Magnesium sulphate and β-alanine enhanced the ability of *Kluyveromyces marxianus* producing bioethanol using oil palm trunk sap

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**ABSTRACT**

The abundance of oil palm trunk waste generated each year has encouraged research in using its sap for fermentation to produce value-added products. One of these value-added products is bioethanol production using yeast strains. In this study, the ability of *Kluyveromyces marxianus* ATCC 46537 to produce bioethanol using oil palm trunk sap (OPTS) was examined. The nutrients (ammonium sulphate, di-ammonium hydrogen phosphate, magnesium sulphate, β-alanine, calcium chloride and potassium dihydrogen phosphate) required to enhance production were screened and optimised. The concentrations of bioethanol and sugars were monitored with high performance liquid chromatography. The results showed that *K. marxianus* could attain maximum bioethanol concentration at 16 h with a higher productivity as compared to *S. cerevisiae*. Magnesium sulphate and β-alanine were found to increase bioethanol production. When 7.93 g/L of magnesium sulphate and 0.90 g/L of β-alanine were supplemented to OPTS, bioethanol production increased 20% with a bioethanol yield of 0.47 g/g and a productivity of 2.22 g/L.h. Therefore, minimum supplementation of OPTS with inorganic nutrients could enhance the bioethanol production of *Kluyveromyces marxianus*.

**Introduction**

Demand for primary energy sources such as crude oil, natural gas, coal, and hydropower are projected to continually increase. Exploitation of natural oil reserves will also continue to increase. Switching to renewable energies such as biomass fuels will reduce dependency on fossil fuels and ensure energy security in the future. Bioethanol is a promising biofuel when produced from renewable resources such as biomass [1]. Numerous advantages can be gained by using bioethanol as a fuel since it helps to extend the lifespan of petroleum reserves and reduces the reliance on imported oil [2]. Bioethanol has higher oxygen content compared to gasoline which results in cleaner combustion [3] and reduced carbon dioxide emissions into the atmosphere [4] thus reducing environmental damage. Most importantly, bioethanol is readily available from common biomass sources [5] such as corn stalks, rice husks, grasses, and sugarcane bagasse.

Malaysia has abundant oil palm biomass arising from replanting activities involving oil palm trees over 20 years old. According to the Malaysian Palm Oil Board, about 10% of the total 5.23 million hectares of oil palm (*Elaeis guineensis*) plantations in Malaysia must be replanted every year due to the decrement of oil productivity and the difficulty of harvesting fruit from old trees [6,101]. This means that about 70 million old palm trees will be felled annually in Malaysia, generating over 15 million tonnes of oil palm trunks [7]. Some of the oil palm trunks are used for plywood manufacturing, but in most cases the trunks are left to rot on the plantation grounds [8]. Only the outer layer of the oil palm trunk is utilised by the plywood manufacturing industry as this is the hardest part of the trunk, whilst the soft inner part is normally discarded. This soft inner part contains a huge amount of sugar-rich sap that can be fermented into bioethanol [9].

The conversion of oil palm trunk sap (OPTS) into bioethanol is possible by using direct microbial fermentation. The potential of OPTS as feedstock for bioethanol, lactic acid and hydrogen production has been examined by Kosugi et al. [9], Yamada et al. [10] and Noparat et al. [11]. Yeast is the typical fermenting microorganism used in this process, particularly *Saccharomyces cerevisiae* [12] due to its excellent fermenting capacity, its high tolerance to ethanol, its relatively tolerant ability to low pH values and its capacity to grow rapidly under anaerobic conditions [13]. Bacteria can also produce bioethanol, but the performance is not as good as yeast. This can be seen from the work of Norhazimah and Faizal [14], who studied the effect of single and co-culture strains on bioethanol production.