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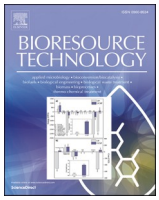

Utilization of agricultural wastes for co-production of xylitol, ethanol, and phenylacetylcarbinol: A review

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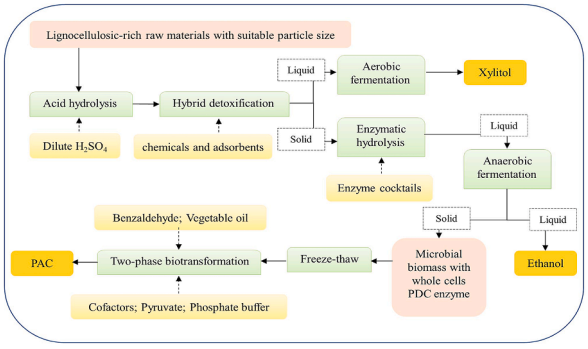
HIGHLIGHTS

- Agricultural wastes as suitable substrates are described.
- Importance of ethanol, xylitol, and phenylacetylcarbinol applications are presented.
- Essential steps for co-production are discussed and reviewed.
- A sustainable, economical, and eco-friendly co-production process is presented.
- Future direction and challenges for industrial scale co-production are described.

GRAPHICAL ABSTRACT



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ABSTRACT

Corn, rice, wheat, and sugar are major sources of food calories consumption thus the massive agricultural waste (AW) is generated through agricultural and agro-industrial processing of these raw materials. Biological conversion is one of the most sustainable AW management technologies. The abundant supply and special structural composition of cellulose, hemicellulose, and lignin could provide great potential for waste biological conversion. Conversion of hemicellulose to xylitol, cellulose to ethanol, and utilization of remnant whole cells biomass to synthesize phenylacetylcarbinol (PAC) are strategies that are both eco-friendly and economically feasible. This co-production strategy includes essential steps: saccharification, detoxification, cultivation, and biotransformation. In this review, the implemented technologies on each unit step are described, the effectiveness, economic

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feasibility, technical procedures, and environmental impact are summarized, compared, and evaluated from an industrial scale viewpoint.

1. Introduction

Cereal grains, particularly corn, rice, and wheat contribute the major proportion of global food production and represent a calorie staple of about 42 % all over the world (Chownk et al., 2019). After cereals, sugarcane is the next most-produced agricultural commodity, accounting for 80 % of global sugar production (Balbino et al., 2023). Extracted sugar is a significant source of carbohydrates in most regions of the world. According to the Food and Agriculture Organization, annual production of cereals and sugarcane is around 2.5 and 1.6 billion tons, respectively (García-Lara and Serna-Saldivar, 2019).

The production of agricultural products generates large amounts of agricultural waste (AW), reaching almost a billion tons per year worldwide (Sharma et al., 2022). However, in the presence of massive AW, traditional management technologies such as open burning and use as soil fertilizer are confronting diverse challenges, including waste and environmental issues. Therefore, an eco-friendly bio-conversion strategy received great attention as it contributes to sustainable waste management and increases the recovery of biobased products from wastes (Piechota et al., 2022).

Previous studies showed that AW could be utilized as raw materials for production of various chemical compounds such as bioplastics, biofuels, biopolymers, biogas, biofertilizers, xylitol, organic acids, and enzymes (Koul et al., 2022). Among these, cellulose-rich materials could serve as valuable raw materials for the manufacture of bioethanol (C_2H_6O) (Mandegari et al., 2018). Like ethanol, xylitol ($C_5H_{12}O_5$) has been widely produced by the bio-conversion of hemicellulose-rich lignocellulosic biomass (Meng et al., 2022; Farias et al., 2022). The incorporation production of xylitol with ethanol through an integrated biorefinery approach is an economic feasibly strategy, which enhances the revenue by 2.3-folds when compared to sole ethanol production (Unrean and Ketsub, 2018; Pant et al., 2022). However, the remnant microbial biomass after ethanol production is usually discarded as waste. In fact, these wastes contain valuable pyruvate decarboxylase (PDC) enzyme which is the key enzyme for decarboxylation step producing acetaldehyde, a penultimate species for ethanol production. PDC can be further employed for the biotransformation process to enhance economic viability. In this regard, the biotransformation of phenylacetylcarbinol (PAC) ($C_9H_{10}O_2$), specifically in *R*-form or *R*-PAC, from pyruvate and benzaldehyde catalyzed by PDC could be achieved.

Valorization of AW through co-production of value-added products such as ethanol, xylitol, and PAC not only mitigates environmental issues by reducing waste but also provides economic advantage by improving profitability. This review is focused on the technology implemented on each unit operation during the co-production process of ethanol, xylitol, and PAC as well as the discussion on effectiveness, economics, and environmental impact of these technologies. Accordingly, the physical and chemical methods employed in saccharification and detoxification processes, microorganisms, and cultivation systems, as well as PDC forms, substrates compositions and operation modes, and two-phase system for subsequent PAC biotransformation are described, compared, and discussed.

2. Ethanol, xylitol, and phenylacetylcarbinol

Ethanol has the largest market share with an annual global production of 29 billion tons and annual sales of 58 billion USD (Rajeswari et al., 2022). Bioethanol is acknowledged as an excellent alternative to fossil fuels such as gasoline and diesel due to its exceptional qualities such as greater octane number, exceptional heat of vaporization, and sustainability. The adoption of bioethanol can mitigate more than 80 % of carbon emissions caused by combustion of fossil fuels. Ethanol-blended gasoline, such as E10 (10 % ethanol) and E85 (85 % ethanol) have been used in several countries to decrease greenhouse gas emissions and dependence on fossil fuels. Ethanol is predicted to become the dominant biofuel for global transportation sectors within the next 20 years (Duarah et al., 2022).

Xylitol has a higher marker price than ethanol (5 USD/kg vs. 0.5 USD/kg) and is one of the highest value bio-based chemicals. It is a sugar alcohol that has gained popularity as a sugar substitute due to its beneficial properties, including its sweetness, low calorie content, and oral health benefits (Gasmi Benahmed et al., 2020). Commercial production of xylitol has expanded due to its health benefits and wide applications in food and pharmaceutical industries. In 2019 the xylitol global market exceeded 880 million USD, and it is further expected to expand beyond 1 billion USD by 2026 (Araújo et al., 2021).

R-PAC, also known by various names, such as 1-hydroxy 1-phenyl-2-propanone, Neuberg's ketol (90–63-1), 1-hydroxy-1-phenylacetone, and a-hydroxy benzyl methyl ketone. It can be considered as a high value chemical (146 USD/kg) and is used as a chiral intermediate precursor to produce ephedrine and pseudoephedrine, epinephrines forms that help loosen smooth muscle, compress blood vessels, and stimulate the central nervous system (Morris et al., 2018). Nowadays, the production of PAC through biotransformation process is getting popular as the methods of direct extraction of ephedrine from *Ephedra* sp. require large cultivation space and chemical synthesis are relatively difficult.

3. Lignocellulosic biomass

3.1. Compositions

AW resulting from corn, rice, wheat, and sugar production includes cob, stalk, husk, leave, bran, straw, top, and bagasse (Table 1). These AWs are composed of cellulose, hemicellulose, and lignin along with other non-structural components such as moisture, ash, and extractives. The three main structural components are present in the following average proportions (% mass basis): 42 % cellulose, 32 % hemicellulose, and 14 % lignin for corn AW; 39 % cellulose, 22 % hemicellulose, and 19 % lignin for rice AW; 33 % cellulose, 27 % hemicellulose, and 17 % lignin for wheat AW; 41 % cellulose, 26 % hemicellulose, and 21 % lignin for sugar AW. Cellulose is the main component followed by hemicellulose, both content account for 60–75 % of the total composition.

3.2. Structure

Lignocellulosic materials, which are composed of complex polysaccharides that must be converted to monosaccharides before they can be utilized by microorganisms. However, this is a challenging task due to their recalcitrant complex three-dimensional structure. Cellulose is a crystalline polysaccharide composed of numerous subunits of glucose linked through β-1,4-glycosidic linkage in same layer, in different layers are connected by hydrogen bond. Hemicellulose is a heteroglycan polysaccharide composed of pentoses and hexoses with branched side chains connecting cellulose and lignin. Lignin is a hydrophobic polymer covering or filling in the gap of cellulose and hemicellulose protects them from microbial and chemical attack and acts as leading barrier in biomass recalcitrance (Sundar and Nampoothiri, 2022). Therefore, destruction of structure and removal of lignin are needed to improve the biomass digestibility by exposing both hemicellulose and cellulose to saccharification process (Tang et al., 2021).

3.3. Particle size

Particle size of lignocellulosic biomass is a crucial determinant for saccharification process, it influences as reaction rate and mass transfer efficiency. Small particles with relatively large surface area per volume ratio are effective in hydrolysis. However, Zhao et al. (2022) found that the effect of the particle size to pretreatment efficiency was weak when the particle size decreased to certain extent, and which require more energy during particle attrition. Particle size is not the smaller the better, optimal balance between particle size and energy consumption is the key factor during assessment of conversion efficiency for lignocellulosic biomass.

Nagarajan et al. (2021) evaluated the impact of corn cob particles sizing in the range of 0.02–2.0 mm on the acid hydrolysis process and found that a significant increase of xylose was obtained for the particle size of 0.5–2.0 mm. In another study, the best degradation efficiency was achieved from corn stalks at the particle size of 0.425–0.85 mm instead of 0.85–2 mm and 0.15–0.425 mm (Zhang et al., 2022a). The particle size reduction process requires intensive energy, maintaining the highest possible particle size without sacrificing sugars conversion efficiency would be preferred in terms of economic viability.

4. Saccharification

4.1. Strategies used to improve saccharification

4.1.1. First-step chemical hydrolysis

Chemical hydrolysis is an essential way to destroy the structure of recalcitrant lignocellulose. In a two-step hydrolysis system, the role of first-step includes disrupting the lignocellulosic matrix, causing the degradation of lignin and hemicellulose. Meanwhile, modification of cellulose crystalline structure allows it to be susceptible to subsequent enzymatic hydrolysis (Zhang et al., 2022c). Table 2 presents an overview of effective alkaline and acid hydrolysis technologies. Evidently, both methods could promote subsequent enzymatic hydrolysis to varying degrees. However, dilute H_2SO_4 hydrolysis is preferred due to its effectiveness, cost-efficiency, and reduced environmental impact.

4.1.2. Second-step enzymatic hydrolysis

In the second-step hydrolysis, alkaline hydrolysis yields a solid fraction that contains cellulose and hemicellulose which can be further hydrolyzed by cellulase and hemicellulase to release glucose and xylose sugars for subsequent ethanol and xylitol production, respectively (Raj and Krishnan, 2020; Pant et al., 2022). On the other hand, dilute acid hydrolyzes hemicellulose to xylose (which is then converted to xylitol). The remaining acidified solid fraction is cellulose-rich, which could be readily broken down by cellulase into glucose for ethanol production. Therefore, acid hydrolysis only requires one-stage enzymatic hydrolysis, and thus is more economical and simpler compared to alkaline hydrolysis, which requires two-stage enzymatic hydrolysis. It is worth noting that the high cost of enzymes of 0.68–1.47 USD/gal ethanol remains a challenge for industrial-scale implementation (Zhao et al., 2021). Thus, enzymatic hydrolysis was mainly (82 %) conducted using commercial enzyme cocktails, which are more economically feasible and commonly used in large-scale processes (Gonçalves et al., 2022; ; Singh et al., 2022c).

5. Inhibitors formation, categorization, and detoxification

5.1. Inhibitors formation and categorization

Under high temperature and acidic conditions, lignin is degraded to aromatic compounds such as vanillin, syringaldehyde, and conferyl aldehyde. Once the pentose sugars (xylose) are released, they undergo further chemical reactions. Xylose is involved in dehydration reactions where removal of water molecules is observed with the subsequent formation of furfural. The conversion of hexose sugars proceeds via isomerization and dehydration routes through either cyclic or acyclic structures to the intermediate hydroxymethyl furfural (HMF). HMF is subsequently hydrolyzed to form both levulinic and formic acids (Guo et al., 2022). Acetic acid is generated when the acetyl groups in the hemicellulose are hydrolyzed due to deacetylation reaction of acetylated pentosane (Agrawal et al., 2021) (see Supplementary Materials). These inhibitors are known to interfere with enzymatic hydrolysis, reduce fermentation efficiency (Agrawal et al., 2021; Guo et al., 2022) or inhibit fermentation totally (Saadatinavaz et al., 2021; Romero-García et al., 2022). According to their chemical nature and origin, these inhibitors can be categorized into three groups of phenolics, furaldehydes, and aliphatic acids (see supplementary materials). Although individual effect of each biomass-derived inhibitor in the hydrolysate is low enough for biocatalysts to tolerate, the combined or synergistic effect of these inhibitors should not be overlooked or considered insignificant.

5.2. Technologies used to improve detoxification

5.2.1. Single detoxification

During previous studies, three detoxification mechanisms were adopted to remove inhibitors. The biological approach exploits the natural abilities of microbial cells and enzymes to degrade, metabolize, or convert inhibitors into less inhibitory or harmless compounds. Physical detoxification, such as adsorption and evaporation, utilizes the absorptivity and volatility nature of some inhibitors to remove them. Chemical detoxification such as overliming by adjusting pH alters the ionization property of the inhibitors to form precipitates. The major methods as well as their advantages and drawbacks are shown in Table 3. The biological method is relatively costly and time consuming compared to the physical and the chemical methods. The capability of the methods to eliminate major inhibitors ranging from high to low is as follows: (acetic acid) resin > evaporation > overliming and activated charcoal; (furfural + HMF) resin and activated charcoal > overliming

Table 1
Structural composition of agricultural wastes from corn, rice, wheat, and sugar production (wt %).

Foods	Wastes	Cellulose	Hemicellulose	Lignin	References
Corn	Cob	35.2–52.0	32.5–45.7	6.8–19.6	(Kucharska et al., 2020; Sun et al., 2022)
	Stalk	36.9–50.4	20.4–21.2	17.4–27.1	(Li et al., 2019; Zhang et al., 2021)
	Husk	29.3–54.7	27.1–39.7	8.8–11.4	(Ponce et al., 2021)
	Leave	33.6–44.4	25.0–40.9	5.2–14.4	(Aboagye et al., 2017; Xu et al., 2019)
Rice	Straw	32.0–38.6	19.7–35.7	6.2–22.3	(Chen et al., 2021)
	Husk	35.0–49.6	10.4–20.0	20.0–25.0	(Kumar et al., 2009; Ponce et al., 2021)
	Bran	38.6–42.0	21.3–26.0	21.1–22.0	(Sivaramakrishnan et al., 2021; Chen et al., 2021)
Wheat	Straw	25.0–45.0	20.0–45.0	8.0–26.0	(Zhang et al., 2022b)
	Husk	23.0–41.7	18.0–21.0	14.0–28.3	(Terzioğlu et al., 2019)
	Bran	30.0–31.1	25.0–34.3	8.0–16.3	(Xiao et al., 2019; Cingöz et al., 2023)
Sugar	Top	33.3–43.0	18.4–28.9	14.0–31.8	(Khaire et al., 2021; Khaire et al., 2022)
	Leave	38.7–53.8	23.6–34.4	13.3–18.9	(Palliprath et al., 2020)
	Bagasse	30.7–45.0	20.0–32.0	17.0–32.0	(Kumar et al., 2021)

Table 2
Comparison of chemical hydrolysis methods with their advantages and disadvantages.

Methods	Catalysts	Conditions	Advantages	Disadvantages
Alkaline hydrolysis	Sodium bisulfite Ammonium hydroxide Sodium hydroxide Potassium hydroxide (KOH)	2–20 % 30–120 ℃ 30 min-48 h	58.3–89.3 % delignification. 79.7–81.0 % enzymatic saccharification efficiency. Alkali hydrolysate can be recycled several times for pretreatment. Low energy requirement.	52.9–75.4 % hemicellulose dissolution. 22.0 % cellulose dissolution. Amorphous cellulose is more sensible to higher concentration of chemicals. 0.48–0.90 g/L furfural and phenolics formation. Wastewater generated by high alkali requires additional treatment cost. Low efficiency for recycled pretreatment.
Acid hydrolysis	Sulfuric acid (H_2SO_4) Hydrogen chloride Maleic acid Humic acid	0.4–1.5 % 120–180 ℃ 15–90 min	77.1–96.2 % hemicellulose solubilization. 63.4–92.9 % enzymatic saccharification efficiency. Structural linkages loosen between hemicellulose and lignin. Maleic acid can be recovered. Extra humic acid can improve lignin removal to 40.6 %. The price of H_2SO_4 is as low as 18.5 times to KOH. No need wastewater handling.	1.60–3.94 g/L acetic acid, 0.45–4.30 g/L furfural and HMF in acid hydrolysate. Energy consumption of H_2SO_4 hydrolysis was 82 % higher than KOH hydrolysis. Low lignin removal.

Source: Goli and Hameeda (2021); Raj and Krishnan (2020); Goshadrou (2019); Patel et al. (2022a); Dionísio et al. (2021); Cai et al. (2021); Liu et al. (2021); Scapini et al. (2021).

Table 3
Detoxification methods and their advantages and drawbacks.

Categories	Methods	Advantages	Drawbacks	References
Biological	*S. cerevisiae* Laccases	Acetic acid, levulinic acid, HMF and furfural were reduced to almost 0. Minimizing environmental issues.	115 h detoxification period.Other compounds present in the hydrolysate (such as salts) might inhibit to a certain extent the enzyme activity. High cost.	(González-García et al., 2019; Romero-García et al., 2022)
Physical	Anion-exchange resin, Macroporous adsorption resin	70–90.2 % acetic acid, 92.6–100 % furfural, and 94.9 % lignin removal.	22.5 % glucose, 15.8 % xylose, and 19.5 % arabinose loss.	(Han et al., 2022; Ma et al., 2022)
	Activated charcoal	HMF and furfural can be completely removed.	14–18 % sugar loss. Only 37 % acetic acid removal. A rise in activated carbon dosage also enhanced the loss of fermentable sugar.	(Araújo et al., 2021; Kusmayadi et al., 2023)
	Evaporation	~60 % acetic acid, ~75–98 % furfural, and 33 % HMF removal. No sugar loss.	Non-volatile inhibitors accumulation. Energy and time consumption.	(Mushtaq et al., 2019; Nascimento et al., 2023)
Chemical	Calcium hydroxide (Ca$(OH)_2$) NaOH	10.8–45.1 % acetic acid, ~60 % furfural, 50 % HMF, 25.3 % phenolic, and 59.5 % 2-cyclopenten-1-one removal	23 % glucose and 9 % xylose loss. Generates gypsum (calcium sulfate). Only minor decreases in guaiacol and phenol.	(dos Santos Vieira et al., 2021; Kubisch and Ochsenreither, 2022)

and evaporation; (phenolics) resin and activated charcoal > overliming, whereas the sugar loss is resin and activated charcoal > overliming > evaporation. As each method has its own unique characteristics, the challenges that lie ahead can include development of a stand-alone detoxification process that efficiently removes inhibitors while minimizing sugars loss or additional negative impacts.

5.2.2. Hybrid detoxification

Currently, the development of hybrid methods is an active area of research. Many researchers have tried to combine two different methods, especially combining physical and chemical methods. For example, the hydrolysate of hybrid poplar which was subjected to single detoxification method of overliming or activated charcoal treatment was not fermentable. However, implementation of combined methods showed a significant improvement in fermentability with decreased sugar loss (Zhang et al., 2018). Similarly, acid hydrolysate of *Parthenium hysterophorus* resulting from sequential chemical detoxification by overliming with $Ca(OH)_2$ followed by activated charcoal treatment exhibited, 86.3 % and 94.2 % reduction in furan and total phenols respectively, and 4.5 % loss of reducing sugars (Bharti et al., 2022). As detoxification is a key step before fermentation, further study is required to address specific inhibitor types and concentrations.

6. Microorganisms, enzymes, and cultivation

6.1. Microorganisms

6.1.1. Xylitol production

Among the microbial strains investigated for industrial xylitol production, species belonging to the genus *Candida* are considered to be promising candidates, namely, *C. tropicalis*, *C. magnoliae*, *C. guilliermondii*, and *C. boidinii* (Kumar et al., 2022a). Among the yeast species listed, *C. tropicalis* is known to be industrially important, inhibitor tolerant, and capable of utilizing pentose and hexose sugars. This strain was used for evaluating scale-up batch production utilizing rice straw hydrolysate with a decent xylitol yield (60 %) (Singh et al., 2021). Thereafter, Singh et al. (2022b) screened 124 yeast isolates using organic wastes from various habitats and found that *C. tropicalis* showed the highest potential for xylitol production as well as inhibitors tolerance with the yield and productivity of 0.90 g/g and 1.5 g/L.h, respectively. Improved xylitol production using *C. tropicalis* and xylose derived from various hemicellulosic hydrolysates has been reported (Erian and Sauer, 2022; Balbino et al., 2023).

6.1.2. Ethanol and phenylacetylcarbinol production

Currently, both *S. cerevisiae* and *C. tropicalis* have been widely employed for ethanol production and PAC biosynthesis at a commercial scale. Agustina et al. (2009) screened 15 microbial strains using a mixture of longan extract and molasses as carbon source and found that *S. cerevisiae* was an appropriate strain for large scale production of ethanol and whole cells biocatalyst. Nunta et al. (2018) found that *S. cerevisiae* produced ethanol at a significantly higher level than *C. tropicalis* using longan juice, whereas the latter yields 2.1-folds higher PDC activity and 1.5-folds higher PAC concentration than former. Thereafter, Nunta et al. (2023) evaluated the production potential of ethanol and PAC using AWs cultivated with *C. tropicalis, C. shehatae*, *S. cerevisiae, and K. marxianus* and found that rice straw cultivated with *C. tropicalis* produced significantly higher ethanol concentration of 15.3 g/L and volumetric PDC activity of 0.303 U/mL. Meanwhile, He et al. (2022) found that some unique functional genes in *C. tropicalis,* which regulate nitrogen and carbon metabolisms, can also be involved in phosphorus metabolism.

6.2. Pyruvate decarboxylase related ethanol and phenylacetylcarbinol production

PDC (EC 4.1.1.1) and alcohol dehydrogenase (ADH; EC 1.1.1.1) are two critical enzymes involved in the conversion of pyruvate to ethanol during fermentation (Panahi et al., 2022). PDC catalyzes the decarboxylation of pyruvate to active acetaldehyde, which is then reduced to ethanol by ADH. PDC abundance has been shown to correlate with ethanol concentration, and its reaction is considered the first, irreversible, and main rate-limiting step in ethanol production in most studies (Luan et al., 2015). PDC can be produced from fungi such as *Aspergillus nidulans*, *A. parasiticus*, *Neurospora crassa* (Troiano et al., 2020), yeast such as *C. utilis*, *C. tropicalis*, *Saccharomyces cerevisiae*, and *Kluyveromyces marxianus* (Gunawan et al., 2007), as well as bacteria such as *Zymomonas mobilis*, *Sarcina ventriculi*, and *Acetobacter pasteurianus* (Liu et al., 2007; Yun and Kim, 2008; Kumar et al., 2022b). Among these, yeast PDC is preferable over bacterial PDC because the latter is more susceptible to inhibition by substrates and products, as well as to deactivation by benzaldehyde (Mushtaq and Mukhtar, 2022).

Zhang et al. (2022d) found that PDC activity and ethanol conversion decreased by 40.91 % and 30.19 % respectively when the *pdc*1 and *pdc*5 double knockout genes of *S. cerevisiae* were employed. Cui et al. (2020) found that adding extra PDC to a final concentration of 3 U/mL significantly reduced ethanol concentration, especially at earlier time points. Although the relationship between ethanol and PDC is inconclusive, PAC production is directly proportional to PDC enzyme activity level up to 5.0 U/mL carboligase (Leksawasdi et al., 2004; Agarwal et al., 2015). The PAC concentration was increased 2-fold when the initial volumetric PDC activity increased 3-fold (Nunta et al., 2023).

6.3. Different systems used to improve cultivation

6.3.1. Microbial cultures and fermentations systems

S. cerevisiae and *C. tropicalis* co-culture strategy as well as glucose and xylose co-fermentation strategy using *C. tropicalis* have been employed to simplify the fermentation process, lower cost, and maintain pentose and hexose sugars utilization efficiency (Table 4). Theoretically, higher ethanol and xylitol yields should be obtained in such a system because *S. cerevisiae* or *C. tropicalis* could utilize glucose to produce ethanol rapidly while allowing *C. tropicalis* to produce xylitol from xylose. However, the utilization of other carbon sources in the presence of glucose is often suppressed in yeast especially xylose (Du et al., 2020; Wu et al., 2021; Dong et al., 2023). The rapid growth of *S. cerevisiae* or *C. tropicalis* caused high ethanol concentration, nutritional deficiency, and anaerobic conditions can limit xylitol production. In addition, the whole cells biomass from co-culture of *C. tropicalis* and *S. cerevisiae* could produce a higher level of PAC in single phase emulsion system comparing with individual strain (Kumar et al., 2023).

6.3.2. Enzymatic saccharification and fermentation systems

Detoxified cellulose-rich residues can be converted to ethanol by undergoing two separate processes of enzymatic hydrolysis and fermentation (SHF). In this regard, an attractive strategy developed by combining two processes, simultaneous saccharification and fermentation (SSF), has the advantage of involving only a single reactor with lower investment cost, substrate inhibition level, energy input, and processing time (Singh et al., 2022a). However, the suboptimal temperature (Banat et al., 2021), presence of insoluble inhibitors (Patel et al., 2022b), as well as enzymes and cells binding and recycling problems resulting in decreased activity and overall process efficiency (Muñoz et al., 2022). Patel et al., 2022b found that bioethanol production by both SSF and SHF of bagasse hydrolysate resulted in similar fermentation performance with ethanol concentration of approximately 55 g/L. Wen et al. (2023) compared SHF and SSF processes using the hydrolysate of delignified and deacetylated poplar and found that the higher ethanol yield of 85.2 % was achieved by SHF compared to 68.6 % of SSF.

6.3.3. Batch, fed-batch, and continuous systems

Batch, fed-batch, and continuous systems have been commonly used in cultivation processes. Jain et al. (2023) found that the fed-batch fermentation can enhance xylitol production and resulted in significant high xylitol concentration, yield, and productivity of 86.76 g/L, 0.87 g/g, and 2.07 g/L.h, respectively compared to batch fermentation. Saini. (2023) compared ethanol production under batch and fed-batch SSF indicating that the latter is an obviously better option for large scale bioethanol production at higher solid loading and biomass containing inhibitory compounds. Nunta et al. (2019) elucidated that the highest ethanol concentration in a 10 L continuous process (34.3 g/L) was significantly higher than that in 100 L batch process (13.2 g/L).

Table 4
Microorganisms and cultivation systems for ethanol and xylitol production.

Microorganisms	Cultivation systems	Ethanol			Xylitol			References
		Titer g/L	Yield g/g	Qp* g/L.h	Titer g/L	Yield g/g	Qp* g/L.h	
C. tropicalis	Co-fermentation	48.0	n.d.	1.00	30.6	n.d.	0.64	(de Souza Queiroz et al., 2021)
C. tropicalis	Co-fermentation	1.50	0.31	0.10	12.0	0.61	0.38	(Antunes et al., 2021)
C. tropicalis	Separate-fermentation	55.6	0.94	0.48	34.5	0.83	0.86	(Raj and Krishnan, 2020)
S. cerevisiae-C. tropicalis	Co-culture	33.4	0.44	1.34	25.1	0.55	0.42	(Zahed et al., 2016)
S. cerevisiae-C. tropicalis	Separate-culture	6.18	0.41	0.09	13.1	0.39	0.12	(Shankar et al., 2020)
S. cerevisiae-C. tropicalis	Separate-culture	8.10	0.45	0.11	11.2	0.37	0.10	(Shankar et al., 2020)
S. cerevisiae-C. tropicalis	Separate-culture	56.1	0.44	0.58	24.0	0.50	0.25	(Unrean and Ketsub, 2018)
S. cerevisiae-C. tropicalis	Separate-culture	6.90	0.43	n.d.	6.15	0.65	n.d.	(Goli and Hameeda, 2021)

* Productivity is calculated from total titer of product per total production time.
n.d. No data or information available.

Batch cultivation was generally applied to an enclosed small scale or a solid-state fermentation process due to the relative simplicity and shorter cultivation time depending on each type of microorganism. In continuous culture, the overall productivity was greater than in batch and fed-batch culture. The impact of substrate inhibition on the product formation can effectively be brought down. In the industrial fermentation process, continuously stirred bioreactors have proven history of practical usages (Anand and Srivastava, 2022).

7. Biotransformation

7.1. Mechanisms

PDC is a thiamine pyrophosphate (TPP) and Mg^{2+} depending non-oxidative enzyme, which is well known for catalyzing the biotransformation of benzaldehyde and pyruvate into PAC (see Supplementary Materials). The active acetaldehyde, the intermediate generated from the pyruvate at the active site of PDC, can undergo two different fates in the PAC biotransformation pathway. One possible fate is it can react with benzaldehyde to produce PAC. The other possible fate is that it can be recombined with released free acetaldehyde molecule to produce a by-product called acetoin (Leksawasdi et al., 2004; Agarwal et al., 2015). In addition, the by-products, benzyl alcohol and PAC-diol (1-phenyl-1,2 propanediol) can be catalyzed by ADH enzyme from the reduction of benzaldehyde and PAC when biotransformation is performed in parallel with microbial cultivation process (Nunta et al., 2018). These side reactions mitigate the PAC production rate by inhibiting the PDC activity and permeability of cells. An ideal PAC biocatalyst should therefore have high PDC activity, low ADH activity with high tolerance to benzaldehyde, benzyl alcohol, and PAC.

7.2. Strategies used to improve biotransformation

7.2.1. Different pyruvate decarboxylase forms

PAC biotransformation could be conducted *in vivo* via direct microbial transformation using growing cells, or *in vitro* using whole cells biomass and partially purified PDC enzyme. Table 5 summarizes different PDC forms and their advantages and drawbacks in PAC biotransformation. In fact, complete conversion of benzaldehyde to PAC is impossible in the live cells system because of the side reactions. The immobilization and encapsulation procedures require intensive labor and capital investment compared to the freeze-thawing method. Meanwhile, the materials need to be evaluated for effectiveness, toxicity, price, and potential environmental pollution. It is noteworthy that compared to partially purified PDC form, the environmental condition for whole cells biomass PDC is more closely related to that of live cells hence allowing for a better *in situ* physiological reaction in producing PAC without the PDC refolding effect from crystallized form or addition of cofactors (Kim et al., 2022).

7.2.2. Substrates compositions and operation modes

Total PAC production is influenced by PDC activity, as well as pyruvate and/or benzaldehyde concentration, the latter being actually the rate-limiting factor. Using partially purified PDC (3.0 U/mL), the Michaelis-Menten kinetics and a sigmoidal-type relationship between initial rates of PAC formation as well as pyruvate and benzaldehyde concentrations are evident in the ranges of 10–250 mM and 0–150 mM, respectively. These substrate ranges are unlikely to cause PDC inhibition (Leksawasdi et al., 2003; 2004). Moreover, the molar ratio of initial pyruvate to benzaldehyde concentrations of 1.2/1.0 was suggested to compensate for possible pyruvate loss to acetaldehyde and acetoin (Leksawasdi et al., 2004; 2005a; 2005b).

A higher PAC concentration can be obtained by either batch or fed-batch mode. Based on the substrate concentration ranges and optimal ratio, PAC concentration of 95.8 mM was achieved by 120/100 mM pyruvate/benzaldehyde batch biotransformation using partially

Table 5
PDC forms and their advantages and drawbacks in PAC biotransformation.

PDC forms	Advantages	Drawbacks	References
Live cells	Easy handling. Inexpensive glucose and other sugars can be employed as carbon source instead of pyruvate. Recycle yeast cells. Membrane protects its endogenous enzymes from substrate inhibition. Presence of intracellular natural factors decreases the need for expensive external cofactors.	Substrate toxicity towards cells viability, PAC production follows the major drop in cell viability. Low efficiency of substrate utilization. Significant amounts of byproducts, especially benzyl alcohol and PAC-diol, could be produced with nearly twice the PAC production rate. Increased cost for the overall process.	(Rogers et al., 1997; Andreu and del Olmo, 2014; Andreu and · lí del Olmo, 2018; Lee et al., 2018)
Whole cells biomass	Simple and economical preparation. Freeze-thaw sequence exerts detrimental effect to ADH structure and activity. PDC stability and activity can be maintained because of the membrane protection.Presence of natural cofactors. No formation of by-products such as acetoin and benzyl alcohol.	Production of some by-products such as acetaldehyde and acetoin.	(Rosche et al., 2005; Gunawan et al., 2007; Nunta et al., 2018; Nunta et al., 2023)
Partially purified PDC	Eliminates certain by-products from side reaction. Recycled and reused during a biotransformation process. Higher substrates utilization rate. Higher PAC formation rate.	High cost of purification and loss of > 90 % of initial PDC activity prior purification step. The vulnerability of exposed PDC enzyme being attacked by toxic benzaldehyde. Necessity of co-factors addition as cofactors are lost through purification process	(Ward and Singh, 2000; Rosche et al., 2002; Khemacheewakul et al., 2021)
Immobilized whole cells and PDC	Easily separated and reused. Storable and recyclable. Reduce exposure to benzaldehyde, improved tolerance of the cells to toxic media and high temperature. Allow high substrate load.	Mass transfer limitation. Inhibitory or toxic products removal from environment. Biomass loading and adhesion strength. Tedious preparation steps.	(Khan and Daugulis, 2011; Doostmohammadi et al., 2016; Seifi et al., 2020)

PDC forms and their advantages and drawbacks in PAC biotransformation.

purified PDC (1.1–1.5 U/mL) (Khemacheewakul et al., 2021). Similarly, a maximum PAC concentration of 300 mM was reached by the fed-batch operation with initial substrate concentrations of 108 mM sodium pyruvate and 90 mM benzaldehyde at an initial carboligase activity of 4 U/mL. However, the increased PAC concentration and associated by-products (acetoin and acetaldehyde) resulted in significant inhibition of PDC during the course of the biotransformation (Leksawasdi et al., 2006).

7.2.3. Two-phase biotransformation system

A two-phase biotransformation system consisting of aqueous and organic solvents has become popular. In this system, benzaldehyde and PAC are partitioned into the organic phase while the buffering aqueous phase contains the PDC enzyme and pyruvate thereby mitigating enzyme deactivation effect (Rosche et al., 2005) (see Supplementary Materials). In addition, it is a useful system for extracting non-polar products, in this case - PAC, when compared to a single aqueous phase system. The non-polar PAC can be separated from polar components such as pyruvate and buffering species, and hence avoiding the relatively more complicated process of extracting PAC from the single-phase system (Leksawasdi et al., 2005a).

7.2.3.1. Organic phase. Studies have investigated various organic solvents considering types of solvents, mass transfer coefficient, partitioning ratio, impact on enzyme activity and cost. These organic compounds are methanol (C1), ethanol (C2), propanol (C3), butanol and ethyl acetate (C4), pentanol, menthyl and tertiary-butyl ether (C5), hexanol (C6), heptanol, heptane, toluene and methylcyclohexane (C7), octanol and octane (C8), nonanol and nonane (C9), decanol and dodecane (C10), hexadecane (C16) and dipropylene glycol (DPG) as well as solvent mixtures of DPG + C1, DPG + C2, DPG + C3, DPG + C7, DPG + C8, or DPG + C9. In fact, certain organic compounds such as C3, C4 and C6 could contribute to enzyme inactivation with evidence of high cross solubility. C8 and C10 exhibited low distribution towards water without observable interphase inactivation (Mack et al., 2021). C5 and C16 played important roles in stabilizing PDC activity (Rosche et al., 2004). The positive effects on PAC production by using a mixture of inexpensive DPG with short chain (C1 - C3) and with longer chain alcohol such as heptanol (C7) and octanol (C8) were also observed (Leksawasdi et al., 2005a; Agustina et al., 2009). Octanol (C8), in particular, was elucidated as the most suitable solvent for PAC production in the two-phase system due to the relatively high partitioning coefficient (log P) of benzaldehyde in octanol (log P = 1.71) (Gunawan et al., 2008). The facilitating value of the log P allowed delivery of high benzaldehyde concentration from organic into aqueous phase.

Ionic liquids (ILs), especially hydrophobic type, with capability to stabilize biocatalyst activity in the absence of deactivation effect is considered an alternative green compound to the organic solvents (Khoo et al., 2021; Imam et al., 2021). The hydrophobic ILs 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) with a relatively high value of log P = 56 for benzaldehyde was chosen as the non-aqueous phase in PAC biotransformation system utilizing *S. cerevisiae* cells. A good biocompatibility in the absence of an inhibitory effect on PDC activity was observed (Kandar et al., 2015). However, ILs could exert detrimental effects to specific biotransformation systems and environments, if not properly chosen. These included some physical and economical limitations that could restrict industrial scale application such as high viscosity and high costing (Yadav et al., 2023). Due to the higher costs of octanol and ionic liquids as well as potential environmental pollution, vegetable oil (1.0–2.0 USD/kg) could be a low cost and eco-friendly organic phase alternative with a significant potential for large-scale application in the two-phase system. For example, palm oil was implemented as the organic phase in PAC biotransformation (Nunta et al., 2018; Nunta et al., 2023), the substrates utilization rate was double that of octanol (Sandford et al., 2005). Sunflower oil was also reported as a suitable organic phase in phytosterol biotransformation (Xu et al., 2014) and bioconversion of limonene to terpineol (Bicas et al., 2010) in a two-phase system.

7.2.3.2. Aqueous phase. 2–2.5 M of 3-[N-morpholino] propanesulfonic acid (MOPS) and phosphate buffers are typically used during PAC biotransformation. MOPS buffer significantly improved long term PDC stability compared to phosphate buffer (Leksawasdi et al., 2003), and the high concentration of MOPS buffer can increase PDC stability and PAC concentration. For instance, PAC production in 20 mM MOPS buffer under controlled pH conditions was relatively low compared to 2.5 M MOPS buffer (Leksawasdi et al., 2005a; Gunawan et al., 2007). However, the heat labile property and cost prohibitive nature (1.09 USD/g) of MOPS are major obstacles in the industrial application.

Phosphate buffer (0.02 USD/g) is a low-cost alternative to MOPS. Khemacheewakul et al. (2018) found that PAC production in 1.0 M (20 mM − 1.0 M) phosphate buffer was able to enhance PAC production up to 28.6 mM with the evidence of additional activation effect to PDC stability and absence of PAC-diol formation. Although phosphate is a competitive inhibitor of yeast PDC and decreasing its affinity for pyruvate, the application of this enzyme in the form of whole cells biomass may not be affected by the high phosphate buffer concentration as PDC is an intracellular enzyme. Another concern regarding the use of phosphate buffer is its environmental impact. Released phosphate ions cause eutrophication that damages marine ecosystems (Ai et al., 2022). Leksawasdi et al. (2021) estimated that implementation of phosphate buffer would cost 0.751 USD/kg of produced PAC in the biotransformation system that utilizes whole cells biomass and recycled buffer. Therefore, with efficient buffer recycling and waste treatment strategies, phosphate buffer could be a suitable low-cost alternative to MOPS.

7.2.3.3. Volume ratio of organic phase and aqueous phase. Since PDC enzyme resides in the aqueous phase while non-polar substrate and products are mainly dissolved in the organic phase, the reaction mainly occurs at the two-phase interface (Guan et al., 2022) (see Supplementary Materials), which affect conversion equilibrium, biocatalytic reactions, and enzyme deactivation rates. By adjusting the organic/aqueous volume ratio or exerting strong mixing, large interfacial area of the emulsion system can be established and therefore enhance mass transfer. Evidently, benzaldehyde utilization and PAC production rates in the emulsion system were 10 and 8-folds faster than those in phase-separated system, respectively (Sandford et al., 2005). The 0.43:1 octanol/MOPS buffer emulsion system yields a higher PAC concentration with unaffected PDC activity compared to 1:1 and 0.67:1, while the 0.25:1 ratio proved impractical due to organic phase separation problem (Gunawan et al., 2008). The optimal ratio of 0.43:1 was also evident in the hexane/citric buffer emulsion system for dianisylideneacetone biotransformation (Schaefer et al., 2013) as well as in the carbon tetrachloride/phosphate buffer for the bioconversion of methyltestosterone to methandienone (Bie et al., 2008). The utilization of a small volume ratio could reduce production cost, especially for expensive solvents. In summary, a suitable volume ratio depends on types of solvents, products, and substrates, as well as their potential interactions with the chosen source of enzyme.

8. Future directions and challenges

8.1. Future directions

Xylitol, ethanol, and PAC are desired high value-add products derived from lignocellulosic biomass. From environmental and economic point of view, a novel co-production strategy can be achieved as shown in the production process flow diagram (see Supplementary Materials). Effective conversion of polysaccharides to fermentable sugars can generally be obtained by two-steps hydrolysis involving

dilute H_2SO_4 and commercial enzyme cocktails. These could provide advantages of less investments on energy, labor, chemical, and downstream process handling. The hybrid detoxification using inexpensive chemicals and adsorbents could improve enzymatic hydrolysis efficiency and fermentability of hydrolysates. Additionally, SHF operation could alleviate the binding problems between lignin with enzymes / cells components commonly found in SSF process. After enzymatic hydrolysis, the solid residue rich in lignin can be further depolymerized and upgraded into value-added fuels and chemicals such as bio-oils for use as aviation and marine biofuels, chemicals harnessing the aromaticity and oxygenation of the lignin moieties (Menezes et al., 2023). The cultivation of suitable yeast in a separate-fermentation system can result in abundant production of xylitol and ethanol with economically viable products over substrate yields as well as relatively high PDC activity. One eco-friendly and economical promising high PAC biotransformation system is vegetable oil / high phosphate buffer two-phase emulsion system which employs whole cells biomass PDC possessing both low ADH activity and lesser degree of PDC inactivation. Although the advantage of this system is clearly pronounced, the management of leftover chemicals might be cumbersome after biotransformation. In fact, certain phase of reaction and chemicals such as vegetable oil and benzaldehyde in two-phase emulsion system could also be reused in a multiple pass system (Kumar et al., 2023). In such a strategy with the objective of maximizing waste utilization and profitability, the incorporated production of three value-added products has a significant potential.

8.2. *Challenges for industrial scale co-production*

Scaled up co-production of ethanol, xylitol, and PAC are necessary steps to ascertain products availability to consumers at relatively reasonable costs. However, there are some aspects that must be resolved or optimized prior to industrial scale production such that the cost effective of the process is realized.

Firstly, improved alternative methods to increase sugar concentrations of the hydrolysates should be considered. High initial sugar concentration is generally essential to achieve high yield and productivity process (Saini et al., 2022). The methods of thermal evaporation and pressure-driven membrane processes such as membrane filtration and reverse osmosis have been used to increase sugar concentration (Sarbatly et al., 2023). Although an evaporation process is a well-established and cost-effective method for concentrating sugar solutions, significant energy input and mass transfer problems could render it not feasible on an industry scale. Low-cost and low-energy requirements render membrane technology an attractive choice for industrial scale adaptation.

Secondly, sugars mixture fermentation (especially between glucose and xylose), sugars purification, and optimal separation methods should be thoroughly investigated. Simple unit operations such as evaporation, distillation, crystallization, and membrane separation have been employed previously. However, only 6 % of the industrial sector implemented these as sophisticated equipments are generally required to separate individual sugar from the hydrolysates (Gonçalves et al., 2022). The persistent sugars mixture problem is still commonly encountered with simple physical separation equipment (Nandal et al., 2020).

Thirdly, the strategies used to lower the total biotransformation cost must be considered. The organic phase was confirmed to be more favorable for PAC production and the possibility of organic recycling has been proposed. The total cost could be mitigated to some extent by recycling certain chemicals such as vegetable oil and benzaldehyde for the second and third passes of the biotransformation (Kumar et al., 2023). However, the laboratory settings to industrial scale are required to develop an appropriate system such that the total operating and capital costs in establishing the series of bioreactor could be minimized further.

9. Conclusions

AW is a suitable substrate for bioconversion of value-added products. The review and analysis of each processing step revealed that ethanol, xylitol, and PAC could be produced through a sustainable, economical, and eco-friendly strategy. This co-production strategy can be further applied to a wide variety of lignocellulosic materials to achieve the objective of zero waste and to increase the economic value of waste. Meanwhile, the environmental issues caused by traditional waste management can be solved. Transitioning from lab-scale to industrial-scale production while maintaining high yields, efficiency, and product quality requires further study.

E-Supplementary Data for this work can be found in e-version of this paper online.

CRediT authorship contribution statement

Juan Feng: Conceptualization, Investigation, Visualization, Writing – original draft. **Charin Techapun:** Writing – review & editing. **Yuthana Phimolsiripol:** Writing – review & editing. **Suphat Phongthai:** Writing – review & editing. **Julaluk Khemacheewakul:** Writing – review & editing. **Siraphat Taesuwan:** Writing – review & editing. **Chatchadaporn Mahakuntha:** Writing – review & editing. **Krisadaporn Porninta:** Writing – review & editing. **Su Lwin Htike:** Writing – review & editing. **Anbarasu Kumar:** Writing – review & editing. **Rojarej Nunta:** Writing – review & editing. **Sumeth Sommanee:** Funding acquisition, Writing – review & editing. **Noppol Leksawasdi:** Formal analysis, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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