



## FEEDSTOCKS USED FOR PRODUCTION OF 2<sup>nd</sup> AND 3<sup>rd</sup> GENERATION

### BIOETHANOL - REVIEW

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**Abstract:** *Global biofuel production has increased significantly over the last decade, but first-generation biofuels have been identified as a major concern, especially their sustainability as they are produced from food crops (such as cereals, sugar cane and vegetable oils). Depending on the feedstocks and cultivation technique, the production of second and third generation biofuels has the potential to provide benefits, such as the recovery of residues and unusable land. Therefore, second and third generation biofuels are indicated to meet the increasing demand for energy and contribute considerably to the development of rural areas and the increasing of bioeconomy. This short review shows that there may be different types of feedstocks (agricultural residues, forest residues, energy crops and algae) which can be used for the production of 2<sup>nd</sup> and 3<sup>rd</sup> generation bioethanol without affecting food security.*

**Keywords:** *cellulose, hemicellulose, starch, lignin, bioethanol*

### 1. Introduction

The feedstocks used to obtain biofuels are plants and cereals that are intended for human consumption. Lignocellulosic biomass (LCB) is easily accessible worldwide and is found in the form of residues and agricultural biomass such as corn straw, wheat straw and rice straw. The production of biofuels aims to protect the environment, to meet new energy requirements, to reduce the import and production of conventional fuels, thus stimulating the development of agriculture [1]. Second-generation biofuels are largely derived from LCB, which includes most plant-based, non-food materials that are inexpensive and found in huge quantities. Currently, the production of second-generation biofuels is not cost-effective because it requires overcoming technical barriers to obtaining them. In terms of bioethanol production, LCB is one of the most abundant and least used resources.

LCB is usually burned for the production of heat and electricity, although it could be used to produce liquid biofuels. However, the production of biofuels from agricultural by-products could only meet a certain proportion of the growing demand for liquid biofuels, crops dedicated to the production of LCB are an important solution for the production of biofuels [2]. Compared to first generation biofuels which are mostly obtained from corn or sugar cane, biofuels obtaining from LCB is more expensive because lignocellulosic materials have a complex structure and require a specific technological process [3, 4]. Microalgae production is the key to the development of the third generation of bioethanol, as they could provide an alternative in terms of biomass production [5, 6]. Algae are also the fastest growing plants on Earth. Depending on how they are recovered, algae can be used to produce biofuels such as biodiesel,

bioethanol, but also other valuable substances [7].

## **2. Feedstocks used in the production of second generation bioethanol (2<sup>nd</sup>G)**

In the food industry, significant quantities of non-food lignocellulosic biomass can be used to produce 2<sup>nd</sup>G bioethanol, thus making it a promising alternative for fossil fuels. Lignocellulosic biomass is one of the most abundant renewable resources on Earth and has a relatively low price [8].

Lignocellulosic feedstocks are renewable and cheap sources. These can come from the forestry, agricultural fields (grains, wheat straw, rice straw and sugar cane), agro-industrial as well as significant quantities of food residues [9].

LCMs are made of cellulose, hemicellulose and lignin which form a complex structure and are resistant to physico-chemical and biological treatments. One of the best strategies is to convert sugars from LCB by enzymatic hydrolysis because it does not require high energy consumption and is a clean process. However, it should be noted that the enzymatic step in the technological process of obtaining bioethanol also has a disadvantage, namely that related to the rigid structure between cellulose and lignin. Therefore, in order to facilitate enzymatic hydrolysis and implicitly to obtain high concentrations of cellulose, a pretreatment step is required, being considered a key step in obtaining an increased bioethanol yield from LCMs [10].

In literature several methods of pretreatment are described and are known as [10]:

- physical pretreatment (milling and milling, microwave oven and extrusion);

- chemical pretreatment (alkaline, acid, organosolvent, ozonolysis and ionic liquid);

- physico-chemical pretreatment (steam explosion, hot water, AFEX ammonia fiber explosion, wet oxidation and CO<sub>2</sub> explosion);

- biological pretreatment.

### **Cellulose**

Cellulose (C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>)<sub>n</sub> is a carbohydrate found in agricultural and woody biomass [10]. It is a linear polymer composed of glucose (D-glucose) molecules that have β-(1,4) -glycoside bonds [11, 12]. Cellulose is insoluble in water therefore, a hydrolysis process must be applied to convert this polysaccharide into glucose molecules [13].

General hydrolysis of cellulose produces only glucose, which can be transformed into different forms of biochemical and chemical substances. Various biochemical and chemical substances such as bioethanol, organic acids, glycerol, sorbitol, mannitol, fructose, enzymes and biopolymers can be obtained through biological processes [14, 15].

Figure 1 shows the enzymatic hydrolysis of cellulose and the enzymes involved in this process.

### **Hemicellulose**

Hemicellulose (C<sub>5</sub>H<sub>8</sub>O<sub>4</sub>)<sub>n</sub> is a short polymer that has a branched structure, comprising sugars such as pentoses (D-xylose and L-arabinose) and hexoses (D-glucose, D-mannose and D-galactose) [16]. Hemicelluloses are found in plants in the form of xyloglucans or xylans. Hemicelluloses are present in woody biomass, softwood and hardwood [17]. Due to its branched structure, hemicellulose is easier to hydrolyze as opposed to cellulose.

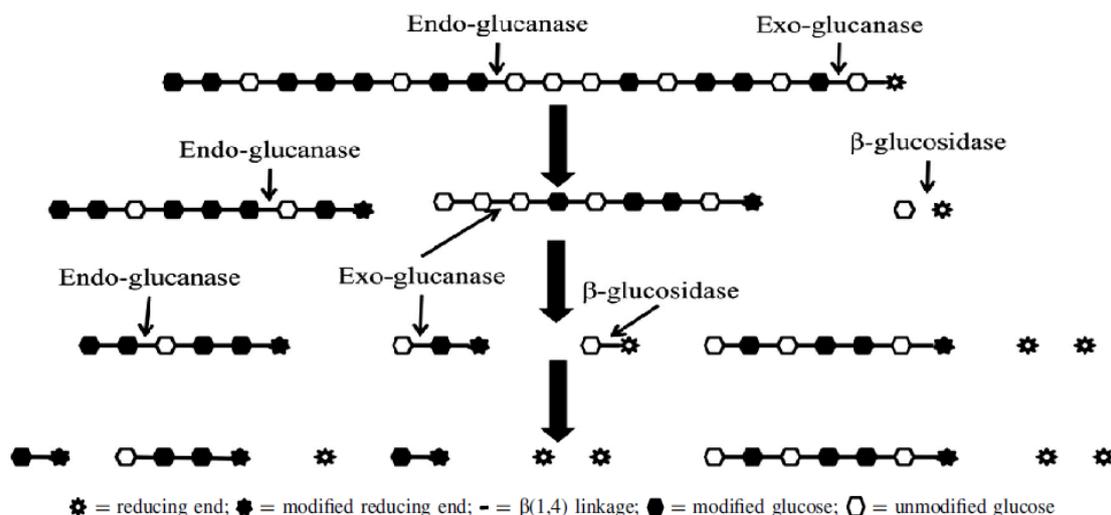


Fig 1. Enzymatic hydrolysis of cellulose [18]

Xylose can be used to obtain xylitol. Xylitol is a non-carcinogenic sweetener, with the same sweetening power of sucrose. Xylose can be transformed with the help of microorganisms into proteins, fuels and solvents. There are certain yeast strains that can ferment xylose and transform it into bioethanol (*Pichia stipitis*, *Candida shehatae*) [15].

### Lignin

Lignin  $[C_9H_{10}O_3 (OCH_3)_{0,9-1,7}]_n$  is an organic compound and has a branched structure consisting of 3 different monomers (coniferyl alcohol, synapyl alcohol and p- coumaryl alcohol) [19]. Lignin is a barrier in the fermentation process of LCB and is resistant to chemical and biological degradation. Also, its presence affects the yield of bioethanol [20].

By utilizing lignin, carbon fibers, emulsifiers, dispersants, sequestrants, surfactants, binders and other chemicals can be obtained [21].

The chemical composition (%) and the main constituents of LCMs are shown in figure 2.

### 2.1. Agricultural residues, municipal solid residues and different types of grass

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Agricultural residues (corn cobs, corn stover, sugarcane bagasse, rice straw, and wheat straw) are important sources for 2<sup>nd</sup>G bioethanol production. The grain harvesting period is relatively short and so these residues are available throughout the year. Each year, between 350 and 450 million tonnes of crops are harvested, resulting in huge quantities of agricultural residues [18]. For example, up to 1 - 3 tons of straw can grow from 1 acre area grown with wheat. From the cost point of view, the price of sugar cane and maize rises to 60.9 USD / tonne respectively, 185.9 USD / tonne, while those for the sugarcane bagasse and corn stover the price is much lower, of only 36.4 USD / tonne and 58,5 USD / tonne respectively [22]. One should know that almost 70% of the cost of bioethanol production is represented by the cost of obtaining the feedstocks [23].

Therefore, for half the costs it would be preferable to use agricultural residues and not to use energy crops. By capitalizing these residues, forestry and arable land held by herbaceous plants (switchgrass, miscanthus) would be reduced.

Municipal solid residues and residues from food industry have been studied for ethanol production [23, 25], because it has an important carbohydrate content, and the protein and mineral content can support the

fermentation process. The study by Matsakas et al. (2014) showed that food residues can be transformed successively into bioethanol after a double fermentation. After the enzymatic hydrolysis and also the fermentation phase completed, an ethanol content of 43 g / L was obtained. Then, a

microwave-assisted hydrothermal pretreatment was applied to the remaining solid residue and again subjected to fermentation. After the second fermentation was completed, an alcohol content of 59 g / L of ethanol was obtained [26].

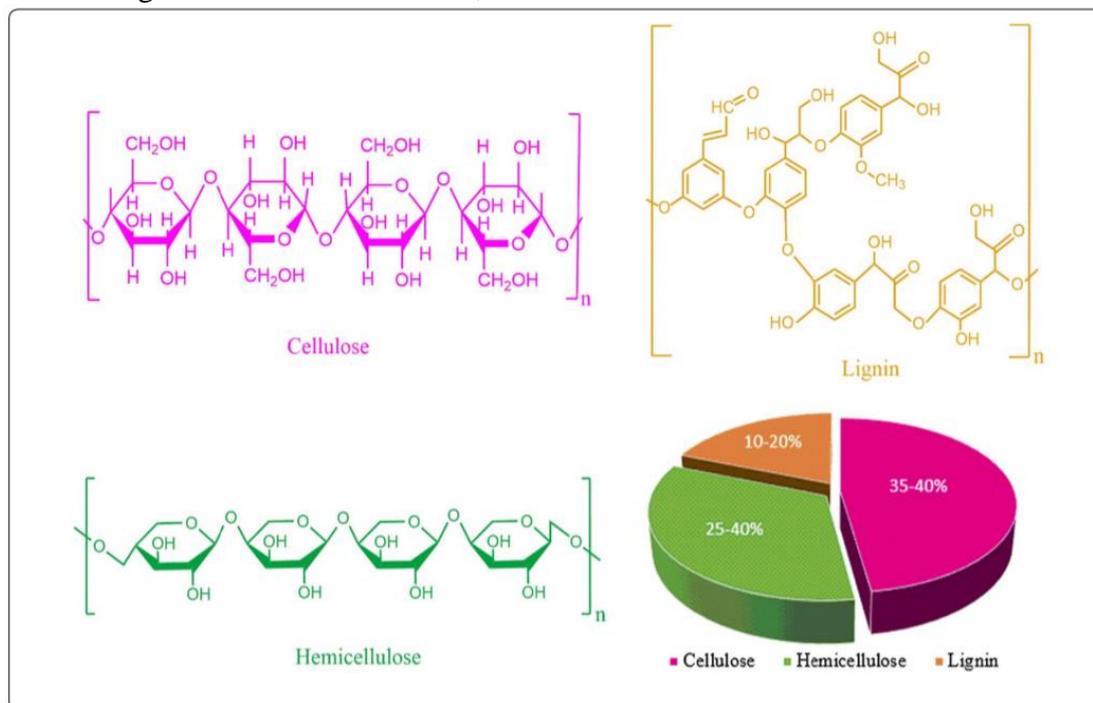


Fig.2. Chemical composition (%) and main constituents of LCM [24]

Switchgrass is a feedstock that has high glucose content, is highly resistant to disease and has high biomass productivity. *Miscanthus giganteus* is another type of grass that can be used for biofuel production, especially as it has a fast growth rate. It is native to Asia, but it is also cultivated in Europe. This grass represents 50 - 70% of the total biomass feedstocks (including forest wood biomass and agricultural residues) that are used for the production of cellulosic biofuels [18]. It was estimated that approximately  $133 \times 10^9$  L of ethanol could be produced if 9.3% of US agricultural land were cultivated with this plant, thus 1/5 of the country's gasoline consumption could be replaced [27]. Scagline-Mellor et al. (2018) argue

that bioethanol yield is higher for miscanthus compared to Switchgrass [28]. In the Mediterranean area, lignocellulosic materials are found that can be used for the production of 2<sup>nd</sup>G bioethanol. These raw materials include: cereal crops, olives, tomatoes, grapes and residues resulting from the processing of grapes, solid residues of olives, "date" palm trunks, perennial lignocellulosic herbs (*Arundodonax*, *Saccharum spont.*, *Aegyptiacum* and *Miscanthus giganteus*) or the cactus species *Luffa cylindrica* and *Luffa prickly pear*.

*Stipa tenacissima* or Esparto grass belongs to the family poaceae; it is a perennial plant that has a fast growth rate. The leaves of this plant have high fiber content [29] and can reach up to 1 m in height. The

*Stipa tenacissima* bushes have a circular and homogeneous shape when they are young, but as they age they dry out and in the center empty spots form. The leaves are thin, ribbon-shaped, smooth, shiny, and solid and at the base they are covered by a hairy sheath. Esparto leaves reach maturity between the fourth and eleventh months after flowering, depending on the geographical area and climatic conditions [30]. *Stipa tenacissima* is spread over an area of about 3 million hectares in Algeria [31] and over 400 thousand hectares in Tunisia, located mainly in the Ksserine, Sidi-Bouزيد, Gafsa and Kairouan regions. For about four decades, the Alfa plant has been considered of great importance for the production of fibers intended for the manufacture of paper. For example, every year Tunisia produces an amount of Alpha pulp in excess of 30,000 tonnes [30, 32].

Given that *Stipa tenacissima* is a plant that has adapted to the semi-arid climate and does not require large quantities of water to grow, it is an important source for bioethanol production. The central-western part of Tunisia faces water shortages, and by cultivating energy plants that require significant quantities of water would put huge pressure on food crops. Specifically, various authors argue that in terms of adaptation, but also environmental sustainability, it would be advisable to grow energy-tolerant drought plants, such as sweet sorghum [33].

Table 1 shows the quantities of some LCMs and their potential for bioethanol production. Table 2 presents a series of LCMs with their main constituents.

**Table 1**  
**The worldwide available quantity of the main agricultural residues and their potential for bioethanol production [34]**

Feedstock for 2 <sup>nd</sup> G bioethanol	Worldwide quantities of agricultural residues (Tg)	Potential bioethanol production (gallons)	Total bioethanol (gallons)	Gasoline equivalent (gallons)
Corn grain residue	20.7	14.38	72.98	52.4
Corn stover	203.62	58.6		
Barley grain residue	3.66	2.46	20.56	14.8
Barley straw	58.45	17.1		
Oat grain residue	0.55	0.39	3.17	2.27
Oat straw	10.62	2.78		
Rice grain residue	25.44	16.8	221.4	159
Rice straw	731.34	204.6		
Wheat grain residue	17.2	11.33	115.13	82.71
Wheat straw	354.35	103.8		
Sorghum grain residue	3.12	2.14	4.93	3.54
Sorghum straw	10.32	2.79		
Sugarcane residue	3.2	1.59	52.89	38
Sugarcane bagasse	180.73	51.3		

## 2.2. Forest wood biomass

Forest wood biomass is known as one of the most promising renewable feedstocks for the production of 2<sup>nd</sup>G bioethanol.

Wood biomass can be obtained from maintenance or forestry exploitation.

This has a high energy value and the acquisition costs are low, therefore it could be used for bioethanol production [41]. In

the US, woody biomass accounts for about 30% of total biomass used annually to generate bioenergy [42]. The wood forest materials used in the USA generally come from 3 species of resinous *Pinus contorta*, *Pseudotsuga menziesii* and *Pinus ponderosa*. These conifer species have a high content of hemicellulose (18 - 33%)

and cellulose (39 - 55%) [42]. Nearly 90% of the dry weight of forest wood biomass is composed of lignin, hemicellulose, cellulose and pectin [43]. Specifically, woody biomass comprises 30 - 60% cellulose, 15 - 40% hemicellulose and 10 - 25% lignin [17, 22, 37].

Table 2

Chemical composition for different LCMs [35, 36]

Feedstock	Carbohydrate compositions (%)			References
	Cellulose	Hemicellulose	Lignin	
Sugarcane tops	35	32	14	[37]
Sugarcane bagasse	32 - 48	19 - 25	23 - 32	[36, 38]
Corn stover	38 - 40	26 - 28	7 - 21	
Corn cob	45	35	15	[38]
Sorghum stalks	27	25	11	[39]
Sorghum straw	32	24	13	
Sweet sorghum Bagasse	34 - 45	18 - 28	14 - 22	[36, 39]
Barley straw	31 - 45	27 - 38	14 - 19	[39]
Rice straw	28 - 38	23 - 32	12 - 14	[36, 39]
Rice husk	37	29	24	[40]
Wheat straw	33 - 41	20 - 32	13-20	[36, 40]
Cotton, flax, etc.	80 - 95	5 - 20	-	[36, 39]
Coir	36 - 43	0.15 - 0.25	41 - 45	[39]
Switchgrass	40 - 45	30 - 35	12	[17]
Leaves	15 - 20	80 - 85	0	[38]
Grasses	25- 43	8 - 50	8 - 30	[17, 23]
Agriculture residues	37 - 50	25 - 50	5 - 15	[17]
Industrial residue from chemical pulp	50 - 80	20 - 30	2 - 10	[17, 36]
Newspaper	40-55	25 - 40	18 - 30	[39]
Paper residues	65	13	1	[40]

There are 35 species of the genus *Populus* that have a fast growth rate and can produce large quantities of woody material that can be used to obtain bioethanol 2<sup>nd</sup>G. The harvest time of forest wood biomass is more flexible as compared to agricultural residues. Forest residues, such as dry trees, wood chips and sawdust, could be an important feedstock that can be converted into bioethanol [17]. The carbohydrate content as well as of other wood extractable substances and the bark of the

various trees are presented in the tables 3, 4, 5 and 6.

### 3. Feedstocks used in the production of third generation bioethanol (3<sup>rd</sup>G)

Currently, the use of algae biomass for 3<sup>rd</sup>G biofuel production is of high interest, as well as investments in the biofuel, PETROLEUM and agri-food industries. It has been proven that major biofuel producing countries, such as the US, Europe and Asia, cannot produce sufficient quantities of corn, soy or rapeseed for their biofuel targets.

Table 3

Proportion of major main constituents of wood [47]

Sample of wood	Holocellulose (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Beech	82.5	46.7	35.8	20.7
Birch	84.2	45.4	38.8	17.7
Alder	77.2	44.1	33.1	22.0
Maple	80.1	44.6	35.5	24.9
Spruce	77.8	50.0	27.8	26.5
Pine	73.1	47.3	25.8	25.6
Oak	69.4	39.1	30.3	22.8

Table 4

Content of carbohydrates and other extractable substances (%) of some trees in North America [44, 45]

Scientific Name	Common Name	X	A	G	Ga	M	Ua	Ac	Lg	Ash
<b>Hardwoods</b>										
<i>Acer rubrum</i>	Red maple	19	0.5	46	0.6	2.4	3.5	3.8	24	0.2
<i>Acer saccharum</i>	Sugar maple	15	0.8	52	<0.1	2.3	4.4	2.9	23	0.3
<i>Betula alleghaniensis</i>	Yellow birch	20	0.6	47	0.9	3.6	4.2	3.3	21	0.3
<i>Betula papyrifera</i>	White birch	26	0.5	43	0.6	1.8	4.6	4.4	19	0.2
<i>Fagus grandifolia</i>	Beech	19	0.5	46	1.2	2.1	4.8	3.9	22	0.4
<i>Platanus occidentalis</i>	Sycamore	15	0.6	43	2.2	2.0	5.1	5.5	23	0.7
<i>Populus deltoides</i>	Eastern cottonwood	15	0.6	47	1.4	2.9	4.8	3.1	24	0.8
<i>Populus tremuloides</i>	Quaking aspen	17	0.5	49	2.0	2.1	4.3	3.7	21	0.4
<i>Quercus falcata</i>	Southern red oak	19	0.4	41	1.2	2.0	4.5	3.3	24	0.8
<b>Softwoods</b>										
<i>Abies balsamea</i>	Balsam fir	6.4	0.5	46	1.0	12	3.4	1.5	29	0.2
<i>Gingo biloba</i>	Ginko	4.9	1.6	40	3.5	10	4.6	1.3	33	1.1
<i>Larix laricina</i>	Tamarack	4.3	1.0	46	2.3	13	2.9	1.5	29	0.2
<i>Picea abies</i>	Norway spruce	7.4	1.4	43	2.3	9.5	5.3	1.2	29	0.5
<i>Picea glauca</i>	White spruce	9.1	1.5	45	1.2	11	3.6	1.3	27	0.3
<i>Picea mariana</i>	Black spruce	6.0	1.5	44	2.0	9.4	5.1	1.3	30	0.3
<i>Picea rubens</i>	Red spruce	6.2	1.4	44	2.2	12	4.7	1.4	28	0.3
<i>Pinus resinosa</i>	Red pine	9.3	2.4	42	1.8	7.4	6.0	1.2	29	0.4
<i>Pinus rigida</i>	Pitch pine	6.6	1.3	47	1.4	9.8	4.0	1.2	28	0.4
<i>Pinus sylvestris</i>	Scots pine	7.6	1.6	44	3.1	10	5.6	1.3	27	0.4
<i>Pinus taeda</i>	Loblolly pine	6.8	1.7	45	2.3	11	3.8	1.1	28	0.3
<i>Pseudotsuga menziesii</i>	Douglas-fir	2.8	2.7	44	4.7	11	2.8	0.8	32	0.4
<i>Thuja occidentalis</i>	Northern white cedar	10.0	1.2	43	1.4	8.0	4.2	1.1	31	0.2
<i>Tsuga canadensis</i>	Eastern hemlock	5.3	0.6	44	1.2	11	3.3	1.7	33	0.2

X- Xylose, A- Arabinose, G- Glucose, Ga- Galactose, M- Mannose, Ua- Uronic acids, Ac- Acetyl, Lg- Lignin

### 3.1. Macroalgae and microalgae

Carbohydrate percentages for seaweed depend on the species and hydrolytic treatment used. These sugars can be fermented by microorganisms and converted into bioethanol and / or

biobutanol [49]. Researches on brown algae have shown that from 50 g / L sugar the ethanol yield is 7.0 - 9.8 g / L, and the fermentation process lasted for 40 hours (acidic medium) [50]. In the case of acid hydrolysis of green macroalgae (*Ulva*), a

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content of 15.2 g sugars/L was obtained [50].  
and the average yield of butanol was 4 g/L

**Table 5**  
**Content of carbohydrates and other extractable substances in the bark of some trees [44, 46]**

Species	X	A	G	Ga	M	Rh	Ua	Ac
<i>Abies amabilis</i>	3.2	3.2	37.4	1.6	8.0	-	5.6	0.8
<i>Picea abies</i>	4.8	1.8	36.6	1.3	6.5	0.3	-	-
<i>Picea engelmannii</i>	3.8	3.3	35.7	2.4	2.9	-	8.0	0.5
<i>Pinus contoria</i>								
Inner bark	3.7	10.6	40.9	4.3	2.5	-	9.9	0.2
Outer bark	3.4	5.5	26.8	4.2	2.5	-	7.7	0.8
<i>Pinus sylvestris</i>	5.8	2.1	30.2	2.4	5.4	0.3	-	-
<i>Pinus taeda</i>								
Inner bark	2.1	5.6	21.3	3.1	2.5	0.3	4.6	-
Outer bark	3.8	1.8	15.8	2.5	2.6	0.1	2.1	-
<i>Betula papyrifera</i>								
Inner bark	21.0	2.7	28.0	1.0	0.2	-	2.2	-
<i>Fagus sylvatica</i>	20.1	3.1	29.7	3.1	0.2	1.2	-	-
<i>Quercus robur</i>	16.4	2.0	32.3	1.3	0.5	0.5	-	-

X- Xylose, A- Arabinose, G- Glucose, Ga- Galactose, M- Mannose, Rh- Rhamnose, Ua- Uronic acids, Ac- Acetyl

**Table 6**  
**The content of fermentable sugars from hydrolyzed biomass and the ethanol content resulting from fermentation [48]**

Sample	Mixed Sugar content		Ethanol content	
	Amount	Percentage (%)	Amount (g/l)	Percentage (%)
Sawdust	18.20	36.40	8.51	17.02
Corn residues	19.24	38.48	8.99	17.98

In recent years it has been found that microalgae are a promising starting material for bioenergy production, because they have a high content of carbohydrates that can be used for the purpose of obtaining bioethanol and biobutanol, respectively a lipid content that could be used to obtain biodiesel. Also, a series of gaseous biofuels, such as biomethane and biohydrogen, can be produced from microalgae or their residues (after obtaining bioethanol and biodiesel) [51]. Unlike plants that do not grow in the aquatic environment, microalgae do not have biopolymers like lignin and hemicelluloses in the chemical structure

[52 - 54]. Under specific conditions, the biomass formed from the microalgae can undergo a hydrolysis step and the carbohydrates can be fermented by the yeasts in bioethanol [55]. In the case of fermentation of microalgae biomass for the release of fermentable sugars it is possible not to use chemical and enzymatic pretreatments. It is known that in the case of cellulose feedstocks by applying these pretreatments, significant amounts of energy are consumed. However, mechanical pretreatments are still needed to disintegrate algal cells by various techniques [56].

Different microalgae species, such as *Chlamydomonas sp.*, *Chlorella sp.*, *Spirulina sp.*, *Spirogyra sp.* Also *Dunaliella sp.* can be used to obtain 3<sup>rd</sup>G bioethanol because they have a starch content of about 64% [57]. Another important aspect is that they have a fast development rate, high photosynthesis activity and high CO<sub>2</sub> absorption capacity [58]. Liyamen and Ricke (2012) concluded that microalgae can produce about 10

times more bioethanol than maize on the cultivation surface. In recent years, with the help of genetic engineering, species of microalgae have been created that have a higher carbohydrate content, resulting in higher yields of 3<sup>rd</sup>G bioethanol. For example, *Chlamydomonas reinhardtii* and *Chlorella vulgaris* JSC-6 cultivated under controlled conditions had a carbohydrate content of 71% and 54%, respectively [59 - 60].

Table 7

Carbohydrate content of different algae and microalgae species [61]

Algal species	Carbohydrate content (%)	Reference
<i>C. vulgaris</i>	20.99 - 55.0	[62, 63]
<i>Chlorella sorokiniana</i>	35.67	[64]
<i>Chlorella minutissima</i>	61	[65]
<i>Chlorella homosphaera</i>	54	[66]
<i>Chlamydomonas reinhardtii</i> UTEX 90	60.0	[67]
<i>Spirulina platensis</i> 30.21	30.21	[64]
<i>Spirulina platensis</i> LEB 52	65	[66]
<i>Scenedesmus dimorphus</i>	21 - 52	[69, 70]
<i>Scenedesmus obliquus</i>	46	[71]
<i>Scenedesmus ecosystem</i>	42 - 53	[72]
<i>Nannochloropsis oceanica</i>	22.70	[73]
<i>Spirogyra sp.</i>	33 - 64	[74]
<i>Porphyridium cruentum</i>	40 - 57	
<i>Ulva lactuca</i>	55-60	[75]
<i>Dunaliella salina</i>	32	[70]
<i>Dunaliella tertiolecta</i>	21.69	[76]
<i>Tetraselmis sp.</i>	24	[77]
<i>Porphyra</i>	40-76	[78]
<i>Palmaria</i>	38-74	[79]

Also, several researchers claim that from microalgae a bioethanol yield can be obtained with values between 0.240 and 0.888 g ethanol / g substrate, at 25 - 30 ° C [56, 80, 81]. Laboratory research has shown that bioethanol yield from biomass formed from microalgae under optimal conditions is about 65% [56].

*Chlorella vulgaris* biomass was enzymatically hydrolyzed, and the resulting carbohydrates were fermented by *Saccharomyces cerevisiae* and converted

to ethanol. The yields obtained for sugars following hydrolysis and ethanol were 0.55 and 0.17 g / g biomass respectively [82]. After extracting from *Schizochytrium sp.* lipids and proteins, the remaining carbohydrates (D- glucose and L- galactose), were transformed by *Escherichia coli* KO11 into bioethanol. Following the fermentation process of the concentration of 25.7 g / L glucose, a yield of 11.8 g ethanol / L was obtained [83].

It is estimated that in a year, between 5,000 - 15,000 gallons of ethanol / acre (46,760 - 140,290 L / ha) can be produced from microalgae [84].

Table 7 shows the carbohydrate content of algae by species. Table 8 shows the bioethanol yield for different algae and microalgae.

**Table 8**

**Bioethanol yield for different species of algae and microalgae [84]**

Feedstock	Bioethanol	Reference
<i>Chlorococcum infusionum</i>	260 g ethanol/Kg algae	[85]
<i>Spirogyra</i>	80 g ethanol/kg algae	[86]
<i>Chlorococcum humicola</i>	520g ethanol/kg microalgae	[87]
<i>Chlamydomonas reinhardtii</i> UTEX 90	11.73 ethanol g/1	[88]
<i>Chlamydomonas reinhardtii</i>	29.2 %	[89]
<i>Chlamydomonas fasciata</i>	19.4	[90]
<i>Chlorella vulgaris</i>	11.66 % ethanol g/1	[91]
<i>Arthrospira platensis</i>	16% g EtOH per g of dry biomass.	[91]

#### 4. Conclusion

By reviewing the current state of research regarding biofuel production, the following conclusions can be drawn:

- Every year, huge quantities of lignocellulosic materials (LCM) are generated from agriculture, which instead of being wasted can be converted into second-generation 2<sup>nd</sup>G bioethanol.
- The forestry sector generates a huge amount of wood biomass which is relatively cheap and can be used for bioethanol production.
- The efficiency of lignocellulosic feedstocks depends mainly on their availability and composition (cellulose, hemicellulose, lignin, ash).
- In order to obtain high yields of carbohydrates, respectively bioethanol 2<sup>nd</sup>G, it is indicated that different pretreatments described in the literature should be applied on lignocellulosic biomass (LCB).
- Recent research has shown that algae / microalgae are a source of biomass from which significant quantities of third-generation bioethanol (3<sup>rd</sup>G) and biodiesel could be produced.

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