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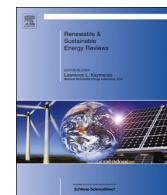
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New biorefineries and sustainable agriculture: Increased food, biofuels, and ecosystem security

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ABSTRACT

The sustainability revolution is the defining challenge of our time to meet increasing needs in the energy–food–water nexus without compromising the ability of next generations. To feed the world, modern agriculture is primarily based on annual grain crops that replace native perennial plant communities on most of the arable land on the planet. This practice may not be sustainable due to high inputs of fresh water, fertilizers, and herbicides; soil erosion; and runoff water pollution. Recent biotechnology breakthroughs enable the fractionation of nonfood lignocellulosic biomass to multiple components, the conversion of nonfood cellulose to starch without sugar loss, the production of in vitro meat without slaughtering livestock, and the production of healthy oil from microbes, suggesting great opportunities of new biorefineries based on nonfood biomass. Perennial plant communities have higher biomass yield per hectare, have easily resource management, store more carbon, maintain better water quality, utilize nutrients more efficiently, tolerate more extreme weather events, and resist pests better than annual crops. Sustainable agriculture based on annual grains and perennial high-biomass yield plants along with new biorefineries could produce a myriad of products from biofuels (e.g., butanol and hydrogen), biomaterials, to food/feed. Sustainable agriculture and new biorefineries could be cornerstones of the coming sustainability revolution based on the most abundant renewable bioresource—biomass.

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Abbreviations: BG, beta-glucosidase; BTW, biomass-to-wheel; CBH, cellobiohydrolase; CBP, cellobiose phosphorylase; DHA, docosahexaenoic acid; Endo, endoglucanase; EPA, eicosapentaenoic acid; FCV, fuel cell vehicle; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; HEV, hybrid electric vehicle; ICE, internal combustion engine; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NPP, net primary production; PEMFC, proton membrane exchange fuel cell; PGP, potato alpha-glucan phosphorylase; SFCV, sugar fuel cell vehicle

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1. Introduction

Humanity is at the beginning of its third major cultural and economic revolution – the sustainability revolution – which will be largely completed in the 21st century [1]. The agricultural revolution, beginning approximately 10,000 years ago, allowed humanity to switch from hunting and gathering to cultivating crops and domesticating animals [2,3]. The industrial revolution in the 18th century began with the efficient utilization of carbon-rich fossil fuels, which were accumulated through millions of years of deposition, and the operation of machinery in place of human and animal labor [2,3].

Similar to the previous two revolutions, the sustainability revolution will lead to transformative changes in daily lives and most aspects of societal organizations. In this revolution, the basic needs of energy and food, as well as environment are inextricably linked, and action in one area has impacts in one or both of the other areas [4]. The sustainability revolution relies heavily on renewable biomass resource that offers an avenue toward food security, a more “green” economy, and even energy independence because biomass is the most abundant renewable bio-resource, approximately five times the world’s energy consumption [4]. In addition, plant biomass is more evenly distributed than fossil fuel resources so to decrease wealth transfer among energy-exporting and energy-importing countries [5].

Farming for food production is the basis of our civilization [6,7]. Henry Kissinger said “Control oil and you control nations; control food and you control the people.” To feed more than seven billion people on Earth, approximately one-third of the world’s arable land is under cultivation, and 70% of the world’s fresh water withdrawals are used for agriculture [4,7,8]. More than three-fourths of the global cropland is sown annually for monoculture grain crops, food legumes, and oilseeds (Fig. 1A), providing calories and nutrients in the human diet, although food biomass accounts for a very small fraction (i.e., ~2%) of the annual net primary production (NPP) [4]. Further aggressive expansion of agricultural lands from forests and grasslands, where perennial plants make up most of the world’s natural terrestrial biomes, is nearly impossible and will impair biodiversity and release a large amount of new CO₂ emissions [9,10]. Modern agriculture is believed to be the largest environmental destroyer to biodiversity in terms of human activities [11].

The irreplaceable role of plant biomass in meeting human needs for food, biofuels, and biobased materials raises serious questions: Is it possible to provide enough food, biofuels, and materials while minimizing the environmental footprint and conserving biodiversity in the sustainability revolution. And how? In this perspective review, we briefly review the histories of agriculture and transportation, present our out-of-the-box visions pertaining to intertwined roles between sustainable agriculture and new biorefineries based on new biotechnology breakthroughs related to synthetic food and advanced biofuels production, and highlight key R&D directions for the coming sustainability revolution.

2. Histories of agriculture and transportation

Because Winton Churchill said “The farther backward you can look, the farther forward you are likely to see,” it is important to briefly review the history of agriculture and transportation as well as present their inherent problems, before out-of-the-box solutions are discussed. Because both food and transportation biofuels have to be produced from renewable biomass resource, it often raises a debate – food versus biofuels. Therefore, the sustainable agriculture whose primary goal is to produce food/feed and biomass is closely related to new roles of biorefineries that may produce a myriad of products.

2.1. Agriculture and its inherent problems

Food is basic to life and survival, to health and fitness for facing challenges, and to the proliferation of and caring for the young [2,7]. The Neolithic agricultural revolution led to one of the most profound changes in the human history, providing a more secure and bountiful way of producing food compared to hunter-gathers [2,7]. As a result, both the world population and the carrying capacity of the land have increased by three orders of magnitude [7]. Higher populations and settlement in villages, towns, and cities enabled work specialization, accelerated the rate of innovation, and increased sociopolitical organization [2].

Because human cannot digest the most abundant cellulose-rich plants, they have to eat starch-rich grain seeds. Agricultural systems began with annual crops that are grown from seeds every year and are harvested for their seeds. The high yields of annual crops are the result of long-term, intense artificial selection for increased allocation of photosynthesis to the seed and decreased intraspecific competition. To maintain monoculture crops and prevent competition from weeds, the annual tilling of the soil is essential. Tillage can be accomplished by labor-intensive hand management on small plots, by farming machinery, by annual flooding, or by controlled watering [7]. Annual cereal crops require churning of the soil, precisely timed inputs and management, and favorable weather (e.g., rainfall, temperature and sun shine) at the correct time. Thanks to shorter growing seasons, annual crops from seeds develop very small and shallow root systems, providing less protection against soil erosion, wasting water and nutrients, storing less carbon below the ground, and tolerating pests less well than native perennial plant communities [11].

The green revolution is an outcome of the extensive use of four technologies: fertilizers, improved hybrid crop plants, mechanization, and irrigation, resulting in continuous increases in the productivity of the farm land in the 20th century [7]. For example, the invention of ammonia synthesis by Fritz Haber, Nobel Chemistry Prize Laureate (1918), and its innovation (that is, the transformation of invention to product) directed by Carl Borch, Nobel Chemistry Prize Laureate (1931), enabled the availability of inexpensive synthetic nitrogen fertilizers. (Note: the ammonia synthesis from purified hydrogen and nitrogen is the most energy efficient way to fix nitrogen compared to other nitrogen-fixing techniques [7]). The key invention of Norman Borlaug, Nobel Peace Prize Laureate (1970), was the

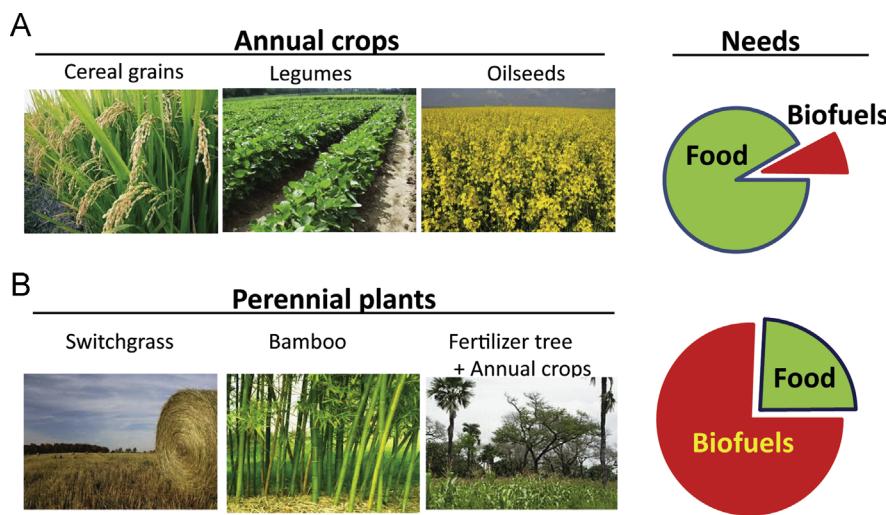


Fig. 1. Current agricultural systems (A) for the production of a variety of food sources based mainly on annual crops (e.g., cereal grains, legumes, and oilseeds), along with perennial pasture, fruit trees, and fishing, as well as for the co-production of biofuels; future perennial plant-based sustainable agriculture (B), which will produce biofuels as a major product and some supplementary food.

Table 1
Comparison of typical yields of annual grain crops.

Annual crops	Annual productivity (tonne/ha/y)					
	Global Average	Ref.	Advanced average	Ref.	Highest record	Ref.
Wheat (C3)	3.1	[175]	8.9 (Dutch) 9.9 (Ireland)	[175]	15.6 (New Zealand)	[175]
Rice (C3)	4.3	[175]	6.95 (China) 9.6 (Egypt) 10.8 (Australia)	[175]	19 (China)	[176]
Maize (C4)	5.0	[175]	11 (Iowa) 9.2 (USA)	[175]	33.8 (Israel)	[175]
Wheat/maize			11.5 (5.9, wheat; 5.6 corn) (Henan, China)	[177]	23.0 (Henan, China)	[177]

development of high-yield and disease-resistant wheat, corn, and rice. Generally speaking, the food price index declined at an average annual rate of 1% in the 20th century [12]. The green revolution led to grain surpluses and lower prices and created a sense of complacency about agriculture and hunger.

However, extraordinarily productive modern agriculture is also ecologically destructive [6]. Agriculture has transformed a large portion of the Earth, as many forests have been cut down and wetlands have been drained to create more farmlands, far more than mining. This land use change has inevitably pushed the native species of animals and plants out, some even to extinction. Some critics say that modern agriculture cannot be sustained because it uses too much fossil fuels and creates numerous environmental problems, such as changes in ecosystems, non-point polluted water runoff, global warming, and decreased biodiversity [13–15].

In this century, both increasing population and food consumption per person are placing unprecedented pressures on modern agriculture and natural resources. Today, approximately one billion people are chronically malnourished while our agricultural systems are degrading land, water, biodiversity and climate on a global scale [16]. To meet the world's future food security and increasing needs for food, biofuels, and materials, the production of utilizable biomass must grow substantially, while the environmental footprint of agriculture must shrink dramatically [4]. A group of scientists has proposed a combined solution, including closing yield gaps on underperforming lands, increasing cropping efficiency, shifting diets, and reducing food waste [16]. Due to the green revolution, the global yields of crops have increased by

approximately three times over the last several decades [17] to 3.1, 4.3 and 5.0 t per ha for wheat, rice, and maize, respectively (Table 1). Because these average values are much lower than the values for advanced agricultural countries and the highest records (Table 1), closing yield gaps may be a major contributor to feeding the world. However, a 2013 analysis suggests that previous projections based on enhanced crop yields are often more optimistic, and there is evidence of yield plateaus or abrupt decreases in grain yields, including rice in eastern Asia and wheat in northwest Europe [18].

2.2. Agriculture in China

Understanding the history and status quo of Chinese agricultural efforts may be informative to crop yield improvement potentials in other countries because China's arable land represents 7% of the total arable land in the world but supports 21% of the world's population. China's limited arable lands have been a chronic problem throughout its 5000-year history, leading to frequent food shortages. The primary aim of the government of China is to ensure current and future food security [19]. It is estimated that total domestic grain demand could increase from 575 million tonnes (Mt) in 2010 to 648 Mt in 2020 and to 700 Mt in 2050 due to population growth and increased grain consumption per person, from 420 to 450 kg per person in 2020 and to 500 kg per person in 2050 [20].

Henan is one of the most important agricultural provinces in China because it not only produces enough food to feed nearly 100

million residents but also exports excess food to other provinces. Henan is considered the cradle of Chinese civilization due to the large number of dynasties that began there, including the first two dynasties, the Xia Dynasty (~2000–1500 BC) and the Shang Dynasty (~1500 BC–1046 BC). Therefore, a saying is “a half of the Chinese history is related to Henan; China's food security relies on Henan.” Before 1984, Henan province failed to produce enough food and had to import food from other provinces. Similar to the green revolution happening around the world, the breakthroughs that made it possible for Henan to switch from a food-importing to food-exporting province were widely used fertilizers, high-yield seeds, and new cropping methods. For example, thanks to its unique climate conditions, Henan province developed the wheat/maize double cropping per year technology. Wheat is a cold-adaptable crop with a growing season that begins with planting in October and ends with harvest in early June. Maize is cold-intolerant, so it must be planted in the spring or early summer. Instead of simple double cropping, relay cropping is used, where maize is started amidst the wheat before it has been harvested to extend the growing season of maize and to increase overall yields. The double and relay cropping allows the capture of up to 66% of the traditional growing season, especially in cool seasons, and increases overall crop yields. Although the primary goal of multiple cropping in other countries may maintain nutrient levels in the soil, such as the intercropping of soybean with maize/wheat cropping, the main goal of double cropping in Henan is to increase grain yields to meet national food security needs. For example, Tian-Cai Guo and his team at the Henan Agricultural University achieved the total productivity of more than 23.0 t of combined maize and wheat per hectare per year on a large scale (*Table 1*).

Approximately 40–50% of the increases in productivity of grains in China in the past 30 years are attributed to the increased use of fertilizers [21]. China is the largest ammonia producer in the world, accounting for approximately a third of the world's ammonia production [22]. A large amount of fertilizers, particularly nitrogen-containing that have to be applied to support high productivity of wheat and maize, are in the range of 527–633 kg/ha [21]. The ratio of grain output to overall energy inputs, including fertilizers, pesticides, fuel, electricity, labor and seeds, is approximately 0.98 [23], suggesting that such high-productivity agriculture may be regarded as the conversion of fossil fuels to edible food without a significant energy gain from solar energy via photosynthesis. In addition, most fertilizers (e.g., ~70%) cannot be utilized by cultivated crops and their runoff causes severe water pollution. Five of China's largest fresh water lakes have substantial dead zones caused by fertilizer runoff [24].

Seemingly successful agricultural efforts in China may be not sustainable due to (i) shrinking farmland due to industrialization and urbanization [19]; (ii) deteriorated farmland due to soil erosion, acidification and pollution [25]; (iii) tightened fresh water supplies [7]; (iv) increasing fertilizer and herbicide inputs; and (v) severe nonpoint water pollution [20,24]. In addition, China produces synthetic nitrogen fertilizers based on dirty and cheap coal. Although the food self-sufficiency rate of China was claimed to be approximately 91% in 2010, it could decrease to 87% and 82% in 2020 and 2030, respectively [26]. Indeed, the current overall food self-sufficiency rate of China may be below 70% when large imports of soybeans (i.e., more than 60 Mt) are taken into account.

Considering relatively high crop yields achieved in China (*Table 1*), it is really challenging to further increase their yields greatly [18]. Will there be out-of-the-box solutions to feed China in the future? Could such solutions be applicable to other countries? (Note: in the beginning of 2015, the Chinese government promotes the use of potato from a vegetable to a new staple crop in addition to rice, wheat, and corn. Planting potato brings multiple benefits: decreasing agricultural water withdrawal greatly compared to other annual crops; growing well on relatively large cold areas; great potentials in potato productivity from

current levels below the world's average; providing high-quality starch and nutrients (e.g. Vc, potassium) as key food diet components.)

2.3. Transportation megatrend

Transportation reflects the level of civilization by linking people from widespread lands together socially and politically [27,28]. It also creates larger markets, making available a greater diversity of goods and services for consumers, and increases the diversity of employment and specialization [2]. A traveling vehicle needs to carry its own fuel as an energy source and an engine that converts stored energy to kinetic energy on wheels [29].

Land vehicles constitute the largest type of transportation energy consumption [1,28]. Prior to the industrial revolution, humanity relied heavily on animal force, but this is costly and has very low energy efficiencies from biomass to kinetic energy [27] (*Fig. 2*). Developing self-propelled vehicles was a long-term goal with some special requirements, including a high energy storage capacity in a small container, a high power/weight ratio engine, affordable fuel, an affordable vehicle, low cost for building the relevant infrastructure, fast charging or refilling of the fuel, and high safety [28–30]. Such strict requirements led to high power/weight internal combustion engines (ICEs) and high-energy density liquid fuels as the dominant transportation approach [28,31]. Karl Benz and Henry Ford are two of the most important inventors [2]. Meanwhile, earlier inventions – the steam cars and electric vehicles invented by Nicolas-Joseph and Thomas Edison, respectively – are largely forgotten due to obvious shortcomings – low energy efficiency, big size, or short driving distance [2,29].

According to the theory of technology inevitability proposed by Kevin Kelly [32], increasing energy utilization efficiency is a megatrend because it decreases the net consumption of primary energy, reduces environmental footprints, and saves money for consumers [2,3,27]. There is no doubt that vehicles equipped with electric motors will replace ICE-powered vehicles because electric motors have very high energy efficiencies plus high power/weight ratios (*Fig. 2*). However, electricity storage remains a large problem because rechargeable batteries have two and one orders of magnitude energy densities less than those of liquid fuels and stored hydrogen, respectively [28]. H₂-fuel cell vehicles (H₂-FCVs) are one of the most investigated powertrain systems by large automobile companies because a high power/weight and high energy efficiency proton membrane exchange fuel cell (PEMFC) stack can generate electricity from stored hydrogen and does not generate pollutants. Currently, HEVs equipped with an ICE and liquid fuel are becoming more and more popular as a transitional solution before large-scale implementation of FCVs [33]. Toyota starts selling up to 1000 FCVs at affordable prices of ~\$50,000–60,000 in 2015, indicating the dawn of the hydrogen age.

3. Intertwined roles of sustainable agriculture and new biorefineries

To increase food, biofuels, and economy security, we would like to suggest the developments of sustainable agriculture based on perennial plants that can produce more biomass per hectare with minimal inputs (*Fig. 1B*) and of new biorefineries (*Fig. 3*) that can produce a number of products from food/feed, biofuels, and biobased materials from nonfood biomass.

3.1. Sustainable agriculture based on dedicated perennial plants

Growing perennial plants other than annual crops has both energy and environmental benefits (*Table 2*). Perennial plants usually have much higher biomass yields due to longer growing seasons (e.g., no germination), more sunlight intercept, and more uptake of water and

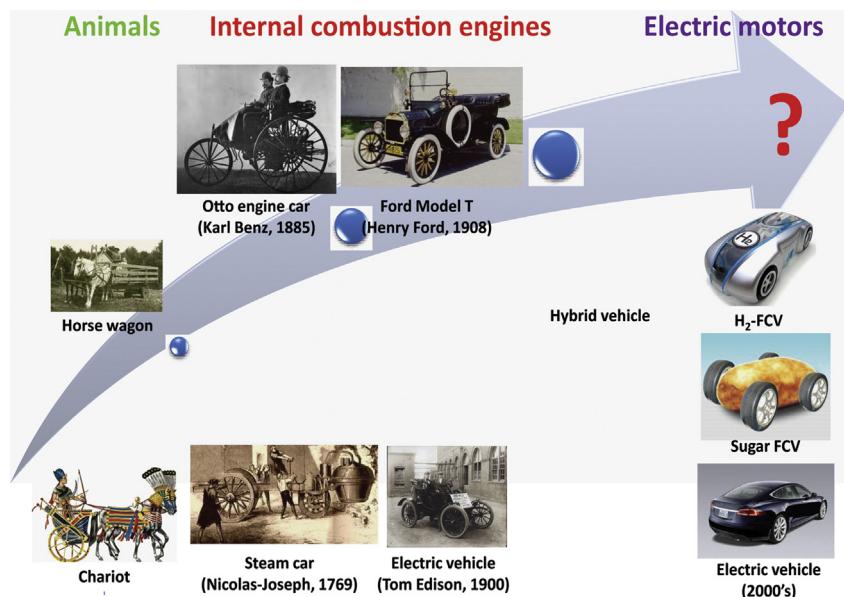


Fig. 2. History of transportation evolution from animal forces to internal combustion engines to future electricity-power systems: battery electric vehicle, H₂ fuel cell vehicle (FCV), or sugar fuel cell vehicle. The steam car invented by Nicolas – Joseph and the electric car invented by Thomas Edison were abandoned due to the better performance of internal combustion engine-powered vehicles.

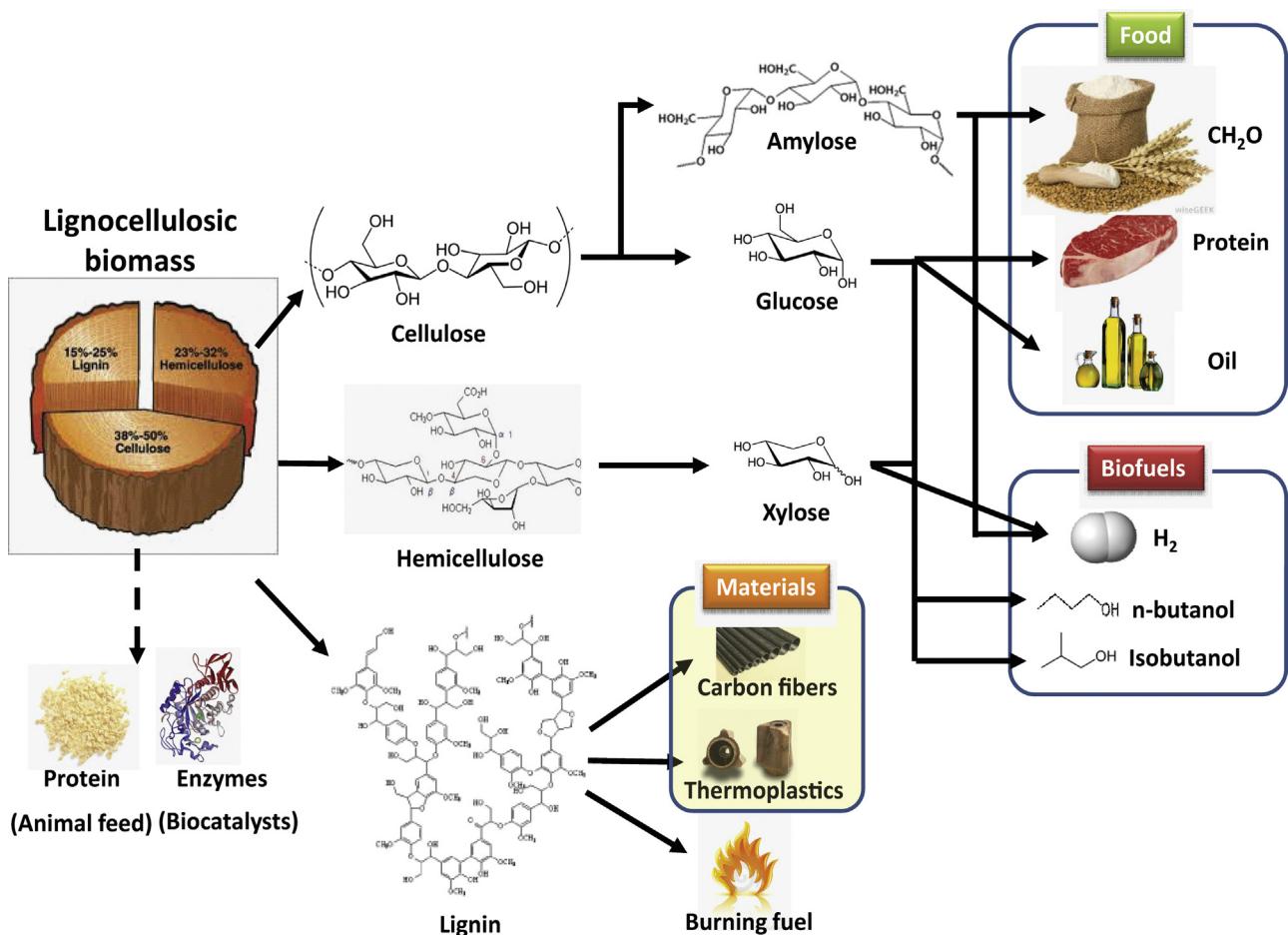


Fig. 3. Scheme of multi-produce biorefineries via lignocellulose fractionation followed by conversions.

nutrients. Perennial plants always have deep and extensive roots systems, which can hold soil to prevent erosion, capture dissolved nitrogen and phosphate before they pollute ground and surface water, and out-compete weeds. For example, even short-root switchgrass

roots have an average length of 3 m [11]. The roots of alfalfa grow rapidly, reaching soil depths of 0.9 m after two months and 1.8 m after five months. In porous soils, root lengths could be as long as 15 m over 20 years. Another drought-resistance fertilizer tree – Acacia

Table 2
Energy and environmental benefits from growing perennial crops versus annual crops.

Benefits	Reasons
Energy benefits (i.e., high ratio of energy output to input)	
High biomass (energy) outputs	Longer growing season More sunlight intercept Uptake of deep soil water and nutrients
Low energy inputs	High nutrient utilization efficiency (i.e., low fertilizer inputs) Nearly no pesticides or herbicides Few seeds needed Few passes of farm machinery Less or almost no irrigation
Environmental benefits	
• Very low soil erosion • Less water consumption • More carbon fixation • Less water pollution • Better biodiversity • Better tolerance of extreme weather and climate change	Deeper root systems Mixed cultures Less seedbed preparation

Table 3
Comparison of different biomass resources and potential bioenergy crops.

Name	Type	Productivity (tonne/ha/y)	Land impact	Typical region	Ref.
Corn stover (stems, leaves and cobs)	Annual agriculture residue	3–7 (collectable) 10 (total)	None, if sufficient stove is left on the field to preserve soil	Midwestern US Henan (China)	[178] [179]
Wheat straw	Annual agriculture residue	5.0		Temperate zone	[178]
Rice straw	Annual agriculture residue	6.7		Tropic, subtropic and temperate regions; Southern China	[178]
Grain sorghum (<i>Sorghum bicolor</i>)	Annual feed grain	7–10		Hot, dry plains	[179]
Energy cane (hybrid of <i>Saccharum officinarum</i> and <i>S. spontaneum</i>)	Perennial grass related to sugarcane	17 (unfertilized) 27–50 (fertilized)	Likely to be grown on land used for pasture, rice, cotton	South-central and southeastern US	[179]
Giant reed (<i>Arundo donax</i>)	Perennial grass	37 (no nitrogen after the first year)	Unknown. Marginal land first. May be an invasive species	Florida, Louisiana	[179]
Common reed (<i>Phragmites australis</i>)	Perennial grass	45–70	Wetland in tropical and temperate regions	Delta regions of rivers	[44]
Napier grass (<i>Pennisetum purpureum</i>)	Perennial bunchgrass	35–45	Likely to be grown on land used for pasture, rice, cotton	Florida and southern region of Texas, Louisiana	[179]
Moso bamboo (<i>Phyllostachys Pubescens</i>)	Perennial evergreen giant grass	~25 Up to 90	Small. Ready plantations	Tropic, subtropic and temperate regions, mountain valleys	[42] [180]
Hybrid poplar (hybrid of <i>Populus</i> genus member)	Fast-growing hardwood	10–25	Small. Ready plantations for paper pulp and lumber	All states in USA, except southern tips of Texas and Florida	[179,181,182]
Switchgrass (<i>Panicum virgatum</i>)	Perennial bunchgrass	8–15	Displace soybeans and wheat and to a lesser extent lay, rice, sorghum, cotton	Midwestern prairie states	[179]
<i>Miscanthus giganteus</i>	Perennial grass	25–60	Displace soybeans and wheat and to a lesser extent lay, rice, sorghum, cotton	Midwestern prairie states	[183,184]
Alfalfa (<i>Medicago sativa</i> L.)	Perennial grass	15–18	None. Established plantations for producing forage crop	Warmer temperature regions	[185]

trees in Africa has a root system to reach up to 80 m below to the surface [34]. In contrast, annual crops such as lowland rice and maize have very shallow root systems of 0.3 and 0.6 m maximum, respectively. Wheat has a slightly longer root system of up to 1.0 m [11]. Root lengths are strongly related to plant tolerance to drought stress and fertilizer utilization efficiency. Some perennial plants (e.g., *Faidherbia albida*, *Tephrosia*) have been co-cultivated with annual grain crops due to their abilities to capture nitrogen from the air and put it into the soil through their roots and falling leaves [35–37]. In addition, deep root systems of perennial plants bring several environmental benefits: low soil erosion, less water consumption, more carbon fixation, less water pollution, and better biodiversity. In a century-long study of factors affecting soil erosion, timothy grass, a perennial hay crop, was approximately 54 times more effective in maintaining topsoil than annual crops [11]. A recent research report clearly

indicates decreased nitrogen losses after the conversion of row crop agriculture to perennial crops [38].

In addition to several well-known bioenergy crops, such as switchgrass, poplar, miscanthus, energy cane, and giant reed [39–41], three promising bioenergy plants – bamboo, Napier grass (elephant grass), and common reed – are highlighted here (Table 3). *Phyllostachys pubescens* (moso bamboo) is a giant woody, tree-like, perennial evergreen grass that grows in a subtropical monsoon climate, although it can withstand temperatures as low as –20 °C [42]. It can be cultivated in marginal lands, such as mountain valleys, mountain foots and gentle slopes. Bamboo productivity is highly dependent on soil, water and climate conditions. The highest average yearly biomass productivity during a ten-year plantation cycle is approximately 25 to up to 90 t of dry culms/ha/y, which can be easily collected [43]. *Phragmites australis* (common reed) is a widespread perennial grass that grows in wetlands

or near inland waterways [44]. Although it is harvested for thatched roofs, ropes, baskets, and pulping feedstock, the common reed is more typically considered an invasive weed due to its vigorous growth and difficulty of eradication. The common reed could be used as a bioenergy crop due to three unique features: (i) high biomass productivity (e.g., ca. 45–71 t/ha/y), (ii) low inputs needed for planting, such as fertilizers and pesticides, and (iii) removal of phosphorus- and nitrogen-containing pollutants in waterways [44]. *Pennisetum purpureum* (Napier grass) is a species of perennial tropical grass native to the African grasslands. It has low water and nutrient requirements and can therefore make use of otherwise uncultivated lands. This wild species has been used primarily for grazing; recently, it has been incorporated into a pest management strategy. Napier grass attracts stemborer moths (a main cause of crop yield loss in Africa) away from maize and is hence the “pull” crop [45]. This strategy is much more sustainable and is more affordable for farmers than insecticide use. In addition, Napier grass improves soil fertility and protects arid land from soil erosion. It is also utilized for firebreaks, windbreaks, in paper pulp production and to produce biomass feedstock.

Multi-cultural perennial plants can provide better wildlife habitat than mono-culture plants [46]. For low-productivity marginal farmland and degraded agricultural land, wildlife-friendly farming based on perennial plants is very likely to be more cost competitive than current farming methods when considering other potential benefits, such as water quality protection and biodiversity conservation. Additionally, restored wetlands on flood-prone farmland can produce extra biomass, increase wildlife abundance, and improve water quality by processes such as denitrification.

3.2. Overview of new biorefineries

New biorefineries based on nonfood biomass could be very complicated to process different biomass feedstocks according to

variations in biomass feedstock and to produce multiple products to meet different needs. It is highly recommended to use available agricultural and forest residues before the large scale cultivation of perennial plants. Approximately three–four billion Mt of non-food agriculture residues, such as rice straw, wheat straw, and corn stover (i.e., stems, leaves, and cobs) are available when annual starch-rich grains (i.e., 2300 Mt per year) are produced [4] (Table 3). Most of these residues are not fully utilized. For example, peasants in China often burn wheat and rice straw, causing serious air pollution, although local governments officially ban this practice. The utilization of agricultural residues in biorefineries could bring several obvious benefits: (i) solving the deposition of agricultural residues, (ii) generating more renewable fuels and chemicals, (iii) decreasing net greenhouse gas emissions, (iv) creating more high-paying manufacturing jobs, and (v) greatly increasing potential food/feed supplies (see Section 3.3). Another indirect benefit is to increase food supplies by decreasing up to 40% of postharvest food waste, especially for developing countries, which lack sufficient crop processing facilities [47–49].

Developing multiple-product biorefineries is similar to the history of the development of modern oil refineries because lignocellulosic biomass is a natural composite containing cellulose, hemicellulose, lignin and other valuable components, such as proteins and heterologous enzymes (Fig. 3). When freshly-harvested biomass feedstocks contain some targeted proteins, such as switchgrass and alfalfa, they could be pre-processed through front-end protein recovery [50,51]. This front-end protein recovery methodology should be carefully chosen and optimized based on the requirements of the targeted proteins; for example, low-quality protein as animal feed additives [51], bulk enzymes used for industrial processes [52], and high-quality proteins for pharmaceuticals and vaccines [53,54]. At the same time, the timing of the biomass harvest will be a trade-off between the maximum protein recovered and biomass yield. Dry lignocellulosic biomass could

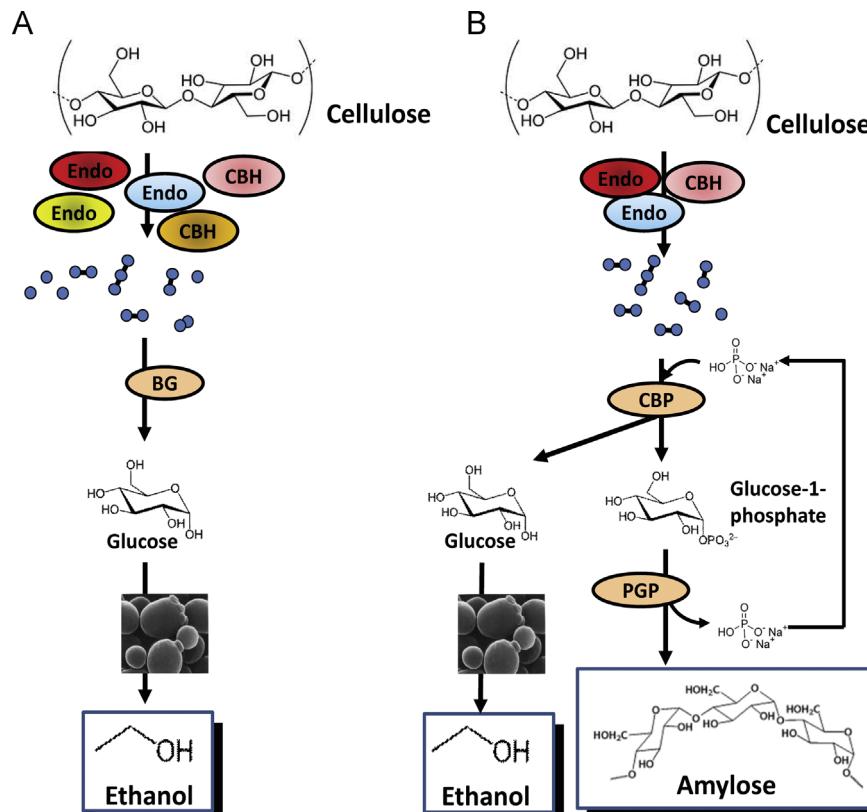


Fig. 4. Schemes of simultaneous saccharification and fermentation (SSF) used in cellulosic ethanol biorefineries (A) and simultaneous enzymatic transformation and fermentation in next generation biorefineries (B). Endo, endoglucanase; CBH, cellobiohydrolase; BG, beta-glucosidase; CBP, cellobiose phosphorylase; and PGP, potato alpha-glucan phosphorylase. Modified from Ref. [59].

be further fractionated into different components – cellulose-rich, hemicellulose- or xylose-rich, and lignin-rich fractions [55–58]. To maximize the economy, different biomass streams could be converted to a myriad of products according to needs, such as food (e.g., starch, microbial oil, in vitro meat), feed (i.e., animal protein additive), bulk enzymes (i.e., cellulase), biofuels (e.g., iso-butanol, hydrogen), biochemicals (e.g., lactate, succinate, synthetic starch), materials (e.g., carbon fibers, thermoplastics), and burning fuel (Fig. 3). The co-production of several relatively high selling-price products (compared to biofuels), such as synthetic functional starch, succinate and thermoplastic polymer replacement, could greatly benefit the economy of the first biorefineries. When the markets of high-selling price products are saturated, more biorefineries could produce two major products: biofuels (e.g., hydrogen) and food/feed simultaneously because of their huge market sizes (e.g., trillions of US dollars for each).

3.3. Synthetic food production in biorefineries

The major nutrients of the human diet are carbohydrates, proteins, and lipids.

3.3.1. Synthetic starch produced from cellulose

Starch is the most important diet component because it provides 50–70% of the calories needed by humans [59]. It is a polysaccharide consisting of a large number of anhydroglucose units joined together primarily by alpha-1,4-glycosidic bonds and alpha-1,6-glycosidic bonds [60]. Natural starch has two forms: linear amylose and branched amylopectin [60]. In contrast, cellulose, a linear glucan linked by beta-1,4-glycosidic bonds, is the supporting material of plant cell walls [61,62]. The annual resource of cellulosic materials is at least 40 times the starch produced by cultivated crops [59]. Humans, unlike cattle, horses, and sheep, cannot utilize cellulose-containing biomass as a food source. Moreover, all cellulose-utilization livestock cannot utilize cellulose feed efficiently, resulting in low feed conversion ratios [63,64].

An in vitro enzymatic pathway has been designed to transform cellulose to synthetic amylose in an aqueous solution (Fig. 4B) [59]. This enzyme cocktail has two modules: (i) partial hydrolysis of cellulose to cellobiose by the optimized mixture of cellobiohydrolase (CBH) and endoglucanase (Endo), and (ii) amylose synthesis by utilizing cellobiose phosphorylase (CBP) and potato alpha-glucan phosphorylase (PGP). In this system, CBP converts cellobiose to glucose 1-phosphate (G-1-P) and

glucose in the presence of phosphate ions; PGP adds one glucose unit from G-1-P at the non-reducing end of amylose, and phosphate is recycled to maintain nearly constant pH and phosphate levels (Fig. 4B). To eliminate glucose inhibition, the ethanol-producing yeast *Saccharomyces cerevisiae* is added to the vessel because the yeast cannot utilize cellobiose and G-1-P [65]. This bioprocessing called simultaneous enzymatic biotransformation and microbial fermentation can transform pretreated biomass to amylose, ethanol and yeast as a single-cell protein in one bioreactor [59]. This process may be regarded as modified simultaneous saccharification and fermentation in second generation cellulosic biorefineries [63] (Fig. 4A), where beta-glucosidase is replaced with the mixture of CBP/PGP. The whole reaction can be conducted without extra energy input or costly coenzyme. Also, all of the glucose units of the cellulosic materials are converted to the desired products – synthetic amylose and ethanol – with no sugar loss.

Synthetic (linear) amylose produced from cellulose is more valuable than (branched) amylopectin and could have a variety of applications, from high-value to low-value products (Fig. 5), as follows. (i) Top-quality synthetic amylose with a well-controlled degree of polymerization can be used as a chromatographic column matrix and drug capsule material in the pharmaceutical industry. (ii) Amylose can be used to make biodegradable plastics, where starch-based plastics account for 50% of the bioplastic market [66]. High-quality amylose is suitable for producing clear, transparent and flexible low-oxygen diffusion plastic sheets and films [67]. (iii) Amylose is an important thickener, water binder, emulsion stabilizer, and gelling agent in the food industry. (iv) Food-grade amylose can be blended with regular cereals and processed to high-amyllose tailored foods for meeting special dietary needs [68,69] because amylose is more slowly digested than amylopectin [69]. These lower glycemic load foods can improve human health and lower the risk of serious non-infectious diseases (e.g., diabetes and obesity) [69,70]. Amylose can be processed to form a resistant starch, which resists digestion and passes through to the large intestine, where it acts as a dietary fiber [71]. Slowly digestible and resistant starch has some healthy benefits, including the prevention and alleviation of metabolic diseases and the prevention of colon cancer [72]. (v) Medium-quality amylose can be used as a high-density hydrogen carrier for the enzymatic production of hydrogen, which could solve the challenges associated with hydrogen production, hydrogen storage, infrastructure, and safety concerns [73–75]. (vi) Low-quality amylose mixed with yeast cells can be used as animal feed for non-ruminant animals, such as pigs and chickens, where yeast cells are a protein source.

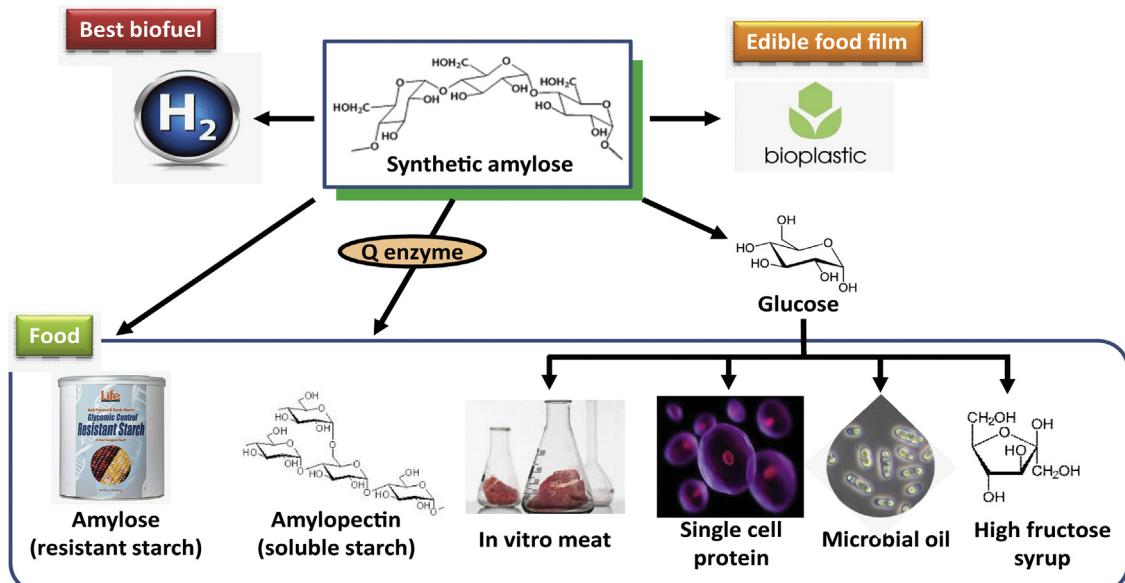


Fig. 5. The central role of synthetic amylose made from cellulose as new food sources (e.g., resistant starch, soluble amylopectin, in vitro meat, single cell protein, microbial oil and high-fructose syrup), biodegradable plastic, and a hydrogen carrier.

Amylose could be converted into other food nutrients. To increase amylose digestion efficiency as food/feed, the addition of the starch-branched enzyme – Q enzyme converts linear amylose to branched amylopectin [76] (Fig. 5). The production of the polysaccharides amylose and amylopectin rather than glucose is essential as key food components because over-consumption of easily utilized soluble sugars (e.g., glucose and fructose) is strongly associated with non-infectious diseases such as diabetes and obesity [72]. Starch may be an important carbon source to produce other food nutrients, such as in vitro meat, single cell proteins, and microbial oil. For example, slowly-utilized starch is a better energy source for cell-free protein synthesis than glucose [77,78].

3.3.2. In vitro meat

Domesticated animals have been raised as a major protein source of diet since the agricultural revolution. The worldwide meat market is approximately 250 billion US dollars per year, including approximately 107 million tons of pork, 57 million tons of beef and veal, and 85 million tons of poultry [79]. However, livestock have very low energy efficiencies from biomass to the desired meat muscle via a food pyramid, consume more fresh water than grain per calorie (i.e., ~10–20,000 kg of fresh water consumed per kg of beef produced vs. ~1000 kg of fresh water per kg of wheat produced [8]), generate a large amount of manure, and release a large quantity of strong greenhouse gasses (e.g., methane) [8,80]. Animals raised in the modern food industry suffer from crowded and unhealthy living conditions. Current meat production system also increases potential risks of infectious diseases transferred from livestock to human beings, such as avian flu, swine flu, and the hepatitis E virus [81]. A vegetarian diet requires only 35% as much water and 40% as much energy as a carnivorous diet [82]. Therefore, a suggested solution to address food security is to change the diet [16].

One of the alternatives for providing proteins is in vitro meat, also known as cultured meat, test tube meat, tube steak, or shmeat, which is lab-created meat grown from a cell culture of animal tissue without raising or slaughtering animals and is different from vegetarian food products produced from vegetable proteins, such as soy and gluten [83,84]. The idea of in vitro meat is the manufacturing of meat products

through tissue engineering technology. In vitro meat could have financial, health, animal welfare, and environmental advantages over traditional meat due to higher energy conversion efficiencies and less resources consumed. Therefore, the cofounders of Google and PayPal are investing in this seemingly costly technology development [85].

The basic procedures of in vitro meat are to (i) painlessly take stem cells from living animals, (ii) multiply them in low-cost (serum-free) culture media, (iii) grow muscle cells on scaffolds, (iv) grind up muscle strips, and (v) add flavors, iron, vitamins, and starch additives (Fig. 6). All these steps may occur without any genetic manipulation. To serve as a credible alternative to livestock meat, in vitro meat should be efficiently produced and should mimic meat in all of its physical sensations, such as visual appearance, smell, texture, and taste. The cost-efficient culture of in vitro meat will primarily depend on culture conditions, such as the source of the medium, its composition, and feedstock-to-meat conversion efficiency. Given the urgency of the problems that the modern meat industry is facing, this endeavor is worth undertaking [84]. In vitro meat, similar to synthetic starch, is currently prohibitively expensive, but it is anticipated that their production costs could be reduced to compete with conventionally obtained meat as technologies and efficiencies of mass production develop.

3.3.3. Microbial oil

Microbial oil is a triglyceride produced by microbes, similar to the vegetable oil produced by plants [86]. Some microorganisms accumulate lipids to a significant level of more than 20% of their dry weight, called oleaginous [87,88]. The lipid content and profile of oleaginous microorganisms differ between species and their growth conditions [87]. Microbial oils include fatty acids, such as palmitic, stearic, and oleic acids, as well as the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [89]. To meet increasing needs for essential fatty acids – omega-3 fatty acids – the oleaginous yeast *Yarrowia lipolytica* was genetically modified to produce EPA at 15% of the dry cell weight [90]. The engineered yeast lipid consists of EPA at 56.6% and saturated fatty acids at less than 5% by weight. This technology platform suggests the feasibility of the production of tailored lipids as a new nutrient source on a large scale [90].

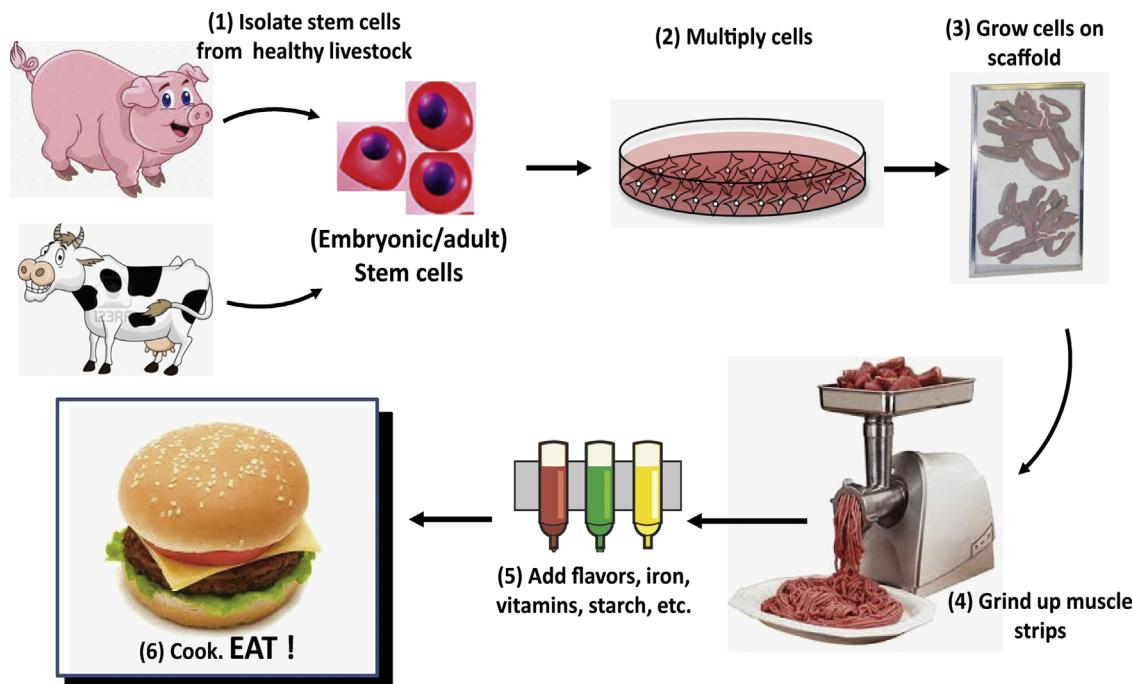


Fig. 6. Scheme of in vitro meat production. Modified from Ref. [83].

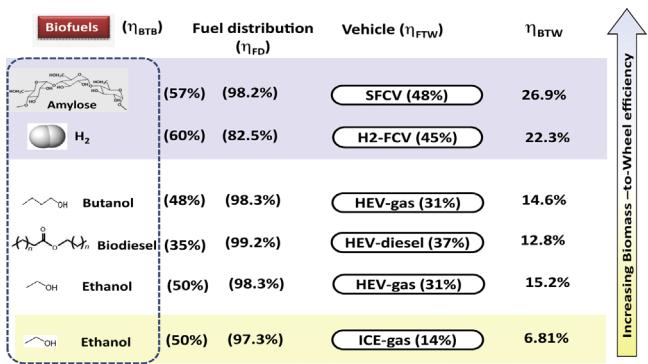


Fig. 7. Energy efficiency comparison of different fuels produced from biomass. The biomass-to-wheel (BTW) efficiency ($\eta_{BTW} = \eta_{BTB} \times \eta_{FD} \times \eta_{FTW}$). The data for energy conversion efficiencies were calculated in Ref. [173].

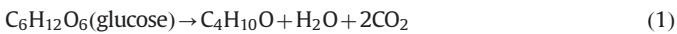
3.4. Advanced biofuels produced in biorefineries

A variety of biofuels have been proposed, such as cellulosic ethanol [91], n-butanol [92,93], iso-butanol [94,95], long chain alcohols [96,97], electricity [98,99], alkanes [100], fatty acid esters [101], hydrogen [74,102,103], hydrocarbons [104,105], and waxes [106]. (Note: In terms of the entire life cycle, the production of any biofuels from biomass sugars is a nearly carbon neutral process [107,108].) A biomass-to-wheel (BTW) efficiency (η_{BTW}), the ratio of the kinetic energy of the wheels of an automobile to the chemical energy of the delivered biomass, is suggested to provide a transparent comparison for numerous biofuels [109]. This energy efficiency analysis suggests transitions from ICE-gas vehicles (i.e., 6.81%) to hybrid electric vehicles (HEVs) (i.e., 12.8–15.2%) to H₂-fuel cell vehicles (FCVs) (i.e., 22.3%) or even sugar-FCV (i.e., 26.9%) [109] (Fig. 7).

To achieve high energy conversion efficiency, microorganisms or cell-free biosystems must have a balance of cofactors, which means that (i) the total amount of reduced cofactors (i.e., NADH and NADPH) generated equals the total amount consumed and that (ii) the NADH generation rates match the NAD(P)H consumption rates [110]. However, most synthetic pathways designed for the production of free fatty acids, fatty acid ethyl esters, alkanes, and waxes, which do not have cofactor balances [100,101,106,111–114]. Thus they will not be scaled up for commercial production due to low energy conversion efficiencies [109,115,116]. Aerobic fermentation balancing cofactors results in decreased product yields. In addition, the energy requirement for aerobic fermentation is high due to intensive aeration, mixing, and cooling. It is very difficult to control low dissolved oxygen levels in large bioreactors (e.g., 100–500 m³) for micro-aerobic fermentations [109,117]. Here both iso-butanol and hydrogen can be produced under strictly anaerobic conditions, featuring high energy-retaining efficiencies.

3.4.1. Iso-butanol

Iso-butanol is one of the most perfect biofuels for meeting needs of current ICEs. It has an energy density similar to n-butanol (i.e., 29.2 MJ/L), approximately 84% of the energy content of gasoline, is of limited miscibility with water, and is completely miscible with gasoline [118]. The branched structure of iso-butanol yields a better octane number than n-butanol. Iso-butanol can be produced by glycolysis followed by the Ehrlich or 2-keto-acid pathway [118,119] (Fig. 8). This pathway decarboxylates keto acid, the intermediate amino acid precursor, into isobutyraldehyde and then reduces it to iso-butanol. The stoichiometric reaction is shown in Eq. 1. Because this reaction does not consume oxygen for balancing the degree of reduction between glucose and iso-butanol, high energy efficiency can be achieved: 95.3% in terms of higher heating values [109].



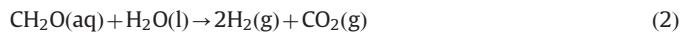
Heterologous pathways for iso-butanol production from sugars (Fig. 8A) have been introduced to a number of microorganisms, such as *E. coli* [96]; *Bacillus subtilis* [120,121], an important industrial microorganism; *Corynebacterium glutamicum* [122], a bacterium known for its high levels of amino acid production; *S. cerevisiae* [123]; and *Clostridium acetobutylicum* [124], a cellulolytic bacterium that produces iso-butanol directly from cellulose. In addition, the iso-butanol-producing pathway has been introduced to several microorganisms for producing the desired product from proteins [125] or CO₂ supplemented by solar energy [126] or electricity [127].

Instead of microbial iso-butanol fermentation, Volker and his coworkers designed a de novo cell-free synthetic pathway for converting glucose to iso-butanol via a minimal number of enzymes (Fig. 8B). Because enzymes can tolerate butanol inhibition better than microbes, this enzyme mixture can produce butanol in the presence of 4% product [95]. Large-scale iso-butanol production in vivo or in vitro could slowly replace ethanol.

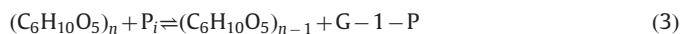
3.4.2. In vitro hydrogen

Hydrogen is the best biofuel for future FCVs mainly due to its cleaner by-product – water – and higher energy conversion efficiency through a PEMFC than an ICE, whose energy efficiency is restricted by the second law of thermodynamics [28] (Figs. 2 and 7). Hydrogen can be produced from biomass and sugars through chemical catalysis (e.g., gasification [128], pyrolysis [129], gasification in critical water [130], and aqueous phase reforming [131]), dark anaerobic fermentation [132], light fermentation [133], microbial electrohydrogenesis [134], and their combinations. However, all these approaches suffer from low product yields, possibly dirty products, or low productivity.

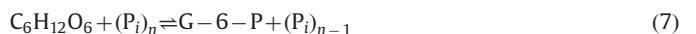
In vitro synthetic biosystems for biomanufacturing are the production of the desired products by assembling a number of purified enzymes or cell lysate and coenzymes [135–146]. In vitro non-natural synthetic pathways can be designed to produce hydrogen by splitting water powered by the chemical energy stored in a number of sugars [143] (Eq. 2), where CH₂O is a chemical shorthand for numerous carbohydrates (e.g., glucose, xylose, starch, cellulose, sucrose, etc.):



Starting from starch (amylose), 13 enzymes from different organisms, such as bacteria, yeast, archaea, plant, and animal, are assembled into an unnatural catabolic pathway (Fig. 9A) [74]. This pathway contains four major modules: (i) a chain-shortening phosphorylation reaction for producing G-1-P catalyzed by alpha-glucan phosphorylase (Eq. 3); (ii) the generation of glucose-6-phosphate (G-6-P) from G-1-P catalyzed by phosphoglucomutase (Eq. 4); (iii) the generation of 12 NADPH from G-6-P through a pentose phosphate pathway and four enzymes in the glycolysis and gluconeogenesis pathways (Eq. 5); and (iv) the generation of hydrogen from NADPH catalyzed by hydrogenase [147] (Eq. 6).



A supplementary pathway may be added to convert one glucose unit generated from one amylose after a series of substrate phosphorylations, catalyzed by alpha-glucan phosphorylase, to glucose-6-phosphate, catalyzed by polyphosphate glucokinase [148] (Fig. 9A).



As a result, all of the glucose units in starch can be converted to hydrogen without the use of ATP. Phosphate ions can be recycled and even condensed by polyphosphate-accumulating microorganisms, used

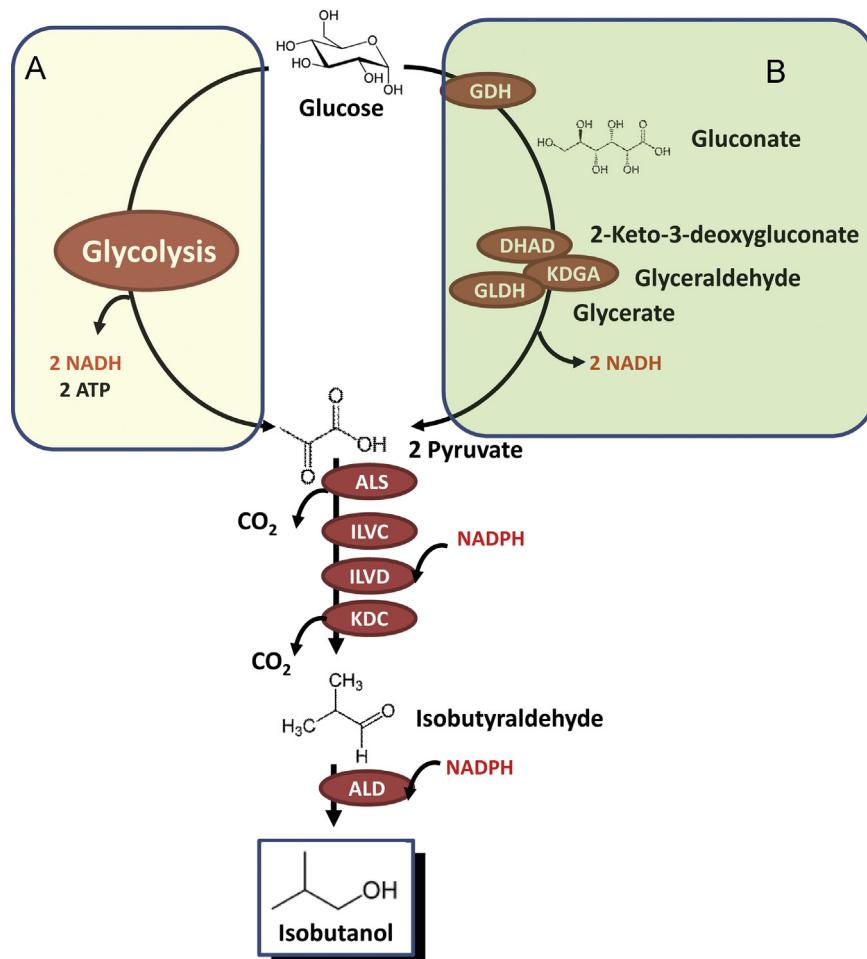


Fig. 8. Synthetic enzymatic pathways for iso-butanol production *in vivo* (A) and *in vitro* (B). Enzymes from pyruvate to iso-butanol are ALS, acetolactate synthase; ILVC, acetoxyhydroxy acid isomeroreductase; ILVD, dihydroxy-acid dehydratase; KDC, 2-ketoacid decarboxylase; ADH, alcohol dehydrogenase. Modified from Ref. [174]. Enzymes for *in vitro* pyruvate generation are GDH, glucose dehydrogenase; DHAD, (gluconate/glycerate) dihydroxy acid dehydratase; ALDH; glyceraldehyde dehydrogenase; KDGA, 2-keto-3-desoxygluconate aldolase. Modified from Ref. [95].

in wastewater treatment processes in Japan [149]. Enzymatic hydrogen production from starch could generate high-speed hydrogen to meet on-demand production for hypothetical sugar fuel cell vehicles [75].

Alternatively, hydrogen can be produced from the biomass sugars cellulose and hemicellulose (Fig. 9B). In this process, a partial hydrolysis of cellulose mediated by endoglucanase and cellobiohydrolase can produce long-chain cellodextrins and glucose. Cellodextrin and cellobiose phosphorylases can produce G-1-P [102]. Similar to the remaining pathway in Fig. 9A, all glucose units and G-1-P can be converted to hydrogen [102]. Xylose ($C_5H_{10}O_5$) is the most abundant pentose because it is a major building block of hemicellulose [150,151]. Because it is not economically feasible to separate xylose from other biomass sugars, the co-utilization of pentose and hexose is essential to the success of the production of biocommodities from biomass sugars [150]. In principle, one mole of xylose combined with H₂O can generate 10 mol of dihydrogen. To maximize dihydrogen production from xylose, another novel cell-free synthetic pathway has been designed (Fig. 9B) in which xylose is isomerized to xylulose by xylose isomerase followed by phosphorylation catalyzed by polyphosphate xylulokinase [152]. Xylulose 5-phosphate is produced and then oxidized by enzymes of the pentose phosphate pathway and glycolysis/gluconeogenesis to generate 10 NADPH and 5 CO₂ [103]. Hydrogen generation from nonfood biomass sugars could be a perfect way to produce low-cost green hydrogen for local users (e.g. fuel cell vehicles) by using scattered biomass resources [30]. Such small-size hydrogen generation systems could require very low

capital investment, partially addressing the infrastructure problem of the hydrogen economy.

The enzymatic production of hydrogen from sugars has several other advantages compared to other fermentative biofuels, including butanol and ethanol: (i) higher energy conversion efficiency, (ii) simpler product separation, and (iii) independence of initial sugar concentration. Nearly all chemical reactions (e.g., ethanol, butanol fermentation) are enthalpy-driven ($\Delta G^\circ < 0$ and $\Delta H^\circ < 0$), i.e., the combustion energy of the product(s) is lower than that of the substrate(s), releasing a fraction of chemical energy as waste heat into the environment. In contrast, enzymatic hydrogen reactions from sugars are entropy-driven ($\Delta G^\circ < 0$ and $\Delta H^\circ > 0$); i.e., the combustion energy of hydrogen is more than that of sugars, generating ~20% hydrogen enthalpy by absorbing low-temperature waste heat. (Note: Similarly, the biological conversion of acetic acid to methane mediated by methanogenesis microorganisms is spontaneous and endothermic [136,153].)

One-pot enzymatic hydrogen production has several advantages over chemical catalysis: (i) higher hydrogen yields, (ii) higher energy efficiency, (iii) ultra-high-purity hydrogen without CO, (iv) lower capital investment for low-temperature and low-pressure bioreactors, and (v) smaller distributed biohydrogen generation systems. However, it has an obvious weakness – slow reaction rates. Recently, enzymatic hydrogen generation rates have been increased by more than 750-fold, to more than 0.3 g H₂/L/h [154]. It is expected that hydrogen reaction rates might be increased by

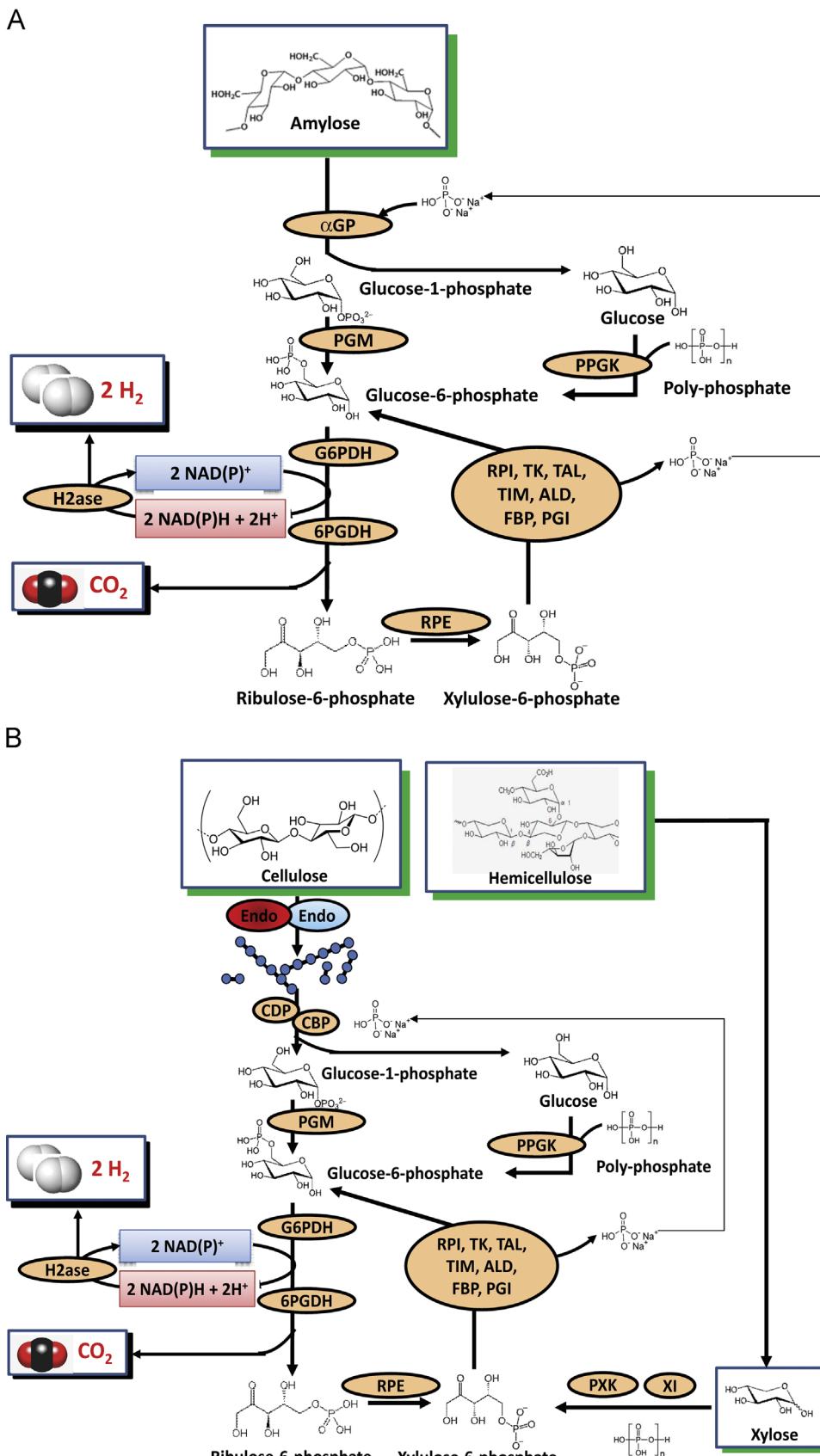


Fig. 9. Synthetic enzymatic pathways for high-yield hydrogen production from synthetic amylose (A) and from biomass sugars (B). Enzymes (A) are α GP, alpha-glucan phosphorylase; PPGK, polyphosphate glucokinase; PGM, phosphoglucomutase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; RPI, ribose 5-phosphate isomerase; RPE, ribulose-5-phosphate 3-epimerase; TK, transketolase; TAL, transaldolase; TIM, triose phosphate isomerase; ALD, (fructose-bisphosphate) aldolase; FBP, fructose bisphosphatase; PGI, phosphoglucose isomerase; and H₂ase, hydrogenase. Enzymes (B) are CDP, cellobextrin phosphorylase; CBP, cellobiose phosphorylase; XI, xylose isomerase; and PXK, polyphosphate xylulokinase. P_i and $(P_i)_n$ are inorganic phosphate and polyphosphate with a degree of polymerization of n .

another order of magnitude over the next several years [136,155]. In partial support of the accelerated rate increases over time, power densities of microbial fuel cells have been improved by more than 1,000,000-fold over the past 20 years [156].

4. Future R&D directions

To provide feedstock for next generation biorefineries, current agricultural and forest residues can be utilized before dedicated perennial plants are cultivated on a large scale. Sustainable agriculture will start with the cultivation of perennial plants on margin and low-yield agricultural lands. Numerous perennial crops, such as energy crops, giant reed, common reed, Napier grass, moso bamboo, hybrid poplar, switchgrass, miscanthus giganteus, alfalfa and so on (Table 3) will be selected based on local climate conditions, such as sunshine, rainfall, temperature, soil quality and their nitrogen fixation ability. Furthermore, to decrease biomass recalcitrance, the discovery of genetic variants in native populations of bioenergy plants and direct manipulation of biosynthesis pathways have produced less recalcitrant feedstocks with favorable properties for biomass pretreatment and downstream conversion [157–159]. Also, to decrease protein production costs, plants can be modified for low-cost production of recombinant proteins [160–162].

At the same time, the most important R&D directions of new biorefineries focus on biomass saccharification, bioconversions of advanced biofuels limited to a few (e.g., butanol and hydrogen), and new applications of lignocellulose-derived products. Several objectives of new biorefineries are

- (i) to cost-effective release soluble sugars from biomass by developing better biomass pretreatments, cellulase engineering, and novel cellulase production systems [55,64,160,163–166];
- (ii) to fractionate lignocellulosic to numerous components, such as proteins, lignin, hemicellulose, and so on for their co-utilization [51,52,55–58,167,168];
- (iii) to exploit new applications of biomass-derived components, such as, synthetic starch as a slowly-released drug carrier or low oxygen diffusion bioplastic film [169], isolated lignin as a precursor for low-cost carbon fibers, engineered plastics and thermoplastic elastomers, polymeric foams, fungible fuels, and commodity chemicals [157];
- (iv) to develop advanced biofuel-producing microorganisms tolerating high product titers and working under anaerobic conditions [109,115];
- (v) to create novel *in vitro* synthetic enzymatic biosystems that can efficiently convert soluble sugars to hydrogen by increasing enzyme stability, decreasing enzyme costs, and utilization of biomimetic coenzymes [145,146,170–172],
- (vi) to improve technologies that can produce artificial food (e.g. starch, meat and oil) from biomass carbohydrates [59,83,84]; and
- (vii) to investigate the short-term and long-term safety, ethics, and health issues of synthetic food/feed.

5. Conclusions

Dramatic changes in agricultural systems could occur in the 21st century to meet the increasing needs of humanity – food, biofuels, and materials because modern agriculture is not sustainable. Sustainable agriculture systems based on perennial plants and a hybrid of perennial plants and annual grain crops will have multi-functions for the production of food/feed and biomass feedstock for new biorefineries as well as ecological service (Fig. 1B). In addition to biobased

products (biofuels, chemicals, and materials), new biorefineries could have additional roles in the production of starch, *in vitro* meat, and microbial oil as new food/feed sources (Fig. 3). New biorefineries would be a cornerstone of the sustainability revolution because it will increase national food and energy security, decrease greenhouse gas emissions, and create new high-paying manufacturing jobs that cannot be outsourced.

In a word, recent biotechnology breakthroughs present great opportunities in revolutionizing more than 10,000 years of agriculture and reviving the new carbohydrate economy in the near future.

Acknowledgments

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References

- [1] Lynd LR. Bioenergy: in search of clarity. *Energy Environ Sci* 2010;3:1150–2.
- [2] Wei J. Great inventions that changed the world Hoboken, NJ, USA: John Wiley & Sons, Inc; 2012.
- [3] Smil V. Energy transitions: history, requirements, prospects. Santa Barbara, CA: ABC-CLIO, LLC; 2010.
- [4] Zhang Y-HP. Next generation biorefineries will solve the food, biofuels, and environmental trilemma in the energy – food – water nexus. *Energy Sci Eng* 2013;1:27–41.
- [5] Smil V. Oil: a beginner's guide. Oxford, England: Oneworld Publications; 2008.
- [6] Balmford A, Green R, Phalan B. What conservationists need to know about farming. *Proc Biol Sci* 2012;279:2714–24.
- [7] Smil V. Feeding the world: a challenge for the twenty-first century. Boston, MA: MIT Press; 2000.
- [8] The World Economic Forum Water Initiative. Water security: the water – food – energy – climate nexus. Washington, DC: Island Press; 2011.
- [9] Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P. Land clearing and the biofuel carbon debt. *Science* 2008;319:1235–8.
- [10] Searchinger T, Heimlich R, Houghton RA, Dong F, Elobeid A, Fabiosa J, et al. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. *Science* 2008;319:1238–40.
- [11] Glover JD, Cox CM, Reganold JP. Future farming: a return to roots. *Sci Am* 2007;297:82–9.
- [12] Fuglie KO, Wang SL. New evidence points to robust but uneven productivity growth in global agriculture. *Amber Waves* 2012;10:1–6.
- [13] Hentall PL, Flowers N, Bugg TDH. Enhanced acid stability of a reduced nicotinamide adenine dinucleotide (NADH) analogue. *Chem Comm* 2001:2098–9.
- [14] Berenguer-Murcia A, Fernandez-Lafuente R. New trends in the recycling of NAD(P)H for the design of sustainable asymmetric reductions catalyzed by dehydrogenases. *Curr Org Chem* 2010;14:1000–21.
- [15] Shinkai S, Hamada H, Kusano Y, Manabe O. Coenzyme models. Part 16. Studies of general-acid catalysis in the NADH model reduction. *J Chem Soc Perkin Trans* 1979;2:699–702.
- [16] Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, et al. Solutions for a cultivated planet. *Nature* 2011;478:337–42.
- [17] Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, et al. Food security: the challenge of feeding 9 billion people. *Science* 2010;327:812–8.
- [18] Grassini P, Eskridge KM, Cassman KG. Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nat Commun* 2013;4:2918.
- [19] Lam H-M, Remais J, Fung M-C, Xu L, Sun SS-M. Food supply and food safety issues in China. *Lancet* 2013;381:2044–53.
- [20] Zhao J, Luo Q, Deng H, Yan Y. Opportunities and challenges of sustainable agricultural development in China. *Philos Trans R Soc Lond B Biol Sci* 2008;363:893–904.
- [21] Zeng J, Chang CH, Wang YP. Study on fertilizer input in China based on food security. *Probl Agric Econ* 2010;5:66–70.
- [22] Fixen PE, Johnston AM. World fertilizer nutrient reserves: a view to the future. *J Sci Food Agric* 2012;92:1001–5.

- [23] Zhao GS, Jiang HR, Wu WL. Sustainability of farmland ecosystem with high yield based on energy analysis method. *Trans Chin Soc Agric Eng* 2011;27:318–23.
- [24] Philpot T. Why China's farming sector is failing. *The Atlantic* 2013.
- [25] Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, et al. Significant acidification in major Chinese croplands. *Science* 2010;327:1008–10.
- [26] Huang J, Yang J, Qiu H. Thoughts on Chinese strategy and policy of food security in the new era. *Probl Agric Econ* 2012;2012:4–8.
- [27] Smil V. *Energies: an illustrated guide to the biosphere and civilization*. Cambridge, MA: The MIT Press; 1999.
- [28] Zhang Y-HP. What is vital (and not vital) to advance economically-competitive biofuels production. *Proc Biochem* 2011;46:2091–110.
- [29] Henry F, Crowther S. *My life and work*. LLC: Garden City Publishing; 1922.
- [30] Zhang Y-HP. Renewable carbohydrates are a potential high density hydrogen carrier. *Int J Hydrogen Energy* 2010;35:10334–42.
- [31] Smil V. Energy myths and realities: bringing science to the energy policy debate. Washington, DC: The AEI Press; 2010.
- [32] Kelly K. *What technology wants*. New York City, NY: Viking; 2010.
- [33] Demirdoven N, Deutch J. Hybrid cars now, fuel cell cars later. *Science* 2004;305:974–6.
- [34] FAO. (<http://www.fao.org/ag/agp/AGPC/doc/gbase/DATA/Pf000095.HTM>).
- [35] Mafongoya PL, Kuntashula E, Sileshi G. Managing soil fertility and nutrient cycles through fertilizer trees in southern Africa. Biological approaches to sustainable soil systems. Taylor & Francis; 2006. p. 273–89.
- [36] Ajayi OC, Place F, Akinnifesi FK, Sileshi GW. Agricultural success from Africa: the case of fertilizer tree systems in southern Africa (Malawi, Tanzania, Mozambique, Zambia and Zimbabwe). *Int J Agric Sustain* 2011;9:129–36.
- [37] Glover JD, Reganold JP, Cox CM. Agriculture: Plant perennials to save Africa's soils. *Nature* 2012;489:359–61.
- [38] Smith CM, David MB, Mitchell CA, Masters MD, Anderson-Teixeira KJ, Bernacchi CJ, et al. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. *J Environ Qual* 2013;42:219–28.
- [39] Clifton-Brown JC, Neilson B, Lewandowski I, Jones MB. The modelled productivity of *Miscanthus × giganteus* (GREEF et DEU) in Ireland. *Ind Crops Prod* 2000;12:97–109.
- [40] Murnen HK, Balan V, Chundawat SPS, Bals B, daCostaSousa L, Dale BE. Optimization of ammonia fiber expansion (AFEX) pretreatment and enzymatic hydrolysis of *Miscanthus × giganteus* to fermentable sugars. *Biotechnol Prog* 2007;23:846–50.
- [41] Sathitsuksanoh N, Zhu Z, Zhang Y-HP. Cellulose solvent- and organic solvent-based lignocellulose fractionation enabled efficient sugar release from a variety of lignocellulosic feedstocks. *Biores Technol* 2012;117:228–33.
- [42] Sathitsuksanoh N, Zhu Z, Ho T-J, Bai M-D, Zhang Y-HP. Bamboo saccharification through cellulose solvent-based biomass pretreatment followed by enzymatic hydrolysis at ultra-low cellulase loadings. *Biores Technol* 2010;101:4926–9.
- [43] Shanmughavel P, Francis K. *Physiology of bamboo*. Jodhpur: Scientific Publishers (India); 2001.
- [44] Sathitsuksanoh N, Zhu Z, Templeton N, Rollin J, Harvey S, Zhang Y-HP. Saccharification of a potential bioenergy crop, *Phragmites australis* (common reed), by lignocellulose fractionation followed by enzymatic hydrolysis at decreased cellulase loadings. *Ind Eng Chem Res* 2009;48:6441–7.
- [45] Khan ZR, Midega CA, Bruce TJ, Hooper AM, Pickett JA. Exploiting phytochemicals for developing a 'push - pull' crop protection strategy for cereal farmers in Africa. *J Exp Bot* 2010;61:4185–96.
- [46] Jordan N, Boody G, Broussard W, Glover JD, Keeney D, McCown BH, et al. ENVIRONMENT: sustainable development of the agricultural bio-economy. *Science* 2007;316:1570–1.
- [47] Gustavsson J, Cederberg C, Sonesson U, van Otterdijk R, Meybeck A. Global food losses and food waste section 32 of International Congress "Save Food" (FAO, Rural Infrastructure and Agro-Industries Division). Interpack2011, Düsseldorf, Germany2011.
- [48] Parfitt J, Barthel M, Macnaughton S. Food waste within food supply chains: quantification and potential for change to 2050. *Philos Trans R Soc B: Biol Sci* 2010;365:3065–81.
- [49] Lynd LR, Woods J. Perspective: a new hope for Africa. *Nature* 2011;474: S20–S21.
- [50] Kim T, Yoo C, Lamsal BP. Front-end recovery of protein from lignocellulosic biomass and its effects on chemical pretreatment and enzymatic saccharification. *Bioproc Biosys Eng* 2013;36:687–94.
- [51] Bals B, Dale BE. Economic comparison of multiple techniques for recovering leaf protein in biomass processing. *Biotechnol Bioeng* 2011;108:530–7.
- [52] Jung S, Lee D-S, Kim Y-O, Joshi C, Bae H-J. Improved recombinant cellulase expression in chloroplast of tobacco through promoter engineering and 5' amplification promoting sequence. *Plant Mol Biol* 2013;83:317–28.
- [53] Hefferon K. Plant-derived pharmaceuticals for the developing world. *Bio-technol J* 2013;8:1193–202.
- [54] Corchero JL, Gasser B, Resina D, Smith W, Parrilli E, Vázquez F, et al. Unconventional microbial systems for the cost-efficient production of high-quality protein therapeutics. *Biotechnol Adv* 2013;31:140–53.
- [55] Sathitsuksanoh N, George A, Zhang Y-HP. New lignocellulose pretreatments by using cellulose solvents: a review. *J Chem Eng Biotechnol* 2013;88:169–80.
- [56] Zhang Y-HP, Ding S-Y, Mielenz JR, Elander R, Laser M, Himmel M, et al. Fractionating recalcitrant lignocellulose at modest reaction conditions. *Biotechnol Bioeng* 2007;97:214–23.
- [57] da Costa Lopes AM, João KG, Rubik DF, Bogel-Lukasik E, Duarte LC, Andreaus J, et al. Pre-treatment of lignocellulosic biomass using ionic liquids: wheat straw fractionation. *Biores Technol* 2013;142:198–208.
- [58] Lan W, Liu C-F, Sun R-C. Fractionation of bagasse into cellulose, hemicelluloses, and lignin with ionic liquid treatment followed by alkaline extraction. *J Agr Food Chem* 2011;59:8691–701.
- [59] You C, Chen H, Myung S, Sathitsuksanoh N, Ma H, Zhang X-Z, et al. Enzymatic transformation of nonfood biomass to starch. *Proc Natl Acad Sci USA* 2013;110:7182–7.
- [60] Nelson DL, Cox MM. *Lehninger principles of biochemistry*. 5th edition. New York: WH Freeman; 2008.
- [61] Himmel ME, Ding S-Y, Johnson DK, Adney WS, Nimlos MR, Brady JW, et al. Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* 2007;315:804–7.
- [62] Ding S-Y, Liu Y-S, Zeng Y, Himmel ME, Baker JO, Bayer EA. How does plant cell wall nanoscale architecture correlate with enzymatic digestibility? *Science* 2012;338:1055–60.
- [63] Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev* 2002;66:506–77.
- [64] Zhang Y-HP, Himmel M, Mielenz JR. Outlook for cellulase improvement: screening and selection strategies. *Biotechnol Adv* 2006;24:452–81.
- [65] De Winter K, Cerdobbel A, Soetaert W, Desmet T. Operational stability of immobilized sucrose phosphorylase: continuous production of α-glucosidase-1-phosphate at elevated temperatures. *Proc Biochem* 2011;46:2074–8.
- [66] van Soest JJG, Vliegenthart JFG. Crystallinity in starch plastics: consequences for material properties. *Trends Biotechnol* 1997;15:208–13.
- [67] Frische R, Wollmann K, Gross-Lannert R, Schneider J, Best B. Special amyloses and their use for producing biodegradable plastics. USA1994. USA patent 5374304.
- [68] Kadokawa J-I. Preparation and applications of amylose supramolecules by means of phosphorylase-catalyzed enzymatic polymerization. *Polymers* 2012;4:116–33.
- [69] Maki KC, Pelkman CL, Finocchiaro ET, Kelley KM, Lawless AL, Schild AL, et al. Resistant starch from high-amylase maize increases insulin sensitivity in overweight and obese men. *J Nutr* 2012;142:717–23.
- [70] Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, et al. High-amylase wheat generated by RNA interference improves indices of large-bowel health in rats. *Proc Natl Acad Sci USA* 2006;103:3546–51.
- [71] Zhang G, Hamaker BR. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. *Crit Rev Food Sci Nutr* 2009;49:852–67.
- [72] Gilbert R, Wu A, Sullivan M, Sumarriba G, Ersch N, Hasjim J. Improving human health through understanding the complex structure of glucose polymers. *Anal Bioanal Chem* 2013;1–12.
- [73] Zhang Y-HP, Huang W-D. Constructing the electricity–carbohydrate–hydrogen cycle for a sustainability revolution. *Trends Biotechnol* 2012;30:301–6.
- [74] Zhang Y-HP, Evans BR, Mielenz JR, Hopkins RC, Adams MWW. High-yield hydrogen production from starch and water by a synthetic enzymatic pathway. *PLoS One* 2007;2:e456.
- [75] Zhang Y-HP. A sweet out-of-the-box solution to the hydrogen economy: is the sugar-powered car science fiction? *Energy Environ Sci* 2009;2:272–82.
- [76] Peat S, Bourne Ej, Barker SA. Enzymic conversion of amylose into amylopectin. *Nature* 1948;161:127–8.
- [77] Wang Y, Zhang Y-HP. Cell-free protein synthesis energized by slowly-metabolized maltodextrin. *BMC Biotechnol* 2009;9:58.
- [78] Caschera F, Noireaux V. Synthesis of 2.3 mg/ml of protein with an all *Escherichia coli* cell-free transcription–translation system. *Biochim* 2014.
- [79] USDA. (http://www.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf).
- [80] Thornton PK. Livestock production: recent trends, future prospects. *Philos Trans R Soc Lond B Biol Sci* 2010;365:2853–67.
- [81] Crisci E, Mussá T, Fraile L, Montoya M. Review: influenza virus in pigs. *Mol Immunol* 2013;55:200–11.
- [82] Marlow HJ, Hayes WK, Soret S, Carter RL, Schwab ER, Sabaté J. Diet and the environment: does what you eat matter? *Am J Clin Nutr* 2009;89:1699S–1703SS.
- [83] Bartholet J. Inside the meat lab. *Sci Am* 2011;304:64–9.
- [84] Post MJ. Cultured meat from stem cells: challenges and prospects. *Meat Sci* 2012;92:297–301.
- [85] Stinson L. See-through sushi, anyone? 13 Insane applications of in vitro meat. (<http://www.wired.com/design/2013/11/a-cookbook-for-in-vitro-meat/>). 2013.
- [86] Leman J. Oleaginous microorganisms: an assessment of the potential. In: Saul IL, Allen IL, editors. *Advances in Applied Microbiology*. Academic Press; 1997. p. 195–243.
- [87] Beopoulos A, Cescut J, Haddouche R, Uribelarrea J-L, Molina-Jouve C, Nicaud J-M. *Yarrowia lipolytica* as a model for bio-oil production. *Prog Lipid Res* 2009;48:375–87.
- [88] Ageitos J, Vallejo J, Veiga-Crespo P, Villa T. Oily yeasts as oleaginous cell factories. *Appl Microbiol Biotechnol* 2011;90:1219–27.
- [89] Kosa M, Ragauskas AJ. Lipids from heterotrophic microbes: advances in metabolism research. *Trends Biotechnol* 2011;29:53–61.
- [90] Xue Z, Sharpe PL, Hong S-P, Yadav NS, Xie D, Short DR, et al. Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. *Nat Biotechnol* 2013;31:734–40.
- [91] Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, et al. Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. *Proc Natl Acad Sci* 2008;105:13769–74.

- [92] Shen CR, Lan El, Dekishima Y, Baez A, Cho KM, Liao JC. High titer anaerobic 1-butanol synthesis in *Escherichia coli* enabled by driving forces. *Appl Environ Microbiol* 2011;77:2905–15.
- [93] Krutsakorn B, Honda K, Ye X, Imagawa T, Bei X, Okano K, et al. In vitro production of n-butanol from glucose. *Metab Eng* 2013;20:84–91.
- [94] Liu X, Bastian S, Snow CD, Brustad EM, Saleski TE, Xu J-H, et al. Structure-guided engineering of *Lactococcus lactis* alcohol dehydrogenase LLAdhA for improved conversion of isobutyraldehyde to isobutanol. *J Biotechnol* 2012;164:188–95.
- [95] Guterl J-K, Garbe D, Carsten J, Steffler F, Sommer B, Reiße S, et al. Cell-free metabolic engineering – production of chemicals via minimized reaction cascades. *ChemSusChem* 2012;5:2165–72.
- [96] Atsumi S, Hanai T, Liao JC. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature* 2008;451:86–9.
- [97] Zhang K, Sawaya MR, Eisenberg DS, Liao JC. Expanding metabolism for biosynthesis of nonnatural alcohols. *Proc Natl Acad Sci* 2008;105:20653–8.
- [98] Campbell JE, Lobell DB, Field CB. Greater transportation energy and GHG offsets from bioelectricity than ethanol. *Science* 2009;324:1055–7.
- [99] Zhu Z, Tam TK, Sun F, You C, Zhang Y-HP. A high-energy-density sugar biobattery via a synthetic enzymatic pathway. *Nat Commun* 2014;5:3026.
- [100] Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB. Microbial biosynthesis of alkanes. *Science* 2010;329:559–62.
- [101] Liu T, Vora H, Khosla C. Quantitative analysis and engineering of fatty acid biosynthesis in *E. coli*. *Metab Eng* 2010;12:378–86.
- [102] Ye X, Wang Y, Hopkins RC, Adams MWW, Evans BR, Mielenz JR, et al. Spontaneous high-yield production of hydrogen from cellulosic materials and water catalyzed by enzyme cocktails. *ChemSusChem* 2009;2:149–52.
- [103] Martín del Campo JS, Rollin J, Myung S, Chun Y, Chandrayan S, Patiño R, et al. High-yield production of dihydrogen from xylose by using a synthetic enzyme cascade in a cell-free system. *Angew Chem Int Ed* 2013;52:4587–90.
- [104] Wang Y, Huang W, Sathitsuksanoh N, Zhu Z, Zhang Y-HP. Biohydrogenation from biomass sugar mediated by in vitro synthetic enzymatic pathways. *Chem Biol* 2011;18:372–80.
- [105] Serrano-Ruiz JC, Dumesic JA. Catalytic routes for the conversion of biomass into liquid hydrocarbon transportation fuels. *Energy Environ Sci* 2011;4:83–99.
- [106] Steen EJ, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, et al. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. *Nature* 2010;463:559–62.
- [107] Richard TL. Challenges in scaling up biofuels infrastructure. *Science* 2010;329:793–6.
- [108] Somerville C, Youngs H, Taylor C, Davis SC, Long SP. Feedstocks for lignocellulosic biofuels. *Science* 2010;329:790–2.
- [109] Huang WD, Zhang Y-HP. Analysis of biofuels production from sugar based on three criteria: thermodynamics, bioenergetics, and product separation. *Energy Environ Sci* 2011;4:784–92.
- [110] Ghosh A, Zhao H, Price ND. Genome-scale consequences of cofactor balancing in engineered pentose utilization pathways in *Saccharomyces cerevisiae*. *PLoS One* 2011;6:e27316.
- [111] Duan Y, Zhu Z, Cai K, Tan X, Lu X. *De novo* biosynthesis of biodiesel by *Escherichia coli* in optimized fed-batch cultivation. *PLoS One* 2011;6:e20265.
- [112] Kalscheuer R, Stolting T, Steinbuchel A. Microdiesel: *Escherichia coli* engineered for fuel production. *Microbiology* 2006;152:2529–36.
- [113] Lu X, Vora H, Khosla C. Overproduction of free fatty acids in *E. coli*: implications for biodiesel production. *Metab Eng* 2008;10:333–9.
- [114] Liu T, Khosla C. Genetic engineering of *Escherichia coli* for biofuel production. *Annu Rev Genet* 2010;44:53–69.
- [115] Bastian S, Liu X, Meyerowitz JT, Snow CD, Chen MMY, Arnold FH. Engineered ketol-acid reductoisomerase and alcohol dehydrogenase enable anaerobic 2-methylpropan-1-ol production at theoretical yield in *Escherichia coli*. *Metab Eng* 2011;13:345–52.
- [116] Dugar D, Stephanopoulos G. Relative potential of biosynthetic pathways for biofuels and bio-based products. *Nat Biotechnol* 2011;29:1074–8.
- [117] Blanch HW. Bioprocessing for biofuels. *Curr Opin Biotechnol* 2012;390–5.
- [118] Peralta-Yahya PP, Zhang F, del Cardayre SB, Keasling JD. Microbial engineering for the production of advanced biofuels. *Nature* 2012;488:320–8.
- [119] Hazelwood LA, Daran J-M, van Maris AJA, Pronk JT, Dickinson JR. The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. *Appl Environ Microbiol* 2008;74:2259–66.
- [120] Li S, Huang D, Li Y, Wen J, Jia X. Rational improvement of the engineered isobutanol-producing *Bacillus subtilis* by elementary mode analysis. *Microb Cell Fact* 2012;11:101.
- [121] 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;1–13.
- [122] Smith K, Cho K-M, Liao J. Engineering *Corynebacterium glutamicum* for isobutanol production. *Appl Microbiol Biotechnol* 2010;87:1045–55.
- [123] Chen X, Nielsen K, Borodina I, Kielland-Brandt M, Karhumaa K. Increased isobutanol production in *Saccharomyces cerevisiae* by overexpression of genes in valine metabolism. *Biotechnol Biofuels* 2011;4:21.
- [124] 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.
- [125] Huo Y-X, Cho KM, Rivera JGL, Monte E, Shen CR, Yan Y, et al. Conversion of proteins into biofuels by engineering nitrogen flux. *Nat Biotechnol* 2011 advance online publication.
- [126] Atsumi S, Higashide W, Liao JC. Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nat Biotechnol* 2009;27:1177–80.
- [127] Li H, Opgenorth PH, Wernick DG, Rogers S, Wu T-Y, Higashide W, et al. Integrated electromicrobial conversion of CO₂ to higher alcohols. *Science* 2012;335:1596.
- [128] Ni M, Leung DYC, Leung MKH, Sumathy K. An overview of hydrogen production from biomass. *Fuel Process Technol* 2006;87:461–72.
- [129] Rezaian J, Cheremisinoff NP. Gasification technologies: a primer for engineers and scientist. CRC press; 2005.
- [130] Rezaian J, Cheremisinoff NP. Biogasification. Gasification technologies: a primer for engineers and scientist. Boca Raton, FL: CRC Press; 2005. p. 119–145.
- [131] Cortright RD, Davda RR, Dumesic JA. Hydrogen from catalytic reforming of biomass-derived hydrocarbons in liquid water. *Nature* 2002;418:964–7.
- [132] Vavilin VA, Rytov SV, Lokshina LY. Modelling hydrogen partial pressure change as a result of competition between the butyric and propionic groups of acidogenic bacteria. *Biores Technol* 1995;54:171–7.
- [133] Ueno Y, Tatara M, Fukui H, Makiuchi T, Goto M, Sode K. Production of hydrogen and methane from organic solid wastes by phase-separation of anaerobic process. *Biokes Technol* 2007;98:1861–5.
- [134] Cheng S, Logan BE. Sustainable and efficient biohydrogen production via electrohydrogenesis. *Proc Natl Acad Sci USA* 2007;104:18871–3.
- [135] Zhang Y-HP. Production of biocommodities and bioelectricity by cell-free synthetic enzymatic pathway biotransformations: challenges and opportunities. *Biotechnol Bioeng* 2010;105:663–77.
- [136] Zhang Y-HP. Simpler is better: high-yield and potential low-cost biofuels production through cell-free synthetic pathway biotransformation (SyPaB). *ACS Catal* 2011;1:998–1009.
- [137] Zhang Y-HP, Myung S, You C, Zhu ZG, Rollin J. Toward low-cost biomanufacturing through cell-free synthetic biology: bottom-up design. *J Mater Chem* 2011;21:18877–86.
- [138] Zhang Y-HP, Sun J-B, Zhong J-J. Biofuel production by in vitro synthetic pathway transformation. *Curr Opin Biotechnol* 2010;21:663–9.
- [139] Billerbeck S, Härlé J, Panke S. The good of two worlds: increasing complexity in cell-free systems. *Curr Opin Biotechnol* 2013;24:1037–43.
- [140] Hodgman CE, Jewett MC. Cell-free synthetic biology: thinking outside the cell. *Metab Eng* 2012;14:261–9.
- [141] Arda I, Hwang E, Zeng A-P. In vitro multienzymatic reaction systems for biosynthesis. *Adv Biochem Eng Biotechnol* 2013;137:153–84.
- [142] Guterl J-K, Sieber V. Biosynthesis “debugged”: novel bioproduction strategies. *Eng Life Sci* 2013;13:4–18.
- [143] Rollin JA, Tam W, Zhang Y-HP. New biotechnology paradigm: cell-free biosystems for biomanufacturing. *Green Chem* 2013;15:1708–19.
- [144] Korman TP, Sahachartsiri B, Li D, Vinokur JM, Eisenberg D, Bowie JU. A synthetic biochemistry system for the in vitro production of isoprene from glycolysis intermediates. *Protein Sci* 2014;25:576–85.
- [145] Zhang Y-HP. Production of biofuels and biochemicals by in vitro synthetic biosystems: opportunities and challenges. *Biotechnol Adv* 2015. <http://dx.doi.org/10.1016/j.biotechadv.2014.10.009>.
- [146] Dudley QM, Karim AS, Jewett MC. Cell-free metabolic engineering: biomanufacturing beyond the cell. *Biotechnol J* 2014 n/a-n/a.
- [147] Sun J, Hopkins RC, Jenney FE, McTernan PM, Adams MWW. Heterologous expression and maturation of an NADP-dependent [NiFe]-hydrogenase: a key enzyme in biofuel production. *PLoS One* 2010;5:e10526.
- [148] Liao HH, Myung S, Zhang Y-HP. One-step purification and immobilization of thermostable polyphosphate glucokinase from *Thermobifida fusca* YX: glucose-6-phosphate generation without ATP. *Appl Microbiol Biotechnol* 2012;93:1109–17.
- [149] Kuroda A, Nomura K, Ohtomo R, Kato J, Ikeda T, Takiguchi N, et al. Role of inorganic polyphosphate in promoting ribosomal protein degradation by the Lon protease in *E. coli*. *Science* 2001;293:705–8.
- [150] Straathof AJJ. Transformation of Biomass into Commodity Chemicals Using Enzymes or Cells. *Chem Rev* 2013.
- [151] Kuhad RC, Gupta R, Khasa Y, Singh A, Zhang Y-HP. Bioethanol production from pentose sugars: current status and future prospects. *Renew Sustain Energy Rev* 2011;15:4950–62.
- [152] Martín del Campo JS, You C, Kim J-E, Patiño R, Zhang Y-HP. Discovery and characterization of a novel ATP/polyphosphate xylulokinase from a hyperthermophilic bacterium *Thermotoga maritima*. *J Ind Microbiol Biotechnol* 2013;40:661–9.
- [153] Thauer K, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* 1977;41:100–80.
- [154] Rollin JA, Ye XH, Martin DCJS, Adams MWW, Zhang Y-HP. Novel hydrogen detection apparatus along with bioreactor systems. *Adv Biochem Eng Biotechnol* 2014. http://dx.doi.org/10.1007/10_2014_274.
- [155] Swartz JR. Cell-free bioprocessing. *Chem Eng Prog* 2013;2013:40–5.
- [156] Logan BE. Exoelectrogenic bacteria that power microbial fuel cells. *Nat Rev Microbiol* 2009;7:375–81.
- [157] Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF, et al. Lignin valorization: improving lignin processing in the biorefinery. *Science* 2014;344.
- [158] Sathitsuksanoh N, Xu B, Zhao B, Zhang YHP. Overcoming biomass recalcitrance by combining genetically modified switchgrass and cellulose solvent-based lignocellulose pretreatment. *PLoS ONE* 2013;8:e73523.

- [159] Fu C, Mielenz JR, Xiao X, Ge Y, Hamilton CY, Rodriguez M, et al. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. *Proc Natl Acad Sci USA* 2011;108:3803–8.
- [160] Ullrich KK, Hiss M, Rensing SA. Means to optimize protein expression in transgenic plants. *Curr Opin Biotechnol* 2015;32:61–7.
- [161] Jung S, Lee D-S, Kim Y-O, Joshi C, Bae H-J. Improved recombinant cellulase expression in chloroplast of tobacco through promoter engineering and 5' amplification promoting sequence. *Plant Mol Biol* 2013;83:317–28.
- [162] Company N, Nadal A, Ruiz C, Pla M. Production of phytotoxic cationic α -helical antimicrobial peptides in plant cells using inducible promoters. *PLoS ONE* 2014;9:e109990.
- [163] Singh A, Taylor Li LE, Vander Wall TA, Linger J, Himmel ME, Podkaminer K, et al. Heterologous protein expression in *Hypocrea jecorina*: a historical perspective and new developments. *Biotechnol Adv* 2015. <http://dx.doi.org/10.1016/j.biotechadv.2014.11.009>.
- [164] Harris PV, Xu F, Kreel NE, Kang C, Fukuyama S. New enzyme insights drive advances in commercial ethanol production. *Curr Opin Chem Biol* 2014;19:162–70.
- [165] Liu G, Qin Y, Li Z, Qu Y. Development of highly efficient, low-cost lignocellulolytic enzyme systems in the post-genomic era. *Biotechnol Adv* 2013;31:962–75.
- [166] Garvey M, Klose H, Fischer R, Lambertz C, Commandeur U. Cellulases for biomass degradation: comparing recombinant cellulase expression platforms. *Trends Biotechnol* 2013;31:581–93.
- [167] Amidon TE, Liu S. Water-based woody biorefinery. *Biotechnol Adv* 2009;27:542–50.
- [168] Diedericks D, van Rensburg E, Görgens J. Fractionation of sugarcane bagasse using a combined process of dilute acid and ionic liquid treatments. *Appl Biochem Biotechnol* 2012;167:1921–37.
- [169] Qi P, You C, Zhang YHP. One-pot enzymatic conversion of sucrose to synthetic amylose by using enzyme cascades. *ACS Catal* 2014;4:1311–7.
- [170] Ninh PH, Honda K, Sakai T, Okano K, Ohtake H. Assembly and multiple gene expression of thermophilic enzymes in *Escherichia coli* for in vitro metabolic engineering. *Biotechnol Bioeng* 2015;112:189–96.
- [171] Paul CE, Arends IWCE, Hollmann F. Is simpler better? Synthetic nicotinamide cofactor analogues for redox chemistry *ACS Catal* 2014;4:788–97.
- [172] Opgenorth PH, Korman TP, Bowie JU. A synthetic biochemistry molecular purge valve module that maintains redox balance. *Nat Commun* 2014;5:4113.
- [173] Huang WD, Zhang Y-HP. Energy efficiency analysis: biomass-to-wheel efficiency related with biofuels production, fuel distribution, and powertrain systems. *PLoS One* 2011;6:e22113.
- [174] Atsumi S, Liao JC. Directed evolution of *Methanococcus jannaschii* citramalate synthase for 1-propanol and 1-butanol biosynthesis from *Escherichia coli*. *Appl Environ Microbiol* 2008;74:7802–8.
- [175] Food and Agriculture Organization of the United Nations. FAOSTAT: production-crops, 2010 data. (<http://faostat3.fao.org/faostat-gateway/go/to/home/E>). 2011.
- [176] Yuan L. A scientist's perspective on experience with SRI in China for raising the yields of super hybrid rice. Assessments of the System of Rice Intensification Cornell International Institute of Food, Agriculture, Development, Ithaca, NY. 23–5.
- [177] National Bureau of Statistics of China. China Statistical Yearbook 2010. (<http://www.stats.gov.cn/tjsj/ndsj/2012/indexeh.htm>). 2011.
- [178] Lal R. World crop residues production and implications of its use as a biofuel. *Environ Int* 2005;31:575–84.
- [179] Bomgardner MM. Chasing cheap feedstocks. *Chem Eng News* 2013;91:11–5.
- [180] Li Z-J, Lin P, He J-Y, Yang Z-W, Lin Y-M. Silicon's organic pool and biological cycle in moso bamboo community of Wuyishan biosphere reserve. *J Zhejiang Univ Sci B* 2006;7:849–57.
- [181] Johansson T, Karačić A. Increment and biomass in hybrid poplar and some practical implications. *Biomass Bioenergy* 2011;35:1925–34.
- [182] DeBell DS, Clendenen GW, Harrington CA, Zasada JC. Tree growth and stand development in short-rotation *Populus* plantings: 7-year results for two clones at three spacings. *Biomass Bioenergy* 1996;11:253–69.
- [183] Heaton EA, Dohleman FG, Long SP. Meeting US biofuel goals with less land: the potential of Miscanthus. *Global Change Biol* 2008;14:2000–14.
- [184] Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W. Miscanthus: European experience with a novel energy crop. *Biomass Bioenergy* 2000;19:209–27.
- [185] Zhang R, Yang H, Bao B, Wang J, Zhang L. Comparison of hay yields accumulated in different years among eight Alfalfa varieties. *Crops* 2010;2010:78–81.