Research Article

Development of Ionic Liquid Utilization in Biorefinery Process of Lignocellulosic Biomass

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Abstract
Ionic liquids have been utilized in various industries as the environment-friendly organic solvents. The tailor-made properties of ionic liquids to meet the requirements of specific tasks lead to the variety of applications in biorefinery. Ionic liquid applications have been developed and integrated in biorefinery of lignocellulosic biomass processing since last decade. Conversion of lignocellulosic biomass to sugars requires both efficient pretreatment and hydrolysis enzymes to produce biofuels and specialty chemicals, and ionic liquids were applied to improve the hydrolysis yields. Ionic liquid pretreatment is one of potential method due to its high efficiency to solubilize cellulose, and its recyclability. The high extractibility of ionic liquid as a solvent was described in many studies and became the new target for R&D sectors. However, the adverse effects of ionic liquids have been discovered bringing out controversy to be discussed. The present review is aimed to provide the insight of applications of ionic liquid and current improvement direction in biorefinery.

Keywords: Lonic liquid, Cellulase, Pretreatment, Lignocellulosic biomass, Consolidated processing, Biorefinery

1 Potential of Lignocellulosic Biomass in Biofuel Production

Due to increasing of industrial and human activities that mainly utilize energy derived from fossil fuel, the greenhouse gases including NO_x, SO_x and CO_x have been released continuously and caused the greenhouse effect worldwide. Today, greenhouse effect becomes serious problem as it tremendously causes environmental problems, for example, flooding, *El nino* and *La nina*. To resolve this devastation, United Nations (UN) called for meeting to set up "United Nations Framework Convention on Climate Change: UNFCCC" to launch the direction of management of how to relieve the greenhouse effect occurrence. In 2015, "Paris agreement" has been signed by UN members to establish the mutual policy to solve this problem, including reduction of temperature increasing to be higher than 2°C, reduction of greenhouse gas, reduction the use of

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fossil fuel and providing incentive in the form of the financial support to the developing countries.

As an UN member, Thai government also joined in this mutual act. And in response to this agreement, Thai Government published the "Renewable Energy Development Plan: REDP" (2008–2022) to promote the production of alternative energy in various forms, including wind energy, water energy, solar energy and biofuels. In this REDP plan, the production of alternative energy will be increased to 20% of total required energy of the whole country that equivalents to 19,799 ktoe (kilotons oil equivalent) in 2022. The sources of alternative energy could be mainly categorized to the forms of electricity, heat and fuels. Biomass is also targeted to be materials for alternative energy production, and it is expected to be utilized to produce 43.91% of total alternative energy demands (8,693 ktoe). Although, many industries have been adopted the REDP plan in their activities, however the total production of energy from biomass still has not yet reached the goal due to limitation of technology, investment and social awareness.

Lignocellulosic biomass, including agricultural wastes, has been produced and disposed during agricultural activities. After harvesting season, most of these residues were left on the fields and burned down causing environmental problems. In 2009, more than 55.61 million tons of agricultural wastes were left unused in Thailand [1]. This lignocellulosic biomass was estimated to yield potential energy capacity up to 732,534 TJ [1]. Lignocellulosic biomass consists of three types of major components, including cellulose, hemicellulose, and lignin. Both cellulose and hemicellulose could be hydrolyzed to monosaccharide or simple sugar that, subsequently, could be fermented to biofuel [2]. Considering to the whole plant, approximately 70% of total dry mass is lignocellulose [3], [4]. Cellulose, a homopolymer of glucoses, takes up to 64% of dry mass of rice straw because it is component of plant cell wall [3], [5]. Similarly, hemicellulose is heteropolymer of hexose and pentose sugars, for example D-xylose, D-arabinose, D-mannose, D-glucose, D-galactose [6]. Regarding to the abundance of sugar monomers available in lignocellulose biomass, it is interesting to develop the strategy to utilize lignocellulosic biomass for biofuel production.

Generally, the biofuel production from lignocellulosic biomass composed of four main steps as following (Figure 1);

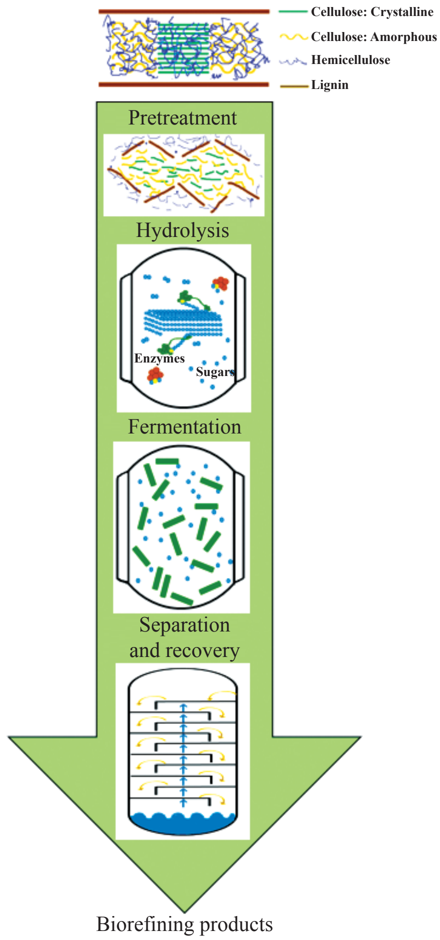

Figure 1: Biofuel production from lignocellulosic biomass.

1. Pretreatment. This step targets to loosen the cellulose microfibrils and increase contact areas to cellulase enzyme. In addition, cellulase inhibitors may be removed in this step.
2. Hydrolysis. This step produces sugars or lignocellulosic monomers by hydrolase enzymes. The product of this step are used as materials in fermentation process.
3. Fermentation. This step converts the products of hydrolysis, i.e. sugars, into different types of bioproducts or biofuels by microbial activities.
4. Recovery, purification and upgrading. This step targets to recover bioproducts or biofuels from fermentation broth, purify and upgrade to meet specification of sale products.

2 Application of Ionic Liquid in Biorefinery Process of Lignocellulosic Biomass

One of most important problem to produce biofuel from lignocelluloses is the resistance of biomass to biodecomposition or hydrolysis [3], [7]. The key success factor for biofuel production is to increase the efficiency of biomass hydrolysis by enhancing the enzyme accessibility via pretreatment method. Pretreatment process helps to loosen the cellulose microfibril to provide the chance for cellulolytic enzyme to attach to the substrate surface [7]. Now, many pretreatment methods have been developed to find the suitable methods for each biomass types, for example, alkaline or acid treatment and liquid hot water pretreatment. During this decade, there are growing numbers of intensive studies to develop pretreatment methods [3], [8]–[12] (Table 1). However, there are still numbers of technological, market and policy barriers that are serious obstacles to the economic feasibility and competitiveness of such process.

Table 1: Ethanol production from pretreated lignocellulosic biomass

Biomass	Pretreatment Method	Yield (g/l)	Reference
Willow	Steam Explosion	4.6	[13]
Aspen	Sulfurdioxide	14.9	[14]
Pine	Sulfurdioxide	32.0	[15]
Sugarcane Bagasse	Dilute Acid	8.67	[16]
Corn Stover	Dilute Acid	15	[17]
Switch Grass	Hot Compressed Liquid Water	16.8	[18]
Alfalfa	Liquid Hot Water	9.6	[19]
Recycled Paper Sludge	Simultaneous Saccharification and Fermentation	35	[20]
Newspaper	Enzymatic Treatment	14.77	[21]

Ionic liquid pretreatment is considered as a new technology in chemical pretreatment. Compared to common organic solvents, ionic liquids display interesting properties and potential advantages, as reasonable chemical inertness, good thermal stability, low volatility, or unique salvation abilities. Similar to concentrated acids, ionic liquids containing chloride, acetate, and other moderately basic anions disrupt the hydrogen bond network of cellulose, and enable its dissolution [22]. Many studies demonstrated that ionic liquid pretreatment has advantages over other pretreatment methods, including high monomeric sugar yields over short pretreatment times, high delignification and low cellulase-inhibitor formation [22]–[24]. Comparatively, pretreated cellulose was hydrolyzed 2–10 times faster compared to untreated cellulose. In 2010, there is another report using ionic liquids with combination of ammonia for rice straw pretreatment, and 82% of cellulose was recovered [25]. The mechanism of ionic liquid pretreatment is caused by impact of ion that attacks hydrogen bond of cellulose by modifying the hydrolgen dipolar and coulombic force [26]. Then, aqueous molecular shells relocate from cellulose to ion of ionic liquid, and cause the rearrangement of intra and inter-molecule interaction. Ionic liquid is also demonstrated to be able to remove lignin, a cellulase inhibitor, as well.

Ionic liquids are groups of new organic salts that exist as liquids at a relatively low temperature (100°C). They have many attractive properties, such as chemical and thermal stability, non-flammability, low vapour pressure, wide electrochemical window and high solvation ability to dissolve various organic and inorganic substances [27]. In contrast to traditional volatile organic compounds, they are called ‘‘green’’ solvents and have led to numerous proposed applications in a variety of fields, including catalysis, extraction, electrochemistry, organic synthesis and polymer chemistry. Furthermore, ionic liquids can be easily modified by changing the structure of the cations or anions. It is estimated that there are more than one thousands of potential cation and anion combinations available. Many ionic liquids have been tested to dissolve cellulose from different types of lignocellulosic biomass and showed different degree of solubility (Table 2).

Table 2: Cellulose solubility in different ionic liquids [28].

Ionic Liquid	T (°C)	Solubility (%w)	Type of Cellulose
1-butyl-3-methyl imidazolium benzoate	70	12	Microcrystalline
1-ethyl-3-methyl imidazolium chloride	90	5	Microcrystalline Avicel
1-ethyl-3-methyl imidazolium acetate	85	13.5	Eucalyptus Pulp
triethyl-2-(2-methoxy ethoxy) ethan ammonium acetate	110	10	Microcrystalline Avicel
1-butyl-3-methyl imidazolium chloride	100	10	Dissolving Pulp
1-ethyl-3-methyl imidazolium chloride	90	5	Microcrystalline Avicel

The first report on cellulose dissolution in ionic liquids was published in 2002 [29]. In this study, ionic liquids combining 1-butyl-3-methyl imidazolium cation with different anions were investigated as solvents of cellulose. It was found out that chloride, as a small hydrogen bond acceptor, was the most effective anion to dissolve cellulose in comparison to large, non-coordinating anions. Since then, many ionic liquids have been reported in the literature with the ability to efficiently dissolve cellulose, such as the ones with halide counter ions like 1-butyl-3-methyl-imidazolium chloride and other counter anions such as phosphate, formate and acetate [30]. In many studies about ionic liquids, it was foreseen that the anion is greatly importance and responsible for the dissolution of cellulose and the role of the cation was not that important. Nowadays, the maximum values of solubility of cellulose were found to be 14.5 wt % for 1-allyl-3-methylimidazolium chloride at 80°C and 16 wt % for 1-ethyl-3-methylimidazolium acetate at 90°C.

3 Pitfalls of Ionic liquid Applications in Hydrolysis Process

Ionic liquids have excellent properties for pretreatment, however there are several drawbacks. The ionic liquid containing halide anions is relatively high viscous, which brings operational complication to the dissolution process. However the ionic liquid with anion such as acetate, formate and phosphate possesses lower viscosity that facilitate its benefit to various applications. Besides, most of ionic liquids are currently not feasible for large scale operation due to relatively high cost, large loading requirements, and need to develop feasible recycling process. Currently, many studies have been investigated the ionic liquid applications in pretreatment of various types of lignocellulosic biomass, and those results suggested the high potential of ionic liquid pretreatment based on efficiency to recover sugar from the biomass [31]–[35].

In nature, cellulose biomass is hydrolyzed and degraded by microorganisms that produce many types of cellulolytic enzymes (Table 3). Both cellulose and hemicellulose can be hydrolyzed and converted to the sugar monomer using a set of enzymes, for example, endo-1-4,-β-xylanase, β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase and acetylxylan esterases [6]. In nature, lignocellulosic biomass are degraded through microbial activities that produce either single cellulase enzyme or multienzyme complexes (so called cellulosome) [36]. However, the topology and chemical properties of biomass are not susceptible to cellulase degradation, for example, surface area is limited to enzyme access and the presence of cellulase inhibitor [12], [37]. The reason for indigestible properties is the outcome of natural selection that plants need to have their own protection from invasion of natural predators or pathogens as well as stress from various environmental conditions.

Table 3: Example of cellulolytic enzymes to hydrolyze different types of biomass

Microbes	Types of Enzymes	Reference
Trichoderma Reesei Zu-02	Cellulase	[38]
Aspergillus niger expressing Helicobacter jecorina Endoglucanase Cel 7B	Cellulase	[39]
Penicillium echinulatum	Endoglucanase, Betaglucosidase, Xylanase	[40]
Neurospora crassa	Endoglucanase, Exoglucanase, Betaglucosidase, Xylanase	[41]
Clostridium sp. TCW1	Endoglucanases, Exoglucanase, B-glucosidase	[42]
Rhodothermus marinus thermus thermophilus	Cellulases, Hemicellulases	[10]

Interestingly, one of important disadvantages is the inhibitory effect of ionic liquid that impairs or reduces the activity of cellulase or the growth of microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* [43], [44]. This inhibition affects to the overall performance of biorefinery as it reduces the success of hydrolysis and fermentation. To recover cellulose from ionic liquid-pretreated mixture, water could be simply added as an anti-solvent, and cellulose will be precipitated easily [25]. The ionic liquid residues remaining in the washed mixture modify the ion environment in the mixture that may not suitable to the function of cellulase enzyme. Therefore, it is recommended to wash the pretreated mixtures several times to make sure that ionic liquid residues are completely removed. It has been previously shown that only 10% v/v of IL residue can reduce efficiency of commercial cellulase enzyme for 70–90% [45]. Moreover, during washing process, there is a loss of costly ionic liquid. The inhibition effect of ionic liquid to cellulase is also the caused by other properties, for example, inhibition in electron transfer at active sites of enzyme, viscous character of ionic liquid, unsuitable pH, ampliphilicity [46].

Several strategies have been developed to overcome these challenges of using ionic liquid-pretreatment with the high efficient hydrolysis in biorefining process, for example developing the enzyme that are tolerant to ionic liquids [47]–[50], identifying metabolic pathways or enzyme properties that improve tolerance to ionic liquids [51], [52]. Park *et al*. 2012 developed cellulase cocktails by combining different classes of glycoside hydrolases obtained from thermophilic bacteria. The formulated cellulase cocktails can tolerate to high temperature condition and 20% v/v ionic liquid-solution [47]. Another study identified *Bacillus* sp MSL2 for cellulase production. The purified cellulase enzyme was tested against an ionic liquid, and its cellulase activity was reduced to 77.7% in 1.0 M 1-ethyl-3-methyl imidazoliumacetate solution, while activity of cellulase isolated from *Bacillus* sp B12 has only 65.72% in 1.0 M 1-ethyl-3-methyl imidazoliumacetate solution [49]. These two types of cellulases were demonstrated to maintain their activities better than CelluClast 1.5L, a commercial cellulase [49]. Cellulase produced from *Paenibacillus tarimensis* and *Aspergillus terreus* UniMAP AA-6 retained their activities > 80% and > 60% in 20% 1-ethyl-3-methyl imidazoliumacetate (~1.2 M), respectively [53], [54]. In another case, cellulase of *Aspergillus fumigatus* has > 50% efficiency in 30% of 1-ethyl-3-methyl imidazoliumacetate (~1.8 M) [48]. These observations suggested that each type of cellulase enzymes has different tolerant level to different types of ionic liquid.

Previously, several studies demonstrated that salt-tolerance could be correlated with ionic liquid-tolerance because; 1) ionic liquid is liquid salt, 2) several evidences showed that salt-tolerant cellulases can tolerate to ionic liquid. For example, *Paenibacillus tarimensis* isolated from Tunisia salt sea produced cellulase that retained its activity up to 40% in 40% 1-butyl-3-methyl imidazoliumchloride solution [55]. Similarly, later on, several salt-tolerant bacterial strains, such as *Bacillus subtilis*, *Bacillus altitudinis* and *Halomonas* spp were isolated and their cellulases showed that more than 75% of cellulase activities still maintained in 1.0 M 1-ethyl-3-methyl imidazoliumacetate solution [56].

4 Conclusion

Ionic liquid pretreatment received intention from R&D section in this decade. From 1-butyl-3-methyl imidazoliumchloride, the first ionic liquid used in lignocellulosic pretreatment [29], until now there are various types of ionic liquids have been used in pretreatment, especially 1-ethyl-3-methyl imidazoliumacetate [24], [25], [57]. Ionic liquid pretreatment modifies cellulose fibril structures from crystalline structure to amorphous structure that is more susceptible to enzymatic hydrolysis. Additionally, ionic liquid can solubilize cellulose and therefore can improve accessibility of enzyme to substrates. However, one of the major limitations of ionic liquid in biorefinery is its inhibitory effect in hydrolysis and fermentation. The development of ionic liquid-tolerant cellulases that can function in ionic liquid-containing solution is a target to meet the requirement of biorefinery process, especially conversion of lignocellulosic biomass to biofuels or bioproducts. These enzymes can be applied in consolidated processing, especially in a “one-pot” reactor that combined pretreatment and hydrolysis steps together, which these two steps are currently considered as rate-limiting step of the whole process.

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