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Review

Consolidated Bioprocessing, an Innovative Strategy towards Sustainability for Biofuels Production from Crop Residues: An Overview

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Abstract: Increased energy demands in today's world have led to the exploitation of fossil resources as fuel. Fossil resources are not only on the verge of extinction but also causing environmental and economic issues. Due to these reasons, scientists have started focusing their interest on other eco-friendly processes to biofuel and recently, second-generation biorefinery is gaining much more attention. In second-generation biorefinery, the main objective is the valorization of lignocellulosic biomass cost-effectively. Therefore, many scientists started different bioprocessing techniques like Consolidated Bioprocessing (CBP) to produce ethanol by using a single or plethora of microorganisms to produce ethanol in a single process. In this review, in-depth study on CBP is assessed as well as biofuel's socio-economic value and a brief study of biorefineries. The study not only involves innovative approaches used in CBP but their effect on society and economic aspects.

Keywords: biomass; biorefinery; circular economy; enzyme; consolidated bioprocessing

1. Introduction

The finite nature and rapid depletion of fossil fuels due to growing global energy demands have negatively impacted the environment as their combustion entails have led to the search for alternative ways of producing fuels from sustainable, renewable, green, and economical energy sources [1–5]. One source of renewable energy production is biomass, which can be transformed into biofuels, which provide many advantages over fossil fuels in terms of mitigating residues generation and greenhouse gas (GHG), increasing energy independence, and improving the economy of agriculturalists [1,2,6,7].

The term biofuel refers to any liquid, gas, or solid fuel predominantly produced from a renewable biomass feedstock. Biofuel examples are bioethanol, biomethanol, biosynthetic gas (biosyngas), biodiesel, biogas (biomethane), biochar, bio-oil, biohydrogen, and Fischer_Tropsch produced liquids [1,8]. Biofuels serve as a bridge between the agricultural and energy markets as agricultural commodities are the significant feedstocks in biofuel production [9].

Recently, crop residues as a potential source of feedstock to produce bioenergy have been gaining importance as they are not in competition with food production for human consumption. They have high availability, wide distribution, and almost zero cost [1,10,11]. Crop residues are generally the waste generated in the harvesting of any agricultural crop. These are typically the parts of the products that are often of no value, including the stem, empty fruit bunches, leaves, and stalks. The amount of agricultural waste is variable and reaches 50% for specific crops [12]. In the production of commodities like sugar, rice, flour, starch, and oil, only a fraction of the main crop is recovered and used. The leftover fraction of the processed products are referred to as agro-industrial crop residue. Generally, these residues are either bagasse, molasses, the spent fibrous pulp left behind from stalks of crops, or seed coats, shells, and husks [1,12]. The optimal use of agricultural wastes has excellent economic and ecological advantages due to the possibility of recycling and producing materials with added value [2]. Wheat straw, rice straw, corn straw, and sugarcane bagasse are the agricultural residues available in the approximate amounts of 354.34, 731.30, 128.02, and 180.73 million tons, respectively. The primary composition of these residues is cellulose (20 to 60%), hemicellulose (20 to 40%), and lignin (10 to 25%) [1,13].

Production and use of biofuels made from crop residues could prevent the over-exploitation of fossil fuel and low waste management problems in the field. However, this potential can only be unlocked if they are cost-competitive to petroleum and starch and sucrose-based biofuels. To reduce capital and processing costs, simplifying the process scheme, and integrating as many unit operations as possible is necessary. Therefore, the consolidated bioprocessing CBP is a strategy where all four steps occur in a single reactor. A single microorganism or microbial consortium converts pretreated biomass to a commodity product such as ethanol without adding saccharolytic enzymes, which would represent an innovative breakthrough for low-cost biomass processing. In principle, a CBP strategy can be applied to produce a broad range of chemicals from natural biomass. It requires degrading recalcitrant biomass substrates into solubilized sugars and metabolic intervention to direct metabolic flux toward desired products at high yield and titer [14–16].

The increase in population brought a greater demand for food and energy; this requires an increase in agricultural production and an increase in agricultural waste, which presents enormous challenges for its proper management. Although crop residues represent a potential source for the generation of biofuels, the environmental impacts that their use could cause must also be critically evaluated [16]. Therefore, this work aims to briefly describe the current situation of biofuels, available raw materials, and their production strategies, especially the CBP approach, a strategy that is aimed at the best option for the sustainable production of biofuels.

2. Biofuels

2.1. Worldwide Scenario of Biofuel Production

We are strongly dependent on fossil fuels due to the extensive use and utilization of petroleum derivatives limiting the use of petroleum resources, resulting in environmental and political issues [17]. Questions regarding ecological stagnation and unpredictability dependent on the future reservoir and increasing oil prices have been inspiring methods relying on alternative energy sources [18]. At a national, regional, and global level, there is acceptance of plants' raw material (i.e., biomass) at an increased level. It can reinstate a large portion of fossil resources as feedstock for the industrial generation of both the energy and non-energy (i.e., chemicals and materials) area [17]. It has been reported to be approximately 2.7% of global transportation (~2.9 TW) that is dominated by first-generation biofuels. Ethanol derived from sugarcane and cornstarch (28 billion gallons in 2018) and biodiesel from rapeseed or soybean oils (8 billion gallons in 2018). These raw materials are also a segment of the food chain supply and do not serve a long-term, high-scale solution; therefore, first-generation biofuels will not be a considerable section of the transportation fuel supply in 2050. Therefore, it is essential to replace the 1G biofuels gradually with lignocellulosic biofuels [18].

2.2. Challenges to Biofuel Production

2.2.1. Policy Initiatives

Several problems are faced in the production of biofuels, among which the production costs above the fluctuating price of fossil fuels stand out, and the ecological challenges during biofuels production. The challenges faced are explained in this section.

Various industry considerations (climate change, pricing ambiguity, and geopolitical uncertainty) are taken into account to explore the utility of substituting biofuels for traditional petroleum-based fuels as a clean energy alternative. Therefore, the U.S. renewable fuel standard (RFS_2) was mainly designed to motivate biofuel production, relying on reducing the greenhouse gas emission related to petroleum fuels [19].

Different types of biofuels produced at a global level have overcome 35 billion gallons during transportation in 2015, and by 2023 will reach 62 billion gallons. The International Energy Agency (IEA) evaluate that biofuels have the probability of supplying continuously from their ongoing share of 3% to about 27% of total transportation by liquid [20].

At this moment, our world´s main objective is to maintain economic development without increasing environmental degradation. Bio-based industries can dramatically alleviate pollution and ecological damage after the industrial revolution [21]. Twenty years ago, Rio's acknowledgment of environment and development took the first step to solve this unorganized scheme for the future. In this session, several countries reviewed the matter of social, scientific, and technological development. The use of agricultural residues was one of the activities affecting all the fields [22]. This valorization consolidates the utilization of second-generation biorefineries.

2.2.2. Market Challenges

One of the dominant objectives in manufacturing renewable fuels is encompassing the target price of 0.79 $\$L^{-1}$ [23], agreed by the Bioenergy Technologies Office (BETO). Scientists can enhance renewable fuel generation's industrial growth by utilizing non-profitable biomass such as forest residues as a feedstock and producing yields from selling co-products [23]. The shortage of fossil fuel has led to increasing concern regarding greenhouse gas emissions and air pollution. This has resulted in rising interest in bioethanol production from natural resources like algal strains, notably from lignocellulosic biomass via enzymatic route. The estimation for biofuel growth by 2020 will be 4% of road transport applications, and according to USDA, global ethanol production will grow to 40% by 2022 [24]. The main aim of the biorefinery is to produce broad-spectrum merchandise in a cost-effective and eco-friendly way. Therefore, second-generation biofuel production is the most feasible process to generate bioethanol in competition with food resources and fertile land. Hence, in the long run, bioethanol production from lignocellulosic material through enzymatic hydrolysis is a valuable process, along with a probability of huge output [25]. It has been reported that 44% cost of biofuel production in 2G ethanol production is from enzymes. The researchers recommended that on-site or near-site enzyme generation encourages access to the remarkable decrease in enzymes' value up to 30–70% carrying its interpreted purification and logistics [25,26]. According to another author, the cost of enzymes at 15% and 35% solid pretreated loading during enzymatic hydrolysis varies from 34.63% to 36.38%, respectively [25,27].

2.2.3. Social and Socio-Economic Issues

The problem that is being faced regarding the depletion of fossil fuels is the involvement in energy security, particularly in the countries that are energy-dependent because of constant clashes in the oil-exporting countries and climate change as a result of the burning of fossil fuels. All these problems are arising due to fossil fuels: society realized biofuels to solve these problems. Specifically, the thriving biofuel sector could administer the opportunity to develop rural areas and generate job opportunities for the local residents, working towards the development of healthy, efficient communities and reducing the emission of greenhouse gases. Recently, some issues regarding biofuel generation and its use have appeared, such as biofuels' effect on energy and food markets, working conditions and workers' rights regarding occupational health, the disparity of biofuel policies, the land change, etc. should also be recognized [28].

2.2.4. Sustainability

For biofuel sustainability, it is necessary to deal with the various complex and conflicting estimates at stake. Hence, the biofuel capability to grant to one particular value cannot guide any definite conclusion regarding biofuel's overall sustainability. The range of the sustainability opinion may differ depending on individuals' inclination, the time scale, and the geographical region. The five pillars of the sustainability theory encompasses social, economic, environmental, legal, and cultural considerations. In a recent study, research proposed estimating various biofuel sustainability objectives for France by 2030, along with a stakeholder-driven perspective; the stakeholders recognize 22 different sustainability standards for biofuels. Therefore, they had a shallow level of agreement between the various professions (feedstock producers, biofuel producers, refining industry, fuel distributors, car manufacturers, end-users, government, and non-governmental organization (NGOs)). The sustainability evaluation initiation, a set of indicators, has been recognized by stakeholders authorized to quantify biofuel's scope to fulfill each of their criteria. There were seventeen biofuel objectives evaluated regarding economic, social, environmental, cultural, and legal considerations, allowing the identification of each biofuel's strengths and weaknesses. Biofuel sustainability evaluation needs a review of a wide variety of different objectives to endure with their multidimensional impacts. Therefore, the biofuel capability to accomplish one particular goal cannot reach any infinite conclusion regarding overall biofuel sustainability, policy efficiency, and if it has a positive or negative impact [29].

Many studies are done on the use of various organic wastes to produce biofuel. These processes have been reported to be successful in response to global challenges like fossil fuel dependency, production cost optimization, and waste management. In addition, the matter of emission control and after so many investigations, everyone concluded that biofuel production from organic waste has an excellent possibility for sustainable and economic development while confirming minimal environmental influence and overall production cost [30].

Despite the advantages that lignocellulosic residues represent for the production of biofuels e.g., value added to crops, reducing GHG emissions and not competing with food, some issues must be critically evaluated [1,16]. Currently, crop residues are used in animal feed or left in the field to avoid erosion, or incorporated by plowing for the recycling of nutrients. Therefore, its use in a massive way could break the balance in the carbon-nitrogen relationship, decrease the amount of nutrients, and affect the soil, causing a severe problem in agricultural land quality [16]. Therefore, it must be established for residues, and the extraction limit of biomass in the corn crop case must also be established. Some data mention that 40% of the residues can be collected in an intensive cultivation system and up to 70% of the residues in a no-tillage system to keep the low risk of material organic and soil structure loss [31].

2.2.5. Biorefineries

Plant-based raw materials (i.e., biomass) are progressively recognized as a possible future resource to supersede large fractions of fossil resources as feedstocks. Therefore, to meet both the energy and non-energy demands for industrial purposes (i.e., chemicals and materials) sectors, fossil fuel exploitation at an outside scale occurs [32]. Bioenergy, biofuels, and biochemicals are the three primary national, regional, and global biorefineries. Building policies to focus on one driver can have destructive effects on others like local coal reserve exploitation, which increased greenhouse gases, resulting in global warming. There are other alternative renewable resources (wind, sun, water, biomass, etc.). However, biomass alone is often a feasible substitute for fossil resources to transport fuels and chemicals, because it is rich in carbon materials present on earth and in fossil fuels [32]. The perception of biorefinery influences a vast scope of technologies competent of separating biomass resources. i.e., wood, grass, corn, etc. into their constituents, i.e., carbohydrates, proteins, triglycerides, etc., resulting in being reconstructed into superior products, which are biofuels and chemicals [32]. Current industrial science trying to approach compounds from a single agriculture residue is among the new investigation exercises worldwide. Biorefineries are eco-friendly and very similar to oil refineries [33]. The objective of oil refineries is to procure comprehensive products from petroleum through a diverse system. The same theory is practiced on biorefineries. The first basic concepts of biorefinery were administered in 1990. Before 1997, images of continuity, environmental awareness, and biorefinery merged with the notion of green technologies [34].

2.3. Generations of Biofuels: A Brief Review

Fuels produced by the biological process are known as biofuel. Primary biofuels are mostly fuelwood, wood chips, pellets, and organic materials, generally used for heating generation, cooking, or electricity purposes in a crude state. Secondary biofuels come from energy crops, agricultural and forestry wastes and by-products, manure, or microbial biomass used extensively in transportation and industrial purposes [35,36]. Innovative research on biomass for secondary biofuel production has been continuous for the last five decades of biofuels production. A wide variety of potential feedstock from all around the world is utilized for biofuels production. Based on the feedstock, biofuels are classified into four groups, namely: first, second, third, and fourth-generation biofuels [37–39].

- First-generation. 1G biofuels are produced from specific parts (usually edible) of oil-based plants and starch and sugar crops. Initially, 1G biofuels showed a promising capability to reduce fossil fuel combustion and lower atmospheric levels of CO_2, which is consumed by crops as they grow [39,40]. Ethanol represents the most common biofuel produced to date. Nowadays, 78% of biofuel's total production contributed by bioethanol produced around 28 billion gallons per year from central corn in the U.S.A. and sugarcane in Brazil [38,41]. However, this generation's biofuels increased production by raising questions due to its production-generating competition food production vs. fuels, arable lands, and biodiversity loss, in addition to being responsible for ecological degradation [37,42]. Studies have shown that biofuels obtained in this manner frequently do not contribute to greenhouse gas reduction, and they require a large amount of energy for their production [43].
- Second generation. 2G biofuels, known as "advanced biofuels," overcome the problem of competition "food versus fuels" by using inedible raw materials, in addition the net carbon (emitted–consumed) from combusting second-generation biofuels is neutral or even negative [42]. Innovative processes producing 2G biofuels from non-food feedstocks sources of biomass included: residues produced by agricultural and food processing systems (discarded biomass), manure, used cooking oil, wood and sawdust to garbage, food waste, and energy crops [35,36,38,42]. Among these sources, the lignocellulosic residues of crops would more likely to be the primary candidates for its abundance, wide availability throughout the world and at times of the year, and its low cost [1,41]. However, the structural heterogeneity in these residues' composition requires more complex production processes, making 2G biofuels not industrially profitable [39,41,42]. Today, the estimated production of 2G biofuels is around 0.4 billion liters/year, i.e., <0.4% of the overall ethanol production. IEA for advanced biofuels estimates a cellulosic ethanol production increase to 0.8 billion liters in 2023 [44].
- Third generation. According to the IEA definition, third-generation biofuels are bio-based fuels produced from aquatic feedstock (usually algae) [36]. Algae are a promising alternative feedstock due to their high lipid and carbohydrate contents, increased carbon dioxide absorption, and the possibility of cultivating wastewater and seawater. Unproductive drylands and marginal farmlands do not compete with food crops on arable land or in freshwater environments [36,38]. Another characteristic that makes algae interesting for biofuels is the low level of lignin. The growth rate is high [38,42] and the possible production of biodiesel, butanol, and methane ethanol. Some green algal species can photolyze mediated biohydrogen (H_2) production [35,45].

However, this type of biomass has disadvantages such as its high initial investment for its production. The biofuel produced from algae is less stable than that produced from other sources, mainly because the oil generated by the algae is highly unsaturated, which means it is more volatile at high temperatures, so it is more likely to degrade. Furthermore, the high water quantity is also a problem when lipids have to be extracted from the algal biomass, requiring dewatering via either centrifugation or filtration before extracting lipids [46].

- Fourth generation. These biofuels, which are still in the developmental stage, use bioengineered microorganisms as microalgae, yeast, fungi, and cyanobacteria. Genetically altered crops used to consume more CO_2 from the environment than they emit. These microorganisms are used to produce different fuels, including ethanol, butanol, hydrogen, methane, vegetable oil, biodiesel, isoprene, gasoline, and jet fuel [39]. Fourth-generation biofuel research started in 2006, and significant results have not been published yet in peer-review journals [35]. Figure 1 shows the schematic representation of the integrated biorefinery for 1G to 4G.

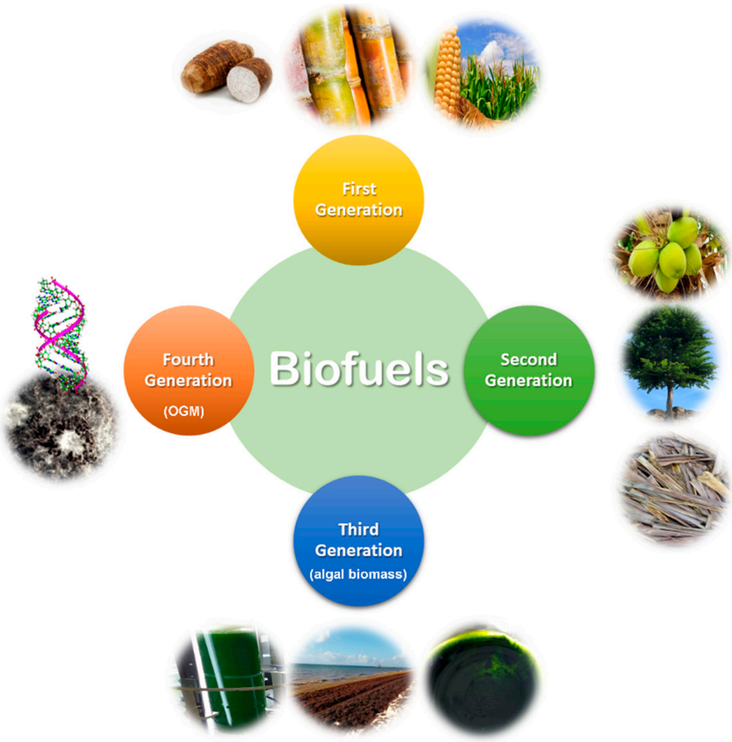

Figure 1. Schematic representation of integrated biorefinery for first, second, third, and fourth generation of biomass.

2.4. Scale-Up

Bioprocessing Techniques

The dominant predicament in cellulase manufacturing is its low enzyme titer. Numerous scholars have endeavored to crack distinctive representations to amplify cellulase enhancement in fermentation technology. It encompasses superior bioprocess performance, employing low-cost material or rudimentary raw materials as a substrate to produce enzymes and genetically obtained microorganisms etc. [47]. There are commonly two kinds of fermentation techniques that focus on enhanced cellulase production: solid-state fermentation (SSF) and submerged fermentation (SMF). These fermentation techniques use microorganisms such as fungus and bacteria. Recently, a new bioprocessing practice developed successively by using bioprocessing techniques in a single reactor. All the bioprocessing practices are discussed in detail in this section, including advancements in new bioprocessing techniques, i.e., CBP and other methods for better enzyme generation.

Currently, scientists mainly focus on the scale-up process of both 2G and 3G biofuel. However, both the sectors are facing problems that are causing interferences in the scaling-up process.

In the 2G biorefinery, in-house enzyme production and enzymatic hydrolysis play a crucial role in reducing the cost and scaling up 2G-ethanol production. From the two types of fermentation, SSF has many advantages but its use is confined at mostly lab scale because of some obstacles in controlling specific parameters and operating variables, which hugely impact microbial growth and metabolite production [25,29]. Moreover, some other drawbacks corresponding to SSF are heat and mass transfer, and high equipment costs [25].

As stated by techno-economic research, the potential to operate biomass at high solid loading during enzymatic hydrolysis will be essential in 2G biorefineries, principally. The equipment cost will come down and can lead to minimizing the requirement process energy and wastewater treatment. However, the enzymatic hydrolysis at high solid loading is somehow not an easy occupation to handle as pretreated solid biomass viscosity increases enormously and starts showing firmly non-Newtonian flow properties. The complications with both mass and heat transfer in the material and mechanical problems are pumping and adequate mixing of the biomass slurry. In SSF, researchers observed ethanol production at high solid loading during simultaneous saccharification and fermentation (SSnF) requires approximately 60% of the energy content while mixing in SSF. All processes are studied at lab scale. Nevertheless, there are several parameters like flow behavior (or even the flow regime) and particular power inputs that change with the reactor scale [48]. According to [49], their study focused on evaluating a prospective fungal strain *Penicillium oxalicum* IODBF5 in the production of cellulase under submerged fermentation. The first shake flask experiment was performed with this strain at 28 °C, 180 RPM and incubated for 8 days and initial cellulase activity reported was 0.7 FPU/mL, which later increased by 1.7 fold i.e., 1.2 FPU/mL in 8 days. After optimizing specific parameters, the same experiment was performed at 7 L reactor level in which the temperature maintained was at 28 °C and pH at 5.0 by adding 1 M HCl or 1 M NaOH. Fungal spores inoculated around 10^9 spores/L, and initial airflow kept was of 1vvm which after 72 h increased to 1.5 vvm and the incubation time was reduced from eight to four days with the same reproducibility. For effective enzymatic hydrolysis with the crude enzyme, the temperature and pH were maintained at 50 °C and 5.0, respectively. At 50 °C, the natural enzyme preserved their 50% and 26% of their activity at 48 h and 72 h, respectively. This property of Truffle was reported by [50] in their study where the capability of *Tuber maculatum* to secrete an extracellular cellulase during SMF was explained for the first time in their research. In SMF basal salt media was used with sodium carboxymethyl cellulose (0.5% *w*/*v*) as a carbon source at pH 7 and the cellulase activity reported was 1.70 U/mL after seven days of incubation. The stability test was performed, and it was observed that the enzyme was stable at 50 °C and pH 5.0. The enzyme was thermostable as well as maintained its 99% activity at 50 °C temperature. Tray Bioreactor, Packed bed Bioreactor (PBB), Rotary Drum Bioreactor (RDB), Fluidized Bed Bioreactor (FBB) and Instrumented Labscale Bioreactor (ILB) are usually used as bioreactors for SSF systems [51]. According to the study performed by [52], cellulase was produced by microorganism *Trichoderma reessei* RUT C-30 under SSF. The substrate used in SSF was wheat bran and cellulose, where cellulose was used as an inducer for further enhanced cellulase production. The combination of wheat bran and cellulose was added in a 250 mL Erlenmeyer flask. The substrate moistened with mineral salt medium and pH was maintained at 4.8 by adding 1 N HCl and 1 N NaOH. This experiment was optimized by involving a two-stage statistical design of experiments, resulting in an increase of CMCase by 3.2 fold i.e., 959.53 IU/gDS. This method was repeated at pilot scale and the reactors used were tray fermenters, and the same lab conditions were used, resulting in 457 IU/gDS yield. Cellulase produced in this experiment was used for enzymatic hydrolysis of alkali pretreated sorghum stover with or without BGL and the BGL used in this experiment was extracted from *Aspergillus niger*. The result from hydrolysis with BGL was reported to be 174% efficient. The hydrolysate produced from hydrolysis of sorghum stover was used in the fermentation process to generate ethanol, which resulted in being around 80% effective. In another study, crude olive pomace and exhausted olive pomace was used in SSF as substrate. They both were pretreated by ultrasound pretreatment, and their comparative study was also performed. In SSF, both wastes were checked and used as a substrate in SSF to yield cellulase and xylanase by fungi. The use of exhausted olive pomace as substrate by *Aspergillus* resulted in higher titers in the screening. They were therefore used in the experiment of ultrasound pretreatment. The outcome of sonication showed a 3-fold increase of xylanase activity and harmed cellulase activity. Furthermore, the liquid part acquired from ultrasound pretreatment was used to maintain substantial moisture, resulting in a positive effect on enzyme activity, which caused a 3.6-fold increase and 1.2-fold increase, respectively [53].

To date, large-scale algal cultivation still presents various mechanical threats that interfere with the process of commercialization as a promising aspect of algal biomass as a renewable feedstock for biofuels production. These threats or challenges are related to upstream or downstream processing.

In upstream processing, these are the algae and algal cultivation process selection process, the energy input for handling closed-photobioreactors, nutrient sources, water reusability, and footprint and susceptibility of algae towards the surrounding.

In downstream processing, this is attributed to harvesting and drying technology for algal cells, efficient algal lipid extraction methods, biodiesel conversion technologies from algae and biodiesel, and probability to transform biofuels production from the algal residue after extracting the lipid. After the technological hurdles, merchandising algal biofuels production's profitable viability is still dubious because algal cultivation and correlated biofuels production technologies are in shortage. Enhance algal biofuels' commercial potential: it is crucial to understand and recognize technical and economically related problems [54].

3. Consolidated Bioprocessing

3.1. Strategies for Development Ideal Consolidated Bioprocessing-Enabling Microorganisms

In searching for CBP functional organism, two different strategies have been applied to endow the microorganism with the capabilities required, such as high hydrolysis rate, tolerance to a compound derived from pretreatment, and proper fermentation.

3.1.1. Native Strategy

The native strategy focuses on studying organisms with the natural capacity to produce different enzymes and use different substrates to improve biofuels' performance. Some approaches to enhance bioprocessing capabilities include adaptive evolution and isolation of new strains for CBP [55].

The native strains proposed for use in CBP are mainly wild-type strains that are generally poorly characterized. To date, genetic manipulation tools are established for only some of them, and in a few cases, their metabolisms are investigated in-depth. Concerning cellulolytic fungi, most genetic engineering efforts are focused on increasing cellulase production. However, there is a growing interest in biofuel production using these organisms. Candidates for the native strategy can be classified into three groups: fungi, bacteria that produce and excrete enzymes, and cellulosome-forming bacteria [56–58].

However, to date, the organisms discovered with the capacity to carry out CBP are well below efficient alcohol production expectations. Therefore, the co-cultivation of two or more different organisms (consortium) taking advantage of their capacities-specific metabolism is a promising method to improve substrate conversion and ethanol yield [56]. A microbial consortium is an association of two or more organisms acting together as a community in a complex system, where all benefits from others' activities can be enjoyed [59]. Consortia can be classified into natural, artificial, and synthetic. Natural consortia are symbiotic due to co-evolution; in contrast, artificial consortia and synthetic consortia are defined as mixed crop systems, differing from each other. In synthetic consortia, genetic modifications are performed to achieve specific interactions between mixed strains [60].

3.1.2. Recombinant Strategy

The recombinant strategy aims to provide the fermenting organisms with the hydrolytic capacity. The hydrolytic organisms to provide them with the fermentation capacity. Therefore, before designing microorganisms for biomass conversion, it is crucial to select host organisms with the desired characteristics, emphasizing strains that can use low-cost substrates, resistance to environmental stress, and a high yield of the desired product [61,62].

The main challenges in the recombinant strategy include the adverse effects of the co-expression of multiple unwanted genes, the modulation of the expression of different genes at the appropriate levels, and the improper folding of proteins, which can prevent their secretion, in addition to inadequate fermentation pathway [61,63].

Recent advances in the development of modified microorganisms through evolutionary, metabolic and genetic engineering approaches have paved the way for using lignocellulosic biomass as a substrate for biofuel production [63].

3.2. Aspects of Consolidated Bioprocessing

3.2.1. Economic Viability of Consolidated Bioprocessing

CBP is a promising strategy for effective biofuel production due to the combination of three processes (enzyme production, saccharification, and fermentation) in CBP. This strategy can reduce the reactor and the enzyme cost, which are the major impediments to low-cost biomass processing [61]. In the conventional ethanol production process, the cost by CBP production can be reduced by 25%. When considering capital, raw materials, utilities, and yield loss expenditures, a comparative cost analysis conducted on ethanol production resulted in a projection of \$0.04 gal^{-1} for CBP. At the same time, saccharification and co-fermentation was projected at \$0.19 gal^{-1} [64].

Despite the potential advantages that CBP represents, some limitations have not allowed bioethanol's industrial production to use this strategy. When using native organisms, the main problem is low yield and productivity despite long fermentation process periods of between 3 and 12 days [55]. In the case of using engineered modified organisms, a significant hurdle for industrial CBP organism development is achieving high levels of enzyme production without compromising ethanol productivity because there are generally problems associated with the co-expression of multiple genes other than those of interest, tolerance to the culture medium, in addition to the high cost inherent in the production of this type of organism [39,65] (Table 1).

Table 1. Advantages and disadvantages of CBP [63].

Advantages of CBP	Disadvantages of CBP
• Simplification of total operation process • Fermentation and saccharification vessels are reduced • Enzymes addition are totally eliminated • Risk of contamination is minimal by reducing carbohydrates and producing ethanol • Co-fermentation of pentose and hexose • Capital investment is highly reduced to the lowest minimum • Less energy is required during the process	• To date, only engineered microbial strains are known to perform optimally in CBP. • The use of recombinant organism is highly restricted in some countries, and there is growing public health concern and environmental risk associated with this. • Wild-type bacterial/fungal species tested to date on CBP produce very low concentrations of ethanol. • CBP typically requires long periods of fermentation

Some pioneering companies that use CBP to produce lignocellulosic ethanol are the American companies Qteros and Mascoma, who used the microorganism *C. phytofermentans*, registered as Q Microbe® (Marlborough, MA, USA). In 2014 Mascoma was acquired by the Canadian company Lallemand and all its assets, and currently continues to work in the production of advanced biofuels [55].

3.2.2. Long Term Economics of Consolidated Bioprocessing

The increasing demand for oil and cleaner energy research has led to an escalation in biofuels production (mainly bioethanol). The worldwide production from 2008 to 2017 presents an annual growth of 5.07% of bioethanol. The ethanol consumption is projected to reach 164 billion liters by 2030, 1.98 times the consumption of 2018. Increased demand for bioethanol is motivated by increased concern about climate change, the annual increase of 3.8% ± 0.5 in the global car fleet, and the fact that ethanol's production and consumption blended with gasoline has been promoted through subsidies, mandates, and financing for research in 65 countries [44,66].

Even though the large-scale techno-economic analysis of consolidated bioprocessing [67] has hardly been reported in the literature, the strategy is presented as a promising technology for biofuels. Different chemical compounds with high value-added compounds, from different types of residual biomass under the circular economy's perspective, are the resource of other biochemical processes. Hence, more beneficial impact is generated long-term. Although it is still a challenge, it is a way to take advantage of waste and convert it into a source to build capital rather than waste it [68]. Any technology that involves the transformation of biomass is aligned with a sustainable model.

3.2.3. Consolidated Bioprocessing (CBP) and Some Case Studies

In recent years, the consolidated bioprocess CBP, initially known as direct microbial conversion (D.M.C.), has been investigated. CBP's critical difference with other biomass bioprocessing strategies lies in the use of a single organism or their consortium for enzyme production, hydrolysis, and fermentation. These are generally carried out at room temperature, which results in the reduction of production costs [64,65,69,70]. Furthermore, the hydrolytic and fermentative processes' compatibility means that a single reactor is required, simplifying its operation. Although CBP has great advantages compared to other production processes, there are issues such as long fermentation periods and low biofuel yields due to the formation of various by-products (organic acids), the sensitivity of microorganisms to alcoholic solvents, and growth limited in the supernatant of hydrolysis, which requires the continuous search for more efficient strategies [64,69].

However, the industrial-scale application of CBP remains challenging so far because of the low efficiency. Derived from CBP: Consolidated Bio-Saccharification (CBS) strategy proposed fermentation separated from the integrated process. CBS produces fermentable sugars as the target product rather than end metabolites such as ethanol. Consequently, fermentation would not be limited by hydrolysis condition. The cellulolytic capability is maximized as well. Thus, CBS provides new insight into lignocellulose bioconversion [11,71]. Figure 2 shows the schematic representation of the consolidated bioprocessing process in terms of biorefinery. The advantages of SSF are linked to the utilization of agro-industrial residues as solid substrate substituting for carbon and energy sources, however enzyme production for industrial purposes still surfaces into certain industrial restrictions [72]. On the contrary, SMF is a universally acknowledged fermentation process for industrial enzyme production as it is more convenient to control all the variables such as pH, temperature, and operational techniques. The CBP strategy proposed in 1996 and increasing evidence supports that CBP may be feasible. CBP's research has focused on developing new and even more effective CBP microorganisms, a critical challenge [73,74]. Several studies were performed for sustainable ethanol production using advanced consolidated bioprocessing. In one research study, the subsequent pairing used in CBP was *Clostridium thermocellum* ATCG 27,405 with mesophilic microaerobe, *Pichia stiptis* NCIM-3498. The biogenic municipal solid waste was pretreated with 0.5% NaOH for reducing the recalcitrance property. It was observed that subsequent CBP (23.99 g/L) was better than CBP alone (18.10 g/L).

In subsequent CBP, exogenous xylanase was added to enhance xylan hydrolysis. Subsequent CBP II biosystem was observed to give maximum ethanol production (36.90 g/L) at pH 5 in a single reactor [75]. In another investigation, the author used yeast engineered with five functional cellulase genes (BGL, XYNII, EGII, CBHI, and CBHII) where ionic pretreated bagasse and laubholz unbleached kraft pulp was used as targeted biomass. The screening was performed to observe that it is essential to breed CBP yeast having the optimized cellulase expressing the ratio for the proposed biomass. In CBP, yeast development is considered a promising and cheap way in consolidated bioprocessing for ethanol production [76]. In one such study, the author used a new approach that is partially consolidated bioprocessing (PCBP). In this process, a mixture of lignocellulosic material was used (mentioned in Table 2). PCBP was used to prepare mixed lignocellulosic substrate by utilizing non-isothermal simultaneous pretreatment. Saccharification methods were used for the hydrolysis of the pretreated substrate. In the saccharification process (*Trichoderma Reesei* RUT C 30) was exploited the combination of laccase (*Pleurotus djamor*) and holocellulase. Then, it was later pursued by the co-fermentation process in a single reactor. The artificial neural network (Feedforward ANN) model was used to optimize all parameters in PCBP that resulted in increased ethanol productivity [77]. In one of the studies, the authors blend two characteristics, i.e., the saccharolytic and fermentation integrated into one microorganism. It diverted a huge interest towards this process for production of ethanol in terms of CBP causing lignocellulosic biorefineries to decrease environmental pollution and economic cost. Therefore, in this study, industrial *S. cerevisiae* strains are used, expressing strong characteristics such as thermotolerance and enhanced resistance to inhibitors. The strain was estimated to be great as it expresses hemicellulolytic enzyme activity on its cell surface. It resulted in increased ethanol productivity with the addition of commercial cellulase [78]. In one of the investigations, a thermophilic anaerobic bacterium was isolated from Himalayan hot spring *Clostridium* sp. DBT-IOC-C19. This strain was considered suitable for consolidated bioprocessing. It expressed a wide range of substrate conversion into ethanol, acetate, and lactate in a single step, where ethanol is considered the main product. DBT-IOC-C19 displayed 94.6% degradation at 5 g/L and 82.74% degradation at 10 g/L of avicel concentration or loading in 96 h of incubation time during fermentation. Rice straw was used as a lignocellulosic substrate for comparative analysis with different strains but total product yield and ethanol production increased in *Clostridium* DBT-IOC-C19 [79]. The same author studied different strains for CBP, e.g., *Clostridium thermocellum* ATCC31924, where crystalline cellulose was used as the sole carbon source. It was reported that cellulosome extracted from the bacterial strain when purified resulted in concentrated cellulase and xylanase enzyme [80]. Ionic liquid pretreated pine needle biomass was used as a substrate in CBP. First, saccharification was performed by using cellulase and xylanase in a single pot. Then, fermentation of hydrolysate was performed by using yeast to yield ethanol [81]. CBP cannot only be used for the production of ethanol, but there are other yields as primary productivity. In one study, the author worked with CBP along with co-cultivation. The author designed a microbial consortium composed of hemicellulose producing bacteria *Thermoanerobacterium, Thermosacchrolyticum* M5, and succinic acid production specialist *Actinobacillus succinogens.* This co-cultivation in CBP resulted in increased production of succinic acid under optimized conditions [82]. The three lignocellulolytic bacteria (*Clostridium thermocellum, C. stercorarium,* and *Thermoanaerobacter thermohydrosulfuricus*) were used as collegial co-culture strains in consolidated bioprocessing. This was performed in terms of a comparative analysis with the monoculture by employing a wide range of lignocellulosic material. The pretreated and unpretreated substrate was used to study the productivity (yield) produced from monoculture and triculture, and co-culture [83]. In another investigation, CBP was used to produce H_2. The cellulose-degrading microorganism was retrieved from the bovine ruminal fluid (BRF). *Clostridium acetobutylium* had great potential in increasing H_2 yield [84]. Figure 3 shows the schematic representation of the consolidated bioprocessing process using strategy hydrothermal pretreatment processing in the production of biofuels.

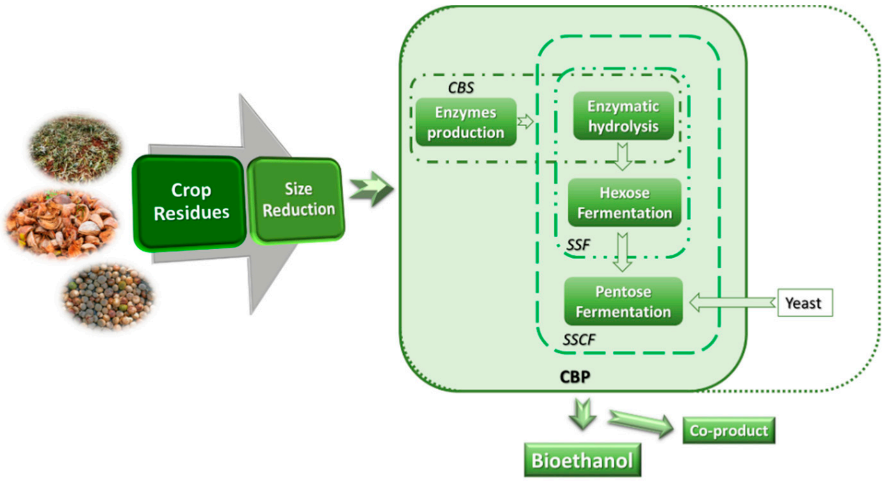

Figure 2. Schematic representation of consolidated bioprocessing processing in terms of biorefinery from lignocellulosic biomass.

Table 2. Consolidated bioprocessing (CBP) for a mixture of lignocellulosic material in the enzyme production.

Microorganism	Substrate	Enzyme	Operational Conditions	Reference
S. cerevisiae MT8-1	Laubholz unbleached kraft pulp and ionic liquid pre-treated bagasse	Cellulolytic enzymes	Incubation time 72–96 h	[76]
Saccharomyces cerevisiae	*Ricinus communis* and *Saccharum spontaneum* and top portions of *Saccharum officinarum*	Laccase, Holo-cellulase	Substrate loading-10% to 30% (*v*/*v*), incubation time (12–24 h), temperature 30–37 °C.	[77]
Thermophilic bacterium Clostridium sp. DBT-IOC-C19	Rice straw, crystalline cellulose	Cellulosomes	Temperature 55 to 65 °C, pH-7 to 8, time-96 h	[79]
C. thermocellum strain ATCC 31,924,	Cellulose	Cellulosomes	pH-8, temperature-55 °C, inoculum size 4% (*v*/*v*) & 0.5% (*w*/*v*) substrate concentration	[80]
B. subtilis G2, *S. cerevisiae* and *P. stipitis*.	Pine needle biomass	Cellulase, Xylanase	pH-5.6, inoculum size-1.5% (*v*/*v*), temperature-30–37 °C, incubation time 24–72 h	[81]
Thermoanaerobacterium thermosaccharolyticum M5 & *Actinobacillus succinogenes* 130Z	Corn cob	Xylanase & β-Xylosidase	Temp-55 °C & 37 °C, pH-6 to 7, incubation time-24 h to 48 h	[82]
CBP thermophile, *Clostridiumthermocellum* ATCC-27405 with mesophilic microaerobe, *Pichia stipitis* NCIM-3498	Biogenic municipal solid waste (BMSW)	Xylanase	Temp (45–70°C), initial pH (6–8), inoculum volume (4–12% *v*/*v*), pretreated BMSW Loading (10–80 g/L cellulose equivalent)	[75]

Table 2. *Cont.*

Microorganism	Substrate	Enzyme	Operational Conditions	Reference
Clostridiumthermocellum, *C. stercorarium*, and *Thermoanaerobacter thermohydrosulfuricus*	cattail (*Typha* spp.) and wheat straw	Cellulase & Hemicellulase	10% (*v*/*v*, monoculture), 5/5% (*v*/*v*, dual cultures), or 3.3/3.3/3.3% (*v*/*v*, tri-culture), temp-45 °C, incubation-4 days	[83]
Clostridium acetobutylicum	Agave biomass	Enzymatic commercial complex Cellic CTec3® Novozymes (Bagsvaerd, Denmark)	10% solid loading, pH-5.5, temp-35 °C, incubation-264	[84]
Industrial *S. cerevisiae* strains	Corn cob	Hemicellulolytic enzymes	pH-5, temp-35 °C, incubation time-48 h	[78]

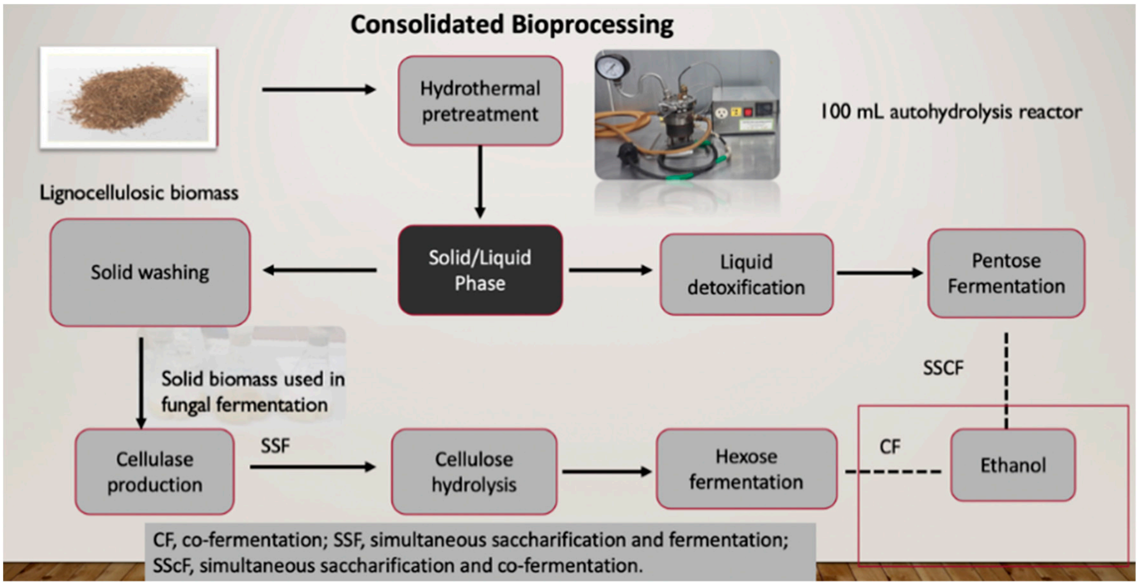

Figure 3. Schematic representation of consolidated bioprocessing processing using as hydrothermal strategy pretreatment. Adapted and modified from [15].

3.2.4. Physiochemical Conversion Routes in CBP with Pretreatment Process

The scientific lead that summons the continuous supply of biomass as the natural way for biomass saccharification with high sugar productivity is still a challenge. The problem is not yet presented in research programs dealing with biomass processing. However, many scientists aspire to work on biomass optimization for several decades. The merged yield from biofuels and chemicals still requires boosting from both technical and economic perspectives. None of the current pretreatment processes has improved, since their results rely on the type of feedstock, downstream process configuration, and many other factors. Consolidated bioprocessing (CBP) associates an individual or consortium of microbes to deteriorate biomass. Physicochemical pretreatment and biological methods are considered a better approach to reduce the recalcitrant property, leading to high sugar yield.

The hydrothermal pretreatment, i.e., liquid hot water pretreatment, does not require fast decompression and does not need any chemicals or catalysts. The temperature lifted between 160–240 °C, which caused an increase in pressure that required preserving the water in the liquid state and activating the lignocellulose composition [85]. The main objective of the liquid hot water pretreatment is to dissociate and solubilize hemicellulose. It makes easy cellulose availability to cellulase and limits inhibitory compounds during the reaction. Nonetheless, for avoiding the generation of inhibitors, the response must be performed at a low pH, i.e., between 4 and 7, reducing monomers' production [85,86]. Scientists recently established that heating lignin and raising the temperature above glass transition temperature could split the bond's existence between carbohydrate lignin. It emerged into the migration of hydrophobic lignin present in liquid media from the cell walls and getting re-precipitated into small spherical droplets assembled on both sides (inside and outside) of fibers [87]. Almost all hemicellulose contents got detached, leaving cellulose fiber bundles behind with free lignin, because of the lignin's realignment on both fibers' sides. After hydrolysis, fast disintegration was reported by researchers, along with a higher degradation temperature [87]. In another study, material balance and multiscale characterization techniques utilized the systematic analysis of the effects of severity factor and pH on the lignin-carbohydrate complex (LCC). Researchers discovered that the severity factor affects xylan removal under a diverse range of temperature profiles, which means a high severity factor causes high xylan removal. The temperature does not affect xylan removal. The liquid phase (hydrolysate) causes pH to drop due to hemicellulose depolymerization and degradation. It results in the accumulation and production of the acetic acid, causing hydrothermal pretreatment, and hemicellulose sugar's high yielding capacity causes increased furfural productivity and xylose loss. Rare, increased hemicellulose sugar degradation causes reduced furfural production. In contrast, approximately 80% of the original xylan was lost [88].

3.2.5. Integrated Technologies Based on Hydrolysis for Biofuel (Ethanol) Production

The quest to achieve a higher yield of biofuel per unit of biomass has led to the integration of different phases of the process; it reduces the cost of capital and makes biofuels more efficient and economically viable. Given that bioethanol is the most produced secondary biofuel today, the search for more efficient configurations for its production has been sought.

Simultaneous Saccharification and Fermentation (SSnF)

Conventionally, ethanol production is carried out in separate phases, first the hydrolysis and later the fermentation. Due to this separation, each one is carried out under optimal conditions [89]. However, the excessive accumulation of sugars during the hydrolysis inhibits the enzymatic activity, reducing the process [63,69,90]. This problem led to the development of simultaneous saccharification and fermentation (SSnF). In this process, hydrolysis and fermentation occur together in the same reactor, reducing enzymatic inhibition due to sugars' presence since the monosaccharides produced are immediately consumed by fermenting microorganisms [65,70,90]. Compared to the conventional process, SSnF achieved a higher hydrolysis rate, and it conducted higher ethanol concentration. This strategy requires less equipment and a more straightforward operation, and the presence of ethanol in the wort makes it less susceptible to contamination by unwanted microorganisms [61,70,90].

However, this method's disadvantage is that the optimal operating conditions for hydrolysis and fermentation are different, implying difficulty for its control and optimization. Enzymatic hydrolysis is performed optimally at temperatures above 50 °C. For most fermenting microorganisms, the optimum temperature for their performance is between 28 and 37 °C; likewise, the optimum pH for the hydrolysis and fermentation stages is different [65,70,90].

Work performed on the search and selection of suitable enzymes and microorganisms for this strategy, and a promising option to overcome this difficulty, is the use of thermotolerant yeasts such as *Kluyveromyces marxianus*, which is a promising species since some of its strains grow at temperatures between 45 and 52 °C [91].

Simultaneous Saccharification and Co-Fermentation (SSCF)

This configuration aims to complete all the sugars released during the pretreatment and hydrolysis of the biomass through mixed yeast cultures that can assimilate both the pentoses and the hexoses produced in the same reactor. This strategy offers this advantage. By continuously removing the final hydrolysis products, causing cellulase and glucosidase activity inhibition can lead to the high productivity of ethanol, giving greater yield compared to the SFS process. One drawback of this method is that the organisms that use hexoses grow faster than those that use pentoses, leading to inhibition by the high ethanol concentration. Genetically modified yeast or bacteria can be used to achieve efficient ethanol production. To carry out the fermentation of both, pentoses and hexoses are required [65,70,92,93].

4. Concluding Remarks and Prospects

When scientists started working on the valorization of lignocellulosic biomass, different bioprocessing technique such SSF and SMF, CBP emerged to play a critical role in the environment and economy. Scientists are using the genetic engineering method on microorganisms to play all the functions with or without pretreatment. CBP is timesaving and cost-effective in biorefineries as it is a one-process technique involving less instruments or one single reactor. In the future, CBP will play a vital role in converting lignocellulosic biomass to ethanol and other essential products in a single process or reactor with or without pretreatment because of some microorganisms capable of doing all at once, making it more economical and timesaving.

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