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1 # Bioconversion of industrial hemp biomass for bioethanol

2 # production: A review

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1   **Abstract**

2   Industrial hemp (*Cannabis sativa* L.) with robust drought-resistant features has excellent

3   agronomic and pharmaceutical characteristics. As the federal prohibition on hemp cultivation was

4   lifted, its valorization in various aspects is highly required. This review aims to summarize the

5   potential of hemp biomass for bioethanol production. Chemical compositions of hemp biomass

6   were evaluated as compared with those of corn fiber, corn stover, and sorghum bagasse. Several

7   representative pretreatment technologies used for hemp biomass were summarized in terms of

8   sugar recoveries, lignin removal, and sugar and ethanol yields. This review presents numerous

9   technical barriers attributed to insufficient fermentable sugar and ethanol concentration during the

10   conversion processes. Also, innovative research approaches (pretreatment optimization, co-

11   fermentation of hexose and pentose, increasing potential sugar loading) in overcoming these

12   challenges were critically reviewed. This review would promote future research on the utilization

13   of hemp biomass for biofuel applications.

14   **Keywords:** Industrial hemp; Lignocellulosic biomass; Pretreatment; Bioethanol production

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1. Introduction

Industrial hemp (*Cannabis sativa* L.) was cultivated in temperate Eurasia for millennia and was first brought to North America in 1606 [1]. Hemp has excellent agronomic, food, and pharmaceutical properties with various applications in industrial fields [2]. In decades, several nations and jurisdictions prohibited the cultivation and processing of industrial hemp due to its principal intoxicating constituent (delta-9 tetrahydrocannabinol (THC)) [3]. The 2018 US farm bill approved industrial hemp varieties that could be cultivated, harvested, and processed with a THC threshold below 0.3% [4]. In the US, hemp production is legal in 46 states except from Idaho, Mississippi, New Hampshire, and South Dakota [4]. As the federal prohibition on hemp cultivation is gradually unbundled, its valorization in various aspects is highly required. Industrial hemp produces both biomass and seeds. Traditionally, hemp biomass's economic value is its fiber-rich stem, which can be used to manufacture fabrics, clothes, and papers [3, 5]. While its seeds with less than 0.3% THC has excellent potential for food and medical applications due to its nutritional and pharmaceutical values [6].

In this review, the primary focus is the potential of hemp biomass for bioethanol production. The biomass yield and chemical compositions of industrial hemp were summarized and compared with other biomass including corn fiber, corn stover, and sorghum bagasse. Then, representative pretreatment technologies used for hemp biomass to enhance the sugar recoveries, lignin removal, sugar and ethanol yields are discussed. This review presents numerous technical barriers attributed

to insufficient fermentable sugar and ethanol concentration during the conversion processes. Finally, innovative research progress (pretreatment optimation, co-fermentation of glucose and xylose, increasing potential sugar loading) in overcoming these challenges were critically reviewed. It is believed that this review would help scientific community and related industry to understand the potential and barriers of hemp biomass for bioethanol application and promoting future research on the utilization of hemp biomass for biofuel production.

2. Hemp biomass

2.1. Biomass yield

Hemp (*Cannabis sativa* L.) is an herbaceous annual belonging to the family Cannabinaceae. It can be cultivated under various climatic conditions due to its resilience to the external environment [15]. In the last three decades, the commercial cultivation of industrial hemp was concentrated in Europe (Table 1). Early European varieties of hemp can be grouped into northern and southern types with distinct characteristics. Northern hemp is characterized by rapid early growth, early flowering, sturdy branching, and high seed yield, whereas southern types tend to be slow-growing, tall, late flowering, and high fiber quality and yield. As shown in Table 1, significant variation in the hemp biomass yields (3.4-31.2 t/ha) was observed, mainly due to the environmental conditions, fertilization applied, plant density, and genotypes [7-10, 12-14]. For example, Campiglia et al. [7] reported that hemp biomass yield ranging from 3.4 to 8.0 t/ha was positively correlated with the vegetative phase's duration. They also found that biomass yield increased as plant density increased.

Ascrizzi et al. [13] found that the crops harvested in Santa Luce showed higher total dry yield and stem yield than those harvested in Cascina, probably due to its greater plant density and higher soil water holding capacity. In addition, Adamovics et al. [16] reported notable variation in biomass yields between cultivars attributed to the differences in genotypes and found that Futura variety achieved the highest biomass yield of 21.3 t/ha.

2.2. Botanical structure

Hemp stem consists of several morphological layers (Fig. 1). The two essential stem fiber, phloem (bast) fiber and xylem (wood) fiber, occur in the hemp stem. The bast fiber (epidermis and phloem layers) contains high cellulose (67-78%) and low lignin (2.9-13%) [18, 19], whereas the woody core (xylem and pith layers) contains about 40% cellulose and 17% lignin [20]. Removal of the cortex by "retting" is a crucial initial step in fiber extraction. Internal to the cortex is the primary phloem fibers that are amalgamated into rope-like, glued together bundles that occurred in the outermost part of the stem, which is the principal fiber of interest. The lignified xylem ring is primarily attributed to the recalcitrance of hemp biomass toward enzymatic and microbial attack [21, 22]. Also, the woody tissue and remnants of pith in the central part of stem consist of the "hurds" which is the primary source of strength.

2.3 Chemical composition

The chemical composition of hemp biomass compared with corn fiber, corn stover, and sorghum bagasse is shown in Table 2. It was reported that compositional variation among the same

varieties was mainly attributed to cultivation, fertilization, and climate conditions [14, 62, 63]. Genotype played an important role in influencing on the chemical composition of hemp biomass: Zhao et al. [42] reported that SS Beta variety contained higher cellulose (42.7%) but lower lignin (15.0%) than Tygra (40.7% and 15.7%); Das et al. [64] found significant variation in glucan (43.8-50.1%), xylan (11.6-14.2%), and lignin (15.4-29.4%) among the 11 hemp cultivars. Cellulose (D-glucose polymer) condenses through β (1-4) glycosidic bonds [65]. Strong hydrogen bonds between and within cellulose strands are ascribed to high crystallinity [42]. Hemp biomass contains relatively higher cellulose (36.5-75.6%) than corn fiber (13.0-18.0%), corn stover (31.0-41.2%), and sorghum bagasse (35.5-41.1%) (Table 2). The high cellulose content in hemp biomass would benefit the fermentable sugar concentration and final bioethanol yield. Hemicellulose (D-pentose polymer) as heterogeneous polysaccharides mainly contains a β-D-xylose monomer. Table 2 also showed the hemicellulose contents of hemp biomass (10.1-32.8%), corn fiber (35.0-45.3%), corn stover (16.5-22.8%), and sorghum bagasse (18.4-25.9%). Compared to corn fiber, corn stover, and sorghum biomass, hemp biomass had a larger range and variation in hemicellulose. Lignin is randomly methoxylated and incorporated by lignols (p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol). The lignin content of hemp biomass ranged from 8.0 to 22.9%, which is relatively lower than corn stover from 12.3 to 25.4% and sorghum biomass from 15.4 to 24.5% but higher than corn fiber from 1.3 to 18.0% (Table 2). Low lignin content in biomass would benefit the bioconversion process due to the weak recalcitrance of biomass [42, 66].

3. Sugar and ethanol production

The production of sugar and ethanol from cellulosic biomass still faces significant technical

challenges. Success depends mainly upon the physical and chemical properties of the biomass,

pretreatment methods, effective enzyme systems, and fermentation microorganisms. In this section,

the review focuses on hemp biomass pretreatment, enzymatic hydrolysis, and ethanol fermentation.

3.1. Biomass pretreatment

The physicochemical crosslinks among macro-polymers in hemp biomass are resistant to

enzymatic attack and microbial digestion. Pretreatment is an essential element to overcome this

barrier by cleaving chemical bonds (Fig. 2). Pretreatment has a function to disrupt and solubilize

the hemicellulose and lignin, making cellulose amenable to enzymes and strains. Sugar recoveries

and lignin removal are essential indicators for the selection of optimal pretreatment conditions [42,

66]. The effects of different pretreatment methods on chemical composition, sugar recoveries, and

delignification of hemp biomass were summarized in Table 3.

Steam explosion pretreatment: The steam explosion has been attracted considerable attention

for hemp biomass pretreatment without the addition of chemicals [33, 68, 72-74]. Barta et al. [25]

reported glucan (> 82%) and xylan (18-66%) recoveries as well as low lignin removal. The

pretreatment was conducted at high temperatures (200-230°C) and caused the solubilization of

most hemicellulose and partial cellulose. High decomposition of hemicellulose can result in

increased glucan content in pretreated biomass and further sugar degradation as inhibitor formation.

1   For example, Pakarinen et al. [39] observed that glucan content in biomass increased from 46.1 to

2   69.6% as hemicellulose decreased from 9.5 to 5.5%. Kreuger et al. [73] reported that 0.8 g

3   hydroxymethylfurfural (HMF) and 2.5 g furfural per 794 g dried hemp stem formed in the

4   hydrolysates. The low delignification was mainly due to derivatives such as furans and insoluble

5   products from the degradation of hemicellulose that could interact with the residual lignin

6   components to form a pseudo-lignin complex [75]. In order to moderate pretreatment temperature

7   and enhance sugar yield, SO_2 and H_2SO_4 were used to assist with the steam explosion [21, 35, 68].

8   Among them, Kuglarz et al. [21] investigated the steam explosion with the addition of H_2SO_4 (0.5-

9   2.0%) at 140-180 °C and observed that glucan (> 95%) and xylan (54-56%) were recovered.

10 Besides, Semhaoui et al. [35] observed that steam with H_2SO_4 pretreatment increased surface area

11 and crystallinity of hemp biomass. However, acids impregnation resulted in large amounts of

12 inhibitor (HMF and furfural) formation in hydrolysates [21, 35, 68], as shown in Table 3. Thus

13 multiple water washing is needed for detoxification after steam explosion pretreatment.

14   **Acid pretreatment**: Dilute acid pretreatment as an industrialized method has been extensively

15 used for enhancing sugar conversion efficiencies of hemp biomass. Among dilute acid pretreatment,

16 H_2SO_4 (0.5-3.0%) was commonly applied to pretreat hemp biomass at 150-180 °C for 10-20 min

17 [23, 24, 28]. During pretreatment, a high proportion of hemicellulose and partial cellulose was

18 solubilized into slurries, resulting in some sugar loss and inhibitor formation [28, 76-78], as shown

19 in Table 3. For instance, Kuglarz et al. [23] conducted the dilute acid pretreatment at 180 °C with

20 H_2SO_4 addition (1-1.5% (w/v)) and observed glucan (> 95%) and xylan (41-51%) recoveries with

1 35-41% of lignin removal. Gunnarsson et al. [24] concluded that H_2SO_4 (1% and 2%) pretreatment

2 at 180 °C resulted in glucan (87-95%) and xylan (11-22%) recoveries but caused HMF (0.15-0.35

3 g/L) and furfural (0.10-0.25 g/L) formation.

4 **Alkaline pretreatment**: Alkaline pretreatment could cleave ester and ether bonds between

5 lignin and cellulose as well as hemicellulose, thus increasing cellulosic accessibility to enzymes.

6 NaOH is mainly employed to pretreat hemp biomass [24, 32, 39, 40]. During NaOH pretreatment,

7 hydroxide ion (OH^-) can neutralize with released acids from the decomposition of cellulose and

8 hemicellulose, thus reducing sugar degradation caused by the catalysis of hydrogen ion (H^+) (Table

9 3). For example, Gunnarsson et al. [24] conducted alkaline (1 and 3%) pretreatment at 121 °C for

10 1 h and obtained glucan (> 96%) and xylan (> 55%) recoveries without inhibitor formation. In

11 other studies, Wawro et al. [32] reported that NaOH (2%) pretreatment at 90 °C for 5 h increased

12 cellulose content from 50.82 to 62.70% and decreased hemicellulose from 27.79 to 20.16% in solid.

13 Stevulova et al. [40] soaked hemp hurds into 1.6 mol/L NaOH solution for 48 h and observed the

14 depolymerization of cellulose. In addition, Gümüşkaya and Usta [71] concluded that alkali sulfite

15 pretreatment at 140-200 °C for 60-150 min increased the crystallite size of cellulose.

16 **Other pretreatments**: Oxidation reagents are generally utilized in pulp bleaching because

17 radicals released from oxidation reagents can significantly disrupt and remove lignin. Gunnarsson

18 et al. [24] reported that H_2O_2 (1 and 3%) coupled with NaOH (pH 11.5) pretreatment at 121 °C for

19 1 h achieved high glucan (> 95%) and xylan (> 60%) recoveries and lignin removal (> 50%). Also,

1 Gandolfi et al. [69] performed organosolv (45% methanol) pretreatment assisted with 3% H_2SO_4

2 at 165 °C for 20 min and achieved more than 75% of hemicellulose recovery and 75% of lignin

3 removal. Furthermore, electron beam irradiation pretreatment increased extractives in biomass due

4 to the chain scission and enhanced enzymatic hydrolysis [29, 36].

5 3.2. Enzymatic hydrolysis

6 Enzymatic hydrolysis and fermentation of cellulose and hemicellulose are critical steps for

7 converting lignocellulosic biomass into bioethanol. Sugar conversion efficiencies and final ethanol

8 concentration are summarized in Table 4. The factors affecting hemp biomass conversion efficiency

9 and ethanol yield are discussed below.

10 **Pretreatment condition**: Pretreatment has a significant impact on enzymatic hydrolysis and

11 fermentation through the re-distribution of chemical composition (cellulose, hemicellulose, and

12 lignin) in hemp biomass and also affects sugar recoveries and degree of recalcitrance. Pakarinen et

13 al. [39] reported that the steam explosion presented a higher carbohydrate conversion rate (78%)

14 than alkali pretreatment (60%). Kuglarz et al. [23] found that alkaline pretreatment had higher

15 sugar yield and ethanol productivity than acid pretreatment under designed pretreatment conditions.

16 Gunnarsson et al. [24] investigated various thermochemical pretreatments using H_2SO_4, NaOH,

17 and H_2O_2 at 121-180 °C and found that 3% H_2O_2 pretreatment achieved the highest overall sugar

18 yield of 73.5%. Zhao et al. [42] compared liquid hot water, H_2SO_4, and NaOH pretreatment at 170 °C

for 30 min and observed that NaOH-pretreated Helena showed the highest sugar (88.9%) and ethanol (96.7%) yields.

Solid loading: Low biomass loading (1.0-7.5%) is the advantage of increasing the accessibility of carbohydrates to enzymes, thus shortening enzymatic hydrolysis and fermentation duration and obtaining high sugar and ethanol yields (Table 4). However, low sugar (< 45 g/L) and ethanol (< 21 g/L) concentration from low biomass loading were unable to meet the minimal ethanol concentration requirement (above 40 g/L) for commercial ethanol distillation. Given 54% of glucan in hemp biomass and 80% of glucan-to-ethanol conversion efficiency, solid loading for ethanol fermentation should be at least higher than 16.3% to meet techno-economical distillation. In addition, increasing solid loading can generally decrease sugar and ethanol yields, thus promoting the pretreatment method based on low solid loading to achieve high sugar and ethanol yields could be controversial and unreasonable statistically.

High solid loading with enhanced fermentable sugars and less water consumption is preferred from cost-efficient and environmental standpoints. However, it still faces some fundamental challenges: 1) high viscous slurries restricts enzymatic accessibility and microbial growing; 2) increased hemicellulose content competes with cellulose for transport systems; 3) hydrophobic interaction between lignin and cellulase reduces enzymatic absorption. Currently, most studies for enzymatic hydrolysis and fermentation of hemp biomass are limited in low solid loading from 2.0 to 7.5% [21, 23-26, 31, 34-36, 68, 69].

Enzyme loading: Combined cellulase and hemicellulase were utilized for enzymatic hydrolysis of pretreated hemp biomass (Table 4). In general, cellulase from 10-30 FPU (filter paper unit)/g-solid and hemicellulase loading from 10-20 IU (international unit) or 140 FXU (fungal xylanase unit)/g-solid were used for enzymatic saccharification of hemp biomass (Table 4). Increasing enzyme loading (dose/solid) would enhance the enzymatic action area to carbohydrates, thus liberating more sugar-based nutritions to microbial fermentation. However, the enzymatic cost is an essential evaluation indicator for the commercial exploration of lignocellulosic bioethanol [79]. A dynamic balance between sugar conversion efficiency and enzyme cost was needed before commercialization.

3.3. Ethanol production

Comparing the potential of hemp biomass with kenaf, switchgrass, and sorghum biomass, hemp biomass with high glucan content would exhibit high theoretical ethanol yields. Sipos et al. [68] observed that dry hemp biomass showed higher ethanol yield (171 g/kg-biomass) and conversion rate (74%) as compared to ensiled hemp (163 g/kg-biomass and 71%) under same pretreatment conditions (impregnation with 2% SO_2 followed by steam pretreatment at 210 °C for 5 min). Zhao et al. [42] compared the potential of four hemp biomass varieties for bioethanol production and found that Tygra variety showed the highest ethanol yield (96.7%) under the same pretreatment and fermentation conditions. Traditional ethanol red yeast (*S. cerevisiae*) is mainly used to ferment C6 glucose into bioethanol [21, 68], but it is incapable of fermenting C6 and C5

monomeric sugars simultaneously. For example, Wang et al. [51] reported that when using C6 yeast

alone, xylose was not consumed and its final concentration reached 17 g/L. To utilize both hexose

and pentose, *Escherichia coli* [80, 81] and engineered yeast [51] have been investigated along with

the increased total ethanol yield and concentration. However, previous studies showed that xylose

competed with the same transport systems with glucose, thus resulting in low sugar conversion

efficiency [82, 83]. For example, Kilian and Van Uden [82] found that glucose competed with

xylose for transport by the low-affinity system and limited xylose transport by the high-affinity

system non-competitively. Meinander and Hahn-Hägerdal [83] reported that glucose, which is

transported with high affinity by the same transport system, restricted xylose conversion by 99%.

To end, the studies targeting engineered strains and *E. coli* on hemp biomass for bioethanol

production are still unavailable. For bioethanol recovery, it is initially recovered from the

fermentation slurries through fractional distillation at atmospheric pressure. Then, the resulting

liquor fraction can be purified by extractive and azeotropic distillation. Solid fraction, including

hemp residues, enzymes, and strains, can be further utilized for thermochemical conversions such

as pyrolysis and combustion. The same technology used for corn ethanol recovery can be used for

hemp ethanol recovery.

4. Technological perspectives

4.1. Optimization of pretreatment

Steam explosion and acid pretreatment showed higher hemicellulose decomposition and inhibitor formation but low lignin removal. In contrast, alkaline and organosolv pretreatment presented higher lignin removal and sugar recoveries but resulted in intensive water-consumption and reagent costs. In order to compensate individual disadvantages, combined pretreatments, such as acid-alkaline [85], acid-methanol [69], acid-ethanol [27], and NaOH-peroxide [24], have been proposed and showed a beneficial effect on further enhancing sugar conversion yields, but economic cost increased simultaneously. Emerging pretreatment technologies from the food industry, such as microwaves, ultrasound, electronic beam irradiation, and pulsed-electric field, have attracted considerable attention for biomass pretreatment [86]. However, their exploration of hemp biomass has not been performed. An optimal pretreatment condition should reduce the recalcitrance of hemp biomass to enzymes and maximize the sugar recoveries and utilization of byproduct. In addition, commercially feasible utilization of innovative and combined pretreatment still needs to couple with detailed process economics before leading to commercial realization and exploitation.

4.2. Simultaneous saccharification and fermentation improvement

Co-fermentation of hexose and pentose: Hemicellulose (xylan) roughly accounts for one-thirds of carbohydrates in hemp biomass (Table 2). However, conventional strain (*S. cerevisiae*) is incapable of digesting xylose, resulting in sugar waste. Substantial studies have been explored to ferment both monomeric glucose and xylose in hydrolysates through the recombination of bacteria

and yeast or engineered yeast [51, 87-89]. Among them, Wang et al. [51] combined traditional C6

yeast (S. *cerevisiae*) with engineered C5/C6 yeast (M11205) and efficiently fermented almost all

glucose and partial xylose. Besides, *E. coli* has been proposed to digest glucose and xylose into

ethanol simultaneously [80, 81]. However, to date, no investigation is conducted on hemp biomass

for bioethanol fermentation using recombined or engineered strains. Therefore, their exploitation

for the conversion of hemp biomass into bioethanol should be invested.

High solid loading: In current corn-based ethanol production, solid loading is up to 25-30%

[90], which is significantly higher than that (2.0-7.5%) from hemp biomass (Table 4) and other

biomass. Fermentation at high solid loading would be advantageous in converting biomass as it

promises high ethanol concentration while reducing water consumption. However, the decrease in

sugar and ethanol yields can offset the advantages of converting at high solids concentration [91].

Thus, the critical point (optimal solid loading) corresponding to the highest ethanol titer is needed

to explore. For an orbital shaker or conventional stirred-tank, high solid loading commonly causes

insufficient mixing due to the high viscosity of slurries. Based on this phenomenon, multi-feed and

fed-batch techniques have been employed to boost ethanol concentration [92-95]. Integration of

first (grain-crop) and second (lignocellulosic biomass) generation has been proposed to accelerate

cellulosic bioethanol's commercialization. Xu and Wang [96] investigated co-fermentation of corn

flour and hydrothermally pretreated corn stover at a ratio of 12:12 and achieved ethanol

concentration of 68.7 g/L and a total ethanol yield of 86.0%. Moreover, Xu et al. [97] obtained

130.2 g/L of ethanol titer by integrating corn flour and hydrolysate liquor from saccharified corn

stover for fermentation. Besides, to enhance enzymatic saccharification and microbial digestion, Tween-80 has been used to block the interaction between lignin and cellulase [98]. Lin et al. [99] reported that an amphiphilic surfactant derived from dehydroabietic acid could improve enzymatic hydrolysis of acid-pretreated biomass. It is believed that the above findings would provide a clue to fabricate, optimize, and integrate the hemp biomass-based bioethanol production pathway.

4.3. Life-cycle assessment and techno-economic analysis

González-García et al. [15] studied life-cycle assessment of hemp hurds for bioethanol production from non-wood pulp mills and concluded that ethanol-based fuels from hemp biomass could offer enhanced environmental performance and decrease reliability in fossil fuels. However, economic and environmental assessment of hemp biomass for bioethanol production in terms of initial cultivation, harvesting, pretreatment, and subsequent distillation has not been carried out. Also, industrial hemp as a versatile crop has various commercial applications. For instance, hemp seeds can be utilized for oil [100], protein [3], and chemical extraction [101]; hemp biomass can be used for biocomposites [18, 102, 103], pyrolysis [104, 105], and bioenergy production [38, 106]. Therefore, comprehensive assessments should be implemented to target comparative analysis of hemp seeds and biomass used in various fields in terms of environmental impacts and economic benefits.

5. Conclusions

Industrial hemp biomass is an excellent alternative candidate for bioethanol production due to its high cellulose content compared to other agricultural residues. Although the potential of hemp biomass for bioethanol production has been investigated intensively, the comprehensive study, including biomass production, optimization of pretreatment and fermentation conditions, and life-cycle-assessment, is very limited. Besides, the economic benefit to growers and related industries largely depends on the growth of planting acreage and government policies.

Declaration of Competing Interest

There are no conflicts to declare.

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Table 1.

Biomass yield of industrial hemp.

Location	Biomass (t/ha, dry matter)	Reference
Central Italy	3.4-8.0	[7]
Southwest Germany	5.2-12.8	[8]
Southern Sweden	7.8-14.5	[9]
Southern Sweden	9.9-14.4	[10]
Netherlands	13.5-15.3	[11]
Latvia	13.5-21.3	[12]
Central Italy	13.1-26.3	[13]
Northern Italy	28.6-31.2	[14]
Range	3.4-31.2	

Table 2.

Chemical composition of lignocellulosic biomass.

Sample	Composition (%, dry basis)			Reference
	Cellulose	Hemicellulose	Lignin	
Hemp biomass	42.0	15.7	13.2	[21]
	46.4	20.1	15.0	[23]
	42.3	18.2	22.9	[24]
	40.1	19.6	21.7	[25]
	36.5	17.0	21.9	[26]
	57.7	17.8	16.8	[27]
	37.0	21.3	13.8	[28]
	38.5	23.9	15.1	[29]
	63.0	14.2	14.6	[30]
	75.6	10.1	10.3	[31]
	50.8	27.8	14.7	[32]
	43.6	15.0	21.5	[33]
	40.1	16.0	14.8	[34]
	58.9	12.7	14.1	[35]
	46.8	15.2	8.0	[36]
	37.4	27.6	18.0	[37]
	63.0	17.0	9.0	[38]
	58.0	13.0	10.0	[38]
	46.1	13.9	18.0	[39]
	44.5	32.8	21.0	[40]
	46.1	18.3	17.7	[41]
	50.8	20.4	18.6	[41]
	51.1	22.1	21.4	[41]
	53.7	21.8	22.2	[41]
	40.1	12.5	14.6	[42]
	42.7	14.3	15.0	[42]
	40.7	13.3	15.7	[42]
	40.1	16.6	17.8	[42]
Range	36.5-75.6	10.1-32.8	8.0-22.9	
Corn fiber	14.0	39.0	5.7	[43]
	13.0	38.8	7.5	[44]
	16.4	45.3	1.3	[45]
	18.0	35.0	18.0	[46]
Range	13.0-18.0	35.0-45.3	1.3-18.0	

Corn stover	41.2	21.0	15.8	[47]
	34.4	22.8	18.0	[48]
	31.0	20.1	25.4	[49]
	31.7	17.1	12.6	[50]
	31.3	16.5	16.6	[51]
	36.1	21.4	17.2	[52]
	37.5	20.8	17.6	[53]
	32.6	27.8	12.3	[54]
	33.1	17.6	17.3	[55]
Range	31.0-41.2	16.5-22.8	12.6-25.4	
Sorghum bagasse	40.4	20.0	19.8	[56]
	41.1	25.9	21.4	[57]
	38.7	22.6	15.4	[58]
	37.8	21.2	16.7	[58]
	37.1	18.5	20.2	[59]
	35.5	20.0	24.5	[60]
	35.6	18.4	18.2	[61]
Range	35.5-41.1	18.4-25.9	15.4-24.5	

Table 3.

Compositional changes, sugar recoveries, and lignin removal of hemp biomass after pretreatment.

Pretreatment	Pretreatment conditions	Results	Reference
Steam	10% solid loading at 200-230 °C for 10 min	Glucan (>82%) and xylan (18-66%) recoveries, lower lignin removal	[25]
Steam	10% solid loading at 200 °C for 5 min	Glucan content increased from 46.1 to 69.6% and xylan content decreased from 9.5 to 5.5%	[39]
Steam with SO_2	2% solid loading at 205-215 °C for 5 min	Glucan (65-67%) and lignin (25-30%) contents in solid, HMF (0.08-0.31 g/L) and furfural (0.29-0.93 g/L)	[68]
Steam with H_2SO_4	10% solid loading at 140-180 °C for 10-20 min	Glucan (>95%) and xylan (54-56%) recoveries, furfural (0.10 g/L) and HMF (0.21-0.25 g/L)	[21]
Steam with H_2SO_4	Acid loading (62.9 g/kg) at 165 °C for 30 min	Surface areas and crystallinity increased, furfural (0.035 g/L) and HMF (0.46 g/L)	[35]
H_2SO_4	10% solid loading at 170 °C for 30 min	Glucan (52-69.0%) and xylan (2.1-2.7%) recoveries and lignin removal (2-31%)	[42]
H_2SO_4	10% solid loading at 180 °C for 10 min	Glucan (>95%) and xylan (41-51%) recoveries and lignin removal (35-41%)	[23]
H_2SO_4	10% solid loading at 180 °C for 10 min	Glucan (87-95%) and xylan (11-22%) recoveries, HMF (0.15-0.35 g/L) and furfural (0.10-0.25 g/L)	[24]
H_2SO_4	10% solid loading at 150-160 °C for 10-20 min	Decomposition of glucan and hemicellulose increased as pretreatment severity enhanced	[28]
Methanol with H_2SO_4	4% solid loading at 165 °C for 20 min	Hemicellulose (>75%) and lignin (> 75%) was removed	[69]

NaOH	10% solid loading at 121 °C for 1 h	Glucan (>96%) and xylan (>55%) recoveries, lignin removal (>60%), no HMF and furfural formation	[24]
NaOH	10% solid loading at 170 °C for 30 min	Glucan (77.5-90.2%) and xylan (24.5-29.7%) recoveries and lignin removal (58.6-75.3%)	[42]
NaOH	10% solid loading at 121 °C for 1 h	Glucan content increased from 46.1 to 83.6% and xylan content decreased from 9.5 to 8.4%	[39]
NaOH	Ground into 2mm and pretreated at 90 °C for 5 h	Cellulose content increased from 50.82 to 62.70%; hemicellulose decreased from 27.79 to 20.16%	[32]
NaOH	Dried hemp hurds was soaked in 1.6 M NaOH or 48 h	Cellulose polymerization and polydispersity index decreased, thermal stability increased	[40]
NaOH and KOH	10% solid loading at 120-140 °C for 1-2 h	Alkali ions were correlated with ash content, char, and lower molecular products	[70]
Na_2SO_3 with NaOH	20% solid loading at 140-200 °C for 60-150 min	The crystallite size of cellulose in alkali sulfite pulp samples increased	[71]
Electron beam irradiation	The sample was irradiated at 150, 300, 450 kGy	Cellulose, xylan, and lignin contents decreased with increasing electron irradiation dose	[29]
Electron beam irradiation	Ground hemp was irradiated at 150-450 kGy	Hot-water and 1% NaOH extraction rates increased, carbonyl groups increased indirectly	[36]

Table 4.

Enzymatic saccharification and fermentation of hemp biomass after pretreatment.

Pretreatment method	Saccharification or fermentation conditions	Results	Reference
Steam	5% solid loading, 25 FPU (NS50013)/g-glucan at 32 °C for 72h	Glucan yield (62-83%) and ethanol yield (38-70%)	[25]
Steam	2% solid loading, 10 FPU (Celluclast) and 500 nkat (Novozyme 188)/g-solid, at 50 °C for 48 h	78% of total carbohydrates conversion	[39]
Steam with SO_2	7.5% solid loading, 20 FPU (Celluclast) and 23 IU (Novozym 188)/g-glucan, at 37 °C for 72h	Glucose yield (373 g/kg) and ethanol titer (21.3 g/L)	[68]
Steam with H_2SO_4	5% solid loading, 30 FPU (Celluclast) and 20 IU (Novozyme 188)/g-glucan, at 37 °C for 48 h	Glucose yield (73–74%), ethanol yield (75–79%) and titer (2.89-10.0 g/L)	[21]
Steam with H_2SO_4	1% solid loading, Celluclast-1.5 L (480 FPU/L), at 50 °C for 24 h	Glucose (2.25-5.90 g/L) and xylose (0.02-1.14 g/L)	[35]
H_2SO_4	7.5% solid loading, 20 FPU (Celluclast) and 15 IU (Novozyme 188)/g glucan, at 50 °C for 48 h	Glucose (35.0-39.1 g/L) and xylose (3.73-3.80 g/L), glucan (68.9-72.2%) and xylan (44.3-50.1%) yields	[23]
H_2SO_4	5% solid loading, 20 FPU (Celluclast) and 15 IU (Novozyme 188)/g glucan, at 50 °C for 48 h	Glucose (23.5-26.6 g/L) and xylose (0.74-1.77 g/L), glucan (69.8-73.9%) and xylan (35.9-47.3%) yields	[24]
H_2SO_4	5% solid loading, 30 FPU (Cellic® CTec3) and 140 FXU (NS22244)/g-solid, at 37 °C for 72 h	Ethanol concentration (11.9-13.8 g/L) and yield (67.2-89.6%)	[42]

Methanol with H_2SO_4	5% solid loading, 20 FPU (Cellic CTec2)/g-solid and at 50 °C/150 rpm for 72 h	60% of cellulose-to-glucose conversion	[69]
NaOH	5% solid loading, 20 FPU (Celluclast) and 15 IU (Novozyme 188)/g glucan, at 50 °C for 48 h	Glucose (25.5-27.2 g/L) and xylose (6.68-8.14 g/L), glucan (78.0-80.1%) and xylan (67.3-85.9%) yields	[24]
NaOH	10 mg enzyme (CTec2 and HTec2) protein/g-biomass, at 50 °C for 72 h	Theoretical ethanol yields (68.2 gallons/dry ton biomass)	[26]
NaOH	5% solid loading, 30 FPU (Cellic® CTec3) and 140 FXU (NS22244)/g-solid, at 37 °C for 72 h	Ethanol concentration (18.2-20.3 g/L) and yield (95.8-96.7%)	[42]
H_2O_2 with NaOH	5% solid loading, 20 FPU (Celluclast) and 15 IU (Novozyme 188)/g glucan, at 50 °C for 48 h	Glucose (25.5-31.3 g/L) and xylose (4.70-5.85 g/L), glucan (83.4-90.0%) and xylan (46.0-59.2%) yields	[24]
Glycerol with NaOH	5% solid loading, 15 FPU (Cellic CTec2)/g glucan, at 50 °C for 48 h	Glucose (84.1-91.9%) and xylose (79.6-91.8 %) yields	[34]
Ionic liquid (Microwave)	15 mL of 16 mg/ml glucose produced from the hydrolysis, at 30 °C for 60 h	75.6% bioethanol yield	[31]
Electron beam irradiation	20 FPU (Celluclast)/g-biomass and Novozym 342 (1/4 of Celluclast addition), at 50 °C for 72 h	Glucan and xylan yields increased by 3.4-6.2% and 7.8-18.4%, respectively	[36]

Figure Captions

Fig. 1. Cross-section of industrial hemp stem (modified from [17]).

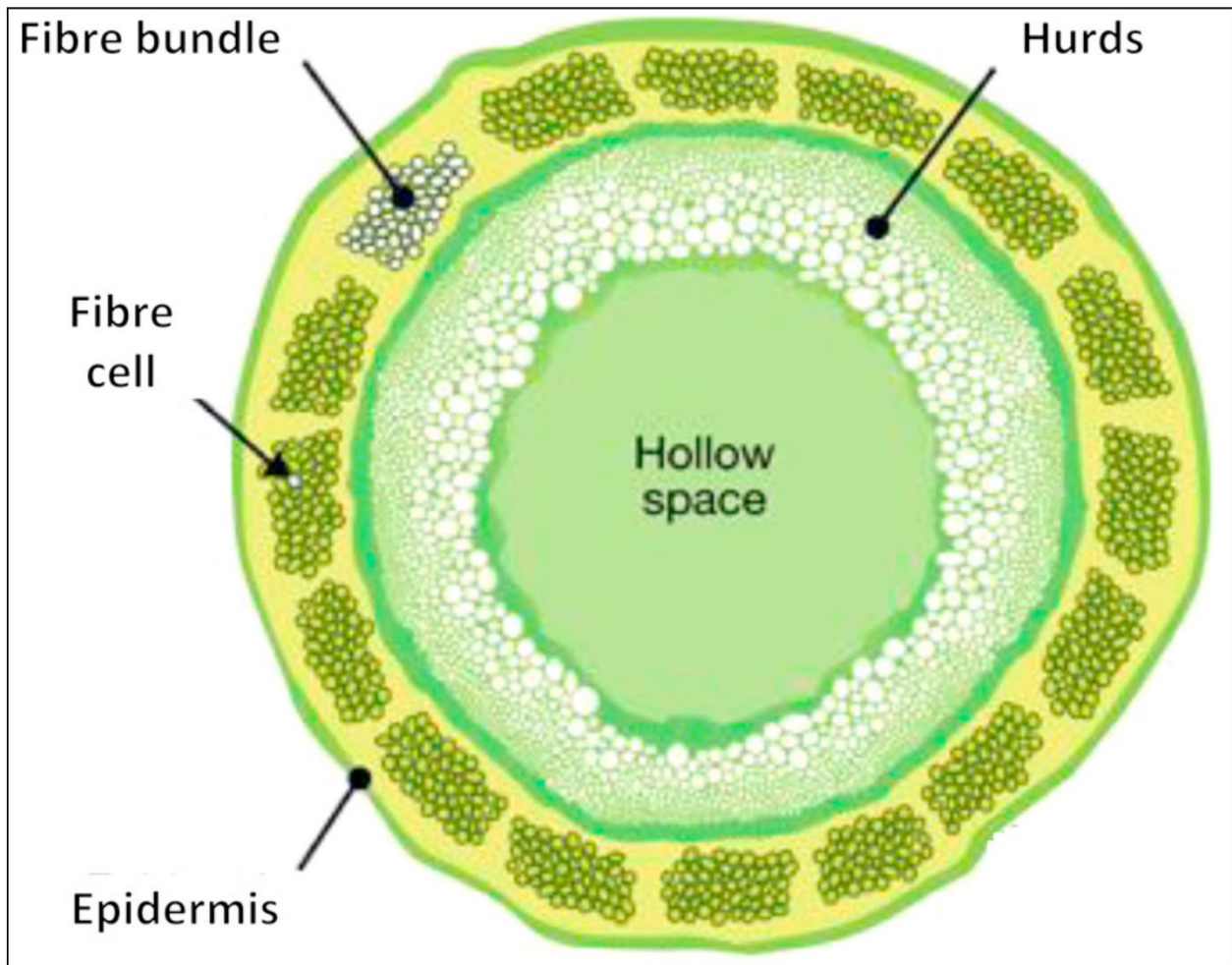**Fig. 2**. The role of pretreatment on hemp biomass (adapted from [67]).

Fig. 1.

Fig. 2.