Biofuels from food processing wastes
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Food processing industry generates substantial high organic wastes along with high energy uses. The recovery of food processing wastes as renewable energy sources represents a sustainable option for the substitution of fossil energy, contributing to the transition of food sector towards a low-carbon economy. This article reviews the latest research progress on biofuel production using food processing wastes. While extensive work on laboratory and pilot-scale biosystems for energy production has been reported, this work presents a review of advances in metabolic pathways, key technical issues and bioengineering outcomes in biofuel production from food processing wastes. Research challenges and further prospects associated with the knowledge advances and technology development of biofuel production are discussed.

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Introduction
Food industry is one of the most important industries in modern society and provides a wide range of products for industrial needs and human food, including production of sugars, grain flours, starches, dairy, fruits, vegetables, meats and breweries. However, the perishability of crop and food products, consumer behaviour and inefficiencies in supply chains results in the generation of significant quantities of food wastes from food processing industries. Food processing wastes (FPW) have the potential to contaminate the environment and their disposal or treatment is costly to food industries. Landfilling, a common practice for disposal of FPW, results in emissions of greenhouse gases and contamination of groundwater [1]. The food industry is also a significant energy user through energy-intensive production processes, product storage and distribution. The composition, quantity and availability of FPW vary depending on the product, and processing scale and technology. FPW are mainly composed of carbohydrate polymers (starch, cellulose and hemicelluloses), lignin, proteins, lipids and organic acids, as summarised in Table 1. Therefore, FPW can be used as carbon and nutrient sources for the bioproduction of fuels and chemicals.

Biofuels are biologically produced from renewable and even waste organic substrates by microorganisms, which are being explored to replace fossil fuels. Biofuels are favourable choice of fuel consumption due to their renewability, biodegradability and generating acceptable quality exhaust gases. Biofuels have emerged as one of the most strategically important sustainable fuel sources and are considered an important way of progress for limiting greenhouse gas emissions, improving air quality and finding new energetic resources. Renewable and carbon neutral biofuels are necessary for environmental and economic sustainability. Conversion of FPW to biofuel for the food industry application is a promising approach to reduce the energy cost for food processing. This paper reviews the recent progress on biofuel production from FPW. We focus on the most studied key biofuels, including gaseous biofuels (methane, hydrogen and hythane) and liquid biofuels (ethanol, butanol and diesel). Future challenges and research perspectives in this technology development field will be discussed.

Gaseous biofuels
Methane
Anaerobic digestion (AD) of FPW is a well-established technology to produce biogases (mainly methane and CO2). The global installed capacity for power generation from biogas production is estimated to be 29.5 GW by 2022 [2]. The methane production can be conducted in a four stage process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [3,4,5**6,7]. Among these steps,
Hydrolysis is the major rate-limiting step due to the complexity of the feedstock, especially for the FPW containing lignocellulosic biomass, animal fats and protein [3,5**]. Pretreatment and pre-hydrolysis are able to accelerate the production of intermediates (fatty acids, fermentable sugars, amino acids, etc.) for synthesizing volatile fatty acid (VFA) [3]. Thermal, ultrasound, alkaline, acid, thermal–chemical and enzymatic methods are commonly employed to hydrolyse carbohydrates, such as slaughterhouse and meat-processing wastes, which are rich in protein and fats [4,8*,9]. However, these pretreatment methods sometimes fail to improve methane production due to over-production of long chain fatty acids and VFA, or inefficient hydrolysis [8*]. Brewer’s spent grain, which mainly contains cellulose, hemicellulose and protein, was enzymatic hydrolysed using cellulase, hemicellulose and protease following thermal–chemical pre-treatment to accelerate the production of fermentable sugars and amino acids prior to methane production [10]. Key parameters associated with the acidogenesis, acetogenesis, and methanogenesis include C/N ratio, pH, VFAs, temperature, long chain fatty acids (LCFAs) and ammonia. Continuing efforts have been made to shorten the long retention time, which takes 15–40 days [5**,*6,7]. A recent review paper reports that nanomaterials are able to improve methane production [7]. In general, the addition of iron oxide enhanced methane production whereas others nanomaterials had mixed effects [7].

**Hydrogen**

Hydrogen (H₂) has been universally recognised as an environmentally friendly and renewable energy resource, and an ideal alternative to fossil fuels, as H₂ has the highest energy density of the known fuels (142 kJ/g), and produces water as the sole by-product of combustion. The global hydrogen market will grow from $87.5 billion in 2011 to $118 billion by 2016 [11]. H₂ production through bioprocesses represents an exiting area of technology development for energy generation. H₂ basically can be produced in light dependent and light independent bioprocesses: the former is biophotolysis and photofermentation, while the latter includes dark fermentation and bio-electrochemical system (microbial fuel cell and microbial electrolysis cell) [12**,13]. Integrated dark–fermentation process was found to be a cost-effective technology for H₂ production using FPW [13]. Among these bioprocesses the dark fermentation is the most promising process because of the relatively low production cost and high production rate [12**,14].

Table 1

| Major food products and processing wastes/by-products | Type | Processing wastes/by-products | Annual yield, million tonnes (year) | Type (major composition) | Annual yield, million tonnes (% of total product yield) |
|------------------------------------------------------|------|------------------------------|-------------------------------------|--------------------------|-------------------------------------------------------|
| Oil (palm)                                           | 54.4 (2013)* | Palm empty fruit bunch (lignocellulose) | 163.2 (~200%) | | |
| Oil (soybean)                                        | 42.6 (2013)* | Soybean meal (protein, carbohydrate) | 170.4 (~400%) | | |
| Oil (rapeseed)                                       | 24.7 (2013)* | Rapeseed meal (protein, carbohydrate) | 32.1 (~130%) | | |
| Rice                                                 | 740.9 (2013)* | Rice hull (lignocellulose, ash) | 148.2 (~20%) | | |
| Wheat                                                | 715.9 (2013)* | Bran (arabinose, cellulose, protein) | 143.2 (~20%) | | |
| Potatoes                                             | 378.4 (2013)* | Potato peel and other processing waste (lignocellulose, starch) | 75.3 (~20%) | | |
| Banana                                               | 106.7 (2013)* | Rejected banana (lignocellulose, pectin, starch) | 32.0 (~30%) | | |
| Apple                                                | 81.0 (2013)* | Rejected apples (fucoseglactoxylooligocan, lignocellulose, glucose, fructose) | 24.3 (~30%) | | |
| Raw sugar                                             | 178.9 (2013)* | Molasses (sucrose, glucose and fructose) | 35.8 (~20%) | | |
| Beer (barley)                                        | 189.1 (2013)* | Brewery waste (carbohydrate, protein, organic acids, high COD) | 851.0 (~450%, wastewater) | | |
| Wine                                                 | 27.4 (2013)* | Brewery waste (carbohydrate, organic acids, high COD) | 164.4 (~600%, wastewater) | | |
| Cheese                                               | 21.3 (2013)* | Whey (lactose and protein) | 191.7 (~900%) | | |
| Beef                                                 | 59.7 (2014)* | Slaughter waste (animal fat, protein) | 29.8 (~50%) | | |
| Pork (2014)                                          | 110.5 (2014)* | Animal fats, protein | 29.8 (~20%) | | |

*a* Data from Food and Agricultural Organisation, www.fao.org.  
*b* Data from the US Department of Agriculture, www.usda.gov.  
*c* Estimated data based on the approximate waste (by-production)/product ratio in literature

Figure 1 outlines possible metabolic pathways in fermentative H₂ production using Clostridium sp. [14]. Anaerobic bacteria break down organic substrates via enzymatic oxidation to generate building blocks and produce metabolic energy for growth. H₂ synthesis is mainly driven by the anaerobic metabolism of pyruvate synthesized during glycolysis. Pyruvate is oxidized to acetyl-coenzyme A (CoA). Oxidation of acetyl-CoA leads to the formation of ATP and H₂ via either formate synthesis or ferrodoxin oxidoreductase [Fd] pathways. Both enteric bacteria and strict anaerobes can produce acetyl-CoA and formate from pyruvate by pyruvate-formate lyase. Formate may be further oxidized by enteric bacteria to H₂ and CO₂. It is unknown whether or not degradation of formate is
complete for stoichiometric H₂ production. So far, a H₂ yield lower than 2 mol/mol glucose has been reported in most studies. The low H₂ yield continues to be the major obstacle to the commercialization of the H₂ technology. From the evolutionary perspective, the low yield is due to the fact that microorganisms capable of producing H₂ have developed their metabolic pathways preferentially for cell growth rather than H₂ synthesis. In all feasible bioprocesses exploited by known microorganisms, H₂ is produced in combination with VFAs and/or alcohols. Therefore, the maximum theoretical yield of 12 mol H₂/mol glucose from the complete conversion of glucose to H₂ and CO₂ is never attained in any known in vivo biosystem. It can be hypothesised that H₂ yield can be improved by eliminating the formation of some of these reduced products (VFAs and alcohols) through redirection of the metabolic pathways towards H₂ synthesis. There is a need, however, for an extensive analysis and detailed understanding of metabolic fluxes and their regulatory circuits leading to H₂ formation. Research is needed to develop a metabolic engineering systematic research approach to address the bottleneck issues, including: firstly, improvement of yield and productivity through an understanding of overall cellular physiology; secondly, extension of the substrate range; and thirdly, deletion or reduction of by-product formation.

Hydrolysis of carbon sources is also important for H₂ production. For example, Fats and lipids in dairy waste and wastewater are not bio-accessible carbon and nutrient sources for H₂ producing microorganisms [14,15]. Hydrolysis of dairy waste materials is able to convert lactose to galactose and glucose from fats and lipids, improving substrate availability and suppression of methanogenic activity [15,16]. In addition, hydrolysis of cheese whey by hydrodynamic cavitation under alkaline condition inhibited the lactic acid bacteria and thus improved H₂ production yield [17].

**Hythane**

Hythane refers to the mixture of biogases, containing and methane and 10–25% H₂ by volume. Hythane has been recognised as a cost-effective biogas energy produced through an AD process using FPW. The main advantage of the bio-hythane process is the low cost and high energy yield. The key bioengineering strategy for hythane production is to control the acidogenesis, acetogenesis and methanogenesis stages to reach a hydrogen/methane ratio suitable for hythane [16,18**].

Biohythane is commonly produced from two-stage processes [19]. In the first stage, operation parameters (pH of 5.5–6.5, thermophilic conditions) favouring the growth of hydrogenogenic bacteria, but inhibiting methanogenic bacteria need to be well controlled, while higher pH (7.0–7.5) and mesophilic condition are used in the second methanogenic stage [18**]. There are a few of update successful studies on the two-stage processes for bio-hythane production using palm oil effluent [20], fruit vegetable waste [21], and starch wastewater [22*].

**Liquid biofuels**

Liquid biofuels are alternative energy sources to replace conventional liquid fuels (diesel and petrol). Liquid biofuels are made from biomass and have qualities that are similar to gasoline, diesel or other petroleum-derived fuels. The advantage of biofuels is that they can substantially reduce greenhouse gas emissions in the transport sector (i.e. between 70% and 90% compared to gasoline) with only modest changes to vehicle technology and the existing fuel distribution infrastructure. Liquid biofuels includes ‘First-Generation’ and ‘Second-Generation’ biofuels [6,23]. The first-generation liquid biofuels are the type of liquid fuels generally produced from sugars and grains or seeds, and requires a relatively simple process. The most well-known first-generation biofuel is ethanol made by fermenting sugar extracted from crop plants and starches. The second-generation liquid biofuels are generally produced by biological or thermochemical processing from lignocellulosic biomass, which are either non-edible residues of food crop production or non-edible whole plant biomass (e.g. grasses or trees specifically grown for production of energy). The main advantage of the production of second-generation biofuels from non-edible feed-stocks is that it limits the direct food versus fuel competition associated with first generation biofuels. Compared to gaseous fuels, lipid fuels are easy and safe to store and transport, and have high heating values per volume. Liquid biofuels attract more interest from end-users and play more important role in replacing fossil
fuels. Ethanol, butanol and biodiesel are the three most studied liquid biofuels. Table 2 summarises some recent achievements on liquid biofuel production using FPW.

### Ethanol

Commercial bioethanol can be produced from many types of agricultural feedstock, including sugarcane, corn, wheat, potatoes, sorghum and cassava. Ethanol production from sugar crops is based on the fermentation of sucrose, followed by distillation to fuel-grade ethanol. Production from sugarcane is particularly easy and efficient because a considerable amount of sucrose is readily available, and crushed stalk (bagasse) can be used to provide heat and power to the process, as well as to other energy uses. If starchy crops are used as the feedstock, an additional hydrolysis is needed to convert starch into sugar, followed by fermentation and distillation. The low efficiency of the starch conversion can be improved (and the costs lowered) using enzymatic hydrolysis and valorising co-products. Bioethanol production is a rather energy-intensive process. Thus, the economic and environmental benefits are sensitive to the technology process, feedstock and co-product prices. Ethanol production catalyzed by yeast is one of the most well-known bioprocesses. Although ethanol has lower energy intensity than methane and H₂, its liquid form and much higher production rate make it attractive as a transport fuel. The United States is the largest fuel ethanol producer and its fuel ethanol production has increased from 3.4 billion gallons in 2005 to ~14.3 billion gallons in 2014 [33].

FPW are important carbon source for fuel ethanol production. Using starch-rich wastes as substrates, ethanol production by *Saccharomyces cerevisiae* can be conducted in either simultaneous saccharification and fermentation (SSF) or separated hydrolysis and fermentation (SHF) process [34]. Enzymes such as α-amylase and glucoamylase are used to accelerate the starch hydrolysis for glucose production. Lactose-rich dairy waste is another well studied carbon source for ethanol production. Yeast strains of *Kluyveromyces* sp. and recombinant *S. cerevisiae* are the mostly studied microorganisms for conversion of lactose to ethanol [35-36]. These strains are able to generate lactose hydrolysis enzymes. Upstream separation techniques such as nanofiltration and electrodialysis were able to enhance ethanol production from delactosed whey permeate [37]. However, these desalination steps will add extra operational cost to ethanol production. The use of fruit juice processing wastes to produce ethanol was also reported. However, ethanol yield was low due to the low concentration of sugars [38-39].

In order to improve ethanol concentration at high substrate loadings, downstream process for ethanol recovery was found capable of reducing microbial inhibition by ethanol. A vacuum recovery technology was coupled with ethanol production using mixed food wastes (mashed

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**Table 2**

| Biofuel/fuel precursor | Food processing waste Type | Carbon source (dry mass) | Pretreatment | Oleaginous microorganism | Microbial oil (Con, g/L) | Microbial oil (Prod, g/L/day) | Refs. |
|------------------------|----------------------------|--------------------------|--------------|--------------------------|--------------------------|-------------------------------|-------|
| Bioethanol             | Instant noodle waste       | ~167 g/L starch          | Oil removal and enzymatic hydrolysis | *Saccharomyces cerevisiae* | 61.1                      | 40.8                          | [23]  |
|                        | Potato peel                | 6% potato peel           | Enzymatic hydrolysis               | *S. cerevisiae*           | 19.6                      | 47                           | [24]  |
|                        | Cheese whey powder         | ~150 g/L lactose         | Dilution                              | Recombinant *S. cerevisiae* | 63.3                      | 12.66                         | [25]  |
|                        | Whey permeate              | 50–108 g/L lactose       | –                                       | *Kluyveromyces marxianus* | >90% (yield)             | –                            | [26]  |
| Biobutanol             | Potato starch              | 60 g/L starch            | –                                       | *Clostridium acetobutylicum* | 9.9                       | 0.14                          | [27]  |
|                        | Inedible dough, bread       | ~50 g/L starch           | –                                       | *C. beijerinckii*          | 9.26–10.47               | 0.19–0.22                     | [28]  |
|                        | and batter liquid          |                          | –                                       | *C. acetobutylicum*        | 7.25                      | 0.17                          | [29]  |
| Microbial oils         | Deproteinised whey permeate| 16.0% lactose            | Enzymatic hydrolysis                  | *Mortierella isabellina*  | 17.13                    | 4.6                           | [30]  |
|                        |                           |                          |                                        |                          |                          |                               |       |
|                        | Cheese whey                | 51.1 g/L COD             | No lactase                            | *C. curvatus*             | 3.65                     | 0.6                           | [17]  |
|                        |                           |                          | Hydrodynamic cavitation Deproteinization | *Yarrowia lipolytica*    | 4.29                     | 0.86                          | [31]  |
|                        | Cheese whey                | 43.9 g/L sugar and 3.1 g/L protein | –                                       | *Aspergillus niger*       | 3.5                      | 0.7                           | [32]  |
|                        | Potato processing wastewater| 23.4 g/L soluble starch | Dilution                              | –                        | –                        | –                             |       |
potatoes, sweet corn and white bread) as substrate at high solid content (35%, w/w), leading to a high ethanol concentration of 144 g/L [34,40]. Membrane distillation technology was used to produce about 120 g/L ethanol from 200 g/L lactose by yeast [41]. However, low ethanol concentration is a major concern in many cases using FPW as sole carbon source due to its low carbohydrate constants.

**Butanol**

Biobutanol or biobased butanol fuel is second generation alcoholic fuel with a higher energy density and lower volatility vs. ethanol. Butanol is an alcohol that can be used as a transport fuel. It is a higher member of the series of straight chain alcohols with each molecule of butanol (C₄H₁₀O) containing four carbon atoms rather than two as in ethanol. There is now increasing interest in use of biobutanol as a transport fuel. n-Butanol is an advanced biofuel because of its higher energy density, lower volatility and hydroscopicity, and less corrosive to existing infrastructure than ethanol [42]. Unlike ethanol, n-butanol can be mixed with gasoline up to 40% as diesel [43]. Biobutanol can be produced from cereal crops, sugar cane and sugar beet, etc., but can also be produced from cellulosic raw materials. Butanol is conventionally produced in an acetone–butanol–ethanol (ABE) process by solventogenic *Clostridia* spp. This is an anaerobic process to convert carbohydrates into acetone, butanol and ethanol. The ABE process using glucose as substrate can be carried out by acidogenesis stage converting glucose to acetic and butyric acids during exponential bacterial growth phase, and followed by the solventogenesis stage converting acetic and butyric acids to ABE during stationary growth phase [43]. The general metabolic pathways for ABE production are shown in Figure 2. The mass ratio of the n-butanol to the total three solvents in a ABE process is ~60–70%. However, cost issues, the relatively low-yield and sluggish fermentations, as well as problems caused by end product inhibition and phage infections, meant that ABE butanol could not compete on a commercial scale with butanol produced synthetically and almost all ABE production ceased as the petrochemical industry evolved.

The use of FPW for n-butanol production is a promising low-cost option for commercial process. The ABE processes generally result in producing n-butanol with a concentration less than 15 g/L. This is because the toxicity of n-butanol to microbial cells impairs the construction of cell membranes and interferes with normal physiological functions [44,45]. In order to improve n-butanol yield, genetic and metabolic engineering approaches have been used to modify *Clostridia* spp. [46,47] and to construct *Escherichia coli* and *S. cerevisiae* strains [48,49,50]. Reducing the metabolic reactions for producing acetone and ethanol, boosting the enzymatic activity for butanol synthesis and increasing the bacterial resistance to butanol are the primary engineering strategy. Construction of synthetic ABE pathways in *S. cerevisiae* is of particular interest because yeast is well-known to tolerate high concentrations of ethanol. A latest study shows that deletion of the major yeast alcohol dehydrogenase gene *ADH1* in *S. cerevisiae* increased butanol production from both endogenous and exogenous pathways [50].

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**Figure 2**

Simplified metabolic pathways for ABE synthesis by *C. acetobutylicum.*

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Conventional butanol recovery by distillation is an energy-intensive and not economical process, due to the low butanol concentration, yield and productivity. A latest review paper summarised other advanced recovery technologies for biobutanol recovery including gas stripping, liquid–liquid extraction, adsorption, and membrane-based technologies [51**]. Extractive fermentation of Clostridium acetobutylicum in a fed-batch process produced 24.8 g biobutanol with the consumption of 105 g glucose in 60 h [52]. The use of a novel in situ gas stripping-pervaporation process integrated with ABE fermentation led to a biobutanol concentration of 75.5 g/L [53*].

Biodiesel from microbial oils

Biodiesel is a renewable and clean-burning diesel replacement. Biodiesel is one of several alternative fuels designed to extend the usefulness of petroleum, and the longevity and cleanliness of diesel engines. The main benefit of biodiesel is that it can be described as ‘carbon neutral’. This means that the fuel produces no net output of carbon in the form of carbon dioxide. Biodiesel is typically made by chemically reacting lipids (e.g., vegetable oil, soybean oil, animal fat) with an alcohol producing fatty acid esters. The largest possible source of suitable oil comes from oil crops such as rapeseed, palm or soybean. Biodiesel has many environmentally beneficial properties. Global biodiesel market is estimated to reach 37 billion gallons by 2016 [54]. Almost all biodiesel is produced using base catalyzed transesterification as it is the most economical process requiring only low temperatures and pressures and producing a 98% conversion yield [54]. The Transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. A triglyceride has a glycerine molecule as its base with three long chain fatty acids attached. The nature of the fatty acids can in turn affect the characteristics of the biodiesel. During the esterification process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the biodiesel and crude glycerol.

Microbial oil accumulation occurs under nutrient, especially nitrogen limiting conditions [56,57**]. Oleaginous bacteria for oil production is generally less sensitive to pH and temperature [57**]. The lipid concentration and productivity vary significantly depending on the substrates, microorganisms, nutrients, and hydrolysis and cultivation methods. Lactose-rich dairy has been extensively studied for microbial lipid production using different microorganisms. Deproteinisation is generally required to improve lipid accumulation while hydrolysis of lactose by lactase sometimes is to improve the fermentation efficiency (Table 2). Microbial lipid production by fungi using starch-rich materials can be performed in SSF process as fungi can produce starch hydrolysis enzymes. However, lipid yield and productivity are still too low for its commercialisation.

Challenges and prospects

1. The study of H₂ production is a long-term research approach to the knowledge advance and technology development for producing H₂ via bioprocesses. Detailed understanding of the biochemical interactions of H₂ producing microorganisms has yet to be realised because of the complexity of the metabolic network, enzymatic interactions and growth environments. Innovative research will add to the knowledge of bioprocess engineering science, novel biotechnology and new research methodology. Further studies need to focus on understanding and modelling of the metabolic flux network as which has a significant impact on enzymatic metabolism and H₂ synthesis, consequently on H₂ productivity and yield. The research outcomes are expected to update fundamental knowledge in terms of metabolic pathways and functions of multiple enzyme interactions within the biochemical network. Metabolic flux analysis and metabolic engineering modelling can be used to improve fundamental insight into the metabolism regulation underlying this network in a H₂ production system. The development and utilization of these powerful bioengineering tools will establish a comprehensive network involving metabolism and process parameters to manage the complexity embedded in cellular metabolism, to explore the impacts of bioprocess conditions on cellular responses, and to deal with the uncertainty of the systematic parameters involved in H₂ production. These research outcomes will benefit the development of a cost-effective bioprocess for H₂ production from waste streams.

2. Biohydrogen production is a practically feasible technology since methane production offsets the H₂ production cost with the full utilization of carbon source. Since H₂ and methane production microorganisms have different optimal cultivation conditions, balance of the microbial communities for a single-stage process and process control for a two-stage are crucial. For an AD process, mixing FPW with other organic
wastes such as agricultural residues (lignocellulosic biomass) and municipal wastes, livestock residues (e.g., manure) can provide extra carbon and nutrient sources, and adjust nutrient ratio, leading to increased product yield. Continuing efforts on process and bioreactor optimization could lead to further progress in the biofuel production.

3. Bioethanol production is a well-established technology. Carbohydrate-rich FPW such as starch-rich and sugar-rich wastes is more suitable for ethanol production because of the high productivity. The development of low cost ethanol recovery technology can improve process economics due to the low ethanol concentration. Coupling bioethanol fermentation with recovery technologies such as membrane pervaporation and gas stripping may lead to the cost reduction.

4. The low product concentration and production rate of butanol and microbial oils are the major hurdles for commercialisation of biobutanol production. Strain improvement through genetic and metabolic engineering techniques to improve the strain’s bioaccessibility to solvents and to strengthen n-butanol production pathway is a promising approach to enhance butanol yield. Coupling butanol recovery technologies such as liquid–liquid extraction and gas-stripping with fermentation is very important engineering process to enhance butanol production. Increasing studies have shown that oleygynous yeast and fungi are promising oil producers for oil production. Strain selection and process control strategies such as adjustment ratios of C/N and C/P, and supplement of exogenous carbon source are the key factors to improve oil yields. Biorefinery of microbial biomass to value-added products will also improve the process economics for biodiesel production.

5. Metabolic engineering is greatly dependent on the significant bioengineering advances in its contiguous bioenergy fields. DNA sequencing and bioinformatics analysis reveal new metabolic pathways and enzyme variants. Advanced analytical tools identify pathway bottlenecks from RNA to metabolite levels, and therefore provides foundation for rational bioenergy engineering. The availability of a series of genetic tools facilitates metabolic pathway optimization. All these research outcomes could advance bioengineering technologies which enable scale up for industrial scale production. The powerful metabolic engineering efforts with the goal of large scale manufacture. The metabolic engineering strategies enable efficient biofuel production platforms, including the evaluation of thermodynamic feasibility of pathways, carbon flux redirection, manipulation of cellular energetics, use of waste carbon sources, engineering substrate uptake mechanism, removal of final products from culture broth to alleviate end-product inhibition and cell toxicity, optimization of process parameters, etc.

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