SUSTAINABLE CHEMICAL PROCESSES

RESEARCH ARTICLE Open Access

Production of ethanol from hemicellulose fraction of cocksfoot grass using *pichia stipitis*

Stephen Ikechukwu Njoku[1,2], Jens Asmus Iversen[1,2], Hinrich Uellendahl[1] and Birgitte Kiær Ahring[1,2*]

Abstract

Background: In this study, cocksfoot grass (*Dactylis glomerata*), an abundant lignocellulosic biomass was pretreated using different operational parameters using wet explosion (WEx) pretreatment for accessing the bioethanol potential of the hemicellulose fraction. Utilization of the hemicellulose liquid hydrolysate to ethanol is essential for economically feasible cellulosic ethanol processes. Fermentation of the separated hemicellulose liquid hydrolysates obtained after the WEx pretreatment was done by *Pichia stipitis* CBS 6054 (*Scheffersomyces stipitis*).

Results: The fermentation of the WEx liquid hydrolysate from the pretreatment at higher severity (180°C, 15 min, 87 psi oxygen and 190°C, 15 min, 0.2% sulfuric acid) was fully inhibited probable by the presence of higher concentrations of inhibitory compounds such as furfural, HMF and acetic acid. The ethanol yield among other WEx conditions was in the range of 89 to 158 mL/kg DM, with the highest yield (92% of theoretical maximum value) found for the lower pretreatment severity at 160°C, 15 min, 87 psi oxygen.

Conclusions: Our findings from this present study demonstrated that the release of hemicellulose sugars in the liquid hydrolysate is maximal when a lower pretreatment severity is applied. This is evident as the highest ethanol yields were found under the pretreatment conditions at lower severity.

Keywords: Wet explosion, Lignocellulosic biomass, Cocksfoot grass, Pretreatment, Ethanol fermentation, Inhibitors, *Pichia stipitis*

Background

Increasing global energy requirements and greater environmental awareness have resulted in increasing focus on alternatives to fossil fuels as energy sources. Lignocellulosic biomass such as agricultural residues, forestry waste and municipal solid waste presents a sustainable and renewable source for the production of liquid biofuels such as bioethanol [1]. As most often being a by-product from food and feed production, lignocellulosic biomass does not compete with the production of edible crops [2,3] and has the potential to be the feedstock for the production of a considerable proportion of transport fuels if cost effective conversion processes are available [4]. The major components in lignocellulosic biomass are cellulose, hemicellulose and lignin. Hemicellulose sugars are the second most abundant carbohydrates in nature and its conversion to ethanol could provide an alternative liquid fuel source for the future [5].

Because of the recalcitrance of the lignocellulosic structure to enzymatic attack, pretreatment of the material is necessary to enhance the accessibility of the enzymes to the substrate [6]. Various thermal and chemical pretreatment methods as well as combinations of both have been proposed to make lignocellulosic biomass susceptible to enzymatic and microbial conversion [7,8]. The resulting slurry from the pretreatment of lignocellulosic biomass contains liquid and solid fractions; the solid fraction mostly contains cellulose and lignin as the major components, while the liquid fraction contains xylose as the main sugar, and small concentrations of other sugars such as glucose and arabinose mainly from hemicellulose liquid hydrolysate. Hence, the optimum utilization of the liquid fractions to ethanol is essential for an economical feasible in biorefinery processes [9]. However, the liquid fractions often contains inhibitors such as furfural from xylose degradation, hydroxymethyl furfural (HMF) from glucose degradation, carboxylic

* Correspondence: bka@wsu.edu
[1]Section for Sustainable Biotechnology, Aalborg University Copenhagen, A.C. Meyers Vænge 15, 2450 Copenhagen, SV, Denmark
[2]Center for Bioproducts and Bioenergy, Washington State University Tri-Cities, 2710 Crimson way, Richland, WA 99354, USA

acids mainly acetic acid from the acetyl group in hemicellulose decomposition, and phenolic compounds from lignin degradation [9] and these are considered to be potential fermentation inhibitors that affect the growth rate of microbes during ethanol fermentations [10].

Microbes such as yeasts and bacteria are essential for the conversion of hemicellulose sugars to ethanol [5]. *Pichia stipitis* (*Scheffersomyces stipitis*) among others is one of the robust xylose-fermenting yeast that has been investigated in many laboratories around the world because of its capability for using pentose sugars beside hexoses with a high ethanol yield [11]. Moniruzzaman, [12] reported ethanol yield of 78% theoretical maximum from exploded rice straw hydrolysate fermented to ethanol by *Pichia stipitis* Y-7124. In a similar manner, Zhu et al. [10] found ethanol yield of around 80% theoretical from steam exploded corn stover acid hydrolyzate fermented to ethanol using *Pichia stipitis* CBS 5776.

The present study investigated ethanol production from hemicellulose hydrolysate of cocksfoot grass using *Pichia stipitis* CBS 6054 (*Scheffersomyces stipitis*) after wet explosion pretreatment. The effect of wet explosion process parameters on the production of fermentation inhibitors such as acetic acid and furfural in the liquid fraction was evaluated.

Results and discussion

Composition of WEx hydrolysates

The main chemical composition of raw material was (g/100g DM): cellulose, 35.73; hemicelluloses, 23.71; and lignin, 18.74. The hydrolysates containing monomeric sugars and fermentative inhibitors used for the fermentations were prepared from the WEx liquid fractions and their compositions are depicted in Table 1.

Fermentation of WEx liquid hydrolysates

The wet explosion liquid hydrolysates or fractions obtained from all the pretreatment conditions were fermented to ethanol by *Pichia stipitis* CBS 6054 (*Scheffersomyces stipitis*). Figure 1A and B shows the changes in ethanol and sugar concentrations among the WEx pretreatment conditions. Based on previous studies on hemicellulose hydrolysate fermentation by the yeast *Pichia stipitis* [13,14], the aeration rate was kept constant at 125 rpm throughout the fermentation, since oxygen is one of the crucial parameters for yeast *P. stipitis* during ethanol fermentation. Oxygen plays an important role in cell growth and generation of energy for xylose transport in *P. stipitis* [13]. However, some studies on liquid hydrolysate fermentation by *P. stipitis* shows that genetically modified *P. stipitis* produces ethanol under anaerobic condition [15,16], but microaerobic conditions are optimal for ethanol production [13]. A rapid consumption of sugars was observed in most of the WEx conditions within the 24 h fermentation time. It is noteworthy that the available glucose in the fermentation broth was first consumed by *P. stipitis* before it started to utilize xylose and its complete uptake occurred in 96 h. The amount of ethanol produced steadily increased within 48h fermentation time and leveled out after 72 h (Figure 1A). A lag phase was not observed during the course of fermentation in most of the pretreatment conditions (Figure 1B), except conditions (*C* and *F*) where metabolic activities was not detected due to high concentrations of fermentation inhibitors especially high contents of acetic acid associated with the above-mentioned conditions. The highest ethanol concentration obtained at the end of the fermentation (17.98 g/L) was achieved for the lower pretreatment severity, *A* (160°C, 15 min, 87 psi oxygen), and it was in accordance with the utilization of sugars which amount to ethanol yield of 157.5 mL/kg DM, corresponding to 92% of theoretical maximum value (Table 2). This is comparable to ethanol yield of 85-90% of the theoretical maximum found for *Pichia stipitis* CSIR-Y633 fermenting xylose sugar [17].

For the pretreatment conditions (*B* and *D*), the ethanol concentration was around 12 g/L, which is not comparable to the ethanol concentration found under condition *A*, but higher than the concentration achieved for condition *E*, which gave only approximately 10 g/L. This shows that the hemicellulose sugars under pretreatment condition *E* (170°C, 15 min, 0.2% sulfuric acid) has to large extend been degraded to other products other than sugars, like furfural during the WEx pretreatment. However, the sugars found under the above-mentioned condition was able to ferment to ethanol, showing that the concentrations of inhibitors under this condition was not a limiting factor for the yeast *P. stipitis*, unlike conditions *C* and *F* (180°C, 15 min, 87 psi oxygen and 190°C, 15 min, 0.2% sulfuric acid) where the yeast *P. stipitis* could not assimilate the sugars probable due to high content of inhibitors.

Table 1 Composition of the WEx hemicellulose hydrolysates from wet exploded cocksfoot grass

Compounds (g/L)	WEx process conditions					
	A	B	C	D	E	F
Hexose sugars	1.83 (0.01)	2.07 (0.04)	1.03 (0.03)	0.78 (0.02)	0.79 (0.01)	2.13 (0.03)
Pentose sugars	35.16 (0.07)	25.86 (0.04)	14.59 (0.05)	27.33 (0.16)	22.96 (0.17)	13.93 (0.13)
Furfural	0.44 (0.02)	0.50 (0.14)	2.90 (0.00)	0.17 (0.12)	0.51 (0.03)	1.00 (0.06)
Hydroxymethyfurfural	0.19 (0.00)	0.21 (0.08)	0.58 (0.15)	0.02 (0.00)	0.09 (0.05)	0.38 (0.11)
Acetic acid	1.72 (0.11)	2.13 (0.04)	5.21 (0.06)	1.32 (0.07)	2.04 (0.13)	3.06 (0.03)

Average of duplicates. Standard deviation shown in parentheses.

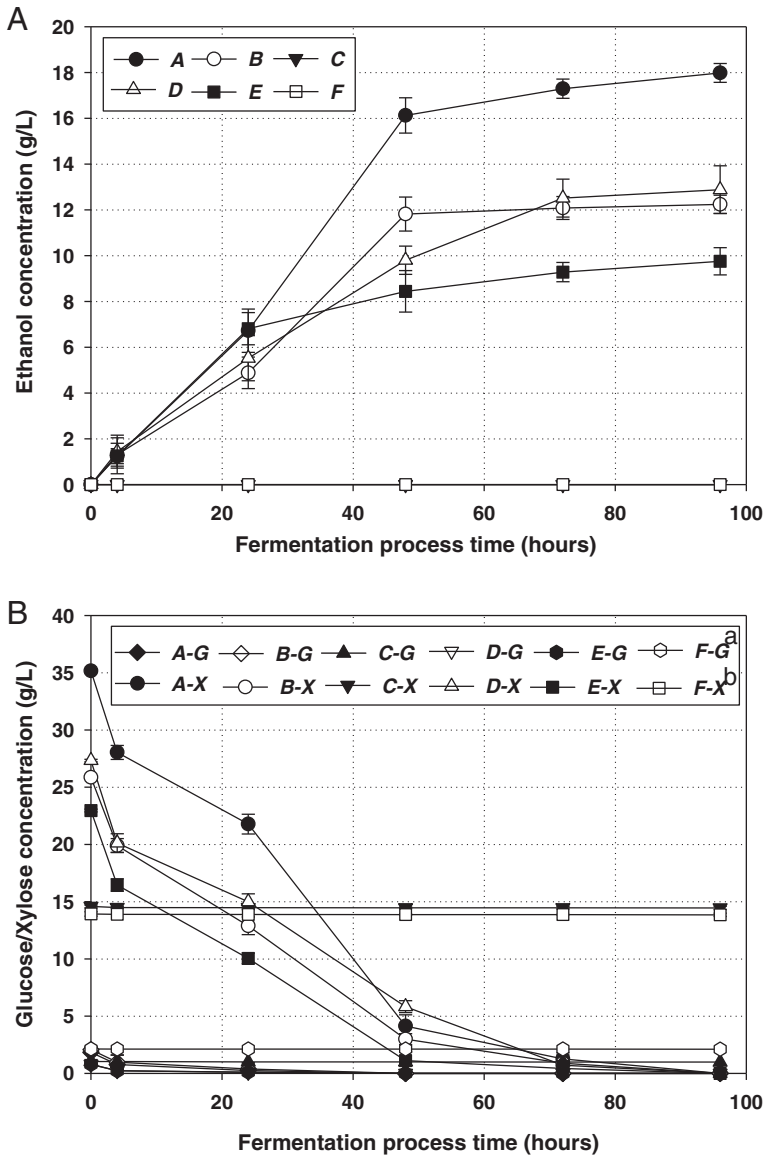

Figure 1 Ethanol production profile. (A) Time course of ethanol production and glucose and xylose consumption **(B)** during ethanol fermentation from hemicellulose hydrolysate by *P. stipitis* CBS 6054 over 96 h, 125 rpm at 30°C and pH 6.0. Values are means of duplicate experiments. [a]G and [b]X notates the glucose and xylose concentration, respectively, after pretreatment at pretreatment condition A-F.

Table 2 Summary of fermentation results among the WEx conditions

Treatment	Final ethanol concentration (g/L)	Ethanol yield (mL/kg-DM)	% of theoretical yield	Final pH
A	17.98 (0.02)	157.50 (0.05)	92.22	6.98 (0.02)
B	12.24 (0.04)	112.30 (0.03)	65.78	6.94 (0.05)
C	0.00 (0.00)	0.00 (0.00)	0.00	6.23 (0.03)
D	12.88 (0.04)	123.70 (0.06)	72.42	7.02 (0.04)
E	9.75 (0.01)	88.50 (0.02)	51.85	6.86 (0.08)
F	0.00 (0.00)	0.00 (0.00)	0.00	6.21 (0.07)

Standard deviation shown in parentheses. Fermentations were performed at 30°C in a shaker incubator at 125 rpm over 96 h.

Pretreatment conditions *B* and *D* (170°C, 15 min, 87 psi oxygen and 170°C, 15 min, 0.2% sulfuric acid), shows a similar ethanol yield, but, was slightly higher in pretreatment condition *D* (Table 2), around 10% higher. The only difference in the above-mentioned conditions was the addition of pure oxygen and sulfuric acid. This is in agreement that pretreatment with addition of dilute acid at a moderate temperature can release up to 100% fermentable hemicellulose sugars and that a balance between solubilization and degradation of hemicellulose sugars is a mechanism in pretreatment with addition of both oxygen and sulfuric acid [1]. The above-mentioned WEx pretreatment conditions achieved ethanol yield of 112.3 and 123.7 mL/kg-DM, which corresponds to 65.8% and 72.4% of theoretical, respectively, (Table 2). In comparison, Zhong et al. [18] reported ethanol yield of 72 and 68% of theoretical maximum, respectively, with *Pichia stipitis* FPL-061 and DX-26 fermenting AFEX-treated rice straw hydrolysate.

The fermentability of WEx hydrolysates under pretreatment conditions *C* and *F* (180°C, 15 min, 87 psi oxygen and 190°C, 15 min, 0.2% sulfuric acid) was fully inhibited, because they contain high concentration of fermentation inhibitors. This demonstrates that lower pretreatment severity is more advantageous for maximizing the production of fermentable hemicellulose sugars thereby reducing the production of inhibitory compounds during pretreatment. The above-mentioned conditions were the most severe pretreatment conditions tested in this study for WEx pretreatment with addition of oxygen or dilute sulfuric acid.

Effect of fermentative inhibitors

The inhibitory effects observed on the fermentation of WEx hydrolysates under pretreatment conditions (*C* and *F*) could be attributed to the presence of furfural at high concentration of about 2 g/L, but the complete inhibition of the fermentation could further be due to the higher concentrations of acetic acid (5.2 and 3.1 g/L, respectively) in the above-mentioned conditions (Table 1). It has been reported elsewhere in the literature [19] that furfural concentration should be at a level of 1.0 g/L in order to present problems for yeast. The formation of acetic acid was more pronounced in the pretreatment condition with high temperature and addition of oxygen pressure. Palmqvist and his co-worker [20] reported in their recent review paper that microorganisms can up to a certain limit survive the stress of these compounds, but cell death would occur if the stress exceeds the limit that cell can bear. The effects of these fermentation inhibitors on ethanol fermentation by *P. stipitis* has been demonstrated in the literature, Bellido et al. [21] found that ethanol yield from hemicellulose hydrolysate decreased with increasing acetic acid concentrations and uptake of xylose was more affected than glucose. This paper further mentioned that cell growth and ethanol yield was considerably affected at 2.5 g/L of acetic acid in synthetic media and complete inhibition of growth and ethanol production occurred at 3.5 g/L. Progressively, HMF and furfural caused delay of sugar consumption, but was eventually assimilated by *P. stipitis* below 2 g/L where inhibition was less profound than with acetic acid. Scordia et al. [22] further reported that fermentation of hemicellulose liquid hydrolysate by *P. stipitis* is mainly inhibited by acetic acid and to lesser extent by the presence of furfural.

However, the liquid hydrolysate originating from any pretreatment of lignocellulosic biomass can be detoxified by removal of inhibitory compounds in order to adapt the yeast to utilize the available sugars to ethanol. Overliming and neutralization are some of the proposed methods to carryout hemicellulose hydrolysate detoxification [23,24]. Performing hemicellulose hydrolysate detoxification is often energy demanding and can elevate the process cost of the ethanol production of hemicellulose sugars. In order to make lignocellulosic ethanol production more economically feasible, the hydrolysate arising from the separated liquid fractions after pretreatment should be able to ferment to ethanol without the need for further detoxification. Therefore, the hemicellulose hydrolysate obtained after the WEx pretreatment was not detoxified.

Based on previous experiments with *P. stipitis* fermentation of hemicellulose hydrolysate [25], the initial pH in the fermentation broth for all the WEx pretreatment conditions were maintained at pH 6.0. At the end of the fermentation, an increase in pH was observed in most of the pretreatment conditions which can be attributed to the consumption of acetic acid by *P. stipitis* (Figure 2). The acetic acid concentration in most of the fermented WEx hydrolysates range from 1.32-2.13 g/L, but at the end of the fermentation, only about 0.1 g/L of acetic acid was found among the fermented WEx hydrolysates. Table 2 shows the final pH range at the end of the fermentation among the pretreatment conditions. A pH range of approximately 7.0 was observed in most the pretreatment conditions, while the acetic acid was significantly consumed, however, the end products generated by *P. stipitis* from the acetic acid consumption was not determined. This is in accordance with the previous investigations on hemicellulose hydrolysate fermentation by *P. stipitis* where the increase in pH was attributed to acetic acid consumption [9,20,22].

Conclusions

This study has demonstrated that wet explosion (WEx) pretreatment with additives (dilute sulfuric acid or oxygen) facilitates the production of fermentable hemicellulose

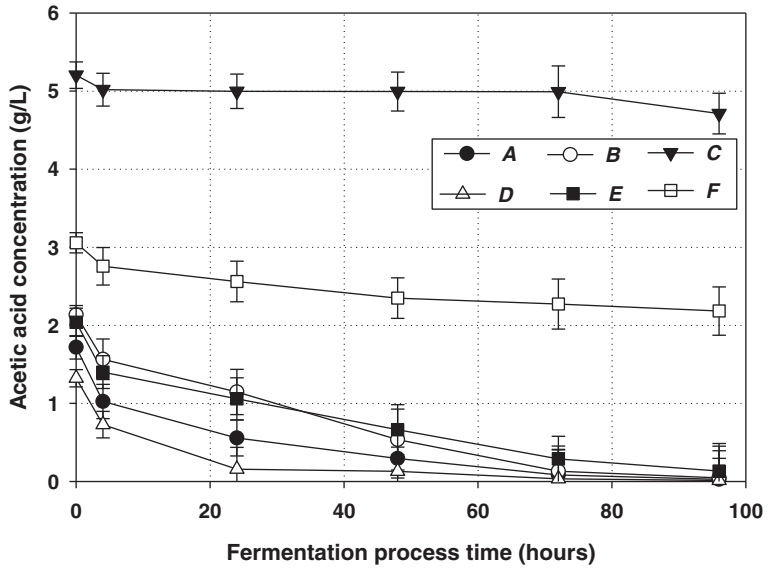

Figure 2 Acetic acid consumption profile. Time course of acetic acid concentrations in the hemicellulose liquid hydrolysates during ethanol fermentation over 96 h using *P. stipitis* CBS 6054 for the different pretreatment conditions A-F.

sugars that was optimally fermented to ethanol by *Pichia stipitis* CBS 6054 (*Scheffersomyces stipitis*) without further detoxification or use of costly enzyme mixtures. It further shows that lower pretreatment severity is an ideal combination of WEx pretreatment parameters for achieving higher ethanol yields from hemicellulose sugars, and at the same time, reduces the formation of fermentation inhibitory compounds. This is evident as the highest ethanol yield of 158 mL/kg DM (92.2% of theoretical) was found under the lower pretreatment severity *A* (160°C, 15 min, 87 psi oxygen). WEx hydrolysates obtained under higher pretreatment severity could, however, not be fermented to ethanol as it contains higher concentrations of inhibitory compounds.

Methods

Wet explosion pretreatment

The Air-dried cocksfoot grass (*Dactylis glomerata*) was hammer milled to a particle size of 2–3 mm, and stored in plastic bags at room temperature prior to pretreatment. A portion of the raw material was ground in a coffee grinder to pass a 1 mm screen and used for chemical composition analysis.

The wet explosion (WEx) pretreatment was performed batch-wise with the following conditions: 160°C-190°C adding (at) 87 psi oxygen pressure (and) or at 0.2% dilute sulfuric acid concentration for 15 min (Table 3), by suspending the raw cocksfoot grass in tap water to reach a dry matter concentration w/w of 25% in a 10 L high-pressure reactor constructed at the Center for Bioproducts and Bioenergy, Washington State University, USA [26]. The reactor was equipped with a gas/liquid inlet for injection of dilute sulfuric acid or oxygen pressure, and a continuous stirrer (2000 rpm). The reactor was heated by a water jacket connected to a heat exchanger controlled by an oil heater. The temperature and pressure inside the reactor were monitored by two temperature sensors and one pressure sensor both mounted in the headspace and in the bottom of the reactor. The acid concentration or oxygen pressure was added into the pretreatment reactor after the desired temperature was reached. After the treatment, the biomass was flashed into a 100 L flash tank connected to the reactor, resulting in a sudden drop in temperature and pressure.

The resulting slurry from the pretreatment was separated into liquid and solid fractions by vacuum filtration. The solid fraction was stored in a freezer (–16°C) for further processing and the filtrated liquid fraction was stored under refrigeration (5°C) and used for ethanol fermentation by *P. stipitis*.

Table 3 Process conditions used for WEx pretreatment of Cocksfoot grass

Treatment	Temp. (°C)	T/R* (min)	Oxygen (psi)	Acid concen.** (%)
A	160	15	87	-
B	170	15	87	-
C	180	15	87	-
D	170	15	-	0.2
E	180	15	-	0.2
F	190	15	-	0.2

*Retention time. **Acid concentration.

Preparation of WEx hydrolysate and fermentation

The hemicellulose hydrolysates used for all the fermentations were the liquid fraction obtained after separating the pretreated samples after WEx pretreatment from the solids, and were directly fermented to ethanol without enzymatic hydrolysis and detoxification. Fermentation was performed under semi-aerobic conditions in sterile 250 mL Erlenmeyer baffled flasks without any nutrient supplementation, covered with an aerobic stopper, and incubated on a rotary shaker at 125 rpm and 30°C for 96 has reported by Agbogbo and Coward-Kelly, [13]. The pH of the hydrolysates was adjusted to 6.0 with 1 M phosphate buffer solution.

Microorganism and media

Pichia stipitis CBS 6054(*Scheffersomyces stipitis*) was conserved and maintained on 20% glycerol at 4°C at the Center for Bioproducts and Bioenergy, Washington State University, USA. *P. stipitis* inoculum medium contained 20 g/L D-xylose, 20 g/L peptone and 10 g/L yeast extract and was prepared aseptically in 250-mL shaking flask as previously described by Agbogbo and Wenger, [9] with 100 mL medium and incubated on rotary shaker at 30°C and 170 rpm for 24 h. All the media were sterilized by autoclaving at 121C for 30 min. The cells were harvested by centrifugation, and the pellet was collected for the hydrolysate fermentation to a final optical density (OD) of 1.0 measured at $OD_{600\ nm}$ corresponding to a cell concentration of approximately 1.7 g/L.

Analytical methods

The fermentation was performed in duplicates and monitored by withdrawing 2 mL of samples for analyses. The initial chemical composition of the raw material was determined according to the procedure developed by the National Energy Laboratory [27], and the dry matter content (DM), volatile solid contents (VS), and ash were determined according to the procedure described by the American Public Health Association [28].

The concentration of sugars, acetic acid and ethanol were determined by high performance liquid chromatography (HPLC) refractive index (RI) equipped with an Aminex HPX-87P column (Bio-Rad Laboratories, CA, USA) at 83 C with deionized water (Thermo Scientific, Barnstead Nanopure, IA, USA) as an eluent with a flow rate of 1.0 mL/min. The optical density (OD) of the yeast cell was measured spectrophotometrically at 600nm. The ethanol yield (Y_{EtOH}) was calculated by dividing the total amount of ethanol produced by the initial dry weight of treated cocksfoot grass. The percent theoretical (stoichiometric) ethanol yield ($\%Y_{EtOH}$) was calculated according to Equation. (1): where 0.51 is the theoretical ethanol yield (in g-ethanol per g-sugar) [29]. This yield is always less than 100% as part of the sugars is converted to cell mass and by-products by the organisms.

$$Y_{EtOH}(\%) = \frac{Y_{EtOH}}{0.51} \cdot 100 \quad (1)$$

Abbreviations

WEx: Wet explosion; HMF: Hydroxymethylfurfural; OD: Optical density; DM: Dry matter; VS: Volatile solid; APHA: American Public Health Association; HPLC: High performance liquid chromatography; AFEX: Ammonia fiber expansion.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SNJ carried out the biomass pretreatment, ethanol fermentation and data analyses, and drafted the manuscript. JAI provided the yeast *Pichia stipitis* and commented on the manuscript. BKA and HU supervised the entire study and contributed to experimental design, manuscript planning, and reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work is financially supported by the Energy Technology Development and Demonstration Programme of the Danish Energy Council, grant no.: 64009–0010.

Received: 16 April 2013 Accepted: 9 July 2013
Published: 6 August 2013

References

1. Taherzadeh MJ, Karimi K: **Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review.** *Int J Sci* 2008, **9:**1621–1651.
2. Chen H, Qiu W: **Key technologies for bioethanol production from lignocellulose.** *Biotechnol Adv* 2010, **28:**556–562.
3. Petersson A, Thomsen MH, Hauggaard-Nielsen H, Thomsen AB: **Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean.** *Biomass Bioenergy* 2007, **31:**812–819.
4. Kristensen JB, Thygesen LG, Felby C, Jørgensen H, Elder T: **Cell-wall structure changes in wheat straw pretreated for bioethanol production.** *Biotechnol for Biofuels* 2008, **1:**1–9.
5. Jeffries TW: **Engineering yeasts for xylose metabolism.** *Curr Opin Biotechnol* 2006, **17:**320–326.
6. Sassner P, Mårtensson CG, Galbe M, Zacchi G: **Steam pretreatment of H_2SO_4–impregnated Salix for the production of bioethanol.** *Bioresource Technol* 2008, **99:**137–145.
7. Galbe M, Zacchi G: **A review of the production of ethanol from softwood.** *Appl Microbiol Biotechnol* 2002, **59:**618–628.
8. Hendriks ATWM, Zeeman G: **Pretreatments to enhance the digestibility of lignocellulosic biomass.** *Bioresource Technol* 2009, **100:**10–18.
9. Agbogbo FK, Wenger KS: **Production of ethanol from corn Stover hemicellulose hydrolysate using *pichia stipitis*.** *Ind Microbiol Biotechnol* 2007, **34:**723–727.
10. Zhu JJ, Yong Q, Xu Y, Chen SX, Yu SY: **Adaptation fermentation of *pichia stipitis* and combination detoxification on steam exploded lignocellulosic prehydrolyzate.** *Nat Sci* 2009, **1:**47–54.
11. Taniguchi M, Tohma T, Itaya T, Fujii M: **Ethanol production from a mixture of glucose and xylose by co-culture of *pichia stipites* and a respiratory-deficient mutant of *saccharomyces cerevisiae*.** *Jour Ferment Bioeng* 1997, **83:**364–370.
12. Moniruzzaman M: **Alcohol fermentation of enzymatic hydrolysate of exploded rice straw by *pichia stipitis*.** *World Jour of Microbiol Biotechnol* 1995, **11:**646–648.
13. Agbogbo FK, Coward-Kelly G: **Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *pichia stipitis*.** *Biotechnol Lett* 2008, **30:**1515–1524.

14. Parekh SR, Parekh RS, Wayman M: **Fermentation of xylose and cellobiose by *pichia stipitis* and *brettanomyces clausenii*.** *Appl Biochem Biotechnol* 1988, **18:**325–338.
15. Shi X-Q, Jeffries TW: **Anaerobic growth and improved fermentation of *pichia stipitis* bearing a *URA1* gene from *saccharomyces cerevisiae*.** *Appl Microbiol Biotechnol* 1998, **50:**339–345.
16. Delgenes JP, Moletta R, Navarro JM: **The effect of aeration on D-xylose fermentation by *P. Tannophilus*, *P. Stipitis*, *K. Marxianus* and *C. Shehatae*.** *Biotechnol Lett* 1986, **8:**897–900.
17. du Preez JC, Bosch M, Prior BA: **Xylose fermentation by *Candida shehatae* and *pichia stipitis*: effects of pH, temperature and substrate concentration.** *Enzyme and Microb Technol* 1986, **8:**360–364.
18. Zhong C, Lau MW, Balan V, Dale BE, Yuan YJ: **Optimization of enzymatic hydrolysis and ethanol fermentation from AFEX-treated rice straw.** *Appl Microbiol Biotechnol* 2009, **84:**667–676.
19. Roberto IC, Lacis LS, Barbosa MFS, de Mancilha IM: **Utilization of sugarcane bagasse hemicellulosic hydrolysate by *pichia stipitis* for the production of ethanol.** *Process Biochem* 1991, **26:**15–21.
20. Palmqvist E, Hahn-Hägerdal B: **Fermentation of lignocellulosic hydrolysates II: inhibitors and mechanisms of inhibition.** *Bioresource Technol* 2000, **74:**25–33.
21. Bellido C, Bolado S, Coca M, Lucas S, Gonzalez-Benito G, Garcia-Cubero MT: **Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by *pichia stipitis*.** *Bioresource Technol* 2011, **102:**10868–10874.
22. Scordia D, Cosentino SL, Jeffries TW: **Second generation bioethanol production from *saccharun spontaneum* L. ssp. *Aegyptiacum* (wild.) hack.** *Bioresource Technol* 2010, **101:**5358–5365.
23. Cantarella M, Cantarella L, Gallifuoco A, Spera A, Alfan F: **Comparison of different detoxification methods for steam-exploded poplar wood as a substrate for the bioproduction of ethanol in SHF and SSF.** *Process Biochem* 2004, **39:**1533–1542.
24. Chandel AK, Kapoor RK, Singh A, Kuhad RC: **Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM 3501.** *Bioresource Technol* 2007, **98:**1947–1950.
25. Ferrari MD, Neirotti E, Albornoz C, Saucedo E: **Ethanol production from eucalyptus wood hemicellulose hydrolysate by *pichia stipitis*.** *Biotechnol and Bioeng* 1992, **40:**753–759.
26. Rana D, Rana V, Ahring BK: **Producing high sugar concentrations from loblolly pine using wet explosion pretreatment.** *Bioresource Technol* 2012, **121:**61–67.
27. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D: **Determination of structural carbohydrates and lignin in biomass.** *Laboratory Analytical Procedure* 2008. http://www.nrel.gov/biomass/pdfs/42618.pdf.
28. American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WAE): **Standard Methods for the Examination of Water and Waste Water: physical and aggregate properties.** In 18th edition. Edited by Greenberg AE, Clesceri LS, Trussel RR. USA: Washington D. C; 1992.
29. Hatzis C, Riley C, Philippidis GP: **Detailed material balance and ethanol yield calculations for biomass-to-ethanol conversion process.** *Appl Biochem Biotechnol* 1996, **96:**0273–2289.

doi:10.1186/2043-7129-1-13
Cite this article as: Njoku *et al.*: Production of ethanol from hemicellulose fraction of cocksfoot grass using *pichia stipitis*. *Sustainable Chemical Processes* 2013 **1**:13.