Asseessment of furfural inhibition on some Saccharomyces strains in lignocellulosic hydrolyzate fermentation

Nguyen Dinh Thi Nhu Nguyen (1), Tran Thi Phuong Thuy (2), Nguyen Thi My Linh

(1) Faculty of Chemical and Environment Engineering, Lac Hong University, Dong Nai, Vietnam
Email: nhunguyen1301@gmail.com; Tel: (84)(90)392-4109

1. Introduction
Liquid biofuel such as bioethanol is getting attention because of climate concern. Since it is eco-friendly, bioethanol can replace traditional fuel source. Bioethanol can be produced from sugar and starch (first generation materials) or low-cost lignocellulosic materials (straw, corn fiber, hard wood, soft wood, paper residues,...) [8]. Lignocellulose includes cellulose, hemicellulose and lignin, in which carbohydrate fractions can be hydrolyzed into monosaccharides (pentose and pentose), those sugars are fermented by microorganisms into ethanol [16].

Saccharomyces cerevisiae, which currently used for production of bioethanol, is not able to metabolize pentose such as xylose in hydrolysates. Other microorganism strain, like Pichia stipitis, is often used to consumed pentose [11]. Efforts were made to develop Saccharomyces strains that can consume pentose [18], like Saccharomyces cerevisiae CEN PK113-7D [18], Saccharomyces cerevisiae VTT C-10883 [15].

Due to complex structure, lignocellulose needs pretreatment steps with dilute or concentrated acids to liberate components for hydrolysis, to remove cellulose and hemicellulose from lignin and to achieve fermentable sugars for yeast’s utilization. This step can generate inhibitors which can be harmful for yeast performance, they are furans (furfural and 5-hydroxymethylfurfural), organic acids (acetic and formic acids) and phenolic compounds (vanillin) [2, 18]. These inhibitors reduce the bioethanol yield and productivity [17].

Among inhibitors, furfural is considered as most important and popular one. It is a product of the hydrolysis of hemicellulose with the catalyst of acid [5, 15, 17]. According to Ask (2013) and Zhengyun (2010), furfural can be converted to less inhibitory compounds [15, 19].

Detoxification should be applied to remove inhibitors, maintaining yeast activity. Methods as nanofiltration [3], using activated carbon [14], or polymeric adsorbents [12].....were applied. Those methods lead to loss of monosaccharide, reduced ethanol yield and lower ethanol concentration, especially with effort to remove all inhibitors in the hydrolyzate.

The goal of this study was determination of lowest furfural’ concentration, that is not obstacle for some Saccharomyces strains. The tolerance limit can be useful for detoxification step. Besides, robust yeast strains for fermentation of lignocellulosic hydrolyzates were screened.

2. Experimental
Materials
Model hydrolyzed lignocellulosic solutions: composition of model hydrolyzed was chosen from the compositions of some straw hydrolysates in studies of Mussatto et al. (2004) [13]; Hasmann (2007) [7]; Oberoi et al. (2010)[9]; Huang et al. (2011) [4] which include glucose (4 g/L); xylose (18 g/L); furfural (0.0 – 0.6 g/L). Model solution was sterilized in autoclave at 121°C in 15 minutes.
**Yeast strains:** *Saccharomyces cerevisiae* ATCC 9080, *Saccharomyces cerevisiae* ATCC 2610, *Saccharomyces kudriavzevii* 9763 (Microbiologics, USA).

**Yeast activation and growth**
Dried yeast cells were activated and maintained in Sabouraud Dextrose Agar (dextrose 40 g/L; peptone 10 g/L; agar 15 g/L). The growth of yeast occurred at 30°C in solid medium. After 2-7 days, it was transferred to a modified Sabouraud Dextrose medium (with KH₂PO₄ 2g/L; yeast extract 1.5g/L; (NH₄)₂SO₄ 1g/L; MgSO₄·7H₂O 0.5g/L), where it remained in shaking incubator at 30°C in 10 hours.

**Fermentation experiments**
Fermentation experiments were carried out in sterile fermentation flask 1000 mL (with stopper and airlock) which contain 500 mL of model hydrolyzed lignocellulosic solution. Each flask was added yeast cells with inoculation of 10⁵ cells/mL of hydrolyzate solution. Each experiment was done triplicate.

**Analysis**
The compounds in samples (glucose, xylose and ethanol) were quantified by HPLC equipped with refractive index detector (RID) and SUGAR SH1011 column. Sulfuric acid 0.01N was used as solvent with flow rate of 1 mL/min. Column’s temperature was 50°C.

**Ethanol yield**
Ethanol yield: Ethanol yield (Y) is ratio of ethanol concentration/initial sugar concentration.

\[ Y = \frac{E}{V} \times 100 \]

With E: ethanol concentration (g/L); V: Volume of hydrolyzate (L); M: Substrate mass in the culture (g) [1].

**3. Results and Discussion**
**Effect of furfural on sugar consumption during fermentation**

Model hydrolyzate solution with monosaccharide concentration of 22 g/L (glucose 4 g/L; xylose 18 g/L) and furfural concentration in range of 0.0 – 0.6 g/L was fermented using each strain of *Saccharomyces cerevisiae* ATCC 9080, *S.cerevisiae* ATCC 9763, *S. kudriavzevii* ATCC 2601. The inhibition of furfural concentrations on yeast fermentation was determined based on remained sugar concentration after 5 days of fermentation, as shown in Image 1, 2 and 3.

With increased fermentation time, sugars (glucose and xylose) are consumed by Saccharomyces strains. Chosen strains can be applied for bioethanol production from lignocellulosic hydrolyzates.
In hydrolyzate with furfural concentration of 0.0 and 0.1 g/L, 32.4 and 32.8% of sugars were consumed after 5 days. Those values for higher furfural concentration (0.2, 0.3 and 0.4 g/L) were 17.7%, 14.1% and 13.5%, respectively. High furfural concentration led to strongly yeast inhibition, when about 92.7% of sugars still remained in the hydrolyzate solution.

For \textit{S. cerevisiae} ATCC 9763, effect of furfural began to be remarkable at the concentration of 0.4 g/L, when just 10.4% of sugar was fermented. This strain could tolerate high furfural concentration than \textit{S. cerevisiae} ATCC 9080.
Yeast strain *S. kudriavzevii* ATCC 2601 had the similar performance with *S. cerevisiae* ATCC 9080, when furfural concentration of 0.4 g/L began to inhibit yeast’s activity. At furfural concentration higher than 0.5 g/L, yeast performance reduced remarkably.

With three used strains of Saccharomyces, ethanol yields were highest with furfural concentration of 0.1 g/L, *S. cerevisiae* ATCC 9080 (0.088 ± 0.008 g/g); *S. cerevisiae* ATCC 9763 (0.083 ± 0.004 g/g) and *S. kudriavzevii* ATCC 2601 (0.097 ± 0.01 g/g), higher than hydrolyzate without furfural, with ethanol yield of 0.070 ± 0.010; 0.047 ± 0.005 and 0.054 ± 0.005 g/g for *S. cerevisiae* ATCC 9080; *S. cerevisiae* ATCC 9763 and *S. kudriavzevii* 2601, respectively. This was suitable with the result of Palmqvist et al. (1999), with furfural concentration of 29 mmol/L, ethanol yield was higher than in hydrolyzate without this inhibitor [6]. However, when furfural concentrations increased, ethanol yield decreased, reached 0.008 ± 0.002 g/g; 0.016 ± 0.003 g/g and 0.028 ± 0.006 g/g for *S. cerevisiae* ATCC 9080, *S. cerevisiae* ATCC 9763 and *S. kudriavzevii* 2601, respectively. In the research of Delgenes et al. (1996), when furfural concentration increased from 0.5 to 2 g/L, ethanol yield decreased from 57 to 11% [10].
4. Conclusions

Since three strains of yeast, *S. cerevisiae* ATCC 9080, *S. cerevisiae* ATCC 9763 and *S. kudriavzevii* ATCC 2601, consumed xylose, they can be used for bioethanol production from lignocellulosic hydrolyzate. Focusing on furfural’s effect, this study determined lowest furfural concentration of 0.1 g/L, which was not harmful for strains applied. Because methods applied for detoxification can lead to sugar loss, this information can be useful for determining limit of removing inhibitor from lignocellulosic hydrolyzate solution.

5. References