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Review

Sugar Beet Pulp as Raw Material for the Production of Bioplastics

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Abstract: The production of bioplastics from renewable materials has gained interest in recent years, due to the large accumulation of non-degradable plastic produced in the environment. Here, sugar beet pulp (SBP) is evaluated as a potential raw material for the production of bioplastics such as polylactic acid (PLA) and polyhydroxyalkanoates (PHAs). SBP is a by-product obtained in the sugar industry after sugar extraction from sugar beet, and it is mainly used for animal feed. It has a varied composition consisting mainly of cellulose, hemicellulose and pectin. Thus, it has been used to produce different value-added products such as methane, hydrogen, pectin, simple sugars, ethanol, lactic acid and succinic acid. This review focuses on the different bioprocesses involved in the production of lactic acid and PHAs, both precursors of bioplastics, from sugars derived from SBP. The review, therefore, describes the pretreatments applied to SBP, the conditions most frequently used for the enzymatic hydrolysis of SBP as well as the fermentation processes to obtain LA and PHAs.

Keywords: enzymatic hydrolysis; fermentation; bioplastics; lactic acid; polyhydroxyalkanoates; pretreatment; biorefinery; sugar beet pulp; solid-state fermentation

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1. Introduction

The use of plastics has increased exponentially in different sectors that affect our daily lives, such as packaging, building, automotive, medical devices, etc. This trend is the result of the good properties of this material, such as its flexibility, lightness, or strength in addition to its simple and productive manufacturing process [1]. However, different characteristics are required for each application and can be changed by switching the component materials. Therefore, the characteristics of plastic can be adjusted for the intended application. The main problem is that plastics are used only for a short period or even just one time, and after that are discarded. If not properly collected, this waste is deposited and accumulates in the environment, contaminating the different ecosystems of all living beings. According to the report data, global plastic production reached 367 million tons in 2020, of which 55 million tons were produced in Europe [2]. On the other hand, only 29 million tons of plastic residue were recovered, corresponding to recycling of only 34.6%. The remainder was used to recover energy (42%) or disposed of in landfill (23.4%) [2]. Consequently, a wide variety of studies are centered on the search for renewable materials with properties similar to plastic but that can be degraded easily after use, thus helping to reduce environmental contamination.

Bioplastics represent an environmentally friendly alternative to plastic. They have the advantage of being biobased, biodegradable or both, which means that they are a product obtained from biomass (biobased) and/or they can be degraded by microorganisms into water and CO_2 (biodegradable) [3]. Thus, bioplastics are classified according to these properties (Figure 1): biobased and non-biodegradable, biobased and biodegradable or fossil-based and biodegradable.

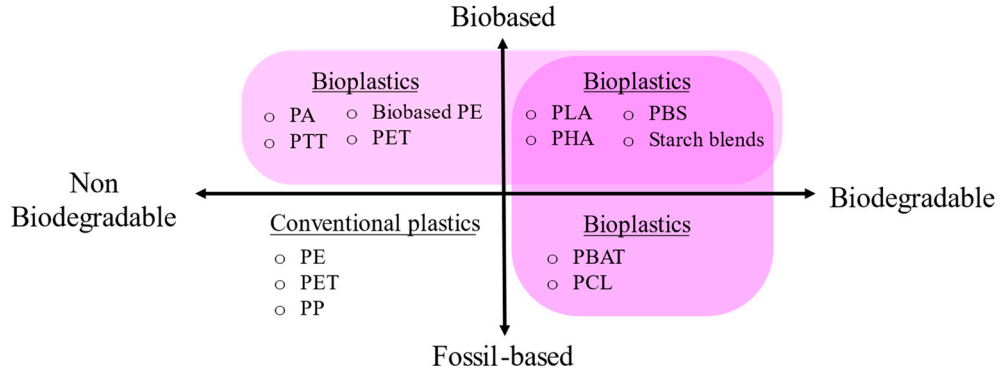

Figure 1. Classification and examples of each type of plastic. PA: polyamide, PTT: polytrimethylene terephthalate; PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene; PLA: polylactic acid; PHA: polyhydroxyalkanoates; PBS: polybutylene succinate; PBAT: polybutylene adipate terephthalate; PCL: polycaprolactone. Adapted from [4].

The most studied examples are polylactic acid (PLA) and PHAs, as they are biobased and biodegradable bioplastics. PLA is a polymer composed of lactic acid (LA) monomers and qualifies as a linear aliphatic thermoplastic polyester [5]. LA molecules contain chiral carbon, for which there are two isomeric forms: L (+) lactic acid and D (−) lactic acid. The properties of PLA can change according to the proportion of L-LA to D-LA. PLA can be used in different areas of our lives such as in medical devices, 3D printers or food packaging [6–9]. LA can be obtained by chemical synthesis or through fermentation. However, fermentation is usually preferred, as it can produce optical pure isomers of LA instead of a racemic mixture of L-LA and D-LA [5]. Through the fermentation pathway, LA is produced from sugars, mainly glucose, by lactic acid bacteria (LAB) through three different metabolic pathways, which influence the required carbon source and the obtained LA yield.

On the other hand, PHAs are a polymer classified as polyesters consisting of units of (R)-hydroxy fatty acid linked by ester bonds [10]. PHAs are generated by some microorganisms, in which they accumulate when the microorganism is under stress due to a lack of nutrients [11]. Various strategies have been studied to induce the accumulation of PHAs inside cells, for instance via limitation of the nitrogen source or oxygen concentration in fermentation using pure cultures. On the other hand, continuous cycles of fest and famine can be used in mixed cultures to select microorganisms able to accumulate PHAs. To date, more than 150 different monomers have been identified, and they can be combined to modify or improve the properties of the bioplastic. In general, they provide excellent benefits, including 100% biodegradability, biocompatibility, non-toxicity and antioxidant and immunotolerant properties [12]. As in the case of PLA, the bioplastic PHA has multiple uses, such as in packaging, coatings, and pharmaceutical and medical applications [13].

These bioplastics have many benefits over petroleum-based plastics. However, the biggest drawback is their production cost, which is 3 to 4 times higher. Different ways of reducing this cost are currently being studied, one of which is through using cheaper raw materials as alternatives [14]. Bioplastics are usually produced from pure sugars or fatty acids and, in some cases, also from first-generation raw materials such as corn or sugarcane [15]. Although high yields are obtained, the use of edible raw materials for bioplastic production raises concerns about food prices and quantity. As an alternative, second-generation feedstocks, such as agriculture or food industry residues, can also be used. These materials do not compete with human food, are found in abundance and are inexpensive. An additional advantage of these materials is they help to reduce the problems associated with the treatment of these wastes.

The majority of second-generation feedstocks are classified as lignocellulosic biomass (LCB) because they are mainly composed of cellulose, hemicellulose and lignin [16,17].

LCB is considered the largest renewable source on Earth [18]. The composition of this biomass depends on its source, but they are generally composed of 35–50% cellulose, 20–35% hemicellulose and 10–25% lignin [19]. As seen in Figure 2, the typical lignocellulose structure is composed of a matrix of crystalline cellulose surrounded by hemicellulose, pectin and lignin polymers [19]. This structure prevents the degradation of cellulose and confers rigidity to cell wall plants and, thus, resistance against insects and pathogens [20].

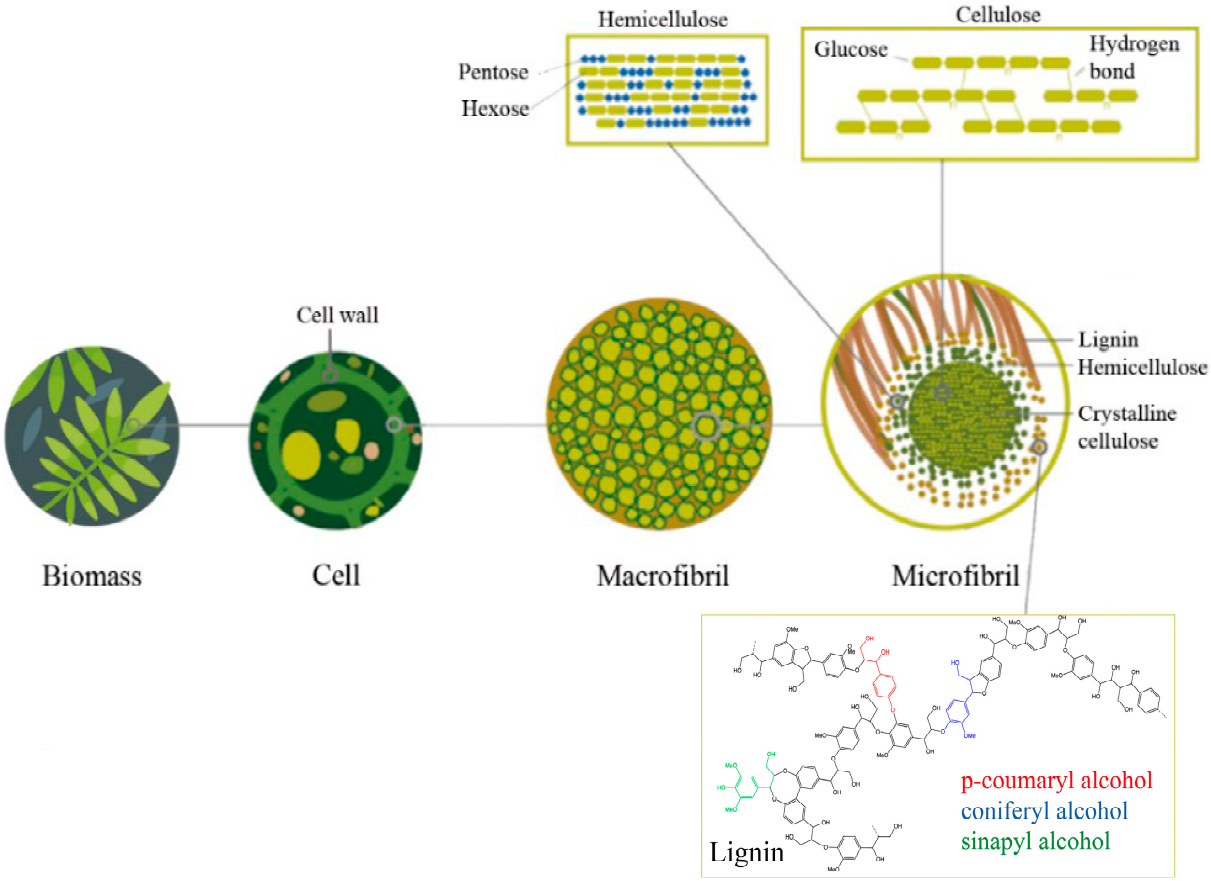

Figure 2. Structure of lignocellulosic biomass. Adapted from [21].

Sugar beet pulp (SBP) is a lignocellulosic by-product of the sugar industry that has traditionally been used for animal feed [22]. However, it has also been used as raw material to produce a wide range of value-added products, such as LA or PHAs, through biotechnological processes. In this review, each of the main stages involved in the production of bioplastics from this by-product are studied in detail (Figure 3). In this way, an evaluation of the different pretreatments applied to SBP to facilitate the subsequent enzymatic hydrolysis has been made. In addition, the influence of the enzymatic cocktail used for the hydrolysis of the biomass is analyzed. Regarding this, and with the aim of reducing the cost of processing the sugar beet pulp, enzymes are sometimes produced directly on the biomass via solid-state fermentation. This novel aspect is also described in detail in the review. Finally, the different strategies used to increase the yield of lactic acid and polyhydroxyalkanoates from SBP hydrolysates will be compared.

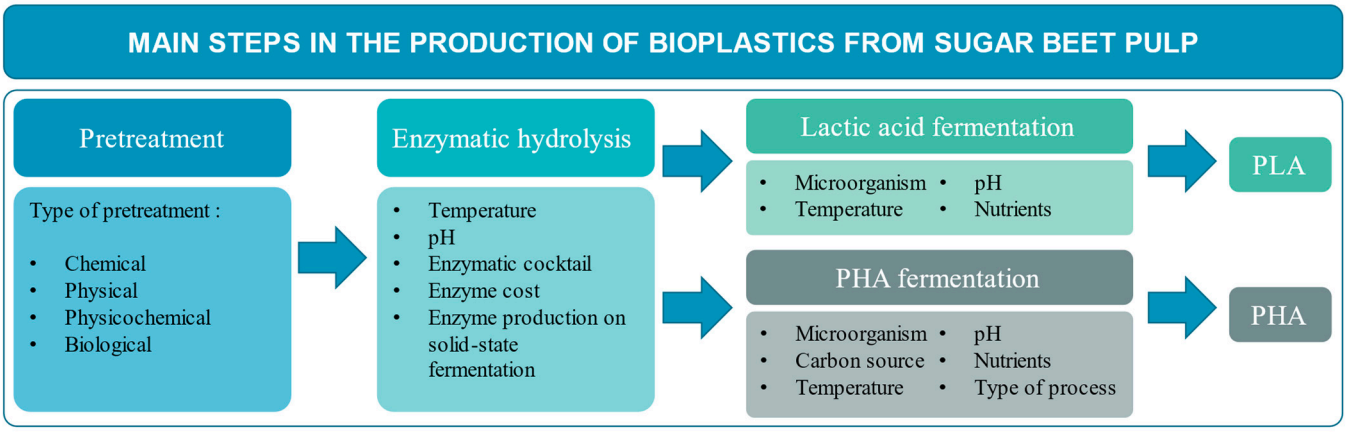

Figure 3. Flowchart showing the main steps in the production of bioplastics from sugar beet pulp, for which the associated issues are addressed in this review.

2. Exhausted Sugar Beet Pulp as a Renewable Feedstock

Sugar beet (*Beta vulgaris* L.) is one of the main sugar crops, along with sugarcane, used to produce sucrose for human consumption. As one of the main sugar crops, sugar beet accounts for about 20% of global sugar production, with sugarcane making up the remaining 80% [22]. This crop thrives in moderate-temperature regions in the northern hemisphere, where the climate is suited to its growth and development. The top sugar beet producers in the world in 2021 were the European Union (112,847.63 tons), the Russian Federation (47,500 tons), and the United States (32,364.15 tons) [23].

The sugar content of sugar beet can vary from 12% to 20%, making it a valuable raw material. The sugar industry can also achieve a financial gain of up to 10% through the use of by-products generated during production. The three main by-products are beet pulp, lime sludge and molasses, which can be used as renewable materials to produce energy or other value-added products [22]. For instance, one ton of sugar beet yields 160 kg of sugar, 500 kg of wet exhausted pulp and 38 kg of molasses [24] (see Figure 4A).

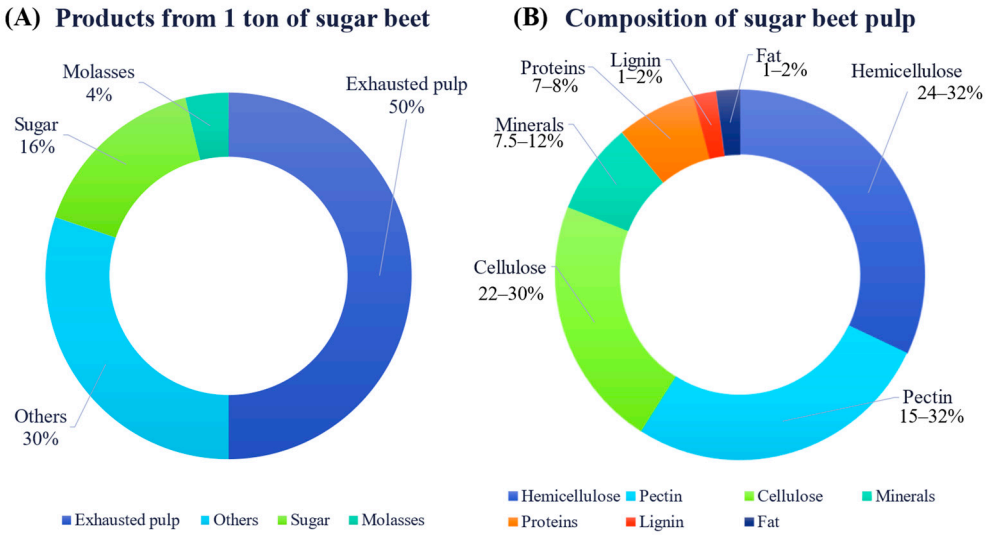

Figure 4. (**A**) Products obtained from 1 ton of sugar beet and (**B**) chemical composition of sugar beet pulp.

2.1. Processing of Sugar Beet

The diagram in Figure 5 shows the steps for producing white sugar from sugar beets. Sugar beetroots, or taproots, are harvested mechanically, separating the leaves from the

bulbs. The bulbs are washed, and the washing sludge is decanted to reuse the water [25]. The roots are then cut into thin strips, called cossettes, which are moved to stainless steel tanks, called diffusers, where they are mixed with hot water (55–75 °C) at a solid/liquid ratio (SLR) of 1:2 *w*/*w* with residence times from 1 to 2 h [22]. The juice obtained, called raw juice, contains mainly sucrose, but other compounds also that have been extracted from the root can hinder the crystallization of sugar. For this reason, the raw juice is purified before crystallization, with calco-carbonic treatment being the most commonly used approach [26,27]. The clean juice obtained after this step, the thin juice, is concentrated with multi-effect evaporators to obtain the final juice (thick juice) [28]. Finally, the thick juice is introduced into the crystallizers, where sugar is produced through crystallization [29,30]. Crystals are later separated from the liquid by centrifugation, dried in continuous rotary dryers and sieved [22]. The residual liquid stream, called molasses, contains 50% *w*/*w* sugars and other substances, such as oligosaccharides and organic acid salts.

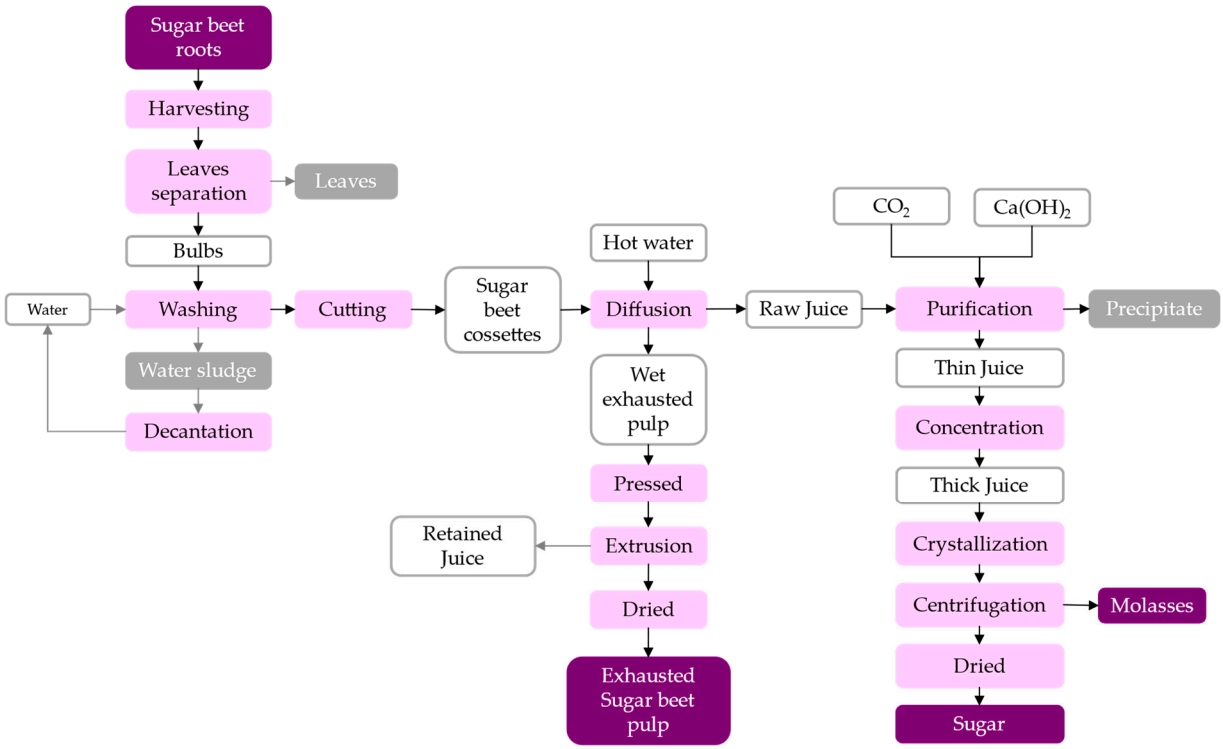

Figure 5. Flowchart of sugar beet processing. Adapted from [22].

The remaining pulp obtained after the diffusion step, named exhausted SBP, is pressed, and the retained juice is recovered. The pulp then contains a humidity of 70% and can be conformed into pellets of approximately 5 cm in length and 0.5 cm in diameter by extrusion [31]. These pellets are preserved by drying to reach a final humidity lower than 10%.

SBP is commonly used as animal feed. However, it has been also used as a raw material for the extraction of pectin and phenolic compounds and for producing several value-added products (VAPs), such as biogas by anaerobic digestion, hydrogen by acidogenic fermentation, or hydrolysates rich in sugars that can be fermented into several bioproducts such as ethanol, lactic acid, succinic acid or polyhydroxyalkanoates, among others. The digestate from the anaerobic digestion of SBB has also been used as an organic fertilizer in agriculture [32–34].

2.2. Chemical Composition of SBP

SBP contains between 87 and 92% of dried matter [35] and is mainly composed of 24–32% of hemicelluloses, 22–30% cellulose, 15–32% pectin, 1–2% lignin, 7–8% protein, 7.5–12% minerals and 1–2% fats [36–38] (see Figure 4B). The pulp composition can vary depending on the process of sugar extraction, the degree of maturation of the root and the sugar beetroot's geographical origin.

Among the sugar industry effluents, beet pulp stands out as a material rich in carbon sources [39]. Hence, component sugars from chemically or enzymatically hydrolyzed polysaccharides from SBP are appropriate raw materials for fermentation. There are several papers reporting on the total sugar composition of SBP after complete hydrolysis as being D-glucose (21–26%), D-galacturonic acid (14–21%) and L-arabinose (21–23%) the majority. The next highest sugar contents were D-galactose (5–6%), L-rhamnose (2.5%), D-xylose (about 2%) and mannose (1%) [40].

In a recent study comparing different agri-food wastes for biopolymer production, the feedstock quality was determined based on having high cellulose and low moisture and lignin content. Low moisture and lignin contents mean lower energy requirements for drying and easier processing, respectively. The residues generated in the harvest and processing stages of the food supply chain of wheat, barley, oats, rapeseed, sugar beet, carrots and onions were analyzed, and the percentage of moisture, cellulose, and lignin ranged from 7–87%, 10–42%, and 2–39%, respectively. Although sugar beet pulp has medium-high cellulose content (around 32%), it showed the lowest lignin (less than 5%). Regarding moisture content, vegetable wastes had a much higher percentage, between 77 and 87%, compared to the cereal residues (less than 20%). However, other factors such as the annual production, seasonality and feedstock cost should be taken into account [41].

3. Production of Bioplastic Precursors from Sugar Beet Pulp

The production of bioplastics, such as PLA and PHAs from SBP, can be performed through a biotechnological process such as the one proposed in Figure 3, which is most applied in the valorization of lignocellulosic biomass. Usually, the biomass is pretreated to enable access to the enzymes used in the saccharification step, where the biomass is hydrolyzed to produce a medium rich in sugars. This medium can be fermented to produce LA or PHAs, which are the precursor molecules to produce PLA and PHAs, respectively.

3.1. Pretreatments Applied to Sugar Beet Pulp

Pretreatment is an important step in the valorization of biomass because it helps to separate lignin and hemicellulose structures from cellulose. In this way, enzymes have easier access to cellulose [42,43]. Depending on the working principle, the pretreatments can be classified as physical, chemical, physicochemical and biological pretreatments [44].

The aim of physical pretreatment is to increase the surface area of the biomass particles. This can be achieved by reducing the particle size with mechanical comminution. Thus, the crystallinity of the cellulose structure is also damaged, which facilitates enzyme accessibility [44,45].

The results achieved with chemical pretreatments will depend on the reagent added, its concentration and the pretreatment duration. Thus, alkalis, such as sodium or potassium hydroxides, are used with the intention to solubilize lignin and hemicellulose [46]. On the other hand, acids, such as sulfuric or hydrochloric acids, are more efficient in degrading only hemicellulose [45]. Moreover, oxidant agents such as alkaline hydrogen peroxide or organic acids are chosen to degrade lignin [42].

Physicochemical pretreatments attempt to solubilize hemicellulose and lignin by changing the temperature, pH and moisture of the biomass [42]. In this way, pretreatments such as steam explosion, ammonia fiber explosion (AFEX), wet oxidation, CO_2 explosion and thermal (liquid hot water) treatment can be performed, where high-temperature exposure is usually combined with high pressure [47].

Biological pretreatments degrade part of the biomass structure by the action of microorganisms [42]. Usually, this is performed using fungi because they can more easily penetrate solids. In this pretreatment, not only might hemicellulose and lignin be degraded but part of the cellulose may also be consumed by the microorganism [45].

Most pretreatments applied to SBP are diluted acid and thermal pretreatment, which produce the highest hydrolysis yields. Other studied SBP pretreatments include size reduction (milling), ultrasound, steam explosion, ensiling and solid-state fermentation, which are all summarized in Table 1. For example, ultrasound pretreatment was applied on SBP using SONOPULS HD 2200 homogenizer in continuous mode with 400 W for 20 min [24]. However, the hydrolysis yield did not increase. On the other hand, for thermal pretreatment of SBP, the hydrolysis yield was increased from 0.70 to 0.85 g of reducing sugars per gram of SBP (g_{RS}/g_{SBP}) [24]. This pretreatment was carried out in an autoclave at 120 °C for 30 or 60 min [24]. Another study performed by Rezic et al. also focused on the effect of these pretreatments on SBP, and similar results were obtained [24,48]. In this case, thermal pretreatment was performed in the autoclave for 20 min, and ultrasound pretreatment was carried out at 200 W for 5 to 45 min [48].

Table 1. Pretreatments applied to SBP. Y_H: hydrolysis yield (g_{RS}/g_{SBP}).

Pretreatment Type		Conditions	Y_H	Reference
Physical	Milling	0.8–1.0 mm	0.71 g/g	[24]
	Milling + Ultrasound	0.8–1.0 mm 50% or 100% amplitude, 20 min, water or 2% *w*/*w* H_2SO_4	0.70–0.76 g/g	[24]
Chemical	Dilute acid	Autoclave, 150 °C, 10 min, 1.1% *w*/*w* H_2SO_4	0.82 g/g	[49]
	Dilute acid	120 °C, 6 min, 0.1 N HCl	0.86 g/g	[50]
	Dilute acid	120 °C, 0.66% H_2SO_4	0.63 g/g	[51]
	Dilute acid	1% H_2SO_4	0.49 g/g	[52]
Physicochemical	Milling + Thermal	Autoclave, 121 °C, 30 or 60 min, 2% *w*/*w* H_2SO_4	0.85 g/g	[24]
	Milling + Thermal	Autoclave, 120 °C, 30 or 60 min, water	0.75 g/g	[24]
	Thermal	Autoclave, 121 °C, 20 min	0.60 g/g	[52]
Biological	Ensiling	Lactobacillus species	0.95 g/g	[53]
	Solid-state fermentation	Aspergillus awamori, 70% moisture, 5 days	0.34 g/g	[52]

Several authors have studied the effect of diluted acid pretreatment on SBP, resulting in the solubilization of pectin and hemicellulose fractions [50]. For instance, SBP was pretreated with sulfuric acid (1.1% *w*/*w*) at 150 °C for 10 min and solid loading of 10% *w*/*w*, and the hydrolysis yield reached 0.82 g_{RS}/g_{SBP} [49]. However, this yield can be improved to 0.86 g_{RS}/g_{SBP} by performing the pretreatment at the optimal conditions found by El-gendy et al. of 120 °C, 0.1 N HCl, 14% *w*/*w* of solid loading and 6 min [50]. Nonetheless, a higher hydrolysis yield (0.92 g_{RS}/g_{SBP}) was achieved when SBP was pretreated with sulfuric acid (0.66%) with a solid loading of 6% at 120 °C [51]. More recently, Marzo et al. performed diluted acid pretreatment on SBP, studying the effect of sulfuric acid concentrations of 0.25, 0.5, or 1% *w*/*v* [52]. The pretreatment was performed by soaking the solid at a solid/liquid ratio of 1:20 (*w*/*v*) with a solution of sulfuric acid and autoclaving the mixture at 120 °C for 20 min. The results showed an increase in the production of glucose as the concentration of acid was increased, reaching a hydrolysis yield of 0.45 g_{RS}/g_{SBP}.

Another lesser-studied pretreatment is the steam explosion. The optimum conditions of pressure and pretreatment time found by Cárdenas–Fernández et al. were 5.3 bar and 24.4 min [54]. With this pretreatment, solubilization of both pectin and the insoluble cellulose fraction was achieved, thereby increasing the ethanol yield due to the increased solubilization of cellulose.

Biological pretreatments such as ensiling or solid-state fermentation have been also studied for SBP. For instance, SBP was pretreated using the ensiling methodology, where

several *Lactobacillus* species were involved [53]. This increased the enzymatic digestibility of SBP by 35% [53]. In this case, the microorganism degraded some fraction of the biomass. As this technique is usually employed for long-term storage of biomass, it seems an interesting option to stabilize SBP and pretreat it at the same time. Additionally, SBP was also pretreated via solid-state fermentation [52]. Fermentation was carried out with the fungus *Aspergillus awamori* at 70% moisture content and incubation at 30 °C for 5 days. The results obtained after the enzymatic hydrolysis of the pretreated SBP showed a decrease in the concentration of total reducing sugars produced compared with the non-pretreated solid. However, the same glucose concentration was obtained, showing that only the hemicellulose and pectin content has been removed by the fungus.

3.2. Enzymatic Hydrolysis of SBP

The production of sugars from SBP can be performed through acid or enzymatic hydrolysis. Acid hydrolysis is carried out with concentrated or diluted acids such as hydrochloric acid or sulfuric acid, which degrade the lignocellulose structure [19]. Although a pretreatment step before acid hydrolysis is not necessary, this step has numerous disadvantages compared to enzymatic hydrolysis. For example, a special material is required for the equipment used in this type of hydrolysis to ensure it can resist the corrosion resulting from the acids [19]. However, one of the main issues of this process is the production of compounds that are inhibitory for the microorganisms, such as furfural or its derivates, which are generated after the degradation of sugars due to acidic conditions. On the other hand, enzymatic hydrolysis is a more environmentally friendly process.

Enzymatic hydrolysis is based on the use of hydrolytic enzymes that hydrolyze the lignocellulose structure into simple sugars. There is a wide variety of enzymes that are necessary to hydrolyze the whole biomass, with the most important being cellulases, hemicellulases and pectinases [55]. The proportion of each will depend on the composition of the biomass that is hydrolyzed. One of the main advantages of enzymatic hydrolysis is the use of mild conditions for temperature (45–50 °C) and pH (4.8–5.0) [56]. Moreover, inhibitory compounds are not generated, and the equipment is not damaged by corrosion. On the other hand, the main problem of enzymatic hydrolysis is the high cost of enzymes and the occasional need for prior pretreatment.

Several authors have studied the enzymatic hydrolysis of SBP with the aim of maximizing sugar production from SBP (Table 2). Some of them studied the influence of the enzymatic cocktail. In this sense, the main enzymes used are commercial cocktails composed of a mixture of cellulase, xylanase and pectinase. The optimum conditions of these cocktails are very similar; thus, the hydrolysis of SBP is generally performed at 50 °C, pH 4.8, 150 rpm and 2% *w*/*w* of solid loading (Table 2). One of the main cocktails used is called "Celluclast® 1.5 L", and it is added to correspond to enzyme activity in the range of 4–30 filter paper units per gram of dry matter (FPU/g). Depending on the added enzyme activity, the hydrolysis yield can be improved from 0.27 to 0.49 g_{RS}/g_{SBP} when the cellulase activity is increased from 5 to 30 FPU/g [57]. However, other cellulase cocktails richer in cellobiase, xylanase or pectinase, such as Novozyme 188, Novozyme 431, Viscozyme L, Pectinex®, Celustar XL or Agropect pomace, can be also used. For example, the hydrolysis yield was increased from 0.15 to 0.70 g_{RS}/g_{SBP} when a cocktail combining cellulase and cellobiase was supplemented with hemicellulases and pectinases [58]. Due to the composition of SBP, the addition of these enzymes solubilizes the fraction of hemicellulose and pectin, making cellulose more accessible to enzymes.

Other authors focused their studies on the pretreatment of SBP to improve the enzymatic hydrolysis yield. For example, the hydrolysis yield was increased from 0.15 to 0.5 g_{RS}/g_{SBP} when SBP was pretreated with ammonia (0.5 mL/g of ammonia, 80 °C, 5 min) and the enzymatic hydrolysis was performed with cellulase and cellobiase [58]. However, the hydrolysis yield decreased from 0.7 to 0.61 g_{RS}/g_{SBP} when SBP was pretreated in the same conditions but the hydrolysis was carried out with cellulase, cellobiase, hemicellulase and pectinase [58]. The hydrolysis yield was also increased from 0.2 g_{RS}/g_{SBP}

for non-pretreated SBP to 0.48 and 0.38 g_{RS}/g_{SBP} when SBP was pretreated with thermal and chemical pretreatment with hydrochloric acid, respectively [59]. The same effect was observed in other papers, where the hydrolysis yield was increased from 0.18 g_{RS}/g_{SBP} for non-pretreated SBP to 0.38, 0.49, 0.23 and 0.25 g_{RS}/g_{SBP}, when hydrochloric acid, ammonia, pectinase or ammonium oxalate was added to pretreat SBP [60].

3.3. Enzyme Production by Solid-State Fermentation

Enzymatic hydrolysis is the stage of the process that most influences the overall cost of the process, due to the high cost of the enzymes. To render the process more profitable, various approaches have been explored, such as looking for new sources of enzymes or alternative techniques to submerged fermentation [61]. One such technique is the production of hydrolytic enzymes by solid-state fermentation (SSF) [62,63]. The advantage of this fermentation is that different types of biomass, such as agri-food industry wastes or bioproducts, can be used as raw material, acting as a source of carbon and nutrients and as solid support for fungal growth and enzyme production [64].

SSF is a heterogeneous fermentation that takes place in the absence or near absence of visible water between particles [65]. It is usually carried out by filamentous fungi that grow on the surface of the solid due to their ability to colonize the interparticle spaces of porous materials [66], although bacteria and yeasts can also be used. Most fungal species employed for this technique belong to the genera *Aspergillus*, *Pleurotus* and *Trichoderma* [67].

SSF is a complex process where parameters such as temperature, aeration rate, pH, initial moisture, particle size, agitation, water activity or inoculum concentration should be optimized, and most will depend on the microorganism selected to produce the enzymes [68]. Depending on the fungal species, the optimum temperature for growth can vary from 20 to 55 °C, while the optimum moisture content can change in the range of 40 and 70% [69]. However, the pH is usually adjusted to 5 [70], and aeration is mainly used in pilot-scale reactors [71].

Table 2. Conditions for enzymatic hydrolysis of SBP. T: temperature; SL: solid loading; EA: enzyme activity; YH: hydrolysis yield (g_{RS}/g_{SBP}).

Reference	T	pH	Agitation	SL	Enzyme Type [1]	EA [2]	Pretreatment	Y_H
[58]	40 °C	4.8	-	5% *w*/*w*	Celluclast® 1.5 L Novozyme 431	4.2 FPU/g d.m. 28.4 CBU/g d.m.	Ammonia 0.5:1, 85 °C, 5 min	0.50 g/g
	40 °C	4.8	-	5% *w*/*w*	Celluclast® 1.5 L Novozyme 431 Viscozyme L	4.2 FPU/g d.m. 28.4 CBU/g d.m. 0.85 HU/g d.m. 60.2 PGU/g d.m.	Ammonia 0.5:1, 85 °C, 5 min	0.61 g/g
	40 °C	4.8	-	5% *w*/*w*	Celluclast® 1.5 L Novozyme 431	4.2 FPU/g d.m. 28.4 CBU/g d.m.	Untreated	0.15 g/g
	40 °C	4.8	-	5% *w*/*w*	Celluclast® 1.5 L Novozyme 431 Viscozyme L	4.2 FPU/g d.m. 28.4 CBU/g d.m. 0.85 HU/g d.m. 60.2 PGU/g d.m.	Untreated	0.70 g/g
[53]	50 °C	4.8	150 rpm	2% *w*/*w*	Celluclast® 1.5 L Novozyme 188	15 FPU/g d.m.15 CBU/g d.m.	Ensilage 90 days	0.19 g/g
[57]	40 °C	4.8	150 rpm	2.5% *w*/*w*	Celluclast® 1.5 L	5 FPU/g d.m.	HCl pH 1.5, 85 °C, 4 h	0.27 g/g
	40 °C	4.8	150 rpm	2.5% *w*/*w*	Celluclast® 1.5 L	30 FPU/g d.m.	HCl pH 1.5, 85 °C, 4 h	0.49 g/g
[72]	50 °C	5	-	10% *w*/*w*	Celustar XL Agropect	0.75 FPU/g d.m.	Untreated	0.3 g/g
[51]	50 °C	4.8	150 rpm	2% *w*/*w*	Celluclast® 1.5 L Novozyme 188 Pectinex®	15 FPU/g d.m. 15 CBU/g d.m. 60 PGU/g d.m.	Diluted acid H_2SO_4, 0.66%, 120 °C, 2% solid loading	0.63 g/g

Table 2. *Cont.*

Reference	T	pH	Agitation	SL	Enzyme Type [1]	EA [2]	Pretreatment	Y_H
[59]	45 °C	4.8	-	2% *w*/*w*	Celluclast® 1.5 L	20 FPU/g d.m.	Untreated	0.20 g/g
	45 °C	4.8	-	2% *w*/*w*	Celluclast® 1.5 L	20 FPU/g d.m.	HCl pH 1.5, 85 °C, 4 h	0.38 g/g
	45 °C	4.8	-	2% *w*/*w*	Celluclast® 1.5 L	20 FPU/g d.m.	Autoclave (2.1 bars, 30 min), water 1:20 (*w*/*v*)	0.44 g/g
[60]	50 °C	4.8	-	5% *w*/*w*	Cellulase	20 FPU/g d.m.	Untreated	0.18 g/g
	50 °C	4.8	-	5% *w*/*w*	Cellulase	20 FPU/g d.m.	HCl 1% *w*/*w*, 80 °C, 6 h	0.38 g/g
	50 °C	4.8	-	5% *w*/*w*	Cellulase	20 FPU/g d.m.	Ammonia 10% *w*/*w*, 80 °C, 6 h	0.49 g/g
	50 °C	4.8	-	5% *w*/*w*	Cellulase	20 FPU/g d.m.	Pectinase 30 U/g, 50 °C, 6 h	0.23 g/g
	50 °C	4.8	-	5% *w*/*w*	Cellulase	20 FPU/g d.m.	Ammonium oxalate 5% *w*/*w*, 80 °C, 6 h	0.25 g/g
[73]	50 °C	5.0	150 rpm	10% *w*/*w*	Celluclast® 1.5 L β-glucosidase xylanase exo-polygalacturonase	2200 FPU/g d.m. 6 CBU/g d.m. 300 HU/g d.m. 110 PGU/g d.m.	Autoclave 120 °C, 20 min	0.71 g/g

[1] Novozyme 188: Cellobiase; Novozyme 431: Cellobiase; Pectinex®: Pectinase; Agropect pomace: Pectinase; Celluclast® 1.5L: Cellulase, xylanase, pectinase, mannanase; Viscozyme L: arabinase, cellulase, β-glucanase, hemicellulase and xylanase; Cellustar XL: xylanase, cellulase, β-glucanase. [2] FPU: filter paper cellulase unit; CBU: cellobiase activity units; PGU: polygalacturonase activity units; HU: hemicellulase activity units; d.m: dry matter.

A noteworthy substrate for this process is lignocellulosic biomass due to its rich composition of cellulose, hemicellulose and pectin. This type of substrate can induce the production of different enzymes by fungi [65,74]. In this sense, SBP seems to be a suitable substrate to produce hydrolytic enzymes. For instance, hydrolytic enzymes, such as xylanase, exo-polygalacturonase and cellulase, were produced via SSF of SBP, reaching 35 U/g of xylanase and 28 U/g of exo-polygalacturonase after 8 days of fermentation with *Aspergillus awamori* [75]. These values were higher than those for production from orange peel waste under the same conditions [75]. Dextranase was also produced by SSF of SBP, reaching peak activity (1057 U/g) after three days of growth of *A. awamori* F-234 [76]. This result was obtained in a study where various residues, such as olive mill solid waste, jojoba mill solid waste and sugar cane bagasse, and different fungi strains, such as *Aspergillus niger* F-93, *Aspergillus fumigatus* F-993, *Penicillium funiculosum* NRC289, *Trichoderma koningii* F-25 and *Aspergillus awamori* F-234, were tested. However, SBP was also studied in combination with other residues such as wheat bran [77]. The results showed that the enzyme production depended on the medium and the fermentation conditions and that polygalacturonase production was induced when wheat bran was mixed with 30% of SBP, reaching 909 U/g with *A. sojae* ATCC 20235 after 8 days at 30 °C [77].

Other studies were performed to increase enzyme production by adding extra nitrogen sources to the raw material. For example, wastewater from monosodium glutamate production was added as a nitrogen and water source to improve pectinase production from the fermentation of SBP with *Aspergillus niger* (CGMCC0455) [78]. Thus, the enzyme activities were increased achieving 15.6×10^{-3} U/g of endopectinase, 3.6×10^{-3} U/g of polygalacturonase and 16×10^{-3} U/g of pectin-lyase [78]. Ammonium sulfate was also tested as a nitrogen source on the production of α-L-arabinofuranosidase by SSF of SBP with *Trichoderma reesei*, reaching 433 U/g of α-L-arabinofuranosidase [79].

To conclude, SSF is a flexible process that can be adapted to produce a wide range of hydrolytic enzymes. However, enzymes are usually extracted and purified to be used in enzymatic hydrolysis, which also raises the overall cost of the process. As an alternative process, some authors have studied the addition of fermented solid directly to the medium to be hydrolyzed, avoiding the enzyme extraction step. Leung et al. were one of the

first to propose this process in the SSF of waste bread [80]. They produced protease and glucoamylase by SSF of waste bread and, subsequently, the fermented solid was added to fresh waste bread to produce a hydrolysate, which then was fermented to succinic acid. The same strategy was followed by other authors, such as Pleissner et al., Kwan et al. or Dessie et al., for different wastes, such as food waste, bakery waste, and fruit and vegetable waste [81–83]. More recently, Marzo et al. used the same strategy on SBP, where the enzymes were produced through the SSF of SBP and then added to fresh SBP to produce a hydrolysate rich in sugars [84]. The hydrolysis yield was increased from 0.45 to 0.55 g_{RS}/g_{SBP} when a fed-batch strategy was applied. Thus, 15 g of fermented solid and 13.75 g of fresh solid were mixed at the beginning of the hydrolysis, and then the same amount of fresh solid was added 3 times every 2.5 h [84].

3.4. Lactic Acid Fermentation

The medium obtained after enzymatic hydrolysis has a high content of sugars, such as glucose. Therefore, it can be a raw material to produce a wide variety of VAPs through submerged fermentation. LA is a common product obtained through the fermentation of sugars and is commonly used in cosmetic formulations and in the food and pharmaceutical industries. For example, in the food industry, it is used as a flavor-enhancing agent and acidifier [85–87]; in cosmetics formulations, it is added for its emulsifying properties and the moisturizing effects produced on the skin; and in the pharmaceutical industry, it is used for the synthesis of dermatologic products and drugs against osteoporosis [88]. Additionally, LA has recently received special attention for being the precursor of PLA, a bio-degradable and bio-based bioplastic [89]. It is also considered a platform chemical to produce different products, such as acrylic acid, pyruvic acid, ethyl-lactate, 2,3-pentanedione, acetaldehyde and propylene glycol [90].

3.4.1. Metabolic Pathways to Produce LA via Fermentation

LA can be produced by a wide variety of microorganisms such as bacteria, fungi, yeast, cyanobacteria or algae [14]. However, the most studied are LAB, which includes genera such as *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissellla* [91]. These LAB require the optimum conditions of temperature (30–45 °C) and pH (6–7) to grow and produce LA [92]. They also require the proper amount of nitrogen, vitamins and minerals for optimal growth. These nutrients may be contained in the hydrolysate obtained from agri-food waste, but they are usually supplemented in the medium. For instance, yeast extract is one of the most used nitrogen sources, however, its supplementation in the hydrolysates increases the total cost of the LA production process [93].

LAB are classified as homofermentative, heterofermentative or facultative heterofermentative, according to the metabolic pathways used to produce LA. Thus, the conversion of sugars to LA by LAB is produced through three different pathways (Figure 5): the Embden–Meyerhof–Parnas (EMP) pathway, the pentose phosphate (PP) pathway and the phosphoketolase (PK) pathway [88].

Homofermentative LAB ferment hexoses via the EMP pathway, producing theoretically 2 mol of LA per mol of glucose. In addition, some strains also convert glucose via the PP pathway instead of the EMP pathway, producing 1.67 mol of LA per mol of glucose [88]. On the other hand, heterofermentative LAB can ferment hexoses and pentoses exclusively via the PK pathway, producing LA, acetic acid or ethanol, and CO_2. Thus, the theoretical yield obtained is 1 mol of LA per mol of glucose or pentose [88]. By contrast, facultative heterofermentative LAB can metabolize hexoses via the EMP pathway and pentoses via the PK pathway, obtaining 2 mol of LA per mol of glucose and 1 mol of LA per mol of pentose [88].

3.4.2. Production of LA from SBP Hydrolysates

Different studies have been performed to produce LA from SBP (Table 3). To increase the LA yield, several authors have varied the pH regulation mode, nutrient supplementation and the type of processing, such as separate hydrolysis and fermentation, simultaneous saccharification and fermentation, fed-batch fermentation or continuous fermentation. For instance, Marzo et al. studied the effect of supplementing the SBP hydrolysate with different nitrogen sources, finding that it is necessary to supplement it with yeast extract to increase the production of LA [94]. Additionally, they also studied pH regulation during the lactic fermentation of SBP hydrolysate with *Lactobacillus plantarum*, reaching 30 g/L of LA with a yield of 0.12 g_{LA}/g_{SBP} by adding 27 g/L of $CaCO_3$ [94]. In another report, the same authors evaluated the pretreatment of SBP, testing acid, alkaline and biological pretreatment. They increased the production of LA to 50 g/L with a yield of 0.5 g_{LA}/g_{SBP} when the SBP was pretreated with 1% H_2SO_4 [52]. Díaz et al. studied different simultaneous saccharification and fermentation strategies to produce LA from SBP with *Lactobacillus casei* [95]. They achieved the maximum concentration of 27 g/L of LA with a yield of 0.13 g_{LA}/g_{SBP} using a fed-batch SSF process with pH control (by adding 30 g/L $CaCO_3$) and nutrient supplementation (by adding MRS medium 0.2 mL/mL).

Table 3. Lactic acid production from SBP. YLA: lactic acid yield (g_{LA}/g_{SBP}).

Reference	Strain	Conditions	Concentration	Y_{LA}
[24]	*Lactobacillus plantarum* HII & *Lactobacillus brevis* PCM 488	SSF with co-culture	60 g/L	0.55 g/g
[96]	*L. coryniformis subsp. torquens* DSM 20005 & *L. preudomesenteroides*	SHF with co-culture	22 g/L	0.78 g/g
[94]	*Lactobacillus plantarum*	SHF	30 g/L	0.12 g/g
[52]	*Lactobacillus plantarum*	SHF with pretreated SBP	50 g/L	0.5 g/g
[95]	*Lactobacillus casei*	Fed-fach SSF	27 g/L	0.13 g/g
[97]	*Bacillus coagulans*	Continuous fermentation	35 g/L	0.71 g/g

Due to the composition of SBP, the hydrolysis of SBP produces a medium rich in glucose, but other sugars such as fructose, mannose, arabinose galactose, raffinose, rhamnose, xylose and galacturonic acid are also included [24]. It is difficult for a single microbial species to completely assimilate such a medium. Lactic acid production from arabinose, galactose and xylose derived from SBP is not as efficient as from glucose [98]. For example, *Bacillus coagulans* was used to produce lactic acid from sugar beet pulp hydrolysates [97]. This strain was able to consume glucose and xylose; however, arabinose was not totally consumed at the end of fermentation. Most homofermentative LAB, such as *Lactobacillus delbrueckii* and *Lactobacillus acidophilus*, can produce lactic acid from glucose but not from sugars derived from hemicellulose, such as arabinose and xylose [99]. For better utilization of the substrate, it is necessary to use LAB able to utilize pentoses. However, pentoses are only utilized by some lactobacilli. This is the case of the facultative heterofermentative microorganism *Lactobacillus casei* 2246, which degrades hexoses, mainly glucose, through the EMP pathway, and pentoses by the PK pathway [95]. This strain has been demonstrated to produce lactic acid from glucose and arabinose from simultaneous saccharification and fermentation of SBP. Moreover, lactic acid fermentation can be improved by using a mixed population of LAB with different assimilation profiles [100]. In fact, the effectiveness of LA production can be improved by 10–30% by using mixed cultures. Hence, Berlowska et al. studied the effect of mixed cultures of LAB species through simultaneous saccharification and lactic fermentation [24]. They performed the fermentation in two steps: the first one with a monoculture able to ferment mainly glucose and the second one with another strain able to consume unfermented sugars (arabinose and xylose). Using this procedure, most sugars were consumed by the mixed culture, reaching a LA concentration of 60 g/L with a yield of 0.55 g_{LA}/g_{SBP} with the strain *Lactobacillus plantarum* HII in the first step

and *Lactobacillus plantarum* HII and *Lactobacillus brevis* PCM 488 in the second. The same strategy was followed by Alexandri et al. after observing the non-complete consumption of the hydrolyzed sugars from SBP [96]. They produced 22 g/L of LA with a yield of 0.78 g_{LA}/g_{SBP} with the strains *L. coryniformis* subsp. *torquens* DSM 20005 and isolate A250 (most likely *L. preudomesenteroides*), adding the second strain when half of the glucose was consumed [96]. Additionally, a similar procedure was performed by Diaz et al., where SBP hydrolysate was fermented first to ethanol and then to LA with the strain *Lactobacillus plantarum* [73]. In that study, the complete use of sugars was achieved by producing ethanol and LA sequentially.

An interesting biorefinery process was presented by Oliveira et al. [97]. This process can be implemented inside the processing of sugar beet to produce sugar. They produce LA from SBP via continuous fermentation with *Bacillus coagulans*, reaching 2781.01 g of LA from 3916.91 g of sugars with a maximum productivity of 18.06 g/L/h.

3.5. Polyhydroxyalkanoates Fermentation

The first identification of poly-3-hydroxybutyrate (P(3HB)) was performed in the strain *Bacillus megaterium* by Lemoigne in 1926 [101]. Afterward, more than 300 bacterial strains have been identified as PHA producers. Some examples include *Cupriavidus necator* (also named *Ralstonia eutropha*), *Pseudomonas aeruginosa*, *Pseudomonas oleovarans* and *Pseudomonas stutzeri* [102]. These bacteria are able to accumulate PHA inside the cell cytoplasm as a reserve of carbon and energy. Therefore, they are produced when the microorganisms cannot grow normally due to a deficiency of nutrients and an excess of carbon.

3.5.1. Metabolic Pathway to Produce PHAs via Fermentation

The production of PHAs is induced by the limitation of nutrients in the medium used for microorganism growth. This limitation activates the pathways of metabolism that bacteria use to produce PHAs [11]. The metabolic pathways involved in the production of PHA depend on the carbon source and the microorganism used. These bacteria can use different carbon sources to produce PHAs, such as carbohydrates or volatile fatty acids (VFAs) [103].

In pure culture fermentation, carbohydrates are metabolized to pyruvate via the Enter–Doudoroff pathway. Afterward, pyruvate is converted to acetyl-CoA and, under growth-limiting conditions, acetyl-CoA is transformed into P(3HB) by the action of three enzymes: 3-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB) and PHA synthase (PhaC) [103]. Through this pathway, the obtained yield will depend on the carbon source. Thus, glucose produces the highest yield (0.40 g PHA/g); however, other sugars such as xylose or arabinose can also be used to produce PHAs, although at lower yields (0.17–0.19 g PHA/g) [104].

The production of PHAs from VFAs follows the same route for metabolism once they are converted to the corresponding acyl-CoA, being the precursors for different hydroxyalkanoate monomers. In this way, P(3HB) is produced from acetate, and 3HV, 3-hydroxy-2-methylvalerate (3H2MV) or 3-hydroxy-2-methylbutyrate (3H2MB) are produced from propionate [11].

PHAs can be produced from pure cultures following a two-step process: growth phase and accumulation phase [11]. Firstly, the strain is cultivated in a medium with a proper ratio of carbon, nitrogen and nutrients. Secondly, one of the essential nutrients (nitrogen, phosphorus or oxygen) is restricted to induce the accumulation of PHA inside the cell. PHAs can also be produced by mixed cultures through the fermentation of VFAs and the process requires two stages [11]. First of all, microorganisms with high PHA storage capacity are selected by imposing cycles where an essential nutrient is restricted. Afterward, the selected microorganisms are cultured to produce PHAs by restricting the same essential nutrient restricted in the previous step. The advantage of using mixed culture is the reduction in production cost due to sterilization is not required and the culture can adapt to various complex feedstocks [105,106].

3.5.2. Production of PHAs from SBP

As previously mentioned, SBP hydrolysate is a complete medium with a great variety of sugars, including glucose, rhamnose, arabinose, galactose, xylose and galacturonic acid. However, there are no published papers on the production of PHA from SBP-derived sugars different from glucose.

Kurt-Kızıldoğan et al. produced poly(3-hydroxybutyrate) (PHB), one of the main PHAs, with *Haloarcula* sp. TG1 from glucose derived from different agricultural wastes [107]. Among them, the highest PHA content (45.6% of the biomass) was achieved using SBP treated with rCKT3eng, a recombinant endoglucanase of *Haloarcula* sp. CKT3 expressed in *E. coli* BL21 (DE3) cells (pH 7.35, 72 h, 37 °C).

The most common pathway in bacteria, yeast and fungi to convert glucose to PHAs is the EMP [108,109]. However, the model organism for PHA production, *C. necator*, lacks the gene for the key enzyme of this pathway, utilizing the Entner–Doudoroff (ED) pathway instead [110]. There are only a few strains able to convert C5 sugars and produce PHAs, but when they do, the conversion efficiencies are very low [111]. It has been demonstrated that some microorganisms are able to perform this conversion from hemicellulose hydrolysates derived from rice straw, sugarcane bagasse, sugar maple wood chips, etc., such as *Bacillus firmus*, *Cupriavidus necator*, *Burkholderia cepacia*, etc. [109]. However, no papers have been found describing PHA production from C5 sugars derived from SBP. Numerous metabolic engineering strategies have been explored for metabolizing pentose sugars available in lignocellulosic hydrolysates. For example, a strain of *C. necator* has been modified to metabolize arabinose through heterologous expression of a set of *E. coli* genes for L-arabinose uptake and metabolism [112].

As stated above, VFAs are another carbon source to produce PHAs. SBP has also been studied as raw material to produce VFAs, with acetic, lactic, caproic and butyric being the main acids produced [113]. These acids are commonly produced through acidogenic fermentation by mixed microbial cultures. This fermentation involves two steps of the anaerobic digestion process, hydrolysis and acidogenesis, and the inhibition of methanogenesis [114]. In this process, the organic matter is hydrolyzed into sugars, amino acids and fatty acids. Afterward, these compounds are converted into VFAs by acidogenic bacteria.

4. Conclusions and Future Directions

Bioplastic production from renewable sources is a topic that has gained interest in recent years. In this direction, SBP is a versatile by-product from the food industry that can be used as raw material to produce value-added products, such as LA and PHAs, through biotechnological processes. Although several studies have been performed on pretreatments and enzymatic hydrolysis of SBP, only a few focused on the production of LA or PHAs, which can be used as precursors of bioplastics. Only one study was found where PHAs are obtained from SBP through a sequential process of hydrolysis and fermentation.

Focusing on another alternative process to produce PHAs, that is from VFAs, there is also a lack of studies where VFAs produced from SBP are used to obtain PHAs. However, there are several studies based on the production of hydrogen and VFAs through the anaerobic digestion of SBP. Hence, it seems that it is a feasible process that has not yet been studied in depth.

Additionally, SBP is composed of similar percentages of cellulose, hemicellulose and pectin. This characteristic makes it an interesting raw material to use in the framework of a biorefinery. Only a few authors have proposed a biorefinery process applied to this material, where pectin, phenolic compounds and sugar-rich hydrolysates can be fermented to ethanol, LA or succinic acid, among others, facilitating the preparation of valuable products. Further research should be carried out in this direction to implement a cost-effective process in the sugar industry.

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Abbreviations

CBU	Cellobiase activity units
PGU	Polygalacturonase activity units
COD	Chemical oxygen demand
d.m.	Dry matter
FPU	Filter paper unit
HU	Hemicellulase activity units
LA	Lactic acid
LAB	Lactic acid bacteria
LCB	Lignocellulosic biomass
MRS	Man, Rogosa and Sharpe broth
PA	Polyamide
PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene succinate
PCL	Polycaprolactone
PE	Polyethene
PET	Polyethene terephthalate
PGU	Polygalacturonase activity units
PHA	Polyhydroxyalkanoate
PLA	Poly-lactic acid
POS	Pectin-derived oligosaccharides
PP	Polypropylene
PTT	Polytrimethylene terephthalate
RS	Reducing sugars
SBP	Sugar beet pulp
SLR	Solid-liquid ratio
SSF	Solid-state fermentation
TS	Total solids
U	Unit of enzyme
VAP	Value-added product
VFAs	Volatile fatty acids
VS	Volatile solid
Y_H	Hydrolysis yield

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