Evolving biocatalysis to meet bioeconomy challenges and opportunities

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ABSTRACT

The unique selectivity of enzymes, along with their remarkable catalytic activity, constitute powerful tools for transforming renewable feedstock and also for adding value to an array of building blocks and monomers produced by the emerging bio-based chemistry sector. Although some relevant biotransformations run at the ton scale demonstrate the success of biocatalysis in industry, there is still a huge untapped potential of catalytic activities available for targeted valorization of new raw materials, such as waste streams and CO_2. For decades, the needs of the pharmaceutical and fine chemistry sectors have driven scientific research in the field of biocatalysis. Nowadays, such consolidated advances have the potential to translate into effective innovation for the benefit of bio-based chemistry. However, the new scenario of bioeconomy requires a stringent integration between scientific advances and economics, and environmental as well as technological constraints. Computational methods and tools for effective big-data analysis are expected to boost the use of enzymes for the transformation of a new array of renewable feedstock and, ultimately, to enlarge the scope of biocatalysis.

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Biocatalysis beyond pharmaceutical and fine chemistry applications

According to A. Bommarius, "Biocatalysis is the general term for the transformation of natural and non-natural compounds by enzymes" [1]. Thus, biocatalysis generally refers to the use of enzymes and microorganisms in chemistry. During the last few decades, biocatalysis has delivered sustainable technologies and selective enzymes that have promoted the transition of chemistry towards processes that are environmentally benign. Enzymes accept a wide variety of complex molecules, including synthetic molecules with structures very different from the substrates found in nature. The practical utilization of enzymes as biological catalysts (biocatalysts) is driven by their versatility, regio-, chemo-, and enantio-selectivity, along with the need for the chemical industry to move towards environmentally compatible catalysts and processes. Conversely, scientific advances in biocatalysis have been boosted primarily by requests coming from the

Abbreviations: kt, kilo tonnes; B, billion; T, trillion; M, million; LCA, life cycle assessment; MFCs, microbial fuel cells; MECs, microbial electrolysis cells; RH, rice husk; DBs, databases; KET, key enabling technology.

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pharmaceutical industry and the fine chemistry sector, which make use of processes often characterized by low atom efficiency and high production of waste [2].

Enzymes used for biocatalytic applications represent a limited share of the global enzyme market, which in 2015 accounted for $8.18 billion [3,4] and is expected to reach $17.50 billion by 2024. Some studies, which have tried to analyse the enzyme market by segments [3–5], indicate that industrial enzymes accounted for 56.9% of the global enzyme market in 2015 [3], corresponding to $4.9 billion [6].The term "industrial enzymes" refers to a wide range of applications in sectors including food and beverage, detergents, animal feed, textile, pulp and paper, nutraceuticals, personal care and cosmetics, and wastewater treatments, thus excluding biocatalysis, which is rather included in the category of "specialty enzymes". This latter segment embraces applications such pharmaceuticals, diagnostics, biocatalysis and biotechnological research, addressing lower volume and higher value products, with pharmaceutical applications being the dominant one and accounting for $1.63 billion in 2015.

It must be underlined that it is quite difficult to analyze the enzyme market, since three companies (Novozymes, Du Pont and DSM) share 70% and several enzyme consumers have their own production facility, especially in the pharma sector, or regulate their supplies by means of joint ventures with producers. At the same time, new players like China and other Eastern countries are becoming important enzyme producers, also gaining new technologies and innovation, and their real role within the market is not always taken into account. Nonetheless, all various analyses agree on the rapid growth of the enzyme market, especially when it is considered that in 2013 it accounted for only $4.412 billion [4]. This considerable expansion of the use of enzymes is motivated by growing environmental concerns, and particularly by the evident benefits coming from the use of enzymes in multiple technical applications in the food, environmental and energy sectors [7,8]. The term "technical enzymes" generally refers to applications in sectors such as bioethanol, pulp and paper, textile and leather, starch processing, cosmetics, waste and water treatment, oilfield and, finally, fine chemistry. The largest share of market revenues is related to enzymes used in the processing of carbohydrates, which comprise glycosidases for biofuel production (Fig. 1). Overall, the food and beverage sector dominates the application market for enzymes. It accounted for 37.5% (more than $1.66 billion) of total market revenue in 2013, while the applications in the formulation of detergents followed with $0.95 billion of revenues [4].

It is evident that there is a wide potential for biocatalytic proteins in fermentation worldwide and for expanding the benefits of biocatalysis to the emerging bio-based chemistry sector, thus boosting bioeconomy as a whole [9].

The urgency to innovate process technologies along with the need to deliver new sustainable and renewable products will affect the future impact of enzymes and biocatalysts on different segments of chemistry. Indeed, new economic (e.g.lack of resources), political (e.g.the United Nations Framework Convention on Climate Change) and regulatory scenarios provide the basis for the gradual replacement of petrochemical feedstock by new platforms of bio-based chemical intermediates and polymer building blocks. Many examples are already evident and available on the global market, produced by fermentation of biomass components or recovered as side products of biomass processing (e.g. glycerol derived from biodiesel synthesis). Indeed, there is a potential synergy between processes leading to biofuels and the success of the new platforms of chemicals or precursors for fine chemicals, and this switch to sustainable resources influences the whole chemistry production chain (market pull).

Given this political and environmental context, the ongoing revolution in life sciences has a huge expanding effect on the possibilities to meet these ambitious goals. An impressive "technology push" derives from the field of genomics: automated sequencing possibilities, fast *in silico* screening and highly efficient use of metagenomics databases expand the opportunities to tailor biocatalyst properties while reducing laborious and expensive laboratory practices [9].

This evolving scenario has inspired the present review article, which aims to demonstrate how biocatalysts are already key enabling tools for the bio-based chemistry. However, their propulsive potential will be fully expressed provided that research in biocatalysis moves beyond the conventional approaches which have been successfully applied so far in the fine-chemistry and pharma sectors. Assembling discrete fragments of innovation appears an inadequate strategy to address the challenges of

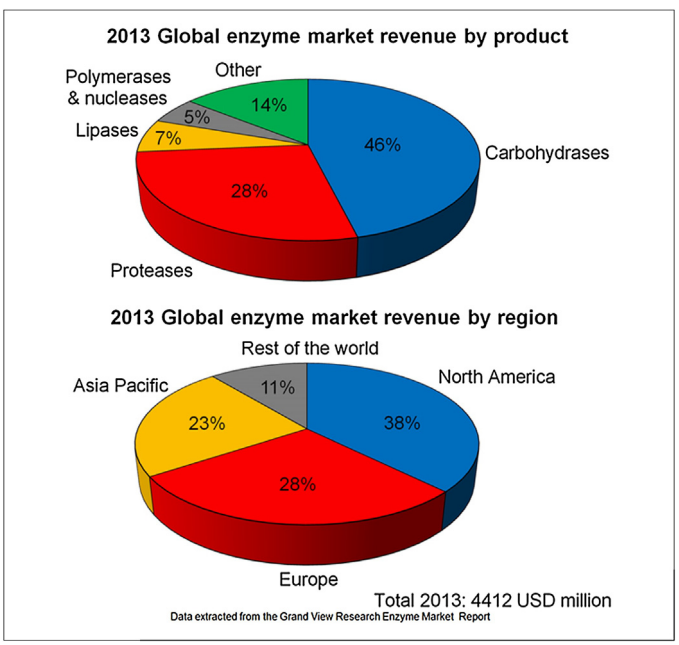

Fig. 1. Global enzyme market revenue by product (top) and by region (bottom) in 2013.

bioeconomy, since stringent technological, economic and environmental constraints must be taken into account throughout research and innovation implementation. New systemic and integrated approaches are required with higher emphasis on the optimization of the biocatalyst as well the process engineering. Following these needs, the exploitation of biological big data together with the development of advanced computational methods for analysis, function automation and optimization are expected to become routine elements driving the biocatalysis research and innovation.

Will biocatalysis boost bio-based chemistry?

A recent report commissioned by the Biobased Industries Consortium has estimated that the European bioeconomy market accounted for €2.1 trillion in 2013, while the annual U.S. bioeconomy market approaches $330 billion [10]. The main EU market shares are represented by the food and beverage sectors (about 50%), followed by the agriculture and forestry segment (21%), with the remainder related to the so-called bio-based business, including chemicals, pharmaceuticals, biofuels and bioenergy, which is the context where biocatalysis is applied [11].

The interest of the chemical industry to develop bioeconomy is global and stimulates alliances with the biotechnological and rural sectors throughout the different bioeconomy value chains [12,13]. The global bio-based chemical market in 2012 accounted for about 9% of the total sales of chemicals and is expected to reach 11% of the worldwide chemical market by 2020, or around €350-400 billion. Overall, the growth of the bio-based market should reach an annual rate of 8% over the preceding decade, with biopolymers, renewable chemicals, and industrial biocatalysts having the highest growth rate [12]. The question is whether and to what extent the technological and scientific potential of biocatalysis will intersect and boost the growth of the bio-based chemistry sector.

Economic analyses indicate that 7% of annual petroleum consumption (88×10^6 barrels per day in 2011) goes to the chemistry sector, which makes use of six fundamental groups of chemicals, including methane, ethylene, propylene, C_4 olefins and a few aromatics [15]. As discussed in a review by M. Franssen [14], biomass and renewable feedstock contain in their chemical structures most of the functional groups that are currently introduced in fossil-based chemicals with high energy and capital costs. On the other hand, enzymes are able to transform natural molecules (Tables 1–4) into an array of functionalized chemicals or

Table 1
Main examples of biocatalysed transformations applicable in the oleochemical sector.

Oils and fats		Biocatalyst	Biotransformation	Product	Reference
Unsaturated fatty acids		Lipase	Epoxydation	Epoxyacids	[78]
Oleic acid		*Candida anctartica* Lipase B	Epoxydation	Epoxy-stearic acid	[79]
Olein fatty acids		*Candida anctartica* Lipase B	Esterification Amidation	Fatty amides	[80]
Rapeseed oil		*Candida anctartica* Lipase B	Epoxydation	Rapeseed oil fatty acids	[81]
Palm olein		*Candida anctartica* Lipase B Lipozyme	Amidation	Fatty amides	[82]
Saturated fatty acids		*Candida anctartica* Lipase B	Transamidation	Alkanolamides (amide surfactants)	[83]
Vegetable and waste oils		Lipases	Transesterification	Biodiesel (FAME)	[84,85]
Vegetable oils	*Jatropha curcas* oil	*Burkholderia cepacia* lipase	alcoholysis	Biodiesel (FAME)	[86]
	Pistacia chinensis seed oil	*Rhizopus oryzae* lipase	Transesterification	Biodiesel (FAME)	[87]
	Babassu and palm oils	*Thermomyces lanuginosus* (TLL); Pseudomonas fluorescens lipase *Candida anctartica* lipase B	Transesterification	Biodiesel (FAME)	[88,89]
	Soybean and rapeseed oil	*Thermomyces lanuginosus* lipase (TLL); *Candida anctartica* lipase B; *Pseudomonas cepacia*; *Rhizopus oryzae* lipase	Transesterification	Biodiesel (FAME)	[90–94]
	Olive oil	*Pseudomonas gessardii* lipase	Hydrolisis	TGA	[95]
	Palm oil	*Rhizopus niveus* lipase; *Candida anctartica* lipase B	Interesterification	Cocoa butter substitute	[96–98]
	Various vegetable oils	Various lipases	Interesterification	Human milk fat substitutes	[99,100]
	Flax-seed oil	Various lipases	Interesterification	Triacylglycerols	[101–103]
Waste oils and fats		Lypozyme RM IM; *Rhizopus oryzae* lipase; Novozym 435; Lipase LS-10A, *Candida* sp. lipase	Transesterification	Biodiesel (FAME)	[104–110]
Slaughterhouse lipid and vegetable oils		*Pseudomonas gessardii* lipase	Hydrolisis	TGA	[111]
Microalgal oil		*Burkholderia cepacia* lipase (immobilized)	Transesterification	Biodiesel (FAME)	[112]
Phytosterols		*Candida rugosa* lipase	Esterification	FFA	[113]
Fish oil		Lipases	Selective concentration of EPA and DHA	Omega-3 concentrates	[114]
		Porcine pancreas lipase	Re-esterification	Monoacylglycerols (MAG)	[115]
Glycerol		Lipases from dry moulds	Esterification	Esters of isopropylideneglycerol	[116]
		Lipase (Novozym 435)	Glycerolysis	Glycerol carbonate	[117]
			Glycerolysis	Glycerol carbonate	[118]
		TEMPO/laccase	Oxidation	Glyceraldehyde, glyceric acid, tartronic acid (for cosmetics and pharmaceuticals),	[119]
Palm Oil Fatty acids and residual fats		Lipase (Novozym 435)	Esterification and transesterification	Biodiesel (FAEE)	[120]

Table 2
Biocatalysed transformations of polysaccharides and sugars.

Carbohydrates	Biocatalyst	Biotransformation	Product	Reference
Cellulose				
Microcristalline cellulose	CLEAs of *Trichoderma reesei* cellulase	Hydrolysis	Glucose	[121]
Cellulose from corn cob (1st step)	Cellulase cellulase from *Trichoderma reesei* (immobilized) – 1st step	Hydrolysis	Glucose	[122,123]
Cellulosic hydrolysate (2nd step)	*Lactobacillus delbrueckii* (immobilized cells) – 2nd step		Lactic acid	
Cellulose from sugar beet pulp	Cellulase	Hydrolysis	Cellobiose	[124,125]
Cellulosic biomass sugars	Glucose isomerase	Isomerization	D-Xylulose	[126]
Peanut-shell hydrolysate	Xylose isomerase	Isomerisation	convert D-xylose to D-xylulose in ethanol production	[127]
Cellulosic biomass Sugar cane biomass Waste woody cellulosic materials Switch grass (*Panicum virgatum* L.)	Cellulases and xylanases	Hydrolysis	Ethanol	[128,129]
Grain products and cane sugar juice or molasse	Xylose isomerase	Isomerisation	convert D-xylose to D-xylulose in ethanol production	[129–133]
Starch	Amilases	Hydrolysis	Maltose, glucose	[134]
	Glucoamylase and *Saccharomyces cerevisiae*	Saccharification and fermentation (SSF)	Ethanol	[135]
Lignocellulosic biomass	Cellulales; hemicellulases	Hydrolysis	Biofuels	[136,137]
Glucose	Glucose isomerase	Isomerisation	Fructose	[138]
Galactose	*Aspergillus oryzae* β-galactosidase	Oligomerisation	Galactooligosaccharides (GOS)	[139,140]
Lactose	β-galactosidase	Transgalactosylation	Galactooligosaccharides (GOS)	[141,142]

Table 3
Enzymes and biotransformations applied to the processing of lignocellulosic raw materials.

Lignin	Biocatalyst	Biotransformation	Reference
Pinewood, timothy grass, and wheat straw	Cellulolytic enzyme mixture (cellulase, b-glucosidase and xylanase)	Hydrolysis	[143]
Eucalyptus, Douglas fir and rice straw	Cellulase	Hydrolysis	[144]
Fir wood chips	Cellulase and β-glucosidase	Hydrolisis	[145]
Spruce, Spruce chips	Laccase-mediator system, laccase	Delignification, Detoxification	[146] [147]
Wasteland weed Saccharum spontaneum	Laccase	Delignification	[148]
Wood pulp	Laccase	Delignification	[149,150]
Sugarcane bagasse	Laccase, manganese peroxidase, lignin peroxidase	Delignification	[151–155]
	Laccase	Detoxification	[156,157]
Ricinus communis	Laccase	Delignification	[158]
Eucalyptus wood	Laccase, Laccase-mediator system	Delignification	[159–164]
Corn stover	Laccase-mediator system, laccase, manganese peroxidase, lignin peroxidase	Delignification	[165,152–154]
	Manganese peroxidase and laccase	Delignification	[166]
Wheat straw	Laccase, Laccase-mediator system, manganese peroxidase, lignin peroxidase	Delignification, Detoxification	[167–175,152,154]
Wood Hydrolisate	Laccase, Lignin peroxidase and combination of the two	Detoxification	[176]
Rice straw	Laccase, manganese peroxidase, lignin peroxidase	Detoxification	[177,178,153,154]
Kraft wood	Laccase	Delignification	[179]
Soft wood	Laccase-mediator system	Delignification	[180,181]
Banana stalk	Laccase, manganese peroxidase, lignin peroxidase	Delignification	[152–154]
Coconut shell	Laccase, manganese peroxidase, lignin peroxidase	Delignification	[155]
Sisal fiber	Laccase, manganese peroxidase, lignin peroxidase	Delignification	[155]

intermediates that nowadays are produced from fossil oil by the petrochemical industry. The main advantage of the use of biocatalysts is that they work optimally at much lower temperatures and milder conditions compared with the conventional chemical processes.

The transformation of biomass and bio-renewables through the introduction of complex functionalities increases the value of chemicals, so that there is more room for enzyme applications in the production of high value chemical products rather than for commodities and biofuels. Nevertheless, it is important to underline that the food and beverages sector, which delivers low cost and high volume products, represents the largest application of enzymes in industry. Indeed, it has been demonstrated that technologies already available for genetic and fermentation optimization allow the reduction of the cost impact of enzymes and that one kg of an enzyme can be produced at a cost around €100 [14]. This concept is also confirmed by a number of large-scale processes employing enzymes for the production of

Table 4
Selected examples of biotransformations of proteins and aminoacids.

Proteins and ammino acids from animal and plant sources	Biocatalyst	Biotransformation	Product	Reference
Soybean flour, Egg white	Protease	Hydrolysis	Aminoacids	[182]
Soybeans, lupin	Protease	Hydrolysis	Proteins	[183–187]
	Carbohydrolase	Hydrolysis	Proteins	[187–189]
Rapeseed	Protease	Hydrolysis	Proteins	[184]
	Carbohydrolase + Protease	Hydrolysis	Proteins	[190]
Sunflower	Protease	Hydrolysis	Proteins	[191]
Peanuts	Protease	Hydrolysis	Proteins	[192,193]
1.1.1 Rice bran	Protease	Hydrolysis	Proteins	[194,195]
	Carbohydrolase + Protease	Hydrolysis	Proteins	[196]
Rubber seed protein concentrate, Wheat gluten	Proteases	Hydrolysis	Free amino acids	[197]
Wheat gluten (step1)	Endo-proteases, *exo*-proteases (step1)	Hydrolysis (step1)	Aminoacids (step1)	[198]
Wheat gluten protein hydrolysate (step2)	Glutaminase (step2)	Deamidation (step2)	Glutamic acid (step2)	
Phenylalanine	Phenylalanine ammonia lyase (PAL)	Deamination	Cinnamic acid	[199]
Alanine	Decarboxylase	Decarboxylation	Ethylamine	[200]
Glutamic acid	Decarboxylase	Decarboxylation	γ-Aminobutyric acid (GABA)	[201–203]
	Amino acid deaminase	Deamination	α- Ketoglutaric acid	[204]
Glutamate	Glutamate dehydrogenase	Oxidation	α-Ketoglutarate	[205]
	NADH oxidase	Reduction		
Lysine	Lysine α-oxidase	Oxidation	5-Aminovaleric acid	[206]
	Lysine monooxigenase	Oxidation	5-Aminovaleric acid	[207]
	Lysine decarboxylase	Decarboxylation	Cadaverine dicarboxylate	[208]
D,L-Methionine	Aspergillus oryzae acylase	Resolution	L-Methionine	[209]
Phenylalanine	Phenylalanine ammonia lyase	Deamination	Cinnamic acid	[210]
Tyrosine	Tyrosine ammonia lyase	Damination	p-hydroxycinnamic acid	[211]
Arginine	Arginine amidinohydrolase, Arginase	Hydrolysis	Ornithine	[212,213]
Aspartic acid	Aspartate α-decarboxylase	Decarboxylation	β-Alanine	[214–216]

commodity chemicals [16]. For instance, through genetic engineering, amylases were reported to have an impact as low as 1 cent per litre on ethanol production from starch.

Economic factors determining the viability of biocatalytic processes

The use of biocatalysts affects both categories of costs, namely capital investment (CapEx) and operational cost (OpEx), so that economic assessments are often the main decision-making tool guiding the choice of biocatalysis at an early stage in a project [18].

A study by Woodley and co-workers [18] discussed the contribution of biocatalyst cost to the total costs of the production processes. Production scale, fermentation yield and expression level are among the crucial factors that determine the economic impact of the biocatalyst. Although, in most cases, biocatalytic processes do not involve operational complexities higher than conventional chemical processes, the development chain can be more complicated and generate higher uncertainty in terms of meeting desired cost thresholds. For instance, many biocatalyzed processes require the development of a tailored catalyst for the target reaction, whereas the timeframe for the development of a preliminary synthetic procedure is generally very limited, especially in the pharma sector. In those cases, the choice of industrial biocatalysis can strongly depend, initially, on the availability of already developed enzymes.

Besides the well-known process metrics, such as product concentration (g/L) and space-time yield (g/L/h), the threshold of minimum productivity for biocatalysts, namely the kg of product produced per kg biocatalyst basis, is the crucial factor which, however, depends on the type of industrial sector. In the case of the pharmaceutical sector, with products characterized by costs above €100 per kg, productivity of free enzymes should be at least 100–250 kg/kg. For specialty chemicals with costs around €5/kg, an acceptable productivity is in the range of 1000–4000 kg product/kg of free enzyme, whereas for bulk chemicals productivity should increase up to 5000–20,000 kg/kg. The diffusion of biocatalysis in the chemical industry attests that these targets are achievable. Nonetheless, low-volume specialized enzymes are profitably applicable only upon a significant improvement of the process or the optimization of the biocatalyst to ensure high productivity.

Enzymes are also used in large-scale processes in crude form or as whole microorganisms, the latter representing a large proportion of biocatalysis, since whole cells can be easily and economically produced through cheap fermentation methods. The use of whole microorganisms is usually implemented when the extraction and purification of the required enzymes are difficult or expensive or when multiple enzymes are required to catalyze a reaction. The expected advantages of the immobilization of whole cells reside in a higher operational stability accompanied by easier downstream processing and scalability of the reaction. Cells are most often immobilized by entrapment inside a wide variety of chemical networks, including polyacrylamide gel, alginate gel, *k*-carrageenan and photo-cross-linkable resins. The pores of the resulting matrices must allow the diffusion of substrates and products, while assuring high immobilization efficiency in terms of retained activity. In particular, acrylamide is produced from acrylonitrile at a scale of 600 Ktons per year in industrial processes that make use of immobilised microorganisms endowed with nitrile hydratase enzymatic activity [17].

Looking at large scale applications of immobilized enzymes, there is a wide number of well-established biocatalyzed processes in the oleochemistry sector, which transform fats and oils into food ingredients but also into emollient esters and biodiesel, through reactions catalysed by immobilized lipases (Table 1). However, the most significant example of large scale application of immobilize biocatalysts is provided by the production of high fructose syrup by means of glucose isomerase (Table 2).

The transformation of the soluble enzymatic protein into an insoluble heterogeneous bio-catalyst presents an advantage when the recovery of the enzyme is required either to prevent contaminations or for recycling the expensive catalyst. However, the immobilization process represents also an extra economic barrier for large-scale applications. The impact of immobilization costs is connected to biocatalyst productivity and, ultimately, to

the recyclability of the enzyme. It is suggested that costs of few hundred $ per kg are acceptable for specialty chemicals, whereas in the bulk chemical sector the economic impact must remain below $10 per kg and if often close to 0.1$ [18,19]. Interestingly, in 1990 immobilized enzymes accounted for almost 20% of enzyme market, while they now represent a much lower fraction [16]. These data are affected by the fact that companies using immobilized enzymes for their processes often have internal enzyme production or purchase the enzyme in free form and then immobilize it in their own facilities.

Although enzyme immobilization is considered an effective route for increasing the stability of biocatalysts, recent trends indicate that enzyme producers or large chemical firms applying enzymes for their processes prefer to invest in enzyme engineering strategies aimed at improving enzyme robustness rather than in enzyme immobilization [20].Nevertheless, immobilization remains an essential choice for many enzyme applications, such as in vegetal oil transformations catalysed by lipases [21,22] carried out in bulk oils. The enzymatic proteins would aggregate when suspended in the hydrophobic medium, whereas immobilization on solid carrier improves the distribution and accessibility of the biocatalyst. In this context, the application of biocatalysts in the oleochemical sector has the potential to be further expanded, provided that robust and cheap immobilized lipases are made available.

Overall, the application of biocatalysts in the transformation of renewable feedstock suffers from stringent economic constraints that make optimization procedures, both in terms of enzyme stability and process design, far more critical when compared to the practice observed in the fine chemistry sector.

From conventional feedstock to waste valorisation

Industrial Biotechnology (IB) and biocatalysis already contribute to bioeconomy within the biorefinery context, namely by transforming different conventional biomass and renewable feedstock into chemicals. Tables 1–4 provide an overview of transformations of oils/fats, polysaccharides, lignocellulosic biomass and proteins made possible by a wide array of enzymes. Research efforts are currently directed not only towards the transformation of biomass through more efficient routes, but also at the identification of new and non-conventional feedstock streams as opportunities. Although some technological breakthroughs are still expected to fill some gaps, such as lignin valorization and exploitation and CO_2 reduction, important technological advances are already available and these innovations have effective potential to reach the market in few years [23,24] upon further optimization.

The food industry is a major driver of biocatalysis growth: lipases are used traditionally for transforming oils and fats in food ingredients such as cocoa butter analogues (Table 1), and nowadays are profitably applied in the synthesis of valuable chemicals, including lubricants, esters for the cosmetic sector and surfactants [25]. More recently [26], Novozymes developed a liquid formulation of a modified *Thermomyces lanuginosus* for the economically attractive conversion of waste oils into biodiesel. Woodley and co-workers demonstrated the scalability of an 80 L fed-batch reactor to a 40 m^3 scale through the design of a 4 m^3 continuous process catalysed by the lipase, which is used for one single cycle. The food industry also makes use of proteases for converting peptides and proteins into shorter peptides (Table 4) used as supplements, ingredients of infant formulas or as pharmacologically active agents [27]. On the otherhand, optically pure amino acids obtained through enzymatic enantioselection are massively used by the fine chemical industry as a chiral-pool, since the stereoselectivity of different enzyme classes allows for the bioconversion of cheap amino acids into chiral building blocks for the manufacture of expensive and chemically complex chiral drugs or fine chemicals.

Biotransformation of sugars (Table 2) represents probably the oldest example of a biorefinery and, also in this case, the first technologies were developed for the food and beverage sector. The amylase-catalyzed hydrolysis of starch dates back to the 1970s and was followed by glucose isomerization to fructose. Currently, they represent the largest enzymatic processes implemented at an industrial scale. The starch industry has capitalized on the know-how generated for polysaccharide processing and later promoted the surge of the first generation biofuel industry. Only in the last two decades have scientific advances enabled the design, production and optimization of a pool of hydrolytic enzymes able to split the chemically heterogeneous glycosidic bonds of cellulose and hemicellulose (Table 2). Conversely, starch based biorefineries are being replaced by second-generation biorefineries fed by non-food-based lignocellulosic feedstock. A joint venture between the engineering company Biochemtex and Novozymes led to development of the PROESA™ technology, which allows conversion of non-food lignocellulosic feedstock into fermentable C_5 and C_6 sugars (http://www.betarenewables.com/en/proesa/biorefinery). The method was implemented for the first time on a 40,000 ton per year scale by the Betarenewable company in Crescentino (Italy) in 2013 and was later transferred for development of industrial scale biorefineries in Slovakia and Brazil.

The lignin fraction resulting as a by-product from biorefinery processes constitutes a further chemical platform for the production of chemicals (Table 3). The abundance of lignin in nature (25–35% of lignocellulosic biomass) makes this biopolymer the ideal source of aromatic building blocks, currently derived from fossil oil (Table 2). Oxidative enzymes (e.g. laccases and peroxidases) have been employed in lignin depolymerisation while laccases have been applied also in lignin detoxification (Table 3) with the aim of removing the toxic components, such as phenolic compounds and furan derivatives. These are produced during the biomass pretreatment and lower final ethanol yield by affecting the cellulolytic enzymes and fermentative microorganisms. Despite extensive research efforts (see Table 3) the recovery of aromatic building-blocks from lignin remains a major challenge because of its amorphous and recalcitrant structure [28]. One of the few commercial uses of lignin is represented by the production of vanillin starting from the residual lignosulfonates of the pulping industry, although the petrochemical route is still more competitive (Table 3).

Nowadays, most lignin (also that derived from second-generation biorefineries using lingo-cellulosic biomass) is burnt for energy production within the pulping industry, whereas chemical routes for the valorization of lignin remain of limited practical relevance [29]. Nevertheless, the problem of supplying bio-based aromatic building-blocks has been circumvented by the production of styrene starting from bio-ethanol, which is first converted into the intermediate butadiene [30]. Moreover, the direct production of aromatics through fermentation has also been reported as feasible [31].

The chemical and, more generally, the bio-based industry seeks feedstock flexibility to lower costs while avoiding competition for the use of soil for food production. That has promoted the interest towards residues and agro-waste streams as a source of biomass and, most importantly, high-value bio-components [32]. This agrees with the concept of “second generation biorefineries”, which should rely on integrated chemical and biotechnological innovations for converting biomass/waste/residues into valuable products in a hierarchical cascade, with the processing chain including returning waste and nutrients to the land. For example, enzymes, such as pectinases, are employed industrially to promote

cell-wall hydrolysis and facilitate extraction of the hydrolysis of antioxidants, pigments, enzymes, dietary fibers, fructans, and an array of nutraceuticals [33].

It should be noted that the food wastes generated worldwide amount at about 1.3×10^9 tons represent an important source of chemicals, nutrients and micro-components [15]. Besides agro-waste, fishing activity and seafood processing lead to 30 up to 70% waste and enzymes such as alcalase and α-chymotrypsin have been applied in the production of hydrolysates from fish-processing co-products, with possible applications in the food and pharma sector (Table 4). The fish waste has an oil/fat content of 19% on a dry weight basis and the latter can be hydrolyzed enzymatically to obtain poly-unsaturated fatty acids under very mild conditions. The biocatalyzed process prevents side-reactions and especially oxidation, thus preserving the precious unsaturated ω-fatty acids chemical structure.

Biocatalysis for CO_2 transformation: turning a threat into a resource

The most abundant and inexpensive source of renewable carbon on Earth is CO_2, with 36.6 billion metric tons of anthropogenic CO_2 emissions per year contributing to greenhouse gas (GHG) and global warming [34]. Nowadays there are mature technologies for CO_2 sequestration with large-scale facilities around the world capturing more than 27 million metric tons of CO_2 every year from power generation plants, and also from industrial sectors such as iron and steel, refining, petrochemical and cement manufacturing. Enzymes have been investigated as a route for reducing the cost barrier of carbon capture technologies, which are the prerequisite for further storage (via gas injection underground for long term storage) or exploitation of CO_2 as a feedstock [35]. One of the many methods currently under development for CO_2 capture is based on the reactivity of amines in absorber columns at 40–60 °C to form carbamates. CO_2 is then released by heating the solution at temperatures above 100 °C. This energy intensive process (about 80% of operational costs) also requires large columns to process massive amounts of CO_2. Since the rate determining step for desorption is the hydration of CO_2 to bicarbonate, studies are in development for the use of immobilized carbonic anhydrase to increase the rate of CO_2 desorption. The enzyme is involved in many biochemical processes in nature, such as detoxification pathways, respiration, pH homeostasis and photosynthesis. Its application causes a reduction in the energy consumed during the desorption step because carbonic anhydrase catalyses the fast hydration of CO_2 at lower temperature. The advantage is twofold: lower energy consumption and smaller volume of absorber columns. Because operational temperatures are relatively high, carbonic anhydrase is generally employed in the immobilized form, which displays higher stability upon prolonged storage and enables recycling of the biocatalyst.

Despite the technological progresses in CO_2 capture and sequestration, currently only a negligible percentage of anthropogenic CO_2 emissions are transformed for practical use. The exploitation of CO_2 as feedstock via reduction to yield C_1 molecules is in principle an attractive proposition [36] but CO_2 is thermodynamically a very stable molecule. The catalytic reduction of CO_2 with H_2 to synthesise methanol is feasible, but the reaction has a standard Gibbs free energy change of reaction ($\Delta_r G^o$) of + 0.84 kcal/mol. The reduction has been carried out experimentally using various metal catalysts [36], which must be activated under harsh conditions both in terms of temperature (150 – 300 °C) and pressure (from 3 to 14 MPa). Moreover, the metal catalysts maintain their activity only in the presence of highly pure feedstock. These factors justify the impracticality of the thermochemical reduction of CO_2 for industrial purposes.

Redox enzymes have the advantage that they are able to catalyse reactions in which conventional chemical catalysts fail. There is evidence that biocatalysts are capable of catalysing the reduction of CO_2 at ambient conditions and some microorganisms can reduce CO_2 simply by reversing the metabolic pathway reactions. The multiple steps reactions usually involve a formate dehydrogenase that catalyses the initial reduction of CO_2.

Unfortunately, the use of isolated redox enzymes is hampered by the high cost of the cofactors (e.g. nicotinamide adenine dinucleotide, NAD +) necessary for CO_2 reduction. Major efforts in the field are directed towards the *in situ* regeneration of cofactors by means of both electrochemical regeneration and cells. Thus, CO_2 has been converted into methanol through a hybrid enzymatic/photocatalytic approach using three dehydrogenases (FateDH, FaldDH, and ADH) [37]. The enzymes, encapsulated into cages of alginate and tetraethoxysilanes, consume 3 mol of NADH, which is then regenerated by exploiting a visible-light-active photocatalytic system made with TiO_2. The electron (hydride) is then transferred from an H-donor (e.g. water–glycerol solutions) to NAD+ with the assistance of a Rh(III)-complex. Globally, the process allows for the production of 100–1000 mol of methanol starting from one mole of NADH [37].

Since the reduction of carbon dioxide requires energy to proceed uphill to the product of reaction, ideally such a source of energy should be renewable and provided either through direct routes, such as photons and electrons, or indirectly by using high-energy chemical molecules as hydrogen. One emerging field of research is the study of bioelectrochemical systems (BESs), which are either able to oxidize organic compounds or to produce hydrogen by reducing protons. The transfer of electrons occurs through interactions that are established between electrodes and these specific biocatalysts [38]. Electrogenic, or electrically-active, bacteria are able to perform 'extracellular electron transfer' (EET) and have been isolated from different environments, including extremes. The use of whole microorganisms in bio-electro-synthetic systems is generally preferred because enzymes adsorbed on electrodes lack long-term stability, although they provide higher reaction specificity and controllability [39].

BESs function as any other electrochemical cell (e.g. a battery), where an anode and a cathode are connected through an external wire that closes the electrical circuit. Optionally, a membrane separates the two electrodes. Electrogenic microorganisms oxidize organic substrates at the anode and then transfer electrons from inside their cell to the electrode. At the same time, the microorganisms release protons into the solution where the two electrodes are submerged, together with CO_2, which can be captured. The electrons flow to the cathode, where a reduction reaction occurs.

A first type of BES is represented by Microbial fuel cells (MFCs), which operate under aerobic conditions. When electrons reach the cathode they combine with oxygen and protons to produce water. MFCs produce electric power, which derives from the external circuit that carries the electronic flow. Alternatively, when the cathode operates under anaerobic conditions, the electrons reduce protons to form hydrogen. This BES configuration is referred to as MEC or Microbial Electrolysis Cell and requires, besides the energy produced by the same microorganisms, some additional energy supply to accelerate the kinetics of substrate conversion or to drive reactions that are thermodynamically unfavourable (Fig. 2).

In principle, electrogenic microorganisms can boost both MFCs and MECs by oxidizing organic components or contaminants present in wastewater [40]. Although MFCs represent a route for generating a large amount of energy from various waste streams, at the state of the art the electricity generated by MFCs is of scarce economic value and cannot compete with other energy sources derived from biomass degradation, such as biogas [38]. On the

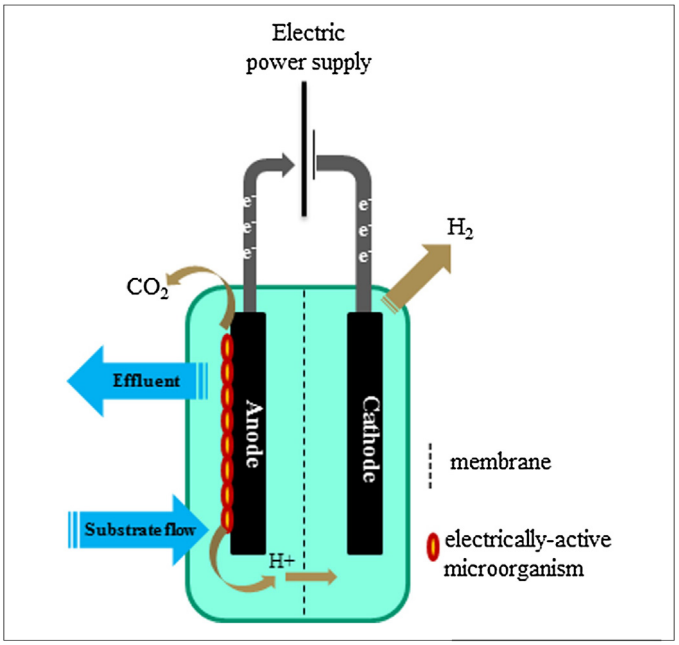

Fig. 2. A schematic representation of a Microbial Electrolysis Cell. An electrically-active microorganism adsorbed on the anode surface oxidizes the chemical components present in a wastewater. The reaction liberates carbonic anhydride, protons and electrons, which flows towards the cathode. Under anaerobic conditions, protons are finally reduced to H_2.

other hand, MECs could be a promising means for producing renewable hydrogen, an attractive and sustainable energy carrier. They have the advantage of requiring a limited amount of energy to treat wastewater and the final energy balance is positive, since the energy contained in the hydrogen produced counterbalances the electrical power supplied for microbial electrosynthesis operation. They also have four-fold higher hydrogen productivity compared to conventional processes based on microbial fermentation and are efficient in the treatment of a diluted concentration of organic components at very mild temperatures ($< 20\ ^{\circ}C$).

The evidence that electric power can drive microbial metabolism has inspired the concept of "Microbial Electrosynthesis" that goes beyond hydrogen production but addresses even the synthesis of multi-carbon chemicals [41]. Nevin and co-workers described the reduction of CO_2 to acetate using a film of *Sporomusa ovata* cells deposited on an electrode, which directly supplied the microorganism with the electrons required for the reduction. The system was conceived as an artificial form of photosynthesis because it was powered by solar energy [41], thus realizing a fully renewable microbial electrosynthesis.

Recent analyses indicate that one major challenge for making bioelectrochemical systems of practical applicability is to elucidate and improve the mechanisms used by microorganisms to transfer electrons to the electrodes. The studies reported so far indicate that two main methods are exploited by microorganisms: in one case, the cells adhere physically to the electrode and there is a direct exchange with the electron surface; other electronically-active microorganisms do not attach on the electrode surface but rather exploit chemical compounds that act as a long-range shuttle.

Biocatalysis for valorization of bio-based building blocks: the case of bio-based polymers

Despite the green chemistry perspective and perfectly fitting into a circular economy context [42], enzymes for industrial-scale applications are in many cases still too expensive to be implemented when compared to traditional chemical catalysts. From an economic perspective, enzymes could easily enter the market for applications that require mild and selective processing not feasible using traditional chemistry [43]. In this context, application of biocatalysis to polyester synthesis and modification can be regarded as an opportunity to increase the value and competitiveness of bio-based polymers.

Projections indicate that the value of the renewable plastic market will increase up to $5.2 billion by 2030 [44]. The growth of the bio-based polymer market is motivated by the need of the plastic industry to decrease the environmental cost of fossil-based plastics. Analyses by the UN Environmental Programme indicate that over 75% of the natural capital cost of plastic use in consumer goods ($75 billion per year) is derived from the extraction and manufacture of plastic feedstock [45]. It appears evident that the problem requires solutions addressing not only the efficient management of plastic waste but also the migration towards more sustainable plastics.

Industrial biotechnology contributes to the replacement of petrol-based polymers and plastics through well-established fermentation technologies able to deliver an array of bio-based monomers usable, for instance, in polyester production. Polylactic acid already represents a success case, with a production of about 180 Ktons per year [46].

Research efforts aim not only to replace the existing fossil-based polymers (drop-in products) but also to design a new generation of polymers and materials that must compete in terms of performance with the well-established fossil-based products. In that respect, different hydrolases can be exploited not only to catalyze *in vitro* synthesis of polyesters under mild conditions, but also to perform targeted hydrolysis, while retaining bulk properties of the polymer. In the latter case, the objective is to insert functional groups onto polymer surface, thus enlarging the spectrum of advanced applications.

A number of studies at laboratory [47–49] or pilot scale [50] have demonstrated the feasibility of enzymatic polycondensation and ring opening polymerization. As an example, the synthesis of aliphatic polyesters carrying vinyl functionalities [51–53] or hydroxyl groups [54,55] has been performed via enzymatic catalysis at temperatures of 50–70 °C. Such mild conditions

prevent the undesirable cross-reactivity of the polymeric chains observed in traditional chemical polycondensations, carried out at temperatures above 150 °C. Moreover, enzymes can be exploited when a mild and limited surface functionalization of polymers is needed. Poly(L-lactic acid), poly(ethylene terephthalate), polyamide 6,6 and polyurethanes [43] are examples of polymers that have been selectively hydrolyzed to create functionalities on their surface able to confer higher hydrophilicity while maintaining the bulk properties of the material. The same polymers were also functionalized [56] with the aim of introducing suitable chemical groups for anchoring bioactive molecules [57] or for conferring properties useful for packaging formulation or clothing applications [58].

Both lipases and cutinases have been employed to catalyze the polycondensation of structurally different bio-based monomers. Cutinases are fungal enzymes responsible in nature for the hydrolysis of cutin, a bulky hydrophobic polyester that protects plant cell walls [59]. Their structure displays an accessible superficial active site that readily accommodates large hydrophobic substrates. Cutinases share with lipases the typical large hydrophobic area on their surface, although there is no evidence of interfacial activation for the cutinases investigated so far. The wide accessibility of the active site of cutinases makes them very promising scaffolds for future engineering studies aimed at the generation of biocatalysts able to introduce targeted functionalities into polymeric films.

Efforts are still needed to transfer the concept of enzymatic synthesis and modification of polyesters to an industrial level. First, it is necessary to enlarge the portfolio of enzymes and biocatalysts. Secondly, studies indicate that more integrated strategies are needed, where process engineering, environmental and cost issues are accounted for and optimized at the same time [60]. Concerning process design, enzymatic polyester synthesis is preferentially carried out in solvent-free systems, thus reducing both environmental and economic costs. The viscosity of reaction mixtures is the undesired consequence, so that mass transfer represents the major limitation to polymer chain growth. Similar problems are also encountered in a number of different solvent-free biotransformations of renewable feedstock, such as in the synthesis of long chain esters starting from fatty acids. It is evident that classical stirred tank reactors employed in chemical manufacture are inappropriate for achieving efficient mixing and mass transfer. Recent studies reported new generations of reactors based on thin-film formation [50] or bubble column reactors [61]. Thin-film processes have the advantage that the biocatalyst is not damaged, as no mixing system is necessary. The thin film is generated by applying centrifugal force to the system and it has been demonstrated that this does not have any detrimental effects on the immobilized enzymes. Since the reaction mixture is spread over a large thin surface, heat and mass transfer are optimal and the removal of volatile by-products is enabled.

Polyester synthesis requires the use of immobilized enzymes to enable the recovery of the expensive biocatalyst (generally lipase B from *Candida antarctica*) and to avoid the contamination of the product with the enzymes. This has been accomplished by anchoring the enzymes covalently on different organic carriers [50,51]. The "thin-film concept" can be conveniently applied to different ester and polyester syntheses catalyzed by immobilized enzymes under solvent-less conditions characterized by high viscosity. The concept was experimentally validated at the 10 kg scale using a turbo reactor [Fig. 3] operated according to a two-step solvent-less continuous process for the polycondensation of adipic acid with 1,4-butanediol [50]. Typically, a turbo reactor consists of a tubular cylindrical body, equipped with a heating jacket and a coaxial bladed rotor. The latter generates a dynamic layer of reactants having a thickness between 1- 20 mm, which moves in a turbulent flow regime in contact with a wall kept at a predetermined temperature. The turbo reactor allows

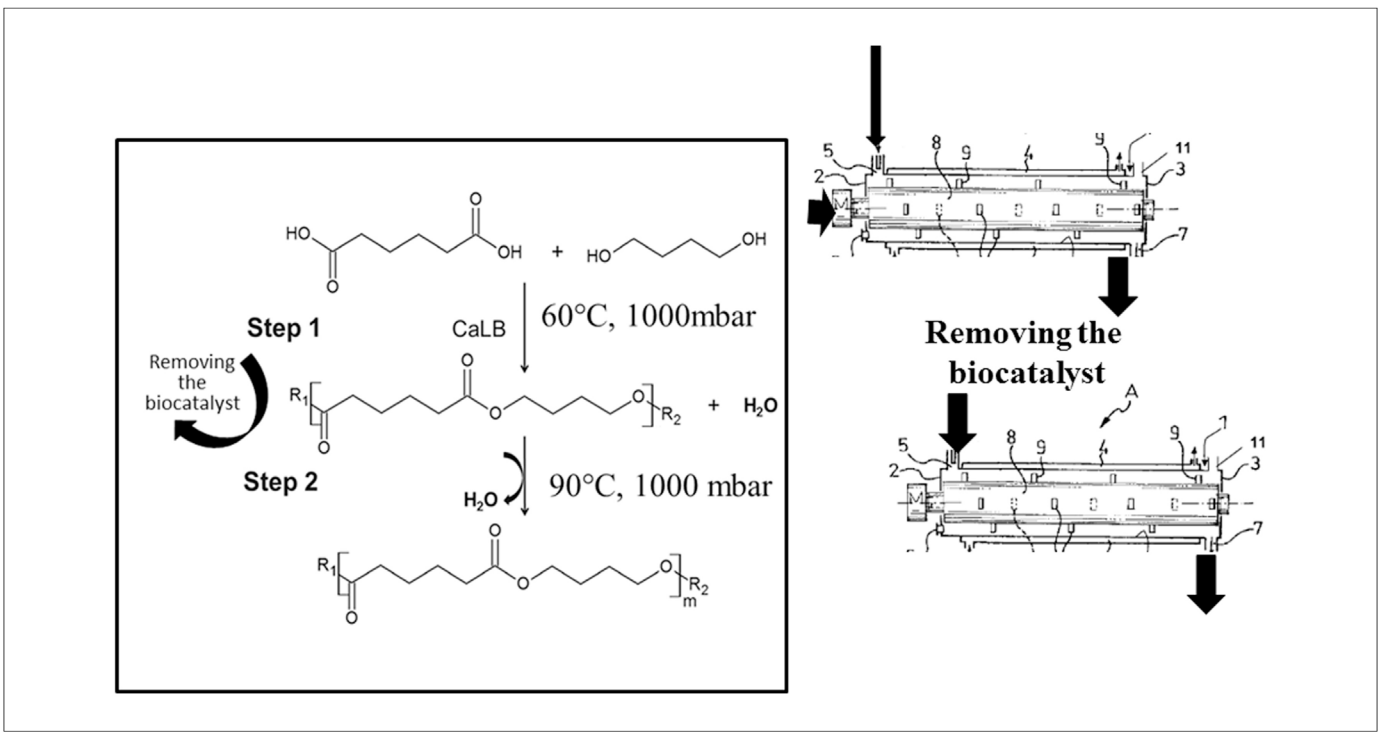

Fig. 3. Scheme of the process for the solvent-free synthesis of poly(1,4-butylene adipate) catalysed by fully recyclable immobilized lipase B from *Candida antarctica* using a turbo reactor on 10 kg scale. The turbo technology creates a thin-film of the viscous reaction mixture. The synthesis is carried out in two steps via a continuous process. The first step of the synthesis involves the biocatalyst and leads to the formation of pre-polymers. After the removal of the biocatalyst, the polycondensation is thermodynamically driven by the evaporation of water, the side product of the reaction.

implementation of a continuous process, where the enzyme catalyzes the first step and leads to the formation of oligomers. At that stage, the product mixture is sufficiently fluid to allow recovery of the biocatalyst, which must be covalently immobilized on solid carriers to avoid product contamination [50]. Afterwards, the temperature increases up to 90 °C and the polymer elongation is driven thermodynamically through the removal of co-product (i.e. water). This technical solution prevents exposure of the biocatalyst to mechanical stress and preserves its long-term activity. It must be noted that the turbo-reactor configuration renders application of vacuum unnecessary, since the water is easily removed from the thin-film even at 90 °C (Fig. 3). In principle, this type of reaction system is applicable to a wide variety of large-scale biotransformations of viscous substrates under solvent-free conditions.

Towards more sustainable and inexpensive industrial biocatalysts

As mentioned before, when dealing with commodity chemicals having low added value, cheap and recyclable biocatalysts are the determinants of success of enzyme applications.

As an example, biodiesel can be produced using biocatalyst technology instead of basic chemical catalysts, with the advantages of reduced energy consumption, prevention of undesirable side products and, conversely, reducing expensive downstream processing. A detailed analysis of enzymatic biodiesel production has concluded that the productivity of the immobilized enzyme is a key requirement for the economic viability of the process. The study suggested a target cost for the biocatalyst of $25 per ton of biodiesel, which is comparable to that of chemical catalysts [62].

One factor that often appears to be underestimated throughout the literature dealing with biocatalysis concerns the environmental impact of the biocatalyst itself and, more specifically, of the immobilized enzymes. In the perspective of achieving bio-based products meeting sustainability certifications, Life Cycle Assessment (LCA) methodology is gaining increasing relevance in the scientific community and is recognized as an effective method for evaluation of environmental burdens associated with productive industrial processes. An interesting LCA study applied a “cradle to gate” approach to evaluate three different processes for enzymatic biodiesel production [62]. The authors estimated the environmental impact of the three catalysts, namely an immobilized biocatalyst, a soluble enzyme and an alkali catalyst. The results showed that the immobilized biocatalyst has a lower environmental impact on biodiesel production compared to the alkali and soluble biocatalysts. In fact, the environmental impact of the immobilized biocatalyst depends strongly on its recyclability and re-use for different cycles [63,64]. Thus, biocatalyst productivity is the major factor affecting both economic and environmental impact of immobilized biocatalysts. In that respect, a productivity of 5–10 tons of product per kg of immobilized enzyme has been indicated as an acceptable target. Another study analysed the impact of enzymes immobilized on fossil based methacrylic carriers and produced industrially for application in the pharmaceutical sector [65]. LCA methodologies pointed out that the major contributions to acidification, eutrophication and photochemical smog formation come from the media used for the enzymatic fermentation (yeast extract soybean protein) and secondly from the immobilization processes. Immobilization was the major contributor in terms of global warming potential, with 16 to 25 kg of carbonic anhydride eq per kg of immobilized biocatalyst. Data indicated that the preparation of the immobilized biocatalyst is energy intensive, consuming from 117 to 207 MJ of non-renewable energy per kg of immobilized enzyme. The global warming potential is attributable to the methacrylic carrier, which emerged as environmentally the most crucial factor associated with the production of the immobilized biocatalyst. That was connected to the use of fossil-based raw materials (i.e., glycidyl methacrylate and ethylene dimethacrylate) in their production.

Another study [18] analysed the contribution of labour, enzyme, equipment and carrier to the final cost of a biocatalyst immobilized on a porous solid carrier. The base case considered a carrier with a cost of €50/kg to obtain a loading of 50 gm of enzyme per kg. As shown in Fig. 4, the cost of the carrier represents the largest contribution to the final cost, although the cost of some carriers available on the market for enzyme immobilization (e.g. methacrylic resins) can be 2–5 fold higher. It is evident that in order to promote a wider uptake of immobilized enzymes for the production of high-volume and low-cost bio-based products,

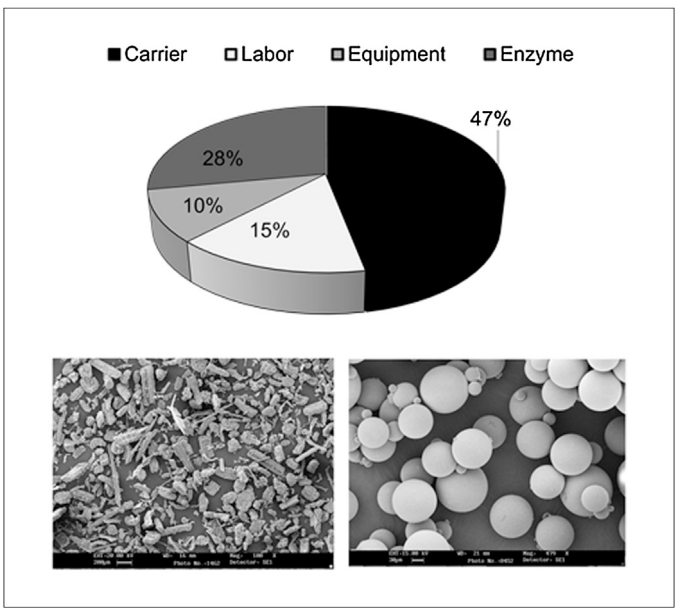

Fig. 4. Top: example of cost distribution in the production of an immobilized enzyme using a commercial porous resin (data from Ref [18]). Bellow: visual comparison (electron micrographs) of renewable fibers (rice husk) used as carrier for enzyme immobilization with commercial methacrylic beads (on the right).

new carriers and immobilization strategies are needed. The conclusions of all these studies also shed light on the recent analyses that demonstrate how immobilized biocatalysts have so far not met the initial optimistic expectations.

Indeed, according to DiCosimo et al. [16] only four types of biotransformation employing immobilized biocatalysts are carried out at a scale larger than 100,000 ton per year [16].

Nevertheless, there is a huge biocatalytic potential that needs to be re-considered and optimized in the perspective of also producing bio-based chemicals and fuels from renewable resources by means of immobilized enzymes [14]. Overall, there is a need for a more holistic analysis of environmental and cost constraints in the development of immobilized biocatalysts for bio-based chemistry applications. It is noteworthy that the cost of commercial methacrylic carriers is in the range of a few hundred € per kg of resin. The literature reports a large number of studies where renewable materials or biopolymers have been used as economical and sustainable alternatives to fossil-based immobilization carriers [66].

Carbohydrate-based biopolymers represent the group that has been most widely investigated. One notable example is provided by the immobilization of penicillin G acylase on chitosan for antibiotic processing on a large industrial scale [22].

Recently, rice husk (RH) has been suggested as a carrier for biocatalyst immobilization. This natural and robust composite material is made of lignin, cellulose, hemicellulose and SiO_2 [60,67]. The milled material requires minimal pre-treatment and is applicable in both physical and covalent immobilization protocols and under various process conditions. Although the material appears considerably less homogeneous in shape and size compared to commercial fossil-based methacrylic carriers (Fig. 4), studies confirmed that lipases covalently immobilized on RH can be recycled and are particularly suitable for application in viscous solvent-less systems. Experimental data has demonstrated the applicability of hydrolases immobilized on RH in the bulk synthesis of emollient esters as well as in solvent free polycondensation of bio-based monomers for the synthesis of polyesters [67]. RH displays remarkable mechanical and chemical robustness and, more importantly, is available worldwide in virtually unlimited amounts (globally 120 Mt per year).

Concerning the greenness of RH as enzyme carrier, some preliminary analysis has underlined that RH can be re-utilized at the end of its proposed industrial application [67]. Thus, it has been bio-degraded *via* anaerobic digestion to produce bio-methane and biogas [68]. As a renewable composite material, RH can be re-used in the building sector and various manufacturing applications, in accordance with the principles of the circular economy [67]. Exhausted RH can be also used as ruminant feed or in pet foods as a source of fiber, or applied as a fertilizer or medium for gardening. RH, and bio-products in general, have the subsidiary advantage that they are subject to less stringent legislative constraints. Thus, even after chemical modification, they are currently exempted from the European REACH registration [69].

Although LCA methodologies can assess the sustainability of biocatalysed processes and bio-based products, it must be noted that this approach implies a very extensive inventory of all inputs and outputs of the production system as well as their environmental impact assessment. In that regard, it would be desirable to develop simpler metrics for use as a decision-making tool at an early stage of product synthesis. Some examples are the E-factor

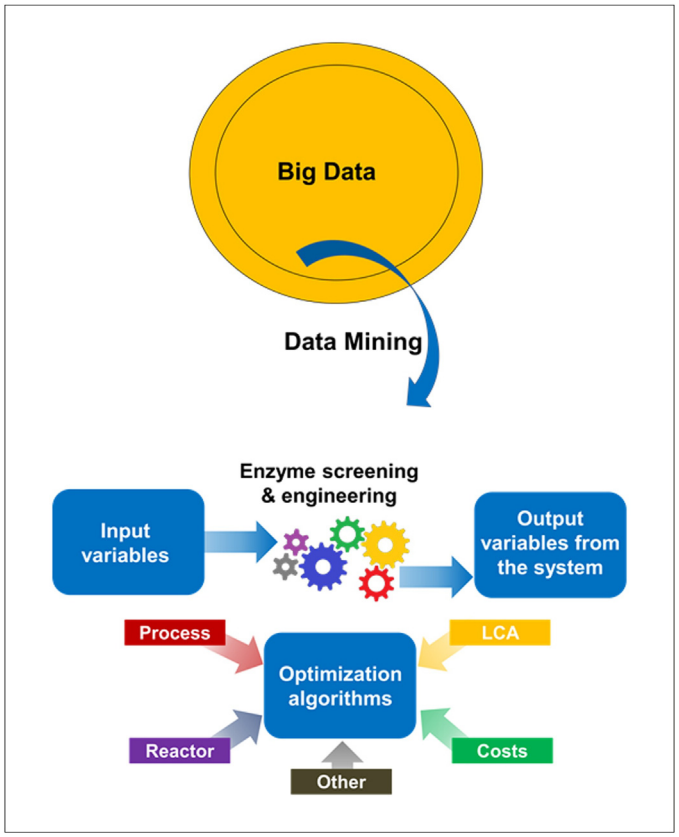

Fig. 5. A systemic vision of integrated strategies for developing biocatalytic processes applicable to bio-based chemistry and biorefineries. Multiobjective optimization algorithms can be used to set constraints and desired targets (technological, environmental and economic) since the beginning of the research. Algorithms can be fed by data obtained through computational mining of data bank (e.g. bioinformatics).

conceived by Roger Sheldon and other atom efficiency criteria [66,70].

Outlook: success will pass through big-data access, multi-sectoral integration and contamination

Nowadays, research and innovation activities are facing a shift towards multi-disciplinary integration. While the complexity of processes is increasing, the information needed to face the new challenges is dispersed among multiple and heterogeneous data sources. The optimal results can be achieved by addressing different domain approaches and analyzing a wide number of variables, objectives and constraints related to various disciplines.

Biocatalysis is an inherently multidisciplinary discipline, embracing know-how spanning from molecular biology to enzyme technology, chemistry and chemical engineering. Impact and success of biocatalysis in bio-based chemistry will strongly depend on the ability of the biocatalysis scientific community to transform the huge amount of *information* currently scattered in a multiplicity of databases (DBs) into useful *knowledge* [71–73]. More specifically, biocatalysis is expected to achieve objectives that require, from one side, tools able to mine big databases and then methodologies for analysing the massive amount of scientific data already available (Fig. 5). A number of extremely powerful and refined bioinformatics tools for data analysis are becoming accessible, in principle, to any end-user, as most are based on open source software [73–75]. However, concerning integration and optimization, biocatalysis is still a relatively novel field compared to other disciplines (e.g. mechanical engineering, material science), which make extensive use of numerical optimization and advanced algorithms able to integrate several actions and software into automated workflows. These computational integration tools allow the design of reproducible and cost-effective processes, while the computational platforms operate by taking unbiased decisions [76]. It must be mentioned that some initial attempts have been made by integrating software and calculation for *in silico* screening and rational design of enzyme mutants [77].

In conclusion, biocatalysis applied within the bio-based sector requires a closer integration, not only of scientific and technological factors but also of constraints and information coming from economic, social, legislative and environmental analysis (Fig. 5). In many cases, scientific advances have already provided data and technological solutions that might reach the market in the coming years, provided they are effectively optimized. Therefore, there is an urgent need for more integrated strategies able to solve highly complex problems that cannot be faced through a simple assembling of discrete steps of innovation.

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