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# PROSPECTS AND STATE-OF-THE-ART IN PRODUCTION OF BIO-BASED SUCCINIC ACID FROM OIL PALM TRUNK

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## ABSTRACT

*Bio-based chemicals possess enormous market potential in realising a circular economy. Industrially, succinic acid (SA) is an important precursor for the establishment of a sustainable biochemical industry. This article reviews the potential of oil palm trunk (OPT) for SA production, from bioconversion aspects such as biomass pretreatment, enzymatic saccharification, and fermentation, to technological advancement and process economics, assisted by* Actinobacillus succinogenes*. For commercial SA exploitation, the focus should be on finding cheap biomass feedstock and optimising unit processes either for a partial or total displacement of expensive chemical paths during fermentation. OPT has been hailed as a viable candidate for the cost-effective production of SA, given its nutrient- and carbohydrate-rich sap and bagasse for improving the intended pretreatment and hydrolysis technologies. Type of operating modes, process configurations and fermentation factors concerning medium, substrate and culture have been identified as keys for advancing SA production from OPT in recent years. Lastly, the potential of OPT as part of a biorefinery feedstock for multiple bioconversion towards effective environmental management is designed to put forth the vision of a circular economy in the palm oil industry.*

**Keywords:** bioconversion, biomass pretreatment, biorefinery, oil palm biomass, succinic acid.

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## INTRODUCTION

Bio-based materials and product value chains have been identified as the most resource-efficient pathway in creating a circular bioeconomy (Lokesh *et al*., 2018). It includes optimal utilisation of biological resources (residues, by-products and side streams) and their conversion into a variety of value-added products such as food, feed, bio-product, biochemical and bioenergy. The transition from an oil-based to a bio-based economy holds great potential in reducing fossil dependency, contributing to socio-economic growth, rural development, environmental benefits and technological advances (da Silva *et al*., 2020). Lignocellulosic plant-based materials are abundant and renewable as versatile resources that can be converted into a wide range of value-added products. The biochemical conversion methods of lignocellulosic biomass have been established as the most suitable for depolymerising its polysaccharides into sugars, which serve as platform molecules for further conversion into desired bio-products via microbial fermentation (Castro *et al*., 2017).

Succinic acid (SA), also called butanedioic acid, remains a promising bio-based platform chemical due to its versatility as a precursor to various commodities and speciality chemicals. SA or its derivatives can be used directly as a source of food additives, pharmaceuticals, surfactants, detergents, solvents, biodegradable plastics and fuels (Nghiem *et al*., 2017). It has been conventionally produced from petrochemical feedstock, *n*-butane, via catalytic hydrogenation of maleic anhydride.

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Given an increase in worldwide environmental awareness associated with the petrochemical process, there has been a shift in world demand towards producing SA via microbial fermentation (Lee *et al*., 2022). However, bio-based SA production cost is unattractive and remains a tall order to compete with the giant petroleum-derived chemicals. Globally, much effort has been made to produce SA using *Actinobacillus succinogenes* from inexpensive and renewable feedstocks, including sugarcane bagasse, corn stover, corn fibre, carob pods (Lu *et al*., 2021), oil palm empty fruit bunch (OPEFB) (Khairil Anwar *et al*., 2021), oil palm frond (OPF), as well as oil palm trunk (OPT) (Luthfi *et al*., 2018).

Malaysia, one of the world's largest palm oil producers, has an oil palm planted area of 5.87 million hectares in 2020 (Parveez *et al*., 2021). Along with the production of crude palm oil, the Malaysian palm oil industry produces a huge amount of oil palm biomass from its plantations and milling activities. OPF, OPT and leaves can be obtained from oil palm plantations during the harvesting period and the rejuvenation of old plants, whereas other residues, such as OPEFB, are generated from palm oil mills (Yong *et al*., 2022). With its abundance, renewability, and low cost, OPT is a potential biomass for bioconversion. Only a small portion of the felled OPT is currently being used for plywood manufacturing and may be sold to interested parties, while the remainder is being shredded and mulched in the field to degrade naturally for nutrient recycling (Naidu *et al*., 2020). This conventional practice provides fertile grounds for the propagation of various pests and vermin, prompting the search for an alternative to exploit and tap the true potentials of underutilised OPT. The bioconversion of OPT into SA seems promising (Bukhari *et al*., 2021b) and could be a practical and profitable way to diversify its utilisation.

As biomass is a lignocellulosic material with varying degrees of recalcitrance, it is desirable to develop an efficient, low-cost, and environmental-friendly pretreatment method to facilitate biodegradation. Each pretreatment method has a different impact on all the subsequent bioconversion steps, in terms of sugar recovery, the toxicity of hydrolysates, enzymatic saccharification and fermentation (Castro *et al*., 2017). Many aspects of bioconversion must be considered for upscaling and commercialisation attempts, such as pretreatment process selection, cost-effectiveness (use of inexpensive and minimal chemicals), and route selection for efficient and easy-to-operate bioconversion. In addition, several problems have been encountered in SA production through microbial fermentation specifically by *A. succinogenes* such as substrate requirements, auxotrophy, inhibitory effect and low production rate. This article reviews the prospect of SA and analyses each steps in bioprocessing OPT for the production of SA by *A. succinogenes*, and provides an insight into its totality and practicality as a potential biomass feedstock for SA production in the future.

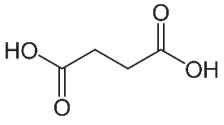
## BACKGROUND AND PROPERTIES OF SUCCINIC ACID

In Europe, SA has historically been used as a general curative and natural antibiotic. Anecdotal evidence has suggested that it could promote neural system recovery, strengthen the immune system, compensate for energy drain in the body and brain, boost alertness, concentration and reflexes, and reduce stress (Vávra, 2009). Other traditional applications of SA include food additives, pharmaceutical intermediates, cosmetics, cement additives, detergents, plasticisers, toners and soldering fluxes (Nghiem *et al*., 2017). Before the emergence of fermentation technology for industrial production, SA was commercially manufactured from the by-product (C4 fractions) of naphtha, *i.e.*, a four-carbon compound (maleic acid or anhydride) via catalytic hydrogenation (*Figure 1*). However, due to price volatility and relatively high carbon footprints, the use of petroleum-based SA has been controversial. With growing public concern in recent years about overcoming this challenge, SA production has begun, driven by the desire to exploit renewable plant-based feedstock (Mancini *et al*., 2019).

Given its broad range of opportunities for various applications, SA has thus far been well-received as a versatile platform chemical. Its potential is reflected in the US Department of Energy's list as being one of the top 12 most promising value-added bio-based chemicals (Werpy and Petersen, 2004). Also known as butanedioic acid ($C_4H_6O_4$) or amber acid, SA is a four-carbon aliphatic dicarboxylic acid, with the two -COOH groups attaching to two different C atoms. It appears as an odourless and colourless crystalline solid. *Table 1* outlines its chemical structure and properties.

**TABLE 1. PHYSICAL PROPERTIES OF SUCCINIC ACID**

| Properties | Value |
|---|---|
| IUPAC ID | Butanedioic acid |
| Formula | $C_4H_6O_4$ |
| Molar mass (g mol$^{-1}$) | 118.09 |
| Density (g cm$^{-3}$) | 1.56 |
| Boiling point (°C) | 235.00 |
| Melting point (°C) | 184.00 |
| Specific gravity | 1.56 |
| $pK_{a1}$ | 4.21 |
| $pK_{a2}$ | 5.64 |



Succinic acid
[CAS 110-15-6]

Source: Lin *et al*. (2005).

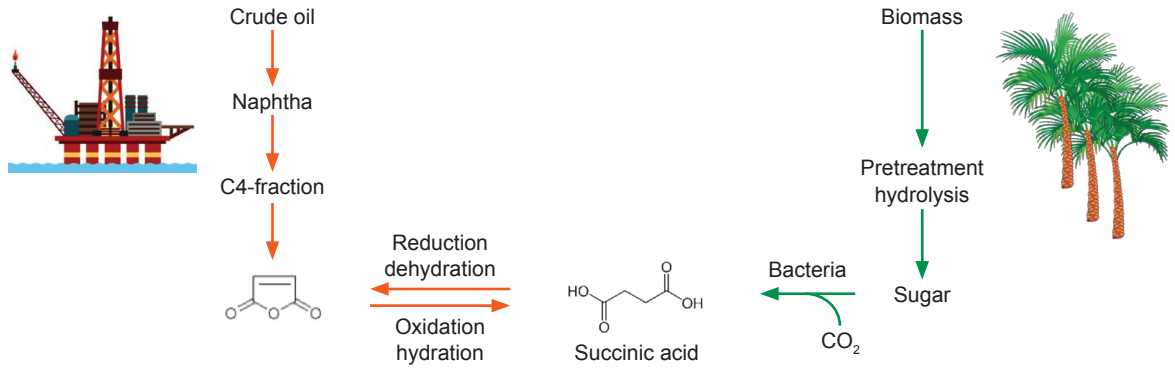



*Figure 1. Production routes for succinic acid (adapted from Mancini* et al.*, 2019).*

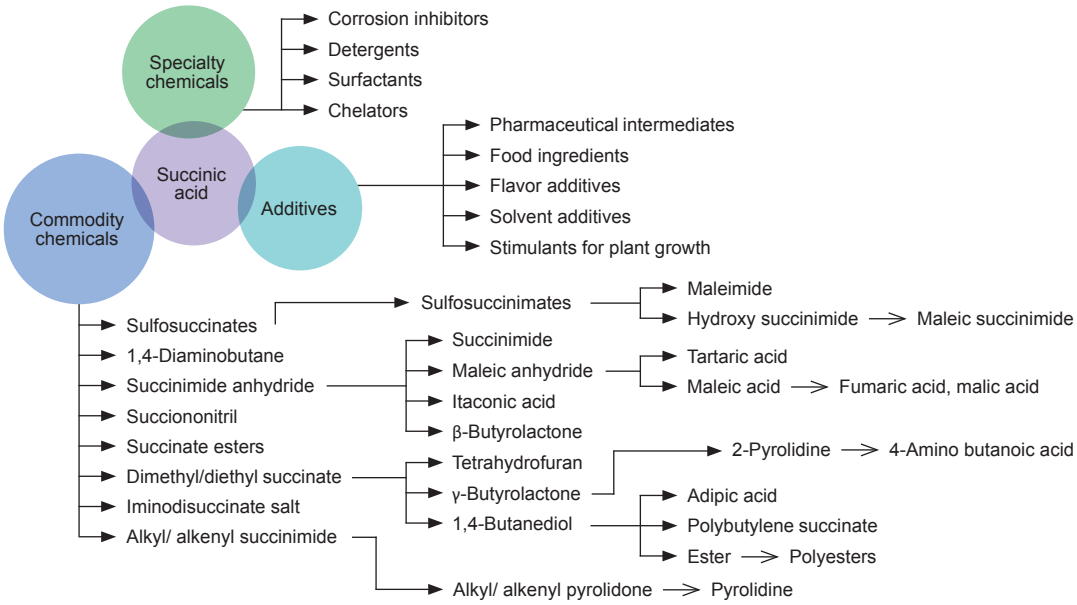



*Figure 2. Various applications of succinic acid and its derivatives. Adapted from Akhtar* et al.*, 2014.*

## MARKET POTENTIAL AND APPLICATIONS OF SUCCINIC ACID

The commercial roles and fields of applications of SA and its derivatives are manifold (*Figure 2*). In detergency, SA is employed to produce additives, detergents, foaming agents and surfactants. In coating, it is applied as an ion chelator for pitting and anti-corrosion purposes. In agriculture, it has the role of a growth regulator for seed treatment and plant rooting (Cao *et al.*, 2013).

SA is used for various applications in the food industry. It acts as an acidity regulator, flavour-conditioning agent, bread-softening agent, flavour or oil micro-encapsulator and sweetener. Additionally, it improves the shelf life of food products, given its antimicrobial properties to inhibit the growth of bacteria, yeasts and moulds (Chimirri *et al.*, 2010). Generally considered safe by the United States Food and Drug Administration (Cao *et al.*, 2013), SA also offers utility potential in the pharmaceutical industry.

In cosmetic formulations, SA offers antioxidant properties and safety in manufacturing derivatives such as emollients, surfactants and emulsifiers (Palagina, 2017). Its high aqueous solubility favours its use as an effective alternative to salicylic acid as a preservative booster and therapeutic agent for anti-acne treatment (Wang *et al.*, 2014). SA has been reported to reduce the intensity of lipid peroxidation, potentially acting as an anti-pollution agent to improve product stability (Xiao *et al.*, 2012). Furthermore, it exhibits promising to energise and revitalise effects, given its role in enhancing mitochondrial activity in skin cells (Huffman *et al.*, 2014).

Recent applications involving SA lie in the polyester-polyurethane markets. Tetrahydrofuran (THF), γ-butylrolactone, and 1,4-butanediol (BDO) are some of the most popular chemicals deriving from SA (Nghiem *et al.*, 2017). THF is used widely as a solvent and feedstock for producing polytetramethylene ether glycol, which in turn is used as a precursor to making polyurethane

polymers. The most prominent role of SA in thermoplastic applications is the production of polybutylene succinate (PBS), a polyester derived from SA and BDO. The substantial demand for SA in PBS for food packaging is associated with non-toxicity, biodegradability and improved heat resistance (VMR, 2021). Moreover, BDO acts as a precursor to various speciality chemicals, which are used as solvents or raw materials in pharmaceuticals and agrochemicals (Cok *et al*., 2014).

In commercial terms, SA is priced at around USD5.9-9.0 $kg^{-1}$ based on its purity. The estimated global SA market size in 2020 has been valued at USD147.42 million. With a compound annual growth rate of around 8.0% from 2021 to 2028, it is encouraging to anticipate a projected value of USD268.8 million by 2028 (VMR, 2021). During this forecast period, the adoption of SA as a promising alternative to adipic acid will be a key driver in the market for the production of polyurethane.

## BIOCATALYSIS OF SUCCINIC ACID

As an intermediate of the tricarboxylic acid (TCA) cycle, SA represents a common metabolic end-product for many anaerobic microorganisms. While the choice of production host is diverse, the natural and native hosts being considered so far are capnophilic rumen microorganisms. On the other hand, non-natural production hosts are chosen based on their genetic accessibility, such as bacteria, yeast, fungi and recently microalgae (*Table 2*). The most commonly used strains include *A. succinogenes*, *Anaerobiospirillum succiniciproducens*, *Bacteroides fragilis*, *Corynebacterium crenatum*, *C. glutamicum*, *Mannheimia succiniciproducens*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* (Akhtar *et al*., 2014; Beauprez *et al*., 2010; Ong *et al*., 2019). Among these, *A. succinogenes* is reported as the most effective living reactor due to its capability to ferment a wide range of substrates (Guettler *et al*., 1999), including the four most abundant plant-based sugars, *i.e*., glucose, fructose, xylose and arabinose.

### *Actinobacillus succinogenes* as a Living Reactor

Originating from the *Pasteurellaceae* family, *Actinobacillus succinogenes* is a Gram-negative rod bacterium first isolated from the bovine rumen (Guettler *et al*., 1999). It is a facultative anaerobe with osmotolerant and pleomorphic properties. Its type strain is ATCC 55618. The complete genome of *A. succinogenes* 130Z was first publicly released via the GeneBank database in 2007, followed by detailed literature in 2010 (McKinlay *et al*., 2010). Several aspects of *A. succinogenes* as shown in *Table 3*, have been considered crucial for being one of the most promising SA-producing strains:

To produce succinate, *A. succinogenes* utilises the phosphoenolpyruvate (PEP) carboxylation pathway. *Figure 3* shows the carbon-flux distribution by *A. succinogenes* leading to the formation of succinate (alongside formate, acetate and ethanol). In this reductive TCA cycle, PEP is formed from the breakdown of glucose to serve as the branch point between the succinate-producing ($C_4$) pathway and the formate, acetate and ethanol-producing ($C_3$) pathway. These two reductive reactions allow for a balanced production of metabolites from PEP (Song and Lee, 2006).

Theoretically, 1.0 mol of SA can be produced from the fixation of 1.0 mol of $CO_2$ and 0.5 mol of glucose, alongside the need for 2.0 mol of NADH. Accordingly, a noteworthy obstacle in securing high succinate yields through the anaerobic pathway is NADH limitation. Therefore, the molar yield of succinate is limited to 1.0 mol per mol glucose, assuming that all the carbon flux undergoes exclusively the anaerobic fermentative pathway (Cheng *et al*., 2013; Vuoristo *et al*., 2016).

**TABLE 2. BIOCATALYSTS INVESTIGATED FOR MICROBIAL CONVERSION OF SUCCINIC ACID**

| Type | Microorganism | Yield (g $g^{-1}$) | References |
|---|---|---|---|
| Bacterium | *Actinobacillus succinogenes**,#, *Anaerobiospirillum succiniciproducens**,#, *Basfia succiniproducens**,#, *Bacteroides fragilis*#, *Mannheimia succiniciproducens**,#, *Enterococcus faecalis*, *Enterococcus flavescens*, *Escherichia coli*#, *Fibrobacter succinogenes*, *Clostridium thermosuccinogenes*, *Corynebacterium crenatum*#, *Corynebacterium glutamicum*#, *Klebsiella pneumoniae*, *Ruminococcus avefaciens*, *Ruminococcus champanellensis*, *Selenomonas ruminantium* | 0.29 - 1.23 | Guettler *et al*. (1999); Beauprez *et al*. (2010); Lee *et al*. (2002) |
| Yeast | *Saccharomyces cerevisiae*#, *Yarrowia lipolytica*#, *Candida brumptii*, *Candida catenulate*, *Candida zeylanoides*, *Pichia kudriavzevii*, *Penicillium simplicissimum*, | 0.22 - 1.17 | Kamzolova *et al*. (2009); Prabhu *et al*. (2020) |
| Fungus | *Aspergillus niger*, *Trichoderma reesei*, *Byssochlamys nivea*, *Paecilomyces varioti*, *Lentinus degener*, *Fusarium* spp., *Penicillium simplicissimum*, *Rhizophus* sp. | 0.18 - 0.95 | Alcantara *et al*. (2017); Bechthold *et al*. (2008) |
| Microalgae | *Micractinium* sp. IC-44 | 0.66 | Sorokina *et al*. (2020) |

Note: *- The most efficient producers; #- the most commonly used.

**TABLE 3. FEATURES OF *Actinobacillus succinogenes* AS SUCCINIC ACID (SA) PRODUCER**

| Feature | References |
|---|---|
| Direct fermentation of water-extracted sugars | Carvalho *et al*. (2016) |
| Capability to use a broad range of carbon sources, including C5 and C6 sugars, disaccharides, and others (glucose, xylose, cellobiose, sucrose, fructose, maltose, lactose, mannose, arabinose, mannitol, and glycerol) | Pereira *et al*. (2018); Yang *et al*. (2020) |
| Performing scalable biorefinery streams | Bradfield *et al*. (2015) |
| Tolerance to high concentrations of glucose<br>Tolerance to impurities in hydrolysates, such as furfural and hydroxymethylfurfural (HMF) | Song and Lee (2006)<br>Diaz *et al*. (2018) |
| Resistance to high concentrations of SA | Guettler *et al*. (1999) |
| Favouring high $CO_2$ availability and reducing power | McKinlay *et al*. (2005); Schindler *et al*. (2014) |
| Fixation and consumption of $CO_2$ | Van Der Werf *et al*. (1997) |
| Non-pathogenicity | McKinlay *et al*. (2010) |
| Ability to form biofilms | Mokwatlo *et al*. (2019) |

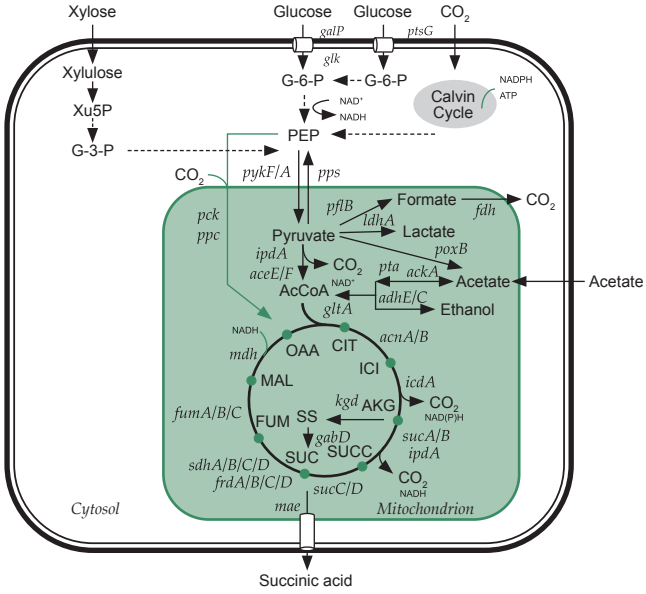



*Figure 3. Production pathways of succinic acid in prokaryotes (*e.g. A. succinogenes, M. succiniciproducens, E. coli, *and* C. glutamicum*). G-6-P: glucose-6-phosphate; G-3-P: glyceraldehyde-3-phosphate; PEP: phosphoenolpyruvate; CIT: citrate; ICI: isocitrate; AKG: α-oxoglutarate; SUCC: succinyl-CoA; SUC: succinate; FUM: fumarate; MAL: malate; and OAA: oxaloacetate (Dai* et al.*, 2020).*

In contrast to the practical application of engineered *E. coli* as a biocatalyst for SA production (Li *et al*., 2016), the metabolic engineering of *A. succinogenes* has been repressed by the lack of appropriate and complementing genetic tools (Dessie *et al*., 2018). Few attempts have thus been undertaken to develop different mutant strains of *A. succinogenes*. Zheng *et al*. (2013) adopted genome-shuffling techniques to improve SA production. However, it was difficult not only to achieve specific controllable traits caused by multiple randomly-occurring mutations but also to determine the exact mode of mutagenesis, with possible resultant irreversible genomic damage. Guarnieri *et al*. (2017) examined the knockout of enzymes involved in the pathways of by-product formation and the over-expression of enzymes in the reductive branch of the TCA cycle leading to SA production. Compared with the wild strain, the knockout mutants exhibited a delayed growth phase, slower sugar consumption, accumulation of unusual byproducts (*i.e.*, lactate), and lower SA production. As a whole, the metabolic engineering of *A. succinogenes* is still in an early incipient stage. Therefore, other perspectives for improving SA production warrant future investigations. These include a selection of efficient pretreatment, optimising fermentation conditions and simplifying overall process steps as strategies to achieve feasible SA processing.

Another challenge of using *A. succinogenes* is the requirement of expensive nitrogen sources such as yeast extract (YE) in fermenting SA. This is due to the presence of auxotrophic cells among the natural SA-producing strains isolated from the rumen, as

such cells rely on amino acids and vitamins readily available in a medium to function and replicate. Thus, complex media enriched with nutrients are necessitated to support the bacterial growth and biological activity of these strains (Beauprez *et al*., 2010). For fermentation using *A. succinogenes*, YE provides nutrients for bacterial growth and SA synthesis. Studies have reported the use of YE as high as 15-20 g $L^{-1}$ in fermenting carob pod extracts (Carvalho *et al*., 2016), sugarcane molasses (Cao *et al*., 2018) and OPF hydrolysate (Luthfi *et al*., 2018). Unfortunately, the high costs of YE are impractical the for large-scale commercialisation of SA. Considerable effort has been devoted to reducing the costs by substituting YE with peanut meal, soybean meal, and cotton meal (Shen *et al*., 2015), corn steep liquor (Tan *et al*., 2016; Xi *et al*., 2013), Spent brewer's yeast hydrolysate (Jiang *et al*., 2010). However, there remain challenges for yield improvement as inferior SA yields have been reported, except for those mediums supplemented with additional biotin, vitamins and iron-containing compounds (Shen *et al*., 2016).

## FEEDSTOCK FOR BIOCATALYSIS OF SUCCINIC ACID

Understanding the underlying mechanism of sugar uptake by the preferred biocatalyst is critical for determining low-cost alternative carbon sources as well as regulating metabolic pathways and fermentative processes for efficient SA production. *A. succinogenes* exhibits an excellent capability to metabolise a broad range of carbon sources due to its natural habitat (rumen of a ruminant), which is rich in various carbohydrate substrates (Dessie *et al*., 2018). In this context, the deployment of lignocellulosic biomass as feedstock for SA production has garnered global attention, considering their advantages - richness in carbohydrates, abundance, sustainability, and affordability. SA has been successfully produced from various lignocellulosic biomass (*Table 4*), such as sugarcane bagasse (Chen *et al*., 2021), corn fibre (Vallecilla-Yepez *et al*., 2021), blue agave (Corona-González *et al*., 2016), carob pod (Carvalho *et al*., 2016), Napier grass (Lee *et al*., 2022), citrus peel (Patsalou *et al*., 2017) and industrial hemp (Kuglarz and Grübel, 2018).

### Lignocellulosic Oil Palm Biomass

Oil palm is regarded as the most economical and productive oil crop, accounting for approximately 77% of Malaysia's total agricultural land, *i.e.*, 5.9 million hectares in 2020 (Parveez *et al*., 2021). The Malaysian palm oil industry represents the largest contributor of lignocellulosic biomass, supplying more than 90% of the country's total biomass (Loh, 2017). Palm oil accounts for only 10% of the total dry matter of the mass harvested, with the remaining 90% made up of various biomass components (*Figure 4*). In terms of production, for each tonne of fresh fruit bunches (FFB) processed, approximately 225 kg of crude palm oil (CPO) is yielded. Besides, about 234 kg of OPEFB, 135 kg of mesocarp fibre (MF), 73 kg of palm kernel shell (PKS) and 67 kg of palm oil mill effluent (POME) are also generated. Furthermore, approximately 46 million tonnes of OPF and 22 million tonnes of OPT were estimated based on replanting and pruning activities in 2020. In terms of long-term soil management, more than 50% of OPF and OPT are allowed to decompose in the plantation sites (Loh, 2017).

As with other lignocellulosic biomass, oil palm biomass predominantly comprises three components, *i.e.*, cellulose, hemicellulose and lignin, alongside minor components such as starch, ash, extractives, water, minerals, silica and proteins (Eom *et al*., 2015b). Fibrous crystalline cellulose forms the core of the lignocellulosic complex, amorphous hemicellulose is found both between microfibrils and macrofibrils of cellulose, and lignin fills interfibrous areas and provides structural support to the lignocellulose matrices (Akhlisah *et al*., 2021).

Cellulose represents the major component of the cell wall of a plant. As a highly stable homopolymer, it consists of linear chains of D-glucose molecules linked by β-1,4-glycosidic bonds with a degree of polymerisation of up to 15 000 (Bajpai, 2016). The even distribution of hydroxides on both sides of its

**TABLE 4. COMPOSTION OF LIGNOCELLULOSIC BIOMASS FOR SUCCINIC ACID PRODUCTION**

| Biomass | Composition (%) | | | References |
|---|---|---|---|---|
| | Cellulose | Hemicellulose | Lignin | |
| Sugarcane bagasse | 41.00 | 24.00 | 24.00 | Chen *et al*. (2021) |
| Corn fibre | 21.00 | 27.50 | 0.80 | Vallecilla-Yepez *et al*. (2021) |
| Blue agave | 42.00 | 22.00 | 18.00 | Corona-Gonzalez *et al*. (2016) |
| Napier grass | 41.18 | 30.15 | 6.68 | Sriariyanun *et al*. (2017) |
| Citrus peel | 33.98 | 9.99 | 6.93 | Rivas-Cantu *et al*. (2013) |
| Industrial hemp | 40.10 | 16.00 | 14.80 | Kuglarz and Grübel (2018) |

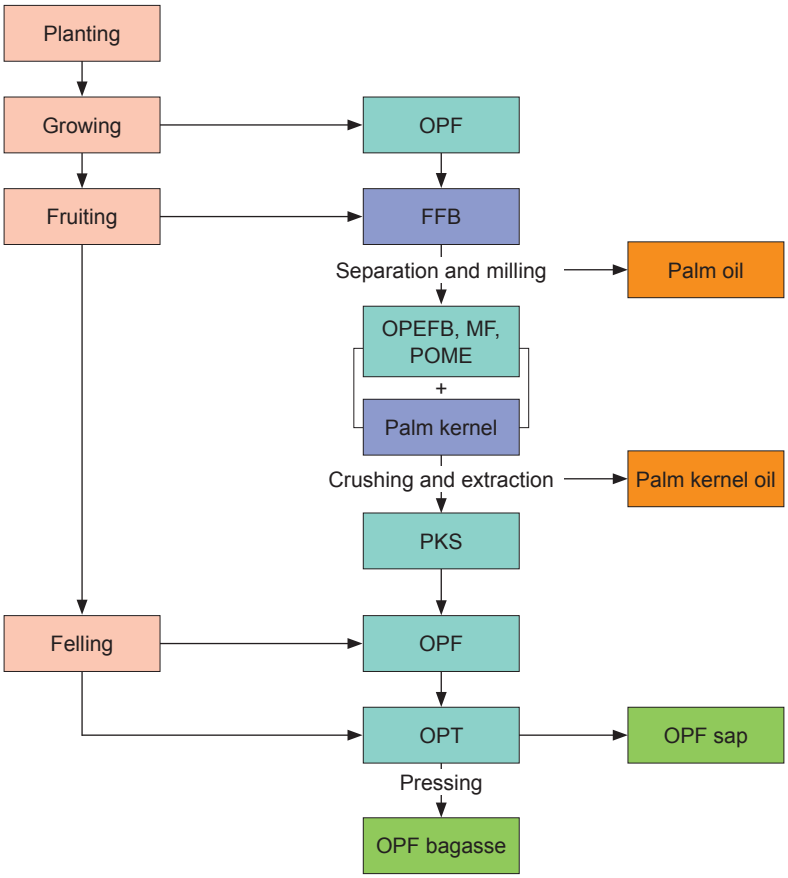



*Figure 4. Biomass produced during the lifetime of an oil palm. Light blue: Biomass residues; yellow: commercialised products, green: biomass of this study. OPF - oil palm frond; FFB - fresh fruit bunch; OPEFB - oil palm empty fruit bunch; MF - mesocarp fibre; POME - palm oil mill effluent; PKS - palm kernel shell; OPT - oil palm trunk. Adapted from Dirkes* et al.*, 2021.*

monomers allows for the formation of intra- and inter-molecular hydrogen bonds. Given such strong hydrogen bonding, cellulose is not only insoluble in most common solvents, including water, but also resistant to hydrolysis (Taherzadeh and Karimi, 2007a).

Hemicellulose, on the other hand, is a highly-branched polysaccharide. As a heteropolymer, it is structurally cross-linked with cellulose and lignin through hydrogen bonds with a degree of polymerisation of fewer than 200 (Asif, 2009). Hemicellulose is composed of a wide range of sugars including pentoses (such as xylose and arabinose) and hexoses (such as glucose, galactose, mannose, glucuronic acid, and galacturonic acid). Other deoxyhexose sugars such as rhamnose and fucose may also be found in trace amounts. The hydroxyl groups of these sugars are sometimes partially substituted with acetyl groups (Bajpai, 2016).

Lastly, lignin is the most abundant naturally occurring non-saccharide compound and a highly cross-linked aromatic heteropolymer distinctively different from other macromolecules of lignocellulosic biomass. Its predominant building unit, phenylpropane, comprises monolignols arranged in a three-dimensional (3D) amorphous structure. The most common monolignols, as precursors, are p-coumaryl (4-hydroxycinnamyl), coniferyl (3-methoxy-4-hydroxycinnamyl), and sinapyl (3,5-dimethoxy-4-hydroxycinnamyl) alcohols (Anwar *et al.*, 2014). These precursors are incorporated into the phenylpropane oligomers and labelled as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, which collectively form the lignin structure (Fisher and Fong, 2014). It is noteworthy that lignin acts as a natural binding agent for both cellulose and hemicellulose in a 3D network, offering structural and mechanical support (Raud *et al.*, 2016). Hence, elevated lignin content is undesirable for bioprocessing as a much-reduced bioavailability of the substrate is to be anticipated due to the resistance of lignin to water and enzymes.

The amounts of carbohydrates and lignin depend on the type of biomass. Generally for oil palm biomass, the constituents are cellulose (44%-54%), hemicellulose (21%-34%), and lignin (10%-25%) (Akhlisah *et al.*, 2021; Diyanilla *et al.*, 2020; Loh, 2017). The high carbohydrate content of this type of biomass (>55%) offers very promising potential in bioconversion technologies (Ishak *et al.*, 2019; Pratiwi *et al.*, 2018).

**Biomass of Focus in This Study - OPT**

The economic lifespan of an oil palm tree ranges from 20 to 30 years, and the trees are usually replanted every 25 years to sustain oil productivity. Previously, the United Nations Environment Programme (UNEP, 2012) projected that about 128 296 ha of oil palms in Malaysia will be potential for OPT harvesting in the next decade. The distribution of potential areas for replanting is shown in *Figure 5*. In 2020, the availability of OPT was estimated to be 9.67 million tonnes (based on 75.5 t $ha^{-1}$ of dry matter) from the total replanting area of 128 088 ha, covering Peninsular Malaysia (36%), Sabah (51%), and Sarawak (13%).

At present, OPT is mostly used as raw material in plywood manufacturing. The limited application has created a surplus, which is cut and shredded into pieces, spread on plantation sites and left to decompose for nutrient recycling. This conventional practice has been adopted since 1985 when open burning is prohibited entirely in Malaysia (Noor, 2003). Given the high lignin content and carbon to nitrogen (C:N) ratio (Loh *et al*., 2013), it may take approximately two years for the biomass to completely decompose (Murai *et al*., 2009), culminating in the rise of greenhouse gas (GHG) levels. Additionally, given the high sugar and moisture contents, the outer parts of OPT are susceptible to insect pests such as rhinoceros beetles, one of the most serious pests to oil palms, while the inner parts are attacked by white-rot fungi (Bahmani *et al*., 2016). It also serves as a carbon source for *Ganoderma* sp. to initiate basal stem rot infection, a major problem in the oil palm industry (Naidu *et al*., 2020). The presence of a large amount of OPT residue disrupts replanting and results in adverse effects on soil functioning and subsequent field maintenance (Uke *et al*., 2021). Under the zero-burning policy, disposal of OPT residue remains a challenge and thus, an urgent need for practical uses.

Given the lignocellulosic nature of OPT, there is a vast possibility that biomass can be harnessed for value-added products. Despite the various R&D options made available for adoption and applications, only a few products/technologies have reached either pilot or commercial stage, *e.g.*, plywood, lumber, flooring, microcrystalline cellulose (MCC), panel products (medium-density fibreboards, particle boards, and cement boards), and animal feed pellets and fuel pellets (Hoong *et al*., 2012; Nordin *et al*., 2004). Some of the inherit characteristics, such as high moisture and fibrous, would have hindered the application of OPT without pretreatment for wood-based industries (Dirkes *et al*., 2021). The prospects of OPT are huge considering now that R&D emphasises its conversion into sugar, chemical derivatives, bioethanol, bioplastic, pulp and paper and dietary supplements (Murata *et al*., 2013). OPT is a unique biomass feedstock for bioconversion. An understanding of its chemical composition is essential to fully tap the benefits.

***Characteristics of sap pressed from OPT***. The moisture content in OPT amounting to 74–80 wt.% of the whole trunk is indicative of a substantial amount of sap (juice) (Bukhari *et al*., 2019b). The sugar composition of pressed OPT sap has been analysed using high performance liquid chromatography (HPLC) (*Table 5*).

The amount of sugars in OPT sap varies between 40 and 141 g $L^{-1}$, with glucose being identified as the most dominant free sugar (*Table 5*). Sugar bioavailability differs within the trunk itself, thus, it is pertinent to determine which part of OPT should be valorised to extract the most sugars. For example, Lokesh *et al.* (2012) used the central section, Komonkiat and Cheirsilp (2013) combined

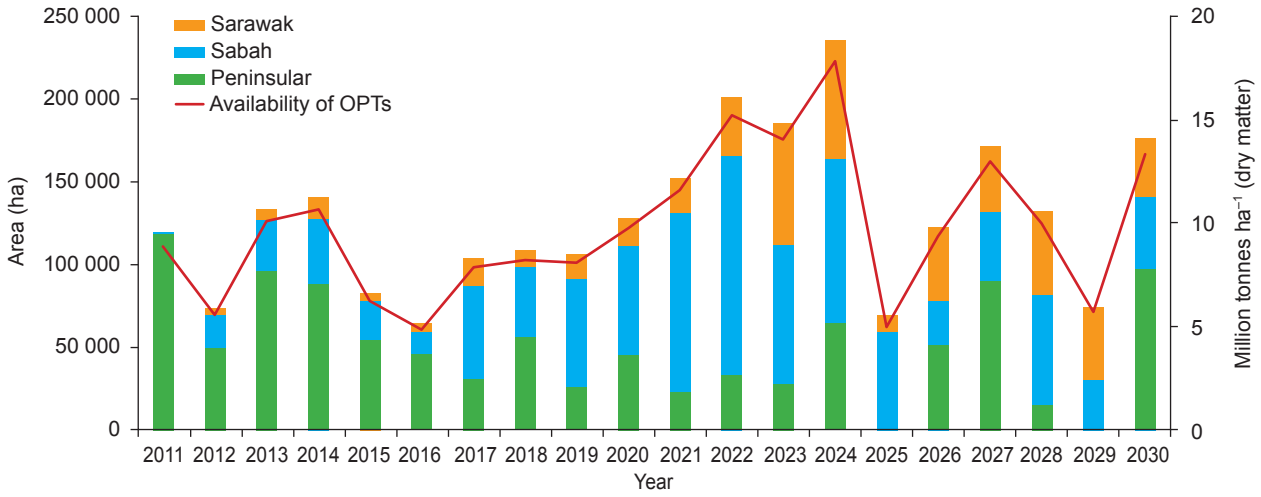



*Figure 5. The potential dry weight of OPT deriving from the replanting sites. The area of a plantation site is estimated by subtracting the total area after 25 years from the total area of the subsequent year. Adapted from UNEP, 2012.*

**TABLE 5. COMPOSITION OF OIL PALM TRUNK SAP**

| Segmentation | Concentration of sugar (g $L^{-1}$) | | | | | References |
|---|---|---|---|---|---|---|
| | Sucrose | Glucose | Fructose | Others* | Total | |
| Inner (central core) | 33.28 | 16.01 | 1.55 | 4.58 | 55.42 | Lokesh *et al*. (2012) |
| - | 13.94 | 42.97 | 4.03 | 3.37 | 64.31 | Norhazimah *et al.* (2013) |
| Combined whole | 9.30 | 16.06 | 7.48 | 7.29 | 40.13 | Komonkiat and Cheirsilp (2013) |
| Middle (7 cm disk) | 31.90 | 67.40 | 38.50 | 3.50 | 141.30 | Kunasundari *et al.* (2017) |
| Combined whole | 10.10 | 26.73 | 5.89 | n.d. | 42.72 | |
| Inner (top 2) | 9.05 | 27.71 | 7.65 | n.d. | 44.41 | |
| Inner (top 1) | 14.81 | 33.12 | 4.07 | n.d. | 52.00 | |
| Inner (bottom 2) | 9.06 | 32.10 | 7.47 | n.d. | 48.63 | |
| Inner (bottom 1) | 9.16 | 28.87 | 4.19 | n.d. | 42.22 | Bukhari *et al*. (2019b) |
| Middle (top 2) | 10.24 | 18.67 | 8.02 | n.d. | 36.93 | |
| Middle (top 1) | 8.65 | 27.19 | 6.92 | n.d. | 42.76 | |
| Middle (bottom 2) | 8.70 | 20.61 | 4.95 | n.d. | 34.26 | |
| Middle (bottom 1) | 11.14 | 25.55 | 3.86 | n.d. | 40.55 | |

Note: n.d. - not determined; * - including galactose, maltose, xylose, arabinose and inositol.

all parts while Kunasundari *et al*. (2017) extracted a 7-cm disk from the middle of the trunk. Different heights and widths of the OPT were discovered to hold varying sugar compositions as well (Bukhari *et al*., 2019b) (*Table 5*). The sugar content increases from; 1) the outer to the inner zone, and 2) the lower to the upper of the OPT. The sap contains a higher sugar concentration in the inner zone (core area), where the parenchyma content is higher than in the outer area (Bukhari *et al*., 2019b). Regardless of the type of sugars, the OPT sap is undoubtedly rich in readily soluble fermentable sugars (mostly hexoses), which would be favoured by microorganisms and can be directly used as fermentative substrates.

Besides, OPT sap contains an abundance of free amino acids, vitamins, and macro- and micro-minerals. These are nutrients favourable for microbial fermentations (Bukhari *et al*., 2019b; Komonkiat and Cheirsilp, 2013; Kosugi *et al*., 2010). Various types of amino acids (*i.e.*, aspartic acid, lysine, arginine, glutamic acid, alanine, proline, methionine, *etc*.) and vitamins (*i.e.*, biotin, folic acid, cobalamin, niacin, pantothenic acid, pyridoxine, thiamine, *etc*.) are available in OPT sap (Bukhari *et al*., 2019b). As such, OPT sap can be considered an ideal feedstock for SA fermentation by *A. succinogenes*. Other bio-products that have been successfully biosynthesised from OPT sap are summarised in *Table 6*.

Unlike cellulosic woody or starchy materials, the use of pressed OPT sap is practically more economical, not only because no complex and expensive pretreatments are required, but also because it contains all the requisite sugars and nutrients for cell growth and product formation. In the biosynthesis of commodity chemicals, the cost of the substrate or the medium is a limiting and crucial factor for industrial-scale implementation. Cost-effective exploitation of OPT sap could help to revolutionise the bio-based industry. As calculated theoretically, approximately 123.2 t of sap could be attained from a ha of oil palm plantation; with a hypothetical sugar content of 4%, about 5 t of sugar could be produced (Dirkes *et al*., 2021). This represents nearly one-quarter of sugar beet yield (24 t sugar per ha), where cultivation is specific for sugar production (Hoffmann and Kenter, 2018). Accordingly, the sugary OPT sap offers viable potential for future implementation in the biotechnological fermentation of SA.

***Characteristics of residual OPT bagasse***. Alongside sap extraction, OPT bagasse (*i.e.*, fibre remaining after OPT pressing) likewise contains copious cellulosic materials that can be hydrolysed to produce monomeric sugars for subsequent fermentation (Bukhari *et al*., 2019a). Its high total structural carbohydrate content in the range of 57%-73% is ideal and favourable for microbial SA production (*Table 7*).

## ESSENTIAL PROCESSES IN BIOCATALYSIS OF SA

### Pretreatment of Lignocellulosic Materials

A notable barrier to recovering valuable materials from lignocellulosic bagasse is the degradation-resistant structure due to the presence of ester- and ether-based cross-linkages between the polysaccharides (cellulose and hemicellulose) and lignin. A pretreatment is therefore necessary to alter

**TABLE 6. BIOTECHNOLOGICAL PRODUCTS PRODUCED FROM OPT SAP**

| Bio-product | Microorganism | Conditions | Titer ($g\ L^{-1}$) | Yield ($g\ g^{-1}$) | References |
|---|---|---|---|---|---|
| Ethanol | *Saccharomyces cerevisiae* ATCC 24860 | 30°C, pH 4.0 and 150 rpm for 24 hr | 47.50 | 0.50 | Adela and Loh (2015) |
| Ethanol | *Saccharomyces cerevisiae* | 32°C, pH 6.0 and 170 rpm for 24 hr | - | 0.55 | Shahirah *et al*. (2015) |
| Ethanol | *Saccharomyces cerevisiae* CCT0762 | 30°C, pH 5.5 and 150 rpm for 24 hr | 29.45 | 0.39 | Mohd Zakria *et al*. (2017) |
| Ethanol | *Kluyveromyces marxianus* ATCC 46537 | 30°C, pH 5.5 and 150 rpm for 24 hr | 29.61 | 0.39 | |
| Butanol | *Clostridium acetobutylicum* DSM 1731 | 37°C, pH 6.0 and 120 rpm | 7.29 | 0.36 | Komonkiat and Cheirsilp (2013) |
| Butanol | *Clostridium beijerinckii* JCM 1390 | | 2.29 | 0.22 | |
| Lactic acid | *Bacillus coagulans* strain 191 | 55°C, pH 6.0 and 200 rpm | 53.9 | 0.88 | Kunasundari *et al*. (2017) |
| Polyhydroxyalkanoate | *Bacillus megaterium* | 30°C, and 250 rpm for 16 hr | 3.28 | - | Lokesh *et al.* (2012) |
| Lipids | *Lipomyces starkeyi* NBRC 10381 | 30°C, pH 6.0 and 190 rpm for 96 hr | 64.40 | 0.19 | Juanssilfero *et al.* (2019) |
| Hydrogen | *Clostridium beijerinckii* | 30°C, for 24 hr | 1 973 mL $L^{-1}$ | | Noparat *et al.* (2012) |

**TABLE 7. COMPOSITION OF OIL PALM TRUNK FIBRE (BAGASSE)**

| Composition (%) | | | | References |
|---|---|---|---|---|
| Cellulose | Hemicellulose | Total carbohydrates | Lignin | |
| 38.85 | 23.84 | 62.69 | 20.36 | Rattanaporn *et al*. (2018) |
| 38.10 | 23.10 | 61.20 | 21.40 | Noparat *et al*. (2017) |
| 33.21 | 25.01 | 58.22 | 25.34 | Pratiwi *et al*. (2018) |
| 40.83 | 32.17 | 73.00 | 21.64 | Tareen *et al*. (2021a) |
| 33.90 | 31.90 | 65.80 | n.d. | Ishak *et al*. (2019) |
| 30.86 | 25.84 | 56.70 | 24.29 | Bukhari *et al.* (2019a) |

Note: n.d. - not determined.

such structural and compositional impediments to hydrolysis and subsequent degradation processes, to improve the digestibility and rate of enzymatic saccharification, and increase yields of targeted products (Luthfi *et al.*, 2017b). Pretreatment is the first step in bioconversion before saccharification. It overcomes biomass recalcitrance, which is caused by factors such as high lignin content, protection of cellulose by lignin, sheathing by hemicellulose, high cellulose crystallinity, degree of polymerisation, the low accessible surface area of cellulose and strong fibre strength (Akhlisah *et al.*, 2021). Pretreatment can be classified into four categories: Physical, physicochemical, chemical and biological (*Table 8*).

It is important to optimise the deployed pretreatment to match the chemical composition and internal structure of the corresponding biomass. Similarly, pretreatment should be prudently selected based on the targeted final products. In general, pretreatment aims to remove lignin, decrease cellulose crystallinity, increase accessible surface areas, and enhance the porosity of the materials, to facilitate subsequent enzymatic saccharification. Inefficient pretreatment leads to not only difficulties in hydrolysing or saccharifying the resultant residues by hydrolytic enzymes, but also the production of a considerable amount of toxic compounds that could possibly inhibit subsequent microbial fermentation. Therefore, pretreatment critically governs the economics of biomass conversion.

Typically, an effective pretreatment method must be able to produce a highly digestible cellulose material that can be easily hydrolysed at a low enzyme loading rate, minimise feedstock preparation and pre-processing prior to pretreatment, maximise recovery of all carbohydrates in usable forms, produce none or trace amounts of lignin-degrading products and other fermentation inhibitors and finally lower energy demand or allow reuse of energy. This will greatly enhance operational costs with low capital investment (Galbe and Zacchi, 2012; Shuai *et al.*, 2010).

**TABLE 8. ADVANTAGES AND LIMITATIONS OF PRETREATMENT METHODS FOR OIL PALM TRUNK**

| Pretreatment method | Advantages | Limitations |
|---|---|---|
| **Physical** | | |
| Mechanical/milling/ grinding | • Reduces cellulose crystallinity<br>• Increases surface area | • High power and energy consumption |
| **Physicochemical** | | |
| Steam explosion | • Causes lignin transformation<br>• Partial hemicellulose solubilisation<br>• Higher yield of glucose and hemicellulose in the two-step method<br>• Industrially developed | • Generation of toxic compounds<br>• Energy-intensive |
| Ammonia based (AFEX, ARP, SAA, LLA, LMAA, PAH) | • Increases accessible surface area<br>• Low formation of inhibitors<br>• Easy to recover and recycle | • Not efficient for raw materials with high lignin content<br>• High cost of a large amount of ammonia<br>• High energy required to maintain process temperature |
| $CO_2$ explosion | • Efficient removal of lignin<br>• Increases accessible surface area<br>• Cost-effective<br>• Does not imply the generation of toxic compounds | • Does not affect lignin and hemicellulose<br>• Very high-pressure requirements |
| Wet oxidation | • Efficient removal of lignin<br>• Low formation of inhibitors<br>• Minimises the energy demand (exothermic) | • High cost of oxygen and alkaline catalyst |
| Hydrothermal/LHW | • Cost-effective<br>• Low formation of inhibitors | • High temperature demands high energy |
| Sulfite-based | • Depolymerising cellulose<br>• Efficient removal of hemicellulose<br>• Sulfonation of lignin | • High temperature demands high energy |
| **Chemical** | | |
| Ozonolysis | • Selective lignin degradation<br>• Does not imply the generation of toxic compounds<br>• Operation at ambient temperature and pressure | • High cost due to a large amount of ozone needed |
| Organosolv | • Causes lignin and hemicellulose hydrolysis | • High cost<br>• Requirement of solvent removal from the system |
| Alkaline | • Removal of lignin and hemicellulose, increase accessible surface area<br>• Low formation of inhibitors | • Long residence time<br>• Less lignin removal<br>• Irrecoverable salts |
| Concentrated acid | • High glucose yield<br>• Ambient temperatures | • High cost of acid and need to be recovered<br>• Reactor corrosion problems<br>• Formation of inhibitors |
| Diluted acid | • Fewer corrosion problems than concentrated acid<br>• Solubilises hemicellulose<br>• Alters lignin structure<br>• Pre-hydrolysing cellulose | • Generation of degradation products<br>• Requirement for neutralisation |
| Deep eutectic solvent | • Low formation of inhibitors<br>• Dissolution of cellulose | • Pre-commercial |
| Ionic liquids | • Minimum degradation of desired products<br>• Operation at low temperature | • Expensive<br>• Requirement of washing before reuse |
| **Biological** | | |
| Fungi, bacteria, archaea | • Degrades lignin and hemicellulose<br>• Mild environmental conditions<br>• Low capital cost<br>• Low energy consumption | • Very low rate of hydrolysis<br>• Loss of carbohydrates as consumedby the microbes<br>• Contamination problems |

Note: AFEX - ammonia fibre explosion/expansion; ARP - ammonia recycle percolation; SAA - soaking in aqueous ammonia; LLA - low-liquid ammonia; LMAA - low-moisture anhydrase ammonia; PAH - pressurised ammonium hydroxide; LHW - liquid hot water. Adapted from Alvira *et al*., 2010; Taherzadeh and Karimi, 2007b; Wang *et al*., 2009.

Exploration work on different pretreatment methods is somewhat limited for OPT (*Table 9*). Existing literature has highlighted physicochemical (hydrotreatment) (Eom *et al*., 2015a) and chemical pretreatment; including dilute acid (Noparat *et al*., 2015; Rattanaporn *et al*., 2018), sulfite (Noparat *et al*., 2017), and deep eutectic solvents (DES) (Zulkefli *et al*., 2017). Such pretreatments enhance the recovery and reveal the available fermentable sugars in OPT that could be referenced in SA production.

As outlined in *Table 9*, hydrothermal pretreatment or liquid hot water/autohydrolysis is one of the most promising among the low-cost pretreatment options, given the use of only water as the reaction medium at temperatures ranging from 160°C to 200°C. The identified optimum conditions in pre-treating OPT is 180°C for 30 min, which could yield the maximum amount of hemicellulosic sugars, with low concentrations of both furfural and HMF (Eom *et al*., 2015b). The hemicellulosic fractions in the prehydrolysates are essential to increase the initial concentration of fermentable sugars. Furthermore, the use of a mixture of cellulase, xylanase and cellobiase during hydrothermal pretreatment of a substrate could encourage the recovery of total sugars (Eom *et al*., 2015a; 2015b). A nearly twofold increase is possible for the direct application of pre-treated whole slurry as a substrate for enzymatic saccharification (sugars recovered: 43.5 g 100 $g^{-1}$), compared to washed pre-treated solids (sugars recovered: 23.3 g 100 $g^{-1}$) (*Table 9*).

Sulfite pretreatment of biomass feedstock (SPORL) consists of a short span of chemical treatment followed by mechanical size reduction (fiberisation). Active reagents include sulfite ($SO_3^{-2}$), bisulfite ($HSO_3^{-1}$), or their combination. A solution of sulfite salt (*e.g*., sodium, magnesium, ammonia, potassium or calcium) reacts first with the feedstock at temperatures ranging from 160°C-190°C and pH 2-4 for about 30 min. It is then fiberised with a disk mill to generate fibrous substrates for subsequent enzymatic saccharification and fermentation. SPORL produces readily-digestible substrates and ensures high recovery of hemicellulosic sugars with only a trace amount of fermentation inhibitors (Shuai *et al*., 2010). Furthermore, sulfonation increases the hydrophilicity of lignin, thus reducing the negative impacts on enzymatic saccharification. OPT pre-treated with SPORL allows >90% saccharification yields in 48 hr with a total fermentable sugar recovery of 62.5% (Noparat *et al*., 2017).

DESs, a potentially green alternative, have been used to pre-treat OPT. Given their similar properties with ionic liquids, DESs have attracted considerable interest for potential cost reduction in bioprocessing. DES composes of two or three charged components with high melting points (New *et al*., 2022). Interestingly, when combined, the components form a mixture with a depressed melting point, making it available as a liquid at ambient temperature (Zulkefli *et al*., 2017). Such a solvent can be easily prepared through atomic economic procedures. With its excellent compatibility and

**TABLE 9. PRETREATMENT METHODS OF OIL PALM TRUNKS IN THE EXISTING LITERATURE**

| Pretreatment method | Conditions | Saccharification | Yield of sugar (g 100 $g^{-1}$) | | References |
|---|---|---|---|---|---|
| | | | Xylose | Glucose | |
| Hydrothermal | 160°C -200°C, 30 min | Cellulase + xylanase + cellobiase | 17.8 | 23.3 | Eom *et al*. (2015b) |
| Hydrothermal | 180°C, 30 min | Cellulase + xylanase | 15.4 | 43.5 | Eom *et al*. (2015a) |
| Sulphuric acid | 3% $H_2SO_4$, 180°C, 40 min | Cellulase + β-glucosidase | - | 80.0%* | Noparat *et al.* (2015) |
| SPORL | 7% $H_2SO_4$ + 6% $Na_2SO_3$, 190°C, 30 min | Cellulase + β-glucosidase | 26.5 | 22.4 | Noparat *et al.* (2017) |
| Deep eutectic solvent | EAC: EG, 100°C, 48 hr | Cellulase + cellobiase | - | 74%* | Zulkefli *et al.* (2017) |
| Oxalic acid (OA) | 15% OA; 100°C, 60 min | Cellulase + cellobiase | - | 14.4 | Rattanaporn *et al.* (2018) |
| OA | 1% OA; 120°C, 180 min | Cellulase | 24.3 | 19.0 | Bukhari *et al*. (2021b) |
| Alkaline hydrogen peroxide | 30% $H_2O_2$, 70°C, 30 min | Cellulase | - | 59.8%* | Tareen *et al*. (2020) |
| Steam explosion + alkaline | 210°C, 4 min + 15% NaOH, 80°C, 90 min | Cellulase + β-glucosidase | - | 88.5%* | Tareen *et al.* (2021a) |

Note: SPORL - sulfite pretreatment to overcome recalcitrance of lignocellulose; EAC: EG - etylammonium chloride: ethylene glycol; * - cellulose-to-glucose conversion.

biodegradability, the presence of a DES ensures the stability of enzymatic activities over an extended period (Thi and Lee, 2019). Among the five DESs screened: choline chloride:glycerol (ChCl:Gly), choline chloride:ethylene glycol (ChCl:EG), choline chloride:urea (ChCl:U), ethylammonium chloride:glycerol (EAC:Gly) and ethylammonium chloride:ethylene glycol (EAC:EG), EAC:EG was found to be the most efficient in OPT pretreatment; having ability to dissolve 58% OPT upon heating at 100°C for 48 hr (Zulkefli *et al*., 2017).

More recently, alkaline hydrogen peroxide (AHP) has been optimised for OPT pretreatment with 3% $H_2O_2$ g $g^{-1}$ of biomass at 70°C for 30 min optimum conditions. Up to 50% lignin can be removed, resulting in 46.2 g $L^{-1}$ glucose with 59.8% enzymatic digestibility in 96 hr (Tareen *et al*., 2020). A more advanced OPT pretreatment under steam explosion conditions (210°C for 4 min) followed by alkaline extraction manages to yield more glucose (71.6 g $L^{-1}$). In optimising alkaline extraction, the Taguchi three-factor design was used to obtain the optimum conditions: 15% NaOH at 90°C for 60 min (Tareen *et al*., 2021b). Through this, the highest cellulose conversion of 88.5% can be afforded following 96-hr saccharification with combined enzymes (Tareen *et al*., 2021b).

Although many pretreatment methods are available, so far only a few are deemed feasible to be industrialised based on environmental and economic considerations (Akhtar *et al*., 2014). Its performance lies with the susceptibility of the glucosidic bonds between hemicellulose and cellulose to acid. Hydronium ions originated from acid catalysts that break the long cellulose and hemicellulose chains down into monomeric sugars (Lloyd and Wyman, 2005). Dilute acid pretreatment gives a high hemicellulose recovery in the liquid fraction while leaving most of the cellulose in the solid residues for subsequent enzymatic saccharification (Noparat *et al*., 2015). They suggested using 3% $H_2SO_4$ at 180°C for 40 min to achieve the highest glucose recovery with ~80% conversion yield. The operating conditions, including reaction time, temperature, and acid concentration are reportedly crucial to govern the treatment efficiency (Zhang *et al*., 2013). Due to the complexity of the lignocellulosic matrices, it is improbable for such pretreatment to break all polysaccharide-lignin linkages, and thus not all monomeric sugars are expected to be recovered.

Pretreatment with inorganic acids such as $H_2SO_4$ has been preferred due to their high catalytic performance and low costs, though disadvantageous in terms of equipment corrosion, low reaction selectivity, and formation of inhibitory by-products (Lee and Jeffries, 2011). To overcome these, organic acids have recently emerged as a more environmentally-sound alternative. Various organic acids have been investigated in pre-treating lignocellulosic biomass: Maleic acid for wheat straw (Barisik *et al*., 2016) and rice straw (Jung *et al*., 2015), oxalic acid for cassava stem (Sivamani and Baskar, 2018) and yellow poplar (Jeong and Lee, 2016). Citric acid, fumaric acid, and lactic acid have also been explored for biomass pretreatment (Sahu and Pramanik, 2018; Tang *et al*., 2018). To date, acetic acid, citric acid (Rattanaporn *et al*., 2018), maleic acid (Jung *et al*., 2014) and oxalic acid (Bukhari *et al*., 2021c) have been deployed in pre-treating OPT bagasse, given promising hydrolysates for subsequent fermentation. Reasonable operational conditions for OPT pretreatment, dilute (mild) acid concentration in particular, need to be enquired about to find the best possible route in OPT bioconversion.

**Enzymatic Saccharification of Pre-treated Materials**

Enzymatic saccharification is the subsequent step after the pretreatment of lignocellulosic biomass to yield fermentable sugars from mainly the released celluloses. Established as the most suitable hydrolysis method, the use of enzymes has demonstrated advantages over concentrated acid hydrolysis such as the relatively mild process conditions, high yields, and absence of corrosion (Duff and Murray, 1996). Enzymatic saccharification can also be performed to measure improvement in sugar conversion, which relates to pretreatment efficiency. In the saccharification of lignocellulosic biomass for the conversion of carbohydrates into sugars, three primary types of cellulases are responsible, as shown in *Table 10*.

**TABLE 10. TYPES AND ACTION OF CELLULASES INVOLVED IN BIOMASS SACCHARIFICATION**

| Enzyme | EC number | Mode of action | References |
|---|---|---|---|
| Endoglucanases (EG, endo-1,4-β-D-glucanohydrolases) | EC 3.2.1.4 | Attack regions of the internal amorphous sites amidst cellulose chains, generating oligosaccharides of various chain lengths | Kumar *et al.* (2008) |
| Exoglucanases (CBH, 1,4-β-D-glucan glucanohydrolases and 1-β-D-glucan cellobiohydrolases ) | EC 3.2.1.91 | Act on the reducing and non-reducing ends of cellulose chains, releasing either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as the major products | Sun and Cheng (2002) |
| β-glucosidase (β-glucosidase glucohydrolases) | EC 3.2.1.21 | Hydrolyse cellodextrin and cellobiose to glucose | Teugjas and Väljamäe (2013) |

Commercial enzymatic cocktails contain diverse enzymes of undisclosed specifications (Kubicek and Kubicek, 2016). During saccharification, glycosidic linkages in hemicellulose molecules are broken, including random cleavages of internal bonds by endoxylanase (Maitan-Alfenas *et al*., 2015). Additionally, some ancillary enzymes could also attack hemicellulose such as glucuronidase, acetylesterase, β-xylosidase, glucomannanase, and gatactomannanase (Duff and Murray, 1996). In general, as cellulose is predominantly found in lignocellulosic biomass, glucose will be the most abundant hexose released in the hydrolysate after saccharification.

The cost of cellulases accounts for a substantial proportion of the total processing costs in the bioconversion of lignocellulosic materials. Cost-saving considerations must be put in place to provide the impetus to reducing enzymatic loadings and recovering used enzymes (Zabed *et al*., 2017). Potential enhancement and detailed mechanism of enzymatic saccharification remain a research focus, where R&D examples are copious in the literature. Endoxylanase for hydrolysing hemicellulose into monomeric sugars (mostly xylose) could be combined with cellulase to boost the hydrolytic performance (Luthfi *et al*., 2016). Additionally, supplementation of cellulase with a small amount of cellobiase (7:1 ratio) during enzymatic saccharification of pre-treated OPEFB was found to improve glucose production by 51%. In another comparative study, the yield of 31.4 g $L^{-1}$ glucose from a combined enzymatic process was higher than that of 20.6 g $L^{-1}$ from cellulase alone (Akhtar and Idris, 2017). Synergism between cellulase (CTec2®) and endoxylanase (HTec2®) has also been reported, as reflected by the optimum sugar yield of 54.2% g $g^{-1}$ of raw OPF (Luthfi *et al*., 2018).

The use of additives such as surface-active agents (surfactants), proteins, and polymers represents another interesting approach to optimise enzymatic saccharification. The potential of such additives has been demonstrated for cost-effectiveness in enhancing cellulose conversion yield, decreasing enzyme loading and reducing hydrolysis time (Monschein *et al*., 2014). Two-pronged mechanisms have been evidenced by their action: The ability to adsorb to lignin, thus avoiding unspecific binding of enzymes to lignin, and modify the substrates, stabilise the enzymes plus improve enzymatic activities (Kristensen *et al*., 2007; Lin *et al*., 2017). However, only a few studies have hitherto investigated the effects of surfactants on the enzymatic saccharification of oil palm biomass. Zain *et al*. (2018) reported that an optimum loading of 1.31% (v $v^{-1}$) of Triton X-100 showed a conversion yield of 88% from the saccharified alkaline-pretreated OPF. Besides Triton X-100, Tween 80 at 0.25% (v $v^{-1}$) was found efficient, with a 23% higher yield in hydrolysing nitric acid-pre-treated OPEFB, compared to the control (Kamsani *et al*., 2018). The effect of surfactants could be potentially exploited to reduce saccharification time or enzyme loading, enabling a reduction in enzyme cost for lignocellulosic bioconversion. In future, the application of surfactants might become economically feasible, especially for high-solid loadings (Monschein *et al*., 2014).

**Fermentation of Lignocellulosic Hydrolysates**

Fermentation refers to microbial metabolism of simple sugars into targeted bio-products, in three different manners, *i.e.*, batch, fed-batch, and continuous mode. For the batch mode, a closed culture system is used, in which the fermentation medium is supplemented with essential ingredients to promote microbial growth and product formation. As the simplest mode, it requires only acids or bases to maintain the pH; no other complicated additives are needed (Zabed *et al*., 2017). The repeated-batch mode refers to the process in which an amount of fermented culture is removed from the system at specific intervals while the remaining portion is used as the inoculum for the next culture (Luthfi *et al*., 2017a). Under the fed-batch mode, the batch and continuous modes are combined for the targeted production. It represents a batch culture system into which substrates and other ingredients are fed, without any removal of the fermented broth (Zabed *et al*., 2017). Lastly, the continuous mode involves the sequential input of the necessary ingredients and the ongoing removal of products from the fermentation vessel. It is widely used in SA production, often contributing to productivity higher than the batch and fed-batch modes (Luthfi *et al*., 2018).

As with previously researched bioethanol production (Kassim *et al*., 2022), the bioconversion of lignocellulose into SA can also be performed through separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP) (Lu *et al*., 2021). *Figure 6* presents the conventional steps in SA production. SHF includes two sequential steps, in which lignocellulose is first enzymatically hydrolysed, and the resultant sugars are then fermented into SA. Its notable advantage is that both steps can be performed (separately) under optimum temperatures, as enzymatic saccharification and microbial fermentation usually exhibit different thermal requirements (Devarapalli and Atiyeh, 2015).

Conversely, SSF integrates those two steps in the same bioreactor (as a one-step process). Compared with SHF, SSF demonstrates more advantages, not only in reducing the amounts of supplemented hydrolytic enzymes but also in increasing product yields. Moreover, enzymatic inhibition arising from

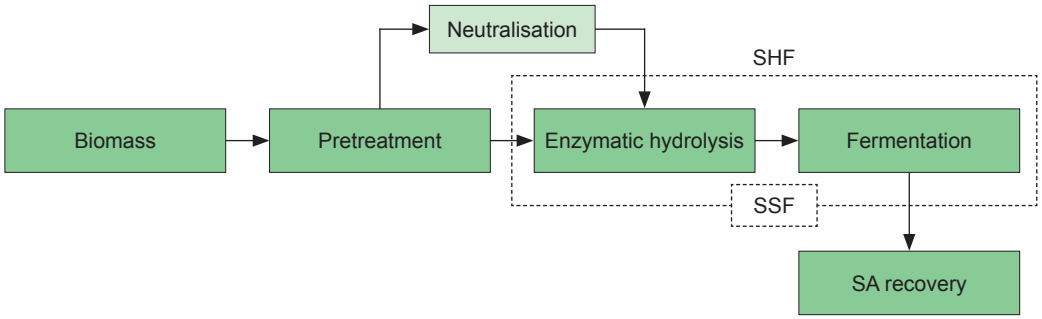



*Figure 6. General schematic for succinic acid production from lignocellulosic biomass. Adapted from Lu* et al.*, 2021.*

the resultant by-products can partly be addressed through SSF. Additionally, the combination of saccharification and fermentation in the same reactor incurs lower equipment costs (Valles *et al*., 2021). To optimise SA production, different fermentation strategies, modes and configurations have been examined. Additionally, factors affecting the fermentative performance, including carbon sources, nitrogen sources, pH regulators, and $CO_2$ availability have also been investigated (Putri *et al*., 2020).

## TECHNOLOGICAL ADVANCEMENT AND PROCESS ECONOMICS

SA production has recently advanced greatly in fermentative technologies targeting high yield per unit of biomass. Attempts to improve process economics must take into consideration the various aspects of bioconversion in their entirety. For example, effective pretreatment is required to improve the enzymatic digestibility of biomass, which ultimately maximises the availability of fermentable sugars for conversion into SA. Against this background, *Table 11* lists current R&D endeavours mainly on the productivity of unit processes by optimising process parameters to gain economic feasibility.

### Exploitation of Renewable Biomass and Selection of Operating Configurations

Investigations have been underway intending to search for new biomass substrates as a viable starting material for SA production. Carvalho *et al*. (2016) demonstrated for the first time that raw carob pods were efficient for SA production, with a favourable sugar yield recovery of 50% (w $w^{-1}$). A volumetric productivity of 1.67 g $L^{-1}$ $hr^{-1}$ and a yield of 0.39 g $g^{-1}$ sugars were obtained via batch fermentation. Exploiting the metabolism of *A. succinogenes*, uncoupling cell growth from SA production, and deploying a fed-batch mode was implemented to boost the SA yield to 0.94 g $g^{-1}$, while maintaining by-products in trace amounts.

Chen *et al*. (2016) used sugarcane bagasse (SCB) hydrolysates as the carbon source, alongside their innovative application of the SCB residues as a cell-adsorption support. The SCB was pretreated with NaOH and $H_2SO_4$ to remove lignin before enzymatic saccharification, resulting in >90% lignin removal for the former with 0.25 M NaOH. Following this, the pretreated SCB was further hydrolysed by a multi-enzyme cocktail containing cellulase, xylanase, β-glucanase, and pectinase. The batch production of SA from 39.9 g $L^{-1}$ of NaOH-pretreated SCB afforded a yield of 0.82 g $g^{-1}$ and a productivity of 1.37 g $L^{-1}$ $hr^{-1}$. Fed-batch was also employed to enhance the process with up to 70.8 g $L^{-1}$ of SA and productivity of 1.42 g $L^{-1}$ $hr^{-1}$. Additionally, the SCB residues were later used as a support for cell adsorption, demonstrating an improved SA productivity of 1.65 g $L^{-1}$ $hr^{-1}$ with good reusability in three batches of fermentation. In another study, SA production from sweet sorghum bagasse was reported for the first time (Lo *et al*., 2020). In this study, a low temperature (50°C) was employed to efficiently release cellulosic sugars after pretreatment with concentrated phosphoric acid; with 29.2 g $L^{-1}$ released glucose to produce 17.8 g $L^{-1}$ of SA, at a yield of 0.61 and a productivity of 0.98.

Salvachúa *et al*. (2016) examined the performance of *A. succinogenes* on xylose-enriched hydrolysates from dilute acid-pre-treated corn stover. The batch production of deacetylated hydrolysates led to 42.8 g $L^{-1}$ of SA, at a yield of 0.74 g $g^{-1}$ and a productivity of 1.27 g $L^{-1}$ $hr^{-1}$. Besides, Shen *et al*. (2016) demonstrated that duckweed was a promising feedstock as its acid hydrolysis outperformed enzymatic saccharification and thermo-hydrolysis. To improve process economics, corn steep liquor powder was supplemented to replace yeast extract as a nitrogen source, with the resultant SA titer of 57.8 g $L^{-1}$ in batch fermentation (Shen *et al*., 2016). Amongst the different configurations such as SHF, SSF, and semi-SSF evaluated, the use of viscosity-reducing enzymes in semi-SSF enhanced SA production to reach 75.46 g $L^{-1}$, at a yield of 0.83 g $g^{-1}$ and productivity of 1.35 g $L^{-1}$ $hr^{-1}$ (Shen *et al*., 2018).

**TABLE 11. RECENT R&D FOR OPTIMISING SUCCINIC ACID PRODUCTION BY *Actinobacillus succinogenes* FROM VARIOUS BIOMASSES (2016-2021)**

| Feedstock | Approach | Major focus | Fermentation kinetics | | | References |
|---|---|---|---|---|---|---|
| | | | *P* (g $L^{-1}$) | *Q* (g $L^{-1}$ $hr^{-1}$) | *Y* (g $g^{-1}$) | |
| Carob pods | SHF, batch | Optimisation of sugar extraction while minimising phenolic compounds | 9.41 | 1.67 | 0.39 | Carvalho *et al*. (2016) |
| | SHF, fed-batch | Reduction of by-products formation | 18.97 | 1.32 | 0.94 | |
| Sugarcane bagasse | SHF, batch | Optimisation of enzymes cocktails | 39.90 | 1.37 | 0.82 | Chen *et al*. (2016) |
| | SHF, fed-batch | Addition of 200 g $L^{-1}$ condensed sugar | 70.81 | 1.42 | 0.82 | |
| | SHF, repeated batch | Utilisation of SCB residue as cell adsorption support | 40.00 | 1.65 | 0.81 | |
| Corn stover | SHF, batch | Fermentation of xylose from DA pretreatment | 42.80 | 1.27 | 0.74 | Salvachúa *et al*. (2016) |
| Duckweed | SHF, batch | Different pretreatments, CSL as nitrogen source | 57.85 | 1.21 | 0.78 | Shen *et al*. (2016) |
| | Semi-SSF, batch | Comparison of SHF, SSF and SSSF | 75.46 | 1.35 | 0.83 | Shen *et al*. (2018) |
| Sorghum bagasse | SHF, batch | Pretreatment using concentrated phosphoric acid at a mild temperature | 17.80 | 0.98 | 0.61 | Lo *et al*. (2020) |
| OPEFB | SSF, batch | Pretreatment using autoclave alkaline (AA) | 20.90 | 0.44 | 0.29 | Akhtar and Idris (2017) |
| | SSF, batch | Pretreatment using DA-microwave alkali | 33.20 | 0.69 | 0.47 | |
| | SSF, batch | Optimisation of SSF via RSM | 42.90 | 0.89 | 0.61 | Akhtar *et al*. (2020) |
| | SSF | Pretreatment using inorganic salt | 38.85 | 0.54 | 0.39 | Khairil Anwar *et al*. (2021) |
| | Semi-SSF | | 50.50 | 0.38 | 0.51 | |
| OPF juice | SHF, batch | Effect of $CO_2$ in serum bottles and bioreactor scale | 29.00 | 0.73 | 0.71 | Tan *et al*. (2018) |
| OPF bagasse | SHF, batch | Pretreatment using alkaline and AA | 36.6 | 0.61 | 0.71 | Luthfi *et al*. (2016) |
| | SHF, repeated batch | Cells immobilisation on CSAC | 44.10 | 1.30 | 0.90 | Luthfi *et al*. (2017a) |
| | SHF, continuous, immobilisation | Cells immobilisation on CSAC | 36.48 | 1.95 | 0.57 | Luthfi *et al*. (2018) |
| | SHF, immobilisation | Cells immobilisation on clay pebbles | 36.30 | 1.48 | 0.77 | Luthfi *et al*. (2020) |
| OPT sap | SHF, batch | Optimisation of SHF | 20.33 | 0.35 | 0.54 | Bukhari *et al*. (2019b) |
| OPT bagasse | SHF, batch | Pretreatment using organic acid | 17.50 | 0.36 | 0.44 | Bukhari *et al*. (2021b) |
| OPT bagasse | SHF, batch | Optimisation of SHF condition, nutrient minimisation | 21.10 | 0.35 | 0.58 | Bukhari *et al*. (2021a) |

Note: SHF - separate hydrolysis and fermentation; SSF - simultaneous saccharification and fermentation; OPEFB - oil palm empty fruit bunch; OPF - oil palm frond; OPT - oil palm trunk; SCB - sugarcane bagasse; DA - dilute acid; CSL - corn steep liquor; CSAC - coconut shell activated carbon; *P* - succinic acid concentration; *Q* - volumetric productivity; *Y* - succinic acid yield.

## Progress for Succinic Acid Production from Oil Palm Biomasses

OPEFB has been investigated for SA production through two pretreatment methods, *i.e.*, dilute acid-microwave alkali (DA-MWA) and autoclave alkali (AA) (Akhtar and Idris, 2017). A higher SA titer of 33.3 g $L^{-1}$ with 0.47 g $g^{-1}$ yield representing a 59% improvement from DA-MWA was obtained compared with that of 20.9 g $L^{-1}$ and 0.29 g $g^{-1}$ from AA. DA-MWA was thus a better pretreatment method followed by SSF at 38°C, pH 6.5, and cellulase loading of 25 FPU $g^{-1}$ OPEFB. The use of DA-MWA through microwave irradiation effectively disrupted the recalcitrant structure of OPEFB. DA-MWA outperformed AA in several aspects: Shorter

reaction time (20 min *vs.* 120 min), lower temperature (90°C *vs.* 121°C), and more effective lignin removal (73% *vs.* 40%) (Akhtar and Idris, 2017). The SA yield was further enhanced to 0.61 g $g^{-1}$ at 36°C, pH 5, and cellulase loading of 38 FPU $g^{-1}$ OPEFB under the optimised SHF operating conditions (Akhtar *et al.*, 2020).

Another pretreatment method using 15% (w $v^{-1}$) $Na_3PO_4.12H_2O$ and 5% (w $v^{-1}$) $ZnCl_2$ was deployed on OPEFB prior to *A. succinogenes* fermentation in a 16-L bioreactor (Khairil Anwar *et al*., 2021). Two different configurations were studied: SSF *vs.* semi-SSF. The hydrolysis reaction at 50°C was commenced first followed by fermentation at 37°C at the same reactor in semi-SSF, offering suitable conditions for microorganism survival. Higher SA concentration (50.5 *vs.* 38.9 g $L^{-1}$) and yield (0.51 *vs.* 0.39 g $g^{-1}$) were revealed in semi-SSF compared to that in SSF.

For SHF, its potential is undeniable as being commonly used for SA production (*Table 11*). Utilising OPF as the substrate, SA production could be further enhanced via this common configuration. The batch-mode production of AA-pretreated OPF produced 36.6 g $L^{-1}$ of SA, with a yield of 0.71 g $g^{-1}$ and a productivity of 0.61 g $L^{-1}$ $hr^{-1}$ (Luthfi *et al*., 2016). When *A. succinogenes* was immobilised on coconut shell activated carbon (CSAC), enhancement of SA production was reported by 23%, compared with that of free cells at 40.2 g $L^{-1}$. Repeated-batch fermentation using CSAC was performed for 5 cycles over 180 hr, achieving an average SA concentration of 44.1 g $L^{-1}$, yield of 0.87 g $g^{-1}$, and productivity of 1.34 g $L^{-1}$ $hr^{-1}$ in the bioreactor (Luthfi *et al*., 2017a). Fermentation in the continuous mode afforded enhanced productivity by 3-fold, reaching up to 1.95 g $L^{-1}$ $hr^{-1}$ (Luthfi *et al*., 2018). Besides, repeated-batch fermentation with clay pebbles for 5 cycles over 130 hr achieved an average SA concentration of 36.3 g $L^{-1}$, yield of 0.77 g $g^{-1}$, and productivity of 1.48 g $L^{-1}$ $hr^{-1}$. The findings also demonstrated that immobilised cells are superior in reducing by-products formation which could be an interesting strategy to ease the subsequent downstream processing (Luthfi *et al*., 2020).

Tan *et al.* (2014) explored utilising OPF juice as a substrate for SA production by *A. succinogenes*. In this study, carbon dioxide ($CO_2$) was reported as another critical parameter in SA production through microbial anaplerotic reactions. High availability of $CO_2$ promotes the flux towards the C4 SA-producing pathway, since the fixation of $CO_2$ into the three-carbon PEP will be needed to form the four-carbon oxaloacetate. When the serum bottle fermentation of extracted OPF juice was supplemented with 400 mM of carbonate as a source of $CO_2$, the yield of SA rose from 0.47 to 0.71 g $g^{-1}$ while its final titer was enhanced by 3.3-fold from 4.4 g $L^{-1}$ to 19.1 g $L^{-1}$. In the bioreactor, the final titer of SA was found to have been affected by $CO_2$ sparging, in which 26.6 g $L^{-1}$ of SA at a sparging rate of 0.1 vvm was improved to 30.7 g $L^{-1}$ at 0.5 vvm. This finding was in line with the optimum sparging rate of $CO_2$ for achieving the highest $CO_2$ dissolved rate (Tan *et al*., 2018).

**OPT as a Promising Feedstock for Succinic Acid Production**

OPT is a highly nutrient-dense carbohydrate-based biomass that can be exploited as a cost-effective and renewable carbon source for bio-chemical industries. OPT contains a high level of structural carbohydrate (~60% of holocellulose) and is ideal for use as a feedstock in SA production. Moreover, it is one of the cheapest biomass which can be obtained at the cost of USD7-USD9 (RM30-RM40) per tonne.

An efficient OPT utilisation strategy can be achieved by separating the sap and fibrous residue (bagasse) using mechanical extraction in order to harness and access the maximum amount of sugars. The OPT sap contains ~5% (w $v^{-1}$) free sugars, such as sucrose, glucose and fructose, which is suitable for direct fermentation (Bukhari *et al*., 2019b). Being a carbon source supplier, OPT sap is advantageous as it also contains a high amount of nitrogen as well as a considerable amount of vitamins and amino acids which are suitable for microbial fermentation. Bukhari *et al*. (2019b) demonstrated for the first time that raw OPT sap was efficient as a sole substrate for SA production. A superior SA yield of 0.54 g $g^{-1}$ was obtained in the medium without YE supplementation as compared to 0.50 g $g^{-1}$ with YE. Hence, OPT fulfils the nutritional requirements of auxotrophic *A. succinogenes* 130Z to meet its physiological demands. Batch fermentation in a bioreactor using OPT sap without YE and minerals resulted in an SA yield of 0.52 g $g^{-1}$, which indicated an achievable cost reduction as a result of avoiding expensive nutrients. The use of OPT sap for bioconversion holds several economic advantages; operational without complex and expensive pretreatment, and relatively easy to handle during fermentation.

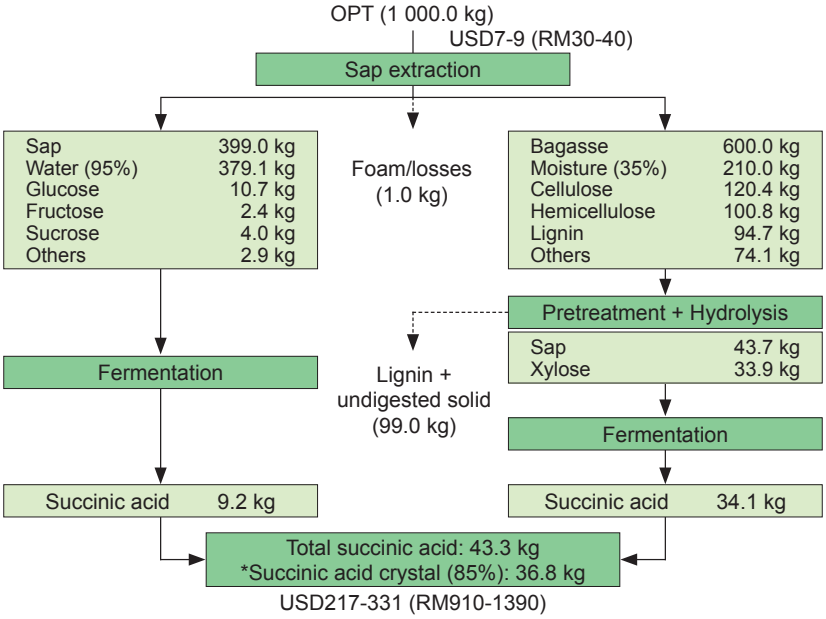
Bukhari *et al*. (2020) investigated the performance of organic acids (*i.e.*, citric acid, formic acid and oxalic acid) in pre-treating lignocellulosic OPT bagasse. Of these, oxalic acid at 5% (w $v^{-1}$) was the most effective in solubilising hemicellulose from OPT bagasse, producing a maximum xylose yield of 16.26 g 100 $g^{-1}$ for 120 min. This yield was comparable to conventional pretreatment using dilute sulphuric acid. However, the SA fermentation by *A. succinogenes* was suppressed (SA yield of 0.13 g $g^{-1}$) at this concentration. Oxalic salts formed at the neutralisation step was the main inhibitor during fermentation. A much higher SA yield of 0.37 g $g^{-1}$ was obtained while reducing the oxalic acid concentration to 1% (w $v^{-1}$) and prolonging the pretreatment time, for which an improved enzymatic

digestibility and fermentability satisfactorily achieved (Bukhari *et al*., 2021c). The results prove the potential of oxalic acid as an alternative to dilute sulphuric acid in biomass pretreatment, which warrants more attention to improve SA yield.

Two different routes have been exploited in SA production from OPT, considering that the microorganism employed has the capability to metabolise a wide range of sugars including glucose (C6) and xylose (C5) simultaneously into SA (Bukhari *et al*., 2021b). In terms of sugar yield, direct application of the whole slurry (Route 2) as substrate afforded a twofold increase compared to the washed pre-treated solid (Route 1). About 43.2 g 100 $g^{-1}$ sugars were recovered from Route 2 in comparison to 22.1 g 100 $g^{-1}$ from Route 1. Mass balance calculations demonstrated that 175.2 g of SA could be obtained from 1 kg of dry OPT bagasse using Route 2, which outperformed Route 1 at 152.6 g SA $kg^{-1}$ OPT. The results showed that saccharification using the whole slurry was evidently more advantageous, given its practical ease of operation (*i.e.*, not requiring solid/liquid separation and washing) and cost-savings (*i.e.*, avoiding additional buffer solution for enzymatic reaction). This study proves that utilisation of both OPT streams for SA production can fully reap the benefit of *A. succinogenes* 130Z.

A cost-effective medium with minimum nutrient supplementation was developed to improve the process economics of SA production by *A. succinogenes* 130Z from OPT bagasse hydrolysate (Bukhari *et al*., 2021b). Different neutralising agents (NaOH, KOH and $NH_4OH$) were screened for pH adjustment to condition the acidic hydrolysates so as not to inhibit the subsequent biocatalytic activities. NaOH was found most promising and should be seriously considered in designing the bioprocess of SA from acid hydrolysates (Bukhari *et al*., 2021a). Next, different nitrogen sources and mineral salts were manipulated for cost reduction potential on SA fermentation (Bukhari *et al*., 2021b). Clearly, YE was essential to be supplemented into acid hydrolysate to facilitate SA synthesis. Interestingly, one-third of the concentration of YE could be significantly reduced while the addition of mineral salts is unnecessary in fermenting OPT bagasse hydrolysate for SA production. As OPT bagasse hydrolysate has sufficient minerals to support SA fermentation, costly nutrients are entirely avoidable. Through this strategy, a batch fermentation of OPT bagasse hydrolysate in a bioreactor can result in SA yield of 0.58 g $g^{-1}$, which corresponds to 211 g of SA from 1 kg of dry OPT bagasse.

Efficient utilisation of OPT biomass is possible through the use of whole OPT, which allows for the maximum use of all available sugars (C5 and C6) for bioconversion into SA. Potentially, OPT sap might be the best source in terms of fermentation nutrients, for cost-effective microbial production. Overall, the total utilisation of OPT is able to produce 43.3 kg of SA from 1 t of OPT (*Figure 7*). Based on mass balance calculations, from 1 t of OPT, a SA production worthy of USD217-USD331 (RM910-RM1390) can be produced. OPT is currently sold at ~USD7-USD9 (RM30-RM40) $t^{-1}$ of the trunk. The added value from OPT raw material to bio-based SA is approximately 30-fold. The findings could prevail and improve economies of scale in utilising OPT biomass as a potential feedstock for SA production.




Source: (Bukhari *et al*., 2019b; 2021a; 2021b)

*Figure 7. Mass balance calculations for succinic acid production from 1 t of oil palm trunk (OPT).*

## CHALLENGES AND WAY FORWARD FOR SUCCINIC ACID PRODUCTION FROM OPT

Overall, the efficient exploitation of OPT to produce bio-based chemicals is able to minimise any environmental burden associated with, the disposal of the residues in particular. OPT serves as a cheap carbon source which would partially replace expensive pure sugar platforms in typical SA fermentation while improving the process economics. Some challenge of SA production from OPT is the inconsistency of feedstock supply and logistics issue; considering OPT is only available during replanting. In the current situation, the palm-plywood manufacturing industry has also generated a large quantity of OPT residues such as veneer waste, OPT end cuts and core logs, which are not utilised at all to benefit the industry. Hence, it is beneficial to explore the potential commercialisation of SA by integrating with the existing palm-plywood factories. Besides, biomass degradation and biodeterioration may occur during the storage of wet OPT over a prolonged period (Harun *et al.*, 2022). In reality, doable biomass projects involving OPT should occupy land closer to the source to minimise the cost of transportation due to high water content and raw material wastage.

Moreover, developing a viable and economical technology in SA production plus integrating the whole value chain, as depicted in *Figure 8*, helps reduce overall production costs and increase competitive edge over petroleum-based counterparts for a wider market. For oil palm biomass, the efficient use as well as selection and management of feedstock based on the biorefinery concept, focusing on multiple productions and high value addition, should be commercially implemented. The utilisation of feedstock as well as by-products gives other opportunities to improve the feasibility of the process. The biorefinery combines the material flows to reach a complete utilisation of the whole biomass components (García *et al.*, 2011). SA would be the main product. However, utilisation and market opportunities for the by-products and compounds from the waste stream should be evaluated. The inhibitors formed during pretreatment (*i.e.*, acetic acid) may be separated from the hydrolysate prior to fermentation, thereby improving the microbial growth and product yield, or may also be utilised as valuable chemicals. The undigested solid residue (containing lignin) generated from the process may be used as a source of energy to sustain the system.

Full exploitation of OPT from the oil palm industry for SA production is envisioned to benefit various sectors through new market creation, thus stimulating the country's economic growth. The resultant additional profits would be socio-economically generous to society as more than 220 000 plantation owners (~16%) in Malaysia are independent smallholders (Parveez *et al.*, 2021). Ultimately, this gears towards achieving sustainable oil palm plantation management by facilitating the removal and utilisation of felled OPT and making replantation feasibly more beneficial within the existing cultivation lands. Most importantly, this can address waste management systems and environmental issues, leading to a more responsive and dynamic industry.

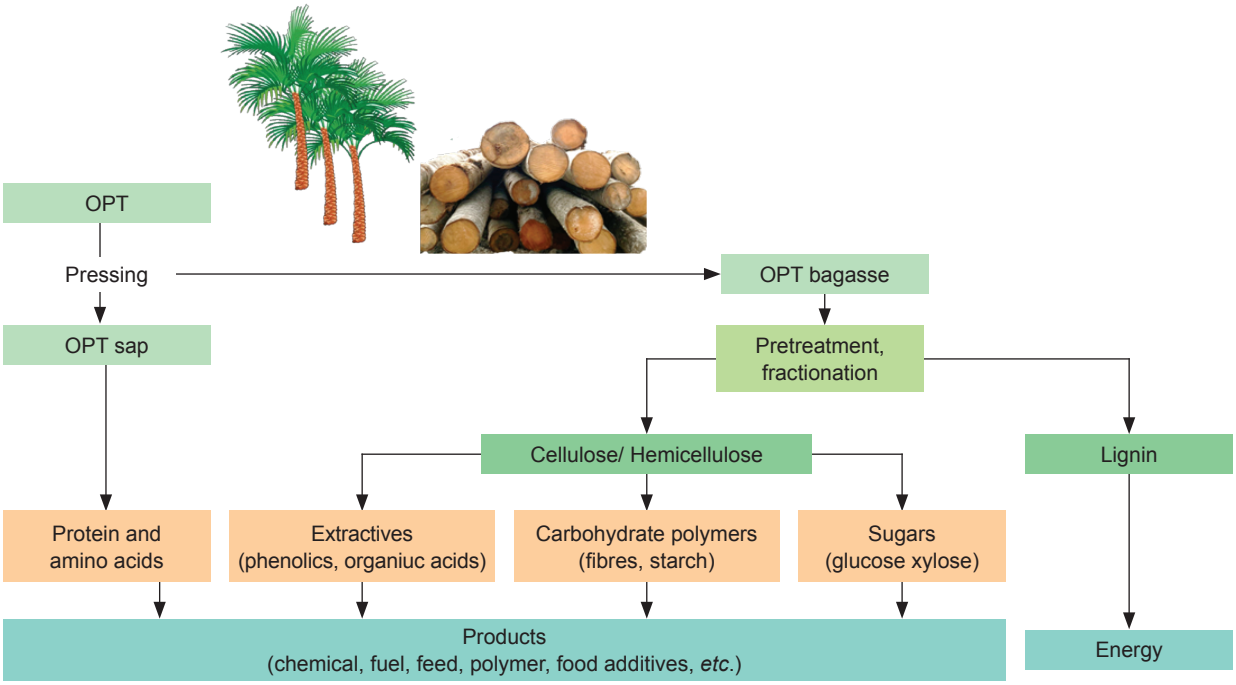



*Figure 8. Essential steps towards biorefinery with multiple products utilising oil palm trunk (OPT).*

## CONCLUSION

Bio-based SA from inexpensive and renewable feedstock such as OPT biomass remains high potential as an alternative C4 building block to various other petroleum-derived counterparts via biotechnological routes. OPT comprises highly nutrient-rich sap and carbohydrate-dense bagasse that can be hydrolysed into fermentable sugars. In enhancing market competitiveness for the case of SA production from OPT, the selection of appropriate bioconversion steps such as inhibitor-free pretreatment, self-sustained enzymatic saccharification, and bacterium-specific fermentation is prudent. *A. succinogenes* performs greatly and has lots of potential but needs to be technologically simplified in terms of overall process and integration, to achieve economical SA production. This review provides useful insights and recommendations for advancing future biomass to chemical commodity development, SA from OPT in particular.

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