<u>Rossyuhaida Mohd Zakria<sup>3</sup></u>, Gek Kee Chua<sup>1</sup>, Jolius Gimbun<sup>1,2</sup>\*, M. N. Nor Shahirah<sup>1</sup>, Sook Fun Pang<sup>1</sup>, Mohd Fazli Farida Asras<sup>3</sup>, Ahmad Ziad Sulaiman<sup>1</sup>, Chin Kui Cheng<sup>1,2</sup>, Wan Asma Ibrahim<sup>4</sup>

<sup>1</sup> Faculty of Chemical Engineering & Natural Resources,

<sup>2</sup> Centre of Excellence for Advanced Research in Fluid Flow (CARIFF),

<sup>3</sup> Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

<sup>4</sup> Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor Darul Ehsan, Malaysia.

\*e-mail address: jolius@ump.edu.my

## 1. Introduction

Malaysia has an abundant amount of oil palm biomass (approx. 15 million tons annually) arising from replanting activities involving old oil palm tree aged above 20 years-old which has lower productivity. In some cases, the outer hard portion of the oil palm trunk is utilised for plywood manufacturing, but the soft inner portion is normally discarded. This soft inner portion contains a huge amount of sugar-rich sap that can be fermented into bioethanol<sup>1</sup>.

Limited study on oil palm trunk sap (OPTS) fermentation using various ethanol-producing strains in exception of those by Norhazimah and Faizal<sup>2</sup>. However, no study concerning OPTS fermentation to bioethanol using *Kluyveromyces marxianus* ATCC 46537, *Saccharomyces cerevisiae* ATCC 9763 and *Saccharomyces pastorianus* ATCC 26602 is available in the literature, and hence screening their suitability is one of the objectives of this work. Furthermore, limited study on the effect of micronutrient addition to bioethanol yield during OPTS fermentation is available in the literature. Thus, the primary objective of the current work is to study the effects of multiple nutrient additions to the bioethanol yield. Subsequently, optimization of the amount of nutrient addition was studied using response surface methodology.

# 2. Experimental

The oil palm trunk sap was obtained from a 30-year old tree from Jengka, Pahang. All the microbial strain was obtained from the American Type Culture Collection, USA, except the baker's yeast, which was obtained from AB MAURI, Malaysia. The inoculum was prepared via incubation in a sterile broth for 18h. The standard optical density of 1.5 - 1.7corresponds to a stationary phase in the microbial growth deduced by studying the microbial growth curve<sup>3</sup>. The fermentation

was performed in a 250 ml Erlenmeyer flask containing OPTS and aseptically inoculated with 10% (v/v)of microorganism suspensions up to 72h incubated at 30 °C and agitated at 150 rpm. The sugar and ethanol content were determined using a high performance liquid chromatography (HPLC). The experimental design and statistical analysis were performed using the design-expert version 8.0.6 software (Stat-Ease Inc., Minneapolis, USA).

# 3. Results and Discussion

The results from the strain screening experiment showed that S. pastorianus produced the highest ethanol yield (62.6%), Κ. followed by marxianus (60.9%), cerevisiae (59.6%) and baker's yeast (55.2%). However, K. marxianus produced a comparatively high ethanol yield at a shorter fermentation time (16 h) compared to the other strains (24 h). Thus, K. marxianus was used for the remainder of this work. Six nutrients. namely, ammonium sulphate, di-ammonium hydrogen phosphate, magnesium sulphate, β-alanine, calcium chloride and potassium dihydrogen phosphate were screened using K. marxianus. The two-level factorial design showed the highest ethanol yield (98.62%)was achieved from the fermentation supplemented by magnesium sulphate and  $\beta$ -alanine. Subsequently, the optimisation study using a response surface methodology found the optimum value of magnesium sulphate was 7.93 g/L and 0.90 g/l for  $\beta$ -alanine (Fig. 1). Under the optimum conditions, the predicted ethanol concentration was 34.58 g/l while the

experimental value (35.50 g/l) was in agreement with the predicted value with 2.66% error.

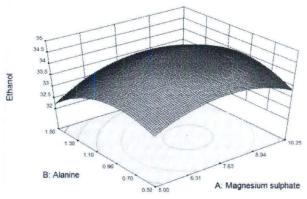


Fig. 1. Effect of magnesium sulphate and  $\beta$ -alanine concentration on ethanol production from OPTS by *K. marxianus*.

# 4. Conclusion

OPTS showed a great potential of producing bioethanol using *K. marxianus* which can produce the highest ethanol yield (98.62%) with the addition of magnesium sulphate and  $\beta$ -alanine. The optimum value of magnesium sulphate was 7.93 g/L and 0.90 g/L for  $\beta$ -alanine, which gives the actual ethanol concentration of 35.50 g/l with a small deviation from the predicted value (2.66%).

#### References

1) A. Kosugi et al., *J. Biosci. Bioeng.*, 110 (2010) 322–325.

 A.H. Norhazimah, C.K.M. Faizal, J. Med. Bioeng., 3 (2014) 297-300.
M.N. Nor Shahirah et al., J. Ind. Eng. Chem., (2014), http://dx.doi.org/

10.1016/j.jiec.2014.08.018.