Use of Lignocellulosic Materials for PHA Production

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Review
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Polyhydroxyalkanoates (PHAs) are very promising materials that might serve as an environmentally friendly alternative to petrochemical plastics. The main obstacle preventing PHAs from entering the market massively is the final cost of the polymer material, a significant portion of which is attributed to carbon substrate. Hence, the researchers have been intensively seeking cheap substrates for sustainable production of PHAs. Lignocellulose represents a very promising substrate for PHAs production – it is abundant, cheap, and it does not compete with human food chain. On the other hand, utilization of lignocellulose materials as substrates for biotechnological processes represents a challenge due to many factors, such as necessary hydrolysis of the biomass to yield fermentable sugars and presence of numerous antimicrobial agents. Therefore, this work summarizes recent advances in biotechnological conversion of lignocellulose materials into PHAs. The review not only deals with the process of fermentation, but it also considers different approaches of lignocellulose hydrolysis and detoxification.

Key words:
polyhydroxyalkanoates, lignocellulose, cellulose, hemicellulose, hydrolysis, detoxification

Introduction

Life of modern civilization is accompanied by accumulation of huge amounts of non-degradable solid waste materials. A major portion of this resistant waste is represented by synthetic polymers of petrochemical origin. The accumulation of plastic wastes has become a very important environmental issue[1]. Conventional synthetic polymers are problematic not only because of their long decomposition time, but they also release various toxic substances during the process of degradation. Hence, there is strong motivation to replace synthetic polymers with materials that can be readily eliminated from our biosphere in an "environmentally friendly" fashion[2]. Furthermore, the industrialized world is currently highly dependent on fossil resources as a supply of energy for industrial processes, and also substrate for the production of a wide range of chemicals and materials. Since the fossil fuels area is finite, its depletion results in a serious global problem. All carbon-based structural materials (such as plastics, foams, coating, and adhesives) owe their properties to long arrays of carbon–carbon bonds. Therefore, one of the challenges of the research today is to find an approach to produce a substitute for petrochemical-based polymers using sustainable renewable sources[3].

Polyhydroxyalkanoates (PHAs) are generally considered as an alternative to petrochemical-based synthetic polymers. These microbial polyesters are synthesized and accumulated as intracellular granules by some microorganisms belonging to the *Bacteria* and *Archaea* domains of life. These storage materials serve as the carbon and energy reserves of the producing microorganisms. PHAs are commonly grouped into two major categories: the short-chain-length (*scl*-) and the medium-chain-length (*mcl*-) PHAs. The repeat units of *scl*-PHAs are composed of hydroxy-acids having three to five carbon atoms, whereas, *mcl*-PHAs contain hydroxy-acids repeat units with six or more carbon atoms. In general, the *scl*-PHAs are more crystalline than the *mcl*-PHAs. As such, *scl*-PHAs usually exhibit thermoplastic-like properties, while *mcl*-PHAs behave like elastomers or adhesives[4]. Due to their physical characteristics, *scl*-PHAs can be used for manufacturing items for packaging or everyday plastics commodities. Therefore, they compete on the market with poly-(olefins) and, in the field of

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"green plastics", also with bio-based poly-(lactate)[5]. However, PHAs are disadvantaged due to their significantly higher production costs, while a major portion of the final costs is represented by the price of carbon substrate. For PHAs production, estimates of the contribution of the substrate cost to total production costs were 28–50 %[6,7]. Therefore, research focuses on inexpensive fermentable raw materials as substrates for biotechnological PHAs production.

Lignocellulose biomass includes agricultural and forestry residues, portions of municipal solid waste, as well as herbaceous and woody crops. With the annual generation of 80 billion tons, lignocellulosic materials have a great potential for the production of a wide variety of industrial and commodity products including paper, lumber, bioethanol, biodegradable polymers (e.g. PHAs) and a range of fine chemicals. Such materials are abundant and competitive in price with petroleum, and, therefore, lignocellulose biomass can provide a sustainable resource also for fermentative production of PHAs. On the other hand, utilization of lignocellulosic materials as substrates for biotechnological purposes is accompanied by many obstacles stemming from the nature of these materials, such as relatively low amount of sugars, cost of hydrolysis process, presence of inhibitors in hydrolysates and cost of their removal in large volumes. Moreover, issues related to substrate accessibility and transportation should be taken into account. Due to these problems, utilization of lignocellulose-based substrates is challenging, and only a limited number of processes seems to be profitable at the moment. Most of the industrial units dealing with lignocellulose biomass utilization work in experimental mode. The fact is that, many technological as well as scientific problems need to be solved and, therefore, the aim of this review is to summarize recent advances in biotechnological production of PHAs from various lignocellulose biomass streams.

Characterization of lignocellulose biomass

Lignocellulosics, comprised of cellulose, pectin, hemicellulose, and lignin, are the most abundant raw materials on Earth; therefore, they are considered as potential substrates for production of various chemical, fuels and materials. Generally, the composition of plant cell wall varies in cellulose (40–80 %), hemicellulose (10–40 %), and lignin (5–25 %) content depending on the type of biomass used[8].

Cellulose is the most widespread organic material in the world. The dominant structure of this polysaccharide of plant cell walls is a linear β-(1→4)-D-glucopyranoside polymer. Due to the fact that cellulose possesses a substantial degree of crystallinity, it functions as a rigid, load bearing component of the cell wall. Furthermore, the rigidity of the cellulose micro-fibril is strengthened within a matrix of hemicelluloses and pectins[9].

Pectin is an acidic cell wall polysaccharide that functions as a sol-like matrix, providing water and ion retention, support and facilitation of cell wall modifying enzymes, cell wall porosity, cell-to-cell adhesion, cell expansion, cell signalling, developmental regulation, and defence[9,10].

Hemicelluloses are highly versatile materials. Generally, they are classified according to the main sugar residue in the backbone, e.g., xylans, mannans, and glucans, with xylans and mannans being the most prevalent. The average degree of polymerization of hemicelluloses is in the range of 80–200. They are usually associated with various other cell-wall components such as cellulose, cell-wall proteins, lignin, and other phenolic compounds by covalent and hydrogen bonding, and by ionic and hydrophobic interactions[9,11,12].

Pretreatment and hydrolysis of lignocellulosic materials

Cellulose and hemicellulose are polysaccharides that can be broken down into sugars and fermented or chemically altered into valuable fuels and chemicals[9]. Hydrolysis technologies may involve a physical treatment, chemical methods such as hydrolysis by concentrated or dilute acid, as well as enzymatic methods whereby often chemical and enzymatic hydrolysis are combined in consecutive steps. The composite formed by cellulose, hemicellulose and lignin is responsible for the remarkable resistance against hydrolysis and enzymatic attack[13]. Of the three components, lignin is the most recalcitrant to degradation, whereas cellulose is more resistant to hydrolysis as compared to hemicelluloses[9,14].

Generally, proper pre-treatment of lignocellulose prior to its enzymatic hydrolysis by cellulases significantly improves glucose yields. The goal of the pre-treatment process is (at least partial) removal of lignin and hemicelluloses (therefore, this type of pre-treatment is also called delignification), reduction of the cellulose crystallinity, as well as increase of the porosity of the lignocellulosic materials. Various types of delignification methods are known, e.g. physical, physicochemical, chemical, biological, and electrical or combination of these[15]. Nevertheless, hydrolysates obtained by enzymatic hydrolysis of delignified materials contain a very low level of inhibitors as compared to those obtained by chemical hydrolysis (described below) of non-pretreated materials. Therefore, they can be considered as superior carbon substrates for various biotechnological processes.

Cellulase refers to a group of enzymes which, acting together, hydrolyse cellulose. Glucose represents the final product of enzymatically driven hydrolysis of cellulose, and its yields can approach 100 % (theoretically)[9,14]. Fungi are the main cellulase-producing microorganisms, though a few bacteria have also been recently reported to yield cellulase activity. Microorganisms of the genera *Trichoderma* and *Aspergillus* are thought to be the most efficient cellulase producers, and crude enzymes produced by these microorganisms are commercially available for industrial and agricultural use[15,16].

Acid hydrolysis of hemicelluloses and cellulose is performed by concentrated or diluted acids: acid catalyses the breakdown of long carbohydrate chains to form shorter chain oligomers and then to sugar monomers. Because of the amorphous form of hemicelluloses, less severe conditions are required for its hydrolysis in comparison with crystalline cellulose. The advantages of acid hydrolysis are that the acid can penetrate lignin without pre-treatment and the rate of acid hydrolysis is faster than enzyme hydrolysis[17]. Sulphuric and hydrochloric acids are the most commonly used catalysts for hydrolysis lignocellulose biomass. This process is carried out at high temperatures to achieve acceptable rates of biomass conversion. On the other hand, the high temperature increases the rates of lignocellulose biomass derived sugars decomposition, thus causing the formation of toxic compounds which further decreases the yields of fermentable sugars. Some bases can also be used for pre-treatment of lignocellulosic materials. The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components. The porosity of the lignocellulosic materials increases with the removal of the crosslinks. Generally, alkaline hydrolysis enhances digestibility of the lignocellulose and reduces inhibitors formation[17,18].

Composition of hydrolysates

Lignocellulosic materials are converted to fermentable sugars by hydrolysis, which subsequently can be fermented yielding a variety of products[19]. Cellulose can be hydrolysed to yield glucose molecules, while hemicelluloses can be broken up, i.e. hydrolysed to yield molecules such as arabinose, mannose, glucose, galactose, xylose and uronic acids[9,11]. Examples of sugars composition of several hydrolysates are shown in Table 2.

The hydrolysis of lignocellulose waste by chemical or enzymatic treatments or combination of both releases several degradation products that prove harmful to microbial fermentation. For example, acid hydrolysis of lignocellulose materials produces several inhibitory compounds that inhibit cells, affect specific growth rates, and thus considerably decrease the yield of the biotechnological process. Based on origin, inhibitors are usually divided into three major groups, i. weak acids, ii. furan derivatives, iii. phenolic compounds. Phenolic and aromatic compounds are released specifically from lignin[9,16]. Some phenolic compounds include extractives which are also derived from released sugars after hydrolysis. The effect of phenolic and other aromatic compounds, which may inhibit both microbial growth and product yield, are very variable, and can be related to specific functional groups. One possible mechanism is that phenolics interfere with the cell membrane by influencing its function and changing its protein-to-lipid ratio. Phenolic compounds are also investigated with regard to inhibition of enzymatic hydrolysis of cellulose; experiments with phenols suggest that one way in which they affect proteins is by inducing precipitation[21].

Lignocellulose hydrolysates contain aliphatic acids, such as acetic acid, formic and levulinic acid. Acetic acid is formed primarily by cleavage of acetyl group of hemicellulose, while formic acid and levulinic acid arise as acid-catalysed thermochemical degradation products from polysaccharides. The last mentioned acid – formic acid is a degradation product of furaldehyde and 5-hydroxymethyl-2-furaldehyde (HMF). Undissociated acids enter the cell through diffusion over the cell membrane and then dissociate due to the neutral cytosolic pH. The dissociation of the acid leads to a decrease in the intracellular pH, which may cause cell death. The last main group of inhibitors present in lignocellulose hydrolysates is the group of furan derivatives. The furan aldehydes furaldehyde and HMF are formed by dehydration of pentose and hexose sugars. These furan aldehydes are known as the most potent inhibitors of microbial cell growth and can inhibit some microorganisms[19,20,21].

Besides furans, phenolics and organic acids mentioned above, there are other products generated in pre-treatment of the lignocellulose. They are extracted from materials by the use of various solvents and play a role in the defence system of the plant, as well as precursors of certain chemicals[23]. These products include tannic and terpene acids, such as caproic acid, caprylic acid, pelargonic acid or palmitic acid. The type and concentration of the acids discharged into hydrolysates depends strongly on the feedstock, hydrolysis temperature, acid concentration and overall severity of the pre-treatment. For example, feedstock with high content of acetylated carbohydrates, such as agricultural residues and hardwood, contain higher amounts of aliphatic acids than softwood[20]. These raw material extractives

cause cell membrane permeation, disturb proton gradient over the inner mitochondrial membrane which inhibits regeneration of ATP in mitochondria, and eventually can lead to cellular death[24].

Hydrolysis of lignocellulosic feedstock might also release inorganic ions (K^+, Na^+,Cl^-) that originate from the raw material, and chemicals added during pre-treatment and possibly from process equipment[20,22,23]. The inorganic ions also come from addition of salts which in turn lead to higher osmotic pressure. If the ions cannot cross the cell membrane, the cell will shrink as a result of hypertonic environment[22,23].

Detoxification of hydrolysates

Several types of detoxification strategies can be used to detoxify slurries and hydrolysates. It is always important to evaluate what substances should be removed prior to cultivation, and on the other hand, which not to remove from the culture media due to their low degree of inhibition or microbial tolerance, and select detoxification procedure accordingly. Generally, detoxification methods are classified according to treatment type into following groups: i. physical methods, ii. chemical methods, iii. biological methods. Principles of the methods as well as their brief description are provided in Table 1.

Lignocellulose biomass as substrate for PHA production

As mentioned previously, lignocellulose represent a highly abundant but also a rather versatile group of materials. Properties and sustainability of these materials with respect to their utilization for biotechnological production of PHAs as well as their hydrolysis and further treatment should be always performed with regards to particular biomass stream and fermentation process.

Apart from substrate selection and preparation, the key importance is usually ascribed to selection of PHAs-producing bacterial strain. Generally, many bacteria have been screened to produce PHAs, however, only a few have been considered as candidates for production of these polyesters on a large scale. The suitability of a bacterium for PHAs production from such a complex and challenging substrate depends on many different factors, e.g. ability to utilize fermentable sugars present in hydrolysates (including pentoses), capability of tolerating or even eliminating potential microbial inhibitors, stability and safety of the organism, growth and accumulation rates, achievable cell densities and PHAs content, extractability of the polymer, and molecular weights of accumulated PHAs[34].

Since PHAs are intracellular products, the total PHAs yields are dependent on biomass concentration. Therefore, reaching high cell density is the first condition of reasonable process of PHAs production. Fed-batch cultivation strategy, which is often used to achieve high cell densities, can in the case of utilization of lignocellulose hydrolysates, be complicated by the phenomenon called carbon catabolite repression (CCR). In presence of sugar mixtures, a preferential consumption of one of the sugars (usually glucose or other hexoses) is observed, while the other sugars (usually pentoses) remain unutilized in the medium. Employing fed-batch cultivation, their concentration in medium can reach inhibiting concentration which significantly decreases productivity of the process[35]. In some bacterial strains, CCR is mediated by proteins of the phospho-transferase system (PTS). Lopes *et al.* studied catabolite repression in PTS mutants of *Burkholderia sacchari* IPT101 to improve total car-

Table 1 – *Overview of detoxification methods*

Type	Detoxification method	Description	Reference
Physical	Evaporation	Removal of volatile compounds such as acetic acid etc.	25, 26
	Membrane separation	Application of membranes that predominantly binds inhibitors	27, 28
Chemical	Neutralization	Partial removal of furaldehyde and phenolics	19
	Over-liming	Neutralization of hydrolysate with calcium hydroxide accompanied by precipitation of calcium sulfate and sorption of inhibitors	19, 29
	Activated charcoal treatment	Inhibitors removal by sorption on activated charcoal	19, 22, 30
	Ion exchange resins	Removal of inhibitors by application of anion and/or cation exchangers	22, 31
Biological	Microbial pretreatment	Application of selected microorganism(s) capable of inhibitors degradation prior to fermentation	22, 24
	Microbial acclimatization	Adaptation of microorganisms to the inhibitors present in lignocellulose hydrolysates	23, 32, 33
	Enzymatic detoxification	Enzyme catalyzed degradation of inhibitors in hydrolysates prior to fermentation	19, 22, 24

bon up-take in sugar mixtures. The wild strain only started consuming xylose after glucose was completely depleted, while one UV mutant was able to consume glucose and xylose simultaneously[36]. Generally, the rate of feeding during fed-batch cultivation should be controlled to overcome accumulation of slowly assimilated sugars in medium. Furthermore, aside from sugars, the rising concentration of inhibitors might represent a problem during fed-batch cultivation and, hence, it is of great importance to remove inhibitors from the medium as much as possible when fed-batch cultivation strategy is to be employed.

Utilization of wood biomass for PHA production

Among various types of lignocellulose, forest biomass represents an enormous reservoir of renewable carbon-rich material. Globally, approximately 80 billion tons of woody biomass is generated per annum, with the production of total plant matter estimated at roughly 180 billion tons annually. While the bulk of the cellulosic component is efficiently exploited by the paper/pulp industry, the hemicellulose and lignin fractions are vastly underutilized process streams, which hold potential as platform intermediates in the production of value-added products, such as PHAs[37]. Hence, maple hemicellulosic hydrolysate obtained by diluted acid treatment was utilized as a renewable feedstock for PHAs production employing *Burkholderia cepacia* by Pan *et al.* To increase the fermentability of wood hydrolysate, several detoxification methods were tested. Over-liming combined with low-temperature sterilization resulted in the highest removal of total inhibitory phenolics (65 %). A fed-batch fermentation exhibited maximum polyhydroxybutyrate (PHB) production after 96 h – 8.72 g L^{-1} broth and 51.4 % of dry cell weight)[38]. In their further work, the authors estimated the contribution of individual inhibitors of wood hydrolysate to total antimicrobial effect. The results indicated that syringic acid was the most important inhibitor among three phenolics analysed, and synergistic inhibition was observed for the combinations of vanillin/syringic acid and vanillic acid/syringic acid. Additionally, strong synergistic effects were observed for the combinations of acetate/phenolics and levulinic acid/furaldehyde[39].

Bowers *et al.* also focused on utilization of wood biomass for PHAs production. In their study, *Pinus radiata* wood chips were subjected to high-temperature mechanical pre-treatment or steam explosion in the presence of sulphur dioxide before being enzymatically treated to produce corresponding hydrolysates. Two PHB-producing bacteria *Novosphingobium nitrogenifigens* and *Sphingobium scionense* were grown on these hydrolysates. The highest PHB yields (dry biomass concentration 1.23 g L^{-1}, PHB content in biomass 32 % and PHB yield 0.4 g L^{-1}) were observed in *Sphingobium scionense*[40]. Furthermore, Silva *et al.* tested PHAs production employing *Brevundimonas vesicularis* and *Sphingopyxis macrogoltabida* using acid-hydrolysed sawdust as a carbon source. The bacterial strains were able to accumulate ter-polymer consisting of 3-hydroxybutyrate, 3-hydroxyvalerate and lactic acid (3-hydroxypropionate), the polymer content in cells reached 72 % of cell dry weight, however, total PHAs yields were limited by low biomass growth[41]. Similar copolymer consisting of 3-hydroxybutyrate and lactic acid was also produced by transgenic *Escherichia coli*. In particular, β-xylosidase and an endoxylanase were engineered into the bacteria, which was additionally designed to express the PHA synthase from *Pseudomonas* sp. 61–3 harbouring a Ser325Th/Gln481Lys mutation [PhaC1Ps(ST/QK)], a propionyl-CoA transferase (PCT) from *Megasphaera elsdenii*, and a β-ketothiolase (PhaA) and NADPH-dependent acetoacetyl-CoA reductase (PhaB) from *Ralstonia eutropha* under a *R. eutropha* constitutive promoter. PHAs production yields using xylan as sole carbon source were minimal; however, when the xylan-based media was supplemented with a single sugar (xylose or arabinose) to permit the accumulation of xylan derived xylose in the media, PHA production yields increased. The highest biomass and PHAs yields obtained within this study were 8.9 and 3.6 g L^{-1}, respectively[42].

Lignocellulose-based agricultural wastes for PHA production

Apart from wood biomass, there is another source of lignocellulose-based materials which can be used as a substrate for PHA production – waste streams of agriculture and food industry. These materials are generated in enormous amounts during processing of agricultural plants. Despite the fact that some of these wastes and by-products can be used for various purposes, such as animal feeding or fertilization, the production of PHAs represents a very interesting and promising strategy for their valorisation yielding high-value product. Hence, there are numerous papers dealing with conversion of these waste materials into PHAs.

Bagasse is the fibrous matter that remains after sugarcane or sorghum stalks are crushed to extract their juice. Since bagasse is a highly abundant and available waste material, there were several attempts to produce PHAs from acid sugarcane bagasse hydrolysate. Silva *et al.* employed *Burkholderia cepacia* and *Burkholderia sacchari.* To improve fermentability of the hydrolysate, three-step detoxification processes (consisting of i. concentration in water bath, ii. over-liming and iii. activated

Table 2 – *Composition of selected hydrolysates of ligonocellulosic materials*

Material	Mechanism of hydrolysis	Sugar concentration (g L^{-1})				Reference
		xylose	glucose	mannose	arabinose	
Soft wood	Enzymatic	3.6	61.8	7.2	0.4	40
Wheat bran	Enzymatic	4.1	12.7	4.1	3.1	54
Oil palm empty fruit brunch	Enzymatic	29.6	48.3	*n.d.*	*n.d.*	52
Rice straw	Chemical	22.6	1.1	*n.d.*	0.5	51
Bagasse	Chemical	24.4	4.1	*n.d.*	2.6	44
Spent coffee grounds	Chemical + Enzymatic	*n.d.*	3.9	23.6	28	49

n.d. – not detected

charcoal treatment) were applied. After the treatment, the highest biomass and PHAs yields were observed in *B. sacchari* 4.4 and 2.7 g L^{-1}, respectively[43]. Even higher PHAs and biomass yields (11.1 g L^{-1} biomass, 6.3 g L^{-1} PHB) on sugarcane bagasse hydrolysate prepared by diluted acid treatment were obtained employing *Ralstonia eutropha*[44], and also *Bacillus thuringiensis* was reported to be capable of sugarcane bagasse hydrolysate utilization and PHB production yielding 10.6 g $L^{-1}$of biomass and 4.2 g L^{-1} PHB[45]. Finally, other types of bagasse – stemming from manufacture of tequila – was used for PHAs production employing *Saccharophagus degradans*, which can readily attach to cellulosic fibres, degrade the cellulose, and utilize this as the primary carbon source while producing PHB. This approach does not require hydrolysis prior to cultivation, which in turns theoretically reduces the cost of up-stream processing. Unfortunately, the study by Munoz and Riley did not reveal PHB yields, thus it is very difficult to estimate efficiency of such a process[46].

Another very promising waste substrate for PHAs production is spent coffee grounds. Coffee is one of the world´s most popular beverages, and has grown steadily in commercial importance in the last 150 years. In 2010, the worldwide annual production of coffee beans exceeded 8 million tons. During the preparation of coffee beverage or the manufacture of instant coffee solid residues known as spent coffee grounds (SCG) are formed. SCG can be considered as a very promising substrate for PHAs production. At first, SCG contain approx. 15 % of oil, which can be simply extracted and converted into PHB by *Cupriavidus necator*[47,48]. The residual solids after oil extraction contain a significant portion of hemicelluloses and cellulose. Therefore, they were hydrolysed (chemical hydrolysis by diluted acid was followed by cellulase treatment) and converted into PHAs employing *Burkholderia cepacia.* Hexoses (predominantly mannose and galactose) were substantially dominating sugars of the hydrolysate, which may be an important factor positively influencing the PHAs production. Moreover, hydrolysate contained levulinic acid (product of hexose degradation), which served as a precursor of 3-hydroxyvalerate resulting in accumulation of P(3HB-*co*-3HV) copolymer[49].

Coir pith, a by-product of coconut fibres and waste material from the coir industry, is stable and not easily degradable due to its high lignin content. Coir pith takes a decade to decompose, thereby posing an environmental hazard and disposal problem. Thus, Prabu and Murugesan investigated PHB production from coir pitch by *Azotobacter beijerinickii*. The waste material was at first partially delignified and further enzymatically hydrolysed by cellulase. The amount of PHB produced by *A. beijerinickii* from coir pitch hydrolysate was 2.4 g L^{-1} [50].

Also, rice straw hydrolysate obtained by chemical hydrolysis using diluted acid was converted into PHA by *Bacillus firmus.* Acid pre-treated black liquor contained sugars and sugar degradation products, such as formic acid, acetic acid, furaldehyde and HMF. The bacterium grew in the hydrolysate medium without detoxification, and it could accumulate 1.9 g L^{-1} biomass with 1.7 g L^{-1} PHB and the PHB content in the cell was 89 %[51].

Oil palm is the most abundant industrial crop in Southeast Asia. Each year, more than 15 million tonnes of oil palm empty fruit bunch are generated by palm oil industries in Malaysia. Oil palm empty fruit bunch consists of cellulose (50.4 %), hemicellulose (21.9 %), lignin (10 %), and ash (17.7 %). Zhang *et al.* investigated PHAs production from oil palm empty fruit bunch. The material was first chemically pre-treated and enzymatically hydrolysed by a cellulase cocktail, and further used as a substrate for PHAs production employing *Bacillus megaterium*. Tryptone was identified as its best nitrogen source, PHB content and production achieved 51.6 % and 12.48 g L^{-1}, respectively, productivity reached 0.260 g L^{-1} h^{-1} [52].

Furthermore, residues of wheat processing such as wheat straw and wheat bran can be considered as very promising potential substrates for PHAs production. World wheat production estimated for the

Table 3 – *Summary of PHAs production from lignocellulose materials*

Production strain	Carbon source	PHA type	Cultivation	Biomass (g L^{-1})	PHA (%)	PHA (g L^{-1})	$Y_{P/S}$	Ref.
Burkholderia cepacia	Xylose	PHB	Flasks, batch	2.6	60	1.6	0.11	62
Pseudomonas strains	Grass biomass	mcl-PHA	Flasks, batch	0.9	33	0.3	n.a.	57
Azotobacter beijerinickii	Coir pitch	PHB	Flasks, batch	5.0	48	2.4	n.a.	50
Burkholderia sacchari	Wheat straw hydrolysate	PHB	Fermenter, Fed-batch	145.8	72	105.0	0.22	55
Recombinant E. coli	Beech wood xylan and xylose	P(LA-*co*-3HB)	Flasks, batch	8.9	40.4	3.6	n.a.	42
Burkholderia cepacia	Wood hydrolysate	PHB	Flasks, batch	n.a.	n.a.	n.a.	n.a.	39
Ralstonia eutropha	Bagasse hydrolysate	PHB	Flasks, batch	11.1	56.5	6.3	n.a.	44
Bacillus firmus	Rice straw hydrolysate	PHB	Flasks, batch	1.9	89	1.7	n.a.	51
Burkholderia cepacia	Wood hydrolysate	P(3HB-*co*-3HV)	Flasks, batch	n.a.	40	2.0	n.a.	36
Recombinant E. coli	Cellulose hydrolysate + propionate	P(3HB-*co*-3HV)	Flasks, batch	4.2	50	2.1	n.a.	61
Bacillus megaterium	Oil palm empty fruit bunch	PHB	Flasks, batch	24.2	51.6	12.5	0.232	52
Burkholderia cepacia	Wood hydrolysate	PHB	Fermenter, Fed-batch	16.9	51.4	8.7	0.19	38
Sacharophagus degradans	Waste from tequila bagasse	PHA	Flasks, batch	n.a.	n.a.	n.a.	n.a.	46
Burkholderia cepacia	Bagasse hydrolysate	PHB	Flasks, batch	4.4	53	2.3	0.29	43
Burkholderia sacchari	Bagasse hydrolysate	PHB	Flasks, batch	4.4	62	2.7	0.39	43
Halomonas boliviensis	Wheat bran + potato waste hydrolysate	PHB	Flasks, batch	8	50	4.0	0.2	54
Ralstonia eutropha	Water hyacinth hydrolysates	PHB	Fermenter, batch	12	58.3	7.0	0.13	59
Burkholderia sacchari	Xylose	PHB	Flasks, batch	5.5	58	3.2	0.26	63
Burkholderia sacchari	Xylose	PHB	Flasks, batch	5.3	50	2.7	0.17	36
Ralstonia eutropha	Enzymatically hydrolysed pulp fibre	PHB	Flasks, batch	8.78	31.9	2.8	n.a.	60
Sphingobium scionense	Enzymatically hydrolysed softwood	PHB	Flasks, batch	1.23	32	0.4	0.22	40
Burkholderia cepacia	Spent coffee grounds hydrolysate	P(3HB-*co*-3HV)	Flasks, batch	5.5	56	3.1	0.24	49
Brevundimona svesicularis	Acid hydrolysed sawdust	P(LA-*co*-3HB-*co*-HV)	Flasks, batch	0.34	78	0.3	n.a.	41
Bacillus thuringiensis	Bagasse hydrolysate	PHB	Flasks, batch	10.6	39.6	4.2	n.a.	45

period 2012–2013 is about 660 million tonnes, of which about 15–20 % is straw. Asia and Europe are the primary production regions, with about 43 % and 32 %, respectively, while North America is the third largest production region with 15 % of global wheat production[53]. Van-Thuoc *et al.* studied PHB production from wheat bran enzymatic hydrolysate using *Halomonas boliviensis*. The PHB yields were improved by addition of butyric acid and sodium acetate, which can be obtained by anaerobic digestion of solid potato waste. The biomass and PHB yields obtained in this study were 8.0 and 4.0 g L^{-1}, respectively[54]. In addition, wheat straw was converted into PHB by *Burkholderia sacchari.* The lignocellulosic hydrolysates were prepared from ground wheat straw using the AFEX (Ammonia Fiber Expansion, treatment of material with ammonia) process as pre-treatment followed by an enzymatic hydrolysis of the cellulose and hemicellulose fractions. Resulting hydrolysate contained glucose, xylose and arabinose as the most predominant sugars. PHB production was performed in fed-batch

cultivation mode where the feeding strategy was set to overcome CCR. Very high maximum polymer concentration of 105 g L^{-1} was gained after 61 hours of cultivation corresponding to an accumulation of 72 % of cell dry weight (CDW). Polymer yield and productivity were 0.22 g g^{-1} total sugar consumed, and 1.6 g L^{-1} h^{-1} respectively[55].

Around 3.4 billion hectares of total land area comprises grasslands, which cover 69 % of the global agricultural area. Grasslands also play an important role in Europe's agriculture, i.e. the total agricultural area constitutes 164 million hectares of cultivated land, of which 76 million hectares are permanent grassland. Grasslands do not require the addition of fertilizers, annual ploughing of soil, and are a carbon sink which generate very low net greenhouse gas emissions. It was estimated that about 19 million hectares of pastures could become available for bioenergy feedstock without compromising Europe's food and feed sectors by 2030[56]. According to Davis *et al.,* grass biomass can be turned into *mcl*-PHAs employing *Pseudomonas* strains. Grass biomass was pre-treated (2 % NaOH 120 °C or hot water 120 °C) and delignified, and further enzymatically digested. Tested *Pseudomonas* strains accumulated 20–34 % of *mcl*-PHA per biomass when these hydrolysates were used as sole carbon and energy source[57]. Furhermore, Koller *et al.* used green grass juice as an additional complex nitrogen and phosphate substrate to improve PHB production by *Ralstonia eutropha*[58]. Also, saccharified water hyacinth hydrolysates were used for PHB production by *Ralstonia eutropha*. Bacteria preferred enzymatic hydrolysate over acid hydrolysate, and PHB production was optimized using response surface methodology. After 72 h of cultivation, 35 g l^{-1} of reducing sugar containing water hyacinth hydrolysate supplemented with 1.5 g L^{-1} $(NH_4)_2SO_4$ in laboratory scale fermenter gave 12 g L^{-1} of dry cell weight and 7 g L^{-1} of PHB[59].

PHAs production was also reported on cellulose hydrolysates. For instance, Zhang *et al.* published PHB production on enzymatically hydrolysed pulp fibre sludge employing *R. eutropha*. By controlling the concentrations of the inorganic salts in the growth medium, PHB content in the cell mass reached 78 %. Efforts were made to find conditions for bacterial growth in the form of a biofilm on a cheap and reusable carrier. A number of positively charged carriers was tested, and the anion exchanger DEAE-Sephadex A-25 was chosen for packed-bed biofilm culture[60]. Chemically hydrolysed cellulose and propionate as a precursor of 3-hydroxyvalerate were used as substrates for P(3HB-*co*-3HV) production by transgenic *Escherichia coli* with superior resistance to HMF. The yields for cell growth and polymers were almost the same with those performed for the case of analytical grade glucose[61].

Conclusions

Lignocellulose materials seem to be very promising substrates for various industrial and biotechnological processes, because utilization of these resources might decrease our dependence on petroleum and reduce the impact of its gradual depletion and increasing price. Despite all the problems related to lignocellulosic biomass utilization for biotechnological processes, the concept of industrial bio-refineries – facilities converting biomass into multiple valuable products – is becoming attractive, and it can be expected that its importance will increase in the following decades. PHAs might represent important products of bio-refineries, which might help these valuable environmentally-friendly polymers to compete with petrochemical-based plastics and therefore, partially replace them in appropriate applications.

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