

# Towards Sustainable Production of Bio-based Lactic Acid *via* a Bio-based Technical Route: Recent Developments and the Use of Palm Kernel Cakes in the Bioconversion

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The continued reliance on non-renewable fossil resources has led to serious environmental issues. In light of these concerns, the transition from non-renewable sources to more sustainable ones have been explored, as exemplified by the production of bio-based lactic acid *via* lignocellulosic biomass bio-refinery process. Malaysia, the second-largest producer of palm oil in the world, generates abundant, cheap, and underutilized oil palm biomass in the form of palm kernel cakes. Comprised of 50% fermentable hexose sugars, palm kernel cakes have emerged as an interesting feedstock substitute in the production of bio-based fine chemicals, *e.g.*, lactic acid. This paper focuses on current work based on selected literature published in the 21<sup>st</sup> century on the exploitation of palm kernel cakes as a novel feedstock in bio-refinery processes after addressing the current global demand and potential commodity applications of bio-based lactic acid. It then discusses current research on potential lactic acid-producing microorganisms, with particular attention to bacteria, and different pretreatment methods for carbohydrate recovery from palm kernel cakes. It also highlights the potential of oil palm biomass, especially palm kernel cakes, as a promising commodity that contributes to sugar platforms in value-added products, *e.g.*, biofuel, bioenergy, ethanol, acids, and fine chemicals.

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## INTRODUCTION

The environmental ramifications of the continued dependence on non-renewable crude oil and the escalating demand for fuel and commodity chemicals have steadily contributed to the rise of greenhouse gas emissions, the current climate crisis, and diminishing fossil-based resources. Attempts to substitute the unstable supply of petroleum-based resources with sustainable chemical platform counterparts are underway (Luthfi *et al.* 2016). One such alternative sustainable chemical platform is lactic acid.

Lactic acid, or 2-hydroxy-propanoic acid ( $C_3H_6O_3$ ), is a colorless and odorless monocarboxylic acid ranked by the National Laboratories of the U.S. Department of Energy as a second-tier building block chemical (Werpy and Petersen 2004). Discovered in the late 1700s, it is a crucial organic acid molecule required for the anaerobic metabolism of living organisms (Costa *et al.* 2020). Lactic acid can be found in the form of isomers L and D-lactic acid (Oliveira *et al.* 2018a). Considered “Generally Recognized as Safe” (GRAS) by the Food and Drug Administration (FDA) (Food and Drug Administration 2015), lactic acid was also listed as one of the most useful building blocks for multiple products (Din *et al.* 2021). It has recently gained substantial attention in various fields of application, *e.g.*, cosmetics, pharmaceuticals, food, chemicals, intermediate building blocks, and the textile, paper, and detergent industries (Abdel-Rahman *et al.* 2021).

Lactic acid can be obtained synthetically from acetaldehyde *via* the lactonitrile pathway. It involves the reaction between acetaldehyde and hydrogen cyanide (HCN), which is catalyzed by a basic catalyst under high temperature and pressure conditions. Sulphuric acid is then further applied to recover and purify the product for the resultant lactic acid (Komesu *et al.* 2017). The energy consumption required for the high temperature and pressure makes up 30% to 40% of the total operational costs of conventional lactic acid production (Komesu *et al.* 2017). Apart from its need for expensive raw materials and inefficient energy usage, this process of producing lactic acid also produces a racemic mixture of D- and L- lactic acid. D-lactic acid has been found to be unsafe for humans and thus incurs a high downstream cost, ultimately increasing the total production cost of lactic acid (Mazzoli 2020).

Alternatively, bio-based lactic acid can be derived from carbohydrate feedstocks found in abundant and inexpensive agricultural residues (Manandhar and Shah 2020). Malaysia as the world’s second-largest producer of palm oil generates abundant, cheap, and sugar-rich oil palm biomass in the form of palm kernel cakes (PKC) (Boonsawang *et al.* 2017). Constituting about 50% fermentable hexose sugars present in the form of galactomannan and glucan, PKC has emerged as a sound and sustainable source for lactic acid production. Lignocellulose feedstock from oil palm biomass contains polysaccharides that can be hydrolyzed *via* chemicals and/or a cocktail of cellulolytic enzymes under mild operating conditions to liberate fermentable sugars in the form of hexose and pentose sugars. Many microorganisms, including lactic acid bacteria, fungi, and yeast, can utilize these sugars and convert them into a large amount of high purity L-lactic acid through fermentation (Idrees *et al.* 2013; Lin *et al.* 2020). It is estimated that 90% of the current global lactic acid supply is derived from bio-based routes and its future demand is expected to increase at an annual rate of more than 12% from 2020 to 2025 (Hassan *et al.* 2019; Marketsandmarkets.com 2020).

The stereospecificity of pure optical L-lactic acid obtained from bio-based processes is determined by the L- or D-lactate dehydrogenases (LDH) gene type and lactate racemases that could interchange between both of the genes. Hence, studies demonstrated that optically pure lactic acid can be obtained *via* the deletion, disruption, and genome shuffling of the LDH gene encoded for the enzyme catalyzing the production of unnecessary isomers. Accordingly, it was found that a high productivity of L-lactic acid can be attained in *L. lactis* strain with UV-mutagenesis due to the enhancement of glucose alongside a reduction in NADH oxidase (Nox) activity of the cell (Hatti-Kaul *et al.* 2018). It is estimated that 90% of the bio-based lactic acid production worldwide incorporates the lactic acid bacteria for fermentation due to its high environmental adaptability, high titers

and productivity, and high tolerance to a wide range of temperatures and pHs (Sauer *et al.* 2008; Oliveira *et al.* 2018a).

## CURRENT GLOBAL DEMAND

The global lactic acid supply is nearly 270,000 t per year, while the demand for lactic acid as an alternative precursor to biodegradable polylactic acid (PLA) has gained considerable interest in recent years (López-Gómez *et al.* 2019). Based on a low price of PLA (\$0.4 to \$9 per kg based on purity and seller), it would be a candidate to replace many other plastic materials (Rajendran and Han 2022). Conversely, global demand for lactic acid has exceeded 1.0 million metric tonnes by 2020 with a projected skyrocketing market value of \$2.1 billion in 2016 to \$9.8 billion by 2025 (Manandhar and Shah 2020). The current market price of lactic acid is comparatively high at \$1.3 to \$4.0 per kg (Mazzoli 2020). According to the Markets and Markets report 2020, the global market of bio-based lactic acid was worth USD 1.1 billion in 2020. It is forecasted to grow to USD 2.1 billion by 2025 at a compound annual growth rate (CAGR) of 12.8% (Marketsandmarkets.com 2020).

In recent years, several major companies from different countries, *e.g.*, NatureWorks LLC (United States), Archer Daniels Midland Company (United States), Corbion-Purac (The Netherlands), and Galactic S. A. (Belgium) are emerging and actively engaged in commercializing bio-lactic acid-based products as new commodities (Cubas-Cano *et al.* 2020). The market of Corbion-Purac is comprised of 74.2% net sales of bakery products, 23.4% of biochemical supplies, and 2.4% others across all of its production plants in the USA, Netherlands, Spain, Brazil, and Thailand (Hörhammer *et al.* 2014). Nature Works LLC is one of the leading companies in lactic acid production with the brand Ingeo™. It can accommodate 140,000 metric tons of Ingeo™ biopolymer for various applications, *e.g.*, safe food packaging, health and personal care, home appliances and fused deposition modeling (FDM) 3D printing filament (Oliveira *et al.* 2018b).

Presently, sugarcane dominates the global lactic acid feedstock with the largest revenue (39.0%) in 2020, followed by yeast extract and other raw feedstocks, *e.g.*, cassava, sweet sorghum, potato, wheat, rye-barley, rice, xylan, and galactan. Given the numerous carbon sources from crop sugars that are used for lactic acid production, and despite promising pure lactic acid productivity, the majority of the carbon sources have issues with the cost, seasonality, and by-products formation, as well as competing with food availability (Oliveira *et al.* 2018b). The issues have a major effect on the overall production cost of lactic acid; to meet the growing demand of lactic acid products, the overall production costs must be less than \$0.8 per kg and the market price should decrease by half the current rate (Ajala *et al.* 2020). The cost of carbon feedstocks makes up 40% to 70% of the total production cost of lactic acid production (López-Gómez *et al.* 2019). Hence, in the long run of cost-effective lactic acid production, lignocellulosic material is an ideal sugar feedstock alternative due to its low price, sustainability, and non-food abundance, hence achieving food security (Abdel-Rahman *et al.* 2021). As oil palm biomass, palm kernel cakes (PKC) are relatively abundant and contain most of the needed macro- and micronutrients for the fermentation with microbes (Riyadi *et al.* 2017).

As compared to other oil palm biomasses, PKC are easily hydrolysable since they contain a low amount of lignins and cellulose and are primarily made up of linear mannan polymers of (1→4) linked β-D-mannopyranose and galactomannan with (1→6) linked α-

D- galactopyranose side groups. Hence, the additional cost for pretreatment can be reduced in terms of readying the lignocellulose for enzyme digestibility, and effectively reduce the total production cost of lactic acid (Raita *et al.* 2016). To date, PKC has been used to produce the following bio-based products, in the increasing ranking order of economic value: biofuel, biohydrogen, biobutanol, bioadhesive, prebiotic poultry diet feed, and fine chemicals (Stegmann *et al.* 2020). However, limited focus has been directed towards the highest economic-value bioproducts from PKC. Hence, the usage of PKC as a potential feedstock for a higher economical value product, *i.e.*, lactic acid in this review, not only brings novelty to fully exploit the beneficial nutrients in PKC, but also elevates the image of locally-harvested biomass, promotes a circular economy and sustainability, and generates a larger national revenue.

## POTENTIAL COMMODITY APPLICATIONS

Bio-based lactic acid is one of the most promising building-block chemicals for the production of secondary conversion chemicals, *e.g.*, acrylic acid, alkyl lactates, propylene glycol, acetaldehyde, 2,3-pentane dione, dioxanes, polyesters, lactide, and polymers (Manandhar and Shah 2020). The secondary chemicals derived from lactic acid, including green solvents, poly-acrylate, specialty chemical intermediates, poly acrylamide, and phthalate polyesters, have been widely used as precursors in various sectors, *e.g.*, safe food supply, environment, industrial, recreation, housing, transportation, textiles, health, and hygiene applications, as summarized in Fig. 1 (Werpy and Petersen 2004).

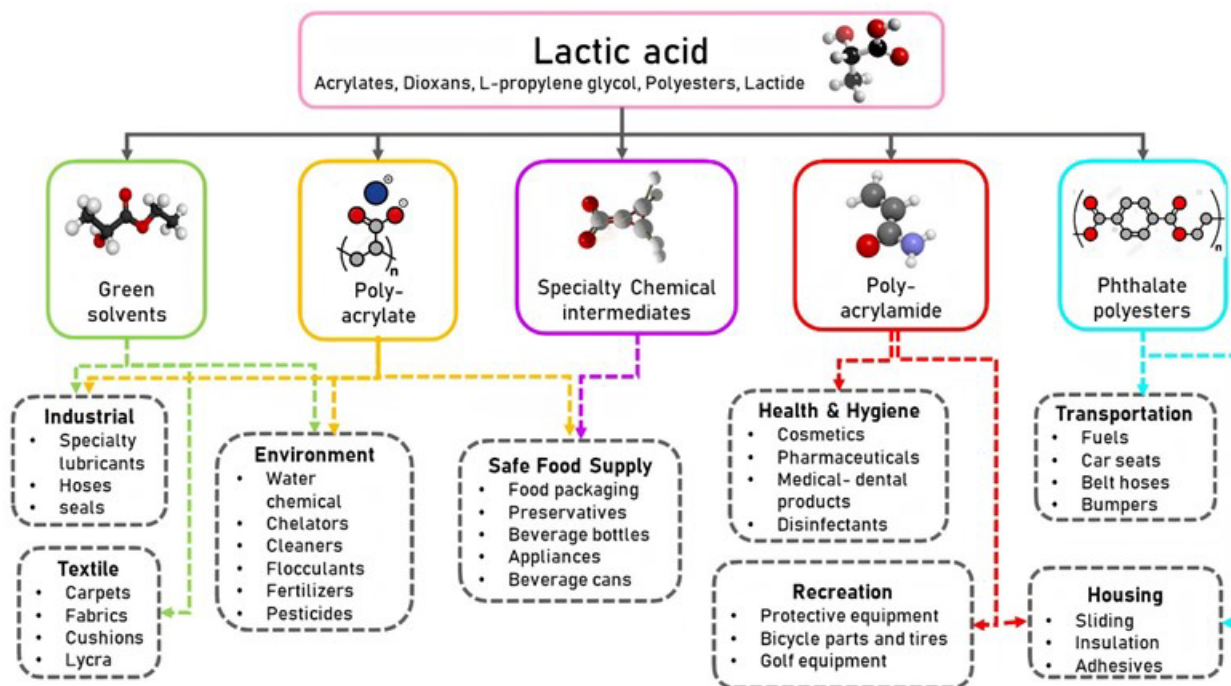


Fig. 1. Applications of bio-based lactic acid

There is also a substantial demand for bio-based lactic acid in the manufacturing of polylactic acid (PLA) polymer (Nagarajan *et al.* 2020). Among all bio-based commodities,

PLA is a fast-rising product with a forecasted CAGR of 16.62% in 2025 (Chandel *et al.* 2020). Polylactic acid polymer is bio-degradable, compostable, and biocompatible. In addition, PLA is widely used in many applications including eco-friendly packages, beverages bottle, prosthetic devices, sutures, and internal drug packaging.

## BIO-BASED LACTIC ACID BIOCATALYSTS

Bio-based lactic acid is a primary product of homofermentative glucose metabolism *via* the Embden–Meyerhof (EM) pathway, which requires glucose-6P isomerase, 6-phosphofructokinase, and fructose-bisphosphate aldolase (Eiteman and Ramalingam 2015). A single mole of glucose yields two moles of pyruvate, which then accepts electrons to form two moles of bio-based lactic acid in a reaction catalyzed *via* lactate dehydrogenase (LDH) (Hatti-Kaul *et al.* 2018). Bio-based lactic acid can be produced from biomass feedstocks *via* aerobically catabolizing starch into lactic acid with the aid of amylolytic fungi, *Rhizopus oryzae* and *Rhizopus arrhizus*, as biocatalysts. Nevertheless, amylolytic fungi have poor lactic acid productivity due to a poor reaction rate in limited mass transfer. Therefore, bio-based lactic acid is primarily produced through the anaerobic processing of carbohydrate substances *via* homolactic microorganisms, *e.g.*, various modified strains of lactic acid-producing bacteria (Juodeikiene *et al.* 2016). In comparison with aerobic processing, anaerobic fermentation could afford high yields even with less energy requirement. However, anaerobes require specialized media and apparatus along with meticulous skills and techniques, especially for cultivation, which makes them economically unattractive (Huang and Tang 2007). Hence, a novel breakthrough in the lactic acid production has been made using facultative anaerobic *A. succinogenes* 130Z in a dual-phase (aerobic-anaerobic) fermentation strategy, which afforded productivity of 22-fold higher than in normal anaerobic conditions (Li *et al.* 2010). The potential characteristics, advantages, and disadvantages of the different lactic acid-producing microorganisms are summarized in Table 1.

Four primary species of bacteria are involved in the production of lactic acid: lactic acid bacteria (LAB), *Bacillus* sp., *Escherichia coli*, and *Corynebacterium glutamicum* (Abdel-Rahman *et al.* 2013). It is estimated that approximately 90% of global lactic acid production stems from LAB fermentation (Sauer *et al.* 2008). However, findings on the potential rumen type bacteria, *Actinobacillus succinogenes*, as a high-titer lactic-acid-producing bacteria are limited, as it has been predominantly used in the production of succinic acid.

### Bacterial Strains

#### *Lactobacillus* sp.

Lactic acid bacteria (LAB) can be categorized into homofermentative and heterofermentative strains. Homofermentative LAB has an aldolase enzyme expressing gene and produces lactic acid as the primary product of glycolysis by converting one glucose molecule into two lactic acid molecules while releasing two ATP molecules. Conversely, heterofermentative LAB utilizes a phosphoketolase pathway to convert a single xylose molecule into one lactic acid and one ethanol or acetic acid molecule (Juturu and Wu 2016). The metabolic pathway of LAB is summarized in Fig 2.

**Table 1.** The Potential, Characteristics, Advantages, and Disadvantages of Bio-Based Lactate Producers

Biological Workhorse	Characteristics	Advantages	Disadvantages	References
<i>Lactobacillus pentosus</i>	Gram positive anaerobic	Ferment glucose, xylose High yield High productivity High tolerant in low pH High growth rates High resistant to different inhibitors	Complex nutrient requirement High production cost	Abdel-Rahman <i>et al.</i> 2013; Castillo Martinez <i>et al.</i> 2013; Boguta <i>et al.</i> 2014
<i>Pediococcus acidilactici</i>	Gram positive anaerobic Non-sporulating	Ferment on glucose and xylose GRAS Non-pathogenic	Lack of electron transport chain Complex nutrient requirement Long fermentation Slow growth Low productivity	Abdel-Rahman <i>et al.</i> 2013; Juturu and Wu 2016
<i>Bacillus coagulans</i>	Gram positive Sporulating Facultative anaerobic Motile Rod-shaped bacterium	Ferment on both pentose and hexose sugars Simple media Rapid growth Lower production cost GRAS High productivity Thermophilic Low risk of contamination	Low tolerant to inhibitor compounds	Ye <i>et al.</i> 2013; Juturu and Wu 2016; Jiang <i>et al.</i> 2016; Aulitto <i>et al.</i> 2017; Ahmad <i>et al.</i> 2019
<i>Actinobacillus succinogenes</i>	Gram-negative bacteria facultative anaerobic Immotile Pleomorphic rod Non-sporulating	Most natural Non-pathogenic GRAS Feeds on wide spectrum of carbon High titer in organic acid Robust in low pH Capnophilic	Highly dependent on CO <sub>2</sub>	Jiang <i>et al.</i> 2014; Luthfi <i>et al.</i> 2017; Pateraki <i>et al.</i> 2016; Chiang <i>et al.</i> 2018; Dessie <i>et al.</i> 2018; Zhang <i>et al.</i> 2019
<i>Escherichia coli</i>	Facultative anaerobic	Rapid metabolisation on pentose and hexose sugars High yield Rapid growth Minimum nutrient demand Low energy consumption Low operational cost	Low tolerance to inhibitors	Boguta <i>et al.</i> 2014; Finn <i>et al.</i> 2017; Wang <i>et al.</i> 2019

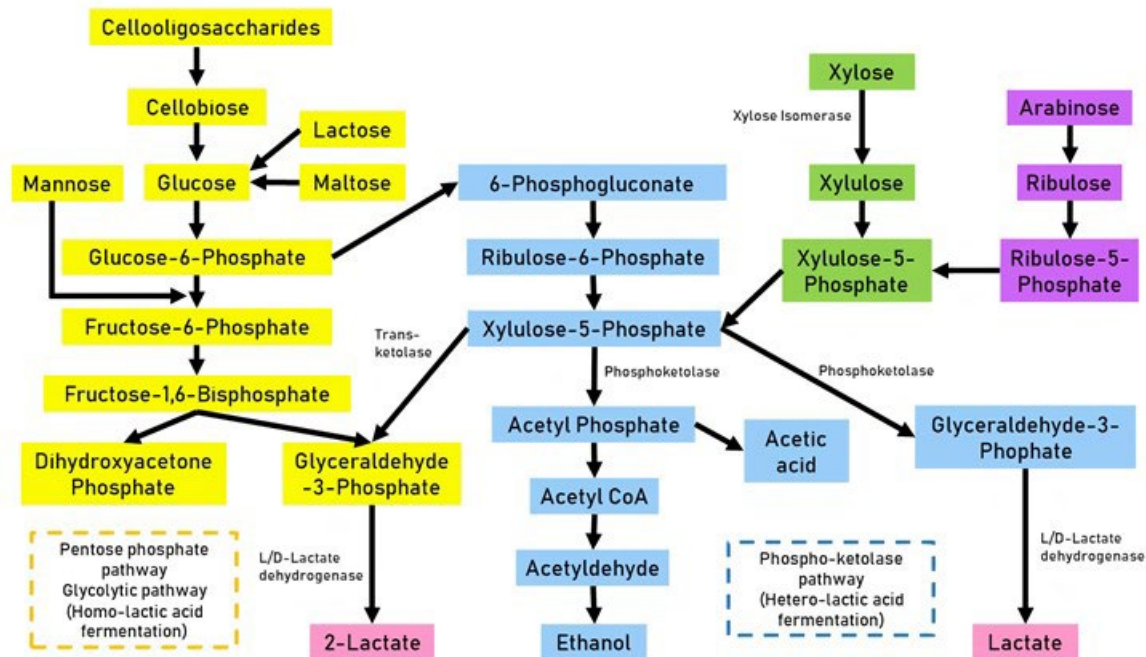


Fig. 2. The metabolism pathway of LAB

*Lactobacillus* sp., belonging to the LAB category, has a variety of genera with more than 150 different species. It is a facultatively anaerobic, Gram-positive, catalase-negative, immotile, and non-spore-forming bacilli (Mejia-Gomez *et al.* 2020). It has diversified catabolic activities from different environments including the gastro-intestinal tract, soil, and decayed matter. These findings contribute to the suitability of *Lactobacillus* sp. as a candidate for the conversion of second-generation lignocellulosic feedstocks into value-added metabolites (Boguta *et al.* 2014). A previous study by Lin *et al.* (2020) reported a titer of  $15.02 \text{ g/L} \pm 0.80 \text{ g/L}$  lactic acid after fermenting a synergy of 6% *L. acidophilus* and *L. plantarum* inocula in a medium of seaweed hydrolysate ( $10.54 \text{ g/L} \pm 1.29 \text{ g/L}$ ) for longer than 24 h at a temperature of  $37 \text{ }^\circ\text{C}$ . Another study found that the simultaneous saccharification and fermentation of *L. pentosus* in banana flesh hydrolysate containing  $56 \text{ g/L}$  of reducing sugars yielded  $50 \text{ g/L}$  of lactic acid (Azaizeh *et al.* 2020).

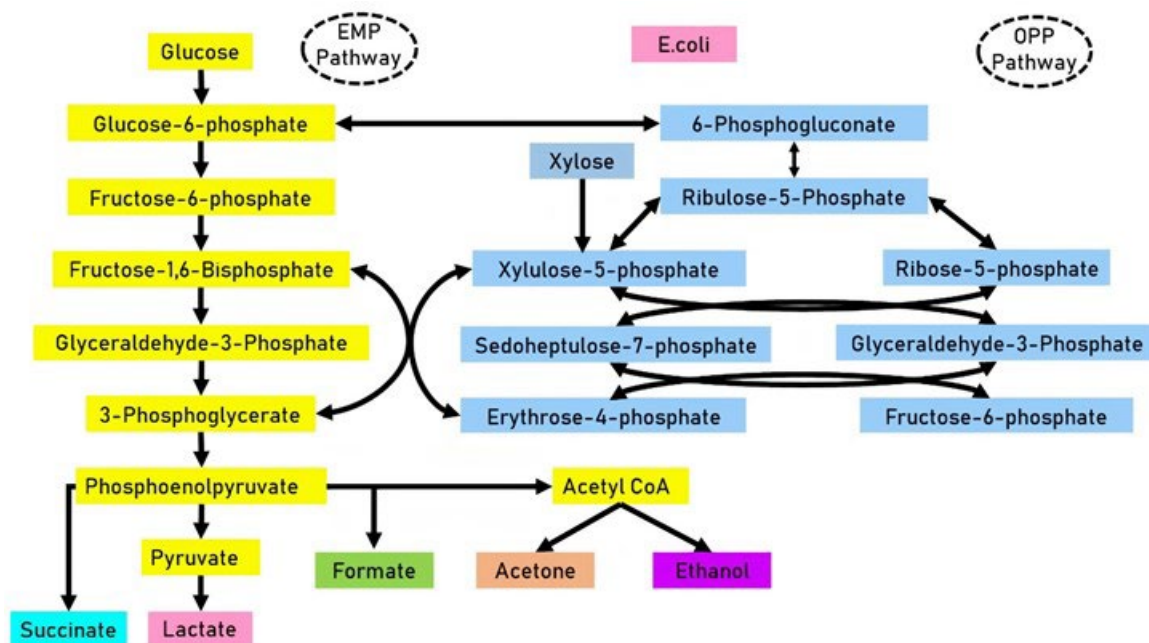
#### *Bacillus* sp.

There are several advantages of using *Bacillus* sp. to lower the production cost of lactic acid. Unlike other LAB species, *Bacillus* sp. requires simple media and cheaper nitrogenous sources including  $(\text{NH}_4)_2\text{SO}_4$  and corn steep liquor as their primary nutrient source (Juturu and Wu 2016). They are also heat-labile and can produce lactic acid under high thermal fermentation conditions (greater than or equal to  $50 \text{ }^\circ\text{C}$ ), hence removing additional sterilization costs (Aulitto *et al.* 2017). They can consume both pentose and hexose sugars, thus maximizing the utilization of all sugars from the lignocellulose (Pleissner *et al.* 2016). However, the species lacks the native genetic tools to metabolize glucose and xylose simultaneously; hence, the metabolism of sugars is inhibited with a carbon catabolite repression (CCR) and thus reduces the output of lactic acid (Li *et al.* 2017). Another study by Jiang *et al.* (2016) incorporated inhibitor tolerant mutagenesis into *B. coagulans* GKN316 to produce  $45.39 \text{ g/L}$  with an 83.15% yield of lactic acid from corn stover hydrolysate. These *Bacillus* species include *Bacillus coagulans*, *Bacillus*

*stearotherophilus*, *Bacillus licheniformis*, and *Bacillus subtilis* (Abdel-Rahman *et al.* 2013).

### *Escherichia coli* (*E. coli*)

*Escherichia coli* strains can rapidly metabolize hexose and pentose sugars, require a minimal demand of nutrients, and are easy to engineer in order to enhance product yield and productivity. Thus, they can be a better cell factory for lactic acid production. Wild-type *E. coli* generates ethanol and multiple organic acids, *e.g.*, lactic acid, acetic acid, succinic acid, and formic acid, as a replacement to the reducing equivalents in glycolysis (Abdel-Rahman *et al.* 2013). The three native glycolytic pathways in *E. coli* are the Embden–Meyerhof–Parnas pathway (EMPP), the oxidative pentose phosphate pathway (OPPP), and the Entner Doudoroff pathway (EDP) (Hollinshead *et al.* 2016). Two molecules of pyruvates, namely ATP and NADH, are released for every glucose molecule catalyzed by ten enzymatic reactions in the EMP pathway. The second half of the EMPP further converts phosphoenolpyruvate to pyruvate, which serves as a precursor for the formation of succinate, lactate, formate, acetate, and ethanol during anaerobic conditions (as shown in Fig. 3) (Wang *et al.* 2019). The OPPP is an oxidative pathway for the synthesis of NADPH. The EDP is only activated in the presence of gluconate. Therefore, the metabolism of *E. coli* most likely depends on the EMP and OPP pathways (Hollinshead *et al.* 2016).



**Fig. 3.** The metabolism pathways of *E. coli*: EMP pathway: Embden–Meyerhof–Parnas pathway; and OPP pathway: oxidative pentose phosphate pathway

The capability of *E. coli* to survive in both aerobiosis and anaerobiosis, as well as fluctuating oxygen conditions has led to easy manipulations of its metabolism and distortions of repeated functions, which have reportedly caused improved performance under anaerobic conditions (Finn *et al.* 2017). Evolution engineering has been underway to improve the anaerobic growth of *E. coli* for the escalation of lactic acid production. This results in a better-evolved, more effective strain as a platform for the anaerobic yield of



organic acids. Collaborative works between systematic and classical molecular biology have found that a mutation of *sucD* and *ilvG*, coupled with manipulations in an early stage of the evolution of RNA transcription and DNA replication machinery genes, have yielded a prominent effect on the anaerobic growth of *E. coli* W3110. A study by Wang *et al.* (2019) demonstrated the competency of an evolutionary engineered *E. coli* WE269 by successfully yielding 96.5% more lactate than a wild-type strain.

### *Actinobacillus succinogenes*

*Actinobacillus succinogenes* is a Gram-negative bacteria generally classified as a safe, non-pathogenic biosafety level 1 (BSL-1) microorganism by the DSMZ and ATCC (Chiang *et al.* 2018). It is an immotile, non-sporulating, facultative anaerobic, pleomorphic, coccobacilli-shaped, capnophilic bacteria, and falls taxonomically under the Pasteurellaceae family (Wang *et al.* 2019). *Actinobacillus succinogenes* is able to consume a wide spectrum of carbon sources, *e.g.*, glucose, xylose, arabinose, mannose, galactose, fructose, sucrose, lactose, cellobiose, mannitol, maltose, glycerol, and sucrose, under anaerobic conditions (Luthfi *et al.* 2017). This is due to the conditions of its natural ecological habitat, *i.e.*, the bovine rumen, which contains an abundance of different carbon sources (Dessie *et al.* 2018). It is worthy of note that *A. succinogenes* is one of the most promising succinate and lactate hosts, due to its robustness in tolerating environments with high organic acid concentrations and other potential growth suppressants (Jiang *et al.* 2014; Chiang *et al.* 2018; Dessie *et al.* 2018; Wang *et al.* 2019). A few studies have recently found that succinic and lactic acid can be produced by *A. succinogenes* in high yields (50% to 87% and 96% to 97%, respectively) (Wang *et al.* 2014). This high lactic acid productivity can be achieved using dual phase cultivation of the strain to shift the carbon flux distribution into lactate (Zhang *et al.* 2019). The presence of oxygen at the beginning of the fermentation promotes cell growth and cell viability in first phase and stimulates the formation of pyruvate and lactate in the latter phase of anaerobic cultivation (Li *et al.* 2010). Moreover, lactate dehydrogenase (LDH) activity also increased by 18-fold in dual phase fed-batches modes cultivation, resulting in 32-fold increase in lactic acid production with a final concentration of 135.6 g/L  $\pm$  0.14 g/L (Wang *et al.* 2016). Hence, *A. succinogenes* is a promising bioproducer for lactic acid, even with no genetic modification.

Figure 4 depicts the metabolic pathway of *A. succinogenes*. The metabolism in the cell relies on the uptake of glucose *via* permease. The glucose is phosphorylated by the phosphoenolpyruvate dependant phosphotransferase system and hexokinase activity to form glucose-6-phosphate. The metabolic conversion of glucose-6-phosphate into phosphoenolpyruvate (PEP) takes place in the glycolytic pathway and OPPP (McKinlay *et al.* 2007). The anaerobic carbon flux of *A. succinogenes* utilizes PEP to split the reaction into two branches. The first branch produces lactate, acetate, ethanol, and the formate-forming C3 pathway, while the second branch leads into the succinate-forming C4 pathway (McKinlay and Vieille 2008). The lactate-forming C3 pathway is controlled by the pyruvate formate lyase 1-activating protein (pflA) and 6-lactate dehydrogenase (encoded LDH) and results in the formation of lactic acid (Zhang *et al.* 2019). The 6-lactate dehydrogenase is the NADH-dependent catabolic enzyme that serves to reduce pyruvate for producing lactic acid (Novy *et al.* 2018; Chroumpi *et al.* 2020).

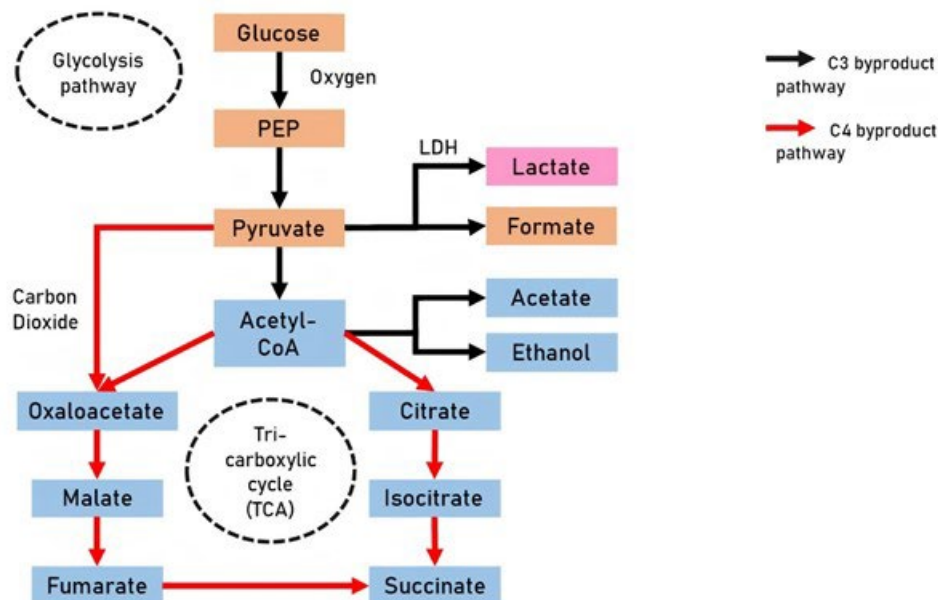


Fig. 4. Metabolism pathway of *A. succinogenes*: LDH: lactate dehydrogenase

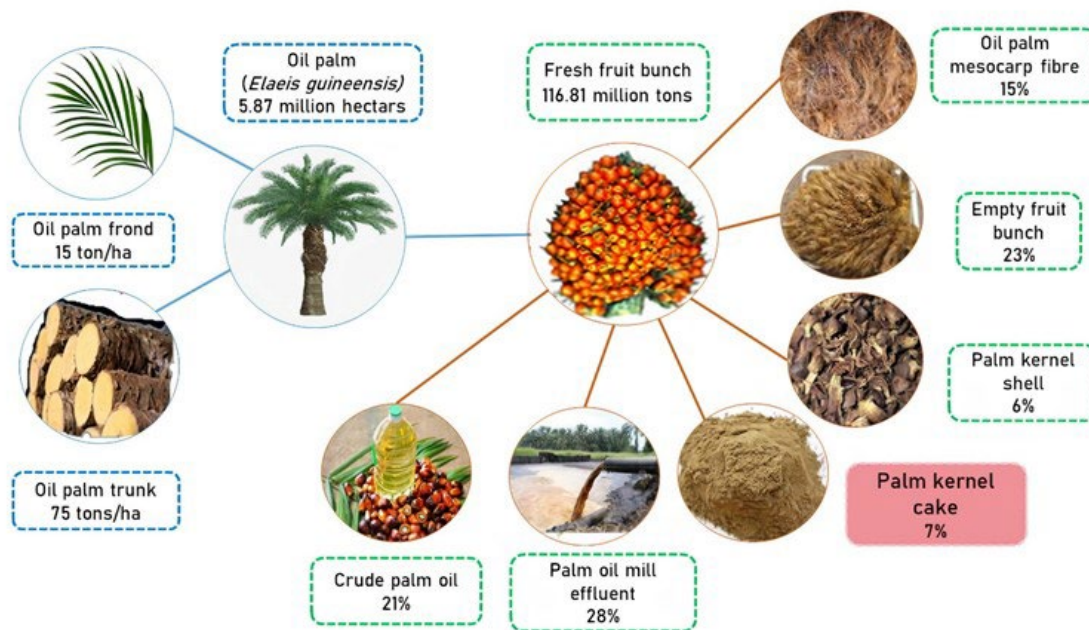
## PERSPECTIVE OF PALM BIOMASS FROM MALAYSIA

Agricultural byproducts comprising of lignocellulosic materials are a promising feedstock for the production of lactic acid because they are abundant, inexpensive, renewable, sustainable, environmentally friendly, and can potentially reduce environmental pollution (Azaizeh *et al.* 2020). Moreover, since lignocellulose biomass is non-edible and is a source of fermentable sugars, it is highly suited for the fermentation of various biochemicals, *e.g.*, bio-based lactic acid, *via* microorganisms. It is a good alternative, as it reduces the competition between the demand for resources required in the production of lactic acid and those used for global food and feed (Aulitto *et al.* 2017). The primary component of lignocellulosic biomass is a cell wall comprised of 30% to 60% cellulose, 20% to 40% hemicellulose, 15% to 25% lignin, and other extractives (Nanda *et al.* 2015; Bukhari *et al.* 2020b).

Malaysia is one of the leading countries involved in promoting biomass usage (Luthfi *et al.* 2017). The annual rate of biomass generation in Malaysia averages at 80 million tonnes. It exceeded 100 million tonnes in 2020 and is expected to rise to 1200 million tonnes by 2035 (Yatim *et al.* 2017; How *et al.* 2019). The various types of biomasses used are rice husks, oil palm biomass, sago biomass, timber residue, coconut trunk fibers, sugar cane residues, and other forms of agricultural residues (Yatim *et al.* 2017). As one of the largest producers of palm oil in the world, Malaysia stores an ample amount of oil palm biomass, which remain mostly untapped. The oil palm biomass including empty fruit bunches (EFB), oil palm trunks (OPT), mesocarp fibres (MF), oil palm fronds (OPF), and palm kernel shell (PKS) have high calorific values. Hence, they can be reutilized in the production of food, pharmaceutical constituents, high-quality chemicals, biofuel, polymers, and bioenergy *via* multiple techniques from a biorefinery processing perspective (Sadhukhan *et al.* 2018; Hassan *et al.* 2019).

## Palm Kernel Cake as an Emerging Biorefinery Feedstock

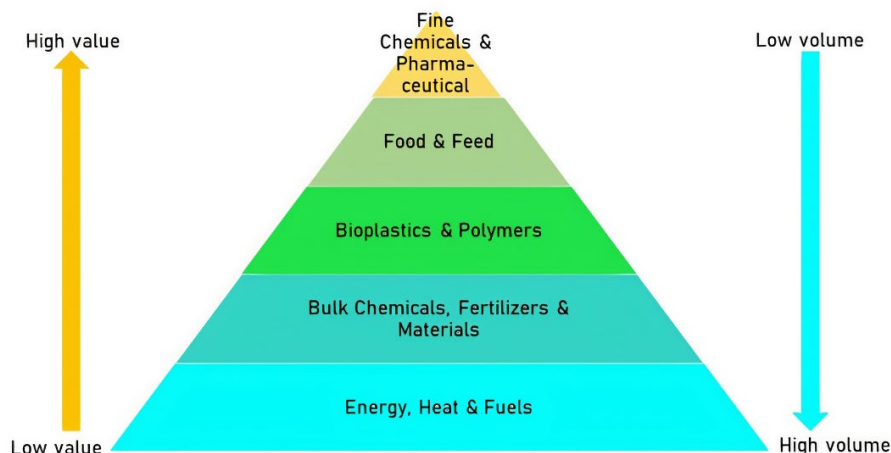
The palm oil industry in Malaysia has a total of 457 actively operating palm oil mills with an entire processing capacity of 116.81 million tons of fresh fruit bunches (FFB) per year (Parveez *et al.* 2021). This is made up of 20% crude palm oil and 80% oil palm lignocellulosic solid biomass on a wet basis (Azman *et al.* 2016; Raita *et al.* 2016). The oil palm residues primarily contain 23% empty fruit bunches, 28% palm oil mill effluents, 7% PKC, 15% oil palm mesocarp fibers, and 6% palm kernel shells, as illustrated in Fig. 5 (Onoja *et al.* 2019). The PKC is the residue of palm fruit after extracting the oil, which makes up approximately 2% to 7% of the total oil palm biomass (Onoja *et al.* 2019; Jana and Kango 2020). Malaysia produced 2.59 million tons of PKC in 2019 and the figure is expected to grow bigger in the near future (Halim *et al.* 2020).



**Fig. 5.** The schematic diagram of oil palm components with their weight percentage on a wet basis

Palm kernel cakes, though inexpensive, plentiful, and hold a vast potential for biochemical conversions, remain ineffectively utilized (Asri *et al.* 2020). Presently, the utilization of PKC primarily focuses on husbandry feed additives, but has minimal nutrient and amino acid contents, low protein digestibility, and is rough-textured (Oladokun *et al.* 2016; Alshelmani *et al.* 2017). As can be seen in Fig. 6, pharmaceutical and fine chemical products are categorized under the highest value biomass applications. This is followed by food and feed products as well as polymers and eco-friendly materials. Energy and fuels are categorized under the lowest-value biomass application in the bio-based product value pyramid (as shown in Fig. 6) (Stegmann *et al.* 2020).

Therefore, the redistribution of PKC to meet the needs of competing bio-based applications can be achieved by reutilizing the PKC in the production of valuable lactic acid. Pretreated PKC has been recently incorporated with enzymatic hydrolysis as feedstock to produce biobutanol, biohydrogen, bioethanol, prebiotic, poultry diet feed additives, bioadhesive, and biofuels (Cerveró *et al.* 2010; Shukor *et al.* 2013; Shukor *et al.* 2014; Azman *et al.* 2016; Jahromi *et al.* 2016; Shukor *et al.* 2016; Tafsin *et al.* 2017; Sari *et al.* 2018).



**Fig. 6.** The value pyramid of bio-based products

These interests emerge due to the potentially low lignin contents in PKC coupled with the richness in polysaccharides, including cellulose (11.6% glucan), hemicellulose (35.2% mannan, 2.6% xylan), and other fermentable C6 sugars (approximately 50%) made of glucose and mannose, which can be further utilized by most of the fermenting microbes (Azman *et al.* 2016; Jahromi *et al.* 2016). The primary carbohydrates in PKC are naturally in the form of mannan, glucomannan, galactomannan, and galacto-glucomannan (Kalidas *et al.* 2017). Similar to hemicellulose, the mannans in PKC are compact and crystallized, yet are still easily hydrolysable due to the presence of a highly linear  $\beta$ -1,4-linked mannan backbone and a low amount of single  $\alpha$ -1,6-linked galactose side groups (Li *et al.* 2018).

The fermentable sugars content in PKC is as high as the sugar content found in empty fruit bunches (EFB) and oil palm trunk (OPT), which are 49% to 56% and 68.9% of the carbohydrates, respectively, which were reportedly used as a carbon feedstock in previous lactic acid production (Sitompul *et al.* 2014; Eom *et al.* 2015; Juturu and Wu 2018). Hence, PKC has a great potential to be one of the most promising emerging feedstocks for lactic acid production (Asri *et al.* 2020). Furthermore, PKC contains most of the macro- and micronutrients required for a cost-effective basal medium for fermentation, including 22000 mg/kg of protein, 6190.9 mg/kg of potassium, 533.5 mg/kg of calcium, 382.5 mg/kg of sodium, 310.4 mg/kg of iron, 161.6 mg/kg of manganese, and 0.84 mg/kg of phosphorus (Shukor *et al.* 2016). Comprising low level of lignin and cellulose, PKC only requires mild pretreatment with lesser formation of inhibitory by-products for superior purity/optical purity of lactic acid.

## PRETREATMENT OF PALM KERNEL CAKES

Pretreatment refers to the disruption of the recalcitrant structure of lignins to expose the underlying carbohydrate components for enzyme accessibility (Haldar and Purkait 2021). A good pretreatment method should be cheap, eco-friendly, and with minimal microorganism inhibitor formation (Cheah *et al.* 2020). The delignification of a lignocellulosic biomass in the pretreatment phase serves to increase the total surface area of the carbohydrates and therefore the simple sugar yield by fragmenting the size of the biomass and decrystallizing the cellulose structure (Rezania *et al.* 2020). Table 2

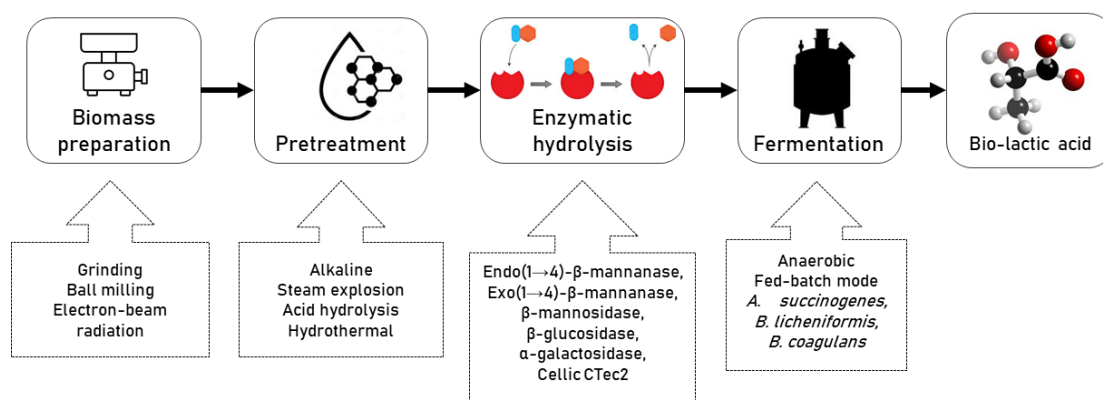
summarizes a variety of pretreatment and hydrolysis methods used in previous works for PKC recovery.

**Table 2.** Pretreatment and Hydrolysis Methods of Palm Kernel Cakes (PKC) Reported in Previous Studies

Optimum		Performance	References
Pretreatment	Hydrolysis		
<p><i>Direct acid hydrolysis</i>  <i>P</i> = 15 psi  <i>T</i> = 121 °C  <i>t</i> = 15 min  <i>S/L</i> = 3.5 to 10 (w/v)            7% (v/v) H<sub>2</sub>SO<sub>4</sub></p>	N/A	Liquid fraction: 27.75 g/L of mannose, 2.75 g/L of glucose, and 2.60 g/L of xylose	Azman <i>et al.</i> 2016
<p><i>Hydrothermal</i>    <i>T</i> = 126 °C  <i>t</i> = 11 min            pH = 5.4  <i>S/L</i> = 1 to 20 (w/v)</p>	<p><i>Mannaway + Gammanase</i>            (Novozymes, Bagsværd, Denmark)  <i>T</i> = 50 °C  <i>t</i> = 96 h            pH = 5.4            agitation = 200 rpm  <i>E/S</i> = 2.3 mg/g PKC 1 to 1 mixture of Mannaway 25 L and Gammanase 1.0 L</p>	Liquid fraction: 340 g mannose/kg of PKC, 365 g of total fermentable C6 sugar (galactose, glucose, and mannose) per kg of PKC Solid fraction: 75% conversion of mannan	Cerveró <i>et al.</i> 2010
<p><i>Hot water treatment</i>    <i>T</i> = 190 °C  <i>t</i> = 30 min            pH = 5.4  <i>S/L</i> = 1 to 7 (w/v)</p>	<p><i>Cellulose: 15 U/g;</i>  <i>Xylanase: 20 U/g;</i>            (Yangshao Biochemical Engineering, Sanmenxia, China)  <i>certain amount loading of endo-mannanase</i>            (Sunson Indus., Ningxia, China)  <i>T</i> = 50 °C  <i>t</i> = 24 h            pH = 5            agitation = 50 rpm  <i>cellulose: 15 U/g;</i>  <i>xylanase: 20 U/g;</i>  <i>certain amount loading of endo-mannanase</i></p>	Liquid fraction: 2.6 g/kg of cellobiose, 38.0 g/kg of glucose, 98.3 g/kg of mannobiose, and a 34 g/kg mannose yield  Solid fraction: 52.8% cellulose, 64.6% hemicellulose, and 16.9% protein recovery	Mi <i>et al.</i> 2016
<p><i>Steam explosion</i>    <i>T</i> = 148 °C  <i>t</i> = 15 min  <i>P</i> = 4.5 bar  <i>S/L</i> = 1 to 5 (w/v)</p>	<p><i>SEB Mannanase</i>            (Advanced Enzyme Technologies, Thane, India) +  <i>CTec2 cellulase</i>            (Novozymes, Bagsværd, Denmark)  <i>T</i> = 50 °C  <i>t</i> = 72 h            pH = 5            agitation = 250 rpm  <i>E/S</i> = 17.9 U/g mannan</p>	Liquid fraction: 28.34 ± 0.06 g/L Fermentable sugars Solid fraction: 107.5% ± 4.2 % of carbohydrate conversion, and 27.37 g/L of mannan conversion	Raita <i>et al.</i> 2016

-	<i>Mannanase enzyme (Habio Bioengineering)</i> T = 45 °C t = 72 h pH = 4.5 agitation = 170 rpm E/S = 5% (w/w) mannanase to 20% (w/v) PKC	Liquid fraction: 71.54 g/L ± 2.54 g/L total sugar, 67.47 g/L ± 2.51 g/L of mannose, and 2.94 g/L ± 0.03 g/L of glucose	Shukor <i>et al.</i> 2016
<i>Acid hydrolysis</i> T = 100 °C to 120 °C t = 1 h S/L = 1 to 0 (v/v) 1% (v/v) Acetic acid	-	Liquid fraction: 8.7% sugar yield	Tafsin <i>et al.</i> 2017
<i>Hydrothermal</i>  T = 121 °C t = 20 min S/L = 1 to 10	<i>β-mannosidase and β-mannanase (Megazyme, Ireland)</i> T = 55 °C t = 12 h pH = 6 agitation = 150 rpm E/S = 20 U/mL of mannopentaose	Liquid fraction: 43.43% mannose, 4.71% ± 0.14% glucose, and galactose 3.78% ± 0.21% xylose yield	Jahromi <i>et al.</i> 2016

The common pretreatment methods found in previous studies can be effectively classified as physical, chemical, or physicochemical based on their operational mechanisms. Physical methods tend to fractionate the structure of the biomass, reducing the particle size and allowing for biomass susceptibility to enzyme digestibility. Methods that fall under this category include grinding, ball milling, microwave-assisted pretreatment, and electron beam irradiation (Anu *et al.* 2020).



**Fig. 7.** The process flow including a variety of pretreatments and the hydrolysis of PKC for bio-lactic acid production

However, these methods require high power and energy and can result in inefficient sugar recovery (Anu *et al.* 2020; Haldar and Purkait 2021). The demerits of the physical methods have thus given rise to the advent of cheaper, faster, and more effective chemical and physicochemical pretreatments. Besides eliminating the lignins, the content of

hemicellulose and cellulose recovery can be maximized. These methods include acid, alkaline, hydrothermal, and steam explosion (Ranjithkumar *et al.* 2017; Rahmati *et al.* 2020). The process flow, which includes a variety of pretreatments and the hydrolysis of PKC, of bio-lactic acid production is simplified in Fig. 7.

### Alkaline Pretreatment

Alkaline pretreatment is a vastly studied pretreatment technique that enhances enzyme digestibility by increasing the surface area of carbohydrates for enzyme digestibility. The application of bases under low pressure and temperature conditions allows them to penetrate the lignocellulose structure, cleave the ester bonds between the hemicellulose and lignin, solubilize the lignin, remove the acetyl groups, and swell the cellulose to achieve an increased surface area (Bensah and Mensah 2013). The process results in a solid portion of cellulose and a liquid stream comprising of solubilized lignins, hemicelluloses, and intact inorganic chemicals. Despite its longer retention time, it is efficient in the delignification of low lignin lignocellulosic biomasses (Guragain *et al.* 2016; Xu *et al.* 2016). Commonly used bases include sodium hydroxide, calcium hydroxide, potassium hydroxide, and ammonium hydroxide (Aftab *et al.* 2019).

Among these bases, sodium hydroxide emerges as the most effective base due to its ability to dissociate in different materials (Baruah *et al.* 2018). The usage of only 1% sodium hydroxide in the pretreatment yielded 83% reducing sugars in corn stover, 78% in brown-midrib (BMR) sorghum stalks, 75% in sorghum stalks, 65% in switchgrass, and 58% in poplar biomass (Guragain *et al.* 2016). When applied to PKC, the total reducing sugars obtained from a 1% sodium hydroxide pretreatment at a temperature of 80 °C for 1 h resulted in 2.15 g/L of total sugars with no growth of *C. acetobutylicum* YM1, *i.e.*, no butanol formation throughout the 72 h of fermentation (Al-Tabib *et al.* 2017). It was reported that the *Clostridium* growth was inhibited by the presence of phenolic compounds released from the delignification of the PKC during the alkaline pretreatment. However, a neutralization step is needed prior to fermentation to remove the inhibitory phenolic compounds, thus increasing the overall production cost of a biochemical (Xu *et al.* 2016). Hence, alkaline pretreatment seems to be less effective for the cellulose and hemicellulose recovery in PKC.

### Hydrothermal Pretreatment

Hydrothermal pretreatment is considered an ideal alternative for the pretreatment of lignocellulosic biomass in large industrial-scale processing because it is safer, more environmentally friendly, energy-saving, has a low capital cost, and contributes to a lower formation of inhibitory compounds (Rizal *et al.* 2018; Padilha *et al.* 2019). Hydrothermal pretreatment involves the use of hot water and steam explosion to break down the recalcitrant structures of lignocellulosic biomass while hydrolyzing the cellulose and degrading the hemicellulose and lignin (Ewanick and Bura 2010; Ahmad *et al.* 2018).

The use of hot water entails a temperature of 170 to 230 °C and a pressure of greater than 5 MPa for a minimum of 20 min with ionizing hydronium ion as a catalyst to depolymerize the hemicellulose (Ali *et al.* 2020). Steam explosion involves heating the lignocellulosic materials to a temperature between 160 and 290 °C, at a pressure of 20 bar to 50 bar for 20 min to 40 min (Ahmed *et al.* 2019).

Due to a sudden drop in pressure, steam explosion causes the autohydrolysis of hemicellulose through the explosion effect, hence improving the enzyme digestibility of the cellulose-rich materials (Raita *et al.* 2016). Autohydrolysis is a form of high-pressured

steam pretreatment by heating lignocellulosic materials at a temperature of 121 °C for 20 min, which releases a blackish-brown fraction of cellulose-rich materials and leaves behind hemicellulose and solubilized lignin (Debiagi *et al.* 2020).

The effectiveness of hydrothermal pretreatment in terms of releasing phenols from lignins while yielding reducing sugars is indicated by the severity factor,  $\log R_0$ . This is associated with the synergistic reactions between the temperature and time (Zakaria *et al.* 2015). A study by Mi *et al.* (2016) found that pretreatment of PKC with hot water at a temperature of 180 °C for 20 min yielded 12.5% reducing sugars without furfural formation. The same study also noted that a similar yield of reducing sugars was obtained when PKC was pretreated with hot steam autohydrolysis at temperatures higher than 121 °C for 20 min. Another study reported that subjecting PKC to a steam explosion at a temperature of 163 °C at 6.5 bar for 20 min resulted in a 15.69% yield of reducing sugars with furfural formation (Raita *et al.* 2016). No considerable difference in the total sugar yield was noted between the raw, untreated PKC, and pretreated PKC after autohydrolysis at a temperature of 126 °C for 11 min (Cerveró *et al.* 2010). The same study also demonstrated that untreated PKC and pretreated PKC *via* hot steam autohydrolysis yielded similar amounts of reducing sugars and butanol. It should be noted that pretreating PKC by heating it at a temperature of 180 °C for 10 min will yield a lower amount of reducing sugars compared to untreated PKC due to the degradation of sugars at high temperatures *via* the Maillard reaction (Al-Tabib *et al.* 2017). A case in point, Tafsir *et al.* (2017) found that the autohydrolysis of PKC at a temperature of 121 °C for 15 min recovered as low as 0.91% to 3.25% of the total sugars. Previous studies applied hydrothermal pretreatment followed by enzymatic hydrolysis on PKC to obtain an optimum sugars recovery range of 28.34% to 43.43%, as depicted in Table 2 (Cerveró *et al.* 2010; Jahromi *et al.* 2016; Mi *et al.* 2016; Raita *et al.*, 2016). In general, the hydrothermal pretreatment of PKC is cost-effective, energy saving, and relatively mild, but the sugars recovery is found to be considerably low compared to the alkaline pretreatment; hence, the sugar recovery has to be further optimized *via* enzymatic hydrolysis.

## Acid Hydrolysis

The recalcitrant structure of crystallized cellulose and ligno-polysaccharide linkages have given rise to a variety of severe treatment methods. Amongst these, acid hydrolysis has been the most favorable chemical pretreatment (Rahmati *et al.* 2020). Acid hydrolysis destroys the linkages of lignins and depolymerizes the hemicelluloses to yield high recovery of monomer sugars. This technique can be performed with mineral acids (hydrochloric acid, sulphuric acid, phosphoric acid, nitric acid) as well as organic acids (oxalic acid, acetic acid, formic acid, propionic acid) (Rezania *et al.* 2020). Pretreatment *via* use of mineral acids can be further subcategorized into concentrated and diluted acid methods. Concentrated acid methods involve the use of greater than 30% acid. These methods are less likely to be applied, due to their costly operational procedures, highly corrosive nature, and formation of inhibitory byproducts, *e.g.*, phenolic compounds, aldehydes, 5-hydroxymethylfurfural (HMF), and furfural (Jönsson and Martín 2016).

Many studies have demonstrated that applying dilute acid hydrolysis (less than 10% acid) on a wide spectrum of lignocellulose biomasses results in high sugar monomer recovery and improved hemicellulose structure in terms of enzyme digestibility (Chen *et al.* 2017). A study by Agbor *et al.* (2011) reported that subjecting biomass to a 0.1% dilute sulphuric acid pretreatment resulted in a greater than 80% glucose yield. In addition, a 1.5% dilute sulphuric acid pretreatment on Bermuda grass and rye straw before they were



subjected to enzymatic hydrolysis yielded 19.71% and 22.93% of reducing sugars, respectively (Ranjithkumar *et al.* 2017). A study by Azman *et al.* (2016) demonstrated that the usage of 7% (v/v) dilute sulphuric acid in the pretreatment of PKC released 27.75 g/L of mannose, 2.75 g/L of glucose, and 2.60 g/L of xylose. Another study found that the use of 2% dilute sulphuric acid in PKC treatment yielded 30 g/L of reducing sugars containing 3.78 g/L of glucose and 26.22 g/L of mannose (Al-Tabib *et al.* 2017).

Even though the use of dilute mineral acid is a cheaper technique, it is still corrosive and forms inhibitory compounds; the demerits are similar to its concentrated counterpart (Ahmad *et al.* 2018). Due to the above issues, organic acids, *e.g.*, oxalic acid, acetic acid, and propionic acid, have emerged as environmentally friendly alternatives (Baruah *et al.* 2018). These organic acids are effective in hydrolyzing a wide spectrum of carbohydrate feedstock over different ranges of temperatures and pHs, are less toxic, provides no inhibition during glycolysis, and are odorless because of their multiple *pKa* values (Ranjithkumar *et al.* 2017). Furthermore, inhibitory compounds, *e.g.*, HMF and furfural, are found as trace elements in the organic acid-pretreated hydrolysate (Bukhari *et al.* 2020). Oxalic acid was demonstrated by a study and found that 92% glucose can be recovered from sugarcane bagasse after being pretreated *via* the microwave-oxalic acid method (Solihat *et al.* 2020). In an experiment studying the effects of hydrochloric acid, maleic acid, oxalic acid, and sulfuric acid on the pretreatment of sugarcane bagasse, it was found that the oxalic acid pretreatment yielded the highest maximum percentage yield of 95.7% xylose (Yan *et al.* 2018). In addition, the same observations were seen in a study by Bukhari *et al.* (2020), which found that the use of oxalic acid yielded 67% xylose, making it the most effective organic acid for the acid hydrolysis of oil palm trunk biomass (OPTB). In a similar study, the acid hydrolysis performance of oxalic acid, formic acid, and citric acid were tested on OPTB with xylose recoveries of 61.2%, 20.1%, and 12.9%, respectively. The excellent performance of oxalic acid is due to the release of H<sup>+</sup> from its dicarboxylic structure, which has superior selectivity for the hydrolysis of hemicellulose into monomer sugars, is less toxic, and does not inhibit the glycolysis of bacteria in the latter fermentation process (Song *et al.* 2020). Oxalic acid has been used in various biomass pretreatments. However, few findings demonstrate the effectiveness of oxalic acid and other organic acids for the pretreatment of PKC. To date, only dilute sulphuric acid and acetic acid have been deployed on PKC, which produced a hydrolysate rich in mannose for subsequent biochemical production (Azman *et al.* 2016; Tafsir *et al.* 2017). Therefore, using oxalic acid pretreatment in PKC seems to be a good tradeoff between high sugars recovery yields (mainly mannan) and avoiding the formation of inhibitory compounds. Judging from the aforementioned aspects, oxalic acid pretreatment is preferred for the processing of PKC to achieve desirable purity and yield of lactic acid without requiring enzymes or additional solvents.

### Enzymatic Hydrolysis

Enzymatic hydrolysis is a key performer in subsequent steps to ensure the highest possible concentration of fermentable sugars (Peinemann and Pleissner 2020). It degrades  $\beta$ -mannan in PKC and releases sugar-rich hydrolysate monomers as a carbon source for further use in the fermentation process (Shukor *et al.* 2016; Sathitkowitchai *et al.* 2018). The hydrolysis of mannan involves four types of enzymes: endo(1 $\rightarrow$ 4)- $\beta$ -mannanase, exo(1 $\rightarrow$ 4)- $\beta$ -mannanase or  $\beta$ -mannosidase,  $\beta$ -glucosidase, and  $\alpha$ -galactosidase. Endo(1 $\rightarrow$ 4)- $\beta$ -mannanase depolymerizes mannan, galactomannan, and glucomannan into oligomers, so that  $\beta$ -mannosidase can cleave them into mannobiose.  $\beta$ -glucosidase and  $\alpha$ -

galactosidase then catalyze the mannanose into mannose monomers (Aguzey *et al.* 2020; Peinemann and Pleissner 2020).

The efficiency of mannan-rich PKC hydrolysis has improved with the development of enzyme cocktails that aim to improve the saccharification and stability efficiency through the reduction of end-product inhibitors and lignin binding (Østby *et al.* 2020). Cellic CTec2 from Novozymes, a core enzyme cocktail, is known to exhibit a high efficiency for the saccharification of cellulose and hemicellulose, thus optimizing operational cost-efficiency for converting pretreated PKC into fermentable sugars (Malgas and Pletschke 2020). Malgas and Pletschke (2020) found that the synergistic effects between mannanase and Cellic CTec2 during mannan hydrolysis increased the fermentable sugar yield by 1.3-fold. Another study also found that enzymatically hydrolyzing 5% pretreated PKC with the synergistic effects of mannanase and Cellic CTec2 at a temperature of 50 °C and a pH of at 5250 rpm for 72 h afforded the highest fermentable sugar yield of 28.34 g/L  $\pm$  0.06 g/L (Raita *et al.* 2016).

Saccharification performance can be further maximized by optimizing the enzyme loading, pH, and temperature of the reaction medium, the substrate concentration, and the duration of incubation (Shukor *et al.* 2016). The longer the incubation time, the higher the yield of reducing sugars. However, the yield of sugar will begin to decrease after the optimum incubation time, due to the formation of inhibitory products that degrade the reducing sugars (Solihat *et al.* 2020).

## FERMENTATION STRATEGY FOR LACTIC ACID PRODUCTION

As discussed in the previous sections of the paper, the biochemical conversion of lignocellulosic sugars into organic acids and metabolites includes pretreatment, enzymatic or acid hydrolysis, and fermentation steps. The resulting products and the overall production performance depend on the fermenting microorganisms (Komesu *et al.* 2017). Numerous fermentation strategies are underway to produce high titer lactic acid using different types of biomasses and lactic acid-bacteria, as summarized in Table 3. It is worth mentioning that a high titer of lactic acid is obtained during fermentation using *Actinobacillus succinogenes* by benefiting from its incomplete TCA cycle (Zhang *et al.* 2019).

A study has demonstrated that fed-batch fermentation can produce lactic acid at 1.65 times greater yield than its batch counterpart (Chen *et al.* 2020). Aeration, another factor in the process of fermentation, plays a critical role in maximizing the yield and productivity of lactic acid. It was reported that the early introduction of oxygen-rich aeration followed by deoxygenated air sparged in the dual-phase cultivation strategy promoted a 22-fold increase in the titers of lactic acid (Li *et al.* 2010). The bi-stage fermentation enhances the C3-lactate formation flux, suppresses the C4-succinate formation flux, and enhances the activity of LDH in *Actinobacillus succinogenes* 18-fold without the need for genetic modification (Zhang *et al.* 2019). The initial aerobic cultivation promotes cell growth and viability, while subsequent anaerobic cultivations enhance the high titers of lactic acid (Wang *et al.* 2016).

**Table 3.** Different Biomass Substrates with the Concentration, Yield, and Productivity of Lactic Acid Fermentation Using Different Microbes

Substrate	Microbe	Fermentation Process	Lactic Acid			References
			Concentration (g/L)	Yield (g/g)	Productivity (g/L/h)	
Wheat straw	<i>Bacillus coagulans</i> , MA-13	Batch	32.05	0.92	2.55	Aulitto <i>et al.</i> 2017
Carob	<i>Bacillus coagulans</i>	Batch	48.7	0.84	2.30	Azaizeh <i>et al.</i> 2020
Algal biomass	<i>Bacillus coagulans</i> Azu-10	Batch	102.2	1.0	3.18	Abdel-Rahman <i>et al.</i> 2021
Glucose	<i>A. succinogenes</i> $\Delta$ <i>pflA</i> strain	Fed-batch aerobic-anaerobic dual phase	43.05	0.3	0.72	Zhang <i>et al.</i> 2019
Ricotta cheese whey	<i>Lactobacillus casei</i> DSM 20011	Batch	43	0.536	1.05	Costa <i>et al.</i> 2020
Corn stover hydrolysate	<i>B. coagulans</i> GKN316	Fed-batch	45.39	0.83	0.4725	Jiang <i>et al.</i> 2016
Glucose	<i>A. succinogenes</i> 130Z	Fed-batch dual phase	135.6 $\pm$ 0.14	0.96 $\pm$ 0.09	2.94 $\pm$ 0.03	Li <i>et al.</i> 2010

## PALM KERNEL CAKE AS A POTENTIAL FEEDSTOCK FOR LACTIC ACID PRODUCTION

Palm kernel cake is low cost and locally available material in Malaysia, but its application is not fully satisfactorily acceptable in animal feeding, as it has low palatability and low nutritional value due to the presence of non-starch polysaccharides (NSPs) and being comprised of 15% to 17% crude protein and 16% crude fibre. It is also lacking in a few essential amino acids, *e.g.*, lysine and methionine (Azizi *et al.* 2021). To date, the usage of PKC covers only 30% of ruminants feed and are still dependent on the cost of other protein supplements and the cost of balancing the amino acid proportions. Even though comprehensive studies are underway on assessing new strategies to improve the nutritive value of PKC as animal feed, such as supplementation with enzymes, extrusion, and conversion through fermentation (Alshelmani *et al.* 2021), higher cost and energy will be required for the feed formulation development. As a result, the feed industry in Malaysia is still heavily dependent on imported feeds, which accounts for RM10 billion per year. According to the Department of Statistics Malaysia (2021), approximately 2.53 million tons of the PKC in Malaysia, which accounts for USD 31 million, is exported to India, China, the European Union (EU), and the Philippines (Parveez *et al.* 2021). Approximately 90% of PKC exports are absorbed by the EU bloc, especially Germany and the Netherlands, as components in their livestock feed formulations (Aspar 2001). The abundant availability of PKC in Malaysia from 42 plants crushers with a total capacity of 7.17 million tons of PKC in 2020 should be effectively exploited by reallocating the utilization of PKC to produce higher economical value products in the form of fine

chemicals (Parveez *et al.* 2021). Bio-based lactic acid is currently in the spotlight as a fine-chemical with fast growing application and market demand. Therefore, taking this opportunity to bring in a bigger revenue to the country, process improvements in the biorefinery of PKC allows the industry to move towards the use of greener technology, which aligns with the current needs of the growing global population, modern urbanization, economical development, and a new sustainable living environment.

## CONCLUSIONS

1. Malaysia is one of the few countries with an untapped and underutilized pool of renewable resources in the form of oil palm biomass.
2. The palm kernel cake (PKC) is identified as an excellent feedstock for the production of bio-based lactic acid, due to its abundance, low costs, and high amount of fermentable sugars.
3. This review also analyzes criteria in the selection of promising fermentative microorganisms for the effective conversion of fermentable sugars into bio-based lactic acid.

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