# Biological Route from Oil Palm Solid Biomass to Sustainable Succinic Acid

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## INTRODUCTION

Succinic acid [International Union of Pure and Applied Chemistry (IUPAC) systematic name: butanedioic acid; historically referred to as amber acid] is a dicarboxylic acid with a $C_4H_6O_4$ molecular formula that can naturally be found in microorganisms, plant and animal tissues (Nghiem *et al.,* 2017). This organic acid consists of four carbon and two functional carboxyl groups (-COOH) which forms colourless and odourless crystals with a distinctive acid taste (Goldberg and Rokem, 2009). Succinic acid is a well-recognised chemical platform used as an intermediate in the industrial synthesis of various chemical compounds (Luthfi *et al.,* 2017). Since 1877, scientists conducting work on the geological sources of amber from several archaeological findings have recognised the ability of succinic acid as an antibiotic and natural remedy (Matuszewska, 2016). It has been commonly used as an alternative to salicylic acid to treat illnesses such as diabetes acidosis, Alzheimer's and Parkinson's diseases, cerebral ischemia and neurological damage (Odin *et al.,* 2002; Pomytkin, 2010). It is believed that the use of succinic acid in medicine increases cellular respiration and glucose metabolism which enables the optimal functioning of the body. Therefore, succinic acid is also known as the 'Spirit of Amber' or the 'Amber of the Modern Age' (Sauer *et al.,* 2008).

Succinic acid was isolated from microbial fermentation for the first time by Georgius Agricola in 1546 (Putri *et al.,* 2020). Large-scale microbial fermentation for the production of bio-succinic acid is currently expected, as the highest production of bio-succinic acid can be achieved by anaerobic microbial fermentation (Putri *et al.,* 2020). In order to establish a bio-based industrial production of succinic acid, the microbes must be able to use a wide variety of sugar feedstocks in order to make use of the cheapest raw materials available (Liu *et al.,* 2013). Furthermore, oil palm biomass are the most abundant renewable resource and have great potential as fermentation substrates (Bukhari *et al.,* 2019b).

## SUCCINIC ACID PRODUCTION PROCESS

Succinic acid is produced from sugar by microbial fermentation or by traditional petrochemical processes (Chang *et al.,* 2016). Some of the commercially available petrochemical-based methods include paraffin oxidation, catalytic hydrogenation of maleic acid from butane, and processing of acetylene, carbon monoxide, or water catalysed by cobalt tetracarbonyl hydride in an acidic medium at high pressure and temperature (Xu and Guo, 2010). Some limitations of the conventional petrochemical pathways are the high energy consumption and greenhouse gas emissions which lead to relatively limited use of succinic acid for the development of various applications.

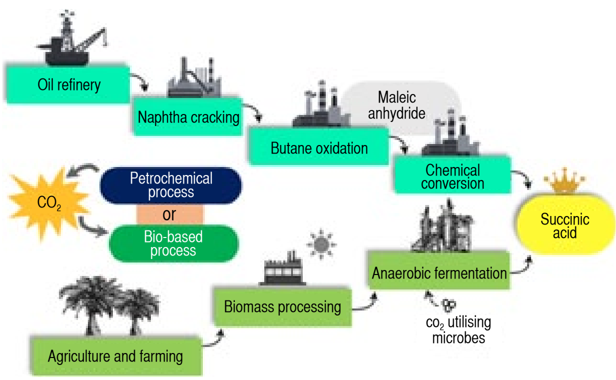


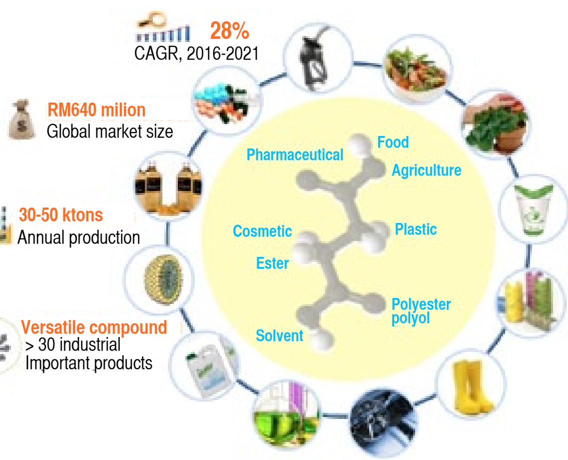

*Figure 1. Petrochemical and bio-based succinic acid production processes.*

In recent decades, the production of bio-based succinic acid by carbon dioxide-utilising bacteria is considered the best alternative to the petrochemical route (Bukhari *et al.,* 2019b; Liu *et al.,* 2013). Bio-production of succinic acid is in line with the mission of the United Nations Framework Convention on Climate Change (UNFCCC) to reduce carbon footprint using green technology in the synthesis of chemical products (Tan *et al.,* 2017).

Realising this reality, the world's leading chemical companies such as Myriant Corporation (USA), Succinity (Spain), Reverdia (Italy), Michigan Institute of Biotechnology (USA), Mitsubishi-Ajinomoto (Japan), MBEL-KAIST (South Korea), Agro-Industrie Recherches et Développements (ARD, France), Mitsui and Co. (Japan), China National BlueStar Co. and PTT Public Company Limited (Thailand) are emerging and actively engaged in exploring alternative white biotechnological routes for the commercialisation of bio-based succinic acid and its derivatives as new commodity (Chang *et al.,* 2016). Succinic acid has been identified as one of the 12 chemical building blocks that could potentially be produced commercially by biological process, according to the US Department of Energy in 2004 (Tan *et al.,* 2014).

On the basis of succinic acid applications, the global market for succinic acid can be segmented into the following sectors (and their respective market share percentages): industrial applications (57.1%); pharmaceuticals (15.91%); food and beverages (13.07%), and others (13.92%) (*Figure 2*) (Chang *et al.,* 2016). Its production capacity now reaches about 30-50 ktons per annum at a compound annual growth rate of 28% (between 2016 and 2021) with global volumes exceeding RM640 million, assuming that the price of succinic acid production will be drastically reduced in the future (Cheng *et al.,* 2012; Tan *et al.,* 2017).

(a)




(b)

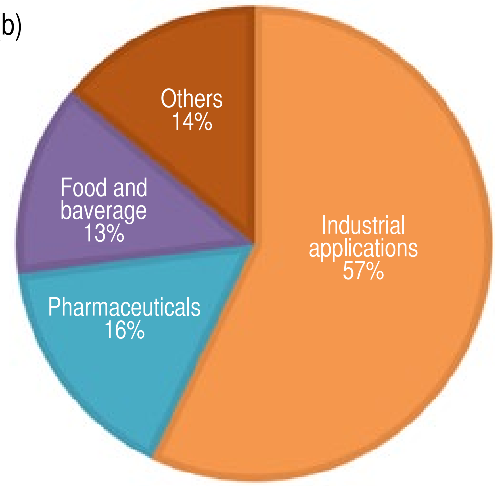



*Figure 2. (a) Succinic acid application and its respective, (b) Market share percentages.*

## BIOMASS PROCESSING

The use of agro-industrial waste for the production of renewable chemicals results in a substantial reduction in the cost of biorefinery and thus supports the current agricultural industry (Bukhari *et al.,* 2019b). Fermentation method that uses bacteria to produce succinic acid from renewable biomass is potentially a more efficient alternative in terms of greenhouse gas savings and optimal energy consumption, while at the same time reducing dependence on limited mineral resources (Tan *et al.,* 2014). Waste or biomass from the palm oil industry is an example of renewable resources in Malaysia and is considered the best fermentative substrate due to its abundant supply and does not compete or interfere with the food chain (Luthfi *et al.,* 2017; Zahari *et al.,* 2012).

The oil palm tree (*Elaeis guineensis*) generally bears fruit within 2.5 years of planting and has an economic life of 25-30 years before replanting. In Malaysia, the oil palm trees are planted in a total area of approximately 5.9 million hectares (Parveez *et al.,* 2020). In line with the unprecedented growth of the palm oil industry, the estimated oil palm waste generation reaches approximately 76 million metric tonnes per year, including oil palm fronds, oil palm trunks, empty fruit bunches, mesocarp fibres, and oil palm shells. Previous work indicated that the total carbohydrates in oil palm solid biomass comprise around 33.00%-68.00% of the total dry weight of biomass (*Table 1*), consisting of repeating monomeric units of glucose, xylose, arabinose and mannose, among others (Chong *et al.,* 2013; Okoroigwe *et al.,* 2014).

**TABLE 1. CARBOHYDRATE CONTENTS IN OIL PALM SOLID BIOMASS**

| Biomass | Oil palm fronds  | Oil palm trunks  | Empty fruit bunches  | Mesocarp fibers  | Oil palm shells  |
|---|---|---|---|---|---|
| Carbohydrate contents | 65.5% | 56.7% | 68.0% | 56.0% | 33.1% |
| Reference | (Luthfi *et al.,* 2016) | (Bukhari *et al.,* 2019) | (Chong *et al.,* 2013) | (Iberahim *et al.,* 2013) | (Okoroigwe *et al.,* 2014) |

Carbohydrate molecules present in the plant cell walls are protected by lignin bound by chemical bonds (Iberahim *et al.,* 2013). Huge quantities of carbohydrates can be obtained when lignin is successfully removed. Therefore, the liberalised sugar monomers from oil palm biomass can be used as a carbon source for succinic acid-producing bacteria (Bukhari *et al.,* 2019a). Monomers are obtained by two bioprocess steps, *i.e.* by (i) pre-treatment, and (ii) hydrolysis with enzymes or acids (Luthfi *et al.,* 2017). In this regard, enzymatic hydrolysis offers advantages such as low energy consumption, non-corrosive, minimal by-products, and higher sugar yields than acid hydrolysis (Alvira *et al.,* 2010). The production of hydrolytic enzymes is now dominated by two major companies; Novozymes (Denmark) and DuPont/Genencor (USA), with a market share of 47% and 21%, respectively (Rodrigues *et al.,* 2015). Some widely used industrial enzymes include Accellerase-1500 and GC-220 from DuPont. Celluclast 1.5 litre, Cellic CTec2, and Cellic CTec3 were produced by Novozymes in collaboration with Sigma-Aldrich/Merck, which helped to further expand the product marketing network (Ioelovich, 2016).

Succinic acid production from oil palm biomass involves a pre-treatment process followed by hydrolysis of carbohydrates into simple sugars and then fermentation to convert sugars into succinic acid (Luthfi *et al.,* 2016). Alkaline pre-treatment with sodium hydroxide, potassium hydroxide, calcium hydroxide (traditionally called slaked lime), and ammonium hydroxide is commonly used to remove lignin as well as alter the inherent recalcitrant structure of the cellulose-hemicellulose matrix (*i.e.* complex carbohydrate structure) to facilitate the accessibility of enzymes to the targeted substrate (Zakaria *et al.,* 2015). Pre-treatment with sodium and potassium hydroxides have proven to be stereo-specific and selectively reduces a minor part of hemicelluloses without significant loss of biomass components (Luthfi *et al.,* 2017). Nevertheless, lime is the cheapest among other alkaline pre-treatments, with an average cost of RM294 $t^{-1}$ hydrated lime as compared to RM1342 $t^{-1}$ NaOH (50%) and KOH (45%) without taking into account recovery costs (Brodeur *et al.,* 2011; Luthfi *et al.,* 2017).

Three groups of enzymes that play a major role in converting cellulose to simple sugars include: (i) endo-1,4-β-glucanase; (ii) exo-1,4-β-glucanase; and (iii) β-glucosidase. The three groups of enzymes described above act synergistically to release cellobiose and cellodextrin from cellulose structures, which are then converted to monomeric sugars (Ioelovich, 2016). The resultant sugar is then used in fermentation for the production of succinic acid.

## OIL PALM SOLID BIOMASS AS A PROMISING CARBON SOURCE

In Malaysia, the palm oil industries are the major contributors to the generation of agricultural biomass. Studies have been conducted to allow efficient use of biomass, such as the conversion of empty fruit bunches, oil palm trunks and oil palm fronds into fermentable sugars and the subsequent production of succinic acid. *Table 2* outlines the available pre-treatment, hydrolysis and succinic acid production technology involving oil palm biomass. Fermentation of empty fruit bunches using *Actinobacillus succinogenes* ATCC 55618 was previously studied (Akhtar and Idris, 2017; Pasma *et al.,* 2013). Prior to fermentation, biomass was pre-treated and enzymatically hydrolysed to produce 0.4–0.7 g $g^{-1}$ of sugar conversion. This variability in sugar conversion was due to the various types of pre-treatment and hydrolysis employed. Microwave-assisted acid or alkali, followed by multiple enzymes (cellulase and Novozyme 188) showed

**TABLE 2. OIL PALM BIOMASS PRE-TREATMENT, HYDROLYSIS AND SUCCINIC ACID PRODUCTION**

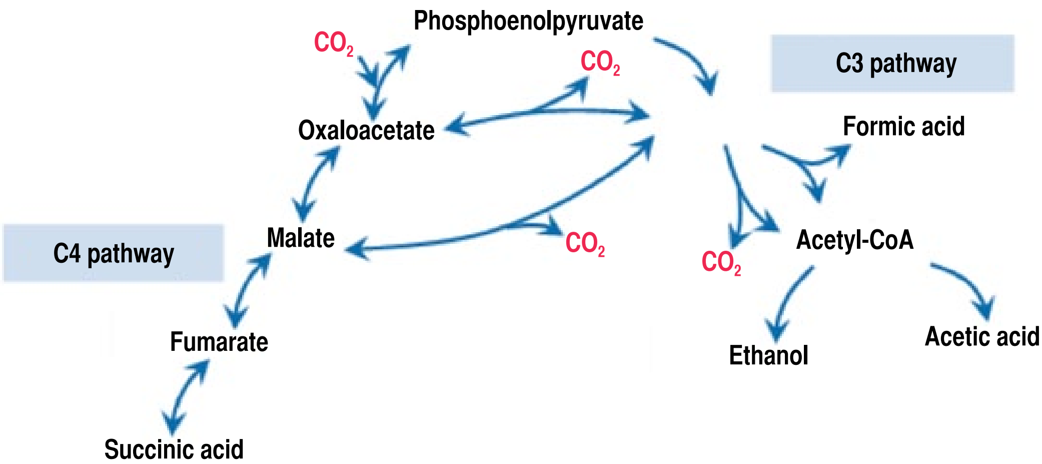
| Biomass | Pre-treatment type | Hydrolysis type | Sugar conversion yield (g $g^{-1}$) | Succinic acid yield (g $g^{-1}$) | Reference |
|---|---|---|---|---|---|
| Empty fruit bunches | Autohydrolysis + hydrogen peroxide | Enzyme: cellulase | 0.73 | 0.33 | (Pasma *et al.*, 2013) |
| Empty fruit bunches | Microwave-assisted acid/alkali | Enzyme: cellulase + Novozyme 188 | 0.45 | 0.47 | (Akhtar and Idris, 2017) |
| Oil palm trunk | Dilute organic acid | Enzyme: Cellic CTec2 | 0.33 | 0.47 | (Bukhari *et al.*, 2020) |
| Oil palm frond | Sodium hydroxide + autohydrolysis | Enzyme: Cellic CTec2 + Cellic HTec2 | 0.53 | 0.71 | (Luthfi *et al.*, 2016) |

lower conversion of sugar but higher yield of succinic acid (Akhtar and Idris, 2017). On the other hand, oil palm trunks hydrolysed with organic acid yielded similar succinic acid (0.47 g $g^{-1}$) due to the low formation of by-products (Bukhari *et al.,* 2020). The highest succinic acid yield of around 0.7 g $g^{-1}$ could be attained with alkaline and autohydrolysis pre-treatment with water, followed by enzymatic hydrolysis using Cellic CTec2 and Cellic HTec2 (Luthfi *et al.,* 2016).

## METABOLIC PATHWAY IN THE PRODUCTION OF SUCCINIC ACID

Researchers looked at how cells create energy from carbon source, by digesting simple sugars in a series of chemical reactions (Tan *et al.,* 2018). This cycle is almost the same for every type of cell, including animals, plants, and bacteria. Rumen-type bacteria such as *Actinobacillus succinogenes* have been identified as the best succinic acid-producing host due to their potential for using various carbon sources as well as carbon dioxide (Song and Lee, 2006). Sugars used by the rumen bacteria are converted to phosphoenolpyruvate (PEP) by glycolysis (Luthfi *et al.,* 2016).

PEP is then converted to pyruvate with the formation of acetic acid, formic acid, and ethanol as the final product (C3 pathway) or oxaloacetate followed by the formation of succinic acid as the final product (C4 pathway), as shown in *Figure 3* (McKinlay *et al.,* 2007; Tan *et al.,* 2018). Carbon dioxide is required to shift the key intermediate PEP from C3 (acetic acid and formic acid) to C4 (succinic acid) pathways with a by-product-to-succinic acid ratio of approximately 1.0 based on redox balance (McKinlay *et al.,* 2007). The presence of carbon dioxide could inhibit the decarboxylation of oxaloacetate and malate. The decarboxylation of C4 to C3 intermediate was sufficiently high to have a major effect on the yield of succinic acid (Tan *et al.,* 2018). Thus, the addition of carbon dioxide leads to an increase in cell activity and to the production of succinic acid as a final product. Since the biological route utilises




Source: Tan *et al.*, (2018).

*Figure 3. Metabolic pathway in the production of succinic acid.*

carbon dioxide, the beneficial effect of carbon dioxide on the production of succinic acid can be translated into an environmentally friendly and sustainable solution for the sequestration of greenhouse gas emissions while, at the same time, allowing better utilisation of oil palm biomass.

## CONCLUSION

It is therefore clear that oil palm biomass can be efficiently utilised by converting it into a sugar solution and then by fermentation for the production of succinic acid. The oil palm solid waste management system can be implemented more effectively by improving biochemical processes for conversion into highly profitable products, such as succinic acid. Indeed, the production of fine chemicals from oil palm biomass can benefit various sectors, thereby stimulating the country's economic growth and, ultimately, protecting the environment with green technology. Complete utilisation of oil palm biomass in large-scale development of valuable fine chemicals would be an ambitious agenda but nonetheless a laudable goal.

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