Journal of Industrial Microbiology & Biotechnology (2001) 26, 145–150

www.nature.com/jim

Ethanol production from hardwood spent sulfite liquor using an adapted strain of *Pichia stipitis*

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Conditions have been optimized for fermentation of pretreated hardwood spent sulfite liquor (HSSL) using an adapted strain of *Pichia stipitis*. The pretreatments, consisting of boiling and overliming with $Ca(OH)_2$ of HSSL, to partially remove inhibitors, and adaptation of the yeast strain to HSSL, were both critical for a successful fermentation. Ethanol concentration was increased from 6.7 to 20.2 g l^{-1} using adapted *P. stipitis* (A) and pretreated HSSL. The maximum ethanol yield ($Y_{p/s}$) and productivity (Q_p) were 0.41 g g^{-1} and 0.44 g l^{-1} h^{-1}, respectively, at an oxygen transfer rate of 2.0 mmol O_2 l^{-1} h^{-1}. The optimized results with this strain were compared to those of other xylose-fermenting yeasts and *Saccharomyces cerevisiae* (SSL-acclimatized) currently used at an industrial plant for the fermentation of spent sulfite liquor. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 145–150.

Keywords: hardwood spent sulfite liquor; adaptation; fermentation; ethanol; *Pichia stipitis*; *Saccharomyces cerevisiae*

Introduction

Spent sulfite liquor (SSL) is a waste product of pulp mills, formed from delignifying wood chips by cooking with acid bisulfite. SSL contains dissolved solids such as lignosulfonates and hemicellulose hydrolysis products, which comprise about 30–35 g l^{-1} of hexoses and pentoses. The composition of the sugar fraction in SSL depends on the type of wood used for pulping [10]. Coniferous "soft" woods yield a high proportion of hexose sugars (predominantly mannose and glucose), whereas deciduous "hard" woods yield a liquor containing predominantly xylose. The effluent has a high biological oxygen demand (BOD) (25000 to 5000 ppm) and presents a disposal problem. To alleviate pollution problems, SSL has been used as a substrate for producing single-cell protein (SCP) (*Candida utilis* or torula yeast) or alcohol.

In some sulfite mills, the hexose sugars in SSL are fermented to ethanol with *Saccharomyces* spp. (SSL-acclimatized), leaving pentose sugars (xylose and arabinose) unfermented. A change-over to a pentose-fermenting yeast could theoretically increase ethanol yield by as much as 25% in the softwood SSL and simultaneously decrease the residual sugar significantly. Width hardwood SSL (HSSL), the potential increase in yield is still higher, because HSSL consists mainly of xylose. The objective of this investigation was to assess the fermentation performance of an adapted strain of *Pichia stipitis* using both synthetic and HSSL media.

Materials and methods

Nomenclature

Abbreviations and terms used are listed in Table 1.

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Received 23 June 2000; accepted 21 October 2000

Microorganisms and media

P. stipitis NRRL Y-7124 was grown at 30±0.5°C and maintained at 4°C on agar slants containing (g l^{-1}): xylose, 20; yeast extract, 3; malt extract, 3; peptone, 5 and agar, 20. Prior to propagation, the culture was adapted by cultivation on (HSSL) medium. *Saccharomyces cerevisiae*, an SSL-acclimatized production from an SSL fermentation plant, was maintained in HSSL medium at 4°C before use.

Medium used for inoculum preparation contained HSSL supplemented with (g l^{-1}): yeast extract, 3; malt extract, 3; and peptone, 5. Inoculum was prepared as described earlier [15]. Cells were harvested, washed twice and resuspended in sterile water to give a cell density of 1.0 g l^{-1}, before adding them to the medium.

Adaptation of the yeast to the HSSL

Adaptation of *P. stipitis* was performed by sequentially transferring and growing cells in media containing increasing concentrations (20%, 40%, 60% and 80%) of HSSL supplemented with (g l^{-1}); xylose, 30; glucose, 10; yeast extract, 2.5; $(NH_4)_2HPO_4$, 2; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.5. Cells harvested from the final medium were designated "adapted strain" (A). It was maintained on agar slants containing (g l^{-1}): yeast extract, 2.5; $(NH_4)_2HPO_4$, 2; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.5; agar, 20 and 50% (v/v) HSSL.

Hardwood spent sulfite liquor (HSSL)

HSSL (20–22% solids) was obtained from M/s Hindustan Pulp and Paper Mill, Nagoan, Assam, India that processed red oak.

Pretreatment of HSSL

HSSL was boiled for 15 min at 100°C, followed by overliming with $Ca(OH)_2$ to pH 10.0. The suspension was filtered and then adjusted to pH 6.5 with sulfuric acid, followed by further filtration. The filtrate was concentrated under vacuum at 25°C to achieve xylose concentrations in the range of (4–5% w/v). The resulting solution was used as a substrate for fermentation medium.

Table 1 List of abbreviations and terms

Term	Unit	Definition
x	g l^{-1}	biomass concentration
s	g l^{-1}	substrate concentration
p	g l^{-1}	product concentration
t	h	time
$Y_{p/s}$	g g^{-1}	ethanol yield coefficient, was calculated as the grams of ethanol produced per grams of sugar consumed
Q_p ave	g l^{-1} h^{-1}	average volumetric ethanol productivity, was estimated by dividing the final ethanol concentration by the time required to achieve complete sugar utilization
Q_p max	g l^{-1} h^{-1}	maximum volumetric ethanol productivity, was estimated at the maximum slope in plots of ethanol concentration vs. elapsed fermentation time
q_p	g g^{-1} h^{-1}	specific ethanol productivity, was estimated by dividing the value of Q_p max by the maximum dry weight concentration
Y_p		theoretical yield of ethanol from xylose (g g $^{-1}$), equal to 0.51
OTR	mmol O_2 l^{-1} h^{-1}	oxygen transfer rate
rpm	rotation min^{-1}	agitation rate
ave		average
max		maximum
SSL		spent sulfite liquor
HSSL		hardwood spent sulfite liquor

Preparation of HSSL and synthetic media

The medium contained either untreated or treated HSSL supplemented with (g l^{-1}): yeast extract, 2.5; $(NH_4)_2HPO_4$, 2; $(NH_4)_2SO_4$, 1; KH_2PO_4, 4; $MgSO_4 \cdot 7H_2O$, 0.5; and trace element solution 1 ml l^{-1}; pH 6.5. Trace element solution contained (g l^{-1}): $CuSO_4 \cdot 5H_2O$, 2.5; $FeCl_3 \cdot 6H_2O$, 2.7; $MnSO_4 \cdot H_2O$, 1.69; $Na_2MoO_4 \cdot 2H_2O$, 2.42; $ZnSO_4 \cdot 7H_2O$, 2.87; $CoCl_2 \cdot 6H_2O$, 2.38 and (conc) H_2SO_4, 3 drops.

Synthetic medium contained the following (g l^{-1}): xylose, 40.2; glucose, 5.4; arabinose, 2.1; mannose, 9. It also contained mineral salts and trace elements in the same concentrations as in the HSSL medium, plus thiaminehydrochloride 2 mg l^{-1}; biotin, 0.02 mg l^{-1}, and citric acid, 8 g l^{-1}. This medium was used to compare the yield and productivity with treated HSSL medium and was also used to evaluate the effect of acetic on ethanol production. The sugars, salts, yeast extract, vitamins and citric acid solutions were sterilized separately.

Batch fermentation

All fermentation were conducted in the batch mode either in 250-ml Erlenmeyer flasks containing 100 ml of medium in a temperature-controlled Climo-shaker or in a BioFlo C30, stirred tank bioreactor (STR), (working volume 300 ml), equipped with agitation, pH, and temperature controls (New Brunswick Scientific, Edison, NJ). The bioreactor was fitted with a reflux cooler to minimize evaporation.

Table 2 Composition and pH of HSSL

Component	Concentration (g l^{-1}) in	
	Untreated	Pretreated[a]
D-Glucose	3.0±0.18[b]	5.4±0.21
D-Mannose	6.5±0.32	9.0±0.35
D-Xylose	26.7±1.13	40.2±1.76
L-Arabinose	1.5±0.22	21.0±0.10
Total dry solids	220.0±3.21	
Lignosulfonates	120.0±3.57	
Acetic acid	9.3±0.28	4.2±0.13
Furfural	0.2±0.03	0.06±0.01
Free sulfur dioxide	0.5±0.28	
PH	1.7	6.5

[a]Concentrated under vacuum.
[b]Each value corresponds to the mean of three experiments, ±SD (standard deviation).

Fermentation conditions

Anaerobic and aerobic conditions were maintained by flushing either nitrogen at a rate of 0.02 vvm (anaerobic) or air at 0.01–0.1 vvm. The pH of the fermentation broth was automatically regulated, to an accuracy of ±0.1 pH units using 2 N NaOH, and the temperature was kept at a constant value of 30±0.5°C. Dissolved oxygen in the fermentation broth was measured with a galvanic-type oxygen electrode, equipped with a 100-kΩ load resistance for improved sensitivity at low dissolved oxygen values. Antifoam (FG-10, Dow Corning, USA) was added to suppress foam during fermentation. The fermentor and the media were sterilized by autoclaving at 121°C for 20 min. Fermentor contents were stirred at 550 rpm. *S. cerevisiae* was grown anaerobically whereas *P. stipitis* (A) was grown anaerobically and semiaerobically. Aliquots of 5 ml were withdrawn periodically to determine cell mass, ethanol, xylitol and concentrations of residual sugars. All experiments were carried out in triplicate.

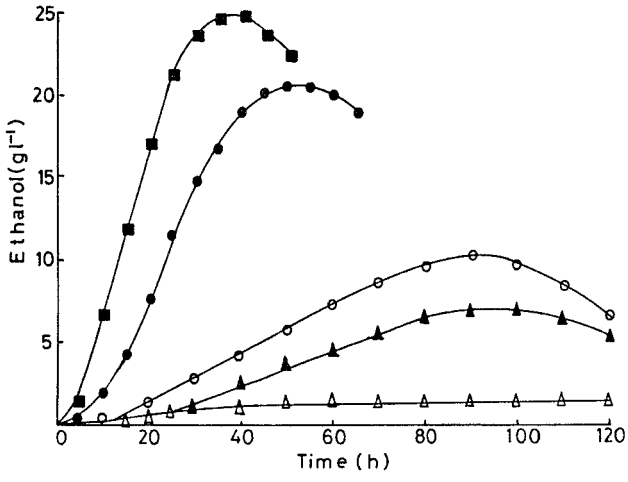

Figure 1 Effect of adapted and parent culture of *P. stipitis* on ethanol production using untreated, treated, and synthetic HSSL media at 30 ± 0.5°C: Untreated HSSL with *P. stipitis* (△); untreated HSSL with *P. stipitis* (A) (▲); treated HSSL with *P. stipitis* (○); treated HSSL with *P. stipitis* (A) (●); synthetic HSSL medium with *P. stipitis* (■).

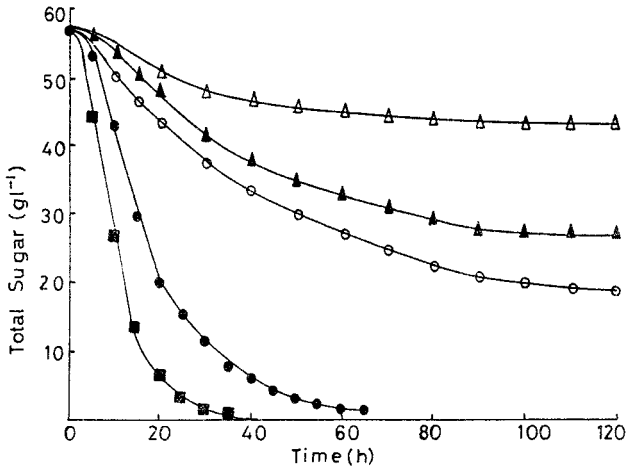

Figure 2 Effect of adapted and parent culture of *P. stipitis* on total sugar utilization using untreated, treated and synthetic HSSL media at 30 ± 0.5°C: Untreated HSSL with *P. stipitis* (△); untreated HSSL with *P. stipitis* (A) (▲); treated HSSL with *P. stipitis* (○); treated HSSL with *P. stipitis* (A) (●); synthetic HSSL medium with *P. stipitis* (■).

Analytical methods

Growth was monitored turbidometrically. Samples were diluted in 0.1 M HCl (to dissolve calcium salts). The samples were centrifuged, and the cells were resuspended in distilled water. The optical density (OD) was measured at 610 nm. OD measurements were calibrated against weighed dried cells. Compositional analyses of fermentation media, cell-free spent media, and HSSL were done using an HPLC equipped with an RI monitor as described by van Zyl *et al* [26]. Total reducing sugars (TRS) were determined colorimetrically using a dinitrosalicylic acid (DNS) reagent [13]. Ethanol concentration in the broth was determined by gas chromatography [14]. Acetic acid and furfural concentrations were determined using a gas chromatograph equipped with a flame ionization detector and a glass column (1.5 mm i.d.×2.0 m) packed with Propack Q, 80–100 mesh (Waters Associates, Milford, MA). Nitrogen was used as a carrier gas. The oven and injection temperatures were 230 and 275°C, respectively. The BOD and sulfite concentrations were determined as described in Ref. [25]. The oxygen transfer rate (OTR) (mmol $O_2\ l^{-1}\ h^{-1}$) was determined by the sulfite oxidation method [3]. The OTR was fixed by adjusting the agitation rate and air sparging velocity. Yeast viability was evaluated using a vital staining method with methylene blue [12] (data not shown).

Chemicals

Malt extract, yeast extract and peptone were obtained from Difco Laboratories (Detroit, MI). D-Xylose, L-arabinose and D-mannose were purchased from Sigma Chemicals (St. Louis, MO).

Results and discussion

Composition of HSSL and pretreated HSSL

The compositional analysis of HSSL and pretreated HSSL are summarized in Table 2. HSSL contained about 37.7 g l^{-1} of monomeric sugars, about 71% of which was xylose. It also contained the inhibitors acetic acid (9.3 g l^{-1}), furfural, and acid-soluble lignin fragments. The pretreated HSSL after concentration under vacuum contained 40.2±1.76 g l^{-1} of D-xylose, the major pentose sugar.

Pretreatment of the HSSL

The pretreatment procedure reduced the negative effects on the fermentation caused by inhibiting substances present in HSSL. Volatile compounds, such as furfural and phenols, are stripped by boiling, whereas overliming with $Ca(OH)_2$ removes other acid components (acetic, tannic and formic acids). Furthermore, the pH increase up to 10.0 due to overliming results in precipitation of heavy metals, mainly Fe and Mn. Furfural is transformed into furfuryl acid, which condenses with other components of HSSL [24]. The mechanism of action of overliming remains unclear [23,24]. Overliming resulted in loss of glucose (12%), xylose (5%), arabinose (7%), mannose (8%) and acetic acid (38%). To reduce losses, the shift to high pH during overliming should be minimized [7].

Warm filtration after overliming was more effective than cold filtration in removing inhibiting substances from HSSL, yielding higher ethanol concentrations (data not shown). This can be explained by the roles of overliming and heat in reducing the concentration of volatile compounds such as furfural, a potential inhibitor of ethanol production [23]. Toxic substances could be precipitated together with the crystals in formation of $CaSO_4$ (gypsum), whose solubility decreases with increasing temperature, assuring a more complete removal of them.

Table 3 Comparison of kinetic parameters for *P. stipitis* (A) and *P. stipitis* Y-7124 fermenting synthetic HSSL and treated and untreated medium[a]

Parameters	Medium for:				
	P. stipitis (A)			*P. stipitis* Y-7124	
	Synthetic HSSL	HSSL, treated	HSSL, untreated	HSSL, treated	HSSL, untreated
Ethanol (g l^{-1})	24.80±0.28	20.20±0.54	6.7±0.19	9.7±0.25	1.2±0.18
Sugar consumed (%)[b]	97.00±1.72	92.00±1.41	44.0±1.85	59.0±2.65	14.0±1.17
$Y_{p/s}$ (g g^{-1})[c]	0.47±0.01	0.41±0.01	0.28±1.01	0.30±0.01	0.16±0.02
Conversion efficiency (%)	94.00±2.31	82.40±1.50	55.0±1.72	59.0±1.90	31.40±0.65
Q_p ($g_p\ l^{-1}\ h^{-1}$)	0.68±0.02	0.44±0.01	0.07±0.01	0.11±0.01	0.01±0.002

[a]Fermentations were performed at 30±0.5°C and at pH 6.5±0.1.
[b]Initial sugar 56.7 g l^{-1}; initial cell density 5 g l^{-1}.
[c]Grams ethanol per gram sugar consumed; arabinose was not taken into account for the calculations.

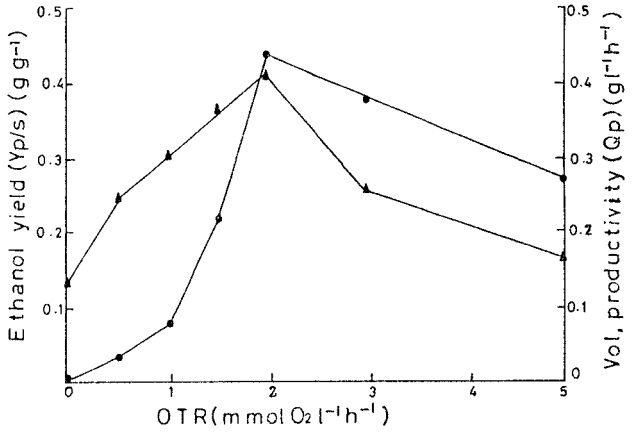

Figure 3 Effect of OTR in HSSL medium on fermentation by *P. stipitis* (A) grown at 30 ± 0.5°C, pH 6.5 ± 0.1 using a Bioflo C-30 stirred tank bioreactor: ethanol yield (▲); productivity (●).

However, conflicting literature exists concerning the extent to which inhibitory components are removed by overliming. For example, Strickland and Beck [23,24] observed that overliming reduces the concentrations of furfural and possibly metal ions, whereas van Zyl *et al* [26] and Amartay and Jeffries [1] reported 17% and 43% decreases in acetic acid concentration, respectively, following neutralization of sugarcane bagasse and corncob acid hydrolyzed hemicellulose hydrolyzates with lime.

Fermentation of HSSL

Ethanol production and sugar utilization rates during fermentation of the untreated and treated HSSL and synthetic HSSL media, with and without adapted cells of *P. stipitis* are presented in Figures 1 and 2. The kinetic parameters are summarized in Table 3. Sequential utilization of sugars was observed, with glucose and mannose being consumed first. After both hexoses were exhausted, xylose assimilation began. A similar sequential utilization of sugars has been reported by others [2,4,8,9,11]. Unlike fermentation of xylose in a semisynthetic medium using *P. stipitis* NRRL Y-7124, the production of xylitol was observed in the HSSL.

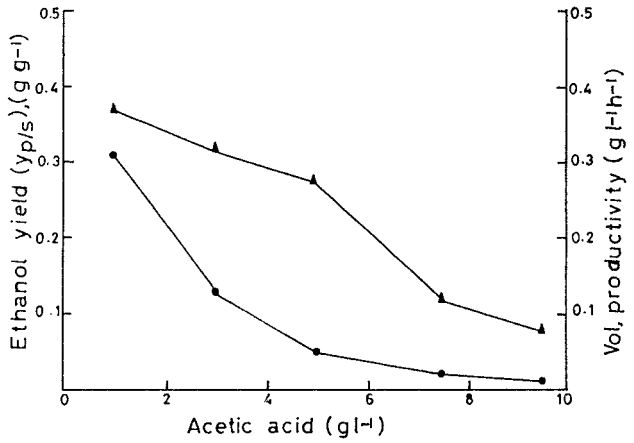

Figure 4 Effect of acetic acid concentration in the synthetic HSSL medium on fermentation by *P. stipitis* (A) grown at 30 ± 0.5°C, pH 6.5 ± 0.1 using a Bioflo C-30 stirred tank bioreactor: ethanol yield (▲); productivity (●).

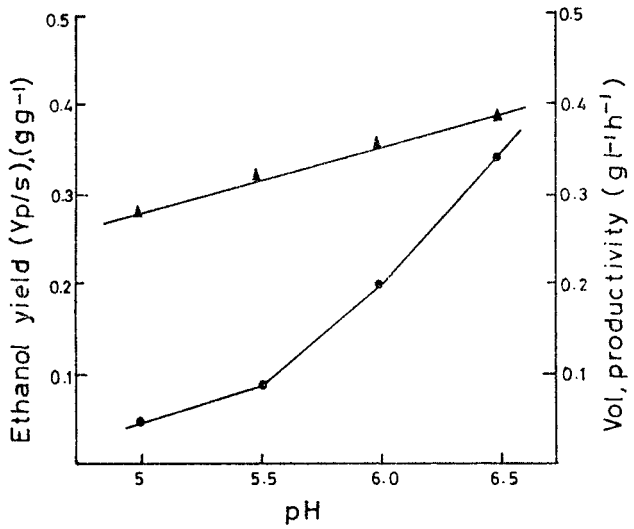

Figure 5 Effect of pH in the synthetic HSSL medium on fermentation by *P. stipitis* (A) grown at 30 ± 0.5°C, pH 6.5 ± 0.1 using a Bioflo C-30 stirred tank bioreactor: ethanol yield (▲); productivity (●).

Pretreatment of HSSL increased the ethanol yield ($Y_{p/s}$) and productivity (Q_p) from 0.16 to 0.30 g g^{-1} and 0.01 to 0.11 g l^{-1} h^{-1}, respectively, using *P. stipitis*. This improvement was probably due to partial removal of inhibitors like acetic acid (38%), furfural, free sulfur dioxide and soluble lignin derivatives present in the HSSL (Table 2). The adapted strain produced ethanol at rates, yields and concentrations significantly higher than the parent strain. The total sugar utilization, ethanol yield ($Y_{p/s}$) and productivity (Q_p) were further increased to 92%, 0.41 g g^{-1}, and 0.44 g l^{-1} h^{-1}, respectively. The ethanol yield was 82% of the maximum theoretical value and is comparable to that observed with *P. stipitis* adapted to wood hydrolyzate by subculturing 70 times [16].

Effect of aeration on ethanol production

The effect of aeration rate on fermentation of HSSL was studied in pH-stat batch culture at various oxygen transfer rates (Figure 3). For the calculations of ethanol yield ($Y_{p/s}$) and sugar

Table 4 Comparison of fermentation parameters for *P. stipitis* (A) and *S. cerevisiae* RLJY-019 in fermenting HSSL medium[a]

Parameters	Yeast/fermentation conditions for:		
	S. cerevisiae Anaerobic pH 5.0	*P. stipitis* (A) Semianaerobic pH 6.5	*P. stipitis* (A) Anaerobic pH 6.5
Ethanol (g l^{-1})	6.80±0.15	20.20±0.43	0.60±0.10
Sugar consumption (%)[b]	25.40±0.55	92.00±1.33	9.70±0.17
$Y_{p/s}$ (g g^{-1})[c]	0.47±0.01	0.41±0.01	0.11±0.01
Conversion efficiency (%)	92.20±0.34	82.00±0.41	21.21±0.08
Q_p (g_p l^{-1} h^{-1})	0.45±0.02	0.44±0.02	0.01±0.004

[a]Fermentations were performed at 30±0.5°C.
[b]Initial sugar 56.7 g l^{-1}; initial cell density 5 g l^{-1}.
[c]Grams ethanol per gram sugar consumed; arabinose was not taken into account for the calculations.

Table 5 Comparison of results for production of ethanol from HSSLs using different yeasts

Organism	pH	Total sugar ($g\ l^{-1}$)	Acetic acid ($g\ l^{-1}$)	Fermentation time (h)	Ethanol ($g\ l^{-1}$)	Yield, $Y_{p/s}$ ($g\ g^{-1}$)	Q_p ($g\ l^{-1}\ h^{-1}$)	q_p ($g\ g^{-1}\ h^{-1}$)	References
P. stipitis CBS 5776	6.5	27.8	6.1	51	10.3	0.37	0.19	0.02	[28]
	6.5	17.0	3.3	17	6.8	0.40	0.40	0.03	[28]
P. stipitis (R) CBS 5776	5.5	54.0	8.3	72	12.3	0.23	0.19	0.02	[17]
	5.5	50.0	0.3	72	18.0	0.36	0.25	0.02	[17]
P. stipitis CBS 5773	6.0	43.0	3.0	20	16.3	0.38	0.82	0.16	[2]
P. tannophilus Y-7124	6.5	62.0	–	120	18.0	0.29	–	–	[18]
C. shehatae (R)	5.5	31.0	–	18	14.0	0.48	0.77	–	[29]
P. stipitis (A)	6.5	56.7	4.2	46	20.2	0.41	0.44	0.04	This work
S. cerevisiae (SSL-acclimatized)	5.5	56.7	4.2	15	6.8	0.47	0.47	0.03	This work

consumption, assimilation of arabinose was excluded, because it is not fermented to ethanol [21]. The highest values for yield ($Y_{p/s}$) and productivity (Q_p) were 0.41 g g^{-1} and 0.44 g l^{-1} h^{-1}, respectively, at an OTR of 2.0 mmol O_2 l^{-1} h^{-1}, using *P. stipitis* (A). Lower values of yields and productivity were obtained at extreme level of aeration. The inverse relation between the degree of aeration and xylitol accumulation reported in the literature [19] was not observed in the present study. Optimum KLaC* values reported in the literature, obtained in semisynthetic media, were 0.7–8.5 mmol O_2 l^{-1} h^{-1} depending on the strain, sugar concentration, and inoculum size [4,9,20,22,27]. The optimum value for OTR found in the present study was (2.0 mmol O_2 l^{-1} h^{-1}) and falls within this range. Similar observations were also reported by Ferrari *et al* [8] with hardwood hemicellulose hydrolyzate. Proper aeration is very important in producing high yields ($Y_{p/s}$) with *P. stipitis* [6,21,26]. Insufficient aeration leads to slow xylose utilization, whereas excessive aeration reduces the ethanol yield because of either product oxidation or cell growth.

Effect of acetic acid concentration and pH on ethanol production

The results obtained in the fermentation of a synthetic HSSL medium, supplemented with variable amounts of acetic acid between 1 and 9.5 g l^{-1}, at pH 5.0, are shown in Figure 4. The addition of 1 and 3 g l^{-1} of acetic acid reduced the ethanol yields ($Y_{p/s}$) and productivity (Q_p), 21.3% and 32%, and 57.5% and 82.2%, respectively. A further increase in the concentration of acetic acid caused a drastic diminution in ethanol yield ($Y_{p/s}$) and productivity (Q_p). Supplementation of the medium 9.5 g l^{-1} acetic acid, similar to the level found in the hydrolysates, inhibited ethanol yield ($Y_{p/s}$) and productivity (Q_p) 82.5% and 97%, respectively. However, increases in ethanol yield ($Y_{p/s}$) and productivity (Q_p) were observed when the fermentation of synthetic HSSL medium containing 5 g l^{-1} acetic acid were carried out at various pH values ranging from 5.0 to 6.5 (Figure 5). A reduction in ethanol concentration, ethanol yield ($Y_{p/s}$) and productivity (Q_p), of 22.6%, 17% and 53.4%, respectively, were observed at pH 5.0 without acetic acid. Acetic acid toxicity was only partially overcome by increasing pH.

Comparison of fermentation performance of P. stipitis (A) and S. cerevisiae

The fermentation parameters for *P. stipitis* (A) and *S. cerevisiae* (SSL-acclimatized) are shown in Table 4. The *P. stipitis* (A) fermentations were carried out both semiaerobically at 2.0 mmol O_2 l^{-1} h^{-1} and anaerobically, whereas *S. cerevisiae* was fermented anaerobically. Fermentation times for the *S. cerevisiae* and *P. stipitis* (A) semiaerobically were 15 and 46 h, respectively. The ethanol yield ($Y_{p/s}$) of *S. cerevisiae* measured as grams ethanol per gram sugar consumed was higher, but the total amount of sugar consumed and ethanol produced were much less than that of the *P. stipitis* (A). In terms of sugar removal, *P. stipitis* (A) under semiaerobic conditions removed 64% more sugar than *S. cerevisiae*. Hence, replacement of *S. cerevisiae* by the xylose-fermenting yeast *P. stipitis* (A) would mean an increase in ethanol concentration and further reduction in the BOD by removal of pentoses.

Fermentation performance of P. stipitis (A) compared to other pentose-fermenting yeasts and S. cerevisiae (SSL-acclimatized)

The fermentation parameters of other xylose-fermenting yeasts and *S. cerevisiae* (SSL-acclimatized) are summarized in Table 5. The different levels of acetic acid in the HSSL and/or the pH that were used during fermentation are important elements that prevent direct comparisons. Both *S. cerevisiae* and *P. stipitis* are severely inhibited by the levels of acids present in SSL [5,26,29]. The sensitivity of *P. stipitis* (A), to acetic acid can be reduced by operating at pH 6.5. However, at higher pH, there is increased opportunity for bacterial contamination. The acetic acid concentration can be further lowered by steam stripping [17,29], but the cost of this extra operation would be high.

Conclusion

HSSL supplemented with mineral salts and nutrients was fermented to ethanol using an adapted strain of *P. stipitis*. Maximum ethanol yield ($Y_{p/s}$) and productivity (Q_p) were 0.41 g g^{-1} and 0.44 g l^{-1} h^{-1}, respectively, at an OTR of 2.0 mmol O_2 l^{-1} h^{-1} and pH 6.5. Acetic acid is an important inhibitory component present in hemicellulose acid hydrolysate. Its toxic effect is due primarily to its undissociated form and increases at a lower pH. Fermentation at a pH well above the p*K*a value (4.8 at 30°C) reduces the inhibitory effect and improves the ethanol yield and productivity.

Acknowledgement

The author gratefully acknowledges help provided by J.S. Sandhu, FNA Director.

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