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REVIEW

Engineering biological systems toward a sustainable bioeconomy

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Abstract The nature of our major global risks calls for sustainable innovations to decouple economic growth from greenhouse gases emission. The development of sustainable technologies has been negatively impacted by several factors including sugar production costs, production scale, economic crises, hydraulic fracking development and the market inability to capture externality costs. However, advances in engineering of biological systems allow bridging the gap between exponential growth of knowledge about biology and the creation of sustainable value chains for a broad range of economic sectors. Additionally, industrial symbiosis of different biobased technologies can increase competitiveness and sustainability, leading to the development of eco-industrial parks. Reliable policies for carbon pricing and revenue reinvestments in disruptive technologies and in the deployment of eco-industrial parks could boost the welfare while addressing our major global risks toward the transition from a fossil to a biobased economy.

Keywords Synthetic biology · Metabolic engineering · Carbon pricing · Sustainability · Bioeconomy

Introduction

> "The best way to predict your future is to create it"—Peter Drucker

We have always lived in a world full of uncertainties and concerns. However, current global interconnectivity also implies higher systemic risks. According to the World Economic Forum, the major global risks are related to unemployment, water crises, severe income disparity, and failure in climate change mitigation as well as fiscal crises in key economies [135]. Economic growth and technological innovations have lifted millions of people out of poverty, raised living standards and erased boundaries between countries and businesses through innovations in communication and transportation. However, they may be undermining their own foundations through the negative side effects on ecosystems, biodiversity and climate. Our economic growth model is linked to the energy consumption, which is linked to increase in water consumption, waste production, greenhouse gas (GHG) emissions and other environmental burdens. In fact, historically, for every 1 % increase in GDP, resource usage has risen on average 0.4 % [89]. Economic development as we know it and resource scarcity are on a collision course. In order to change this dramatic equation, the systemic nature of our most significant global risks calls for innovations and business models that can sustain economic growth without the current environmental drawbacks.

Shifting from oil-based economy to a biobased economy, or simply bioeconomy, can have an impact not only on climate mitigation but also on the other social and economic risks. The bioeconomy growth is closely linked to the evolution of the biotechnology industry. The ability to engineer biological systems has been possible due to scientific breakthroughs leading to the emerging of disciplines such

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as metabolic engineering and synthetic biology. Synthetic biology is the 'design and construction of new biological systems that do not exist in nature through the assembly of well-characterized, standardized and reusable components' [113]. Metabolic engineering is broadly defined as 'the development of methods and concepts for analysis of metabolic networks, typically with the objective of finding targets for engineering of cell factories' [113]. Both concepts, generally called engineering of biological systems, draw on a wide range of disciplines and methodologies to design new biological functions, assemble biological systems and increase their performance. Engineering of biological systems can lead to innovations and business models that can bridge the gap between exponential growth of knowledge about biology and the creation of a new sustainable value chain for a broad range of economic sectors including energy, chemicals, materials, water treatment, materials and agriculture. This article will discuss the challenges and opportunities faced by biotechnology to create sustainable solutions toward a global bioeconomy.

Petrochemistry: brief history and outlook

The fossil fuel industry includes the global processes of exploration, extraction, refining, transporting, chemical conversion of compounds and marketing fossil products. Fossil fuels are of great importance because they can be burned (oxidized to carbon dioxide and water), producing significant amounts of energy per unit weight. The use of fossil fuels is vital to the maintenance of industrial civilization in its current configuration, and thus a critical concern for any nation.

The energy industry and transportation sector are by far the most important consumers of fossil fuels. From a total of 12 billion tons of oil equivalent used every year, about 10 billion tons (calculated as oil equivalents) are used by those sectors and only 3 % of all fossil fuels are used in the chemical industry. The chemical industry utilizes raw materials derived from coal (e.g., syngas), fractions of the oil (e.g., naphtha) and natural gas (e.g., ethane or syngas from methane) for the production of broad range of chemicals [137]. The American Chemistry Council (ACC) divides the chemical industry into five main segments [2]: basic chemicals, pharmaceuticals, specialty chemicals, agrochemicals and consumer products. Basic chemicals include inorganic chemicals, polymers and plastics. Also called commodities chemicals, these chemicals are produced in large volumes. Pharmaceuticals include in vitro and other diagnostic substances, vaccines, plasmas and other biological products; vitamins and pharmaceutical preparations for both human and veterinary use. Specialty chemicals are low-volume, high-value compounds sold on the basis of performance, some examples include paint, adhesives, electronic chemicals, oilfield chemicals and flavors and fragrances; agrochemicals include fertilizers and crop protection chemicals, i.e., pesticides. Consumer products include soap, detergents, bleaches, laundry aids, toothpaste, shampoos, skin care products, cosmetics, deodorants, perfume, among others.

The world petrochemical industry has drastically changed in the last 20–30 years. The USA, Western Europe and Japan dominated over 80 % of world basic chemical production prior to 1980. However, world-scale construction of petrochemical facilities in countries with vast reserves of crude oil and natural gas (e.g., Saudi Arabia and Iran) has increased the supply and share of petrochemical production earmarked for the export market, while countries with rapidly growing populations like Thailand, Brazil, India, Malaysia, Indonesia and China were driven for self-sufficiency. As a consequence, USA, Western Europe and Japan accounted for only 37 % of world primary petrochemicals production in 2010. Regardless of the geopolitical structure in the industry, fossil fuels are responsible for more than 80 % of the total primary energy (TPE) and chemicals are a $3 trillion global enterprise (~5 % of world GDP) [137].

BOX 1: Oil and biorefinery value chains overview

The petrochemical industry value chain starts in the extraction of fossil fuels (e.g., offshore oil drilling). In the refinery, the raw materials are processed using different methods to separate their major fractions (e.g., natural gas is composed of mainly methane, ethane, propane and butane; oil is composed of a more complex mixture containing alkanes, naphthenic, aromatic and asphaltic compounds). Oil can be distilled in several fractions like LGP, naphtha and diesel. In a petrochemical site, naphtha can be steam cracked into lower olefins like ethylene, propylene and aromatics, named benzene, toluene and xylene. Those are called "basic petrochemicals" (Fig. 1). As to natural gas, methane can be separated for energy production and ethane can be converted by steam cracking into ethylene, by which almost no higher olefins or aromatics is formed.

Depending on the type of raw material (e.g., light naphtha, heavy naphtha or natural gas), the mix of "basic petrochemicals" will be different. For example, steam cracking of ethane produces 80 % of ethylene while steam cracking of naphtha will yield only 30 %. On the other hand, by cracking naphtha, it is possible to produce a broader range of "intermediate chemicals" products such as butadiene, isoprene and aromatics

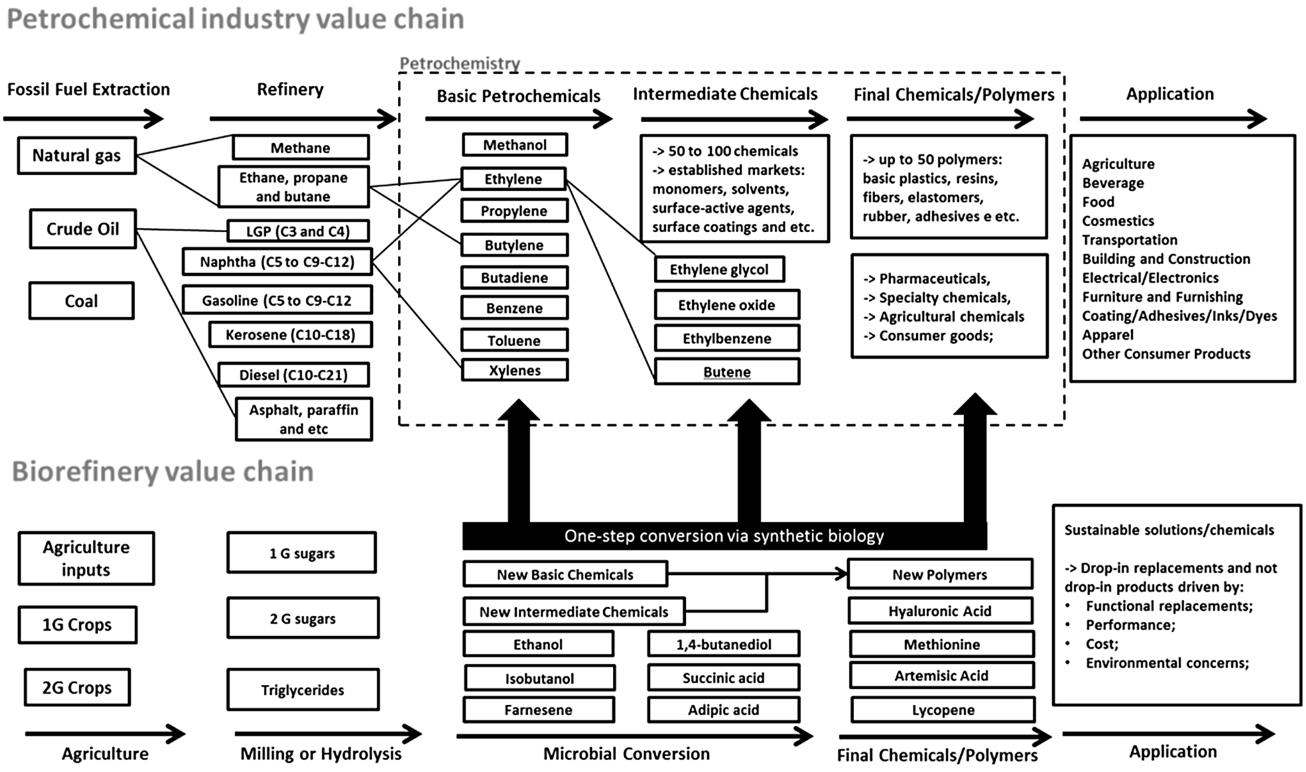


Fig. 1 Petrochemical and biorefinery value chains

(Fig. 1). However, the chemicals not produced by ethane cracking derived from natural gas can be made by “on-purpose” units using a cost-competitive ethylene (Fig. 1). For example, ethylene can be dimerized to butane and further dehydrogenated to butadiene.

Ethylene is the major basic chemical for production of polyethylene and intermediate chemicals like ethylene glycol, ethylene oxide, ethylbezene or butene. Additionally, those chemicals can be further converted to “final chemicals” and polymers to be used in a wide range of applications. The production of most industrially important chemicals involves catalysis. Research into catalysis is a major field in applied science and involves many areas of chemistry, notably organometallic chemistry and materials science.

Hence, there are regional aspects of raw material availability that influence the petrochemical value chain. China, for example, is expanding its petrochemical industry toward coal due to its significant reserves; on the other hand, the new technologies of extraction of natural gas trapped in shale formation (so called shale gas) are changing the energy matrix of USA toward natural gas.

The biorefinery value chain usually starts in agriculture, including crops cultivation and inputs such as fertilizers and seeds. Sugar and triglycerides extracted from crops and trees can be converted to a broad array of chemicals. Sugar can be converted to basic petrochemicals such as styrene [107], isobutylene [105], isoprene [156] or butadiene [101]. Additionally, sugar can be directly converted to “intermediate chemicals” such as 1,4-butanediol [161] and adipic acid [162], “specialty chemicals” like succinic acid [12] and isoprenol [171]; or even to pharmaceuticals like artemisic acid [125] or taxadiene [70] and consumer goods such as amino acids [132] and hyaluronic acid [98]. Besides drop-in products, other functional replacements could be produced, like farnesane [153] or isobutanol [7] for diesel and gasoline substitution, respectively.

The petrochemical industry has also been impacted by globalization of the world economy. Mergers, acquisitions and joint ventures of major petrochemical companies have led to fewer producers of commodity petrochemicals with a broader geographical reach. Additional factors that influence strategic movements are value chain integration, economies of scale, cyclical markets, fossil raw materials price and supply and, finally, environmental concerns.

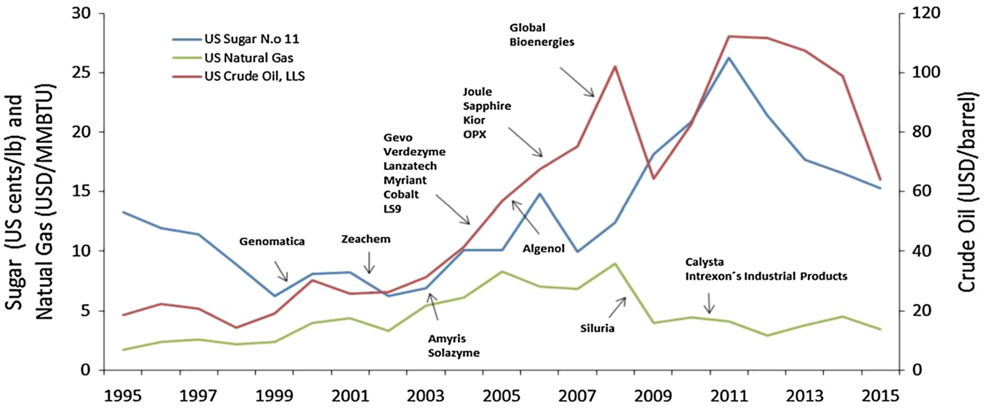


Fig. 2 Historical prices for crude oil (LLS/FOB), sugar (N.o 11/FOB) and natural gas (burner tip/delivered/US Gulf Coast). Free onboard (FOB) indicates that the seller is responsible for getting the goods onto a ship designated by the buyer. Sugar N.o 11 is a futures FOB contract for the physical delivery of raw cane sugar

Major petrochemical companies are integrated forward in the value chain to produce downstream chemicals and polymers and integrated backward to produce basic raw materials or raw materials extraction. These strategies are focused on improving business margins and guaranteeing raw material supply.

Additionally, petrochemical producers rely on the economies of scale of large production facilities to enhance their competitive advantages. This results in a cyclical process, in which the margins of this industry become sensitive to supply and demand that are associated with the addition of world-scale plants. The cyclical markets are generally characterized by periods of tight supply, leading to high operating rates and margins, followed by periods of oversupply primarily resulting from the additions of world-scale petrochemical plants, leading to reduced operating rates and lower margins.

The price and supply of crude oil and natural gas are intimately intertwined with national strategies, global policies and power. At the beginning of this century, crude oil prices were on the rise and a barrel was traded for nearly \$139 at their peak in 2008, a similar trend has been observed for natural gas prices (Fig. 2). That was caused by a myriad of factors including reports showing a decline in petroleum reserves [67], worries over peak oil [67], Middle East tension, increased worldwide oil demand and oil price speculations. However, at the same period of the 2008 international crisis, the development of new extracting technologies for shale gas, shale oil, bituminous sands and in deep water oil enabled the utilization of new energy reserves, increasing supply, especially in non-OPEP countries. For example, this year, USA is expected to overtake Russia and Saudi Arabia to become the world's largest producer of oil and gas combined. In 2000, shale gas provided only 1 % of US natural gas production; by 2010, it was over 20 % and the US government's Energy Information Administration predicts that by 2035, 46 % of the United States' natural gas supply will come from shale gas [141]. The petrochemical industry will follow this tendency of shifting to natural gas to provide raw materials (e.g., ethane) for ethylene and on-purpose production of basic and intermediate chemicals (BOX 1). The combination of economic crises, the development of new extraction technologies (e.g., shale fracking) and production in non-OPEP countries have led to a scenario of low demand and high supply of fossil fuels.

For the most of the twentieth century, growing reliance of petroleum was almost universally celebrated as an asset, a symbol of human progress. However, with the rise of environmental concerns, there is a rising number of mandatory legislation to decrease GHG emissions and reduction in fossil fuels, especially in Japan, USA and Europe. However, with the increased maturity of shale gas technologies, companies were able to access a more inexpensive raw material with lower carbon footprint. In 2012, US carbon dioxide emissions dropped to the 1994 level, achieving approximately 70 % of the CO_2 emission reductions targeted under Kyoto Agreement. With the continued displacement/retirement of coal plants, shale gas became a major factor in CO_2 emission reduction in US, which has the potential to provide even more GHG-related benefits in the near future [26].

In a recovering economy with a competitive oil/gas industry which is driving the reduction in GHG emissions, the development of renewable technologies has become a real challenge. Even though shale gas has been pledged to be a short-term solution for reduction in CO_2 emissions, current sustainability issues urge for renewable/clean technologies.

Shale Revolution and the Challenges for Industrial Biotechnology

The raw material competitiveness to produce commodity chemicals is defined by their availability, logistics and cost of production. In the last decades, another factor has become relevant—sustainability. For example, despite the competitiveness of shale gas, due to possible impact on

Table 1 Availability and price for the main industrial biotechnology raw materials

	Estimated world production (million ton)	Price
Glycerol	2–3 (refined glycerin)	Crude FOB Midwest [72] 231–319 USD/t
Sugar	170–180	N.o 11 FOB [36] 350–375 USD/t
Fatty acids	6–8	C21 FOB S.E. ASIA [72] 980–1050 USD/t
Lignocellulosic biomass (agricultural residues and wastes)	6.000 [27]	30–45 USD/dryton [52]

groundwater contamination, France has chosen not to use shale gas until the environmental security of its extraction has been proven [117]. Other countries have created mechanisms to encourage the use of renewable energy sources, such as the Renewable Fuel Standard in USA [123]. Additionally, some companies are committed to creating a more sustainable value chain [37].

The main raw materials for renewable commodities chemicals are: sugars, triglycerides, fatty acids and glycerol. The higher prices of fatty acids make them more suitable for value-added chemicals such as specialty chemicals. Glycerol stands out for its low price; however, its availability and logistics limit its utilization in major production centers in Asia, mainly Malaysia and Indonesia. Sugars are worldwide produced in large volumes from different sources: from sugarcane in Brazil, India and Indonesia; from corn in USA or from sugar beet in Europe. Historically, sugar has been used in biotechnology for a wide product range, from commodities to pharmaceuticals such as ethanol, vitamins, aminoacids, enzymes and beverages, just to name a few products (Table 1).

Braskem has been using Technology Roadmap in order to understand the dynamic scenario of renewable technologies. Information about 90 products produced by more than 400 companies has been evaluated in order to try to identify the best opportunities of investments in renewable sector. Using this approach, it is observed that ~80 % of the companies monitored are using sugar and starchy biomass (e.g., dextrose from corn) for microbial biocatalysis (BOX 2). The main sugar producer countries are Brazil, India, China and Thailand, and the main corn producers are USA and China, which also positioned those countries as potential leaders in the bioindustry.

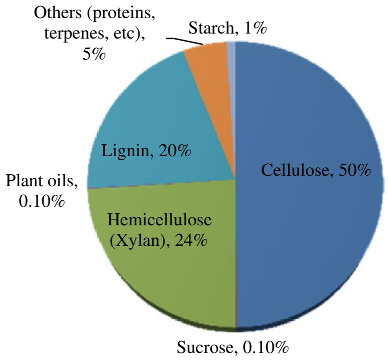


Fig. 3 Composition of biomass [58]

BOX 2: Biocatalysis

Biocatalysis is the application of enzymes and microbes in synthetic chemistry. The field of biocatalysis has reached its present industrially proven level through several waves of technological research and innovations [19]. Biocatalysts provide significant advantages over traditional chemical catalysts: (1) biocatalysts can potentially achieve high yields and 100 % selectivity at milder process conditions; (2) microbial biocatalysts or multi-enzymatic catalysis are able to contain an entire set of reactions enabling complex conversions; thus, (3) biocatalysts can provide a reduction in CapEx (capital expenditure) and OpEx (operational expenditure) requirements potentially providing economic viability at smaller scales. (4) Additionally, the biocatalysts are renewable organisms/enzymes that can be produced from renewable sources and (5) can be selected or engineered to convert different substrates to different end-products.

Modern tools of bioinformatics, metagenomics, protein structure, "omics" and protein engineering as well as advances in DNA sequencing and synthesis aid the development of biocatalysts and their tailor-designed integration into industrial processes. Consequently, engineering of biological systems find wide application in the production of pharmaceutical intermediates, novel materials and diagnostics, as well as fine, performance and commodity chemicals [19].

Table 2 Potential of energy crops and 2G generation ethanol for increasing biorefinary production scale

	Hectares	Sugarcane crushed (MM t/year)	1G sugar Production (kt/year)	1G ethanol production (kt/year)	2G ethanol production (kt/year)
Average commercial (74 t/ha/year)	75.000	3.5	250	125	215
Commercial maximum (148 t/ha/year)	75.000	7.1	500	250	400
Experimental maximum (212 t/ha/year)	75.000	10.2	700	350	575

Considering that (1) 50 % of sugarcane and straw is used for energy production or and the other 50 % is kept in the field, (2) 14 % of sugarcane juice/ton of sugarcane and (3) 70 % of the maximum theoretical yield for the conversion of lignocellulose sugars to ethanol

During the rise of oil and natural gas prices in the 2000s discussed previously, sugar prices were relatively stable with growing agricultural productivity (Figs. 2, 3). Apart from that, climate change concerns, corporate social responsibility projects from major chemicals/polymer consumers and seeming biotech technical maturity created a perfect environment for investment in industrial biotechnology by large petrochemical companies and venture capitalists in the early 2000s. It is no coincidence that during the oil price escalation between 2000 and 2008, the foundation of main current industrial biotech startups can be observed (Fig. 2). During this period, the opportunity observed between the high cost of oil and the low cost of sugars enabled a high valuation of many companies related to the development of renewable technologies.

The 2008 economic crises also represented a turning point for the biofuels hype. From mid-2008, the drastic changes in the macroeconomic scenario are influenced by oil supply/demand and sugar prices. From 2010 to 2013, the oil market has remained stable (~100 US$/barrel). At about US $50 a barrel, crude oil prices are down by more than half from their June 2014 peak of US $107. Additionally, the development of a global ethanol market has created a tight regulation between the price of sugar, ethanol, gasoline and oil [49, 159]. Moreover, speculations on land prices, stricter environmental/labor regulations and rising prices of agricultural inputs have caused to increase the cost/prices of sugar. Thus, the sugar prices (No. 11) rose from an annual average (1995–2008) of 10 cents/lb to an annual average (2008–2014) of 20 cents/lb. In the last year, the sugar prices have dropped to an average of 15 cents/lb.

In recent years, some industrial biotech startups focused on biofuels went bankrupt or entered a difficult financial situation because they were unable to demonstrate techno-economic feasibility. Other companies, which had the technological flexibility, outlined new strategies for their products, especially to obtain value-added products. Amyris, which emerged to be a diesel company, has become a multi-molecule company to explore different specialty chemicals derived from farnesene or other molecules which could be produced within the isoprenoid pathway. At its peak, Amyris's shares were valued at more than $30 per share and sporting a market cap in excess of $1 billion. The company now trades at around $200 million.

Another approach used by biotech entrepreneurs was to migrate to biocatalysis of inexpensive methane derived from shale gas. After 2008, Calysta and Intrexon's Industrial Products were created focused on methane conversion to chemicals using microbial catalysis, such as lactic acid, farnesene and isobutanol, which were already produced in industrial scale using sugar as raw material (Fig. 2).

In addition to the recent increase in the cost of sugar, renewable technologies have a logistical limitation related to freight and handling cost of transporting biomass to the biorefinery. The biomass has a much lower energy density than fossil fuels, for example, straw (baled) has a power density of 2 GJ/m^3 compared to 31 GJ/m^3 of coal. Solid wood (8 GJ/m^3) and wood pellets (11 GJ/m^3) could represent alternatives for raw materials with higher energetic density [48]. Thus, there is a distance limit (radius) where it is economically feasible to transport biomass to the refinery, which limits the production capacity of the plant. In Brazil, for example, sugarcane is produced within the radius of 25 km [114]. For this reason, a world-scale ethanol mill in Brazil crushes about 3.5 million tons of sugarcane per year producing about 125 thousand tons per year (kty) of ethanol and 250 kty sugar (Table 2), which is an order one magnitude lower than new ethylene crackers(>1.000 kty). Therefore, it is necessary to increase productivity and decrease cost of biomass transportation logistics in order to enable economies of scale in the production of renewable fuels and commodity chemicals.

Other limitations of biorefineries include the feedstock limitation during the off-season. In general, petrochemical plants operation rate is not limited by feedstock supply, they can run up to 8.000 h/year. However, ethanol/sugar mills are currently limited by the crops production season. In Brazil, the average operation of a sugarcane/ethanol mill is 200–250 days (4.800–6.000 h/year). In corn mills, there is an extra cost for corn storage along the year. This limitation has a great impact on the plant productivity and return on investment.

Lastly, agriculture is heavily dependent upon NPK fertilizers that are three main macronutrients essential for plant growth and current productivities. Nitrogen (N) represents 78 % by volume of the atmosphere; however, its transformation to ammonia is highly demanding both in energy and hydrogen (usually from natural gas). Phosphorous (P) are extracted from a finite resource of phosphate rocks. Three countries control more than 85 % of the known global phosphorus reserves [34, 50]. Therefore, it is important to keep in mind that main inputs for industrial agriculture are fossil fuel based or from finite sources. Moreover, more than half of the fertilizer applied cannot reach the plant, but is washed off by rain and irrigation water [11, 64]. This part of lost fertilizer causes not only large economic losses but also very serious environmental pollution.

In conclusion, the new economic scenario and the so-called shale gas revolution have had a great impact on the time-to-market of many renewable/clean technologies, especially in the energy and commodities sectors. Additionally, current drawbacks of agriculture-based industries, such as biomass energy density and operation time, constrain the competitiveness of biobased industries for production of bulk chemicals. However, a new set of biobased technologies have reached industrial scale and are promising to strengthen the competitiveness of renewable technologies. The role of second-generation technologies and synthetic biology in leveraging bioeconomy will be discussed in the following topics.

Lignocellulose: a new sugar supply

Since 1975, the overall yield of the Brazilian ethanol industry has steadily increased by 2–3 % per year, reaching over 6500 l of ethanol per hectare of sugarcane. This was based on increasing agriculture and industrial efficiency. Agricultural improvements include selection of new sugarcane varieties with increased amounts of sugarcane biomass per hectare. While the amount of total sugar per ton of sugarcane has steadily increased (1–2 % per year), the industrial yield reached a plateau near the maximum theoretical yield of ethanol conversion from sugar [43].

Classical plant breeding has been the main approach toward sugarcane improvement. The complexity of the sugarcane genome, its narrow genetic base [97, 126] and the time required for a new variety to reach commercialization (12–15 years) are constraints of this method. Although it is not clear whether sugarcane improvement through classical breeding is reaching a yield limit, there are specific desired traits that cannot be introduced into sugarcane by traditional breeding. Thus, many advances on sugarcane improvement will depend on sugarcane transformation. This is also true for other sugar sources, such as corn and sugar beet. Current constraints of high-throughput sugarcane transformation include the low transformation efficiency, stability and the long time required for its commercial release [69]. Current average commercial sugarcane yield is about 74 t/ha/year, and experimental maximum yield is approximately 212 t/ha year fresh weight. However, this level of yield remains lower than the 381 t/ha year maximum theoretical yield that have been calculated from models of physiological processes contributing to plant growth [151]. Despite the difficult to predict that this experimental/theoretical potential can be translated to commercial fields, this means that there is still room for using synthetic biology to increase agricultural yields.

BOX 3: Second-generation (2G) technologies

Current first-generation biofuels/chemicals are made from the sugar and vegetable oils found in arable crops and trees, which can be easily extracted using conventional milling technology. In comparison, second-generation biofuels/chemicals are made from lignocellulosic or woody crops or agricultural residues (e.g., as sugarcane bagasse or corn stover) or energy crops. Lignocellulosic biomass varies among species but generally consists of ~25 % lignin and ~75 % carbohydrate polymers in dry weight (cellulose and hemicellulose) and it is the largest known renewable carbohydrate source (Fig. 3) [58]. Major challenges for biological conversion are posed by biomass recalcitrance. The cellulosic and hemicellulosic portions of biomass can be separated from the lignin and depolymerized to obtain the constituent sugars, mainly glucose, xylose and arabinose. To overcome the biomass recalcitrance, feedstock deconstruction is therefore required. For that purpose, there are different second-generation routes currently under development, for example, biomass gasification followed by syngas fermentation/catalysis or biomass cellulosic enzymatic hydrolysis followed by sugar fermentation. Up to date, biomass enzymatic hydrolysis and microbial sugar conversion is the preferred technology for ongoing industrial projects; however, a myriad of technologies are competing in that space in biomass pre-treatment, enzymes supply, microorganism capable of consumption of glucose, xylose and hydrolysis by-products, fermentation strategies and downstream technologies (e.g., Dupont, ClariantorBeta Renewables 2G ethanol technologies).

We currently consume 12 billion of oil equivalent (1 ton of oil equivalent represents approximately 42 gigajoule). Faaijand co-author estimated that about 100 EJ/year of agricultural residues and wastes could be supplied at costs of around USD 2–3/GJ (approximately USD

30–45/ton of dry matter) by 2050 [51, 52]. Therefore, the available biomass could only supply one-fifth of all primary energy demand, indicating that other renewable energy sources (e.g., solar and wind energy) are required to fully substitute fossil fuels. Depending on the feedstock choice and the cultivation technique, second-generation production has the potential to provide benefits such as consuming waste residues and making use of marginal lands. This way, the new fuels can offer considerable potential to promote rural development and improve economic conditions in emerging and developing regions [47]. However, while second-generation crops and production technologies are more efficient, their production could become unsustainable if they compete with food crops for available land. Thus, their sustainability will depend on whether producers comply with criteria like minimum lifecycle GHG reductions, including land use change and social standards [47 ,51]. The International Energy Agency assessed the potential of agricultural and forestry residues as potential feedstock for 2G biofuels. In 2030, 10 % of global residues could then yield around 4.1 % of the projected transport fuel demand. Using 25 % of global residues could be converted to 10.3–14.8 % of the projected transport fuel demand [47].

Nevertheless, additional significant gain of efficiencies is expected to come from agricultural improvements and the so-called second-generation technologies (BOX 3). To maximize the energetic potential of sugarcane and other biomass, an increasing number of scientists are working toward the development of new and improved crops and biomass deconstruction technologies. To unleash the potential of 2G technologies major aspects need to be improved: (1) crops productivity, including plants that can thrive with little fertilization/irrigation on marginal lands; (2) cellulolytic enzymes cost and enzyme-free technologies; (3) robust microbial conversion of 2G sugars and hydrolysis by-products, and, (4) reduce investment (CapEX) requirements for biomass deconstruction. In Table 2, the potential of increasing production scale via increasing sugarcane productivity or via 2G crops is presented. It is possible to increase the current 1G ethanol production more than fourfold by increasing productivity and using 2G sugars. Additionally, edible sugar production could be increased almost threefold. This is the same capacity level of a global scale petrochemical ethylene plant (1.000 kty). The scale of production controls overall manufacturing costs in chemical process industry. The twofold increase in plant capacity is known to reduce manufacturing cost by 20–25 % [95].

In 2014, at least two second-generation ethanol plants have inaugurated the lignocellulose sugars utilization in industrial scale. The joint-venture POET-DSM Advanced Biofuels have opened the 20 million gallon Project Liberty in Emmetsburg (USA). Granbio also opened a 21.6 million gallon commercial-scale facility on Alagoas state in Brazil. And finally, the joint-venture DuPont Danisco Cellulosic Ethanol is expected to open a 30 million gallon plant in Nevada (USA) which will become the world's largest cellulosic ethanol facility in the world [92].

In addition to increasing land productivity and the utilization of different raw materials/residues, it is also important to increase the product portfolio. There are examples of facilities which produce a range of products, such as Cargill Biorefinery at Castro/Brazil which produces corn, starches, sweeteners and amino acids; or in Cargill Biorefinery at Blair/USA producing sugar, lactic acid, PLA, ethanol, corn oil; or the "Les Sohetes" Biorefinery (Pomacle/France) which produces ethanol, cosmetics ingredients and succinic acid from sugar beets and wheat [5]. However, synthetic biology is already expanding this product mix, increasing profit margins and decreasing commercial risks of biorefineries. For example, the joint-venture Paraiso Bioenergia and Amyris (Brotas/Brazil) is producing ethanol, sugar and farnesene as an intermediate for specialty chemicals and fuels [56, 150]; or the joint-venture Solazyme and Bunge (Moema/Brazil) producing ethanol, sugar and tailored triglyceride oils for the use in oleochemical and fuel applications [60].

New biochemical routes and reduced R&D risks

For many years, microbial production of chemicals was limited by nature's chemical repertoire: fatty acids, amino acids, organic acids, alcohols, antibiotics, etc. Microbes were randomly mutated and selected to reach higher yield and productivity. The advent of recombinant DNA technology in 1972 [76] opened a new realm of biology for exploration [84]. Scientists soon realized that the ability to move genes between organisms offered the opportunity to produce non-native proteins in genetically tractable hosts, leading to the production of human insulin in *Escherichia coli* in 1982 [79]. Metabolic engineering emerged in the early 1990s with a focus on the integrated treatment of metabolic pathways in order to engineer microbes to reach better fermentation performances using rational approaches [10, 84, 140]. In 2004, the publication of the DOE: "Top 12 products from Biomass" highlighted a number of molecules with properties to become drop-in substitutes of petrochemicals. However, those molecules were still limited to biochemical pathways found in nature. Metabolic engineering had a pivotal role to increase yield and productivity in genetic-modified hosts for naturally occurring pathways such as *n*-butanol, acetone, 2,3-butanediol, succinic acid, lactic acid, 1,3-propanediol, 3-hydroxypropionic acid and

Table 3 Basic and specialty chemicals produced in a one-step conversion from renewable sources to the final chemical via new synthetic biology routes

Compound	Institution/Company	Main applications	References
Vitamin C	Genencor and Eastman	Vitamin supplement	[146]
Isoprene	Genencor and Goodyear Braskem, Amyris and Michelin Ajinomoto and Bridgestone	Elastomers	[156]
Isobutylene	Global Bioenergies	Polymers and chemicals	[105]
Adipic acid	Verdezyne Genomatica Institut für Bio-und Geowissenschaften	Thermoplastics and plasticizers	[118] [1] [119]
1,4-Butanediol	Genomatica	Polymers and chemicals	[161]
Styrene	Arizona State University	Thermoplastics and elastomers	[107]
Hexamethylene diamine	Genomatica	Thermoplastics	[1]
Caprolactam	Genomatica	Thermoplastics	[1]
1,5 diaminopentane (cadaverine)	Technische Universitat Braunschweig	Thermoplastics	[85]
Fatty alcohols	Wuhan Institute of Biotechnology	Detergents, surfactants and cometics	[99]
Phenol	TNO Quality of Life	Precursor of chemicals	[155]
Methyl ketones	Joint BioEnergy Institute	Solvent	[62]
FAEEs	LS9	Diesel	[138]
Farnesane	Amyris	Diesel	[124]
Butadiene	Genomatica and Braskem Global Bioenergies and Synthos	Elastomers	[101] [24]
Propylene	Global Bioenergies	Polypropylene	[105]
Ethylene glycol	MIT	Polyethylene terephthalic	[139]
Ethylene	Chalmers University of Technology	Thermoplastics and precursor of chemicals	[78]
Methionine	Metabolic Explorer	Feed supplement	[18]
Methacrylic acid	Genomatica	Thermoplastics	[25]
2,5-Furandicarboxylic acid	Synthetic Genomics	Potential substitute for terephthalic acid	[82]
Indigo	Genencor	Dyes	[154]
1,3-Propanediol	Genencor	Thermoplastics	[46]

many other compounds. However, those processes were usually dependent on a microbial catalysis and a catalytic step for production of the final production, e.g., 3-hydroxypropionic acid fermentation and further catalytic dehydration to acrylic acid or 2,3-butanediol fermentation and further catalytic dehydration to butadiene.

Since the past decade, engineering of biological system has been evolving significantly toward the creation of novel pathways and the optimization of its performance. Expanding the metabolic pathways for biosynthesizing the target chemicals requires not only the enumeration of a series of known enzymes, but also the identification of biochemical gaps whose corresponding enzymes might not actually exist in nature. Several approaches for potentially filling the gaps have been applied, including exploring the enzyme promiscuity, design of novel enzymes and exploration of hypothetical proteins [136]. In the current challenging scenario, one-step conversion to the final product, conversion yields close to the theoretical maximum became of crucial importance for the economical production of renewable commodities chemicals. Companies that started their “Strain Development for New Metabolic Pathways” (BOX 4) with R&D goals of 60 or 70 % of maximum theoretical yield and moderate productivity had to redefine their goals to reach yields closer to the maximum theoretical yield and more aggressive productivity. This has a significant impact of timelines, costs and successful rate on metabolic engineering development plans. For this reason, many companies delayed their demonstration or industrial plants or were just unable to demonstrate economic and technological feasibility. The production of green hydrocarbons for fuels or commodities chemicals, such as alkanes or farnesane, were especially affected because of its low theoretical yields and current sugar prices. On the other hand, synthetic biology has enabled the development of solutions to increase yields and/or new pathways for the synthesis of more complex and value-added molecules. Recently, production of drop-in chemicals in a high-efficiency one-step process has become possible,

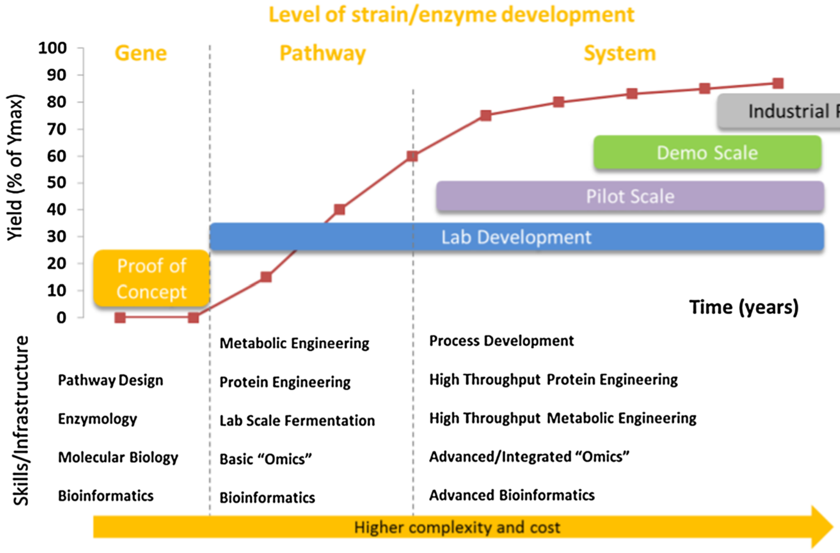


Fig. 4 Schematic representation of different R&D levels in the development of a new metabolic pathway—from discovery to industrial scale

while petrochemical processes still demand several catalytic steps and high energy use (Table 3).

In addition to developing one-step biological routes for the production of drop-in commodities chemicals, synthetic biology has also an important role in developing high-yield metabolic pathways and CO_2 recycling. Most of the listed chemicals are derived from sugars using glycolysis to produce acetyl-coenzyme A (CoA) which is used as a precursor for the biosynthetic pathways. Glycolysis involves the partial oxidation and splitting of sugars to pyruvate, which in turn is decarboxylated to produce acetyl-CoA for various biosynthetic purposes [17]. The decarboxylation of pyruvate loses a carbon equivalent and limits the theoretical carbon yield to only two moles of acetyl-CoA per mole of hexose. This native route is a major source of carbon loss in biorefining and microbial carbon metabolism [17]. James Liao Group from UCLA, design and construct a non-oxidative, cyclic pathway that allows the production of stoichiometric amounts of acetyl-CoA from sugar without carbon loss [17]. The new non-oxidative glycolysis enables complete carbon conservation in sugar catabolism to acetyl-CoA. In another approach, the combination of the CO_2-fixing reaction of phosphoenolpyruvate kinase and other genetic modifications allows the production of succinate, fumarate and malate with maximum theoretical yields higher than 100 % (g/g) from glucose [165]. However, depending on the metabolic pathway, CO_2 production cannot be avoided; therefore, methods for recycling carbon dioxide have been developed. Rubisco-based engineered *E. coli* allows in situ CO_2 recycle to synthesize products during the microbial catalysis. While the CO_2 emission is lowered, the yield of fermentation products can be increased [173]. Other approaches include Wood–Ljunghahl pathway [120] or the 3-hydroxypropionate-4-hydroxybutyte cycle [121]. A number of other schemes can be envisioned to rewire the metabolic pathway for de novo carbon fixation pathways [3, 13, 44, 59]. The "BOX 5" looks at the use of CO_2 and electricity in microbial catalysis to increase the overall biorefinery yield.

Nevertheless, the competitiveness of bulk chemicals production depends on yields and productivities close to the maximum theoretical yield (BOX 4). Therefore, the strain developments for new metabolic pathways are still costly and long-term projects. From the proof-of-concept to the industrial scale, a metabolic engineering process can extend from 5 to more than 10 years. This results not only in a high R&D risk but also in a high market risk. 1,3-propanediol is one of the few examples of an intermediate chemical which has been successful in making the cost advantage transition from the petro-based chemicals. This accomplishment took 7 years to commercialization and another 3 years to achieve market penetration [93]. In another example, Genomatica took 5 years to reach demonstration scale from the first proof-of-concept of the ability for a microorganism to produce 1,4-butanediol in 2008.

BOX 4: Strain development of new metabolic pathways

Strain development for the production of chemicals from engineered organisms can be organized into different levels (Fig. 4). The first level focuses on the selection and expression of necessary enzymes. For the development of new metabolic pathways, it is necessary to design a new set of metabolic reactions, identify possible enzymes for those reactions and test their activities in vitro or in vivo. Next, pathway level optimization is achieved by reducing pathway toxicity, knocking out obvious competing pathways, enhancing substrate and cofactor availability and enhancing driving forces [91]. However, further optimization of pathway expression requires high-throughput technologies with system analysis (system level) to enable the production with yields and titers next to the maximum theoretical yield. The "omics" technology and in silico models are useful to identify non-obvious competing pathways and additional beneficial enzymes for overexpression [91]. These systematic approaches have been reviewed previously [54, 167] and will not be discussed in detail here. Additionally, automated high-throughput screening platforms and system analysis are the core to reduce development times and cost. The development of techniques for rapid directed genome editing [40, 103, 152], automated DNA assembly [66], synthesis of entire synthetic viable genomes [61], computer-aided design programs [122, 131] have increased current design and construction skills. The combination of those technologies has the potential to substantially reduce development times and costs for biological engineering, and, therefore, decrease the investment risk in renewable technologies. 'One can envision a future when a microorganism is tailor-made for production of a specific chemical from a specific starting material, much like chemical engineers building refineries and other chemical factories from unit operations'—Jay Keasling [83].

Naphtha or ethylene cracking produced ethylene and propylene as main basic chemicals but also different by products such as butadiene and isoprene. In the unit operation, it is possible to obtain a wide range of chemicals that can be further separated and converted to other chemicals/polymers. On the other hand, biocatalysis can potentially achieve 100 % of selectivity in milder process conditions and different molecules are synthesized within the same metabolic pathway. This feature could be used to create synergies between different projects and to de-risk and increase value of an R&D portfolio. Value-added products could be produced in early development stages with lower yields, while bulk chemicals could be produced in latter stages with higher yields. Amyris uses its platform to convert plant sugars into a variety of hydrocarbon molecules derived from the isoprenoids pathway. Within its isoprenoid platform, Amyrisis is able to explore hundreds of products, including farnesene for diesel and cosmetics application, isoprene for rubber production and artemisic acid for malaria treatment [157]. In a different approach, Global Bioenergies uses the same isobutylene protein engineering platform for production of different olefins [105].

As the technologies for these biobased chemicals evolve, the gap between biobased and fossil fuel-based production costs is shrinking. Additionally, there are supply risks on the petrochemical industry that can be an opportunity to biotechnology. As mentioned before, oil industry is switching from petroleum-derived naphtha to lighter natural gas-based feedstocks, which reduces the output of valuable C3 (propylene), C4 (butadiene, isobutene and butenes), C5 (isoprene, pyperylene and DCPD) and aromatics (benzene, toluene and xylene). These coproducts, in turn, are the main starting materials for a variety of chemical intermediates and polymers. This could result in an opportunity to processes based on renewable "on-purpose" "one-step" production of certain chemicals that could be in short supply, such as butadiene, 1,4-butanediol, isoprene, adipic acid, caprolactam and its derivatives [81]. In the following topic, we will discuss the role of synthetic biology in the consolidation of industrial steps via "one step" and "on-purpose" microbial conversion to a myriad of compounds.

Biological engineering of "consolidated bioprocessing"

Brazil has the most renewable energy matrix of the industrialized world, with 45.9 % of its production from sources such as water resources, biomass and ethanol, as well as wind and sun energies. The hydroelectric power plants correspond to the generation of over 75 % of the country's electricity. It should be noted that the world energy matrix is composed of 13 % of renewable sources in the case of industrialized countries and 6 % in the developing countries [22]. In Brazil, the energy content of sugarcane corresponds to 18 % of the country's energy matrix and represents a higher share than hydroelectricity [134]. This value includes the ethanol energy content for fuels application and the bagasse which is currently burned in boilers to generate electricity for the mills and, if there is a surplus, for the national grid. In the 2011/2012 harvest, Brazil produced approximately 23 billion liters of 1G ethanol that can be used by almost 60 % of all Brazilian light vehicles [134]. Additionally, the ethanol is used to buffer gasoline prices volatility and carbon footprint, by blending 27 % of ethanol in the gasoline. In consequence of the free market competition between renewable and petrochemical fuels in Brazil, the correlation between ethanol and gasoline prices is remarkable [49, 159].

This interconnectivity increases with the advance of bioenergy, biofuels and biochemicals. As it was observed in the petrochemical industry, in order to guarantee margins and avoid raw material price volatility, the renewable value chain is also becoming integrated, backward to the production of 1G and 2G feedstocks and forward to the production of chemicals and polymers. Dupont for example is investing in technologies that will allow full integration in the renewable value chain—by developing new plant feedstock, cellulolytic enzymes and genetic-modified microorganism for ethanol, butanol (Butamax—a joint venture between Dupont and BP) or isoprene/polyisoprene production (a joint venture with Goodyear). Oil companies and traditional 1G ethanol producer, such as Total and Abengoa, also move toward integration with second-generation technologies.

In addition to alternative feedstocks, engineered biological systems could have an interesting role in integration of the value chain. A biological catalyst is able to contain an entire set of reactions "in one miniature bioreactor" enabling complex conversions. The direct conversion of sugars to complex chemicals creates a possibility to forward integration of biorefineries in a wide range of chemical sectors. Biorefineries could produce fuels, bulk chemicals, plastic, pharmaceuticals, cosmetics, etc. Additionally to that, the direct conversion of complex chemicals can have a significant impact in production costs and environmental impact. The following example shows the impact of reducing industrial steps in the competitiveness of renewable terephthalic acid (PTA) used mainly in polyethylene terephthalate (PET) bottles. PTA is produced at about 40 million ton per year by petrochemical methods based on the catalytic oxidation of *p*-xylene.

Despite the fact that there is no commercially available process for manufacturing bio-PTA, several renewable routes are envisioned for its production. For example, the PTA production via isobutanol fermentation requires at least four industrial steps (one fermentation step and three catalytic steps). Fermented isobutanol has to be chemically dehydrated to produce isobutene, and *p*-xylene is produced via isobutene dimerization. Finally, terephthalic acid is produced by oxidation of *p*-xylene by oxygen in air (Fig. 5). By having a direct biochemical conversion of carbohydrate to isobutene, isobutene gas would be produced directly from a fermenter, and isobutanol recovery and its chemocatalytic dehydration could be bypassed. Gevo is producing isobutanol in commercial facility in Luverne [94] and had already demonstrated the production of p-xylene and PTA in partnership with Toray and Coca-Cola. Global Bioenergies have demonstrated the direct production of isobutylene from sugar in laboratory scale, and the pilot plant is under construction [148].

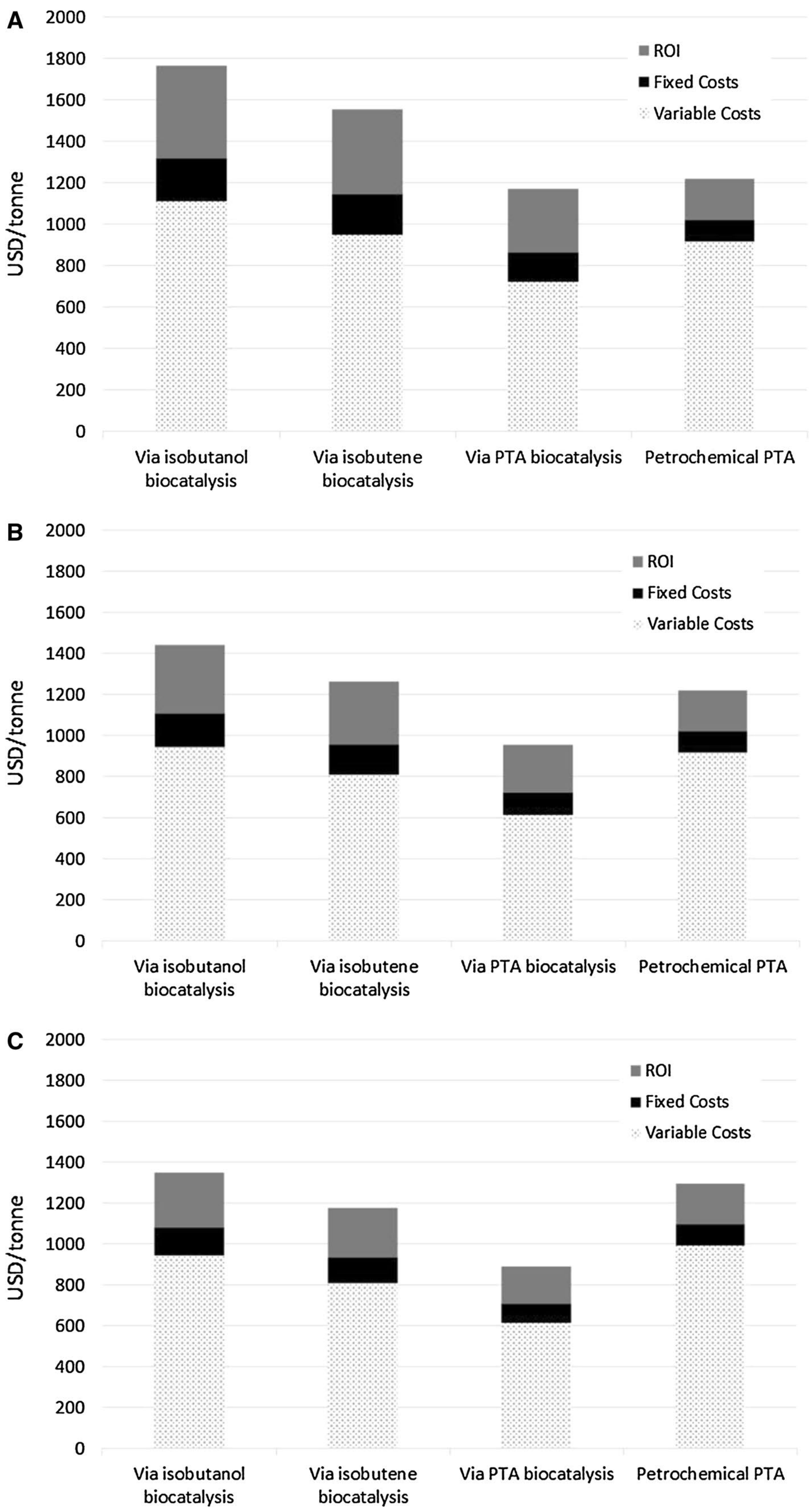
As outlined before, if a one-pot biochemical conversion of carbohydrate to PTA would become possible, several recovery and chemocatalytic steps would be bypassed. However, in the Genomatica patent application, at least three new enzymatic reactions are proposed with no empirical demonstration. Based on information of patents, computational simulation, articles and internal analysis, the capital expenditure (CapEX) and operational expenditure (OpEX) were estimated. The output of this analysis is not an economic evaluation of different technologies, but a relative analysis between different routes under specific premises described in the additional information. The major goal is not to evaluate economic feasibility but to exemplify the potential of synthetic biology to consolidate industrial steps (Fig. 5) via new enzymatic reactions. In this article, this case will be used to exemplify the impact of different strategies and incentives to increase the competitiveness of renewable technologies.

Fig. 5 Estimate of minimum selling price for different terephthalic acid (PTA) technologies for the first semester of 2014. For detailed information, please check additional information. The production of PTA via isobutanol include the CapEX and OpEX of a (1) 300 thousand tons per year (kty) plant for isobutanol fermentation of sugarcane juice, (2) 230 kty dehydration plant to produce isobutylene, (3) 205 kty dimerization plant to p-xylene and (4) 300 kty oxidation plant to PTA. The production of PTA via isobutene includes a (1) 230 kty plant for the direct fermentation of sugar juice to isobutene, (2) 205 kty dimerization plant to p-xylene and (3) 300 kty oxidation plant to PTA. The PTA direct microbial conversion of sugarcane juice includes a 300 kty plant. For the petrochemical, PTA production considers a 500 kty plant for the oxidation of p-xylene. **a** Estimated impact of engineering of biological system on bioprocess consolidation. A greenfield and standalone plant using sugarcane juice as raw material is considered. **b** Estimated impact of the PTA plant integration in an eco-industrial park. A green PTA plant integrated in an eco-industrial park is considered. The integration benefits are a 25 % Capex reduction and 15 % reduction costs in raw material and utilities (which include possible by-products credits). **c** Estimated impact of a carbon price policy on PTA production. A green PTA plant integrated in an eco-industrial park and an additional reduction of 20 % of CapEx due to "tax and invest" policies is considered. It was also considered a price of 43.5 USD/ton of CO_2 emissions for the petrochemical PTA. Additionally, it was considered that the CO_2 emissions from the renewable technologies are zero or even negative ▶

As observed in Fig. 5a, there is a significant difference in scale between the petrochemical process (via *p*-xylene) and the fermentation routes. As shown before, this is due to the logistic limitation of sugarcane transportation. A new world-scale petrochemical PTA plant has a capacity of 500 kty up to 1.000 kty. On the other hand, it is estimated that 4 million tons of sugarcane could produce about 300 kty of PTA using sugarcane juice as raw material. Additionally, the minimum selling price for the petrochemical PTA is corroborated by the current PTA prices—1.300,00 USD/ton of PTA [72].

Besides direct microbial conversion from sugar to chemicals, Fig. 6 summarizes some other strategies available to bring new renewable technologies to the market. All those technologies could have different applications and work synergistically to increase overall competitiveness of biorefineries.

Consolidated bioprocessing technology (CBP) can decrease CapEX and OpEX by consolidating the production of enzymes, cellulose enzymatic hydrolysis and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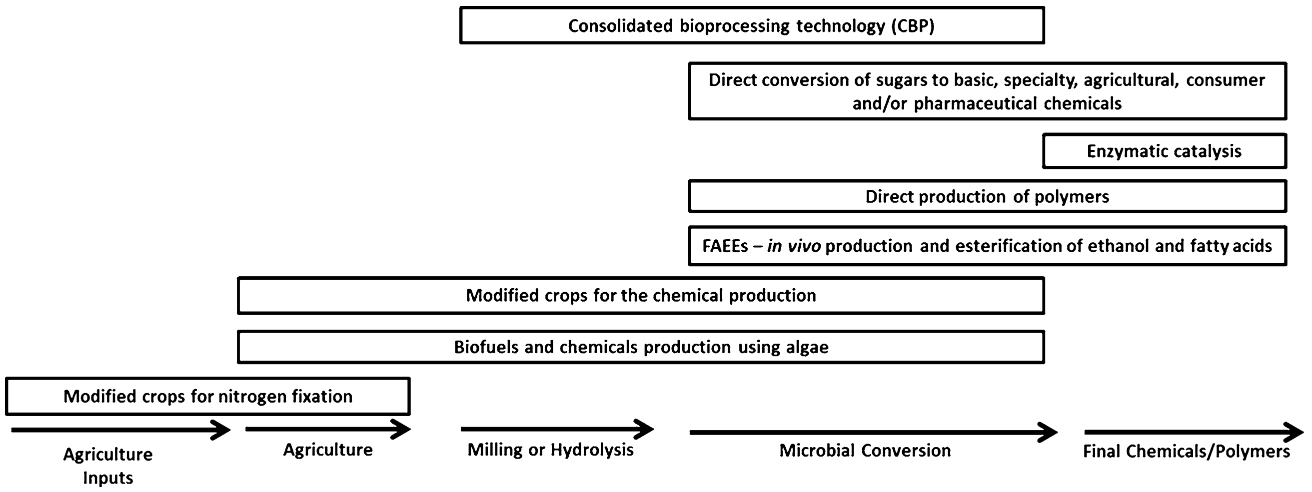

Fig. 6 Schematic representation of some examples of value chain consolidation via biotechnology

following fermentation, being considered one of the lowest cost configurations for cellulose hydrolysis and fermentation of 2G chemicals [100]. CBP requires the development of engineered microorganisms that are capable of simultaneously making the necessary cellulolytic enzymes and converting the sugars released by those enzymes into the desired end-products [100]. In this article, it is proposed a broad meaning of CBP which is related with the consolidation of any industrial step via engineering of biological system, as observed in the PTA case (Fig. 6). In other interesting example, Keasling and coworkers [138] showed that the use of synthetic biology for the biodiesel production could potentially bypass many steps in the current biodiesel value chain. Biodiesel production currently requires the production of fatty acids (e.g., from palm tree or soy beans) and further esterification with methanol or ethanol, to produce FAMEs or FAEEs (fatty acid methyl esters or fatty acid ethyl esters). Keasling and coworkers engineered recombinant *E. coli* strains to produce free fatty acids through cytosolic expression of a native *E. coli* thioesterase and deletion of fatty acid degradation genes. After the introduction of ethanol production genes from *Zymomonas mobilis* and expression of endogenous wax-ester synthase, direct production of FAEEs was achieved at 674 mg/L. Furthermore, hemicellulases were expressed in the biodiesel-producing cells which represents a step toward producing these compounds directly from lignocellulosic biomass. This is an emblematic example how engineering of biological system can empower consolidated bioprocessing to decrease the number of industrial steps required for the production of chemicals and, consequently, increasing competitiveness and sustainability.

Table 4 represents a not exhaustive list of new synthetic biology routes for production of all classes of chemicals. It is interesting to notice that all those pathways were discovered in the last decade. Additionally, engineering of biological systems could result in the simplification of downstream process such as polymerization. For example, polylactic acid (PLA) is a promising biomass-derived polymer, but is currently synthesized by a two-step process: fermentative production of lactic acid followed by chemical polymerization. Sang Yup Lee group [80] reported the production of PLA homopolymer and its copolymer, poly(3-hydroxybutyrate-co-lactate), by direct microbial conversion.

So far, technologies based on sugar conversion and microbial catalysis have been described. However, synthetic biologists are also developing the direct production of chemicals in crops or algae. Genetic-modified crops have been extensively modified to include new traits including resistance to chemical treatments (e.g., resistance to herbicides) or improving the nutrient profile. However, scientists are expanding the traits to be engineered in plants. Chromatin, Inc. announced that it has created sorghum plants containing elevated levels of the energy-rich compound farnesane [20]. Like sugarcane, sorghum has traditionally been used as a source of sugars that can be subsequently converted to biofuels by microbes. By creating farnesane within sorghum, it becomes possible to bypass microbial fermentation and directly harvest biofuels from the crop itself.

With the same goal of bypassing microbial conversion of sugar, photosynthetic microalgae can become a major source of food, feed ingredients and chemicals. Microalgae have several potential advantages over land-based crops. Their simple unicellular structure and high photosynthetic efficiency allow for a more suitable host for metabolic engineering in comparison with plants. Algae can be grown on marginal land using brackish or salt water and do not compete for resources with conventional agriculture. Their cultivation could be coupled with the uptake of CO_2 from industrial waste streams and the removal of excess nutrients from wastewater [68]. In addition to oil production, potentially valuable coproducts such as pigments, antioxidants, nutraceuticals, fertilizer or feeds could be produced [106]. James Liao group showed that algae could be engineered to produce isopropanol [87], isobutyraldehyde [8], isobutanol [8], and 1-butanol [90]. Despite these advantages, algal fuel or bulk chemicals are not currently in widespread use, largely due to their high cost of production [31]. However, ExxonMobil started a $600M research program in collaboration with Craig Venter's Synthetic Genomics aimed at creating algal strains for more efficient bioproduction. In a different direction, the microbial electrosynthesis can be an interesting approach to bypass photosynthesis for production of renewable chemicals and energy (BOX 5).

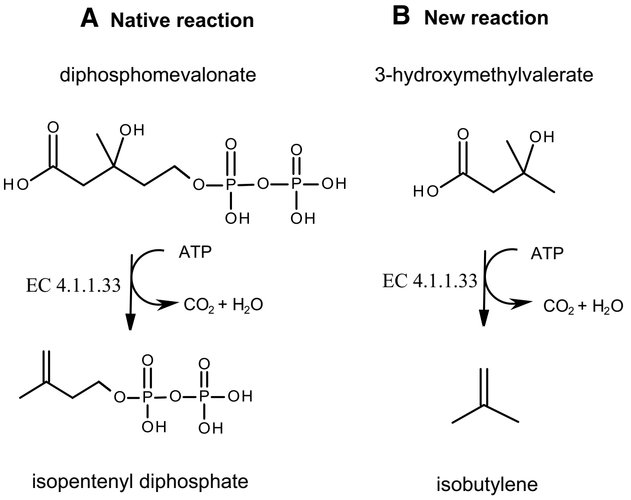

Fig. 7 Exploring the promiscuity of diphosphomevalonate decarboxylase (EC 4.1.1.33). Reaction A is the native reaction from the isoprenoid pathway, and reaction B was discovered by Global Bioenergies for production of isobutylene

BOX 5: Microbial electrosynthesis

As described in the "BOX 1", the usual biorefinery value chain starts with crops or algae cultivation which means that all the renewable processes described so far are derived from photosynthesis. However, photosynthesis is still an inefficient process, plants generally operate at 1 % or lower efficiency in converting solar energy into stored chemical energy [14]. Moreover, the energy stored in plant or algae requires significant processing to produce biofuels and chemicals. Microbial electrosynthesis bypasses photosynthesis altogether by utilizing microorganisms that can directly use energy from electricity or H_2 to the production of chemical compounds such as chemical, hydrogen or fuels from CO_2 [112]. During microbial electrosynthesis, electrons supplied via an electric current are used by the microorganisms to reduce carbon dioxide to yield industrially relevant products. Potential advantages of microbial electrosynthesis over biomass-based strategies include the 100-fold higher efficiency of photovoltaics in harvesting solar energy, eliminating the need for arable land, avoiding potential environmental degradation associated with intensive agriculture [102, 111]. It was been demonstrated the production of acetic acid [112], isobutanol and 3-methyl-1-butanol [96] using microbial electrosynthesis. Despite its potential, microbial electrosynthesis is a nascent concept, and much more information on the microbiology and the engineering of this process is required [112]. Another approach is the production of a chemical intermediate, such as formic acid, from electricity and CO_2 to further microbial catalysis to chemicals. Ginkgo Bioworks approach to electrosynthesis is to use formic acid (produced by DNV) as a vector of electrical energy for microbial conversion. Additionally, Ginkgo Bioworks created a new central metabolic pathway for formic utilization in *E. coli*, allowing the production of pyruvate and acetyl-CoA derived chemicals from formic acid, including isooctane for gasoline applications [57]. The combination of engineered microbes of Ginkgo and DNV technology for formic acid production from electricity and CO_2 represents a short route toward scale-up of microbial electrosynthesis.

Additionally to increase competitiveness and sustainability, "consolidated bioprocessing" could allow traditional industries such as petrochemical and sugar industry to access new markets which otherwise could be blocked by intellectual property, raw material access or other market barriers. However, a large number of very interesting molecules remain difficult to produce by direct microbial conversion, as they cannot cross the membrane, are highly toxic, have a low final concentration and/or yield, or are co-synthesized with many by-products [109]. In order to overcome several limitation of in vivo catalysis, other approaches have been developed.

Enzymatic catalysis and in vitro synthetic biosystems

Biotransformations have become an indispensable tool in the fine chemical industry for catalyzing chemoselectively,

Table 4 New pathways for the direct conversion of sugar to pharmaceuticals, specialty chemicals and consumer products via microbial catalysis

Compound	Example of application	Reference
Taxadiene	Taxol precursor (potent anticancer drug)	[70]
Echinomycin	Antitumor, antibacterial and antiviral	[168]
Anthracyclines	Antibiotics	[42]
Artemisic acid	Drug for malaria treatment	[125]
Miltiradiene	Treatment of many cardiovascular diseases	[42]
Aureothin	Antibiotics	[133]
Revesteratrol	Natural Antioxidant	[145]
Vanillin	Ingredient	[23]
Patchouli	Fragrance in household product	[159]
Lycopene	Antioxidant	[9]
Ubiquinone	Medicine, food and cosmetics	[163]
Isoprenol	Specialty chemicals	[171]
Prenol	Fragrance	[115]
Hyaluronic acid	Skin care	[98]
Glycolic acid	Skin care or polymer application PGA	[164]
Omega 3	Dietary supplement	[63]
Salvianic acid A	Antioxidant and therapeutic potential on cardiovascular diseases	[160]
Beta-carotene	Precursor of vitamin A and colorant	[170]
Heparosan	Precursor to heparin (a drug to prevent blood coagulation)	[166]
(2*S*)-Pinocembrin	Building block for the synthesis of a variety of other flavonoid molecules	[158]
Shikimate	Chiral template for the synthesis of the antiviral drug Tamiflu used in the treatment of swine/avian flu	[28]
L-Phenylalanine	Food industry, Sweetener aspartame	[12]
3-hydroxy-γ-butyrolactone	synthesis of a variety of pharmaceuticals, polymers and solvents	[45]
Violacein and deoxyviolacein	Therapeutic against pathogenic bacteria and viruses as well as tumor cells	[127]
1,2,4-butanetriol	Production of 1,2,4-butanetriol trinitrate (military propellant)	[149]
Taurine	Amino acid for clinical treatment such as cardiovascular diseases, hepatic disorders, alcoholism and cystic fibrosis	[33]
Silk Proteins	Materials and textile applications	[53]

regioselectively and enantioselectively reactions [142] where they are typically used as one in a series of chemical transformations. In the mid and late 1990s, Pim Stemmer and Frances Arnold pioneered molecular biology methods by which enzymes may be tuned for specific applications using a systematic process of mutation and selection known as directed evolution [39]. As a result of the advances made in this field, remarkable new features can now be engineered into enzymes, such as the ability to accept previously inert substrates (see examples in "BOX 6" of a transaminase for sitagliptin synthesis) or to change the nature of the product that is formed (see example in "BOX 6" of the utilization of a diphosphomevalonate decarboxylase for alkene production or amino acid metabolism that makes alcohols). In the past, an enzyme-based process was designed around the limitations of the enzyme; today, the enzyme is engineered to fit the process different reactions (e.g., oxygenation, halogenation, dehydrogenation, group transfers, hydrolysis, condensation and bond formation) [39]. These reactions have been reviewed previously [39] and will not be discussed in detail here.

BOX 6: Protein engineering

An interesting case is the chemical manufacturing route to Sitagliptin, a dipeptidyl peptidase-4 inhibitor treatment for type II diabetes, developed by Merck and Codexis. By designing and generating new enzyme variants, Codexis was able to identify a novel enzyme that provided detectable initial activity. This enzyme was then improved more than 25,000-fold in order to generate the highly active, stable and enantioselective enzyme from a starting activity that did not previously exist in the natural world. The application of synthetic biology and enzyme in organic synthesis is currently marketed under the trade name Januvia® [147].

As outlined before, Global Bioenergies developed a process for the direct conversion of isobutylene from sugars. The development of a new enzymatic reaction for the simultaneous dehydration and decarboxylation of 3-hydroxyisovalerate to isobutylene using a diphosphomevalonate decarboxylase (EC 4.1.1.33) created the possibility of a new method for producing isobutylene (Fig. 7). The additional development of an enzyme capable of condensation acetone and acetyl-CoA in 3-hydroxyisovalerate had a key role in the development of a metabolic pathway for the conversion of sugars to isobutylene [104, 105]. This research showed the possibility of the direct conversion of sugar to short chain terminal alkenes by microorganisms, such as propylene and butene. In an interesting approach, James Liao group utilized a broad substrate range 2-keto-acid decarboxylases to produce a broad range of branched alcohols such as isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol from carbohydrates. This strategy diverts the 2-keto acid intermediates from an amino acid biosynthetic pathway to alcohol synthesis [7].

Microbial catalysis can also be combined with enzymatic catalysis for production of complex chemicals. An industrial case is the improvement of an existing process for commercial production of cephalexin, a synthetic antibiotic, by DSM. Starting with a penicillin-producing microbial strain, DSM introduced and optimized two enzyme-encoding genes for a one-step direct fermentation of adipoyl-7-ADCA, which could then be converted into cephalexin via two enzymatic steps. The new process replaced a 13-step chemical process, resulting in significant cost, energy and residues savings [16].

In this century, only a few researchers propose to put more than four biocatalytic components in one vessel to implement very complicated reactions comparable to microbial cell factories [4, 130]. In vitro synthetic biosystems could enable higher yield and productivity in comparison with microbial catalysis. Those systems do not require ATP or NADH synthesis for microbial biomass production or maintenance. For this reason, it has been showed the possibility of production under stoichiometric yields of hydrogen, 1,3-propanediol, lactate and other chemicals [169].

In conclusion, scientists are increasing their control over the complex milieu of living cells. With the improvement of the processes outlined here, synthetic biologists will thus have an increasing palette of systems to draw on to carry out target reactions with increased efficiency [39].

Biorefinary and industrial symbiosis

Industry clusters exist in large numbers all over the world. The clusters vary in size and comprise one type of industry or a combination of industries. The colocated companies could have several forms of inter-company links [29]. In an ideal industry symbiosis, waste material and energy are utilized between/among the factors of the system and the consumption of raw material and energy inputs as well as the generation of waste and emissions are thereby reduced [30]. An industry symbiosis can also include the exchange of information or services, on site production of bioenergy and electricity, training and system planning [41]. Industrial symbioses systems are sometimes also called eco-industrial parks [41].

On the other hand, the growth rate of GHG emissions in the future will depend on three main factors: (1) the growth rate of the global economy, (2) the energy intensity of the economy and (3) the carbon intensity of our energy supply. The development of eco-industrial parks could be a tool for decoupling economic growth with carbon intensity of our energy supply. By having a renewable supply chain for the sustainable production of commodities chemicals while producing bioenergy, electricity and an infinity array of others products, eco-industrial parks could sustain social, economic and environmental development (the triple bottom line).

Figure 8 is a schematic representation of the interconnectivity of different biological engineering-based technologies to create an eco-industrial park. As outlined in this article, the value chain could start with different raw materials such as 2G crops, chemical producing crops or biofuels producing algae. Hence, raw material could be designed/selected to meet the resources constrains. For example, in regions with tight water supply, brackish or salt water resistant algae/microbes or even the utilization of microbial electrosynthesis could be used as host for chemical catalysis. In Fig. 8, sugar and triglycerides produced can be extracted and the lignocellulose raw material can be burnt to generate energy/electricity or hydrolyzed into sugars. Sugars can be used for production of a range array of biofuels, chemicals, food, pharmaceuticals, feed and enzymes, as described previously. Produced enzymes can be used for lignocellulose hydrolysis, downstream enzymatic catalysis or sold as end-products, e.g., for detergent or laundry applications. Chemicals can be sold as end-products or converted to other chemicals and polymers. The major by-products of those processes, e.g., liquid waste streams, microbial residues and CO_2, can be used among other processes for the conversion of energy and chemicals. For example, vinasse or stillage is a liquid residue produced at an impressive rate of 12–15 liters per liter of ethanol. Since Brazil's ethanol production in 2012/2013 was estimated at 25 billion liters, approx. 370 billion liters of vinasse was at disposal. Vinasse is generally used as fertilizer but due to the high chemical oxygen demand (COD), low pH and high potassium content, its use as a fertilizer should be limited [110]. Alternatively, vinasse

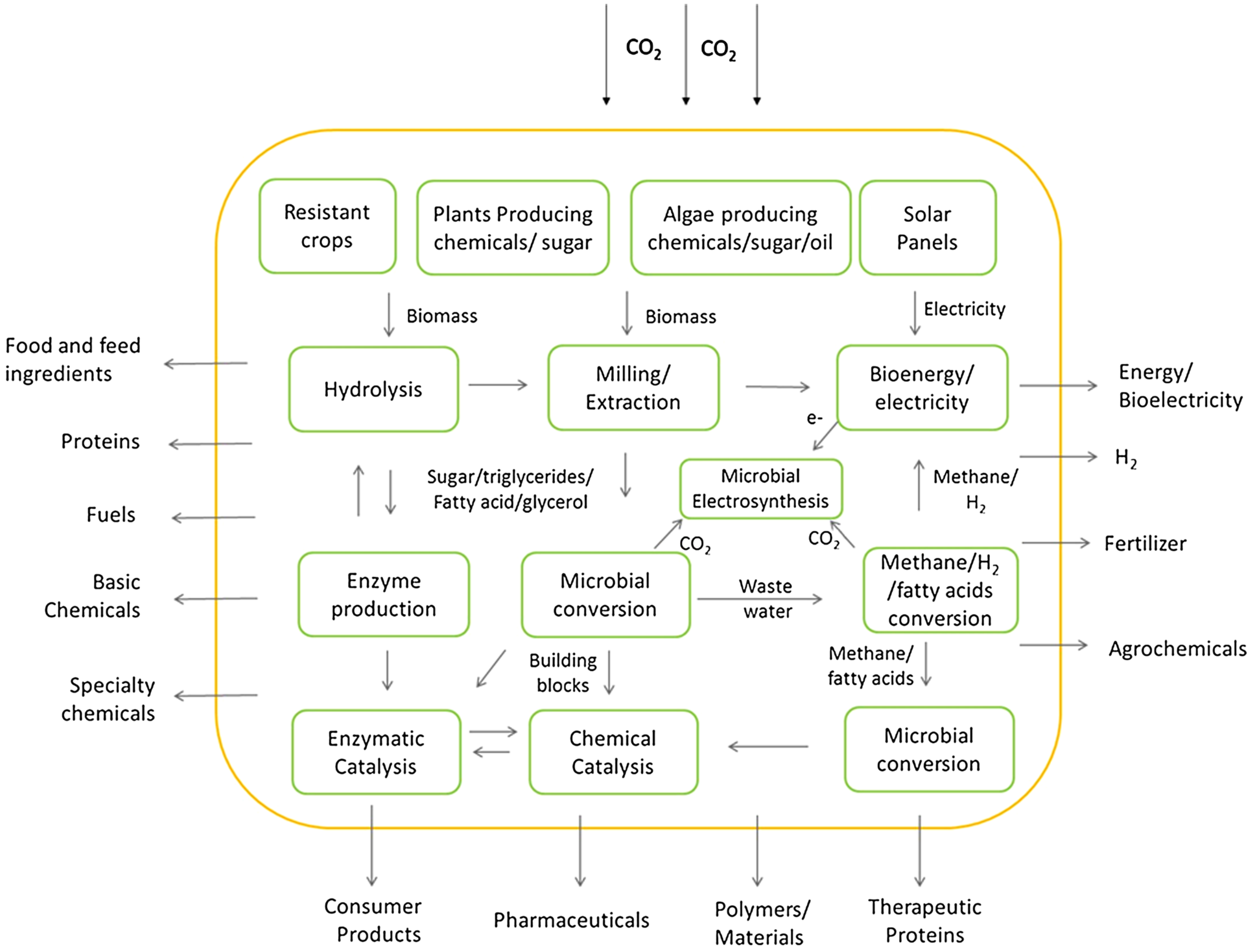

Fig. 8 Schematic representation of an eco-industrial park showing that companies could focus in different parts of the biorefinary value chain. Agricultural companies could focused in the development/commercialization of different traits of crops. Enzyme companies are involved in the hydrolysis of 2G sugars and other catalytic reactions. Biofuels companies could concentrate in 1G or 2G biofuels production, while other companies could produce value added chemicals such as agrochemicals, pharmaceuticals and specialty chemicals. Other traditional chemical catalysts manufacturers could be interesting in using renewable building blocks for the conversion of other chemicals or the polymerization of renewable thermoplastics, elastomers and/or advanced materials. Waste stream enterprises could focused on the conversion of waste streams (e.g. from biofuels or biochemicals production) to short-chain fatty acids, hydrogen and/or methane, which means that waste streams could be upgraded to resources again. In that regard, short-chain fatty acids/methane could be used as raw material for microbial conversion. Additionally, methane and solid residues could be burnt during electricity and steam production by energy companies

could be used as a raw material for production of hydrogen [153], short-chain fatty acids [55, 144], proteins [38], algal oil [143] or even bioplastics [15]. As comments before, algae could be designed for the direct synthesis of different chemicals [8, 87, 90, 106]. The vinasse microbial catalysis also impact on the COD reduction, potassium removal and its further utilization as a fertilizer without the current drawbacks [110]. Furthermore, methane or H_2 from biodigestion could be used for electricity/energy production which in turn can stimulate 2G production because the lignocellulose could be released from burning. Alternatively, methane could be used in microbial catalysis production of chemicals and biofuels, especially if more efficient methane biological metabolism is developed [65]. However, microbial short-chain fatty acids or algal oil produced from vinasse could be more suitable raw materials for microbial conversion or biodiesel production, respectively.

Taking advantage of available inexpensive and renewable electricity, CO_2 can be converted to chemicals via microbial electrosynthesis (BOX 4). Solar panels could produce additional electricity for the eco-industrial park and for the conversion of CO_2 to chemicals using bioelectrochemical systems. This would have a significant impact on the overall yield of chemicals from biomass while also decreasing the carbon footprint toward cradle-to-gate zero-carbon emission. Additionally, inputs for agriculture could be recycled. Residual microbial biomass/enzymes can also represent valuable by-products, e.g., ethanol residual yeast is dried and sold as animal feed. Finally, the rational use and recovery of NPK macronutrients are fundamental for the sustainability of the eco-industrial parks. Several approaches for rational use and recycling nitrogen [32, 73, 129] phosphorous [50, 128] and potassium have been extensively studied [34, 173].

Recent developments in engineering of biological system allow bridging the gaps to create an eco-industrial parks for the production of food, feed, chemicals, biofuels, energy and electricity. However, the sustainable production is based on the development of complementary technologies related to energy efficiency, wastewater treatment, soil conservation, biomass logistics, discovery of new drugs and advanced materials, to name just a few. Those processes could also be related to eco-industrial parks and, it is not a coincidence that engineering of biological systems could have an impact on related areas that are not the main focus of this article. For example, synthetic biology could offer a more efficient use of natural resources by creating symbiotic microbes that could decrease water [74] and nitrogen [73, 129] plant requirement. Moreover, engineered microbes could serve as a platform for drug discovery [86] and drug delivery systems [35]. And the list goes on.

Eco-industrial parks can be envisage as based on various feedstocks that can be processed by a myriad of conversion technologies and companies for the production for a diverse portfolio of products and services (e.g., wastewater treatment and lignocellulose hydrolysis). All those processes will benefit from raw material logistics, common facilities, utilities, wastewater treatment, cooling towers and other shared equipment's, including bioreactors. The eco-industrial park will benefit for economy of scale (reduction in fixed cost and CapEx), bioenergy and bioelectricity. Returning for the PTA example, the integration of the renewable PTA technology in an eco-industrial park could have a great impact on its economics, especially CapEx, energy cost and fixed costs. CapEx reduction is possible due to shared utilities and logistics. OpEX reduction is possible due to lower cost of energy and electricity production due to a shared boiler facility, for example. An additional revenue could be obtained with the utilization of wastewater, residual biomass and CO_2 for the further production of energy/chemicals (or at least related to an investment reduction for wastewater or residual biomass treatment). Additionally, it is envisioned that utilization of high-yield crops and lignocellulosic sugars could have a significant impact on production scale and sugar cost, respectively. In Fig. 5b, it is estimated a potential impact of producing PTA in an eco-industrial park. It is considered a 25 % reduction in CapEx and a 15 % reduction in raw material and utilities prices, which reflect in an increased competiveness for bio-PTA technologies.

In conclusion, eco-industrial parks represent a potential solution to decouple economic growth from environmental burdens and to increase the competitiveness of renewable technologies. In order to explicit this difference between fossil-based technologies and renewable technologies, it is necessary to create mechanism to account for externalities.

Carbon pricing

Externalities arise when certain actions of producers or consumers have unintended external (indirect) effects on other producers or/and consumers. Externalities may be positive or negative. Positive externality arises when an action by an individual or a group confers benefits to others. A technological spillover is a positive externality and it occurs when an invention not only benefits the firm but also the society as a whole. Negative externalities arise when an action by an individual or group produces harmful effects on others. Air pollution, for example, is a negative externality. The production and consumption of fossil fuels add costs to society in the form of detrimental impacts on resource availability, the environment and human health. Fossil fuels GHG emissions have many other externalities related with global warming such as ocean acidification, health care, agriculture and climate disasters. But these costs are not reflected in fossil fuel prices. Additionally, the benefits in air quality and climate change of the fossil fuels replacement for biofuels are also not reflected in biofuels prices.

International policies such as carbon pricing and emissions trading put a cost on the practice of emitting greenhouse gases and create an economic incentive to reduce emissions and the development of renewable and/or clean technologies [116]. Limiting emissions of GHGs is vital in order to reduce the risks of major changes to the climate and its societal consequences. Recent reports from the Intergovernmental Panel on Climate Change (IPCC) indicate the need to limit the increase in global mean temperature under 2 °C above pre-industrial level. Additionally, UN-IPCC make clear the importance of putting a price on carbon to keep this limit [75]. In the last UN Climate Leadership Summit, governments, businesses and investors have signaled their support for carbon pricing. A growing number of companies and governments are already working within carbon pricing systems and are developing expertise in managing their emissions and incorporating greenhouse gas reduction targets in their business/strategic planning [88]. In 2013, over 100 companies worldwide publicly disclosed that they already use carbon pricing as a tool to manage the risks and opportunities to their current operations and future profitability. Companies have disclosed using an internal price on carbon (USD per ton of CO_2 equivalent) as a planning tool to help identify revenue opportunities, risks, and as an incentive to drive maximum energy efficiencies to reduce costs and guide capital investment decisions [27]. ExxonMobil is assuming a cost of $60 per metric ton by 2030. BP and Royal Dutch Shell currently use $40 per metric ton, and Total uses $34/t [27]. In those companies, the internal price of carbon is on average $43.5 per metric ton of CO_2.

Long-term and reliable policies are required to push bioeconomy forward and to create an investment scenario that reflects our current societal challenges and the long-term externalities caused by the use of fossil fuels. Making smart use of the revenues generated by a carbon tax (or cap and trade program) is just as important as the price of carbon itself [71]. A CO_2 pricing strategy that invests carbon revenues to clean energy R&D and eco-industrial parks to generate emissions reductions appears to offer a powerful combination of improved economic dynamics toward sustainability. For example, the utilization of the CO_2 tax for CapEX reduction for the the deployment of renewable technologies could alleviate part of the current challenges in this field. Returning to the PTA example, if we account the cost of CO_2 emissions to the previous techno-economic evaluation of PTA production routes, the production of PTA from petrochemical xylene accounts for direct and indirect emissions of approximately 1,7 ton CO2 equivalent/ton of PTA [6]. It was considered a CO2 price of $43.5 per metric ton of CO_2; nevertheless, carbon prices may be politically constrained to as low as $2–$8 per ton of CO_2. [77]. On the other hand, the renewable PTA production from sugarcane could have a positive impact on GHG emissions by fixing carbon on the chemical/material produced and avoiding emissions of the petrochemical counterpart. As mentioned before, "tax and invest" strategy could represent an additional CapEX reduction for renewable PTA production—it was considered a 20 % reduction. Figure 6c represents the impact of a carbon tax and invest policy in a PTA production in an eco-industrial park. This could be a scenario, in which several renewable technologies become much more attractive and would push in investment away from more conventional fossil-based technologies toward a bioeconomy. In Fig. 5c, it is observed that this scenario could bring additional competitiveness to all renewable technologies evaluated. It is important to notice that the isobutanol fermentation is already on industrial scale while isobutylene microbial conversion is still pilot scale. Based on the described results and assumptions, it is shown that additional policies could trigger the substitution of fossil-based technologies using available renewable technologies [94, 148].

Conclusions

> "A revolution is coming – a revolution which will be peaceful if we are wise enough; compassionate if we care enough; successful if we are fortunate enough – but a revolution is coming whether we will it or not. We can affect its character; we cannot alter its inevitability". Robert F. Kennedy

The world is not on track to meet the target agreed by governments to limit the long-term rise in the average global temperature. Global GHG emissions are continuously growing (1.1 % per year) and [21], in May 2013, carbon dioxide (CO_2) levels in the atmosphere exceeded 400 parts per million for the first time in several hundred millennia. Science tells us that our climate is already changing and that we should expect extreme weather events (such as storms, floods and heat waves) to become more frequent and intense [75]. Nevertheless, GHG emissions grew at a faster rate over the decade from 2000 to 2010 than they did over the previous three decades according to the latest report from the IPCC [75]. Growing population and increase of living standards in developing countries are the main drivers for rising CO_2 emissions and fossil fuel usage in the next decades. Therefore, the supply of sustainable and affordable energy is one of the main challenges for mankind and might trigger a sustainability revolution.

However, the revolution has been toward natural gas, especially in substitution of coal in USA. In the OECD, natural gas will overtake oil as the dominant fuel by 2031, reaching a share of 31 % in primary energy by 2035 [75]. Natural gas industry is driving the reduction in GHG emissions and has been pledged to be a short-term solution for CO_2 reduction. Beating all the odds, the oil prices have dropped in half in the last two quarters. With new discoveries, stability in parts of the Middle East and increasing drilling efficiency, global oil output is rising in the next several years, adding to pressure on oil prices and in the valuation of renewable technologies.

On the other hand, ever since the 1970s, the world appears to have been using less energy to produce a given unit of economic activity. Energy usage is gradually decoupling from economic growth.

However, the global warming risks demand to take drastic measures to reduce significant emissions of CO_2. According to the last IPCC report, GHG emissions in 2050 will have to be 40–70 % lower than what they were in 2010. By the end of the century, GHG emissions will need to be at zero, or even possibly, it will be required to remove carbon dioxide from the atmosphere [75].

Several breakthroughs will be required for the society decarbonization in order to mitigate climate change. As outlined here, engineering biological systems could serve as one of the pillars for developing those sustainable solutions. Engineering biological system will have a strong role in value chain consolation through the creation of more efficient and sustainable routes for the production of food, biofuels, energy, chemicals and materials, especially in the context of eco-industrial parks. Strengthening the eco-industrial parks requires overcoming collective action challenges through international cooperation between enterprises, governments and civil society. Reliable policies for carbon pricing are fundamental to include externality costs of fossil-based products. In turn, CO_2 tax reinvestment in disruptive technologies and in the deployment of eco-industrial parks could support the transition from a fossil to a biobased economy. With the right incentives and timing, biological solutions for global problems could create a new eco-industrial architecture boosting the welfare and addressing our major global risks.

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