Renewable and Sustainable Energy Reviews 82 (2018) 3863–3885

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

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

Metabolic engineering of microorganisms for biofuel production

Parastoo Majidian[a], Meisam Tabatabaei[a,b,*,1], Mehrshad Zeinolabedini[a], Mohammad Pooya Naghshbandi[c], Yusuf Chisti[d,**]

[a] Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[b] Biofuel Research Team (BRTeam), Karaj, Iran
[c] Department of Microbial Biotechnology, School of Biology, College of Science, University of Tehran, Tehran, Iran
[d] School of Engineering, Massey University, Palmerston North, New Zealand

ARTICLE INFO

Keywords:
Metabolic engineering
Biofuels
Surface display
Microalgae
Cyanobacteria
Yeasts

ABSTRACT

Microorganisms directly and indirectly contribute to production of diverse biofuels. Heterotrophic microorganisms are being used for commercial production of biofuels such as biogas and fuel alcohols from organic matter. Photosynthetic microorganisms convert inorganic carbon and water to potential fuels (e.g. fuel alcohols, biohydrogen) and fuel precursors (e.g. biomass, starch, lipids). Only a few microbial processes are used for commercial production of biofuels, but this will certainly change with the enhanced production capabilities being achieved through microbial metabolic engineering. Processes that previously required multiple steps of feedstock pretreatment and subsequent conversion to fuel are being consolidated into single-step microbial processes using metabolically engineered species. Microorganisms with the ability to produce fuels from feedstock they could not use previously, are being engineered. This review discusses some of the metabolic engineering approaches being used to enhance the commercialization potential of microbial biofuels including fuel alcohols, biodiesel and biohydrogen. At present, all biogas production relies on native populations of methanogens and this does not seem likely to change in the near term. Potential fuels from microalgae, cyanobacteria and other photosynthetic bacteria, whether native or engineered, have distant prospects of commercial use. Metabolically engineered yeasts surface displaying various hydrolytic enzymes appear to hold the greatest potential for near term commercial use in generating bioethanol from starch, pretreated lignocellulose and other polysaccharides. The bacterium *Zymomonas mobilis* metabolically engineered to make bioethanol from pentose sugars is already being commercialized. Other similar examples are likely to emerge as more engineered microorganisms become available.

1. Introduction

The global energy consumption continues to increase in keeping up with increasing industrialization and improving quality of life. Fossil fuels are currently our main sources of energy, but renewable energy carriers including biofuels have been receiving increasing attention [1,2]. Biofuels include mainly bioethanol, biodiesel, biogas, and biomass. Displacing a part of the fossil fuels with renewable biofuels has the potential to reduce emissions of greenhouse gases and improve the environmental quality [3–6].

All biofuels represent energy captured from sunlight. More specifically, photosynthesizing microorganisms and higher plants capture sunlight and use it to convert carbon dioxide and water to biochemicals. Some of these biochemicals may be used directly as fuels. In other cases, they may serve as feedstock for making fuels either through the action of other microorganisms [7] or via chemical processes. For example, starch and cellulose produced via photosynthesis may be converted into bioethanol through the action of non-photosynthesizing microorganisms. Similarly, photosynthetically-produced biomass and oils can be converted into fuels such as biodiesel and gasoline using chemical technologies. Microbial biomass generated via photosynthesis may eliminate the need for arable land for fuel production and, hence, reduce potential impact of biofuels on production of food and fodder [8,9].

Production of biofuels through microbial photosynthesis and microbial bioconversion of an organic feedstock can be greatly improved by genetic and metabolic enhancements of the relevant microorganisms. Traditionally, microbial strains for industrial applications have

* Corresponding author at: Biofuel Research Team (BRTeam), Karaj, Iran.
** Corresponding author.
E-mail addresses: meisam_tab@yahoo.com, meisam_tabatabaei@abrii.ac.ir (M. Tabatabaei), Y.Chisti@massey.ac.nz (Y. Chisti).
[1] (www.brteam.ir)

https://doi.org/10.1016/j.rser.2017.10.085
Received 6 August 2016; Received in revised form 10 July 2017; Accepted 28 October 2017
Available online 10 November 2017
1364-0321/

been improved by selection, random mutagenesis and screening processes [10,11]. However, these processes are largely uncontrolled, slow, and unpredictable. In some cases, it may be impossible to obtain a desired phenotype through mutagenesis and selection if the relevant biochemistry is missing in a microorganism [12]. Therefore, the enhancement of microorganisms for various purposes increasingly relies on rational engineering including genetic and metabolic engineering [12,13]. Genetic and metabolic engineering are being used to modify the surfaces of the cells to display novel proteins and other substances for improving biocatalytic capability of the cells [14,15]. For example, microorganisms displaying specific enzymes on the surface may improve enzymatic conversion of starch into sugars for fermentation to fuel alcohols. Moreover, surface display of desired proteins in engineered cells allows easy reproduction of the displayed biocatalyst and easy separation of the product from the catalyst [16,17]. Cells displaying a suite of the relevant surface enzymes and having the relevant intracellular metabolic pathways could greatly improve the ability to convert certain feedstocks to biofuels. Metabolic engineering can provide microorganisms with improved titers and yields of products; with the capacity to use an expanded range of carbon substrates; and with the ability to better withstand high titers of potentially toxic products and adverse environmental conditions [18]. Specific biochemical pathways can be enhanced by manipulating the production and regulation of specific enzymes and deletion of pathways that may be counterproductive. Entirely new metabolic pathways taken from one microorganism may also be engineered into a superior host organism [14]. Overall, metabolic engineering allows precise modification of microbial species without causing an accumulation of unfavorable mutations [14].

Metabolic engineering together with genetic engineering are among core technologies for enhanced production microbial biofuels. Continuing developments in synthetic biology, genetic engineering and manipulation of enzyme expression allows biofuel-producing pathways to be inserted in a microbial host [19]. This review discusses some of the metabolic engineering approaches used in production of main types of biofuels including bioethanol, biobutanol, other alcohols, biodiesel, and biohydrogen. Developments in fuel production using engineered cyanobacteria, other bacteria, microalgae, and yeasts are discussed. The prospects of the use of system metabolic engineering for generating superior biofuel-producers strains are assessed.

2. Metabolic engineering for biofuels production

Product diversity, yield, concentration, and productivity may be enhanced in metabolically-engineered microorganisms for enhanced fuel production [20–22]. Moreover, downstream recovery of a certain biofuel may also be simplified through metabolic engineering. Furthermore, the cost of fuel production may be reduced by engineering microorganisms to use cheap substrates [23]. Some metabolic pathways and other routes used in microbial production of fuel precursors and common fuels are summarized in Fig. 1 [24,25].

2.1. Bacteria

Numerous bacteria are able to convert various substrates into fuels such as biohydrogen, bioalcohols, and biogas. Novel bacteria with enhanced production potential for fuels are continually being sought from nature, in particular from thermophilic environments. Recent advances in the use of bacteria for producing alcohols and hydrogen are discussed in the following sections. Methanogenic archaea and production of biogas have been extensively reviewed elsewhere [26–31].

2.1.1. Bioethanol

2.1.1.1. Zymomonas mobilis. Bioethanol is the most commonly produced biofuel and is used as an additive to gasoline. Owing to its clean burning characteristics, fuel ethanol can significantly reduce emissions of greenhouse gases including carbon dioxide [32]. The anaerobic bacterium *Zymomonas mobilis* is perhaps the best known native producer of bioethanol [33,34]. Compared to yeasts, *Z. mobilis* more efficiently converts glucose to bioethanol, but cannot naturally use most other sugars.

Z. mobilis and the classical ethanologenic yeast *Saccharomyces cerevisiae* both share a natively-expressed homoethanol pathway. However, they differ in terms of their glycolysis; *S. cerevisiae* uses the Embden-Meyerhof-Parnas (EMP) pathway while *Z. mobilis* uses the Entner-Doudoroff (ED) pathway [35]. The ED pathway is 50% more efficient than the EMP pathway leading to less ATP consumption during the ethanologenic process. In addition, *Z. mobilis* is capable of consuming glucose faster than *S. cerevisiae* apparently because of its high cell specific surface area. These factors collectively result in a higher ethanol productivity in *Z. mobilis* in comparison with *S. cerevisiae* [36]. Ethanol production is mainly controlled by the PET operon encoding pyruvate decarboxylase and alcohol dehydrogenase. On a given amount of glucose, *Z. mobilis* generally produces less biomass than yeast [34] and this is considered an advantage. In addition to ethanol, other by-products may also be produced [36].

The inability of Z. *mobilis* to convert pentose sugars to ethanol is its main drawback as cheap pentose sugars are abundantly available in lignocellulosic feedstocks. This problem has been recently addressed by metabolic engineering of pentose utilization capability in *Z. mobilis* [37]. Ethanol production using this technology is being commercialized by DuPont. A recombinant *Z. mobilis* strain TMY-HFPX with multiple improved characteristics was developed and shown to achieve an ethanol concentration of up to 136 g/L in a solution containing 295 g/L glucose (90% of theoretical yield) without requiring exogenous amino acids and vitamins [38]. The enhanced strain reportedly harbored multiple gene modules, i.e., the *xylA/xlyB/tktA/talB* operon for xylose utilization, the *metB/yfdZ* operon for lysine and methionine biosynthesis, the thioesterase gene *tesA* to enhance free fatty acid biosynthesis for increasing ethanol tolerance, a proton-buffering peptide operon for acid stress tolerance, and a small heat shock protein operon for heat stress tolerance [38].

2.1.1.2. Escherichia coli. The bacteria *Escherichia coli* and *Bacillus subtilis* have been most widely used to construct ethanol producing strains because the molecular biology of these microorganisms is well understood. They are capable of utilizing diverse substrates and are amenable to metabolic engineering for exclusive production of ethanol [39–44]. *E. coli* was probably the first microorganism to be successfully modified for ethanol production through metabolic engineering [45]. This initial success was further built upon by introducing the relevant foreign genes in *E. coli* and disrupting pathways for competing products. Thus, the *E. coli* W (wild) was transformed into *E. coli* KO11, a novel strain capable of producing ethanol [33]. *E. coli* KO11 contained genes from *Z. mobilis* for encoding pyruvate decarboxylase and alcohol dehydrogenase (PET operon). In addition, the gene for production of fumarate reductase was deleted. *E. coli* KO11 was successfully adapted to produce ethanol at nearly 95% of the theoretical yield in a complex medium and showed increased ethanol tolerance relative to the original host. However, this strain was unable to grow in ethanol concentration of 3.5% and required complex nutritional supplements which contributed to the cost of ethanol production [33].

In a different investigation, ethanol tolerance of the strain KO11 was increased by 10% by adaptive evolution and selection through long term exposure to media supplemented with increasing ethanol content. The adapted strain was LY01. However, both LY01 and its parent KO11 required complex nutritional supplements and this added to the expenses of ethanol production. Therefore, *E. coli* KO11 was further engineered to strain SZ110 to allow ethanol production in less nutritionally-demanding media [45]. The strain SZ110 was engineered by eliminating lactate dehydrogenase gene and inserting pyruvate formate

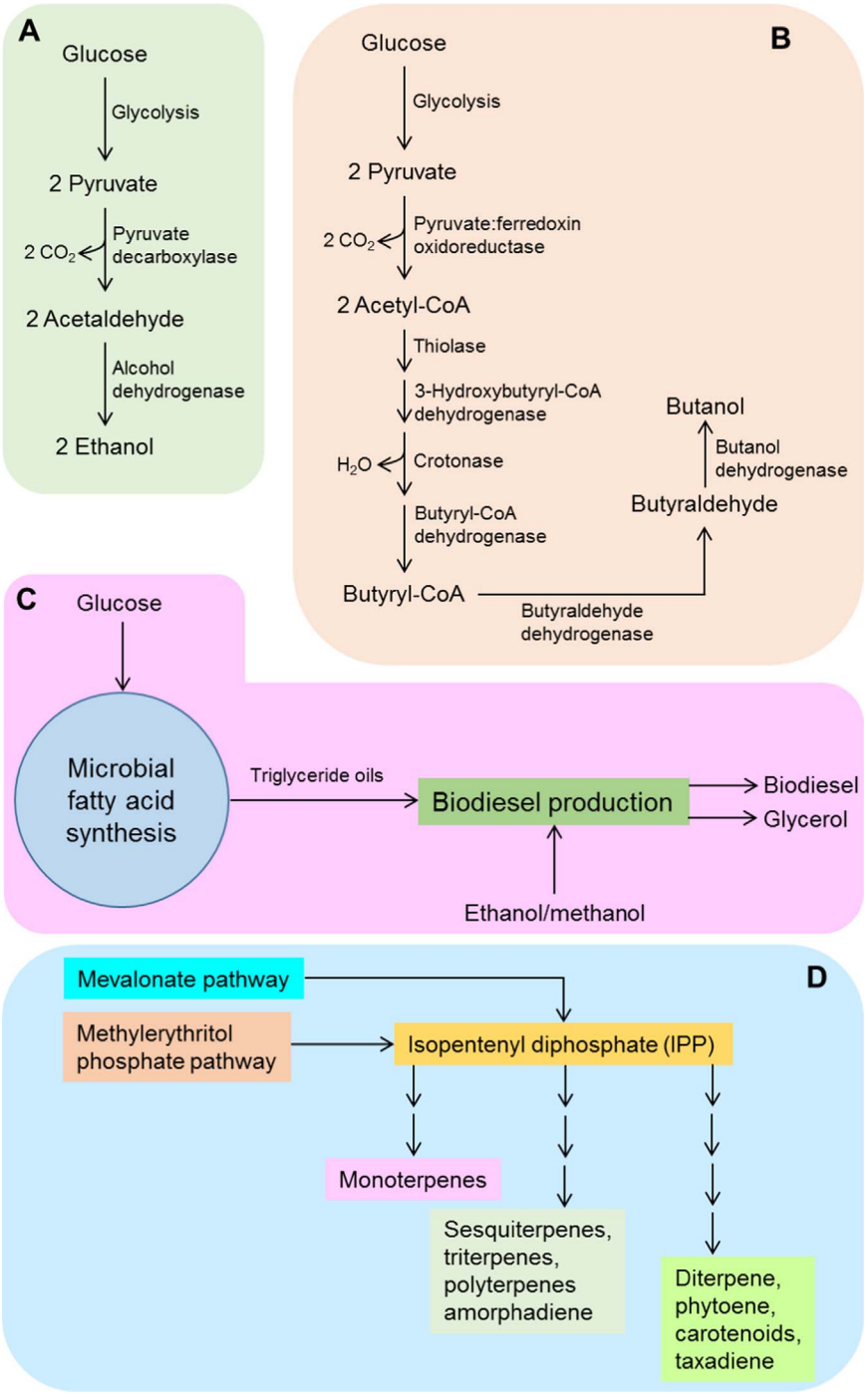

Fig. 1. Major pathways for the production of: ethanol (A); butanol (B); biodiesel (C); and isoprenoid fuel precursors [24] (D).

lyase and PET operon from *Z. mobilis* [33]. Further engineering of SZ110 by transposon-mediated mutagenesis and metabolic evolution provided the strain LY168 [46]. First, the transposon containing promoterless *pdc*, *adhA*, and *adhB* genes were randomly inserted into the chromosome of SZ110. Functional selections were subsequently performed by serial transfers in mineral salts media without antibiotics [47]. Eventually, the combination of genetic engineering and long term adaptation resulted in microbial biocatalysts that produced up to 45 g ethanol/L in 48 h in a simple mineral salts medium. Overall, the strain LY168 successfully grew and produced ethanol in media containing only mineral salts, a high concentration of sugar and the osmoprotectant betaine. The strain LY168 could reportedly produce 0.5 g ethanol/g xylose, or close to the maximum theoretical yield of 0.51, in a minimal medium provided with betaine [33]. The key difference between the strains LY168 and KO11 was how the expression of the homoethanol pathway was regulated. In strain KO11, the *Z. mobilis pdc* and *adb* genes were regulated by *pfl* promoter with multiple layers of transcriptional control, while in strain LY168, the genes encoding the homoethanol pathway were integrated within the gene encoding 23S ribosomal RNA subunit *rrlE*, concurrent with the direction of transcription.

Recently, the glycolytic pathway of *E. coli* KO11 was engineered by knocking out phosphoglucose isomerase (*pgi*) to channel carbon flux from glucose through the Entner–Doudoroff (ED-P) and pentose phosphate (PP-P) pathways to lactate and acetate production [48]. However, the engineered strain grew quite slowly under non-aerated conditions in minimal media containing 40 g/L glucose. To improve KO11 Δpgi's capacity to grow, the recombinant strain was evolved for 60 days

Table 1
Ethanol yield of some genetically engineered thermophilic bacteria.

Microorganism	Substrate	Genotype[a]	Mode	Ethanol yield (mol/mol)	Reference
Clostridium thermocellum	Cellobiose	ΔpyrF, Δpta::gapDHp-cat	Batch	0.59	[55]
C. thermocellum	Avicel	ΔpyrF, Δpta::gapDHp-cat	Batch	0.71	[55]
C. thermocellum adhE*(EA) Δldh	Cellobiose	Δhpt, Δldh	Batch	0.37	[56]
C. thermocellum	Avicel	Δhpt, Δldh, Δpta (evolved)	Batch	1.08	[56]
C. thermocellum/Thermoanaerobacterium saccharolyticum	Avicel	Δhpt, Δldh, Δpta (evolved) and Δpta, Δack, Δldh	Batch	1.26	[56]
T. saccharolyticum TD1	Xylose	Δldh	Batch	0.98	[56]
T. saccharolyticum ALK2	Cellobiose	Δpta, Δack, Δldh	Continuous	ND	[57]
T. saccharolyticum HK07	Cellobiose	Δldh, Δhfs	Batch	0.86	[58]
T. saccharolyticum M0355	Cellobiose	Δldh, Δack Δpta	Batch	1.73	[59]
T. saccharolyticum M1051	Cellobiose	Δldh, Δack Δpta, ureABCDEFG	Batch	1.73	[57]
Geobacillus thermoglucosidasius TM242	Glucose	Δldh-, pdh up, pflB-	Batch	1.73	[60]
G. thermoglucosidasius TM242	Glucose	Δldh-, pdh up, pflB-	Batch	1.84	[60]
G. thermoglucosidasius TM242	Xylose	Δldh-, pdh up, pflB-	Batch	1.34	[60]
Thermoanaerobacter mathranii BG1L1	Wheat straw	Δldh	Continuous	1.53–1.67	[61]
T. mathranii BG1G1	Glucose + glycerol	Δldh, GldA	Batch	1.68	[62]
T. mathranii BG1G1	Xylose + glycerol	Δldh, GldA	Batch	1.57	[62]
T. mathranii BG1G1	Xylose + glycerol	Δldh, GldA	Continuous	1.53	[62]

[a] ack, acetate kinase; GldA, glycerol dehydrogenase A; hfs, hydrogenase; hpt, hypoxanthine phosphoribosyl transferase; pdh, pyruvate decarboxylase; pyrF, orotidine-5-phoshate decarboxylase; pfl, pyruvate formate lyase; ure, urease; ND, not determined.

and renamed KO11 E35. Compared with its parental strain KO11, the KO11 E35 had higher activities of glucose-6-phosphate dehydrogenase and the ED-P enzymes. Subsequently, KO11 E35 was further improved by deleting the *pta*, *ack*, and *ldh* genes leading to a homoethanologenic derivative called KO11 PPAL strain. This strain possessed specific ethanol production rates similar to those achieved by KO11, but diverted less glucose to biomass production. This led to larger yields of ethanol on glucose and biomass. These improved characteristics were ascribed to the strain's capability to channel most of the carbon flux from pyruvate to ethanol as well as increased expression of heterologous pyruvate decarboxylase and alcohol dehydrogenase from *Z. mobilis* [48].

In a different study, the homoethanol producer *E. coli* SE2378 (*Δldh Δpfl*) was obtained via mutagenesis and had an ethanol productivity of 2.24 g/(h g-cells) at nearly 80% of the theoretical ethanol yield [46]. Another homoethanol producer *E. coli* SZ420 (*ΔfrdBC Δldh ΔackA ΔfolA-pfl ΔpdhR::pflBp6-aceEF-lpd*) was made by deleting the competing fermentation pathways in *E. coli* B and enabling overexpression of pyruvate dehydrogenase complex (aceEF-lpd, a typical aerobically-expressed operon) under anaerobic conditions [47]. SZ420 could convert xylose to ethanol at nearly 90% of the theoretical yield [47]. Adaptive evolution of nontransgenic *E. coli* KC01 (*ldhA pflB ackA frdBC pdhR::pflBp6-aceEF-lpd*) to obtain a homoethanol producer was also reported [49]. The resulting bacterium converted xylose to ethanol with 94% of theoretical yield [49].

As discussed above, *E. coli* is attractive for metabolic engineering for fuel production owing to the fact that its metabolic pathways are easily deleted or replaced. However, from a bioprocessing point of view, ethanol production using engineered *E. coli* has limitations in comparison with eukaryotic production systems. *E. coli* can be highly susceptible to phage infection and the utility of its spent biomass is limited in view of enteric origins [50].

2.1.1.3. Bacillus subtilis. Construction of *B. subtilis* BS35 was reported through disruption of the native lactate dehydrogenase (LDH) gene (*ldh*) by chromosomal insertion of the *Z. mobilis* pyruvate decarboxylase gene (*pdc*) and alcohol dehydrogenase II gene (*adhB*) under the control of the *ldh* native promoter [43]. The BS35 reportedly produced ethanol and butanediol, but the rates of cell growth and glucose consumption were reduced by 65–70% in comparison with the wildtype. BS35 was further modified to BS36 (BS35 Δ*alsS*) by eliminating the butanediol production pathway. This increased the ethanol yield to 89% of the theoretical value, but rates of cell growth and glucose consumption still remained low. The strain BS37 (BS35 Δ*alsS udhA*$^{+}$) was then developed from BS35 by inactivation of *alsS* through chromosomal integration of *E. coli* transhydrogenase gene (*udhA*). BS37 showed ethanol titers of up to 8.9 g/L in long-term cultivation [44].

2.1.1.4. Thermophilic bacteria. An important problem with mesophilic bacteria such as *E. coli* and *B. subtilis* is their poor ability to hydrolyze carbohydrate polymers, poor tolerance to extreme pH values, and inability to withstand high salt concentrations [7]. Therefore, bioprocesses based on these microorganisms are easily contaminated with other unwanted species, making them poorly suited for use in large-scale production operations.

Although bioethanol is commercially produced from sugars such as sucrose and starch hydrolysates derived from food crops [51,52], this form of production competes with food supply and arable land for production of food and fodder [53]. Therefore, processes capable of using non-food lignocellulosic biomass as a source of sugars for making ethanol are highly desirable. While ethanol production from lignocellulosic biomass is possible, it requires expensive pretreatment of the biomass to release fermentable sugars [54]. Hence, microorganisms with the ability to hydrolyze lignocellulosic materials and simultaneously convert the produced sugars into ethanol are of interest. Genetically engineered thermophilic bacteria are likely to be most useful in this context [34]. A thermophilic operation has the potential to not only enhance the rate of biomass hydrolysis and fermentation of sugars into ethanol, but also to reduce the potential of contamination of the fermentation process by unwanted microbes. Genetically engineered thermophilic bacteria have been extensively investigated for production of ethanol as summarized in Table 1.

2.1.2. Butanol and higher alcohols

Microbial production of primary higher alcohols such as 1-propanol, 1-butanol, and other linear-chain fatty alcohols has been widely investigated [63]. Butanol, a four carbon primary alcohol ($C_4H_{10}O$), is considered to be superior to ethanol as a fuel because of its higher energy density, lower vapor pressure, and being less hygroscopic and corrosive [64–67]. Moreover, due to relative similarity of its properties to gasoline, it is well compatible with the currently in-place gasoline infrastructure [68]. Therefore, butanol and other higher alcohols have the potential to directly substitute gasoline. Unmodified gasoline car engines fueled with pure butanol have successfully operated over long

periods [69]. Butanol is traditionally produced via the acetone-butanol-ethanol (ABE) fermentation, one of the first large-scale industrial fermentation processes [70]. Member of the bacterial genus *Clostridium* have been widely investigated as natural producers of butanol [68,71]. However, metabolic engineering of clostridial strains is challenging due to a complex metabolic regulation system and difficulties in their genetic manipulation [72]. In addition to clostridial strains, genetically engineered *E. coli* strains are also increasingly attracting attention as butanol producers [73,74].

2.1.2.1. Clostridium sp. Fermentative production of butanol by *Clostridium* sp. involves two distinct phases of acidogenesis and solventogenesis [75]. In the acidogenic phase, cells grow and convert the substrate to acetic and butyric acids and, therefore, during this phase pH declines to below 5. In the solventogenesis, the organic acids are transformed into acetone, butanol, and ethanol in the ratio of 3:6:1 [66,68]. Acetone and ethanol are inevitable byproducts of a conventional ABE fermentation. The metabolic engineering of clostridia has been attempted with a focus on selective overproduction of butanol to increase its yield and reduce the cost of removing the unwanted byproducts. This has mainly involved the use of integrative plasmids to inactivate the key genes of the biosynthetic pathways of unwanted byproducts [76,77]. Also, as n-butanol is extremely toxic to host cells, a lot of effort has focussed on developing more robust strains through metabolic engineering or mutagenesis approaches [68].

Clostridium acetobutylicum M5, a derivative of *C. acetobutylicum* ATCC 824, is basically incapable of producing butanol due to a loss of the megaplasmid pSOL1 [78]. This bacterium was later engineered to achieve enhanced butanol production [76,77,79]. For instance, overexpression of the *adhE1* gene under the control of the *ptb* promoter led to butanol production of up to 0.84 g butanol/g ABE. This was much higher than ~0.6 g butanol/g ABE produced by wildtype clostridia. A different production enhancement approach involved the disruption of the *adc* gene encoding acetoacetate decarboxylase in *C. acetobutylicum* which enhanced selective production of butanol to 0.82 g butanol/g ABE in a medium supplemented with methyl viologen.

Harris et al. metabolically engineered *C. acetobutylicum* PJC4BK and claimed a butanol concentration of up to 16.7 g/L compared with 11.7 g/L for wildtype *C. acetobutylicum* [80]. The PJC4BK was made by disruption of the *buk* gene encoding butyrate kinase, the enzyme involved in butyrate formation pathway [81]. Final butanol titer can also be increased by using other methods such as enhancing the product tolerance in a microorganism through construction of mutants [82]. For instance, Tomas et al. used overexpression of the molecular chaperone GroESL to enhance the activity of the solventogenic enzymes, increase butanol tolerance and eventually boost the final butanol titer [83]. Major achievements in clostridial 1-butanol production have been recently reviewed by Moon et al. [72].

2.1.2.2. E. coli. Solventogenic clostridia are strict anaerobes and grow slowly compared with other commonly known aerobic bacteria [12]. This adversely impacts the economics of butanol production by the conventional ABE fermentation. Furthermore, genetic manipulation of *Clostridium* sp. has proven to be difficult and this has shifted the focus towards engineering the clostridial fermentative pathway in bacteria such as *E. coli* [74,84] and *B. subtilis* [11,85,86] for butanol production. In an effort by Atsumi et al., a functional butanol biosynthesis pathway was reconstructed in *E. coli* by introducing the *thl*, *hbd*, *crt*, *bcd*, *etfAB* and *adhE2* genes from *C. acetobutylicum* [73]. This allowed a butanol titer of 139 mg/L to be attained under anaerobic conditions [73]. In another study, a metabolically-engineered *E. coli* harboring the *thiL*, *crt*, *bcd*, *etfAB*, *hbd*, and *adhE2* genes of *C. acetobutylicum* produced 1.2 g/L of butanol from 40 g/L glucose [87].

Despite these achievements, a major bottleneck to further improvements in butanol production remains the lack of sufficient understanding of how the metabolic shift from acid to solvent production is regulated at the molecular level. The relevant inducing signals, the regulators and their interactions, and the connections among the regulatory networks are poorly understood [88–90].

2.1.2.3. Other bacteria. Other bacteria including *Corynebacterium glutamicum* [91–94] and *Ralstonia eutropha* [95] have also been used to produce higher alcohols from 2-ketoacids, the metabolic intermediates of amino acid biosynthetic pathway. A *C. glutamicum* made by the deletion of the *ilvE*, *aceE*, and *pqo* genes encoding transaminase B, pyruvate dehydrogenase subunit E1, and pyruvate: quinone oxidoreductase, respectively, and by the overexpression of the *ilvBNCD* genes was shown to possess an elevated ability to produce isobutanol from glucose [92].

An engineered *R. eutropha* strain was confirmed to convert carbon dioxide to higher alcohols in an electromicrobial process [96] This process involved the use of electrical current in a specially designed bioreactor. The engineered bacterium could produce nearly 90 mg/L of isobutanol and 50 mg/L of 3-methyl-1-butanol using CO_2 as the sole carbon source. Autolithotrophic bacteria such as *R. eutropha* can use electricity instead of hydrogen as source of electrons [96] and, therefore, may be promising for biofuel production processes if there is a net energy benefit. Table 2 summarizes the studies reported on production of biobutanol and higher alcohols by the use of metabolically-engineered microorganisms. The metabolic pathways involved in the production of higher alcohols are summarized in Fig. 2.

2.1.3. Biohydrogen

Hydrogen burns cleanly leaving only water. It is therefore, believed to be a promising future fuel [119–122]. Metabolic engineering of bacteria for production of biohydrogen has received much attention, but commercial production does not seem to be viable at present. In bacteria, hydrogen may be produced by photofermentation and dark fermentation. Photoheterotrophic hydrogen production requires an organic carbon source as well as sunlight and is carried out by anoxygenic photosynthetic bacteria such as the purple nonsulfur bacteria [123]. Nitrogenase is the key enzymes involved in the process and anaerobic conditions are required for hydrogen production [124,125]. The hydrogen production activity of nitrogenases tends to be low and the expression of these enzymes is repressed by ammonium ions [126,127]. Moreover, various other reactions competitively scavenge electrons from nitrogenase leading to reduced hydrogen yield and photochemical efficiency [124,128,129].

Biohydrogen production by dark fermentation of various substrates is mainly carried out by facultative and obligate anaerobic bacteria [130]. Dark fermentation results in higher rates of hydrogen production relative to photofermentation [123,131]. Both mesophilic and thermophilic dark fermentation processes exist depending on the bacterial species involved. Both wildtype and metabolically-engineered bacteria have been studied for hydrogen production [125,126]. Mesophilic bacteria such as *Clostridium butyricum*, *Enterobacter aerogenes*, and *E. coli* have attracted particular attention [132–134]. Table 3 summarizes the studies conducted on hydrogen production using metabolically engineered bacteria.

2.1.3.1. E. coli. Yoshida et al. reported on a genetically modified *E. coli* for hydrogen production by dark fermentation [135]. The bacterium was engineered by inactivating the formate-hydrogen lyase (FHL) repressor (*hycA*) and the negative regulator for FHL. In addition, the bacterium was modified to overexpress FHL and its activator genes (*fhlA*). The resulting organism showed a high volumetric hydrogen productivity of 300 L H_2/L /h in a high cell-density fermentation (93 g biomass dry weight/L) with formate as the substrate. Subsequently, the bacterium was further modified through disruption of *ldhA* encoding lactate dehydrogenase and *frdBC* encoding fumarate dehydrogenase in attempts to improve hydrogen yield [136]. Using this strain, up to

Table 2
Engineered microorganisms used for production of biobutanol and higher alcohols.

Product	Host	Genotype (knockout; overexpression)	Metabolic engineering approach	Substrate	Medium	Titer (g/L)	Cultivation	Comment	Reference
1-Propanol	*E. coli* BW25113	Δ*ilvA* Δ*ilvB; LlkivD ScADH2MjcimA*^{mut} *leuABCD*	Directed evolution of *Methanococcus jannaschii* citramalate synthase (CimA) in *E. coli*	Glucose	Defined	2.78	Shake flask	1-Butanol 0.39 g/L	[98]
1-Propanol	*E. coli* W3110	Δ*lacI* Δ*lysA* Δ*metA* Δ*tdhA* Δ*iclR* Δ*ilvIH* Δ*ilvBN* Δ*rpoS thrA*^{C1034T} *lys*^{CC1005T} P*thr*::P*tac* P*ppc*::P*trc* *ilvA*^{C1139T,G1341T,C1351G,T1352C} *thrABC MjcimA ackA adhE*^{mut}	Introduction of a feedback resistant *ilvA*, overexpressing the *cimA* gene encoding citramalate synthase and the *ackA* gene encoding acetate kinase A/propionate kinase II; introduction of a modified *adhE* gene encoding an aerobically functional AdhE, and deletion the *rpoS* gene encoding the stationary phase sigma factor	Glucose, glycerol	Semi defined	10.8	Bioreactor	Aerotolerant AdhE; 20 g/L initial glucose; no feeding	[99]
Propanol	*B. subtilis* BSUDL-03	–	Introduction of PalsSD-controlled auto-inducible 2-ketoisovalerate biosynthetic pathway and a Pspac-controlled IPTG-inducible Ehrlich pathways in *Bacillus subtilis*	Glucose	Defined	3.83 ± 0.46	fed-batch Pro fermentation system (DASGIP)	–	[93]
Isopropanol	*E. coli* ATCC 11303	None; *lacI*q C*athl atoDA* C*aadc adh*B-593	Production of synthetic pathway by combination of different genes from *C. acetobutylicum* thl (acetyl-coenzyme A [CoA] acetyltransferase), *E. coli* atoAD (acetoacetyl-CoA transferase), *C. acetobutylicum* adc (acetoacetate decarboxylase), and *C. beijerinckii* adh (secondary alcohol dehydrogenase)	Glucose	Semi defined	5	Baffled shake flask	Yield of 0.15 g/g; acetone accumulated on depletion of glucose	[100]
Isopropanol	*E. coli* ATCC 11303	None; *lacI*q C*athl atoDA* C*aadcadh*B-593	Use of *E. coli* TA76 and optimization of fermentation conditions and isopropanol removal by gas stripping	Glucose	Semi defined	143	Stirred flask	Yield of 0.23 g/g; gas stripping; 240 h	[101]
Isopropanol	*E. coli* ATCC 11303	None; C*athl atoDA* C*aadc adh*B-593 T*fbgl*-blc (fused)	Firstly, use of genetically-engineered *E. coli* to produce isopropanol directly from cellobiose via cellobiose degradation by β-glucosidase (BGL) on the cell surface. Then, introduction of the synthetic pathway for isopropanol production into the strains and comparison of their isopropanol production in the presence of glucose	Cellobiose	Semi defined	4.1	Shake flask	Yield of 0.08 g/g	[102]
Isopropanol	*E. coli* JM109	None; *Cathl CactfAB Caadc adhB-593*	*E. coli* JM109 Harboring isopropanol-producing pathway consisting of five genes encoding four enzymes, thiolase, coenzyme A (CoA) transferase, and acetoacetate decarboxylase from *C. acetobutylicum* ATCC 824, and primary–secondary alcohol dehydrogenase from *C. beijerinckii* NRRL B593	Glucose	Complex	13.6	Baffled shake flask	Yield of 0.17 g/g; acetone yield of 0.03 g/g	[103]

(*continued on next page*)

Table 2 (*continued*)

Product	Host	Genotype (knockout; overexpression)	Metabolic engineering approach	Substrate	Medium	Titer (g/L)	Cultivation	Comment	Reference
Mixed alcohols	*C. acetobutylicum* ATCC 824	Δ*buk::ermC; adc ctfAB* $adh_{B\text{-}593}$	Primary/secondary alcohol dehydrogenase gene from *C. beijerinckii* NRRL B-593 (i.e., *adhB-593*) introduced in *C. acetobutylicum* ATCC 824	Glucose	Semi defined	20.4	Bioreactor	Yield of 0.30 g/g; isopropanol 4.4 g/L, butanol 14.1 g/L. With gas stripping	[11]
Mixed alcohols	*C. acetobutylicum* ATCC 824Δ*buk::ermC; adc ctfAB* $adh_{B\text{-}593}$	Δ*buk::ermC; adc ctfAB* $adh_{B\text{-}593}$	A synthetic acetone operon (act operon; adc-ctfA-ctfB) used to increase flux to isopropanol	Glucose	Semi defined	35	Bioreactor	Yield of 0.26 g/g; isopropanol 4.1 g/L; butanol 25.1 g/L. With gas stripping	[11]
Mixed alcohols	*C. acetobutylicum* ATCC 824	Δ*buk* Δ*CA_C1502; adc ctfAB* $adh_{B\text{-}593}$	Different synthetic isopropanol operons were constructed and introduced on plasmids in a butyrate minus mutant strain (*C. acetobutylicum* ATCC 824 Δcac15ΔuppΔbuk)	Glucose	Semi defined	20.4	Bioreactor	Yield of 0.33 g/g; with gas stripping	[104]
Mixed alcohols	*C. acetobutylicum* Rh8	None; $adh_{B\text{-}593}$	Overexpression of a single secondary alcohol dehydrogenase from *C. beijerinckii* NRRL B593 in strain Rh8 under the control of thl promoter	Glucose	Semi defined	23.9	Bioreactor	Random mutagenized strain; yield of 0.31 g/g; isopropanol, 7.6 g/L; butanol, 15 g/L	[105]
Mixed alcohols	*C. acetobutylicum* BKM19	Δ*buk::ermC;* $adh_{B\text{-}593}$ $hydG_{B\text{-}593}$	Developed a hyper ABE producing BKM19 strain which converted acetone to isopropanol. Transformation of BKM19 strain with the plasmid pIPA100 containing the *sadh* (primary/secondary alcohol dehydrogenase) and *hydG* (putative electron transfer protein) genes from the *C. beijerinckii* NRRL B593 cloned under the control of the thiolase promoter	Glucose	Semi defined	28.5	Bioreactor	Pilot-scale fermentation; random mutagenized strain; yield of 0.37 g/g; isopropanol, 3.5 g/L; butanol, 15.4 g/L; ethanol, 9.6 g/L	[70]
1-Butanol	*C. acetobutylicum* ATCC 824	Δ*pta* Δ*buk;* $adhE^{D485G}$	Simultaneous disruption of the *pta* and *buk* genes, encoding phosphotransacetylase and butyrate kinase, overexpression of the *adhE1D485G* gene, encoding a mutated aldehyde/alcohol dehydrogenase	Glucose	Semi defined	18.9	Bioreactor	Simultaneous disruption of the *pta* and *buk* genes, encoding phosphotransacetylase and butyrate kinase; overexpression of the *adhE1D485G* gene, encoding a mutated aldehyde/alcohol dehydrogenase	[106]
1-Butanol	*C. acetobutylicum* ATCC 824	Δ*pta* Δ*buk*; $adhE^{D485G}$	As above	Glucose	Semi defined	130	Bioreactor	Volumetric productivity of 1.32 g/L h; with in situ recovery; yield of 0.31 g/g	[106]
1-Butanol	*C. acetobutylicum* ATCC 824	Δ*adc*; *Ecgsh adhE ctfAB thl hbd crt bcd*	Inactivation of *adc* gene to eliminate the production of acetone; expression of *gsh*AB genes in *adc* locus to improve the strain's robustness	Glucose	Semi defined	14.9	Bioreactor	Yield of 0.34 g/g; 3.3 g/L ethanol	[107]
1-Butanol	*C. tyrobutyricum* ATCC 25755	Δ*ack*; Ca*adhE2*	Overexpression of aldehyde/alcohol dehydrogenase 2 (*adhE2*, Genebank no. AF321779) from *C. acetobutylicum* ATCC 824, which converted butyryl-CoA to butanol, under the control of native thiolase (*thl*) promoter	Glucose	Semi defined	10	Anaerobic bottle	Yield of 0.27 g/g; 5.8 g/L butyrate; manual control of pH with NaOH	[108]
1-Butanol	*C. tyrobutyricum* ATCC 25755	Δ*ack*; Ca*adhE2*	As above	Mannitol	Semi defined	16	Anaerobic bottle	Yield of 0.31 g/g; 1.0 g/L butyrate; manual control of pH with NaOH	[109]

(*continued on next page*)

Table 2 (*continued*)

Product	Host	Genotype (knockout; overexpression)	Metabolic engineering approach	Substrate	Medium	Titer (g/L)	Cultivation	Comment	Reference
1-Butanol	*C. tyrobutyricum* ATCC 25755	None; Ca*adhE2*	Expression of *adhE2* gene in *C. tyrobutyricum* under the control of a native thiolase promoter using four different conjugative plasmids (pMTL82151, 83151, 84151, and 85151) each with a different replicon (*pBP1* from *C. botulinum* NCTC2916, *pCB102* from *C. butyricum*, *pCD6* from *C. difficile*, and *pIM13* from *Bacillus subtilis*)	Mannitol	Complex	20.5	Anaerobic bioreactor	Yield of 0.33 g/g; productivity of 0.32 g/(L h); 1 g/L butyrate; manual control of pH with NaOH	[109]
1-Butanol	*E. coli* DH1	None; Re*phaAB* A*caphaJ* T*dter* C*aadhE2 aceEF lpd*	Construction of two plasmid system as first, synthetic genes encoding PhaA, PhaB and Crt as a single operon driven by the relatively weak arabinose promoter, and second, plasmid containing an operon comprising *ccr* and *adhE2* using a strong T7lac promoter (*pET-ccr.adhE2*)	Glucose	Complex	3.4	Shake flask	Anaerobic after induction	[110]
1-Butanol	*E. coli* DH1	None; Re*phaA* C*ahbd* C*acrt* T*dter* Ca*adhE2 aceEF lpd*	As above	Glucose	Complex	4.7	Shake flask	Yield of 0.28 g/g; shift to anaerobic conditions after induction	[110]
1-Butanol	*E. coli* BW25113/F'	Δ*ldhA* Δ*adhE* Δ*frdBC* Δ*pta*; *atoB* C*ahbd* C*acrt* C*aadhE2* C*bfdh* T*dter*	Construction of a modified clostridial 1-butanol pathway in *E. coli* to provide an irreversible reaction catalyzed by trans-enoyl-coenzyme A (CoA) reductase (Ter) and created NADH and acetyl-CoA driving forces to direct the flux	Glucose	Complex	15	Anaerobic bioreactor	Yield of 0.36 g/g; no gas stripping	[111]
1-Butanol	*E. coli* BW25113/F'	Δ*ldhA* Δ*adhE* Δ*frdBC* Δ*pta; atoB*C*ahbd* C*acrt* C*aadhE2* C*bfdh* T*dter*	As above	Glucose	Complex	30	Anaerobic bioreactor without gas stripping	Yield of 0.36 g/g; without gas stripping	[111]
1-Butanol	*E. coli* MG1655	*fadR_ crp** Δ*arcA* Δ*adhE* Δ*pta*Δ*frdA* Δ*yqhD*; *atoC yqeF fucO*	Introduction of *fadR* and *atoC(c)* mutations in *E. coli*, deletion of *arcA* gene to prevent ArcA-mediated repression of most operons encoding the β-oxidation cycle	Glucose	Defined	14	Baffled shake flask		[112]
Isobutanol	*E. coli* BW25113	Δ*adhE* Δ*ldhA* Δ*frdBC* Δ*fnr* Δ*pta* Δ*pflB*; Bs*alsS ilvCD* Ll*kivD* Sc*ADH2*	Overexpression of five KDCs gene (*Pdc6, Aro10, Thi3* from *S. cerevisiae*, *Kivd* from *Lactococcus lactis18*, and *Pdc* from *C. acetobutylicum*) with alcohol dehydrogenase 2 (Adh2) of *S. cerevisiae* 19	Glucose	Semi defined	22	Shake flask	Yield of 0.35 g/g	[74]
Isobutanol	*B. subtilis*	Δ*ldh*; Cg*ilvCD alsS* Ll*kivD*Sc*ADH2*	Introduction of an efficient heterologous Ehrlich pathway controlled by the promoter P43 into *B. subtilis* for the isobutanol biosynthesis	Glucose	Complex	2.62	Bioreactor		[113]
Isobutanol	*B. subtilis*	Δ*ldh*; Cg*ilvCD alsS* Ll*kivD*Sc*ADH2*	Construction of a P alsSD-controlled auto-inducible 2-ketoisovalerate biosynthetic pathway and a P spac-controlled IPTG-inducible Ehrlich pathway in *B. subtilis* to modulate gene expression	Glucose	Complex	3.83	Bioreactor	Auto-inducible 2-ketovalerate synthetic operon	[93]

(*continued on next page*)

Table 2 (*continued*)

Product	Host	Genotype (knockout; overexpression)	Metabolic engineering approach	Substrate	Medium	Titer (g/L)	Cultivation	Comment	Reference
Isobutanol	*C. cellulolyticum* ATCC 35319	None; Ll*kivD* Ec*yqhD* Bs*alsS* Ec*ilvCD*	Cloning of the genes encoding *B. subtilis* –acetolactate synthase, *E. coli* acetohydroxyacid isomeroreductase, *E. coli* dihydroxy acid dehydratase, L. *lactis* ketoacid decarboxylase, and *E. coli* and L. *lactis* alcohol dehydrogenases into a pAT187 derivative plasmid under the control of constitutive ferredoxin (Fd) promoter from *C. pasteurianum*	Cellulose (Sigmacell type 50)	Defined	0.66	Not specified	7–9 d; strong expression of *alsS* may have been deleterious	[114]
Isobutanol	*C. glutamicum* ATCC 13032	Δ*pyc* Δ*ldhA*; Bs*alsS* Ll*kivD ilvCD adhA*	Overexpression of *alsS* of *B. subtilis*, *ilvC* and *ilvD* of *C. glutamicum*, *kivd* of L. *lactis*, and a native alcohol dehydrogenase (adhA)	Glucose	Complex	4.9	Shake flask	Yield of 0.09 g/g	[92]
Isobutanol	*C. glutamicum* ATCC 13032	Δ*aceE* Δ*pqo* Δ*ilvE* Δ*ldhA* Δ*mdh*; *ilvBNCD* Ec*pntAB* Ll*kivD adhA*	Inactivation of the pyruvate dehydrogenase complex, pyruvate: quinone oxidoreductase, transaminase B, and additional overexpression of the *ilvBNCD* genes, encoding acetohydroxyacid synthase, acetohydroxyacid isomeroreductase, and dihydroxyacid dehydratase in *C. glutamicum*	Glucose	Semi defined	13	Bioreactor	Yield of 0.20 g/g; productivity of 0.32 g/(L h); shifted to anaerobic conditions	[91]
Isobutanol	*R. eutropha* H16	Δ*phaCAB* Δ*ilvE* Δ*bkdAB* Δ*aceE*; *adh*(con) *ilvBHCD* Ll*kivD*	Overexpression of plasmid-borne, native branched-chain amino acid biosynthesis pathway genes in combination with various mutant strains of *R. eutropha* with isobutyraldehyde dehydrogenase activity. Overexpression of heterologous ketoisovalerate decarboxylase gene for biosynthesis of isobutanol and 3-methyl-1-butanol	Fructose	Defined	0.27	Shake flask	Coproduced 40 mg/L 3-methyl-1-butanol	[109]
Isobutanol	*E. coli* DH1	*fadE*; '*tesA fadD* A*cacr1*	Elimination of the first two competing enzymes associated with β-oxidation, FadD and FadE	Glucose	Defined	0.06	Baffled shake flask		[115]
Isobutanol	*E. coli* MG1655	Δ*araBAD* Δ*fadE* Δ*fadAB* Δ*ackA* Δ*pta*; Uc*fatB fadD* Ma*acr2* (Maqu_2507)	Overexpression of an acyl-ACP thioesterase (BTE), an acyl-CoA ligase (FadD), and an acyl-CoA/ aldehyde reductase (MAACR) genes in an engineered strain of *E. coli* to produce a high level of 1-dodecanol and 1-tetradecanol	Glucose	Defined	1.65	Bioreactor	Yield of 0.134 g/g; mainly C12 and C14 alcohols	[116]
Isobutanol	*E. coli* BL21 (DE3)	Δ*fadE*; *fadD* Ma*acr2* (Maqu_2220) '*tesA*	Introduction of two fatty acyl-CoA reductases encoded by *Maqu_2220* and *Maqu_2507* genes from *Marinobacter aquaeolei* VT8 into *E. coli*	Glucose	Defined	1.73	Bioreactor	Yield of 28.3 mg/g	[117]
Isobutanol	*E. coli* BL21 (DE3)	Δ*fadE*; Se*aar*	Introduction of fatty acyl-ACP reductase gene of *Synechococcus elongatus* to an *E. coli* strain	Glycerol	Defined	0.75	Bioreactor	Yield of 0.02 g/g	[118]

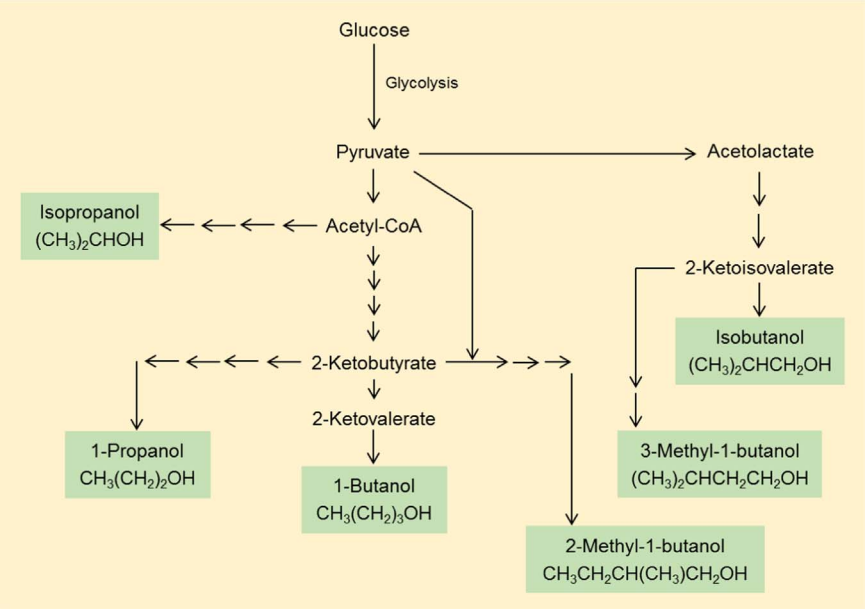

Fig. 2. Metabolic pathways for production of some higher alcohols. Based on Choi et al. (2014) [97].

1.82 mol of hydrogen could be produced from 1.08 mol of glucose.

In another study, a combinatorial engineering of the FHL complex and carbon metabolism was implemented in *E. coli* to obtain improved hydrogen yield [137]. Disruption of the *hycA* gene enabled a 2-fold improvement in FHL activity. Subsequent deletion of two uptake hydrogenases (1, hya; and 2, hyb) improved hydrogen yield from 1.2 to 1.48 mol/mol glucose confirming the critical impact of uptake hydrogenases on anaerobic fermentation of glucose. Disruption of multiple genes (*ldhA*, *frdAB*, *hycA*, *hya*, and *hyb*) further improved hydrogen yield on glucose to 1.8 mol/mol despite a high partial pressure of hydrogen in the culture system. With a low partial pressure of hydrogen, the same multiple mutated strain produced 2.11 mol H_2/mol glucose [137]. This was a little higher than the theoretical maximum yield attainable with *E. coli* strains relying on the formate-dependent pathway.

Following a similar approach, hydrogen yield in *E. coli* BW25113 Δ*hyaB* Δ*hybC* Δ*hycA* Δ*fdoG/pCA24 N-FhlA* was improved from 0.47 to 0.70 mol H_2/mol of glucose through overexpression of the FHL complex and inactivation of the uptake hydrogenases [138]. Seol et al. (2011) further produced genetically engineered recombinant *E. coli* SH5 with multiple deletion mutations in uptake hydrogenases (*hya* and *hyb*), *hycA*, and the carbon metabolic pathways (*ldhA* and *frdAB*) and claimed a hydrogen productivity of up to 2.4 L H_2/L /h [138].

A low yield of hydrogen on the carbon substrate is a general problem of fermentative hydrogen production. Co-production of ethanol and hydrogen has been proposed to overcome this. Pursuing this approach, Seol et al. developed *E. coli* BW25113 Δ*hycA* Δ*hyaAB* Δ*hybBC* Δ*ldhA* Δ*frdAB* Δ*pta-ackA* Δ*pfkA* (designated as SH*_ZG) overexpressing *zwf* and *gnd* encoding the key enzymes in the pentose-phosphate (PP)

Table 3
Examples of metabolic engineering for biohydrogen production in bacteria.

Strain	Metabolic engineering approach	Yield (mol H_2/mol glucose)	Rate (mmol H_2/(g cell h)	Reference
E. coli W3110	Wildtype	ND	~ 90	[135]
E. coli W3110 ΔhycA/fhlA	Inactivated FHL repressor and overexpressed FHL	ND	~ 260	[135]
E. coli W3110	Wildtype	1.08	9.5	[135]
E. coli W3110 ΔldhA ΔfrdBC	Inactivated lactate dehydrogenase and fumarate dehydrogenase	1.82	13.4	[135]
E. coli BW25113	Wildtype	1.20	30.6	[136,137]
E. coli SH1 (BW25113 ΔhycA)	Inactivated negative regulator for FHL	1.17	58.2[a]	[137]
E. coli SH2 (SH1 ΔhyaAB)	Inactivated uptake hydrogenase 1	1.37	46.2[a]	[137]
E. coli SH3 (SH2 ΔhybBC)	Inactivated uptake hydrogenase 2	1.48	49.2[a]	[137]
E. coli SH4 (SH3 ΔldhA)	Inactivated lactate dehydrogenase	1.61	45.6[a]	[137]
E. coli SH5 (SH4 ΔfrdAB)	Inactivated fumarate reductase	1.80/2.11[b]	52.2[a]	[137]
E. coli BW25113/pCA24N	Wildtype containing empty vector (pCA24N)	0.47	3.7[c]	[138]
E. coli BW25113 ΔhyaB ΔhybC ΔhycA ΔfdoG/pCA24NFhlA	Inactivated uptake hydrogenases and overexpress FHL complex	0.70	12[c]	[138]
E. coli BL21(DE3) pA pYdbK	Wildtype harboring *E. coli* ydbK encoding a putative pyruvate: flavodoxin/ferredoxinoxidoreductase	0.67	ND	[141]
E. coli BL21(DE3) ΔiscR pAF pYdbK	Inactivated negative transcriptional regulator of the isc operon and expressed Fd-dependent hydrogenase (hydA) of *C. acetobutylicum*, maturation factors of hydA, and [4Fe–4 S]-ferredoxin of *C. pasteurianum*	1.88	ND	[141]
Clostridium paraputrificum M-21	Overexpressed [FeFe]-hydrogenase (HydA)	2.4	ND	[142]
Clostridium tyrobutyricum JM1	Overexpressed hydrogenase (HydA)	1.8	ND	[143]
Thermococcus kadakaraensis KOD1	Gene cloning and knockout gene	3.3	ND	[144]

ND, Not determined.
[a] Measured using formate as a substrate.
[b] Measured under low H_2 partial pressure.
[c] Measurement based on protein (mmol H2/(g protein h)).

pathway [139]. Although the resulting strain successfully co-produced ethanol (1.38 mol/mol) and hydrogen (1.32 mol/mol) from glucose, without acetate, the generation of pyruvate at a high rate of 0.18 mol/mol apparently reduced the efficiency of the co-production [139]. Subsequently, Sekar et al. (2016) attempted to enhance the co-production rate of the strain SH*_ZG by eliminating pyruvate accumulation and overexpressing the *zwf* and *gnd* genes. They designated the new strain as SH9*_ZG. It co-produced H_2 and ethanol at 1.88 and 1.40 mol/mol, respectively, under anaerobic conditions [140].

2.1.3.2. Thermophilic bacteria. Success with metabolically-engineered mesophiles has led to renewed interest in engineering of thermophilic bacteria for hydrogen production [145–147]. The most common thermophilic hydrogen producers include *Pyrococcus furiosus*, *Thermococcus kodakaraensis*, and species of the genera Clostridium and Thermoanaerobacterium and the families Thermotoga and Caldicellulosiruptor [146,148–154].

For example, overexpression of [Fe]-hydrogenase (HydA) in *Clostridium paraputrificum* M-21 improved hydrogen yield on glucose from 1.4 to 2.4 mol/mol [151]. Similarly in a different investigation, overexpression of HydA in *C. tyrobutyricum* JM1 increased hydrogen yield on glucose from 1.2 to 1.8 mol/mol [141]. Liu et al. inactivated the *ack* gene encoding acetate kinase of *C. tyrobutyricum* and achieved a nearly 1.5-fold increase in hydrogen yield compared to the wildtype strain [143].

The above achievements notwithstanding, use of metabolic engineering requires an in-depth understanding of the often complex metabolic pathways and regulatory circuits involved in hydrogen production [123].

2.2. Yeasts

Yeasts are promising hosts for production of industrial products and have been widely used for commercial production of recombinant enzymes and proteins [155]. The relatively large spheroidal cells of yeasts often simplify bioprocessing relative to many bacteria and mycelial fungi. Moreover, they can be grown at ambient temperature and have simple nutritional requirements. On the other hand, the ability of yeasts to thrive at low pH values helps in minimizing contamination at large scale industrial operations where rigorous sterilization of the media and bioreactor may not be feasible [156]. Unlike many bacteria, yeasts are relatively resistant to potential phage contaminations [156]. Capacity of yeasts for sexual reproduction provides opportunities for improving them through cellular engineering [157].

In view of the abovementioned characteristics, yeasts have attracted much attention as cell factories for production of biofuels, in particular bioethanol. The yeast *S. cerevisiae* has attracted special interest as it is perhaps the best known yeast with a long history of industrial use [156,157]. *S. cerevisiae* is generally recognized as safe (GRAS) and many recombinant technologies have been developed for it [158]. Much of the sugarcane derived bioethanol in Brazil [51] and the corn starch hydrolysate-derived product in the United States [52] depends on yeasts for fermentation.

Several recent reviews have provided an overview of the many metabolic engineering schemes that have been used in *S. cerevisiae* [159–165]. Some of the key developments relating to production of biofuels in *S. cerevisiae* are summarized in Table 4. Surface-engineered yeasts used for biofuels production are discussed in a separate section.

2.2.1. Bioethanol

2.2.1.1. S. cerevisiae. The well-known yeast *S. cerevisiae* has been the mainstay of bioethanol production from sugars from the earliest times [174]. Compared to wildtype bacteria such as *E. coli*, yeasts better tolerate relatively high levels of alcohols although certain bacteria are also highly alcohol-tolerant. In view of its potential, much information exists on the biology, metabolism, and genetics of *S. cerevisiae*. This information has been used to improve strains for production of fuel ethanol. As a result, ethanol yield on sugars now often approaches the theoretical limit of 0.51 g ethanol/g of glucose. Up to 93% of the glucose used may be converted to ethanol while the remainder is mostly converted to cell biomass [175].

Native *S. cerevisiae* can effectively use sugars but cannot use sugar polymers such as starch and cellulose to produce ethanol. Therefore, a prior hydrolysis of the sugar polymers is essential prior to use in yeast fermentation. However, this prior hydrolysis step adds to the cost of processing while a competitive fuel must essentially be extremely cheap. This has led to successful efforts to engineer a hydrolytic capability in *S. cerevisiae* so that polymeric substrates could be used directly [176]. *S. cerevisiae* engineered to surface display minicellulosomes has allowed simultaneous saccharification and fermentation of crystalline cellulose leading to an ethanol titer of 1.4 g/L [177]. In other developments, *S. cerevisiae* was engineered to ferment sugars that it previously could not. For instance, Ha et al. reported an engineered strain capable of cofermenting mixtures of xylose and cellobiose with an ethanol productivity of nearly 0.65 g/L/h [178]. Engineering of the abilities to ferment new sugars and hydrolyze extracellular sugar polymers have not remained confined to *S. cerevisiae* and other microorganisms with these capabilities have also been developed.

A major by-product of ethanol fermentation is glycerol. Glycerol is required to maintain an osmotic balance in yeast cells. Deletion of *gpd1* and *gpd2*, encoding cytosolic NADH-dependent glycerol-3-phosphate dehydrogenases, has reduced or completely eliminated the production of glycerol and allowed more carbon to be channeled to ethanol synthesis; however, such double deletion strains do not grow well anaerobically [175]. Much efforts has focussed on overcoming this problem [179].

2.2.1.2. Native xylose-fermenting yeasts. Genetic and metabolic engineering of native xylose fermenting yeasts such as *Pichia stipites* [180] and *Spathaspora passalidarum* [181] hold much promise.

2.2.1.3. Thermotolerant yeasts. Use of thermotolerant yeasts offers important advantages in production of bioethanol [163,182–184] and these yeasts too can be further enhanced by metabolic engineering. The thermotolerant yeast *Kluyveromyces marxianus* ferments glucose to ethanol and can grow at nearly 50 °C. In addition, *K. marxianus* can use a variety of substrates such as cellobiose, xylose, xylitol, arabinose, glycerol, lactose, and inulin. Hu et al. claimed that in ethanol production from inulin hydrolysates, *K. marxianus* provided a significantly higher ethanol yield than native *S. cerevisiae* [185].

The thermotolerant yeast *P. kudriavzevii* was reported to ferment cassava hydrolysate to ethanol at 40 °C to achieve an ethanol concentration of 78.6 g/L in about 24 h [186]. Ethanol productivity of 3.28 g/L/h with a yield of 85.4% of theoretical was reported for this process [186]. As the knowledge available on some of the lesser-known yeasts improves, they could be metabolically-engineered for much improved ethanol production processes than available now.

2.2.1.4. Halophilic yeasts. Certain hydrolysates of sugar polymers are naturally high in salt and require suitably tolerant yeasts to ferment. For instance, hydrolysates of seaweed biomass contain high salt concentrations. A marine yeast (*Candida* sp.) has been shown to ferment polysaccharide-based seaweed hydrolysate to ethanol in the presence of 6.25–11.25% salt [187].

2.2.1.5. Other yeasts. The sugar alcohol glycerol is a byproduct of the processes used in biodiesel production. The yeast *Pachysolen tannophilus* was reportedly able to ferment glycerol [188]. A final ethanol titer of 17.5 g/L was reported in a medium supplemented with 5% (v/v) crude glycerol as the substrate. This corresponded to 56% of the theoretical yield [188].

Table 4
Some biofuels produced in engineered yeasts.

Strain	Product	Key enzymes expressed and pathway	Titer (g/L)	Substrate	References
S. cerevisiae TMB3057	Ethanol	XR-XDH strain overexpressing XKS1 and PPP Δ*gre3*	50	Xylose	[166]
S. cerevisiae TMB3066	Ethanol	*Piromyces* XI strain overexpressing XKS1 and PPP Δ*gre3*	50	Xylose	[167]
S. cerevisiae RWB202-AFX	Ethanol	*Piromyces* XI evolved isolate	20	Xylose	[168]
S. cerevisiae INVSc1/pRS406XKS/ pILSUT1/pWOXYLA	Ethanol	Orpinomyces XI strain overexpressing XKS1 and SUT1	50	Xylose	[169,170]
S. cerevisiae ADAP8	Ethanol	Evolved Orpinomyces XI strain overexpressing XKS1 and SUT1	20	Xylose	[170]
S. cerevisiae CEN.PK 2-1C	Isobutanol	Overexpression of genes in valine metabolism as ILV2, ILV3, ILV5, and BAT2	4.12×10^{-3}	Glucose	[171]
S. cerevisiae BY4741	Isobutanol	Elimination of genes encoding members of the pyruvate dehydrogenase complex such as LPD1, overexpression of enzymes responsible for transhydrogenase-like shunts such as pyruvate carboxylase, malate dehydrogenase, and malic enzyme	1.62	Glucose	[172]
S. cerevisiae S288C(MATa/α)	Isobutanol	Mitochondrial expression of *ILV*, *KivD*, *ADH* genes	635×10^{-3}	Glucose	[173]
S. cerevisiae BY4742	*n*-Butanol	*n*-Butanol biosynthetic pathway in which isozymes from a number of different organisms (*S. cerevisiae*, *E. coli*, *Clostridium beijerinckii*, and *Ralstonia eutropha*) were substituted for the clostridial enzymes	2.5×10^{-3}	Galactose	[173]

2.2.2. Higher alcohols

2.2.2.1. S. cerevisiae. As pointed out earlier, higher alcohols are more attractive as transport fuels compared with ethanol [189]. In this context biobutanol has generated the highest level of interest and microorganisms are being engineered for its production. A *S. cerevisiae* engineered to produce biobutanol from galactose achieved a titer of 2.5 mg/L [117]. In a study by Chen et al., overexpression of genes in the valine pathway of *S. cerevisiae* enabled isobutanol production [171]. Brat et al. further improved the ethanol titer to 0.63 g/L by relocating the valine biosynthetic enzymes into the cytosol [190]. In a different study, Kondo et al. genetically enhanced the Ehrlich pathway and altered the carbon flux in *S. cerevisiae* to achieve isobutanol titers as high as 0.143 g/L [191]. Compartmentalization of the synthesis pathway in yeast mitochondria enabled titers of 0.64 g/L for isobutanol together with 0.1 g/L of isopentanol and 0.12 g/L of 2-methyl-1-butanol [192]. All this suggests that yeasts can be successfully engineered to produce certain higher alcohols. Biobutanol production using metabolically engineered *S. cerevisiae* is already being commercialized by Gevo, Inc., in the United States. Sugars produced from starch or woody biomass by enzymatic hydrolysis are used as feedstock.

2.3. Cyanobacteria

Cyanobacteria are photosynthetic prokaryotic bacteria previously known as blue-green algae, but are actually not algae at all as microalgae are microscopic photosynthetic eukaryotes. Cyanobacteria and microalgae are sunlight-driven cell factories that convert carbon dioxide and water to sugars and eventually to diverse biochemicals [193]. Many types of fuels and fuel precursors can be produced from microbial biomass grown via photosynthesis (Fig. 3). As sunlight, carbon dioxide and water are often readily available, cyanobacteria and microalgae have attracted much attention for producing fuels and other products [194–200]. Continuing developments notwithstanding, there are many hurdles to commercialization of fuels from microalgae and cyanobacteria [201].

Cyanobacteria may have a higher photosynthetic efficiency compared to land plants [202]. Furthermore, cyanobacteria do not compete for land with terrestrial crops [203]. Being prokaryotes, cyanobacteria are structurally simpler than microalgae and unlike algae, can be genetically engineered relatively easily. In view of this, cyanobacteria are attracting attention as subjects for metabolic and genetic engineering [193]. More than 126 cyanobacterial genomes have been sequenced [204] and the necessary platforms for genetic manipulation of cyanobacteria have been established. Overall, the major foci of metabolic engineering of cyanobacteria include direct production, product secretion, and process optimization [200]. Metabolic engineering aspects of cyanobacteria have been extensively reviewed [195,205,206]. Studies have spanned production of biohydrogen [169,171,207–209], ethanol [207,210–213], isobutyraldehyde [214], isoprene [213,215], β-caryophyllene [226], sucrose [217], butanol [218], fatty alcohols [219,220], and fatty acids [221,222]. Biofuels and other chemicals produced in metabolically-engineered cyanobacteria are summarized in Table 5.

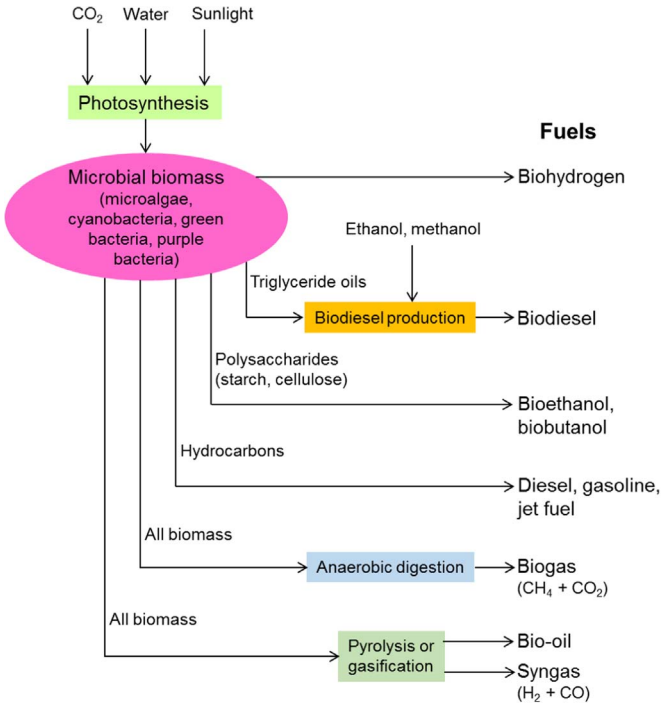

Fig. 3. Potential fuels and fuel precursors obtainable via microbial photosynthesis.

2.3.1. Biohydrogen

Many studies have reported biohydrogen production using cyanobacteria [126,233–237]. The electrons released in the primary reactions of oxygenic photosynthesis can be directly channeled to hydrogen production [237]. More specifically, hydrogen production in cyanobacteria occurs via two pathways: 1) as a byproduct of nitrogen fixation by cyanobacterial nitrogenases; and 2) through the action of bidirectional (or reversible) hydrogenases [205]. Hydrogen production via nitrogenases requires ATP whereas bidirectional hydrogenases do not

Table 5
Titers of some biochemicals produced in engineered cyanobacteria.

Compound	Organism	Titer or productivity[a]	Manipulated gene(s)	Reference
Acetone	*Synechocystis* sp. PCC6803	36 mg/L	–	[223]
2,3-Butanediol	*Synechococcus elongatus* sp. PCC7942	2.4 g/L	–	[224]
1-Butanol	*S. elongatus* sp. PCC7942	30 mg/L	*ter*, *nphT7*, *bldh*, *yqhD*, *phaJ*, *phaB*	[218]
1-Butanol	*Synechococcus* sp.	14.5 mg/L	*hbd*, *crt*, *adhE2*, *ter*, *atoB*	[225]
Ethanol	*Synechococcus* 7942	230 mg/L	*pdc*, *adh*	[210]
Ethanol	*Synechocystis* 6803	552 mg/L	*pdc*, *adh*	[211]
Ethanol	*Synechocystis* sp. PCC6803	5.5 g/L	*pdc*, *adh*	[226]
Ethylene	*Synechocystis* sp. PCC6803	171 mg/L/d	–	[227]
Fatty acids	*Synechocystis* sp. PCC6803	197 mg/L	*tesA*, *accBCDA*, *fatB1*, *fatB2*, *tesA137*	[221]
Fatty alcohol	*Synechocystis* 6803	200 ± 8 μg/L	*far*	[213]
Fatty alcohol	*Synechocystis* 6803	20 ± 2 μg/L/OD	*far*, *aas*	[226]
Fatty alcohol	*Synechocystis*	2.87 mg/g DCW	Δ*sll0208*, Δ*sll0209*	[220]
Isobutanol	*Synechococcus* 7942	18 mg/L	*kivd*, *yqhD*	[214]
Isobutanol	*S. elongatus* sp. PCC7942	450 mg/L	*alsS*, *ilvC*, *ilvD*, *kivd*, *yqhD*	[214]
Isobutyraldehyde	*S. elongatus* sp. PCC7942	1.1 g/L	*alsS*, *ilvC*, *ilvD*, *kivd*, *rbcls*	[214]
Isoprene	*Synechocystis* sp. PCC6803	50 mg/g DCW/d	–	[216]
2-Methyl-1-butanol	*S. elongatus* sp. PCC7942	2 mg/L	*kivd*, *yqhD*, *cims*	[228]
Alkanes	*Synechocystis* 6803	162 ± 10 μg/L/OD	*accBCDA*	[219]
Alkanes	*Synechocystis*	2.3 mg/L/OD	*sll0208*, *sll0209*	[229]
Hydrogen	*Synechococcus* 7942	2.8 μmol/h /mg Chl-a	*hydEF*, *hydG*, *hydA*	[230]
Hydrogen	*Arthrospira maxima*	400 μmol/L /h/		[231]
Methane	*A. maxima*	0.4 L/d		[232]

[a] Chl-*a*, chlorophyll *a; DCW, dry cell weight; OD, optical density.*

require ATP and therefore, they are more efficient for hydrogen production [238]. Nevertheless, both these routes are challenging in practice for commercially-viable production despite the claimed advantages of cyanobacterial hydrogen production relative to the other microbial producers [237]. Although diverse cyanobacteria are reportedly able to produce hydrogen under various culture conditions, *Anabaena* spp. are particularly good producers (e.g. 68 μmol H_2/mg chl-*a*/h) [234].

Novel hydrogen producing cyanobacteria are being sought. Culture strategies as well as genetic and metabolic engineering of specific cyanobacteria to improve hydrogen production are receiving much attention [239–242]. The main challenges faced by cyanobacterial hydrogen production are the oxygen sensitivity of the hydrogenase enzymes involved in the process and the generally low hydrogen productivity as consequence of the competition for electrons by other pathways [208,242]. Oxygen-free operation can be difficult as photosynthesis naturally evolves oxygen. Thus, there is an inherent incompatibility between oxygenic photosynthesis and hydrogen production. Protein engineering has been attempted to reduce oxygen sensitivity of hydrogenases as well as to increase the photosynthetic efficiency by truncating the light harvesting antenna size [208]

In addition to the above-mentioned challenges, the availability of the reducing agents such as ferredoxin and NADPH is another bottleneck as these are needed also for other metabolic activities such as respiration [243]. Therefore, for enhancing hydrogen production, a part of the available electrons must be redirected to hydrogen producing enzymes and oxygen-tolerant hydrogenases must be engineered [205].

Both wildtype and engineered cyanobacteria are being extensively researched for hydrogen production. For instance, attempts are being made to eliminate pathways that consume reducing agents. Mutants of *Synechococcus* 7002 lacking lactate dehydrogenase have demonstrated 5-fold greater hydrogen production compared with the wildtype [242]. In a different investigation, Bandyopadhyay et al. described *Cyanothece* sp. ATCC 51142, a unicellular nitrogen-fixing cyanobacterium with the ability to generate high levels of hydrogen (465 μmol H_2/mg of chlorophyll h) in the presence of glycerol under aerobic conditions [244].

In addition to microbial engineering, novel bioprocesses involving temporal or spatial separation of aerobic photosynthesis and anoxic hydrogen production are being developed [245]. Notwithstanding these advances, cyanobacterial hydrogen production requires further improvement to become economically viable.

2.3.2. Bioethanol

Starch produced by cyanobacteria may be hydrolyzed and fermented into bioethanol or longer chain alcohols using the same technology as employed with corn and cassava starch. Some cyanobacteria are naturally capable of fermenting the accumulated starch into ethanol and excreting the ethanol in the extracellular environment [243]. Ethanol production pathway in cyanobacteria involves an initial conversion of pyruvate to acetaldehyde through decarboxylation and this is followed by the reduction of the produced acetaldehyde to ethanol [193]. Ethanol can also be produced directly from carbon dioxide, water, and sunlight in metabolically-engineered cyanobacteria.

In general, dark fermentation (i.e. an absence of photosynthesis) is not a primary route of energy production in most cyanobacteria [243,246] and occurs at a limited rate [243]. Nevertheless, attempts have been made to increase the rate of natural dark fermentation through genetic modification. For instance, *Synechococcus* sp. PCC 7942 was modified by inclusion of the coding sequences for pyruvate decarboxylase and alcohol dehydrogenase II from *Z. mobilis* [210]. These genes were reportedly expressed under the control of the cyanobacterial rbcLS operon promoter alone and also in combination with the *E. coli* lac promoter and the ethanol productivity of the transformed strain reached 54 nmol per OD730 unit/L/d [209]. Ethanol productivity could be further increased/(mg chl h) when the CI-PL temperature inducible promoter was used [212]. In another study, the model cyanobacterium *Synechocystis* sp. PCC 6803 was transformed via a double homologous recombination system to integrate the pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adh*) genes from *Z. mobilis* into the cyanobacterial chromosome under the control of the strong light-driven psbAII promoter [211]. The ethanol productivity achieved using this engineered cyanobacterium was 5.2 mmo per OD730 unit/L/d [211].

Increasing NADPH production was hypothesized to increase production of biomass and ethanol in cyanobacteria [247]. This hypothesis was proved by over expressing the endogenous *zwf* gene encoding glucose-6-phosphate dehydrogenase of pentose phosphate pathway in the model cyanobacterium *Synechocystis* sp. PCC 6803. Overexpression of *zwf* led to increased NADPH production and a consequent increase in production of biomass and ethanol compared with the wild type under both autotrophic and mixotrophic conditions [247]. In one study, the

freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 was subjected to transformation by introducing the pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase B (*adhB*) genes of *Z. mobilis* into its genome, but only one construct produced bioethanol at detectable levels [248].

2.3.3. Higher alcohols

Keto acid pathway offers a route to propanol and butanol. 2-Ketobutyrate is an intermediate in the biosynthesis of citramalate and citramalate synthase has been found in cyanobacteria [249,250]. Therefore, there is the possibility of producing propanol and butanol from 2-ketobutyrate in cyanobacteria. For instance, engineering of isobutanol biosynthetic pathway and overexpression of Rubisco in *Synechococcus elongatus* PCC 7942 enabled an isobutyraldehayde productivity of 6230 μg/L /h and an isobutanol productivity of 3000 μg/L /h [214]. In a different study, *Synechocystis* PCC 6803 was genetically modified with the *ispS* gene of *Pueraria montana* leading to the production of isoprene [216]. Isoprene, a potential biofuel, is a hydrocarbon with higher energy content than ethanol and is easier to separate from an aqueous broth compared with ethanol.

Some cyanobacteria naturally produce alkanes as well [250]. As pure hydrocarbons, alkanes are energy-rich potential fuels. This could open up new options for producing fuels in engineered cyanobacteria in the future.

2.4. Microalgae

Microalgae are photosynthetic cell factories capable of producing starch, oils, and other biochemicals [22]. Compared with higher plants, microalgae grow more rapidly as they utilize sunlight better. Therefore, microalgae have the potential to provide huge quantities of starch and oils for conversion to various types of fuels [251,252]. Microalgae with a modified keto acid pathway may be a promising platform for producing isobutanol as well [253,254]. Long chain alcohols (C5–C8) have also been successfully produced in microalgae by modifying the cells for overproduction of 2-keto acids, the intermediates of the amino acid biosynthesis [254,255]. These intermediates were later converted to butanol derivatives by the heterologous expression of 2-keto acid decarboxylase and alcohol dehydrogenase [71,254].

In general, microalgae have attracted the most attention as producers of oils for fuels [8,197]. Oil productivity of microalgae is far greater than that of oil palm, one of the most productive commercial oil crops [256–260]. Moreover, microalgae can be grown on land which is not suitable for agriculture using seawater and brackish water. Their numerous advantages notwithstanding, commercialization of oil production from algae faces difficult challenges that are unlikely to be overcome in the short-term [201]. The costs of production of the biomass and its downstream processing are high [259]. Hence, economic viability of large-scale production of microalgae-based biofuels is questionable. Metabolic and genetic engineering are important to a future commercialization of algal fuels. Extensive effort is underway in these areas. The genomes of more than 30 microalgae have already been sequenced [259].

2.4.1. Biodiesel

Microalgae often produce some of the same triglyceride oils found in oil crops such as canola and sunflower. As such, the triglyceride component of the algal oil may be readily converted to biodiesel. As mentioned earlier, under suitable conditions, the oil content of microalgae can be as high as 30–70% of their dry biomass and this has been the source of interest in biodiesel derived from microalgae [8,261–265].

Much of the existing effort on large-scale production of algal oils has focused on native algae. Nonetheless, attempts are being made to metabolically engineer algae for an enhanced production capability. The green alga *Chlamydomonas reinhardtii* has been the main focus of genetic and metabolic engineering work simply because its genome is known and it has a long history of use in research as a model alga. Genes associated with biosynthesis and accumulation of triglyceride oil in *C. reinhardtii* have been isolated and characterized to some level [266,267]. Because triacylglycerols (TAGs) are derived from either acylation of diacylglycerol (DAG) via a de novo pathway (acyl CoA-dependent pathway) or recycling of membrane lipids (acyl CoA-independent pathway), most of the metabolic engineering strategies have focused on manipulating the rate-limiting steps of these two pathways hoping to increase the metabolic flux for TAG production. For example, Li et al. claimed a 10-fold increase in the production of TAGs in a starchless mutant of *Chlamydomonas* by the inactivation of ADP-glucose pyrophosphorylase [266]. The resulting mutant BAFJ5 lacked the small subunit of ADP-glucose pyrophosphorylase and accumulated up to 32.5% by weight of neutral lipids and 46.5% by weight of total lipids [268].

Many of the genes involved in lipid synthesis in microalgae have been subjected to both knockout and overexpression in order to understand their role and identify the most efficient strategies for increasing the lipid content of the biomass. The enzymes acetyl CoA carboxylase (ACCase) and type-II fatty acid synthase (FAS) have been reported to be the main rate-limiting enzymes in fatty acid synthesis pathway [259]. ACCase is involved in forming malonyl CoA from carboxylate acetyl CoA and FAS is involved in elongating fatty acid chains. Therefore, it may be possible to enhance lipid production rates by increasing the activity of these enzymes via genetic engineering [264]. In pursuing this approach, expression vectors containing the gene encoding ACCase have been constructed and a transformation protocol has been developed for overexpression of ACCase in *Cyclotella cryptica* and other algal species [269]. However, ACCase overexpression has not increased lipid content [270]. It may be that ACCase activity is a limiting step in lipid biosynthesis mainly in cells that normally do not store large amounts of lipids [254].

In an attempt to increase lipid production without affecting growth, the marine diatom *Thalassiosira pseudonana* was engineered by knockdown of a multifunctional lipase/phospholipase/acyltransferase [271]. The resultant strains had up to ~3-fold lipid content relative to the wildtype during exponential growth. Metabolic engineering has also been used to alter the lipid profile in the marine diatom *Phaeodactylum tricornutum* [272]. The engineered diatom produced TAG with increased levels of shorter chain length fatty acids (lauric acid, C12:0; myristic acid, C14:0) which are more desirable for biodiesel production [271].

The above improvements notwithstanding, economic production of microalgal biodiesel is unlikely in the near future.

2.4.2. Biohydrogen

Like cyanobacteria, purple non-sulfur photosynthetic bacteria, some dark fermenting microorganisms and microalgae can also be used to produce hydrogen [273–281]. Three pathways are known to exist for hydrogen production in microalgae (Fig. 4). Two of these pathways require light while the third functions in the dark.

Biohydrogen is appealing specially for electricity production by fuel cells [281]. In many cases hydrogen is naturally produced in microalgae as a secondary metabolite to balance the redox energetics [282,283]. Hydrogen production has been studied to various depths in *C. reinhardtii* [280,284,285], *Scenedesmus obliquus* [286–288], *Chlorella fusca* [281], *Chlorella* sp. [289,290], *Chlorella vulgaris* [291], *Chlorella protothecoides* [292], and *Platymonas subcordiformis* [293,294]. Although hydrogen is naturally produced in microalgae under suitable culture conditions and hydrogenase activity can be externally induced by adding DCMU (a PSII electron chain uncoupler), hydrogen production cannot be maintained together with active photosynthesis because oxygen inactivates hydrogenase [281]. Under suitable conditions, hydrogen production may be augmented by channeling the electron flux to hydrogenases so that protons are biocatalytically converted to

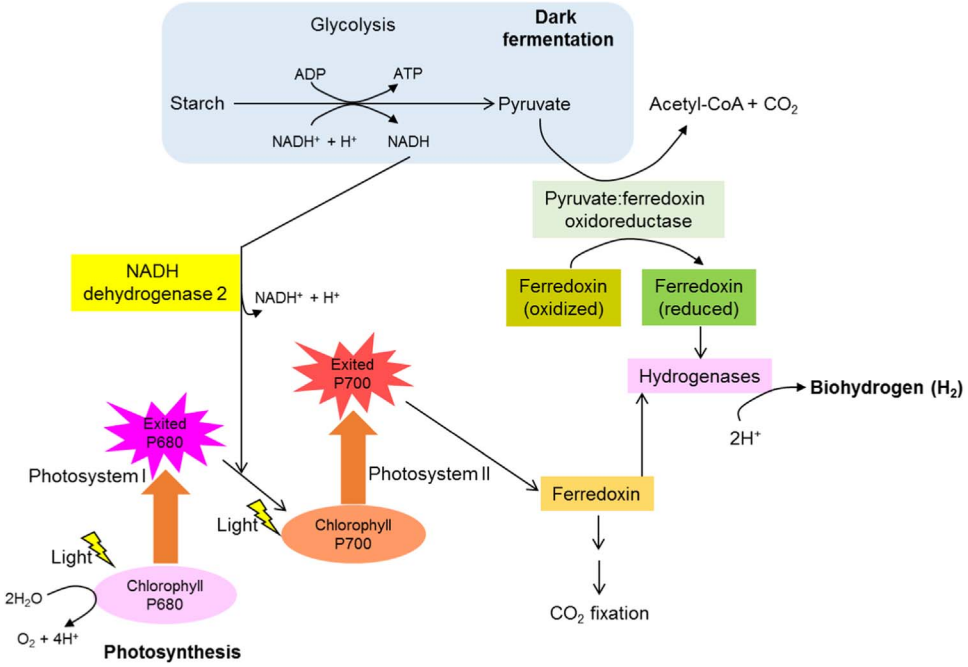

Fig. 4. The production of biohydrogen in microalgae. In the alga *Chlamydomonas reinhardtii* at least three pathways exist for hydrogen production. Two of these pathways involve light: (1) in a direct pathway, activity of the photosystem II supplies electrons to hydrogenases through the photosynthetic electron transport chain to drive production of hydrogen; and (2) in an indirect pathway, hydrogen is produced without the involvement of photosystem II through a process that depends on photosystem I. In the third pathway, the dark fermentation, glycolysis of starch drives hydrogen production. Based on Dubini and Gonzalez-Ballester (2016) [152]. ADP – adenosine diphosphate; ATP – adenosine triphosphate; NADH – nicotinamide adenine dinucleotide reduced form; $NADH^+$ – nicotinamide adenine dinucleotide oxidized form.

hydrogen ($2H^+ + 2e^- \rightarrow H_2$). To achieve this in *C. reinhardtii*, Kruse et al. blocked the cyclic electron transfer around photosystem I to reduce diversion of electrons away from hydrogenases. This improved hydrogen production rate by 5- to 13-fold relative to the control under a range of conditions [278]. In a different investigation, Rumpel et al. enhanced hydrogen production rate by 5-fold by redirecting electrons from photosystem I to hydrogenases [295]. Similarly, expression of an exogenous ferredoxin from *C. acetobutylicum* in addition to the native ferredoxin of *C. reinhardtii*, led to an enhanced electron flow to the hydrogenase HydA via siphoning of electrons from the fermentation of internal reducing equivalents (such as glycogen) [296]. This enhanced hydrogen production by nearly 2-fold under light-dependent anoxic conditions [296]. A ferredoxin-NADP+ reductase (FNR)-down-regulated mutant of *C. reinhardtii* had a 2.5-fold higher hydrogen production activity relative to the parent strain under sulfur deprivation [297]. Other similar approaches have also been reported [298].

3. Cell surface engineering for biofuel processes

Surface-engineered cells carry specific non-native proteins on their external surface. These surface proteins may extend from the plasma membrane through the cell wall, or they may be bound to the cell-surface components [16]. Surface displayed proteins are generally confined to particular domains on the surface of the cell.

The yeast *S. cerevisiae* has a rigid cell wall which is about 200 nm thick [299] and mainly consists of mannoproteins and β-linked glucans. The β-glucans rich layer occurs just above the plasma membrane [299]. Some of the proteins displayed on the surface of the yeast cells have secretion signal peptides at the N-terminal and glycosylphosphatidylinositol (GPI) anchors at the C-terminal. Protein synthesis occurs in the cell and the resulting proteins are then translocated into the lumen of the endoplasmic reticulum (ER) and on to the Golgi apparatus. Subsequently, membrane-enclosed vesicles carry the protein from the Golgi to the plasma membrane [300]. GPI-anchored proteins are further moved to the exterior of the plasma membrane via the secretory pathway. They are then released from the plasma membrane by the action of a phosphatidylinositol-specific phospholipase C (PI-PLC) and transferred to the outermost surface of the cell wall [299].

Either a single protein or a cocktail of proteins may be surface-displayed [301]. The displayed protein may be a hydrolytic enzyme, for example, to breakdown a polymeric substrate such as lignocellulosic biomass into small molecules that can be taken up by the cell as substrate (Fig. 5). Multiple displayed proteins may act synergistically to carry out a certain extracellular transformation before the product of such a transformation is internalized by the cell [299,301].

3.1. Yeasts and other microorganisms

Engineered microbial cells armed with surface-displayed bioactive proteins are potentially powerful biocatalysts for production of biofuels and other products [302]. Surface display technologies were initially established for phages and bacteria [26,301] and have since been applied more broadly. Numerous proteins and peptides have been successfully displayed with retention of their bioactivity [16]. Simultaneous display of multiple types of molecules on the same cell has become possible to greatly expand the usefulness of the surface-engineered microorganisms [15]. Compared to most bacteria, yeasts offer a larger surface for display and are easier to recover from a broth after

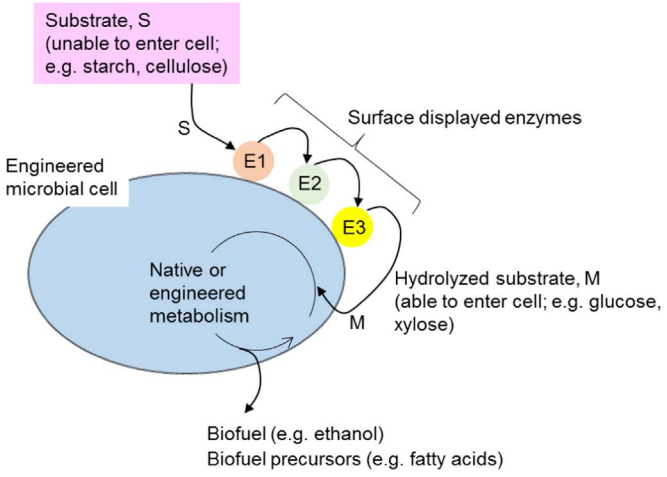

Fig. 5. Metabolically engineered microbial cell with surface displayed enzymes E1–E3. These enzymes extracellularly convert the substrate S to products (i.e., M) that can be taken up by the cell. These products, or modified substrates, then enter the metabolic pathways in the cell to eventually generate a biofuel or fuel precursor.

use.

Among yeasts, surface arming was first developed in *S. cerevisiae*. Subsequently, similar display systems were used to transform other yeasts, including *Pichia pastoris* [303] and *Yarrowia lipolytica* [304]. With suitably displayed enzymes capable of hydrolyzing extracellular sugar polymers to simple sugars, a yeast can use substrates that it could not previously use. Such surface displayed yeasts allow consolidation into a single operation the otherwise separate steps of polymer hydrolysis and sugar fermentation (Fig. 5). This kind of consolidated bioprocessing is certain to greatly improve the economics of conversion of lignocellulosic substrates to ethanol [305].

Direct production of ethanol from starch involving combined saccharification-fermentation steps and surface engineered yeast has been shown to work. The biocatalyst used was *S. cerevisiae* with exo glucoamylase (a starch-degrading enzyme) of *Rhizopus oryzae* displayed on its surface [306,307]. The production efficiency of ethanol from starch was further enhanced by co-displaying of exo glucoamylase and endo α-amylase from *Bacillus stearothermophilus* [308,309]. A surface engineered yeast expressing β-glucosidase and cellulase of *Aspergillus aculeatus* was shown to assimilate cellooligosaccharides [307]. Surface display of α-amylase of *Streptococcus bovis* on a glucoamylase displaying yeast was reported by Shigechi et al. (2004) [310]. The engineered yeast was shown to thrive and produce ethanol in a medium with starch as the sole carbon source.

Yeasts capable of directly fermenting cellulose require co-display of multiple enzymes including endoglucanases (EGs), cellobiohydrolases (CBHs), and β-glucosidases (BGLs) [311]. EGs act randomly on the amorphous region of the cellulose chain to produce reducing and nonreducing ends. CBHs are needed to release cellobiose from the exposed ends while BGLs hydrolyze the released oligosaccharides to glucose. A surface engineered yeast co-displaying endoglucanase II (EG II) and cellobiohydrolase II (CBH II) of *Trichoderma reesei* and BGL1 of *A. aculeatus* was shown to produce ethanol directly from cellulose that had been pre-swollen with phosphoric acid [312]. In the sake yeast *S. cerevisiae*, ethanol production from β-glucan was achieved by cell surface display of EG and BGL of *Aspergillus oryzae* [313].

In cells engineered to co-display multiple enzymes, an important consideration is the control of the relative ratios of the various enzyme activities displayed [192,302]. Attempts have been made to optimize the ratios of the surface displayed enzyme activities to improve synergism [314,315]. Although such optimization is based mostly on empiricism, it has been shown to improve ethanol production from cellulosic substrates such as newsprint [316].

Some clostridia produce cellulosome, a complex of scaffolding proteins and the multiple enzymes needed for hydrolysis of cellulose and hemicellulose [317]. This complex is formed through interaction of a cohesin module in the scaffolding proteins and a dockerin module in cellulosomal enzymes [318]. Tsai et al. engineered a yeast to display a mini-cellulosome [319]. The scaffolding proteins of this displayed cellulosome consisted of three cohesin domains derived from *Clostridium thermocellum*, *Clostridium cellulolyticum*, and *Ruminococcus flavefaciens*. The scaffolding proteins were displayed on the surface of *S. cerevisiae* using the α-agglutinin-based display system. The incubation of the scaffolding protein-displaying yeast with three recombinant cellulases (EG, CBH, and BGL) fused with a dockerin domain produced by *E. coli* led to a cellulosome displaying yeast able to synergistically hydrolyze cellulose [319].

A consortium of microorganisms displaying different proteins may also be co-cultured to achieve hydrolysis–fermentation of substrates such as cellulose. Goyal et al. developed a yeast consortium consisting of four engineered yeasts that were co-cultured [320]. In their experiments, one of the yeasts displayed cellulosome scaffolding protein and the other three yeasts secreted three different dockerin-fused enzymes. This resulted in the formation of a mini-cellulosome on the surface of the yeast displaying the scaffolding protein.

Hemicellulose, including xylan, also occurs in cellulosic biomass. In addition to cellulose, the hemicellulose components of the biomass must also be effectively hydrolyzed to allow full utilization of the biomass for production of ethanol. Xylan is hydrolyzed to xylo-oligosaccharides by endo-β-xylanase and the xylo-oligosaccharides are further hydrolyzed to D-xylose by β-xylosidase. A *S. cerevisiae* co-displaying xylanase II (XYN II) of *Trichoderma reesei* and β-xylosidase (XylA) of *A. oryzae* was engineered using the α-agglutinin-based display system and shown to hydrolyze xylan to xylose [318]. In a further development, xylose reductase (XR) and xylitol dehydrogenase (XDH) of *Pichia stipitis* and xylulokinase (XK) of *S. cerevisiae* were produced in the yeast co-displaying XYN II and XylA. The resulting yeast simultaneously saccharified xylan and fermented xylose to ethanol [321,322].

The enzyme xylose isomerase catalyzes the two-step isomerization of xylose to xylulose. In a study, bioactive xylose isomerase of *C. cellulovorans* was surface-displayed on *S. cerevisiae*. The resulting yeast could grow in a medium containing xylose as the sole carbon source and produced ethanol directly from xylose [323].

Lignin is another constituent of lignocellulosic biomass. Lignin impedes the action of cellulases by masking the cellulose fibers [324]. Therefore, various biomass pretreatments are used to remove the lignin prior to enzymatically hydrolyzing the cellulosic residue in a second step [324,325]. The enzyme laccase from various white-rot fungi can degrade lignin. A *S. cerevisiae* was engineered via the α-agglutinin-based display system to surface display laccase I of *Trametes* sp. [325]. Hydrothermally-processed rice straw was treated with the laccase-displaying yeast and subsequently treated with a yeast co-displaying EG II, CBH II, and BGL1. This two-step treatment improved ethanol yield from rice straw [325].

4. Future outlook of metabolic engineering for biofuel production

Although metabolic engineering is a key enabling technology for transforming microorganisms into efficient cell factories [325–327], identifying suitable enzyme targets and metabolic pathways for modification or replacement are not easy. Successful metabolic engineering relies on methods that aid assembly and optimization of novel pathways in microbes. Diverse factors contribute to the performance of a metabolic pathway and problems linked to abundance of mRNA and enzyme activities may not be identified simply by monitoring product titers. Synthetic biologists and metabolic engineers rely on a variety of methods to identify the limiting parts of a pathway [328–333]. Bioproducts not normally made by microorganisms in nature may be synthesized using emerging development in metabolic engineering [334–339]. Such systems metabolic approaches are contributing to development of biofuel producer species as summarized in Table 6. Emerging techniques include high-throughput sequencing for deciphering genomes [340,341]; highly sensitive transcriptomics, proteomics [342–344] and metabolomics methods; [345,346] and advances in fluxomics [347] and computational tools. These technologies are enhancing the potential of metabolic engineering to influence fuel production.

5. Concluding remarks

Metabolic engineering approaches are proving useful for production of biofuels and biofuel feedstocks in diverse microorganisms including bacteria, yeasts, cyanobacteria, and microalgae. Compared to the traditional random mutagenesis for strain improvement, the metabolic engineering is rational, rapid, and extremely powerful. More specifically, entire new pathways can be introduced into suitable microorganisms to enable production of biofuels and precursors of interest. Alternatively, parts of specific biochemical pathways may be engineered to optimize production of a biofuel. Commercialization of biofuels will inevitably require microbial cell factories that are substantially superior to wildtypes and some of the engineered strains now available. The surface display technology will allow microorganisms to

Table 6
System metabolic engineering approaches for the production of biofuels.

Strategy	Target	Strain	Achievement	Reference
Introduction of an engineered n-butanol pathway comprising genes of *atoB*, *hbd*, *crt*, *bcd*, and *adhE2* along with comparative proteomics analysis	*n*-butanol	*C. acetobutylicum*	Yields of 40 and 120 mg/L n-butanol from cellobiose and crystalline cellulose, respectively	[348]
Transcriptome analysis and overexpression of YOR1, SNQ2, PDR5, and PDR15	Alkanes	*S. cerevisiae*	Intracellular C10 and C11 alkanes amounts were lowered by 33% and 94.4%, respectively	[349]
Heterologous expression of ABC2 and ABC3 transporters		*S. cerevisiae*	Tolerance to decane increased 80-fold	[350]
Quantitative proteomic analysis	Ethanol and butanol	*C. acetobutylicum* ATCC 824	Reduction of ethanol (0.47 g/L for cellobiose; 0.27 g/L for cellobiose plus lignin) and butanol production (0.13 g/L for cellobiose; 0.04 g/L for cellobiose plus lignin)	[351]
Heterologous expression of mevalonate pathway by principal component analysis of proteomics (PCAP) approach	Bisabolene	*E. coli*	The titer of Isoprenoid-Based Biofuels was increased 40%	[344]
Metabolomics and proteomics methods and engineering of the heterologous isoprenoid pathway	Isoprenoid-based C5 alcohols (3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, and 3-methyl−1-butanol)	*E. coli*	The titers of 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol and 3-methyl-1-butanol were increased by 2.23 g/L, 150 mg/L, and 300 mg/L, respectively.	[352]
Targeted proteomics, via selected-reaction monitoring (SRM) mass spectrometry	Sesquiterpene, amorpha-4,11-diene	*E. coli*	The final amorpha-4,11-diene titer was improved by over threefold (> 500 mg/L)	[353]
Integrated analysis of gene expression and metabolic fluxes	Medium-chain-length poly(3-hydroxyalkanoates) (mcl-PHA)	*Pseudomonas putida* KT2440	A higher PHA content (29.7 wt% of CDW) was achieved at low dilution rate as compared with a high rate (12.8 wt% of CDW)	[354]

make use of substrates that they previously could not use. This capability will improve the economics of production of some biofuels. Genetic and metabolic engineering in combination with synthetic biology and systems biology are the key to generating highly capable cell factories for production of biofuels. Based on the present state of development of metabolically engineered microorganisms discussed in this review, the following can be inferred:

1. Commercial production of cellulases and other hydrolytic enzymes using engineered microorganisms is well established for producing feedstock sugars from polymeric substrates (lignocellulose, starch, xylans) for microbial fermentation to mainly bioethanol and biobutanol. Production of commercial fuel alcohols relies heavily on these enzymes, but lignocellulosic ethanol remains relatively expensive as a separate process is required for producing the enzymes.
2. Yeasts engineered to ferment simple sugars to isobutanol instead of the conventional ethanol are already being commercialized. Sugars derived from enzymatic hydrolysis of starch, or woody biomass, are used as the fermentation feedstock.
3. Yeasts engineered to surface display hydrolytic enzymes appear to be highly promising and have good prospects for near-term commercial use in production of bioethanol directly from starch and pretreated cellulosic biomass.
4. The bacterium *Zymomonas mobilis* metabolically engineered to ferment the wood sugar xylose is being used in commercial processes for making bioethanol from lignocellulosic biomass.
5. Production of biogas, already well established commercially, will continue to rely on native microbial populations in the near term because of the complexities of engineering methanogenic bacteria.
6. Microalgae, cyanobacteria and other photosynthetic bacteria, whether native or engineered, do not seem to have a near term potential for generating commercializable biofuels.

In brief, genetically and metabolically engineered microorganisms are now having an impact on the commercial biofuels scene and this is likely to increase rapidly in the future.

Acknowledgements

The authors would like to thank Iranian Biofuel Society (IBS), Agricultural Biotechnology Research Institute of Iran (ABRII), and Biofuel Research Team (BRTeam) for supporting this work.

References

[1] Khalife E, Tabatabaei M, Najafi B, Mirsalim SM, Gharehghani A, Mohammadi P, Aghbashlo M, Ghaffari A, Khounani Z, Shojaei TR, Salleh MAM. A novel emulsion fuel containing aqueous nano cerium oxide additive in diesel–biodiesel blends to improve diesel engines performance and reduce exhaust emissions: Part I–experimental analysis. Fuel 2017;207:741–50.
[2] Montgomery H. Preventing the progression of climate change: one drug or polypill? Biofuel Res J 2017;4:536.
[3] Hajjari M, Tabatabaei M, Aghbashlo M, Ghanavati H. A review on the prospects of sustainable biodiesel production: a global scenario with an emphasis on waste-oil biodiesel utilization. Renew Sustain Energy Rev 2017;72:445–64.
[4] Sarkar D, Shimizu K. An overview on biofuel and biochemical production by photosynthetic microorganisms with understanding of the metabolism and by metabolic engineering together with efficient cultivation and downstream processing. Biores Bioprocess 2015;2:1–19.
[5] Rahimi V, Karimi K, Shafiei M, Naghavi R, Khoshnevisan B, Ghanavati H, Mohtasebi SS, Rafiee S, Tabatabaei M. Well-to-wheel life cycle assessment of Eruca Sativa-based biorefinery. Renew Energy 2017;117:135–49.
[6] Rajaeifar MA, Ghanavati H, Dashti BB, Heijungs R, Aghbashlo M, Tabatabaei M. Electricity generation and GHG emission reduction potentials through different municipal solid waste management technologies: a comparative review. Renew Sustain Energy Rev 2017;79:414–39.
[7] Jin H, Chen L, Wang J, Zhang W. Engineering biofuel tolerance in non-native producing microorganisms. Biotechnol Adv 2014;32:541–8.
[8] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.
[9] Talebi AF, Dastgheib SMM, Tirandaz H, Ghafari A, Alaie E, Tabatabaei M. Enhanced algal-based treatment of petroleum produced water and biodiesel production. RSC Adv 2016;6:47001–9.

[10] Derkx PM, Janzen T, Sørensen KI, Christensen JE, Stuer-Lauridsen B, Johansen E. The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. Microb Cell Fact 2014;13:S5.
[11] Lee J, Jang YS, Choi SJ, Im JA, Song H, Cho JH, Seung DY, Papoutsakis ET, Bennett GN, Lee SY. Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for isopropanol-butanol-ethanol fermentation. Appl Environ Microbiol 2012;78:1416–23.
[12] Park JH, Lee SY. Towards systems metabolic engineering of microorganisms for amino acid production. Curr Opin Biotechnol 2008;19:454–60.
[13] Liao JC, Mi L, Pontrelli S, Luo S. Fueling the future: microbial engineering for the production of sustainable biofuels. Nat Rev Microbiol 2016;14:288–304.
[14] Liu D, Evans T, Zhang F. Applications and advances of metabolite biosensors for metabolic engineering. Metab Eng 2015;31:35–43.
[15] Kuroda K, Ueda M. Arming technology in yeast—novel strategy for whole-cell biocatalyst and protein engineering. Biomolecules 2013;3:632–50.
[16] Tafakori V, Torktaz I, Doostmohammadi M, Ahmadian G. Microbial cell surface display; its medical and environmental applications. Iran J Biotechnol 2012;10:231–9.
[17] Hubbe MA. Catalysts inspired by life. Biofuel Res J 2016;3:430.
[18] Zhang F, Rodriguez S, Keasling JD. Metabolic engineering of microbial pathways for advanced biofuels production. Curr Opin Biotechnol 2011;22:775–83.
[19] Martien JI, Amador-Noguez D. Recent applications of metabolomics to advance microbial biofuel production. Curr Opin Biotechnol 2017;43:118–26.
[20] De Bhowmick G, Koduru L, Sen R. Metabolic pathway engineering towards enhancing microalgal lipid biosynthesis for biofuel application—a review. Renew Sustain Energy Rev 2015;50:1239–53.
[21] Talebi AF, Tohidfar M, Tabatabaei M, Bagheri A, Mohsenpor M, Mohtashami SK. Genetic manipulation, a feasible tool to enhance unique characteristic of *Chlorella vulgaris* as a feedstock for biodiesel production. Mol Biol Rep 2013;40:4421–8.
[22] Tao H, Guo D, Zhang Y, Deng Z, Liu T. Metabolic engineering of microbes for branched-chain biodiesel production with low-temperature property. Biotechnol Biofuels 2015;8:92.
[23] a) Gustavsson M, Lee SY. Prospects of microbial cell factories developed through systems metabolic engineering. Microb Biotechnol 2016;9:610–7;
b) Immethun CM, Hoynes-O'Connor AG, Balassy A, Moon TS. Microbial production of isoprenoids enabled by synthetic biology. Front Microbiol 2013;4:75.
[24] Jang YS, Park JM, Choi S, Choi YJ, Cho JH, Lee SY. Engineering of microorganisms for the production of biofuels and perspectives based on systems metabolic engineering approaches. Biotechnol Adv 2012;30:989–1000.
[25] Yu D, Kurola JM, Lähde K, Kymäläinen M, Sinkkonen A, Romantschuk M. Biogas production and methanogenic archaeal community in mesophilic and thermophilic anaerobic co-digestion processes. J Environ Manag 2014;143:54–60.
[26] Šustr V, Chroňáková A, Semanová S, Tajovský K, Šimek M. Methane production and methanogenic archaea in the digestive tracts of millipedes (diplopoda). PLoS One 2014:e102659.
[27] Tabatabaei M. Molecular phylogeny and characteristics of methanogens from a palm oil mill anaerobic tank [Ph.D thesis]. University Putra Malaysia (UPM); 2009.
[28] Tabatabaei M, Rahim RA, Abdullah N, Wright ADG, Shirai Y, Sakai K, Sulaiman A, Hassan MA. Importance of the methanogenic archaea populations in anaerobic wastewater treatments. Process Biochem 2010;45:1214–25.
[29] Suryawanshi PC, Chaudhari AB, Kothari RM. Mesophilic anaerobic digestion: first option for waste treatment in tropical regions. Crit Rev Biotechnol 2010;30:259–82.
[30] Jabłoński S, Rodowicz P, Łukaszewicz M. Methanogenic archaea database containing physiological and biochemical characteristics. Int J Syst Evol Microbiol 2015;65:1360–8.
[31] Tabatabaei M, Zakaria MR, Rahim RA, Wright ADG, Shirai Y, Abdullah N, Sakai K, Ikeno S, Mori M, Kazunori N, Sulaiman A. PCR-based DGGE and FISH analysis of methanogens in an anaerobic closed digester tank for treating palm oil mill effluent. Electron J Biotechnol 2009;12:12–3.
[32] Khalife E, Tabatabaei M, Demirbas A, Aghbashlo M. Impacts of additives on performance and emission characteristics of diesel engines during steady state operation. Prog Energy Combust Sci 2017;59:32–78.
[33] Ajit A, Sulaiman AZ, Chisti Y. Production of bioethanol by *Zymomonas mobilis* in high-gravity extractive fermentations. Food Bioprod Process 2017;102:123–35.
[34] Scully SM, Orlygsson J. Recent advances in second generation ethanol production by thermophilic bacteria. Energies 2014;8:1–30.
[35] Akponah E, Akpomie OO, Ubogu M. Bio-ethanol production from cassava effluent using *Zymomonas mobilis* and *Saccharomyces cerevisiae* isolated from rafia palm (*Elaesis guineesi*) sap. Eur J Exp Biol 2013;3:247–53.
[36] Yang S, Fei Q, Zhang Y, Contreras LM, Utturkar SM, Brown SD, Himmel ME, Zhang M. *Zymomonas mobilis* as a model system for production of biofuels and biochemicals. Microb Biotechnol 2016;9:699–717.
[37] Agrawal M, Dunn KL, Rao CV. *Zymomonas mobilis* for the conversion of lignocellulosic biomass to fuels and chemicals. In Engineering of microorganisms for the production of chemicals and biofuels from renewable resources. Springer International Publishing; 2017. p. 67–92.
[38] Wang H, Cao S, Wang WT, Wang KT, Jia X. Very high gravity ethanol and fatty acid production of *Zymomonas mobilis* without amino acid and vitamin. J Ind Microbiol Biotechnol 2016;43:861–71.
[39] Zheng YN, Li LZ, Xian M, Ma YJ, Yang JM, Xu X, He DZ. Problems with the microbial production of butanol. J Ind Microbiol Biotechnol 2009;36:1127–38.
[40] Koppolu V, Vasigala VK. Role of *Escherichia coli* in biofuel production. Microbiol Insights 2016;9:29.
[41] Kim JH, Block DE, Shoemaker SP, Mills DA. Atypical ethanol production by carbon catabolite derepressed lactobacilli. Bioresour Technol 2010;101:8790–7.
[42] Nikel PI, de Lorenzo V. Robustness of *Pseudomonas putida* KT2440 as a host for ethanol biosynthesis. New Biotechnol 2014;31:562–71.
[43] Romero S, Merino E, Bolívar F, Gosset G, Martinez A. Metabolic engineering of *Bacillus subtilis* for ethanol production: lactate dehydrogenase plays a key role in fermentative metabolism. Appl Environ Microbiol 2007;73:5190–8.
[44] Soo CS, Yap WS, Hon WM, Ramli N, Shah UKM, Phang LY. Improvement of hydrogen yield by ethanol producing *Escherichia coli* recombinants in acidic condition. Electron J Biotechnol 2017;26:27–32.
[45] Zhou S, Yomano LP, Shanmugam KT, Ingram LO. Fermentation of 10% (w/v) sugar to D (−)-lactate by engineered *Escherichia coli* B. Biotechnol Lett 2005;27:1891–6.
[46] Kim JR, Jung SH, Regan JM, Logan BE. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour Technol 2007;98:2568–77.
[47] Zhou S, Iverson AG, Grayburn WS. Engineering a native homoethanol pathway in *Escherichia coli* B for ethanol production. Biotechnol Lett 2008;30:335–42.
[48] Huerta-Beristain G, Cabrera-Ruiz R, Hernandez-Chavez G, Bolivar F, Gosset G, Martinez A. Metabolic engineering and adaptive evolution of *Escherichia coli* KO11 for ethanol production through the Entner–Doudoroff and the pentose phosphate pathways. J Chem Technol Biotechnol 2017;92:990–6.
[49] Wang Y, Manow R, Finan C, Wang J, Garza E, Zhou S. Adaptive evolution of nontransgenic *Escherichia coli* KC01 for improved ethanol tolerance and homoethanol fermentation from xylose. J Ind Microbiol Biotechnol 2011;38:1371–7.
[50] Huffer S, Roche CM, Blanch HW, Clark DS. *Escherichia coli* for biofuel production: bridging the gap from promise to practice. Trends Biotechnol 2012;30:538–45.
[51] Goldemberg J. Ethanol for a sustainable energy future. Science 2007;315:808–10.
[52] Hill J, Nelson E, Tilman D, Polasky S, Tiffany D. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proc Natl Acad Sci USA 2006;103:11206–10.
[53] Sanchez OJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 2008;99:5270–95.
[54] Rehman MSU, Kim I, Chisti Y, Han JI. Use of ultrasound in the production of bioethanol from lignocellulosic biomass. Energy Educ Sci Technol 2013;30:1391–410.
[55] Tripathi SA, Olson DG, Argyros DA, Miller BB, Barrett TF, Murphy DM, McCool JD, Warner AK, Rajgarhia VB, Lynd LR, Hogsett DA, Caiazza NC. Development of pyrF-based genetic system for targeted gene deletion in *Clostridium thermocellum* and creation of a pta mutant. Appl Environ Microbiol 2010;76:6591–9.
[56] Biswas R, Prabhu S, Lynd LR, Guss AM. Increase in ethanol yield via elimination of lactate production in an ethanol-tolerant mutant of *Clostridium thermocellum*. PLoS One 2014;9:e86389.
[57] Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, Hogsett DA, Lynd LR. Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. Proc Natl Acad Sci USA 2008;105:13769–74.
[58] Shaw AJ, Hogsett DA, Lynd LR. Identification of the [FeFe]-hydrogenase responsible for hydrogen generation in *Thermoanaerobacterium saccharolyticum* and demonstration of increased ethanol yield via hydrogenase knockout. J Bacteriol 2009;191:6457–64.
[59] Argyros DA, Tripathi SA, Barrett TF, Rogers SR, Feinberg LF, Olson DG, Foden JM, Miller BB, Lynd LR, Hogsett DA, Caiazza NC. High ethanol titers from cellulose by using metabolically engineered thermophilic, anaerobic microbes. Appl Environ Microbiol 2011;77:8288–94.
[60] Cripps RE, Eley K, Leak DJ, Rudd B, Taylor M, Todd M, Boakes S, Martin S, Atkinson T. Metabolic engineering of *Geobacillus thermoglucosidasius* for high yield ethanol production. Metab Eng 2009;11:398–408.
[61] Georgieva TI, Mikkelsen MJ, Ahring BK. Ethanol production from wet-exploded wheat straw hydrolysate by thermophilic anaerobic bacterium *Thermoanaerobacter* BG1L1 in a continuous immobilized reactor. Appl Biochem Biotechnol 2008;145:99–110.
[62] Yao S, Mikkelsen MJ. Metabolic engineering to improve ethanol production in *Thermoanaerobacter mathranii*. Appl Microbiol Biotechnol 2010;88:199–208.
[63] Cheon S, Kim HM, Gustavsson M, Lee SY. Recent trends in metabolic engineering of microorganisms for the production of advanced biofuels. Curr Opin Chem Biol 2016;35:10–21.
[64] da Silva Trindade WR, dos Santos RG. Review on the characteristics of butanol, its production and use as fuel in internal combustion engines. Renew Sustain Energy Rev 2017;69:642–51.
[65] Amiri H, Azarbaijani R, Yeganeh LP, Fazeli AS, Tabatabaei M, Salekdeh GH, Karimi K. Nesterenkonia sp. strain F, a halophilic bacterium producing acetone, butanol, and ethanol under aerobic conditions. Sci Rep 2016;6:18408.
[66] Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS. Fermentative butanol production by *Clostridia*. Biotechnol Bioeng 2008;101:209–28.
[67] Karimi K, Tabatabaei M, Sárvári Horváth I, Kumar R. Recent trends in acetone, butanol, and ethanol (ABE) production. Biofuel Res J 2015;2:301–8.
[68] Xue C, Zhao J, Chen L, Yang ST, Bai F. Recent advances and state-of-the-art strategies in strain and process engineering for biobutanol production by *Clostridium acetobutylicum*. Biotechnol Adv 2017;35:310–22.
[69] Kótai L, Szépvölgyi J, Szilágyi M, Zhibin L, Baiquan C, Sharma V, Sharma PK. Biobutanol from renewable agricultural and lignocellulose resources and its perspectives as alternative of liquid fuels. In: Fang Z, editor. Liquid, gaseous and solid biofuels- conversion techniques. InTech; 2013. p. 199–262.
[70] Jang YS, Malaviya A, Lee J, Im JA, Lee SY, Lee J, Eom MH, Cho JH, Seung DY. Metabolic engineering of *Clostridium acetobutylicum* for the enhanced production of isopropanol-butanol-ethanol fuel mixture. Biotechnol Progr 2012;29:1083–8.
[71] Huang H, Liu H, Gan YR. Genetic modification of critical enzymes and involved

genes in butanol biosynthesis from biomass. Biotechnol Adv 2010;28:651–7.
[72] Moon HG, Jang YS, Cho C, Lee J, Binkley R, Lee SY. One hundred years of clostridial butanol fermentation. FEMS Microbiol Lett 2016;363:fnw001.
[73] Atsumi S, Cann AF, Connor MR, Shen CR, Smith KM, Brynildsen MP, Chou KJY, Hanai T, Liao JC. Metabolic engineering of *Escherichia coli* for 1-butanol production. Metab Eng 2008;10:305–11.
[74] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 2008;451:86–9.
[75] Kolek J, Patáková P, Melzoch K, Sigler K, Řezanka T. Changes in membrane plasmalogens of *Clostridium pasteurianum* during butanol fermentation as determined by lipidomic analysis. PLoS One 2015;10:e0122058.
[76] Jiang Y, Xu C, Dong F, Yang Y, Jiang W, Yang S. Disruption of the acetoacetate decarboxylase gene in solvent-producing *Clostridium acetobutylicum* increases the butanol ratio. Metab Eng 2009;11:284–91.
[77] Lee JY, Jang YS, Lee J, Papoutsakis ET, Lee SY. Metabolic engineering of *Clostridium acetobutylicum* M5 for highly selective butanol production. Biotechnol J 2009;4:1432–40.
[78] Liao C, Seo SO, Celik V, Liu H, Kong W, Wang Y, Blaschek H, Jin Y, Lu T. Integrated, systems metabolic picture of acetone-butanol-ethanol fermentation by *Clostridium acetobutylicum*. Proc Natl Acad Sci USA 2015;112:8505–10.
[79] Sillers R, Chow A, Tracy B, Papoutsakis ET. Metabolic engineering of the non-sporulating, non-solventogenic *Clostridium acetobutylicum* strain M5 to produce butanol without acetone demonstrate the robustness of the acid-formation pathways and the importance of the electron balance. Metab Eng 2008;10:321–32.
[80] Harris LM, Desai RP, Welker NE, Papoutsakis ET. Characterization of recombinant strains of the *Clostridium acetobutylicum* butyrate kinase inactivation mutant: need for new phenomenological models for solventogenesis and butanol inhibition? Biotechnol Bioeng 2000;67:1–11.
[81] Willson BJ, Kovács K, Wilding-Steele T, Markus R, Winzer K, Minton NP. Production of a functional cell wall-anchored minicellulosome by recombinant *Clostridium acetobutylicum* ATCC 824. Biotechnol Biofuels 2016;9:109.
[82] Heap JT, Kuehne SA, Ehsaan M, Cartman ST, Cooksley CM, Scott JC, Minton NP. The ClosTron: mutagenesis in *Clostridium* refined and streamlined. J Microbiol Methods 2010;80:49–55.
[83] Tomas CA, Welker NE, Papoutsakis ET. Overexpression of groESL in *Clostridium acetobutylicum* results in increased solvent production and tolerance, prolonged metabolism, and changes in the cell's transcriptional program. Appl Environ Microbiol 2003;69:4951–65.
[84] Baez A, Cho KM, Liao JC. High-flux isobutanol production using engineered *Escherichia coli*: a bioreactor study with in situ product removal. Appl Microbiol Biotechnol 2011;90:1681–90.
[85] Lee JH, Kim SB, Kang SW, Song YS, Park C, Han SO, Kim SW. Biodiesel production by a mixture of *Candida rugosa* and *Rhizopus oryzae* lipases using a supercritical carbon dioxide process. Bioresour Technol 2011;102:2105–8.
[86] Lee SY, Jang YS, Lee JY, Lee J, Park JH, Im JA, Eom MH, Lee J, Lee SH, Song H, Cho JH, Seung DY. Enhanced butanol production obtained by reinforcing the direct butanol-forming route in *Clostridium acetobutylicum*. mBio 2012;3:e00314–12.
[87] Inui M, Suda M, Kimura S, Yasuda K, Suzuki H, Toda H, Yamamoto S, Okino S, Suzuki N, Yukawa H. Expression of *Clostridium acetobutylicum* butanol synthetic genes in Escherichia coli. Appl Microbiol Biotechnol 2008;77:1305–16.
[88] Lütke-Eversloh T, Bahl H. Metabolic engineering of *Clostridium acetobutylicum*: recent advances to improve butanol production. Curr Opin Biotechnol 2011;22:634–47.
[89] Jang YS, Lee J, Malaviya A, Seung DY, Cho JH, Lee SY. Butanol production from renewable biomass: rediscovery of metabolic pathways and metabolic engineering. Biotechnol J 2012;7:186–98.
[90] Zheng J, Tashiro Y, Wang Q, Sonomoto K. Recent advances to improve fermentative butanol production: genetic engineering and fermentation technology. J Biosci Bioeng 2015;119:1–9.
[91] Blombach B, Riester T, Wieschalka S, Ziert C, Youn JW, Wendisch VF, Eikmanns BJ. *Corynebacterium glutamicum* tailored for efficient isobutanol production. Appl Environ Microbiol 2011;77:3300–10.
[92] Smith KM, Cho KM, Liao JC. Engineering *Corynebacterium glutamicum* for isobutanol production. Appl Microbiol Biotechnol 2010;87:1045–55.
[93] Li S, Jia X, Wen J. Improved 2-methyl-1-propanol production in an engineered *Bacillus subtilis* by constructing inducible pathways. Biotechnol Lett 2012;34:2253–8.
[94] Su H, Lin J, Wang G. Metabolic engineering of *Corynebacterium crenatium* for enhancing production of higher alcohols. Sci Rep 2016:6.
[95] Lu J, Brigham CJ, Gai CS, Sinskey AJ. Studies on the production of branched-chain alcohols in engineered *Ralstonia eutropha*. Appl Microbiol Biotechnol 2012;96:283–97.
[96] Li H, Opgenorth PH, Wernick DG, Rogers S, Wu TY, Higashide W, Malati P, Huo YX, Cho KM, Liao JC. Integrated electromicrobial conversion of CO_2 to higher alcohols. Science 2012;335. [1596–1596].
[97] Choi YJ, Lee J, Jang YS, Lee SY. Metabolic engineering of microorganisms for the production of higher alcohols. mBio 2014;5:e01524–14.
[98] Atsumi S, Liao JC. Directed evolution of *Methanococcus jannaschii* citramalate synthase for biosynthesis of 1-propanol and 1-butanol by *Escherichia coli*. Appl Environ Microbiol 2008;74:7802–8.
[99] Choi YJ, Park JH, Kim TY, Lee SY. Metabolic engineering of *Escherichia coli* for the production of 1-propanol. Metab Eng 2012;14:477–86.
[100] Hanai T, Atsumi S, Liao JC. Engineered synthetic pathway for isopropanol production in *Escherichia coli*. Appl Environ Microbiol 2007;73:7814–8.
[101] Inokuma K, Liao JC, Okamoto M, Hanai T. Improvement of isopropanol production by metabolically engineered *Escherichia coli* using gas stripping. J Biosci Bioeng 2010;110:696–701.
[102] Soma Y, Inokuma K, Tanaka T, Ogino C, Kondo A, Okamoto M, Hanai T. Direct isopropanol production from cellobiose by engineered *Escherichia coli* using a synthetic pathway and a cell surface display system. J Biosci Bioeng 2012;114:80–5.
[103] Jojima T, Inui M, Yukawa H. Production of isopropanol by metabolically engineered *Escherichia coli*. Appl Microbiol Biotechnol 2008;77:1219–24.
[104] Dusséaux S, Croux C, Soucaille P, Meynial-Salles I. Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for the high-yield production of a biofuel composed of an isopropanol/butanol/ethanol mixture. Metab Eng 2013;18:1–8.
[105] Dai Z, Dong H, Zhu Y, Zhang Y, Li Y, Ma Y. Introducing a single secondary alcohol dehydrogenase into butanol-tolerant *Clostridium acetobutylicum* Rh8 switches ABE fermentation to high level IBE fermentation. Biotechnol Biofuels 2012;5:44.
[106] Jang YS, Lee JY, Lee J, Park JH, Im JA, Eom MH, Lee J, Lee SH, Song H, Cho JH, Seung DY, Lee SY. Enhanced butanol production obtained by reinforcing the direct butanol-forming route in *Clostridium acetobutylicum*. mBio 2012;3:e00314–12.
[107] Hou X, Peng W, Xiong L, Huang C, Chen X, Chen X, Zhang W. Engineering *Clostridium acetobutylicum* for alcohol production. J Biotechnol 2013;166:25–33.
[108] Yu M, Zhang Y, Tang IC, Yang ST. Metabolic engineering of *Clostridium tyrobutyricum* for n-butanol production. Metab Eng 2011;13:373–82.
[109] Yu M, Du Y, Jiang W, Chang WL, Yang ST, Tang IC. Effects of different replicons in conjugative plasmids on transformation efficiency, plasmid stability, gene expression and n-butanol biosynthesis in *Clostridium tyrobutyricum*. Appl Microbiol Biotechnol 2012;93:881–9.
[110] Bond-Watts BB, Bellerose RJ, Chang MC. Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. Nat Chem Biol 2011;7:222–7.
[111] Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC. Driving forces enable high-titer anaerobic 1-butanol synthesis in *Escherichia coli*. Appl Environ Microbiol 2011;77:2905–15.
[112] Dellomonaco C, Clomburg JM, Miller EN, Gonzalez R. Engineered reversal of the [bgr]-oxidation cycle for the synthesis of fuels and chemicals. Nature 2011;476:355–9.
[113] Li S, Wen J, Jia X. Engineering *Bacillus subtilis* for isobutanol production by heterologous Ehrlich pathway construction and the biosynthetic 2-ketoisovalerate precursor pathway overexpression. Appl Microbiol Biotechnol 2011;91:577–89.
[114] Higashide W, Li Y, Yang Y, Liao JC. Metabolic engineering of *Clostridium cellulolyticum* for production of isobutanol from cellulose. Appl Environ Microbiol 2011;77:2727–33.
[115] Steen EJ, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, Del Cardayre SB, Keasling JD. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 2010;463:559–62.
[116] Youngquist JT, Schumacher MH, Rose JP, Raines TC, Politz MC, Copeland MF, Pfleger BF. Production of medium chain length fatty alcohols from glucose in *Escherichia coli*. Metab Eng 2013;20:177–86.
[117] Liu A, Tan X, Yao L, Lu X. Fatty alcohol production in engineered *E. coli* expressing Marinobacter fatty acyl-CoA reductases. Appl Microbiol Biotechnol 2013;97:7061–71.
[118] Liu R, Zhu F, Lu L, Fu A, Lu J, Deng Z, Liu T. Metabolic engineering of fatty acyl-ACP reductase-dependent pathway to improve fatty alcohol production in *Escherichia coli*. Metab Eng 2014;22:10–21.
[119] Hosseinpour S, Aghbashlo M, Tabatabaei M, Younesi H, Mehrpooya M, Ramakrishna S. Multi-objective exergy-based optimization of a continuous photobioreactor applied to produce hydrogen using a novel combination of soft computing techniques. Int J Hydrog Energy 2017;42:8518–29.
[120] Brentner LB, Peccia J, Zimmerman JB. Challenges in developing biohydrogen as a sustainable energy source: implications for a research agenda. Environ Sci Technol 2010;44:2243–54.
[121] Aghbashlo M, Hosseinpour S, Tabatabaei M, Hosseini SS, Najafpour G, Younesi H. An exergetically-sustainable operational condition of a photo-biohydrogen production system optimized using conventional and innovative fuzzy techniques. Renew Energy 2016;94:605–18.
[122] Singh A, Rathore D. Biohydrogen production: sustainability of current technology and future perspective. Springer; 2016.
[123] Oh YK, Raj SM, Jung GY, Park S. Current status of the metabolic engineering of microorganisms for biohydrogen production. Bioresour Technol 2011;102:8357–67.
[124] Pattanamanee W, Chisti Y, Choorit W. Photofermentive hydrogen production by *Rhodobacter sphaeroides* S10 using mixed organic carbon: effects of the mixture composition. Appl Energy 2015;157:245–54.
[125] Pattanamanee W, Choorit W, Deesan C, Sirisansaneeyakul S, Chisti Y. Photofermentive production of biohydrogen from oil palm waste hydrolysate. Int J Hydrog Energy 2012;37:4077–87.
[126] Pattanamanee W, Choorit W, Kantachote D, Chisti Y. Repeated-batch production of hydrogen using *Rhodobacter sphaeroides* S10. Int J Hydrog Energy 2012;37:15855–66.
[127] Wang Y, Zhou P, Tong J, Gao R. Advances in the genetic modification of *Rhodobacter sphaeroides* to improve hydrogen production. Renew Sustain Energy Rev 2016;60:1312–8.
[128] Singh A, Sevda S, Abu Reesh IM, Vanbroekhoven K, Rathore D, Pant D. Biohydrogen production from lignocellulosic biomass: technology and sustainability. Energies 2015;8:13062–80.
[129] Hosseini SE, Wahid MA. Hydrogen production from renewable and sustainable energy resources: promising green energy carrier for clean development. Renew Sustain Energy Rev 2016;57:850–66.
[130] Hallenbeck PC, Ghosh D. Advances in fermentative biohydrogen production: the way forward? Trends Biotechnol 2009;27:287–97.

[131] Chen WM, Tseng ZJ, Lee KS, Chang JS. Fermentative hydrogen production with *Clostridium butyricum* CGS5 isolated from anaerobic sewage sludge. Int J Hydrog Energy 2005;30:1063–70.
[132] Chittibabu G, Nath K, Das D. Feasibility studies on the fermentative hydrogen production by recombinant *Escherichia coli* BL-21. Process Biochem 2006;41:682–8.
[133] Khanna N, Kotay SM, Gilbert JJ, Das D. Improvement of biohydrogen production by *Enterobacter cloacae* IIT-BT 08 under regulated pH. J Biotechnol 2011;152:9–15.
[134] Yoshida A, Nishimura T, Kawaguchi H, Inui M, Yukawa H. Enhanced hydrogen production from formic acid by formate hydrogen lyase-overexpressing *Escherichia coli* strains. Appl Environ Microbiol 2005;71:6762–8.
[135] Yoshida A, Nishimura T, Kawaguchi H, Inui M, Yukawa H. Enhanced hydrogen production from glucose using ldh-and frd-inactivated *Escherichia coli* strains. Appl Microbiol Biotechnol 2006;73:67–72.
[136] Kim S, Seol E, Oh YK, Wang GY, Park S. Hydrogen production and metabolic flux analysis of metabolically engineered *Escherichia coli* strains. Int J Hydrog Energy 2009;34:7417–27.
[137] Maeda T, Sanchez-Torres V, Wood TK. Metabolic engineering to enhance bacterial hydrogen production. Microb Biotechnol 2008;1:30–9.
[138] Seol E, Manimaran A, Jang Y, Kim S, Oh YK, Park S. Sustained hydrogen production from formate using immobilized recombinant *Escherichia coli* SH5. Int J Hydrog Energy 2011;36:8681–6.
[139] Seol E, Sekar BS, Raj SM, Park S. Co-production of hydrogen and ethanol from glucose by modification of glycolytic pathways in *Escherichia coli*—from Embden–Meyerhof–Parnas pathway to pentose phosphate pathway. Biotechnol J 2015;11:249–56.
[140] Sekar BS, Seol E, Raj SM, Park S. Co-production of hydrogen and ethanol by pfkA-deficient *Escherichia coli* with activated pentose-phosphate pathway: reduction of pyruvate accumulation. Biotechnol Biofuels 2016;9:95.
[141] Akhtar MK, Jones PR. Construction of a synthetic YdbK-dependent pyruvate: H 2 pathway in *Escherichia coli* BL21 (DE3). Metab Eng 2009;11:139–47.
[142] Jo JH, Jeon CO, Lee SY, Lee DS, Park JM. Molecular characterization and homologous overexpression of [FeFe]-hydrogenase in *Clostridium tyrobutyricum* JM1. Int J Hydrog Energ 2010;35:1065–73.
[143] Liu X, Zhu Y, Yang ST. Construction and characterization of ack deleted mutant of *Clostridium tyrobutyricum* for enhanced butyric acid and hydrogen production. Biotechnol Progr 2006;22:1265–75.
[144] Kanai T, Imanaka H, Nakajima A, Uwamori K, Omori Y, Fukui T, Atomi H, Imanaka T. Continuous hydrogen production by the hyperthermophilic archaeon, *Thermococcus kodakaraensis* KOD1. J Biotechnol 2005;116:271–82.
[145] Auria R, Boileau C, Davidson S, Casalot L, Christen P, Liebgott PP, Combet-Blanc Y. Hydrogen production by the hyperthermophilic bacterium *Thermotoga maritima* Part II: modeling and experimental approaches for hydrogen production. Biotechnol Biofuels 2016;9:268.
[146] Abreu AA, Tavares F, Alves MM, Pereira MA. Boosting dark fermentation with co-cultures of extreme thermophiles for biomethane production from garden waste. Bioresour Technol 2016;219:132–8.
[147] Talluri S, Raj SM, Christopher LP. Consolidated bioprocessing of untreated switchgrass to hydrogen by the extreme thermophile *Caldicellulosiruptor saccharolyticus* DSM 8903. Bioresour Technol 2013;139:272–9.
[148] Cao GL, Ren NQ, Wang AJ, Guo WQ, Yao J, Feng YJ, Zhao QL. Statistical optimization of culture condition for enhanced hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16. Bioresour Technol 2010;101:2053–8.
[149] Bielen AA, Verhaart MR, van der Oost J, Kengen SW. Biohydrogen production by the thermophilic bacterium *Caldicellulosiruptor saccharolyticus*: current status and perspectives. Life 2013;3:52–85.
[150] De Vrije T, Bakker RR, Budde MA, Lai MH, Mars AE, Claassen PA. Efficient hydrogen production from the lignocellulosic energy crop Miscanthus by the extreme thermophilic bacteria *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. Biotechnol Biofuels 2009;2:12.
[151] Dubini A, Gonzalez-Ballester D. Biohydrogen from microalgae. In: Bux F, Chisti Y, editors. Algae biotechnology: products and processes. New York: Springer; 2016. p. 165–93.
[152] Muniro SA, Zinder SH, Walker LP. The fermentation stoichiometry of *Thermotoga neapolitana* and influence of temperature, oxygen, and pH on hydrogen production. Biotechnol Progr 2009;25:1035–42.
[153] Zeidan AA, Van Niel EW. A quantitative analysis of hydrogen production efficiency of the extreme thermophile *Caldicellulosiruptor owensensis* OL T. Int J Hydrog Energy 2010;35:1128–37.
[154] Morimoto K, Kimura T, Sakka K, Ohmiya K. Overexpression of a hydrogenase gene in *Clostridium paraputrificum* to enhance hydrogen gas production. FEMS Microbiol Lett 2005;246:229–34.
[155] Kim IK, Roldão A, Siewers V, Nielsen J. A systems-level approach for metabolic engineering of yeast cell factories. FEMS Yeast Res 2012;12:228–48.
[156] Jeffries TW. Engineering yeasts for xylose metabolism. Curr Opin Biotechnol 2006;17:320–6.
[157] Sheng J, Feng X. Metabolic engineering of yeast to produce fatty acid-derived biofuels: bottlenecks and solutions. Front Microbiol 2015;6:554.
[158] Caspeta L, Castillo T. Systems metabolic engineering of *Saccharomyces cerevisiae* for production of biochemicals from biomass. In engineering of microorganisms for the production of chemicals and biofuels from renewable resources. Springe Int Publ 2017:31–65.
[159] Sellis D, Callahan BJ, Petrov DA, Messer PW. Heterozygote advantage as a natural consequence of adaptation in diploids. Proc Natl Acad Sci USA 2011;108:20666–71.
[160] Buijs NA, Siewers V, Nielsen J. Advanced biofuel production by the yeast *Saccharomyces cerevisiae*. Curr Opin Chem Biol 2013;17:480–8.
[161] Runguphan W, Keasling JD. Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid-derived biofuels and chemicals. Metab Eng 2014;21:103–13.
[162] Tang X, Lee J, Chen WN. Engineering the fatty acid metabolic pathway in *Saccharomyces cerevisiae* for advanced biofuel production. Metab Eng Commun 2015;2:58–66.
[163] Petrovič U. Next-generation biofuels: a new challenge for yeast. Yeast 2015;32:583–93.
[164] Qin J, Zhou YJ, Krivoruchko A, Huang M, Liu L, Khoomrung S, Siewers V, Jiang B, Nielsen J. Modular pathway rewiring of *Saccharomyces cerevisiae* enables high-level production of L-ornithine. Nat Commun 2015;6:8224.
[165] Teo WS, Ling H, Yu AQ, Chang MW. Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid short- and branched-chain alkyl esters biodiesel. Biotechnol Biofuels 2015;8:177.
[166] Karhumaa K, Sanchez RG, Hahn-Hägerdal B, Gorwa-Grauslund MF. Comparison of the xylose reductase-xylitol dehydrogenase and the xylose isomerase pathways for xylose fermentation by recombinant *Saccharomyces cerevisiae*. Microb Cell Fact 2007;6:5.
[167] Hahn-Hägerdal B, Karhumaa K, Jeppsson M, Gorwa-Grauslund MF. Metabolic engineering for pentose utilization in *Saccharomyces cerevisiae*. Adv Biochem Eng Biotechnol 2007;108:147–77.
[168] Van Maris AJ, Winkler AA, Kuyper M, De Laat WT, Van Dijken JP, Pronk JT. Development of efficient xylose fermentation in *Saccharomyces cerevisiae*: xylose isomerase as a key component. Adv Biochem Eng Biotechnol 2007;108:179–204.
[169] Madhavan A, Tamalampudi S, Srivastava A, Fukuda H, Bisaria VS, Kondo A. Alcoholic fermentation of xylose and mixed sugars using recombinant *Saccharomyces cerevisiae* engineered for xylose utilization. Appl Microbiol Biotechnol 2009;82:1037–47.
[170] Madhavan A, Tamalampudi S, Ushida K, Kanai D, Katahira S, Srivastava A, Fukuda H, Bisaria VS, Kondo A. Xylose isomerase from polycentric fungus Orpinomyces: gene sequencing, cloning, and expression in *Saccharomyces cerevisiae* for bioconversion of xylose to ethanol. Appl Microbiol Biotechnol 2009;82:1067–78.
[171] Chen X, Nielsen KF, Borodina I, Kielland-Brandt MC, Karhumaa K. Increased isobutanol production in *Saccharomyces cerevisiae* by overexpression of genes in valine metabolism. Biotechnol Biofuels 2011;4:2089–90.
[172] Matsuda F, Ishii J, Kondo T, Ida K, Tezuka H, Kondo A. Increased isobutanol production in *Saccharomyces cerevisiae* by eliminating competing pathways and resolving cofactor imbalance. Microb Cell Fact 2013;12:119.
[173] Steen EJ, Chan R, Prasad N, Myers S, Petzold CJ, Redding A, Ouellet M, Keasling JD. Metabolic engineering of *Saccharomyces cerevisiae* for the production of n-butanol. Microb Cell Fact 2008;7:36.
[174] Nevoigt E. Progress in metabolic engineering of *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 2008;72:379–412.
[175] Kurylenko O, Semkiv M, Ruchala J, Hryniv O, Kshanovska B, Abbas C, Dmytruk K, Sibirny A. New approaches for improving the production of the 1st and 2nd generation ethanol by yeast. Acta Biochim Pol 2016;63:31–8.
[176] Young E, Lee SM, Alper H. Optimizing pentose utilization in yeast: the need for novel tools and approaches. Biotechnol Biofuels 2010;3:24.
[177] Fan LH, Zhang ZJ, Yu XY, Xue YX, Tan TW. Self-surface assembly of cellulosomes with two miniscaffoldins on *Saccharomyces cerevisiae* for cellulosic ethanol production. Proc Natl Acad Sci USA 2012;109:13260–5.
[178] Ha SJ, Galazka JM, Kim SR, Choi JH, Yang X, Seo JH, Glass NL, Cate JH, Jin YS. Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation. Proc Natl Acad Sci USA 2011;108:504–9.
[179] Nielsen J, Larsson C, van Maris A, Pronk J. Metabolic engineering of yeast for production of fuels and chemicals. Curr Opin Biotechnol 2013;24:398–404.
[180] Agbogbo FK, Coward-Kelly G. Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*. Biotechnol Lett 2008;30:1515–24.
[181] Long TM, Su YK, Headman J, Higbee A, Willis LB, Jeffries TW. Cofermentation of glucose, xylose, and cellobiose by the beetle-associated yeast *Spathaspora passalidarum*. Appl Environ Microbiol 2012;78:5492–500.
[182] Arora R, Behera S, Sharma NK, Kumar S. A new search for thermotolerant yeasts, its characterization and optimization using response surface methodology for ethanol production. Front Microbiol 2015;6:889.
[183] Caspeta L, Nielsen J. Thermotolerant yeast strains adapted by laboratory evolution show trade-off at ancestral temperatures and preadaptation to other stresses. mBio 2015;6:e00431–15.
[184] West TP, Kennedy II DE. Isolation of thermotolerant yeast strains for ethanol production: a need for new approaches. J Microb Biochem Technol 2014;6:7.
[185] Hu N, Yuan B, Sun J, Wang SA, Li FL. Thermotolerant *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* strains representing potentials for bioethanol production from *Jerusalem artichoke* by consolidated bioprocessing. Appl Microbiol Biotechnol 2012;95:1359–68.
[186] Yuangsaard N, Yongmanitchai W, Yamada M, Limtong S. Selection and characterization of a newly isolated thermotolerant *Pichia kudriavzevii* strain for ethanol production at high temperature from cassava starch hydrolysate. Antonie Van Leeuwenhoek 2013;103:577–88.
[187] Khambhaty Y, Upadhyay D, Kriplani Y, Joshi N, Mody K, Gandhi MR. Bioethanol from macroalgal biomass: utilization of marine yeast for production of the same. BioEnergy Res 2013;6:188–95.
[188] Liu X, Jensen PR, Workman M. Bioconversion of crude glycerol feedstocks into ethanol by *Pachysolen tannophilus*. Bioresour Technol 2012;104:579–86.

[189] Dürre P. Biobutanol: an attractive biofuel. Biotechnol J 2007;2:1525–34.
[190] Brat D, Weber C, Lorenzen W, Bode HB, Boles E. Cytosolic re-localization and optimization of valine synthesis and catabolism enables increased isobutanol production with the yeast *Saccharomyces cerevisiae*. Biotechnol Biofuels 2012;5:65.
[191] Kondo T, Tezuka H, Ishii J, Matsuda F, Ogino C, Kondo A. Genetic engineering to enhance the Ehrlich pathway and alter carbon flux for increased isobutanol production from glucose by *Saccharomyces cerevisiae*. J Biotechnol 2012;159:32–7.
[192] Avalos JL, Fink GR, Stephanopoulos G. Compartmentalization of metabolic pathways in yeast mitochondria improves the production of branched-chain alcohols. Nat Biotechnol 2013;31:335–41.
[193] Oliver NJ, Rabinovitch-Deere CA, Carroll AL, Nozzi NE, Case AE, Atsumi S. Cyanobacterial metabolic engineering for biofuel and chemical production. Curr Opin Chem Biol 2016;35:43–50.
[194] Dhaman Y, Roy P. Challenges and generations of biofuels: will algae fuel the world. Ferment Technol 2013;2:119.
[195] Ducat DC, Way JC, Silver PA. Engineering cyanobacteria to generate high-value products. Trends Biotechnol 2011;29:95–103.
[196] Dutta K, Daverey A, Lin JG. Evolution retrospective for alternative fuels: first to fourth generation. Renew Energy 2014;69:114–22.
[197] Han SF, Jin WB, Tu RJ, Wu WM. Biofuel production from microalgae as feedstock: current status and potential. Crit Rev Biotechnol 2014;35:255–68.
[198] Lü J, Sheahan C, Fu P. Metabolic engineering of algae for fourth generation biofuels production. Energy Environ Sci 2011;4:2451–66.
[199] Parmar A, Singh NK, Pandey A, Gnansounou E, Madamwar D. Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresour Technol 2011;102:10163–72.
[200] Woo HM. Solar-to-chemical and solar-to-fuel production from CO_2 by metabolically engineered microorganisms. Curr Opin Biotechnol 2017;45:1–7.
[201] Chisti Y. Constraints to commercialization of algal fuels. J Biotechnol 2013;167:201–14.
[202] Melis A. Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci 2009;177:272–80.
[203] Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 2008;19:235–40.
[204] Shih PM, Wu D, Latifi A, Axen SD, Fewer DP, Talla E, Calteau A, Cai F, Tandeau De Marsac N, Rippka R, Herdman M, Sivonen K, Coursin T, Laurent T, Goodwin L, Nolan M, Davenport KW, Han CS, Rubin EM, Eisen JA, Woyke T, Gugger M, Kerfeld CA. Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. Proc Natl Acad Sci USA 2013;110:1053–8.
[205] Angermayr SA, Hellingwerf KJ, Lindblad P, de Mattos MJT. Energy biotechnology with cyanobacteria. Curr Opin Biotechnol 2009;20:257–63.
[206] Huang HH, Camsund D, Lindblad P, Heidorn T. Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology. Nucleic Acids Res 2010;38:2577–93.
[207] Khanna N, Lindblad P. Cyanobacterial hydrogenases and hydrogen metabolism revisited: recent progress and future prospects. Int J Mol Sci 2015;16:10537–61.
[208] Khetkorn W, Khanna N, Incharoensakdi A, Lindblad P. Metabolic and genetic engineering of cyanobacteria for enhanced hydrogen production. Biofuels 2013;4:535–61.
[209] Shestakov SV, Mikheeva LE. Genetic control of hydrogen metabolism in cyanobacteria. Russ J Genet 2006;42:1272–84.
[210] Lai MC, Lan EI. Advances in metabolic engineering of cyanobacteria for photosynthetic biochemical production. Metabolites 2015;5:636–58.
[211] Dexter J, Fu P. Metabolic engineering of cyanobacteria for ethanol production. Energy Environ Sci 2009;2:857–64.
[212] Dexter J, Armshaw P, Sheahan C, Pembroke JT. The state of autotrophic ethanol production in cyanobacteria. J Appl Microbiol 2015;119:11–24.
[213] Niederholtmeyer H, Wolfstädter BT, Savage DF, Silver PA, Way JC. Engineering cyanobacteria to synthesize and export hydrophilic products. Appl Environ Microbiol 2010;76:3462–6.
[214] Atsumi S, Higashide W, Liao JC. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat Biotechnol 2009;27:1177–80.
[215] Bentley FK, Zurbriggen A, Melis A. Heterologous expression of the mevalonic acid pathway in cyanobacteria enhances endogenous carbon partitioning to isoprene. Mol Plant 2014;7:71–86.
[216] Lindberg P, Park S, Melis A. Engineering a platform for photosynthetic isoprene production in cyanobacteria, using *Synechocystis* as the model organism. Metab Eng 2010;12:70–9.
[217] Reinsvold RE, Jinkerson RE, Radakovits R, Posewitz MC, Basu C. The production of the sesquiterpene β-caryophyllene in a transgenic strain of the cyanobacterium *Synechocystis*. J Plant Physiol 2011;168:848–52.
[218] Lan EI, Liao JC. Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. Metab Eng 2011;13:353–63.
[219] Tan X, Yao L, Gao Q, Wang W, Qi F, Lu X. Photosynthesis driven conversion of carbon dioxide to fatty alcohols and hydrocarbons in cyanobacteria. Metab Eng 2011;13:169–76.
[220] Yao L, Qi F, Tan X, Lu X. Improved production of fatty alcohols in cyanobacteria by metabolic engineering. Biotechnol Biofuels 2014;7:94.
[221] Liu X, Sheng J, Curtiss III R. Fatty acid production in genetically modified cyanobacteria. Proc Natl Acad Sci USA 2011;108:6899–904.
[222] Ruffing AM. Improved free fatty acid production in cyanobacteria with *Synechococcus* sp. PCC 7002 as host. Front Bioeng Biotechnol 2014;2:17.
[223] Zhou J, Zhang H, Zhang Y, Li Y, Ma Y. Designing and creating a modularized synthetic pathway in cyanobacterium *Synechocystis* enables production of acetone from carbon dioxide. Metab Eng 2012;14:394–400.
[224] Oliver JW, Machado IM, Yoneda H, Atsumi S. Cyanobacterial conversion of carbon dioxide to 2, 3-butanediol. Proc Natl Acad Sci USA 2013;110:1249–54.
[225] Lan EI, Liao JC. ATP drives direct photosynthetic production of 1-butanol in cyanobacteria. Proc Natl Acad Sci USA 2012;109:6018–23.
[226] Gao Z, Zhao H, Li Z, Tan X, Lu X. Photosynthetic production of ethanol from carbon dioxide in genetically engineered cyanobacteria. Energy Environ Sci 2012;5:9857–65.
[227] Ungerer J, Tao L, Davis M, Ghirardi M, Maness PC, Yu J. Sustained photosynthetic conversion of CO_2 to ethylene in recombinant cyanobacterium *Synechocystis* 6803. Energy Environ Sci 2012;5:8998–9006.
[228] Oberhardt MA, Palsson BO, Papin JA. Applications of genome-scale metabolic reconstructions. Mol Syst Biol 2009;5:320.
[229] Shen CR, Liao JC. Photosynthetic production of 2-methyl-1-butanol from CO_2 in cyanobacterium *Synechococcus elongatus* PCC7942 and characterization of the native acetohydroxyacid synthase. Energy Environ Sci 2012;5:9574–83.
[230] Ducat DC, Sachdeva G, Silver PA. Rewiring hydrogenase-dependent redox circuits in cyanobacteria. Proc Natl Acad Sci USA 2011;108:3941–6.
[231] Wang W, Liu X, Lu X. Engineering cyanobacteria to improve photosynthetic production of alka(e)nes. Biotechnol Biofuels 2013;6:69.
[232] Ananyev G, Carrieri D, Dismukes GC. Optimization of metabolic capacity and flux through environmental cues to maximize hydrogen production by the cyanobacterium *Arthrospira* (*Spirulina*) *maxima*. Appl Environ Microbiol 2008;74:6102–13.
[233] Abed RMM, Dobretsov S, Sudesh K. Applications of cyanobacteria in biotechnology. J Appl Microbiol 2009;106:1–12.
[234] Dutta D, De D, Chaudhuri S, Bhattacharya SK. Hydrogen production by cyanobacteria. Microb Cell Fact 2005;4:36.
[235] Sakurai H, Masukawa H. Promoting R & D in photobiological hydrogen production utilizing mariculture-raised cyanobacteria. Mar Biotechnol 2007;9:128–45.
[236] Tamagnini P, Leitão E, Oliveira P, Ferreira D, Pinto F, Harris DJ, Heidorn T, Lindblad P. Cyanobacterial hydrogenases: diversity, regulation and applications. FEMS Microbiol Rev 2007;31:692–720.
[237] Hall DO, Markov SA, Watanabe Y, Rao KK. The potential applications of cyanobacterial photosynthesis for clean technologies. Photosynth Res 1995;46:159–67.
[238] Gupta V, Ratha SK, Sood A, Chaudhary V, Prasanna R. New insights into the biodiversity and applications of cyanobacteria (blue-green algae)-prospects and challenges. Algal Res 2013;2:79–97.
[239] Lau NS, Matsui M, Abdullah AAA. Cyanobacteria: photoautotrophic microbial factories for the sustainable synthesis of industrial products. BioMed Res Int 2015;2015:754–934.
[240] McKinlay JB, Harwood CS. Photobiological production of hydrogen gas as a biofuel. Curr Opin Biotechnol 2010;21:244–51.
[241] McNeely K, Xu Y, Bennette N, Bryant DA, Dismukes GC. Redirecting reductant flux into hydrogen production via metabolic engineering of fermentative carbon metabolism in a cyanobacterium. Appl Environ Microbiol 2010;76:5032–8.
[242] Park JH, Lee SY, Kim TY, Kim HU. Application of systems biology for bioprocess development. Trends Biotechnol 2008;26:404–12.
[243] Quintana N, Van der Kooy F, Van de Rhee MD, Voshol GP, Verpoorte R. Renewable energy from cyanobacteria: energy production optimization by metabolic pathway engineering. Appl Microbiol Biotechnol 2011;91:471–90.
[244] Bandyopadhyay A, Stöckel J, Min H, Sherman LA, Pakrasi HB. High rates of photobiological H_2 production by a cyanobacterium under aerobic conditions. Nat Commun 2010;1:139.
[245] Kaushik A, Sharma M. Exploiting biohydrogen pathways of cyanobacteria and green algae: an industrial production approach. Biohydrogen production: sustainability of current technology and future perspective. India: Springer; 2017. p. 97–113.
[246] Bensaid S, Ruggeri B, Saracco G. Development of a photosynthetic microbial electrochemical cell (PMEC) reactor coupled with dark fermentation of organic wastes: medium term perspectives. Energies 2015;8:399–429.
[247] Choi YN, Park JM. Enhancing biomass and ethanol production by increasing NADPH production in *Synechocystis* sp. PCC 6803. Bioresour Technol 2016;213:54–7.
[248] Pfannerer F, Krennhuber K, Jäger A, Kahr H. New constructs for ethanol production via cyanobacteria. Agron Res 2016;14:174–80.
[249] Wu B, Zhang B, Feng X, Rubens JR, Huang R, Hicks LM, Pakrasi HB, Tang YJ. Alternative isoleucine synthesis pathway in cyanobacterial species. Microbiology 2010;156:596–602.
[250] Schirmer A, Rude MA, Li X, Popova E, Del Cardayre SB. Microbial biosynthesis of alkanes. Science 2010;329:559–62.
[251] Liu J, Song Y, Qiu W. Oleaginous microalgae Nannochloropsis as a new model for biofuel production: review & analysis. Renew Sustain Energy Rev 2017;72:154–62.
[252] de Farias Silva CE, Bertucco A. Bioethanol from microalgae and cyanobacteria: a review and technological outlook. Process Biochem 2016;51:1833–42.
[253] Subramanian V, Dubini A, Seibert M. Metabolic pathways in green algae with potential value for biofuel production. In: Gordon R, Seckbach J, editors. The science of algal fuels. New York: Springer; 2012. p. 399–422.
[254] Radakovits R, Jinkerson RE, Darzins A, Posewitz MC. Genetic engineering of algae for enhanced biofuel production. Eukaryot Cell 2010;9:486–501.
[255] Lamsen EN, Atsumi S. Recent progress in synthetic biology for microbial production of C3–C10 alcohols. Front Microbiol 2012;3:196.
[256] Tabatabaei M, Tohidfar M, Jouzani GS, Safarnejad M, Pazouki M. Biodiesel production from genetically engineered microalgae: future of bioenergy in Iran. Renew Sustain Energy Rev 2011;15:1918–27.
[257] Quinn JC, Turner CW, Bradley TH. Scale-up of flat plate photobioreactors

considering diffuse and direct light characteristics. Biotechnol Bioeng 2012;109:363–70.
[258] Weyer KM, Bush DR, Darzins A, Willson BD. Theoretical maximum algal oil production. Bioenergy Res 2010;3:204–13.
[259] Banerjee C, Dubey KK, Shukla P. Metabolic engineering of microalgal based biofuel production: prospects and challenges. Front Microbiol 2016:7.
[260] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol 2008;26:126–31.
[261] Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chem Eng Proces 2009;48:1146–51.
[262] Talebi AF, Mohtashami SK, Tabatabaei M, Tohidfar M, Bagheri A, Zeinalabedini M, Mirzaei HH, Mirzajanzadeh M, Shafaroudi SM, Bakhtiari S. Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. Algal Res 2013;2:258–67.
[263] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. J Biotechnol 2006;126:499–507.
[264] Sahay S, BraganzaV J. Microalgae based biodiesel production–current and future scenario. J Exp Sci 2016;7:31–5.
[265] Gaurav N, Sivasankari S, Kiran GS, Ninawe A, Selvin J. Utilization of bioresources for sustainable biofuels: a review. Renew Sustain Energy Rev 2017;73:205–14.
[266] Miller R, Wu G, Deshpande RR, Vieler A, Gärtner K, Li X, Moellering ER, Zauner S, Cornish AJ, Liu B, Bullard B, Sears BB, Kuo MH, Hegg EL, Shachar-Hill Y, Shiu SH, Benning C. Changes in transcript abundance in *Chlamydomonas reinhardtii* following nitrogen deprivation predict diversion of metabolism. Plant Physiol 2010;154:1737–52.
[267] Msanne J, Xu D, Konda AR, Casas-Mollano JA, Awada T, Cahoon EB, Cerutti H. Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae *Chlamydomonas reinhardtii* and *Coccomyxa* sp. C169. Phytochem 2012;75:50–9.
[268] Li Y, Han D, Hu G, Dauvillee D, Sommerfeld M, Ball S, Hu Q. *Chlamydomonas* starchless mutant defective in ADP-glucose pyrophosphorylase hyper-accumulates triacylglycerol. Metab Eng 2010;12:387–91.
[269] El-Sheekh MM. Genetic engineering of eukaryotic algae with special reference to *Chlamydomonas*. Turk J Biol 2005:29.
[270] Dunahay TG, Jarvis EE, Roessler PG. Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophila*. J Phycol 1995;31:1004–12.
[271] Trentacoste EM, Shrestha RP, Smith SR, Glé C, Hartmann AC, Hildebrand M, Gerwick WH. Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth. Proc Natl Acad Sci USA 2013;110:19748–53.
[272] Radakovits R, Eduafo PM, Posewitz MC. Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. Metab Eng 2011;13:89–95.
[273] Rollin JA, del Campo JM, Myung S, Sun F, You C, Bakovic A, Senger RS. High-yield hydrogen production from biomass by in vitro metabolic engineering: mixed sugars coutilization and kinetic modeling. Proc Natl Acad Sci USA 2015;112:4964–9.
[274] Pakpour F, Najafpour G, Tabatabaei M, Tohidfar M, Younesi H. Biohydrogen production from CO-rich syngas via a locally isolated *Rhodopseudomonas palustris* PT. Bioprocess Biosyst Eng 2014;37:923–30.
[275] Hankamer B, Lehr F, Rupprecht J, Mussgnug JH, Posten C, Kruse O. Photosynthetic biomass and H_2 production by green algae: from bioengineering to bioreactor scale-up. Physiol Plant 2007;131:10–21.
[276] Hemschemeier A, Melis A, Happe T. Analytical approaches to photobiological hydrogen production in unicellular green algae. Photosynth Res 2009;102:523–40.
[277] Kruse O, Rupprecht J, Bader KP, Thomas-Hall S, Schenk PM, Finazzi G, Hankamer B. Improved photobiological H_2 production in engineered green algal cells. J Biol Chem 2005;280:34170–7.
[278] Lee HS, Vermaas WF, Rittmann BE. Biological hydrogen production: prospects and challenges. Trends Biotechnol 2010;28:262–71.
[279] Melis A, Zhang L, Forestier M, Ghirardi ML, Seibert M. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. Plant Physiol 2000;122:127–36.
[280] Melis A, Happe T. Hydrogen production. Green algae as a source of energy. Plant Physiol 2001;127:740–8.
[281] Gimpel JA, Specht EA, Georgianna DR, Mayfield SP. Advances in microalgae engineering and synthetic biology applications for biofuel production. Curr Opin Chem Biol 2013;17:489–95.
[282] Srirangan K, Pyne ME, Chou CP. Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. Bioresour Technol 2011;102:8589–604.
[283] Philipps G, Krawietz D, Hemschemeier A, Happe T. A pyruvate formate lyase (PFL1) deficient *Chlamydomonas reinhardtii* strain provides evidence for a link between fermentation and hydrogen evolution in green algae. Plant J 2011;66:330–40.
[284] Scoma A, Krawietz D, Faraloni C, Giannelli L, Happe T, Torzillo G. Sustained H_2 production in a *Chlamydomonas reinhardtii* D1 protein mutant. J Biotechnol 2012;157:613–9.
[285] Show KY, Lee DJ, Tay JH, Lin CY, Chang JS. Biohydrogen production: current perspectives and the way forward. Int J Hydrog Energy 2012;37:15616–31.
[286] Florin L, Tsokoglou A, Happe T. A novel type of iron hydrogenase in the green alga Scenedesmus obliquus is linked to the photosynthetic electron transport chain. J Biol Chem 2001;276:6125–32.
[287] Juantorena AU, Lastres O, Hernandez G, Bustos A, Sebastian PJ, Eapen D. Hydrogen production by microorganisms and its application in a PEMFC. Int J Energy Res 2012;36:902–10.
[288] Papazi A, Andronis E, Ioannidis NE, Chaniotakis N, Kotzabasis K. High yields of hydrogen production induced by meta-substituted dichlorophenols biodegradation from the green alga *Scenedesmus obliquus*. PLoS One 2012;7:e49037.
[289] Chader S, Hacene H, Agathos SN. Study of hydrogen production by three strains of *Chlorella* isolated from the soil in the Algerian Sahara. Int J Hydrog Energy 2009;34:4941–6.
[290] Song W, Rashid N, Choi W, Lee K. Biohydrogen production by immobilized *Chlorella* sp. using cycles of oxygenic photosynthesis and anaerobiosis. Bioresour Technol 2011;102:8676–81.
[291] Amutha KB, Murugesan AG. Biological hydrogen production by the algal biomass *Chlorella vulgaris* MSU 01 strain isolated from pond sediment. Bioresour Technol 2011;102:194–9.
[292] He M, Li L, Zhang L, Liu J. The enhancement of hydrogen photoproduction in *Chlorella protothecoides* exposed to nitrogen limitation and sulfur deprivation. Int J Hydrog Energy 2012;37:16903–15.
[293] Guan Y, Deng M, Yu X, Zhang W. Two-stage photo-biological production of hydrogen by marine green alga *Platymonas subcordiformis*. Biochem Eng J 2004;19:69–73.
[294] Ran C, Yu X, Jin M, Zhang W. Role of carbonyl cyanide m-chlorophenylhydrazone in enhancing photobiological hydrogen production by marine green alga *Platymonas subcordiformis*. Biotechnol Progr 2006;22:438–43.
[295] Rumpel S, Siebel JF, Farès C, Duan J, Reijerse E, Happe T, Lubitz M, Winkler M. Enhancing hydrogen production of microalgae by redirecting electrons from photosystem I to hydrogenase. Energy Environ Sci 2014;7:3296–301.
[296] Sun Y, Chen M, Yang H, Zhang J, Kuang T, Huang F. Enhanced H_2 photoproduction by down-regulation of ferredoxin-NADP+ reductase (FNR) in the green alga *Chlamydomonas reinhardtii*. Int J Hydrog Energy 2013;38:16029–37.
[297] Meuser JE, D'Adamo S, Jinkerson RE, Mus F, Yang W, Ghirardi ML, Seibert M, Grossman AR, Posewitz MC. Genetic disruption of both *Chlamydomonas reinhardtii* [FeFe]-hydrogenases: insight into the role of HYDA2 in H_2 production. Biochem Biophys Res Commun 2012;417:704–9.
[298] Sakuragi H, Kuroda K, Ueda M. Molecular breeding of advanced microorganisms for biofuel production. BioMed Res Int 2011;2011:416931.
[299] Barlowe CK, Miller EA. Secretory protein biogenesis and traffic in the early secretory pathway. Genetics 2013;193:383–410.
[300] Ueda M, Tanaka A. Cell surface engineering of yeast: construction of arming yeast with biocatalyst. J Biosci Bioeng 2000;90:125–36.
[301] Shibasaki S, Maeda H, Ueda M. Molecular display technology using yeast-arming technology. Anal Sci 2009;25:41–9.
[302] Smith MR, Khera E, Wen F. Engineering novel and improved biocatalysts by cell surface display. Ind Eng Chem Res 2015;54:4021–32.
[303] Wang Q, Li L, Chen M, Qi Q, Wang PG. Construction of a novel *Pichia pastoris* cell-surface display system based on the cell wall protein Pir1. Curr Microbiol 2008;56:352–7.
[304] Yuzbasheva EY, Yuzbashev TV, Laptev IA, Konstantinova TK, Sineoky SP. Efficient cell surface display of Lip2 lipase using C-domains of glycosylphosphatidylinositol-anchored cell wall proteins of *Yarrowia lipolytica*. Appl Microbiol Biotechnol 2011;91:645–54.
[305] Lynd LR, Van Zyl WH, McBride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: an update. Curr Opin Biotechnol 2005;16:577–83.
[306] Cherf GM, Cochran JR. Applications of yeast surface display for protein engineering. Methods Mol Biol 2015;1319:155–75.
[307] Murai T, Ueda M, Kawaguchi T, Arai M, Tanaka A. Assimilation of cellooligosaccharides by a cell surface-engineered yeast expressing β-glucosidase and carboxymethylcellulase from *Aspergillus aculeatus*. Appl Environ Microbiol 1998;64:4857–61.
[308] Labarre C, Schirawski J, Zwet A, Fitzgerald GF, Sinderen D. Insertional mutagenesis of an industrial strain of *Streptococcus thermophilus*. FEMS Microbiol Lett 2001;200:85–90.
[309] Teparic R, Mrsa V. Overview of systems and techniques for surface display of recombinant proteins in yeast *S. cerevisiae*. Appl Food Biotechnol 2015;3:3–14.
[310] Shigechi H, Koh J, Fujita Y, Matsumoto T, Bito Y, Ueda M, Satoh E, Fukuda H, Kondo A. Direct production of ethanol from raw corn starch via fermentation by use of a novel surface-engineered yeast strain codisplaying glucoamylase and α-amylase. Appl Environ Microbiol 2004;70:5037–40.
[311] Kricka W, Fitzpatrick J, Bond U. Metabolic engineering of yeasts by heterologous enzyme production for degradation of cellulose and hemicellulose from biomass: a perspective. Front Microbiol 2014;5:174.
[312] Fujita Y, Ito J, Ueda M, Fukuda H, Kondo A. Synergistic saccharification, and direct fermentation to ethanol, of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme. Appl Environ Microbiol 2004;70:1207–12.
[313] Kotaka A, Bando H, Kaya M, Kato-Murai M, Kuroda K, Sahara H, Hata Y, Kondo A, Ueda M. Direct ethanol production from barley β-glucan by sake yeast displaying *Aspergillus oryzae* β-glucosidase and endoglucanase. J Biosci Bioeng 2008;105:622–7.
[314] Ito J, Kosugi A, Tanaka T, Kuroda K, Shibasaki S, Ogino C, Ueda M, Fukuda H, Doi RH, Kondo A. Regulation of the display ratio of enzymes on the Saccharomyces cerevisiae cell surface by the immunoglobulin G and cellulosomal enzyme binding domains. Appl Environ Microbiol 2009;75:4149–54.
[315] Yamada R, Taniguchi N, Tanaka T, Ogino C, Fukuda H, Kondo A. Research cocktail δ-integration: a novel method to construct cellulolytic enzyme expression ratio-optimized yeast strains. Microb Cell Fact 2010;10:32.
[316] Nakanishi A, Bae J, Kuroda K, Ueda M. Construction of a novel selection system for

endoglucanases exhibiting carbohydrate-binding modules optimized for biomass using yeast cell-surface engineering. AMB Express 2012;2:1–8.
[317] Shoseyov O, Shani Z, Levy I. Carbohydrate binding modules: biochemical properties and novel applications. Microbiol Mol Biol Rev 2006;70:283–95.
[318] Hyeon JE, Kang DH, Han SO. Signal amplification by a self-assembled biosensor system designed on the principle of dockerin–cohesin interactions in a cellulosome complex. Analyst 2014;139:4790–3.
[319] Tsai SL, Oh J, Singh S, Chen R, Chen W. Functional assembly of minicellulosomes on the *Saccharomyces cerevisiae* cell surface for cellulose hydrolysis and ethanol production. Appl Environ Microbiol 2009;75:6087–93.
[320] Goyal G, Tsai SL, Madan B, Da Silva NA, Chen W. Simultaneous cell growth and ethanol production from cellulose by an engineered yeast consortium displaying a functional mini-cellulosome. Microb Cell Fact 2011;10:89.
[321] Katahira S, Fujita Y, Mizuike A, Fukuda H, Kondo A. Construction of a xylan-fermenting yeast strain through codisplay of xylanolytic enzymes on the surface of xylose-utilizing *Saccharomyces cerevisiae* cells. Appl Environ Microbiol 2004;70:5407–14.
[322] Katahira S, Mizuike A, Fukuda H, Kondo A. Ethanol fermentation from lignocellulosic hydrolysate by a recombinant xylose- and cellooligosaccharide-assimilating yeast strain. Appl Microbiol Biotechnol 2006;72:1136–43.
[323] Ota M, Sakuragi H, Morisaka H, Kuroda K, Miyake H, Tamaru Y, Ueda M. Display of *Clostridium cellulovorans* xylose isomerase on the cell surface of *Saccharomyces cerevisiae* and its direct application to xylose fermentation. Biotechnol Progr 2013;29:346–51.
[324] Palamae S, Palachum W, Chisti Y, Choorit W. Retention of hemicellulose during delignification of oil palm empty fruit bunch (EFB) fiber with peracetic acid and alkaline peroxide. Biomass Bioenergy 2014;66:240–8.
[325] Nakanishi A, Bae JG, Fukai K, Tokumoto N, Kuroda K, Ogawa J, Nakatani M, Shimizu S, Ueda M. Effect of pretreatment of hydrothermally processed rice straw with laccase-displaying yeast on ethanol fermentation. Appl Microbiol Biotechnol 2012;94:939–48.
[326] Hasheminejad M, Tabatabaei M, Mansourpanah Y, Javani A. Upstream and downstream strategies to economize biodiesel production. Bioresour Technol 2011;102(2):461–8.
[327] Sheng L, Kovács K, Winzer K, Zhang Y, Minton NP. Development and implementation of rapid metabolic engineering tools for chemical and fuel production in *Geobacillus thermoglucosidasius* NCIMB 11955. Biotechnol Biofuels 2017;10:5.
[328] Long MR, Ong WK, Reed JL. Computational methods in metabolic engineering for strain design. Curr Opin Biotechnol 2015;34:135–41.
[329] Shabestary K, Hudson EP. Computational metabolic engineering strategies for growth-coupled biofuel production by *Synechocystis*. Metab Eng Commun 2016;3:216–26.
[330] Roointan A, Morowvat MH. Road to the future of systems biotechnology: crispr-cas-mediated metabolic engineering for recombinant protein production. Biotechnol Genet Eng Rev 2017:1–18.
[331] Lee JW, Kim TY, Jang YS, Choi S, Lee SY. Systems metabolic engineering for chemicals and materials. Trends Biotechnol 2011;29:370–8.
[332] Park JH, Lee SY. Towards systems metabolic engineering of microorganisms for amino acid production. Curr Opin Biotechnol 2008;19:454–60.
[333] Lee JW, Na D, Park JM, Lee J, Choi S, Lee SY. Systems metabolic engineering of microorganisms for natural and non-natural chemicals. Nat Chem Biol 2012;8:536–46.
[334] Chubukov V, Mukhopadhyay A, Petzold CJ, Keasling JD, Martín HG. Synthetic and systems biology for microbial production of commodity chemicals. NPJ Syst Biol Appl 2016;2:16009.
[335] Koppolu V, Vasigala VK. Role of *Escherichia coli* in biofuel production. Microbiol Insights 2016;9:29.
[336] Gong T, Liu R, Zuo Z, Che Y, Yu H, Song C, Yang C. Metabolic engineering of *Pseudomonas putida* KT2440 for complete mineralization of methyl parathion and γ-hexachlorocyclohexane. ACS Synth Biol 2016;5:434–42.
[337] Beckers V, Poblete-Castro I, Tomasch J, Wittmann C. Integrated analysis of gene expression and metabolic fluxes in PHA-producing *Pseudomonas putida* grown on glycerol. Microb Cell Fact 2016;15:73.
[338] Nogales J, Gudmundsson S, Thiele I. Toward systems metabolic engineering in cyanobacteria: opportunities and bottlenecks. Bioengineered 2013:4158–63.
[339] Park SJ, Jang YA, Noh W, Oh YH, Lee H, David Y, Baylon MG, Shin J, Yang JE, Choi SY, Lee SH, Lee SY. Metabolic engineering of *Ralstonia eutropha* for the production of polyhydroxyalkanoates from sucrose. Biotechnol Bioeng 2015;112:638–43.
[340] Weber T, Charusanti P, Musiol-Kroll EM, Jiang X, Tong Y, Kim HU, Lee SY. Metabolic engineering of antibiotic factories: new tools for antibiotic production in actinomycetes. Trends Biotechnol 2015;33:15–26.
[341] Medema MH, Cimermancic P, Sali A, Takano E, Fischbach MA. A systematic computational analysis of biosynthetic gene cluster evolution: lessons for engineering biosynthesis. PLoS Comput Biol 2014;10:e1004016.
[342] Wang Y, Chen L, Zhang W. Proteomic and metabolomic analyses reveal metabolic responses to 3-hydroxypropionic acid synthesized internally in cyanobacterium *Synechocystis* sp. PCC 6803. Biotechnol Biofuels 2016;9:209.
[343] Tang X, Feng H, Zhang J, Chen WN. Comparative proteomics analysis of engineered *Saccharomyces cerevisiae* with enhanced biofuel precursor production. PLoS One 2013;8:e84661.
[344] Alonso-Gutierrez J, Kim EM, Batth TS, Cho N, Hu Q, Chan LJ, Petzold CJ, Hillson NJ, Adams PD, Keasling JD, Garcia Martin H, Lee TS. Principal component analysis of proteomics (PCAP) as a tool to direct metabolic engineering. Metab Eng 2015;28:123–33.
[345] George KW, Chen A, Jain A, Batth TS, Baidoo EE, Wang G, Adams PD, Petzold CJ, Keasling JD, Lee TS. Correlation analysis of targeted proteins and metabolites to assess and engineer microbial isopentenol production. Biotechnol Bioeng 2014;111:1648–58.
[346] Liebeke M, Dorries K, Zuhlke D, Bernhardt J, Fuchs S, Pane-Farre J, Engelmann S, Volker U, Bode R, Dandekar T, Lindequist U, Hecker M, Lalk MA. Metabolomics and proteomics study of the adaptation of *Staphylococcus aureus* to glucose starvation. Mol Biosyst 2011;7:1241–53.
[347] Wu SG, Wang Y, Jiang W, Oyetunde T, Yao R, Zhang X, Shimizu K, Tang YJ, Bao FS. Rapid prediction of bacterial heterotrophic fluxomics using machine learning and constraint programming. PLoS Comput Biol 2016;12:e1004838.
[348] Gaida SM, Liedtke A, Jentges AHW, Engels B, Jennewein S. Metabolic engineering of *Clostridium cellulolyticum* for the production of n-butanol from crystalline cellulose. Microb Cell Fact 2016;15:6.
[349] Ling H, Chen B, Kang A, Lee JM, Chang MW. Transcriptome response to alkane biofuels in *Saccharomyces cerevisiae* identification of efflux pumps involved in alkane tolerance. Biotechnol Biofuels 2013;6:10.
[350] Chen B, Ling H, Chang MW. Transporter engineering for improved tolerance against alkane biofuels in *Saccharomyces cerevisiae*. Biotechnol Biofuels 2013;6:21.
[351] Raut MP, Couto N, Pham TK, Evans C, Noirel J, Wright PC. Quantitative proteomic analysis of the influence of lignin on biofuel production by *Clostridium acetobutylicum* ATCC 824. Biotechnol Biofuels 2016;9:113.
[352] George KW, Thompson MG, Kang A, Baidoo E, Wang G, Chan LJ, Adams PD, Petzold CJ, Keasling JD, Lee TS. Metabolic engineering for the high-yield production of isoprenoid-based C5 alcohols in *E. coli*. Sci Rep 2015;5:11128.
[353] Redding-Johanson AM, Batth TS, Chan R, Krupa R, Szmidt HL, Adams PD, Keasling JD, Lee TS, Mukhopadhyay A, Petzold CJ. Targeted proteomics for metabolic pathway optimization: application to terpene production. Metab Eng 2011;13:194–203.
[354] Beckers V, Poblete-Castro I, Tomasch J, Wittmann C. Integrated analysis of gene expression and metabolic fluxes in PHA-producing *Pseudomonas putida* grown on glycerol. Microb Cell Fact 2016;15:73.