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Genetic improvement of microorganisms for applications in biorefineries

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Abstract

The development of biorefineries directed to the production of fuels, chemicals and energy is important to reduce economic dependence and environmental impacts of a petroleum-based economy. Microorganisms are essential in several industrial bioprocesses nowadays, and it is expected that new microbial bioprocesses will play a key role in biorefineries. However, the bioconversion process requires a robust and highly productive microorganism. In this scenario, several strategies to genetically improve microorganisms to overcome the bioprocesses challenges have been considered. In this work, we review microorganisms importance in the biorefineries concept, highlight the desirable traits they must hold in order to be employed, and discuss the main strategies to improve such traits. The focuses of this work are on four main targets in the improvement of microorganisms: driving carbon flux towards the desired pathway, increasing tolerance to toxic compounds, increasing substrate uptake range and new products generation.

Keywords: Microorganisms; Biorefineries; Bioprocess; Metabolic engineering; Genetic engineering; Evolutionary engineering

Introduction

The interest in renewable and sustainable biotechnological processes for energy, biofuels and chemicals production has been increasing over the years. Economical and environmental factors have been pushing the chemical industry, for instance, to invest in new means to get the same products in a more sustainable and economical way. It is estimated that by 2025, 15% of global chemical sales will be bio-derived [1]. In this context, the development of biorefineries appears as an important alternative to the common known petroleum-based processes and products. Biorefineries can be defined as "the sustainable processing of biomass into a spectrum of marketable products (food, feed, material, chemicals) and energy (fuels, power, heat) [2]. Chemical, physical and biological processes can be employed in a biorefinery to convert biomass into a large spectrum of products of interest [2]. The biorefinery concept is attractive because it would allow production of high added-value compounds and/or big volumes of biofuels, with market competitive prices, while reducing waste disposal and energy costs. In addition, always taking into consideration the sustainability of the process and its indirect impacts (such as water use, impact on soil and biodiversity and competition for food). Few biorefineries have been in operation for several years, for instance, the pulp and paper based biorefinery Borregaard, in Norway [3], but there is still immense potential to be developed in several countries [2].

Microorganisms are main characters in industrial bioprocesses, being directly responsible for the production of the desired chemical, or indirectly providing important components for the processes. Indeed, there are several industrial processes in operation around the world based on microorganisms for production of food additives, enzymes and chemicals; for instance, the bioethanol industry from sugarcane in Brazil. Due to their versatility, microorganisms are also expected to play an essential role in conversion processes employed in biorefineries.

The development of biorefineries brings new opportunities and challenges to the industrial application of microorganisms. New substrates may be used and a variety of products formed; however, strains adapted to the industrial processes need to be developed. Based on the feedstock characteristics and desired products, a microbial strain and a production process optimization are needed to achieve an ideal conversion process (Figure 1). This led research groups from several institutions

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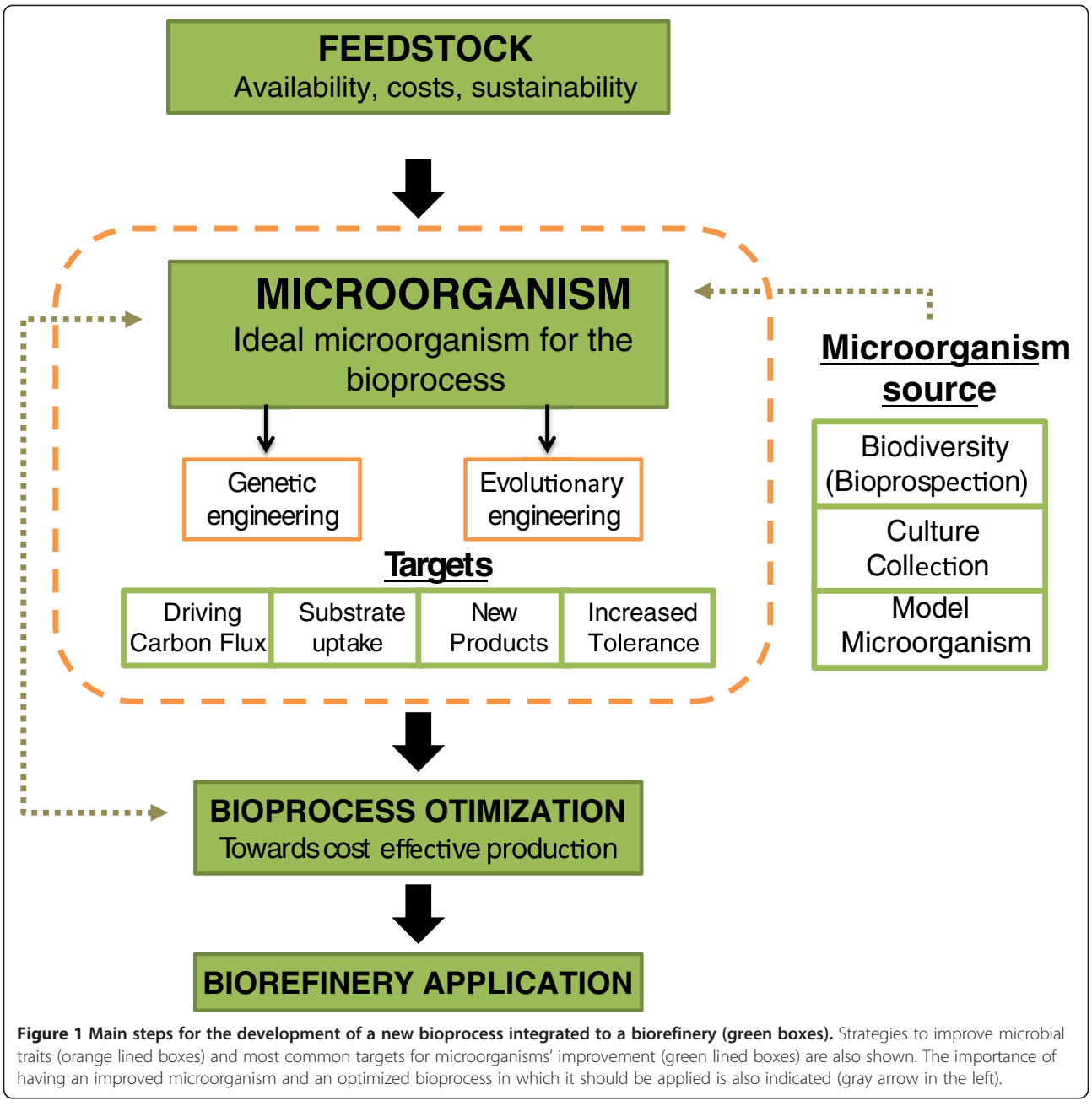

Figure 1 Main steps for the development of a new bioprocess integrated to a biorefinery (green boxes). Strategies to improve microbial traits (orange lined boxes) and most common targets for microorganisms' improvement (green lined boxes) are also shown. The importance of having an improved microorganism and an optimized bioprocess in which it should be applied is also indicated (gray arrow in the left).

worldwide to start developing microbial strains for application in biorefineries and nowadays there are many promising experimental processes being developed. This work focuses on the importance of microorganisms in industries, summarizes and discusses the main targets for microbial improvement and the strategies currently employed to generate and improve strains to achieve commercial, technological and environmentally viable industrial processes (Figure 1). In addition, we highlight and exemplify general strategies to develop microorganisms that are able to produce fuels and chemicals from renewable feedstocks.

Review

Microorganisms currently used and new from biodiversity

In order for a microorganism to be applied in a bioprocess, it must present specific traits, which would allow its maximum performance, i.e. high production yields and rates, even when submitted to one or a series of challenges. These may include substrate and product toxicity, variations or extreme pH values, high or variable temperature and pressure values, presence of competitors (biological or chemical contamination), inability to use all components of the substrate, and others [4]. It will be rather difficult to find a microorganism that has naturally all necessary traits to be

employed in an industrial bioprocess. Therefore, genetic improvements of microorganisms have become an essential step in the development of such processes (Figure 1).

Genetically, physiologically and biologically well-characterized microorganisms, such as the yeast *Saccharomyces cerevisiae,* the bacteria *Escherichia coli* and other microorganisms which are also already employed commercially, are frequently the initial choice for the development of novel biocatalysts for industrial application. Previous knowledge about such microbes would ease the task of genetic improvement and the industrial utilization of new strains.

The yeast *S. cerevisiae* is the eukaryotic model microorganism and it is commonly utilized in bioethanol, brewery and bakers industries worldwide. In addition, several new bioprocesses for fuels and chemicals production are being developed based on this yeast [4] (Figure 2). It presents high level of ethanol tolerance (i.e. product inhibition is absent or minimal), ability to grow under different aeration conditions, including strictly anaerobic (which makes the process more easily controlled), have little nutrition requirements, shows high tolerance to toxic compounds and low pH tolerance, which also contributes to prevent bacterial contamination [5]. The accumulated knowledge about *S. cerevisiae* surpasses any other eukaryotic species. In fact, *S. cerevisiae*'s genome was the first eukaryotic genome to be completely sequenced [6]. More recently, systems biology tools are abundantly available and further studies have been improving the understanding about this organism. Indeed, *S. cerevisiae* has been genetically modified to produce a variety of chemical products (Table 1 and Figure 2).

While *S. cerevisiae* is the eukaryotic model organism, *E. coli* stands as the prokaryotic one. Similarly to the yeast, its genetics, physiology and biology are well-known, and genetic manipulation tools are already well established for it. *E. coli*'s complete genome was published in 1997 [24]. *E. coli* has been genetically modified to produce many different chemical compounds (Table 1 and Figure 2) [25] demonstrating its biotechnological potential. Having many characteristics of interest to industry, such as efficient growth under industrial conditions, low nutritional requirements, anaerobic growth, capacity to use many different carbon sources including carbohydrates, polyols, and fatty acids [26] , this bacterium was already engineered to produce ethanol from lignocellulose [27] and it is currently employed in a bioethanol pilot plant in Florida [28].

The accumulated knowledge about such "model" microorganisms, in the most different subjects of studies, and familiarization with their requirements and performance, facilitates the task of genetic improvement and eases the industrial utilization of new strains. However, identification of new microorganisms, new genes and enzymes from the microbial biodiversity, still remains essential to reveal new traits and capabilities to favor development of biotechnological applications [29]. This becomes more relevant because estimates point

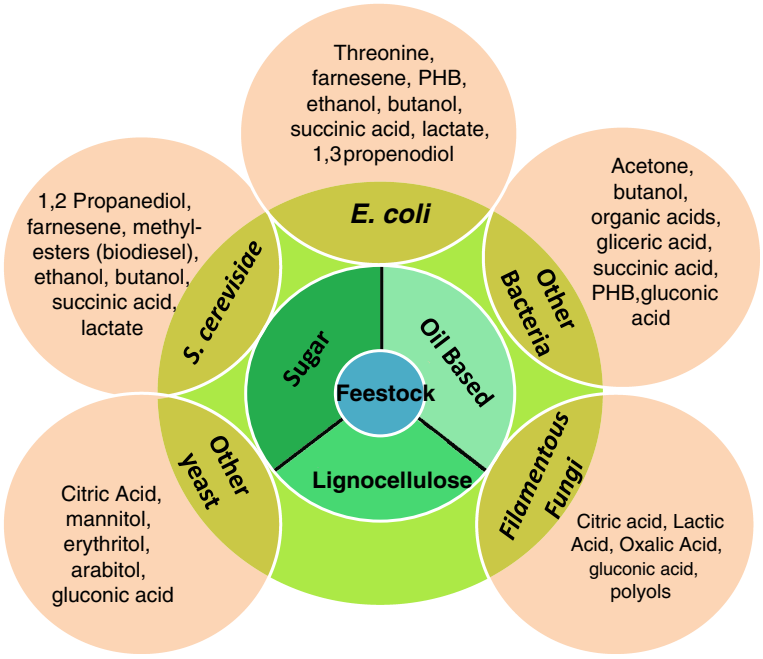

Figure 2 Examples of biofuels and chemicals produced from renewable feedstock by microbial strains. Detailed information about microbial strains and their production capacity can be found at 3, 8, 21, 29, 32, 34 and references there in.

Table 1 Selected examples of four major target categories where genetic engineering strategies were applied to improve product formation by microorganisms

Organism	Product	Main substrate	Yield*	Productivity	Concentration	Outcomes	Main genetic modifications	Reference
Driving carbon flux towards the desired pathway								
E. coli SY4	Ethanol	Glycerol	0.42 g g^{-1}	0.15 g L^{-1} h^{-1}	7.8 g L^{-1}	Yield improved 69 fold. Engineered strains efficiently utilized glycerol in a minimal medium without rich supplements	Deletion of genes to minimize the synthesis of by-products	[7]
*E. coli LA02*Δ*dld*	Lactic acid	Glycerol	0.80 g g^{-1}	1.25 g g^{-1} h^{-1}	32 g L^{-1}	Low-value glycerol streams to a higher- value product like D-lactate. Yield improved seven fold	Overexpression of pathways involved in the conversion of glycerol to lactic acid and blocking those leading to the synthesis of competing by-products	[8]
E. coli	Acetate	Glucose	0.456 g g^{-1}	1.38 g g^{-1} h^{-1}	53 g L^{-1}	Reduction of the fermentation by products concentration by 1, 25 (succinate) to 33 fold (lactate). Yield improved over seven fold	Deletion of genes involved in the succinate formation as fermentation product	[9]
Y. lipolytica	Succinic acid	Glycerol	0.45 g g^{-1}	n.d	45 g L^{-1}	Succinic acid production yield increased over 20 fold	Deletion in the gene coding one of succinate dehydrogenase subunits	[10]
Y-3314								
Mannheimia succiniciproducens	Succinic Acid	Glucose	0.76 g g^{-1}	1.8 g g^{-1} h^{-1}	52.4 g L^{-1}	Nearly complete elimination of fermentation by-products, (acetic, formic, and lactic acids) and carbon recovery increased to 58% to 77% by fed-batch culture	Disruption of genes responsible for by product formation (*ldhA, pflB, pta,* and *ackA*)	[11]
Increasing of tolerance to toxic compounds								
C. acetobutylicum	Butanol	Glucose	n.d.	n.d.		Increased tolerance and extended metabolism response to butanol stress.	Overexpression of spo0A, responsible for the transcription of solvent formation genes	[12]
C. acetobutylicum	Butanol	Glucose	70.8%	n.d.	13.6 g L^{-1}	Reduction of acetone production from 2,83 g L^{-1} to 0,21 g L^{-1} and enhanced butanol yield from 57% to 70.8	Disruption of the acetoacetate decarboxylase gene (*adc*) avoiding acetone production and optimization of medium	[13]
S. cerevisiae	Ethanol	Glucose plus HMF (inhibitor)	0.43 g g^{-1}	0.61 g g^{-1} h^{-1}	n.d	Four times higher specific uptake rate of HMF and 20% higher specific Ethanol productivity	Overexpression of alcohol dehydrogenases ADH6 or ADH1-mutated	[14]
S. cerevisiae	Ethanol	Spruce hydrolystae	n.d	0.39 g g^{-1} h^{-1}	n.d	HMF conversion rate and ethanol productivity for the engineered strains four to five times and 25% higher than for the control strain.	Overexpression of alcohol dehydrogenases ADH6 or ADH1-mutated	[14]

Table 1 Selected examples of four major target categories where genetic engineering strategies were applied to improve product formation by microorganisms *(Continued)*

E. coli XW068(pLOI4319)	Lactate	Xylose plus HMF	85% of the theoretical maximum	n.d.	n.d	Furfural tolerance increased by 50%. Minimal growth and lactate production occurred after 120 h for the control strain	Overexpression of NADH-dependent propanediol oxidoreductase (FucO)	[15]
Increasing substrate uptake range								
E. coli	Ethanol	Xylose	0.48 g g^{-1}	2.00 g g^{-1} h^{-1}	43 g L^{-1}	Rapid co-fermentation due to reduced repression of xylose metabolism by glucose, and 60% less time required for fermentation of 5-sugars mix to ethanol.	Deletion of methylglyoxal synthase gene (*mgsA),* involved in sugar metabolism	[16]
Lactobacillus plantarum	Lactic Acid	Corn starch	0.89 g g^{-1}	4.51 g g^{-1} h^{-1}	86 g L^{-1}	First direct and efficient fermentation of optically pure D- lactic acid from raw corn starch reported	Deletion of L-lactate dehydrogenase gene (ldhL1) and expression of *Streptococcus bovis* 148 α-amylase (AmyA)	[17]
S. cerevisiae	Ethanol	Xylose	0.43 g g^{-1}	0.02 g g^{-1} h^{-1}	7.3 g L^{-1}	Higher ethanol yields than XR/XDH carrying strains	Overexpression of *Piromyces sp* xylose isomerase (XI)	[18]
S. cerevisiae	Ethanol	Xylose	0.33 g g^{-1}	0.04 g g^{-1} h^{-1}	13.3 g L^{-1}	Higher specific ethanol productivity and final ethanol concentration than XI carrying strains	Overexpression of xylose reductase (XR) and xylitol dehydrogenase (XDH) enzymes from *Scheffersomyces stipitis*	[19]
E. coli	Butanol	Glucose	6.1%	0.02 g g^{-1} h^{-1}	1.2 g L^{-1}	Anaerobic production of butanol by a microorganism expressing genes from a strict aerobic organism	Expression of *C. acetobutylicum* butanol pathway sinthetic genes in *E. coli*	[20]
Generation of new products								
E. coli	Fatty acid ethyl esters (FAEEs)	Glucose	7%	n.d.	30.7 g L^{-1}	Tailored fatty ester (biodiesel) production	Heterologous expression of a "FAEE pathway" engineered in *E. coli*	[21]
S. cerevisiae	Butanol	Galactose	n.d	n.d	2.5 mg L^{-1}	First demonstration of n-butanol production in *S. cerevisiae*	N-butanol biosynthetic pathway engineered in *S. cerevisiae*	[22]
E. coli K12	1,3-propandiol	Glycerol	90.2%	2.61 g g^{-1} h^{-1}	104.4 g L^{-1}	Substantially high yield and productivity efficiency of 1,3-PD with glycerol as the sole source of carbon	Heterologous overexpression of genes from natural producers of 1,3-PDO	[23].

*expressed in g product per g substrate or% of maximum theoretical; n.d. not determined; n.c. not calculated.

that there are 10^{30} microorganism cells in the Earth. That is more than stars in the universe. And like in the universe, we only know a small fraction of those, and have characterized even fewer. Indeed, the vast microbial diversity in microorganism collections world-wide still remains unexploited and in the wild unknown species relies an enormous unknown potential.

The discovery of new genes, pathways, enzymes and characteristics in newly discovered and described wild organisms, can be applied in the development of new production processes. For instance, *Pichia ciferrii* was recognized as a potential producer of sphingolipids, including sphingosine, since genes encoding enzymes of the biosynthetic pathway were identified. However, no detectable amounts of sphingosine were produced by the wild type strain. Thus, metabolic engineering strategies including the implementation and improvement of a metabolic pathway for the conversion of sphinganine to sphingosine were used to develop a final strain capable of producing approximately 240 $mg.L^{-1}$ triacetylated sphingosine (TriASo) in shake flasks and up to 890 $mg.kg^{-1}$ in lab-scale fermentation. Further improvement of such strain could lead to even higher concentrations of sphinganine and sphingosine for cosmetic and pharmaceutical applications [30]. These results are still preliminary for industrial application, but they clearly demonstrate the potential of bioprospecting for developing bioprocesses.

Most certainly, encountering the complete ideal wild microorganism to be used in a specific biorefinery is a challenging mission. Scarcely a wild microorganism will have all desired traits to be employed in a biorefinery. Thus, genetic engineering strategies shall be used to design an ideal host, improving substrate uptake range and product formation, increasing tolerance, yields and rates and allowing production of new chemicals by a specific strain. In the next sections, strategies to develop such microorganisms for industrial processes applications are presented and discussed.

Genetic improvement of microorganisms

Bioprocesses require microbial strains that are able to tolerate several different stresses while keeping high yields and productivity. In addition, in order to develop and keep viable bioprocesses, the microbial strains employed or envisioned to be used need constant genetic improvement for achieving or keeping high production rates. For instance, even though the yeast *S. cerevisiae* is used for more than 30 years in Brazilian bioethanol industry, each year improved strains for the process are selected [31]. On the other hand, wild strains that contain desirable characteristics for biotechnological application usually have very low production rates or are very sensitive to the industrial conditions. Thus, different strategies have been applied to genetically improve microorganisms to solve problems such as the ones listed above, and directly or indirectly increase productivity and consequently the profitability of the bioprocess. Four major target categories where genetic and evolutionary engineering strategies may be applied to improve product formation by microorganisms are: i) driving carbon flux, ii) increase tolerance to toxic compounds, iii) increase of substrate uptake range and iv) generation of new products (Figure 1). Following, each of these targets is discussed.

Driving carbon flux

Naturally, microorganisms have their metabolic pathway optimized to sustain maximal growth and outcome competitors in the environment. Thus, production of a desired chemical usually is reduced during cell growth (expenses of carbon and energy sources) and by-product formation. Thus, a common target for modifications that directly affects microorganism's productivity is driving carbon flux through a specific pathway towards the desired product.

Microorganisms from the most different groups, from bacteria and yeast to filamentous fungi, have been genetically modified to increase production of a desired biofuel or chemical compound. Nowadays, strains that are able to produce a variety of chemical compounds in concentrations as high as above 90% m/m of the theoretical maximum are available (Table 1) (Figure 2). The strategies to increase product formation generally include a series of modifications in the microorganism metabolism, achieved by overexpression or knockout of enzymes in the producing pathway [13,32], changing redox balancing of the cell by redirecting carbon fluxes from NADPH- to NADH consuming reactions [33-36], engineering global transcription machinery [37] and others (Table 1). All these types of modification were employed, for instance, to obtain *S. cerevisiae* strains that are able to produce ethanol from sugars that are present in lignocellulosic hydrolysates [38]. *S cerevisiae* strains able to ferment lignocellulosic hydrolysates rich in xylose and produce ethanol with yields up to 0.44 g ethanol/g sugar (86% of theoretical maximum) were obtained [38].

Increased tolerance to the substrate

Another common trait that may hamper product formation by microbial strains is their low tolerance to substrate or fermentation end-product. Indeed, the fermentation medium may impose a harsh environment for the microorganism and consequently, an important trait to define the strain to be used in an industrial process is its tolerance level to toxic compounds. When tolerant strains are not available for the desired process, genetic engineering strategies may be applied to improve strain response for inhibitory compounds. A good example of such is the

improvement of strains for production of biofuels and chemicals from lignocellulosic hydrolysates.

Lignocelulose is composed of the polymers cellulose and hemicellulose, and the macromolecule lignin. Prior to fermentation, lignocellulosic biomass must be submitted into a pretreatment to reduce its recalcitrance. In the next step, the hydrolysis, cellulose and hemicellulose are broken down into their sugar monomers, those which should later be converted into the final product [38]. The problem is that during pretreatment and hydrolysis not only sugars are solubilized, but also, compounds that inhibit microbial metabolism may be released and formed during these steps [39]. Indeed, compounds like furaldehydes (5-hydroxymethyl-2-furaldehyde – HMF; - and 2-furaldehyde – furfural), organic acids (acetic, levulinic and furoic) and phenolic derivatives are commonly found in lignocellulosic hydrolysates. However, concentration of such compounds varies according to biomass and process conditions employed. As these inhibitors can affect microbial growth, decrease product yield and productivity; prolong lag phase of microbial growth, and reduce cellular viability [39,40], several evolutionary or metabolic engineering strategies have been employed to develop strains able to tolerate them. Evolutionary engineering mimics the evolutionary mechanisms of nature, in which through variation, strains are selected according to the response to the pressure they are submitted to [40].

Evolutionary engineering strategies have been applied, for example, to generate strains with higher tolerance to specific compounds (furfural, for instance), or to lignocellulosic hydrolysates, by selecting strains with the ability to remain viable and keep growth even in presence of such compounds. Through multiple selection cycles, in presence of increasing concentrations of the selection pressure, i.e. the toxic compound, mutants with higher tolerance can be selected. To increase genetic variation in the population to be submitted to the selection, pressure mutagenic agents like UV light and EMS (Ethyl methanesulfonate) can be applied. Evolutionary engineering strategies have been commonly employed to obtain *S. cerevisiae* [41], *P. stipitis* [42][43], *S. passalidarum* [44] mutants which are able to ferment lignocellulosic hydrolysates with higher rates than the native strains. For instance, the yeast strain TMB3400 was grown in minimal medium containing 3 mM furfural. Once cells reached late exponential phase they were transferred to a fresh media amended with furfural. Upon shorter lag phases the furfural concentration was increased continuously. Finally, after approximately 300 generations, single colonies were obtained, and the best isolated strain showed a lag phase of 17 h instead of 90 h for parental strain in media supplemented with 17 mM furfural. In addition, viability tests in furfural containing medium showed that the evolved strain remained viable, whereas the parental strain showed continuously decreasing colony- forming unit capacity after 10 h [41]. The main disadvantage of evolutionary engineering resides in the fact that the genetic trait responsible for the improvement has to be identified posteriorly and thus cannot be directly transferred to another strain.

Yeast tolerance to lignocellulosic hydrolysate inhibitors has also been improved by genetic engineering strategies (Table 1) [40]. The general strategy involves identification of genes that confer resistance to inhibitors and their posterior overexpression in the desired microorganism. Yeast oxide-reductases enzymes, like alcohol dehydrogenase 6 (Adh6), and Adh1, able to convert HMF and furfural to their corresponding alcohols, when overexpressed have been shown to improve yeast growth and fermentation rates not only in medium supplemented with theses inhibitors but also in lignocellulosic hydrolysates. Genes related to regeneration of cofactors NAD(P)H, and transcription factors related to stress response have also been demonstrated to increase yeast tolerance towards lignocellulosic hydrolysate inhibitors [45]. Despite the time frame required to identify genes or enzymes that confer increased tolerance, the genetic engineering strategies are advantageous because the trait can be transferred from one strain to another promptly.

Increase of substrate uptake range

The increased interest to produce fuels and chemicals from renewable resources, especially from lignocellulosic feedstocks and crude glycerol residue from biodiesel industry, made the expansion of substrate utilization another important target for genetic improvement of microorganisms [7]. Screening and genetic engineering of wild- and well-known microbial strains to increase production of fuels an chemicals from substrates previously not- or poorly utilized have gained much attention lately [2,46].

A better utilization of lignocellulosic feedstocks for fuels and chemicals production requires xylose utilization. This pentose sugar is present in several biomasses and it is the second most abundant sugar in many of them. In sugar cane bagasse, for instance, xylose corresponds to up to 30% of the sugars present in the biomass [47]. Thus, xylose utilization in biotechnological processes is desirable and might contribute considerably to the economic viability of the process. In this context, second generation bioethanol production from xylose with *S. cerevisiae* is one of the most evaluated bioprocess. As this yeast is widely used in alcohol industries, including first generation bioethanol production in Brazil, but it is not able to ferment pentoses, many strategies to construct xylose-fermenting *S. cerevisiae* strains have been employed. Among these, introduction and improvement of xylose catabolic pathways; increase sugar uptake rate by overexpression of

transporters; changing redox metabolism; and others as reviewed by Van Maris et al. [5] and Hahn-Hägerdal et al. [38]. Nowadays, several yeast strains able to convert xylose to ethanol are available, either with reductase -dehydrogenase or xylose isomerase pathway, with yields around 90% from the theoretical maximum, (Table 1) [48].

Production of biodiesel by (trans)esterification of oils and fats results in approximately one ton of crude-glycerol from every ten tons of biodiesel produced. As biodiesel production increased worldwide, glycerol availability did too and its prices in the market decreased. Thus, microbial processes to convert glycerol into renewable fuels and chemicals have been considered. Indeed, several groups demonstrated the potential of bacteria utilization , as well as yeast and filamentous fungi for production of ethanol, butanol, 1,3-propanediol, polyols and other chemicals from glycerol (Table 1) (Figure 2). This subject has been recently reviewed by Almeida et al [46] and Yang [49].

New products

In addition to increased production rates by redirecting carbon fluxes, increase of substrate uptake ranges and improving tolerance to inhibitory compounds, genetic engineering strategies can be employed to generate microorganisms able to produce biofuels and chemicals not naturally formed by their genetic and biochemical machinery. In this case, enzymes and pathways from one organism can be transferred to the desired microbial host, which ultimately will produce the desired compound. Nowadays, there are several examples of engineered microorganisms for production of compounds such as building block chemicals (compounds from which a big number of molecules of interest can be obtained) rather than bioethanol in this category (Table 1).

Acids derived from lignocellulosic sugars have a large potential as precursors of plastics and as building block compounds [49]. Among these there is xylonic acid, an organic acid with five carbons, derived of xylose, which is naturally produced by bacteria from the genre *Acetobacter*, *Aerobacter*, *Pseudomonas Gluconobacter* and *Erwinia*. Although wild type bacteria are efficient in the xylonic acid production, they still have high nutritional requirements, and low cell biomass production yields, which makes their utilization in industrial processes difficult. Consequently, for the last three years, genetic engineering strategies were used to build recombinant xylonic acid producing strains of *E. coli, S. cerevisiae, Kluyveromyces lactis* and *Pichia kudriavzevii* [50-55]. These microorganisms were chosen as possible hosts for presenting high growth rates, simple nutritional requirements and specially yeasts, for presenting good tolerance to inhibitors found in lignocellulosic hydrolysates, as commented above [45]. Indeed, the identification of genes from different microorganisms, that code for the enzymes involved in the conversion of xylose to xylonic acid allowed construction of strains able to produce xylonic acid with yields above 90% of theoretical maximum and at high concentrations [50-55].

The number of compounds naturally produced by *E. coli* is limited, and this bacterium is not a natural biofuel producer. However, advances in metabolic engineering techniques have allowed the development of strains capable of producing a big variety of biofuels from different carbon sources, such as glucose, xylose, glycerol, and fatty acids [26,56]. An interesting example is the construction of a *E. coli* strain able to produce butanol when expressing the fermentative metabolic pathway of *Clostridia acetobutylicum*. The expression of six genes from this pathway (*thl, hbd, crt, bcd, etfAB and adhE2*) in *E. coli* was necessary to obtain a strain able to produce 139 mg L^{-1} butanol from glucose under anaerobic conditions [26,56]. In an independent study, Inui and co-workers also inserted different combinations of genes from *C. acetobutylicum* butanol pathway (*thL, hbd, crt, bcd–etfB–etfA, and adhe*) in *E. coli*. The best resulting strain was able to produce 1184 mg L^{-1} of butanol. Although the amount of butanol produced by the generated strains is around 10 times lower than what is obtained by *Clostridia*, these experiments show that *E. coli* is a viable host for the production of biobutanol and the power of genetic engineering [20]

Conclusion

Several microbial-based bioprocesses are currently used in industry, and new ones should be established within the biorefinery context. To meet specific demands of the industry, which requires microbial strains able to produce fuels and chemicals from different renewable resources in high yields and productivity, researchers have been constructing and genetically improving microbial strains. The focus of these improvements can be grouped in four main categories: i) driving carbon flux towards the desired pathway, ii) increasing tolerance to toxic compounds, iii) increasing substrate uptake range, and iv) generation of new products. Thanks to the advances of genomic and molecular analysis techniques, and systemic analysis tools, microorganisms able to produce a variety of biofuels and chemicals from lignocellulose and other substrates, with production capacities in magnitudes orders higher than native ones, are currently available in the literature. Further studies concerning such microorganisms and their potential, are expected to contribute significantly to the development of bioprocesses within the biorefinery concept.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions
This work was carried out in collaboration between both authors. JRMA defined the review theme; BGP and JRMA wrote, read and approved the final manuscript.

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