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ORIGINAL ARTICLE

Production of xylitol and ethanol from acid and enzymatic hydrolysates of *Typha latifolia* by *Candida tropicalis* JFH5 and *Saccharomyces cerevisiae* VS3

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Abstract
Currently, there is an increased usage of lignocellulosic biomass for production of various renewable bioproducts. In the present study, an aquatic weed *Typha latifolia* (commonly known as cattail) was used as substrate for xylitol and bioethanol production. Initially, dilute alkali pretreatment with sodium bisulfite (2% w/v $NaHSO_3$; at room temperature for 18 h) led to maximum of 75.30 ± 1.45% delignification with 1.11 ± 0.32% sugar loss, while dilute acid hydrolysis (at 121 °C for 60 min with2% H_2SO_4) of alkali-pretreated *T. latifolia* released 13.30 ± 0.30% g/L xylose sugars with 0.83 ± 0.08 g/L phenolics and 0.50 ± 0.08 g/L furfurals. After chemical detoxification (overliming and activated charcoal treatment) of acid hydrolysate, the phenolics and furfurals concentration reduced to 72.95% and 80.38%, respectively. Furthermore, enzymatic saccharification of cellulosic-rich residue of alkali acid–treated *T. latifolia* showed maximum 658.40 ± 10.14 mg/g sugar yield with 79.70 ± 1.42% saccharification efficiency. Subsequently, fermentation of detoxified acid hydrolysate with pentose utilizing yeast *Candid tropicalis* JFH5 produced 6.15 ± 0.17 g/L xylitol with 0.65 g/g yield. Furthermore, enzymatic saccharification of cellulosic-rich biomass released 11.20 ± 0.92 g/L sugars; which on subsequent fermentation with *Saccharomyces cerevisiae* VS3 produced 6.90 ± 0.30 g/L ethanol with 0.43 g/g yield.

Keywords *Typha latifolia* · Xylitol · Bioethanol · Saccharification · *Candida tropicalis* · *Saccharomyces cerevisiae* · Fermentation

1 Introduction

The increased consumption of energy and decreased fossil fuel reserves have stimulated researchers around the world to search for alternative, renewable, and sustainable energy sources. Bioproducts such as xylitol and ethanol produced from lignocellulosic material are eco-friendly and renewable; moreover, produces less greenhouse gas emissions compared to fossil fuels [1, 2]. Generally, any lignocellulosic biomass is composed of two sugar polymers viz. cellulose, hemicellulose, and aromatic polymer lignin. Cellulose and hemicelluloses act as a carbon source for the production of value-added chemicals such as ethanol and xylitol, while lignin remains most underutilized aromatic polymer which is recalcitrant in nature [3, 4].

Among various lignocellulosic materials, weedy lignocelluloses are the most promising biomass feedstocks employed for production of value-added products. Weeds such as *Typha latifolia*, *Eichhornia crassipes*, *Lantana camara*, *Prosopis juliflora*, and *Saccharum spontaneum* are cheaply available weedy lignocelluloses that grow abundant on agriculturally degraded lands or water bodies and do not require additional economic input [5]. *Typha latifolia*, also known as common cattail, is an invasive perennial leafy aquatic tropical herb which grows up to height of 3 m in height. It grows and spreads very fast in canals, ponds, stream banks, and slow-flowing waters, and considered a weed because of its faster growing rate and its invasion to arable lands and irrigation systems [6]. *T. latifolia* can be potential carbon feedstock for the production of value-added products such as xylitol and bioethanol, owing to its high holocellulose composition, productivity

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(50 – 60 ton/ha/year), easy delignification (high *S*/*G* ratio), high adaptability, and pest resistance [7].

Xylitol is a five-carbon sugar alcohol, has been recognized as one of the 12 important value-added chemicals to be produced from biomass. It is an artificial sweetener employed as a sugar substitute in dietary and pharmaceutical industries, with various beneficial properties such as low energy content, anticarcinogenic and possesses insulin-dependent metabolism [8, 9]. At present, industrial production of xylitol involves chemical hydrolysis and hydrogenation of xylan rich hemicellulose fraction, which is a costly and labor-intensive process. Alternatively, bioconversion of xylose to xylitol via fermentation-based process is an eco-friendly, less energy-intensive, and overall cost-effective process [8–10].

In any lignocellulose conversion, pretreatment of biomass stands most important and challenging step. An ideal pretreatment method should eliminate maximum lignin fraction from biomass with lower sugar loss, and on subsequent saccharification has to give maximum sugar yield from holocellulose fraction [11]. The initial step in biotechnological production of xylitol involves pretreatment of lignocellulosicbiomass, and dilute acid hydrolysis of pretreated biomass to release maximum amount of pentose sugars into hydrolysate, which can be fermented to xylitol using pentose utilizing yeast [12]. The purpose of conducting acid hydrolysis of biomass is to release sugars from the hemicellulosic fraction and as well as improving the access of the cellulolytic enzymes to cellulose. Dilute acid treatment can be applied either to pretreat the lignocellulosic biomass preceding enzymatic saccharification or to directly depolymerize the lignocellulosic polymers to fermentable sugars [13]. During dilute acid hydrolysis method, numerous lignocellulosic inhibitors are formed which exhibit undesirable effects on fermentation. To overcome these issues, various physicochemical detoxification methods are carried out to decrease the inhibitor concentration in hydrolysate [9]. Physicochemical detoxification methods like overliming with Ca $(OH)_2$, adsorption with activated charcoal, alteration of pH, ion exchange resins, and biological detoxification are most commonly used methods of detoxification of lignocellulosic hydrolysates [9, 14, 15].

Various bacterial, fungal, and yeast strains are known to produce xylitol, and many of them belong to *Candida* genus. These include *Candida tropicalis*, *C. guilliermondii*, *C. boidinii*, *Debaryomyces hansenii*, and *Pachysolen tannophilus*, which were found to be efficient xylitol producers. Among them, *C. tropicalis* is considered an inhibitor tolerant, pentose sugar utilizing yeast, which produces xylitol from hemicellulosic hydrolysate of the agro-wastes [16, 17], while for bioethanol fermentation, *Saccharomyces cerevisiae*, a hexose (glucose) fermenting yeast, is extensively used, which is known to withstand harsh fermentation conditions including thermotolerance, high ethanol and inhibitor tolerance [2].

The major interest of the current study is to evaluate *T. latifolia* as a potential lignocellulosic substrate for xylitol and bioethanol production. The present work aims to fully exploit hemicellulosic and cellulosic fractions of *T. latifolia*, and its possible bioconversion to xylitol and bioethanol, respectively. In previous studies, low yields of xylitol and ethanol were reported due to inability to maintain optimal fermentation condition for xylitol and ethanol production. This is because of variations in process parameters such as aeration, temperature employed during xylitol and ethanol production [9]. Hence, to improve the xylitol and ethanol yields, fermentation of hemicellulosic and cellulosic hydrolysate was separately carried out with *C. tropicalis* and *S. cerevisiae*, respectively. Though there are previous studies on bioethanol production from *T. latifolia*, to the best of our knowledge, this is the initial report on bioconversion of alkali-pretreated *T. latifolia* to xylitol using *C. tropicalis* yeast strain. Figure 1 shows the experimental overview of the present study for the bioconversion of *T. latifolia* to xylitol and bioethanol employing *C. tropicalis* JFH5 and *S. cerevisiae* VS3 yeast strains.

2 Materials and methods

2.1 Preparation and compositional analysis of raw material

T. latifolia was collected from Mandhapur, Siddipet Dist., and Telangana state of India. The straw was oven dried (60 °C for 2 days) and processed to attain a particle size of 1 – 5 mm. The milled biomass was washed under tap water to remove extraneous matter, and subsequently oven dried (50 ± 0.5 °C) to attain moisture content below 10%. The processed material was sealed in plastic bags and kept at room temperature until further use. The cellulose, lignin, and hemicellulose contents of *T. latifolia* were determined according to standard laboratory protocols as described by Slutier et al. [18].

2.2 Chemical pretreatment of T. latifolia

Chemical delignification of *T. latifolia* was carried out by treating the biomass (5 g; 10% w/v solid loading) with different alkaline chemicals for overnight (18 h) at room temperature. The different alkaline chemicals used for delignification include 1% (v/v) ammonium hydroxide (NH_4OH), 1% (w/v) of calcium hydroxide [Ca $(OH)_2$], sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium dithionite ($Na_2S_2O_4$), and sodium bisulphite ($NaHSO_3$). Among alkaline chemicals studied, best alkaline chemical was selected

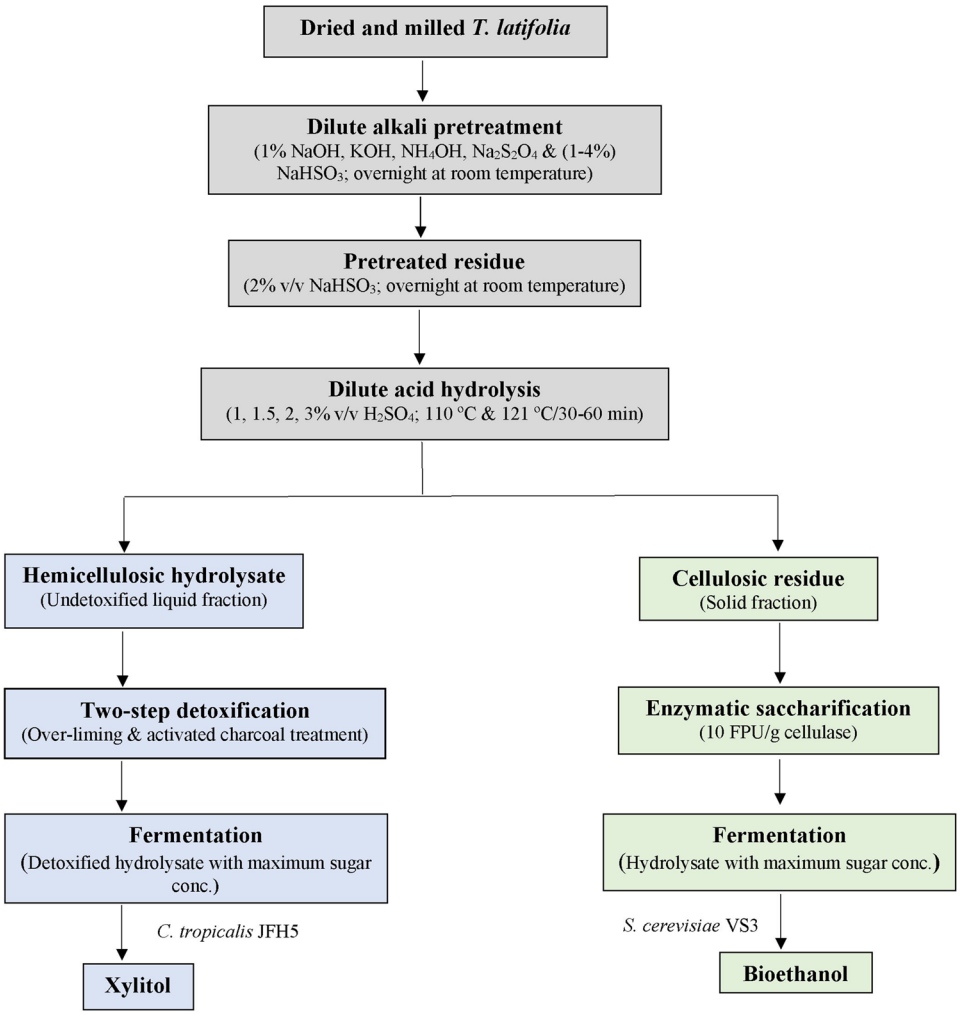

Fig. 1 Experimental overview of xylitol and bioethanol production from pretreated T. latifolia with *C. tropicalis* and *S. cerevisiae* yeast strains

based on maximum delignification and further improved its delignification efficiency at different concentrations. After pretreatment, the slurry of delignified biomass was filtered with muslin cloth, and the separated residue was washed repeatedly with water until neutral pH. The leftover residue was dried in an oven and further used for dilute acid hydrolysis.

2.3 Dilute acid hydrolysis of T. latifolia

Five grams of delignified *T. latifolia* was subjected to dilute acid hydrolysis using various concentrations of sulphuric acid [1.0%, 1.5%, 2.0%, and 3.0% (v/v)], and autoclaved at temperatures (110° and 121 °C) for 30–60 min reaction time. After hydrolysis, the slurry was separated into liquid hydrolysate and solid cellulosic rich residue. The liquid acid hydrolysate was quantified for sugars, phenolics, and furfurals, while residues biomass was neutralized with tap water, oven-dried, and later used for enzymatic hydrolysis.

2.3.1 Detoxification of acid hydrolysate of *T. latifolia*

The acid hydrolysate of *T. latifolia* has been subjected to chemical detoxification, according to the protocol as described by Naseeruddin et al. [19]. Initially, overliming with calcium hydroxide was performed until pH of the hydrolysate reached to 10.5 ± 0.05. Later, inhibitors were precipitated by incubating for 1 h with stirring at regular intervals. Subsequently, the slurry was vacuum filtered, and the filtrate pH was set to 6 with 6 N sulphuric acid. The vacuum filtration was repeated again to eliminate traces of salt precipitates. Finally, the resultant hydrolysate was again detoxified with activated charcoal (2.5%, w/v), with constant stirring at room temperature for 1 h. The resultant mixture was vacuum filtered and centrifuged at 3000 rpm for 20 min. After sequential detoxification, the concentration of sugars and inhibitors was estimated by HPLC, and subsequently used for fermentation studies.

2.4 Enzymatic hydrolysis of cellulosic residue

Delignified, acid-treated residue of *T. latifolia* was used for enzymatic saccharification. Saccharification was carried out at 10% (w/v) biomass loading, using citrate phosphate buffer 0.05 M, (pH 5.0). Initially, cellulosic residue was soaked in citrate buffer for 1 h, and then, cellulase enzyme (Accellerase 1500, Genencor, USA) with 30 FPU/g enzyme loading was added, while the surfactant Tween 80 was added to improve the enzymatic reaction. At 50 °C, 150 rpm, the enzymatic saccharification was carried out for 72 h time period. Samples were collected at regular time intervals and centrifuged at 6000 g for 10 min, and the collected supernatant was analyzed for total reducing sugars.

Saccharification efficiency (%) = 1/4 × reducing sugar concentration obtained × 0.98 × 100 / potential sugar concentration in the pretreated residue.

2.5 Microorganisms and maintenance

Candida tropicalis JFH5 was isolated from fruit pulp samples. The yeast strain was characterized biochemically and genetically identified based on 5.8 s rRNA, 28 s rRNA genes (accession number KP703846.1), and internal transcribed sequences ITS1 and ITS2 in GenBank database (www.ncbi.nlm.nih.gov/BLASTn). The yeast was maintained on agar slants containing (g/L): xylose, 3; yeast extract, 1; peptone, 2; agar, 20 at pH 5.0 and stored at temperature 30 °C. The yeast isolate was cultivated in medium containing (g/L): xylose 30, peptone 20, yeast extract 10, KH_2PO_4 1, NH_4SO_4, 0.5; $MgSO_4.7H_2O$, 1. The pH was adjusted to 5.5 and later incubated at 30 °C on shaker incubator at 150 rpm for overnight. The cells were collected after they attained exponential phase and centrifuged for 5 min at 13000 g. The pellet obtained was washed with distilled water and further used as inoculum for fermentation of xylitol.

Saccharomyces cerevisiae VS3 is lab isolate, kindly provided by Kiran Sree et al. [20]. The yeast strain was maintained on agar slants containing yeast extract, peptone dextrose agar (YEPD). The medium consisted of (g/L): yeast extract, 10; peptone, 20; dextrose, 20; and agar, 25; and pH was maintained at 5.0. Stock cultures of yeast were maintained at 4 °C and sub cultured after every 2 weeks. The yeast inoculum preparation consists of 30 g/L dextrose, 10 g/L yeast extract, 20 g/L peptone at pH 5.0.

2.6 Fermentation of detoxified, hemicellulosic hydrolysate to xylitol

The detoxified, hemicellulosic acid hydrolysate with the highest concentration of sugar was employed for xylitol fermentation by pentose fermenting yeast *C. tropicalis* JFH5. The hydrolysate of *T. latifolia* was supplemented with (g/L): yeast extract 1, peptone 2.5, $MgSO_4$ $7H_2O$ 1, $CaCl_2$ 1, KH_2PO_4 1.5, NH_4NO_3 1, and pH of media was adjusted to 6.0. The hemicellulosic hydrolysate was sterilized at110 °C for 15 min; after cooling the media, *C. tropicalis* (10% v/v; OD_{600} 0.6) was aseptically inoculated into the fermentation media. The xylitol fermentation was carried out at 30 ± 0.50 °C for 96 h time period at 150 rpm. Samples were collected after every 12 h interval and centrifuged at 10,000 g for 15 min at 4 °C. The cell free supernatant was filtered and analyzed for xylose and xylitol by HPLC.

2.7 Fermentation of cellulosic sugars of pretreated *T. latifolia* to ethanol

For bioethanol production, enzymatic hydrolysate of *T. latifolia* containing the highest sugar concentration was supplemented with (w/v): 0.1% peptone, yeast extract, NH_4Cl, KH_2PO_4 and 0.05% of $MnSO_4.5H_2O$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $ZnSO_4.7H_2O$, and $FeCl_3.2H_2O$. The media pH was adjusted to 5.5, and autoclaved at 110 °C for 20 min, cooled, and further inoculated with *S. cerevisiae* (10% v/v; OD_{600} 0.6) and fermented at 30 ± 0.50 °C for 48 h by agitating at 150 rpm. The fermentative broth was sampled at regular intervals of 12 h, cold centrifuged at 10,000 g for 15 min, and the supernatant was determined for sugar consumption and ethanol production.

2.8 Analytical methods

2.8.1 Estimation of total reducing sugars and inhibitors

The total reducing sugars in hydrolysate was quantified by DNS method [21], while phenolics released after delignification and acid hydrolysis was estimated by UV–Visible spectrophotometer using Folin-Ciocalteus method as per Singleton et al. [22]. Finally, furans released during acid hydrolysis of biomass were determined spectrophotometrically by protocol as described by Martinez et al. [23].

2.8.2 Estimation of sugars and sugar alcohol (xylitol) and ethanol

For HPLC analysis of xylose and xylitol, samples were filtered by cellulose acetate filters (0.22 µm) prior to HPLC analysis. The column employed was Zodiac Carb 70C (Zodiac Lifesciences, India), deionized water was used as elutent at 70 °C, with a flow rate of 0.6 mL per minute, using refractive index detector (RID), while ethanol was analyzed using gas chromatography (Shimadzu GC-2011, Japan) with ZB-Wax column (30 mm × 0.25 mm) and a flame ionization detector (FID). The column temperature was maintained at 150 °C with run time of 5.5 m using nitrogen gas as the carrier (16 kPa). The injector temperature and the detector

temperature were maintained at 175 °C and 250 °C, respectively, with flow rate of 40 ml/min, and spilt ratio of 1/50, with velocity of H_2 flow at 60 ml/min.

2.8.3 Structural analysis of pretreated T. latifolia by scanning electron microscopy and X-Ray diffraction studies

To reveal the morphological changes caused in the biomass by pretreatment, scanning electron microscopy (SEM) analysis of pretreated and untreated cattail was performed. The dried samples were mounted on aluminum stubs and later sputter-coated with a gold 160 layer (JEC 300). The microphotographs of the sputter-coated biomass were acquired using JSM-6610LV (JEOL, Japan) scanning electron microscope. Finally, the crystalline nature of pretreated T. latifolia was studied by obtaining X-Ray diffraction (XRD) patterns on XRD 175 diffractometer (X-Calibur-S Single Crystal) with Cu kappa platform as X-ray source. The 2θ value ranged from 5 to 45°. Calculation of crystallinity index (CrI) was done using equation: $CrI = [(I_{002} - I_{am})/ I_{002}] \times 100$, where CrI indicates % of crystallinity index, while I_{200} represents maximum intensity of diffraction for 2θ between 22 and 23°, and I_{am} indicates intensity of diffraction for 2θ between 18 and 19° for cellulose I.

3 Results and discussion

3.1 Chemical composition of T. latifolia

The raw material (dried stem) of *T. latifolia* was analyzed using standard laboratory methods to determine the major components such as cellulose, hemicellulose, and lignin. The raw substrate used in this study was found to contain 37.58 ± 1.48% cellulose, 22.90 ± 0.92% hemicellulose, 18.36 ± 0.45% lignin, 5.75 ± 0.46 ash, and 9.80 ± 0.28% extractives. The occurrence of 60.48% (based on the dry weight of the substrate) high percentage of hemicellulose fraction and holocellulose makes *T. latifolia* a potential and renewable substrate for xylitol and other value added bioproducts such ethanol production. The cellulose, hemicellulose, and lignin content of *T. latifolia* obtained in the present study is in agreement with previous study reports of Aysu et al. [5]. The content of cellulose (34.5%) and lignin (26.4%) of *T. latifolia* reported by Zhang et al. [24] was not similar the current study. The change in the feedstock composition may be due to season, geographic location, processing, and analysis methods used for determination of chemical composition [2]. In the current study, *T. latifolia* was found to contain 18.36% lignin content, which necessitates an appropriate and effective pretreatment method to eliminate lignin and enhance the biomass digestibility.

3.2 Dilute alkali pretreatment of *T. latifolia*

In the present study, dilute alkali pretreatment of *T. latifolia* was carried out with different alkaline chemicals to determine maximum delignification of biomass. Delignification results were compared in percentage of delignification efficiency. The sugar loss for each pretreatment was also reported with the aim of minimizing hemicellulose removal from biomass delignification. Figure 2a displays the percentage of delignification of *T. latifolia* with different alkaline chemicals with 1% (w/v or v/v) concentrations at room temperature for overnight time period. On initial

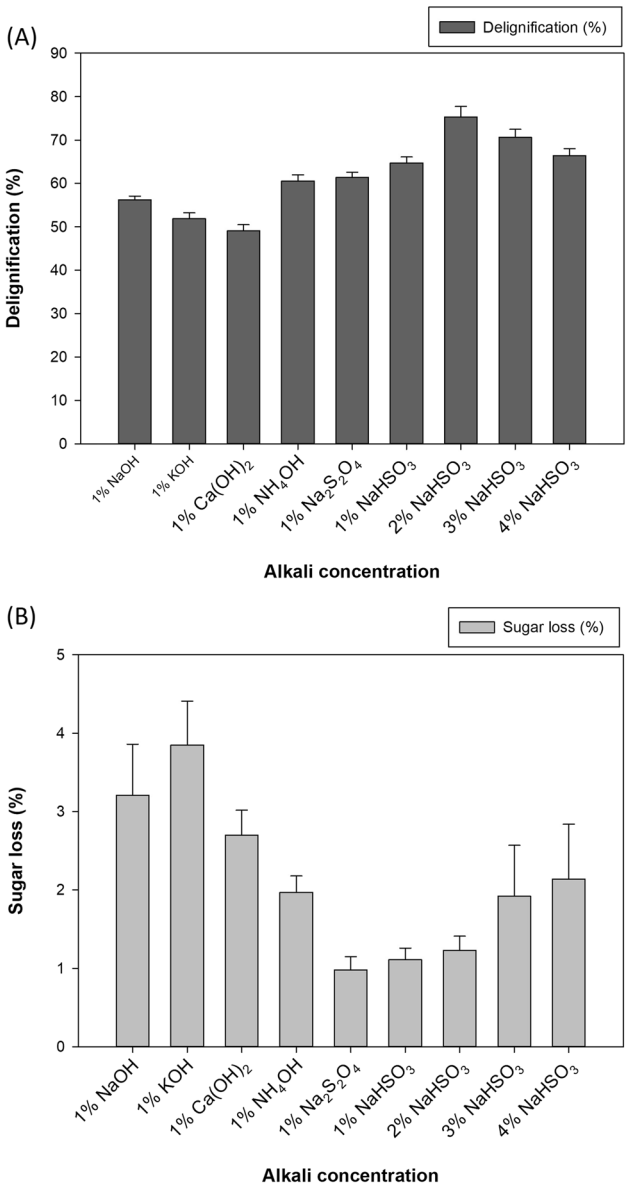

Fig. 2 Dilute alkali pretreatment of *T. latifolia* using NaOH, KOH, $Ca(OH)_2$, NH_4OH, Na_2S2O_4, and $NaHSO_3$. **a** Delignification (%), **b** sugar loss (%)

screening with different alkaline chemicals, the delignification of *T. latifolia* was ranged between 49.10 ± 1.40% [1% w/v Ca $(OH)_2$] and 64.70 ± 1.18% (1% w/v $NaHSO_3$). Sodium bisulphite ($NaHSO_3$) showed significant ($p < 0.05$) lignin removal compared to other alkaline chemicals studied. Furthermore, $NaHSO_3$ delignification efficiency was further studied at different concentrations (1–4% w/v). It is found that 75.30 ± 1.45% maximum lignin removal ($p < 0.05$) was reported with 2% w/v $NaHSO_3$ for 18 h time period. It was also observed that there was a significant decrease in lignin removal at 3–4% w/v $NaHSO_3$ concentrations with increased sugar loss. During dilute alkaline pretreatment of *T. latifolia*, percentage of sugar loss was studied (Fig. 2b), which was ranged between 0.98 ± 0.03% (1% v/v $Na_2S_2O_4$) and 3.85 ± 0.76% (3% KOH). After pretreatment, the amount of holocellulose in *T. latifolia* was changed between 60.48 ± 0.20% (raw material) and 81.50 ± 0.64% (2% $NaHSO_3$) of the dry weight of pretreated residue. Our dilute alkaline pretreatment data was comparable with aquatic weed *Saccharum spontaneum*, carried out by Chaudhary et al. [25], who reported maximum of 51% delignification using 7% w/v NaOH for 48 h time period.

3.2.1 Dilute acid hydrolysis of alkali-treated *T. latifolia*

To maximize the xylose sugars for xylitol production, dilute acid hydrolysis of alkali-pretreated *T. latifolia* was performed with different acid concentrations (1.0, 1.5, 2.0, and 3.0% v/v H_2SO_4); temperatures and reaction times (110 °C/30–60 min & 121 °C/30–60 min). The efficiency of acid hydrolysis (%), with corresponding xylose sugar concentration and release of lignocellulosic inhibitors viz. phenolics and furfurals, was studied during dilute acid hydrolysis (Table 1). During dilute acid hydrolysis of pretreated *T. latifolia*, xylose sugar concentration was ranged from 05.25 ± 0.28 g/L (1.0% H_2SO_4; 110 °C for 30 min) to maximum of 13.80 ± 0.30 g/L (2.0% H2SO4; 121 °C for 60 min) (Table 1), while inhibitors phenolics and furfural concentrations ranged between 0.48 ± 0.09 (1.0% H_2SO_4, 110 °C for 30 min) and 0.90 ± 0.07 g/L (3.0% H_2SO_4, 121 °C for 60 min); 0.20 ± 0.01 (1.0% H_2SO_4, 110 °C for 30 min) and 0.58 ± 0.08 g/L (3.0% H_2SO_4, 121 °C for 60 min), respectively. The maximum xylose sugar concentration (13.80 ± 0.30 g/L) with 61.36 ± 1.40 hydrolysis efficiency was obtained at 121 °C for 60 min using 2.0% H_2SO4, with 0.83 ± 0.08 g/L phenolics and 0.50 ± 0.08 g/L furfurals. However, at higher acid concentration (3.0% H_2SO_4) and temperature (121 °C for 60 min), the xylose sugar concentration (10.05 ± 0.94) was decreased due to harsh reaction conditions, resulting in high concentration of phenolics and furfurals. Furthermore, after sequential detoxification (overliming and adsorption with activated charcoal) of acid hydrolysate of *T. latifolia*, the concentration of phenolic and furfurals reduced by 72.95 ± 0.23% and 80.38 ± 0.22%, respectively. Lopez-linares et al. [26] carried out dilute acid hydrolysis (2% v/v H_2SO_4) of rapeseed at 130 °C for 1 h and reported 10.30 g/L xylose sugar concentration, which is in agreement with

Table 1 Total sugars, hydrolysis efficiency (%), and inhibitors profile from dilute acid hydrolysis of pretreated *T. latifolia* at different parameters

Temperature/reaction time	Acid conc. (% H_2SO_4 v/v)	Xylose concentration (g/L)	Hydrolysis efficiency (%)	Phenolics (g/L)	Furans (g/L)
110 °C/30 min	1.0	$05.25^{D} \pm 0.28$	$23.18^{D} \pm 1.08$	$0.48^{C} \pm 0.09$	$0.20^{C} \pm 0.01$
	1.5	$06.40^{C} \pm 0.45$	$29.82^{C} \pm 0.92$	$0.62^{B} \pm 0.05$	$0.30^{B} \pm 0.07$
	2.0	$08.68^{A} \pm 0.85$	$39.75^{A} \pm 1.20$	$0.71^{A} \pm 0.02$	$0.37^{B} \pm 0.04$
	3.0	$07.72^{B} \pm 0.72$	$34.50^{B} \pm 1.35$	$0.77^{A} \pm 0.08$	$0.42^{A} \pm 0.03$
110 °C/60 min	1.0	$08.15^{B} \pm 0.37$	$36.82^{C} \pm 1.63$	$0.55^{D} \pm 0.09$	$0.27^{C} \pm 0.09$
	1.5	$08.95^{B} \pm 0.45$	$40.45^{B} \pm 0.86$	$0.68^{C} \pm 0.05$	$0.32^{B} \pm 0.08$
	2.0	$09.75^{A} \pm 0.36$	$44.09^{A} \pm 1.02$	$0.76^{B} \pm 0.04$	$0.40^{A} \pm 0.05$
	3.0	$09.48^{A} \pm 0.62$	$40.91^{B} \pm 0.80$	$0.81^{A} \pm 0.07$	$0.45^{A} \pm 0.02$
121 °C/30 min	1.0	$10.57^{A} \pm 0.89$	$47.73^{C} \pm 0.98$	$0.64^{C} \pm 0.02$	$0.32^{C} \pm 0.08$
	1.5	$10.66^{A} \pm 0.16$	$48.18^{B} \pm 1.32$	$0.72^{B} \pm 0.05$	$0.36^{C} \pm 0.05$
	2.0	$10.95^{A} \pm 0.58$	$49.55^{A} \pm 1.40$	$0.79^{B} \pm 0.08$	$0.44^{B} \pm 0.04$
	3.0	$10.18^{A} \pm 0.40$	$46.36^{D} \pm 1.20$	$0.85^{A} \pm 0.06$	$0.50^{A} \pm 0.07$
121 °C/60 min	1.0	$11.70^{C} \pm 0.95$	$50.91^{D} \pm 0.85$	$0.71^{C} \pm 0.07$	$0.39^{C} \pm 0.06$
	1.5	$12.52^{B} \pm 0.25$	$57.73^{B} \pm 1.21$	$0.78^{C} \pm 0.05$	$0.42^{B} \pm 0.05$
	2.0	$13.80^{A} \pm 0.30$	$61.36^{A} \pm 1.40$	$0.83^{B} \pm 0.08$	$0.50^{A} \pm 0.08$
	3.0	$10.05^{D} \pm 0.94$	$56.75^{C} \pm 0.87$	$0.90^{A} \pm 0.07$	$0.58^{A} \pm 0.04$

Values superscripted in each treatment by A–D are ranking the highest to the lowest of significant; same alphabets are insignificant according to Tukey grouping method test significance difference test ($p < 0.05$)

our current study. Our dilute acid hydrolysis data is also well supported by Leonel et al. [27], carried out dilute acid (100 mg H_2SO_4 per one-gram substrate) pretreatment of apple pomace at 121 °C for 20 min and reported 11.1 g/L xylose sugars with 0.30 g/L, 0.15 g/L furfurals and hydroxy-methyl furfural (HMF), respectively.

3.2.2 Enzymatic hydrolysis of alkali- and acid-treated *T. latifolia* cellulosic residue

The neutralized cellulosic-rich residue obtained after alkali and acid treatment of *T. latifolia* was used for enzymatic saccharification studies to release maximum cellulosic sugars for bioethanol production. Enzymatic saccharification of pretreated *T. latifolia* was determined by studying sugar yield (mg/g pretreated substrate) and saccharification efficiency (%). As illustrated in Fig. 3, enzymatic hydrolysis of alkali acid–treated *T. latifolia* showed maximum 658.40 ± 10.14 mg/g sugar yield with 79.70 ± 1.42% saccharification efficiency at 30 FPU/g cellulase after 72 h time period. It was observed that during enzymatic hydrolysis of alkali acid–treated *T. latifolia*, there is significant ($p < 0.05$) increase in sugar yield until the end of saccharification period. Naseeruddin et al. [19] carried out sequential acid and enzymatic hydrolysis of *Prosopis juliflora* with sodium dithionite ($Na_2S_2O_4$) and reported 68.56% saccharification efficiency. Keshav et al. [28] studied combined acid-enzymatic saccharification of steam exploded cotton stalk and reported 8.50 g/L of total sugar concentration with 84.20% saccharification efficiency at 20 FPU/g cellulase loading, after 72 h time period.

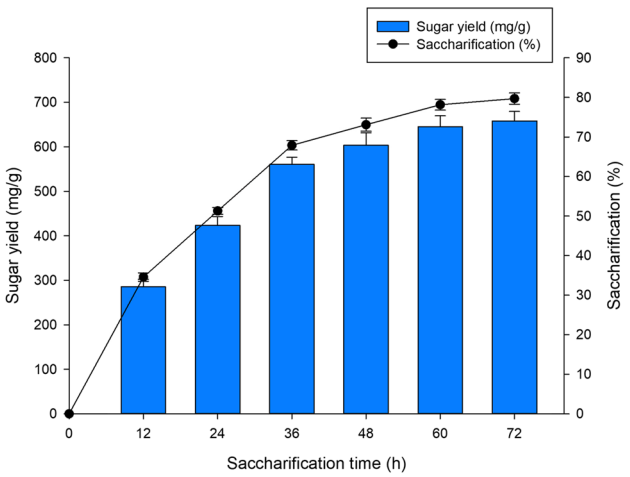

Fig. 3 Enzymatic saccharification of alkali acid–treated *T. latifolia* (Enzymatic hydrolysis was conducted at 10% (w/v) solid loading in 50 mM acetate buffer (pH 5.0), at 30 FPU/g cellulase loading for 72 h time period)

3.3 Structural characterization of pretreated *T. latifolia*

3.3.1 Structural analysis by SEM

The morphological and structure changes caused during alkaline pretreatment and dilute acid hydrolysis of *T. latifolia* were studied and compared with native (untreated) biomass using scanning electron microscopy (Fig. 4a–4d). It is clearly evident from the Fig. 4a that untreated *T. latifolia* appears to be intact, smooth, fibrous structures arranged in orderly fashion, while alkali-pretreated biomass showed (Fig. 4b) rough, defibrated, and irregular surface with cracks in lignocellulosic matrix, which might be the indication of lignin degradation in biomass. Furthermore, acid- and enzyme-saccharified *T. latifolia* biomass was appeared to be more diverse in structural changes compared to pretreated biomass with more pores and swelling in lignocellulosic matrix of *T. latifolia* (Fig. 4c–4d). The similar types of SEM observations were also observed by Keshav et al. [11] and Chandel et al. [29].

3.3.2 Structural analysis by XRD

The percentage of crystallinity index (CrI) is the amount of crystalline material present in lignocellulosic material, and generally specifies the contact of crystalline portion of biomass surface. Among the lignocellulosic components of native and pretreated *T. latifolia*, only cellulose component of biomass known to possess crystalline structure and others such as hemicellulose and lignin are of an amorphous nature. Figure 5 shows diffractograms and crystallinity index of the treated and untreated samples of *T. latifolia*. It was observed that after acid hydrolysis of pretreated biomass, the percentage of crystallin index was increased, which corresponds to observation of more intense peaks in the diffractograms, which indicates the removal of amorphous components (lignin and hemicellulose) of biomass. The increased crystallinity was generally due to hemicellulose removal, which is generally observed during acid treatment and/or hydrolysis of lignocellulosic biomass. Our XRD results are in agreement with Xavier et al. [30], who reported a 78.50% increase in crystallinity after acid pretreatment, while the untreated biomass sample showed the lowest rate (44.89%) of crystallinity verifying the occurrence of extra amorphous regions.

3.4 Production of xylitol from detoxified hemicellulosic hydrolysate by C. tropicalis JFH5 strain

The detoxified hemicellulosic hydrolysate containing 12.85 ± 0.38 xylose sugar concentration was used for xylitol production with *C. tropicalis* JFH5 strain, and fermentation

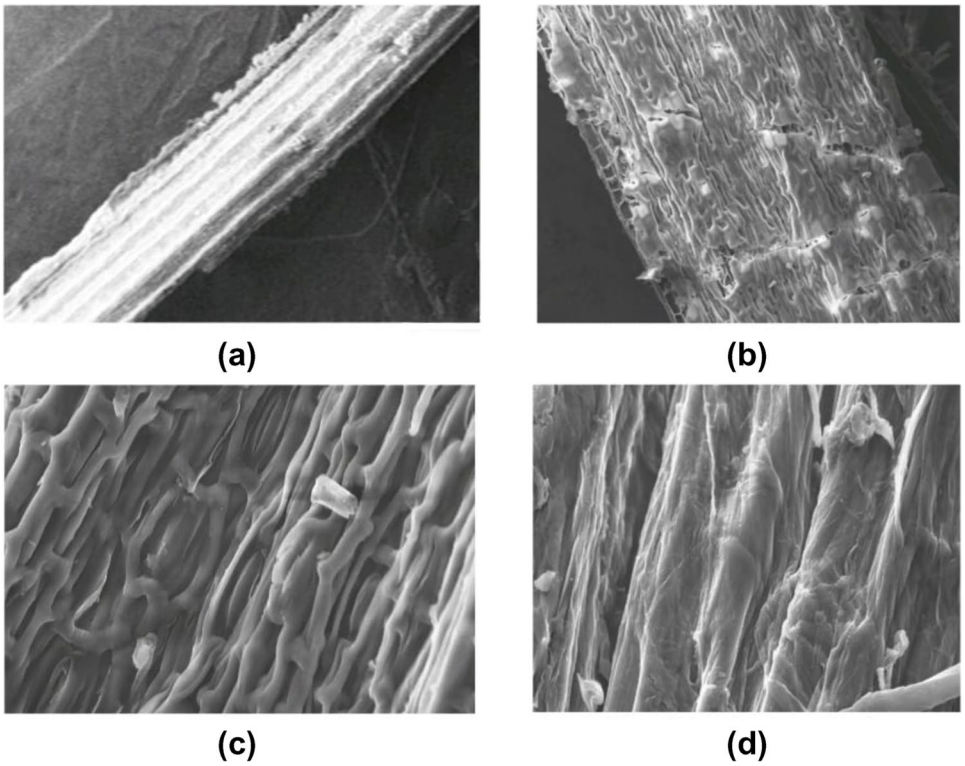

Fig. 4 Scanning electronic microscopic (SEM) images of *T. latifolia* samples. **a** Untreated *T. latifolia* biomass. **b** (2%) Sodium bi sulphite pretreated T. *latifolia* biomass. **c** Acid hydrolysed T. *latifolia* at 121 °C for 60 min with 2% Dilute H_2SO_4. **d** Enzyme-saccharified *T. latifolia* with 30FPU/g cellulase enzyme

was carried out until 96 h time period. Figure 6a shows the fermentation profile of xylitol production and as well xylose sugar consumption of *C. tropicalis* JFH5 at different time periods. It was observed that production of xylitol was significantly ($p < 0.05$) increased with increasing time period until 72 h; thereafter, xylitol production was significantly decreased. The maximum xylitol production 6.15 ± 0.17 g/L with a yield of 0.65 g/g was attained at 72 h time period, with 9.45 ± 0.80 xylose sugar consumption (Fig. 6a). The xylose-to-xylitol fermentation efficiency of *C. tropicalis* was found to be 71.59%. The xylitol yield obtained in the present study was found to be similar with studies performed by Singh et al. [31], who reported 0.60 g/g xylitol yield from rice straw acid hydrolysate using *C. tropicalis* MTCC 6192 strain. In another study, Jia et al. [32] performed xylitol production from corn cob hemicellulosic acid hydrolysate using *C. tropicalis* strain, and reported 0.77 g/g xylitol yield. Vallejos et al. [33] studied and reported xylitol yield of 0.46 g/g from hemicellulosic hydrolysate of sugarcane bagasse using *C. tropicalis* yeast strain.

3.5 Bioethanol production from enzymatic hydrolysate by S. cerevisiae VS3 strain

The enzymatic hydrolysate of *T. latifolia* containing total sugars (18.90 ± 0.55 g/L) was employed for ethanol production with *S. cerevisiae* VS3 strain, and fermentation was carried out until 72 h time period. Figure 6b shows sugar consumption, ethanol production, and yield at different time periods. It was observed that ethanol production was significantly ($p < 0.05$) increased with increasing time period until 48 h, thereafter, production of ethanol was ($p < 0.05$) decreased. The decrease in ethanol concentration in fermentation media after 48 h time period might be due to concomitant utilization of accumulated sugars and ethanol by the adapted yeast strain in fermentation media [2]. The maximum ethanol production 6.90 ± 0.30 g/L with 0.43 g/g yield was achieved at 48 h time period, with 15.80 ± 0.68 sugar consumption (Fig. 6b). In the present study, sugar-to-ethanol fermentation efficiency of *S. cerevisiae* strain was found to be 85.71%. Furthermore, the ethanol yield obtained in the present study is in agreement with previous studies conducted by Keshav et al. [2], reported 0.44 g/g ethanol yield from cotton stalk enzymatic hydrolysate using same yeast strain.

Xavier et al. [30] evaluated simultaneous production of xylitol and ethanol from sisal fiber hemicellulosic hydrolysate with *C. tropicalis* CCT 1516 strain, and reported maximum ethanol (0.27 g/g) and xylitol (0.32 g/g) yields after 60 h time period. Sehnem et al. [34] studied xylitol production from rice hull hydrolysate using furaldehyde-tolerant yeast strain *Wickerhamomyces anomalus*, and reported 0.86 g/g xylitol yield. In the same study, they have also reported 0.37 g/g ethanol yield from soybean hull hydrolysate using furaldehyde-tolerant strains of *S. cerevisiae*. In another study, Du et al. [35] studied two-stage fermentation of ethanol and xylitol from

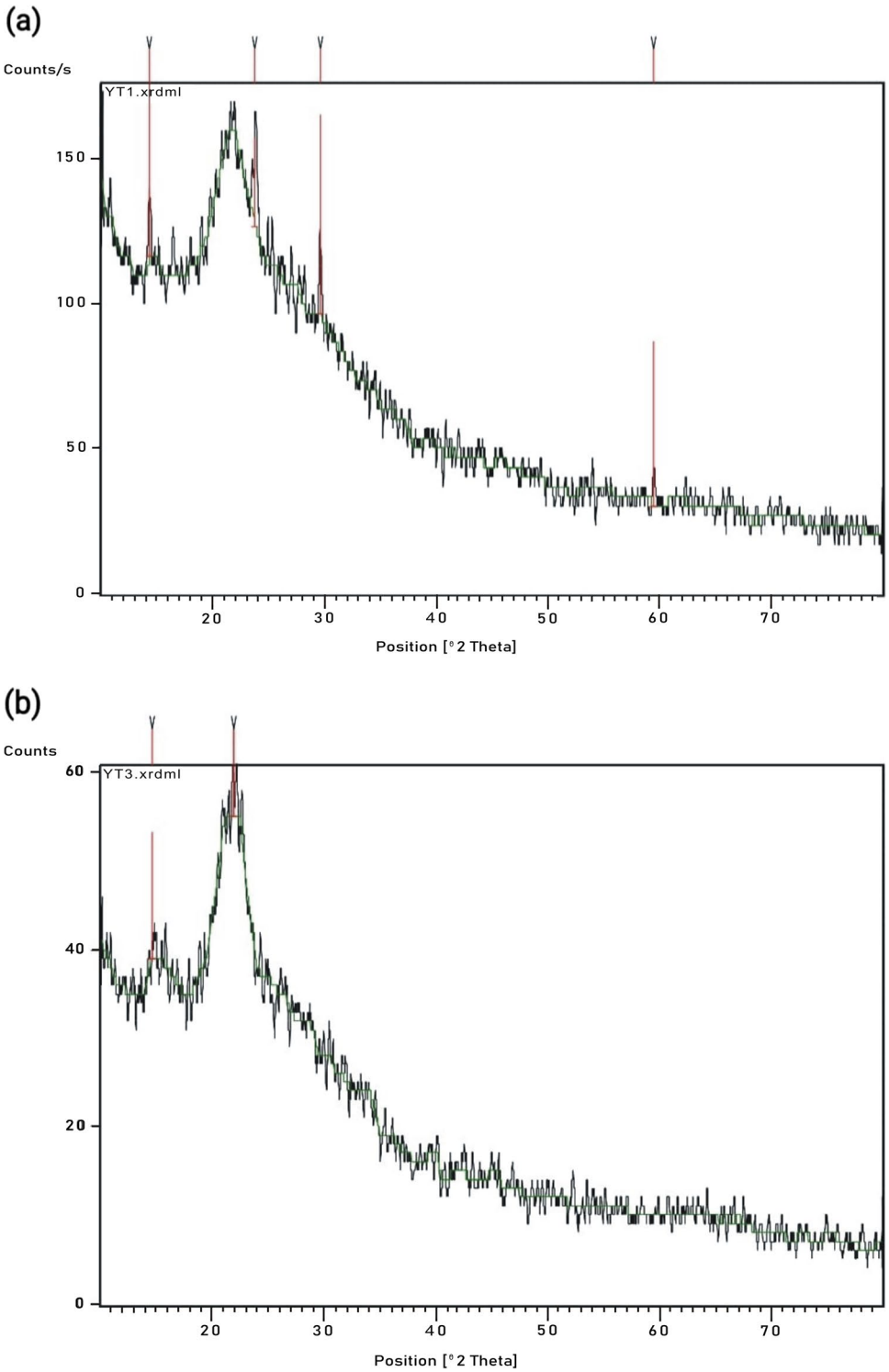

Fig. 5 **a** XRD patterns of untreated *T. latifolia*. **b** Acid-treated *T. latifolia*

non-detoxified corn cob hydrolysate using *Kluyveromyces marxianus* yeast strain, and reported 0.41 g/g ethanol yield and 0.82 g/g xylitol yield.

4 Conclusions

In the present work, *T. latifolia* (cattail) was utilized as potential substrate for xylitol and bioethanol production. To improve the digestibility of biomass, *T. latifolia* was subjected to alkali pretreatment, which on subsequent acid saccharification

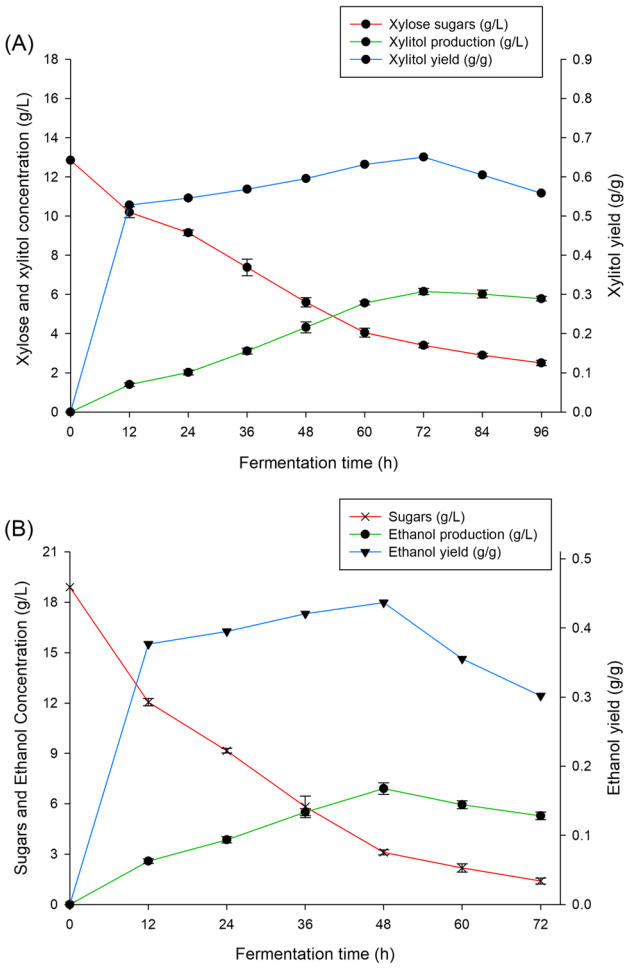

Fig. 6 **a** The time course of xylose sugar consumption, xylitol production, and yield of *C. tropicalis* JFH5 using detoxified hemicellulosic hydrolysate. **b** The time course of sugar consumption, ethanol production, and yield of *S. cerevisiae* VS3 using *T. latifolia* enzymatic hydrolysate

produced xylose rich hemicellulosic hydrolysate. Fermentation of detoxified hemicellulosic hydrolysate with pentose utilizing yeast *C. tropicalis* produced 0.65 g/g xylitol yield. Furthermore, enzymatic saccharification of alkali acid–treated *T. latifolia* biomass residue with high sugar concentration, on subsequent fermentation with *S. cerevisiae*, produced 0.44 g/g ethanol yield. Our study results confirmed that *T. latifolia* could be a potential and renewable substrate rich in hemicellulosic and cellulosic sugars, which could be ideal for lignocellulosic biorefinery for production of value-added bioproducts such xylitol and bioethanol. Our fermentation results also indicates that application of pentose-utilizing yeast *C. tropicalis* and hexose utilizing S. *cerevisiae* is ideal microbial cell factories for effective utilization of all the sugars present in lignocellulosic hydrolysate, and has the potential in large-scale production of biochemicals and biofuels.

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