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Fresh oil palm frond juice as a renewable, non-food, non-cellulosic and complete medium for direct bioethanol production

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ABSTRACT

Oil palm frond (OPF) is the largest biomass source in the palm oil industry. Fresh OPF juice can be readily obtained by just pressing the fresh OPF, similar to sugarcane juice. OPF juice contains sugars and other nutrients such as nitrogen, magnesium, calcium, zinc, phosphorus and sulphur, making it a potential medium for bioethanol fermentation. In this study, the potential of fresh OPF juice as a complete non-food medium for direct bioethanol production was evaluated. A promising yield of 0.38 g bioethanol per g sugars consumed was obtained after 24 h of fermentation of fresh OPF juice without nutrient supplementation and without pH correction, which is comparable to synthetic medium at 0.40 g/g. This value is also comparable to the 0.4 g/g yield obtained from sugarcane juice in the Brazilian bioethanol industry. Therefore, this study provides an opportunity for the use of fresh OPF juice as a new renewable, non-food and non-cellulosic feedstock for the bioethanol industry.

1. Introduction

The primary feedstock for first generation bioethanol is obtained from sugarcane followed by other starchy crops such as corn, wheat, sugarbeet and sorghum (Balat and Balat, 2009). Bioethanol from starch and food feedstocks are uneconomical and raise ethical concerns as they compete with the food security. Although second generation bioethanol derived from lignocellulosic materials are widely recognised as promising alternative sources of energy, ineffective pretreatment and high cost of hydrolytic enzymes are the main issues hindering the commercialisation of bioethanol. Therefore, efforts for bioethanol production have been focused on the potential of non-edible feedstocks enriched with sugars (Limayem and Ricke, 2012).

The palm oil industry generates abundant biomass throughout the year comprising of oil palm fronds (OPF), oil palm trunks (OPT), empty fruit bunches (EFB), palm kernel shell (PKS), mesocarp fibre (MF) and palm oil mill effluent (POME). Among the oil palm solid biomass, OPF contributes the largest portion. In the oil palm plantations, OPF are regularly cut during harvesting of fresh fruit bunches or pruning of the oil palm trees. It is estimated that per each tonne of fresh fruit bunch harvested, one tonne of fresh OPF is removed from the oil palm trees (MPOC, 2010). The mass balance of oil palm products and biomass from oil palm trees is shown in Fig. 1. OPF currently receive less attention as the plantation owners believed that whole OPF is important for nutrient recycling and soil conservation to the oil palm tree, hence the current practise is to leave the OPF at the plantation. However, Zahari et al. (2012) reported that only petiole or basal part of the OPF contains large amount of sugars and can be exploited to produce value added products. On the other hand, application of whole OPF as feed for ruminant livestock has been the subject of research since 1991 (Islam, 1999; Khamseekhiew et al., 2002; Zahari et al., 2002). However, the application of OPF as ruminant feed is not very convenient due to its low feed quality resulting from high fibre content, low nitrogen

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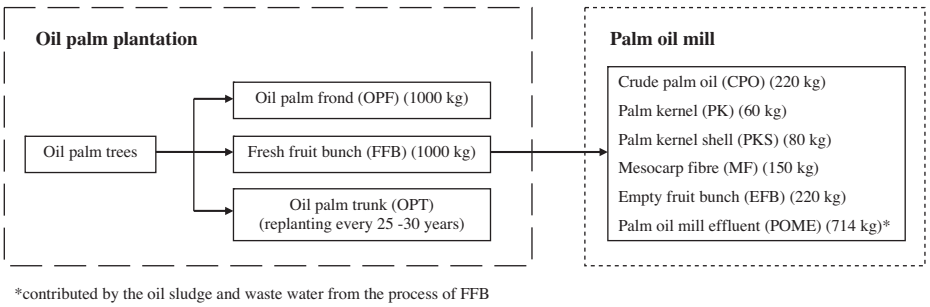

Fig. 1. Mass balance of oil palm products (CPO and PK) and oil palm biomass (OPF, EFB, OPT, PKS, MF and POME) from oil palm trees.

content and low digestibility which finally leads to low feeding intake of the ruminant livestock. Nevertheless, due to its huge quantity and availability throughout the year, OPF has been widely used as a roughage source and as a component in ruminant feed with an ideal percentage for livestock. The optimum level of OPF inclusion for ruminant feeding is only 30% (Kum and Zahari, 2011). Abu Hassan (1996) reported that animal performance is affected when the content of OPF is more than 60%. Other findings have shown the potential of sugar recovery from the lignocellulosic fibre of OPF for second generation bioethanol (Goh et al., 2010a,b). Basically, there are four stages in second generation bioethanol production; pretreatment, enzymatic hydrolysis, bioethanol fermentation and distillation. An applicable pretreatment is vital for optimum sugars recovery from biomass. However, the main shortcomings of lignocellulosic bioconversion into fermentable sugars are the high cost of pretreatment technology and chemical waste generation from the pretreatment.

Hence, apart from the lignocellulosic route, a direct route to derive bioethanol from OPF is by fermenting the squeezed sugar juice from OPF petiole, as a new, green and renewable energy feedstock (MIA, 2013). Previous studies have shown that 40–50% (w/w) of juice can be extracted from fresh OPF petiole using a simple pressing machine (Roslan et al., 2014; Zahari et al., 2012). The OPF juice could provide good nutritional content for bacterial growth during fermentation due to the presence of high amount of sugars (glucose, sucrose and fructose), minerals and nutrients. Other findings have reported that sterile OPF juice with supplementation of other nutrients has potential to be used as substrate for poly(3-hydroxybutyrate) and bioethanol (Zahari et al., 2012, 2014). Therefore, the aim of this study was to assess the potential of fresh OPF juice as a complete fermentation feedstock for bioethanol production without sterilisation, without pH adjustment and without additional nutrients. Comparison of bioethanol production was made with fresh sugarcane juice. This work is expected to provide useful information to assist interested parties in evaluating the potential development of first generation bioethanol facility from OPF juice.

2. Materials and methods

2.1. Oil palm frond and sugarcane juices

Fresh oil palm frond was obtained from oil palm trees planted at Taman Pertanian Universiti, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The petiole or basal part was collected, while the leaves part was left at the oil palm plantation as natural fertiliser and soil cover. The petiole was pressed and squeezed by three roller hydraulic press machine (No. 1 Mini mill 7.5 kW × 4P × 1/195, 415 v × 50 Hz, Matsuo Co. Ltd., Kagoshima, Japan) to obtain the juice. The juice was centrifuged at 15,000 × *g*, 4 °C for 15 min to remove solid materials (Zahari et al., 2012). The OPF juice was stored in −20 °C freezer for further experiments. On the other hand, fresh sugarcane juice was obtained from a stall in Seremban, Negeri Sembilan, Malaysia. The juice was centrifuged and stored in a similar manner.

2.2. Bioethanol fermentation using OPF and sugarcane juices

The commercial bakers yeast, *Saccharomyces cerevisiae* (Mauripan Baking Industry, Malaysia) was inoculated on yeast potato dextrose (YPD) agar, which consisted of glucose (20 g/l), peptone (20 g/l), yeast extract (10 g/l) and technical agar (10 g/l). This culture was incubated at 30 °C for 24 h. A loopful of yeast was pre-cultured in medium containing 5 g yeast extract and 20 g glucose per litre. The inoculum was cultivated at 30 °C for 12 h before centrifuging at 8000 rpm for 5 min to obtain the cell pellet and introduced into the production medium.

The production media comprised fresh OPF juice and sugarcane juice. Both media were fermented separately to observe the ability to produce bioethanol in batch system, without nutrient supplementation and sterilisation. The initial pH for OPF and sugarcane juices was 4.84 and 5.03, respectively. The pH of the juices was not corrected for fermentation. A control medium was prepared to mimic OPF juice by using a mixture of commercial sugars comprising of glucose (48 g/l), sucrose (12 g/l), fructose (8 g/l), with other nitrogenous and mineral components such as peptone (20 g/l), yeast extract (10 g/l), KH_2PO_4 (1.0 g/l), $MgSO_4{\cdot}7H_2O$ (0.1 g/l), $CaCl_2{\cdot}2H_2O$ (0.1 g/l) and $(NH_4)_2SO_4$ (1.5 g/l). The fermentation was conducted in 250 ml flasks at 30 °C, 150 rpm for 48 h. Samples were withdrawn and centrifuged at 10,000 rpm for 5 min. The obtained cell free supernatant was used for the determination of bioethanol produced and sugars consumed by the yeast.

2.3. Analytical methods

The analysis for elemental constituents in the juices (carbon, nitrogen, sulfur) was determined using CNHS analyser (LECO, CNHS932, USA) whereas macro and micronutrients were determined using inductively coupled plasma (ICP) (Perkin Elmer, 7300 DV, USA) (Omar et al., 2011). Sucrose, glucose, fructose and bioethanol in the OPF and sugarcane juices were determined by high performance liquid chromatography (HPLC) (Shimadzu LC-20A series, Japan) using the Shodex sugar Na^+ (KS-802) column (8.0 mm × 300 mm) with a refractive index detector operated at 80 °C. The mobile phase was 100% water at a flow rate of 0.6 ml/min. The components were identified by comparing their retention times with those standards under analytical conditions and quantified by external standard method. A pH metre was used to determine the pH of both juices.

2.4. Calculation

Sugar utilisation (1), bioethanol yield (*Y*) (2), volumetric productivity of bioethanol (3) and fermentation efficiency (FE) (4)