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Review

Effect of Lignocellulose Related Compounds on Microalgae Growth and Product Biosynthesis: A Review

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Abstract: Microalgae contain valuable compounds that can be harnessed for industrial applications. Lignocellulose biomass is a plant material containing in abundance organic substances such as carbohydrates, phenolics, organic acids and other secondary compounds. As growth of microalgae on organic substances was confirmed during heterotrophic and mixotrophic cultivation, lignocellulose derived compounds can become a feedstock to cultivate microalgae and produce target compounds. In this review, different treatment methods to hydrolyse lignocellulose into organic substrates are presented first. Secondly, the effect of lignocellulosic hydrolysates, organic substances typically present in lignocellulosic hydrolysates, as well as minor co-products, on growth and accumulation of target compounds in microalgae cultures is described. Finally, the possibilities of using lignocellulose hydrolysates as a common feedstock for microalgae cultures are evaluated.

Keywords: lignocellulose; hydrolysis; microalgae; cultivation; extraction; bioproducts

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1. Microalgae: A Source of Valuable Compounds

Microalgae include several groups of microorganisms that belong to the *Prokaryota* or *Eukaryota*, typically found in fresh water or marine systems in single cell forms or in groups. They are capable of performing photosynthesis, producing approximately half of the atmospheric oxygen while using the greenhouse gas carbon dioxide to grow photoautotrophically [1]. Microalgae contain valuable compounds such as lipids, proteins and pigments (Table 1) which have substantial potential for commercial applications. Microalgae cells accumulate lipids which include triacylglycerides (TAGs), polyunsaturated fatty acids (PUFAs) and sterols [2]. These lipids constitute storage materials or membrane structural components in microalgae and can be used for biofuel, food supplement and pharmaceutical production. Indeed, fossil fuels are nowadays still the main source of carbon based fuels, the exploitation of which causes emission of greenhouse CO_2. Biodiesel production from oil crops is seen as currently the best alternative, but still presents the main drawback of competing with food production for arable land. Therefore, production of biodiesel from TAGs present in microalgae can become an environmentally friendly alternative as microalgae produce oil and fix CO_2 from atmosphere without the necessity of implementing vast arable areas for cultivation [3]. On the other hand, consumption of PUFAs in the human diet can help prevent the development of cardiovascular and mental diseases [4]. Fish are a rich source of PUFAs, but uncontrolled fishing has led to a substantial decrease in the worldwide fish population [5]. Production of PUFAs from microalgae may overcome this problem. On the other hand, sterols from microalgae are important part of the diet for juvenile scallops or prawns in aquaculture hatcheries [6]. Moreover, the high protein content in microalgae makes them a possible fodder for agricultural livestock [7]. In addition, microalgae cells also possess pigments such as chlorophylls and carotenoids. Chlorophylls harvest solar light in the process of photosynthesis while carotenoids are accessory pigments that increase the range of sun light used for photosynthesis (β-carotene) or protect the photosynthetic mechanism against photodamage induced due to environmental stress conditions (astaxanthin, lutein) [8]. Those pigments can be used as food colorants and cosmetic additives against UV light or as pharmacological agents because of their wound healing and anticancer properties [9,10].

In order to make production of value-added compounds from microalgae an economically feasible process, it is necessary to produce high amount of microalgae biomass. Microalgae are cultivated in open ponds or different types of photobioreactors (see below, Section 5).

However, high culture densities are not achievable in scaled-up systems due to light limitations [11]. Many strains of microalgae are able to consume sugars, alcohols and organic acids as a source of carbon during heterotrophic or mixotrophic cultivation [12–14]. During heterotrophic growth, microalgae are cultivated in the dark, assimilate organic substances from the medium to cover energy requirements and release carbon dioxide. During mixotrophic cultivation, microalgae consume CO_2 using light energy as well as external organic compounds from environment. High microalgae biomass concentrations can be achieved during cultivation in large-volume bioreactors when organic substances are applied [15]. The ability of microalgae to grow on organic substrates raises the possibility of cultivating microalgae on lignocellulose feedstock and thus reduce cultivation costs and increase productivity. Lignocellulose is the world's most abundantly available raw plant material that can become a promising feedstock for microorganisms such as bacteria, yeasts and fungi to produce high value added products and biofuels [16–18].

Table 1. Taxonomy classification of microalgae with cellular component content *.

Taxonomy	**Microalgae**						
Domain	Eucaryota						
Division	Chlorophyta						
Class	Chlorophyceae					Trebouxiophyceae	
Order	Sphaeropleales	Volvocaes [1]		Chlamydomonadales		Chlorellales	
Family	Scenedesmaceae	Haematococcaceae	Dunaliellaceae	Chlamydomonadaceae		Chlorellaceae	
Genus	Acutodesmus	Haematococcus	Dunaliella	Chlamydomonas		Chlorella	
Species	*Scenedesmus obliquus*	*Haematococcus pluvialis*	*Dunaliella salina*	*Chlamydomonas reinhardtii*	*Chlorella prothotecoides*	*Chlorella zoofingiensis*	*Chlorella vulgaris*
Content	Proteins 51%	Pigments 1.5%	Pigments 11%	Lipids 65%	Lipids 62%	Lipids 54%	Proteins 46%
Reference	[19]	[20]	[21]	[22]	[23]	[24]	[25]
Domain	Eucaryota						
Division	Heterokontophyta	Dinophyta [2]	Euglenophyta	Heterokontophyta	Chlorophyta		
Class	Bacillarophyceae	Dinophyceae	Euglenophyceae	Eustigmatophyceae	Chlorophyceae		Chlorophyceae [3]
Order	Naviculales	Dinotrichales [2]	Euglenales	Eustigmatales	Sphaeropleales	Chlorococcales	Chlorococcales [3]
Family	Phaeodactylaceae	Crypthecodiniaceae	Euglenaceae	Monodopsidaceae	Selenastraceae	Chlorococcaceae	Dictyosphaeriaceae [3]
Genus	Phaeodactylum	Crypthecodinium	Euglena	Nannochloropsis	Monoraphidium	Neochloris	Botryococcus
Species	*Phaeodyctylum tricornutum*	*Crypthecodinium cohnii*	*Euglena gracilis*	*Nannochloropsis oculata*	*Monoraphidium contortum*	*Neochloris oleoabundans*	*Botryococcus braunii*
Content	Lipids 20%	Lipids 20%	Lipids 29%	Lipids 32%	Lipids 30%	Lipids 52%	Lipids 65%
Reference	[26]	[27]	[28]	[29]	[30]	[31]	[32]
Domain	Procaryota						
Division	Cyanobacteria						
Class	Cyanophyceae						
Order	Nostocales [3]	Chroococcales [4]	Chroococcales	Chroococcales [5]		Oscillatoriales [6]	
Family	Nostocaceae [3]	Spirulinaceae [4]	Microcystaceae	–		Oscillatoriaceae	
Genus	Anabaena	Spirulina	Microcystis	Thermosynechococcus		Oscillatoria	
Species	*Anabaena azollae*	*Spirulina platensis*	*Microcystis aeruginosa*	*Thermosynechococcus elongates*		*Oscillatoria acuminata*	
Content	Proteins 40%	Proteins 67%	Lipids 28%	Lipids 20%		Lipids 25%	
Reference	[33]	[34]	[35]	[36]		[35]	

* Cellular content values are expressed as % of dry weight and may vary markedly depending on growth conditions such as light intensity, CO_2 concentration, N deprivation, temperature or the presence of organic substrates; Source: AlgaeBase, Integrated Taxonomic Information System (ITIS) Report, PATRIC (Pathosystems Resource Integration Center). [1] According to ITIS Report Order: Volvocales, according to AlgaeBase Order: Chlamydomonadales; [2] According to ITIS Report Division: Pyrrophycophyta, Order: Gonyaulacales, according to AlgaeBase: Division: Dinophyta, Order: Dinotrichales; [3] According to ITIS Report; [4] According to AlgaeBase; [5] According to PATRIC; [6] According to Algae Base Order: Oscillatoriales, according to ITIS Report Order: Nostocales.

In this publication, the effect of lignocellulose feedstocks on microalgae growth and production of target compounds from microalgae culture is evaluated.

2. Composition and Treatment of Lignocellulose Materials

Lignocellulosic materials can be found in a large variety of plants such as coniferous trees (softwood), broad leave trees (hardwood), grasses and agricultural residues (Table 2). Lignocellulose is composed of three main biopolymers: namely cellulose, hemicelluloses and lignin. Cellulose, is a non-branched polymer consisting of D–glucopyranose units (hexoses) connected via β-(1,4)–glycosidic linkages. Hemicellulose is a complex carbohydrate polymer containing pentoses (mainly xyloses in the case of xylan—the main constituent of hardwood, grasses and agricultural wastes) and hexoses (typically mannoses in the case of mannan found principally in softwood), as the main sugars, bonded with β-(1,4)–glycosidic linkages. Unlike cellulose, many compounds such as saccharide residues and organic acids (glucuronic acid GluA), organic acid groups (acetyl) or lignin components are attached to the main sugar chain giving hemicellulose a branched structure. Finally, lignin is a complex biopolymer that consists of phenylpropanoid units such as hydroxyphenyl, guaiacyl and syringyl, which are connected to each other via various ether and carbon—carbon bonds. Cellulose chains are arranged in bundles and interlinked with hemicellulose. Lignin is cross-linked with hemicellulose and occupies space between cellulose bundles [37,38]. Plant materials also contain starch and small amounts of pectins, proteins, minerals, lipids, terpenoids, polyphenols and alkaloids. Starch is composed of amylose and amylopectin and serves as a storage material. When photosynthesis can not take place, glucose from starch provides energy that is used by plants to perform survival functions [39]. Starch constitutes 0.4% of straw [40], but its content in wheat bran can be 34% [41]. Pectin is a polysaccharide possessing in structure with D–galacturonic acid (GalA) as main units that are connected via α-(1,4)–glycosidic linkages. Additionally, regions composed of galacturonic acids are connected together via rhamnose (Rha) to which galactose (Gal) and arabinose (Ara) chains are also attached [42]. In plant material, mineral elements: Ca, K, Mg, Na, P, Fe, Mn and Si, are combined with organic molecules or are present in a form of inorganic salts [43,44]. Lipids in lignocellulose comprise a wide range of different compounds including fatty acids, glycerides, sterols and waxes [45]. Terpenoids such as monoterpenes and diterpenes can be found in softwood residues [46,47] and triterpenes are constituents of hardwood bark [48]. Polyphenols are found in softwood and hardwood and are represented by a wide range of compounds including gallotannins and ellagitannins, proanthocyanidins, flavonoids, lignans and stilbenes [49–51]. A variety of alkaloids can also be present in grasses [52] or the wood of tropical plants [53]. Lignocellulose sugars constitute a feedstock for bacteria, yeast and fungi to produce a variety of target compounds. Xylitol, a sweetener, was produced from xylose by *Candida guilliermondii* [17], while *Saccharomyces cerevisae* [54] and *Clostridium beijerinckii* [55] convert sugars to ethanol and butanol, respectively—second generation biofuels. Itaconic acid, a building block for fibers and rubbers, was produced by *Ustilago maydis* from xylose or glucose [18] and hydrogen, a third generation biofuel, is obtained from sugars with the use of *Caldicellulosiruptor saccharolyticus* [16]. However, the presence of three major polymers and other minor substances in lignocellulose, their rigidity and strong structure make the access to valuable carbohydrates very complicated. A number of methods have been developed to successfully breakdown this recalcitrant polymer structure and efficiently hydrolyse lignocellulosic materials (Figure 1).

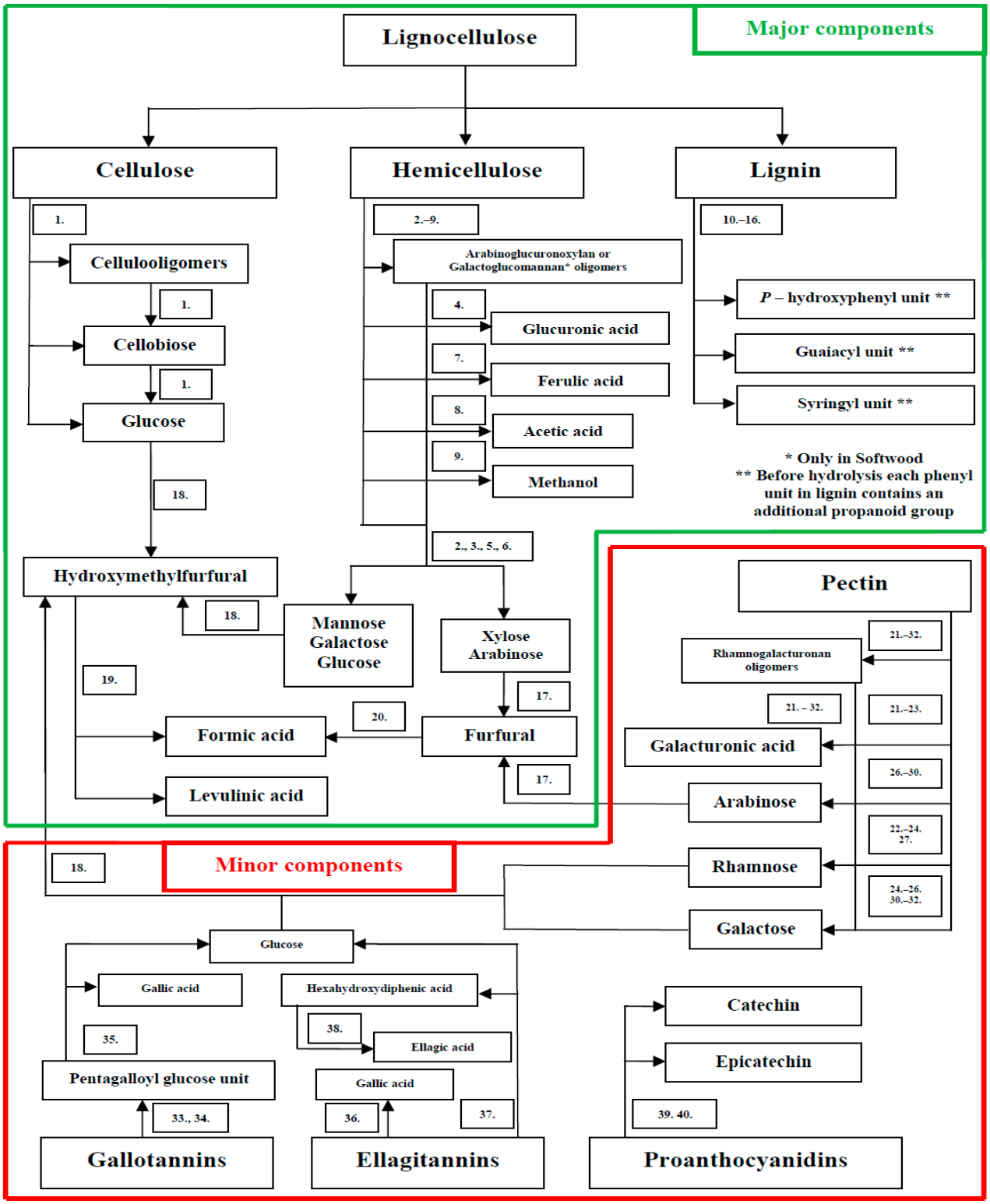
Table 2. Exemplary composition of different lignocellulosic materials.

Component *	*Triticum Aestivum* Bran	Corn Stover	*Cynodon Dactylon* Grass	*Hordeum Vulgare* Brewer's Spent Grain	*Oryza Sativa* Straw	*Picea Abies* Softwood	*Saccharum Officinarum* Bagasse	*Salix* Hardwood
Glucan [1]	10.5	36.1	30.4	16.7	35.9	40.9	35.8	43.0
Xylan	18.3	21.4	22.6	19.9	19.0	5.1	21.2	14.9
Mannan	–	1.8	0.0	–	–	10.1	0.79	3.2
Galactan	1.1	2.5	1.8	–	–	1.9	0.74	2.0
Arabinan	10.1	3.5	4.9	8.4	3.1	1.0	1.94	1.2
Klason lignin	5.0	17.2 [3]	18.8	22.9	13.6	27.7 [3]	16.6	24.2
AS lignin [2]	–		4.4	4.8	3.3		1.6	2.4
Reference	[41]	[56]	[57]	[58]	[59]	[60]	[61]	[62]

***** Values are expressed as % of dry material; [1] From cellulose; [2] AS—Acid Soluble; [3] A sum of Acid Soluble and Klason lignin.

Figure 1. Lignocellulose structure breakdown: hydrolysis of specific bonds and possible pathways for release of lignocellulose derived compounds. **1.** Hydrolysis of O–glycosidic bond β (C1→C4) between Carbon 1 of Glu and Carbon 4 of another Glu. **2.** Hydrolysis of O–glycosidic bond β (C1→C4) between Carbon 1 of one Xyl and Carbon 4 of another Xyl. **3.** Hydrolysis of O–glycosidic bond β (C1→C4) between Carbon 1 of Man or Glu and Carbon 4 of Man or Glu. **4.** Hydrolysis of O–glycosidic bond (C1→C2) between Carbon 1 of GluA and Carbon 2 of Xyl. **5.** Hydrolysis of O–glycosidic bond α (C1→C2) or α (C1→C3) between Carbon 1 of Ara and Carbon 2 or 3 of Xyl. **6.** Hydrolysis of O–glycosidic bond α (C1→C6) between Carbon 1 of Gal and Carbon 6 of Man. **7.** Hydrolysis of feruloyl group Fr–O–C on Carbon 5 of Ara. **8.** Hydrolysis of acetyl group Ac–O–C on Carbon 2 or 3 of Xyl. **9.** Hydrolysis of methyl group Met–O–C on Carbon 4 of GluA. **10.** Hydrolysis of ether bond β–O–C4 between Carbon β in propanoid group of one unit and Carbon 4 in phenyl structure of the second unit. **11.** Hydrolysis of ether bond α–O–C4 between Carbon α in propanoid group of one unit and Carbon 4 in phenyl structure of the second unit. **12.** Hydrolysis of ether bond C4–O–C5 between Carbon 4 in phenyl structure of one unit and Carbon 5 in phenyl structure of the second unit. **13.** Hydrolysis of carbon bond β–C5 between Carbon β in propanoid group of one unit and Carbon 5 in phenyl structure of the second unit. **14.** Hydrolysis of carbon bond β–C1 between Carbon β in propanoid group of one unit and Carbon 1 in phenyl structure of the second unit. **15.** Hydrolysis of carbon bond C5–C5 between Carbon 5 in phenyl structure of one unit and Carbon 5 in phenyl structure of the second unit. **16.** Hydrolysis of carbon bond β–β between Carbon β in propanoid group of one unit and Carbon β in propanoid group of the second unit. **17.** Dehydration of pentose structure with the loss of 3 molecules of water. **18.** Dehydration of hexose structure with the loss of 3 molecules of water. **19.** Hydrolysis of ring in hydroxymethylfurfural structure and its conversion to levulinic acid and formic acid. **20.** Hydrolysis of formyl group and its oxidation to formic acid. **21.** Hydrolysis of α O–glycosidic bond (C1→C4) between Carbon 1 of GalA and Carbon 4 of another GalA. **22.** Hydrolysis of O–glycosidic bond (C1→C2) between Carbon 1 of GalA and Carbon 2 of Rha. **23.** Hydrolysis of O–glycosidic bond (C1→C4) between Carbon 1 of Rha and Carbon 4 of GalA. **24.** Hydrolysis of O–glycosidic bond (C1→C4) between Carbon 1 of Gal and Carbon 4 of Rha. **25.** Hydrolysis of O–glycosidic bond (C1→C4) between Carbon 1 of Gal and Carbon 4 of Gal. **26.** Hydrolysis of O–glycosidic bond (C1→C3) between Carbon 1 of Ara and Carbon 3 of Gal. **27.** Hydrolysis of O–glycosidic bond (C1→C4) between Carbon 1 of Ara and Carbon 4 of Rha. **28.** Hydrolysis of O–glycosidic bond (C1→C5) between Carbon 1 of Ara and Carbon 5 of Ara. **29.** Hydrolysis of O–glycosidic bond (C1→C3) between Carbon 1 of Ara and Carbon 3 of Ara. **30.** Hydrolysis of O–glycosidic bond (C1→C6) between Carbon 1 of Ara and Carbon 6 of Gal. **31.** Hydrolysis of O–glycosidic bond (C1→C3) between Carbon 1 of Gal and Carbon 3 of Gal. **32.** Hydrolysis of O–glycosidic bond (C1→C6) between Carbon 1 of Gal and Carbon 6 of Gal. **33.** Hydrolysis of meta ester bond between hydroxyl group and carboxyl group of different gallic acids. **34.** Hydrolysis of para ester bond between hydroxyl group and carboxyl group of different gallic acids. **35.** Hydrolysis of ester bond between carboxyl

group of gallic acid and Carbon 1,2,3,4 or 6 of glucose in pentagalloyl glucose unit. **36.** Hydrolysis of ester bond between carboxyl group of gallic acid and Carbon 1 of glucose. **37.** Hydrolysis of two ester bonds between two carboxyl groups of hexahydroxydiphenic acid and C2 and C3 or C4 and C6 of glucose. **38.** Lactonization of hexahydroxydiphenic acid to ellagic acid. **39.** Hydrolysis of carbon bond C4–(C8 or C6) between Carbon 4 of one flavanol unit and Carbon 8 (or 6) of the second unit. **40.** Hydrolysis of ether bond C2–O–C7 between Carbon 2 of one flavanol unit and Carbon 7 of the second unit.



Lignocellulose biomass at first is pretreated with mechanical (mill, screw press) [63] or physical (steam explosion, *etc.*) [56] methods to "open up" the lignocellulose structure and make the material more accessible for further chemical hydrolysis. Hot water extraction [57] and dilute acid treatment [58,64,65] are implemented to release sugars and organic acids localized in the hemicellulose structure. Xylans in hemicellulose can also be selectively extracted by organic solvents [18] such as oxalic acid combined with methyltetrahydrofuran (2-MTHF) or hydrolyzed enzymatically by xylanases into oligomers and simple sugars [66]. Alkaline treatment with NaOH or $Ca(OH)_2$ [16] and Na_2CO_3 [59] breaks linkages in the lignin structure and removes phenolic compounds. Biological treatment of lignin with fungi was also reported as these microorganisms are able to decompose lignin via enzymatic action of ligninases [67]. Lignin can be also "extracted" by dilute acid [68], oxidation/alkaline [60], ethanolysis/alkaline [61], acetic/formic acid [69] and ionic liquid [70] treatment. Pretreatment methods increase the accessibility of cellulose towards cellulosic enzymes that subsequently hydrolyze cellulose into simple sugars as final products [16,57,70,71]. However, during chemical treatment of lignocellulose, sugars can undergo degradation to furans: 2-furfural (2-F) and 5-hydroxymethylfurfural (5-HMF) [72]. Further decomposition of these furans leads to the formation of formic acid. Additionally, 5-hydroxymethylfurfural can be cleaved to levulinic acid [72,73]. Final selection of hydrolysis methods depends on the material type, expected degree of hydrolysis, targeted products, environmental and economic considerations.

3. Effect of Lignocellulose Components on Microalgae Cultures

Compounds available in lignocellulosic hydrolysates can be an attractive feedstock for microalgal cultivation as positive effects of sugars, acetates and phenolics on microalgae growth have been reported. Also the influence of numerous minor co-products such as sugar acids, alcohols, furans and their degradation products, fatty acids, terpenoids, polyphenols, alkaloids as well as impurities on microalgae cultures has been mentioned in many publications (Summary in Section 4).

3.1. Sugars

Growth of *Chlorella zofingiensis* and astaxanthin synthesis were confirmed during heterotrophic cultivation with 50 g/L of glucose, mannose or galactose. However, *Chlorella* cultivation with 50 g/L of galactose gave four times less biomass density and 27% less astaxanthin content when compared to growth with glucose or mannose [74]. In another study, heterotrophic growth of *Chlorella* strains was improved on 10 g/L glucose with a growth rate increase of 40%–85% compared to autotrophic cultures. However, the lipid content in *Chlorella* cells cultivated in the dark was decreased by 14%–39% compared to photoautotrophic cultivation [75]. Heterotrophic cultivation of *Neochloris oleoabundans* also showed the ability of this strain to grow on 10 g/L glucose or 10 g/L cellobiose with a biomass productivity that was 32% higher for glucose if compared to cellobiose. However, no *Neochloris* growth was observed when using xylose or arabinose as carbon sources [31]. When *Chlorella sorokiniana* was cultivated, addition of 8 g/L glucose resulted in a 3-fold and 5-fold increase in growth rate and almost 2-fold and 4-fold increase in total fatty acid content for *Chlorella* culture grown on heterotrophic and mixotrophic mode, respectively [76]. Also mixotrophic cultivation of *Chlorella sorokiniana* with 18 g/L glucose gave a 60% higher biomass density, but the lutein content in *Chlorella* cells was 30%

smaller when compared to autotrophic cultivation [77]. Cultivation of *Phaeodactylum tricornutum* in mixotrophic mode and using 0.5–1 g/L glucose increased the growth rate by 38% and cell lipid content by 144%–161% in comparison to autotrophic control [78]. *Chlorella* strains upon xylose enhancement were able to grow on pentose sugars, but only in the presence of light, with *Chlorella* showing improved growth on xylose and no growth changes on arabinose. What is more, addition of glucose improved xylose utilization in *Chlorella* culture [79]. Rhamnose was reported to support *Chlorella vulgaris* growth at 1.64 g/L, with *Chlorella* culture density at the end of cultivation reaching the same level as in case of using 1.5 g/L xylose and being 20% smaller when compared to 1.8 g/L glucose [80].

3.2. Acetates

Acetates in lignocellulosic hydrolysates come from acetyl groups which are localized on the main hemicellulose chains. Acetates can constitute 2.9%–4.2% [62], [69] of lignocellulosic materials and are easily released together with hemicellulose sugars during hot water or dilute acid treatment [64]. Some strains of microalgae are able to use acetate as an organic carbon source. A proper combination of light intensity and acetate concentration (2.46 g/L) resulted in enhancement of *Haematococcus pluvialis* growth in mixotrophic cultures. However, overdoses of acetate (above 4.1 g/L) caused cell bleaching and had a lethal effect on *Haematococcus pluvialis* cells [81]. Comparably, in another study acetate (2.5 g/L) increased growth of mixotrophically cultivated *Haematococcus pluvialis* by 24% and cell carotenoid content by 80%. Increases in acetate concentration up to 10–20 g/L caused growth inhibition, but carotenoid content in *Haematococcus* cells increased three times when compared to control [82]. Addition of acetate (up to 3.28 g/L) for mixotrophic cultivation of *Chlorella sorokiniana* gave 20% more biomass and cell lutein content increased. An increase in acetate concentration (4.1–4.9 g/L) caused that biomass density remained at the same level as in case of 3.28 g/L acetate, but cell lutein content decreased, when compared to control or acetate concentrations up to 3.28 g/L [77]. *Chlamydomonas reinhardtii* can use acetate in the dark as the only carbon and energy source, thereby leading to high microalgae densities [83]. *Chlamydomonas reinhardtii* can also grow in the presence of acetate under mixotrophic conditions. *Chlamydomonas* cultivation in 1 g/L acetate (TAP) medium gave almost 2-fold increase in growth rate, when compared to photoautotrophic cultivation without acetate. Additionally, acetate cultivation resulted in larger cell size as well as higher chlorophyll cell content and oxygen production (by 31% and 52%, respectively). On the other hand, although *Chlamydomonas* cultivation on acetate showed a 34% higher growth rate, chlorophyll cell content was smaller by 24% and oxygen production was 2.2-fold smaller when compared to photoautotrophic cultivation, with an additional 5% CO_2 supply [84].

3.3. Methanol

Methanol is generated in lignocellulose hydrolysates as a result of proton attack on methyl groups attached to glucuronic acid in hemicellulosic structures [64]. Methanol at a concentration of 7.9 g/L increased *Chlorella* biomass culture by 90% and lipid accumulation by 40%, under mixotrophic cultivation with additional 5% CO_2 supply. Mixotrophic *Chlorella* cultivation with 7.9 g/L methanol, but without 5% CO_2 decreased biomass culture by 65% and lipids accumulation by 61%, in comparison to photoautotrophic cultivation with 5% CO_2 [85]. In another study, mixotrophic cultivation of *Scenedesmus obliquus* with 3.9 g/L methanol resulted in an increase in biomass by 340%, when

compared to photoautotrophic control. However no *Scenedesmus* growth enhancement, with respect to control, was detected during heterotrophic cultivation on methanol [86]. Methanol concentrations such as those mentioned above cannot be obtained directly from lignocellulose, but external addition of methanol could greatly improve mixotrophic growth of microalgae on lignocellulosic hydrolysates.

3.4. Sugar Acids

Glucuronic acid—a constituent of hemicellulose—and galacturonic acid—a constituent of pectin—can also be released from plant materials due to chemical treatment. Acid treatment of *Eucalyptus* wood gave a hydrolysate containing 1.5 g/L of glucuronic acid and 1 g/L of galacturonic acid [87]. In animals, glucuronic acid is an intermediate in the L-ascorbic acid biosynthesis pathway. In plants, galacturonic instead of glucuronic acid participates in the biosynthesis of L-ascorbic acid [88]. Addition of 2.5 g/L galacturonic acid to the microalga *Ochromonas danica* cultivated on 1 g/L glucose in the presence of light resulted in 3.3 fold increase in ascorbic acid production when compared to experiments, where *Ochromonas* was cultivated mixotrophically on glucose. Addition of 2.5 g/L glucuronic acid failed to enhance ascorbic acid synthesis [89]. However, mixotrophic cultivation of microalga *Euglena gracilis* caused 2-fold and 4-fold increase in ascorbic acid production with 2.5 g/L glucuronic and galacturonic acid, respectively, as a comparison to the control grown in the presence of light, but without any added sugars or sugar acids. *Euglena* was thus shown to possess both "animal–like" and "plant–like" pathways for ascorbic acid synthesis [90].

3.5. Phenolics

Phenolic compounds are known to exert inhibitory activity against microorganisms. Growth and ethanol production were inhibited in *Candida shehatae* by 33% and 53% and in *Pichia stipitis* by 88% and 91%, respectively, when 0.5 g/L vanillin was used [91]. Microalgae show various growth responses when exposed to phenolic compounds. Strains of microalgae such as *Chlorella saccharophila* and *Scenedesmus quadricauda* showed full resistance against catechol but *Chlorella zofingiensis*, *Coelastrum microporum* and *Mesotaenium caldarorium* were completely inhibited in the presence of catechol. Additionally, the green microalga *Scenedesmus quadricauda* was able to metabolize 0.4 g/L phenolic compounds of different structure with 95% removal of catechol, *p*-hydroxybenzoic acid, *p*-coumaric acid and caffeic acid and 85% removal of ferulic acid [92]. In another study, the effect of three isomers of hydroxybenzoic acid (13.8 mg/L) on the growth of *Chlorella vulgaris* was tested. *o*-Hydroxybenzoic acid was shown to possess stimulatory effect on *Chlorella* growth together with an increased amount of protein, sugar, pigment and nucleic acid content in microalgae cells. *p*-Hydroxybenzoic acid also showed a growth-enhancing effect but to a smaller extent than *o*-hydroxybenzoic acid. *m*-Hydroxybenzoic acid exerted an inhibitory effect on growth of *Chlorella vulgaris* [93]. The stimulating activity of *p*-hydroxybenzoic acid was mentioned in a few reports. *p*-Hydroxybenzoic as well as vanillic acid and syringic acid had stimulating effect towards *Chlorella pyrenoidosa* growth. Particularly, there was a shift from inhibition to stimulation for *p*-hydroxybenzoic acid at 41–55 mg/L and vanillic acid at 50–67 mg/L [94]. *p*–Hydroxybenzoic acid had stimulatory effects at lower concentration (up to 138 mg/L) and inhibitory effects at high concentration (1.36 g/L) on the growth of *Pseudokirchneriella subcapitata*. *o*–Hydroxybenzoic acid

was toxic (13.8–138 mg/L) to *Pseudokirchneriella*, but the presence of *p*-hydroxybenzoic acid decreased the negative effect of *o*–hydroxybenzoic acid [95].

A few mechanisms can be proposed to explain the effect of phenolic compounds on microalgae growth. Phenolic compounds are known to have regulatory effects on enzyme activity, structure of cellular membranes and synthesis of macromolecules [93,94]. On the other hand, phenolic compounds under aerobic conditions are biodegraded to basic organic molecules and inorganic carbon dioxide which can be consumed by algae [96]. Metabolism of phenols was also reported for *Coniochaeta ligniaria*, a fungus strain which was able to purify dilute acid hydrolysate of cornstover from phenolic compounds [97]. Such phenolic compounds were found in lignocellulose hydrolysates obtained after spruce, willow or brewer´s spent grain treatment [68,98,99] (Table 3).

Table 3. Phenolic compounds in hydrolysates from lignocellulosic materials.

Phenolic Compound	Concentration in Hydrolysate (mg/L)	Treatment Method	Material	References
Vanillin	36, 430	Dilute acid treatment Steam explotion + SO_2 impregnation	Spruce Willow	[68,98]
Vanilic acid	3, 33	Alkaline hydrolysis Dilute acid treatment	Brewer's spent grain Spruce	[68,99]
Catechol	440	Steam explotion + SO_2 impregnation	Willow	[98]
Ferulic acid	145	Alkaline hydrolysis	Brewer's spent grain	[99]
p-Hydroxybenzoic acid	27, 81	Alkaline hydrolysis Dilute acid treatment	Brewer's spent grain Spruce	[68,99]
p-Coumaric acid	139	Alkaline hydrolysis	Brewer's spent grain	[99]
Syringic acid	8	Alkaline hydrolysis	Brewer's spent grain	[99]

3.6. Furans

Furfural and hydroxymethylfurfural (5-HMF) are furans which are formed from sugars during dilute acid treatment of lignocellulose [100]. The concentration of furans in lignocellulosic hydrolysates was reported to range between 0.26 and 5.7 g/L for furfural and from 0.49 to 7.3 g/L for HMF [68,101]. So far, information about the effect of furans on microalgae growth has been rather scarce. Furfural up to 2 g/L and HMF up to 5 g/L were reported to cause strong inhibition of growth and ethanol production in *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipites* and *Candida shehatae* cultures [91]. Furfural and hydroxymethylfurfural were examined for their effect on *Spirulina maxima* growth. Both types of furans exerted an inhibitory effect on *Spirulina* growth with full inhibition for furfural at 0.67 g/L and for HMF at 1.13 g/L. Inhibition of photosynthesis was detected and shown by the decrease in oxygen production. Additionally, it was concluded that furans could interfere in metabolic processes and cause lysis of *Spirulina* cells [102]. Recently, it has been reported that furfural up to 0.6 g/L can cause 30% biomass reduction during mixotrophic acetate-based cultivation of *Chlamydomonas reinhardtii* [103].

3.7. Levulinic Acid

Levulinic acid is generated upon cleavage of HMF [73] and its effect on microalgae was presented in a few reports. It was stated that levulinic acid at concentrations above 1.16 g/L inhibited growth and chlorophyll synthesis in photoautotrophically cultivated *Sceletonema costatum*, *Chlorella vulgaris* and *Agmenellum quadruplicatum* cells [104–106]. Inhibition of growth and chlorophyll synthesis was accompanied by accumulation of aminolevulinic acid—an intermediate for chlorophyll synthesis, as levulinic acid inhibits enzymatic conversion of 5-aminolevulinic acid in chlorophyll synthesis pathway.

3.8. Fatty Acids

Lipids constitute the extractable fraction of lignocellulose and their overall content in wood of oleaginous trees such as *Eucalyptus* is up to 0.2% [45,107]. Fatty acids content in *Eucalyptus* wood is about around 0.03%–0.04%, with palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) as the most common representatives [107]. These fatty acids can be found in effluents released during pulping and bleaching treatment of wood and were tested in terms of their effect on *Selenastrum capricornutum* growth [108]. Oleic acid (C18:1) was the strongest inhibitor, as this fatty acid decreased *Selenastrum* growth by 50% at a concentration of 0.47 mg/L. Palmitic acid (C16:0) and linoleic acid (C18:2) also caused 50% inhibition, but at higher concentrations, 3.87 mg/L and 1.55 mg/L, respectively. On the other hand, a triglyceride of oleic acid had almost no inhibitory effect on *Selenastrum* growth, even at a concentration of 5 mg/L. In another study [109], growth of *Monoraphidium contortum* and *Chlorella vulgaris* was also inhibited by 50% in the presence of the fatty acids mentioned above, but inhibitory concentrations differed considerably. For *Monoraphidium* growth, linoleic acid (8 mg/L) was a stronger inhibitor than palmitic acid (9.2 mg/L) or oleic acid (12.1 mg/L). For *Chlorella* growth, linoleic acid (9.4 mg/L) also exerted stronger inhibitory effects than oleic acid (12.4 mg/L), but palmitic acid (59.1 mg/L) was shown to be a very poor inhibitor. Additionally, a leakage of K^+ ions from *Monoraphidium* and *Chlorella* cells was detected upon exposure to the tested fatty acids and it was suggested that fatty acids caused damages to the membranes of microalgae cells.

3.9. Terpenoids

Terpenoids are a large class of hydrocarbons based on the isoprene structure and include monoterpenoids, diterpenoids and triterpenoids (Table 4).

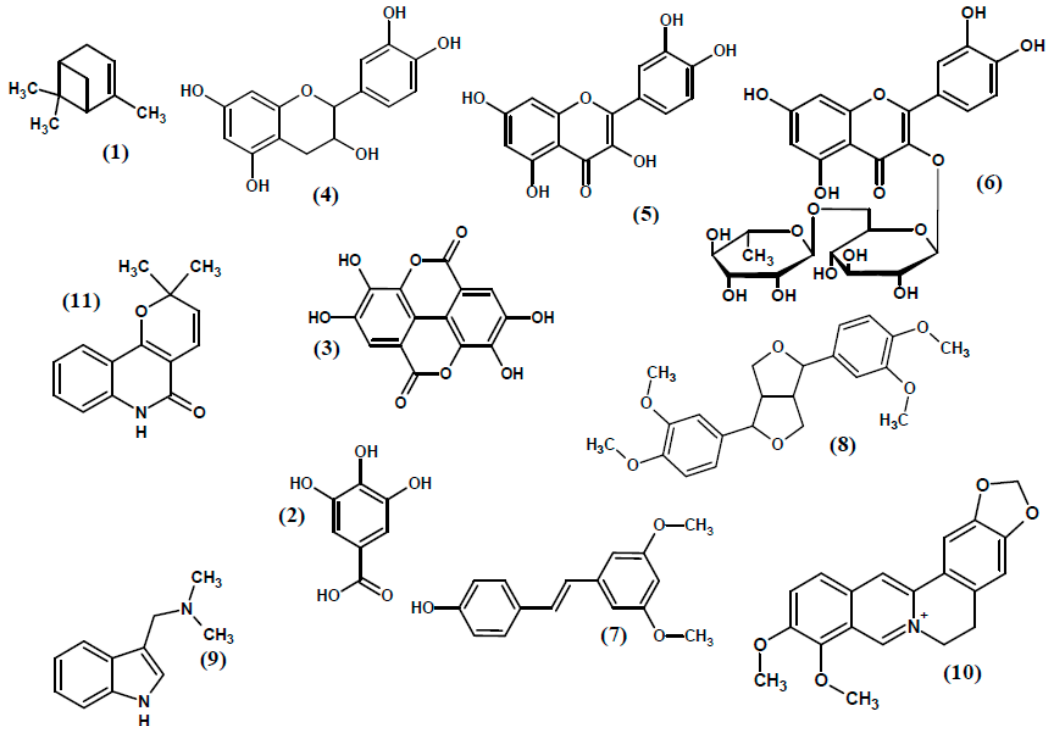
Monoterpenoids such as α–pinene, β–pinene and limonene can be obtained from fir (*Abies*) residues by steam distillation [46]. Diterpenoids such as abietic acid or palustric acid were extracted from Scots pine (*Pinus sylvestris*) or Norway spruce (*Picea abies*) residues with the use of acetone [47]. Triterpenoids such as betulin can be found in an extract from White Birch (*Betula papyrifera*) upon mixed organic solvent—water extraction [48]. (+)-Limonene, (−)-α–pinene and (−)-β–pinene were tested in terms of their effect on *Chlorella pyrenoidosa* growth, but no inhibition was observed [110]. However, α–pinene was reported to be an efficient bio-solvent used, instead of *n*-hexane, for lipid extraction from *Chlorella vulgaris* [111].

Table 4. Extraction of terpenoids, polyphenols and alkaloids from natural sources.

Name	Group	Source	Extraction Solvent	Content	Ref.
α–Pinene (1)	Monoterpenoids	*Abies alba* wood	Water	0.2% [A]	[46]
		Abies alba knots		26.4% [A]	
β-Pinene	Monoterpenoids	*Abies balsamea* knots	Water	0.4% [A]	[46]
		Abies alba knots		2.3% [A]	
Limonene	Monoterpenoids	*Abies alba* knots	Water	2.1% [A]	[46]
Abietic acid	Diterpenoids	*Pinus sylvestris* wood	Acetone	0.65%–1.43% [A]	[47]
		Pinus sylvestris knots		2.1%–3.9% [A]	
		Picea abies wood		0.017% [A]	
		Picea abies knots		0% [A]	
Palustric acid	Diterpenoids	*Pinus sylvestris* wood	Acetone	0.25%–0.67% [A]	[47]
		Pinus sylvestris knots		0.43%–1.7% [A]	
		Picea abies wood		0.045% [A]	
		Picea abies knots		0.014% [A]	
Betulin	Triterpenoids	*Betula papyrifera* bark	EtOAC—Ethanol-Water	15.4% [A]	[48]
Gallic acid (2)	Gallotannins	*Terminalia paniculata* bark	Water-Chloroform	0.068% [B]	[112]
Ellagic acid (3)	Ellagitannins	*Terminalia paniculata* bark	Water-Chloroform	0.061% [B]	[112]
Catechin (4)	Proanthocyanidins	*Acacia catechu* wood	Water	4.5% [A]	[113]
Quercetin (5)	Flavonoids	*Terminalia paniculata* bark	Water-Chloroform	0.019% [B]	[112]
Rutin (6)	Flavonoids	*Terminalia paniculata* bark	Water-Chloroform	0.049% [B]	[112]
Pinosylvin	Stilbenes	*Pinus sylvestris* wood	Acetone	0.12%–0.98% [A]	[47]
		Pinus sylvestris knots		0.91%–3.5% [A]	
Resveratrol	Stilbenes	*Picea mariana* bark	Water	0.01% [A]	[114]
Pterostilbene (7)	Stilbenes	*Pterocarpus marsupium* wood	EtOAc	No data	[115]
Secoisolariciresinol	Lignans	*Araucaria araucana* wood	Methanol	32.99% [C]	[116]
Lariciresinol	Lignans	*Araucaria araucana* wood	Methanol	10.09% [C]	[116]
Pinoresinol	Lignans	*Araucaria araucana* wood	Methanol	7.32% [C]	[116]
Eudesmin (8)	Lignans	*Araucaria araucana* wood	Methanol	18.24% [C]	[116]
Gramine (9)	Alkaloids	*Hordeum vulgare* shoots	No data	0.7% [A]	[117]
		Phalaris arundinacea samples	Chloroform	0.011% [A]	[52]
Berberine (10)	Alkaloids	*Phellodendron* bark	Water or Methanol	No data	[118]
Flindersine (11)	Alkaloids	*Flindersia australis* wood	No data Ethanol	No data	[119]
		Hortia colombiana wood		0.009% [A]	[120]

[A] expressed as % of dried tested material; [B] expressed as % of water bark extract; [C] expressed as % of aqueous MeOH extract.

Table 4. *Cont.*



3.10. Polyphenols

Polyphenols available mainly in wood and bark can be divided into hydrolysable tannins, condensed tannins or smaller flavonoids, stilbenes and lignans (Table 4). Hydrolysable tannins contain esters of gallic acids with glucose (gallotannin) or esters of hexahydroxydiphenic acids and gallic acids with glucose (ellagitannins) as basic units [121]. Condensed tannins are proanthocyanidins [122] composed of flavanol units (catechin or epicatechin). Flavonoids found in bark are quercetin (flavonol) or rutin (quercetin glycoside) [112]. Stilbenes are diphenylethylene substances [123] such as pinosylvin present in wood or knots [47], resveratrol present in bark [114] or pterostilbene present in wood [115]. Lignans available in wood are derivatives of phenylpropanoid dimers and include secoisolariciresinol, lariciresinol, pinoresinol and eudesmin [116].

Release of tannins can be achieved by means of water extraction [113,124], also with addition of inorganic salt (Na_2CO_3, $NaHSO_3$) [125], as well as hexane, ethyl acetate, ethanol [126], methanol [127] or water–ethanol [124] and water–chloroform mixtures [112]. The extract prepared from *Terminalia paniculata* bark after water–chloroform extraction treatment contained gallic acid, ellagic acid, quercetin and rutin [112]. The effect of gallic acid, a simple phenol molecule that forms ester bonds with glucose in the gallotannin structure, was tested on the cyanobacterium *Nostoc* sp. [128]. Gallic acid at 10 mg/L caused 40% growth inhibition, 84% protein content decrease and 98% chlorophyll content reduction. Additionally, the enzymatic activity of glutamine synthetase and nitrate reductase in *Nostoc* cells was inhibited by 30% and 68%. In cyanobacteria, nitrate (NO_3^-) is reduced by nitrate reductase and nitrite reductase into ammonium, which is combined with 2-oxoglutarate via glutamine synthetase/glutamate synthase action to form glutamic acid [129]. This amino acid possesses a primary role in the synthesis of other amino acids—building blocks for peptides and proteins, and is also converted to 5-aminolevulinic acid—a precursor of chlorophyll synthesis [130].

Inhibition of enzymes involved in glutamic acid synthesis pathway caused inhibition of chlorophyll and pigment production. Ellagic acid, a lactonized product of hexahydroxydiphenic acid present in ellagitannin, was tested in terms of its effect on the cyanobacterium *Microcystis aeruginosa* [131]. Growth of *Microcystis* was inhibited by 50% in the presence of 5 mg/L ellagic acid. As a comparison, gallic acid caused 50% growth inhibition of *Microcystis* at a concentration of 1 mg/L. Quercetin was reported to inhibit the photosynthetic mechanism in the diatoms *Thalassiosira pseudonana*, *Phaeodactylum tricornutum* and *Thalassiosira weissflogii*, 67%, 62% and 55%, respectively, at 6 mg/L. In contrast, the green microalgae *Chlamydomonas* sp. and *Dunaliella tetriolecta* were not inhibited even in the presence of 12 mg/L quercetin [132]. Rutin, a glycoside composed of quercetin and rutinose, showed to exert 50% inhibition of *Sceletonema costatum* growth at 0.4 mg/L [133]. Catechin, a flavanol present in condensed tannins, can be produced from *Acacia catechu* wood upon water extraction [113]. An exposure of *Microcystis aeruginosa* and *Pseudokirchneriella subcapitata* to catechin (25–100 mg/L) caused an increased formation of reactive oxygen species (ROS) in cells of both tested strains cultivated in dark or light conditions. Catechin, upon cell uptake, was suggested to be converted to quinone with generation of ROS, which can damage structural cell components. ROS formation in cells exposed to catechin was higher in light than in dark conditions. Presumably, oxygen (O_2) and reducing power (NAD(P)H) generated during photosynthesis can enhance catechin to quinone conversion and ROS formation [134]. Pinosylvin, a dihydroxyl derivative of stilbene, was found in acetone extracts from Scots pine (*Pinus sylvestris*) wood and knots [47]. Resveratrol, a trihydroxyl derivative of stilbene, can be released from Black Spruce (*Picea mariana*) bark upon hot water extraction [114]. Pterostilbene, a dimethylated derivative of resveratrol, can be extracted from *Pterocarpus marsupium* wood with the use of ethyl acetate [115]. Inhibitory effect of pinosylvin, resveratrol and pterostilbene was tested on *Selenastrum capricornutum* and cyanobacterium *Oscillatoria perornata* growth [135]. Pterostilbene negatively affected *Selenastrum* and *Oscillatoria* growth at concentrations of 2.5 mg/L and 25.6 mg/L, respectively, thereby showing higher *Selenastrum* susceptibility. On the other hand, pinosylvin (21.2 mg/L) or resveratrol (22.8 mg/L) did not affect growth of tested strains. Eudesmin, a tetramethylated furofuran derivative of phenylpropanoid dimers, was present in aqueous methanol extract from *Araucaria araucana* wood [116]. Eudesmin negatively affected the growth of *Oscillatoria perornata* at 3.86 mg/L and *Selenastrum capricornutum* at 38.6 mg/L, thereby showing the higher susceptibility of *O. perornata*. As a contrast, eudesmin exerted no effect on *Oscillatoria agardhii* even at 38.6 mg/L [136].

3.11. Alkaloids

Gramine, an indole alkaloid (Table 4) present in young barley shoots (up to 0.7%) [117] or reed canary grass (up to 0.01%) [52], is synthetized in a self-defense mechanism against animal grazers [137]. *Microcystis aeruginosa* cultivated in the presence of various gramine concentrations and exposure times showed structure breakage (2 mg/L, 24–60 h) and DNA fragmentation (1 mg/L, 5 days; 8 mg/L, 1 or 5 days) [138]. Growth of *Chlorella vulgaris* was also inhibited by 50% when 65 mg/L gramine was added [139]. Berberine, an isoquinoline alkaloid extracted from *Phellodendron* bark [118], inhibited growth of *Scenedesmus quadriqauda*, *Microcystis aeruginosa*, *Synechococcus nidulans* and *Aphanothece clathrata* by 50% at 0.75 mg/L, 0.27 mg/L, 0.57 mg/L and 0.64 mg/L, respectively.

On the other hand, 50% inhibition of *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* growth could be achieved only if berberine concentrations higher than 1 mg/L, were applied [140]. Flindersine, a pyranoquinoline alkaloid isolated from wood of *Flindersia australis* [119] and *Hortia colombiana* [120], caused 50% inhibition of *Oscillatoria perornata* and *Selenastrum capricornutum* growth at a dosage of 3.6 and 4 mg/L, respectively. No effect on *Oscillatoria agardhii* growth was detected, even with 22.7 mg/L [136]. The mode of inhibitory action of alkaloids against microalgae may be attributed to their ability to cause oxidative damage in cells. Berberine, in an experiment on *Microcystis aeruginosa* growth, was reported to inhibit activity of superoxide dismutase (SOD), the enzyme responsible for converting O_2^- into H_2O_2 and O_2. O_2^- is a ROS causing damage to macromolecules in cellular structures. Because berberine inhibited by 43% SOD activity, cell O_2^- content was elevated up to 7 times [141]. However, concentration of berberine used in that study (0.2 g/L) was much higher than in another growth inhibitory report [140].

3.12. Impurities

Some compounds can be present in lignocellulosic hydrolysates as contaminants from environment (heavy metals) or as remnants after chemical pretreatment (ionic liquids). Their possible effect on microalgae should be also taken into consideration.

3.12.1. Heavy Metal Ions

Heavy metal ions can be released into lignocellulose hydrolysate from corroded equipment used for hydrolysis [142] or from hydrolyzed lignocellulose materials, as plants accumulate heavy metals [143] during cultivation on polluted areas (post-industrial terrains, roadsides *etc.*). Metal ions such as lead (Pb), chromium (Cr), cadmium (Cd) and nickel (Ni) have an influence on microalgae growth. Pb at a concentration of 0.5 mg/L caused 50% inhibition of *Selenastrum capricornutum*, *Chlorella pyrenoidosa*, *Chlorella ellipsoidea* and *Chlorella vulgaris* cultivated in phosphate limited medium [144]. The presence of 10 mg/L Ni reduced growth of the cyanobacterium *Synechococcus* sp. and was accompanied by cell morphological changes and elevated Ni content [145]. Cd at a concentration of 17 mg/L inhibited the growth by 51% and photosynthetic oxygen evolution by 30% in *Scenedesmus armatus* culture cultivated in the presence of 0.1% CO_2, but the increase in CO_2 to 2% improved protection of *Scenedesmus* cells against cadmium, as only 8% inhibition of oxygen evolution and 27% growth inhibition was observed [146]. Cr at 0.97 mg/L was also shown to strongly suppress growth and the photosynthetic mechanism in *Chlorella vulgaris* cells [147].

3.12.2. Ionic Liquids

Ionic liquids are a class of new organic solvents used for hydrolysis of polymers present in lignocellulose. Treatment of legume straw with 1-butyl-3-methylimidazolium chloride (BMIM Cl) gave two solid fractions, a residue fraction with decreased by 31% lignin content and a lignin-cellulose flocculated fraction containing no hemicellulose [148]. The use of 1-ethyl-3-methylimidazolium chloride (EMIM Cl) with H_2SO_4 resulted in 73% and 77% conversion of cellulose and hemicellulose from hydrolyzed *Miscanthus* grasses [149]. If solid fractions containing ionic liquid remnants [150] are

further hydrolyzed with enzymes, ionic liquid molecules can be released into hydrolysates. The presence of ionic liquids in lignocellulose hydrolysate should be avoided, as inhibitory effect of EMIM Cl and BMIM Cl was also reported for microalgae [151]. Growth of *Chlorella vulgaris* was inhibited by 56% with 1.46 g/L EMIM Cl and by 66% with 0.17 g/L BMIM Cl. *Oocystis submarina* growth was suppressed by 70% with 1.83 g/L EMIM Cl and by 67% with 0.26 g/L BMIM Cl. Growth of diatom *Cyclotella meneghiniana* was reduced by 66% with 14.6 mg/L EMIM Cl and by 68% with 1.74 mg/L BMIM Cl. 1-Butyl-3-methylimidazolium chloride proved to be a stronger inhibitor of microalgae than 1-ethyl-3-methylimidazolium chloride and the diatom *Cyclotella* was shown to be more sensitive to ionic liquids than the green microalgae *Chlorella* or *Oocystis*. Additionally, a decrease in growth inhibition to less than 10% with the increase in salinity of the growth medium used for *Chlorella*, *Oocystis* and *Cyclotella* cultivation was observed. It was suggested that salts created ion pairing with methylimidazolium cation molecules, thereby diminishing their interaction with negatively charged components of cell wall structure and alleviating growth inhibitory effects.

4. Effect of Lignocellulose Hydrolysates on Microalgae

Many lignocellulose related compounds have been tested separately in terms of their effect (Table 5) on microalgae cultures, but so far only few works have been reported about the direct effect of lignocellulosic hydrolysates on microalgae. In one study [152], rice straw, upon organosolvent treatment and a further hydrolysis with cellulosic enzymes to produce glucose from cellulose, was used as a feedstock for mixotrophic cultivation of *Chlorella pyrenoidosa*. Interestingly, the biomass productivity of *Chlorella* growing on rice straw hydrolysate medium containing 11 g/L sugars was three times higher than for *Chlorella* cultivated on synthetic medium containing 11 g/L glucose. It was suggested that non-sugar substances present in the rice straw hydrolysate could be responsible for acceleration of *Chlorella pyrenoidosa* growth. However, lipid content in *Chlorella* cells was only slightly higher (56.3%) for the growth on rice straw hydrolysate when compared to lipid content in *Chlorella* (50.3%) grown on glucose enriched medium. In another report [19], wheat bran material was biologically treated with fungal strains to produce reducing sugars which were used to enhance the growth of *Chlorella vulgaris* and *Scenedesmus obliquus*. Experiments showed that 0.25%–1.5% of wheat bran hydrolysate in the medium improved the growth of microalgae strains cultivated under mixotrophic or heterotrophic mode. Additionally, the presence of wheat bran hydrolysate increased carbohydrate and protein content in *Chlorella* and *Scenedesmus* cells when mixotrophic or heterotrophic cultivation was applied. Lipid content in *Chlorella* and *Scenedesmus* cells growing on wheat bran hydrolysate under mixotrophic conditions were higher than under heterotrophic mode. Chlorophyll content in microalgae cultures growing mixotrophically was up to 10 times higher than in the same cultures growing heterotrophically. However, during mixotrophic cultivation on wheat bran hydrolysate, chlorophyll content in *Chlorella* culture was higher in comparison to the control but chlorophyll content in *Scenedesmus* culture was smaller than in the control and decreased with the increase of wheat bran hydrolysate content. Recently, it has been mentioned that *Chlamydomonas reinhardtii* is able to excrete cellulosic enzymes which hydrolyze exogenous cellulose into cellobiose [153]. Consequently, cellobiose was consumed by *Chlamydomonas* under mixotrophic CO_2 limiting conditions, but the effect of cellobiose was better seen under heterotrophic conditions.

Table 5. Effect of lignocellulosic hydrolysate related compounds on microalgae under various cultivation conditions: a summary.

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
Glucose Mannose Galactose	50 g/L [a]	*Chlorella zofingiensis*	No	Not mentioned	Growth confirmed Astaxanthin synthesis confirmed	[74]
Glucose	10 g/L	*Chlorella vulgaris*	No	6 days	Increased growth [1] Decreased lipid content [1]	[75]
Glucose Cellobiose	10 g/L [a]	*Neochloris oleoabundans*	No	5 days	Growth confirmed	[31]
Xylose Arabinose	10 g/L [a]	*Neochloris oleoabundans*	No	5 days	No effect on growth	[31]
Glucose	8 g/L 8 g/L	*Chlorella sorokiniana*	Yes No	6 days 6 days	Growth acceleration [1] Increased total fatty acid content [1] Growth acceleration [1] Increased total fatty acid content [1]	[76]
Glucose	18 g/L	*Chlorella sorokiniana*	Yes	10 days	Increased biomass density [1] Decreased lutein content [1]	[77]
Glucose	0.5–1 g/L	*Phaeodactylum tricornutum*	Yes	10 days	Increased growth [1] Increased lipid content [1]	[78]
Xylose	0.15 g/L	*Chlorella*	Yes	2 weeks	Increased growth [2]	[79]
Glucose Rhamnose Xylose	1.8 g/L [a] 1.64 g/L [a] 1.5 g/L [a]	*Chlorella vulgaris*	No	15 days	Growth confirmed	[80]
Acetate	2.46 g/L over 4.1 g/L	*Haematococcus pluvialis*	Yes	8 days	Growth confirmed Decreased growth [3]	[81]
Acetate	2.5 g/L 10–20 g/L	*Haematococcus pluvialis*	Yes	10 days	Increased growth [1] Increased carotenoid content [1] Decreased growth [1] Increased carotenoid content [1]	[82]

Table 5. *Cont.*

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
Acetate	up to 3.28 g/L 4.1–4.9 g/L	*Chlorella sorokiniana*	Yes	10 days	Increased biomass concentration [1] Increased lutein content [1] Increased biomass concentration [1] Decreased lutein content [1]	[77]
Acetate	1 g/L	*Chlamydomonas reinhardtii*	Yes	2 days	Increased growth [1] Chlorophyll content increased [1] Cell size increased [1] Oxygen production increased [1] Increased growth [4] Chlorophyll content decreased [4] Cell size unchanged [4] Oxygen production decreased [4]	[84]
Methanol	7.9 g/L + 5% CO_2 7.9 g/L without 5% CO_2	*Chlorella sp.*	Yes Yes	45 days 45 days	Increased biomass growth [4] Increased lipid content [4] Decreased biomass growth [4] Decreased lipid content [4]	[85]
Methanol	3.9 g/L	*Scenedesmus obliquus*	Yes No	40 h 24 h	Biomass growth enhancement [1] No growth enhancement [1]	[86]
Glucuronic acid	2.5 g/L [b]	*Ochromonas danica*	Yes	6 h	No increase in ascorbic acid synthesis [5]	[89]
Glucuronic acid	2.5 g/L	*Euglena gracilis*	Yes	4 h	Enhanced ascorbic acid synthesis [6]	[90]
Galacturonic acid	2.5 g/L [b]	*Ochromonas danica*	Yes	6 h	Enhanced ascorbic acid synthesis [5]	[89]
Galacturonic acid	2.5 g/L	*Euglena gracilis*	Yes	4 h	Enhanced ascorbic acid synthesis [6]	[90]
Catechol	0.05 µg [c]	*Chlorella zofingiensis* *Coelastrum microporum* *Mesotaenium caldarorium*	Yes	Not mentioned	Growth inhibition [7a]	[92]
Catechol	0.05 µg [c]	*Chlorella saccharophila* *Scenedesmus quadricauda*	Yes	Not mentioned	No effect on growth [7b]	[92]

Table 5. *Cont.*

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
Catechol *P*-hydroxybenzoic acid *P*-coumaric acid Caffeic acid Ferulic acid	0.4 g/L [a]	*Scenedesmus quadricauda*	Yes	5 or 10 days	Removal of compounds from growth medium	[92]
O-hydroxybenzoic acid *P*-hydroxybenzoic acid	13.8 mg/L [a]	*Chlorella vulgaris*	Yes	6–9 days	Growth stimulation [8] Increased pigment content [8] Increased protein content [8] Increased RNA and DNA content [8]	[93]
M-hydroxybenzoic acid	13.8 mg/L	*Chlorella vulgaris*	Yes	6–9 days	Growth inhibition [8]	[93]
P-hydroxybenzoic acid	13.8–55 mg/L	*Chlorella pyrenoidosa*	Yes	16 days	Growth stimulation [8]	[94]
Vanillic acid	16.8–67 mg/L	*Chlorella pyrenoidosa*	Yes	16 days	Growth stimulation [8]	[94]
Syringic acid	19.8–79 mg/L 99 mg/L	*Chlorella pyrenoidosa*	Yes	16 days	Growth stimulation [8] Culture death	[94]
P-hydroxybenzoic acid	13.8–138 mg/L 1.36 g/L	*Pseudokirchneriella subcapitata*[+]	Yes	72 h	Growth stimulation [8] Growth inhibition [8]	[95]
O-hydroxybenzoic acid	13.8–138 mg/L	*Pseudokirchneriella subcapitata*[+]	Yes	72 h	Growth inhibition [8]	[95]
2-Furfural	0.67 g/L	*Spirulina maxima*	Yes	144 h	Growth inhibition [8] Photosynthesis inhibition [8]	[102]
2-Furfural	0.6 g/L + acetate	*Chlamydomonas reinhardtii*	Yes	Not mentioned	Growth inhibition [9]	[103]
5-HMF	1.13 g/L	*Spirulina maxima*	Yes	144 h	Growth inhibition [8] Photosynthesis inhibition [8]	[102]
Levulinic acid	1.16–11.6 g/L	*Sceletonema costatum*	Yes	96 h	Growth inhibition [8] Aminolevulinic acid accumulation [8] Chlorophyll synthesis inhibited [8]	[105]

Table 5. *Cont.*

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
Levulinic acid	1.16–5.8 g/L	*Chlorella vulgaris*	Yes	24 h	Growth inhibition [8] Aminolevulinic acid accumulation[8] Chlorophyll synthesis inhibited [8]	[104]
Levulinic acid	6.96 g/L	*Agmenellum quadruplicatum*	Yes	14 h	Growth inhibition [8] Aminolevulinic acid accumulation [8] Chlorophyll synthesis inhibited [8]	[106]
Palmitic acid C16:0	3.87 mg/L	*Selenastrum capricornutum*	Yes	72 h	Growth inhibition [8]	[108]
Palmitic acid C16:0	59.1 mg/L	*Chlorella vulgaris*	Yes	24 h	Growth inhibition [8] K^+ leakage from cells	[109]
Palmitic acid C16:0	9.2 mg/L	*Monoraphidium contortum*	Yes	24 h	Growth inhibition [8] K^+ leakage from cells	[109]
Oleic acid C18:1	0.47 mg/L	*Selenastrum capricornutum*	Yes	72 h	Growth inhibition [8]	[108]
Oleic acid C18:1	12.4 mg/L	*Chlorella vulgaris*	Yes	24 h	Growth inhibition [8] K^+ leakage from cells	[109]
Oleic acid C18:1	12.1 mg/L	*Monoraphidium contortum*	Yes	24 h	Growth inhibition [8] K^+ leakage from cells	[109]
Linoleic acid C18:2	1.55 mg/L	*Selenastrum capricornutum*	Yes	72 h	Growth inhibition [8]	[108]
Linoleic acid C18:2	9.4 mg/L	*Chlorella vulgaris*	Yes	24 h	Growth inhibition [8] K^+ leakage from cells	[109]
Linoleic acid C18:2	8.0 mg/L	*Monoraphidium contortum*	Yes	24 h	Growth inhibition [8] K^+ leakage from cells	[109]
α–Pinene β–Pinene Limonene	10 g/L [d]	*Chlorella pyrenoidosa*	Yes	2 days	No effect on growth [7b]	[110]
α–Pinene	Analytical grade	*Chlorella vulgaris* stored as dried paste	–	7–8 h of extraction	Extraction of lipids from *Chlorella*	[111]

Table 5. *Cont.*

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
Gallic acid	10 mg/L	*Nostoc sp.*	Yes	5 days	Growth inhibition [8] Protein content reduction [8] Chlorophyll content reduction [8] Inhibition of glutamine synthetase activity [8] Inhibition of nitrate reductase activity [8]	[128]
Gallic acid	1 mg/L	*Microcystis aeruginosa*	Yes	15 days	Growth inhibition [8]	[131]
Ellagic acid	5 mg/L	*Microcystis aeruginosa*	Yes	15 days	Growth inhibition [8]	[131]
Quercetin	6 mg/L	*Thalassiosira pseudonana* *Phaeodactylum tricornutum* *Thalassiosira weissflogii*	Yes	Not mentioned	Photosynthetic mechanism inhibited [8]	[132]
Quercetin	12 mg/L	*Chlamydomonas sp.* *Dunaliella tetriolecta*	Yes	Not mentioned	No inhibition of photosynthetic mechanism [8]	[132]
Rutin	0.4 mg/L	*Sceletonema costatum*	Yes	3 days	Growth inhibition [8]	[133]
Catechin	25–100 mg/L	*Microcystis aeruginosa* *Pseudokirchneriella subcapitata*	Yes No Yes No	2 h 2 h 2 h 2 h	Formation of ROS˜ in cells Formation of ROS˜ in cells Formation of ROS˜ in cells Formation of ROS˜ in cells	[134]
Pinosylvin	21.2 mg/L 21.2 mg/L	*Selenastrum capricornutum* *Oscillatoria perornata*	Yes Yes	4 days 4 days	No effect on growth [8] No effect on growth [8]	[135]
Resveratrol	22.8 mg/L 22.8 mg/L	*Selenastrum capricornutum* *Oscillatoria perornata*	Yes Yes	4 days 4 days	No effect on growth [8] No effect on growth [8]	[135]
Pterostilbene	2.5 mg/L 25.6 mg/L	*Selenastrum capricornutum* *Oscillatoria perornata*	Yes Yes	4 days 4 days	Growth inhibition [8] Growth inhibition [8]	[135]
Eudesmin	3.8 mg/L 38.6 mg/L 38.6 mg/L	*Oscillatoria perornata* *Oscillatoria agardhii* *Selenastrum capricornutum*	Yes Yes Yes	4 days 4 days 4 days	Growth inhibition [8] No effect on growth [8] Growth inhibition [8]	[136]
Gramine	2 mg/L 1 mg/L 8 mg/L	*Microcystis aeruginosa*	Yes	24–60 h 5 days 1 day or 5 days	Breakage of cell wall structure [8] DNA fragmentation [8] DNA fragmentation [8]	[138]
Gramine	65 mg/L	*Chlorella vulgaris*	Yes	10 days	Growth inhibition [8]	[139]

Table 5. *Cont.*

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
Berberine	1 mg/L	*Pseudokirchneriella subcapitata*[+]	Yes	4 days	Not stated	[140]
	1 mg/L	*Chlorella vulgaris*	Yes	4 days	Not stated	
	0.75 mg/L	*Scenedesmus quadricauda*	Yes	4 days	Growth inhibition [8]	
	0.27 mg/L	*Microcystis aeruginosa*	Yes	4 days	Growth inhibition [8]	
	0.57 mg/L	*Synechococcus nidulans*	Yes	4 days	Growth inhibition [8]	
	0.64 mg/L	*Aphanothece clathrata*	Yes	4 days	Growth inhibition [8]	
Berberine	0.2 g/L	*Microcystis aeruginosa*	Yes	3 days	Inhibition of SOD activity [8] Increased O_2^- content in cells [8]	[141]
Flindersine	3.6 mg/L	*Oscillatoria perornata*	Yes	4 days	Growth inhibition [8]	[136]
	22.7 mg/L	*Oscillatoria agardhii*	Yes	4 days	No effect on growth [8]	
	4 mg/L	*Selenastrum capricornutum*	Yes	4 days	Growth inhibition [8]	
Lead Pb (added as $PbCl_2$)	0.5 mg/L	*Selenastrum capricornutum* *Chlorella pyrenoidosa* *Chlorella ellipsoidea* *Chlorella vulgaris*	Yes	7 days	Growth inhibition [8]	[144]
Cadmium Cd (added as $CdCl_2$)	17 mg/L	*Scenedesmus armatus*	Yes	24 h	Growth inhibition [10a] Inhibition of photosynthetic mechanism [10a] Growth inhibition [10b] Inhibition of photosynthetic mechanism [10b]	[146]
Nickel Ni (added as $NiCl_2$)	10 mg/L	*Synechococcus sp.*	Yes	10 days	Growth inhibition [8]	[145]
Chromium Cr (added as K_2CrO_4)	0.97 mg/L	*Chlorella vulgaris*	Yes	96 h	Growth inhibition [8] Photosynthetic mechanism inhibited [8]	[147]
EMIM Cl	1.46 g/L	*Chlorella vulgaris*	Yes	72 h	Growth inhibition [8]	[151]
EMIM Cl	1.83 g/L	*Oocystis submarina*	Yes	72 h	Growth inhibition [8]	[151]
EMIM Cl	14.6 mg/L	*Cyclotella meneghiniana*	Yes	72 h	Growth inhibition [8]	[151]
BMIM Cl	0.17 g/L	*Chlorella vulgaris*	Yes	72 h	Growth inhibition [8]	[151]
BMIM Cl	0.26 g/L	*Oocystis submarina*	Yes	72 h	Growth inhibition [8]	[151]

Table 5. *Cont.*

Compound	Concentration	Microalgae	Light	Cultivation Time	Effect on Microalgae	Ref.
BMIM Cl	1.74 mg/L	*Cyclotella meneghiniana*	Yes	72 h	Growth inhibition [8]	[151]
Rice straw hydrolysate	11 g/L sugars [e]	*Chlorella pyrenoidosa*	Yes	60 h	Increased growth [11] Increased lipid content [11]	[152]
Wheat bran hydrolysate	0.25%–1.5% [f]	*Chlorella vulgaris*	Yes	6 days	Increased biomass growth [12] Increased protein content [12] Increased pigment content [12]	[19]
Wheat bran hydrolysate	0.25%–1.5% [f]	*Chlorella vulgaris*	No	6 days	Increased biomass growth [13] Increased protein content [13] Increased pigment content [13]	[19]
Wheat bran hydrolysate	0.25%–1.5% [f]	*Scenedesmus obliquus*	Yes	8 days	Increased biomass growth [12] Increased protein content [12] Decreased pigment content [12]	[19]
Wheat bran hydrolysate	0.25%–1.5% [f]	*Scenedesmus obliquus*	No	8 days	Increased biomass growth [13] Increased protein content [13] Decreased pigment content [13]	[19]

[1] when compared to photoautotrophic cultivation; [2] when compared to "non xylose enhanced" strains; [3] when compared to experiments with lower acetate concentrations; [4] when compared to photoautotrophic cultivation with 5% CO_2 supplied; [5] when compared to mixotrophic cultivation with 1% glucose; [6] when compared to experiments without any sugars or sugar acids added; [7a] diameters of inhibition zone observed on filter paper disk; [7b] no inhibition observed on filter paper disk; [8] when compared to experiments without tested compound added; [9] when compared to mixotrophic acetate-based cultivation; [10a] when compared to control during cultivation with 0.1% CO_2; [10b] when compared to control during cultivation with 2% CO_2; [11] when compared to mixotrophic conditions with synthetic medium containing glucose; [12] when compared to photoautotrophic cultivation without wheat bran hydrolysate in growth medium; [13] when compared to cultivation in dark without wheat bran hydrolysate in growth medium; [a] compounds tested separately; [b] as an addition to 1 g/L of glucose; [c] expressed as weight on filter paper disk; [d] concentration in ethanol used to saturate paper disks on agar plates; [e] sugars from rice straw hydrolysate in growth medium; [f] % of wheat bran hydrolysate in growth medium; [+] formerly known as *Selenastrum capricornutum*; [~] Reactive Oxygen Species.

5. Strategies for Implementing Lignocellulose Extracts into Microalgae Cultivation Systems

In this review, a new approach to use lignocellulose hydrolysates as a feedstock for microalgae culture is presented. Such an approach requires many processing steps, including lignocellulose hydrolysis, detoxification of lignocellulosic hydrolysates with their implementation in microalgae cultivation systems and downstream processing of microalgae cultures. Many systems such as open ponds, or closed-up photobioreactors or bioreactors have been developed to cultivate microalgae [11]. Open ponds are the simplest cultivation systems where algae growing in ponds covering wide areas, are exposed to sun irradiation and convert light into biomass. Closed-up photobioreactors are systems where parameters such as pH, temperature, O_2 tension, concentration of CO_2 added or nutrient availability can be strictly controlled during cultivation. Such photobioreactors can be situated outdoors with light energy supplied from sun or can be placed indoors where light energy is provided by artificial lamps. In bioreactors, similar to photobioreactors, all cultivation parameters are controlled but a lack of light source makes this system only suitable for cultivation of microalgae that can use organic carbon sources, instead of light. Lignocellulosic hydrolysates contain organic carbon, in the form of sugars and acetate, which can be added to the bioreactor to support heterotrophically cultivated microalgae. Hydrolysates can be also implemented in photobioreactor cultivation, as addition of organic carbon in mixotrophic cultures was proved to increase biomass growth, if compared to photoautotrophic cultures. Whether production of compounds from microalgae cultures enriched with lignocellulosic hydrolysates can be incorporated on a commercial scale, depends strictly on economic factor. A long chain of processing steps generates costs due to energy input for material transport, hydrolysis, detoxification and cultivation system operations. Also the cost of chemical usage and labor have to be taken into consideration. All costs contribute to the final price of a desirable product and if this price is too high, the product cannot appear on the market. Therefore, efforts should be made to design systems that can provide efficient production process along with reduced energy input and maintenance costs. Microalgae are commercially cultivated in open ponds because of their economic feasibility and simplicity of maintenance. However in open systems, the light provided to microalgae cells is not sufficient and as a result, biomass density is not higher than 0.5 g/L [11]. Nowadays microalgae research is focused on closed-up photobioreactors, where better light utilization by microalgae cells results in achievable biomass densities between 5 and 25 g/L, however due to light limitation, this is not possible in scale-up systems [154]. Heterotrophic cultivation of microalgae in light independent bioreactors can also give biomass densities as high as 100 g/L in scale-up systems, when organic carbon source is supplied [15]. Implementation of lignocellulosic hydrolysates into open pond systems is rather doubtful, as bacteria can contaminate systems, consume supplied organic carbon, grow and overcome the microalgae cultures. This major problem could be solved by addition of organic solvents such as methanol or ethanol, together with lignocellulosic hydrolysates, into open systems. Methanol, which enhanced *Chlorella* [85] and *Scenedesmus* [86] growth and ethanol, which improved growth of *Euglena gracilis* [155], would also provide a sterility factor to prevent contamination by wild strains. However, not all microalgae strains are capable of utilizing methanol or ethanol and at higher concentration these solvents could also become inhibitory for microalgae [156,157]. Combination of some of processing steps into one process could be an interesting approach. Hydrolysates from lignocellulose hydrolysis can contain inhibitory substances such as furans, levulinic acid, fatty acids, *etc.*

Hydrolysate sugars can support microalgae growth, but inhibitors present in the same hydrolysate can cause negative effects, making implementation of lignocellulosic hydrolysate a useless process. In order to overcome this barrier, detoxification methods such as evaporation, precipitation, active charcoal or ion exchange resin treatment are used [158]. A promising method could be adaptation of microalgae to inhibitors as in case of a *Chlamydomonas reinhardtii* strain, which was cultivated for 170 days in the presence of gradually increasing concentrations of a bio-oil fraction that contained furfural and phenolics. As a result, an increased tolerance of *Chlamydomonas* towards toxic substances was achieved [103]. Some microalgae are also capable of removing phenolics from the growth medium [92]. Therefore, the possibility of using microalgae cultures as a detoxification treatment for lignocellulosic hydrolysates could be considered. Such hydrolysates containing phenolic compounds could be implemented into growth media during cultivation of microalgae in open ponds or enclosed photobioreactors. However, microalgae cultivated in open ponds are at risk of contamination from wild strains. Hence closed-up photobioreactors, where culture sterility can be maintained with less difficulty, seem to be more suitable for detoxification process with the use of microalgae. On the other hand, the presence of wild strains can be beneficial for detoxification process, as microalgae in consortium with bacteria can also degrade phenols [159,160]. The concentration of the added phenolic fraction should be also taken into consideration, in order to not cause lethal effects on the microalgae. Phenolics could be also implemented at lower concentration to increase biomass production, as these compounds were mentioned to stimulate growth of microalgae [93]. Again closed up systems are more suitable for this purpose, as the amount of the implemented phenolic fraction can be optimized in response to detoxification rate and culture growth rate, during cultivation on batch or fed–batch mode [161]. Heterotrophic cultivation in bioreactors can also be harnessed for detoxification, as the microalga *Ochromonas danica* was able to grow in a medium containing phenol or phenol with sugars, in the dark, with complete phenol removal from the medium [162]. Finally, the capability of producing cellulosic enzymes by *Chlamydomonas reinhardtii* [153] allows consideration of new possibilities of cultivating microalgae on lignocellulose without the need of using lignocellulose pretreatment methods or specific commercial hydrolysis enzymes.

6. Conclusions

Lignocellulose extracts have potential to enhance the growth of microalgae and stimulate accumulation of specific products in mixotrophic or heterotrophic cultures, but the selection of suitable strains and adjustment of cultivation conditions should be properly combined. Sugars and acetates present in lignocellulosic hydrolysates can be used by microalgae cells as carbon sources. Improved growth of some strains and production of target compounds in the presence of sugars or acetates can be expected in some strains [76,78,82], but in other strains increased growth can only be achieved at the expense of decreased content of target compounds in microalgae cells [75,77]. The concentration of organic substances should be also taken into consideration as acetates at higher concentrations can inhibit growth or product synthesis [77,81,82]. Moreover, cultivation conditions such as availability of light strongly influences production of specific substances in microalgae cultures [19]. In addition to sugar and acetates, other substances such as phenolics and furans are constituents of lignocellulose hydrolysates [98–101]. Phenolic compounds can be stimulatory or inhibitory

for microalgae, but the final effect depends strictly on phenolic structure and concentration, as well as on microalgae strains used [93–95]. Furans show inhibitory activity toward microalgae [102,103], but this effect has been scarcely investigated. Furans originate from sugars during lignocellulose treatment and it seems necessary to either maintain furan concentration below the inhibitory threshold or apply a pre-adaptation step to increase the resistance of microalgae strains to furans in the cultivation medium enriched with lignocellulose derived compounds [103]. Finally, the composition of lignocellulosic hydrolysates is dependent on the lignocellulose treatment method implemented. Methods and process hydrolysis conditions should be selected in such a way to achieve optimal feedstock substrates for microalgae cultivation, without generation of growth inhibitors.

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Conflict of Interests

The authors declare no conflict of interest.

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