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INVITED REVIEW PAPER

Lignocellulosic and marine biomass as resource for production of polyhydroxyalkanoates

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Abstract−Polyhydroxyalkanoates (PHAs) are considered as sustainable 'green/bio plastics' because they have potential to replace their depleting petroleum-based competitors in the recent future. To reach this goal, PHAs must be able to compete with the established petroleum-based plastics in both technical and economic aspects. The current PHA production is based on high-priced substrates of high nutritional value and simple carbon sources such as glucose, sucrose, starch, or vegetable oils. Non-food based carbon-rich complex polysaccharides of lignocellulosic and marine biomass can be used as alternative and suitable feedstock through consolidated bioprocessing (CBP). CBP is a promising strategy that involves the production of lytic enzymes, hydrolysis of biomass, and fermentation of resulting sugars to desired products in a single process step. CBP offers very large cost reductions if microorganisms possessing the abilities are found or microbial processes are developed to utilize substrate and simultaneously produce products. This review focuses on possible available complex polysaccharides of lignocellulosic and marine biomass that can be used as resources to produce PHAs in biorefineries, including CBP.

Keywords: Polyhydroxyalkanoates, Bioplastics, Consolidated Bioprocessing, Lignocellulose, Marine Biomass

INTRODUCTION

Biomass derived from plants or marine algae is the only foreseeable sustainable source of fuels and materials available. Cellulose, agar, carrageenan, laminarin, mannitol, alginate, fucoidan, etc., are some of the most abundant components of this biomass. Lignocellulose, agar, and alginate are attractive for their relatively low cost, plentiful supply, and uncompetitive feature with a food source. The central technological impediment to more widespread utilization of these important resources is the general absence of low-cost technology for overcoming the recalcitrance of this biomass. A promising strategy to overcome this impediment involves the production of lytic enzymes, hydrolysis of biomass, and fermentation of resulting sugars to desired products in a single process step called 'consolidated bioprocessing' (CBP). CBP offers very large cost reduction if microorganisms possessing the capabilities are found or microbial processes are developed to utilize substrate and simultaneously produce products. CBP has been a subject of increased research effort in recent years [1,2].

The recalcitrance hurdle can, in principle, be overcome by combined action of pre-treatment and catalytic degradation. The catalytic degradation is achieved via two ways: acidic and enzymatic. The acidic processes generally prohibit bacterial growth and hamper downstream fermentative process [3,4]. Enzymatic hydrolysis is another approach of biomass conversion in which higher amounts of sugars can be extracted with low levels of inhibitors. A major obstacle for the implementation of economically feasible lignocellulosic biorefineries is the excessively high cost of enzymes. Around $0.10-0.40/gal of total metabolite production cost is generally accounted for cellulolytic enzymes [5]. Processes in which cellulosic biomass is fermented to desired products in one step without adding externally produced enzymes are of obvious appeal. Indeed, such CBP is widely recognized as the ultimate configuration for low cost hydrolysis and fermentation of cellulosic biomass [6].

A CBP-enabling microbe must be able to both solubilize a practical biomass substrate and produce desired products under industrial/biorefinery conditions. Since microbes with these properties are difficult to find in nature, developing CBP-enabling microorganisms is being made through two strategies: engineering naturally occurring cellulolytic microorganisms to improve product-related properties, such as yield and titer, and engineering non-cellulolytic organisms that exhibit high product yields and titers to express a heterologous cellulase system enabling cellulose utilization. Other strategies could be exploitation of microbe synergy as well as the use of thermophilic organisms and/or complexed cellulase systems. Cellulose-adherent cellulolytic microorganisms are likely to successfully compete for products of cellulose hydrolysis with non-adhered microbes, including contaminants, which could increase the stability of industrial processes based on microbial cellulose utilization.

The CBP strategy has received the most attention with respect to ethanol production and is being implemented commercially for ethanol production [1]. It is in principle applicable to production of a broad range of products from plant and algal biomass. In this review, we endeavor to provide an overview of biorefinery and possible complex carbon sources available to produce PHA by a

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CBP strategy.

BIOREFINERIES

In the twenty-first century, petroleum-based products will steadily be replaced by products obtained from renewable materials. Now, there is a strong trend back from the oil-based economy to a more bio-based economy [7]. This change is driven by the concern about climate change caused by greenhouse gas emissions, the predicted depletion of petroleum resources, and the desire for a secure and independent energy supply [8-12]. In biorefineries, intermediates and end products are produced from renewable resources such as biomass [13]. Equivalent to petrochemical refineries, biorefineries are envisaged to use all basic compounds of biomass (cellulose, starch, algal carbohydrate, oils, etc.) and to convert them into biofuels, biomaterials (e.g. bioplastics), and biochemicals [8,13,14]. Chemical, enzymatic, and microbial processes can be employed to transform carbohydrates, the major fraction of biomass, into building-block chemicals, which form the basis for the production of bio-based fuels, chemicals, and materials [8,13,14]. Examples of key building block chemicals include alcohols and carboxylic acids [8]. Many of these building blocks can be produced by microbial fermentation, but also final products such as bioplastics can be directly produced with microorganisms [15]. Since these processes usually employ simple carbon sources and pure cultures of microorganisms, the cost of production increases. Moreover, these processes often have to be performed in multiple steps. These conditions result in expensive equipment, complicated procedure, and simple carbon source consumption, making industrial biotechnology unfavorable for the large scale production of relatively cheap bulk biochemicals and biomaterials. Therefore, use of complex polysaccharides can be an alternative way to reduce the production cost in biorefineries.

COMPLEX CARBON SOURCES

1. Complex Polysaccharides in Terrestrial Environments

Lignocellulose, which is a complex form of cellulose, hemicelluloses (including xylans, arabinans, and mannans), and lignin, are the most abundant renewable resources on earth because plant biomass, especially the plant cell wall, primarily occurs in the form of lignocelluloses [16-19]. It also contains smaller amounts of pectins, inorganic compounds, proteins, and extractives, such as waxes and lipids, which also have potential value. The exact composition of lignocellulose depends on the species, the plant tissue and the growth conditions. Generally, lignocellulosic biomass consists of 35-50% cellulose, 20-35% hemicellulose, and 10-25% lignin [20,21]. The components of different lignocellulose feedstock are presented in Table 1.

Table 1. Composition of some lignocellulosic biomass

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood	40-55	24-40	18-25
Softwood	45-50	25-35	25-35
Corn stover	45	35	15
Wheat straw	30	50	15
Rice straw	32.1	24	18
Bagasse	25-45	28-32	15-25
Oil palm	50.4	21.9	10
Grass	25-40	25-50	10-30
Paper	85-99	0	0-15

1-1. Cellulose

Cellulose, which is the most abundant organic compound in the biosphere, is the largest single component of lignocellulose. Cellulose microfibrils act as the structural backbone of the plant cell wall. They are somewhat irregular and contain regions of varying crystallinity. The degree of crystallinity depends on how tightly ordered the hydrogen bonding is between its component cellulose chains. Areas with less-ordered bonding, and therefore more accessible glucose chains, are referred to as amorphous regions. The relative crystallinity and fibril diameter are characteristic of the biological source of the cellulose [22-24]. The irregularity of cellulose fibrils results in a great variety of altered bond angles and steric effects, which hinder enzymatic access and subsequent degradation. Because of this variability, cellulose degradation requires a variety of enzymes, presumably with wide variations in the shape of the substrate-binding pockets and/or active sites [25,26].

1-2. Hemicellulose

Hemicellulose is a group of polysaccharides that makes up around 30 wt% of the biomass. These carbohydrate polymers are of lower molecular weight than cellulose (degree of polymerization around 100-200) [27]. Hemicellulose is composed of both hexose and pentose sugars; the C6 sugars of glucose, mannose, galactose and the C5 sugars of xylose and arabinose. Hemicellulose polymers can be branched and may be decorated with functionalities such as acetyl and methyl groups, cinnamic, glucuronic, and galacturonic acids. Hemicellulose is thought to bind non-covalently to the surface of cellulose fibrils. It acts as an amorphous matrix material, holding the stiff cellulose fibrils in place. It has been suggested that the substitution with hydrophobic groups such as acetyl and methyl groups enhances the affinity of hemicellulose to lignin and thus aids the cohesion between the three major lignocellulosic polymers [27]. The most common hemicellulose sugar in grasses and hardwood is xylose. Due to its non-crystalline nature, hemicellulose is more susceptible to depolymerization than cellulose, an aspect of its behavior that is exploited by many deconstruction strategies. Like cellulose, hemicellulose is also an important source of fermentable sugars for biorefining applications.

2. Complex Polysaccharides in Marine Environments

The marine environment represents a virtually untapped source of energy, which could, theoretically, meet the total global demand for energy [28]. In environmental aspects, terrestrial biomass-based biorefinery can rather exacerbate climate change when taking into account the life cycle of its final products. Fargione et al. [29] and Dominguez-Faus et al. [30] reported that direct and indirect land use change for energy crop cultivation induces a significantly high carbon debt and high water consumption. Although many researchers have been trying to utilize lignocellulosic biomass that is not used for food, this biomass can still incur the same environmental

consequences associated with land use and water consumption [29,30]. Thus, terrestrial biomass-based biorefinery seems not to be sustainable at present due to environmental as well as economic impacts.

In marine environments, the annual production of over 25 billion tons of complex polysaccharides is recycled to usable carbon [26]. These complex polysaccharides, including agar, chitin, alginate, and cellulose, function as structural and/or energy-storage polymers in planktonic organisms, algal blooms, benthic invertebrates, as well as terrestrial and aquatic plants. Marine plant biomass has many advantages over terrestrial plant biomass as a feedstock [28]. For example, macroalgae, so-called seaweeds, have the high potentials to fully and partly displace terrestrial biomass and produce sustainable bioenergy and biomaterials. Macroalgae do not need land and freshwater for their cultivation [31]. Based on the presence or lack of phyto-pigments other than chlorophyll, marine macroalgae can be classified into three major classes: brown algae (Phaeophyceae), red algae (Rhodophyceae), and green algae (Chlorophyceae)

2-1. Brown Macroalgae

Brown macroalgae include almost 1800 species of multicellular algae with a characteristic olive-green to dark brown color. Abundant amount of yellow-brown pigment fucoxanthin, which masks the green color of chlorophyll, is produced by these species. This group includes the largest and most complex of the macroalgae, the Laminaria, which may reach lengths of 100 m and grow as much as 50 cm/day. Laminaria are found at depths below the low tide level in temperate and polar regions and are farmed extensively in Asia as food products, especially in China, Japan, and Korea [32].

Brown macroalgae contains up to 67% carbohydrates by dry weight [33]. The composition of brown macroalgae such as Laminaria includes laminarin and mannitol [32] as given in Table 2. Brown macroalgae also contain alginate and cellulose, which are two structural polysaccharides abundant in the cell wall for mechanical strength to prevent ripping during currents and tidal fluctuations. Although the sugars like laminarin and mannitol can be easily extracted from milled seaweed for bioconversion, the full potential of biofuel or biochemical production from brown macroalgae has not yet been realized, because industrial microbes are not able to metabolize the alginate component [34,35]. Alginate can be a good carbon source for production of PHA or biofuel [33,36] because alginates are quite abundant in nature. In brown algae, they are produced as a structural component, comprising of up to 40% of dry weight [36].

2-2. Red Macroalgae

Red macroalgae include almost 6000 species of algae having a characteristic red or pink color from the pigments phycocyanin and phycoerythrin, which allow growth in relatively deep waters [28]. Red algae are found in the intertidal and subtidal zones of the sea at depths up to 40 m or occasionally as deep as 250 m. The composition of red macroalgae varies from species to species but generally consists of cellulose, glucan, and galactan (Table 1). The red algae are known for high carbohydrate content with *Gelidium amansii* as one of the most abundantly available red seaweed species, appearing along the warm and shallow coastal area of many sub-tropical countries. The *G. amansii* mainly consists of polysaccharide complexes of fibrin (cellulose) and agar (galactan) whose basic monomer is glucose and galactose, respectively [37]. The major repeating unit of agar is agarobiose, which is a disaccharide composed of 1,3-linked D-galactose and 1,4-linked 3,6-anhydrous-L-galactose (3,6-AHG) [38]. Accordingly, Kim et al. [39] reported that the dilute-acid hydrolysis of *G. amansii* to produce sugars can be performed in a batch-type autoclave, and the main products are D-galactose, 3,6-AHG, and D-glucose. Among them, the galactose and glucose are classified as fermentable mono sugars and the 3,6-AHG as non-fermentable. Since the physical morphology of agar components is softer than that of cellulose, the hydrolyzed products, galactose and 3,6-AHG, are to be firstly released at relatively mild hydrolysis conditions. The 3,6-AHG, one of the main sugar component of *G. amansii*, is also known as acid-labile. Hence, it is very prone to decompose into 5-HMF and, subsequently, into organic acids such as levulinic acid and formic acid that act as inhibitors in the fermentation process [38]. Therefore, CBP could be useful for exploitation of this abundantly available carbon source.

2-3. Green Macroalgae

Green macroalgae include an estimated 1500 species, of which only about 15% are marine and the remainder live in freshwater or terrestrial environments. Because of the need for more light for photosynthesis, green seaweeds live mostly in the shallowest waters, including the intertidal pools that fill and drain with the tides. They are common in bays or estuaries where salt water and fresh water mix together. In most cases, the composition of green macroalgae includes starch for food reserves with cellulose and pectin as the main structural polysaccharide in the cell wall [28].

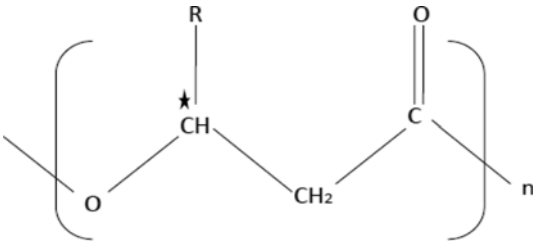
Most of these complex polysaccharides are insoluble and relatively recalcitrant, requiring dedicated multi-enzyme systems for their degradation [40,41]. Microorganisms are very diverse and live in every part of the biosphere. They play an important role in ecosystems such as nutrient recycling, biological decomposition, and production of vital metabolites [42,43]. Marine bacteria are useful and industrially significant [26,44,45]. Some marine bacteria are known to mediate the degradation of agar, alginate, and cellulose [26], but production of metabolites such as PHAs from these abundantly available carbon sources is still rare.

Table 2. Seaweed class and carbohydrate composition

Seaweed	Class	Carbohydrate composition
Gelidium amansii	Red	Agar, Carrageenan, Cellulose
Laminaria japonica	Brown	Alginate, Mannitol, Laminarin, Fucoidan, Cellulose
Ulva pertusa	Green	Starch, Cellulose

POLYHYDROXYALKANOATES

PHAs are biopolyesters synthesized by microorganisms as energy storage material [46,47]. The occurrence of PHA in bacteria has been known since 1920s. Lemoigne reported the formation of poly(3-hydroxybutyrate) (PHB) inside bacteria [48]. PHAs have attracted much commercial and research interests due to their bio-



R=methyl	C_4	Poly(3-hydroxybutyrate)	(3HB)
R=ethyl	C_5	Poly(3-hydroxyvalerate)	(3HV)
R=propyl	C_6	Poly(3-hydroxyhexanoate)	(3HC)
R=butyl	C_7	Poly(3-hydroxheptanoate)	(3HH)
R=pentyl	C_8	Poly(3-hydroxoctanoate)	(3HO)
R=hexyl	C_9	Poly(3-hydroxnonanoate)	(3HN)
R=heptyl	C_{10}	Poly(3-hydroxdecanoate)	(3HD)
R=octyl	C_{11}	Poly(3-hydroxyundecanoate)	(3HUD)
R=nonyl	C_{12}	Poly(3-hydroxydodecanoate)	(3HDD)
R=decyl	C_{13}	Poly(3-hydroxytridecanoate)	(3HTD)

Fig. 1. Chemical structure of PHA. The nomenclature and carbon number for PHA compounds is determined by the functional alkyl R group. Star denotes chiral center of PHA-building block.

degradability, biocompatibility, chemical diversity, and manufacture from renewable carbon resources [49]. A PHA molecule is typically made up of 600 to 35,000 (*R*)-hydroxy fatty acid monomer units [50]. Each monomer unit harbors a side chain *R* group that is usually a saturated alkyl group (Fig. 1), but can also take the form of unsaturated, branched, and substituted alkyl groups, although these forms are less common [51]. The monomer composition and the chain length of the polymer, as well as the length of the side chains influence the properties of the polymer. Depending on the total number of carbon atoms within a PHA monomer, PHA can be classified as either short-chain length PHA (scl-PHA; 3 to 5 carbon atoms), medium-chain length PHA (mcl-PHA; 6 to 14 carbon atoms), or long-chain length PHA (lcl-PHA; 15 or more carbon atoms) [50]. About 150 different PHA monomers have been identified and this number continues to increase with the introduction of new types of PHA through the chemical or physical modification of naturally-occurring PHA [52], or through the creation of genetically-modified organisms to produce PHA with specialized functional groups [53]. These features gave rise to diverse PHA properties that can be tailored for various applications ranging from biodegradable packaging materials to medical products. PHA is also considered as pharmaceutically active compound and currently investigated as potential anti-HIV drugs, anti-cancer drugs, antibiotics, etc. [54].

Accumulation of PHAs is a natural process for bacteria to store carbon and energy, when nutrient supplies are imbalanced. They are accumulated when growth is limited by depletion of nitrogen, phosphorous, or oxygen and an excess amount of a carbon source is still present [55]. While the most common limiting nutrient is nitrogen, for some bacteria such as *Azotobacter* spp., the most effective limiting nutrient is oxygen [56]. The first PHA to be discovered and therefore the most studied is PHB. Bacteria in their metabolism produce acetyl-coenzyme A (acetyl-CoA), which is converted into PHB by three biosynthetic enzymes. In the first step, 3-ketothiolase (PhaA) combines two molecules of acetyl-CoA to form acetoacetyl-CoA. Acetoacethyl-CoA reductase (PhaB) allows the reduction of acetoacetyl-CoA by NADH to 3-hydroxybutyryl-CoA. Finally, PHB synthase (PhaC) polymerizes 3-hydroxybutyryl-CoA to PHB, coenzyme-A being liberated. Only (R)-isomers are accepted as substrates for the polymerizing enzyme [57]. During normal bacterial growth, the 3-ketothiolase will be inhibited by free coenzyme-A coming out of the Krebs cycle. When entry of acetyl-CoA into the Krebs cycle is restricted (during non-carbon nutrient limitation), the surplus acetyl-CoA is channeled into PHB biosynthesis [58].

Bioplastics are plastic-like materials that are biodegradable/compostable and made from renewable materials. Some biodegradable plastics are made from non-renewable materials (e.g., polycaprolactone (PCL), Ecoflex® from BASF), while some plastics derived from renewable materials are not biodegradable (e.g., polyamide). Bioplastics currently on the market are starch-based materials (e.g., produced by Novamont, Italy), cellulose-based materials (e.g., cellophane), polylactic acid (e.g. by NatureWorks, USA; Purac, the Netherlands; Toyota, Japan), and PHAs (e.g. Metabolix, USA; PHB Industrial SA, Brazil; Tianan Biologic Material, China; Biomer, Germany). These materials differ greatly in their properties such as moisture permeability, crystallinity, glass transition temperature, and melting point. Therefore, they are not necessarily competing with each other but may be used for different applications. Even though PHAs have unique properties like high heat and moisture resistance compared to other bioplastics, their market penetration is currently very small. This will definitely change once the large scale production starts using cheap and abundantly available carbon source such as lignocelluloses and marine macroalgae.

CURRENT STATUS OF PHA PRODUCTION FROM COMPLEX POLYSACCHARIDES

Cellulose, hemicelluloses, and seaweeds are polysaccharides that can be broken down into sugars and fermented or chemically altered into valuable fuels and chemicals. Hydrolysis technologies may involve physical treatments, chemical methods such as hydrolysis by concentrated or dilute acid, and enzymatic methods, whereby often chemical and enzymatic hydrolysis are combined in consecutive steps. The aim of the pre-treatment process is removal of lignin and hemicelluloses, reduction of the cellulose crystallinity, as well as increase of the porosity of the lignocellulosic materials, which helps to enhance degradation by cellulase. Cellulase refers to a group of enzymes which act together to hydrolyze cellulose. Fungi are the main cellulase-producing microorganisms, though a few bacteria have also been reported to yield cellulase activity [59]. Table 3 summarizes fermentation parameters for PHA production from lignocellulosic biomass and seaweeds. Lignocellulosic materials are potential substrates for low-cost PHA production by CBP but only a few such studies have been reported so far (Table 3). Munoz and Riley [60] used tequila bagasse for PHA production employing *Saccharophagus degradans*, which can readily attach to cellulosic fibers, degrade the cellulose, and utilize this as

Table 3. Summary of PHA production from complex carbon sources

PHA producing strain	PHA type	Production by CBP	Carbon source	Pretreatment	Enzyme used for hydrolysis	PHA conc. (g/L)	Reference
Azotobacter beijerinickii	PHB	No	Coir pith	delignified by autoclaving	Commercially obtained	2.4	[72]
Bacillus firmus	PHB	No	Rice straw hydrolysate	2% H_2SO_4 Pretreated	N	1.7	[73]
Bacillus megaterium	PHB	No	Oil palm empty fruit bunch	Alkaline hydrogen peroxide	On-site produced cellulase	12.5	[65]
Bacillus mycoides	P(3HB-co-3HV)	No	Rice husk hydrolysate	Acid hydrolysis	N	0.39	[74]
Bacillus thuringiensis	PHB	No	Bagasse hydrolysate	Acid hydrolysis	N	4.2	[75]
Brevundimona svesicularis	P(LA-*co*-3HB-*co*-HV)	No	Acid hydrolyzed sawdust	Acid hydrolysis	N	0.3	[76]
Burkholderia cepacia	PHB	No	Wood hydrolysate	Acid hydrolysis	N	8.72	[77]
Burkholderia cepacia	PHB	No	Bagasse hydrolysate	n.i.	n.i.	2.33	[64]
Burkholderia cepacia	PHB	No	Wood hydrolysate	n.i	n.i	n.a.	[77]
Burkholderia cepacia	P(3HB-*co*-3HV)	No	Wood hydrolysate	n.i.	n.i.	2.0	[78]
Burkholderia cepacia	P(3HB-*co*-3HV)	No	Spent coffee grounds hydrolysate	Acid treatment	Commercially obtained	3.1	[79]
Burkholderia sacchari	PHB	No	Bagasse hydrolysate	n.i.	n.i.	2.73	[64]
Burkholderia sacchari	PHB	No	Wheat straw hydrolysate	AFEX	N	105	[62]
E. coli TG1b (pSYL107)	PHB	No	Xylose and soybean hydrolysate	Commercially obtained	Commercially obtained	4.4	[63]
Escherichia coli LS5218	P(3HB-*co*-3HV)	No	Cellulose hydrolysate	n.i.	Ruthenium-catalyzed cellulose hydrolysate	3.3	[68]
Halomonas boliviensis	PHB	No	Wheat bran+ potato waste hydrolysate	N	On-site produced cellulase	4.0	[80]
Halomonas hydrothermalis	PHA	No	Seaweed derived crude levulinic acid	n.i.	n.i.	1.07	[66]
Paracoccus sp. LL1	PHB	No	Corn stover hydrolysate	Alkaline hydrogen peroxide	On-site produced cellulase	9.71	[7]
Pseudomonas strains	mcl-PHA	No	Grass biomass	2% NaOH	Commercially obtained	0.3	[81]

Table 3. Continued

PHA producing strain	PHA type	Production by CBP	Carbon source	Pretreatment	Enzyme used for hydrolysis	PHA conc. (g/L)	Reference
Ralstonia eutropha	PHB and polyesters	No	Bagasse hydrolysate	Acid hydrolysis	N	3.9	[82]
Ralstonia eutropha	PHB	No	Water hyacinth hydrolysates	Acid treatment	Commercially obtained	7.0	[83]
Ralstonia eutropha	PHB	No	Enzymatically hydrolyzed pulp fiber	n.i.	Commercially obtained	2.8	[84]
Recombinant E. coli	P(LA-*co*-3HB)	No	Beech wood xylan and xylose	Without treatment	N	3.6	[85]
Recombinant E. coli	PHB	Yes	CMC	N	N	0.05	[61]
Sacharophagus degradans	PHA	Yes	Waste from tequila bagasse	Without treatment	N	n.a	[60]
Sphingobium scionense	PHB	No	Enzymatically hydrolyzed softwood	HTMP or SEW	Commercially obtained	0.4	[86]
Sphingopyxis macrogoltabida	P(3HB-*co*-3HV)	No	Sawdust	Acid hydrolysis	N	0.23	[76]

n.i.: not indicated, N: nil, P(3HB-co-3HV): copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate, AFEX: ammonium fiber expansion, HTMP; high-temperature mechanical pre-treatment, SEW: steam exploded wood

the primary carbon source while producing PHB. This approach does not require hydrolysis prior to cultivation, which in turn theoretically reduces the cost of up-stream processing. Unfortunately, Munoz and Riley [60] did not reveal PHB yields; thus it is very difficult to estimate the efficiency of such a process. Gao et al. [61] showed PHA production in single step (CBP) by using recombinant *Escherichia coli*, but low PHA concentration of 0.05 g/L was obtained. Till date, only two studies done by Munoz and Riley [60] and Gao et al. [61] showed production of PHA in single step from complex carbon source. Numerous reports are available by utilizing chemical hydrolysis, enzymatic hydrolysis, or chemical and enzymatic hydrolysis in combined steps (Table 3). For example, high PHA concentration of 105 g/L was obtained by *Burkholderia sacchari* from wheat straw hydrolysate [62]. Another study by Lee [63] reported PHA concentration of 4.4 g/L from xylose supplemented with soybean hydrolysate using a recombinant *E. coli*. By process parameter optimization, Silva et al. [64] obtained PHA concentration of 0.23 g/L from acid hydrolyzed sawdust using *Sphingopyxis macrogoltabida* LMG 17324. Zhang et al. [65] achieved 12.48 g/L of PHA from oil palm empty fruit bunch, which was first chemically pre-treated and enzymatically hydrolyzed by a cellulase cocktail, and further used as a substrate for PHA production employing *Bacillus megaterium*. The range of PHA concentration reported using cellulose hydrolysate varied from 0.2 to 105 g/L. Although there are numerous efforts on developing core technologies (production, harvest, storage, depolymerization, and bio/chemical conversion) for producing PHA from terrestrial plant biomass, production of PHA from marine biomass has received less attention. Bera et al. [66] showed PHA production from marine algae derived components, but PHA concentration was low. Utilization of algae for PHA production in efficient way is still a challenge for researchers. Production of PHAs from plant and algal biomass has been tried in a variety of wild type and recombinant microorganisms [67-69]. However, these systems require hydrolysis pretreatment of biomass in a step prior to fermentation, so that it can be readily taken up by the microorganisms [70]. The pretreatment processes are often at high temperatures and low pH conditions. These are usually prohibitive for bacterial growth, generate potentially toxic compounds, and hamper downstream fermentation process, resulting in lower yields and increased production costs [3,4,71]. Fig. 2 shows two different strategies of PHA production from plant or marine biomass. The first strategy involves production of cellulase by fungi separately, hydrolysis of pretreated biomass using the generated cellulase, and use of hydrolyzed sugar for production of PHAs. However, the second approach that couples the hydrolysis and fermentation steps (i.e., CBP) can be an attractive strategy for the production of PHAs from plant and algae derived carbon sources (Fig. 2).

FUTURE PROSPECTS

The production of PHAs from complex polysaccharides is not

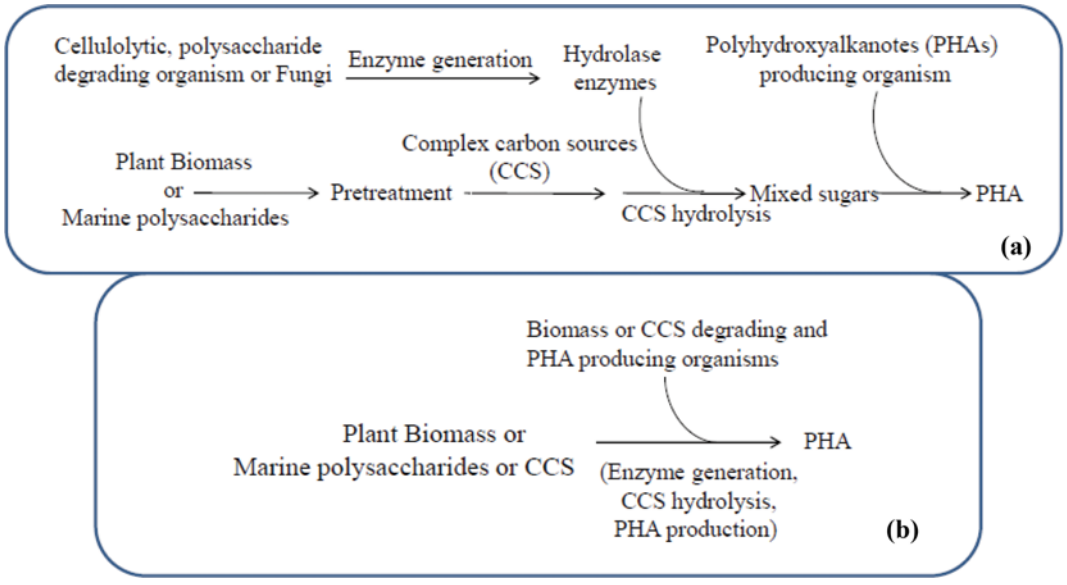


Fig. 2. Current status of PHA production from plant or marine biomass. (a) Typically, biomass hydrolysis is carried out in a separate process step via cellulose and hemicellulose hydrolyzing enzymes to generate sugars and PHA is obtained by PHA producing organisms. (b) Consolidated bioprocessing combines enzyme generation, biomass hydrolysis, and PHA production in a single stage.

well characterized. In fact, surprisingly little is known about how and which plant or marine biomass could be useful for production of PHAs. Currently, around 50% of production cost of PHAs depends on the carbon source used for fermentation, making it costlier than petroleum-derived plastics. For PHAs to replace conventional synthetic plastics, much effort should be given to reducing production cost. Screening/isolation of novel or reported bacteria and investigation of their ability to polymer accumulation from complex polysaccharides can serve this purpose. Genetic modification of bacteria utilizing complex carbon sources could be another way to produce PHA in high yield. CBP can provide new paradigm in PHA production from complex carbon sources using these microorganisms.

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