Addressing key challenges in fermentative production of xylitol at commercial scale

Ravella, Rao; Walker, David James; Gallagher, Joe; Winters, Ana; Bryant, David

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Chapter 9
Addressing Key Challenges in Fermentative Production of Xylitol at Commercial Scale: A Closer Perspective

Sreenivas Rao Ravella, David J. Warren-Walker, Joe Gallagher, Ana Winters, and David N. Bryant

Abstract Xylitol has been recognized by the US Department of Energy (DOE) as one of the top 12 value-added chemicals obtained from biomass, with a world market of 200,000 tonnes per year. The global xylitol market is expected to reach a value of US$ 1 Billion by 2026 growing at a compound annual growth rate (CAGR) of 5.8% during 2021–2026. Historically, the commercial xylitol production process has been dependent on the chemical hydrogenation of xylose. Several xylitol production plants, mainly in China that use the chemical process have had to reduce their production capacity to address regulations governing sustainability and environmental standards. In this chapter, key challenges and possible solutions for fermentative xylitol production at commercial scale are discussed in terms of: (1) Feedstock supply for commercial production plants; (2) Industrial biomass pretreatment; and (3) Lessons learned from industrial operations. These are drawn together to identify technology gaps and scaling-up challenges in light of the capital expenditure required to build a state-of-the art xylitol industrial biotechnology (IB) production facility and the potential to reduce climate change impact and contribute towards achieving net-zero targets.

Keywords Xylose · Xylitol commercialisation · Scale-up · Biorefining · Steam explosion · Fermentation

9.1 Introduction

More than 130 counties aim to be climate neutral by 2050, with 14 enshrining this commitment in law, 30 in policy documents, 15 in pledges with the remainder undergoing further discussions (https://www.eciu.net/netzerotracker). In order to facilitate

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S. R. Ravella (✉) · D. J. Warren-Walker · J. Gallagher · A. Winters · D. N. Bryant
Institute of Biological Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth SY23 3EE, UK
e-mail: rsr@aber.ac.uk

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the global transition toward climate neutrality, ambitious action plans and objectives related to bioenergy, the circular economy and biorefineries in particular are required. The European Commission emphasised the latter point out in a recently published report on the opportunities afforded from biorefining processes in the EU (EU Biorefinery—outlook to 2030). This EU outlook presented scenarios on how demand and supply for bio-based chemicals and materials could grow to 2030, and actions required to increase the deployment of biorefineries in the EU.

The International Energy Agency (IEA) in, IEA Bioenergy Task 42 "Biorefining in a Circular Economy" identified 4 key areas to classify and describe biorefinery systems: (1) Platforms (e.g. core intermediates such as C5/C6 carbohydrates, syngas, lignin, pyrolytic liquid) (2) Products (e.g. energy carriers, chemicals and material products) (3) Feedstock (i.e. biomass, from dedicated production or residues from forestry, agriculture, aquaculture and other industry and domestic sources and; (4) Processes (e.g. thermochemical, chemical, biochemical and mechanical processes) e.g. (Table 9.1).

Currently, there are more than 224 commercial biorefineries operating across Europe, of which 181 use 'first generation' (1G) feedstock, i.e. sugars, starch or oils. However, comparably fewer, approximately 40, refine lignocellulosic (woody biomass) or "second generation" (2G) feedstock (Hassan et al. 2019). Table 9.2 list examples of commercial 2G biorefineries, their products, capacity and operational status.

Commercially, xylitol, a tooth and diabetic friendly sweetener, is produced by chemical hydrogenation of xylose, a C5 sugar refined from biomass (i.e. birchwood and/or corn cobs etc.) (Makinen 2000; Werpy and Petersen 2004; Ravella et al. 2012). While the scientific literature is awash with fermentative xylitol processes, >900 articles in the past 5 years, using native or engineered yeast (*Candida, Pichia,*

Table 9.1 List of lignocellulosic feedstock stream exploded and processed to release xylose and arabinose in hemicellulosic hydrolysates at Aberystwyth University's BEACON Biorefining Centre, UK (http://www.beaconwales.org)

Feedstock	Xylose: arabinose ratio	Xylose content in biomass (%)
Corn Stover	4.8	25
Willow	6.5	16
Wheat Straw	4.2	30
Sugarcane bagasse	6.5	24
Miscanthus	6.2	25
Brewers spent grain	1.8	20
Oat Hulls	13	24
Birch	11	27
Silver Birch	11	27
Corn cobs	9.0	28
Rye Grass Fibre	3.3	15

Table 9.2 Examples of commercial and demonstration 2nd Generation (2G) lignocellulosic biorefineries

Main investor	Processing technologies	Feedstock	Product	Commercial operations	Location
Beta renewables	Versalis' PROESA® steam explosion pretreatment	Wheat straw and hardwood	2G ethanol 59,000 MT/yr	2013 Deactivated 2021 new venture with Saipem launched	Crescentino (Italy)
Bharat petroleum corporation limited (BPCL)	Tata Projects	Rice and corn straw 488 MT/day	2G ethanol 80 MT/day	Active since/initiated?2022	Bargarh, Odisha, India
Croatian oil and gas company INA	Axens Futurol™ technology http://www.axens.net		2G ethanol 55,000 MT/yr	2020 planning stage	Sisak, Croatia
Assam bio refinery private limited (ABRPL)	Formicobio™ technology—3G technology http://www.chempolis.com	Bamboo 300,000 MT/year	2G ethanol 51,000 MT/yr, furfural 2100 MT/Yr, acetic acid 9000 MT/yr, Biocoal 156,000 MT/yr	2021 planning stage	India
Clariant	Commercial scale Sunliquid® technology, Valmets Biotrac SE process	Wheat/barley Straw/corn Stover 750–800 MT/day	2G ethanol 50,000 MT/year	2022 planning stage	Podari, Romania
Clariant	Demonstration-scale Sunliquid® technology, Valmets Biotrac SE process	Local Agricultural Residues	2G ethanol 150 MT/day	2022 planning stage	Slovakia
Clariant	Pilot-scale Sunliquid® technology, Valmets Biotrac SE process	Wheat/barley/rice straw, corn stover, miscanthus, SCB 12–16 MT/day	2G ethanol pilot plant <3 MT/day	2012 operational	Germany

(continued)

Table 9.2 (continued)

Main investor	Processing technologies	Feedstock	Product	Commercial operations	Location
CTC Sugarcane Technology Centre-Piracicaba, Brazil	Andritz steam explosion pretreatment technology http://www.andritz.com	SCB	Pilot plant for C5 sugar extraction	Deactivated technology for sale	Brazil
LIBERTY Project POET-DSM http://www.poetdsm.com/liberty	Andritz pretreatment technology http://www.andritz.com	285,000 dry tonnes of corn stover	25 million gallons of 2G ethanol	Stopped in 2019, now focusing on R&D to improve process	USA
Bioflex Agroindustrial part of GranBio www.granbio.com	SE pretreatment	Sugarcane straw	2G ethanol 47,000 MT/year	2014 (20 months to construct $265 million plant?)	Brazil
Raízen		SCB	2G ethanol > 31,000 MT/year	2014 technology for sale	Brazil
Fortress Global Enterprises Inc	S2G BioChem and Mondelēz International 2G sugar technology,	Maple, aspen, poplar, birch	Xylitol demonstration 2,000 MT/year commercial > 20,0000 MT/yr	2018 Demonstration plant initiated, commercial plant pending	Sarnia (Ontario, Canada)
DuPont	DWB concept	Wood	Xylose and xylitol	2012 operational	Austria
Sappi	Valmets ‘Xylex’ technology	Wood	Xylose and Xylitol	2018 operational	South Africa
Oy Karl Fazer Ab		Oat hulls 20,000 MT/yr	Xylitol 4000 MT/yr	2019 construction started	Lahti, Finland
Borregaard Biorefinery	Advanced biorefinery (20 integrated plants)	Wood	2G ethanol, Cellulose fibre and vanillin	1950 operational	Sarpsborg, Norway

(continued)

Table 9.2 (continued)

Main investor	Processing technologies	Feedstock	Product	Commercial operations	Location
SP Energy Technology Center	Bio4Energy	Lignocellulose	Demonstration plant	Operational	Sweden
CLaMber Biorefinery	SE pretreatment and fermentation	Agri waste	Demonstration plant 2G sugars	2015 operational	Spain
ZeaChem Demonstration Biorefinery	Thermal Hydrolysis and fermentation Zea2™	Agri waste and wood 10 tonnes/day	2G ethanol and acetic acid	2014 operational	USA
Stora Enso http://www.storaens.com	B2X technology	SCB	Demonstration plant SCB to xylose	Closed 2021	USA

MT = metric tonnes; SCB = sugarcane bagasse; SE = steam explosion; DWB = Dupont's wood based

Debaromyces etc.) or bacteria, broad commercialisation of an industrial biotechnology (IB) process remains tantalisingly elusive. In the UK and Mexico, the new start-ups ARCITEK Bio Ltd (https://www.arcitekbio.co.uk/) and XiliNat (https://www.xilinat.com/) are on the road to commercialising fermentative processes for xylitol production, but are still at a nascent stage. Recently, however, Sweet Appeal Natural Products LLC announced their first commercial sale of xylitol in China that was produced through a fermentation process using corncobs as a feedstock (https://www.sweet-appeal.com). The focus of this chapter is to explore the current commercial status for IB production of xylitol and highlight the challenges and barriers to market.

In order to supply industrial xylitol manufacture at around 10 k tonne per annum, the amount of biomass required is in the order of >100 k tonnes per annum. Future estimates indicate that up to 40 new second generation biorefineries may be constructed and come on-line in Europe by 2030, thereby significantly increasing the market demand for biomass feedstock (EU Biorefinery—outlook to 2030). While the majority of these biorefineries will focus on products other than xylitol, there may be those where xylose is a side stream that will no doubt be suitable for production of xylitol. Integrating production technologies to manufacture multiple products may very well lead to economic and environmental sustainability gains afforded through the energy recovery, economies of scale and minimisation of waste. Furthermore, a key area that can improve the sustainability of xylitol production and reduce climate change impact will be advances in biomass pretreatment technologies that lower energy demands and maximise 2G sugar release (Silva and Chandel 2012).

As with chemical catalysis, there are several challenges to achieving profitable fermentative xylitol production at commercial scale. These include ensuring a regular supply of biomass and developing an economically viable process including pretreatment, bioconversion of xylose to xylitol and downstream processing (DSP) to obtain a pure product, as well as valorisation of all side streams. Pertinent to fermentative production of xylitol is the potential to use crude xylose hydrolysate streams containing contaminants and/or fermentation inhibitors (e.g. phenolics, organic acids, furfurals). These are formed during pretreatment to extract xylose and purification is a costly processing step essential for chemical catalysis. However, the ability to utilise crude hydrolysates is dependent on the microbial capacity to tolerate or detoxify these as they can significantly impair product titres, rates, and yield. Again, this area has been extensively investigated with tolerance being improved using adaptive evolution or synthetic biology approaches but little headway has been made in the commercialisation of these approaches.

Each of the above steps, or unit operations, require optimisation and integration at pilot and demonstration-scale in order to avoid logistical issues that could result in down-time or failure of commercial manufacturing campaigns. The financial cost associated with integrating unit operations at pre-commercial levels can impede growth of small to medium sized enterprises (SME's) and extend the time taken for the innovative technologies/products to reach market. Across Europe however, several regional or national facilities are available to SME's to help develop laboratory scale fermentation process(es) up to pilot and demonstration-scale. Coupled with financial assistance afforded by schemes such as innovation vouchers, the financial barrier is lower leading towards gaining future investment and validation of processes, such as IB production of xylitol, at a commercially relevant level. Some key challenges and possible solutions in fermentative production of xylitol are discussed in the sections below.

9.2 Feedstock Supply

Availability of lignocellulosic biomass at national and international level needs to be assessed when planning construction of a biorefinery (Martínez-Pérez et al. 2007; Akgul et al. 2012; USDA 2011; Alexander et al. 2015; Hodgson et al. 2016; S2Biom 2016; Dahmen et al. 2019; Schröder et al. 2019; Lüders et al. 2020). The EU funded S2Biom project (https://www.s2biom.wenr.wur.nl/) predicted 476 million tonnes of lignocellulosic biomass will be required to fulfil the needs of all biobased industries in Europe by 2030 (S2Biom 2016). Furthermore, the project predicted at least 1 billion tonnes of lignocellulosic biomass will be produced in Europe on an annual basis by 2050. Zaimes et al (2015) discussed numerous supply chain issues, mostly associated with Life Cycle Analysis (LCA) and outcomes of feedstock choices.

A sustainable biorefining industry is dependent on both sustainable feedstock and a sustainable value chain to ensure that continuity of supply meets demand (Dale 2017). As with a xylitol production facility using chemical conversion, an IB xylitol plant based on fermentation process requires at least 90,000–270,000 dry tonnes of feedstock per annum to produce approximately 10,000–30,000 tonnes of xylitol. Furthermore, losses of up to 50% xylitol can be incurred during downstream processing, purification and crystallization, resulting in more biomass being required than may be projected initially. Table 9.1 gives examples of feedstock that can be utilised to provide a hemicellulosic stream for xylitol production. An important consideration in feedstock choice is not only xylose content and yield per hectare but also the xylose to arabinose ratio (X:A) of the hemicellulose as both the chemical and IB xylitol processes can produce arabitol from arabinose. As a food additive xylitol must meet purity criteria set out in Regulation (EU) 231/2012 amended by Regulation (EU) 724/2013 that state the final product must contain <1% of other polyols. As arabitol is an epimer of xylitol it’s separation can confound DSP leading to significant product losses. Microbial biocatlaysts have been engineered to reduce arabitol production during xylitol fermentation offering process improvement benefits, however consumer acceptance of this technology remains to be seen (Yoon et al. 2011).

The biomass supply chain needs to be assessed, on a case-by-case basis. Some refineries can be self-sufficient utilising feedstock produced “in house” for xylitol production. For example, the Finnish corporation, Fazer, is building a xylitol production plant in Lahti that will utilise oat hulls derived from processing of oats in its neighbouring oat milling plant. During the past few years, Fazer has invested approximately 40 million euros in constructing a xylitol manufacturing facility that will utilise a chemical hydrogenation process (https://www.fazer.com/about-us/fazerxylitol/). Sugar manufacturing companies can also provide sugarcane bagasse (SCB), an abundant side-stream, as a feedstock for xylitol production that has a high xylan content and can be readily hydrolysed to xylose. The integration and co-production of microcrystalline cellulose alongside an IB xylitol process using SCB is currently being investigated in the BBSRC Newton-Bhabha Innovate UK project, BIOREVIEW (http://www.bioreviewproject.org/). Another example of an industry that produces a waste stream that can supply xylose for xylitol production is the paper and pulp manufacturing sector. Moreover, recent advances in paper and pulp production, allows recovery of a hemicellulosic C5 stream that can be used for large-scale xylitol production. For example, Danisco® Xylitol branded as XIVIA™ is produced using the DuPont Wood Based integration concept (DWB) (https://www.dupontnutritionandbiosciences.com/).

9.3 Steam Explosion Pre-Treatment

2G biomass is inherently recalcitrant and generally requires application of an appropriate pretreatment for the release and hydrolysis of structural carbohydrates from feedstock (William et al. 2017). Various physico-chemical methods are used to deconstruct the complex lignocellulosic cell-wall matrix of plants (i.e. the crosslinked composite of cellulose, hemicellulose and lignin) into multiple components. These treatments release carbohydrates as monomers or oligomers and increase the surface area of polymers for subsequent hydrolysis by cellulolytic enzyme cocktails (Chandel et al. 2020). Steam explosion (SE) is a widely used, scalable and effective pretreatment adopted in lignocellulosic biorefineries (https://www.valmet.com/). During this pre-treatment, the biomass is subjected to high-pressure steam at a temperature of 160–260 °C enabling water molecules to penetrate the biomass. Following rapid decompression, xylose is liberated from the biomass forming a soluble xylose rich hydrolysate (Walker et al. 2018). SE has a long commercial history, is a scalable technology and in the past few decades has been applied by some commercial lignocellulosic plants (Table 9.2).

Over the past decade several companies have built pilot, demonstration, and commercial SE rigs for lignocellulosic biorefineries (Table 9.2). Valmet (https://www.valmet.com/) have supplied a range of scaled BioTrac pretreatment systems to locations globally; a demonstration-scale system in Straubing, Germany and, a commercial scale system in Romania for 2G bioethanol production, both to Clariant. Valmet's BioTrac system, can process more than 250,000 tons of lignocellulosic feedstock (wheat and barley straw) annually and their SE pilot-scale pretreatment systems have been supplied to India and Sweden. In 2021 Valmet was appointed to rebuild the pretreatment system for RE Energy's biorefinery in Kalundborg, Denmark that will process straw to produce 2G bioethanol and lignin. Furthermore, Valmet supplied a demonstration-scale plant for second generation sugar extraction at the Sappi Ngodwana Mill in South Africa, specifically for the extraction of hemicellulosic sugars and lignin from its dissolving wood pulp (DWP) process (http://www.sappi.com). The C5 sugar stream was used to produce xylitol and furfural production using Plaxica's proprietary "Xylex" technology.

In the UK, Nova Pangea Technologies (http://www.novapangaea.com) employ pilot and demonstration-scale SE pretreatment technology that produces C5 sugar streams as part of their REFNOVA process that also produces both lignin char and glucose from the thermolysis of the remaining pulp. Interestingly, despite considerable commercial effort for fuel production there are no 2G ethanol plants that we are aware of separating C5 streams for the co-production of xylitol, despite the positive technoeconomic effect of the higher xylitol selling price lowering the Payback Selling Price of ethanol from €1.62/kg to €0.79/kg (Bioenergy, IEA—2020; ISBN 978-1-910,154-69-4; De Bari et al. 2017).

9.4 Lessons Learned from Industrial Operations

Information relating to the hurdles encountered during the development and deployment of modern integrated biorefineries can be hard to come by and are often cloaked in industrial secrecy, "know-how", with certain processing requirements being both product and market sector specific. However, there are unit operation and process design considerations that require common solutions for all. For example, Slupska and Bushong (2019) highlight 4 key areas of learning that arose from the commercialisation of cellulosic ethanol at POET/DSM's Project LIBERTY plant, with a starting cost of USD $227 million, which would be equally applicable to a modern-day xylitol production facility. These are related to: Biomass collection; interdependence of unit operations; new operation areas; and saccharification and fermentation. The Borregaard Biorefinery in Norway, is arguably the oldest, commercial integrated biorefinery where 20 manufacturing plants are integrated within one production site and controlled with advanced monitoring systems to produce several by-products (http://www.etipbioenergy.eu/images/Factsheet_Borregaard_final.pdf). In this regard, the challenges associated interdependence of unit operations has been successfully addressed at this facility (Rodsrud et al. 2012).

Some additional lessons learned that have been reported by biorefining operations include:

- Continuous feeding of biomass into pretreatment reactors was challenging using agricultural feedstock as they cannot be handled as easily or in the same manner as wood chips Slupska and Bushong (2019).
- Steam explosion technology is a scalable option for 2G biorefining, however several plants have struggled to operate continuously and have temporarily halted or completely terminated operations and are now looking for new investors (i.e. Beta Renewables, CTC-Piracicaba, Brazil (Table 9.2).

- Commercially available, “off the shelf” equipment may not necessarily integrate easily into new processes. For example, SE systems installed at commercial scale in 2G ethanol plants have required integrating with feedstock collection systems to avoid unnecessary downtime to clear blockages associated with removal of molten net wrapping, used to bale the feedstock, from the reactor (Slupska and Bushong 2019).
- Demonstration plants were useful for Integrated process testing—this is of great value for the development of new technologies, to address integration of processes such as hemicellulose hydrolysate separation or extraction of C5 sugars from 2G lignocellulosic ethanol plants.
- Process changes to current 2G lignocellulosic technology to separate C5 streams depends on further process development, integration of C5 hydrolysate stream separation and concentration technologies for high xylose titres in the hydrolysate.

9.5 CASE STUDY: Challenges of Biomass Pretreatment by Steam Explosion

The BEACON Biorefining Centre (http://www.beaconwales.org) at Aberystwyth University (AU) (Fig. 9.1), have operated batch wise, pilot-scale SE since 2013, investigating multiple biomass feedstock for xylitol production and other industrial

Fig. 9.1 BEACON Pilot plant with pilot-scale steam explosion rig and pilot-scale membrane purification systems (http://www.beaconwales.org)

research applications (Table 9.1). They have applied this pretreatment technology to extract hemicellulosic sugars from multiple biomass feedstocks and demonstrated that a combination of SE and acid treatment is effective for extraction from corn cob powder, SCB and grass fibres.

Through process optimisation studies, xylose recovery yields of up to 90% were achieved while minimising co-production of fermentation inhibitors, such as furan-2-carboxaldehyde (furfural), from hydrothermal dehydration of C5 sugars (Walker et al. 2018). The latter outcome is equally important with respect to maximising C5 yield, as the presence of inhibitors in the hydrolysate represents a xenobiotic challenge for microbial bioconversion of xylose to xylitol during the fermentation process (Ravella et al. 2012; Hernández-Pérez et al. 2019). Depending on microbial tolerance these may need to be eliminated or minimized through additional unit operations, such as over liming to remove furans, or more advanced processes such cross-flow ultrafiltration to separate and isolate xylose from the complex hydrolysate matrix. Each of these solutions bear an economic burden, with lime-based detoxification incurring the additional cost of waste disposal and considerable sugar loss. Indeed, the challenge of pretreatment and integration of multiple unit operations alongside the associated logistics were among the key lessons learned by POET and DSM in the commercialisation of cellulosic ethanol at Project LIBERTY (Slupska and Bushong 2019).

The production of inhibitors formed during the SE pretreatment process can also be feedstock dependent and negatively affect fermentation. Exemplifying this point the *Candida tropicalis* strain isolated at IBERS, AU demonstrated good xylitol productivity using xylose in crude Miscanthus and wheat straw hydrolysates, but not from corn stover where furfural levels where up to 100% greater at equivalent pretreatment severities (Somani et al. 2018; Walker et al. 2018). Taking this into consideration, an industrial biorefinery operating an IB fermentative xylitol production process needs to address feedstock choice, continuity of supply, pretreatment and the challenge of process integration.

During process development at AU, the ability to produce sufficient hydrolysate for larger scale xylitol fermentation, >50 L, using a static, batch fed pilot-scale SE rig, presented a challenge to researchers. The Cambi hydrothermal pretreatment system (http://www.cambi.com) (Fig. 9.1) has a reactor capacity of 30 L, where only 15–16 kg feedstock (<1.5 kg per run) could be processed per day to produce 40–50 L of dilute hydrolysate. As a result, it took several months of work to produce sufficient hydrolysate volume for larger scale fermentation trials (up to 160 L) to provide data relating to the scalability of the process.

In collaboration with Bangor University (BU), UK, (BBSRC Newton-Bhabha Innovate UK project BIOREVIEW), BU were able to increase hydrolysate production using a modified continuous steam refining rig for processing wood chips into fibre. Using this system, it was possible to process 100–200 kg of Miscanthus and SCB per day thereby producing the requisite hydrolysate volume for larger scale fermentations. However, optimising the process required addressing several problems. In agreement with Slupska and Bushong (2019) handling, imbibing and feeding biomass into a continuous dynamic pretreatment process differed between wood chips and agricultural feedstock. The latter was more difficult to process and took substantial development to achieve consistent results. For instance, optimised process parameters for batch SE, that released the majority of xylose from SCB, resulted in near complete destruction of the feedstock in the continuous system. Similarly, due to the larger volumes of material and the physical disruption from milling, recovering liberated xylose from the fibre in larger quantities proved both logistically and technically challenging due to the extremely fine particle size.

Process development for the IB production of xylitol has demonstrated that scaling up from a lab to pilot-scale process involves several unit operations, over and above continuous pretreatment for C5 hydrolysate production, namely; counter current washing of pretreated material to maximise xylose recovery; effective solid and liquid separation; and sugar concentration (Fig. 9.2). Although these processes were performed batch-wise, industrially each operation would need to be integrated as a continuous process (Fig. 9.3) and therefore be subject to the interdependence of unit operation complexities highlighted by Slupska and Bushong (2019). Following, optimisation at pilot-scale, thorough validation at demonstration-scale is required prior to commercial production.

Pretreatment operations in a commercial facility would, by necessity, be performed on a continuous basis to accommodate xylose release from several tonnes

Fig. 9.2 Pilot-scale processing of pretreated biomass at Aberystwyth University biorefining centre. **a** Liquid/solid separation by screw press; **b** counter current washing solids to maximise C5 recovery; **c** recovered hydrolysate; **d** C5 sugar concentration by membrane filtration

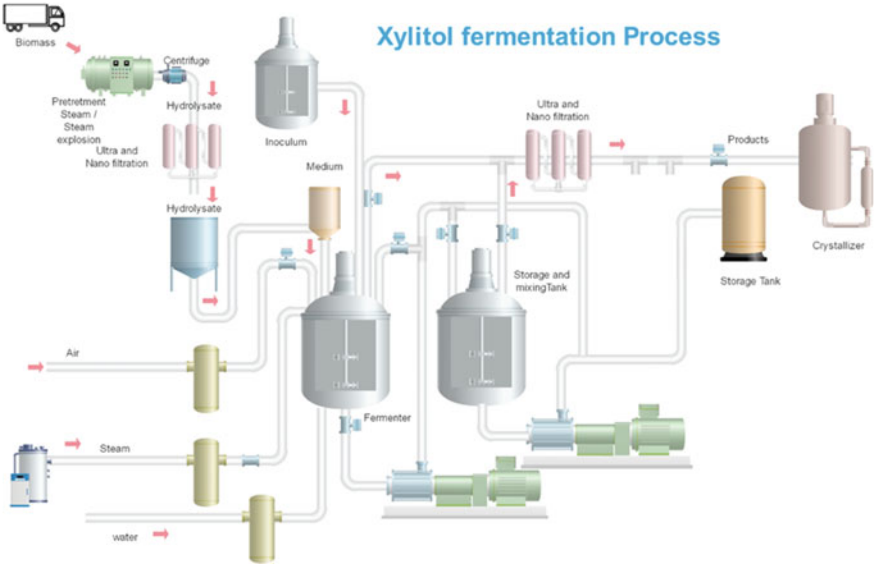

Fig. 9.3 Generalised end-to-end industrial biotechnology xylitol production model

of feedstock per hour. It is important to recognise however, that SE pretreatment systems are big ticket items in terms of CAPEX. Moreover, the transition to a larger-scale, dynamic process represents a new operation area in comparison to static batch-wise processes, thereby presenting financial and logistical scale-up integration challenges.

9.6 Identifying Technology Gaps, Process Changes and Challenges in Scaling-Up

Commercial production of xylitol through a fermentation process involves pretreatment of lignocellulosic biomass, extraction and concentration of a C5 sugar stream (xylose), fermentation of xylose to xylitol using microbes, purification of xylitol from fermentation broth, and finally crystallisation (Fig. 9.3).

Developing a technology process change is a continuous learning experience and these process changes, such as transition from batch-wise, to fed-batch or continuous fermentation, must be evaluated at pilot-scale with further evaluation at demonstration- and commercial- scale. While small to medium sized enterprises (SME’s) can access pilot and demonstration-scale biorefining facilities available across Europe and the UK to scale-up sustainable technologies, in global terms, access to such biorefining facilities is limited. The installation, operation and maintenance of pilot/demonstration-scale plants is expensive and requires national and regional investment to assist financing SME access and reduce the fiscal barrier facing fermentative xylitol and other biobased technologies on the road toward commercialization.

Over the past two decades most of the published studies based on lab scale fermentation have been performed using either pure xylose that achieve modest titres, productivity rates and/or yield. Again, one must consider whether these studies are practical in terms of scaling up a fermentative xylitol technology that would be competitive with the incumbent chemically catalysed hydrogenation used commercially. As an example, the Fazer xylitol facility (Table 9.2) currently under construction at Lahti, Finland intends to produce xylitol at 4000 metric tonnes (MT)/year, which if operating for 300 days equals 0.56 T of xylitol product per hour. Now consider a lab-scale batch or fed fermentation process using pure xylose as a substrate that is being proposed as a competitive alternative where the resulting xylitol titre was 187 g L^{-1}, with a yield of 0.75 g xylitol g $xylose^{-1}$ and a volumetric productivity of 3.9 g xylitol L^{-1} h^{-1} (Kim et al. 2002). At a commercial fermentation scale of 100 m^3 production would equal 0.39 MT/h for 48 h with associated discharge, cleaning time and downstream processing costs. Note also that 0.56 MT/h is the amount of final product and conservatively we can assume a figure of 30% product losses occurred during downstream processing. The competitive volumetric productivity target then becomes 0.8 MT/h, approximately double the volumetric productivity developed at lab scale. Obviously, it’s possible to increase the amount produced per hour by increasing production capacity, however this would result in increased CAPEX and OPEX with the effect of reducing profitability and potential investor interest.

A fermentative process can use crude hydrolysate and not require xylose purification prior to bioconversion to xylitol, which can offer a technoeconomic advantage over chemical processes. As discussed previously, biomass pretreatment to liberate xylose results in the production of fermentation inhibitors, such as furan-2-carboxaldehyde, and requires the generation and use of inhibitor tolerant yeast and/or bacteria. This area has been widely explored and many reviews are available and in-depth discussion is beyond the scope of this chapter. The key point here is that a costly substrate purification process can be avoided, however improved xylitol productivities are also required to achieve industrial scale–up. In a recent review on *Candida spp.* yeast for xylitol production from agricultural residues and grasses, only one out of 25 studies reported a titre greater than 100 g L^{-1}, productivity of 2.8 g L^{-1} h^{-1} and yield of 0.86 g/g of xylose in 39 h from sugarcane bagasse hydrolysate (West 2021). As discussed in the Fazer competitive scenario, these data suggest that fermentative processes for xylitol production need to be significantly intensified.

Using a combination of commercially realistic production goals, process intensification and the development of high yielding microbial strains, industrially competitive fermentative xylitol production processes could be achieved. For instance, yield has been increased to 1 g xylitol/ g xylose by generating yeast strains deficient in xylitol dehydrogenase that converts xylitol into xylulose for subsequent metabolism in the pentose phosphate pathway (Ko et al. 2006). In an integrated process this would also result in reducing the amount of biomass needing pretreatment in the range of 14–25% with associated OPEX cost savings. However, for process intensification the order of importance to improve production performance are: (1) volumetric productivity of 8–10 g L^{-1} h^{-1}; (2) titre of >10%; and (3) yield of 1:1. Future studies focussing on achieving, or ideally exceeding, these specified targets using crude hydrolysate will help develop a fermentative technology with the commercial potential to bear the costly scale-up development process.

9.7 Capital Investment for Lignocellulosic Biorefineries

Recently, techno-economic analysis (TEA) and LCA for hemicellulosic sugar production from residual 2G feedstock in an integrated small-scale biorefinery was performed (Lopes et al. 2022) (Fig. 9.4). The analysis estimated that production of 2000 tons of xylitol from 30,000 tonnes/year of corn stover, a capital expenditure (CAPEX) of 88.12 million USD was required with an operational cost of 4.66 million USD per year. These financial estimates are in broad agreement with the commercial experience of Oy Karl Fazer Ab (https://www.fazergroup.com/) to finance the reverse integration of a xylitol production plant using oat hulls from their oat milling operations. In 2019, the European Investment Bank (EIB) lent Fazer EUR 40 million in finance towards covering research and development (R&D) and

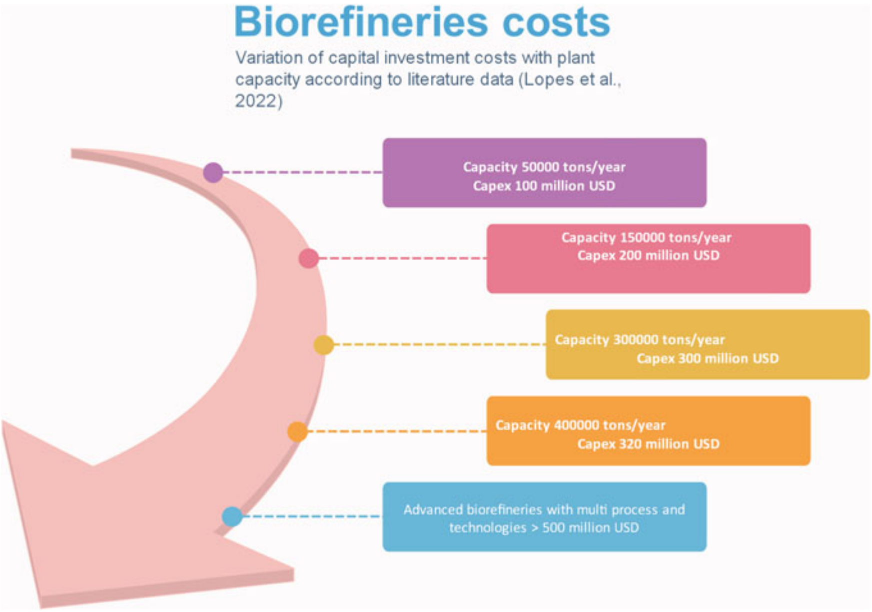

Fig. 9.4 Biorefineries CAPEX cost per tonnes of Lignocellulose biomass processed modified from Lopes et al. 2022

capital expenditure costs, with the total costs approximating to EUR 87 million for a facility to produce 4000 tonnes xylitol per year. In comparison, from 2020 Fortress Global were in the process of operating a 2000 tonnes/year xylitol demonstration unit at its Fortress Specialty Cellulose Mill (FSCM-https://www.fortressge.com/products/) in collaboration with Mondelez International, Inc. (https://www.mondelezinternational.com/). Following successful commissioning and validation of the xylitol demonstration plant, the FSCM has the capacity to supply feedstock for over 20,0000 tonnes per year. Fortress intend to capitalise on this with a further USD $150 million investment for a full-scale plant expected to yield up to $40 million in earnings before interest, taxes, depreciation, and amortization (EBITDA) per year. Within their production process, contaminating alditols, such as arabitol, removed during downstream processing will be valorised by hydrogenation to glycols (IEA Bioenergy: Task 42: Bio-Based Chemicals A 2020 Update).

Another TEA study by Longwen Ou et al. (2020) evaluated the economics of a biorefinery to process 450,000 dry MT of Miscanthus per year to produce sugars and xylitol from the hemicellulose fraction, this refinery cost estimate was with a CAPEX of 379 million USD to 423 million USD based on xylitol from C5 sugars and polyol production from lignin. Franceschin et al., (2011) estimated that a demonstration plant based on 5 MT/h biomass feed rate and 0.374 MT/h xylitol production would cost in the order of 30 million Euros (Fig. 9.5). Whereas the predicted cost of a commercial plant (Fig. 9.6) producing 1.5 MT/h was >100 million Euros.

The recently published study *EU Biorefinery Outlook to 2030* predicts that lignocellulosic biorefining will be important for the bioeconomy and will cost between 81 and 325 million Euros to build demonstration-scale biorefineries, and require investment of 3–13 billion euros for the construction of new commercial scale biorefineries by 2030.

9.8 Understanding the Sustainability of the Process

Sustainability of lignocellulosic biorefineries and xylitol production has been an active area of research over the last decade. The BIOCORE project (http://www.biocore-europe.org/) assessed and analysed the industrial feasibility of the biorefining concept for processing lignocellulosic biomass (forest biomass), and agricultural co-products (e.g. wheat straw, rice straw) alongside different processes to produce a wide variety of products, e.g. biofuels, chemical intermediates, polymers and the sweetener xylitol. The environmental impacts of the processes and products were assessed through LCA, where the analysis determined that significant environmental benefits could be gained by establishing future 2G biorefineries, with potential opportunities arising from process optimization. Among the by-products investigated were a combination of xylitol and itaconic acid or polyester resins. The European project "BIOCORE" recommended multiple by-products based on environmental sustainability assessments of the biorefinery concepts from lignocellulosic biomass (LCB) (O'Donohue 2014).

Fig. 9.5 Xylitol production through demonstration plant (Franceschin et al. 2011)

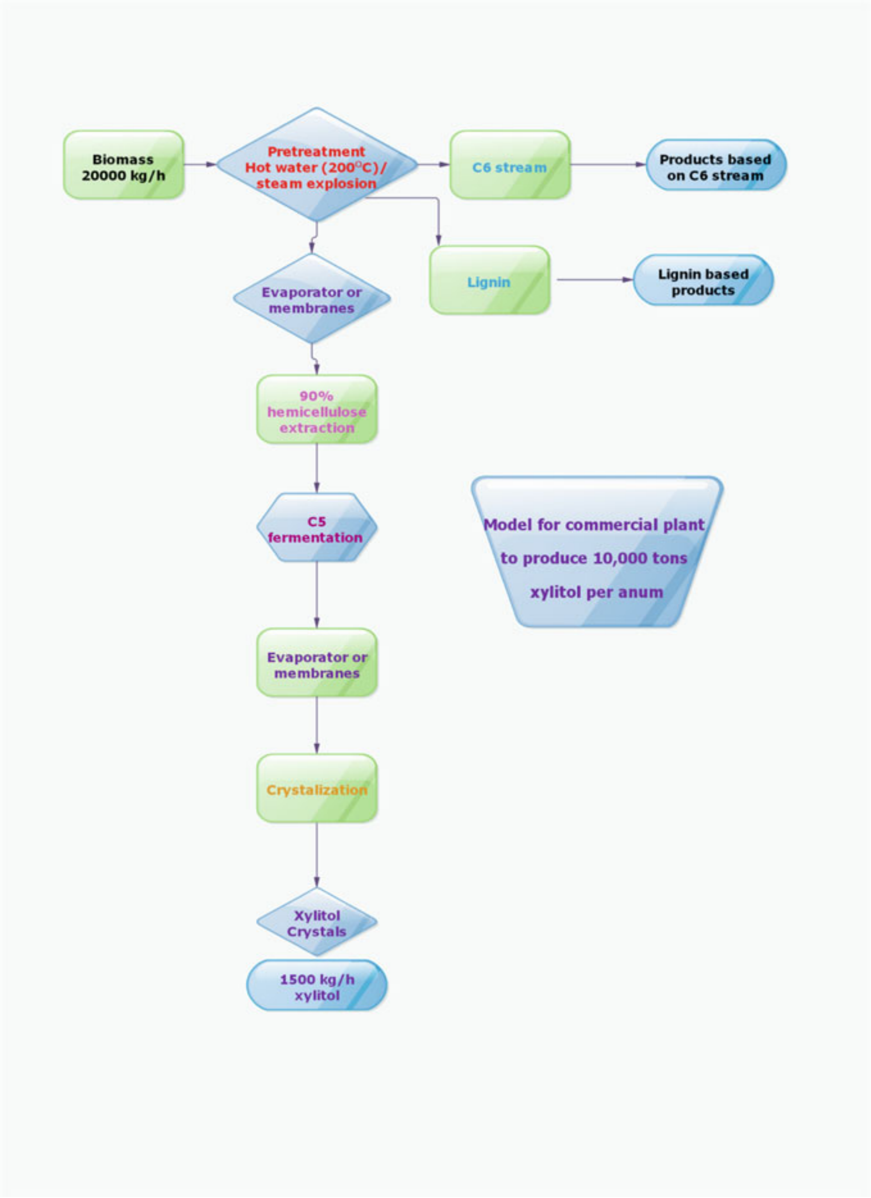

Fig. 9.6 Xylitol production through commercial plant

There have also been lessons learnt about the sustainability of xylitol production from existing commercial enterprises. In China, the commercial xylitol manufacturing process uses corncob as a feedstock for hydrolysis, Biomass Hydrolysis Process (BHP), that generates both water and C6 sugar-based waste, the indiscriminate disposal of which has resulted in environmental pollution. Since 2007, the introduction of new environmental regulations by the Chinese government has resulted in limiting effluent from xylitol production (https://www.foodmanufacture.co.uk/Article/2007/04/27/Still-sweet-on-xylitol#). As a result, these regulations have reduced production capacity of Chinese xylitol producers in order to meet the new wastewater directive.

In contrast, DuPont's (http://www.dupontnutritionandbiosciences.com) Wood Based (DWB) integrated concept to produce xylitol is a more environmentally begin process as the carbon footprint of DWB Xylitol is 90% lower than when production is based on BHP. As with the BIOCORE project, the environmental impacts of the processes and products were assessed. The DWB method requires significantly less energy (85% lower), has less impact on toxicity for both land (94% less) and water (99% less). In the DWB process, the xylose producing facility is integrated with a pulp and paper plant. Moreover, the feedstock originates from sustainably managed forests with the benefit of DuPont's XIVIA™ being more sustainable compared with xylitol manufactured by BHP. These assessments were based on 15 different parameters while evaluating both processes. The major differences were calculated based on Kg CO_2 eq released during the manufacturing of xylitol from wood compared to corncobs.

The IEA Bioenergy Task 42 "Biorefining in a Circular Economy", concluded that to achieve a sustainable biobased and circular economy, several points need to be addressed including reducing fossil fuel dependency, limiting greenhouse gas emissions, designing new processes, developing new technologies, recycling chemicals, and mainly the deployment of new biorefineries in rural locations to develop these areas.

Use of lignocellulosic biomass in integrated biorefineries will improve the environmental sustainability of the green bioeconomy. The above-mentioned BHP and DWB methods produce xylose which is converted to xylitol. During the transition toward production of biobased fuels and chemicals, integrated biorefining technologies enabling C5 stream use for IB production of xylitol and other commodities will improve economic and environmental impact (Fig. 9.7).

9.9 Conclusions and Perspectives

In summary, a xylitol plant requires development of a sustainable process that can be applied at commercial scale, ideally integrated with production of other lignocellulose derived products. The following have been identified as the critical points to be addressed:

Key challenges and solutions

>100000 dry tons of biomass for xylose stream to produce >10000 tons of xylitol per year

High CAPEX for steam explosion pretreatment, maintanance as most of the demo and commercial plants struggle to optimise and run the systems.

Availability or extreme difficult to buy C5 hydrolysate to test run fermentations at pilot and demo scale. Also difficulty to produce hydrolysate required for largae scale fementation test runs from lignocellulose biomass using batch or continuous pilot steam explosion rigs

Yeast or bacteria needed to produce high titer can surpass 100g/L xylitol production during fermentation and with a high productivity. Also withstand inhibtors in the hydrolysates. Most of the studies performed at lab scale, that information not sufficient while scaling up the biotech process. 30 to 50% downstream losses.

Assessment of biomass and organising supply chain logistics, transport to site and storage

Steam explosion pretreatment is important for integrated lignocellulosic biorefineries to produce xylitol and other by products

Process optimization will help extraction of xylose in to the hydrolysates from lignocellulose biomass

Biotech process can be integrated in to newly built adavance lignocellulosic biorefineires, adavance biorefineries more sustainable and biotech process technology can be tranferable where C5/Xylose streams exsits. optimise downstream process loss.

Fig. 9.7 Key challenges and possible solutions for developing biotech process for xylitol production

Preliminary process development:

1. Continuity and accessibility of feedstock supply
2. Pretreatment that minimises co-production of fermentation inhibitors
3. Concentration of hydrolysate
4. Selection of micro-organism
5. Fermentation process that maximise rate, titre and yield
6. Purification of product
7. Integration of process steps to maximise efficiency
8. Process validation at both pilot and demonstration-scale need consideration from the beginning
9. Protecting intellectual property and securing investment

Process development needs:

1. Assess biomass availability (>100,000 dry tons per year), transport, supply, biomass size reduction including shredding of bales, chopping, and storage of biomass.

2. Steam explosion-based pretreatment is a high CAPEX process for lignocellulosic biorefineries that may be offset by producing higher value products. Additionally SE or other pretreatment processes should be able to process a variety of feedstock.
3. Availability of large quantity of hydrolysates for large scale fermentation developmental runs is a challenge that is reliant on pretreament interdependency.
4. IB processes are dynamic based on physical, chemical and biological parameters. Fermentation process dynamics will change during scale up and therefore need robus validation at pilot-scale before progressing to demonstration trials.
5. Currently during downstream processing of fermentation broth xylitol results in losses of around 30 to 50%, optimization and step-wise improvements will increase economic viability.

Commercialization needs:

1. De-risk venture capital and commercial failure by evaluating the total process at demonstration scale to enable investor confidence in high CAPEX builds as biorefineries are multimillion-dollar outlays.
2. Pretreatment equipment installation takes time and investment, i.e. the SE rigs need to be ordered, built, and installed based on plant specifications, and feedstock handling specifications. Manufacturing and installations take time as these systems are not able to be purchased readymade.
3. Develop sustainable processes that address the circular economy by producing multiple and higher value products in lignocellulosic biorefineries with standardised LCA low carbon supply chains.
4. Determination to commercialize a new technology.

Despite the above mentioned challenges and barriers, fermentative xylitol production is a promising technology that can be part of lignocellulosic biorefineries, as exemplified in the world's first commercial IB xylitol process started in China. In turn, this should galvanise sugarcane producing countries, such as Brazil and India to capitalise on their in-house sugar mill facilities, feedstock and infrastructure for integrated IB xylitol production (Hernández-Pérez et al. 2019).

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