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Enabling a circular economy

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Biocatalysis and biomass conversion: enabling a circular economy

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This paper is based on a lecture presented to the Royal Society in London on 24 June 2019. Two of the grand societal and technological challenges of the twenty-first century are the 'greening' of chemicals manufacture and the ongoing transition to a sustainable, carbon neutral economy based on renewable biomass as the raw material, a so-called bio-based economy. These challenges are motivated by the need to eliminate environmental degradation and mitigate climate change. In a bio-based economy, ideally waste biomass, particularly agricultural and forestry residues and food supply chain waste, are converted to liquid fuels, commodity chemicals and biopolymers using clean, catalytic processes. Biocatalysis has the right credentials to achieve this goal. Enzymes are biocompatible, biodegradable and essentially non-hazardous. Additionally, they are derived from inexpensive renewable resources which are readily available and not subject to the large price fluctuations which undermine the long-term commercial viability of scarce precious metal catalysts. Thanks to spectacular advances in molecular biology the landscape of biocatalysis has dramatically changed in the last two decades. Developments in (meta)genomics in combination with 'big data' analysis have revolutionized new enzyme discovery and developments in protein engineering by directed evolution have enabled dramatic improvements in their performance. These developments have their confluence in the bio-based circular economy.

This article is part of a discussion meeting issue 'Science to enable the circular economy'.

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1. Introduction: the circular bio-based economy

The linear take–make–consume–dispose flow of materials and 'planned obsolescence' that formed the cornerstones of the twentieth-century consumer society are not conducive to tackling the grand challenges that society now faces in the twenty-first century. Mounting concern with global problems such as climate change, depletion of natural resources, the decimation of the earth's biodiversity and massive environmental pollution are motivating a paradigm shift to thinking and acting within a circular economy (CE) concept (figure 1) [1,2].

A CE is restorative or regenerative by design [3]. It endorses the preservation of natural resources and the reduction or, preferably, elimination of waste by designing products such that their recycling and reuse is inherently facilitated. It also strives to dramatically reduce our dependence on fossil resources—coal, oil and natural gas—and waste generation by exploiting renewable resources in a so-called bio-based economy. It is not entirely new. The iconic environmentalist, Barry Commoner, already observed in 1971 [4]: 'Here is the great fault of the life of man in the ecosphere. We have broken out of the circle of life, converting its endless cycles into man-made linear events: oil is taken from the ground, distilled into fuel, burned in an engine, converted thereby into noxious fumes which are emitted in the air.'

The CE is implicit in the bio-based economy which is concerned with the use of renewable biomass to replace the unsustainable use of fossil resources as the raw material for the manufacture of fuels, commodity chemicals and materials, such as plastics. This is synonymous with the concept of sustainable development. Thus, in order to be sustainable, a technology must fulfil two conditions: (i) natural resources should be used at rates that do not unacceptably deplete supplies over the long term and (ii) residues should be generated at rates no higher than can be assimilated readily by the natural environment [5,6].

Figure 2*a* depicts the petrochemical carbon cycle in which carbon dioxide is converted via photosynthesis into plant biomass that is subsequently laid down in geological reservoirs where, over a period of millions of years, it becomes coal, natural gas and oil. Liquid transportation fuels are produced from oil in petrochemical refineries. Their subsequent combustion produces carbon dioxide which is returned to the atmosphere, but in a five orders of magnitude shorter time. The result is depletion of fossil resources and, in the shorter term, increased carbon dioxide levels in the atmosphere, which are widely believed to be a direct cause of climate change.

In order to be sustainable, the underlying processes in the carbon cycle need to be brought into balance (figure 2*b*) by converting plant biomass, which can be replaced in a matter of a few years, directly to biofuels and chemical products. Hence, the conversion of renewable biomass to biofuels, chemicals and biomaterials in integrated bio-refineries forms the basis of a carbon neutral, bio-based economy (figure 3). The term 'bio-based' refers to the use of renewable biomass as the raw material rather than the technology used for its conversion, which can involve chemical or biotechnological processing.

2. Carbon neutral technologies and waste valorization

A switch from fossil-based resources to renewable biomass as a feedstock for the manufacture of biofuels, commodity chemicals and biomaterials, such as bio-based plastics is essential for the envisaged decarbonization of society [7–9]. An additional benefit could be the substitution of existing products by inherently safer, bio-based alternatives with reduced environmental footprints, such as recyclable and/or biodegradable plastics [10,11].

Renewable biomass consists primarily of carbohydrates which can be divided into storage polysaccharides, such as starch and inulin and the disaccharide, sucrose, which comprise first generation (1G) biomass, and structural polysaccharides exemplified by (ligno)cellulose, hemicellulose, pectin and chitin which constitute second generation (2G) feedstocks. Aquatic carbohydrates, derived from micro- and macro-algae, consist of a variety of polysaccharides that differ in structure from their terrestrial counterparts and are potential feedstocks for third generation (3G) bio-refineries.

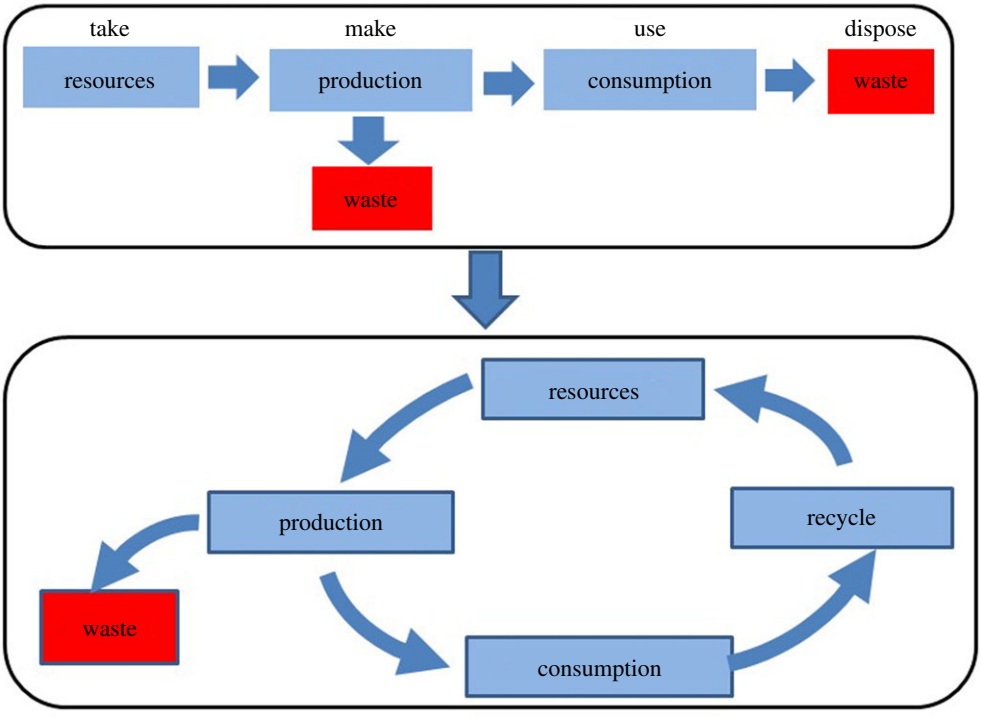


Figure 1. The linear versus the circular economy. (Online version in colour.)

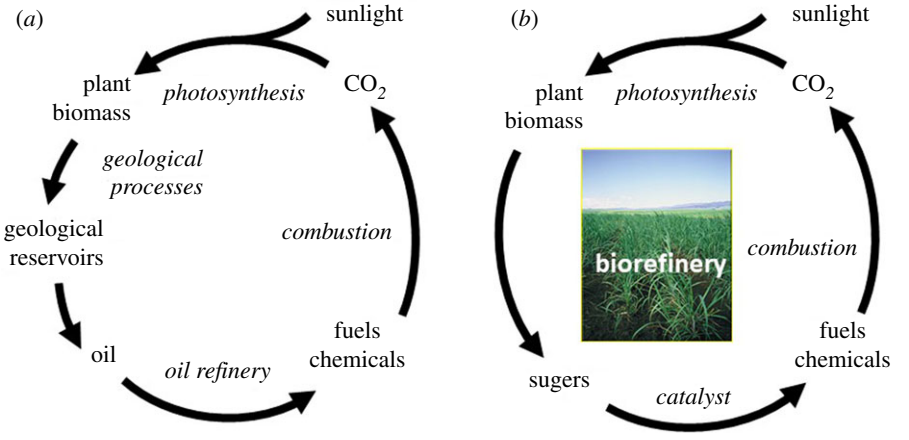


Figure 2. (*a*) The petrochemical carbon cycle and (*b*) the bio-based circular economy. (Online version in colour.)

Currently, the bio-based economy is based almost exclusively on 1G feedstocks which can relatively easily be depolymerized hydrolytically to glucose which is further converted to biofuels, mainly bioethanol, and commodity chemicals. The major producers are the United States with 56% (61 billion litres; 48 million tonnes) consisting of corn starch-based ethanol and Brazil with 28% (30 billion litres; 24 million tonnes) sucrose-based ethanol and the EU with a paltry 5% (5.4 billion litres; 4.2 million tonnes) mainly from wheat starch in 2018, leaving 11% for the rest of the world [12]. However, the use of starch from corn and wheat and sucrose from sugar cane and beet, and triglycerides from edible oil seeds are not perceived as sustainable options in the longer

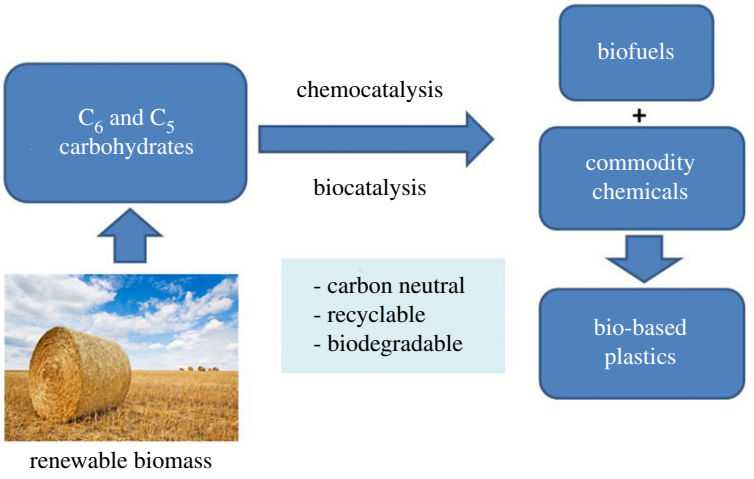


Figure 3. The bio-based economy. (Online version in colour.)

term because of competition, directly or indirectly, with food production. In the European Union emphasis is, therefore, firmly on the use of 2G biomass, comprising waste lignocellulose and waste oils and fats as feedstocks, for the future production of biofuels and commodity chemicals.

This would seem, in the first instance, to be in contradiction with the CE concept which emphasizes the elimination of waste. However, some organic waste in the form of lignocellulose, such as agricultural and forestry residues, is unavoidable and this waste constitutes a suitable feedstock for valorization in integrated bio-refineries [13]. Interestingly, lignocellulose waste is formed as inevitable waste in the production of both food and 1G feedstocks. In the latter case, there is much to be gained from conversion, for example of both the sugar and the sugar cane bagasse or sugar beet pulp, or both the cassava starch and cassava pulp [14] in what is sometimes called 1.5 G biomass processing. For example, global production of lignocellulosic waste in the form of sugar cane bagasse, corn stover, wheat straw and rice straw amounts to hundreds of millions of tonnes per annum [15] and far exceeds the annual production of the top petrochemicals, ethylene (150 million tonnes), propylene (90 million tonnes) and para-xylene (40 million tonnes). Conversion of this waste to value-added products is at the heart of a bio-based circular economy [16–18].

Similarly the enormous amounts of so-called food supply chain waste (FSCW) [19], formed in the harvesting, processing and use of agricultural products in food and beverages is largely unavoidable but can be converted to value-added products in food waste bio-refineries [20,21]. The growing need for creating value by valorization of unavoidable waste is widely recognized and is embodied in the European Commission's 'Roadmap to a resource efficient Europe' [22].

3. Biocatalysis is green and sustainable

Most organic chemists are familiar with many of the benefits of enzymatic catalysis which make them green and sustainable: high chemo-, regio- and enantio-selectivities under mild conditions (physiological pH, ambient temperature and pressure) affording products of high purity in processes which are more efficient in the consumption of energy and resources and generate less waste than conventional routes. Enzymes are biocompatible (sometimes even edible), biodegradable and essentially non-hazardous. Additionally, they are derived from inexpensive renewable resources which are readily available and not subject to the large price fluctuations which undermine the long-term commercial viability of catalysts derived from scarce precious metals [23]. Moreover, it avoids the costly removal of trace amounts of noble metals from end products to a level that meets regulatory requirements [23].

However, this begs the question: if biocatalysis is so attractive why was it not broadly applied in industrial organic synthesis in the last 50 years? The main reasons were limited commercial availability of many (sub) classes of enzymes and the poor performance of most enzymes under the typically demanding conditions of industrial organic synthesis. Thus, in the 1980s and 1990s, applications generally involved hydrolases—mainly lipases and proteases—that were applied in other industries such as laundry detergents and food processing and amidases used in the manufacture of a few D- and L-amino acids, for example [24]. Even pioneering applications of hydrolases with non-natural substrates were carried out with such hydrolases [25]. In industrial processes, substrate and product concentrations far exceed those usually experienced in Nature and enzymes are confronted with molecules that are often structurally very different to their natural substrates [26]. Moreover, substrates and products are frequently hydrophobic and enzymes need to be tolerant to organic solvents that are added to enhance substrate solubility. Generally speaking, for commercial viability, product concentrations should be at least $100\,g.l^{-1}$; Space–Time Yields greater than $10\,g.l^{-1}\,h^{-1}$ and catalyst productivities greater than 100 g of product per g of enzyme [27]. This was enabled by dramatic developments in new enzyme discovery (more enzymes) and enzyme performance (better enzymes).

(a) Enzyme discovery in the age of (meta)genomics

Historically, enzyme discovery involved preparing microbial cultures from environmental samples and was seriously hindered by the fact that 99% of microorganisms are 'unculturable'. This changed irrevocably with the advent of (meta)genomics, that is the sequencing and annotating (identifying the individual genes and their functions) of genomes of numerous individual organisms contained in genetic material derived from environmental samples [28]. The first two bacterial genomes were sequenced in 1995 [29]. The subsequent introduction of next generation sequencing (NGS) technologies [30] dramatically reduced the cost of genome sequencing (figure 4). The National Human Genome Research Institute (NHGRI) has tracked these costs at sequencing centres that they funded [31].

In 2014, *ca*. 30 000 bacterial genomes had already been sequenced. This exponentially expanding genomic data, contained in publicly accessible databases, can be mined *in silico* to search for sequence homology with existing enzymes of known function, thus enabling the identification of countless new enzymes. At the same time, the cost of gene synthesis has decreased substantially, although not as dramatically as that of sequencing [32]. It is now possible to identify an interesting gene sequence by (meta)genome bioprospecting of public databases, order its synthesis on line, and have a plasmid, ready for cloning in an appropriate microbial host, such as *Saccharomyces cerevisiae* or *Escherichia coli*, delivered in two weeks or less. In the past, only a tiny fraction of microbial diversity was accessed, strongly suggesting that many desirable enzyme activities are out there just waiting to be discovered for industrial applications [33] using metagenomics-based screening protocols [34]. Indeed, combination with powerful bioinformatics for rapid analysis of DNA sequence data is essential to keep up with the unstoppable march of genomic data acquisition.

(b) Better enzymes through directed evolution

Engineering proteins via directed evolution is undoubtedly the single most important enabler of the rapid growth in industrial biocatalysis in the last two decades. In the 1990s the so-called rational design strategy, based on structural and mechanistic information, was superseded by *in vitro* random mutagenesis protocols [35] employing the error-prone polymerase chain reaction epPCR [36] to generate libraries of mutant proteins. However, in Darwinian evolution, beneficial homologous combinations result from reassortment of mutations and this can be imitated, *in vitro*, using DNA shuffling [37].

A disadvantage of DNA shuffling is that it requires the screening of enormous libraries of mutants. Subsequently, smaller, 'smart' libraries of mutants, which could be rapidly screened,

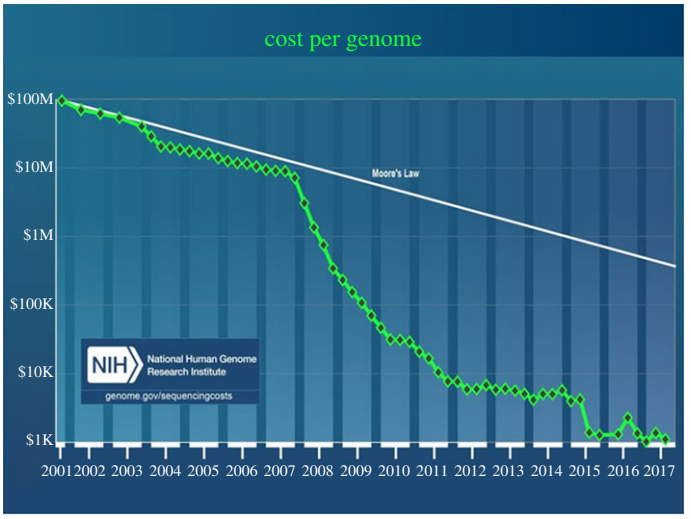


Figure 4. Reduced costs of next generation sequencing. (Online version in colour.)

were generated by combining elements of rational design and random mutagenesis [38]. For example, ProSAR [39] augments DNA shuffling with statistical analysis of protein sequence–activity relationships to identify individual mutations responsible for improved functions. The spectacular improvements obtained using ProSAR with the bacterial halohydrin dehalogenase (HHDH), a key enzyme in the commercial synthesis of an intermediate for atorvastatin (Lipitor), included a 4000-fold increase in volumetric productivity [40]. Furthermore, directed evolution techniques continue to evolve, and as huge amounts of data are involved, it is an ideal situation for exploiting machine learning [41].

There is no doubt that the use of directed evolution techniques, such as DNA shuffling, to produce highly engineered enzymes with dramatically improved performance, has paved the way for the widespread application of biocatalysis in industrial organic synthesis, particularly in the synthesis of active pharmaceutical ingredients. In addition to the above described atorvastatin, biocatalysis has been successfully applied to the industrial synthesis of sitagliptin [42] and a host of other APIs (active pharmaceutical ingredient) [43,44]. Indeed, this astounding success story provoked the observation that we are now in the Golden Age of Biocatalysis [45]. However, because of the small volumes involved, the monumental impact that biocatalysis has had in the pharmaceutical industry pales in quantitative significance with what we are concerned with here, namely the transition to a carbon neutral, circular economy. Only large volume products, such as biofuels and industrial monomers, will have a significant effect on reducing the carbon footprint of chemicals manufacture as a whole. Moreover, it is worth noting that whether or not a raw material is renewable is hardly an issue in the pharmaceutical industry.

(c) Better circularity with enzyme immobilization

Enzymes are soluble in water and cannot be easily recovered from aqueous waste streams. Consequently, most enzymes are currently applied on a single use, throw-away basis which is not conducive to cost-effective processing or a circular economy. Hence, significant cost savings can be achieved by immobilization of the enzyme(s) as a solid, heterogeneous catalyst that is insoluble in water and can be separated by filtration or centrifugation and recycled multiple times. Enzyme immobilization involves adsorption on, covalent attachment to, or encapsulation in a suitable carrier, or by cross-linking [46].

Immobilization as cross-linked enzyme aggregates (CLEAs; figure 5) [47] is an example of the latter, carrier-free method which has the advantage of higher catalyst productivities (kg product

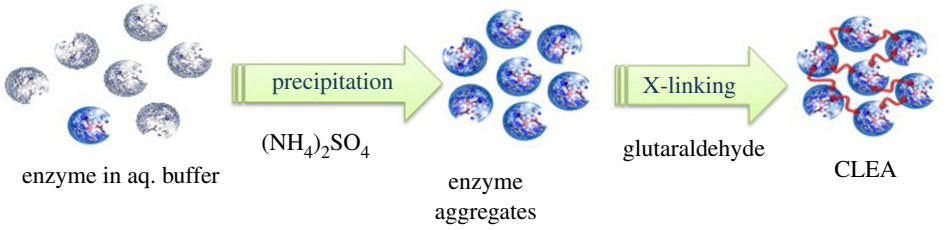


Figure 5. Enzyme immobilization as cross-linked enzyme aggregates (CLEAs). (Online version in colour.)

per kg biocatalyst) and avoids the extra cost of a suitable carrier. CLEAs are readily prepared from crude cell lysates obtained from fermentation broth, thus avoiding the costs of purification. In addition, they are highly stable towards leaching in aqueous media and generally exhibit improved storage and operational stability with regard to denaturation by heat, organic solvents and autolysis. In a more recent elaboration of the technology, the cross-linking is performed in the presence of magnetic (nano)particles to produce magnetizable CLEAs which can be separated magnetically from mixtures containing other solids [48].

4. Primary conversion of first and second generation polysaccharides

Irrespective of whether the final product is a biofuel or a commodity chemical the first step is depolymerization of the polysaccharide feedstock. Production of bioethanol from the 1G feedstock starch is depicted in figure 6. It involves a two-step enzymatic hydrolysis of starch to glucose involving liquefaction catalysed by α-amylase (EC 3.2.1.1.) and saccharification catalysed by glucoamylase (EC 3.2.1.3). This is then followed by fermentation of the resulting glucose with *S. cerevisiae* (brewer's yeast). The enzymatic hydrolysis and fermentation can be carried out as a separate hydrolysis and fermentation (SHF) or in a one-pot, simultaneous saccharification and fermentation (SSF) [49]. An advantage of the latter is that the glucose is immediately consumed by the fermenting organism thus avoiding product inhibition in the enzymatic hydrolysis step.

The 2G feedstock lignocellulose is much more difficult to process. It consists of roughly 65% polysaccharides (40% cellulose and 25% hemicellulose) and 25% lignin. There are basically two ways to depolymerize lignocellulose: thermochemical and hydrolytic (figure 7) [50]. Thermochemical processing involves pyrolysis or gasification to a mixture of carbon monoxide and hydrogen (syn gas) analogous to coal gasification processes developed following the first oil crisis in 1974 [51]. The syn gas can be further processed using established technologies such as methanol synthesis or the Fischer–Tropsch conversion to hydrocarbons. An interesting alternative is to ferment the syn gas using acetogenic bacteria to afford biofuels and commodity chemicals [52]. However, a serious bottleneck in syn gas fermentation is created by the mass transfer limitations as a result of the limited solubility of the CO and H_2 in the aqueous medium [53].

The current method of choice is enzymatic hydrolysis [54] in which a pre-treatment step, such as a steam explosion, ammonia fibre expansion (AFEX) [55] or lime treatment, is required to open up the recalcitrant lignocellulose structure and render the targeted glycoside (ether) and ester bonds accessible to the enzyme cocktails [56–58].

(a) Pre-treatment of lignocellulose

Pre-treatment is generally conducted in water, in which cellulose, hemicellulose and lignin are present as suspended solids. In the Organosolv process (figure 8), by contrast, lignocellulose is subjected to elevated temperatures (185–210°C) in water/organic solvent (e.g. ethanol) mixtures [59]. Organic acids formed *in situ* catalyse the cleavage of the lignin–polysaccharide complex. Cellulose is removed by filtration and ethanol by distillation resulting in precipitation of the lignin

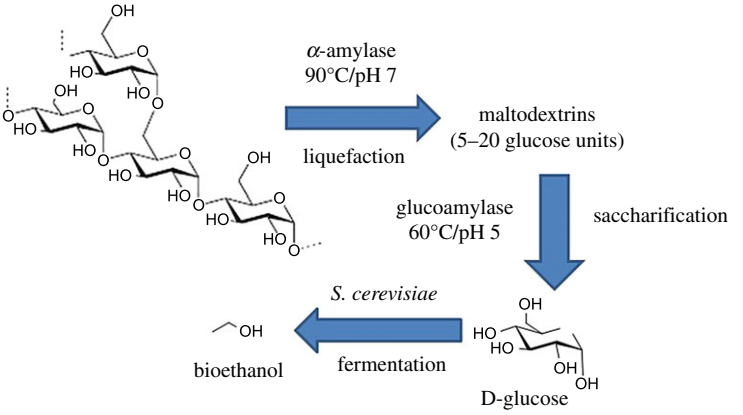
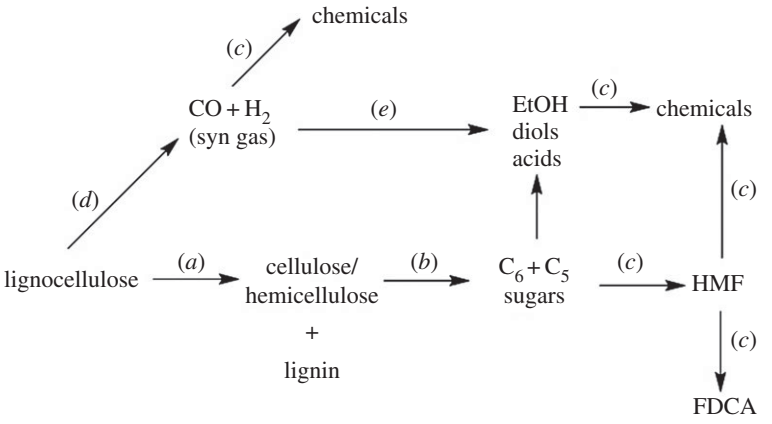


Figure 6. First generation bioethanol from starch. (Online version in colour.)



(*a*) pretreatment (*b*) enzymatic hydrolysis (*c*) chemocatalysis
(*d*) gasification (*e*) fermentation

Figure 7. Primary conversion of lignocellulose.

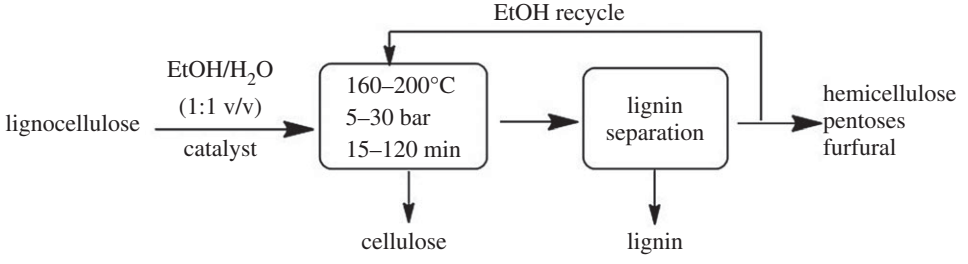


Figure 8. Organosolv pre-treatment of lignocellulose.

to leave a filtrate containing hemicellulose and/or the hydrolysis products. However, the use of a volatile organic solvent at such high temperatures presents a potential safety hazard. An interesting alternative, therefore, is to use a mixture of water with high-boiling (crude) glycerol to produce a mixture of sugars, lignin and high purity glycerol [60,61].

Recently, increasing attention is being focused on the relatively new utilization of ionic liquids (ILs) in lignocellulosic biomass pre-treatment (Ionosolv pre-treatment) and the state of the art has recently been reviewed [62]. A significant advantage of ILs is that they are eminently suitable for conducting enzymatic reactions with highly polar substrates such as carbohydrates and the activities of cellulolytic enzymes are maintained in many ILs [63]. There are basically two IL pre-treatment strategies: complete dissolution of the biomass in the IL or selective extraction of lignin and hemicellulose [64]. Dissolution of the entire biomass allows for direct coupling with enzymatic hydrolysis but generally requires rather costly ILs, such as 1-ethyl-3-methylimidazolium acetate, [EMIM] [OAc].

Promising results have been obtained using the fractionation strategy with low-cost protic ionic liquids (PILs) which are readily prepared by neutralizing simple amines with acids such as acetic or sulfuric acid. For example, Hallett & co-workers [65–67] used triethylammonium hydrogen sulfate, [TEA][HSO_4], and NN-dimethylbutylammonium hydrogen sulfate, [DMBA][HSO_4], to fractionate lignocellulose into soluble lignin and hemicellulose and insoluble cellulose pulp. The latter was hydrolysed enzymatically to glucose, in up to 100% yield, even from recalcitrant feedstocks such as softwoods.

Singh & co-workers [68] described a one-pot conversion of lignocellulose to ethanol via pre-treatment, enzymatic saccharification and fermentation (PSF) in the PIL, ethanolamine acetate [EOA][OAc]. The latter is produced by simply mixing two commodities: ethanolamine and acetic acid. Prices of such PILs are estimated to be in the region of USD 1 per kg or less. Interestingly, the fermentation step in these studies is conducted with *S.cereviseae* that can only accept hexoses as substrate. Hence, ethanol yield and concentrations could be significantly further improved by employing engineered yeasts [69] that are able to convert the pentoses—xylose and arabinose—derived from the hemicellulose fraction of lignocellulose. Indeed, for commercial viability, it is of the utmost importance to convert all of the components of biomass—cellulose, hemicellulose and lignin—to value-added products.

(b) Lignin first valorization of lignocellulose

Another recent development in lignocellulose valorization is the so-called lignin first strategy [70,71]. Historically, the primary objective of lignocellulose biorefining was to valorize the polysaccharide fractions, in particular to produce high-quality cellulose for paper manufacture. Lignin was the ugly sister of the lignocellulose family, only fit for providing the energy necessary for the overall process and to be removed in the early stages of biorefining. Harsh processing conditions were applied, resulting in irreversible degradation of the lignin fraction, yielding an intractable solid that is only suitable for use as an inexpensive energy source. By contrast, it is now generally accepted that for economic viability, it is essential to valorize all three components of lignocellulose. Lignin is potentially an important source of renewable aromatic building blocks as an alternative to fossil resources-based benzene, toluene and xylenes (BTX). However, in order to unlock this potential, a paradigm shift was needed and this led to the emergence of the lignin first concept which relies on active lignin stabilization during lignocellulose fractionation.

The lignin first strategy gives priority to lignin valorization from the outset by employing so-called reductive catalytic fractionation (RCF) [72] otherwise known as catalytic upstream biorefining (CUB). In this strategy, solvent extraction of lignin is accompanied by lignin depolymerisation and reductive stabilization of reactive intermediates by catalytic hydrogenolysis, using molecular hydrogen or an external hydrogen donor. This results in the formation of a highly depolymerized lignin oil consisting of a limited set of aromatic monomers [73] as possible platform chemicals [74,75].

An alternative to reductive catalytic fractionation is to convert lignin by fermentation to commodity chemicals, for example to *cis*, *cis*-muconic acid [76,77], a potential platform chemical for the manufacture of polyesters, polyamides and polyurethanes. It can also be converted to

terephthalic acid via isomerization to *trans*, *trans*-muconic acid and Diels–Alder reaction with ethylene [78].

(c) Enzymatic hydrolysis of cellulose and hemicellulose

Enzymatic hydrolysis of cellulose and hemicellulose to fermentable sugars requires the involvement of a complex cocktail of cellulolytic and hemicellulolytic enzymes [54,79]. The pre-treatment costs and the cost of the enzyme cocktail contribute significantly to the overall cost of 2G bioethanol. The enzyme costs have decreased significantly over the last decade and are still decreasing, as a result of optimization of the production and performance of the cellulolytic enzyme cocktail [80].

The hydrolysis of cellulose involves catalysis by at least six enzymes, exo-1,4-β-glucanase (EC 3.2.1.91), endo-1,4-β-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.176), β-glucosidase (EC 3.2.1.21) and copper-dependent lytic polysaccharide monooxygenases [81], which catalyse the oxidative cleavage of polysaccharides. Hemicellulose has a more complicated structure than cellulose and requires a diverse suite of enzymes to affect its hydrolysis to its constituent sugars, mainly xylose and mannose. These enzymes are divided into core enzymes, which catalyse cleavage of the polysaccharide backbone, and ancilliary enzymes, which catalyse the removal of functional groups. Examples of core enzymes are endo-β-1,4-xylanase (EC 3.2.1.8), xylan-1,4-β-xylosidase (EC 3.2.1.37), endo-1,4-β-mannanase (EC 3.2.1.78) and β-1,4-mannosidase (EC 3.2.1.25). Ancilliary enzymes include β-glucuronidase (EC 3.2.1.139), acetylxylan esterase (EC 3.2.1.55), ferulic acid esterase (EC 3.1.1.73) and p-coumaric acid esterase (EC 3.1.1-).

In vivo these enzymes are contained in cellulosomes [82], multi-enzyme complexes produced by many cellulolytic fungi and bacteria. Cellulosomes have a distinct advantage compared to simple mixtures of the free enzymes owing to the close proximity of the enzymes. *In vitro* this proximity effect can be mimicked in combi-CLEAs. For example, a xylanase-mannanase combi-CLEA was successfully applied in the conversion of lime pre-treated sugar cane bagasse and milled corn stover [83]. The authors concluded that combi-CLEAs are ideal candidates for achieving cost-effective application of lignocellulytic enzymes. Similarly, a combi-CLEA of xylanase, β-1,3-glucanase and cellulase was more operationally stable than the free enzymes and retained more than 97% of its activity on storing at 4°C for 11 weeks, compared to 65% for the free enzymes [84]. The combi-CLEA was successfully used in the hydrolysis of ammonia-cooked sugar cane bagasse and could be recycled six times. Various groups have reported the successful immobilization of a cellulase cocktail as CLEAs [85–90].

The application of immobilized enzymes in the hydrolysis of starch and cellulose presents an extra challenge: the solid catalyst may have to be separated from other suspended solids present in the reaction mixture. This is readily achieved [91], in a cost-effective manner on a large scale, in starch hydrolysis using magnetizable CLEAs of glucoamylase and magnetic separation equipment commonly used in the mining industry. Similarly, the immobilization of cellulolytic enzymes as magnetizable CLEAs has been reported [92]. The resulting glucose and pentose sugars are subsequently converted to bioethanol by fermentation. The enzymatic hydrolysis and fermentation can be carried out separately or in a one-pot, simultaneous saccharification and fermentation (SSF) [93]. In the latter option a ‘smart’ magnetizable CLEA in combination with magnetic separation can be used to separate the immobilized enzyme(s) from the yeast in the fermentation broth.

5. Catalytic conversion of fermentable sugars to commodity chemicals and bio-based polymers

In an integrated biorefinery, fermentable sugars produced by hydrolysis of polysaccharides, such as waste lignocellulose, are converted to commodity chemicals [94]. This is directly comparable to the conversion of hydrocarbons, such as lower olefins and aromatics, to commodity chemicals in

traditional, petrochemical refineries. Conversion to commodity chemicals, such as lower alcohols, diols and mono- and di-carboxylic acids involves primarily fermentation processes and much progress has been made in this area as a direct result of metabolic pathway engineering [95,96], the whole cell equivalent of protein engineering. It can involve the optimization of existing biochemical pathways or the introduction of new pathways.

A large majority of these commodity chemicals comprises industrial monomers, the raw materials for a plethora of polymer products, such as plastics. This coincides with another global problem of growing concern that the circular economy seeks to alleviate: massive contamination of our natural environment by single-use plastics.

(a) The environmental impact of single-use plastics

Their large-scale production and use dates back less than 70 years but a world without plastics is unimaginable today. The commonly used plastics are produced from fossil resource derived hydrocarbons, notably ethylene, propylene and aromatics (BTX). The largest volume non-fibre plastics are polyethylene, PE (36%), polypropylene, PP (21%) and polyvinylchloride, PVC (12%), followed by polyethylene tetraphthalate (PET), polystyrene (PS) and polyurethanes (PURs), each comprising less than 10%. Polyesters, mostly PET, account for most of the fibre production and together these seven groups account for 92% of all the plastics ever made [97]. Approximately 42% of all non-fibre plastics, consisting primarily of PE, PP and PET involve single-use applications in packaging.

None of these plastics biodegrade in the natural environment but sunlight can cause their fragmentation into 'microplastics', the long-term environmental impact of which is largely unknown. Cumulative waste generation of primary and secondary (recycled) plastic waste, in the period 1950–2015, amounted to 6300 million tons [97]. Roughly, 800 million tons (12%) of this waste was incinerated and 600 million tons (9%) was recycled, only 10% of which was recycled more than once. Roughly, 4900 million tons, amounting to 60% of all the plastics ever produced were discarded and accumulated in landfills or in the natural environment. Projection of current global use patterns and waste management trends leads to the prediction that 12 000 million tons of plastic waste will have accumulated in landfills and the natural environment by 2050. The conclusion is clear: application of non-biodegradable plastics on a single-use basis is unsustainable and has to be replaced by a circular economy scenario in which plastics are designed for recyclability and/or biodegradability.

(b) Bio-based building blocks and polymers

Bio-based polymers can be divided into two categories: drop-in replacements and new bio-based polymers [98]. The former are chemically identical to their petrochemical counterparts and are, at least partially, derived from renewable biomass. Examples include PE from bioethanol and PET from bioethylene glycol. Drop-in bio-based PET is the overall market leader [98] and currently consists almost entirely of partly bio-based PET (20% bio-based carbon content) produced from bioethanol (2 C atoms) and fossil p-xylene (8 C atoms) as shown in figure 9. Considerable research effort is being devoted to the synthesis of bio-based terephthalic acid in order to enable the production of 100% bio-based PET.

Avantium, by contrast, developed polyethylene furandicarboxylate (PEF), a bio-based alternative to PET that is produced from ethylene glycol and furan-2,5-dicarboxylic acid (ester). The latter is prepared by chemo- [99] or biocatalytic oxidation [100,101] of HMF (5-hydroxymethylfurfural) that is in turn obtained by acid catalysed dehydration of glucose (figure 10). In addition to being 100% bio-based, i.e. carbon neutral, PEF also has superior mechanical, thermal and gas barrier properties compared to PET [102]. Similarly, a bio-based polyester has been produced from FDCA and bio-based 1,3-propane diol [103].

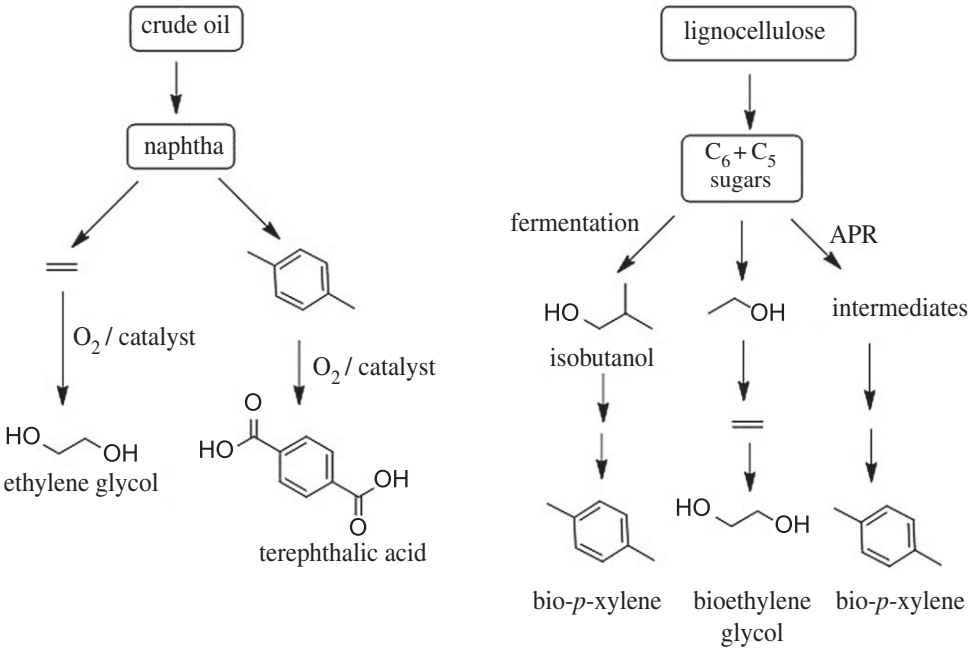


Figure 9. Production of fossil- versus bio-based (PET).

(c) Enzymatic synthesis of (bio-based) polyesters and polyamides

The sustainability of polymers can be further enhanced by employing greener enzymatic methodologies for their manufacture. This is not feasible with some polymers, such as polyolefins, but polyesters [104–106] and polyamides [106,107], for example, are eminently suited to enzymatic production. An example of the former is provided by poly(butylene succinate) (PBS) which is biodegradable and derived from two raw materials—1,4-butane diol and succinic acid—which are produced from renewable biomass. Practical applications, e.g. in biodegradable thermoplastics, require a high molecular weight (greater than 20 000) polymer. This has been produced using organometallic catalysts at elevated temperatures (greater than 190°C) but suffers from problems of discoloration and difficult removal of residual amounts of metals from the polymer. By contrast, PBS with a molecular weight of 38 000 was produced by Novozyme435 catalysed reaction of diethyl succinate with 1.4-butane diol at 95°C in diphenyl ether as solvent [108]. The latter was necessary in order to create a single liquid phase system.

Similarly, bio-based polyesters and polyamides can be produced by Nov435 catalysed reaction of furan dicarboxylic acid (FDCA) esters with diols [11] or diamines [109]. The diol produced by hydrogenation of HMF (BHMF) can similarly be converted to polyesters by Nov435 catalysed reaction with esters of dicarboxylic acid including FDCA (figure 11).

Lactic acid is an example of a commodity chemical that has traditionally been produced by fermentation and polylactic acid (PLA) is both 100% bio-based and biodegradable but only under certain conditions (it is industrially compostable). It is the most well established bio-based polymer with a current production of 195 000 tons per annum in 2015 which is predicted to grow substantially in the future [110]. The structurally related polyhydroxyalkanoates (PHAs) are examples of new bio-based polymers that are biodegradable, even in cold seawater. PHAs are involved in energy and carbon storage in acetogenic bacteria, such as *Plasticicumulans acidivorans*, and can comprise up to 90% of the dry weight of the microorganism [111]. In contrast with the production of industrial monomers from waste lignocellulose, such bacteria can be used to produce PHAs directly from essentially any organic waste, including that from paper mills [112],

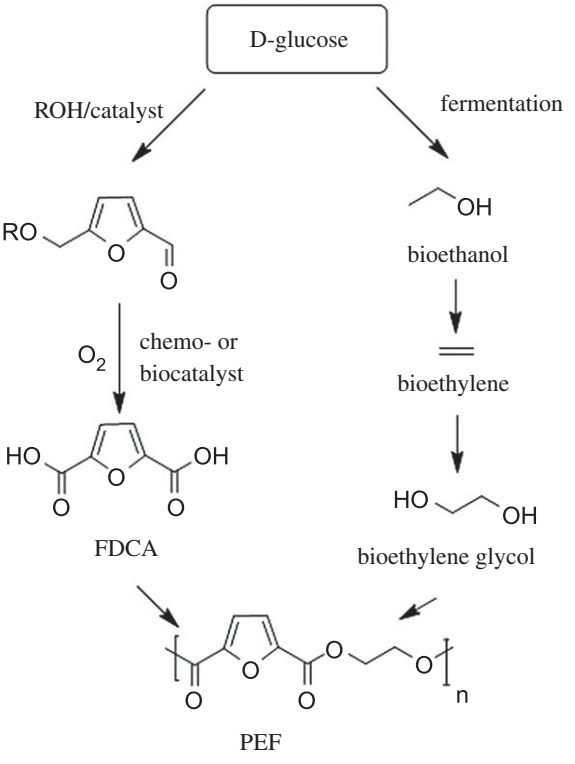


Figure 10. Production of carbon neutral PEF dicarboxylate.

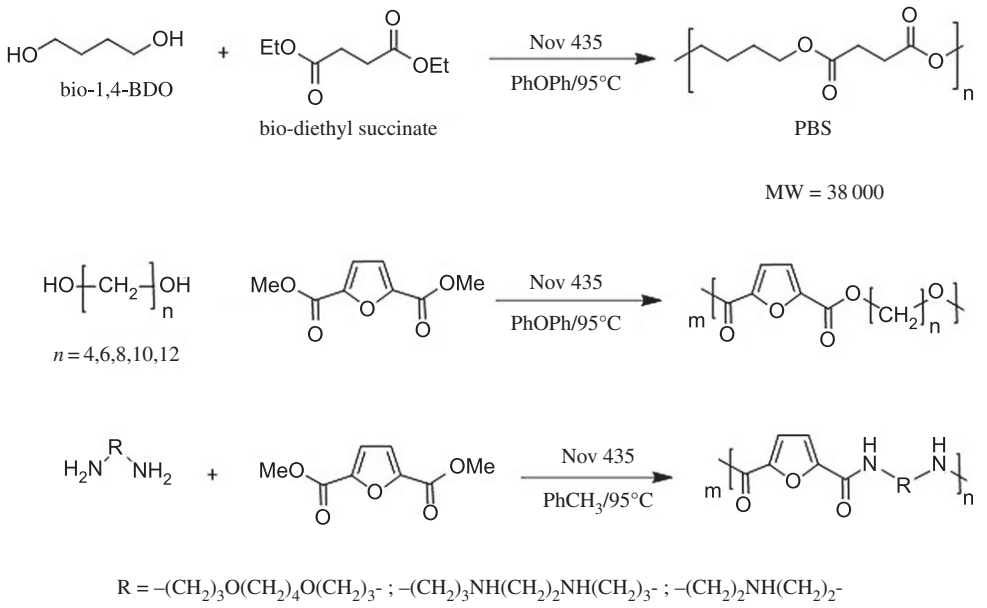


Figure 11. Nov435 catalysed synthesis of bio-based polyesters and polyamides.

municipal waste [113] and food supply chain waste [114]. The market is currently very small but is expected to grow tremendously in the future [110].

6. Conclusion and outlook

Biocatalysis and biomass conversion and combinations thereof are playing an important underpinning role in the drive towards a sustainable circular economy, based on renewable raw materials, as an alternative to the unsustainable use of fossil resources. This paradigm shift is largely driven by the pressing need to mitigate climate changes resulting from increasing concentrations of fossil resources-derived carbon dioxide in the atmosphere and to reduce the massive environmental damage caused by the use of non-biodegradable plastics on a single-use basis.

We expect that further advances in metagenomics, directed evolution of enzymes and metabolic pathway engineering of whole microbial cells, aided by advances in bioinformatics, including machine learning [115], will enable the development of more cost-effective biocatalytic processes. With regard to the feedstock, the valorization of unavoidable waste lignocellulose and triglycerides remains an attractive option that fits well with the concept of a circular economy. However, extensive areas of arable land and/or forest and immense quantities of fresh water are required to support plant growth. By contrast, aquatic biomass such as algae [116] are not in competion with food crops for arable land and fresh water and growth rates are much higher than for terrestrial plants. Moreover, since algae accumulate both carbohydrates and trigycerides they are, in contrast with lignocellulose, suitable for both bioethanol and biodiesel production and their very low lignin and hemicellulose content circumvents the need for difficult and expensive pre-treatment. However, in the short term, there may be more profitable things to do with algal oils than burning them as transportation fuels [117].

In short, we believe that current and future developments, at the interface of chemistry and biology, or from an industrial viewpoint, chemical and biotechnology, will enable a truly sustainable bio-based circular economy.

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