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**Original Paper**

# Ethanol production by *Escherichia coli* from detoxified lignocellulosic teak wood hydrolysates with high concentration of phenolic compounds

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**Abstract:** Teak wood residues were subjected to thermochemical pretreatment, enzymatic saccharification, and detoxification to obtain syrups with a high concentration of fermentable sugars for ethanol production with the ethanologenic *Escherichia coli* strain MS04. Teak is a hardwood, and thus a robust deconstructive pretreatment was applied followed by enzymatic saccharification. The resulting syrup contained 60 g $l^{-1}$ glucose, 18 g $l^{-1}$ xylose, 6 g $l^{-1}$ acetate, less than 0.1 g $l^{-1}$ of total furans, and 12 g $l^{-1}$ of soluble phenolic compounds (SPCs). This concentration of SPC is toxic to *E. coli*, and thus two detoxification strategies were assayed: (1) treatment with *Coriolopsis gallica* laccase followed by addition of activated carbon and (2) overliming with $Ca(OH)_2$. These reduced the phenolic compounds by 40% and 76%, respectively. The detoxified syrups were centrifuged and fermented with *E. coli* MS04. Cultivation with the overlimed hydrolysate showed a 60% higher volumetric productivity (0.45 $g_{ETOH}$ $l^{-1}$ $hr^{-1}$). The bioethanol/sugar yield was over 90% in both strategies.

**Keywords:** Teak wood, *Escherichia coli*, Bioethanol, Detoxification, Soluble phenolic compounds

## Introduction

Mexico is one of the main producers of agroindustrial crops such as corn, agave, rice, sugar cane, and a variety of fruits. The wood industry has recently gained importance due to the excellent economic benefits that it provides. Specifically, the teak wood (*Tectona grandis L.*) industry has grown significantly. The interest in this material, and consequently its price, has grown due to its hardness, beautiful veins, natural oils, and rubber (Fierros, 2012). This industry has also generated important amounts of residues such as branches, chunks, and sawdust resulting from the cutting process. These residues are rich in cellulose (up to 50% of the total weight) and contain significant amounts of hemicellulose (up to 25% of the total weight) (Rizanti et al., 2018), making teak wood residues attractive as a lignocellulosic feedstock for the production of biofuels such as bioethanol.

Among biofuels, bioethanol is an important commodity in transportation and industry and is a sustainable, renewable, and eco-friendly energy source (Sebayang et al., 2016). Bioethanol can be produced from lignocellulosic biomass by fermenting depolymerized sugars with microorganisms. The biosynthesis of ethanol using hydrolysates from agroindustrial residues has additional economic and environmental benefits (Balat & Balat, 2009). Therefore, several methodologies have been applied to pretreat these feedstocks to obtain slurries containing fermentable sugars (Kumar et al., 2009). Lignocellulose has three main components: highly crystalline cellulose, hemicellulose, and lignin. These are organized into macrofibrils that confer structural stability to the plant cell wall (Zhao et al., 2012). Therefore, a pretreatment that deconstructs the chemical and physical structure of lignocellulose is necessary to obtain syrups enriched in xylose (with some glucose and other sugars depending on the lignocellulosic material) and amorphous cellulose (Montiel et al., 2016). The pretreated cellulose can be hydrolyzed (saccharified) into free glucose molecules. Most of the fermentable glucose is obtained from cellulose. Thus, enzymatic cocktails of cellulolytic hydrolases (endocellulases, exocellulases, $\beta$-glucosidases) are commonly used for this purpose (Adsul et al., 2020).

Nonetheless, some components of the lignocellulosic hydrolysates from wood, for example, lignin derivatives including soluble phenolic compounds (SPCs) and wood polysaccharide degradation products such as organic acids and furan derivatives, act as inhibitors of bacterial fermentation (Jönsson et al, 1998; Jurado et al., 2009). Therefore, an additional detoxification step prior to fermentation of the hydrolysates is usually required to improve the fermentability of such syrups. A broad array of detoxification strategies have been reported (Palmqvist & Hahn-Hägerdal, 2000), including biological, physical, and chemical methods such as the use of oxidoreductase-type enzymes (peroxidases and laccases) from fungi (Jönsson et al., 1998), roto-evaporation (Palmqvist et al., 1996), ultrafiltration (Toledano et al., 2010), chromatographic separation (Ouyang et al., 2011), adsorption with activated or black carbon (Lee et al., 2011), organic solvent extraction (Wilson et al., 1989), and alkali treatment (Martinez et al., 2001). These approaches

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could be applied over lignocellulosic hydrolysates as the sole treatment or as a combination in sequential steps (Llano et al., 2017).

Overliming is an alkali detoxification treatment involving a pH increase with $Ca(OH)_2$ to pH 9–11 resulting in precipitation of toxic compounds and a fermentable syrup with gypsum (Martinez et al., 2000; Martinez et al., 2001). It is widely used because of its low cost (Llano et al., 2017), high efficiency for removal of inhibitors (Palmqvist & Hahn-Hägerdal, 2000), and the production of high-value-added compounds from lignin such as calcium lignosulfonate as coproducts (Torres et al., 2020). However, fermentable sugars, mainly xylose, are degraded at basic pH values with losses of around 10–20% of the initial sugar (Martinez et al., 2000; Mohagheghi et al., 2006). On the other hand, detoxification with oxidoreductases has shown up to 80% removal of lignin derivatives without significant negative effects on hydrolysate content and generating phenolic polymers, which are also high-value-added molecules (Jönsson et al., 1998; Moreno et al., 2012).

Yeasts are the most used microorganisms to produce ethanol. However, wild-type *Saccharomyces cerevisiae* strains cannot efficiently consume xylose and other pentoses contained in the hydrolysate slurries, which are an important fraction of the lignocellulosic-derived sugars suitable for transformation into ethanol (Zaldivar et al., 2001). Therefore, other microorganisms, mainly bacteria such as *Escherichia coli*, have been engineered to produce this biofuel (Lewicka et al., 2014). *E. coli* is a robust microorganism capable of consuming different carbon sources (hexoses and pentoses). It grows in mineral media with low nutritional requirements, and a wide range of well-known molecular tools are available to modify its genome (Martinez et al., 2017). We previously constructed a metabolically engineered ethanologenic *E. coli* strain MS04 (Fernández-Sandoval et al., 2012) that can produce ethanol from hexoses and pentoses with yields up to 90% in mineral media and lignocellulosic hydrolysates (Vargas-Tah et al., 2015).

This study aimed to evaluate the effectiveness of saccharification and detoxification over thermochemically pretreated teak wood hydrolysates (TWHs) focusing on their use as fermentable media for bioethanol production using ethanologenic *E. coli* MS04. Fermentation in laboratory-simulated hydrolysates (LSHs) was also performed as a control. The results show that TWHs containing a high concentration of SPCs, which are toxic to ethanologenic microorganisms, and a mixture of glucose and xylose are fermented to ethanol with high yields after the corresponding pretreatment, saccharification, and detoxification.

## Materials and Methods

### Strain

The ethanologenic strain used in this work was the Gram-negative bacteria *E. coli* MS04 (MG1655, Δ*pflB*, Δ*adhE*, Δ*frdA*, Δ*xylFGH*, *gatC-S184L*, Δ*midarpA*, Δ*reg* 27.3 kb, and Δ*ldhA*) (Fernández-Sandoval et al., 2012), which was metabolically engineered to produce ethanol as the main fermentation product.

### Culture Media

#### *Simulated hydrolysates*

Laboratory-simulated hydrolysates were based on AM1 mineral medium, previously developed in our laboratory (Martinez et al., 2007), supplemented (per liter) with 0.1 g sodium citrate, 2 g sodium acetate, 30 µg kanamycin, 50 g glucose, and 20 g xylose. AM1 medium contains per liter: 2.63 g $(NH_4)_2HPO_4$, 0.87 g $NH_4H_2PO_4$, 0.246 g $MgSO_4{\cdot}7H_2O$, 0.15 g KCl, 0.117 g betaine, and 1.5 ml trace elements. The trace element solution contains per liter: 1.6 g $FeCl_3$, 0.2 g $CoCl_2{\cdot}6H_2O$, 0.1 g $CuCl_2$, 0.2 g $ZnCl_2{\cdot}4H_2O$, 0.2 g $Na_2MoO_4$, 0.05 g $H_3BO_3$, and 0.33 g $MnCl_2{\cdot}4H_2O$.

#### *Deconstruction of teak wood*

The hydrolysates of teak wood (TWHs) were prepared according to a preliminary version of a methodology developed by our group (Hernández-Luna et al., 2016), involving thermochemical hydrolysis in a 5-l Parr-type reactor containing a gas–liquid–solid system of 18% wt/wt teak wood powder, 7% wt/wt $SO_2$, and the balance water. Typical reaction conditions were 140°C and 450 rpm for 90 min.

#### *Enzymatic saccharification*

Enzymatic saccharification was performed in 0.2-l minifermenters fitted with a peg mixer (Caspeta et al., 2014) with a cellulase-lyophilized cocktail (43 FPU $g^{-1}$, 100 $U_{xylanase}$ $g^{-1}$) (NEO Biotech Co., Ltd., Shaanxi Province, China). Sodium citrate was added to a 50 mM final concentration in the slurry from the deconstructed TWH after adjusting the pH to 4.8 with 10 N KOH and adding 15 FPU $g_{glucan}^{-1}$ of the enzymatic cocktail. The release of reducing sugars was monitored for 36 hr.

#### *Detoxification of TWHs*

Detoxification of TWH was performed after the saccharification process using two methodologies:

*Overliming*: The pH was adjusted to 11 with solid $Ca(OH)_2$ at 30°C and it was held for 40 min under agitation. The resulting syrup was then centrifuged at 4500 × *g* for 20 min (room temperature), and the supernatant was recovered; 5 N $H_2SO_4$ was used to adjust the pH to 7.

*Detoxification with oxidoreductases and activated carbon* (AC): A fungal laccase from *Coriolopsis gallica* (LC*g*, 2450 $U_{ABTS}$ $ml^{-1}$) (Pickard et al., 1999) was used without mediators. The detoxification process was performed in 1-l reactors (Applikon ADI 1010/ADI 1025, Delft, The Netherlands) with 800 ml of saccharified TWH, pH 4.5, 800 rpm, 30°C, and 0.8 $l_{air}$ $min^{-1}$ (aeration rate to provide oxygen for the laccase activity). The reaction started with the addition of 510 $U_{ABTS}$ $ml^{-1}$ of LC*g* (final concentration) (Jönsson et al., 1998), and the phenolic compound reduction was measured after 2 hr. Once the enzymatic reaction was finished, 5% wt/wt of AC was added to absorb any remaining inhibitors (Llano et al., 2017). This was held 60 min under the same conditions used for the LC*g* catalysis. Precipitated polyphenols and AC were removed by centrifugation (20 min, 4300 × *g*).

### Fermentation in Simulated and TWHs

#### *Inoculum preparation*

Fermentation in both LSH and TWH used the ethanologenic *E. coli* strain MS04. The preinoculum was prepared by growing cells from a cryovial (1:1 glycerol 80% and strain MS04 at $OD_{600}$ ~ 2.0 in mineral media) in test tubes containing 4 ml of Luria Bertani medium. The cells were incubated for 12 hr at 37°C and 300 rpm. The inoculum was prepared by transferring the content of the test tubes to 200 mL minifermenters with AM1 mineral media supplemented with 20 g $l^{-1}$ of xylose. The inoculum was grown for approximately 24 hr at 37°C, 150 rpm, and pH 7 (controlled with 2 N KOH) until an $OD_{600}$ between 1.5 and 2 was reached. The cells were harvested by centrifugation (4°C, 10 min, 4300 × *g*) and resuspended in fresh mineral media to inoculate the fermenters with simulated hydrolysate or TWH at an initial $OD_{600}$ of 1 (0.37 $g_{DCW}$ $l^{-1}$).

### *Fermentation*

Fermentation used the same bioreactor system as used before for saccharification. Before inoculation, the pH of the slurry containing saccharified and detoxified TWH was adjusted to 7 with 10 N KOH and concentrated solutions of betaine (an osmoprotectant). Ammonium phosphate buffer and kanamycin were added to a final concentration of 1 mM, 5 mM, and 30 μg $l^{-1}$, respectively. The cultures were incubated at 37°C, 150 rpm, and pH 7 (controlled with 2 N KOH) until complete substrate (glucose and xylose) depletion or until sugar consumption stopped. Control experiments were carried out in AM1 mineral medium.

## Analytical methods

Cell growth for inoculum preparation was determined spectrophotometrically from optical density measurements at 600 nm (DU 70, Beckman Instruments, Inc., Fullerton, CA, USA), and converted to dry cell weight per liter using a calibration curve: 1 optical density at 600 nm = 0.37 $g_{DCW}$ $l^{-1}$. The samples were centrifuged, and the cell-free culture broth was frozen for subsequent analysis. Xylose and acetate concentrations were measured by high-pressure liquid chromatography (Waters U6K, Millipore Co., Milford, MA, USA) using an Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm; Bio-Rad Laboratories, Hercules, CA, USA), a 5.0 mM $H_2SO_4$ solution as the mobile phase (0.5 ml $min^{-1}$) at 60°C, a photodiode array detector at 210 nm (Model 996, Waters, Millipore Co.), and a refractive index detector (Model 2410, Waters, Millipore Co., Milford, MA, USA). Glucose concentrations during saccharification and in the cultivation medium were measured using a biochemical analyzer (YSI model 2700, YSI Inc., Yellow Springs, OH, USA). The ethanol produced from fermentations was quantified by gas chromatography (Agilent, 6850 series GC System, Wilmington, DE, USA) as reported elsewhere (Fernández-Sandoval et al., 2012). The concentrations of lignin-derived soluble polyphenols were estimated by the Prussian blue method as reported elsewhere (Graham, 1992) using tannic acid as a reference phenolic compound.

## Fermentation Parameters

The kinetic and stoichiometric parameters for ethanol yield from consumed sugars (based on a theoretical yield of 0.51 $g_{ethanol}$ $g_{sugars}^{-1}$, $Y_{EtOH}$), volumetric productivity of ethanol ($Q_{EtOH}$), volumetric uptake rate of glucose ($Q_{glu}$), and volumetric uptake rate of xylose ($Q_{xyl}$) were calculated according to the equations defined by Schuler & Kargi (2002). Data in figures and tables show the standard deviation.

# Results and Discussion

## Pretreatment of Teak Wood Residues

Teak wood residues from cuttings were powdered and hydrolyzed under high pressure and temperature in a gas–liquid–solid system. After the hydrolysis pretreatment, the resulting syrups contained on average: 8 g $l^{-1}$ of glucose, 18 g $l^{-1}$ of xylose, 2 g $l^{-1}$ of arabinose, 6 g $l^{-1}$ of acetic acid, and 12 g $l^{-1}$ SPC. The concentration of furans was lower than 0.1 g $l^{-1}$, and no other fermentable sugars were detected.

The high crystallinity of cellulose makes the hydrolysis of the polymer difficult; hence, the thermochemical deconstruction mainly breaks the noncovalent bonds of crystalline cellulose, generating amorphous cellulose and a few free glucose molecules. However, since hemicellulose is amorphous, the hydrolysis of xylan should be easier than the hydrolysis of glucan. Therefore, an additional treatment involving hydrolysis of amorphous cellulose could achieve higher glucose concentrations.

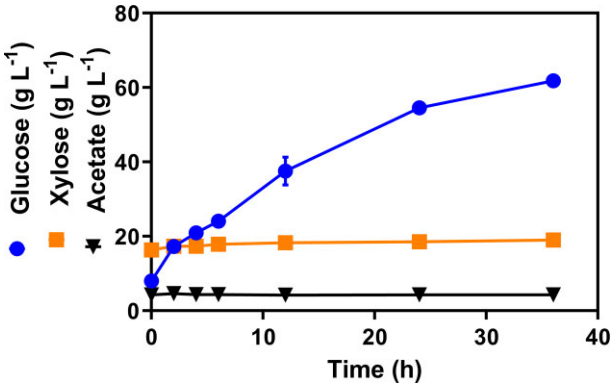


**Fig. 1.** Kinetics of enzymatic saccharification of teak wood hydrolysate (TWH). Error bars indicate standard deviation from mean values.

## Enzymatic Saccharification of Pretreated TWHs

Thermochemically pretreated TWH was saccharified with a commercial cellulase cocktail to break the bonds of cellulose and release glucose that, collectively with xylose, is a fermentable sugar for ethanol production. The reaction was followed until the concentration of released glucose did not change significantly, as shown in Fig. 1.

Fig. 1 shows that the only compound released during enzymatic saccharification was glucose as produced from cellulose hydrolysis. At 36 hr, the glucose and xylose concentrations reached 60 and 20 g $l^{-1}$, respectively. Higher times of saccharification did not result in higher glucose concentrations. The total concentration of fermentable sugars was close to 80 g $l^{-1}$ and is desirable and comparable to hydrolysates from other residues such as corn stover and agave bagasse (Vargas-Tah et al., 2015). Thus, teak wood is a promising material for use as a feedstock in the production of second-generation bioproducts.

## Fermentation of Nondetoxified Saccharified Teak Wood Hydrolysate

Cultivation under nonaerated conditions was performed in minifermenters in order to evaluate the potential of nondetoxified TWH as a culture medium for production of biofuels. Here, half of the saccharified slurries were centrifuged to separate the remaining solids suspended in the broth and determine their effect on ethanol production. Likewise, a control fermentation in mineral media supplemented with similar amounts of glucose, xylose, and acetate was performed to evaluate the cellular viability of the strain in a medium analogous to TWH. The kinetics of glucose and xylose consumption, as well as ethanol production, for TWH and the control in mineral media are shown in Fig. 2a–c, respectively.

Fig. 2a,b shows that there was no effect of the suspended solids on the ethanol production with *E. coli* MS04 because glucose consumption and ethanol production are very similar in both conditions. Although the ethanol yield from the consumed glucose was higher than 90% for the evaluated conditions (Table 1), we note that fermentation in nondetoxified TWH was less efficient than in mineral media because glucose and xylose were consumed simultaneously and depleted at 30 hr. The residual glucose was around 50% of the initial value in nondetoxified TWH at 60 hr of cultivation; xylose was not consumed. Therefore, the final ethanol titer

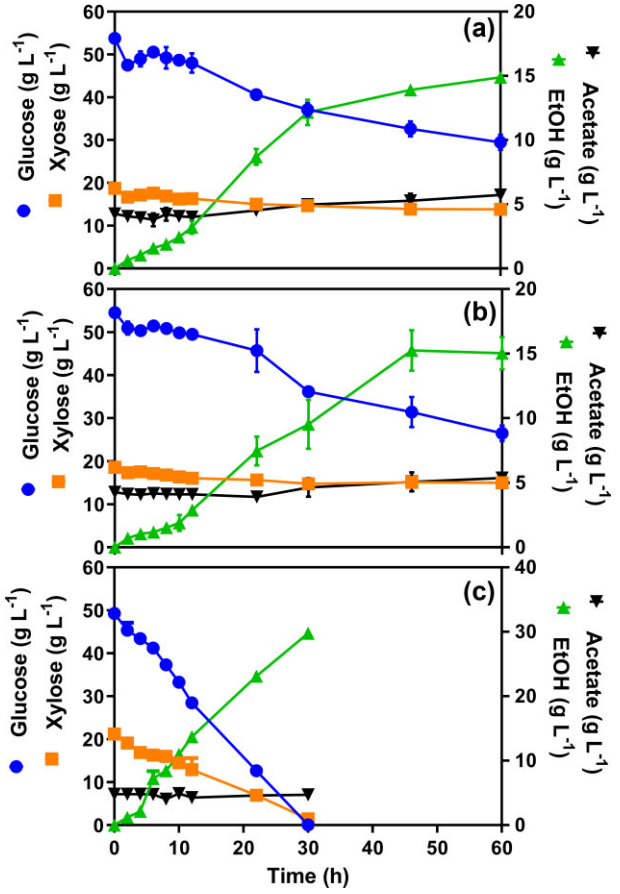


**Fig. 2.** Glucose and xylose consumption, ethanol production (EtOH), and acetate concentration in (a) teak wood hydrolysate (TWH) without suspended solids, (a) TWH with suspended solids, and (c) simulated hydrolysate (control). Error bars indicate standard deviation from mean values.

**Table 1.** Kinetic and Stoichiometric Parameters for ethanol fermentation in Nondetoxified Teak Wood Hydrolysate (TWH) and Laboratory-Simulated Hydrolysate (LSH)

| Parameter | LSH | TWH with solids | TWH without solids |
|---|---|---|---|
| $Y_{EtOH}$ (%) | 85 (2) | 93 (2) | 100 (7) |
| $Q_{glu}$ ($g_{glu}$ $l^{-1}$ $hr^{-1}$) | 1.64 (0.04) | 0.47 (0.05) | 0.41 (0.04) |
| $Q_{xyl}$ ($g_{xyl}$ $l^{-1}$ $hr^{-1}$) | 0.66 (0.05) | 0.06 (0.01) | 0.082 (0.003) |
| $Q_{EtOH}$ ($g_{EtOH}$ $l^{-1}$ $hr^{-1}$) | 0.99 (0.03) | 0.25 (0.02) | 0.25 (0.01) |
| EtOH (g $l^{-1}$) | 29.7 (0.9) | 15.0 (1.3) | 14.9 (0.5) |

in TWH (15 g $l^{-1}$) was half of the control (30 g $l^{-1}$). Consequently, the productivity decreased four times for nondetoxified TWH.

Acetate production increased approximately 25% with fermentation; however, the resulting concentration does not inhibit bacteria. This has been observed for the *E. coli* MS04 strain due to its metabolic characteristics (Fernández-Sandoval et al., 2012).

It is well known that lignocellulosic hydrolysates contain compounds toxic to bacteria such as acetic acid, furans, and phenolic lignin derivatives (Jurado et al., 2009; Okuda et al., 2008; Palmqvist & Hahn-Hägerdal, 2000). In this context, the ethanologenic strain used in this study (*E. coli* MS04) was previously evolved in the laboratory to tolerate up to 10 g $l^{-1}$ of acetic acid (Fernández-Sandoval et al., 2012), avoiding bacterial inhibition that could be caused by

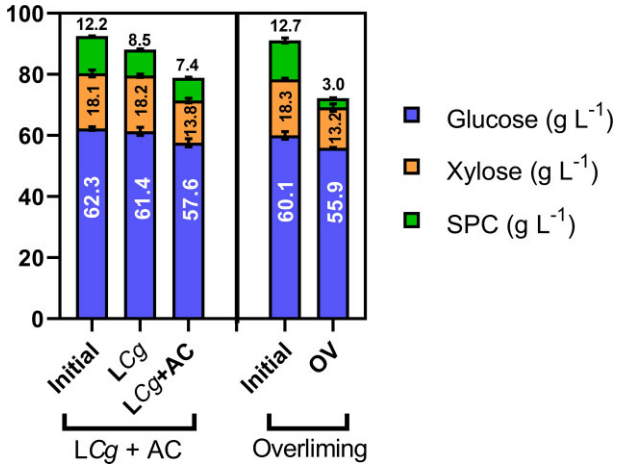


**Fig. 3.** Concentrations of fermentable sugars and SPCs before and after application of detoxification procedures. Error bars indicate standard deviation from mean values. AC = activated carbon; OV = overliming.

6 g $l^{-1}$ of this compound in TWH. The furan concentration was too low to cause inhibition. However, soluble phenolic lignin derivatives are toxic because they disrupt the cell membrane, affecting cell growth and consumption of sugars (Clark & Mackie, 1984; Palmqvist et al., 1999). According to the characterization and composition analysis of teak wood residues, approximately 23% of the dried biomass is lignin (Hernández-Luna et al., 2016); thus, SPCs should be removed from the slurries to improve the consumption of glucose and xylose under these fermentation conditions.

## Detoxification of TWHs

Saccharified TWHs were treated to eliminate or at least reduce the detrimental levels of SPCs. Two different strategies were followed: (1) treatment with laccase from *C. gallica* (LC*g*)—an oxidoreductase that oxidizes phenolic molecules into amorphous polymers that precipitate in the medium (Moreno et al., 2012; Tinoco et al., 2001)—followed by addition of AC in order to desorb a higher amount of toxins (Llano et al., 2017) and (2) treatment with lime (overliming) to precipitate toxic compounds such as SPCs and furans (Martinez et al., 2001).

The effects of each detoxification step on SPCs and fermentable sugars are shown in Fig. 3. The treatment with LC*g* (LC*g* bar) did not seem to affect the concentration of fermentable sugars, although the SPC concentration decreased by 40%. Nevertheless, the increases in laccase concentration and incubation time did not result in higher SPC detoxification (data not shown). Compared with other reports of laccase detoxification of lignocellulosic hydrolysates (Jönsson et al., 1998; Jurado et al., 2009), the percentage of SPC detoxification achieved here was 30–60% lower. However, initial SPC concentrations in the reports above were 80–90% lower and hydrolysate compositions were also different because they depend on the agroindustrial waste used and the decomposition pretreatment steps. Thus, it is important to perform a robust biocatalytic study of the causes and possible solutions to the relatively low detoxification percentages of LC*g*. However, this task is out of the scope of this work. Importantly, inactivation of laccases during detoxification of phenols has been widely reported and may be caused by enzyme entrapment within the synthetized polyphenols (Ba et al., 2013), attack of free radicals (products of the laccase catalytic reaction) to aromatic residues on the active site (Avelar et al., 2018), and the medium composition (Jurado et al., 2009).

Here, 5% wt/wt AC was added to the medium to achieve higher detoxification degrees. Concentrations of SPC and monosaccharides were measured after 2 hr. Fig. 3 shows that the SPC concentration after the AC treatment (LC*g* + AC bar) decreased by only 13%, while the content of glucose and xylose was reduced by 6% and 24%, respectively. Detoxification by absorption with AC has been reported to be efficient in terms of furans and SPCs, but this step also reduces the concentration of sugars by almost 50% (Llano et al., 2017). According to these results, the addition of AC should be avoided from the detoxification step.

The effectiveness of lime treatment for detoxification of lignocellulosic hydrolysates has been reported (Llano et al., 2017; Martinez et al., 2000, 2001), although the concentration of sugars, mostly pentoses, can be seriously affected by degradation. Additionally, in the specific case of teak wood deconstructed with the treatment described in this work, the overliming treatment produces insoluble lignosulfonates, which are often used in the cement industry and are precursors of fine chemicals (Aro & Fatehi, 2017; Bjørsvik & Minisci, 1999). In this work, solid $Ca(OH)_2$ was added to saccharified TWH until pH 11. The concentrations of SPCs and sugars before and after overliming are shown in Fig. 3 (right side). As expected, reductions of 7% and 27% in glucose and xylose concentrations, respectively, were found, similar to the total sugar loss caused by AC addition. On the other hand, the concentration of SPC decreased by 76% with overliming, resulting in a more effective treatment than LC*g*. Longer holding times are not recommended because sugar losses increase (Martinez et al., 2001; Millati et al., 2002).

A combination of detoxification techniques is often used for better results in ethanol production from lignocellulosic biomass (Moreno et al., 2017). In this work, overliming followed by LC*g*–AC treatment, or vice versa, was effective to achieve higher percentages of detoxification. However, the addition of LC*g* after overliming is impractical because this enzyme tends to get entrapped within the synthetized polyphenols and other materials present in the medium, such as calcium lignosulfonates (Ba et al., 2013). Furthermore, the necessary decrease of pH from 11 to 4.5 for laccase activity leads to the generation of gypsum and other undesired byproducts. The use of LC*g* prior to overliming avoids these issues; nonetheless, it involves a series of additional processing steps because it becomes necessary to remove the polyphenols produced by LC*g* for efficient purification of calcium lignosulfonates. An additional step implies higher production costs.

## Fermentation of Detoxified TWHs

TWHs saccharified and detoxified by LC*g*–AC and overliming were used as culture media for ethanol production with *E. coli* MS04. The fermentation system and culture conditions were similar to those used for fermentation with nondetoxified hydrolysates (see the section *Fermentation of Nondetoxified Saccharified TWH*). Fig. 4 shows the kinetic behavior for consumption of sugars and production of ethanol for both treatments. First, we note that the consumption of sugars was incomplete for THWs detoxified by the LC*g*–AC treatment: About half of the initial glucose remained at 96 hr; xylose was barely consumed. This behavior is very similar to that observed for nondetoxified hydrolysates, thus indicating that the detoxification degree achieved with this methodology is not sufficient to generate an accurate fermentable slurry. In addition, both sugar uptake and ethanol production rates (Table 2) were slightly lower than those obtained for nondetoxified TWH (Table 1) probably due to absorption of nutrients on AC.

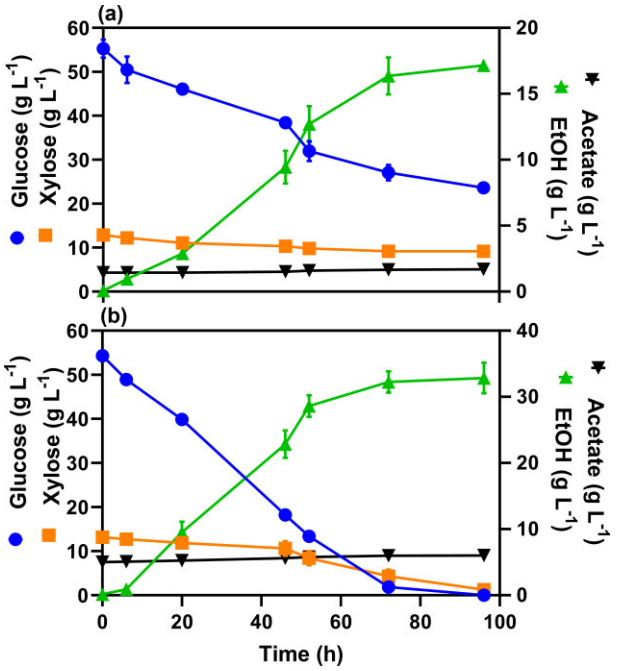


**Fig. 4.** Glucose and xylose consumption, ethanol (EtOH) production, and acetate concentration in (a) LC*g*–AC (activated carbon) detoxified teak wood hydrolysate (TWH) and (b) overliming detoxified TWH. Error bars indicate standard deviation from mean values.

**Table 2.** Kinetic and Stoichiometric Parameters for Ethanol Fermentation in Detoxified Teak Wood Hydrolysate (TWH)

| Parameter | LC*g*–AC-detoxified TWH | Overliming-detoxified TWH |
|---|---|---|
| $Y_{EtOH}$ (%) | 95 (1) | 96 (6) |
| $Q_{glu}$ ($g_{glu}$ $l^{-1}$ $hr^{-1}$) | 0.33 (0.01) | 0.75 (0.01) |
| $Q_{xyl}$ ($g_{xyl}$ $l^{-1}$ $hr^{-1}$) | 0.038 (0.001) | 0.12 (0.01) |
| $Q_{EtOH}$ ($g_{EtOH}$ $l^{-1}$ $hr^{-1}$) | 0.18 (0.01) | 0.45 (0.03) |
| EtOH (g $l^{-1}$) | 17.2 (0.4) | 32.9 (3.3) |

*Note.* AC = activated carbon.

In contrast, the fermentation of TWH detoxified by lime addition was complete (Fig. 4b) and led to an almost complete depletion of sugars at 72 hr. Therefore, the overliming detoxification treatment seems to be sufficient to generate a fermentable syrup from teak wood. The sugar consumption rates for glucose and xylose were 54% and 80% lower than values reported for LSH, respectively (see Tables 1 and 2); these values were 56% and 70% higher than that obtained for LC*g*–AC treatment, respectively. Similarly, the ethanol production rate was half that for LSH and 40–60% lower than those reported in previous studies for fermentation of other lignocellulosic hydrolysates with *E. coli* MS04 such as corncobs and corn stover, but with 10-fold more inoculum (Pedraza et al., 2016; Vargas-Tah et al., 2015).

It is well known that high cellular densities are associated with higher volumetric productivities (Shiloach & Fass, 2005). In the case of ethanol/sugar yields, the values over 95% achieved here have been reported for this strain in lignocellulosic hydrolysates. Therefore, overliming is a relatively efficient detoxification strategy for TWHs. However, the slurries obtained from the deconstruction $SO_2$ thermochemical pretreatment from teak wood contain inhibitors of fermentation—namely, SPCs—that are three

**Table 3.** Ethanol Production From Different Hardwood Hydrolysates Using Ethanologenic Microorganisms

| Ethanologenic microorganism | Hardwood (HW) | Pretreatment conditions/HW load[a] | Inocula ($g_{DCW}$ $l^{-1}$) | Detox treatment | SPC before detox (g $l^{-1}$) | SPC after detox (g $l^{-1}$) | Initial sugars (g $l^{-1}$) | EtOH (g $l^{-1}$) | $Y_{EtOH}$ (%) | $Q_{EtOH}$ (g $l^{-1}$ $hr^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* B pLOI297 | Aspen | Extrusion with $SO_2$ | 0.5 | Overliming | NR | NR | 31 | 15 | 94 | 0.26 | Lawford and Rousseau (1991) |
| *Pichia stipitis* NRRLY-7124 | Red oak | 0.5% $H_2SO_4$ 4 hr, 12 atm, 4 min, 100°C, 15 min | 0.2 | None | NR | NR | 39.4 | 14.5 | 72 | 0.21 | Nigam (2001) |
| *E. coli* KO11 | Maple | 1% $H_2SO_4$, 160°C, 10 min | 0.2 | Overliming | NR | NR | 84 | 35.4 | 80 | 0.4 | Okuda et al. (2007) |
| *P. stipitis* NCIM–3498 | *Prosopis juliflora* (mezquite) | 3% $H_2SO_4$, 120°C, 60 min/10% | 0.15 | Overliming and AC | 10 | NR | 36 | 7.13 | 78 | 0.3 | Gupta et al. (2009) |
| *Saccharomyces cerevisiae* MTCC-36 and *P stipitis* NCIM-3498 | *Shorea robusta* (sal) | 1% HCl, 121°C, 30 min/10% | 0.2 | None | 1.41 | 0 | 19.1 | 9.43 | 97 | 0.39 | Raina et al. (2020) |
| *S. cerevisiae* MEC1133 | Paulownia | (1) Autohydrolysis 210°C/17% (2) 1.5% $H_2SO_4$, 115°C, 75 min | 1.5 | Overliming | 0.65 | 0 | 47.6 | 12.5 | 63 | 0.26 | Domínguez et al. (2021) |
| *P stipitis* CECT-1922 | | | 1.5 | Overliming | 0.65 | 2 | 46.8 | 14.2 | 61 | 0.30 | |
| *E. coli* MS04 | *Tectona grandis* (Teak) | 7% $SO_2$, 140°C, 90 min/18% | 0.37 | None | 12.7 | — | 73 | 15 | 93 | 0.25 | This work |
| | | | 0.37 | Overliming | 12.7 | 3 | 67.5 | 32.9 | 96 | 0.45 | |

*Note.* NR = not reported; SPC = soluble phenolic compounds.
[a]Percentages are expressed as wt/wt.

to four times higher than the average report for lignocellulosic hydrolysates achieved from dilute acid pretreatment (Mills et al., 2009).

Versus other processes for ethanol production from hardwood hydrolysates, the productivity and ethanol titer achieved here are considerably higher than most reported values (Table 3). It is well known that hardwoods contain a high concentration of lignin-derived compounds (Domínguez et al., 2021). Most of them are toxic for microorganisms and constitute a bottleneck for the utilization of sugar-rich hardwoods in fermentation processes, especially when they are compared with other lignocellulosic biomasses. Some of the hydrolysates reported in Table 3 were subjected to less severe conditions of hydrolysis such as lower temperatures and holding times followed by detoxification with overliming or overliming + AC (Gupta et al., 2009). Nevertheless, the productivity obtained in this work was 1.13–2.5-fold higher even when the SPC concentration after detoxification was 4.6-fold higher than the value reported by Domínguez et al (2021).

The ethanol titer reached in this work depends on the initial concentration of monosaccharides and ethanol yield. It was 2.2- to 4.6-fold higher with *E. coli* strains MS04 and K011 than most values reported for the fermentation of other hardwood hydrolysates shown in Table 3. This demonstrates not only the robustness of the pretreatment and saccharification processes for obtaining a syrup with high sugar concentration, but also the efficacy of ethanologenic *E. coli* to ferment a partially detoxified medium containing 6 g $l^{-1}$ of acetate and a relatively high amount of phenolic compounds (3 g $l^{-1}$ of SPC). This calls for further studies to improve the ethanol production process from this material in terms of ethanol titer and productivity. This is an important task because teak has a high concentration of polymerized sugars, and *E. coli* MS04 has had successful fermentation from hardwood hydrolysates in terms of ethanol titer and yield versus other ethanologenic bacteria and yeasts.

## Conclusion

Teak wood residues were deconstructed thermochemically with sulfur dioxide as catalyst. The slurry was sequentially saccharified, yielding a total concentration of fermentable sugars close to 80 g $l^{-1}$. However, the deconstruction method produced over 12 g $l^{-1}$ of SPCs, which is toxic to any ethanologenic microorganism, hindering the sugar consumption as demonstrated in this study with the ethanologenic *E. coli* strain MS04. The syrup was processed by laccase-activated carbon and overliming treatments, reducing the phenolic compounds by 40% and 76%, respectively, but also containing 6 g $l^{-1}$ of acetate. Fermentation of the overlimed TWH by *E. coli* MS04 resulted in higher ethanol titer, yield, and productivity than most previously reported results for other hardwood hydrolysates using yeast or ethanologenic bacteria. These results demonstrate not only the effectiveness of the pretreatment and saccharification processes to hydrolyze teak hardwood, but also the efficacy of the ethanologenic *E. coli* MSO4 to ferment a partially detoxified medium containing high concentrations of phenolic compounds and acetate. Furthermore, the SPCs, from the overliming treatment, were recovered as insoluble Ca lignosulfonates by centrifugation, a valuable coproduct used in the cement industry or in the synthesis of fine aromatic chemicals. To the best of our knowledge, this is the first relevant approach for bioethanol production from residual teak wood biomass.

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## Conflict of Interest

All authors declare no conflict of interest.

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