Potential of Oil Palm Trunk (OPT) Sap as Biofuels and Bioproducts via Fermentation

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INTRODUCTION

Plant biomass and agro-industrial wastes have great potential to become candidate for low cost substrates for biotechnological processes. One such potential biomass feedstock in Malaysia is oil palm trunk (OPT). Felled OPT during replanting activity is available at an estimated 7.57 million tonnes (based on 74.48 t ha^{-1} of dry OPT) from about 101 698 ha of committed areas due for replanting in 2018 (MPOB, 2018). OPT contains large quantity of sap as indicated by high moisture content (70%-85%) (Murata *et al.,* 2013; Adela and Loh, 2015). The sap from felled OPT can be easily extracted using a simple mechanical pressing machine. The OPT sap, with high level of sugar, is readily fermentable into various desired products. Furthermore, its inherited and abundant amount of free amino acids, vitamins and minerals are supplements favourable for fermentation process (Kosugi *et al.,* 2010; Komonkiat and Cheirsilp, 2013; Bukhari *et al.,* 2019).

Fermentation involves the use of microorganisms, *i.e.,* bacteria, yeasts, fungi or a combination thereof to convert a substrate to the desired product. These microorganisms can synthesize various important compounds applicable in many industries, including food, chemical, pharmaceutical and energy production. Fermentation is initiated by introducing a selected microbial strain to a substrate of interest and further cultivating it under environmental conditions that favour cell growth and product formation. Fermentation can be classified into solid-state and submerged cultures; dependent on aerobic/anaerobic process with or without oxygen for different metabolic pathway of any selected strain (Chisti, 2010).

One of the key factors to be considered in deploying fermentation at an industrial level is cost of substrate in the form of digestible carbon source. Carbon source is the major component of primary focus in fermentation (Tan *et al.,* 2016); thus, renewable, sustainable and cheap options derived from lignocellulosic or starchy biomass are very much sought after and have been exploited as a fermentation substrate. Nitrogen, the second major component in fermentation can be supplied either as inorganic nitrogenous salts or organic nitrogenous compounds. A fermentation substrate typically requires micronutrients such as vitamins as well as trace elements such as phosphorus, iron, zinc and sulphur (Chisti, 2010). This requirement which helps to achieve an optimum yield of the targeted product has contributed to higher overall production cost for commercial exploitation (Civelek Yoruklu *et al.,* 2019).

Unlike other lignocellulosic biomass, the utilisation of OPT sap as a fermentation feedstock should be of industrial interest as no additional chemical or enzymatic treatment is required. This indeed significantly reduces major processing costs. In addition, there are no potential inhibitors and salts such as weak acids, furan derivatives and phenolic compounds resulted from chemical pretreatment of lignocellulosic biomass that tend to affect subsequent fermentation (Rumbold *et al.,* 2010; Che Maail *et al.,* 2014). A few previous studies have shown that OPT sap is suitable to be efficiently utilised as a fermentation substrate for production of ethanol (Adela and Loh, 2015; Mohd Zakria *et al.,* 2017), butanol (Komonkiat and Cheirsilp, 2013), bioproducts such as bioplastics (Lokesh *et al.,* 2012) and a range of carboxylic acids (*e.g.* lactic

acid and succinic acid) (Chooklin *et al.,* 2011; Kunasundari *et al*., 2017; Bukhari *et al.,* 2019).

CHARACTERISTICS OF OIL PALM TRUNK (OPT) SAP

It is of paramount importance to determine the chemical composition of OPT sap in evaluating its suitability as a fermentation substrate. A good substrate for fermentation must have sufficient sources of carbon, nitrogen and oxygen (in the case for aerobic culture) to support microbial growth and product formation. Supplementation of carbon source is generally accomplished in the form of sugars. Accordingly, the OPT sap contains high concentration of sugars with glucose being predominant (26.7 g litre^{-1}, 62%), followed by sucrose (10.1 g litre^{-1}, 24%) and fructose (5.9 g litre^{-1}, 14%) (*Table 1*). A somewhat similar sugars profile was reported elsewhere (Kosugi *et al.,* 2010): glucose (49.5 g litre^{-1}, 88%), sucrose (3.8 g litre^{-1}, 7%) and fructose (3.1 g litre^{-1}, 5%). The presence of nitrogen source at a high concentration of 1.27 g litre^{-1} (Bukhari *et al.,* 2019) in OPT sap might be attributed to a copious amount of amino acids available in the sap; the dominant ones being aspartic acid, lysine, arginine and glutamic acid (*Table 2*).

TABLE 1. SUGARS CONCENTRATION IN OPT SAP

Sugar	Concentration (g litre^{-1})
Sucrose	10.10 ± 2.08
Glucose	26.73 ± 5.06
Fructose	5.89 ± 1.79
Total	42.72 ± 5.78

Source: Bukhari et al., (2019)

TABLE 2. AMINO ACIDS CONCENTRATION IN OPT SAP

Amino acid	Concentration (µg g^{-1})
Aspartic acid	2630 ± 35
Serine	850 ± 42
Alanine	940 ± 42
Proline	950 ± 70
Valine	220 ± 35
Methionine	360 ± 49
Lysine	2255 ± 102
Isoleusine	75 ± 3
Leusine	95 ± 10
Phenylalanine	90 ± 14
Tryptophan	90 ± 0
Threonine	410 ± 28
Glutamic acid	1090 ± 183
Arginine	1970 ± 205

Source: Bukhari et al., (2019)

TABLE 3. MINERALS CONCENTRATION IN OPT SAP

Mineral	Concentration (µg g^{-1})
Calcium	152.33 ± 62.76
Iron	42.76 ± 37.66
Magnesium	278.76 ± 3.15
Manganese	6.37 ± 5.56
Phosphorus	184.81 ± 143.97
Potassium	4444.85 ± 8.27
Selenium	0.03 ± 0.00
Sodium	284.68 ± 194.91
Zinc	2.01 ± 0.04

Source: Bukhari et al., (2019)

TABLE 4. VITAMIN CONCENTRATION IN OPT SAP

Vitamin	Concentration (µg g^{-1})
Ascorbic acid	36.95 ± 18.46
Biotin	200 ± 0.00
Cobalamin	0.08 ± 0.00
Folic acid	0.07 ± 0.00
Niacin	7.70 ± 0.00
Pantothenic acid	4.88 ± 3.29
Pyridoxine	5.20 ± 0.00
Retinol	87.31 ± 0.00
Thiamine	1.12 ± 0.40
α-tocopherol)	3.25 ± 3.90

Source: Bukhari et al., (2019)

TABLE 5. ORGANIC AND PHENOLIC COMPOUNDS IN OPT SAP

Compounds	Concentration (µg ml^{-1})
Fumaric acid	78 ± 5.7
Malic acid	286 ± 15.1
Succinic acid	65 ± 3.9
Citric acid	190 ± 10.0
p-hydroxybenzoic acid	260 ± 14.1
Phthalic acid	11 ± 0.51
Iso-citric lactone	1.6 ± 0.01
3,4-dimethoxybenzoic acid	30 ± 1.2
Aconitric acid	15 ± 0.9
Vanilic acid	93 ± 6.0
Syringic acid	290 ± 19.0
p-coumaric acid	67 ± 2.7
Ferulic acid	39 ± 1.4

Source: Kunasundari et al., (2017)

Besides, various vitamins are also found in OPT sap; majority as biotin, retinol and ascorbic acid (*Table 3*). Other elements such as potassium, sodium, magnesium, calcium, iron and manganese are also available (*Table 4*).

OPT sap appears slightly acidic (~pH 5) due to presence of syringic acid, malic acid, p-hydroxybenzoic and citric acid (Kunasundari *et al.,* 2017). The availability of various amino acids, vitamins and minerals in OPT sap is indicative of an excellent substrate for microbial fermentation.

BIOFUELS PRODUCTION

Biofuels are gaining interest scientifically and publicly driven by factors such as fluctuation of oil price, urge for an increased energy security and responsibility to mitigate greenhouse gas emissions from fossil fuels. Producing ethanol via fermentation of sugars to be blended with gasoline is a wise strategy able to reduce dependence on fossil fuels while reducing carbon footprints (Valdivia *et al.,* 2016). A decade ago, Kosugi *et al*., had pioneered in exploring the potential of OPT sap for ethanol production (Kosugi *et al.,* 2010). Ethanol was successfully produced from the sap using *Saccharomyces cerevisiae* Kyokai no. 7, without nutrients supplementation, at a comparable rate and yield to the reference medium *i.e.* glucose as a carbon source. Later, Norhazimah and Faizal (2014) attempted to utilise different strains to produce ethanol from OPT sap and further optimise the fermentation conditions *i.e.* temperature and agitation. They found that 30°C was the best temperature for growth of strains *S. cerevisiae* (local), *S. cerevisiae* Kyokai no. 7 (ATCC 26622), *S. cerevisiae* JCM 2220 (ATCC 9804), *Zymomonas mobilis* JCM 10190 (ATCC 29191) and *Zymobacter palmae* JCM 21091 (ATCC 51623); except for *Pichia stipitis* JCM10742 (ATCC 58376) which grew well at 32.5°C. The study also demonstrated that *S. cerevisiae* is the most efficient strain that can produce ethanol from OPT sap.

Using similar yeast species (*S. cerevisiae* ATCC 55618), Adela and Loh (2015) optimised the fermentation conditions, *i.e.* pH, temperature, inoculum size, nitrogen source, dilution effect and growth medium to enhance the production of ethanol. The optimum conditions obtained were pH of 4.0, temperature of 30°C and inoculum size of 10% (v/v), without the requirement of exogenous nitrogen supplementation and substrate dilution. Regardless of any addition of nitrogen source (*i.e.* yeast extract, meat extract, peptone, urea, and ammonium chloride), the yeast rapidly fermented all sugars into ethanol. A representative ethanol fermentation profile of OPT sap, without the addition of nutrients is shown in *Figure 1*. A fermentation period of 24 hr was best for ethanol production with 47.5 g litre^{-1} ethanol and 0.5 g g^{-1} yield at 1.98 g litre^{-1} hr^{-1} formation rate (*Table 6*). This study demonstrates that OPT sap has sufficient nutrients to support fermentation by *S. cerevisiae*, and inhibiting substances during fermentation can be avoided as it affords the best ethanol production performance compared to that in reference medium *i.e.* yeast extract-peptone-dextrose (YPD) medium.

Some of the OPT sap samples have a lower sugar concentrations due to biodeterioration upon storage. Hence, those saps must be condensed via flat membrane filtration, then fermented using more robust thermotolerant yeast, *e.g. Kluyveromyces marxianus* TISTR5925 (Murata *et al*., 2015). *K. marxianus* offers some advantageous features for ethanol production over *S. cerevisiae* as it can withstand higher temperature. Ethanol production conducted at higher temperature between 35°C-40°C could significantly improve the energy efficiency of the process as less energy is anticipated due to a decreased fermentation tank size and distillation tower cooling costs.

As an alternative to ethanol, butanol has become more attractive due to some of its advantageous properties such as higher volumetric energy content, higher hydrophobicity, better blending ability, better compatibility with combustion engines, less corrosion and higher octane rating (Qureshi and Blaschek, 2001). A study by Komonkiat and Cheirsilp (2013) revealed that OPT sap is a promising substrate to produce butanol by *Clostridium acetobutylicum* DSM 1731, attaining an optimum butanol production of 14.4 g litre^{-1} and yield of 0.35 g g^{-1}, without any supplementation of nutrients (*Table 6*).

LACTIC ACID PRODUCTION

Lactic acid has many applications in food, cosmetic, pharmaceutical and chemical industries (Ye *et al.,* 2013). In recent years, lactic acid has gained considerable attention because of its potential use as a precursor for production of polylactic acid (PLA), a biodegradable polymer used to develop commodity plastics. PLA is attractive because of its mechanical properties that resemble those of conventional plastics such as polyethylene, polypropylene and polystyrene (Sudesh and Iwata, 2008). Kosugi *et al*., proved that sugars contained in OPT sap were readily converted into lactic acid by homolactic acid bacterium, *Lactobacillus* lactis ATCC19435 with production efficiency comparable to the reference Modified Strullu and Romand (MSR) medium using glucose as a substrate.

Similar to ethanol fermentation, the cells for lactic acid grow well too without requiring additional nutrients; achieving a theoretical yield of 90% based on total sugars consumption (Kosugi *et al.,* 2010). Later, Chooklin *et al.,* (2011) evaluated a different species, *Lactobacillus casei* TISTR 1500, in producing lactic acid. However, this bacteria could only exhibit higher biomass and lactic acid yields when MRS medium was supplemented to OPT sap fermented at 37°C and pH 5.5 using 20 g litre^{-1} of total sugars (Chooklin *et al.,* 2011).

Kunasundari *et al*., (2017) isolated a new bacteria capable of producing lactic acid, namely *Bacillus coagulans* strain 191, and fermented it in OPT sap under non-sterile conditions. The deployed pretreatment to remove excess phenolics and minerals in the OPT sap via alkaline precipitation improved the sugar fermentability, which subsequently afforded a very high lactic acid yield of 92% and productivity of 2.64 g litre^{-1} hr^{-1} (Kunasundari *et al.,* 2017). Furthermore, the use of a thermophilic bacterium has facilitated the non-sterilised lactic acid fermentation in OPT sap, which is susceptible to microbial contamination. Heating step can be omitted using non-sterilised operation condition hence, lower energy is required (Kunasundari *et al.,* 2017). Additionally, the degradation of sugars and decoloration of raw material, as a result of sterilisation, can also be avoided (Qin *et al.,* 2009).

SUCCINIC ACID PRODUCTION

Succinic acid is a versatile precursor to various commodity and specialty chemicals. It has conventionally been produced in small quantities from the petrochemical, n-butane, via catalytic hydrogenation of maleic anhydride. Succinic acid or its derivatives can be used directly as a source of food additives, pharmaceuticals, surfactants, detergents, solvents, biodegradable plastics and fuels. Succinic acid's greatest market potential, though, would be its use as an intermediary commodity chemical feedstock for producing bulk chemicals, stronger-than-steel plastics, ethylene diamine disuccinate (a biodegradable chelator) and diethyl succinate (a green solvent for replacement of methylene chloride) (Zeikus *et al.,* 1999). Succinic acid can be produced from renewable feedstocks via fermentation of glucose using natural producers or engineered microorganisms, such as *Actinobacillus succinogenes, Anaerobiospirillum succiniciproducens, Mannheimia succiniciproducens, Corynebacterium glutamicum* and recombinant *Escherichia coli* (Akhtar *et al.,* 2014). Among these strains, A. *succinogenes* is the most promising due to its ability to naturally produce succinic acid at high concentration from a broad range of carbon sources (including glucose, cellobiose, lactose, xylose, arabinose and fructose) (Shen *et al.,* 2015).

Recently, our group reported on the possibility of converting OPT sap into succinic acid using *A. succinogenes* 130Z (ATCC24860) (Bukhari *et al.,* 2019). In this study, we have optimised the most suitable fermentation conditions (*i.e.* pH, temperature) and the appropriate medium components (*i.e.* carbon, nitrogen) in achieving the maximum succinic acid production. The optimised fermentation conditions were at pH 6.5-7.5, temperature of 37°C, carbonate loading of 30-40 g litre^{-1}, without nitrogen supplementation under anaerobic conditions. The performance of *A. succinogenes* to synthesize 26% more succinic acid in OPT sap as compared to its reference medium using technical grade sugars manifested the suitability of the feedstock in succinic acid fermentation as it contains all the required nutrients favourable for *A. succinogenes* growth (*Figure 2*). Succinic acid production in OPT sap reached a maximum

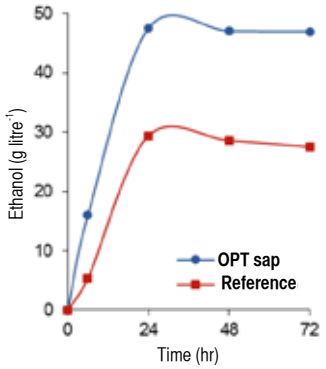

Figure 1. Time course of ethanol production by S. cerevisiae using oil palm trunk (OPT) sap. Reference fermentation was carried out in yeast extract-peptone-dextrose (YPD) medium (1% yeast extract, 2% peptone and 2% dextrose).

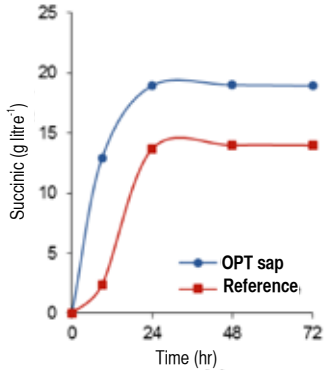

Figure 2. Time course of succinic acid production by A. succinogenes using oil palm trunk (OPT) sap. Reference fermentation was carried out in defined medium [0.02% $MgCl_2$•6H2O, 0.02% $CaCl_2$•2H2O, 0.3% KH_2PO_4, 0.1% NaCl, 1.5% yeast extract and 4.0% $MgCO_3$].

TABLE 6. FERMENTATION-BASED PRODUCTS EMPLOYING OIL PALM TRUNK SAP

Bio-product	Microorganism	Initial sugar (g litre^{-1})	Product titer (g litre^{-1})	Reference
Ethanol	*Kluyveromyces marxianus* TISTR 5925	96.3	45.4	(Murata *et al.,* 2015)
Ethanol	*S. cerevisiae* ATCC 26422	55.0	30.0	(Kosugi *et al.,* 2010)
Ethanol	*S. cerevisiae* ATCC 26422	67.0	38.1	(Norhazimah and Faizal, 2014)
Ethanol	*S. cerevisiae* ATCC 24860	93.9	47.5	(Adela and Loh, 2015)
Butanol	*Clostridium acetobutylicum* DSM1731	50.0	14.4	(Komonkiat and Cheirsilp, 2013)
Lactic acid	*Lactobacillus casei* TISTR 1500	20.0	8.9	(Chooklin *et al.,* 2011)
Lactic acid	*Lactobacillus casei* ATCC 19435	16.7	17.0	(Kosugi *et al.,* 2010)
Lactic acid	*Bacillus coagulans*	78.7	42.7	(Kunasundari *et al.,* 2017)
Succinic acid	*A. succinogenes* ATCC 55618	34.0	19.0	(Bukhari *et al.,* 2019)

concentration of 20.3 g litre^{-1} within 24 hr compared to only 13.7 g litre^{-1} in the reference medium. This may be attributed to the additional nutrients compositions in OPT sap *i.e.* vitamins and minerals.

Apart from better yield, the fermentation in OPT sap also generated less by-products *i.e.* acetic acid and formic acid. A total of 11.63 g litre^{-1} of by-products was accumulated in substrates containing yeast extract compared to only 4.73 g litre-1 in non-supplemented sap (Bukhari *et al.,* 2019). As acetic acid and formic acid have close properties with succinic acid, their presence lead to difficulties during purification step (Tan *et al.,* 2016). The lower accumulated by-products from the non-supplemented OPT sap allows for an easier and cheaper downstream process.

CONCLUSION

The major advantages in using OPT sap for bioconversion are (1) inexpensive, as no additional chemical or enzymatic treatment is required, (2) excellent properties (high concentrations of sugars, nitrogen, amino acids, vitamins, and minerals), (3) high/satisfactory yield of desired products, and (4) low by-products formation, thereby making the fermentation process economical. Considering more than 7.5 million tonnes of OPT are felled annually in Malaysia, the OPT sap is indeed an important resource for downstream processing of biofuels and bioproducts.

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