromicale G. Globe artichoke crop residues and their potential for bioethanol production by dilute acid hydrolysis. *Biomass Bioenerg.* 2020;134: 105471. <https://doi.org/10.1016/j.biombioe.2020.105471>.
35. Huang C-F, Jiang Y-F, Guo G-L, Hwang W-S. Development of a yeast strain for xylitol production without hydrolysate detoxification as part of the integration of co-product generation within the lignocellulosic ethanol process. *Biores Technol.* 2011;102(3):3322–9.
36. Moreno AD, Ibarra D, Ballesteros I, González A, Ballesteros M. Comparing cell viability and ethanol fermentation of the thermotolerant yeast *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* on steam-exploded biomass treated with laccase. *Biores Technol.* 2013;135:239–45.
37. Sarawan C, Suinyuy TN, Sewsynker-Sukai Y, Gueguim Kana EB. Optimized activated charcoal detoxification of acid-pretreated lignocellulosic substrate and assessment for bioethanol production. *Biores Technol.* 2019;286: 121403. <https://doi.org/10.1016/j.biortech.2019.121403>.
38. Chandel AK, Narasu ML, Chandrasekhar G, Manikyam A, Rao LV. Use of *Saccharum spontaneum* (wild sugarcane) as biomaterial for cell immobilization and modulated ethanol production by thermotolerant *Saccharomyces cerevisiae* VS 3. *Biores Technol.* 2009;100(8):2404–10.
39. Keshav PK, Shaik N, Koti S, Linga VR. Bioconversion of alkali delignified cotton stalk using two-stage dilute acid hydrolysis and fermentation of detoxified hydrolysate into ethanol. *Ind Crops Prod.* 2016;91:323–31. <https://doi.org/10.1016/j.indcrop.2016.07.031>.
40. Kumar A, Singh L, Ghosh S. Bioconversion of lignocellulosic fraction of water-hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to ethanol by *Pichia stipitis*. *Biores Technol.* 2009;100(13):3293–7.
41. Cunha FM, Badino AC, Farinas CS. Effect of a novel method for in-house cellulase production on 2G ethanol yields. *Biocatal Agric Biotechnol.* 2017;9:224–9. <https://doi.org/10.1016/j.bcab.2017.01.007>.
42. Quevedo-Hidalgo B, Monsalve-Marín F, Narváez-Rincón PC, Pedroza-Rodríguez AM, Velásquez-Lozano ME. Ethanol production by *Saccharomyces cerevisiae* using lignocellulosic hydrolysate from *Chrysanthemum* waste degradation. *World J Microbiol Biotechnol.* 2013;29(3):459–66.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.