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REVIEW

Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*

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Abstract Rising crude oil prices and environmental concerns have renewed interest in renewable energy. Cellulosic ethanol promises to deliver a renewable fuel from non-food feedstocks. One technical challenge producing cellulosic ethanol economically is a robust organism to utilize the different sugars present in cellulosic biomass. Unlike starch where glucose is the only sugar present, cellulosic biomass has other sugars such as xylose and arabinose, usually called C5 sugars. This review examines the most promising naturally occurring C5 fermenting organism, *Pichia stipitis*. In this work, the properties that make *P. stipitis* unique from other organisms, its physiology and fermentation results on lignocellulosic substrates have been reviewed. *P. stipitis* can produce 41 g ethanol/l with a potential to cleanup some of the most concentrated toxins. These results coupled with the less stringent nutritional requirements, great resistance to contamination and its thick cell walls makes *P. stipitis* a viable organism for scale-up. However, *P. stipitis* has a slower sugar consumption rate compared to *Saccharomyces cerevisiae* and requires microaerophilic condition for ethanol production. Finally, future studies to enhance fermentation capabilities of this yeast have been discussed.

Keywords Biomass · Ethanol · Inhibitors · Wood · Xylose

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Introduction

Increase in global energy demand has caused oil prices to reach record levels in recent times. High oil prices together with concerns over CO_2 emissions have resulted in renewed interests in renewable energy. In using a renewable energy source such as ethanol, the carbon is recycled through photosynthesis. Ethanol is a renewable fuel which can be used in cars either as neat alcohol (100% ethanol) or in different combinations with gasoline (E10, E15, E85). The US and Brazil are the leading producers of fuel ethanol in the world. Most of the ethanol produced in the US, currently over 7 billion gallons, is made from corn grown mostly in the Midwest regions of the US. However, studies predict only 15 billion gallons can be produced from corn at a sustainable level (http://www.ncga.com/ethanol/pdfs/2006/HowMuchEthanolCan%20ComeFromCorn.v.2.pdf). The recent doubling in corn prices without an increase in the ethanol price has reduced profit margins for producing ethanol from corn. The need for nonfood feedstock makes cellulosic substrates attractive for producing ethanol.

Cellulosic biomass refers to agricultural residues such as corn stover, wheat straw, bagasse, plant residues, municipal solid wastes, and manure. These are waste materials or low value feedstock with enormous potential for ethanol production. Recently, the US government passed an energy bill mandating 36 billion gallons of ethanol by 2022, of which 21 billion gallons will be produced from cellulosic biomass. Developing an efficient technology to convert the sugars in cellulose to ethanol in an economical process is a challenge currently worked on by industry, research institutions and academia.

Although there are different processes for producing ethanol from biomass, the most common approach is shown in Fig. 1. The feedstock is milled and pretreated to open up the biomass structure. Cellulosic biomass is a recalcitrant substrate consisting mainly of cellulose, hemicellulose and lignin (Mosier 2005). Pretreatment may involve high temperatures and chemicals to make the cellulose and hemicellulose susceptible to enzymatic action. Enzymes are added to hydrolyze the biomass to simple sugars such as glucose and xylose. Fermentation is then performed where the sugars are converted to ethanol. Fermentation is followed by distillation where ethanol is recovered and the waste water is treated for reuse.

Some technical challenges in developing an economical process for ethanol from cellulosic biomass include the requirement for a high yield feedstock (tons/acre), storage and transport of feedstock, pretreatment cost, reducing or eliminating chemicals in pretreatment, lowering the cost and quantity of enzymes, an efficient organism for using the available sugars (DOE/SC-0095). Other challenges include developing an efficient ethanol recovery system and cleanup of the waste water stream. Unlike corn where glucose constitutes all the sugars present, glucose constitutes $\sim$60% of the total sugars available in cellulosic biomass (Lee 1997). Fermentation of the available sugars in cellulosic biomass presents a unique challenge because of the presence of other sugars such as xylose and arabinose (C5 sugars). In addition, pretreatment chemicals and toxins generated during pretreatment presents additional problems that are not seen in the current corn to ethanol process.

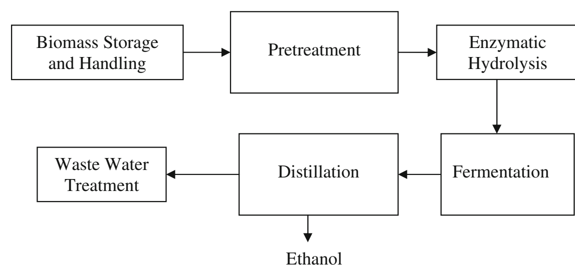

Fig. 1 Simplified schematic process for producing ethanol from biomass

Developing an efficient organism to ferment the C5 sugars has been pursued for the past few decades. Organisms to ferment the C5 sugars in cellulosic biomass can be divided into two subgroups, namely naturally occurring and genetically engineered microorganisms. The naturally-occurring microorganisms include *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus*. Genetically-engineered organisms with C5 fermenting capabilities include *Saccharomyces cerevisiae*, *E. coli*, *Zymomonas mobilis* (Skoog and Hahn-Hagerdal 1988). There are other reviews on C5 organisms in general (Skoog and Hahn-Hagerdal 1988; Jeffries and Jin 2000). This review looks at the most promising naturally occurring C5 fermenting organism, *P. stipitis*. In this work, the unique properties of *P. stipitis* compared to other naturally occurring C5 organisms, its physiology and fermentation results on pretreated cellulosic biomass has been reviewed.

Nature of the yeast

Pichia stipitis belongs to a group of yeasts isolated from decaying wood and the larvae of wood inhabiting insects (Toiviola et al. 1984). The ecological niche of this yeast provides it with capabilities to utilize most of the sugars present in wood. *P. stipitis* has developed various cellulases and hemicellulases to break down wood into monomeric sugars (Jeffries et al. 2007). One of those enzymes is β-glucosidase which breaks down cellobiose into glucose monomers; therefore *P. stipitis* has the ability to ferment cellobiose. In yeasts, such as *S. cerevisiae*, ethanol is produced when sugar concentrations are relatively low, even under aerobic conditions. This phenomenon is known as the Crabtree effect. Unlike *S. cerevisiae*, *P. stipitis* is a respiratory yeast, which does not produce ethanol under aerobic conditions, even in the presence of excess sugars (Klinner et al.

2005). The choice to produce ethanol or cell mass by *P. stipitis* depends on the O_2 supply to the cells. At high aeration rates, only cell mass is produced and at low aeration rates, ethanol is produced (du Preez 1994).

P. stipitis is able to ferment glucose, xylose, mannose, galactose and cellobiose (Parekh and Wayman 1986). It also has the ability to produce cell mass from L-arabinose but not ethanol (Nigam 2002). *P. stipitis* exhibits both low-affinity and high-affinity proton symport systems that operate simultaneously (Kilian and Uden 1988). The low-affinity transport system is shared between glucose and xylose for sugar transport. Glucose inhibits xylose transport by noncompetitive inhibition in the low-affinity proton symport system (Kilian and Uden 1988). The low-affinity transport is used when sugar concentrations are high and the high affinity systems are used when sugar concentrations are low. Repression of xylose uptake occurs in fermentation media containing glucose and xylose (Chandra et al. 1988). Therefore, glucose is the preferred sugar by *P. stipitis* in ethanol production. The rate of glucose consumption is higher than xylose under similar growth conditions (Agbogbo et al. 2006). The transport of sugars into the cells is the rate limiting step in the utilization of sugars for ethanol production in *P. stipitis* (Ligthelm et al. 1988).

In most bacteria, D-xylose conversion proceeds via direct isomerization to D-xylulose using xylose isomerase (Fig. 2a). In yeasts and most fungi, the conversion is carried out by two oxidoreductases, xylose reductase (XR) and xylitol dehydrogenase (XDH). XR is NADPH cofactor specific whereas XDH is NAD^+ cofactor specific. The difference in cofactor preference of XR and XDH leads to the formation of xylitol under anaerobic conditions (Fig. 2b). Xylitol is therefore a byproduct in ethanol fermentation and its production reduces the final ethanol yield. *P. stipitis* is one of the few types of yeast that is able to ferment xylose to ethanol under anaerobic conditions because it possesses both NADH and NADPH specific XR cofactor. The benefit of XR using NADH is that there is a total cofactor balance when this cofactor is used, and therefore no xylitol is produced (Fig. 2c). Kinetic studies indicate that NADPH is the preferred coenzyme because its affinity is about double the value for NADH (Verduyn et al. 1985). Under anaerobic conditions, xylose fermentation by *P. stipitis* must proceed by NADH-linked XR for a total cofactor balance. The ability of *P. stipitis* and *P. tannophilus* to use NADH for XR provides these yeasts with the ability to produce less xylitol in xylose conversion compared to other xylose fermenting yeasts under anaerobic conditions (Verduyn et al. 1985).

Studies on *P. stipitis* and *P. tannophilus* suggest that *P. stipitis* produces less xylitol than *P. tannophilus* even though they both have the ability to use NADH as a cofactor for XR (Debus et al. 1983). The reason for this difference is because *P. stipitis* has a complex oxidative respiratory system that contains cytochrome and non-cytochrome electron transport chains in the mitochondria. The non-cytochrome electron transport chain is the salicyl hydroxamic acid (SHAM) sensitive pathway. The existence of the SHAM sensitive pathway in *P. stipitis* provides a redox sink for coping with cofactor in-balance, and therefore results in less

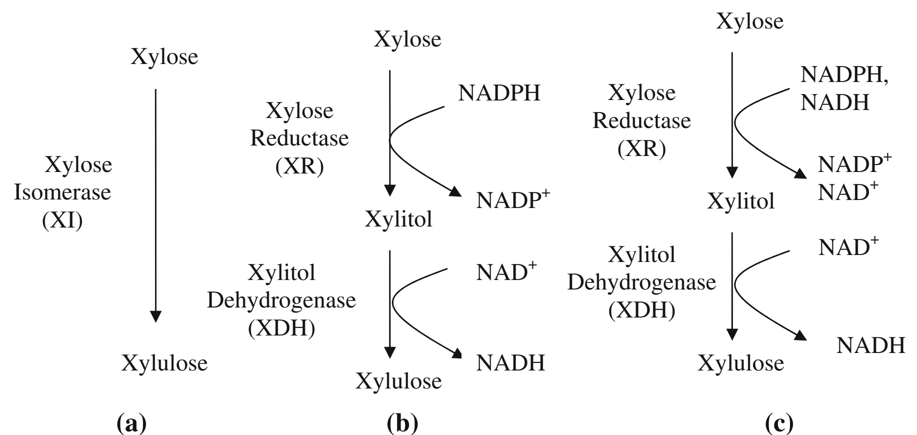

Fig. 2 Xylose fermentation pathway

xylitol production by *P. stipitis* (Jeppsson et al. 1995) compared to other yeasts lacking this pathway. It is for the same reason that XR and XDH genes cloned from *P. stipitis* into recombinant *S. cerevisiae* produce considerable amounts of xylitol, although transformants expressed the same level of XR and XDH enzyme activities as in *P. stipitis* (Hahn-Hagerdal et al. 1994). Deletion of the cytochrome c gene from *P. stipitis* resulted in a mutant that uses the SHAM-sensitive respiratory pathway for aerobic energy production. The mutant produced had 50% lower growth rates than the parent strain on fermentable sugars, and had a 21% higher yield on ethanol compared to parental strains (Shi et al. 1999). Further work showed that the non-proton translocating NAD(P)H dehydrogenase are linked to the SHAM-sensitive terminal oxidase in xylose metabolizing cells and serves a regulatory function in the complex redox network of *P. stipitis* (Shi et al. 2002).

Recently, the genome sequence for *P. stipitis* has been published (http://www.jgi.doe.gov/pichia) (Jeffries et al. 2007). The genome sequence shows that *P. stipitis* uses an alternative nuclear codon that substitutes serine for leucine when CUG is specified (Laplaza et al. 2006). The sequence showed numerous genes for bioconversion such as xylanase, endo-1,4-β-glucanase, exo-1,3-β-glucosidase, β-mannosidase and α-glucosidase (Jeffries et al. 2007). The presence of these genes in *P. stipitis* offers very useful traits for simultaneous saccharification and fermentation of cellulose and hemicellulose. Amino acid supplementation, controlled oxygen limitation and sequential double induction have been found to improve xylanase production in *P. stipitis* (Gorgens et al. 2005). Colocation of genes with related functions has been found to occur with a high frequency in *P. stipitis*. This suggests that proximal relationships evolved through selective pressure in *P. stipitis* (Jeffries et al. 2007). The colocation of genes for related functions may enhance the gene activities and their mutual function (Jeffries et al. 2007).

The physiology of the yeast

The optimal temperature for *P. stipitis* fermentation is between 25 and 33°C and the optimal pH is 4.5–5.5 (du Preez et al. 1986). Nutrients in fermentation media play an important part in the growth and ethanol production in *P. stipitis*. Studies on *P stipitis* NRRL Y-7124 using a defined medium provided with nitrogen, vitamin, amino acids, purines and pyrimidines shows that some of these components could enhance growth and ethanol production in *P. stipitis* (Slininger et al. 2006). Ethanol production increased with addition of amino acids and nitrogen was required for non-growth associated ethanol production (Slininger et al. 2006). Ammonium salts increased the ethanol productivity and the ethanol to biomass yield in *P. stipitis* (Guebel et al. 1992; Agbogbo and Wenger 2006). Magnesium has also been shown to play an important role in redox balance and therefore has an effect on xylitol production (Mahler and Nudel 2000). Low levels of Mg resulted in xylitol accumulation and a high intracellular NADH content (Mahler and Nudel 2000). Corn steep liquor is a viable nutrient source for *P. stipitis* fermentation when used as a sole nitrogen source compared to amino acids, vitamins and other nutrients (Amartey and Jeffries 1994). A summary of the effect of various nutrients on growth and ethanol production is shown in Table 1.

Oxygen plays an important role in cell growth, redox balance, functioning of the mitochondria and generation of energy for xylose transport in *P. stipitis* (Skoog et al. 1990). Fermentation in *P. stipitis* is not induced by high sugar concentrations, but inactivated by aerobic conditions (Passoth et al. 1996). The investigation by Passoth et al. revealed that the branching point between respirative and fermentative metabolism which includes enzymes such as pyruvate decarboxylase, alcohol dehydrogenase and aldehyde dehydrogenase were induced by a reduction in oxygen tension (Passoth et al. 1996). Some studies have shown that *P. stipitis* produces ethanol under anaerobic conditions (Delgenes et al. 1986), but microaerobic conditions are optimal for ethanol production (Grootjen et al. 1990). The different measurements used to quantify the level of oxygenation are rev/min in shake-flasks whilst vvm (volume/volume min), OTR (O_2 transfer rate) and DO (dissolved O_2) are used in fermentor experiments (Skoog and Hahn-Hagerdahl 1987). These differences make it difficult to compare data from different O_2 transfer systems. The optimal DOT values are well below 1% of saturation, which is below the sensitivity limit for dissolved O_2 electrodes suitable for use in microbial fermentation (du Preez 1989).

Table 1 Effect of different nutrients on growth and ethanol production in *P. stipitis*

Nutrient	Strain	Effect on cell growth	Effect on ethanol concentration	References
Ammonium	NRRL Y-7124 CBS 6054	Slightly decreased	Increase	Guebel et al. (1992), Agbogbo and Wenger (2006)
Amino acids	NRRL Y-7124	Arg, Hist, Isoleu and Prol increased cell growth. Ala, Glutamic acid, Leucine and Tyrosine decreased cell growth	Ala, Arg, Asp, Glu, Gly, Hist, Leu, and Tyr increased ethanol concentration. Isoleucine reduced ethanol concentration	Slininger et al. (2006)
Calcium	NRRL Y-7124 CBS 6054	Increased growth at low concentration (0.34 mM). Decreased growth at high concentrations (1 mM)	Slightly lower ethanol concentration and ethanol to biomass yield	Guebel and Nudel (1994), Agbogbo and Wenger (2006)
Carbon dioxide	CBS 5773 CBS 5776	Decrease in growth rate	Decrease in fermentation rate and final ethanol concentration	Linden et al. (1993)
Corn steep liquor	CBS 6054	Increase in cell growth	Increase in ethanol fermentation rate and final ethanol concentration	Amartey and Jeffries (1994)
Magnesium (1–4 mM)	NRRL Y-7124	Increase in cell growth	Increase in ethanol concentration	Mahler and Nudel (2000), Guebel and Nudel (1994)
Malt extract, yeast extract and ammonium sulfate	CBS 6054	Decrease in cell growth	Decrease in ethanol fermentation rate and final ethanol concentration	Amartey and Jeffries (1994)
Peptone, yeast extract, $MgSO_4$, and KH_2PO_4	CBS 6054	Decrease in cell growth	Increase in ethanol fermentation rate and final ethanol concentration	Amartey and Jeffries (1994)
Purines and pyrimidines	NRRL Y-7124	No significant effect on growth	No effect on ethanol production	Slininger et al. (2006)
Yeast nitrogen base with supplements	CBS 6054	Decrease in cell growth	Decrease in ethanol fermentation rate and but an increase in final ethanol concentration	Amartey and Jeffries (1994)
Vitamins (thiamine, riboflavin, calcium panthotenate, niacin, pyridoxamine, thioctic acid, folic acid, biotin, B_{12})	NRRL Y-7124	Generally improved cell growth	No effect on ethanol production	Slininger et al. (2006)

The initial xylose concentration has an effect on the fermentation parameters of *P. stipitis* with maximum ethanol productivities occurring at a xylose concentration of 50 g/l (du Preez et al. 1985). Sugar inhibition studies show that volumetric ethanol productivity is inhibited at initial xylose concentrations of between 76 and 99 g/l and ethanol yield decreased when xylose concentration was above 145 g/l (Roberto et al. 1991). Chamy and coworkers found no evidence of xylose inhibition when xylose concentration was 200 g/l (Chamy et al. 1994). High sugar concentrations increase osmotic stress on organisms and therefore reduces growth and fermentation rate. Therefore, the observation by Chamy and coworkers on xylose inhibition may be due to experimental conditions which reduce the sugar stress.

Delgenes et al. (1988) showed that *P. stipitis* produces ethanol initially at 50 g/l, even though this totally inhibits the strains activity under microaerobic conditions. Ethanol can damage the cell membrane by altering the cell membrane organization and permeability. Ethanol is known to dissipate the proton-motive force across the plasma membrane leading to cytoplasmic acidification and cell death. The ethanol tolerance of *P. stipitis* was shown to have a correlation with the ATPase activity of the plasma membrane (Meyrial et al. 1995). The ATPase is used for proton transport across the cell membrane to reduce cytoplasmic acidification. Glucose grown cells of *P. stipitis* showed higher specific ATPase activity compared to xylose grown cells, and therefore the ethanol tolerance of glucose grown cells were higher than xylose grown cells (Meyrial et al. 1995).

Fermentation of lignocellulosic substrates

P. stipitis has been used as an ethanologen to ferment various types of pretreated biomass such as red oaks, wheat straw, sugarcane bagasse, rice straw, corn cob, corn stover, aspen wood, pine wood and poplar wood (Table 2). Fermentations using these hydrolyzates have been performed using mainly shake flasks and bioreactors ranging from 125 ml to 5 l (Table 2). The ethanol produced ranged from 6 to 41 g/l, at a yield of 0.31–0.48 g ethanol/g sugars consumed (Table 2). The percentage of sugars consumed was in the range of 78–100% and fermentation times between 5 and 100 h. In most of these studies, the pretreated substrate was detoxified by methods such as steam stripping (Parekh et al. 1987), overliming (Amartey and Jeffries 1996; Eken-Saracoglu and Eden 2000), molecular sieves and mixed bed of resins (Tran and Chambers 1985). In other cases, the hydrolyzate was used without detoxification by adjusting the pH to around 6.0 (van Zyl et al. 1988; Agbogbo and Wenger 2007).

The effect of different inhibitor concentrations on *P. stipitis* has been studied (Tran and Chambers 1986; Delgenes et al. 1996; Liu et al. 2004, 2005). In all these studies, model compounds of inhibitors generated in acid prehydrolysis hindered fermentation in *P. stipitis* (Tran and Chambers 1986). Some compounds in pretreated lignocellulosic substrates such as vanillin (Delgenes et al. 1988), and syringaldehyde (Tran and Chambers 1986) were identified as some of the most toxic components. Recently it was demonstrated that *P. stipitis* reduces the aldehyde group in the furan ring of HMF and furfural (Liu et al. 2004, 2005). These results together with the observation of a decrease in acetic acid concentration during fermentation on *P. stipitis* (Agbogbo et al. 2007), presents a potential for in situ detoxification of the inhibitors generated during pretreatment.

Conclusion and future prospects

P. stipitis has certain unique qualities that make it the most promising naturally occurring organism to utilize the sugars present in lignocellulosic substrates. One of these qualities is the enzyme systems developed for breaking down and using wood sugars. *P. stipitis* has developed two mechanisms to minimize xylitol production. The first mechanism is the ability to use two XR cofactors (NADH, NADPH), which *P. stipitis* shares with other organisms such as *P. tannophilus*. The second mechanism, which is unique to only *P. stipitis* is the existence of the SHAM sensitive pathway which provides a redox sink for coping with cofactor imbalance. Recently, the genome of *P. stipitis* has been sequenced and this provides the blueprints for making genetic manipulations.

P. stipitis has a slower sugar consumption rate compared to *S. cerevisiae*. Understanding the limitations for sugar transport in *P. stipitis* will help improve

Table 2 Fermentation results of using *P. stipitis* on pretreated biomass substrates

Substrate	Pretreatment type	Reactor type and size	Ethanol (g/l)	Yield (g/g)	Aeration rate	Sugar utilized (%)	Fermentation time (h)	References
Aspen wood chips	SO_2 pretreatment	250 ml Erlenmeyer flasks	41	0.47	125 rpm	95	96	Parekh et al. (1988)
Aspen wood chips	SO_2 pretreatment	250 ml Erlenmeyer flasks	29	0.41	125 rpm	78	96	Parekh et al. (1988)
Aspen wood chips (hardwood)	Dilute H_2SO_4	3.4 l Continuous Dynamic Immobilized Cell Bioreactor	9.2	0.45	40 rpm	88	5	Parekh et al. (1987)
Corn cob	Dilute H_2SO_4	–	13.3	0.41		95[a]	48	Amartey and Jeffries (1996)
Corn cob	Dilute H_2SO_4	250 ml shake flasks	10.4	0.34	80 rpm	85[a]	96	Eken-Saracoglu and Arslan (2000)
Corn stover	Dilute H_2SO_4	125 ml Erlenmeyer flasks	25	0.42	150 rpm	92	72	Agbogbo et al. (2007)
Corn stover	Dilute H_2SO_4	125 ml Erlenmeyer flasks	15	0.37	150 rpm	97	72	Agbogbo and Wenger (2007)
Corn stover	Dilute H_2SO_4	250 ml flasks	14.5[a]	0.39–0.41	150 rpm	–	20[a]	Fenske et al. (1998)
Crude softwood sulfite spent liquor	Pulp and paper mill liquor	3.4 l Continuous Dynamic Immobilized Cell Bioreactor	18	0.45	40 rpm	89	14	Parekh et al. (1987)
Mixed aspen and pine	SO_2 catalyzed	3.4 l Continuous Dynamic Immobilized Cell Bioreactor	10.3	0.47	40 rpm	94	4	Parekh et al. (1987)
Pine wood chips (softwood)	Dilute H_2SO_4	3.4 l Continuous Dynamic Immobilized Cell Bioreactor	10	0.48	40 rpm	90	4	Parekh et al. (1987)
Poplar	Dilute H_2SO_4	250 ml flasks	12[a]	0.31–0.38	150 rpm	–	50[a]	Fenske et al. (1998)
Red oak spent sulfite liquor	Pulp and paper mill liquor	300 ml Stirred tank bioreactor (STR) 250 ml Erlenmeyer flasks	20.2	0.41	2 mmol O_2 $l^{-1}h^{-1}$	89[a]	46	Nigam (2001b)
Red oak wood chips	Dilute H_2SO_4 (0.5 v/v)	250 ml Erlenmeyer flasks	14.5	0.4	4.5 mmol O_2 l^{-1} h^{-1}, 150 rpm	93.5	100	Nigam (2001a)
Red oak wood chips	Dilute H_2SO_4	500 ml fermentor	9.9	0.46[a]	200 ml/min, 250 rpm	98[a]	–	Tran and Chambers (1986)
Rice straw	Steam Explosion	3 l fermentor	6	0.40	2 vvm 500 rpm	100	20	Moniruzzaman (1995)
Steam stripped sulfite spent liquor	Pulp and paper mill liquor	3.4 l Continuous dynamic immobilized cell bioreactor	18.5	0.46	40 rpm	94	7	Parekh et al. (1987)
Sugarcane bagasse	Dilute H_2SO_4	2 l Multigen F-2000 bioreactor 250 ml Erlenmeyer flasks	15	0.38[b]	100 ml/min 300 rpm	–	60[c]	Van Zyl et al. (1988)

Table 2 continued

Substrate	Pretreatment type	Reactor type and size	Ethanol (g/l)	Yield (g/g)	Aeration rate	Sugar utilized (%)	Fermentation time (h)	References
Switchgrass	Dilute H_2SO_4	250 ml flasks	14[a]	0.36–0.42	150 rpm	–	36[a]	Fenske et al. (1998)
Wheat straw	Dilute H_2SO_4 (1.85% w/v)	5 l Microferm Fermentor	22.3	0.43	0.02 vvm	85.8	50	Nigam (2001c)

[a] Calculated from data and graphs in the paper

[b] Yield calculation based on initial sugar concentration

[c] Calculated from maximum productivity

the fermentation rate. Moreover, understanding the sugar repression systems in *P. stipitis* will help improve sugar utilization rates. Further work should also focus on increasing the ethanol tolerance and concentrations in *P. stipitis* at industrial relevant conditions. More work on understanding ATPase activity could help increase the ethanol tolerance in *P. stipitis*. Evolution towards higher promoter strength for higher transcript levels could be advantageous in enhancing fermentation rates. Searching for other xylose fermenting organisms from woody environments could potentially lead to the discovery of new and potent organisms. Finally, determining how other inhibitor compounds change during fermentation in *P. stipitis* could potentially reveal new insights.

Fermentation results on *P. stipitis* show that 61 g ethanol/l can be produced in synthetic media (Slininger et al. 2006), and 41 g/l (Parekh et al. 1987) in pretreated aspen wood. These results, coupled with the fact that *P. stipitis* can utilize corn steep liquor as a nutrient source (Amartey and Jeffries 1994), provides unique opportunities for looking at the capabilities of this yeast in cellulosic ethanol production. The ability of *P. stipitis* to consume acetic acid, reduce the furan ring in HMF and furfural creates an opportunity for this yeast to cleanup some of the most concentrated toxins in cellulosic biomass conversion. This could be very beneficial in waste water treatment since there will be a reduction in the quantity of toxins to be treated. Fermentation of lignocellulosic substrates using *P. stipitis* at pilot scale could potentially reveal new insights on using this organism in industrial applications.

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