Energy &
Environmental Science

Cite this: DOI: 10.1039/c0ee00069h

www.rsc.org/ees

ANALYSIS

Analysis of biofuels production from sugar based on three criteria: Thermodynamics, bioenergetics, and product separation†

Wei-Dong Huang[ab] and Y.-H. Percival Zhang[*acd]

Received 9th May 2010, Accepted 2nd December 2010
DOI: 10.1039/c0ee00069h

We compare the production of four biofuels – ethanol, butanol, fatty acid ethyl ester (palmitate ethyl ester, PEE), and hydrogen from renewable carbohydrate (glucose) based on the energy-retaining efficiency that is greatly influenced by thermodyanimcs, bioenergetics, and product separation. Ethanol and butanol are produced in anaerobic fermentations; PEE is produced in semi-aerobic fermentation; hydrogen is produced by cell-free synthetic enzymatic pathway biotransformation (SyPaB), where enzymes are produced from carbohydrate by microbial fermentations. A decreasing order in theoretical energy efficiency determined by thermodynamics is hydrogen, ethanol, butanol, and PEE. Bioenergetics analysis suggests that a small fraction of carbohydrate (*e.g.*, 5–15%) is allocated to the synthesis of cell mass in anaerobic fermentations (*e.g.*, ethanol and butanol), a significant fraction (*e.g.*, 20–30% or higher) has to be allocated to the synthesis of cell mass for semi-aerobic fermentations (*e.g.*, PEE production), and a very small fraction (*e.g.*, less than 1%) is used to produce the enzyme mixtures. A decreasing order in product separation energy is hydrogen, secreted PEE, ethanol, butanol, and intracellular PEE. Hydrogen production by SyPaB would be most appealing because its energy-retaining efficiency is ~49% higher than ethanol, ~55% higher than butanol, and ~87% higher than PEE, even without considering higher hydrogen-fuel cell efficiency than those of biofuel-internal combustion engines. Our analysis suggests that it may be difficult to produce some advanced biofuels economically through aerobic fermentations due to low energy efficiency, as compared to ethanol, butanol, and hydrogen.

[a]*Biological Systems Engineering Department, Virginia Polytechnic Institute and State University (Virginia Tech), 210-A Seitz Hall, Blacksburg, Virginia, 24061, USA. E-mail: ypzhang@vt.edu; Fax: (+540) 231-3199; Tel: (+540) 231-7414*
[b]*Environmental Division, College of Earth and Space Science, University of Science and Technology of China, Hefei, China 230026*
[c]*Institute for Critical Technology and Applied Science (ICTAS), Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, USA*
[d]*DOE BioEnergy Science Center (BESC), Oak Ridge, Tennessee, 37831, USA*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c0ee00069h

1. Introduction

The production of transportation fuels from renewable energy sources is one of the most important challenges for the sustainable development of humankind. For the transportation sector, several special requirements have been met, for example, high energy storage capacity in a small container (*e.g.*, ~50 liters), high power output (*e.g.*, ~20–100 kW per vehicle), affordable fuel costs (*e.g.*, $ ~ 20/GJ), affordable vehicles, low costs for rebuilding the relevant infrastructure, fast charging or refilling of

Broader context

Biotransformation mediated by microorganisms or enzymes have comparative advantages over chemical catalysis, such as low energy consumption, modest reaction condition, high selectivity, and low costs of bioreactor. But microbial fermentation or enzyme production must consume a significant fraction of carbohydrate for the production of biocatalysts so to decrease potential yield of the desired product. Numerous biofuels can be produced from sugar, such as ethanol, butanol, long-chain alcohols, fatty acid esters, wax, alkanes, hydrogen, electricity, and so on. Some can be easily separated from broth or cells but some is not; some has higher energy densities than others. Which is (and is not) vital to developing advanced biofuels is a key question for future biofuels R&D. When more than 50% of the combustion energy in sugar (*e.g.*, 0.18/kg or $10.8/GJ) is lost during its bioconversion and separation, the production of such biofuel may be economically prohibited. Since aerobic fermentation consumes a significant fraction of carbohydrate to the synthesis of cell mass, there are great challenges in the economical production of biofuels through aerobic fermentations.

the fuel (*e.g.* several minutes per time), safety, and so on. Therefore, it is believed that future transportation fuels will mainly consist of biofuels (*e.g.*, ethanol, butanol, hydrogen) for most light-duty vehicles, electricity for a small fraction of light-duty vehicles for short-distance commuters, and high energy density liquid biofuels (*e.g.*, butanol, fatty acid esters and hydrocarbons) for jet planes.[1,2] Most future transportation fuels will be produced from renewable non-food biomass because lignocellulose is the most abundant and low-cost renewable bioresource.[3–5] Such transition from the fossil fuel-based economy to the carbohydrate economy would bring benefits to the economy, environment, and national energy security.[3,6] An increased biomass energy conversion efficiency means decreasing the impacts of both the carbon footprint and the water footprint on the environment.[7]

Different scenarios of biofuels production have been proposed starting from plant biomass or eventually from solar energy (Fig. 1). Through biomass sugar platform, potential biofuels include cellulosic ethanol, butanol, fatty acid esters (ester-diesel), hydrogen, methane, alkane, and more. Through syngas made by a thermochemical pathway, potential biofuels are ethanol, hydrogen, methanol, dimethylether, FT-diesel, electricity, and so on. The scope of this analysis is restricted to biotransformation from sugars to biofuels (Fig. 1) because the production of biofuels through biotransformation mediated by microorganisms or enzymes has numerous advantages over catalysis, such as high energy efficiency, high chemical selectivity (*i.e.*, high yield of desired product), modest reaction condition, low-cost of bioreactor, and so on. Also, sugar platform has great flexibility for producing other value-added biochemicals, such as isoprene, lactic acid, acetic acid, in the future.

Although ethanol is produced on a large scale, numerous advanced biofuels are being investigated, such as hydrogen,[8–10] butanol and long chain alcohols,[11–13] electricity,[14–16] fatty acid esters,[17–19] waxes,[17,20,21] and so on. Numerous perspective papers[5,20–25] and research papers[11,17,26,27] have been published in high-profile journals, but which biofuel will become predominant in the future is in debate.[28–33] Since thermodynamics drives economics in commodity production,[34] it is vital to compare energy-retaining efficiency, a combustion energy ratio of the separated biofuel to sugar through biotransformation and separation.

The production of biofuels from non-food biomass involves numerous steps: biomass growing, harvesting, and collection; lignocellulose pretreatment/fractionation; biomass saccharification; carbohydrate fermentation/biotransformation to a desired biofuel; biofuel separation; waste treatment; and system integration (Fig. 1). Numerous research papers have been published based on the whole life cycle analysis.[28,31–33] Since the results of these analyses are based on a number of assumptions, controversies arise pertaining to whether such assumptions are solid or the input data are out-of-date.[33,35,36] To avoid the uncertainties of some inputs in upstream steps, such as biomass growing and collection, fertilizer and pesticide consumption, and biomass saccharification, our small-scale (not life cycle) analysis would help identify the key limiting step for biofuels R&D, evaluate risks/benefits of potential biofuels R&D at the beginning stage, and focus the limited funding resource to more important projects. Thermodynamics of the the biochemical pathway determines a theoretical yield of biofuel without synthesis of biocatalyst; bioenergetics determines a maximum practical biofuel yield through biotransformation because a fraction of the carbohydrate is allocated (consumed) to synthesis of the biocatalyst; product separation energy determines a fraction of energy spent for the production of useable biofuels.

In this analysis, we assess the energy-retaining efficiency of four biofuels from biomass carbohydrate. They are liquid water miscible ethanol and butanol, liquid water immiscible fatty acid esters, and gaseous hydrogen. Among them, ethanol is the gold reference for assessing other potential "advanced" biofuels. The same methodology can be applied to other advanced biofuels. The advantages and limitations of different biofuels and their future applications through internal combustion engine or fuel cell/electric motor are not discussed here, since several excellent papers and analysis have covered this topic.[4,20,21,25,37] Based on the above three criteria alone without complicated techno-economical models that are based on numerous assumptions and uncertain inputs,[5,38–40] we suggested re-considering feasibility of some advanced biofuels R&D by using the above three criteria at the beginning, followed by more detailed techno-economical analysis and life cycle analysis.

2. Biochemical pathway for biofuel production

Ethanol, a high-octane liquid fuel blend, can be produced through microbial anaerobic fermentation by numerous microorganisms, such as *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Under anaerobic conditions, *S. cerevisiae* can convert one glucose to two pyruvate, two ATP, and two NADH through the glycolysis (Embden-Meyerhof) pathway (eqn (1) & Fig. 2)

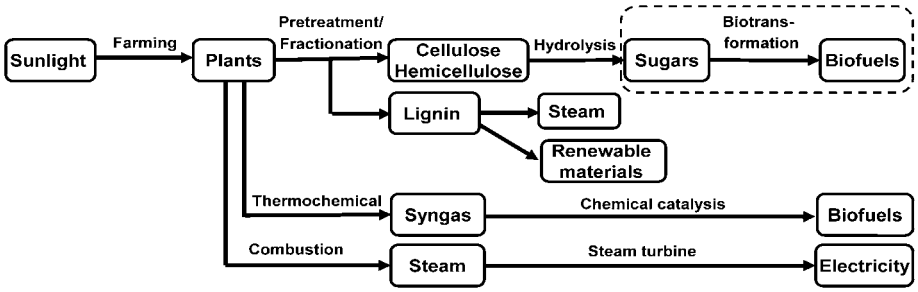

Fig. 1 Different pathways for biofuels production from lignocellulosic biomass. The current analysis focuses on the sugar-to-biofuels biotransformation in the dotted line box for the production four types of representative biofuels.

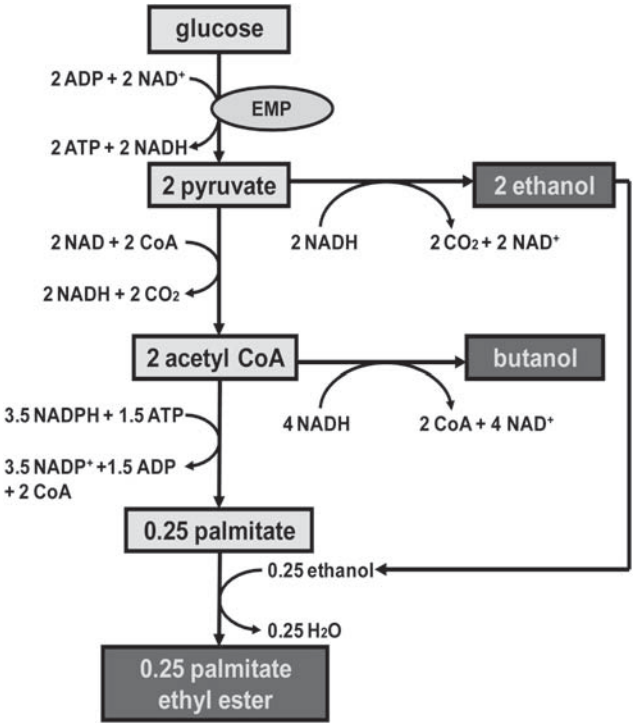

Fig. 2 Pathways of ethanol (by yeast), butanol, and palmitate ethyl ester (PEE) production from glucose.

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i + 2\ \text{NAD}^+ \rightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+ + 2\ \text{H}_2\text{O} \quad (1)$$

Pyruvate can be converted to acetaldehyde by pyruvate decarboxylase and then to ethanol with consumption of NADH mediated by alcohol dehydrogenase. The overall ethanol fermentation mediated by *S. cerevisiae* can produce two ethanol and two ATP from one glucose with a balanced NADH (Table 1). In contrast, *Z. mobilis* can implement the same chemical reaction with only one net ATP generated through the Entner-Doudoroff pathway.[41,42]

Butanol, a four-carbon alcohol, has several advantages over ethanol, such as higher energy density, more hydrophobicity, and lower evaporation heat, but its production suffers from low yield and low titer.[43,44] Acetone, butanol, and ethanol (ABE) fermentation converts glucose to two pyruvates through glycolysis, and then to acetyl CoA and NADH mediated by pyruvate-ferredoxin oxidoreductase (eqn (2)).[43] Two acetyl CoA are linked together to one acetoactyl-CoA and then converted to one butanol mediated by a number of biohydrogenation reactions (eqn (3)).[43] The overall butanol fermentation can produce one butanol per glucose along with two moles of ATP (Table 1).

$$\text{pyruvate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \quad (2)$$

$$2\ \text{acetyl CoA} + 4\ \text{NADH} + 4\ \text{H}^+ \rightarrow \text{butanol} + 2\ \text{CoA} + 4\ \text{NAD}^+ \quad (3)$$

James Liao and his co-workers have re-designed the non-natural butanol-producing pathway through the amino acid synthesis pathway.[11] This new pathway has a balanced NADH and generates 2 ATP per glucose.

Fatty acid esters can be produced by linking free fatty acids and alcohols.[17,19] Fatty acids are a key component of the cell membrane, a primary metabolite that is directly involved in normal cells' growth, development, and reproduction. Fatty acids are synthesized from acetyl CoA through a series of condensation and reduction processes.[45] Taking palmitate (16-carbon saturated fatty acid) as an example, one molecule of palmitate requires 8 moles of acetyl CoA, 7 moles of ATP and 14 moles of NADPH (eqn (4)),[45,46] as shown in Fig. 2. Palimitate and ethanol can be converted to palmitate ethyl ester (PEE) mediated by acyltransferase (eqn (5)).

$$8\ \text{acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 6\ \text{H}^+ \rightarrow \text{palmitate} + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ \text{H}_2\text{O} + 7\ \text{ADP} + 7\ P_i \quad (4)$$

$$\text{palmitate} + \text{ethanol} \rightarrow \text{palmitate ethyl ester (PEE)} + \text{H}_2\text{O} \quad (5)$$

Therefore, a combination of eqn (1), (2), (4), and (5) results in eqn (6) for PEE production,

$$4.5\ \text{glucose} + 16\ \text{NAD}^+ + 14\ \text{NADPH} + 2\ \text{ADP} + 2P_i \rightarrow \text{PEE} + 16\ \text{NADH} + 14\ \text{NADP}^+ + 9\ \text{CO}_2 + 9\ \text{H}_2\text{O} + 2\ \text{ATP} \quad (6)$$

In order to achieve high-yield PEE production, it is vital to (1) balance NADH and NADPH by efficiently converting the reduced NADPH, which is mainly used for anabolism, to the

Table 1 Stoichiometric reactions for biofuels production and thermodynamic data

Reaction	ATP/mol mol^{-1}	NAD(P)H/mol mol^{-1}	$\Delta_r G°$/kJ mol^{-1}	$\Delta_r H°$/kJ mol^{-1}
Ethanol fermentation: $C_6H_{12}O_6$ (aq) → 2 C_2H_6O (l) + 2 CO_2 (g)	1-2	0	−236	−68
Butanol fermentation: $C_6H_{12}O_6$ (aq) → $C_4H_{10}O$ (l) + 2 CO_2 (g) + H_2O (l)	2	0	−273	−124
Palmitate ethyl ester (PEE): $C_6H_{12}O_6$ (aq) + 2/9 O_2 → 2/9 $C_{18}H_{36}O_2$ + 2 H_2O + 2 CO_2 (g)	4/9	4/9[a]	−524	−263
H_2 production by SyPaB: $C_6H_{10}O_5$ (aq) + 7 H_2O (l) → 12 H_2 (g) + 6 CO_2 (g)	0	0	−27	+628

[a] Assume that NADPH and NADH can be exchanged with an efficiency of 100%.

reduced NADH, which is mainly used for catabolism, and (2) consume the unbalanced reduced coenzymes through oxidative phosphorylation.

In nature, most hydrogen is biologically produced through anaerobic fermentation from carbohydrate.[47–50] One glucose can produce four dihydrogens, two acetic acids, and four ATP through mixed acid pathway fermentation (eqn (7)).[6] That is, the theoretical hydrogen yield through anaerobic fermentation is four, called "the Thauer limit".[50]

$$\text{glucose} + 4\ \text{ADP} + 4\ P_i \rightarrow 2\ \text{acetic acid} + 4\ H_2 + 2\ CO_2 + 4\ \text{ATP} \quad (7)$$

In order to break the Thauer limit, *in vitro* synthetic enzymatic pathways have been demonstrated for generating high-yield hydrogen from starch or cellulosic materials and water through synthetic pathway biotransformation (SyPaB).[8,9] As shown in Fig. 3, these synthetic pathways contain (i) production of glucose-1-phosphate (G-1-P) through a chain-shortening phosphorylation reaction catalyzed by α- or β-glucan phosphorylase (eqn (8)), (ii) conversion of G-1-P to glucose-6-phosphate (G-6-P) catalyzed by phosphoglucomutase (eqn (9)), (iii) pentose phosphate pathway along with the partial glycolysis and gluconegenesis pathways containing 10 enzymes for producing 12 NADPH and 6 CO_2 per G-6-P (eqn (10)), and (iv) hydrogen generation from NADPH catalyzed by hydrogenase (eqn (11)).

$$(C_6H_{10}O_5)_n + P_i \leftrightarrow (C_6H_{10}O_5)_{n-1} + \text{G-1-P} \quad (8)$$

$$\text{G-1-P} \leftrightarrow \text{G-6-P} \quad (9)$$

$$\text{G-6-P} + 12\ NADP^+ + 7\ H_2O \leftrightarrow 12\ NADPH + 12\ H^+ + 6\ CO_2 + P_i \quad (10)$$

$$12\ NADPH + 12\ H^+ \leftrightarrow 12\ H_2 + 12\ NADP^+ \quad (11)$$

The combination of eqn (8)–(11):

$$C_6H_{10}O_5 + 7\ H_2O \rightarrow 12\ H_2 + 6\ CO_2 \quad (12)$$

Clearly, no microorganism can implement the reaction (eqn (12)) because it does not generate any ATP or reduced cofactors for supporting the microorganism's growth and duplication.

3. Thermodynamics

The first law of thermodynamics teaches us that energy can be changed from one form to another, but cannot be created or destroyed. The second law of thermodynamics states that the entropy of an isolated system tends to increase over time, approaching a maximum value at equilibrium. The production of biofuel is a process by which the chemical energy stored in carbohydrate is converted to the chemical energy in a desirable biofuel. Because most chemical reactions are enthalpy-driven ($\Delta_rH^\circ < 0$), a fraction of the chemical energy of the reactant is dissipated to the environment as heat.

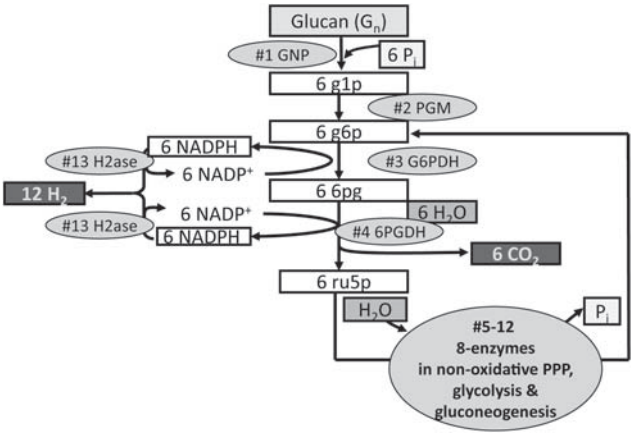

Fig. 3 Synthetic pathway for hydrogen production from glucan and water. PPP, pentose phosphate pathway. The enzymes are: GNP, glucan phosphorylase; PGM, phosphoglucomutase; G6PDH, G-6-P dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; and H2ase, hydrogenase. #5–12 enzyme are phosphoribose isomerase, ribulose 5-phosphate epimerase, transketolase, transaldolase, triose phosphate isomerase, aldolase, fructose-1, 6-bisphosphatase, and phosphoglucose isomerase. The metabolites and chemicals are: g1p, glucose-1-phosphate; g6p, glucose-6-phosphate; 6pg, 6-phosphogluconate; ru5p, ribulose-5-phosphate; and P_i, inorganic phosphate.

Table 1 shows the thermodynamics values of the four biofuel-producing processes. The negative values of standard Gibbs free energy suggest that all the reactions occur spontaneously. The reactions for the production of ethanol, butanol, and PEE have negative values of Δ_rH°, suggesting that a fraction of the chemical energy stored in carbohydrate is dissipated like most chemical reactions. Among the three, PEE fermentation is the least energy efficient, losing 10% of its combustion energy (Table 2). Hydrogen production through SyPaB is an entropy-driven reaction[8,9] because both products are gas and the gaseous products have much higher entropy values than those of the aqueous reactants. A positive Δ_rH° value of +628 kJ mol^{-1} suggests that this reaction can absorb a significant amount of (low-temperature) heat and convert it to chemical energy in the form of hydrogen. Such low-temperature heat can be accessed from the environment or any low-temperature waste heat

Table 2 Biofuel yield based on mole and weight as well as theoretical energy efficiency

	Formula	MW	Molar yield/mol mol^{-1}	$Y_{P/S}^{The}$/g g^{-1}	Combustion energy[a]/kJ	$\eta_{P/S}^{The}$[b]
Ethanol	C_2H_6O	46.07	2	0.511	2734	97.4%
Butanol	$C_4H_{10}O$	74.12	1	0.411	2676	95.3%
PEE	$C_{18}H_{36}O_2$	284.48	2/9	0.351	2526	90.0%
H_2	H_2	2.02	12	0.148	3430	122.1%

[a] combustion energy of biofuel produced from per mole of glucose. [b] a ratio of the combustion energy of biofuel divided by the combustion energy of glucose or its equivalent.

sources, for example, the proton exchange membrane fuel cells or waste hot water from power stations.

Table 2 shows the theoretical biofuel yields based on weight, mole, and energy efficiencies ($\eta_{P/S}^{The}$). One glucose unit can produce two ethanol, one butanol, 2/9 PEE, or 12 dihydrogen. Therefore, mass yields (w/w) are 0.511 (ethanol), 0.411 (butanol), 0.351 (PEE), and 0.148 (hydrogen). The energy efficiencies in decreasing order are 122.1% (hydrogen), 97.4% (ethanol), 95.3% (butanol), and 90.0% (PEE) (Table 2). Clearly, hydrogen generation by SyPaB is the most appealing because low-temperature waste heat is converted for generating hydrogen energy. Ethanol production has the second highest retaining efficiency, followed by butanol and PEE. PEE production has the lowest efficiency mainly because some reduced cofactors have been oxidized for keeping their balance (Table 1).

4. Bioenergetics

Carbohydrate is used as both a carbon source to support microorganism growth or enzyme production by microbial fermentation and as an energy source for biofuel production. Since fermentable carbohydrate from lignocellulosic biomass contains much lower organic nutrients as compared to those from corn kernels or sugarcane, a significant fraction of carbohydrate has to be allocated to the synthesis of microorganisms or enzymes. In laboratory experiments, yeast extract or peptone is often used as the source for both carbon and nitrogen sources for supporting the synthesis of cell mass. But they are too costly for industrial biofuel production.[51] Also, because carbohydrate cost accounts for more than 50% of the final price of low-value biofuel,[44,52] it is vital to estimate carbohydrate allocation to biocatalyst synthesis and biofuel production.

For a microbial fermentation, synthesis of cell mass can be estimated based on ATP generation.[53,54] Without any by-product and cell mass, a stoichiometric reaction for biofuel production based on glucose can be written as eqn (13) (see Table 1),

$$\text{glucose} \rightarrow Y_{P/S}^{The}\ (\text{biofuel}) + Y_{CO2/S}\ CO_2 + \text{b ATP} + \text{c NAD(P)H} \quad (13)$$

where $Y_{P/S}^{The}$, theoretical biofuel yield based on glucose in terms of mol biofuel/mol glucose (see their values in Table 2); $Y_{CO2/S}$, CO_2 yield based on glucose in terms of mol CO_2/mol glucose; and b and c are stoichiometric coefficients for ATP and NAD(P)H generation, in terms of mol mol^{-1} glucose, respectively. $c > 0$ for aerobic fermentation; $c = 0$ for anaerobic fermentations of ethanol and butanol.

When extra reduced coenzymes are produced in microbial biofuel fermentation, they can be oxidized for ATP generation. The overall net ATP generation is

$$\text{glucose} + \text{c/2 } O_2 \rightarrow Y_{P/S}^{The}\ (\text{biofuel}) + Y_{CO2/S}\ CO_2 + (\text{b} + 2.5\ \text{c})\ \text{ATP} \quad (14)$$

The synthesis of cell mass based on ATP plus a mole of glucose can be written as

$$\text{a glucose} + (\text{b} + 2.5\text{c})\ \text{ATP} \rightarrow \text{X cell mass} + \text{d } CO_2 \quad (15)$$

where a is mole of glucose consumption for the synthesis of cell mass when one mol glucose is converted to biofuel completely; X is mol C of cell mass synthesized, cell mass has a general formula of $CH_{1.8}O_{0.5}N_{0.2}$,[55] $MW_X = 24.6$, and the degree of reduction (γ_X) of cell mass = 4.2; and d is the stoichiometric coefficient for CO_2 production.

The combination of eqn (13) and 15 results in

$$(1 + \text{a})\ \text{glucose} + \text{c/2 } O_2 \rightarrow Y_{P/S}^{The}\ (\text{biofuel}) + \text{X cell mass} + (Y_{CO2/S} + \text{d})\ CO_2 \quad (16)$$

The value of X can be calculated as

$$X = \frac{(b + 2.5c)}{Y_{X/ATP}^{app} \cdot MW_X} \quad (17)$$

where $Y_{X/ATP}^{app}$is the apparent cell mass based on ATP (g cell mass/mol ATP).

The value of *a* (mol glucose) used for synthesis of the cell mass can be calculated as

$$a = X \cdot \frac{\gamma_X}{\gamma_S} \quad (18)$$

where γ_S is the degree of reduction of glucose, being 24.

Therefore, allocation of carbohydrate to cell mass ($A_{X/S}$, unitless) can be calculated as

$$A_{X/S} = \frac{a}{1 + a} \quad (19)$$

So, the maximum practical biofuel yield based on carbohydrate ($Y_{P/S}^{Max}$, mol biofuel/mol glucose) is calculated as

$$Y_{P/S}^{Max} = Y_{P/S}^{The} \cdot (1 - A_{X/S}) \quad (20)$$

In anaerobic ethanol fermentation, one mole of glucose can produce one mole of ATP through the Entner-Doudoroff pathway in *Z. mobilis*, two moles of ATP through the Embden-Meyerhof pathway in *S. cerevisiae*. In anaerobic fermentation, the maximum ATP gain is four moles of ATP.[50] In aerobic fermentations, one mole of glucose can generate 30–32 ATP through complete oxidation of glucose.[46] Semi-aerobic fermentations usually produce more ATP (*i.e.*, more cell mass) than anaerobic fermentations and less ATP than aerobic fermentations.[56,57]

$A_{X/S}$ values are estimated based on cell mass yield based on glucose ($Y_{X/S}$, g cell mass/g glucose) in the literature as

$$A_{X/S} = Y_{X/S} \cdot \frac{MW_S}{MW_X} \frac{\gamma_X}{\gamma_S} = 1.31 \cdot Y_{X/S} \quad (21)$$

where MW_S is the molecular weight of glucose, being 180.

Fig. 4 shows the effects of $Y_{X/ATP}^{app}$ changes on $A_{X/S}$ values for different fermentations from anaerobic fermentations (1, 2, or 4 ATP per glucose) to semi-aerobic (6 ATP per glucose) to aerobic fermentation (30 ATP per glucose). Although theoretic values of $Y_{X/ATP}$ may be as high as 30 g cell mass/mol ATP,[58] apparent $Y_{X/ATP}^{app}$ values might change greatly due to ATP consumption for maintenance and/or ATP dissipation.[53,59]

Vigorous aerobic fermentations that can produce approximately 30 ATP per glucose usually have $Y_{X/S}$ values from ~0.43 to 0.56 for single cell protein production[60–62] and aerobic waste

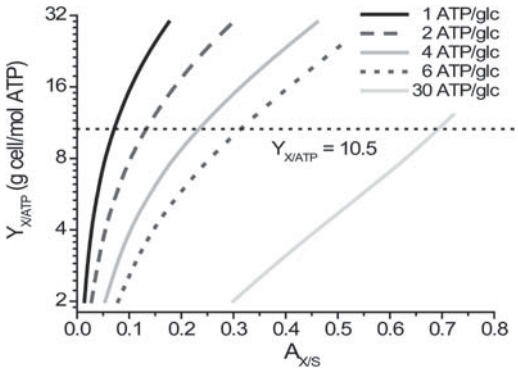

Fig. 4 Calculated allocation of sugar to cell mass ($A_{X/S}$) curves depending on $Y_{X/ATP}$ values for different fermentations with different ATP gains (1 ATP/glucose, *e.g.*, *Z. mobilis* anaerobic fermentation; 2 ATP/glucose, *S. cerevisiae* anaerobic fermentation; 4 ATP/glucose, acetic acid anaerobic fermentation; 6 ATP/glucose, semi-aerobic fermentation; and 30 ATP/glucose, aerobic fermentation).

water treatment.[63] Anaerobic ethanol fermentations by *Z. mobilis* (1 ATP/glucose) and *S. cerevisiae* (2 ATP/glucose) have $Y_{X/S}$ values of 0.024–0.038[64] and 0.11,[59] respectively. Typical anaerobic mixed acid fermentations that produce 2–4 ATP per glucose have $Y_{X/S}$ values from 0.10–0.15.[53,65] Semi-aerobic fermentations that can generate more ATP than anaerobic fermentation and less than vigorous aerobic fermentations have $Y_{X/S}$ values of 0.20–0.30.[56,66] The above values imply that $Y_{X/ATP}^{app}$ should be *ca.* 10.5 g cell mass/mol ATP (Fig. 4), as recommended in the literature.[54,55] According to this $Y_{X/ATP}^{app}$ value, Fig. 4 predicts that $A_{X/S}$ values for semi-aerobic PEE-producing fermentation might range from 0.23 to 0.31 or even higher.

In order to increase biofuel yield, a practical way is to decrease $Y_{X/ATP}^{app}$ values through optimizing fermentation process that can uncouple ATP generation and ATP consumption for synthesis of cell mass. For example, in Brazil high-titer ethanol fermentation is usually conducted in continuous fermentation with cell recycling.[67] High concentration ethanol inhibits cell growth by disrupting cellular membrane integrity,[68] resulting in ATP dissipation, less cell mass formation, and higher ethanol yield.[67,69] Although high-titer ethanol slows the cell-specific ethanol production rate, the much higher volumetric cell mass concentration through cell recycling results in higher volumetric productivity than that without cell recycling. In a batch operation for ethanol production, two-step fermentation is usually conducted. In the first step, oxygen is provided from fast synthesis of cell mass by consuming a small fraction of carbohydrate; in the second step, anaerobic fermentation is conducted for ethanol production without significant synthesis of cell mass. High-titer ethanol can nearly inhibit yeast growth at the end of fermentation, resulting in very low apparent $Y_{X/ATP}$. It is why the resting cell biotransformation usually has a very high product yield because almost no carbohydrate is allocated to synthesis of cell mass.[70] Similar, butanol fermentation can be conducted by a two-step fermentation for enhanced product yield. But it is notable that sometimes it may be difficult to uncouple ATP production and ATP consumption, especially for the production of PEE because their production is (partially) associated with cell growth and dissolved oxygen should be controlled precisely for preventing over-synthesis of cell mass in this semi-aerobic fermentation.

Increasing biofuel yield can be implemented by (1) decreasing formation of by-products by metabolic engineering, (2) decreasing carbohydrate allocation to synthesis of biocatalysts (microorganisms or enzymes), and (3) generating less ATP by selecting the special pathway. For example, *Z. mobilis* has higher ethanol yield than *S. cerevisiae*.[71]

For SyPaB, allocation of carbohydrate to the production of the enzyme mixture depends on two important factors: weight-based total turnover number of enzymes, and their recombinant protein yield based on carbohydrate ($Y_{P/S}$, g protein/g glucose).[70] Higher $Y_{P/S}$ results in lower carbohydrate allocation to enzymes, and typical values of recombinant protein yield in *E. coli* range from 0.01 to 0.2. Our analysis suggests that when all enzymes reach a threshold TTN_W value of ~100,000, less than 1% of sugar is allocated to the enzyme mixtures regardless of $Y_{P/S}$ values from 0.01 to 0.2.[70]

5. Product separation

Biofuels must be separated from the aqueous fermentation broths before their applications. Four biofuels can be classified into three groups: (1) liquid ethanol and butanol, which are miscible with water; (2) liquid PEE, which is immiscible in water; and (3) gaseous hydrogen mixed with another gas – CO_2.

Ethanol in a fermentation broth is usually separated by distillation followed by dehydration through a molecular sieve. Fig. 5 shows the effects of ethanol concentration on distillation energy spending.[72] Increasing ethanol concentration greatly decreases its separation costs, especially when ethanol concentration is lower than 4% w/w, a critical value of ethanol separation. When ethanol concentration is lower than this value, regular distillation cannot economically separate ethanol. Four percentage of ethanol separation requires an energy input of 35% of its combustion energy. When ethanol concentration is 12%, distillation energy equals 12.6% of its combustion energy. At the same alcohol mass concentration, energy consumption for butanol separation is lower than for ethanol.[73] But butanol fermentation titers are 1.5–2%,[11,74] much lower than those of ethanol (4%–12%), resulting in higher butanol separation spending.[75]

Fatty acids or their ethyl esters are immiscible in water so that they are easily separated by a liquid/liquid separator. But fatty acids or esters may be stored in cells or be secreted across the cellular membrane to the fermentation broth. Separation of intracellular fatty acids from microorganisms is like that from microalgae, including numerous sequential steps: centrifugation/filtration, dehydrogenation, and oil extraction. Oil separation from algae is energy intensive, requiring energy input ~27.9 MJ kg^{-1} product, accounting for *ca.* 71% of the combustion energy of the product.[76] An alternative way is to secrete water-immiscible fatty acids or esters into the aqueous broth, so that the energy input for oil/water separation by centrifugation is estimated to be *ca.* 0.62% of the energy of the products.[77] But the secretion mechanism of fatty acids is not clear. Also it is important to study the effects of high concentration fatty acids on the host's growth and product formation because nonpolar

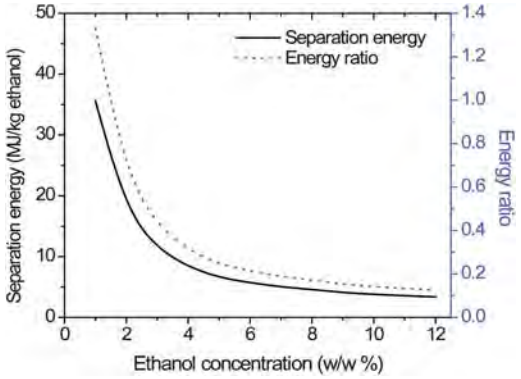

Fig. 5 Relationship between ethanol distillation energy and ethanol concentration based on the data.[72]

solvents (PEE) may hurt nonpolar compounds (*e.g.*, cell membrane).

The gas produced by SyPaB contains 66.7% H_2 and 33.3% CO_2.[8,9] Hydrogen and CO_2 can be separated by membrane technology, pressure swing adsorption (PSA), or a hybrid of both.[78] Separation cost of H_2 and CO_2 is estimated to be comparable to that for methane and CO_2 (*i.e.*, ~4.8% loss).[79] Since produced hydrogen is high purity, mixed with an inert gas –CO_2, this hydrogen/CO_2 mixture can be directly used by PEM fuel cells with a slight loss in fuel cell efficiency (*ca.* 1%), estimated by thermodynamics calculation.

6. Overall energy-retaining efficiency

The overall energy retaining efficiency from sugar to the purified biofuel (η) can be calculated as

$$\eta = \eta_{P/S}^{The} \cdot (1 - A_{X/S}) \cdot R \cdot (1 - \eta_S) \quad (22)$$

where $\eta_{P/S}^{The}$ is theoretical biofuel efficiency based on sugar (unitless); *R*is a ratio of carbohydrate to the desired biofuel relative to a sum of the biofuel and other by-products, which can be enhanced by metabolic engineering;[24] and $\eta_S$is energy loss for the product separation (see Section 5).

Fig. 5 shows the energy-retaining efficiencies for ethanol, butanol, PEE, and hydrogen from biomass carbohydrate. Based on current technologies, ethanol has the highest energy efficiency (*ca.* 72%) among three liquid biofuels, which can explain why only ethanol can be produced economically. For butanol fermentation, two major obstacles need be solved – (i) increasing the butanol titer so as to reduce butanol separation energy loss and (ii) increasing butanol yields based on carbohydrate. For PEE fermentation, it is obvious that it is nearly economically infeasible to produce it intracellarly because of its intrinsic high separation energy. In order to economically produce PEE, PEE must be secreted across the cell membrane and the R value must be as high as ~0.9. Current PEE technologies are far from commercialization feasible because of low R and high $A_{X/S}$. Through intensive efforts in synthetic biology, the overall efficiency of PEE would be 64%, much lower than future scenarios for ethanol (81%) and butanol (78%), because a significant fractionation of carbohydrate has been consumed for the synthesis of cell mass (*i.e.*, ~0.20, see Fig. 4). If this η value of PEE (64%) is reached, PEE production would be economically viable, partially competitive with ethanol and butanol. That is, PEE final production cost is projected to be *ca.* $ ~ 20/GJ, based on sugar costs (0.18/kg, $10.6/GJ).[4,44] Different from the three liquid biofuels, hydrogen production by SyPaB has the highest energy-retaining efficiency (101%) and would have a projected efficiency of ~121% after technology improvement (Fig. 6). The two major obstacles to hydrogen production are costs of enzymes and coenzymes and their lifetime because the enzymes cannot be self-renewed. These obstacles are being addressed.[44,80] SyPaB technology has its clear advantages: high product yield, fast reaction rate, high energy-retaining efficiency, easy process control, engineering flexibility, *etc.*[44] In fact, discovery of thermoenzymes that have TTNs > 10 million are highly doable for industrial biotransformation, for example, *Clostridium thermocellum* phosphoglucomutase,[81] *Thermotoga maritima* 6-phosphogluconate dehydrogenase,[82] *etc.* In the food industry, immobilized thermostable glucose isomerase has reached a TTN value of more than 250 million.[83] Also, an immobilized thermophilic enzyme – *C. thermocellum* phosphoglucose isomerase on the surface of a cellulosic material – has exhibited TTN values of 1,000 million (submitted).

In this analysis, we compare the production of four biofuels from biomass sugars and provide the upper limits of their energy-retaining efficiency. Only ethanol is economically produced now mainly due to its highest η value. Two major obstacles to butanol production – low butanol yield and low titer – are being solved.[37] For PEE fermentation, more obstacles must be solved: (i) efficient secretion of PEE across the cellular membrane, (ii)

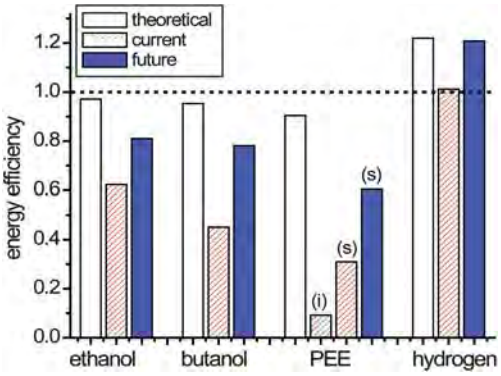

Fig. 6 Theoretical, current, and future energy-retaining efficiencies for the production of ethanol, butanol, PEE, and hydrogen based on biofuel yield relative to by-products, allocation of carbohydrate to biocatalysis synthesis, and biofuel separation loss. Current and future R ratios are 96% and 99% for ethanol, 70% and 95% for butanol, 70% and 90% for PEE, and 95% and 100% for hydrogen, respectively. Current and future $A_{X/S}$ values are 10% and 2% for ethanol, 12% and 2% for butanol, 50% and 20% for PEE, and 10% and 0.1% for hydrogen, respectively. The η_S values are 14% for 12% (w/w) ethanol (current and future); 23.3% and 12% for 2% (current) and 5% butanol (future), respectively; 71% for intracellular (i) PEE (current), 2% for secretory (s) PEE (current), 1% for secretory PEE (future); 3% for hydrogen (current), and 0.85% for hydrogen (future). More data for the efficiency calculation is available in the supporting materials (Table S1).†

a dramatic increase in PEE yield (*i.e.*, R value), (iii) a good balance between NADH regeneration and NADPH consumption, (iv) a narrow distribution of PEE chain length, (v) precise control of dissolved oxygen levels for semi-aerobic fermentation to avoid over-synthesis of the cell mass (*i.e.*, lower $A_{X/S}$), and (vi) good mixing and careful aeration in large bioreactors. Because energy efficiency is the most important criterion for energy assessment according to the International Energy Agency, hydrogen production by SyPaB would be most appealing because its energy-retaining efficiency is 49% higher than ethanol, 55% higher than butanol, and 87% higher than PEE, even without considering much higher conversion efficiency for hydrogen fuel cells than internal combustion engines for liquid biofuels. The similar analysis would be applied to the production of other "advanced" biofuels.

Nomenclature

a	mole of glucose consumption for the synthesis of cell mass (mol glucose)
b, c, d	stoichiometric coefficients for ATP generation, NAD(P)H generation, and CO_2 generation, mol mol^{-1} glucose
X	stoichiometric coefficient for cell mass (mol C) synthesis
$A_{X/S}$	allocation of carbohydrate to cell mass, unitless
MW_S	molecular weight of glucose, 180 g mol^{-1} glucose
MW_X	molecular weight of cell mass ($CH_{1.8}O_{0.5}N_{0.2}$), 24.6 g mol^{-1} cell mass
R	ratio of glucose to the desired biofuel relative to a sum of the biofuel and other by-products.
$Y_{P/S}^{The}$	theoretical biofuel yield based on glucose, in terms of mol biofuel/mol glucose
$Y_{X/ATP}^{app}$	apparent cell mass based on ATP (g cell mass/mol ATP)
$Y_{P/S}^{Max}$	maximum practical biofuel yield based on glucose, mol biofuel/mol glucose
$Y_{CO2/S}$	CO_2 yield based on glucose in terms of g CO_2/g glucose
γ_S	degree of reduction of glucose, unitless, being 24
γ_X	degree of reduction of cell mass, unitless, being 4.2
η_S	energy loss for the product separation, unitless
η	overall energy-retaining efficiency, unitless
$\eta_{P/S}^{The}$	theoretical biofuel efficiency based on sugar, unitless

Subscript

X	cell mass
S	substrate (carbohydrate, glucose)
P	product (biofuel)

Acknowledgements

This work was supported mainly by the DOE Bioenergy Science Center (BESC), partially by the Air Force Office of Scientific Research and MURI, the USDA Biodesign and Bioprocess Center, ICTAS, and DuPont Young Faculty Award.

References

1 Y.-H. P. Zhang, *Int. J. Hydrogen Energy*, 2010, **35**, 10334–10342.
2 C. E. Thomas, *Int. J. Hydrogen Energy*, 2009, **34**, 6005–6020.
3 Y.-H. P. Zhang, *J. Ind. Microbiol. Biotechnol.*, 2008, **35**, 367–375.
4 Y.-H. P. Zhang, *Energy Environ. Sci.*, 2009, **2**, 272–282.
5 L. R. Lynd, M. S. Laser, D. Bransby, B. E. Dale, B. Davison, R. Hamilton, M. Himmel, M. Keller, J. D. McMillan, J. Sheehan and C. E. Wyman, *Nat. Biotechnol.*, 2008, **26**, 169–172.
6 L. R. Lynd, P. J. Weimer, W. H. van Zyl and I. S. Pretorius, *Microbiol. Mol. Biol. Rev.*, 2002, **66**, 506–577.
7 R. Dominguez-Faus, S. E. Powers, J. G. Burken and P. J. Alvarez, *Environ. Sci. Technol.*, 2009, **43**, 3005–3010.
8 X. Ye, Y. Wang, R. C. Hopkins, M. W. W. Adams, B. R. Evans, J. R. Mielenz and Y.-H. P. Zhang, *ChemSusChem*, 2009, **2**, 149–152.
9 Y.-H. P. Zhang, B. R. Evans, J. R. Mielenz, R. C. Hopkins and M. W. W. Adams, *PLoS One*, 2007, **2**, e456.
10 T. Maeda, V. Sanchez-Torres and T. K. Wood, *Microb. Biotechnol.*, 2008, **1**, 30–39.
11 S. Atsumi, T. Hanai and J. C. Liao, *Nature*, 2008, **451**, 86–89.
12 S. Atsumi, A. F. Cann, M. R. Connor, C. R. Shen, K. M. Smith, M. P. Brynildsen, K. J. Y. Chou, T. Hanai and J. C. Liao, *Metab. Eng.*, 2008, **10**, 305–311.
13 K. Zhang, M. R. Sawaya, D. S. Eisenberg and J. C. Liao, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 20653–20658.
14 S. K. Chaudhuri and D. R. Lovley, *Nat. Biotechnol.*, 2003, **21**, 1229–1232.
15 D. R. Lovley, *Curr. Opin. Biotechnol.*, 2008, **19**, 564–571.
16 B. E. Logan, *Nat. Rev. Microbiol.*, 2009, **7**, 375–381.
17 E. J. Steen, Y. Kang, G. Bokinsky, Z. Hu, A. Schirmer, A. McClure, S. B. del Cardayre and J. D. Keasling, *Nature*, 2010, **463**, 559–562.
18 X. Lu, H. Vora and C. Khosla, *Metab. Eng.*, 2008, **10**, 333–339.
19 R. Kalscheuer, T. Stolting and A. Steinbuchel, *Microbiology*, 2006, **152**, 2529–2536.
20 J. L. Fortman, S. Chhabra, A. Mukhopadhyay, H. Chou, T. S. Lee, E. Steen and J. D. Keasling, *Trends Biotechnol.*, 2008, **26**, 375–381.
21 M. A. Rude and A. Schirmer, *Curr. Opin. Microbiol.*, 2009, **12**, 274–281.
22 T. Liu and C. Khosla, *Annu. Rev. Genet.*, 2010, **44**.
23 H. Li, A. F. Cann and J. C. Liao, *Annu. Rev. Chem. Biomol. Eng.*, 2010, **1**, 19–36.
24 H. Alper and G. Stephanopoulos, *Nat. Rev. Microbiol.*, 2009, **7**, 715–723.
25 S. K. Lee, H. Chou, T. S. Ham, T. S. Lee and J. D. Keasling, *Curr. Opin. Biotechnol.*, 2008, **19**, 556–563.
26 A. Schirmer, M. A. Rude, X. Li, E. Popova and S. B. del Cardayre, *Science*, 2010, **329**, 559–562.
27 A. J. Shaw, K. K. Podkaminer, S. G. Desai, J. S. Bardsley, S. R. Rogers, P. G. Thorne, D. A. Hogsett and L. R. Lynd, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 13769–13774.
28 J. E. Campbell, D. B. Lobell and C. B. Field, *Science*, 2009, **324**, 1055–1057.
29 Y.-H. P. Zhang and J. A. Rollin, *Nature Precedings*, 2009, **2009**, 3725.3721.
30 R. Melamu and H. von Blottnitz, *Int. J. Hydrogen Energy*, 2009, **34**, 1126–1134.
31 Z. Hu, G. Pu, F. Fang and C. Wang, *Renewable Energy*, 2004, **29**, 2183–2192.
32 J. Goldemberg, *Science*, 2007, **315**, 808–810.
33 A. E. Farrell, R. J. Plevin, B. T. Turner, A. D. Jones, M. O'Hare and D. M. Kammen, *Science*, 2006, **311**, 506–508.
34 V. Smil, *Energy in Nature and Society*, MIT Press, Cambridge, MA, 2008.
35 T. Searchinger, R. Heimlich, R. A. Houghton, F. Dong, A. Elobeid, J. Fabiosa, S. Tokgoz, D. Hayes and T.-H. Yu, *Science*, 2008, **319**, 1238–1240.

36 T. D. Searchinger, S. P. Hamburg, J. Melillo, W. Chameides, P. Havlik, D. M. Kammen, G. E. Likens, R. N. Lubowski, M. Obersteiner, M. Oppenheimer, G. Philip Robertson, W. H. Schlesinger and G. David Tilman, *Science*, 2009, **326**, 527–528.
37 M. R. Connor and J. C. Liao, *Curr. Opin. Biotechnol.*, 2009, **20**, 307–315.
38 T. L. Richard, *Science*, 2010, **329**, 793–796.
39 T. Eggeman and R. T. Elander, *Bioresour. Technol.*, 2005, **96**, 2019–2025.
40 J. Hill, E. Nelson, D. Tilman, S. Polasky and D. Tiffany, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 11206–11210.
41 T. W. Jeffries, *Nat. Biotechnol.*, 2005, **23**, 40–41.
42 M. Zhang, C. Eddy, K. Deanda, M. Finkestein and S. Picataggio, *Science*, 1995, **267**, 240–243.
43 D. T. Jones and D. R. Woods, *Microbiol. Rev.*, 1986, **50**, 484–524.
44 Y.-H. P. Zhang, *Biotechnol. Bioeng.*, 2010, **105**, 663–677.
45 J. M. Berg, J. L. Tymoczko and L. Stryer, *Biochemistry* (fifth edition), W.H. Freeman & Co., New York, 2002.
46 D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry (5th edition)*, WH Freeman, New York, 2008.
47 W. Lubitz, E. J. Reijerse and J. Messinger, *Energy Environ. Sci.*, 2008, **1**, 15–31.
48 R. M. Navarro, M. C. Sánchez-Sánchez, M. C. Alvarez-Galvan, F. d. Valle and J. L. G. Fierro, *Energy Environ. Sci.*, 2009, **2**, 35–54.
49 A. Sartbaeva, V. L. Kuznetsov, S. A. Wells and P. P. Edwards, *Energy Environ. Sci.*, 2008, **1**, 79–85.
50 K. Thauer, K. Jungermann and K. Decker, *Bacteriol. Rev.*, 1977, **41**, 100–180.
51 B. E. Wood, L. P. Yomano, S. W. York and L. O. Ingram, *Biotechnol. Prog.*, 2005, **21**, 1366–1372.
52 L. R. Lynd, C. E. Wyman and T. U. Gerngross, *Biotechnol. Prog.*, 1999, **15**, 777–793.
53 Y.-H. P. Zhang and L. R. Lynd, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 7321–7325.
54 M. Shuler and F. Kargi, *Bioprocess Engineering: Basic Concepts* (2nd Ed.), Prentice-Hall 2001.
55 E. T. Papoutsakis, *Biotechnol. Bioeng.*, 1984, **26**, 174–187.
56 T. Walther, P. Hensirisak and F. A. Agblevor, *Bioresour. Technol.*, 2001, **76**, 213–220.
57 M. F. S. Barbosa, M. B. de Medeiros, I. M. de Mancilha, H. Schneider and H. Lee, *J. Ind. Microbiol. Biotechnol.*, 1988, **3**, 241–251.
58 J. B. Russell and G. M. Cook, *Microbiol. Rev.*, 1995, **59**, 48–62.
59 J. J. Heijnen and J. P. Vandijken, *Biotechnol. Bioeng.*, 1992, **39**, 833–858.
60 K. C. Ivarson and H. Morita, *Appl. Environ. Microbiol.*, 1982, **43**, 643–647.
61 T. Hongpattarakere and A. H-Kittikun, *World J. Microbiol. Biotechnol.*, 1995, **11**, 607–609.
62 B. J. Abbott and A. Clamen, *Biotechnol. Bioeng.*, 1973, **15**, 117–127.
63 W. Gujer, M. Henze, T. Mino and M. C. M. van Loosdrecht, *Wat. Sci. Technol.*, 1999, **39**, 183–193.
64 O. Sreekumar, N. Chand and S. C. Basappa, *J. Biosci. Bioeng.*, 1999, **88**, 334–338.
65 Y.-H. P. Zhang and L. R. Lynd, *J. Bacteriol.*, 2005, **187**, 99–106.
66 D. K. Oh and S. Y. Kim, *Appl. Microbiol. Biotechnol.*, 1998, **50**, 419–425.
67 A. E. Wheals, L. C. Basso, D. M. G. Alves and H. V. Amorim, *Trends Biotechnol.*, 1999, **17**, 482–487.
68 L. O. Ingram and T. M. Buttke, *Adv. Microb. Physiol.*, 1984, **25**, 253–300.
69 C. E. Wyman, *Appl. Biochem. Biotechnol.*, 2001, **91–93**, 5–21.
70 Y.-H. P. Zhang, J.-B. Sun and J.-J. Zhong, *Curr. Opin. Biotechnol.*, 2010, **21**, 663–669.
71 F. W. Bai, W. A. Anderson and M. Moo-Young, *Biotechnol. Adv.*, 2008, **26**, 89–105.
72 E. Kvaalen, P. C. Wankat and B. A. McKenzie, AE-17. ALCOHOL DISTILLATION: BASIC PRINCIPLES, EQUIPMENT, PERFORMANCE RELATIONSHIPS, AND SAFETY http://www.ces.purdue.edu/extmedia/ae/ae-117.html.
73 C. R. Fischer, D. Klein-Marcuschamer and G. Stephanopoulos, *Met. Eng.*, 2008, **10**, 295–304.
74 T. C. Ezeji, N. Qureshi and H. P. Blaschek, *Chem. Rec.*, 2004, **4**, 305–314.
75 A. Friedl, N. Qureshi and I. S. Maddox, *Biotechnol. Bioeng.*, 1991, **38**, 518–527.
76 L. Lardon, A. Helias, B. Sialve, J. P. Stayer and O. Bernard, *Environ. Sci. Technol.*, 2009, **43**, 6475–6481.
77 J. Sheehan, V. Camobreco, J. Duffield, M. Graboski and H. Shapouri, *Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in an Urban Bus*, National Renewable Energy Laboratory, Golden, CO, 1998.
78 H. Yang, Z. Xu, M. Fan, R. Gupta, R. B. Slimane, A. E. Bland and I. Wright, *J. Environ. Sci.*, 2008, **20**, 14–27.
79 N. Johansson, MS thesis, Lund university, 2008.
80 J. D. Ryan, R. H. Fish and D. S. Clark, *ChemBioChem*, 2008, **9**, 2579–2582.
81 Y. Wang and Y.-H. P. Zhang, *J. Appl. Microbiol.*, 2010, **108**, 39–46.
82 Y. Wang and Y.-H. P. Zhang, *Microb. Cell Fact.*, 2009, **8**, 30.
83 D. Vasic-Racki, in *Industrial Biotransformations*, ed. A. Liese, S. Seebald and C. Wandrey, Wiley-VCH, KGaA, Weinheim, 2006, pp. 1–37.