Lignocellulosic Biorefineries: The current state of challenges and strategies for efficient commercialization

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Abstract

Lignocellulosic biomass is the most abundant and sustainable feedstock available globally. As a source of the polysaccharides, cellulose and hemicellulose, it can be converted into biofuels and other platform chemicals. This article highlights some important aspects that need to be focused upon for the commercial development of lignocellulosic biorefineries. Although, lignocellulosic biomass offers clear value in terms of its green advantages and sustainability, there has been very low commercial success at industrial production levels. This can be attributed to a few key factors such as an irregular biomass supply chain, inefficient or complex pre-treatment and saccharification technologies, and scale up challenges leading to high capital and operating expenditures. Moreover, techno-economic studies performed on lignocellulosic biorefineries have revealed that process complexity is the most detrimental factor prohibiting scale-up. Although there have been several research efforts funded both by the public and private sectors, biomass valorization into biofuels and chemicals remains a technical and economical challenge. This review examines the global drivers towards the advancements of lignocellulosic biorefineries, technical and operational challenges for industrialization and future directions towards overcoming them.

Research Highlights:

- Commercialization of lignocellulosic biomass refineries needs efficient value chain
- Sustainable feedstock supply can be gained with biomass source diversification
- New pre-treatment technologies can enhance yield and accelerate industrial scale-up
- Novel enzyme cocktails can improve extraction and hydrolysis of polysaccharides
- Industrial scale-up requires development of efficient co-processing techniques

Keywords: Circular bioeconomy; Lignocellulose; Biomass valorization; Biorefineries; Waste to energy; Industrialization

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Abbreviations

EU	European Union
US	United States
USD	United States Dollar
2G	Second generation
FAO	Food and Agriculture Organization
GHG	Greenhouse gases
IEA	International Energy Agency
UN	United Nations
SDG	Sustainable development goals
BRDi	Biomass R&D Initiative
MSP	Minimum selling price
NaOH	Sodium Hydroxide
KOH	Potassium Hydroxide
CAPEX	Capital Expenditure
OPEX	Operational Expenditure

1 Introduction

The development of the global economy across all industrial sectors including energy, agriculture, and food is facing unprecedented challenges imposed by regulatory guidelines to combat climate change and decrease reliance on fossil fuels. The over-utilization of these non-renewable resources pressure on the environment and their long-term sustainability. Thus, many countries across the globe have laid out their roadmaps for achieving sustainable development through advancement of the bioeconomy. The launch of European Commission's bioeconomy strategy in 2012 [1] (revised in 2018) and the inclusion of bioeconomy as a key growth driver in US's FY2021 Research and Development priorities [2] is an indicator of the seriousness with which the focus is shifting towards this sector. In fact, the turnover from the bioeconomy was reported to be USD 400 billion in the US (2017) and nearly €2.3 trillion in the EU (2018) [1,2]. This has resulted in an upsurge of deployment of green biobased refineries which primarily utilize lignocellulosic biomass as the feedstock to sustainably produce end products.

Biorefineries valorize lignin, cellulose and hemicellulose into useful products such as biofuels and other platform chemicals [3]. However, there is no standardization in the conversion of lignocellulosic biomass into these end products due to a wide range of factors which include biomass source, type and recalcitrance level [3]. There are multiple valorization techniques available that can be deployed to refine the lignocellulosic biomass into over 200 value-added chemicals [4]. Figure 1 represents the common routes to derive these bioproducts from biomass. Sugar monomers can be derived from the polysaccharide components of the lignocellulosic biomass i.e. cellulose and hemicellulose [5], which can then undergo anaerobic digestion and fermentation to be converted into useful end-products such as biochemicals, biogas and biofuels. The transformation of the polysaccharides into monomeric sugars dictates the cost-effectiveness and efficiency of the bioconversion process.

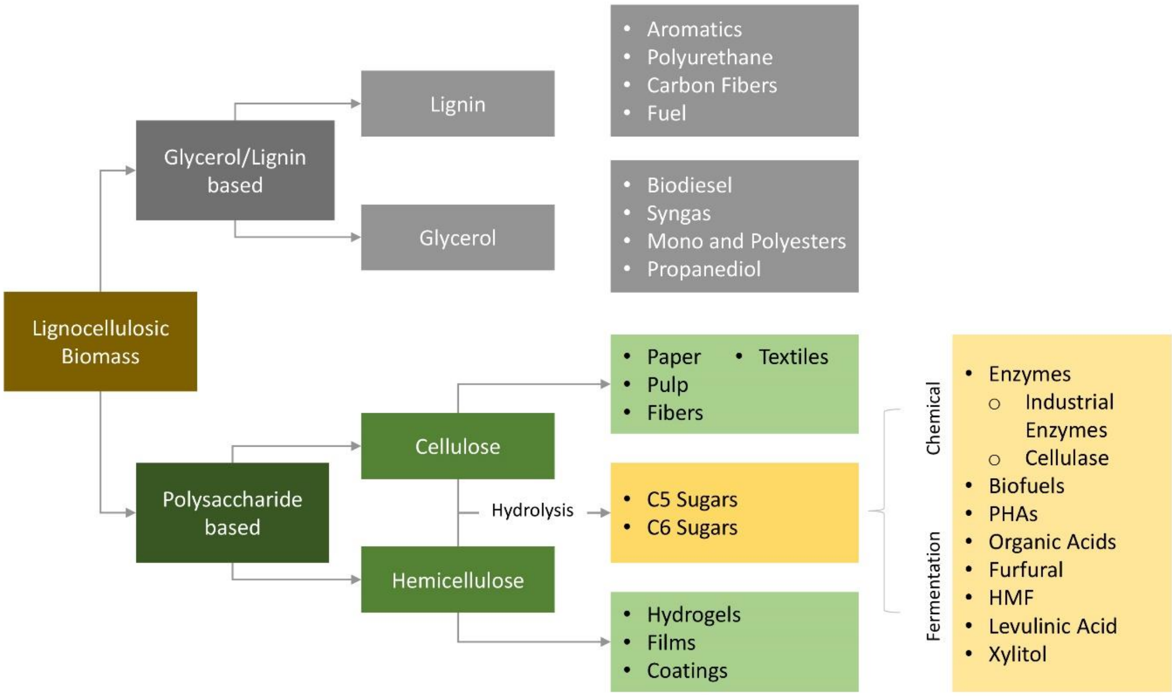

Figure 1. Valorization of lignocellulosic biomass into bioproducts and chemicals

There are several key challenges that impact the product yield and energy input required in the bioconversion process which need to be addressed in order to setup full-scale biochemical and bioenergy production from biomass. Lignocellulosic biomass is made up of polysaccharides (cellulose and hemicellulose), closely surrounded in a matrix lignin polymer structure making it a complex resource [3]. The structure varies depending upon the source of the biomass feedstock used in the biorefineries. One common feature, however, is that the presence of the lignin polymer significantly lowers the ability of the necessary enzymes to attack the cellulose to hydrolyse it into sugars, resulting in low yields [5]. An additional step of pre-treatment thus becomes essential to breakdown this recalcitrant structure and allow for separation and further treatment of the biomass components. Researchers have proposed many pre-treatment techniques over the years to break down the lignocellulosic structure to make it susceptible to hydrolysis [6-8]. Pre-treatment helps in efficiently generating second generation (2G) sugars which are natural intermediates during the chemical and biological conversion and are the key ingredient in a biorefinery [9]. However, pre-treatment is an energy and cost intensive process and varies largely with the feedstock and the desired end-product [5,7]. Hence it is essential to consider this step while evaluating the overall techno-economic viability and sustainability of the lignocellulosic biorefinery.

The feedstocks that were primarily used in first generation biorefineries included sweet sorghum, beet sugar, sugarcane, soybean, rye, barley, wheat, or corn. The extraction of sugars from these feedstocks was done using several methods such as squeezing, water-based extraction, steam jet cooking etc. [10]. The extracted sugars were further treated via catalytic transformations or biological methods into biofuels (butanol, ethanol) and platform chemicals (propionic acid, lactic acid etc.) [11]. Additionally, various other products formed in the biorefineries included feed material (dry distillery grains and soluble, processed cake) and food (corn syrup, corn liquor, and oil) [12, 13]. Using further treatment methods on the products resulted in the production of surfactants, pains and dyes, detergents, adhesives, paper, and biopolymers [14, 15]. But the growing concerns around using food grade feedstock into biorefineries led to the development of second-generation biorefineries that focused on using non-edible portions of the food production value chain. These feedstocks include materials such as forestry waste, agro-industrial waste, municipal solid waste, crop residues etc. [16]. These feedstocks can be subjected to a range of thermo-chemical and biochemical processes such as gasification, pyrolysis, torrefaction, and enzymatic hydrolysis to produce biofuels and sugars [17]. However, these techniques with many challenges such as high energy requirements, utilization of harmful chemicals and are overall not sustainable processes.

Other challenges in the industrialization of lignocellulosic biorefineries include the viability of the supply chain (from the point of generation of the feedstock until it gets into a processing facility), scale-up of laboratory-scale experiments into commercial-scale processes and the technical maturity of the technologies used in biorefineries. This review aims to discuss the steps that are being taken globally through regulatory and governing bodies to push further the agenda of the circular economy and sustainable production of energy and chemicals using green resources. This review highlights current progress made in addressing the aforementioned challenges providing a viable roadmap for the future of lignocellulosic biorefineries by reconciling technical advancements with the market drivers and challenges.

2 Biorefinery Industry Development Drivers

Lignocellulosic biomass feedstock can be sourced from primary/secondary residues from agroforestry or from non-food/food crops. The annual growth of the bio-based product market in the EU is estimated to be 4% reaching nearly €50 billion by 2030 [18]. Bio-based product market growth is fuelled by the global shift towards green, naturally sourced and sustainable products that have minimal or no impact on the environment. This will result in an increase of the lignocellulosic biomass feedstock market share to ~25% by 2030 [19].

2.1 Global environmental challenges

Traditional fossil fuels are energy dense and have proven to be an effective resource as energy sources for the growth, development and industrialization of this planet [20]. With the projected global population level predicted to reach 9.2 billion by 2050, demand for energy and raw materials will continue to increase, straining natural resources beyond their available capacity [21]. Additionally, feeding this population will also incur a growth in cultivable land which is estimated to be around 1 billion ha (by 2030) by the Food and Agriculture Organization (FAO) [22]. A little acted upon side-effect of this continuous utilization of resources is the disruption of ecological balance endangering biodiversity, loss of aqueous systems and atmospheric quality [23]. Moreover, climate change accelerated by the production of greenhouse gases (GHGs) and generation of substantial amounts of wastes by the production and consumption of products and goods further add to this imbalance. The International Energy Agency (IEA) reported the total amount of CO_2 emissions in 2020 to be 30.6 Gt [24] which is far from a goal of 'net zero' by 2050 as set by the United Nations (UN) [25]. The amount of global waste generated is reported to be approximately 2.01 billion tonnes annually and is poised to reach 3.4 billion tonnes by 2050, of which at least 33% is not managed properly adding a significant challenge of their effective disposal into the environment and causing downstream damage [26]. Out of this biomass, lignocellulosic waste (food and green, wood, paper and cardboard) forms almost 63% of the total mass [18], which could be a valuable resource for the biorefinery industry, not only reducing the stress on the environment but also advancing the cause of circular bioeconomy. These trends have attracted the attention of the governments across the globe and 2015 witnessed the establishment of 17 sustainable development goals (SDGs) for 2030. These goals emphasize responsible production and consumption, clean water, clean energy and climate action among others [27]. Additionally, the UN estimates that if the right steps are taken with urgency across the globe, global warming could be limited to 1.5^{o}C [25], which further requires that biobased economy be established seriously and soon.

2.2 EU Framework for sustainable development

Taking global agreements into account, the EU has devised a roadmap towards the reduction of dependence on non-renewable energy sources and increasing the production and utilization of sustainable green resources. The EU launched its 2012 European Bioeconomy Strategy to create a platform for innovation and efficient utilization of resources in a sustainable circular manner. The aim of the bioeconomy is to cover industries that are dependent on bio-based resources such as plants, animals and biomass, including organic waste. The framework supports the SDGs as well as the Paris Agreement on climate change [28]. In addition to this, the EU has also created the European Green Deal to establish Europe as the first climate neutral continent with no net GHG emissions by 2050 at the same time making EU's economy stable and sustainable [29]. In order to achieve these goals, the EU agreed on a GHGs reduction target of 40% and renewable energy share of 27% by 2030 [30]. This has invigorated and incentivized focus the development of biorefineries in the EU making them more prevalent and economically viable through deployment of suitable techniques to valorize bio-based feedstock into products.

2.3 U.S. Bioeconomy development focus

The growth of bioeconomy in the US started in the early 2000s with the enactment of the Biomass Research and Development Act of 2000 establishing an Interagency Biomass R&D Board, a Technical Advisory Committee, and the Biomass R&D Initiative (BRDi) [31]. The focus was further strengthened with the enforcement of Energy Independence and Security Act in 2007 setting a target for biofuel production of 140 million m^3 annually by 2022 [32]. In 2012, the US federal government published the National Bioeconomy Blueprint with five strategic objectives – investment into bioeconomy innovation, scale-up of technologies from laboratory to industry, regulatory frameworks to reduce barriers to deployment of environment friendly technologies, academic support, and develop public-private partnerships [33]. These supporting frameworks created nearly 4 million job opportunities in 2017 leading to the production of 2,500 certified bioproducts and ~65.4 billion litres of biofuels contributing to 2.5% of the US economy [34]. The US has also set itself a vision of becoming a bioeconomy powerhouse through the Billion Ton Bioeconomy Initiative tripling its bioeconomy size by 2030 [31]. This initiative will lay the foundation to innovatively utilize the abundant biomass resources to produce bio-based products while maximizing economic, social, and environmental benefits.

3 Biorefinery value chain – economical and technical considerations

A move towards the establishment of a sustainable bioeconomy will need to overcome several inherent challenges faced at various points in the lignocellulosic biorefinery value chain. Figure 2 gives an overview of the lignocellulosic biorefinery value chain and indicates the potential roadblocks that will be discussed in the following sections along with their potential solutions for industrial application.

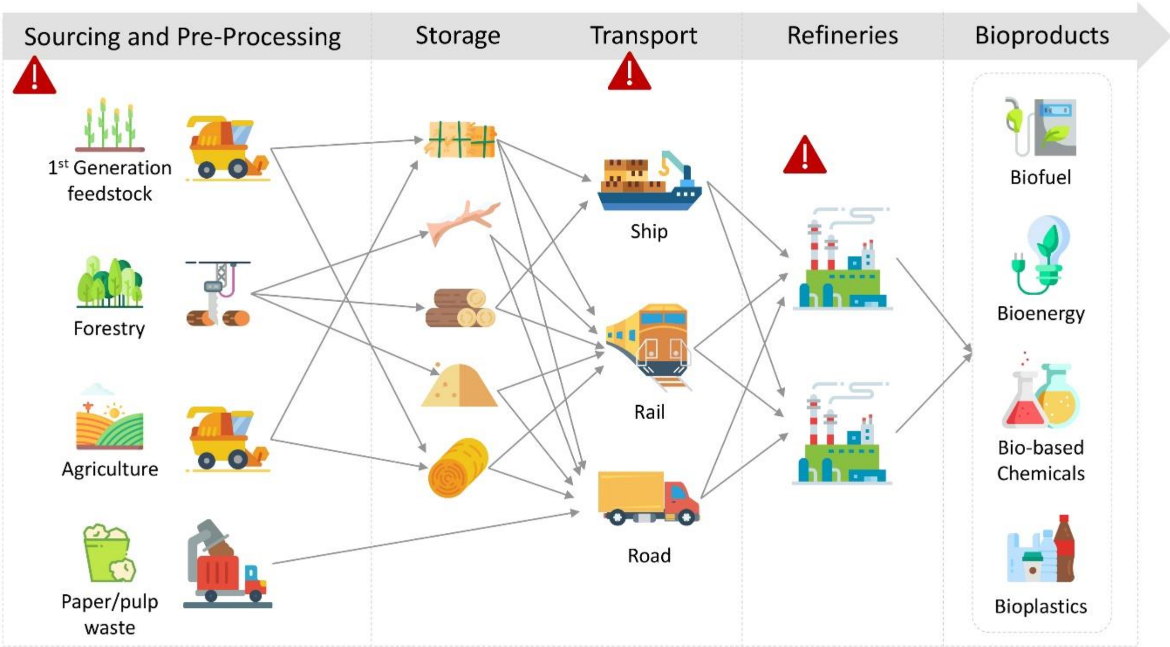

Figure 2. Overview of the lignocellulosic biorefinery value chain and the potential roadblocks to successful implementation

3.1 Feedstock and its availability

Since biorefining is a capital-intensive endeavour, the biorefinery's ability to manufacture products at a sustainable rate and price-competitive value is highly dependent on the easy and low-cost availability of the lignocellulosic feedstock. There is a large variety of lignocellulosic biomass sources that can act as feedstock depending upon their availability around the year. Various countries utilize the most abundant biomass supply available to them as the primary feedstock. For instance, Canada's forest industry generates a large volume of residues which are used to produce ~52 billion litres of ethanol and biodiesel annually [35]. Countries such as USA and Brazil use corn stover and sugarcane bagasse residues respectively on a large scale. Beyond that, the US uses switchgrass, poplar and miscanthus as other sources of lignocellulosic biomass in order to diversify its feedstock, whereas Brazil started to use corn under its RonovaBio program in 2020 [36]. Countries such as India and China, which are primarily agronomies, use a variety of feedstock sources that are generated during agricultural production and processing including sugarcane bagasse [37]. India produced nearly 683 million tonnes of crop residues in 2018, the majorly of which is used as feedstock for fuel, industrial production and fodder. However, nearly 87 million tonnes have been burned in the croplands as waste [38], leading to significant air pollution. In the EU, diverse biomass sources have been used over the years including agriculture, forestry, and fisheries. Figure 3 is a Sankey diagram providing an overview of the lignocellulosic biomass flow in the EU. Much of the feedstock is produced within EU and is utilized majorly to produce biomaterials and bioenergy [39].

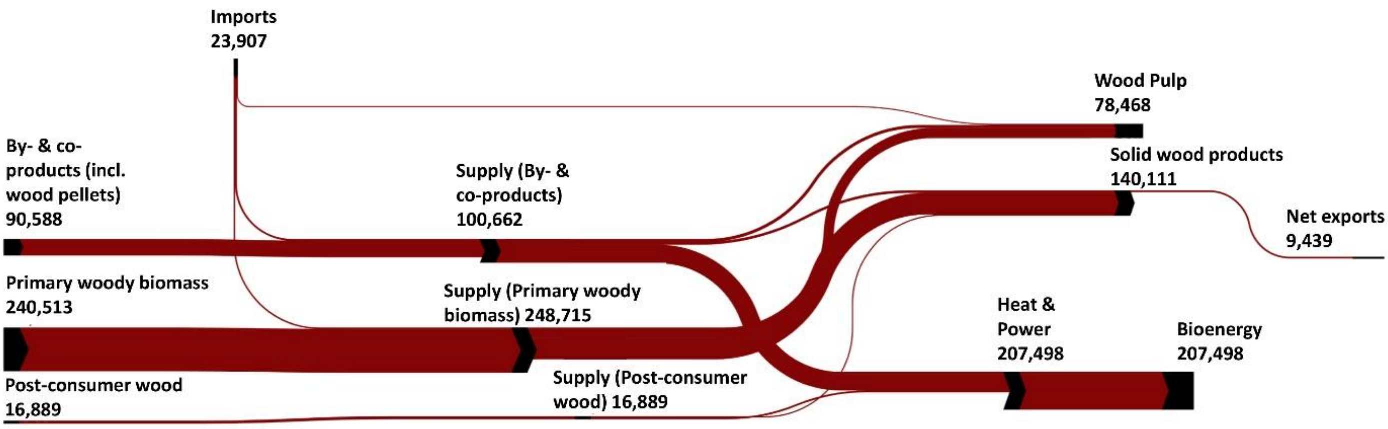

Figure 3. Biomass flow from lignocellulosic sources in EU (2017) [39]

Food crop-based feedstocks i.e., first-generation feedstocks are primarily used in biofuel production. For instance, sugar and starch crops (sweet sorghum, sugarcane, corn) can serve as raw materials for bioethanol production whereas oil seeds (sunflower, palm, soybean) can be used in biodiesel production [40]. Second generation feedstock from non-food sources such as switchgrass, sawdust, bagasse, corn stover, rice husk, apple pomace, orange peel and other agri-food waste not only add to the variety of the feedstock source but also helps in reutilization of biomass waste and advancement of the circular bioeconomy [41]. Lignocellulosic biomass feedstock can be obtained from other sources such as non-food energy crops (willow, poplar, other hardwood sources) [42], grassy crops (miscanthus, energy cane, sorghum) [43] and paper and pulp industry waste [44]. An emerging avenue or third generation of biomass feedstock is marine-based biomass e.g. seaweed [45] and microalgae [46] which has attracted the attention of the research community in the recent past due to its high growth rate and photosynthetic ability as well as the fact that its production does not rely on traditional arable land. An evaluation by S2Biom project on biomass availability across EU from 2013 to 2016 to produce bioenergy reported lignocellulosic biomass to be a sustainable source of feedstock to support biorefineries in the years to come [18].

These lignocellulosic biomasses resources, especially first-generation feedstocks, have achieved commercial success in biofuel production while second and third generation resources are either under study or are undergoing techno-economic evaluations. There are multiple factors such as the complexity and structural integrity of the biomass that impacts the treatment methodology applied in the biorefinery and thus plays a key role in the commercial viability of plant operations [47]. Table 1 summarizes various techno-economic studies performed on diverse lignocellulosic biomass sources. It is observed that the price of biomass is usually <$1/kg for second generation feedstocks i.e., waste from other industries, while the process itself may lead to a higher final minimum selling price (MSP) due to factors such as pre-treatment, higher energy requirement and special equipment to carry out the process. One of the ways to mitigate this could be to achieve economies of scale as demonstrated by Okeke and Mani [60] who were able to bring down the MSP of liquid fuel generated from biogas via Fischer-Tropsch synthesis (from $1.49/kg to $0.54/kg) by increasing the plant capacity by tenfold.

Table 1. Summary of techno-economic studies performed on lignocellulosic biomass

Source	**Biomass**	**Pre-treatment**	**Output**	**Cost Price ($/kg)**	**Minimum Selling Price ($/kg)**	**Reference**
Forest	Hardwood	Fast pyrolysis	Biodiesel	0.053	1.09	[48]
	Wood chips		Biobutanol	0.03	0.138	[49]
	Softwood	Mechanical micro-sizing	Reduced sugars	0.058	0.33	[50]
Agriculture	Wheat straw	Steam explosion	Ethanol	0.053	1.03–1.23	[51]
	Cotton stalk	Thermochemical	*n*-Butanol	0.4	0.66	[52]

	Cotton straw	Fast pyrolysis	Levoglucosan	0.15	3.0	[53]
	Switchgrass Sugarcane Corn grain	Ball milling	Jet fuel	115-125 20-30 55-95	1.38 0.96 1.01	[53] [54]
Industrial	Pine wood	Thermal Fast Pyrolysis	Cycle oil Gasoline	0.085 0.12	0.35 2.23	[55] [56]
Sea	Seaweed	Fast pyrolysis	Biofuel Ethanol	0.1 0.098	1.5–1.8 1.17	[57] [58]
Field trail	Miscanthus	Thermochemical	Bioethanol	0.08-0.10	0.65–0.71	[59]

In fact, nearly 50% of the production cost of ethanol production from cellulosic biomass can be attributed to feedstock procurement cost [61]. Another important consideration could be diversification of the feedstock being processed at the biorefinery, reducing dependence on the availability of one type of biomass lowering operational risk. Hence it is important to accurately evaluate the supply of biomass during the planning of a biorefinery setup.

3.2 Supply Chain and Transportation

Generally, the biomass supply chain includes sorting, transport, storage, and processing (Figure 2). In the lignocellulosic biomass valorization value chain, transportation in the supply chain is a crucial step that dictates the cost of production and eventually the MSP of the end products. The agreement between the biomass suppliers and the processing plants is essential for year-round availability of biomass at competitive rates [62]. The key steps used to maximize the efficiency of transportation are biomass size minimization, drying and compaction [63]. An estimation of the cost associated with the production, processing and transportation puts it in the range of 40-60% [64] and can be accurately analysed through Network Analysis extension available in ArcGis tool [65]. The success of second-generation feedstock based biorefineries requires a large-scale development of biomass transport and storage for a sustainable production of ethanol or biochemicals. The processing of biomass can happen either in a decentralized setup (local small-scale pretreatment and processing) or centralized setup (large scale industrial production). The disadvantage of a decentralized processing is high processing cost and erratic biomass availability, whereas a centralized setup suffers from huge transportation and storage costs of biomass [66]. Hence the decentralized setup should be applied at sites where biomass and waste production are significantly consistent e.g. food processing plants [66, 67], whereas as centralized setup can benefit from economies of scale and bring down the overall of cost of production significantly lower as well as achieving higher operational efficiencies [68].

The presence of moisture in biomass is a key consideration for storage and transport as 20% or higher moisture content makes the biomass aerobically unstable. Storing this type of biomass for the longer term can cause a loss of dry matter eventually reducing yield, fungal spore growth and self-ignition. The location of the factory is another critical factor in logistics planning and if it is far from the biomass source site, transportation costs increase leading to an overall higher production cost. Most techno-economic studies assume the availability of biomass at the processing plant or in the vicinity. But the inclusion of longer distances in the cost calculations increases the MSP of the end-product making it less economically attractive. Transportation cost also varies with the bulk density of the lignocellulosic biomass e.g. corn has higher bulk density than agri-residues (721 v/s 50–100 kg dry matter m^{-3} respectively), resulting in additional energy requirements at each step in the biorefinery value chain [69]. A study on the transportation of pre-processed wood chips increased their bulk density and the overall efficiency of the operations [70]. Various modes such as road, rails, or ships are deployed in transportation, and has been observed that in US alone trucking accounted for 90% of biomass transport to biorefineries [69]. It´s estimated that cost of transporting biomass ($/ton/km) is lowest through ships (0.01), followed by rail (0.017) and road (0.07), although the handling costs in ships is higher against rail and road [71].

The main goal of transportation planning for a biorefinery must include the minimization of overall energy input and transportation cost. In order to achieve this, key factors that need to be considered include bulk density, handling and storage, fuel requirements, and distance between source and processing plant [63]. These parameters can vary from one refinery to another depending on the kind of lignocellulosic biomass being processed. The Biomass Exchange Model (Figure 4) could be a potential solution to alleviate some of the challenges posed by the distributed nature of lignocellulosic biomass feedstock generation [72]. This is particularly helpful in tackling timely availability of feedstock, establishing a reliable chain and mapping the nearest available supplier which in turn reduces the overall cost of the operations.

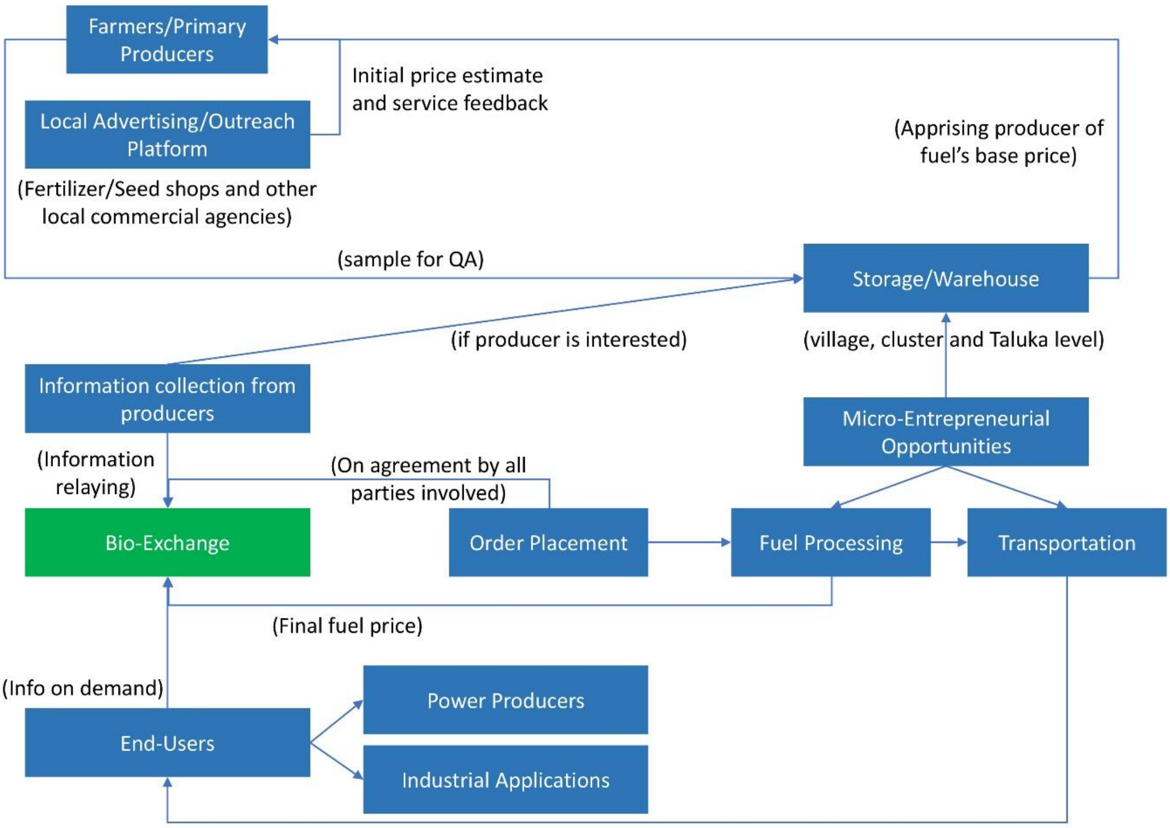

Figure 4. Biomass exchange model for sustainable sourcing of lignocellulosic biomass [72]

In this model the small-scale local markets serve as the points of advertisements for the availability of the biomass. There could be potential distribution nodes i.e. warehouses created at the local level to store the feedstock sourced from various suppliers, hence making it easier for the suppliers and the biorefineries to supply and collect biomass respectively. The biomass exchange serves as the hub of information for availability of the feedstock at various warehouses and the biorefineries can request the biomass exchange to facilitate the enquiry, pricing negotiation and order placement.

3.3 Pre-treatment of lignocellulosic biomass

A necessary step towards the effective utilization of lignocellulosic biomass is pre-treatment which breaks down the recalcitrant structure of the biomass, liberating the cellulose and hemicellulose from the matrix structure of lignin. This step eases the saccharification process and enhances the yield of the final product [73]. Various physical (e.g. extrusion, milling), chemical (e.g. ionic liquids, alkali, concentrated acid hydrolysis), physicochemical (e.g. ammonia fibre expansion, CO_2 explosion and steam explosion), as well as biological (e.g. fungi, bacteria and enzymes) pre-treatment processes have been developed over the years to enhance lignocellulosic biomass degradability [73].

However, most conventional pre-treatment techniques have different drawbacks including high energy and cost consumption, inhibitory intermediates and by-product formation diminishing the overall attractiveness of the biomass valorization [5]. The pre-treatment step has been estimated to account for nearly 40% of the overall processing cost in bioethanol production due to various processing conditions [74]. For instance, most physical pre-treatment processes require high energy and special equipment, increasing the overall cost and limiting the industrial scalability from lab-scale [75]. Conversely, chemical pre-treatment needs comparatively lower energy, but utilizes digesters and chemicals which again increases cost and overall greenness of the process [76]. Usually, these pre-treatments are performed in conjunction with thermal processes such as high pressure and temperature to accelerating the efficiency of biomass breakdown [75]. Additionally, the pre-treatment usually requires the hydrolysate to be neutralized of inhibitors before the saccharification adding to the overall cost of valorization [77]. Toxic derivatives of the inhibitors formed during the process pose environmental hazard and must be treated before they can be disposed, adding to the total cost. Hence, there is a growing inclination towards adoption of biological pre-treatments which are considers environmentally safe, need lower energy, no by-products formation and overall cheaper. The microbes deployed in the pre-treatment can also be used in the production of enzymes thus lowering the cost of enzyme procurement. However, it requires large bioreactors for large-scale hydrolysis, the microbial growth is slow as is the enzymatic hydrolysis rate, thus impeding the adoption of these methods [78]. Table 2 summarizes the techno-economic operational parameters of the pre-treatment categories along with the breakdown mechanism of the lignocellulosic biomass.

Table 2. Comparison of techno-economic operational parameters for of lignocellulosic biomass pre-treatments [78-82]

	Pre-Treatment method		
	Chemical	Biological	Physicochemical
Methods	Dilute sulfuric acid Alkaline hydrolysis Organosolv process Ionic liquids (ILs) Deep Eutectic Solvents (DES) γ-Valerolactone (GVL)	Microbes (fungi, bacteria, actinomycetes) Enzymes (laccases, peroxidases etc.)	Liquid hot water Steam explosion AFEX Pyrolysis
Breakdown mechanism	Fractionation of lignin releasing the polysaccharides for further hydrolysis and generation of monomeric sugars	Decomposes the cellulose and hemicellulose to yield intermediate compounds that can be further hydrolysed by enzymes	The delamination of cell wall microfibrils enhances the digestibility of lignocellulosic biomass
Advantages	• High conversion rate • Higher product/sugar yields on hydrolysis • Very high delignification • Moderate reaction conditions	• No chemical requirement • Lower energy consumption • Mild reaction conditions	• Highly effective on lignocellulosic biomass • Non-toxic and less corrosive
Disadvantages	• Harmful chemicals are not sustainable • Higher water usage • Loss of hemicellulose and lignin	• Continuous monitoring • Very large setups • Lower hydrolysis sugar outputs • Slow process	• Costly setup due to high temperature and pressure requirements • Special reactor design

	• Additional considerations for handling corrosion		
Energy input	H	VL	H
Process efficiency	H	L	M
CAPEX	H	M	H
OPEX	H	M	M
Environmental impact	H	VL	L
Inhibitor generation	H	VL	N/A
Odour generation	H	H	L
Biomass solubilization	H	H	N/A
Applicability to lignocellulosic biomass	H	M	M

N/A – Not Applicable; H – High; M – Medium; L – Low; VL – Very Low

It is difficult to identify and apply a universal pre-treatment process across lignocellulosic biomass due to a range of factors that impact their characteristics and mechanisms. For instance, assessment of biofuel production from corn stover using various pre-treatment techniques established dilute acid to be the most profitable option at $39.2 million/year in biofuel production [83]. The production cost for corn stover, spruce, and wheat straw were found to be $1.19/L, $1.11/L, and $1.25/L respectively [84]. In another study, Zhao et al. [85] estimated the total cost of bioethanol production from corn stover via dilute acid pre-treatment and enzymatic hydrolysis. They found that the total cost of bioethanol production stood at $1.6/L which was still too costly to rival fossil fuel derived ethanol prices thus necessitating incentives or subsidies to drive down MSP and adoption. Additionally, the possibility to recover and reuse by-products and chemicals from the pre-treatment process can enhance the cost effectiveness of the overall process. For instance, enzymes can be recycled for reuse after the degradation process is complete albeit at a reduced efficacy to bring down the overall process cost. Continuous removal of inhibitors such as furfurals, weak acids and phenolics using membrane, evaporation, and biochar, etc. in ionic liquid and acidic pre-treatment processes can improve yields [86].

Another technique to improve the breakdown performance is to combine various lignocellulosic pre-treatment techniques that can operate in conjunction with each other to improve yield of sugars and the overall process efficiency [87-89]. The combination of the pre-treatments brings together the advantages of individual techniques and applies a synergistic impact on the lignocellulosic biomass for a superior conversion. However, as discussed earlier the overall conversion efficiency is impacted by the biomass complexity and structure, thus making it difficult to administer a universally applicable combination that can effectively pre-treat a wide range of lignocellulosic biomass. Additionally, multiple pre-treatment techniques would necessitate fulfilling additional requirements such as chemicals and equipment thus increasing the overall cost.

3.4 Lignocellulosic biomass saccharification

Pre-treatment is usually followed by an enzymatic hydrolysis step which converts the carbohydrates into soluble sugars. While there have been attempts at non-enzymatic hydrolysis, enzymatic hydrolysis has proven to be highly effective and cost efficient at hydrolysing the biomass into reducing sugars achieving higher than 90% conversion of the polysaccharides. Figure 5 gives a cost breakdown between various steps involved in the lignocellulosic biomass conversion. Nearly half of the expenditure goes towards the pre-processing, transportation and storage of the biomass [90], whereas the cost of enzymatic hydrolysis contributes to nearly 25% of the overall production cost [91], making it essential to optimize the step to achieve profitable levels of hydrolysate production. Developing a highly efficient cocktail of enzymes that can hydrolyse the polysaccharides from the lignocellulosic biomass remains a major challenge. The enzymes involved in the hydrolysis of cellulose and hemicelluloses are cellulases and hemicellulases respectively. Additionally, cellulose degradation can be performed through polysaccharide monooxygenases which can carry out oxidative chain breakage to degrade cellulose [92]. The heterogeneity of hemicellulose requires hemicellulases to be a complex mix of enzymes including xylosidases, mannanases, xylanases, and arabinofuranosidases for hydrolysis. The lignocellulosic biorefinery employs cellulases to hydrolyse cellulose which is driven by the combined action of exo-β-(1,4)-glucanases, endo-β-(1,4)-glucanases and β-D-glucosidases releasing D-glucose and D-cellobiose from the substrate [93]. Table 3 lists few selected examples of enzymatic hydrolysis performed on lignocellulosic biomass to yield sugars and other products.

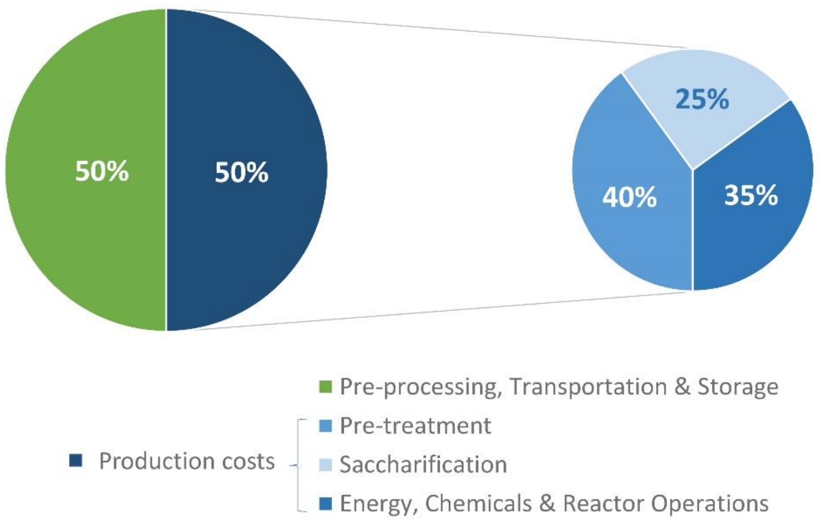

Figure 5. Cost breakdown of the steps involved in lignocellulosic biomass conversion

Table 3. Enzymatic hydrolysis performed on pre-treated lignocellulosic biomass

Lignocellulosic Biomass	**Pre-treatment**	**Hydrolysis conditions**	**Sugar recovery**	**End-product**	**Reference**
Corn stover	Biological with Phlebia brevispora NRRL-13018	Enzyme cocktail (Celluclast, Novozyme Fiberzyme), pH 5.0, 45°C, 72h	442 ± 5 mg/g of substrate	36 ± 0.6 g ethanol from 150g substrate	[94]
	Extractive ammonia, 30 min, 120°C	8% glucan w/v loading, enzyme loading of 7.5mg/g glucan	44 wt% of the biomass lignin present in ammonia-soluble fraction	Ethanol yield of 18.2 kg/100 kg of substrate	[95]

	NaOH pre-treatment, 0.125% w/w Anthroquinone on corn stover, 55mg/g NaOH, 120°C	20% solid loading, 20 mg/g Cellic Ctec 3 enzyme loading	glucose (> 90%) and xylose (> 70%) yields	>85% cellulose conversion	[96]
	Deacetylation and mechanical refining, dilute NaOH (0.1 M)		230 g/l monomer sugars	10.7% v/v ethanol	[97]
Sugarcane bagasse	Alkaline pre-treatment	130°C, 1.5% NaOH, 30 min 170°C 1.5% NaOH, 30 min	Glucose yield of 293 kg/tonne bagasse		[98]
	Autohydrolysis, 180°C, 20 min	5 FPU/g enzyme (Cellic CTec2+HTec2 (Novozymes).	84.4% sugar recovered		[99]
Wood chips	Neutral sulphonation	10% solid loading; 20 mg/g Novozymes (Cellic CTec 3)	>80% sugar recovery		[100]
Wheat straw	Organosolv, 160°C, 40 min	9 mg g^{-1} Cellic CTec2 in substrate	Cellulose conversion of 89%	65% ethanol and 51% pulp yield	[101]
Rice husk	KOH	Substrate loading of 2.5%, Enzyme cocktail (Novozyme 188, Celluclast), 72 h.	87% Sugar yield	Polyhydroxyalkanoates (PHA)	[102]

The use of commercial cellulases is cost prohibitive during ethanol production in the biorefinery. Currently fungal strains of *Aspergillus* and *Trichoderma* sp. are used to produce commercial cellulases for hydrolysis [103]. There is an immediate requirement to produce low-cost high-volume enzymes that can completely deconstruct lignocellulosic biomass. Enzymatic cocktails can be an economically viable solution in biorefineries for 2G ethanol production [104]. While initially deployed on model cellulosic substrates *viz.* Avicel and filter paper, new enzyme cocktails have been used in the hydrolysis of sugarcane bagasse, wheat straw, and corn stover [105]. Méndez Arias et al. [106] demonstrated a 91% hydrolysis of sugarcane bagasse using an optimized enzyme cocktail consisting of crude extracts from *Trichoderma harzianum*

(15%), *Aspergillus niger* (35%), and *Penicillium funiculosum* (50%), which was higher than the hydrolysis achieved using individual strains. In another study, 72% cellulose hydrolysis in hydrothermally pre-treated bagasse was achieved using an enzyme cocktail obtained from a mixture of *Aspergillus niger* (10%) for β-glucosidase, engineered *Bacillus subtilis* (10%) for endoglucanase, and *Trichoderma reesei* (80%) [107]. The hydrolysis of pre-treated sugarcane bagasse using commercial cellulase Celluclast 1-5L admixed with 30% *Aspergillus oryzae* crude extract is observed to be significantly enhanced, which could be caused by the feruloyl esterase and xylanase enzymes in the extract of *A. oryzae* [108]. A synergistic action of hemicellulases and other enzymes is required to efficiently convert hemicellulose into pentose and hexose sugars [109].

Additionally, the hemicellulose hydrolysate obtained during the treatment of the biomass must be fermented and utilized for the complete saccharification of the lignocellulosic biomass [110]. However, ethanol production through fermentation only converts the hexose sugars while leaving behind the pentose sugars along with proteins and other carbon sources as residues [111]. Although there have been studies that report various xylose fermenting fungal and recombinant bacteria, the ethanol yield from them is quite low and are not suitable for large scale ethanol production setups. Thus, to improve ethanol yield and it is important to explore genetically enhanced cellulolytic and fermentative microorganisms [112]. Ligninolytic enzymes derived from white rot fungi can be an option to enhance the performance of enzyme cocktails, but their industrial application is prohibited by the low enzyme concentration production. Lytic polysaccharides mono-oxygenases (LPMOs) are mono-copper non-hydrolytic enzymes which have been observed to degrade lignocellulosic biomass. LPMOs cleave the glycosidic bonds in cellulose and hemicellulose in the presence of reducing agents. The cellulosic activity of *T. reesei* CBH1 is enhanced in the presence of *Aspergillus aculeatus* AA16 LPMO [113]. LPMOs can be used to enhance the performance of commercial cellulase cocktails in converting the cellulose in lignocellulosic biomass. An added advantage of using LPMOs is the reduction in the amount of hydrolytic enzyme needed to convert the polysaccharides into reducing sugars [114]. Oxidative enzyme derived from *Streptomyces coelicolor* have also been reported to enhance the hydrolysis of sugarcane bagasse [109].

Statistical tools can be employed to design and validate the performance and cost of enzyme cocktails obtained by mixing extracts derived from various microorganisms [115]. An estimation of the cellulase enzyme production cost was performed using an open-access process model. Using the *Trichoderma reesei* (*T. reesei*) fungus with a cellulase production of 100g/L in 8 days, the enzyme production cost was estimated to range from $0.34/gallon (loading of 5FPU/g) to $1.47/gallon (loading of 10 FPU/g) [116]. This shows that cost associated with utilization of enzymes is independent of the biorefinery operational parameters and is a significant contributor to the overall biomass processing cost. The cost of enzyme production and utilization is driven up by low cellulase production and specificity, high dosage, and lack of appropriate enzyme cocktail mixtures needed to efficiently hydrolyse the biomass. This requires developing organisms with novel properties and new enzymes with high cellulolytic activity. Additionally, pre-treatment of the lignocellulosic biomass with proteins that can disrupt the cellulose crystalline structure by attacking loosenins, swollenins, and expansins can make the biomass prone to enzymatic treatment [117]. Thus, there is a need to explore and evaluate a range of enzyme sources to concoct novel enzyme mixtures that can hydrolyse lignocellulosic biomass of various types and sources. Techniques such as bioprospecting of microorganisms, metagenomic analysis, and heterologous expression can be employed to attain enhanced enzymatic activities which could drive down the cost of enzymatic hydrolysis.

3.5 Scale-up towards commercialization

With the growth of global demand for bio-based products, it is important to scale-up the biorefineries from lab-scale to industrial setups. But, setting up of lignocellulosic biorefinery is exposed to similar technical challenges as any other refinery plant because of the scale of biomass that needs to be processed to achieve industrial scale productions of bioproducts. The scale up of lignocellulosic biorefinery operations requires efficient management of resources, process optimizations and innovations [118]. Hence it is important to identify the right parameters that need to be considered while scaling up form laboratory scale to pilot and eventually commercial scale (Figure 6). The reproducibility of the results obtained at bench-scale or pilot level is not necessarily achievable on large volume of lignocellulosic biomass due to the number of parameters that impact the yield and compounding of the deterrents at the large scale. Hence, it is important to develop techno-economic models that build upon processing flows, process models and simulation to perform risk and cost sensitivity, life cycle assessments, and forecasting [61]. Additionally, the success of a scaled-up biorefinery is also dependent on the correct combination of process design with biomass feedstock choice. For instance, steam explosion pre-treatment of sugarcane bagasse yields better C6 sugars recovery in fiber and C5 sugar recovery in liquid. Corn stover has been shown to yield better sugar outputs when pre-treated with ammonia hydroxide. It can be concluded that biomass type, composition, and pre-treatment conditions have deep impacts on the design and scale-up of biorefineries [118].

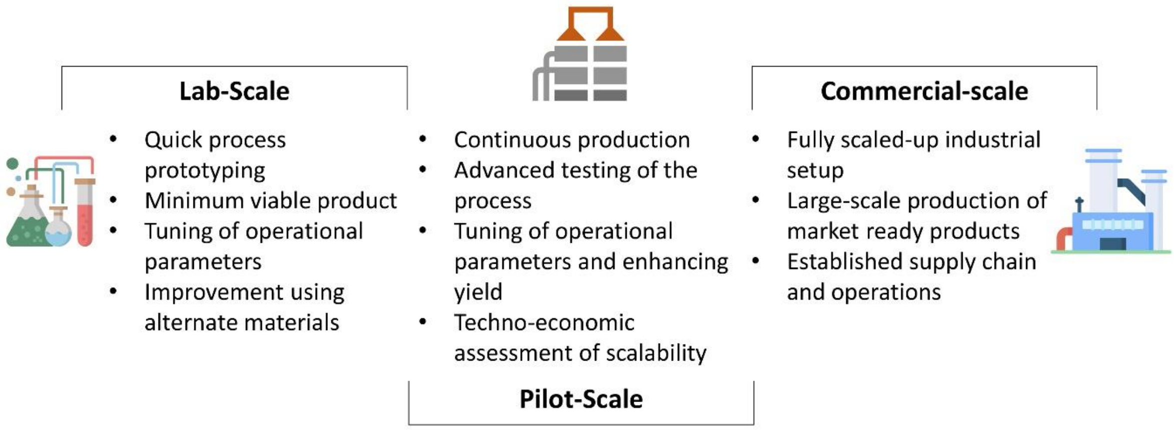

Figure 6. Scaling up a biorefinery: from laboratory to pilot and commercial scale

Successful commercialization is also dependent on achieving operational efficiencies through process automation. There are several opportunities for automation along the biorefinery value chain e.g. automated handling and sorting of biomass, transfer to pretreatment setup, automatic initiation of enzymatic hydrolysis based on present conditions and also during product separation and packaging. While there has been significant progress in the production of ethanol from corn and bagasse, there is still some way to go before complete process maturity and automation is achieved at commercial scale [119]. Furthermore, co-location of biorefineries with existing agri-processing such as beverages and sugar factories can significantly enhance the scalability of the biorefinery while also reducing the overall operational cost by automating the transfer of waste from mills to the biorefinery [120]. Automation can also improve the operational efficiency of multi-product biorefineries as it can enable reduced energy consumption, ease of material handling and removing unnecessary manual interventions [37].

Generally, capital expenditure (CAPEX) would drive the scale-up strategy followed by the operational expenditure (OPEX) of biomass handling and processing. It is important to justify the CAPEX and explore the opportunities to reduce it to the maximum extent possible to prevent a commercial failure. One of the ways to do this would be to utilize the existing infrastructure available in companies already involved in bio-based chemicals production to reduce upfront investments of a greenfield implementation. For instance, Gevo Inc. has created an isobutanol-ethanol side-by-side dry-mill process using yellow dent corn [121]. Other projects that have been commercially deployed to produce biofuels from lignocellulosic biomass include BetaRenewables (2G ethanol), DuPont Biosciences (2G ethanol), Virent (bio-gasoline), and Algenol (4G ethanol), [118]. However, several of the biomass-based companies such as Green Biologics and KiOR Inc. have faced scale-up challenges and have been shut down. Green Biologics was not able to achieve the production levels required to break even and was eventually declared bankrupt due to lack of further funding. KiOR Inc. could only achieve production of drop-in fuel from biomass of 10 t/day as opposed to its target of 500 t/day due to structural design issues. Thus, it is important to continuously innovate and improve the production process to make it sustainable and economically viable.

The biorefinery setup involves multiple complex steps that are required for the conversion of biomass into fuel and chemicals. The level of complexity can vary depending on the features of the biorefinery, higher the number of features higher the complexity index of the biorefinery, and this can be used to build a Biorefinery Complexity Profile [BCP] [122]. For each feature of the biorefinery a Technology Readiness Level (TRL) assessment can be performed on a scale of 1-9 with 1 being "basic research" and 9 being "system proven and ready for full commercial deployment". These features can include overall TRL, feedstocks, processes, platforms, and products. A commercial scale setup can easily be created for a low complexity biorefinery i.e. TRL level 9 [122]. BCP can be used a yardstick to evaluate various biorefineries and their potential in terms of economic and technological risks. The current state of biorefineries puts traditional biorefineries (corn, sugarcane, wood-based) at a TRL of 9, lignocellulosic biorefineries (woody waste, straw etc.) at level 6-8 and marine biorefineries (microalgae, seaweed) at level 5-6 [123]. The utilization of microbial techniques adds up to the complexity of the setup due to scaling challenges associated with microbial factories. For example, product toxicity often limits production to levels below commercial viability, leaving processes untenable [124]. Furthermore, substrate toxicity, especially in impure, complex biomasses, can significantly impede production [125]; this can be a challenge with the use of lignocellulosic biomass. Additionally, chemical treatment of lignocellulosic biomass in biorefineries leads to the formation of by-products that require additional processing adding to the overall run cost as well as potential detrimental impact on the environment.

4 Research Gap and Future Research Directions

There is a considerable growth in the utilization of lignocellulosic biomass in the production of biofuels. There are several advanced biofuel facilities, at different levels of development, across Europe, Asia and North, - and South America that are using lignocellulosic biomass. Although considerable investment from public and private sources have been made into research and development associated with biomass valorization, there is a need for further investment to mature this technology for commercial success [126]. Nonetheless, there needs to be considerable progress made in the biomass treatment and valorization technologies in order to achieve commercialization and economic viability. This will require bringing down the overall cost associated with the setup and operations as lignocellulosic biorefineries require specialized processes and equipment to convert the biomass into end products [127]. There are several individual processes involved in the entire value chain of the lignocellulosic biomass processing and end products production providing ample opportunities for process integration. Avenues of cost optimization include developing more efficient and effective pre-treatment processes [128], improving enzyme efficiency to reduce the amount of enzymes needed [114, 115], total conversion of polysaccharides into sugars [110], converting both C5 and C6 sugars in fermentation [129], and process integration to reduce CAPEX and OPEX [38].

A SWOT (strength, weakness, opportunity, and threat) analysis of biorefineries is discussed in Table 4 elucidating its advantages and challenges it faces to be competitive against conventionally produced fuels and chemicals.

Table 4: SWOT analysis for future development of biorefineries

Strength	Weakness
• Clean, green, and sustainable development of products and energy • Supports the circular economy principles of maximizing resource utilization and leaving very minimal waste • Enables industries such as agriculture, chemicals, and energy to collaborate and create opportunities of sustainable growth • Reduced dependence on fossil fuels thereby reducing climate impact • Enables local and rural employment increasing self-sufficiency and additional revenue source for individuals and industries	• Technical maturity of various valorization techniques is still at lab-scale or pilot scale • Higher investment due to new processes and technology development requirement • Lower existing synergies between feedstock supply and demand • Lack of infrastructure support to enable seamless collaboration between various stakeholders in the biorefinery value-stream • Mass processing of biomass waste is not possible due to difference in treatment and operational parameters depending on biomass source and type
Opportunity	**Threat**
• Clustering of industries such as food processing, chemicals and energy production can create immense synergies reducing the overall cost of production • Immense potential to bring down GHG emissions • Higher knowledge capital creation on the reuse of waste streams • Innovative application of waste to energy and waste to chemicals processes • Reduction in overall waste generation supporting the sustainable development goals of the UN • Environmental and public policies are supporting the growth and investment into the bioeconomy sector	• Conventional production has achieved economies of scale with highly competitive pricing • Cost of biomass-based production using biomass and waste is still significantly high resulting in high cost of end-products • Process improvement and automations have reached high level of maturity in conventional production • Sustainable supply of biomass for biorefineries is threatened by logistical challenges and biomass availability throughout the year • Biofuels although green in nature are threatened by renewable energy such as solar, wind and tidal

The variance in the biomass types and sources determines the technique required for its pretreatment and valorization. There is no ‘one size fits all’ approach in lignocellulosic biomass valorization. There is also a lack of consolidated understanding on the process optimization for various biomass types and valorization techniques. This presents a roadblock in the scale up of lignocellulosic biorefineries commercialization. Thus, there is a need to create a central database of lignocellulosic biomass treatment techniques and the respective optimized process parameters based on conducted research at lab and pilot scale. This would be immensely helpful in create highly optimized and scalable biorefinery setup.

An integrated biorefinery which uses the waste or residual lignocellulosic biomass along with by-products to produce co-products such as heat, energy, fertilizer, bio-chemicals etc. can add to the overall economic value of the biorefinery [130]. The cost-benefit of this setup is realized when high-value low-volume co-products are produced along with the primary product [131]. This would allow for a wide range of feedstocks to be utilized more efficiently than their current utilization [132]. The most useful and obvious co-products include electricity, heat and fuel. Keeping in line with the green focus of the circular bioeconomy, the lignocellulosic bioconversion and its optimization must include a systematic enhancement to performance of microorganisms and enzymes, with a focus on the environmental impact and cost. Despite the obvious advantages of biological treatment technologies involving microorganisms and enzymes they are slow and highly dependent on the pH, temperature, humidity and growth rate. There is research underway to tackle these challenges such as discovery of new ligninolytic and cellulolytic strains and co-culture systems that have high performance under stress [133, 134] as well as optimization of operational parameters for better performance [135]. An advanced technique *viz.* phenotypic microarray allows a rapid screening of lignocellulosic biomass hydrolysate fermentability by measuring the metabolic output of yeast. Using this technique, acid pre-treatment was determined as the most suitable approach for wheat straw as opposed to alkaline and autohydrolysis pre-treatment [136]. There are huge costs associated with the utilization of enzymes, especially if in their commercial applications.

The high cost of enzymes can be tackled with the exploration and genetic modification of various bacterial and fungal species that have better lignocellulolytic performances for biomass treatment and saccharification. Function-based metagenomic approaches can offer insights into the DNA of microorganisms and can allow cloning of genes into suitable hosts, thus providing an opportunity to increase the expression and secretion of biomass-degrading enzymes [137]. Another approach is to use enzyme engineering *in vitro* through random mutagenesis to develop industrially applicable biomass-degrading enzymes [137]. Companies such as Novozymes and DuPont have made success into developing commercial enzymes. For instance, enzyme cocktail-Cellic CTec 3 and Cellic HTec 3 developed by Novozymes has shown the most promise in commercial applications for enzymatic hydrolysis of biomass. But competitiveness in the enzymes market is essential to monopolistic behaviour and reduced prices. Co-fermentation or simultaneous saccharification and fermentation process performs the enzymatic hydrolysis and fermentation in the same step allowing for microorganisms to use the released sugars simultaneously [138].

Despite advancements in the process optimizations there needs to be further research attempted to enhance the ability to recover and reuse chemicals and by-products from the pretreatment step to improve overall cost-effectiveness. For instance, fungal enzymes could be recycled without much reduction in their effectiveness after degrading lignin in lignocellulosic biomass. Another challenge to be tackled is the mitigation of inhibitory effect of furfurals, weak acids and phenolics formed during pretreatment step. Utilization of biochar, ionic resins and membranes could be a potential route to remove these inhibitors [86]. Some of the inhibitors such as acetic acid formed during the process are useful reagents for recycling enzymes during the pretreatment process. Effective detoxification in the pretreatment step is critical to the success of the commercialization journey of biorefineries. This also would be helpful in reducing the energy requirement of the process.

Although liquid biofuels derived from lignocellulosic biomasses have achieved commercial scalability, biogas production remains limited. This is primarily due to the setup requirement (large digester adding to the setup cost), need to maintain optimal operational conditions for microbial growth (37°C with long retention time), and low output volume at lower temperatures. Thus, there is a need to develop advanced techniques to establish simple and cost-effective processes for biogas production from lignocellulosic biomass. Kutsay et al. [139] in their study developed a detailed process design of commercial biogas plant based on thermal expansion pre-treatment which increased biomethane production by 50% along with effective waste treatment and energy recycling within the plant. Another important step towards commercialization is to study the operational parameters of pilot scale setups and extrapolating the findings in detailed techno-economic assessments to optimize the upstream and downstream processes to build models for commercial biorefineries for lignocellulosic biomass. Downstream processing or product recovery in lignocellulose biorefineries post-fermentation can be achieved through crystallization, purification and concentration, and centrifugation or distillation. The lignin residue at the end of the process could be reutilized in the boilers to generate energy due to its high energy density [53].

5 Conclusion

Global environmental challenges are pushing nations to attempt large strides towards establishment of modern bioeconomy. This strategy is fuelled by biorefineries and circular economy concept which advocate the utilization of bio-based feedstock such as lignocellulosic materials and wastes. The abundance of lignocellulosic biomass available from forests, agriculture and industrial sources has encouraged its application in the production of renewable biofuels. There is an extensive research ongoing to overcome the challenges in its pre-treatment and saccharification such as cost minimization and combining various methods to achieve scalable operations. Although biorefineries have primarily targeted energy production, their focus is now including bioproduct manufacturing as a promising addition to their operations. Lignocellulosic biorefineries have the potential to become sustainable sources of value-added chemicals and products such as organic acids, PHA, biofuels, and bioplastics etc. at competitive prices. However, the inherent recalcitrant nature of lignocellulose adds to the complexity and cost in achieving full-fledged commercial scale. Although chemical and physicochemical pre-treatment methods have proven to be effective in converting the biomass into reducing sugars, they still are bogged down by environmental impacts, high investments, and inhibitors formation. Biological treatments such as such as microbial and enzymatic pre-treatments are eco-friendly but time consuming (15–40 days), impacting the overall cost-effectiveness. Thus, developing a simple, sustainable, and cost-effective pre-treatment setup (single or combination) will accelerate lignocellulosic biorefineries on the path to commercialization. Additionally, the recovery and reutilization of by-products, enzymes and chemicals into valuable products and other steps of the production process will reduce waste and add to the process efficiency. While performing techno-economic assessments for industrial scale-up some of the key factors to be focused on include biomass availability, pre-processing, transportation and handling. Additional considerations also include fixed costs (infrastructure and equipment cost), variable costs (labour, maintenance, power and chemical costs), plant runtime, waste processing and disposal.

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Conflict of Interest

The authors declare no conflict of interest.

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