International Conference on Low Carbon Asia
11-12 October 2015, Johor, Malaysia.
ICLCA 2015

ISOBUTANOL AND 3-METHYL-1-BUTANOL PRODUCTION BY *SACCHAROMYCES CEREVISIAE* IN AEROBIC CONDITION

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Keywords: Biofuel, Fermentation, *Saccharomyces cerevisiae*, Isobutanol, 3-Methyl-1-Butanol

EXTENDED ABSTRACT

Alternative fuels from renewable sources are receiving public and scientific attention due to continuous depletion of petroleum fuel-reserves and environmental problem such as global warming and climate change. Higher alcohols (C3-C5) possess many advantages thus become suitable candidate in replacing gasoline as transprtation fuel. This paper investigates the production of isobutanol and 3-methyl-1-butanol as well as the toxicity of these alcohols towards *Saccharomyces cerevisiae*. This yeast was able to produce both alcohols with the highest concentration of 92 mg/ L (isobutanol) and 245 mg/L (3-methyl-1-butanol). *Saccharomyces cerevisiae* was capable to grow in more than 2% isobutanol but unable in concentration of 3-methyl-1-butanol higher than 1%.

Introduction

The effort of deriving renewable fuel has intensified dramatically due to increasing awareness about global warming and climate change. Fossil fuels have been found as major contribution in greenhouse gases as it produced methane (CH_4) and nitrous oxide (N_2O) as well as raises the atmospheric concentration of harmful carbon dioxide (CO_2). Another major concern in global movement towards reducing the use of fossil resources is the diminishing supply of fossil fuel-reserves as today is the fuel dependent era (Kumar and Gayen, 2011). The increasing industrialization and motorization of the world has led to the risen demand of petroleum-based fuels consequently increasing the crude oil price thus directly affected the global economic development and growth.

Recently, longer chain or branched alcohols have gained more interests than bioethanol as the candidate to replace gasoline as transportation fuel (Connor and Liao, 2011). Isobutanol and 3-methyl-1-butanol have been recognized for their potentiality as fuel additive or substitute due to their attractive physical properties. Having favourable fuel characteristics such as higher energy density, lower hygroscopicity and less volatility have make these biofuels superior fuel extender compared to bioethanol. *S. cerevisiae* (baker's yeast) is able to produce small amounts of isobutanol and 3-methyl-1-butanol as by-product during degradation of amino acids. The production of alcohol naturally gives an opportunity to avoid the expression of alcohol synthesis genes into the production host as this process is complicated and require lots of effors, however the yield produced is quite low (Chen *et al.*, 2011).The study presented in this paper aims to investigate the ability of *S.cerevisiae* in producing isobutanol and 3-methyl-1-butanol and to evaluate the resistance of this yeast towards these alcohols.

Methods

Batch fermentation were performed in 250 ml shake flasks with a working volume of 150 ml synthetic medium at 30 ^{0}C in a shaking incubator with 170 rpm agitation speed for 96 hours. During the course of fermentation, 2 ml samples were collected regularly for analyses of isobutanol, biomass and glucose. The alcohol tolerance was experimented by inoculating the yeast in the growth medium contained several percent of isobutanol and 3-methyl-1-butanol. The alcohol tolerance was

determined based on microbial growth obtained through turbidity measurements using UV-visible spectrophotometer.

Results and Discussion

The highest production of both isobutanol and 3-methyl-1-butanol occurred during 48 hours of fermentation with the yield of 92 mg/ L and 245 mg/L respectively (Figure 1). It can be concluded that the both alcohol production occurred during exponential growth period. This is because alcohol is a primary metabolite which is directly involved in growth, development and production.

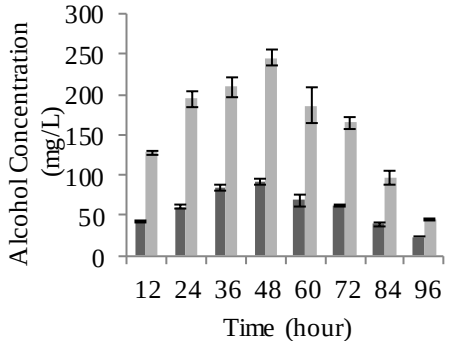

Figure 1: Isobutanol and 3-Methyl-1-Butanol Production (■)Isobutanol (■) 3-Methyl-1-Butanol

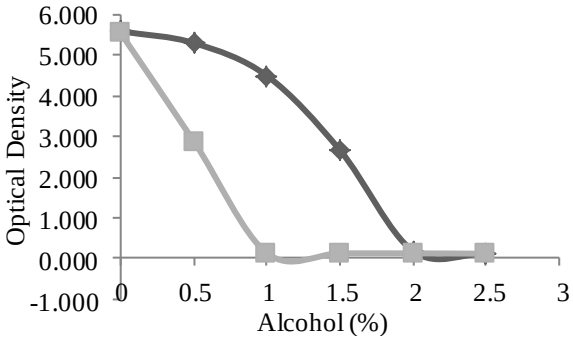

Figure 2: Tolerance of Isobutanol and 3-Methyl-1-Butanol Towards *S.cerevisiae* (♦) Isobutanol (■)3-Methyl-1-Butanol

Alcohol tolerance is one of the crucial factors affecting the production of alcohol. The accumulations of alcohol titers destroy the cell membrane of the microorganism hence resulting to reduce in growth of the species which eventually affects the alcohol production (Liu and Qureshi, 2009). The growth of *S.cerevisiae* in the presence of different concentration of isobutanol and 3-methyl-1-butanol were tested in order to investigate the effect of alcohol toxicity towards yeast (Figure 2). *S.cerevisiae* unable to grow when the concentration of 3-methyl-1-butanol higher than 1% compared to isobutanol (2%). This indicated that the effect of 3-methyl-1-butanol on the cells was more severe.

Conclusion

The results shows the ability of *S.cerevisiae* in producing isobutanol and 3- methyl-1-butanol without heterologous pathways. *S. cerevisiae* has been proven as good yeast in alcohol tolerance as it grew in more than 2% isobutanol but not in 3-methyl-1-butanol. This result concluded that 3-methyl-1-butanol is more toxic towards yeast compared to isobutanol.

Acknowledgements: The authors are grateful and would like to express their sincere gratitude to the Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia (UTM) and Ministry of Education (MOE) for the kind supports and funding for this research through FRGS grant R.J130000.7844.4F511.

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