Effect of Furfural and 5- Hydroxy Methyl Furfural on Growth of Ethanol Fermenting Yeast *Pichiastipites NCIM 3498*

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Abstract: Today much emphasis is being given to the production of bioethanol from lignocellulosic biomass. Among the agricultural crop residues, wheat straw is the second largest biomass production in the world after rice straw (Kim and Dale, 2004). During bioethanol production some chemical inhibitors are formed. Furfural and 5- Hydroxy methyl furfural are the most important inhibitors during ethanol fermentation by yeast. These inhibitors are formed during pretreatment of lignocellulosic biomass due to high temperature and acid concentrations. In present study Pichiastipites NCIM 3498 was grown under different concentrations of furfural, HMF and in combination of both ranging from 5 to 50 mM. Result showed that up to 30 mM there was no significant inhibition in ethanol yield and growth of Pichiastipites NCIM 3498 at 72 hrs incubation.Pichiastipites NCIM 3498 showed drastic decrease in ethanol yield and growth after 30 mM of furfural and HMF concentrations. Furfural was found to be more toxic than HMF as observed by total viable cell count (number of colonies) in plates. At 50 mM concentration total viable cell count in plates were negligible. These findings provide the information about the furfural and HMF on yeast cells tolerance of Pichiastipites NCIM 3498 to the maximum thresholds of inhibitors formed during pretreatment of lignocellulosic biomass.

Keywords: Wheat straw;Pichiastipitis; Furfural; 5- Hydroxy methyl furfural; Magnifying colony counter; viable cell count.

1. INTRODUCTION

Renewable lignocellulosic materials are attractive low-cost feedstock for ethanol production(Mosier, 2005; Prasad *et al*., 2007). Lignocellulosiccrop residuesconsisted of cellulose, hemicelluloses andlignin and a small amount of extractives and ash. Generally, lignocellulosic biomassproduced approximately 70 to 80% carbohydrates (Prasad *et al*., 2007). If properly hydrolyzed, these carbohydrates can serve as an ideal feedstock for ethanol production (Mosier et al.,2005).Dilute acid pretreatment is probably the most commonly applied method among the chemical pretreatment methods for maximising fermentable sugar for ethanol production (Agbogbo and Wenger, 2006). Furfural and 5-hydroxymethylfurfural (HMF) is representative by-product

(Fig.1) and the most potent inhibitory compounds generated from acid pretreatment of lignocelluloses to simple sugars for fermentation.

During sugar degradation with acid pretreatment, furfural is mainly derived from pentose dehydration and HMF is formed from dehydration of hexoses.

Most ethanol fermenting yeasts, including industrial strains, are susceptible to various inhibitory compounds derived from acid pretreatment and especially to the presence of furfural and HMF (Cantarella et al., 2004; Klinke et al., 2004). These compounds damage microorganisms by reducing enzymatic and biological activities (Hsu et al., 2010), thus reducing the overall efficiency for bioconversion of lignocellulosics to ethanol.Previous studies have made known the kinetics and inhibition effect of furfural, and in some cases also HMF, on the enzymes alcohol dehydrogenase (ADH), aldehyde dehydrogenase (AlDH), and pyruvate dehydrogenase (PDH). In the current work, impact offurfural and HMF on microbial growth and ethanol yield was studied.

2. MATERIALS AND METHODS

Organisms and Growth Conditions

The yeast *PichiastipitisNCIM 3498*wasselected for study. The*PichiastipitisNCIM 3498* strain is sub-cultured withnutrient media (g/L): Glucose, 10.0; Yeast extract, 3.0; Malt extract, 3.0; Peptone, 5.0; Distilled water, 1.0 L; pH adjusted to7.0-7.5. Then we prepared nutrient agar and liquefied agar was poured in petri plates with 20 ml in each plate. Allow to harden at room temperature. Plates are now ready to inoculate. After inoculation sticking was done using 10^6 serial dilution and kept in BOD incubator for 48 hours at 30ºC.*Pichia stipitisNCIM 3498* was grown under different concentrationsof furfural, HMF and in combination of both ranging from 5 to 50 mM. Colonies were counted with the help of magnifying colony counter.

3. RESULTS AND DISCUSSION

Result showed that concentrations of furfural, HMF and in combination of both up to 30 mM, there was no significant inhibition in growth (total viable cell count) of *PichiastipitisNCIM 3498* at 72 hrs period of incubation. *PichiastipitisNCIM 3498* showed drastic decrease in total number of colonies after 30 mM of furfural and HMF concentrations. Furfural was found to be more toxic than HMF as observed by total viable cell count (number of colonies) in plates. At 50 mM concentration total viable cell count in plates were negligible (Fig.2).

Results indicated that concentrations of furfural, HMF and in combination of both up to 30 mM, there was no significant inhibition observed in ethanol yield (g/g sugar utilized) by *Pichiastipitis NCIM 3498* at 72 hrs period of incubation. Yeast strain*Pichiastipitis NCIM 3498* showed drastic

decrease in ethanol yield after 30 mM of furfural and HMF concentrations (Fig.3). Delgenes*et al.* (1996) and Carolina*et al.* (2014) also reported similar findings of these inhibitory compounds on growth and ethanol fermentationby P. *stipitis, C. shehatae, Zymomonasmobilisand Saccharomycescerevisae*.

4. CONCLUSION

These findings provide the information about the furfural and HMF on yeast cells tolerance of *Pichiastipites NCIM 3498* to the maximum thresholds of inhibitors formed during pretreatment of lignocellulosic biomass.

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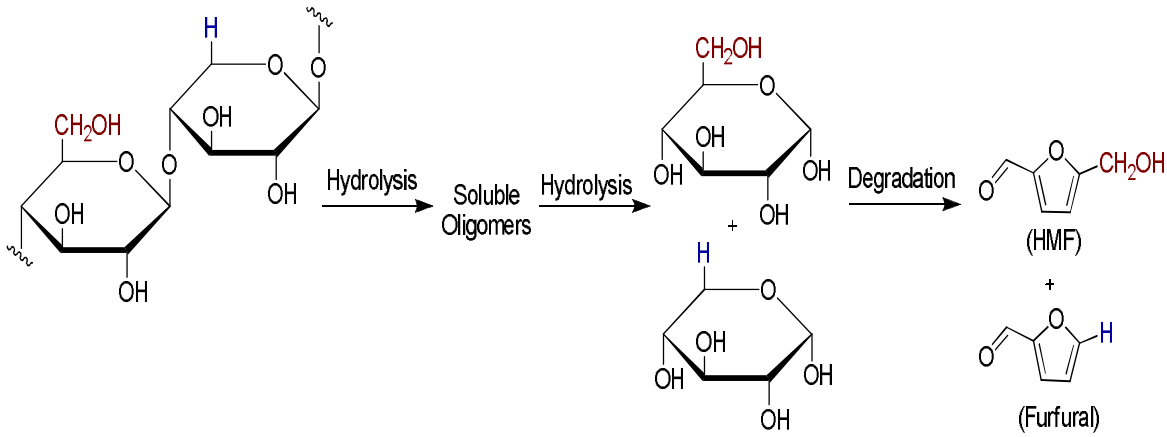

Fig 1. Inhibitory products from pretreatment of lignocellulosic materials

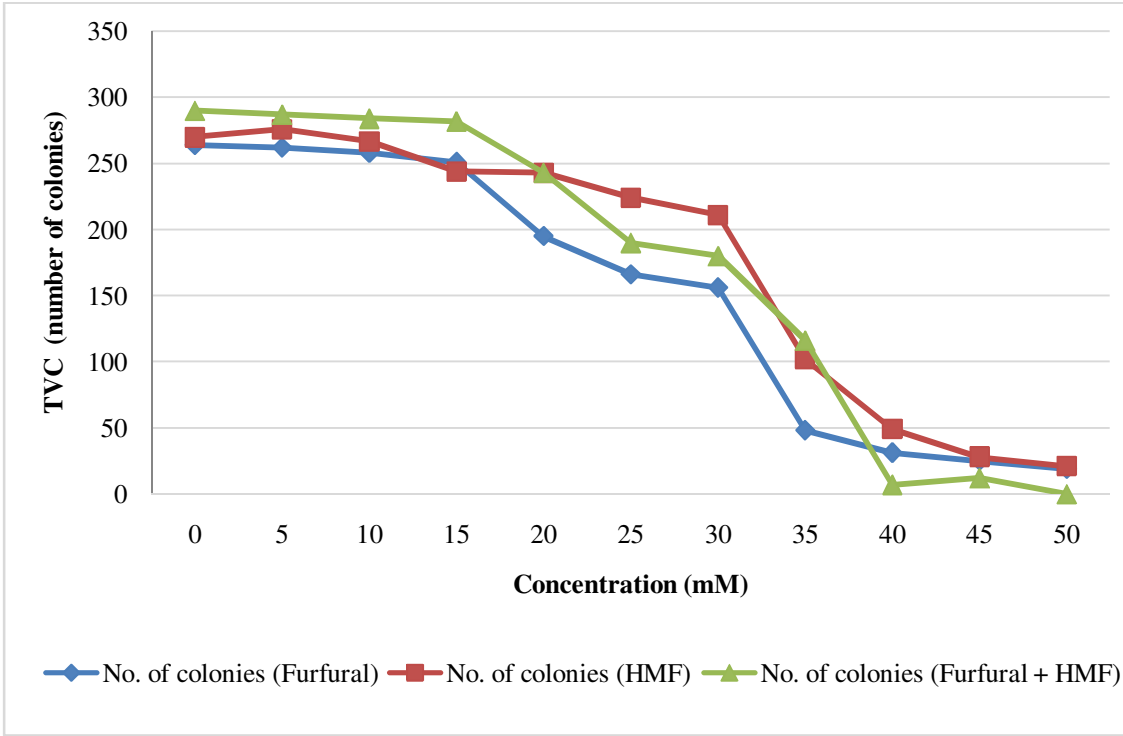

Fig. 2.Effect of Furfural and 5- Hydroxy methyl furfural concentration on growth of *PichiastipitisNCIM 3498*

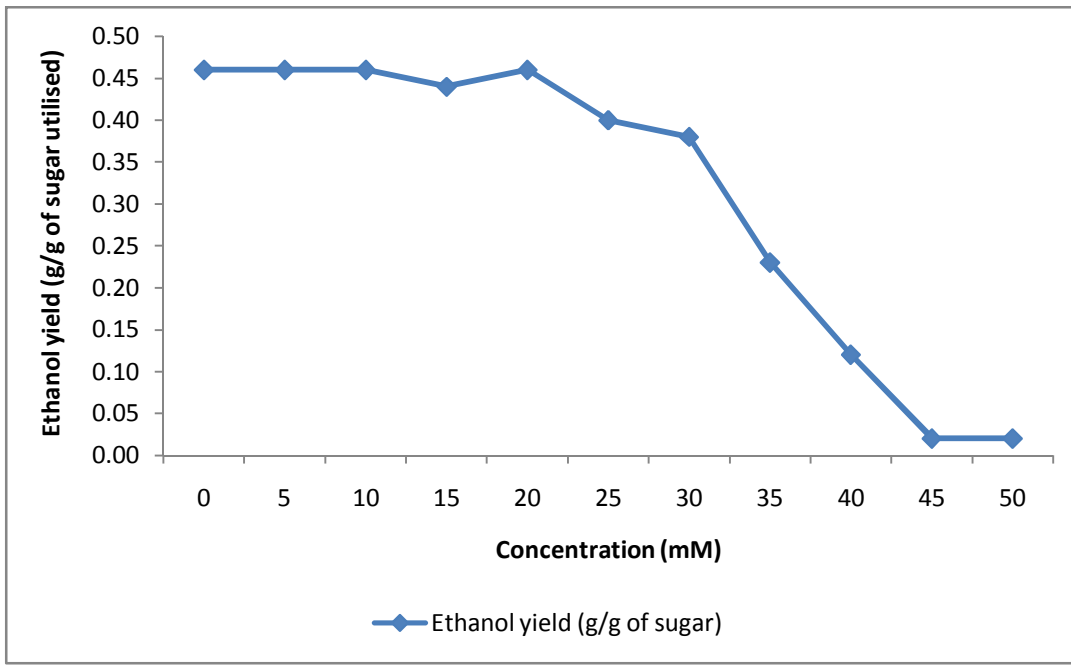

Fig. 3. Effect of Furfural and 5- Hydroxy methyl furfural concentration on ethanol yield by *Pichiastipitis NCIM 3498*