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Potential of Weed Biomass for Bioethanol Production

Siripong Premjet

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Abstract

Lignocellulosic biomass from weedy plants represents a potential alternative feedstock for economic production of bioethanol. Large numbers of weedy plant species are growing all over the world. Characteristics such as high dry matter yield, low water and nutrient requirements for growth, and cellulose contents make weedy plants very attractive as feedstock for bioethanol production. However, like other lignocellulosic feedstock, the complex structure presents resistance and recalcitrance to processes of conversion to bioethanol. Several weedy plants have been studied to determine their physical characteristics and suitability for bioethanol production. Different conversion techniques have been employed to increase monomer sugars and hence bioethanol yield. This chapter discusses processes and current research activities in bioconversion of weed biomass to bioethanol.

Keywords: bioethanol, fermentation, lignocellulosic biomass, pretreatment, weedy plants

1. Introduction

Rapid economic and population growth have resulted in drastic increase in energy consumption especially in the transportation sector. To meet growing demand for fuel energy, most countries around the world depend heavily on imported petroleum fuel [1]. However, concerns have been raised about gradual depletion of fossil fuels and environmental pollution as a result of its combustion [2]. This has necessitated the search of alternative sustainable and eco-friendly source(s) of fuel energy. As part of the search, many governments worldwide are promoting the use of biofuels such as bioethanol and biodiesel as alternative transportation fuel [3].

Bioethanol is currently the most widely used liquid biofuel [4]. It is an eco-friendly and renewable fuel produced from plant-based starches and sugars [5]. Global production of bioethanol is mainly from food-related crops such as corn, cassava, sugarcane, rice, and sweet potatoes [3]. However, these feedstock are directly consumed by humans as food or as animal feed. Continuous use of these crops for bioethanol production may put pressure on productive agricultural lands and result in higher food prices [6]. Concerns about sustainability of bioethanol production from food-related crops have raised attention to the potential of lignocellulosic biomass for bioethanol production [7].

Lignocellulosic biomass is inexpensive and abundant worldwide. It includes agricultural and forestry waste, grasses, and other nonfood plants [8]. This type of biomass is a rich source of biopolymers, chemicals, and sugars [9]. Current research into bioethanol production is mainly focused on assessing the potential of nonfood crops as feedstock and improving the efficiency of their conversion [10]. Lignocellulosic biomass from invasive weeds is a good feedstock for the economic production of bioethanol [2]. These weedy cellulosic substrates do not need extra expenses as they grow on agriculturally degraded land or water bodies [11]. Large numbers of such invasive species are found all over the world. The potential of weed biomass for the production of bioethanol has been explored and discussed in this chapter.

2. Lignocellulosic biomass from weedy plants: chemical composition and potential for bioethanol production

The major components of lignocellulosic biomass are cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are the main carbohydrates in lignocellulosic biomass. The contents of these components vary significantly depending on the type of biomass and source [6]. Cellulose is a crystalline and linear structure made up of units of glucose strongly linked together by β -1-4-glycosidic bonds. These linkages give cellulose very high crystalline structure making it resistant to degradation. It is the most abundant organic polymer on earth. Hemicellulose on the other hand, consists of linear and highly branched mixture of pentoses (xylose and arabinose) and hexoses (glucose, galactose, and mannose). Lignin is a highly branched polyphenolic polymer, which gives stability to biomass structure [12]. Cellulose and hemicellulose, the major substrates for bioethanol production, form the main components of the total dry weight of lignocellulosic biomass [7]. These fractions are linked together by covalent and hydrogen bonds, which are further strongly bonded to lignin. This gives lignocellulosic biomass a very complex structure, which is very resistant to degradation. Digestibility of lignocellulosic biomass is therefore affected by the degree of complexity and composition [11]. The structure and composition of different lignocellulosic biomass differ and this greatly affects the efficiency of their conversion to bioethanol.

Lignocellulosic biomass from weedy plants is one of the most sustainable alternative feedstock for bioethanol production [12]. Annual and perennial weedy plants are found all over the world at all seasons. They invade large areas of land and water bodies causing environmental and socioeconomic problems [2]. They grow rapidly on marginal lands under extreme conditions such as drought, low nutrient and high temperatures, hence requiring no

additional economic input such as fertilizer and pesticides [7]. Weed biomass contains large amounts of chemicals and materials, which can be extracted for several industrial applications [13]. These plants have been reported to produce high dry matter yield and contain high and low percentages of cellulose and lignin contents, respectively [14]. The high dry matter

Scientific name	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	EtOH TY (L/Ton)
<i>Imperata cylindrica</i>	44.4 ± 0.1	31.1 ± 0.0	6.7 ± 0.0	6.9 ± 0.0	548.4 ± 1.4
<i>Amaranthus viridis</i>	37.4 ± 0.1	34.2 ± 0.0	5.1 ± 0.1	16.9 ± 0.2	521.0 ± 0.9
<i>Sida acuta</i>	56.0 ± 0.3	16.0 ± 0.4	6.8 ± 0.1	5.1 ± 0.1	520.3 ± 5.4
<i>Rottboellia cochinchinensis</i>	41.6 ± 0.7	28.6 ± 0.4	7.5 ± 0.1	10.5 ± 0.3	509.7 ± 8.1
<i>Sorghum halepense</i>	44.4 ± 0.1	25.8 ± 0.2	6.6 ± 0.5	8.8 ± 0.3	508.8 ± 2.6
<i>Eragrostis amabilis</i>	39.7 ± 0.4	29.6 ± 0.2	7.2 ± 0.2	5.5 ± 0.1	502.9 ± 4.7
<i>Cyperus imbricatus</i>	35.6 ± 0.1	32.3 ± 0.3	4.7 ± 0.3	7.4 ± 0.1	493.6 ± 3.2
<i>Cenchrus echinatus</i>	35.8 ± 0.6	31.8 ± 0.4	6.3 ± 0.3	13.9 ± 0.2	491.4 ± 7.4
<i>Cyathula prostrata</i>	50.0 ± 0.3	17.0 ± 0.3	10.9 ± 0.1	10.3 ± 0.1	484.8 ± 4.4
<i>Eriochloa procera</i>	37.0 ± 0.0	29.5 ± 0.1	5.3 ± 0.3	13.1 ± 0.0	483.2 ± 1.1
<i>Brachiaria mutica</i>	37.7 ± 0.01	28.8 ± 0.8	5.6 ± 0.8	10.9 ± 0.2	482.8 ± 6.7
<i>Sporobolus indicus</i>	35.6 ± 0.0	29.9 ± 0.1	6.6 ± 0.0	9.1 ± 0.3	476.2 ± 1.0
<i>Leucaena leucocephala</i>	55.2 ± 0.0	10.1 ± 0.1	16.1 ± 0.1	2.6 ± 0.6	471.9 ± 1.2
<i>Echinochloa crus-galli</i>	34.7 ± 0.2	30.1 ± 0.1	4.6 ± 0.0	8.9 ± 0.5	470.8 ± 2.0
<i>Cyperus iria</i>	33.4 ± 0.2	31.0 ± 0.0	6.3 ± 0.0	5.4 ± 0.1	468.9 ± 1.3
<i>Typha angustifolia</i>	47.1 ± 0.1	16.9 ± 0.4	10.0 ± 0.3	11.3 ± 0.1	462.9 ± 3.9
<i>Dactyloctenium aegyptium</i>	32.0 ± 0.1	31.6 ± 0.1	7.7 ± 0.0	9.5 ± 0.4	462.4 ± 0.3
<i>Achyranthes aspera</i>	53.7 ± 0.1	10.2 ± 0.1	8.5 ± 0.1	11.7 ± 0.3	461.0 ± 1.5
<i>Pennisetum polystachyon</i>	40.0 ± 0.0	23.3 ± 0.1	6.2 ± 0.2	7.5 ± 0.3	459.2 ± 0.6
<i>Cyperus compactus</i>	32.8 ± 0.3	29.0 ± 0.8	4.6 ± 0.5	11.2 ± 0.1	448.9 ± 8.5
<i>Aeschynomene Americana</i>	48.3 ± 0.2	13.4 ± 0.0	15.4 ± 0.3	7.4 ± 0.4	446.2 ± 1.3
<i>Celosia argentea</i>	44.3 ± 0.3	17.2 ± 0.2	9.7 ± 0.9	10.0 ± 0.1	445.3 ± 3.2
<i>Dicliptera roxburghiana</i>	41.9 ± 0.3	17.5 ± 0.3	8.7 ± 0.4	15.2 ± 0.0	429.8 ± 4.3
<i>Crotalaria pallida</i>	49.6 ± 0.2	9.1 ± 0.2	11.7 ± 0.1	4.3 ± 0.2	423.6 ± 2.7
<i>Scoparia dulcis</i>	36.5 ± 0.3	19.1 ± 0.1	6.6 ± 0.0	4.5 ± 0.6	402.6 ± 2.9
<i>Urena lobata</i>	43.5 ± 0.3	11.4 ± 0.7	9.6 ± 0.1	7.5 ± 0.3	396.7 ± 4.4
<i>Cyperus cyperoides</i>	29.7 ± 0.6	24.6 ± 0.2	10.9 ± 0.6	8.8 ± 0.1	394.0 ± 5.3

Source: [14] EtOH TY = Theoretical ethanol yield.

Table 1. Chemical composition and theoretical ethanol yields of weed biomass.

yield and cellulose contents of weedy plant species make them ideal feedstock for bioethanol production. They also have an added advantage as feedstock for bioethanol production since they do not compete with food crops for productive agricultural lands [15]. Moreover, due to seasonal nature of agricultural wastes, lignocellulosic biomass from weed species is very important in ensuring continuous production of bioethanol throughout the year [16]. A wide range of weedy species are grown naturally on marginal lands all over the world that can be used as feedstock for bioethanol production. Perennial grasses and short rotation forest plants are among these weedy species growing worldwide [17]. The possibility of converting biomass from invasive weeds to fuel bioethanol is currently an area of great research interest around the world. The physical characteristics and bioethanol production potential of several weedy species have been studied.

Parthenium hysterophorus, a common invasive weed species was studied in India as a potential feedstock for bioethanol production. Chemical composition analysis of this weed species revealed 53.63% holocellulose and 10.44% lignin contents, making it an attractive feedstock for production of bioethanol [18]. *Cannabis sativa*, a versatile weedy plant, grows naturally in large areas in Pakistan. It produces large amount of biomass due to its rapid growth rate. *Cannabis sativa* contains 55% cellulose and only 5% lignin. It has been reported as a potential cheap and eco-friendly feedstock for bioethanol production in Pakistan [19]. *Pennisetum purpureum*, commonly known as Napier grass or elephant grass, *Vetiveria zizanioides* also known as vetiver grass, *Digitaria decumbens*, *Paspalum atratum*, *Cynodon* sp., and *Pennisetum polystachyon* are all weedy species found in Asia that have been studied and proposed as feedstock for bioethanol production [12].

In an earlier research, different types of weedy plants were identified in six provinces in lower Northern Thailand (**Table 1**). Majority of these weed biomass were found to contain high cellulose but low lignin contents. The cellulose contents of most of these weed biomass is higher or similar compared to well-known lignocellulosic materials from agricultural residues including corn stalk bagasse (43.4%) [20], corncob ($31.5 \pm 1.2\%$) [21], wheat straw ($35.2 \pm 0.3\%$) [22], paddy straw (32.6%) [23], soybean straw (34.40%) [24], and sugarcane bagasse (27.3%) [25]. High theoretical bioethanol yields were also estimated for these weed biomass based on the contents of cellulose and hemicellulose. Bioethanol yield of between 548.4 ± 1.4 and 394.0 ± 5.3 L/ton was realized from some of the weed species [14]. Majority of these weed species are potential substrate for bioethanol production.

3. Biological conversion of weed biomass to bioethanol

Bioethanol is produced from three main renewable resources namely starch, sugars, and lignocellulosic biomass. The production of bioethanol from starch and sugar (first generation bioethanol production) differs significantly from that of lignocellulosic biomass. The process of bioethanol production from sugar-related crops involves direct extraction of sugars followed by fermentation to bioethanol. However, starch carbohydrates are extracted from starch-based crops and hydrolyzed into monomer sugars with subsequent fermentation of

sugars to bioethanol [26]. Unlike first generation bioethanol production where carbohydrates are easily converted to bioethanol, carbohydrate portions in weed biomass are not freely available for the conversion to bioethanol. Biological conversion of weed biomass to bioethanol involves various processes (**Figure 1**). The major steps involved in the conversion process include pretreatment of biomass to make it easily digestible in subsequent processes. The cellulose and hemicellulose contents are then hydrolyzed to monomer sugars followed by the fermentation of sugars to bioethanol. Finally, bioethanol is purified through distillation or other processes such as dehydration to conform to world bioethanol specifications [27].

3.1. Pretreatment of weed biomass

Like most lignocellulosic biomass, the recalcitrance of weed biomass is a major problem in their conversion to bioethanol. This is due to the crystalline structure of cellulose coupled with lignin and hemicellulose strongly bonded to each other and serving as a protective cover to cellulose. The pretreatment of weed biomass is thus very important in releasing fermentable sugars for bioethanol production [6]. It helps to break the bond between lignin and hemicellulose, hence destroying the protective cover of cellulose. It also helps to decrease cellulose crystallinity making it more susceptible to enzymatic hydrolysis and fermentation [12]. Different pretreatment methods can be used on various types of weed biomass for bioethanol

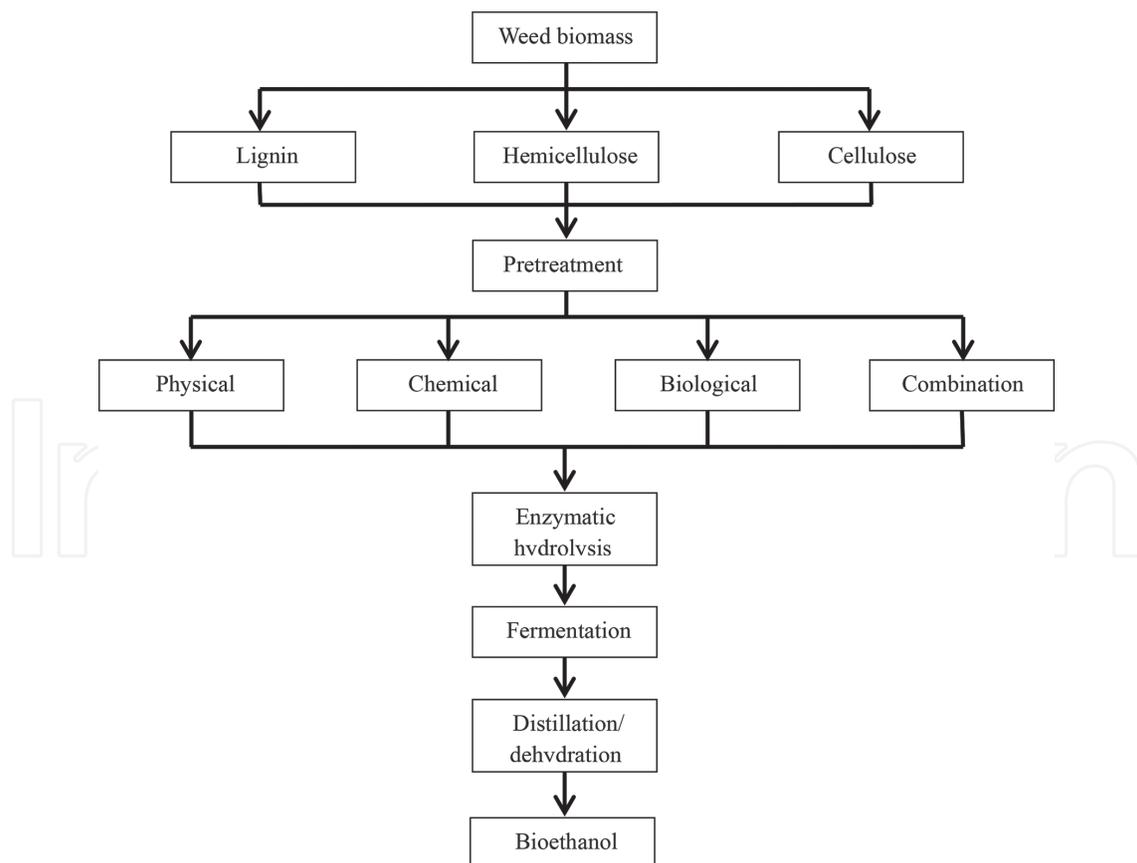


Figure 1. Schematic diagram of major steps in weed biomass conversion to bioethanol.

production. However, the cost of pretreatment, production of inhibitors, type of weed biomass, energy requirements, and efficiency are major factors that need to be considered in the choice of pretreatment method [28]. Pretreatment may be physical, chemical, biological, or a combination of these [29].

3.1.1. Physical pretreatment

Physical pretreatment includes methods aimed at reducing particle size of biomass. These methods consist of mechanical operations such as chipping, milling, and grinding. These processes help to increase the porosity and surface area of biomass to enhance its conversion to bioethanol [9]. Mechanical operations are usually carried out as a preparatory step during the conversion process [12]. Other methods including different kinds of irradiation and ultrasonic pretreatment have been developed to physically enhance accessibility to cellulose during the conversion process. Physical pretreatment, however, requires high amount of energy contributing to high cost of bioethanol production [9].

3.1.2. Chemical pretreatment

Chemical pretreatment is the most common and studied pretreatment method for the conversion of lignocellulosic biomass to bioethanol. Different chemicals including alkali, ionic liquids, organic solvents, oxidizing agents, and acids can be used [30]. Acid pretreatment is one of the most promising methods and has been extensively studied. It mainly results in solubilization of hemicelluloses but less effective in lignin removal [27]. The type of acid, concentration, volume, and pretreatment temperature are some factors that affect the efficiency of this technique [9]. Acid pretreatment may be carried out with either concentrated or dilute acid. However, dilute acid is normally preferred as concentrated acid, which is toxic and corrosive, and results in the production of high levels of inhibitors including furfural derivatives, acetic acid, phenolics, and other aromatic compounds [31]. Pretreatment with acid may be conducted at high temperature for a short time or low temperature for a longer period [32]. Various types of acids including hydrochloric, phosphoric, nitric, oxalic, formic, acetic, and maleic have been studied as chemicals for pretreatment of lignocellulose biomass. Despite its effectiveness, acid pretreatment is toxic and generates inhibitory compounds that negatively affect enzymatic hydrolysis and fermentation processes [9]. It is therefore crucial to remove these compounds, a process that adds to the cost of bioethanol production.

Alkaline pretreatment on the other hand breaks the intermolecular bonds between lignin and hemicelluloses and reduces cellulose crystallinity [33]. During alkaline pretreatment, biomass is treated with alkali chemicals such as sodium, calcium, ammonium, and potassium hydroxides at varying temperatures with or without pressure [5]. Alkaline pretreatment enhances accessibility of enzymes to cellulose by mainly solubilizing lignin contents of biomass. It results in less sugar degradation and produces low inhibitors compared to acid pretreatment [20]. However, alkaline pretreatment results in the production of salts are very difficult to recover [6].

Ozone, a strong oxidizing agent is very effective for the removal of lignin in lignocellulosic biomass. This type of chemical pretreatment is normally done at room temperature and results in

no inhibitor formation [30]. Organic solvents such as methanol, ethanol, ethylene glycol, glycerol, acetic acid, formic acid, phenol, and dioxane are also very effective in extracting lignin and hemicellulose [29]. Ionic liquids have been identified as promising solvents for pretreatment because of their ability to dissolve lignin and carbohydrates. A variety of ionic liquids including those containing cholinium cations and linear carboxylate anions have been identified for their ability to enhance digestibility of lignocellulosic biomass. An advantage of ionic liquid is the recovery of separate lignin and carbohydrate fractions after pretreatment. However, ionic liquids are very expensive and can inhibit enzymatic hydrolysis and fermentation processes [34].

3.1.3. Biological pretreatment

Biological pretreatment of lignocellulosic biomass involves using different types of microorganisms including fungi, bacteria, and actinomycetes [9]. These organisms have the ability to produce ligninolytic enzymes such as peroxidases (lignin peroxidase and manganese peroxidase) and laccases. These two groups of enzymes play significant role in lignin degradation during biological pretreatment. The most common microorganism for biological pretreatment is filamentous fungi. White-rot fungi have been identified as the most effective microorganism for the biological pretreatment of lignocellulosic biomass [35]. A number of white-rot fungi including *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Cyathus stercoreus*, *Pycnoporus cinnabarinus*, *Ceriporia lacerata*, and *Ceriporiopsis subvermispora* are able to produce lignin degrading enzymes for the effective delignification of lignocellulosic biomass. Biological pretreatment does not generate toxic substances, is mild, requires low energy, and more environmentally friendly compared to other pretreatment techniques [23]. Nonetheless, the process is very slow and requires carefully controlled conditions as well as large space making it not attractive for commercial bioethanol production. Some microorganisms also tend to degrade cellulose and hemicellulose in addition to lignin [31].

Biological pretreatment may also be carried out with ligninolytic enzyme extracts. This has been reported to prevent degradation of carbohydrates that is associated with microbial pretreatment [31]. These enzymes are extracted from lignin degrading microorganisms, purified and used for the pretreatment process. Crude enzyme extracts have, however, been reported to contain other factors such as proteins and mediators. The presence of these factors enhance the activity of these enzymes making them more effective compared to purified ones. The major problem associated with enzymatic delignification is low enzyme production and activity. Enhancing the culturing conditions may however help to increase the activity and the yield of these enzymes [36].

The effect of pretreatment on biomass varies depending on the method and type of lignocellulosic biomass. Development of effective pretreatment conditions is thus crucial for converting weed biomass to bioethanol. To release monomer sugar units from weed biomass, researchers have studied the effect of different kinds of pretreatment on different types of weed biomass (**Table 2**). Ratsamee [10] pretreated purple guinea grass (*Panicum maximum* cv. TD 53) with dilute sulfuric acid (H_2SO_4) and calcium hydroxide ($Ca(OH)_2$) to improve cellulose digestibility. Pretreatment with the two chemicals resulted in a significantly higher glucose contents in the biomass after enzymatic hydrolysis. However, purple guinea grass biomass

pretreated with calcium hydroxide yielded slightly higher glucose concentration after hydrolysis. Wongwatanapaiboon [17] assessed the potential of bioethanol production from different types of grasses by pretreating them with alkaline peroxide ($H_2O_2 + NaOH$). Following alkaline peroxide pretreatment and enzymatic hydrolysis with cellulase and xylanase, total reducing sugar in the range of 521–559 mg/g biomass was obtained. Chandel [37] reported maximum total reducing sugar yields of 310 ± 9.80 , 541.2 ± 9.53 , and 646.23 ± 8.99 mg/g biomass after enzymatic hydrolysis of wild sugarcane (*Saccharum spontaneum*) biomass pretreated with dilute sulfuric acid (H_2SO_4), dilute sodium hydroxide (NaOH), and aqueous ammonia (aq. Ammonia), respectively. In an earlier research, pretreatment of *Achyranthes*

Weed biomass	Pretreatment conditions	Enzymatic hydrolysis	Sugars after pretreatment/hydrolysis	Reference
<i>Panicum maximum</i> cv. TD53	3% H_2SO_4 , autoclave at 121°C for 30 mins	Accellerase™ 1000 (9FPU/g)	10.1 g/L glucose	[10]
	4% $Ca(OH)_2$, autoclave at 121°C for 5 mins		11.9 g/L glucose	
<i>Paspalum atratum</i>	7.5% $H_2O_2 + NaOH$	Cellulase (60 U/g) + xylanase (1200 U/g)	506 mg/g biomass	[17]
<i>Pennisetum purpureum</i> Schum.			529 mg/g biomass	
<i>Pennisetum purpureum</i> cv. Mott			559 mg/g biomass	
<i>Pennisetum purpureum</i> × <i>Pennisetum americanum</i>			556 mg/g biomass	
<i>Saccharum spontaneum</i>	1.5% H_2SO_4 (v/v)	Cellulase (15 FPU/g)	310 ± 9.80 mg/g biomass	[37]
	1.0 M NaOH	Cellulase (25 FPU/g)	541.2 ± 9.53 mg/g biomass	
	15% aq. ammonia	Cellulase (25 FPU/g)	646.23 ± 8.99 mg/g biomass	
<i>Achyranthes aspera</i>	80% H_3PO_4	Cellulase (30 FPU/g) + β -glucosidase (60 U/g)	8.0 g/L glucose	[38]
<i>Sida acuta</i>	75% H_3PO_4		8.6 g/L glucose	
<i>Arundo donax</i>	1% (v/v) H_2SO_4 , autoclave at 121°C for 30 mins	Cellulase (135 FPU/g) + Cellobiase (75 FPU/g)	724.0 mg/g biomass	[2]
<i>Saccharum spontaneum</i>	followed by 1.5% NaOH + ultrasound irradiation		851.7 mg/g biomass	
<i>Mikania micrantha</i>			592.0 mg/g biomass	
<i>Lantana camara</i>			662.2 mg/g biomass	
<i>Eichhornia crassipes</i>			758.6 mg/g biomass	
<i>Leucaena leucocephala</i>	<i>Phanerochaete chrysosporium</i>	Cellulase (30 FPU/g)	1.2 g/L glucose	[39]

Table 2. Pretreatment and enzymatic hydrolysis of weed biomass.

aspera and *Sida acuta* with different concentrations of phosphoric acid (H_3PO_4) helped to increase glucose concentration (8.0 and 8.6 g/L, respectively) of the biomass after enzymatic hydrolysis with a combination of cellulase and β -glucosidase [38]. Preliminary studies on biological pretreatment of *Leucaena leucocephala* with *Phanerochaete chrysosporium* also resulted in an increase in glucose concentration (1.2 g/L) of pretreated biomass after hydrolysis with cellulase enzyme [39]. Borah [2] carried out acid hydrolysis with sulfuric acid (H_2SO_4) followed by delignification with sodium hydroxide (NaOH) and ultrasound irradiation of five weed species as feedstock for bioethanol production. After enzymatic hydrolysis, the average yield of total fermentable sugars (hexose and pentose) from all five weed species was reported to be 43.85 g/100 g of biomass, representing 27.36 g theoretical bioethanol yield. It can be inferred from **Table 2** that the optimum conditions of pretreatment differ significantly for each weed biomass.

3.2. Enzymatic hydrolysis

Pretreatment of lignocellulosic biomass is followed by acid or enzymatic hydrolysis to break down cellulose and sometimes hemicellulose into fermentable sugars such as glucose and xylose [12]. Enzymatic hydrolysis is however eco-friendly and preferred to the noneco-friendly harsh acid hydrolysis [33]. The total amount of fermentable sugars produced is dependent on the type of lignocellulosic biomass and efficiency of pretreatment process [12]. Enzymatic hydrolysis of biomass is carried out in different forms. In some cases, pretreated biomass is initially hydrolyzed by enzymes followed by fermentation of sugars to bioethanol in a process called, separate hydrolysis, and fermentation (SHF). This process requires two separate distinct process conditions for both enzymatic hydrolysis and fermentation. A major setback back to this process is the accumulation of sugar during enzymatic hydrolysis step, which can inhibit enzymatic activities [12]. The production of monomer sugars and fermentation of these sugars may also be carried together in a process known as simultaneous saccharification and fermentation (SSF) [11]. The tendency of monomer sugar accumulation is as less as individual sugars released are converted to bioethanol at the same time. This process may however be very complex with respect to process conditions, which can lead to a decrease in bioethanol yield. Specific operating conditions must therefore be established to enhance both enzymatic hydrolysis and fermentation processes [12]. An emerging method is consolidated bioprocessing (CBP) in which a microorganism or group of microorganisms are used to convert untreated biomass to bioethanol. The microorganism(s) have special inherent abilities to secrete enzymes that degrade biomass and ferment sugars released to bioethanol. This method is very promising, however, research activities is still at an infant stage [12].

Cellulase enzymes are used for enzymatic hydrolysis of cellulose after pretreatment. Enzymes for hydrolysis may be obtained from commercial enzyme producers. In some cases, the enzymes may be produced, harvested, and use for hydrolysis. These enzymes are produced by both bacteria and fungi; however, most commercial cellulases are produced from fungi [33]. Cellulases are made up of three set of enzymes including endoglucanase (1,4- β -D-glucan glucanohydrolase, EC 3.2.1.3), exoglucanase (1,4- β -D-glucan cellobiohydrolase, EC 3.2.1.91), and cellobiase (β -glucosidase; EC 3.2.1.21). Endoglucanase cuts cellulose chains into fragments

of glucose, cellobiose, and cellotriose while exoglucanase cleaves it into cellobiose units [11]. Cellobiase, however, breaks cellobiose units into glucose that can be fermented to bioethanol. Majority of cellulases obtained from fungi lacks β -glucosidase and must be supplemented with β -glucosidase during enzymatic hydrolysis to enhance efficiency [33]. Cellulase activity is dependent on the concentration and source. Different dosages of cellulases are used during enzymatic hydrolysis. This may depend on the composition of pretreated biomass as well as the type of pretreatment technique used. Enzymatic hydrolysis of cellulose requires mild conditions including pH of between 4.8 and 5.0 and temperature of approximately 50°C. High hydrolysis efficiency is however achieved with an optimized temperature, time, pH, enzyme load, and biomass concentration [4].

The hemicellulose component may also be hydrolyzed with hemicellulases into monomer sugars for fermentation to bioethanol [7]. Compare to cellulose, hemicellulose hydrolysis is very complex because of its composition (mixture of pentoses and hexoses). Multiple enzyme system including endo-xylanase, exo-xylanase, and β -xylosidase together with auxiliary enzymes α -arabinofuranosidase, α -glucuronidase, acetyl xylan esterase, and ferulic acid esterase are involved in hemicellulose hydrolysis [26].

Enzymatic cocktails comprising cellulases and hemicellulases have been used to hydrolyze various pretreated weed biomass for bioethanol production (Table 2).

3.3. Fermentation

Following enzymatic hydrolysis, the supernatant containing various sugars (pentoses and hexoses) is fermented to bioethanol. Different types of microorganisms including fungi and bacteria can be used to ferment sugars from weed biomass to bioethanol. *Zymomonas mobilis* [40], *Kluyveromyces* sp. [41], and *Saccharomyces cerevisiae* [4] are common microorganisms for fermentation of glucose to bioethanol. *S. cerevisiae* is the most common microorganism for commercial bioethanol production. However, *Pachysolen tannophilus*, *Pichia stipitis*, and *Candida shehatae* are well-known for their ability to ferment xylose to bioethanol [33]. However, the activity of *S. cerevisiae* is affected by several factors including high temperature, osmotic stress, bioethanol concentration, and contamination from bacteria [41]. These conditions inhibit microbial growth during fermentation process, thus affecting the yield of bioethanol production. Furthermore, the inability of *S. cerevisiae* to ferment pentoses also affects bioethanol yield during fermentation. However, studies are continuously being conducted to isolate and identified *S. cerevisiae* strains that are able to tolerate these stress conditions to improve bioethanol yield during fermentation. Microbial strains from *Pichia* sp., *Candida* sp., *Schizosaccharomyces* sp. and *Pachysolen* sp. have also been identified for fermentation of pentoses to bioethanol. Recombinant DNA technologies have been exploited to develop strains that are resistant to stress and also have the ability to ferment pentoses, all aimed at increasing bioethanol yield [4].

Fermentation of bioethanol is normally undertaken in a bioreactor with three major different processes namely batch, fed-batch, and continuous [4]. During batch process of bioethanol production, the fermentation ingredients including substrate, culture medium, and nutrients

are fed to the bioreactor only at the start of the process. No feeding is done till the process is over after which bioethanol is harvested. The substrate, medium, and nutrients may however be fed and bioethanol removed continuously during continues fermentation process. The fed-batch process is a combination of the batch and continues processes. During this process, fermentation ingredients are continuously fed to the bioreactor but bioethanol is only harvested at the end of the process [26]. Bioethanol produced after fermentation is further purified through distillation and other cutting-edge processes such as pervaporation [7]. Different types of microorganisms have been studied for their ability to ferment weed biomass to bioethanol (Table 3).

Wongwatanapaiboon [17] reported a significantly higher bioethanol yield from alkaline peroxide pretreated *Vetiveria zizanioides* cv. Sri Lanka and *Vetiveria zizanioides* cv. Ratchaburi. Using the fermenting organisms *Saccharomyces cerevisiae* TISTR 5339 and *P. stipitis* CBS 5773, 32.72 and 30.95% of theoretical ethanol yield was reported for pretreated *Vetiveria zizanioides* cv. Sri Lanka and *Vetiveria zizanioides* cv. Ratchaburi biomass respectively. Tavva [18] reported similar bioethanol yield for *Torulaspora delbrueckii* R3DFM2, *Schizosaccharomyces*

Weed biomass	Pretreatment	Fermenting microorganism	EtOH production	Reference
<i>Vetiveria zizanioides</i> cv. Sri Lanka	Alkaline peroxide	<i>Saccharomyces cerevisiae</i> TISTR 5339 + <i>P. stipitis</i> CBS 5773	0.14 ± 0.01 g/L	[17]
<i>Vetiveria zizanioides</i> cv. Ratchaburi			0.14 ± 0.01 g/L	
<i>Parthenium hysterophorus</i>	Sulfuric acid	<i>Torulaspora delbrueckii</i> R3DFM2	0.24 g/g biomass	[18]
		<i>Schizosaccharomyces pombe</i> R3DOM3	0.27 g/g biomass	
		<i>Saccharomyces cerevisiae</i> R3DIM4	0.27 g/g biomass	
<i>Saccharum spontaneum</i>	Aqueous ammonia	<i>Pichia stipitis</i> NCIM3498	0.40 ± 0.01 g/g biomass	[37]
	Sulfuric acid		0.38 ± 0.02 g/g biomass	
	Sodium hydroxide		0.39 ± 0.02 g/g biomass	
<i>Lemna minor</i>	Alkaline	<i>Saccharomyces cerevisiae</i>	0.218 g/g biomass	[13]
<i>Lemna gibba</i>			0.197 g/g biomass	
<i>Pistia stratiotes</i>			0.215 g/g biomass	
<i>Eichhornia</i> sp			0.189 g/g biomass	
<i>Pennisetum polystachion</i>	Sodium hydroxide	<i>Saccharomyces cerevisiae</i> (TISTR 5596)	16.0	[42]
<i>Panicum maximum</i> cv. TD 53	Calcium hydroxide	<i>Saccharomyces cerevisiae</i> TISTR 5596	5.9 g/L	[10]

Table 3. Ethanol production from weed biomass.

pombe R3DOM and *Saccharomyces cerevisiae* R3DIM4 fermentation of sulfuric acid pretreated *Parthenium hysterophorus*. The efficiency of bioethanol production by the three microbial strains was reported as 78.84, 87.82, and 87.17%, respectively. Chandel [37] used *Pichia stipitis* NCIM3498 to ferment hydrolyzate obtained from aqueous ammonia, sulfuric acid and sodium hydroxide pretreated *Saccharum spontaneum*. The results show maximum bioethanol production from hydrolyzate for all the pretreated biomass. Gusain and Suthar [13] converted alkaline pretreated aquatic weeds into bioethanol using *Saccharomyces cerevisiae*. Bioethanol yields of between 0.189 and 0.218 g/g biomass were reported for the four different species of aquatic weeds. Prasertwasu [42] fermented hydrolyzate from sodium hydroxide pretreated *Pennisetum polystachion* with *Saccharomyces cerevisiae* (TISTR 5596) and reported high bioethanol yield after 24 hours. Ratsamee [10] also reported maximum bioethanol yield after fermenting hydrolyzate from calcium hydroxide pretreated *Panicum maximum* cv. TD 53 with *Saccharomyces cerevisiae* TISTR 5596 for 48 hours.

4. Conclusion and future perspectives

Weed biomass is a promising feedstock for economic bioethanol production. The abundance of weed biomass worldwide is an assurance of its sustainability as a feedstock. Current research on the conversion of weed biomass to bioethanol is focused on pretreatment techniques. Different pretreatment techniques have been explored to convert weed biomass into bioethanol. Maximum bioethanol yields have been reported after fermentation of hydrolyzates from pretreated weed biomass. However, current technologies are still inadequate for bioethanol production from weed biomass to compete with starch and sugar based bioethanol in terms of production yield and cost. Production of cellulosic bioethanol from weedy plants is only at the laboratory scale. Further research to establish cost effective and efficient conversion processes including pretreatment technique(s) for a wide range of weed biomass is needed. Predictive models will also aid in the selection, design, optimization, and process control pretreatment technologies that match biomass feedstock with appropriate method and process configuration. On the other hand, active research is going on to ensure commercial production of bioethanol from weed biomass. This includes improvements in pretreatment technologies, specific activities of enzymes as well as isolation of new fermentation microorganism from natural environment. With strong support from various governments, bioethanol production from weed biomass will play a major role in meeting energy demand globally.

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Conflict of interest

The author has no conflict of interest to declare.

Author details

Siripong Premjet

Address all correspondence to: siripongp@nu.ac.th

Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand

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