

Review on Pretreatment Methods and Ethanol Production from Cellulosic Water Hyacinth

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Lignocellulosic biomass resources are renewable materials that can be converted to fermentable sugars and subsequently into ethanol. Water hyacinth (*Eichhornia crassipes*) is a cellulosic aquatic plant that has high carbohydrates, low lignin content, and notable reducing sugars content in its structure. Based on the literature review in the case of water hyacinth, the most frequently used pretreatment methods were acid and alkali, while ionic liquid and microwave-assisted methods were used rarely. The dominant sugars were glucose, xylose, galactose, arabinose, and mannose. Based on the findings, *cellulase* and *S. cerevisiae* were mostly used for enzymatic hydrolysis and fermentation of water hyacinth to ethanol, respectively. This review presents the recent studies in pretreatment, hydrolysis, and fermentation of water hyacinth biomass into ethanol.

Keywords: Bioethanol; Cellulosic biomass; Lignocellulosic materials; Pretreatment; Water hyacinth

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INTRODUCTION

Concern about the greenhouse effect is an important reason for interest in renewable energy sources. Ethanol due to its potential as an alternative automotive fuel has attracted worldwide attention (Ganguly *et al.* 2012). Economic and environmental concerns about the depletion of fossil fuels have driven many countries to become interested in the use of biofuels as a source of renewable and cheap energy, replacing fossil fuels (Rezania *et al.* 2015a). Non-food lignocellulose-rich materials such as plant biomass are counted as a source of renewable energy (Saini *et al.* 2015). As stated by Nouredini and Byun (2010), agricultural residues, forest residues, wood, grass, waste paper, and municipal wastes are the biggest potential feedstock (lignocellulosic biomass) for ethanol production. Due to the abundance of lignocellulosic biomass, it can be considered as a suitable material for bioconversion to ethanol (Zabed *et al.* 2016). Many lignocellulosic biomasses, such as rice straw, sugarcane bagasse, wheat straw, cotton stalk, bamboo, and sugarcane tops are abundantly available as agro-residues (Sindhu *et al.* 2016).

On the other hand, the reduction of CO₂ in the atmosphere mitigates climate change, which depends on the usage of bioethanol instead of fossil fuels (Hosseini and Wahid

2013). The main advantages of producing second-generation rather than first-generation biofuels are the utilization of non-edible parts of biomass, which provides a better land use efficiency, higher contribution to the mitigation of CO₂, and higher ethanol yield (Jambo *et al.* 2016). The main disadvantage of using lignocellulose rather than starch is its higher ethanol production cost. This is due to the two non-ecofriendly and high-energy steps, namely pretreatment and enzymatic hydrolysis (Phitsuwan *et al.* 2013).

As Brown and Brown (2013) estimated, up to 25 million gallons of ethanol can be produced from cellulosic biomass-to-ethanol conversion technology annually. As reported by Naik *et al.* (2010), by 2022 the USA needs to provide 36 billion gallons of ethanol for its consumption, while they estimated that 21 billion can be obtained from cellulosic feedstock and 15 billion gallons from corn kernels. Researchers are trying to find suitable methods with lower cost to produce ethanol by optimization using different feedstocks, pretreatment technologies, and enzymatic hydrolysis/fermentation processes (Schell *et al.* 2016). As mentioned by many studies, pretreatment, enzymatic hydrolysis, and microbial fermentation are the three main steps for conversion of lignocellulosic materials to bioethanol (Uday *et al.* 2016).

COMPOSITION OF LIGNOCELLULOSIC BIOMASS

Cellulose, hemicellulose, and lignin are three main constituents of lignocellulosic biomass. Cellulose and hemicellulose are composed of a mixture of carbohydrate polymers (Kumar and Murthy 2011). Lignocellulosic biomass, which has cellulose (30 to 35%), hemicellulose (20 to 30%), and lignin (10 to 20%), is an alternative feedstock for bioethanol production (Achinah and Euverink 2016). As reported by Barakat *et al.* (2013), 80% of the total weight of lignocellulosic residues comes from carbohydrates and lignin, with some variation due to some factors such as type of species, growth conditions, tissue, and cell wall maturity of plants.

In lignocellulosic biomass, a matrix of cellulose and lignin is surrounded by hemicellulose chains (Klein *et al.* 2016). Cellulose is a homopolymer of glucose, while hemicellulose is built from a variety of five- and six-carbon monomers. Lignin is a complex amorphous polymer with high molecular weight that is tightly bound to carbohydrates. Moreover, it plays the role of cement for the cross-linking between cellulose and hemicellulose to form a rigid three-dimensional structure of the cell wall (Sarkar *et al.* 2012). In untreated biomass, cellulose, hemicelluloses, and lignin are linked to form a strong structure that is difficult to process for ethanol production (Harun *et al.* 2011). Difficult degradation of lignin is due to its rigid structure, which is one of the limitations of using lignocellulosic-biomass materials in fermentation (Taherzadeh and Karimi 2008). Lignin removal is a difficult process that requires some chemicals and enzymes. The choice of a suitable feedstock with lesser lignin can improve the economic potential of biofuel production compared with lignin-rich biomass (Bhatt and Shilpa 2014). Table 1 shows the composition of different lignocellulosic biomass.

Based on Table 1, different types of lignocellulosic biomass have varying amounts of cellulose (10% to 51%), hemicellulose (17% to 43%), and lignin (17% to 40%). This amount of lignin is assumed as high for production of ethanol. Different biomass substrates consist of differently arranged lignin structures, which significantly influences the chemical and physical processing of biomass during pretreatment for cellulose exposure (Ke *et al.* 2013). Moreover, by increasing the availability of the cellulose, carbohydrates,

and biomass digestibility by different pretreatments, bioconversion into bio-products can be more feasible (Ghaffar *et al.* 2015).

Table 1. Composition of Different Lignocellulosic Biomass (Untreated Form)

Lignocellulosic biomass	Cellulose	Hemicellulose	Lignin	Reference
Sugarcane top	29.85	18.85	25.69	(Sindhu <i>et al.</i> 2011)
Cornstalk	34.45	27.55	21.81	(Ma <i>et al.</i> 2011)
Bagasse	30	35	18	(Sarkar <i>et al.</i> 2012)
Sugarcane bagasse	44	27	24	(de Souza <i>et al.</i> 2013)
Sweet sorghum bagasse	36.9	17.8	19.5	(Umagiliyage <i>et al.</i> 2015)
Wheat straw	38.7	19	17.3	(Valdez-Vazquez <i>et al.</i> 2015)
Rice straw	35.8	21.5	24.4	(Imman <i>et al.</i> 2015)
Rapeseed	51.3	17.3	44	(Pei <i>et al.</i> 2016)
Corn stover	36.3	31.4	17.2	(Saha <i>et al.</i> 2016)

CELL WALL COMPOSITION OF WATER HYACINTH (*Eichhornia crassipes*)

Water hyacinth (*Eichhornia crassipes*) is a free-floating aquatic plant that originates from Brazil and Ecuador. It belongs to the Pontederiaceae family and is related to the lily family Liliaceae. It reproduces both asexually, through stolons, and sexually through seeds, which is difficult to control and the seed can remain dormant for up to 20 years (Rezania *et al.* 2015b). Water hyacinth (WH) biomass has monosaccharide and polysaccharide structures that contain different types of sugars and starch. The polymeric carbohydrates in WH are primarily cellulose and hemicellulose. Interestingly, not much data on bioethanol production from aquatic plants is available, except for WH (Rezania *et al.* 2015a). As reported by Lara-Serrano *et al.* (2016), in WH, the lowest cellulose content is found in the stem, while the highest is in the roots. High contents of cellulose and hemicellulose with low lignin, impressive growth rate and no competition on land use has led WH to be regarded as a suitable lignocellulosic material for bioenergy generation (Rezania *et al.* 2016; Feng *et al.* 2017).

The evaluation of WH composition is important due to the variation of carbohydrate and lignin content in different studies. As reported by Kumar *et al.* (2009), WH has a high percentage of cellulose and hemicellulose (44% to 66.9% of dry weight basis), and a low lignin content, from 3.5% to 9.5%, which is sufficient to extract fermentable sugars with various pretreatments. However, the presence of lignin can make it resistant to degradation due to the compact structure between cellulose and hemicellulose. Pretreatment should be performed to improve the digestibility of WH, as the hydrolysis process is difficult and expensive (Gao *et al.* 2013b). Many studies have reported on the composition of WH during the ethanol production process, as shown in Table 2.

Table 2. Composition of Water Hyacinth (Carbohydrates and Lignin)

Component	Ahn <i>et al.</i> 2012	Xia <i>et al.</i> 2013	Ganguly <i>et al.</i> 2013	Singh and Bishnoi 2013	Cheng <i>et al.</i> 2014	Yan <i>et al.</i> 2015	Lin <i>et al.</i> 2015	Zhang <i>et al.</i> 2015	Das <i>et al.</i> 2016a	Das <i>et al.</i> 2016b	Ruan <i>et al.</i> 2016
Cellulose	34.19	23.31	35	19.2	24.15	31.81	28.9	18.07	24.7	31.44	24.5
Hemicellulose	17.66	22.11	33	40.0	27.23	25.64	30.8	28.21	32.2	44.68	34.1
lignin	12.22	12.58	15.5	4.8	12.39	3.55	4.6	7.03	3.2	19.99	8.6

*The component values are expressed in g per 100 g dry matter.

Different studies obtained different amounts of carbohydrates and lignin from WH biomass. Biotic and abiotic factors such as differences in species, growth state, and time of harvesting have also influenced carbohydrate and lignin content. As Table 2 shows, the cellulose content ranged from 18% to 35%, hemicellulose content ranged from 17% to 45%, and lignin content from 3% to 20%. Although the lignin content of WH shows some variation, the average is lower than other lignocellulosic materials, 17% to 40%, which makes it more suitable for ethanol production.

PRETREATMENT METHODS

For the separation of carbohydrates and lignin, a pretreatment step is necessary; however, due to the high cost and difficulty, the biochemical conversion of lignocellulosic biomass to ethanol is still limited. After the separation, the carbohydrate portion can be fermented into alcohols (Anca-Couce 2016). Selection of suitable pretreatment method can enhance the digestibility and reduce the limitations of enzymatic hydrolysis in a feasible and economical way (Sun *et al.* 2016).

Pretreatment processes can also have a significant impact on configuration, efficiency, and cost of downstream operations (Zheng *et al.* 2014; Shirkevand *et al.* 2016). In addition, a fundamental understanding of various pretreatment technologies can help to match the best pretreatment method/combination for a specific biomass feedstock (Mood *et al.* 2013). According to Arenas-Cárdenas *et al.* (2016), biomass characteristics, biomass availability, financial resources, and low negative environmental impacts can be considered to select the best pretreatment method.

Recently, Sindhu *et al.* (2016) showed that the advantages and effectiveness of combined pretreatments are greater than the chemical pretreatments methods. This is due to the improvement of enzymatic hydrolysis and biofuel production of combined pretreatments when compared with a single pretreatment process. Alkali pretreatment can be used in combination with acid, as it is a proper method for delignification (Mood *et al.* 2013). Mishima *et al.* (2008), used 20 chemical pretreatments to improve the efficiency of enzymatic hydrolysis of WH. The results indicated that the most effective method for improving the enzymatic hydrolysis is alkaline/oxidative pretreatment. The pretreatment process also can increase enzyme accessibility to biomass and yields of fermentable sugars (Zheng *et al.* 2014). Yield of fermentable sugars can reach to 90% with some pretreatment methods which is less than 20% without any pretreatment (Alizadeh *et al.* 2005).

Pretreatment of lignocellulosic biomass in a cost-effective way is a major challenge for bioethanol production (Singh *et al.* 2015). Different cost-effective pretreatment methods have been identified based on the types of lignocellulosic biomass and

productivity (Srivastava *et al.* 2015). As demonstrated by Shafiei *et al.* (2015), in acid pretreatment, many types of acids such as sulfuric, nitric, or hydrochloric acids can be used. In this method the major parameters are particle size, retention time, acid concentration, liquid to solid ratio and temperature. However, the solubilization of hemicellulose and cellulose in alkali method is lesser than acid pretreatment (Bhatt and Shilpa 2014).

In the case of WH, pretreatment is normally carried out using acid/alkali treatment. Enzymatic hydrolysis yields of glucose and total reducing sugars, as well as fermentation yields of ethanol are considered as measures of the effectiveness of these pretreatment methods (Guragain *et al.* 2011). In a study by Sukumaran *et al.* (2009), the reducing sugar concentration was two times higher than that found using acid pretreatment for WH biomass using alkali pretreatment,.

Table 3. Advantages and Disadvantages of Selected Pretreatment Methods

Type of pretreatment	Advantage	Disadvantage
Acid (H ₂ SO ₄)	Removal of Lignin and hemicellulose, High hemicellulose solubility, Widely usage of dilute acid pretreatment due to its effectiveness, high sugar recovery efficiency (> 90%) for both xylose and glucose, cellulose accessibility for enzymatic saccharification	Concentrated-acid process is corrosive and dangerous, Specialized non-metallic constructions is needed, Formation of inhibitors at low pH, Losses of sugar content, Neutralization and salt disposal
Alkali (NaOH)	Major removal of lignin and a part of hemicellulose, Decrease in polymerization degree and crystallinity	low digestibility in softwoods, Large amount of water is needed for washing, Long pretreatment resident time, High chemical recovery cost
Ionic liquid (IL)	Less crystallinity of regenerated cellulose and accessible external and internal surfaces of cellulose, Lignin recovery and reuse after removal, Disruption of lignin and hemicellulose network	High cost of chemicals, Recovery of solubilized cellulose/hemicellulose Toxicity of some ionic liquids Sugar separation from ILs and recycling
Combined methods (microwave-assisted)	Improved enzymatic hydrolysis, Effective removal of lignin and hemicellulose Maximum utilization of lignocellulosic components	High energy demands, Special equipment is needed, Production of toxic waste which can limit further downstream processing, Inability to remove hemicelluloses and lignin

Adopted from (Sarkar *et al.* 2012; Brandth *et al.* 2013; Mood *et al.* 2013; Baeyens *et al.* 2015; Elgharbawy *et al.* 2016; Singh *et al.* 2016; Sun *et al.* 2016)

Due to the tough structure of lignin, more severe pretreatment conditions are required to dissolve these lignocellulosic materials in Ionic Liquids (ILs) (Sun *et al.* 2016). Moreover, the increased rate of cellulose hydrolysis *via cellulase* in ILs leads to more production of fermentable sugars that can be converted into fuels (Menon and Rao 2012). In addition, a higher fermentable sugar yield was obtained by aqueous ILs pretreatment than pure IL pretreatment under the same conditions (Fu and Mazza 2011). A study by Cheng *et al.* (2015) demonstrated that simultaneous processes of pretreatment and wet

storage conserved 70% carbohydrates and removed 40% lignin from WH. As found by Xu *et al.* (2016), initially, 38.9% to 63.6% of lignin was removed from pretreated WH with surfactant-free ILs, meanwhile cellulose was well protected and retained. Gao *et al.* (2013a) found that 27.9% and 49.2% of lignin was removed after pretreatment of WH by 1-butyl-3-methylimidazolium chloride ([Bmim]Cl)/DMSO.

Microwave-assisted pretreatments have also been used to improve the enzymatic hydrolysis of lignocellulosic materials (Klein *et al.* 2016). As demonstrated by Moretti *et al.* (2014), microwave-assisted chemical pretreatments are more effective than conventional heating chemical pretreatments. Generally, pretreatment methods are classified in four different categories including physical, chemical, physicochemical, and biological. Chemical methods are used widely due to higher yield efficiency, although these methods are harmful for the environment. Table 3 shows a few pretreatment processes with a yield of fermentable sugars that is suitable for ethanol production from any type of lignocellulosic biomass.

SUGAR PRODUCTION FROM WATER HYACINTH

Many studies have employed lignocellulosic biomass as a feedstock for fermentable sugar production, which is the key element in sustaining bio-products such as bioethanol. A popular way of sustaining bioethanol is using fermentable sugars for producing bio-productions taken from starch crops. The result has been able to help satisfy the huge demand for a cheap and sustainable source of feedstock for fermentable sugar production (Mood *et al.* 2013). Cheng *et al.* (2015) shows that the presence of carbohydrates in biomass after wet storage and pretreatment affects the process of enzymatic saccharification and has resulted in decreases in the sugar yield. The availability of different sugars is related to using various types of enzymes for the degradation of lignocellulosic structure (Uday *et al.* 2016).

Enzymatic hydrolysis is necessary to make carbohydrates accessible for ethanol production. During enzymatic hydrolysis, WH produces more pentose sugars rather than hexose sugars. As reported by Aswathy *et al.* (2010), glucose and xylose are the major fermentable sugars in WH hydrolysate. They also found that the maximum reducing sugar yield obtained in hydrolysis of acid pretreated WH was only 136 mg/g and 639.42 mg/g in alkali pretreated WH. After acid pretreatment of WH by H₂SO₄ (2% (v/v) at 110 °C for 90 min, the maximum yield of fermentable sugars was 0.54 g/g WH (Fileto-Pérez *et al.* 2013).

In a study by Xia *et al.* (2013), microwave-acid pretreatment improved enzymatic saccharification of WH and 483 mg/g WH reducing sugars with 94.6% sugar yield was obtained. Xu *et al.* (2016), obtained the reducing sugar yield of WH pretreated with IL microemulsions at 70 °C for 6 h at 563.7 mg/g, followed by a hydrolysis yield of 86.1%. Hence, at optimal hydrolysis conditions (*T*: 190 °C, time = 10 min and *cellulase* dosage = 5 wt%), microwave pretreated WH produced 0.296 g/g total volatile solids reducing sugar yield (Lin *et al.* 2015). According to Mishima *et al.* (2008), the major WH sugar contents was found in the roots, except for arabinose, which was in the leaves. As found by Manivannan and Narendhirakannan (2015), the amounts of glucose, xylose, and total reducing sugars range from 0.07 to 0.41 g/g WH. Table 4 shows the characterization of different types of sugars in WH biomass.

Table 4. Characterization of Different Types of Sugars in WH

Reference	Glucose	Xylose	Galactose	Arabinose	Mannose	Cellobiose	Lactose
(Mishima <i>et al.</i> 2008)	√	√	√	√	√		
(Mukhopadhyay, and Chatterjee 2010)	√	√	√	√	√		
(Ahn <i>et al.</i> 2012)	√	√	√	√	√		
(Xia <i>et al.</i> 2013)	√	√	√	√		√	
(Cheng <i>et al.</i> 2014)	√	√	√	√	√	√	√
(Das <i>et al.</i> 2014)	√	√		√			
(Lin <i>et al.</i> 2015)	√	√	√		√		√

FERMENTATION AND ETHANOL PRODUCTION

In this section, various types of microorganisms and enzymes that frequently contribute to ethanol production from WH are discussed. In addition to the multitude of pretreatment methods, there are two types of enzymatic hydrolysis and fermentation methods, including Separate Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF). The suitable fermentation method was based on the characteristics of the fermenting microorganism. Some parameters, such as type of biomass, type of pretreatment, inoculum size, moisture content, and pH are the main parameters that affect the SSF process (Mansour *et al.* 2016). The fermentation organism must have the potential to ferment with available saccharides present in hydrolysates, while being able to withstand inhibitors (Ganguly *et al.* 2012). For instance, Jayakody *et al.* (2016) found a novel inhibitor-tolerant *S. cerevisiae* strain that was able to overcome the barriers to industrialization of cellulosic ethanol production.

Cellulase is composed of endoglucanases, exoglucanases, and β -glucosidases, which have the potential for enzymatic hydrolysis of cellulose. Cellulases are mostly produced by fungi, for instance *Trichoderma reesei*, *Aspergillus*, *Schizophyllum*, and *Penicillium* (Baeyens *et al.* 2015). In ethanol production, some native or wild-type microorganisms, used in fermentation, include *Saccharomyces cerevisiae*, *Escherichia coli*, *Zymomonas mobilis*, *Pachysolen tannophilus*, *C. shehatae*, *Pichia stipitis*, *Candida brassicae*, and *Mucor indicus*. Meanwhile, *S. cerevisiae* and *Z. mobilis* are the best known yeast and bacteria, respectively (Talebniya *et al.* 2010). Baeyens *et al.* (2015) showed that *Candida shehatae* and *Pichia stipitis* yeasts have a good potential at low pH levels, while they have a low tolerance for various inhibitors, including the ethanol product.

The most common and traditional microorganism used in industrial bioethanol production is the yeast *S. cerevisiae*. Considerable efforts have been dedicated to engineering this microorganism to metabolize xylose (Dionisi *et al.* 2015). *S. cerevisiae* can ferment only hexoses, which probably accounts for the low ethanol production. However, it cannot apply for pentose sugars, which may constitute up to 40% of WH. To overcome this problem, recombinant DNA technology (genetic engineering) is recommended by Srivastava *et al.* (2015),

The advantages of *Zymomonas mobilis* over *S. cerevisiae* with respect to producing bio-ethanol are (1) higher sugar uptake and ethanol yield; (2) lower biomass production; (3) higher ethanol tolerance; and (4) amenability to genetic manipulations. Schell *et al.* (2016) showed that *Z. mobilis* has better performance in SHF because the microorganism does not perform well at the low glucose concentrations typically seen during SSF. The ability of ethanol production from WH has been investigated and reviewed in some studies (Ganguly *et al.* 2012; Rezania *et al.* 2015a). Table 5 shows recent studies regarding ethanol production from WH biomass.

Table 5. Recent Studies of Ethanol Production from WH

Reference	Pretreatment method	Fermentation mode	Microorganism and enzyme	Reducing sugar / finding	Ethanol yield
(Guragain <i>et al.</i> 2011)	1% (v/v) (H ₂ SO ₄)	SHF in flask	<i>S. cerevisiae</i> and cellulase from <i>Trichoderma reesei</i>	Glucose and total sugars yield of acid pretreatment were 445 and 714 mg/g of WH.	Ethanol concentration was 0.45 mg/mg glucose.
	Using EMIMDP and BMIMA in IL pretreatment			Glucose and total sugars yield of acid pretreatment were 332 and 584 mg/g of WH.	Ethanol concentration was 0.40 mg/mg glucose
(Ahn <i>et al.</i> 2012)	Alkaline-oxidative (A/O) pretreatment	Batch and continuous	<i>S. cerevisiae</i> (KCTC 7928)	Final glucose concentration was 16.42 (g/L).	Ethanol productivity of continuous fermentation was 0.77 (g/l h), which was 1.57 times higher than that of batch.
(Singh and Bishnoi 2013)	2.75% NaOH and 1-hour pretreatment time	Solid state fermentation in bioreactor	<i>A. niger</i> used for saccharification and <i>S. cerevisiae</i> , and <i>P. stipitis</i> used for fermentation	Sugar consumption were 51, 65 and 82% by <i>S. cerevisiae</i> , <i>S. stipitis</i> and co-culture of both respectively.	Ethanol produced from <i>S. cerevisiae</i> , <i>S. stipitis</i> and by co-culture of both, with 4.3, 6.2 and 9.8 g/L, respectively.
(Das <i>et al.</i> 2014)	Three different pretreatments: wet oxidation, phosphoric acid (H ₃ PO ₄)-acetone, and ammonia fiber explosion (AFEX)	SSF in flask	Using <i>S. cerevisiae</i> and <i>Candida shehatae</i>	TRS was for wet oxidation equal to 1.1 g/L and a yield of 0.107 (g / g WH), for phosphoric acid equal to 1.30 g/L and a	Highest ethanol titer of 1.52 g/L by AFEX as compared with wet oxidation (1.23 g/L) and

				yield of 0.168 (g/g) and for AFEX pretreated WH had 1.4 g/L and a yield of 0.187 (g/g)	phosphoric acid-acetone pretreatments (1.31 g/L).
(Cheng <i>et al.</i> 2014)	Microwave with 1% dilute H ₂ SO ₄	SSF in beaker	<i>P. Stipitis</i> and <i>Pachysolen tannophilus</i> and hydrolysis by <i>Trichoderma reesei cellulase</i>	Highest TRS was 482.8 g/g WH	Highest ethanol yields 22 g/g (raw biomass of WH) with 76.3% of the theoretical ethanol yield. Maximum production rate was 0.19 (g/ L/h).
(Manivannan and Narendhirakannan 2015)	Varying concentrations of H ₂ SO ₄ (0.1, 0.5, 1, 1.5 or 2 %) at a ratio of 1:8	SHF	<i>C. intermedia</i> , <i>P. stipitis</i> , <i>P. tannophilus</i> and <i>S. cerevisiae</i>	1.96-3.79 g/L was yield of glucose and xylose, 3.79-5.27 g/L of total reducing sugars.	Ethanol production by: <i>P. tannophilus</i> (0.043), <i>P. stipitis</i> . (0.037), <i>C. intermedia</i> (0.021), <i>S. cerevisiae</i> (0.015 g/g)
(Yan <i>et al.</i> 2015)	Pretreatment by 1.5% (v/v) H ₂ O ₂ and 3% (w/v) NaOH	SHF and SSF in flask	Enzymatic hydrolysis by <i>cellulase</i> using newly isolated <i>Kluyveromyces marxianu K213</i> and control <i>S. cerevisiae</i>	Reducing sugars were (223.53 mg/g dry) compared to 48.67 mg/g dry in the untreated sample.	Maximum ethanol (7.34 g/L) obtained in SHF using <i>K. marxianu K213</i> that was 1.78-fold greater than angel yeast <i>S. cerevisiae</i> (4.94 g/L).
(Zhang <i>et al.</i> 2015)	(1% H ₂ SO ₄ at 100 °C for 30min (0.5% NaOH at 40 °C for 30min and microwave-alkaline (150 W microwave combined with 0.5% NaOH for 0.5 min)	SSF	Using <i>cellulase</i> and <i>S. cerevisiae</i>	In optimized condition, 402.93mg/g and (197.60mg/g in hydrolysates, and 205.33 mg by residue hydrolysis) reducing sugar was produced.	The optimized condition was at 38.87 °C in 81.87h when inoculated with 6.11mL yeast and 1.291g/L bioethanol was produced.

(Das <i>et al.</i> 2015)	Sodium hydroxide with a biomass loading of 10% (w/v), 5% (w/v) concentration of NaOH, soaked for 1 hour and treatment time of 10 minutes at 130 °C.	SHF in flask	Cellulase from <i>Trichoderma reesei</i> and xylanase from <i>Trametes versicolor</i> for saccharification and <i>Pichia stipitis</i> , <i>Candida shehatae</i> and <i>S. cerevisiae</i> for fermentation	Maximum TRS (0.5672 g/g) was obtained using 9.92 (% w/w) substrate concentrations.	Maximum ethanol was 10.44 g/L using <i>Pichia stipitis</i> , followed by 8.24 and 6.76 g/L for <i>C. shehatae</i> and <i>S. cerevisiae</i> .
(Das <i>et al.</i> 2016a)	(10 %, w/v) WH with dilute H ₂ SO ₄ (2 %, v/v)	SHF	Mixture of <i>S. cerevisiae</i> (MTCC 173) and <i>Z. mobilis</i> (MTCC 2428)	The maximum sugar yield was (425.6 mg/g)	Ethanol production was 13.6 mg/mL
(Das <i>et al.</i> 2016b)	Microwave-assisted alkali and organosolv	SSF in flask and bioreactor	GH5 isolated from <i>C. thermocellum</i> + recombinant hemicellulase GH43 + <i>S. cerevisiae</i> + <i>C. shehatae</i>	In optimized condition TRS yield was 12.35 ± 0.07 g/L and 16.12 ± 0.09 g L ⁻¹ , in flask and bioreactor, respectively.	Optimized shake flask and bioreactor SSF yielded ethanol titer of 9.78 and 13.7 g/L, respectively.

As shown in Table 5, although most studies used combined pretreatment methods, there has been no published study regarding ethanol production from IL pretreated WH. For IL pretreatment, researchers mostly have focused on the evaluation of reducing sugars and lignin removal. The reason might be due to the reaction of water molecules with IL solvents during fermentation, which is related to the higher moisture content of WH (25%) in comparison to wheat straw (10%) as reported by Li *et al.* (2016) and rice straw (10%) as reported by de Assis Castro *et al.* (2016). Similarly, microwave-assisted pretreatments were not very favorable for pretreatment of WH. The reason could be the negative effect of irradiation on the WH structure. For instance, irradiation can disrupt the cell wall structure that may reduce the amount of reducing sugars in WH biomass.

CONCLUSIONS

This review shows that WH is a competent cellulosic biomass. Because it has a high carbohydrate and low lignin content in comparison to other biomass types, it can be regarded as highly suitable for the production of ethanol as a second generation biofuel. Based on the literature, for conversion of WH to ethanol, *S. cerevisiae* is used frequently rather than *C. shehatae*, *Pichia stipites*, and *Z. mobilis*. Moreover, for enzymatic hydrolysis of WH a wide range of *cellulase* types are used. Meanwhile, in light of the three major steps in ethanol production from biomass are pretreatment, enzymatic hydrolysis, and fermentation, the commercialization of ethanol production has encountered a major limitation due to the high cost of pretreatment. Comprehensive development and optimization are therefore required to make production of ethanol from lignocellulosic

biomass in a cost effective manner. As earlier reported by (Sukumaran *et al.* 2009), the costs of enzyme for hydrolysis and saccharification are key barriers for commercialization of ethanol production from biomass. In addition, due to low cost and high availability, lignocellulosic biomass can be used as long term alternative source for ethanol production (Baeyens *et al.* 2015). Furthermore, the usage of cost-effective raw materials such as lignocellulosic residuals in effective fermentation methods (*e.g.* SSF), the economic aspects of ethanol production can be improved. Recently Sindhu *et al.* (2016) found that the reduction of pretreatment cost and enzyme saccharification with proper reactor design can improved the cost of ethanol production.

For commercialization of ethanol production from WH, in addition to the technical mentioned barriers considered in this article, harvesting and transportation costs also should be considered and minimized. Hence, the yield of ethanol production from water hyacinth is 0.12 g/g ethanol, which is lower than rice straw 0.18 g/g ethanol and wheat straw 0.2 g/g ethanol.

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