Engineering Microbiology 4 (2024) 100139

Contents lists available at ScienceDirect

Engineering Microbiology

journal homepage: www.elsevier.com/locate/engmic

Review

Research advances on the consolidated bioprocessing of lignocellulosic biomass

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ARTICLE INFO

Keywords:
Lignocellulosic biomass
Biorefinery
Consolidated bioprocessing
Biochemicals

ABSTRACT

Lignocellulosic biomass is an abundant and renewable bioresource for the production of biofuels and biochemical products. The classical biorefinery process for lignocellulosic degradation and conversion comprises three stages, i.e., pretreatment, enzymatic saccharification, and fermentation. However, the complicated pretreatment process, high cost of cellulase production, and insufficient production performance of fermentation strains have restricted the industrialization of biorefinery. Consolidated bioprocessing (CBP) technology combines the process of enzyme production, enzymatic saccharification, and fermentation in a single bioreactor using a specific microorganism or a consortium of microbes and represents another approach worth exploring for the production of chemicals from lignocellulosic biomass. The present review summarizes the progress made in research of CBP technology for lignocellulosic biomass conversion. In this review, different CBP strategies in lignocellulose biorefinery are reviewed, including CBP with natural lignocellulose-degrading microorganisms as the chassis, CBP with biosynthetic microorganisms as the chassis, and CBP with microbial co-culturing systems. This review provides new perspectives and insights on the utilization of low-cost feedstock lignocellulosic biomass for production of biochemicals.

1. Introduction

Biomass is an abundant and renewable resource on earth and is divided into two types: food-based biomass resources and lignocellulosic biomass resources. The first generation of biofuels is produced from food-based resources (corn starch, sugarcane sugar, and sunflower oil et al.), which created the issue of the competition with food production. In contrast, the second generation of biofuels is produced from non-food biomass such as lignocellulosic biomass derived for instance from crop residues, forest residues and municipal solid waste. The overall amount of lignocellulosic biomass exceeds 145 billion tons per year worldwide [1]. Given the abundance and environmental friendliness of lignocellulosic biomass [2], it has the potential to be converted into biofuels, such as bioethanol, and high-value-added biochemicals [3]. The utilization of lignocellulosic biomass feedstocks facilitates the transition from a linear to a circular economy, thus meeting global sustainability requirements [4]. However, only 3 % of lignocellulosic biomass is effectively utilized [5], and further research is required for the efficient utilization of lignocellulosic biomass.

The recalcitrant nature of lignocellulosic biomass, which is caused by structural complexity and heterogeneity, is a major obstacle to its decomposition and utilization. Lignocellulose is composed of three ma-

Abbreviations: AECC, alkali-extracted deshelled corn cobs; AFEX, ammonia fiber explosion; AI, artificial intelligence; BGLs, β-glucosidases; CBB, Calvin-Benson-Bassham; CBHs, cellobiohydrolases; CBM, carbohydrate-binding modules; CBP, consolidated bioprocessing; CBS, consolidated bio-saccharification; CMC, carboxymethyl cellulose; DMCC, Direct microbial conversion of biomass with CO_2 fixation; ED, Entner-Doudoroff; EGs, endoglucanases; FAEE, fatty acid ethyl ester; FPS, farnesyl pyrophosphate synthase; ILs, ionic liquids; Ldh, L-lactate dehydrogenase; LPMOs, lytic polysaccharide monooxygenases; MCC, microcrystalline cellulose; ML, machine learning; NRPs, non-ribosomal peptides; PASC, phosphoric acid swollen cellulose; PEFA, polyol esters of fatty acids; PGASO, promoter-based gene assembly and simultaneous overexpression; PHA, polyhydroxyalkanoate; Pta, phosphotransacetylase; PYC, pyruvate carboxylase; RAC, regenerated amorphous cellulose; rTCA, reduced tricarboxylic acid cycle; SCFAs, short-chain fatty acids; SECS, steam-exploded corn stover; TAL, triacetic acid lactone; XI, xylose isomerase; XK, xylulose kinase.

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https://doi.org/10.1016/j.engmic.2024.100139
Received 28 July 2023; Received in revised form 26 January 2024; Accepted 29 January 2024
Available online 2 February 2024

jor components: cellulose, hemicellulose, and lignin, which account for more than 90 % of the total plant cell wall content. Cellulose, formed by the polymerization of glucosyl units with β−1,4 glycosidic bonds, is the most abundant biopolymer on the earth [6]. The linear cellulose chains further form a highly crystalline microfibrillar structure through strong intra- and intermolecular hydrogen bonds [7,8], which poses significant challenges for its efficient degradation. Hemicellulose are heteropolymers with a certain degree of branching, consisting of different hexoses (galactose, mannose, rhamnose, fucose), pentoses (xylose, arabinose) and glucuronic acid [9]. The chemical structure and content of hemicelluloses vary significantly within different biomass types of the same plant as well as among different plants [10]. For example, xylose is the main component of hemicelluloses in the cell walls of grasses and broadleaf trees, whereas mannose is the main component of hemicelluloses in the cell walls of cork and coniferous trees [11,12]. The diversity and heterogeneity of hemicellulose are also the main obstacles to its degradation and utilization. Lignin is a complex, non-crystalline, three-dimensional reticulated phenolic polymer, and its main role is to provide structural support and to form a natural, impermeable barrier against microbial attack and oxidative stress [13]. In plants, cellulose, hemicellulose, and lignin form a supramolecular system in which lignin acts as a binder for cellulose and enhances the mechanical strength of the plant cell wall. In general, the complex composition of lignocellulosic biomass poses a major obstacle for the efficient separation of carbohydrates from lignin and their subsequent utilization, limiting the development of lignocellulose biorefinery technologies and significantly reducing the efficiency of biofuels and high value-added chemicals production. The main approaches for lignocellulosic biomass biorefinery are the classical three-stage biorefinery process and the consolidated bioprocessing (CBP) technology, among which CBP combines the sugar production and fermentation in a single step to produce various bioproducts from lignocellulosic biomass. The present review summarizes three different CBP strategies, including CBP construction with lignocellulose-degrading microorganisms as the chassis, CBP construction with biosynthetic microorganisms as the chassis, and the construction of CBP with microbial co-cultures. This review provides insights and new perspectives on the utilization of lignocellulose as feedstock for the production of biochemicals.

2. The classical three-stage biorefinery process

The classical lignocellulose biorefinery process can be divided into three separate steps: pretreatment, enzymatic saccharification, and fermentation (Fig. 1).

Pretreatment of the feedstock is required for effective enzymatic saccharification of lignocellulose. The pretreatment of lignocellulose aims to separate various components of biomass (especially removing lignin from cellulose and hemicellulose) and also disrupts the hydrogen bonds and van der Waals interactions of cellulose microfibrils, and thus helps loosen the rigid lignocellulosic biomass structure [14]. A wide range of pretreatment techniques, including physical, chemical, and biological pretreatment methods, has been developed to disrupt the structure of lignocellulosic biomass. Physical pretreatment techniques are primarily used to reduce the particle size and increase the specific surface area of lignocellulose through mechanical crushing [15] and high-temperature hydrothermolysis treatment [16]. In recent years, irradiation methods such as microwave and ultrasonic techniques have been widely used to release intracellular cellulose in a short time under high-energy radiation [17]. However, these physical pretreatment methods usually require huge energy input and thus cannot be easily implemented on a large scale [18]. Chemical pretreatment methods utilize special chemicals to disrupt the structure of lignocellulose by selectively dissolving the specific components of lignocellulose. Chemical pretreatment techniques (e.g., acid, alkali, oxidation, and organic solvent pretreatment) can effectively increase the biomass surface area and improve lignocellulose degradation [19,20]. However, both physical and chemical pretreatment methods generate toxic and inhibitory compounds like furfural and hydroxymethylfurfural, which interfere with enzymatic saccharification and fermentation [21–23]. Thus, a detoxification step becomes necessary before using pretreated biomass for saccharification and fermentation. In addition, toxic liquid effluent released from pretreatment and detoxification also causes severe environmental pollution [24]. The increased cost required for detoxification and waste disposal limits its large-scale industrial application [25]. Compared to physical and chemical pretreatment methods, biological pretreatment methods have the advantages of lower operating costs, higher yields, and fewer inhibitory by-product formation but are less efficient and

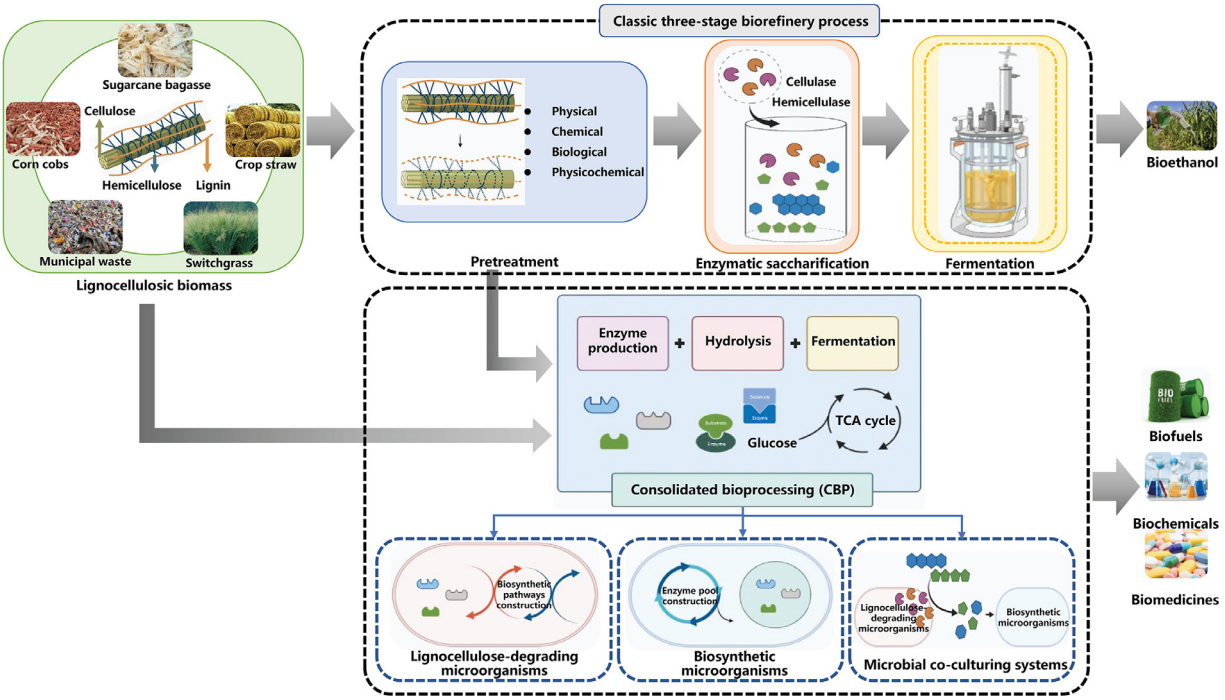

Fig. 1. A schematic diagram of the classic three-stage biorefinery process and consolidated bioprocessing technologies.

time-consuming. Biological pretreatment techniques require selected microorganisms or enzymes to degrade lignin and hemicellulose. For example, white rot, brown rot, and soft rot fungi are capable of secreting lignin peroxidase, manganese peroxidase, and laccase, which can effectively degrade lignin [26]. There are also pretreatment technologies that combine several physical, chemical, and biological methods [21,22]. Physico-chemical pretreatment includes a combination of physical and chemical methods such as steam explosion, liquid hot water, CO_2 explosion, and ammonia fiber explosion (AFEX) [27]. Physicochemical pretreatment methods remove lignin at a higher rate and efficiently disrupt the cellulose polymer by reducing cellulose crystallinity [28]. For example, the steam explosion pretreatment method can effectively reduce cellulose crystallinity and enhance glucose yield from enzymatic hydrolysis; therefore, this method is considered the most cost-effective pretreatment method [29,30]. In addition, supercritical fluids pretreatment is also considered an economical and environmentally friendly process that can replace the conventional pretreatment processes [31].

The pretreatment process loosens the rigid and complex structure of the lignocellulosic biomass, which facilliates the enzymatic saccharification of cellulose and hemicellulosic polysaccharides by lignocellulolytic enzymes. In the enzymatic saccharification stage, the efficient hydrolysis of cellulose requires the synergistic action of cellobiohydrolases (CBHs), endoglucanases (EGs), and β-glucosidases (BGLs) [21,32,33]. Among these, the CBHs move processively along the cellulose chains and release cellobiose units from either the reducing ends or non-reducing ends, while the EGs randomly hydrolyze internal glycosidic bonds within the cellulose chain generating oligosaccharides of different lengths and thus increasing the acting sites for CBHs; finally the BGLs hydrolyze cellobiose into glucose [34]. The enzyme cost and catalytic efficiency of the lignocellulolytic enzymes determine the cost-effectiveness and feasibility of the overall biorefinery process [35,36]. The enzymatic degradation of hemicellulose in lignocellulosic biomass is carried out by hemicellulase enzymes including xylanase, mannanase, and arabinosidase. In addition to the hydrolytic enzymes, recently discovered lytic polysaccharide monooxygenases (LPMOs) and several other oxidoreductases also play important roles in the efficient degradation of lignocellulosic biomass [37,38].

Enzymatic saccharification of the pretreated lignocellulosic biomass yields a series of monosaccharides, which can be used for downstream fermentation to produce the desired products, mainly biofuels such as bioethanol. In the study by Zhao et al. (2019), corn stover pretreated with Na_2CO_3 and H_2O_2 was enzymatically hydrolyzed by a lignocellulolytic enzyme cocktail composed of CBHs, EGs, BGLs, and xylanases. The resulting hydrolysate was fermented by a highly efficient ethanol-producing *Saccharomyces cerevisiae* WXY12 strain, yielding 46.87 g/L of bioethanol with a 27.4 % theoretical conversion rate [39]. Hemicellulose is also used for producing functional sugars, like oligosaccharides, xylose, arabinose, and chemicals such as xylitol, acetic acid, and furfural [40–42]. One of the key challenges for the efficient production of biofuels and chemicals through biorefinery is to simultaneously ferment all the released pentoses and hexoses into the target products [43]. Although significant progress has been made in fermenting both pentoses and hexoses using engineered microorganisms, some challenges still need to be addressed, such as the production of by-products and poor product tolerance of the strains. Therefore, the fermentation performance of selected microbial strains also restricts the industrialization of biorefineries.

In the current stage, several demonstration plants have been established with the three-stage biorefinery technology. However, the operational costs, particularly those of cellulases, are still found to be high in practice [44]. The cost of cellulase production has been reduced to about 10–20 US $ per kg of the enzyme [45], but enzymatic saccharification of lignocellulosic biomass is still the main limiting step due to the low cellulase activity and high cost of cellulase production. Thus, lignocellulosic biomass biorefineries are not yet available for large-scale industrial applications and still need to be continuously improved.

3. Consolidated bioprocessing technology

Consolidated bioprocessing is considered another approach worth exploring for the production of biofuels and high-value-added chemicals from lignocellulosic feedstock. CBP is characterized by combining sugar production and fermentation in a single step (Fig. 1), which accomplishes cellulase production and secretion, lignocellulose whole-component hydrolysis, and biosynthesis of chemicals in one bioreactor [46]. Thus, the CBP approach reduces the cellulase production cost along with the elimination of separate enzymatic hydrolysis, which would significantly decrease the overall biorefinery process cost. Therefore, CBP represents another avenue worth exploring for lignocellulose biorefinery [47].

CBP with a single microbial strain as the chassis has been extensively studied due to its relatively well-defined metabolism [48,49]. A rapid growth rate, extensive substrate utilization, high product productivity, and robust resistance are required as a microbial CBP chassis. In the monomicrobial systems, CBP strains must be able to degrade lignocellulosic feedstocks and synthesize the desired products. Microorganisms with these properties are rarely found in nature, therefore CBP strains need to be constructed either with natural lignocellulose-degrading microorganisms as the chassis or microorganisms with biosynthetic pathways as the chassis [50].

3.1. Construction of CBP strains with lignocellulose-degrading microorganisms as the chassis

In nature, various bacterial and fungal microorganisms have been found to be capable of degrading lignocellulose. However, these lignocellulose-degrading microorganisms generally lack the metabolic pathways for the synthesis of the desired products. Therefore, the construction of CBP with lignocellulose-degrading microorganisms as the chassis requires the expression of product synthesis pathways and metabolic engineering to enhance their synthesis (Table 1). Simultaneously maintaining a high level of lignocellulose degradation capacity while increasing the productivity of the target product are the main challenges of this strategy.

3.1.1. Lignocellulose-degrading bacteria as the chassis for CBP construction

Due to the rapid proliferation and strong tolerance to various environmental conditions, bacteria have received extensive attention in lignocellulose degradation [51]. Most of the lignocellulose-degrading bacteria are anaerobic, such as *Clostridium, Ruminococcus, Pseudomonas, Bacillus, Proteus*, and *Serratia* [52–54]. These anaerobic lignocellulose-degrading bacteria generally weave lignocellulolytic enzymes into a complex structure called cellulosomes [55,56], which consists of two main parts: a catalytically active, multi-enzyme subunit with a dockerin domain and a non-catalytic scaffolding protein with a cohesin domain [57]. Cellulases are assembled into a multi-enzyme complex by specific binding of the dockerin domain to the cohesin domain on the scaffold protein, which can be further anchored onto the cell surface. In addition, scaffold proteins contain carbohydrate-binding modules (CBM) that bind specifically to cellulose, leading to a targeted effect on substrates [58,59]. Thus, the effective synergistic degradation of lignocellulosic biomass is achieved through a combination of spatial proximity of different types of cellulases (proximity effect) and targeting of the multi-enzyme complex to the substrate (targeting effect). Cellulosomes thus have a highly ordered spatial structure that allows multiple synergistic effects between enzyme and enzyme, enzyme and cell, and enzyme and substrate. Therefore, cellulosomes are superior to the display of free cellulases directly on the cell surface in terms of cellulose degradation ability.

Clostridium thermocellum is an anaerobic, thermophilic Gram-positive bacterium that has been widely used for lignocellulosic biomass conversion due to its natural cellulose-degrading capacity, which is comparable to commercially available cellulases [60,61]. It usually grows

Table 1
Synthesis of bio-based chemicals using metabolically engineered natural lignocellulose-degrading microorganisms.

Microorganism	Engineering approach	Substrate	Product	Reference
C. cellulolyticum M1570	Deletion of key genes involved in the production of byproducts acetic acid and lactic acid, and adaptive evolution	19.6 g/L Avicel	5.6 g/L ethanol	[62]
C. thermocellum CT24	Heterologous expression of the isobutanol biosynthetic pathway	33.6 g/L Avicel	5.4 g/L isobutanol	[63]
C. thermocellum LL1668	Heterologous expression of the *n*-butanol biosynthetic pathway	47.5 g/L Avicel and 4 g/L ethanol	357 mg/L *n*-butanol	[64]
C. phytofermentans ATCC 700394	None	0.5 % (w/w) AFEX-pretreated corn stover	2.8 g/L ethanol	[68]
C. cellulovorans	Heterologous expression of different aldehyde/alcohol dehydrogenases	Cellulose	1.11 g/L butanol and 0.20 g/L ethanol	[69]
C. cellulovorans	Heterologous expression of *adhE1* and *ctfA-ctfB-adc genes* from *C. acetobutylicum* ATCC 824 for constructing a coenzyme A dependent acetone-butanol-ethanol pathway, and adaptive evolution	AECC	3.47 g/L *n*-butanol	[70]
T. reesei QM9414	Heterologous expression of the ethylene biosynthetic pathway	2 % (w/v) wheat straw	4012 nL/h/L ethylene	[78]
T. reesei	Deletion of the xylitol dehydrogenase gene (*xdh1*) and the l-arabinitol-4-dehydrogenase gene (*lad1*)	2 % (w/v) organosolv-pretreated barley straw and 2 % (w/v) d-xylose	13.22 g/L xylitol	[79]
T. reesei Rut-C30	Overexpression of the erythrose reductases gene (*err1*)	1.7 % (w/v) alkaline organosolv-pretreated wheat straw	5 mg/L erythritol	[83]
M. thermophila JG424	Overexpression of the PEP carboxylase gene (*ppc*) and the malate dehydrogenase gene (*mdh*); heterologous expression of the HCO_3^- transporter gene (*bicA*) and the carbonic anhydrase gene (ca) from *Synechococcus.* sp. PCC7002	75 g/L Avicel	83.3 g/L malic acid and 15.4 g/L succinic acid	[87]
M. thermophila CP-51	Heterologous expression of genes encoding CBB cycle enzymes RuBisCO (*cbbM*) from *Rhodospirillum rubrum* and PRK (*prk*) from *Spinacia oleracea*; deletion of the pyruvate decarboxylase gene (*pdc*), lactate dehydrogenase gene (*ldh*), and PEP carboxykinase gene (*pck*)	Corncob and CO_2	0.53 g/g malic acid	[88]

at high temperatures (50–60 °C) and thus exhibits a higher lignocellulosic biomass degradation efficiency than mesophilic bacteria. Particularly, the enzymes produced by *C. thermocellum* have a strong tolerance to harsh conditions, such as the presence of phenolic compounds produced during the pretreatment of lignocellulose. In addition, *C. thermocellum* has a natural ability to produce ethanol. Therefore, *C. thermocellum* has great potential to be developed as a CBP chassis. Argyros et al. (2011) deleted the genes encoding l-lactate dehydrogenase (Ldh) and phosphotransacetylase (Pta) in *C. thermocellum* by an established reverse selection system to eliminate the production of the by-products acetate and lactate, thereby increasing the flux towards ethanol. The engineered strain was further improved by adaptive evolution of 2000 h. Using Avicel as the carbon source, the engineered strain was shown to produce 5.6 g/L ethanol, which is a 4.2-fold increase compared to that of the wild-type strain [62]. *C. thermocellum* itself cannot produce butanol and isobutanol. Lin et al. (2015) used different promoters to drive the expression of isobutanol biosynthetic genes in *C. thermocellum,* and the engineered strain produced 5.4 g/L of isobutanol from Avicel under optimized conditions, reaching 41 % of the theoretical yield [63]. In addition to isobutanol, Tian et al. (2019) engineered a *C. thermocellum* strain to convert Avicel to produce 357 mg/L of butanol by heterologously expressing the enzymes for butanol production, key enzyme engineering, and supplying additional ethanol [64]. Garcia et al. (2020) designed an advanced genome-scale metabolic model for *C. thermocellum*. This model offers a more comprehensive and accurate representation of the organism's metabolism by integrating genetic, genomic, and metabolic data from various sources. It not only supports metabolic flux simulations but also serves as a system-level framework for data integration. Using this model, these authors studied *C. thermocellum*'s redox metabolism and identified the significance of NADPH as a cofactor, offering insights into potential engineering targets for improving the production of reduced products, such as ethanol, in *C. thermocellum* [65].

Another promising CBP chassis strain belonging to the genus *Clostridium* is *C. phytofermentans* ATCC 700,394. Among the sequenced genomes of *Clostridium* spp., its genome encodes the largest number of lignocellulolytic enzymes [66], which can degrade cellulose and hemicellulose into fermentable sugars. Moreover, unlike *C. thermocellum*, which is unable to consume xylose, *C. phytofermentans* can consume almost all types of sugars present in lignocellulose and produce ethanol and acetate as the main products [66,67]. Using corn stover pretreated with AFEX with a particle size of 0.5 mm as the feedstock, Jin et al. (2011) showed that *C. phytofermentans* ATCC 700,394 produced 2.8 g/L of ethanol after 10 days of fermentation under optimum conditions [30 °C, 5 % (v/v) inoculum, and initial pH 7.0] [68]. Bao et al. (2019) engineered *Clostridium cellulovorans* by introducing three different aldehyde/alcohol dehydrogenase genes *bdhB, adhE1*, and *adhE2* from *Clostridium acetobutylicum* for the production of ethanol and *n*-butanol. Co-expression of *adhE1* and *bdhB* in *C. cellulovorans* enhanced *n*-butanol production as compared to ethanol, and the highest butanol/ethanol ratio of 7.0 and 5.6 (g/g) was achieved through fermentation by using glucose and cellulose, respectively [69]. For further improvement in *n*-butanol production, Wen et al. (2019) developed an evolved strain by integrated metabolic and evolutionary engineering. The engineered *C. cellulovorans* strain produced *n*-butanol by utilizing alkali-extracted deshelled corn cobs (AECC), achieving the highest titer of 3.47 g/L [70].

3.1.2. Lignocellulose-degrading fungi as the chassis for CBP construction

Cellulases used in industrial applications are mainly produced by filamentous fungi [71], such as *Aspergillus niger, Aspergillus nidulans, Aspergillus oryzae, Trichoderma reesei, Humicola insolens*, and *Myceliophthora thermophila* [72]. Among them, *T. reesei* has a high cellulase secretion capacity, and the industrial *T. reesei* mutant strains are reported to produce up to 100 g/L cellulases [73]. However, a CBP strain requires both strong lignocellulose-degrading capabilities and efficient product synthesis pathways. Although *T. reesei* can metabolize all types of monosaccharides present in lignocellulose, its ethanol yield is low, and it also produces other undesired by-products, such as acetic acid and lactic acid [74]. However, due to the widespread use of *T. reesei* in commercial enzyme production, the well-established large-scale fermentation techniques, and the availability of genetic manipulation tools [75], it is considered a promising CBP chassis strain. The major challenge of *T. reesei* as a CBP strain is that the expression of cellulase and glycolysis-related genes is susceptible to be inhibited by hypoxic conditions [76,77], which is essential for ethanol production. In addition, the transcription of cellulase genes is also inhibited in the presence of ethanol, which is another challenge that still needs to be addressed. Nevertheless, many studies

have used *T. reesei* as a CBP chassis strain to produce biochemicals such as ethylene, xylitol, and erythritol from lignocellulose. However, in most cases, their product yields are far from commercial requirements. Chen et al. (2010) utilized three strong promoters, the *cbh1* promoter from *T. reesei*, the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) promoter from *A. nidulans*, and the 3-phosphoglycerate kinase I (*pgk1*) promoter from *T. reesei* to drive the expression of the ethylene synthase gene *efe* from *Pseudomonas syringae* pv. *glycinea* in *T. reesei* QM9414; 14 transformants achieved ethylene production from wheat straw. The highest ethylene production rate was observed with *T. reesei* transformant C30–3 using the *pgk1* promoter, reaching a value of 4012 nL/h/L [78]. Blocking the downstream metabolism of xylitol in the *T. reesei*d-xylose metabolic pathway enabled the strain to produce xylitol from hemicellulose. Dashtban et al. (2013) deleted the xylitol dehydrogenase gene (*xdh1*) and the l-arabinitol-4-dehydrogenase gene (*lad1*) of the *T. reesei*d-xylose metabolic pathway, which resulted in the synthesis of xylitol. Using sodium hydroxide and organic solvent ethanol pretreated barley straw as the carbon source with a replenishment of 2 % d-xylose, the yield of xylitol reached 13.22 g/L [79].

T. reesei Rut-C30, a hyper-cellulolytic mutant strain, has a higher capacity for cellulase production. Furthermore, in the presence of lignocellulose, Rut-C30 exhibits a pellet-like morphology in the early stages of fermentation [80] and can grow in a dense pellet form rather than in an extended mycelial form by the addition of the surfactant Triton X-100 [81], thus achieving an even higher enzyme production. Compared to the strain QM9414 from the Natick pedigree, Rut-C30 also shows a greater ethanol tolerance [82]. Jovanovic et al. (2014) overexpressed the erythritol reductase gene (*err1*) of *T. reesei* Rut-C30, and the resulting engineered strain produced about 10-fold higher amounts of erythritol (5 mg/L) than the wild-type strain using wheat straw pretreated with an alkaline organic solvent process as the feedstock [83]. However, this yield was still far from that of erythritol production from glucose and thus warrants further studies.

The thermophilic fungi *M. thermophila*, belonging to the genus *Myceliophthora*, is capable of secreting a large amount of thermostable cellulase [84], which can efficiently degrade lignocellulose, including cellulose and hemicellulose. Notably, the growth rates of *M. thermophila* with cellulose and glucose as carbon sources are almost identical. Its cellulose utilization rate is about 5 times that of *T. reesei* and 2.5 times that of *C. thermocellum* [85]. Moreover, the optimal growth temperature of *M. thermophila* is 45 °C [86], which is close to the optimal catalytic temperature of cellulases (50 °C), thus making *M. thermophila* an ideal CBP chassis for simultaneous lignocellulose decomposition and product biosynthesis. Using *M. thermophila* as the chassis, Li et al. (2020) constructed a CBP platform, which directly transformed unpretreated lignocellulose to malic acid and succinic acid in one step without the addition of additional lignocellulolytic enzymes [87]. In this study, a reduced tricarboxylic acid cycle (rTCA) pathway was established, and a malic acid transporter was introduced, which enabled the recombinant strain to produce malic acid from lignocellulose corncob. Subsequently, the rTCA pathway was further enhanced by overexpression of the phosphoenolpyruvate carboxylase gene (*ppc*) and the malate dehydrogenase gene (*mdh*). In addition, CO_2 fixation was enhanced by heterologous expression of the HCO_3^-transport protein gene (*bicA*) and carbonic anhydrase gene (ca) from *Synechococcus* sp. PCC7002. Consequently, the engineered strain produced 83.3 g/L of malic acid from 75 g/L of Avicel in shake flasks with a yield of 1.11 g/g, which was 1.26 times higher than that of the parental strain. The production of succinic acid reached 15.4 g/L, which is the highest level ever reported using lignocellulose as feedstock. Li et al. (2021) constructed a novel biorefinery system, DMCC (Direct microbial conversion of biomass with CO_2 fixation), in *M. thermophila* through metabolic engineering by incorporating two CO_2 fixation modules, i.e., the pyruvate carboxylase (PYC) module and Calvin-Benson-Bassham (CBB) pathway. The metabolic-engineered *M. thermophila* CP-51 strain showed an increase in the malic acid titer by 40 %, 10 %, and 7 %, in xylose, glucose, and cellulose, respectively, as compared to the parent strain. Using lignocellulosic feedstock, the *M. thermophila* CP-51 strain with the DMCC system obtained a malic acid yield of up to 0.53 g/g. This study suggested that a constructed DMCC system can produce 1 ton of malic acid from 1.89 t of raw lignocellulosic feedstock by fixing 0.14 t atmospheric CO_2 [88].

3.2. Construction of CBP using microorganisms with biosynthetic pathways as the chassis

Introducing genes encoding lignocellulolytic enzymes into microorganisms with biosynthetic pathways represents another avenue for CBP construction (Table 2). The main challenge of this strategy is to achieve the efficient heterologous expression of cellulases or hemicellulases to accomplish the conversion of lignocellulose. CBP construction using microorganisms with biosynthetic pathways as the chassis has been applied to many different microorganisms, including *Escherichia coli* [89], *Zymomonas mobilis* [90], *Klebsiella oxytoca* [91], and *S. cerevisiae* [92–94].

3.2.1. Bacteria with biosynthetic pathways as the chassis for CBP construction

E. coli is the most common bacterial host organism for recombinant protein production and also a commonly used chassis for CBP. Bokinsky et al. (2011) conducted innovative CBP studies using engineered *E. coli* and ionic liquids (ILs) pretreated lignocellulose. They engineered *E. coli* strains by heterologous expression of an intracellular cellulase gene (*cel*) from *Bacillus* sp. D04 and a xylanase gene (*xyn10B*) from *Clostridium stercoranium*. The recombinant *E. coli* strain grew well in ILs-pretreated willow jelly, eucalyptus, and yard waste without exogenous addition of lignocellulolytic enzymes. Further introduction of the biofuel biosynthetic pathway into this engineered *E. coli* strain achieved the production of 71 mg/L fatty acid ethyl ester (FAEE), 28 mg/L *n*-butanol, and 1.7 mg/L pinene using ILs-pretreated lignocellulosic biomass [89].

B. subtilis is a most representative industrial microorganism with many valuable characteristics such as low nutrient requirements, fast growth rate, easy cultivation, high protein secretion capacity, and is a biosafe strain [95–97]. *B. subtilis* is widely used in aerobic fermentation for the production of enzymes (e.g., nattokinase, α-amylase) [98], vitamins [99], antibiotics [100], pyrimidine nucleoside [101], hyaluronan [102], etc. Under anaerobic conditions, *B. subtilis* can produce two predominant products, lactic acid and 2,3-butanediol, in addition to acetic acid. *B. subtilis* 168 encodes a secreted EG of glycoside hydrolase family 5 (BsCel5) and an intracellular BGL, but lacks a CBH [103]. Due to the insufficient expression level of EG in *B. subtilis*, it cannot grow on cellulose. Therefore, heterologous expression of the key cellulase genes is required to enable *B. subtilis* to produce chemicals from various lignocellulosic biomass [104]. Zhang et al. (2011) enabled *B. subtilis* to grow on regenerated amorphous cellulose (RAC) and well-pretreated lignocellulose without the addition of other organic nutrients (e.g., yeast extract, peptone, amino acids) by overexpressing the endogenous EG gene (*cel5*) in a non-cellulose-utilizing *B. subtilis*. Subsequently, the specific activity of BsCel5 on RAC was improved by two rounds of directed evolution, and the expression and secretion levels of BsCel5 in *B. subtilis* were also enhanced to improve the lignocellulose-degradation capacity of the *B. subtilis* strain. In addition, the authors deleted the α-acetyl lactate synthase gene (*alsS*) involved in the 2,3-butanediol biosynthetic pathway in the recombinant *B. subtilis* strain to eliminate the synthesis of the minor product 2,3-butanediol and aiming to increase the yield of the major product lactate. The resulting strain produced 3.1 g/L of lactate from RAC, achieving a yield of 60 % of the theoretical maximum [105,106].

The production of the chiral lactic acid monomer by fermentation using inexpensive lignocellulosic biomass instead of starchy feedstock will reduce the production cost of biodegradable plastic polylactic acid. However, many inhibitors (e.g., furfural and phenolics) produced during lignocellulose pretreatment can inhibit the growth and metabolism

Table 2
Synthesis of bio-based chemicals using microorganisms with biosynthetic pathways.

Microorganism	Engineering approach	Substrate	Product	Reference
E. coli MG1655 Δ*fadE*	Heterologous expression of the intracellular cellulase gene (*cel*) from *Bacillus.* sp. D04 and the xylanase gene (*xyn10B*) from *C. stercoranium*; heterologous expression of the FAEE biosynthetic pathway	55 g/L ILs-pretreated switchgrass	71 mg/L FAEE	[89]
E. coli DH1 Δ*adhE*	Heterologous expression of the intracellular cellulase gene (*cel*) from *Bacillus*. sp. D04 and the xylanase gene (*xyn10B*) from *C. stercoranium*; heterologous expression of the *n*-butanol biosynthetic pathway	55 g/L ILs-pretreated switchgrass	28 mg/L *n*-butanol	[89]
E. coli MG1655	Heterologous expression of the intracellular cellulase gene (*cel*) from *Bacillus*. sp. D04 and the xylanase gene (*xyn10B*) from *C. stercoranium*; heterologous expression of the pinene biosynthetic pathway	55 g/L ILs-pretreated switchgrass	1.7 mg/L pinene	[89]
B. subtilis	Overexpression of the endoglucanase gene (*cel5*); deletion of the α-acetyl lactate synthase gene (*alsS*) involved in the biosynthesis of the minor product 2,3-butanediol	RAC	3.1 g/L lactate	[105]
P. acidilactici XH11	Long-term adaptive evolution (111 days)	Undetoxified acid-pretreated corncob slurry	61.9 g/L d-lactic acid	[110]
K. oxytoca SZ21	Heterologous expression of the endoglucanase gene (*celY, celZ*) from *E. chrysanthemi*; heterologous expression of the ethanol biosynthetic pathway	6.85 g/L amorphous cellulose	4.67 g/L ethanol	[111]
S. cerevisiae	Heterologous expression of the β-glucosidase gene (*bglI*) from *S. fibuligerabeta* and the endoglucanase gene (*egII*) from *T. reesei*	Pretreated corn cobs	4.05 g/L ethanol	[93]
S. cerevisiae	Heterologous expression of the endoglucanase gene (*egII*) and the cellobiohydrolase gene (*cbhII*) from *T. reesei*, the β-glucosidase gene (*bglI*) from *A. aculeatus* and the cellodextrin transporter gene (*cdtI*) from *N. crassa*	PASC	4.3 g/L ethanol	[94]
S. cerevisiae	Deletion of the gene encoding cell wall mannoprotein (*cwp2*) and cell wall-associated secretory glycoprotein (*ygp1*), respectively	5 % (w/v) cellobiose	11.3 g/L ethanol	[92]
S. cerevisiae CRD5HS	Adaptive evolution	Pretreated corn stover and corn cob	85.95 and 94.76 g/L ethanol	[128]
P. pastoris	Construction of mini-cellulosomes on the cell surface	CMC	5.1 g/L ethanol	[135]
K. marxianus KR7	Heterologous expression of five cellulase genes (*cbhII, cbhI, egIII, eglA, npabgs*), one cellodextrin transporter gene (*cdtI*), and one selection marker gene (*kanMX*)	MCC	0.6 g/L ethanol	[136]
R. toruloides ABFPUB_26	Heterologous expression of *ent*-kaurene synthase	Corn stover hydrolysate	1.4 g/L *ent*-kaurene	[142]
R. toruloides	Heterologous expression of indigoidine synthase	Unfiltered sorghum hydrolysate	2.9 g/L indigoidine	[143]
R. toruloides	Heterologous expression of 2-pyrone synthase	Unfiltered sorghum hydrolysate	3.9 g/L TAL	[144]
M. circinelloides	Mc-XI: overexpression of xylose isomerase Mc-XK: overexpression of xylulokinase	Corn straw hydrolysate prepared by dilute acid pretreatment and enzymatic hydrolysis	2.17–2.28 g/L lipid	[106]

of lactic acid-producing strains, which will result in lower lactic acid production [107]. *Pediococcus acidilactici* strains have been shown to be highly tolerant to inhibitors [108], and are also shown to be capable of metabolizing all the lignocellulose-derived sugars (glucose, xylose, mannose, galactose, and arabinose) for lactic acid production [109]. Therefore, *P. acidilactici* has great potential as a chassis strain for lactic acid production from lignocellulose. Qiu et al. (2022) significantly improved the tolerance of *P. acidilactici* XH11 to four typical aldehyde inhibitors (5-hydroxymethylfurfural, furfural, vanillin, and 4-hydroxybenzaldehyde) through a long-term adaptive evolutionary strategy, which allowed the strain to produce 61.9 g/L of d-lactic acid from undetoxified acid-pretreated corncob slurry [110].

K. oxytoca has the natural ability to metabolize cellobiose and cellotriose, and would thus be a potential CBP chassis after metabolic engineering. Zhou et al. (2001) heterologously expressed ethanol synthetic enzymes (*pdc, adhB*) from *Z. mobilis* and endoglucanase genes (*celY, celZ*) from *Erwinia chrysanthemi* in *K. oxytoca* M5A1, resulting in a recombinant strain that secreted over 20,000 $U{\cdot}L^{-1}$ of extracellular EG, which is more than 10 times the level of enzyme production previously reported for *S. cerevisiae* as well as other engineered bacterial strains during fermentation for ethanol production. Combined with its ability to metabolize cellobiose and cello-triose, the recombinant strain was able to directly convert amorphous cellulose into 4.67 g/L of ethanol without the addition of cellulases from other organisms, achieving 76 % of the theoretical yield [111].

Z. mobilis has a unique Entner-Doudoroff (ED) metabolic pathway and exhibits "uncoupled growth" [112,113], which means that its cells can consume sugar rapidly, regardless of its need for growth. This bacterium has a high glucose uptake and catabolism rate, which is 5 times faster than yeast [114], however, it does not have the natural ability to metabolize the pentoses released during the hydrolysis of lignocellulosic biomass [90]. *Z. mobilis* also has a wide pH range tolerance (pH 3.5–7.5) [115] as well as high glucose (400 g/L) and notable ethanol (16 % v/v) tolerance [116], making it a promising bioethanol producer [117,118]. *Z. mobilis* has now been engineered to metabolize all types of major biomass sugars [112,119]; heterologous cellulases have also been expressed in *Z. mobilis* to endow it with the ability to degrade lignocellulose [120,121]. In addition, He et al. (2021) achieved the heterologous expression of ethylene synthase from *P. syringae* pv. *phaseolicola* in *Z. mobilis*. With further modifications of the central carbon metabolism, the ZM532-*efe* strain achieved an ethylene yield of 5.8 $nmol/OD_{600}/mL$ using enzymatically hydrolyzed corn straw as the sole carbon source [122]. This study demonstrated the potential of *Z. mobilis* as a CBP chassis, after further introduction of lignocellulolytic enzymes.

3.2.2. Fungi with biosynthetic pathways as a chassis for CBP construction

S. cerevisiae is a well-known industrial host due to its high tolerance to low pH, high temperature, and various inhibitors [123]. Moreover, versatile genetic manipulation tools have been developed for *S. cerevisiae* which facilitate the assembly of biosynthetic pathways involv-

ing multiple genes [124]. Therefore, *S. cerevisiae* is considered to be a promising chassis strain for the consolidated bioprocessing of lignocellulose.

S. cerevisiae has a high capacity for ethanol production, whereas its capability to express heterologous cellulases is often poor [125], limiting its application as a CBP chassis. Davison et al. (2019) heterologously co-expressed the *β*-glucosidase gene (*bglI*) from *Saccharomycopsis fibuligerabeta* and the endoglucanase gene (*egII*) from *T. reesei* in the cellulase hypersecretory strain *S. cerevisiae* YI13. The resultant recombinant strain was able to convert 56.5 % of the cellulose present in pretreated corn cobs into glucose and produce 4.05 g/L of ethanol via fermentation [93]. Although *S. cerevisiae* cannot take up cello-oligosaccharides, some fungi can take up and assimilate oligosaccharides via the cellodextrin transporter [126]. To improve the efficiency of cellulose degradation by *S. cerevisiae*, Yamada et al. (2013) co-expressed the EG gene *egII* and the CBH gene *cbhII* from *T. reesei*, the BGL gene *bglI* from *Aspergillus aculeatus*, and the cellodextrin transporter gene *cdtI* from *Neurospora crassa* in *S. cerevisiae*. The engineered strain produced 4.3 g/L of ethanol from phosphoric acid swollen cellulose (PASC) following 72 h of fermentation, achieving 37 % of the theoretical yield, which was 1.7 times higher than that of the strain expressing only cellulase (2.5 g/L) [94]. Arnthong et al. (2022) disrupted the gene encoding the cell wall mannoprotein (*cwp2*) and the cell wall-associated secretory glycoprotein gene (*ygp1*), respectively, in *S. cerevisiae*, and the activity of BGL in the corresponding mutant strains was increased by 63 % and 24 %, respectively, compared to the original strain BGL-6_Kl. The ethanol production from cellobiose by the *ygp1*-deficient strain was increased by 59 % to 11.3 g/L compared to BGL-6_Kl [92]. This study demonstrated the important role of synergistic optimization and proteins related to cell wall function in improving the production of biobased products, via yeast strains, from lignocellulose. Recently, inspired by cellulosomes, synthetic biologists began to display designed cellulosomes onto yeast cell surfaces which can efficiently depolymerize cellulose and hemicellulose components of lignocellulosic biomass in an energy-limited environment [127]. Nevertheless, one of the drawbacks of *S. cerevisiae* is its inability to metabolize xylose. Numerous efforts have been devoted to the metabolic engineering of *S. cerevisiae* to ferment xylose. For this, we direct the reader to the excellent review by Qiu et al. (2023) [43]. Notably, Chen et al. (2023) identified xylose isomerase (XI) by big data mining and constructed four *S. cerevisiae* strains that can efficiently utilize xylose. The developed *S. cerevisiae* CRD5HS strain achieved an ethanol titer of 85.95 and 94.76 g/L from pretreated corn stover and corn cob, respectively, without detoxification or washing the pretreated biomass [128].

Pichia pastoris is one of the most commonly used hosts for the eukaryotic expression of heterologous proteins due to its high level of heterologous protein expression [129,130], its fast growth rate, and strong pH adaptability (pH 3.0–7.0); it is also less susceptible to ethanol accumulation and suitable for large-scale high-density fermentation [131,132]. Additionally, *P. pastoris* is one of the few yeasts that can ferment common sugars present in biomass (i.e., glucose and xylose) [133]. Therefore, *P. pastoris* is an attractive chassis for CBP construction. However, natural *P. pastoris* produces little or no cellulases and hemicellulases, and only a few strains can directly ferment xylan to ethanol [134]. Thus, *P. pastoris* has been genetically modified to enhance its lignocellulosic biomass degradation capacity. Dong et al. (2020) constructed mini-cellulosomes on the cell surface of *P. pastoris* and used the engineered yeasts to directly convert carboxymethyl cellulose (CMC) to ethanol with a titer of 5.1 g/L. In addition to this, *P. pastoris* with mini-cellulosomes was lyophilized as composite cellulases without affecting enzyme activity, which has great potential for industrial applications [135].

Chang et al. (2013) also isolated a *Kluyveromyces marxianus* KY3 strain, which can metabolize both hexoses and pentoses for ethanol production [136]. It has also been shown that the *K. marxianus* KY3 exhibits high heat resistance, a high growth rate, a wide growth temperature and pH range, as well as a broad substrate profile and efficient heterologous protein expression capacity [137]. In addition, the authors developed a technique called "promoter-based gene assembly and simultaneous overexpression (PGASO)" which was employed to simultaneously integrate five cellulase genes (*cbhII, cbhI, egIII, eglA, npabgs*), one cellodextrin transporter gene (*cdtI*), and one selection marker gene (*kanMX*) into the genome of the KY3 strain. The resultant strain KR7 was shown to convert microcrystalline cellulose (MCC) to 0.6 g/L of ethanol, which was a 2.5-fold increase in yield compared to the control strain [136]. Although the yield of ethanol obtained in *K. marxianus* was low compared to yeast strains of other genera so far, it has great potential as a new CBP chassis strain.

Rhodosporidium toruloides, an oil-producing yeast belonging to *Basidiomycota*, is a promising chassis for the conversion of lignocellulose into biobased products [138]. *R. toruloides* has the ability to grow to high cell densities with diverse substrates and is also resistant to strong osmotic stresses [139]. Furthermore, *R. toruloides* also showed strong tolerance to inhibitors present in lignocellulose hydrolysates and is capable of utilizing all types of monosaccharides commonly found in lignocellulosic biomass feedstocks for growth [140]. Significant progress has been made in the development of genetic manipulation tools for *R. toruloides*, laying an important foundation for a wide range of bioengineering applications [141]. *R. toruloides* has been engineered to produce a variety of bioproducts. Geiselman et al. (2020) constructed a heterologous synthetic pathway for non-native diterpene *ent*-kaurene in *R. toruloides*, and achieved the synthesis of *ent*-kaurene. The supply of the precursor geranylgeranyl diphosphate was found to be the limiting factor for the synthesis of *ent*-kaurene. The exploration and introduction of a more effective farnesyl pyrophosphate synthase (FPS) and the balanced expression of FPS and *ent*-kaurene synthase enabled the engineered strain to produce 1.4 g/L of *ent*-kaurene from corn stover hydrolysate in a 2 L bioreactor [142]. *R. toruloides* is also employed for the production of heterologous non-ribosomal peptides (NRPs). Wehrs et al. (2019) heterologously expressed the indigoidine synthase gene (*BpsA*) from *Streptomyces lavendulae* and the 4′-phosphopantetheinyl transferase gene (*sfp*) from *Bacillus subtilis* in *R. toruloides*, which resulted in the production of 2.9 g/L of blue pigment indigoidine from a sorghum lignocellulosic hydrolysate [143]. In addition to NRPs, Otoupal et al. (2022) achieved the production of the polyketide product triacetic acid lactone (TAL, 2.0 g/L) in *R. toruloides* by heterologously expressing the codon-optimized 2-pyrone synthase gene (*2-ps*) from *Gerbera hybrida* using sorghum straw hydrolysates. Further implementation of the strain in a one-pot separation-free process, which carried out the pretreatment, saccharification, and fermentation, enabled the production of 3.9 g/L TAL in a 2 L bioreactor from sorghum straw hydrolysates, which represents the highest titer of TAL obtained from lignocellulosic biomass [144]. These studies highlight the potential of *R. toruloides* as a CBP chassis for the conversion of lignocellulose into bio-based products by further introducing heterologous lignocellulolytic enzyme genes into its genome.

The lipid-producing fungus *Mucor circinelloides* is a model organism for the study of lipid accumulation and lipid production. *M. circinelloides* can metabolize a variety of sugars (e.g., glucose and xylose) present in lignocellulose hydrolysates, making it one of the ideal microorganisms for the conversion of lignocellulose into functional lipids [145]. Zhang et al. (2021) increased xylose consumption and lipid production by overexpressing the genes encoding xylose isomerase (XI) and xylulose kinase (XK) in *M. circinelloides*. Compared to the control strain, the fatty acid content of the two constructed strains (Mc-XI and Mc-XK) increased by 19.8 % and 22.3 %, respectively. In addition, the uptake of xylose from corn stover hydrolysate by the engineered strains was significantly increased by 71.5 % (Mc-XI) and 68.8 % (Mc-XK), respectively. Using the corn stover hydrolysates as feedstock, the engineered strain achieved a production of 2.17–2.28 g/L of lipid in a 2 L bioreactor [106]. Further enhancing the lignocellulose-degrading capability of *M. circinelloides* will enable it to be a CBP chassis for lipid production from lignocellulosic biomass.

Table 3
Synthesis of bio-based chemicals from lignocellulose using microbial co-culturing CBP systems.

Cellulolytic microorganism	Biosynthetic microorganism	Substrate	Product	Reference
T. thermosaccharolyticum M5	*A. succinogenes* 130Z	80 g/L unpretreated corn cobs	12.51 g/L succinic acid	[46]
Streptomyces. sp. SirexAA-E	*P. megaterium*	5 g/L *Miscanthus* biomass	40 mg/g PHA	[149]
C. cellulovorans DSM 743B	*C. beijerinckii* NCIMB 8052	30.1 g/L AECC	3.94 g/L *n*-butanol	[150]
C. cellulovorans DSM 743B	*C. beijerinckii* NCIMB 8052	55.1 g/L AECC	1.05 g/L isobutanol and 6.22 g/L *n*-butanol	[151]
T. reesei	*R. delemar*	40 g/L MCC	6.87 g/L fumaric acid	[155]
T. reesei Rut-C30	*U. maydis*	270 g/L α-cellulose	33.8 g/L itaconic acid	[157]
T. reesei C10	*S. cerevisiae* LGA-1C3S2	50 g/L SECS	6.42 g/L d-glucaric acid	[158]
T. reesei Rut-C30	*E. coli* NV3 pSA55/69	20 g/L AFEX-pretreated corn stover	1.88 g/L isobutanol	[159]
C. phytofermentans	*S. cerevisiae* cdt-1	100 g/L α-cellulose	22 g/L ethanol	[162]
T. reesei	*L. pentosus*	5 % (w/w) MCC	34.7 g/L lactic acid	[164]
T. reesei	*L. pentosus* and *C. tyrobutyricum*	Beechwood	196 kg/t butyric acid	[165]

3.3. Construction of CBP with microbial co-culturing systems

In nature, the effective degradation of lignocellulose occurs through the synergistic action of multiple bacteria, fungi, protists, and wood-feeding animals [146]. Inspired by this, CBP with microbial co-culturing systems is receiving increased attention [47] (Fig. 1, Table 3).

The simultaneous expression of both lignocellulolytic enzymes and biobased product synthetic enzymes will increase the metabolic burden of a specific microorganism. Unlike a single microorganism, in microbial co-culture systems, the expression of lignocellulolytic enzymes and bio-product synthetic enzymes can be accomplished in different microorganisms, thus being able to relieve the cellular metabolic burden through functional specialization [147]. In CBP with a microbial co-culture system, the degradation of lignocellulose is performed by upstream strains, while the production of bio-based chemicals is achieved by downstream strains. In particular, the rapid consumption of fermentable sugars by downstream strains can alleviate the substrate inhibition of lignocellulolytic enzymes and thus facilitate the hydrolysis of lignocellulose by upstream strains [148]. The three main types of microbial co-culture systems are bacteria and bacteria, fungi and fungi, and fungi and bacteria co-culture systems. Consequently, the establishment of a stable and efficient synthetic microbial community for industrial manufacturing is an important research topic.

3.3.1. Co-culturing systems with bacteria and bacteria

A specific division of labor between cells in a co-culture system is important for efficient lignocellulose degradation and conversion. In the study by Lu et al. (2020), they created a co-culture system consisting of the hemicellulase-producing *Thermoanaerobacterium thermosaccharolyticum* strain M5 and a succinic acid-producing *Actinobacillus succinogenes* strain. Under optimized conditions, this CBP co-culture system successfully achieved succinic acid production from xylan and unpretreated corn cobs [46]. In this CBP co-culture system, *T. thermosaccharolyticum* secreted xylanase and β-xylosidase to degrade xylan to xylose, which was used by *A. succinogenes* for succinic acid production. In addition, the rapid consumption of xylose by *A. succinogenes* also alleviated the inhibition of the xylanase activity. These two strains thus exhibited a good synergistic effect, making the whole fermentation process more efficient. By optimizing the fermentation conditions, such as inoculation time and pH, this CBP co-culture system produced 32.50 g/L and 12.51 g/L of succinic acid from 84 g/L of xylan and 80 g/L of corn cobs, respectively.

The synthesis of polyhydroxyalkanoate (PHA) using lignocellulosic biomass is a sustainable way to achieve the production of bioplastic-based PHA. The highly cellulolytic strain *Streptomyces* sp. SirexAA-E can hydrolyze cellulose and hemicellulose but cannot produce PHA. Kumar et al. (2023) co-cultured *Streptomyces* sp. SirexAA-E with *Priestia megaterium*, which cannot utilize plant polysaccharides for growth but is capable of producing PHA. Under optimized conditions (30 °C, pH 7, 5 g/L of *Miscanthus* biomass, an inoculation ratio of 1:4 (v/v) of *Streptomyces*. sp. SirexAA-E and *P. megaterium*), this co-culture system produced 40 mg PHA/g of *Miscanthus* biomass [149].

Microorganisms in a synthetic microbial co-culture system of CBP generally do not undergo long-term coevolution, and therefore the growth and metabolism of members of the CBP co-culture system need to be artificially coordinated. Genetic engineering and adaptive evolution are usually performed to improve the adaptability between members of a CBP co-culture system. Wen et al. (2020) constructed a dual *Clostridium* co-culture system consisting of *C. cellulovorans* DSM 743B and *Clostridium beijerinckii* NCIMB 8052, which can directly utilize AECC to produce *n*-butanol [150]. In this co-culture system, the cellulolytic microorganism *C. cellulovorans* DSM 743B could degrade cellulose into fermentable sugars and also produce butyric acid, which supported the growth of *C. beijerinckii* and its production of butanol. Moreover, the consumption of fermentable sugars and butyric acid alleviated the feedback inhibition on cellulase activity and toxicity to the strain, respectively. The production of butanol requires a low ambient pH (pH 4.5–5.5) conditions; however, at any pH values below 6.4, the cellulolytic *C. cellulovorans* grows poorly and thus cannot produce enough fermentable sugars from lignocellulose for the growth of both strains and the production of butanol. Therefore, the authors further engineered *C. cellulovorans* to improve its tolerance to a low pH value. Without pH control, the engineered co-culture system produced 3.94 g/L of butanol in 83 h, which was 5 times more than the control under the same conditions. Wen et al. (2022) further introduced the isobutanol biosynthetic pathway into *C. beijerinckii*, allowing it to produce both butanol and isobutanol [151]. After medium optimization, the recombinant *C. beijerinckii* strain was able to produce 194 mg/L of isobutanol and 7.16 g/L of butanol from glucose. Overexpression of acetaldehyde/ethanol dehydrogenase (*adhE1*), ketoisovalerate decarboxylase (*kivD*), and aldehyde reductase (*yqhD*) in *C. cellulovorans* enabled the strain to synthesize 156 mg/L of isobutanol and 1.81 g/L of butanol within 120 h from 39.5 g/L of AECC. Finally, the co-culture of the above two recombinant strains yielded 1.05 g/L and 6.22 g/L of isobutanol and butanol, respectively, which were 6.73 and 3.44 times higher than the monoculture.

3.3.2. Co-culturing systems with fungi and fungi

Filamentous fungi display high lignocellulose-degrading ability and exuberant metabolisms, which provide great potential in the biorefinery of lignocellulosic biomass [152–154]. Therefore, CBP co-culture systems consisting of fungi and fungi have also been widely studied. A fungal CBP co-culture system consisting of the lignocellulose-degrading fungus *T. reesei* and the fumaric acid-producing strain *Rhizopus delemar* was established by Scholz et al. (2018). In this fungal co-culture, cellulases produced by *T. reesei* degraded lignocellulosic biomass to release fermentable sugars, which were immediately converted to fumaric acids by *R. delemar* in the same bioreactor. The titer of fumaric acids produced by this fungal co-culture reached 6.87 g/L using 40 g/L of MCC as the substrate [155]. No addition of cellulases or expensive supplements such as yeast extract are required in the above process, which can significantly reduce the production cost. *Ustilago maydis* has a strong ca-

pacity for the production of itaconic acid. Although *U. maydis* possesses its own lignocellulolytic enzymes [156], the cellulase activity of *U. maydis* is so low that it cannot efficiently produce itaconic acid directly from lignocellulose. Schlembach et al. (2020) co-cultured *U. maydis* with the cellulolytic fungus *T. reesei*, which can grow in a similar environment at 30 °C under aerobic conditions. In this co-culture system, *T. reesei* was responsible for the degradation of lignocellulose, while *U. maydis* was responsible for the production of itaconic acid. With a clear division of labor between the two fungi, this co-culture system produced 33.8 g/L of itaconic acid from 270 g/L of α-cellulose in a fed-batch fermentation [157].

Fang et al. (2022) designed an microbial co-culture system consisting of *T. reesei* C10 and an engineered *S. cerevisiae* strain LGA-1 [158]. *T. reesei* C10 could produce more cellulases and thus release more fermentable sugars from the lignocellulose, while the *S. cerevisiae* strain LGA-1 was engineered to metabolize cellobiose for the biosynthesis of d-gluconic acid. This *T. reesei-S. cerevisia*e co-culture system managed to produce 6.42 g/L of d-glucaric acid from 50 g/L of steam-exploded corn stover (SECS). Both cellulase production by *T. reesei* and d-glucaric acid production by *S. cerevisiae* were carried out under aerobic conditions, which simplified the process in commercial applications. Thus, this *T. reesei-S. cerevisia*e co-culture system provides a promising CBP platform for the direct conversion of lignocellulose to d-glucaric acid. However, the yield is not yet sufficient for industrialization and warrants further investigation.

3.3.3. Co-culturing systems with fungi and bacteria

Regarding the co-culture of fungi and bacteria, Minty et al. (2013) developed a powerful fungal-bacterial consortium for the conversion of lignocellulose into valuable products [159]. This consortium was composed of two "specialists", the cellulolytic specialist *T. reesei* Rut-C30, which secretes cellulases to hydrolyze lignocellulose into soluble sugars, and the fermentation specialist *E. coli* NV3 pSA55/69, which metabolizes soluble sugars to synthesize bio-based products. The *E. coli* strain was metabolically engineered to produce isobutanol [160,161]. The authors used this synthetic fungal-bacterial consortium to achieve the direct conversion of AFEX-pretreated corn stover to 1.88 g/L of isobutanol, reaching up to 62 % of the theoretical maximum [159].

A co-culture system developed by Zuroff et al. (2013) consisting of the cellulase-producing *C. phytofermentans* and the cellodextrin-fermenting yeast *Candida molischiana* or *S. cerevisiae* cdt-1 is another example of a system exhibiting division of labor between cellulolytic and sugar fermentation microorganisms. By controlling the volumetric transport rate of oxygen, a symbiotic relationship was established between *C. phytofermentans* and the yeast species. Both yeasts were able to provide respiratory protection to the obligatory anaerobic bacterium *C. phytofermentans* in exchange for soluble sugars released by lignocellulose hydrolysis. The yeasts were able to convert these soluble sugars to ethanol, thus enabling direct ethanol production from α-cellulose [162]. However, the lignocellulose degradation by *C. phytofermentans* was relatively low under high substrate loading; thus, additional EGs were added to the co-culture of *C. phytofermentans* and *S. cerevisiae* cdt-1, achieving the conversion of 100 g/L of α-cellulose into approximately 22 g/L of ethanol, which is significantly higher than that of *C. phytofermentans* (6 g/L) and *S. cerevisiae* cdt-1 (9 g/L) monocultures.

The lignocellulose-degradation rate remains the key rate-limiting step in CBP. Therefore, accelerating the rate of lignocellulose-degradation and increasing the release rate of fermentable sugars is critical for the application of CBP. In general, fungi have a higher lignocellulose-degradation capacity than bacteria [163]. However, the distinction in fungal and bacterial growth conditions, such as temperature, oxygen demand, and pH, is also a critical issue that needs to be addressed for fungal and bacterial co-culture systems. Shahab et al. (2018) took advantage of metabolic compartmentalization and spatial structure to construct a fungal-bacterial co-culture system consisting of the aerobic fungus *T. reesei* and the facultative anaerobic bacterium *Lactobacilli pentosus* in a biofilm reactor, achieving the synthesis of 34.7 g/L of lactic acid with 5 % (w/w) MCC as the substrate [164]. This is the first reported production of lactic acid from lignocellulose using microbial co-cultures. In this co-culture system, aerobic *T. reesei* formed a biofilm on the surface of an oxygen-permeable, dense tubular membrane, through which oxygen could diffuse into the fungal biofilm by locally defined aeration of the tubular membrane. *T. reesei* consumed oxygen and produced cellulases and hemicellulases under aerobic conditions, and these cellulolytic enzymes were secreted into the fermentation slurry, which could effectively degrade lignocellulose and release soluble sugars. Since all the oxygen was consumed in the biofilm, facultative anaerobic *L. pentosus* fermented the different sugars to produce lactic acid under anaerobic conditions. Further addition of *Clostridium tyrobutyricum* to the co-culture system of *T. reesei- L. pentosus* achieved the production of 196 kg of butyric acid per ton of beechwood [165]. In addition, replacing *C. tyrobutyricum* with the specialized anaerobic bacteria *Veillonella criceti* and *Megasphaera elsdenii*, which synthesize various short-chain fatty acids (SCFAs) using lactic and acetic acid as substrates, respectively, achieved the conversion of lignocellulose to SCFAs [165]. The utilization of oxygen by *T. reesei* and *L. pentosus* created a lower redox condition in the co-culture system, for the growth of anaerobic microorganisms, making the co-culture stable.

4. Outlook and challenges

Although CBP is considered a promising strategy for the production of biofuels and biochemicals from lignocellulosic biomass in a single bioreactor, CBP still faces some practical challenges such as the recalcitrant nature of lignocellulosic biomass, the tolerance of strains to the toxic compounds generated during lignocellulosic degradation, the engineering of strains for the high yield of various products, and the complexity of the metabolic interactions between different microorganisms used in consortium. In the monomicrobial systems, the construction of CBP with natural lignocellulose-degrading microorganisms as the chassis requires the multiple steps of strain engineering for the introduction of synthetic pathway and strain metabolic engineering, which requires efficient genomic editing techniques. This is particular time-consuming for lignocellulose-degrading fungi. The construction of CBP with biosynthetic microorganisms as the chassis requires the heterologous expression of a suite of cellulases, hemicellulases and LPMOs to accomplish the efficient degradation of lignocellulosic biomass, which is also challenging due to the low expression levels of lignocellulolytic enzymes. Considering that the efficient degradation of lignocellulose requires enzyme cocktails with multiple proteins and the product biosynthetic pathway also requires multiple genes, the development of CBP microbial co-culture systems has strong potential. In this regard, a specific division of labor between strains in the co-culture systems is critical for effective lignocellulose degradation and product biosynthesis. Establishing robust synthetic microbial communities with both high lignocellulose-degradation and bio-product synthesis capabilities is an important future research direction, which requires an in-depth understanding of the interactions between enzymes, metabolic pathways, and microorganisms.

As CBP combines multiple steps in a single bioreactor, the switching of bio-product synthesis is not flexible. Liu et al. (2020) proposed a consolidated bio-saccharification (CBS) strategy using cellulosomes as biocatalysts and achieved the integration of hydrolytic enzyme production and saccharification of lignocellulosic biomass [166]. The CBS strategy can separate the downstream fermentation steps to some extent, providing more flexibility than the consolidated bioprocessing technology in fermentations for different biochemicals. Liu et al. integrated the CBS strategy with the fermentation of the deep-sea yeast strain *Rhodotorula paludigena* P4R5 for the synthesis of polyol esters of fatty acids (PEFA). The authors further developed a semi-continuous process without complicated product separation, which resulted in the production of 41.1 g/L of PEFA from corn cob hydrolysis residues [167].

Recently, they isolated a thermophilic lactic acid-producing *Geobacillus stearothermophilus* 2H-3 and combined it with the developed CBS, achieving the production of 51.36 g/L of lactic acid from various agricultural wastes, including corn stover, corncob residue, and wheat straw [168]. In the future, strains used in CBS may be co-cultured with downstream fermentation strains to develop an efficient co-culture system for the economic production of biochemicals from lignocellulose.

The recent digital revolution based on machine learning (ML) and artificial intelligence (AI) tools is transforming many research fields in biotechnology [169,170] and will also be helpful for biorefinery development in key enzyme engineering, optimization of degradation enzyme cocktails, improvement of enzyme production and secretion, strain metabolic engineering, and the construction of co-culture consortia based on iterative Design-Build-Test-Learn cycles [171,172]. Models based on an artificial neural network have been implemented to predict the sugar yields of inorganic salt-based pretreatments of lignocellulosic biomass; they exhibited high coefficients of determination (R^2 of 0.097), facilitating the initial screening of lignocellulose bioprocess development [173]. A novel model for the optimization of enzyme cocktails has been recently established based on deep-learning methods. With no need for reliance on expert-level prior reaction mechanism knowledge, the developed model speeded up the optimization and screening of enzyme cocktails for the efficient degradation of complex lignocellulose substrates [174]. Recently, Huang et al. (2023) explored the potential of establishing machine learning models to simulate lignocellulosic biomass-based mixed sugar fermentation, which facilitates strain comparison, product titer evaluation, and fermentation profile construction. These AI tools in combination with the developments in synthetic biology and metabolic engineering will greatly facilitate the development and improvement of CBP to achieve the economical production of desired products from lignocellulosic biomass [175,176].

Taken together, CBP combines multiple-steps of biorefinery in a single bioreactor and represents a promising route to achieve the conversion of lignocellulose into high-value-added bio-products. Importantly, the CBP technology avoids the addition of exogenous hydrolytic enzymes, which can significantly reduce the cost of lignocellulose biorefinery. CBP technologies with cellulolytic or biosynthetic microorganisms, such as chassis and microbial co-culture systems, show great potential and are expected to be able to provide alternative pathways worth exploring for lignocellulose biorefinery. In addition, it is worthwhile to investigate the potential of CBP for the production of fuel and different industrial chemicals from low-cost renewable lignocellulosic biomass with the aid of synthetic biology and artificial intelligence.

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.

CRediT Authorship Contribution Statement

Zhongye Li: Writing – original draft, Investigation, Writing – review & editing. **Pankajkumar R. Waghmare:** Writing – review & editing, Writing – original draft. **Lubbert Dijkhuizen:** Writing – review & editing. **Xiangfeng Meng:** Writing – review & editing, Funding acquisition. **Weifeng Liu:** Writing – review & editing, Funding acquisition.

Acknowledgments

This work was supported by grants from the National Key R&D Program of China (No. 2019YFA0905700), the National Natural Science Foundation of China (31970071), the Young Scholars Program of Shandong University, and the Major Basic Research of Shandong Provincial Natural Science Foundation (ZR2019ZD19).

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