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## Scaling up xylitol bioproduction: Challenges to achieve a profitable bioprocess

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### ABSTRACT

Xylitol is a GRAS (*Generally Recognized as Safe*) polyol commonly used in the food industry and able to promote several benefits to the health. In addition, it can also be used as a building block molecule for the manufacture of different high-value chemicals. Currently, the commercial production of xylitol occurs by chemical route through the catalytic hydrogenation of xylose from lignocellulosic biomass. Since this is an expensive process due to the severe reactional conditions employed, the biotechnological route for xylitol production, which comprises the biological conversion of xylose into xylitol, emerges as a potential lower-cost alternative to obtain this polyol due to the milder process conditions required. However, the biotechnological route still presents important bottlenecks and challenges that impairs the process scaling up. Modern strategies and technologies that can potentially improve xylitol bioproduction include adaptive evolution of microbial strains to enhance their tolerance to inhibitors and the xylose uptake rate during the fermentation step; development of engineered microorganisms to result in higher xylose-to-xylitol bioconversion yields; as well as xylitol purification techniques to improve the recovery yields. Moreover, techno-economic analysis of the overall production chain is essential to identify the process viability for large-scale implementation as well as the steps requiring improvements. These are some key factors discussed in this review, which aims to provide insights for the development of a more economically competitive, less energy demanding and scalable new technology for xylitol production.

### 1. Introduction

The concern with global resources and climate change is leading to a transformation in the production systems. Around the world, industries are modifying and/or adapting their processes to guarantee a sustainable future for the society - where the natural resources and the environment are respected and preserved. The overarching goal is a reduction of the impacts generated by the exploitation of natural resources through the promotion of strategic initiatives that aim to guarantee environmental and social protection. In this sense, the development of biotechnological processes able to replace the chemical processes currently used in a large scale for the production of renewable energy and chemicals, has attracted great attention as they have potential to be more sustainable production systems [1]. Although the

concept is promising, developing efficient and competitive biotechnological processes able to replace the chemical routes with economic benefits is a task that requires significant efforts in all the steps of the production chain. So far, the biotechnological production of only a few products, including ethanol and some organic chemicals, has successfully reached the industrial scale. Increasing the spectra of products to be produced biotechnologically is, therefore, an area of research in great expansion.

In this context, one of the products that has received important attention for production via biotechnological route is xylitol, a high-value biomolecule with numerous applications in different industrial sectors. Xylitol is a polyol with specific properties, which allow its application especially in food, nutraceutical, cosmetic, and pharmaceutical industries [2,3]. Currently, it is mainly used in the food industry

**Abbreviations:** ALE, Adaptive Laboratory Evolution; GHG, Greenhouse Gases; GRAS, Generally Recognized As Safe; LCA, Life Cycle Assessment; LHW, Liquid Hot Water; NAD<sup>+</sup>/NADH, Nicotinamide Adenine Dinucleotide; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; PHB, Poly-3-Hydroxybutyrate; PPP, Phosphate Pentose Pathway; ST, Sugar Transporter; XDH, Xylitol Dehydrogenase; XR, Xylose Reductase.

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due to its sweetening power similar to the conventional sugar but with lower caloric content. The daily intake of xylitol can promote several benefits to the health. Moreover, xylitol is metabolized independently of insulin, which makes it a safe product for consumption by diabetic people [4]. More recently, xylitol molecule has stand out in the field of materials science, due to its thermal properties and energy storage capacity [5–7]. Also, studies demonstrate xylitol as a platform molecule to produce chemicals, fuels, and energy (H<sub>2</sub>). Its use as an emerging building block has been evaluated in feedstock integrated biorefineries, further contributing to its valorization mainly through the catalysis reaction to produce 1,2-propandiol, ethylene glycol, glycerol, lactic acid, among others [8]. As a result of its several features and new applications, the xylitol demand has been increasing in the last years, and its global market reached US\$ 921 Million in 2020 [9].

Xylitol currently in the market is produced by chemical synthesis through catalytic hydrogenation of xylose, a process that requires the use of a high-cost catalyst and severe reaction conditions with high energy expenditure due to the high temperature and pressure employed. This production route involves a series of purification steps, and consequently, xylitol is a more expensive product when compared to other sugars and sugar-alcohols currently in the market. Such higher price negatively impacts the use of xylitol as a biomolecule for other industrial processes and applications.

The biotechnological production of xylitol emerges as a promising, less expensive and eco-friendly alternative to avoid the high-cost catalyst and severe reaction conditions used in the chemical synthesis. In addition, this route can provide energetic benefits, either by saving energy during the production process or by supporting the coproduction of energy in a biorefinery. Since the bioconversion step is operated under milder conditions compared to the chemical route, it is expected to have a lower energy demand, which is beneficial to the global process. Moreover, the energy expenditure of this process can be balanced by cogenerating energy from the combustion of the solid waste generated. However, currently the xylitol bioproduction is still not as efficient and cost-competitive to replace the chemical route on a large scale and further improvements are needed to make this process feasible for industrial implementation.

Numerous studies have been done to improve the efficiency and costs of the biotechnological process for xylitol production. Recent advances in the field of adaptive evolution, genetic engineering of microorganisms, and techno-economic studies of the entire conversion chain (upstream, fermentation and downstream steps) have indicated promising approaches to make the biotechnological route a reality in the future. The challenges and recent trends for the xylitol bioproduction process are discussed in more details in the next sections.

## 2. Xylitol production routes: what is known and what needs to be improved

### 2.1. Classical production of xylitol: the chemical route

Currently, the commercial xylitol is manufactured from the chemical reduction of xylose through catalytic hydrogenation. This process consists in the hydrolysis of lignocellulosic biomass, followed by the xylose purification from the hemicellulosic fraction [10,11]. An important inconvenient of hemicellulosic hydrolysates is that they are complex media composed of xylose as main sugar, but also contain a variety of other components including other monomeric sugars, a few organic acids, sugar degradation products and lignin-derived phenolic compounds. Besides the non-sugar compounds, other monomeric sugars present on lignocellulosic biomass (glucose, galactose, and arabinose) should also be removed to avoid the generation of unwanted polyols and by-products that may hinder the xylitol purification in the subsequent step. As a result of the extensive purification steps, an ultrapure xylose solution is submitted to catalytic hydrogenation reaction, at high temperature and high pressure, using Raney nickel as catalyst [12], which is

able to provide up to 98% conversion yield [13].

The final step in the chemical route of xylitol production is the downstream process, consisting of the stages of recovery, purification (chromatographic separation), and crystallization at low temperatures [14,15]. The recovery of xylitol starts by removing the catalyst and concentrating the hydrogenated solution. The purification step uses mainly activated carbon adsorption and ion exchange chromatography for decolorization and inorganic salt removal, respectively. The crystallization requires highly purified and concentrated solution to obtain a pure crystal, which will be dried and packaged for commercialization.

The need for high energy expenditure during the hydrogenation processes, coupled with the high cost of the Raney nickel catalyst, deactivation of the metallic catalyst, plus the complexity of the purification procedures are some factors that stand out when assessing the costs of the chemical process for producing xylitol [11]. These important aspects negatively impact the economic viability of xylitol produced by chemical synthesis resulting, in a high-price end product. As an alternative to the bottlenecks highlighted in the chemical process, the biotechnological route has been proposed, but there are still challenges to overcome before it becomes commercially viable.

### 2.2. The biotechnological route as an alternative for xylitol production

The production of xylitol through the biotechnological route has emerged as a potential alternative to the chemical route. The main characteristics of the biotechnological route are the operation under mild conditions of temperature and pressure. In addition, the xylose conversion into xylitol is performed by enzymes or microorganisms, being the microbial conversion the most often used (Fig. 1). This process is considered environmentally friendlier compared to the chemical route and presents an extensive literature worldwide, with several studies using different feedstocks, fermentation conditions, and operational parameters.

The main steps of biotechnological route for xylitol production consists of: 1. Lignocellulosic biomass pretreatment; 2. Hemicellulosic hydrolysate concentration and detoxification; 3. Xylose into xylitol bioconversion; 4. Xylitol purification and recovery [3]. The first step, biomass pretreatment, is important to breakdown the recalcitrant structure of the lignocellulose and release xylose, the sugar to be used as precursor for xylitol production.

There are some pretreatments options to deconstruct lignocellulosic materials for xylose recovering. For example, non-selective reactions can be used such as acid hydrolysis, alkaline hydrolysis, organosolv, water-based treatments, or mechanical disruption [16]. Another option is the use of ionic liquids, which has the advantage for being eco-friendly [17]. However, the wide use of ionic liquids is still dependent on cost-benefit analysis to assess the feasibility for implementation in large-scale pretreatment plants.

Similar to the chemical route, dilute-acid pretreatment is one of the most employed methods to release xylose from lignocellulosic biomass for xylitol production through the biotechnological route, since it is a highly efficient method for hemicellulose disruption [16]. However, although highly efficient to release xylose from hemicellulose, the addition of acids is a disadvantage of this method since it may cause corrosion problems in the reactor, reducing the useful life of the equipment. Due to this problem, the hydrothermal (or liquid hot water (LHW)) pretreatment has been considered as an interesting option to replace the dilute-acid method as it uses only water at high temperatures and does not require the addition of acids, being also a more environmentally friendly alternative [18]. Regardless of the method, the liquid fraction obtained after biomass pretreatment corresponds to the hemicellulosic hydrolysate rich in xylose to be used for xylitol bioproduction. On the other hand, unlike the chemical route, the biotechnological route does not require the extensive steps for xylose purification since the microbial conversion step is a biologically selective process [3].

The dilute acid pretreatment depolymerizes mostly the

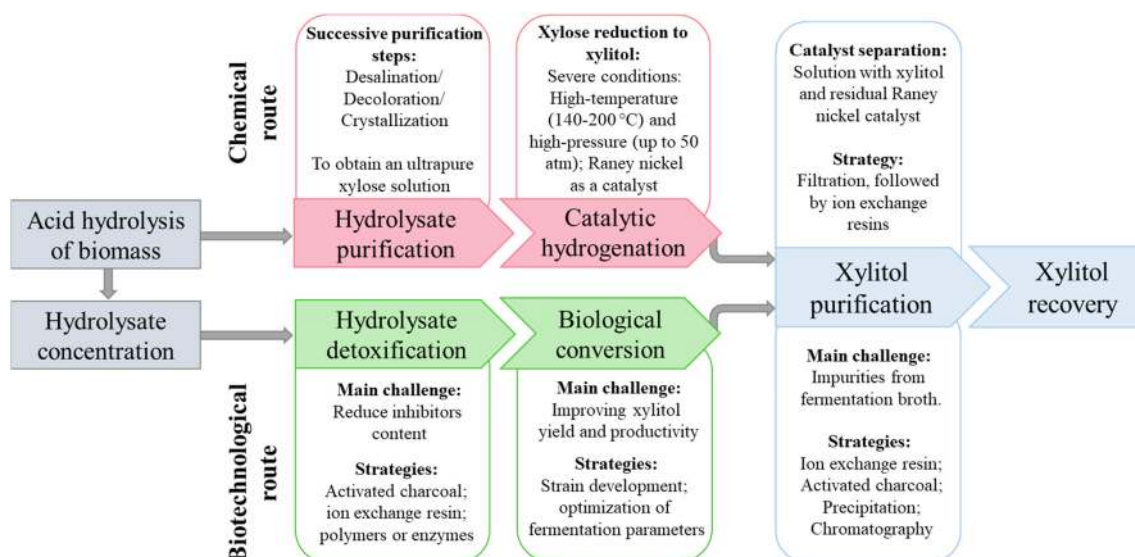


Fig. 1. Xylitol production from lignocellulosic biomasses by chemical and biotechnological routes: features, challenges and strategies.

hemicellulosic fraction of lignocellulosic biomasses, releasing sugars and non-sugar compounds, such as xylose, arabinose, glucose, and acetic acid. This process causes the formation of furfural and 5-hydroxymethyl-furfural due to the dehydration of pentoses and hexoses. Besides, cellulose and lignin are also deconstructed, releasing glucose and several phenolic compounds, respectively [19]. Such non-sugar compounds act as inhibitors of the microbial metabolism and strongly affect the microbial performance during the fermentation. Therefore, after obtaining the xylose-rich hemicellulosic hydrolysate, a detoxification step is required to eliminate or at least significantly reduce the concentration of non-sugar compounds present in the hydrolysate. Reducing the concentration of these inhibitory compounds is a key step to enhance the yeast fermentative performance to convert xylose into xylitol. To do that, several strategies have been proposed, which include adsorption in activated charcoal [20] or in ion exchange resins [21], use of enzymes such as lignin peroxidases [22], nanofiltration and reverse osmosis

membranes [23], biopolymers [24], among others. Nevertheless, detoxification is an extra step to be included in the xylitol production chain, and will, therefore, contribute with extra costs to the overall process. Moreover, the resins, enzymes, membranes, and biopolymers, for example, to be used for detoxification are also expensive, thus contributing to a higher final cost of the xylitol produced via biotechnological route. Finding a low-cost solution to overcome the presence of inhibitors in the hemicellulosic hydrolysate to obtain an efficient microbial performance during the fermentation is one of the main points requiring efforts for the development of a robust bioprocess for xylitol production.

Many species of microorganisms can produce xylitol naturally in the environment. This is the case of some species of filamentous fungi, yeasts, and bacteria [25–27]. Among these, yeasts stand out since several species have already been reported as being able to achieve high yields at different scales of production. The most studied yeasts for

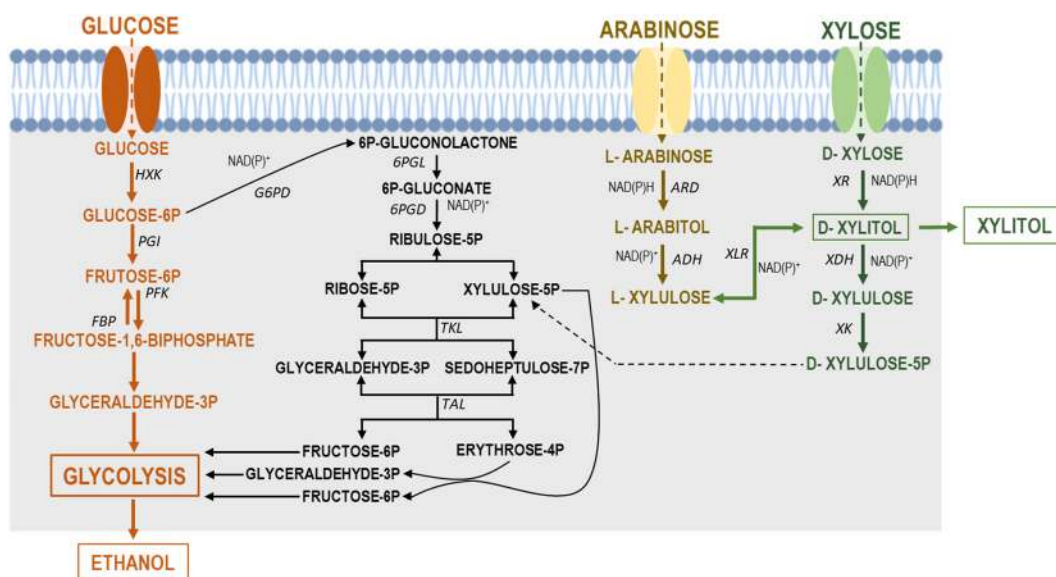


Fig. 2. Schematic presentation of xylose and glucose assimilation pathway in xylose-fermenting yeasts. Xylose is transported into the cell by the transporter proteins, and subsequently reduced to xylitol. Key enzymes were indicated: XR, xylose reductase; XDH, xylitol dehydrogenase; XK, xylulokinase, ARD, arabinose reductase; ADH, arabinol dehydrogenase; XLR, xylulose reductase. Pentose phosphate pathway: G6PD, glucose 6-phosphate dehydrogenase; 6PGL, 6-phosphogluconolactonase; 6PGD, 6-phosphogluconate dehydrogenase; TKL, transketolase; TAL, transaldolase. Glycolysis: HXK, hexokinase; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; FBP, fructose 1,6-bisphosphatase.

xylitol production belong to the genus *Candida* (mainly *C. guilliermondii*, *C. parapsilosis*, and *C. tropicalis*), as well as the yeasts *Scheffersomyces stipitis*, *Debaryomyces hansenii*, and *Kluyveromyces marxianus*. In yeasts, the xylose-to-xylitol conversion is dependent on the enzyme xylose reductase (EC 1.1.1.21, XR), which needs a reduced cofactor NADH/NADPH. The produced xylitol can be accumulated or further converted into xylulose by the enzyme xylitol dehydrogenase NAD<sup>+</sup>-dependent (EC 1.1.1.9, XDH). Xylulose is phosphorylated to xylulose-5-phosphate and converted into glycolytic intermediates, such as glyceraldehyde-3-phosphate (GA3P) and fructose-6-phosphate (F6P), via the phosphate pentose pathway (PPP) (Fig. 2). So, in order to promote the xylitol accumulation and avoid its further conversion into different metabolites, the expansion of genetic engineering tools is of great importance. Strain development is therefore an important approach to be considered for the development of an efficient industrial process for biotechnological production of xylitol.

Besides having a suitable microbial strain, establishing the process conditions is fundamental to achieve high xylitol yield during the fermentation, which will have a strong impact on the process economics and large-scale viability. Therefore, many studies have been done with the aim of understanding the effect of the process variables on the fermentation performance, as well as to establish the optimum conditions of pH, temperature, oxygen availability, cell concentration, nutrient supplementation, among other variables able to result in maximum xylitol production. Among these, the oxygen availability measured by volumetric oxygen transfer coefficient ( $K_{L,a}$ ) is considered one of the most important parameters regulating this process, since it may deviate the microbial performance to product or cell formation [28, 29].

Xylitol purification and recovery from the fermentation broth is the last step of the biotechnological route for xylitol production. This is still an expensive step in the overall production chain and significantly impairs the large-scale implementation of this bioprocess. The main challenge of this downstream process is the presence of high amounts of impurities in the fermentation broth, which include residual sugars, fermentation by-products, phenolic compounds, salts, proteins, among others [30,31]. When compared to the other steps of the biotechnological route for xylitol production, the downstream process has received less attention and significant efforts are still required to develop a cost-efficient technology for separation and purification of the product with a high yield. So far, successive purification steps have been proposed, which increase the production costs and cause partial loss of xylitol. Traditional methods for xylitol purification such as ion exchange resins, activated charcoal, chromatography, liquid-liquid extraction, and nanofiltration, for example, result in about 40–60% recovery yield and the final product is obtained with 98% purity [14,15,32,33]. In fact, the purification of the fermented broth containing xylitol should be done by a combination of different methods in order to achieve a significant removal of impurities (residual sugars, color, proteins and peptides), as each method is more efficient for a particular purpose. Ion exchange chromatography, for example, is efficient to remove salts; while activated charcoal is especially used for decolorization of the fermentation broth. Nanofiltration membranes have been recently tested for xylitol purification, resulting in high removal of color (96%), proteins and peptides (85.7%) from the fermentation broth [34]. Use of supercritical CO<sub>2</sub> for xylitol recovery from fermented hydrolysate medium resulted in a recovery yield of 40.5%, but xylitol was recovered with 99.5% purity [35]. In fact, the use of supercritical fluid is a promising alternative for xylitol recovery from fermented broth since the product can be recovered with very high purity. In this case, efforts are needed to improve the recovery yield by testing different process parameters such as different temperature ranges, use of other solvents, or even by adding further extraction steps.

Taking into account the most recent developments in this topic, it is evident the importance of developing a suitable process for xylitol recovery and purification able to support the application of the

biotechnological route in a large scale. Since this is the final step in the production chain, its efficiency will also depend on the results obtained during the previous steps. For example, obtaining a fermentation broth with high concentration of xylitol and low concentration of interferers will benefit the purification stage. So, special attention should be given to the composition of the fermentation medium as well as on the performance of the microbial strain, which should be robust enough to produce xylitol with high yield even from a medium containing a mixture of compounds, as is the case of lignocellulosic hydrolysates.

### 3. Development of robust strains for use on xylitol production

The microbial strain to be used for xylose-to-xylitol bioconversion is a key point to be investigated in order to improve the product formation and the economic viability of the biotechnological route. In this sense, a promising approach is to develop cell factories with enhanced ability to produce xylitol from complex hydrolysate media, able to result in high product yield and low formation of by-products. This would improve the efficiency of the bioprocess while would minimize the costs required for the downstream step. Knowledge in genetic engineering and adaptive evolution of microbial strains, associated to modern genetic tools and equipment could support such developments, as discussed below.

#### 3.1. Strain improvement by adaptive laboratory evolution to overcome hydrolysate toxicity

Adaptive laboratory evolution (ALE) of microorganisms is a potential strategy to improve the microbial performance during the fermentation since it promotes the adaptation of cells with particular traits to specific stress conditions, being a useful tool to enhance the ability of strains to grow in medium containing lignocellulosic inhibitors (furan derivatives, phenolic compounds and organic acids), for example [1,36]. In this case, the inhibitor concentration is the selective pressure to be applied during the ALE process to obtain strains with higher tolerance to these compounds [37]. By improving the strains performance/tolerance, the hydrolysate detoxification step could be avoided, which would bring economic benefits to the biotechnological process for xylitol production. So, ALE is a promising approach to be used in favor of the development of a cost-efficient xylitol bioproduction process.

The ALE process can be performed by sequential transfer of the microbial culture to a new medium containing increased concentration of inhibitory compounds, or by using a chemostat system, increasing the concentration of inhibitors in the feed solution. So far, few studies have reported the use of ALE to improve the performance of microbial strains for xylitol production. However, promising results have been achieved for different yeasts. ALE of a recombinant xylose utilizing *Saccharomyces cerevisiae* using a chemostat system and spruce hydrolysate as cultivation medium, for example, resulted in a strain with higher xylitol yield (0.74 g/g) than the wild-type strain (0.61 g/g) [36]. Relevant results have also been reported when ALE was applied to improve the performance of *C. tropicalis* to produce xylitol from hydrolysate-based media [38]. Besides being efficient to overcome the toxicity of biomass hydrolysates, ALE can also be used to improve cell growth and xylose uptake rate, as observed during the adaptation of *C. tropicalis* for xylitol production using successive batch cultivations in a medium containing xylose as sole carbon source [39]. *K. marxianus* NIRE-K1 evolved in medium containing 2% of xylose as sole carbon source also presented a better performance for xylose consumption (more than 80%) and xylitol production (xylitol yield was 1.65 higher compared to the non-adapted yeast) [40].

The ALE process has been efficiently used to obtain robust yeast strains for the production ethanol [41,42], which is a biobased product successfully produced in industrial scale. Application of this technique for the production of other biobased products, such as xylitol, for example, is a promising and emerging area of research.

### 3.2. Strain development for an improved xylitol production

Different genetic approaches have been considered for the development of efficient strains for xylitol production (Table 1). Yeasts that can naturally metabolize xylose and excrete xylitol have been targets in studies of gene identification to exploit genetic diversity. Such characteristics are attractive considering the recombinant DNA technologies available and the number of plasmids and strains constructed for xylitol bioproduction already reported in the literature [43–45]. As soon as the first studies on the topic started, it was possible to predict that this strategy would be widely explored for this purpose [46]. Using *S. cerevisiae* as a host, these authors based on the yeast *S. stipitis* CBS 6054, a pioneer in studies involving xylose assimilation, for expression of the *Xyl1* gene, which encodes the enzyme xylose reductase. As a result, 95% efficiency of xylose conversion into xylitol was reported. Since then, the *Xyl1* gene was identified and characterized in several other naturally xylose-fermenting yeast species as, for example, *C. tropicalis* [47], *C. shehatae* [48], *C. parapsilosis* [49] and *K. marxianus* [50]. Interestingly, fermentations performed in 2.5-L bioreactor with the engineered strain *K. marxianus* KCTC17555 showed promising results of xylitol productivity and yield of 1.00 g/L/h and 0.75 g/g, respectively [50].

Although *S. cerevisiae* does not grow on xylose as the sole carbon source, this yeast has the gene *NADPH-dependent aldose reductase (Gre3)* in its genome, which encodes an enzyme that can convert xylose into xylitol – the enzyme being expressed under stress induced conditions. Recent studies revealed that *Gre3* overexpression in *S. cerevisiae* facilitates xylose fermentation and concomitant xylitol production [45,51]. However, this enzyme requires continuous regeneration of the NADPH cofactor, which can be obtained by using co-substrates such as glycerol.

Quantitative real-time PCR and transcriptome analysis has shown a low expression of XR in naturally xylose metabolizing yeasts [52]. An increase in enzymatic activity can be achieved through XR gene overexpression, thus favoring the reaction of xylose reduction into xylitol, increasing the volumetric productivity of this bioproduct. As well as increasing XR expression has shown to be a key point for improving the conversion of xylose to xylitol, studies have suggested that xylitol accumulation is favored by reducing the activity of the NAD-dependent enzyme xylitol dehydrogenase (XDH) encoded by the *Xyl2* gene [53,54]. This occurs because XHD is the enzyme responsible for converting xylitol into xylulose. Engineered strains of *C. tropicalis* with a mutated *Xyl2* gene resulted in xylitol production with high yield and productivity without the need to add co-substrates [53]. In addition, research conducted with *K. marxianus* revealed that gene strategy of disruption in *KmXyl2* was efficient for xylitol production with high yield (>98%) [55]. This phenomenon is related to the decrease in the oxidation of

xylitol to xylulose, thus leading to the accumulation of this polyol.

The emergence of competitive genetic tools has supported important advances involving the microbial production of xylitol. This is the case of the CRISPR/Cas9 genome editing technology for simultaneous deletion – insertion strategy in strain development [56,57]. In a fed-batch fermentation of hemicellulosic hydrolysate in 15-L bioreactor, multi-copy integration of *Xyl1* gene from *Neurospora crassa* in engineered *Escherichia coli* resulted in an improved xylitol titer of 131.6 g/L with productivity of 2.09 g/L/h [56]. The CRISPR/Cas9 system was also used for overexpression of *C. tropicalis Xyl1* in *S. cerevisiae*. As a result, xylitol productivity and yield of 0.83 g/L/h and 0.99 g/g, respectively, were obtained using glucose: xylose ratio of 1:2.5 [57].

Besides the functional characterization, knowledge of defined structures and identification of well-conserved regions are also relevant to provide information on the catalytic activities of this class of enzymes [58]. The protein crystal structure of *C. tenuis* and *S. stipitis* XR [59–61], for example, revealed important properties related to an increased xylose consumption. These studies allow identifying specific secondary structures related to cofactor binding and catalytic residues. Molecular docking simulations based on identification of 10 important residues involved in xylose binding hydrophobic pocket (Trp20, Asp47, Trp79, His110, Phe111, Phe128, Phe221, Leu224, Asn306, and Trp311) allowed a better understanding on the catalytic mechanism and efficiency of XR in yeast [60].

### 3.3. Genetic engineering of sugar transporters and mixed-substrate fermentation

Xylose uptake, especially from complex media, is one of the main factors affecting the xylitol bioproduction. It is known that xylose assimilation transport systems may limit the xylose utilization as well as the dynamic metabolic flux balance [62]. In this sense, the study of sugar transporter proteins (ST), their mechanisms of regulation and transport kinetics, is fundamental for the establishment of an efficient and optimized bioprocesses.

The monosaccharide transport affinity in yeasts is classified based on the identification of the transport system. Sugar transporter proteins can act by facilitated diffusion mechanism - that is according to the concentration gradient and regardless of energy expenditure - or through the proton-symport system, which, unlike the previous model, transports the solute against its concentration gradient with energy expenditure and need to the movement of protons [63–65].

To improve the xylose consumption, several ST genes have been isolated from different yeast species. Among the ST gene families, first studies started from the identification of the hexose transporter genes (*Hxt*) in *S. cerevisiae* [66]. The expression of these genes is highly

**Table 1**  
Genetically modified yeast strains, scale, and fermentative parameters of xylitol production.

Yeast	Strain	Genotype	Fermentation conditions	Titer (g/L)	Productivity (g/L/h)	Yield (g/g)	Reference
<i>Meyerozyma guilliermondii</i>	–	$\Delta$ Xyl2, MgXyl1	50 mL, batch	5.3	0.07	0.27	[103]
<i>Saccharomyces cerevisiae</i>	SCX-1	CDT-1, gh1-1, Xyl1	100 mL, batch	18.8	0.16	0.99	[104]
<i>Saccharomyces cerevisiae</i>	SCX-5	CDT-1, gh1-1, Xyl1	100 mL, batch	19.2	0.16	1.00	[104]
<i>Kluyveromyces marxianus</i>	YZJ017	$\Delta$ Xyl1, $\Delta$ Xyl2, NcXyl1	250 mL, batch	50.1	1.04	1.01	[55]
<i>Kluyveromyces marxianus</i>	YZJ015	$\Delta$ Xyl1, NcXyl1	250 mL, batch	71.4	1.49	0.83	[55]
<i>Kluyveromyces marxianus</i>	36907-FMEL1	KmXyl1	250 mL, batch	53	0.36	0.67	[105]
<i>Candida glycerinogenes</i>	WL2002-5 PURGPDx1	SsXyl1	250 mL, batch	65.1	0.54	0.87	[106]
<i>Saccharomyces cerevisiae</i>	C16	CtXyl1	500 mL, batch	29.0	0.83	0.99	[57]
<i>Saccharomyces cerevisiae</i>	W4	SsXyl1	500 mL, batch	16.5	0.66	0.76	[57]
<i>Candida tropicalis</i>	LXT2	$\Delta$ Xyl2, NcXyl1, CoXT2	500 mL, batch	50.0	1.14	1.00	[43]
<i>Saccharomyces cerevisiae</i>	SYBE004	Tal1, Tkl1	0.7 L-Bioreactor, batch	12.0	0.36	0.48	[107]
<i>Saccharomyces cerevisiae</i>	XP-RTK	ScGre3, SsSut1	1 L-Bioreactor; Fed-batch	16.3	0.21	–	[45]
<i>Saccharomyces cerevisiae</i>	CEN.PK113-5DGPDP-XR	CtXyl1	2 L- Bioreactor, Fed-batch	35.9	1.16	0.91	[108]
<i>Kluyveromyces marxianus</i>	17555-JBP2	KmXyl1	2.5 L-Bioreactor, batch	47.8	1.00	0.75	[50]
<i>Saccharomyces cerevisiae</i>	PE-2-GRE3	ScGre3	3.7 L Bioreactor, PSSF	24.3	–	0.88	[51]
<i>Debaryomyces hansenii</i>	DBX11, DBX12	$\Delta$ Xyl2	4 L-Bioreactor, batch	56.2	2.34	0.96	[109]
<i>Candida tropicalis</i>	XZX-B4ZG	$\Delta$ Xyl2II, YlGnd	5 L- Bioreactor, batch	97.1	0.82	–	[110]

regulated according to the concentration and availability of glucose to the yeast, which modulates the consumption of the other carbon sources [67]. Within this gene family, transporter proteins expressed under conditions of low glucose concentrations can also transport the xylose molecule. The high-affinity and moderate hexose transporters proteins, HXT7 and HXT4, for example, have been widely explored for the construction of a xylose consumption recombinant *S. cerevisiae* strain [68–70]. However, the glucose transporter HXT7 shows about 100-fold lower affinity for xylose compared to glucose [69].

Considering naturally xylose fermenting yeasts as a potential source of high-affinity transporters, putative xylose-specific transporter genes were identified in different yeast species based on genome sequencing and available data. The identification of glucose/xylose facilitator 1 (GXF1) and glucose/xylose symport 1 (GXS1) proteins in *C. intermedia* revealed the importance of substrate consumption on xylose metabolism [64]. Other gene families related to xylose uptake have also been studied such as *S. stipitis* sucrose transporter proteins (SUT's) and xylose transporter proteins (XUT's). *CiGxf1* and *CiGxs1*, *S. stipitis Sut1* and *Xut1*, 4, 6 and, 7 are some examples of characterized yeast xylose transporters reported in the literature [71–73]. Through evolution and phylogenetic data, other putative ST genes were also recently identified in *C. intermedia* and *C. sojae* [74,75]. Although promising, the development of recombinant strains with genes that encode transporter proteins to xylose consumption towards the xylitol production still comprises only a small part of the studies on this topic. In some studies, the effects exerted by glucose during the fermentation of glucose/xylose mixtures have been related to the regulation caused by the transport of monosaccharides, for example.

Co-substrate fermentation could be an interesting strategy to improve the xylose assimilation by yeasts. The use of co-substrates, such as glucose, has been studied for NADPH regeneration; a cofactor required for converting xylose into xylitol in yeasts [76,77]. However, the ratio of these substrates in enzyme activity is a parameter of great importance to be considered in order to enhance the xylitol productivity and yield. For glucose, for example, the effect of different glucose: xylose ratios: 1:25, 1:12, 1:5, and 1:2.5, was tested during the cultivation of *C. guilliermondii* in sugarcane bagasse hemicellulosic hydrolysate, and the ratio 1:5 was found as being the best condition for xylitol production [78]. The use of glycerol and sucrose as co-substrates for xylitol production has also been evaluated in a perspective of using industrial wastes/by-products as low-cost alternatives to conventional sugars, with additional benefits to the bioprocess in terms of sustainability. Addition of sucrose to xylose based media was found favorable to xylitol production [79]. The same was observed when gluconic acid (20 g/L) was used as a co-substrate, which resulted in a 100% xylitol yield according to the ratio used (increased concentrations of gluconic acid reduced the production of xylitol). A fed-batch cell culture resulted in a xylitol titer of 44.8 g/L [80].

The proportion of carbon sources to be used for fermentation is indeed an important point to be considered in order to maximize the production of xylitol. Glucose at high concentrations, for example, can exhibit catabolic repression, reducing the xylose metabolism [81]. One of the causes of this effect is the transport system mechanism and its higher affinity for glucose than xylose. The substrate transport system in yeasts is controlled according to the available carbon source and the cell's sensitivity to the concentration of these sugars. The yeast *S. cerevisiae* has anchored in its plasma membrane, proteins characterized for their function as sensors; that is, they can differentiate low and high concentrations of glucose in the extracellular medium [82]. The proteins called “sensors” include SNF3 and RGT2, which belong to the family of hexose transporters. Their function is related to the induction of genes that encode specific carrier proteins, thus promoting efficient substrate import [83,84].

Arabinose is a pentose sugar commonly found in mixture with xylose in hemicellulosic hydrolysates. Selection of yeasts able to grow in media containing mixture of these two pentoses, but without producing

arabitol, is another strategy that can potentially improve the xylitol production from hemicellulosic hydrolysates. This procedure has been tested to obtain a mutant *C. tropicalis* able to consume arabinose only for cell growth, and xylose for xylitol production, with the final aim of obtaining a final arabitol-free xylitol solution [85]. Although few explored, this concept can potentially offer a solution to the presence of arabinose in the final fermentation broth, benefiting the subsequent downstream step.

Several techniques and molecular strategies can be used to maximize the xylitol bioproduction. Obtaining strains more resistant to inhibitors present in hemicellulosic hydrolysates, more efficient to convert xylose into xylitol, and with high capacity of carbohydrate uptake are among the strategies to increase the xylitol production yield. Although the biotechnological route is well established, the steps of this process still require improvements to become more economically competitive when compared to the chemical route.

#### 4. Techno-economic and environmental assessments: potential perspectives to improve the feasibility of xylitol bioproduction

Despite efforts on developing robust strains and establishing cost-efficient downstream methods to improve the cost-competitiveness of xylitol bioproduction, techno-economic and environmental assessment are also important to indicate potential steps which requires attention, as well as to provide an estimation on appropriate investment costs, profit, balance of mass and energy of the process, environmental impacts, among other parameters relevant for industrial application [86]. Since xylitol is produced from hemicellulose, which is one of the three main fractions of lignocellulosic biomass composition, integration of the xylitol production in a biorefinery could be an economically feasible strategy for large scale implementation of xylitol bioproduction [87].

A sustainable biorefinery requires the maximum use of biomass components to produce a wide spectrum of energy and chemicals [88]. A number of valuable bioproducts can be obtained through the integral utilization of biomass, which may support the faster growth of the biobased economy [89]. Modeling and simulating the integration of high-added value products in a biorefinery contributes with important information on the perspectives for industrial implementation. In this context, techno-economic studies have considered different biorefinery scenarios for xylitol production using different agro-industrial residues/by-products as raw materials, including sugarcane bagasse, corn stover, brewer's spent grains, among others (Table 2).

Simulation studies have shown that two of the most expensive steps of the biotechnological process for xylitol production are the hydrolysate concentration and the crystallization of the product, due to the high energy demand required [90–92]. Developing efficient technologies to recover xylitol as well as recycling the used chemicals would be potential alternatives to reduce the production costs. An option to decrease the energy consumption could be the integration of heat exchangers, which would save the energy of the process due to a more efficient consumption of energy and water. It has been reported, for example, that the implementation of 14 heat exchangers in an ethanol and xylitol biorefinery resulted in 68.9% energy saving as steam and 64.4% reduction in cooling water consumption [90]. In addition, the application of a cogeneration system could supply the energy demand of the process through the combustion of solid residues. However, in some simulations, the burning of solid waste for cogeneration was not positive, due to the depreciation and additional capital cost invested [93, 94]. In the case of a multiproduct biorefinery, the net profit margin decreased 47% when the cogeneration system was added [95].

Recently the techno-economic assessment of a corn stover biorefinery for xylitol production integrated with second generation ethanol showed that integration is more appropriate than just ethanol production, since it requires a total investment cost of 144.9 M€, compared to 153.1 M€ for ethanol only [94]. Regarding multiproduct systems, Hernández et al. [95] simulated the production of xylitol,

**Table 2**

Production cost of xylitol in different biorefinery scenarios using lignocellulosic biomass as a feedstock.

Biomass	Products	Feedstock input	Production costs	Xylitol unit production cost	Reference
Oil Palm Rachis (OPR)	Xylitol, biogas, lactic acid, ethanol	25 ton/day	23.32 mi US\$/year	0.46 US\$/kg	[111]
Spent blackberry pulp SBP	Xylitol, phenolic compounds extract, ethanol	2000 kg/h	9.71 mi US\$/year	22.0 US\$/kg	[93]
Banana peel	Xylitol, ethanol, biogas, cogeneration	1 ton/h	5.50 mi US\$/year	1.71 US\$/kg	[86]
Lignocellulosic biomasses	Xylitol	4.1 ton/h	67.69 mi €/year	1.83 €/kg	[98]
Corn stover	Xylitol and ethanol	33 ton/h	89.9 mi €/year	–	[94]
Brewer's spent grain	Xylitol, lactic acid, phenolic acids, activated carbon	100 ton/h	242.12 mi US\$/year	0.81 US\$/kg	[101]
Sugarcane bagasse	Xylitol	15,000 ton/year	29.05 mi US\$/year	–	[92]
Olive tree crops	Xylitol, ethanol, antioxidants, electricity	40,000 ton/year	11.4 mi €/year	1.48 €/kg	[112]
Olive stone	Xylitol, furfural, ethanol, PHB	80,000 ton/year	60.36 mi US\$/year	3.12 US\$/kg	[95]

furfural, ethanol and poly-3-hydroxybutyrate (PHB) from olive stone. Only xylitol and PHB proved to be feasible, showing a high profit margin of 53%, which was negative for ethanol and furfural. In another situation, Clauser et al. [96] compared two scenarios for small-scale (15,000 tons/year) sugarcane bagasse biorefinery: 1. production of xylitol and ethanol; 2. production of xylitol and pellets. The integration of xylitol and pellets had a total investment cost 8.2 mi US\$ cheaper than ethanol since the energy consumption for pellets corresponded to 10% of the total energy of the scenario 1.

A sensitivity analysis of oil palm empty fruit bunches biorefinery evaluated several scenarios by varying the prices of ethanol, xylitol and lignin. In this analysis, it was concluded that to achieve a positive yearly profit, the ethanol production must be carried out together with the co-production of xylitol and lignin, which are high added-value products [97]. Mountraki et al. [98] reported the sensitivity analysis of xylitol production with a break-even price of 1.76 €/kg and positive Net Present Value (NPV) at 1.90 €/kg for the catalytic route, while the biocatalytic route presented 2.28 €/kg of break-even price and NPV at 3.07 €/kg. Although the economic evaluation indicated the catalytic route as the most suitable, this analysis needs to be carefully performed considering different scenarios, scales, feedstocks, and products, which may significantly affect the final prices and conclusions.

In addition to the economic studies of a biorefinery, it is also very important to evaluate the potential environmental impacts of a process through Life Cycle Assessment (LCA) [99]. There is still limited literature regarding LCA of the biotechnological production of xylitol and future studies should better explore this analysis to demonstrate the potential of this technology to promote environmental sustainability. Dasgupta et al. [100] pointed out the chemical inputs, fuel, electricity, and emissions involved in xylitol production as the main drivers of environmental impacts. The authors also performed the biocatalytic production of xylitol from corncob biomass and the LCA studies revealed that ~95% of the environmental impacts were marine aquatic and freshwater eco-toxicity, with a carbon footprint of 8.68 kg CO<sub>2</sub> in the overall process. Other strategies that can reduce environmental impacts caused in bioprocesses are the energy and mass integration, and the cogeneration of power and heat, due to the reduction in the greenhouse gases (GHG) emissions [95,101].

Besides the integration of different products in a biorefinery, the features employed in each step of production may also influence the viability of the process in a large scale. Fed-batch fermentation for xylitol production, for example, resulted in higher product titer than the batch operation mode, which significantly improved the economic potential of the process [102]. Last but not least, analyses of biorefinery scenarios including mass and heat integration, as well as cogeneration, have also been demonstrated to increase the productivity of biorefineries and to be an environmentally friendly alternative, since they allow recycling wastewater and solid residues [93,95,101].

## 5. Conclusions and future perspectives

The worldwide demand for xylitol has grown annually. This trend can be related to the increase in cases of diseases related to metabolic

disorders such as diabetes and obesity, associated with the excessive consumption of conventional sugar by the population. In addition, the use of xylitol as a building block chemical in different industrial segments to obtain high added-value products also contributes to the market growth, and consequently, to its large-scale production.

The biotechnological route for xylitol production has some advantages compared to the commercial chemical route, since it allows improvements and adaptations of the process by the development of high-throughput technologies. Process adjustments can be extensively investigated from the initial to final steps, i.e., since the deconstruction of the lignocellulosic biomass until the crystallization for xylitol recovery. That is the case, for example, of investigating adaptive evolution and genetic engineering strategies for microbial improvement to obtain enhanced resistance to inhibitory compounds present in the hydrolysate medium, as well as for an improved consumption and bioconversion of the carbon source, xylose, into xylitol. Modern techniques are constantly being developed in these areas, which can be employed to improve the yields of this biotechnological process.

Although some challenges still need to be overcome to make the bioproduction of xylitol a reality on a large scale, techno-economic assessment of the global production route, as well as the investigation of potential scenarios considering the integration of the production of xylitol with other biobased products and bioenergy, are relevant approaches to be considered in future studies in this area. In fact, the combination of different disciplines including biological sciences with sustainability assessment (techno-economic and life cycle analyses), for example, can lead to important indications for improvement of this bioprocess. Moreover, the LCA can also provide information on the environmental impact of the developed technology, being a relevant parameter to be considered for industrial implementation of this technology.

## Author contributions

Sarah S. Queiroz and Fanny M. Jofre: Data curation, Writing – original draft; Solange I. Mussatto: Conceptualization, Writing-Reviewing and Editing, and Supervision; Maria das Graças A. Felipe: Conceptualization, Supervision and Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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