# Challenges of Chemical Pretreatment of Lignocellulosic Biomass for Biofuel Production

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**Structured Abstract:**

**Purpose:** Chemicals have been utilised to deconstruct the structural and chemical complexity of lignocellulosic biomass for application in the production of biofuel. This paper appraises the impacts and challenges of these applications.

**Method:** Review of relevant literature.

**Findings:** Alkali and acid pretreatments are effective in destroying the lignin complexes and releasing carbohydrate for subsequent hydrolysis. The use of acids has been impeded by generation of inhibitory by-products during digestion or fermentation; while NaOH pretreatment is accompanied by salt build up and elevation of digestate pH, which is detrimental to methanogenesis. A disadvantage associated with acids and alkali is the destruction, instead of separation of lignin polymers because of their potential use as biopolymers. Organosolv and ionic liquid (IL) pretreatments are emerging procedures having efficiencies comparable to acids and alkali with insignificant production of inhibitory or refractory substances. Ionic liquid and organosolv merited for recovery of lignin and the IL/solvent used could be recycled.

**Originality:** Careful selection of pretreatment temperature is beneficial to biofuel production because at extreme temperatures, impediments are likely. High energy expenditure is associated with most of the pretreatment, thus threatening their feasibilities. Exploration of new and cheaper pretreatment substances with optimum properties than existing substances is recommended.

**Key words:** Pretreatment, Lignocellulose, Chemical, Challenges, Digestion

## Introduction

At present, raw materials for biofuel constitutes 40-80% of overall production cost (Chang *et al*., 2010) signifying the need for exploring cheap sources. The utilisation of renewable energy sources is becoming increasingly necessary in attempts to attain the changes required to address the effects of global warming (McKendry, 2002). Biofuels (biogas, ethanol and biodiesel) have the potential of supplying enormous and environmental friendly energy likely to replace fossil fuel. The cost and technology have impeded the viability of these sources.

Pretreatment of crop residues receives attention because of the potentials of biomass as substrates in biofuel production. This attention is particularly to deconstruct the complexes of lignin, hemicellulose and cellulose into digestible sugars that could be attacked by microorganisms. Furthermore, the use of residues and wastes is recommended to avoid undesirable land-use change, and food security effects due to application of crops to produce biofuel (Smeets, 2015). Food crops produce energy at minimal cost, raising argument that questions whether these applications may not affect global food production, availability and affordability. This brings the need to develop cost-effective technologies to deal with recalcitrance of lignocellulosic substrates in bioenergy production. Improved and cheap pretreatment procedures of biomass that allow their use in co-digestion with animal wastes are promising attempts to attain these challenges.

Anaerobic digestion (AD) and fermentation are cost-effective bioconversion technologies of all the available technologies (Zheng *et al.,* 2014). The digestion or fermentation of single homogenous substrate from animal wastes is associated with ammonium toxicity due to rapid degradation of organic nitrogen and remains a consistent challenge in biofuel production (Pan *et al.*, 2015). Generally, in mono digestion, there was high degradation of lipids and accelerated conversion of long-chain fatty acids which are important inhibitors (Rasit *et al.*, 2015; Wu *et al.,* 2016). Co-digestion of animal wastes and AL is likely to impede the production of $NH_4$, by improving the equilibrium of carbon/nitrogen ratio (Pan *et al.*, 2015; Ward *et al.*, 2008).  More so, incorporation of AL in co-digestion supplements substrate requirements during summer when animal slurries are not sufficient (Adouani *et al.*, 2016). On the overall, co-digestion with the biomass improves the efficiency and yields of AD (Yang *et al.*, 2015). Chen *et al.* (2010) compared mono and co-digestion of vermicompost and corn stalks and found that co-digestion improves biodigestibility and methane production. Despite these merits, the application of lignocellulose in biofuel production is challenged by its chemical composition and physical structure.

Before discussing any pretreatment technology, it is vital to briefly note the slim difference between biogas production through volatile fatty acid (VFA) platform and fermentation (sugar platform) for bioethanol or hydrogen production. Anaerobic digestion is a "process under controlled conditions where free oxygen is absent and temperatures are suitable for naturally occurring mesophilic or thermophilic anaerobic and facultative bacteria and archaea species, which convert the inputs to biogas and whole digestate" (Suanon *et al.*, 2017). It is

carried out through four stages: hydrolysis, acidogenesis (fermentation), acetogenesis and methanogenesis. While fermentation for bioethanol production involves the processes of hydrolysisand acidogenesis (Domínguez *et al*., 2017; Zhen *et al*., 2017). The AD is strict anaerobic process while fermentation could be partially aerobic. The two processes operate under entirely opposite pH requirements. Anaerobic methane production requires a pH range of 6.8-7.6, but ethanol production tolerates as low pH value as 3 (Gulhane *et al.* 2017; Mattila *et al.*, 2017). The implication is that pretreated substrates suitable for bioethanol production may not always be suitable in the production of biogas. The objectives of this paper are to assess chemical pretreatment methods of agricultural residues and to highlight impacts and challenges encountered in each method and proffer possible solutions.

**Biomass Composition**

Biomass is hydrocarbon material consisting of carbon, hydrogen, oxygen, small traces of sulphur, nitrogen and significant quantities of inorganic species (Yaman, 2004). Agricultural wastes (plant residues and by-products) are collectively referred as lignocellulosic biomass. They have cell walls containing organic components: cellulose, hemicellulose and lignin making-up 75% of the biomass dry matter (Van Acker *et al.,* 2016; Xie *et al.*, 2011).

**Cellulose**

Cellulose is a polysaccharide and water insoluble substance with the general formula $(C_6H_{10}O_5)_n$. It has an average molecular weight of 300,000-500,000 and constitutes approximately 50% of the cell wall (Yaman, 2004), whose structure is shown in Figure I. Its size is defined by average number of monomer (glucose) units referred as degree of polymerization. Cellulose is a linear polymer with cellulobiose as the repeating unit and, the intra- and inter-molecular hydrogen bonds stiffen the cellulose chain and promote crystallinity (Hu, 2014).
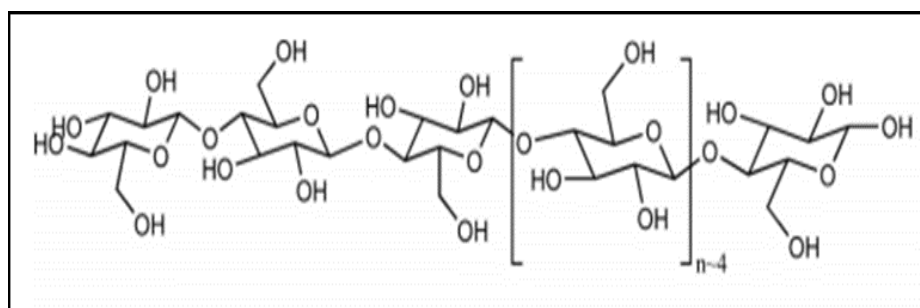


Figure I Chemical structure of cellulose (Mora-Pale *et al*., 2011)

**Hemicellulose**

Figure II shows the chemical structure of hemicellulose $(C_5H_8O_4)_n$, a complex polysaccharides found with cellulose in the cell wall. Unlike cellulose, hemicellulose is soluble in dilute alkali and consists of branched structures, varying considerably among different woody and herbaceous species. Hemicelluloses usually carry 50–200 monomeric units and a few simple sugar residues (Yaman, 2004).
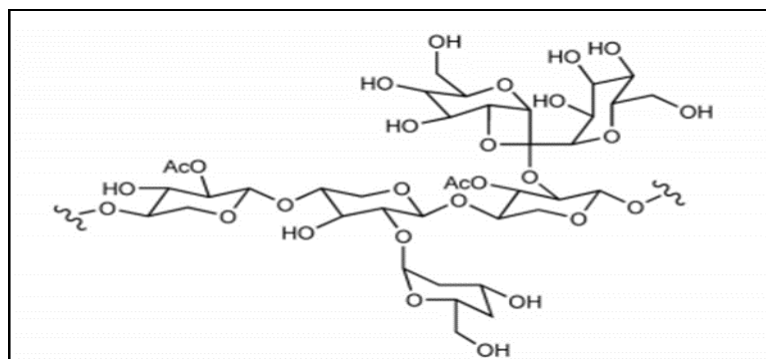


Figure II Chemical structure of Hemicellulose (Mora-Pale *et al.*, 2011)

**Lignin**

The lignin (Figure III) is highly branched, substituted, mononuclear aromatic polymers in the cell wall bounding adjacent cellulose fibres to form a lignocellulosic complex. This complex resist conversion by microbial systems and many chemical agents. The lignin contents on dry basis in both soft and hardwoods ranged from 20-40% by weight and from 10-40% by weight in various herbaceous species, such as bagasse, corncobs, peanut shells, rice hull and straws (Biswas *et al.*, 2015a; Yaman, 2004).
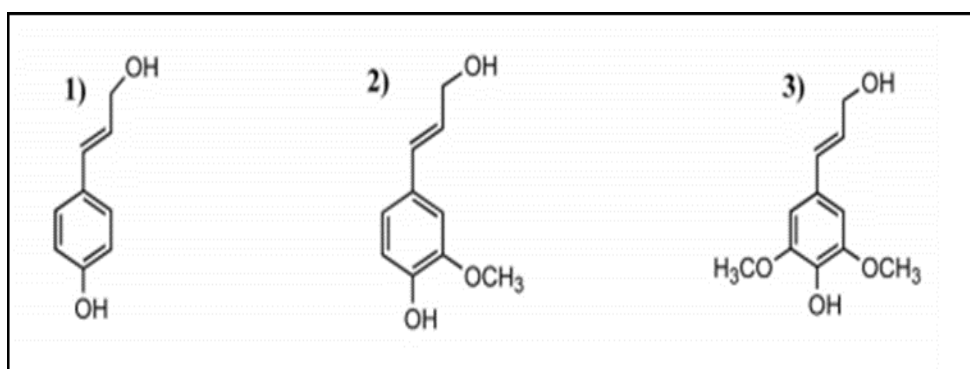


Figure III Chemical structure of lignin (Mora-Pale *et al.*, 2011)

Three forms of lignin monomers represented are: (1) p-hydroxyphenyl alcohol; (2) coniferyl alcohol; and (3) synapyl alcohol.

The recalcitrant lignin, hemicellulose and cellulose are present in almost all plant residues. However, this complex differs from plant to plant (Table I) and on the extent of maturity of the plant/tree (Tsapekos *et al.*, 2016).

Table I Carbohydrate content of some agricultural residues

| Lignocellulosic Materials | Cellullose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|
| Hardwoods stems | 40–55 | 24–40 | 18–25 |
| Softwood stems | 45–50 | 25–35 | 25–35 |
| Nut shells | 25–30 | 25–30 | 30–40 |
| Corn cobs | 45 | 35 | 15 |
| Grasses | 25–40 | 35–50 | 10–30 |
| Paper | 85–99 | 0 | 0–15 |
| Wheat straw | 30 | 50 | 15 |
| Sorted refuse | 60 | 20 | 20 |
| Leaves | 15–20 | 80–85 | 0 |
| Cotton seed hairs | 80–95 | 5–20 | 0 |
| Newspaper | 40–55 | 25–40 | 18–30 |
| Waste papers from chemical pulps | 60–70 | 10–20 | 5–10 |
| Primary wastewater solids | 8–15 | N/A | 24–29 |
| Swine waste | 6.0 | 28 | N/A |
| Solid cattle manure | 1.6–4.7 | 1.4–3.3 | 2.7–5.7 |
| Coastal Bermuda grass | 25 | 35.7 | 6.4 |
| Switch grass | 45 | 31.4 | 12.0 |

(Source: Stamatelatou *et al.*, 2012)

The molecular structure of the lignocellulosic biomass is poorly accessible to microorganisms and their enzymes. Its high crystalline structure and/or low surface area may be responsible for its resistance to hydrolysis (Montgomery and Bochmann, 2014). Although, pretreatment may end up altering only one component (Zhao *et al.,* 2012b), the complex can be broken, and the lignin fraction separated by pretreatment (Agbor *et al*., 2011). Biomass pretreatment not only affect the enzymatic hydrolysis of the substrates but dictates the downstream processing, commercial scalability, and pathway of biorefinery process (Zhu *et al.*, 2010). Therefore, there is need for more data and improvement to guarantee a better understanding and easy extraction of the lignin complexes.

## Pretreatment

Bioconversion of AL into biofuels is significantly impeded due to their chemical and structural complexities. This challenged the use of lignocelluloses as feedstock in biogas production. Cellulose and hemicellulose transformed into sugars are easily converted to biofuels through established technologies, by adjusting the surrounding matrix before enzymatic conversions of the polymers (Zheng *et al.*, 2009). Pretreatment is thus a process which converts lignocellulosic biomass from its recalcitrant form into cellulose enzyme systems, and makes hydrolysis more effective (Zheng *et al.*, 2009). Alternatively, pretreatment is described by Zhao *et al.* (2012a) as the process which disrupts the compact structure of lignocellulosic biomass and exposes cellulose fibres, attainable through mechanical comminution, chemical alterations of biomass compositions, biological decomposition, or a combination of these methods. Pre-treatment could be any process that modifies indirect factors and improve direct factors which increases the availability of cellulose and hemicellulose for microbial activities (Zhao *et al.*, 2012b). Structural complexities are the main factors limiting hydrolysis of lignocelluloses (Zhao *et al.*, 2012b). Esteghlalian *et al.* (2001), Yejun and Hongzhang (2007), Chang and Holtzapple (2000), Himmel *et al.* (1994) and Alvira *et al.* (2010) concluded that digestibility of lignocellulosic substrates is a function of factors such as contents of lignin, hemicelluloses, acetyl group and cell wall proteins, crystallinity, particle size and cell wall thickness. Figure IV shows the typical depiction of what happens before and after pretreatment of lignocellulose.
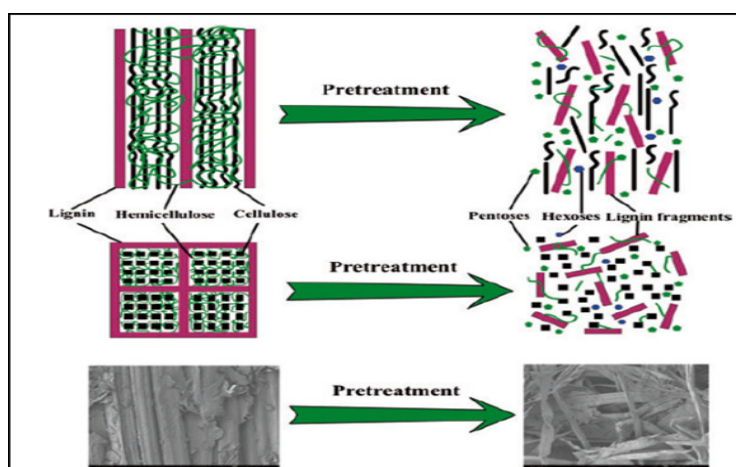


Figure IV Structural deconstruction of lignocellulose by pretreatment (Zhao *et al.* 2012a)

A variety of potential inhibitors are produced during pretreatment of AL, a major challenge especially if chemicals are involved. Therefore, Du *et al.* (2010) suggested that qualitative

and quantitative examination of pretreatment hydrolysates are vital in identifying potential correlations between pretreatment chemistries and microbial inhibition in the downstream bioconversion processes. As technologies for bioconversion of lignocellulose become more established and shifted from laboratories to the context of real application, the significance of the management of inhibitions is likely to increase. Because the application of the recalcitrant feedstocks becomes increasingly relevant to obtain high product yields even with high solid loadings (Jönsson and Martín, 2016).

Montgomery and Bochmann, (2014) reported that the toxicity of inhibitors to yeasts is known, but it is not completely clear if they inhibit all AD microorganisms, or they are simply very difficult to degrade anaerobically. An indication that the impacts of these inhibitions on *Clostridia*, *Bacteroidetes* and the archaeal communities usually responsible for digestion is not clearly understood (Ferguson *et al.,* 2014). The inhibitory effect on methane producing microorganisms have not been reported adequately in the literature (Cybulska *et al.,* 2016), probably due to its insignificance as suggested by Schroyen *et al.* (2014).

**Types of Pretreatment**

In principle, pretreatment processes are grouped into 4 broad categories. The physical pretreatment includes mechanical, thermal, ultrasound and electrochemical techniques. Chemical pretreatment comprised the acid, alkaline, ionic liquids and oxidative techniques. Biologically, enzymes and wood degrading organisms such as white, brown, soft-rot fungi and bacteria are applied to modify the chemical and structural composition of the biomass for easy digestion. Steam explosion, extrusion and thermochemical are considered combined techniques (Montgomery and Bochmann, 2014; Pan *et al.*, 2015; Zheng *et al.*, 2009). The next section discusses five forms of chemical pretreatment: alkaline, acid, ionic liquids (ILs), organosolv and combined thermochemical pretreatments. Generally, before most of these chemicals are applied, size reduction is often carried out.

**Impacts and challenges chemical pretreatment of lignocellulose**

**Chemical Pretreatment**

Chemical pretreatment usually follows size reduction which obviously distorts the recalcitrance and improves the surface area. Montgomery and Bochmann (2014) report that chemical pretreatment has not been carried out at large scale for biogas production. However,

it has widely been used in the production of ethanol. In this section, the impacts and challenges of chemical pretreatments namely, alkaline, acidic, organosolv, IL and combined thermochemical pretreatment are discussed.

**Impacts and Challenges of Alkaline Pretreatment**

Evidences suggest that alkaline pretreatments are cost-effective and therefore preferred chemical pretreatment agents (Ariunbaatar *et al.* 2014; Kamali *et al.*, 2016). Alkali such as sodium hydroxide (He *et al.,* 2008), potassium permanganate (Ma *et al.*, 2015), aqueous sodium sulphide (Qing *et al.* 2016) and potassium hydroxide (Jaffar *et al.*, 2016) are effective pretreatment substances. In an excellent examination, Carrere *et al.* (2016) concluded that alkali and biological pretreatments are more dependable for application on lignocelluloses. High NaOH concentrations during pretreatment improves digestion and methane production. Specifically, a dose of 6% (w/w) appears the best treatment but some authors have reported variable doses and concentrations (Forster-Carneiro and Isaac, 2012; Janke *et al.,* 2015). Capabilities of alkali to degrade lignin, neutralize organic acids and mitigate inhibition during digestion are interesting (Antonopoulous *et al.,* 2016; Forster-Carneiro and Isaac, 2012). If VFA platform is used, NaOH is useful in determining the production of VFAs which is directly correlated with NaOH concentration (Park *et al.*, 2015). In slight contrast, Singh *et al*. (2015) reported that alkali is specifically effective on low-lignin biomass (hardwood, herbaceous crops and agricultural residues) than high-lignin biomass. This highlights the limitation of alkali pretreatment. Figure V (a-c) is the scanning electron microscopy (SEM) images of the untreated biomass showing vascular elements packed in bundles with relatively flat and clean surfaces.
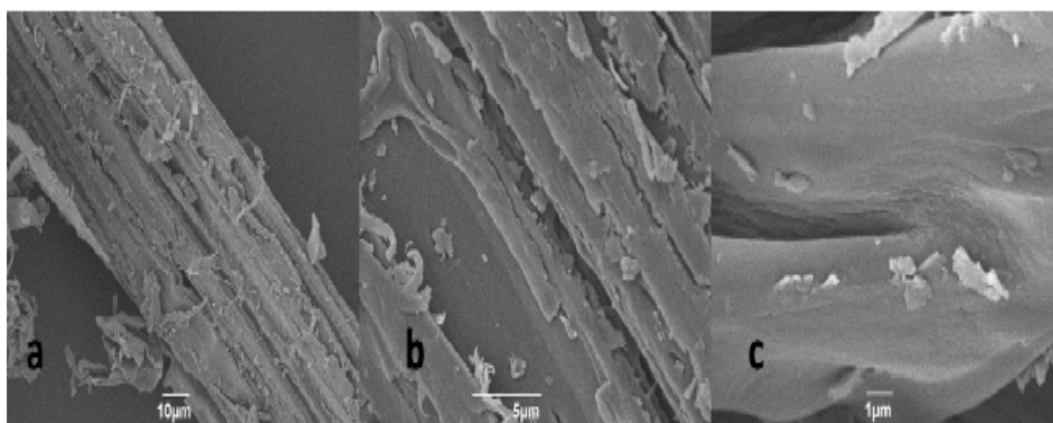


Figure V SEM images of untreated *Miscanthus* (Gabhane *et al.,* 2015)

The SEM images in Figure VI are NaOH pretreated biomass at two different concentrations showing damaged coatings. The pretreatment is morphologically pronounced.
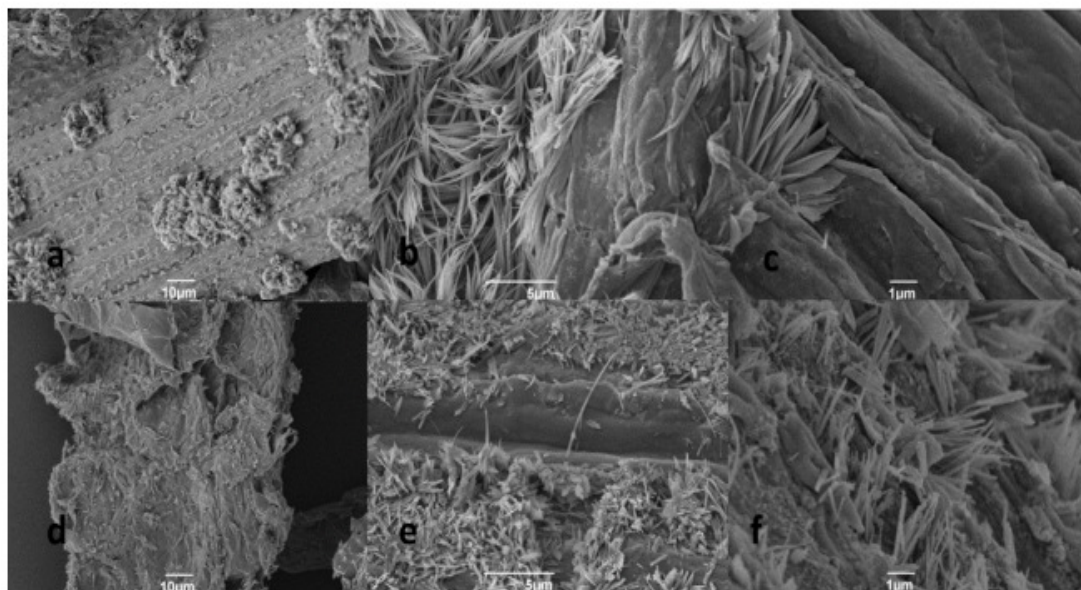


Figure VI SEM images of Miscanthus treated with 0.2 M (a-c) and 0.4 M NaOH (d-f)
(Gabhane *et al.,* 2015)

Investigation by He *et al.* (2008) explored the influence of solid-state NaOH pretreatment on rice straw during biogas production. Fourier transform infrared, hydrogen-1 nuclear magnetic resonance spectrometry, x-ray diffraction and gas permeation chromatography were used to assess changes in chemical and physical characteristics of lignin, hemicellulose and cellulose. Results showed that biogas produced by rice straw treated with 6% NaOH increased by 27.5%. Degradation of 16.4% cellulose, 36.8% hemicellulose and 28.4% lignin were observed and water-soluble substances were by 122.5%. Moreover, the ester bond of lignin-carbohydrate complexes was destroyed, releasing more cellulose. Linkages of interunit and functional groups of lignin, cellulose and hemicellulose were broken down, leading to significant changes of chemical structures. Pretreatment reduces large molecular weight and 3-dimensional network of lignin to small molecular weight and linear structures. The changes in chemical compositions and structures, and physical characteristics made the biomass more susceptible to biodegradation and were responsible for optimisation of biogas production (He *et al.,* 2008). In support of these findings, similar study by Su *et al.* (2015) using alkaline hydrogen peroxide, report high lignin deconstruction, cellulose and hemicellulose depolymerisations and improved biogas production.

On the contrary, Mahdy *et al*. (2014) evaluated low temperature auto-hydrolysis at 50$^o$C incubated at 24 and 48-hour with (0.5, 2 and 5% w/w NaOH) doses. It was found that improvement in methane production was not significant because organic matter solubilised were more of exopolymers released during pretreatment than intracellular materials. In support of Mahdy, Monlau *et al*. (2015) indicate that alkaline pretreatment fails to improve methane yield and, hemicellulose was more effectively digested than the cellulose, while lignin was not affected. This inconsistency is probably due to the differences in the pretreatment temperatures and doses of NaOH / other alkali used in the studies.

These studies show that any alkali could be used in pretreatment, NaOH being the most popular. There are many undesirable processes such raise of substrates pH and lately during digestion, salt build-up may be significant and probably inhibit methanogenesis due to imbalance of ammonium and ammonia (Montgomery and Bochmann, 2014). Another major weakness of alkali pretreatment is the destruction of lignin instead of separation, which allows lignin to be used as biopolymers. Moreover, cost may limit the use alkali as pretreatment substance. However, it may be beneficial in the treatment of high lignin and acidic substrates (Montgomery and Bochmann, 2014), in contrast to the assertion of Singh *et al*. (2015). This argument suggests that pretreatment procedures should be carefully evaluated and selected for effectiveness.

**Impacts and Challenges of Acid Pretreatment**

A great deal of previous and recent studies on acid pretreatment of lignocelluloses focus on their impacts on chemical composition and physical structures (Amnuaycheewa *et al*., 2016; Mirmohamadsadeghi *et al*., 2016; Petersson *et al*., 2007; Taherzadeh and Karimi, 2008). Figure VII is a schematic diagram of dilute acid pretreatment of lignocellulose and component extraction.
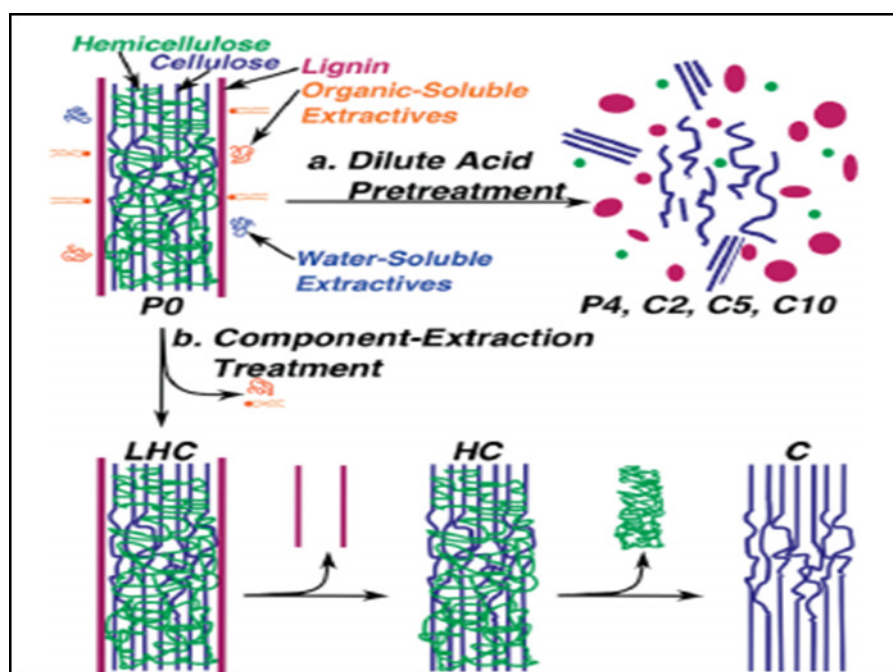
Figure VII Sketch of dilute acid pretreatment and component extraction (Pingali *et al.,* 2010)

Kim *et al.* (2003) pretreated corn stover with aqueous ammonia in a flow-through column reactor, a process named ammonia recycled percolation and found substantial (70-85%) removal of lignin after 20 minutes. Findings indicated that the process solubilised about 40-69% of hemicellulose, leaving cellulose unaffected. In slight contrast, concentrated phosphoric acid was used in the pretreatment of pine, poplar and berry (Mirmohamadsadeghi *et al*., 2016). Methane production was not improved by pretreatment of hardwood but significantly improved for pine. Compositional analyses showed remarkable reduction of hemicellulose and lignin contents, and accessible surface area was massively improved identical to the images in Figure VIII when $H_2SO_4$ was used. In a similar study, there was observed significant delignification after pretreatment with organic solvents and $H_2SO_4$ as catalyst (Teramura *et al*., 2016). Notwithstanding, Carrere *et al*. (2015) was critical on the use of acid pretreatment of lignocelluloses but preferred alkali and fungi. Moreover, Battista *et al*. (2016) compared the basic and acid pretreated coffee and found that the basic pretreated coffee produced the highest biogas constituting about 79% v/v of methane under mesophilic condition. Major weakness of this claim is that the type of acid and base used were not stated and the basic pretreatment reported by the author is generic. It could mean size reduction or use of alkali which may be due commercial confidentiality.

The study by Kim *et al.* (2003) demonstrated the efficiency of ammonia in improving digestion. However, pretreatment temperature was not mentioned and the energy input normally affects cost of pretreatment. Additionally, digestibility was estimated using

empirical means instead of setting up pilot study to assess the real digestibility of the substrate.
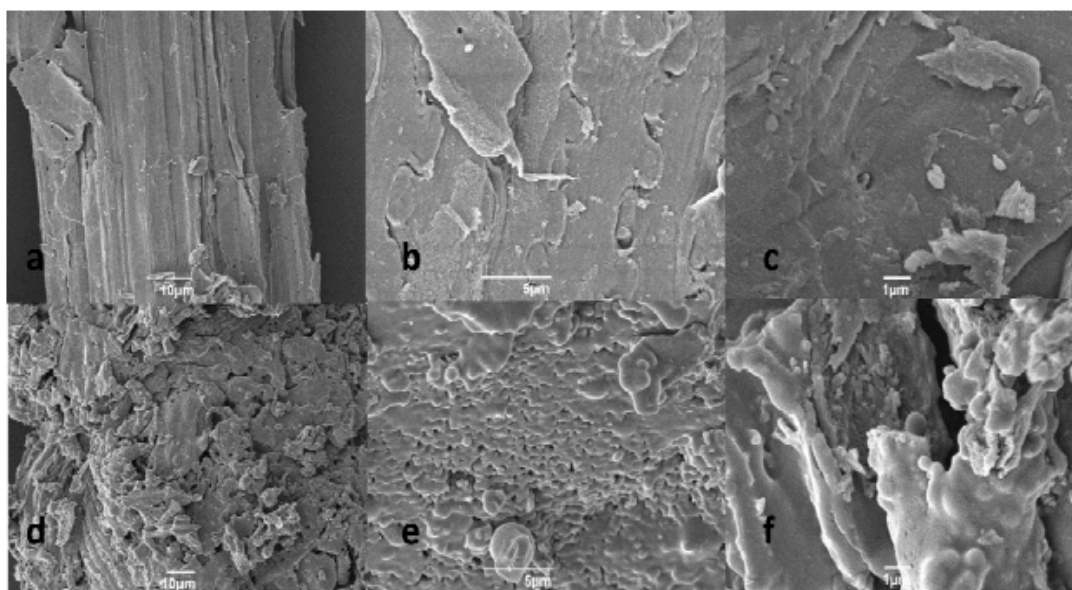


Figure VIII $H_2SO_4$ pretreated Miscanthus with 0.2 M (a-c) and 0.4 M (d-f) (Gabhane *et al.,* 2015)

Notwithstanding the range of benefits attributed to this method, there are many challenges likely to be encountered. Firstly, it could be noticed from the literature that acid do have little or no impact on cellulose, prompting the use cellulase or other substances to break them further. The presence of significant quantity of Klason lignin as constituent of most lignocelluloses is a source of bottleneck associated with acid pretreatment. The pseudo lignin chemically behaves like lignin. These substances (klason lignin and pseudo lignin) in totality are not soluble during pretreatment under acidic condition. Any formation of lignin-like material is more likely to be unfavourable for enzymatic hydrolysis (Sannigrahi *et al.* 2011). Therefore, destruction of acid soluble lignin and inability to breakdown or destroy some forms of lignin are potential concerns of the use of acids (Montgomery and Bochmann, 2014).

The formation of pseudo lignin by combination of carbohydrates and degradation products (furans, phenols, aldehydes and other insoluble degradation products) is detrimental to the process triggering irreversible cellulase loss (Gao *et al.*, 2014; Jönsson, 2016; Kumar *et al.*, 2012). It was put forward that benzoquinones and small aliphatic aldehydes likely contribute to inhibition (Jönsson, 2016), but this paper argued that the claim needs additional examination. The use of 1% $HNO_3$ led to higher furfural and lower hydroxylfurfural

production and at 0.5% $HNO_3$, the trend reversed. It was reported that furfural is more effective inhibitor than hydroxylfurfural and at 0.5% $HNO_3$ there was higher production of VFA as opposed to 1% $HNO_3$ (Park *at al*., 2015). This suggests that the use of 0.5% $HNO_3$ is likely to be appropriate concentration than any other.

Conversely, recent study indicated that residual lignin can boost the capability of oxidizing enzymes to effect higher conversion of recalcitrant complexes to sugars, obviously with no adverse consequence of lignin content during hydrothermal pretreatment of sugarcane bagasse, corn stover, and wheat straw (Rodríguez-Zúñiga *et al.*, 2015). Moreover, adjustment of pretreatment conditions could mitigate this scenario (Kumar *et al*., 2012) and co-digestion is suggested as possible remedy to inhibitory effects (Ward *et al*., 2008).

Perhaps the most serious disadvantage of the use of acid is its corrosiveness which necessitates the use of very expensive non-corrodible materials in the manufacture of pretreatment materials and digesters. Moreover, suggestions indicated the need to recycle the acids due to economic reasons (Kim, 2011; Lee *et al*., 2011; Wang *et al*., 2011). Sighn*et al.* (2015) reported that these effects could be minimised by combination of concentrated acid and alkali or dilute acid and alkali or alternatively, the use of dilute acids instead. However, cost of pretreatment substances may limit the use of this proposal. Furthermore, dilute acid alone may be appropriate especially in the production of bioethanol because the process tolerates low pH.

**Impact and Challenges of Ionic Liquid (IL) Pretreatment**

The application of ILs which are usually liquids at ambient temperature have been studied (Gao *et al.,* 2013a; Tadesse and Luque, 2011; Zheng *et al.,* 2014). Substances such as 1-*n*-ethyl-3-methylimidazolium acetate (Singh *et al*., 2009), 1-ethyl-3-methylimidazolium acetate (Fu and Mazza, 2011; Lee *et al.*, 2009; Li *et al*., 2010), 1-ethyl-3-methyl imidazolium diethyl phosphate (Li *et al.*, 2009), 1-butyl-3-methylimidazolium chloride, 1-ethyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium hydrogen sulphate (Nguyen *et al.* 2010) and many other ILs have been used in the pretreatment of different lignocelluloses. Impacts of several IL pretreatments on sugar yields from biomass is shown in Figure IX.

The more recent discoveries of dissolution of lignocellulosic materials in ILs, partially separating the main biopolymers, suggest further paths towards realising a truly sustainable chemical and energy economy based on the concept of a bio-refinery which provides

chemicals, materials, and energy (Sun *et al.*, 2011). Although, this dissolution is reported useful for sugar platform (fermentation), the swelling and ballooning effects of ILs are useful for VFA platform in digestion (Jeihanipour *et al.* 2010). Ionic liquid pretreatments are not significantly affected by particle size and feedstocks mixtures (Simmons, 2013) thus reducing the process cost. However, particle size and moisture contained in the biomass were found to affect the dissolution of biomass in the IL (Brandt, 2011). But it is not clear to what extent the moisture or particle size impeded the dissolution, or their impact is synergistic.
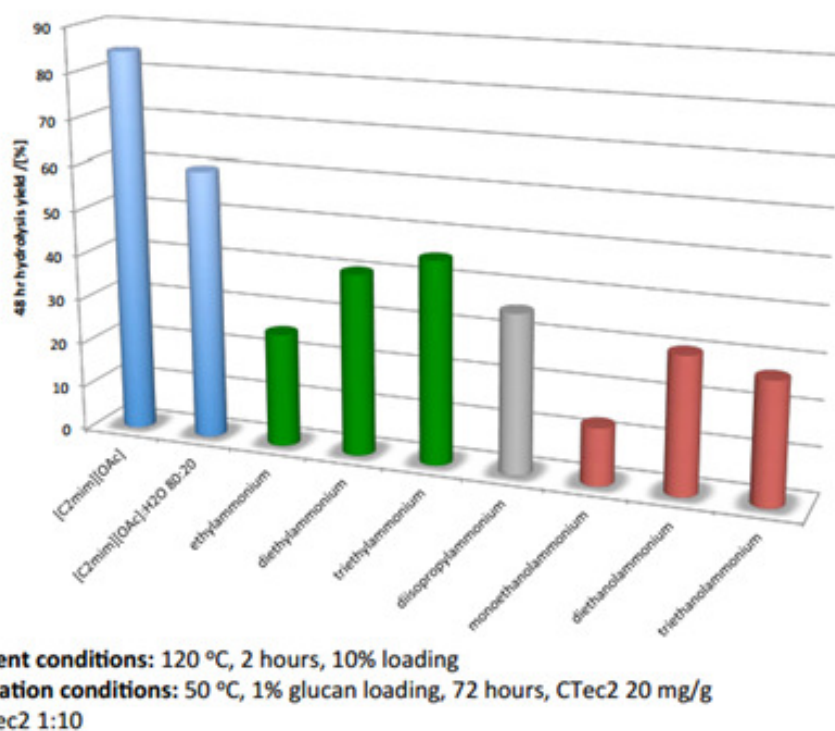


**Pretreatment conditions:** 120 °C, 2 hours, 10% loading
**Saccharification conditions:** 50 °C, 1% glucan loading, 72 hours, CTec2 20 mg/g glucan, HTec2 1:10

Figure IX Effects of various IL substances on sugar yield (Simmons, 2013)

Parthasarathi *et el.* (2016) investigate the impact of tetrabutylammonium [TBA]$^+$ and hydroxide [OH]$^-$ ions on biomass and found approximately 90% glucose yield at 50$^o$C. Energy demand analysis and process modelling indicate 75% reduction of energy requirement in comparison with other methods. It was further argued that [TBA]$^+$ and [OH]$^-$ pretreatment is more effective than other ILs such as 1-ethyl-3-methylimidazolium acetate under milder conditions and less than half of the IL required for effectiveness. These findings indicate the need for re-shaping the current approach in ILs pretreatment studies. This claim is a remarkable progress (considering its affordability and low energy expenditure). However, it is important to substantiate the claim for application in biorefineries.

Previous research comparing dilute acid and IL pretreatments of switchgrass found that IL significantly reduces crystallinity, lignin content and increases surface area (Li *et al.,* 2010), which are consistent with the assertions of Simmons (2013). These advantages enhance enzyme hydrolysis (16.7 fold) and give glucan yield of 96% in 24 h. A study on pretreatment of Eucalyptus *dunnii* bark residues with IL by Reina *et al.* (2016) show similar outcomes. The results of Li *et al.* (2010) appear dependable because adequate analytical methods used likely improve the accuracy of the results and the issues raised regarding inhibitions of bacterial processes in acidic treatments is avoided. Studies by Cheng *et al*. (2011), You *et al.* (2016), Pérez-Pimienta *et al.* (2017), and Weerachanchai *et al.* (2012) report similar findings. The best temperature with this method is around 120°C. At higher temperature (>120$^{\text{o}}$C), there was observed decline of regenerated biomass yield, sugar conversion, lignin extraction, and higher crystallinity index (Cheng *et al*., 2011; Weerachanchai *et al.,* 2012). On the contrary, Doherty *et al.* (2010) obtain no significant difference between the IL maple wood and the untreated samples in terms of crystallinity, lignin removal and conversion of fermentable sugars. These are likely to be due to viscosity and IL strength (Engel *et al.,* 2010). However, it could be addressed using co-solvents (Simmons, 2013), and extraction though at an expense.

Several researchers like Gao *et al.* (2013b), Elgharbawy *et al*. (2016), Li *et al,* (2016) and many studies established a range of advantages of this method; nevertheless, the IL cost, process integration, recovery and recycling are persistent challenges (George *et al.*, 2015; Simmons, 2013). Based on these, Mora-Pale *et al*. (2011) warned that failure to make IL pretreatment economically attractive threatens its application. However, some ILs could contest with a few low-cost pretreatment chemicals in terms of efficiency and process cost. Moreover, recent study indicated that recycled ILs showed similar effectiveness compared with fresh ILs (Widjaja *et al*., 2015), therefore, recycling techniques should be developed to be cheap compared with purchase of new ILs.

Recycling techniques need to be put in place which must allow a cost-effective process. A recycling procedure suggested by Sathitsuksanoh *et al.* (2015) involves the use of additional additives (alcohol) and its application appears to solve viscosity problem as well. Moreover, the technology of aqueous biphasic systems developed to recover IL, with recovery in the range of 54.3–94.6% appears well done (Gao *et al*., 2013b). One-pot lignocellulosic processing using JBEI (Joint BioEnergy Institute) platform technologies, and IL-Ketone-

alcohol system of IL recovery shown in Figure X presented by Simmons could resolve integration of process design and recovery issues.
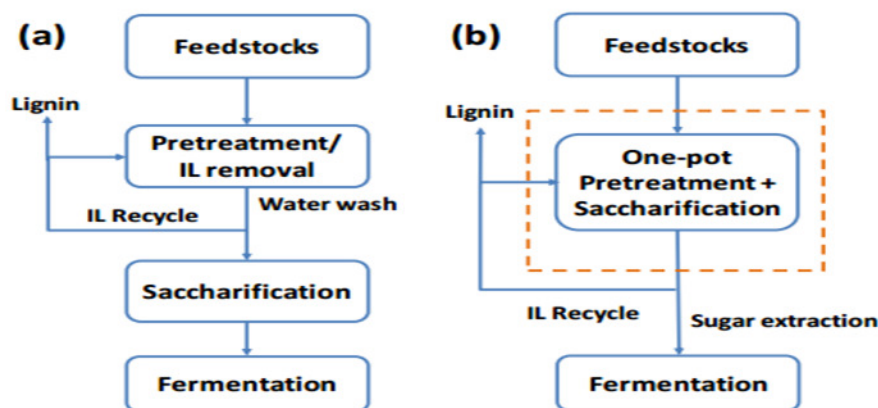


Figure X a) Ordinary sugar platform bioconversion process, b) The optimized one-pot lignocellulosic platform (Simmons, 2013)

According to Vancov *et al.* (2012), after cellulose regeneration from the IL and subsequent evaporation of antisolvent, the IL could be reused up to 4-5 times without affecting sugar yields. Sangian *et al.* (2015) confirmed this assertion when ILs were recycled and compared against fresh ILs to pretreat high lignin coconut coir dust. Lee *et al.* (2009) noted the significance of the reuse of ILs for commercial processing of biomass and attempted to re-apply 1-ethyl-3-methylimidazolium acetate ([Emim] [CH3COO]) in pretreatment. The [Emim] [CH3COO] was initially used to pretreat wood flour and then washed with water to remove the extract from the residual wood flour solids. The water was removed by evaporation from the mixture of water and [Emim] [CH3COO], which contained mostly lignin. The [Emim] [CH3COO] solution (without further purification) was then used to pretreat wood flour resulting in the accumulation of lignin. Although lignin continuously accumulated by repeating this process, the extraction efficiencies remained largely unaffected. Subsequent extraction of lignin and reuse of [Emim] [CH3COO] are facilitated by the high solubility of lignin in [Emim] [CH3COO]. The data in Figure XI is obtained by Nguyen *et al.* (2010) indicating higher prospects for recycling of ILs since they are considered expensive. However, authors were not able to explain their procedures to arrive at such interesting results.
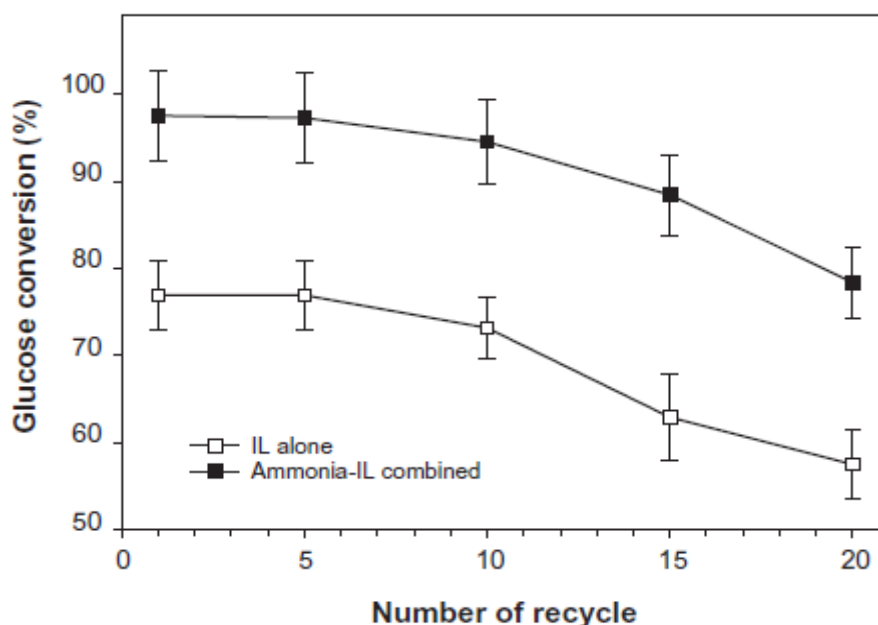
Figure XI Efficiency of the recycled 1-ethyl-3-methylimidazolium acetate ([Emim] Ac) (Nguyen *et al*., 2010).

An alternative solution to cost and probably other drawbacks of ILs is the discovery of new ILs with optimal properties (George *et al.* 2015; Simmons, 2013). A techno-economic analysis of an optimized one-pot configuration (OOPC) shows significant economic and environmental benefits for cellulosic biorefineries. The OOPC achieved a reduction of the IL required by 90%, reduction of wastewater generation by 85%, and reduction of overall cost of ethanol production by 40%. Moreover, net energy use, greenhouse gas emission, chemicals for wastewater treatment and waste generation were effectively minimised (Xu *et al*., 2016).

Irrespective of what the recycling procedure appears, the use of additional substance likely increases the cost of pretreatment. Additionally, there may be the drawback of making the process a bit more, if not significantly complex than ordinarily using ILs. An argument put forward by Allison *et al.* (2016) that there are unknown factors causing IL pretreatments adversely affect digestion of pretreated materials should not be overlooked. This further suggests that there could be significant possibility of increasing the inhibitions unless thorough chemical analyses of these additives prove otherwise.

**Impacts and Challenges of Organosolv Pretreatment**

Organosolv is the pretreatment of lignocellulosic feedstocks in organic solvents or their aqueous solutions in the temperature range of 100–250°C (Zhao *et al.* 2012b). Pretreatment

potentials of organic solvents such as acetic acid, ethanol, methanol (Kabir *et al*., 2014), acetone and butanol (Amiri *et al.,* 2014), ethylene glycol, glycerol acetic acid, and formic acid (Zhang *et al.,* 2016) and many other solvents have been investigated. Separation of biomass components into cellulose, lignin, and hemicellulose with cleanliness and easy solvent recovery and reuse are unique merits of organosolv (Zhang *et al.*, 2016; Zhao and Liu, 2012). Notwithstanding, the cost and ease of recovery of solvent should be considered (Taherzadeh and Karimi, 2008; Zhang *et al.,* 2016). Figure XII shows the sketch of ethanol production with biomass pretreated with organic solvents.
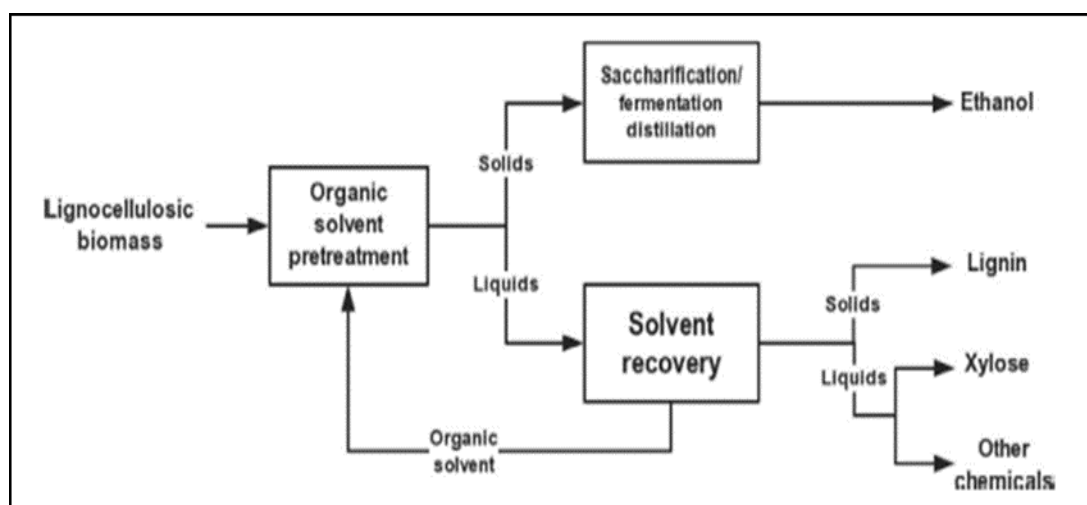


Figure XII Production process with organosolv pretreatment (Zhang *et al*., 2015)

The ability of biomass-derived *n*-butanol to fractionate lignocellulose into its components have been studied (Lancefield *et al*., 2017). Effective removal of hemicellulose and lignin was achieved with *n*-butanol containing 5% water and 0.2 M HCl, leaving cellulose suitable for hydrolysis. Complete conversion of the hardwood beech was achieved using cellulase. The mild conditions (<120 °C) and high solvent inhibit lignin degradation allowing isolation of high quality lignin which is four-fold higher than those obtained from previous studies. Another study (Sun *et al.*, 2016) in support of Lancefield *et al.,* obtained 70% lignin and hemicellulose, and 94% cellulose yields. The pretreatment deconstructs the recalcitrant architecture thereby altering the fibre size, surface area, average size and roughness. Interestingly, key chemical bond dissociation of the complexes is achieved. Vargas *et al*. (2016) reported similar findings while examining organosolv pretreatment of barley straw under comparable conditions with Lancefield *et al*. (2017).

Kabir *et al.* (2014) obtained >50% increase of methane production when acetic acid, ethanol, and methanol were separately used to pretreat forest residues. These findings are in line with those obtained by Ostovareh *et al.* (2015) during pretreatment of corn stalks with ethanol. The above literatures suffer a setback due to the use of catalyst with most of the studies using acids either to minimize solvent requirement or facilitate delignification. Many organic or aqueous-organic solvents such as oxalic, salicylic, and acetylsalicylic acid could be used without addition of catalysts at temperatures of 150–200°C (Taherzadeh and Karimi, 2008). Excessive use of solvent is the major drawback of the work of Lancefield *et al.* (2017).

The studies reviewed thus far provide evidence that organosolv pretreatment is suitable in the pretreatment of biomass for bioethanol production. However, it may be difficult for the treated biomass to be used in biogas production, unless the acidity of the biomass is neutralised because methanogenesis is impeded at lower substrate pH. Purchase of buffering substances adds to the cost of pretreatment, a situation that should be avoided. Moreover, careful washing and solvent removal with centrifuge or recycling protocol may mitigate the acidic effect. Another major drawback of organosolv pretreatment is the high energy demand making the process more expensive (Taherzadeh and Karimi, 2008; Zhao and Liu, 2012); although, Parthasarathi *et el.* (2016) argued that there is possibility of reducing process energy requirement. Moreover, the simplicity of solvent recovery (evaporation and condensation) may balance the cost of energy requirement. The solvent recycling through evaporation and condensation is energy intensive too, except if recycling should utilise the pretreatment heat and may require little input of energy.

**Impacts and Challenges of Thermochemical Pretreatment**

This is the application of high temperature in combination with chemicals such as acids, alkali, ILs or organic solvents to deconstruct the lignocellulose complex. Temperature plays significant role in improving the efficiency of chemical pretreatment. However, selection of appropriate temperature should be carefully done to avoid impeding the process (Montgomery and Bochmann, 2014). A formation of dark-coloured xylose and lignin products have been noticed at very high temperatures. These complexes include heterocyclic and phenolic compounds (Montgomery and Bochmann, 2014).

The effects of a combined chemical and high temperature pretreatment have been studied (Elumalai *et al.*, 2016; Jin *et al.*, 2016; Passos *et al.*, 2016). Arisutha *et al.* (2016) developed

several pretreatment procedures using $Ca(OH)_2$, NaOH, ammonium carbonate, $H_2SO_4$, maleic acid and hydrothermal (auto-hydrolysis, hot-compressed water treatment, liquid hot water treatment) with $H_2SO_4$ as catalyst.Results indicated that thermochemical pretreated bamboo produces the highest yields (biogas; 820 ml/day and methane; 72%). Similarly, Capecchi *et al.* (2016) focused on thirteen treatments using switchgrass: steam explosion ($195^{o}C$ for 5, 10 and 15 min), $Ca(OH)_2$ at concentration of 0.4% and 0.7%, $Ca(OH)_2$ at high concentration ($205^{o}C$ for 5, 10 and15 min), $H_2SO_4$ (0.2% at $195^{o}C$ for 10 min) followed by steam explosion. Results showed that mild lime ($Ca(OH)_2$) was less aggressive and shows more residual solid, resulting in higher energy output per unit biomass. The decrease in water use, less inhibitory products, and post process recovery of $Ca(OH)_2$ as $CaCO_3$ signify additional benefits of this type of thermochemical pretreatments. Another recent study assesses pretreatment of catalpa sawdust using NaOH, $Ca(OH)_2$, $H_2SO_4$, and HCl at $100^{o}C$ and found that thermo-$Ca(OH)_2$ increases sugar yields by 1185.7% compared to control and it is the best of all the treatments (Jin *et al.*, 2016). These findings are consistent with the results of Capecchi *et al.* (2016). One of the major drawback of Arisutha's work is that, analytical techniques used were not clearly explained and thus discourages repeatability of procedures. Additionally, higher doses of chemicals were used by Jin while, lower doses and 45 min less of residence time led to similar outcome in Capecchi's work, even though the pretreated biomass are not the same.

Fernandes *et al.* (2009) concluded that $Ca(OH)_2$ improves biodigestibility and methane yield of high lignin substrates at higher temperatures. Likewise, Elumalai *et al.* (2016) and Tsapekos *et al.* (2016) reported findings corroborating positive impacts of alkali at high temperatures. The process enhances degradability by more than 4-fold and 6% NaOH at 55°C is the most effective pretreatment method which increases methane production by 26% (Tsapekos *et al.* 2016). Wet explosion (WEx) is another thermochemical pretreatment method with oxygen injection and explosive decompression which can be tailored to suit variety of feedstock. The WEx method has successfully been applied in fermentation and AD processes using manure, agricultural and forest residues. It is like steam explosion, except that oxygen is added (Biswas *et al.*, 2015a). A sketch of impact of WEx pretreatment on lignocelluloses is shown in Figure XIII. Similarly, Ahring *et al.* (2015) reported an improved methane production for substrates pretreated with steam explosion. Recently, maximum digestion was achieved at 190°C at 30 min and oxygen loading of 7.5%. Glucose yield at pH

5.5 was 63.3% with remarkable recovery of cellulose (99.9%), lignin (96.3%), xylose (69.2%) and mannose (76.0%), was obtained (Biswas *et al.,* 2015b).
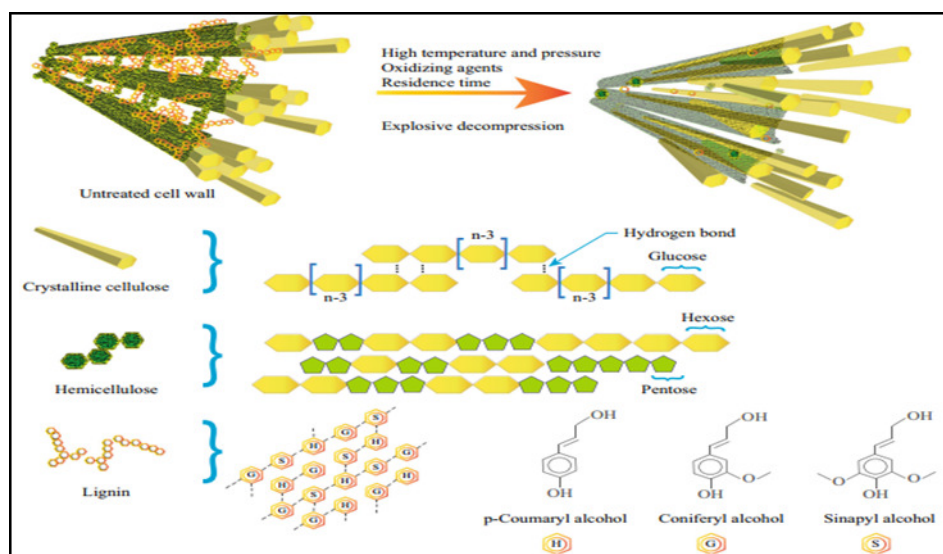


Figure XIII Effect of wet explosion pretreatment on the cell wall structure of lignocellulose (Biswas *et al*., 2015b).

The major limitation of these processes is the energy burden, arguing that ambient temperature or solar assisted processes should be explored. Moreover, the adverse effects of high temperature pretreatment was long recognised (Stuckey and McCarty, 1984) and recent studies confirmed this assertion (Carlsson *et al.*, 2012; Lagerkvist and Morgan-Sagastume, 2012; Rafique *et al*., 2010). More recently, Martín *et al.* (2017) observed that recovered lignin during acid pretreatment at temperatures of 150, 160 and 170$^{o}$C, were higher than in the untreated biomass. This is attributed to the formation of pseudo-lignin, a Klason-positive aromatic substance caused by thermal decomposition of polymeric carbohydrates. Jard *et al.* (2013) indicated a decline in methane production for samples pretreated at 180-200$^{o}$C, credited to the formation of refractory compounds. For example, furfural and hydroxylfurfural are generated during nitric acid pretreatment due to reaction of pentose and hexose at high temperature (Park *et al.* 2015). However, higher conversion efficiency was obtained within this temperature range recently (Chen *et al.*, 2017).

**Conclusion**

This paper reviewed the impacts and challenges of various chemical pretreatments of lignocellulosic biomass in the production of biogas or bioethanol. Previous studies have shown that acids and alkali pretreatments are effective in delignification and recovery of

cellulose and hemicellulose sugars. Acids are often less effective on cellulose and accompanied by generation of refractory by-products especially when extreme temperatures are involved. A disadvantage with acids and alkali is the destruction, instead of separation of lignin which may be recycled as biopolymers. Moreover, alkali is accompanied by salt build-up and elevation of digestate pH while acids are associated with low substrate pH, both are detrimental to acidogenesis and methanogenesis respectively.

The applications of organic solvents and ILs are more likely to replace the use of alkali and acids due to easy recovery of pretreatment substances, separation of high quality lignin and less generation of inhibitory substances. They are however considered expensive.

All the pretreatments are designed at certain temperatures. This paper argued that thermochemical pretreatment is merely a nomenclature and not an independent procedure entirely.

## Recommendations

Further research should consider the development of cheaper pretreatment substances in addition to the existing substances being studied or used.

Also, future work should focus on the discovery of new ILs with optimum properties and minimizing the viscosity and strength of ILs during pretreatment.

## References

Adouani, N., Pons, M. N., Hreiz, R., & Pacaud, S. (2016). Dynamic modelling of an anaerobic digester for wastes at the territory level. IFAC-Papers Online, *49*(7), 1169-1174.

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., & Levin, D. B. (2011). Biomass pretreatment: fundamentals toward application. *BiotechnologyAdvances*, *29*(6), 675-685.

Ahring, B. K., Biswas, R., Ahamed, A., Teller, P. J., & Uellendahl, H. (2015). Making lignin accessible for anaerobic digestion by wet-explosion pretreatment. *Bioresource technology*, *175*, 182-188.

Allison, B. J., Cádiz, J. C., Karuna, N., Jeoh, T., & Simmons, C. W. (2016). The effect of ionic liquid pretreatment on the bioconversion of tomato processing waste to

fermentable sugars and biogas. *Applied Biochemistry and Biotechnology*, *179*(7), 1227-1247.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource technology*, *101*(13), 4851-4861.

Amiri, H., Karimi, K., & Zilouei, H. (2014). Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production. *Bioresource technology*, *152*, 450-456.

Amnuaycheewa, P., Hengaroonprasan, R., Rattanaporn, K., Kirdponpattara, S., Cheenkachorn, K., & Sriariyanun, M. (2016). Enhancing enzymatic hydrolysis and biogas production from rice straw by pretreatment with organic acids. *Industrial Crops and Products*, *87*, 247-254.

Antonopoulou, G., Vayenas, D., & Lyberatos, G. (2016). Ethanol and hydrogen production from sunflower straw: The effect of pretreatment on the whole slurry fermentation. *Biochemical Engineering Journal*, *116*, 65-74.

Arisutha, S., Baredar, P., Deshpande, D. M., & Suresh, S. (2016). Effects of Thermo-chemical Pre-treatment on Bamboo for Biogas Production. *Indian Chemical Engineer*, *58*(1), 79-88.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., & Lens, P.N. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, *123*, 143-156.

Battista, F., Fino, D., & Mancini, G. (2016). Optimization of biogas production from coffee production waste. *Bioresource technology*, *200*, 884-890.

Biswas, R., Uellendahl, H., & Ahring, B. K. (2015a). Wet explosion: a universal and efficient pretreatment process for lignocellulosic biorefineries. *BioEnergy Research*, *8*(3), 1101-1116.

Biswas, R., Teller, P. J., & Ahring, B. K. (2015b). Pretreatment of forest residues of Douglas fir by wet explosion for enhanced enzymatic saccharification. *Bioresource Technology*, *192*, 46-53.

Brandt, A. (2012). Ionic liquid pretreatment of lignocellulosic biomass (Doctoral dissertation, Imperial College London).

Capecchi, L., Galbe, M., Wallberg, O., Mattarelli, P., & Barbanti, L. (2016). Combined ethanol and methane production from switchgrass (Panicumvirgatum L.) impregnated with lime prior to steam explosion. *Biomass and Bioenergy*, *90*, 22-31.

Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., & Ferrer, I. (2016). Review of feedstock pretreatment strategies for improved anaerobic digestion: from lab-scale research to full-scale application. *Bioresource Technology*, *199*, 386-397.

Chang, H. N., Kim, N. J., Kang, J., & Jeong, C. M. (2010). Biomass-derived volatile fatty acid platform for fuels and chemicals. *Biotechnology and Bioprocess Engineering*, *15*(1), 1-10.

Chang, V. S., & Holtzapple, M. T. (2000). Fundamental factors affecting biomass enzymatic reactivity. In Twenty-first symposium on biotechnology for fuels and chemicals (pp. 5-37). Humana Press.

Chen, B. Y., Zhao, B. C., Li, M. F., Liu, Q. Y., & Sun, R. C. (2017). Fractionation of rapeseed straw by hydrothermal/dilute acid pretreatment combined with alkali post-treatment for improving its enzymatic hydrolysis. *Bioresource Technology*, *225*, 127-133.

Chen, G., Zheng, Z., Yang, S., Fang, C., Zou, X., & Luo, Y. (2010). Experimental co-digestion of corn stalk and vermicompost to improve biogas production. *Waste Management*, *30*(10), 1834-1840.

Cheng, G., Varanasi, P., Li, C., Liu, H., Melnichenko, Y. B., Simmons, B. A., Kent, M. S. & Singh, S. (2011). Transition of cellulose crystalline structure and surface morphology of biomass as a function of IL pretreatment and its relation to enzymatic hydrolysis. *Biomacromolecules*, *12*(4), 933-941.

Cybulska, K., Kołosowski, P., Wrońska, I. & Dobek, T. (2016). Assessment of the biodiversity of samples used for isolation of microbial strains capable of converting straw destined as a substrate for biogas plant. *Journal of Ecological Engineering*, *17*(1), 114-118.

Doherty, T. V., Mora-Pale, M., Foley, S. E., Linhardt, R. J., & Dordick, J. S. (2010). Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. *Green Chemistry*, *12*(11), 1967-1975.

Domínguez, E., Romaní, A., Domingues, L., & Garrote, G. (2017). Evaluation of strategies for second generation bioethanol production from fast growing biomass Paulownia within a biorefinery scheme. *Applied Energy*, *187*, 777-789.

Du, B., Sharma, L. N., Becker, C., Chen, S. F., Mowery, R. A., Van Walsum, G. P., & Chambliss, C. K. (2010). Effect of varying feedstock–pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates. *Biotechnology and Bioengineering*, *107*(3), 430-440.

Elgharbawy, A. A., Alam, M. Z., Moniruzzaman, M., & Goto, M. (2016). Ionic Liquid pretreatment as emerging approaches for enhanced enzymatic hydrolysis of lignocellulosic biomass. *Biochemical Engineering Journal*, *109*, 252-267.

Elumalai, S., Agarwal, B., & Sangwan, R. S. (2016). Thermo-chemical pretreatment of rice straw for further processing for levulinic acid production. *Bioresource Technology*, *218*, 232-246.

Engel, P., Mladenov, R., Wulfhorst, H., Jäger, G., & Spiess, A. C. (2010). Point by point analysis: how ionic liquid affects the enzymatic hydrolysis of native and modified cellulose. *Green Chemistry*, *12*(11), 1959-1966.

Esteghlalian, A. R., Srivastava, V., Gilkes, N., Gregg, D. J., & Saddler, J. N. (2001). An overview of factors influencing the enzymatic hydrolysis of lignocellulosic feedstocks. In ACS symposium series (Vol. 769, pp. 100-111). Washington, DC; *American Chemical Society*; 1999.

Ferguson, R. M. W., Villa, R., & Coulon, F. (2014). Bioengineering options and strategies for the optimization of anaerobic digestion processes. *Environmental Technology Reviews*, *3*(1), 1-14.

Fernandes, T. V., Bos, G. K., Zeeman, G., Sanders, J. P. M., & Van Lier, J. B. (2009). Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. *Bioresource Technology*, *100*(9), 2575-2579.

Forster-Carneiro, T., & Isaac, R. (2012). Anaerobic Digestion. Biogas Production: Pretreatment Methods in Anaerobic Digestion, *1*.

Fu, D., & Mazza, G. (2011). Aqueous ionic liquid pretreatment of straw. *Bioresource Technology*, *102*(13), 7008-7011.

Gabhane, J., William, S. P., Vaidya, A. N., Das, S., & Wate, S. R. (2015). Solar assisted alkali pretreatment of garden biomass: Effects on lignocellulose degradation, enzymatic hydrolysis, crystallinity and ultra-structural changes in lignocellulose. *Waste Management*, *40*, 92-99.

Gao, J., Chen, L., Yan, Z., & Wang, L. (2013a) Effect of IL pretreatment on the composition, structure and biogas production of water hyacinth (Eichhorniacrassipes). *Bioresource Technology*, *132*, 361-364.

Gao, J., Chen, L., Yuan, K., Huang, H., & Yan, Z. (2013b). IL pretreatment to enhance the anaerobic digestion of lignocellulosic biomass. *Bioresource Technology*, *150*, 352-358.

Gao, X., Kumar, R., Singh, S., Simmons, B. A., Balan, V., Dale, B. E., & Wyman, C. E. (2014). Comparison of enzymatic reactivity of corn stover solids prepared by dilute acid, AFEX™, and ionic liquid pretreatments. *BiotechnologyforBiofuels*, *7*(1), 1.

George, A., Brandt, A., Tran, K., Zahari, S. M. N. S., Klein-Marcuschamer, D., Sun, N., Sathitsuksanoh, N., Shi, J., Stavila, V., Parthasarathi, R., Singh, S., Holmes, B.M., Welton, T., Simmons, B.A., & Singh, S. (2015). Design of low-cost ionic liquids for lignocellulosic biomass pretreatment. *Green Chemistry*, *17*(3), 1728-1734.

Gulhane, M., Pandit, P., Khardenavis, A., Singh, D., & Purohit, H. (2017). Study of microbial community plasticity for anaerobic digestion of vegetable waste in Anaerobic Baffled Reactor. *Renewable Energy*, *101*, 59-66.

He, Y., Pang, Y., Liu, Y., Li, X., & Wang, K. (2008). Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. *Energy & Fuels*, *22*(4), 2775-2781.

Himmel, M. E., Baker, J. O., & Overend, R. P. (1994). Enzymatic conversion of biomass for fuels production. Washington, DC: *American Chemical Society*.

Hu, F. (2014). Pseudo-lignin chemistry in pretreatment of biomass for cellulosic biofuel production. *PhD Thesis*,

Jaffar, M., Pang, Y., Yuan, H., Zou, D., Liu, Y., Zhu, B., Korai, R.M., & Li, X. (2016). Wheat straw pretreatment with KOH for enhancing biomethane production and fertilizer value in anaerobic digestion. *Chinese Journal of Chemical Engineering*, *24*(3), 404-409.

Janke, L., Leite, A., Batista, K., Weinrich, S., Sträuber, H., Nikolausz, M., Nelles, M. & Stinner, W. (2016). Optimization of hydrolysis and volatile fatty acids production from sugarcane filter cake: Effects of urea supplementation and sodium hydroxide pretreatment. *Bioresource Technology*, *199*, 235-244.

Jard, G., Dumas, C., Delgenes, J. P., Marfaing, H., Sialve, B., Steyer, J. P., & Carrère, H. (2013). Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalgaPalmariapalmata. *Biochemical Engineering Journal*, *79*, 253-258.

Jeihanipour, A., Karimi, K., & Taherzadeh, M. J. (2010). Enhancement of ethanol and biogas production from high-crystalline cellulose by different modes of NMO pretreatment. *Biotechnology and Bioengineering*, *105*(3), 469-476.

Jin, S., Zhang, G., Zhang, P., Li, F., Fan, S., & Li, J. (2016). Thermo-chemical pretreatment and enzymatic hydrolysis for enhancing saccharification of catalpa sawdust. *BioresourceTechnology*, *205*, 34-39

Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, *199*, 103-112.

Kabir, M. M., Rajendran, K., Taherzadeh, M. J., & Horváth, I. S. (2015). Experimental and economical evaluation of bioconversion of forest residues to biogas using organosolv pretreatment. *Bioresource Technology*, *178*, 201-208.

Kamali, M., Gameiro, T., Costa, M. E. V., & Capela, I., 2016. Anaerobic digestion of pulp and paper mill wastes–An overview of the developments and improvement opportunities. *Chemical Engineering Journal*, *298*, 162-182.

Kim, T. H. (2011). Sequential hydrolysis of hemicellulose and lignin in lignocellulosic biomass by two-stage percolation process using dilute sulfuric acid and ammonium hydroxide. *Korean Journal of Chemical Engineering*, *28*(11), 2156-2162.

Kim, T. H., Kim, J. S., Sunwoo, C., & Lee, Y. Y. (2003). Pretreatment of corn stover by aqueous ammonia. *Bioresource Technology*, *90*(1), 39-47.

Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial & Engineering Chemistry Research*, *48*(8), 3713-3729.

Lagerkvist, A., & Morgan-Sagastume, F. (2012). The effects of substrate pre-treatment on anaerobic digestion systems: a review. *Waste Management*, *32*(9), 1634-1650.

Lancefield, C. S., Panovic, I., Deuss, P. J., Barta, K., & Westwood, N. J. (2017). Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: towards complete biomass valorisation. *Green Chemistry*, *19*(1), 202-214.

Lee, J. W., Zhu, J. Y., Scordia, D., & Jeffries, T. W. (2011). Evaluation of ethanol production from corncob using Scheffersomyces (Pichia) stipites CBS 6054 by volumetric scale-up. *Applied Biochemistry and Biotechnology*, *165*(3-4), 814-822.

Lee, S. H., Doherty, T. V., Linhardt, R. J., & Dordick, J. S. (2009). Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnology and Bioengineering*, *102*(5), 1368-1376.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H. V., Auer, M., Vogel, K. P., Simmons, B. A., & Singh, S. (2010) Comparison of dilute acid and IL pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. *Bioresource Technology*, *101*(13), 4900-4906.

Li, H. Y., Chen, X., Wang, C. Z., Sun, S. N., & Sun, R. C. (2016) Evaluation of the two-step treatment with ILs and alkali for enhancing enzymatic hydrolysis of Eucalyptus: chemical and anatomical changes. *Biotechnology for Biofuels*, *9*(1), 166.

Li, Q., He, Y. C., Xian, M., Jun, G., Xu, X., Yang, J. M., & Li, L. Z. (2009). Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. *Bioresource Technology*, *100*(14), 3570-3575.

Ma, L., Cui, Y., Cai, R., Liu, X., Zhang, C., & Xiao, D. (2015). Optimization and evaluation of alkaline potassium permanganate pretreatment of corncob. *Bioresource Technology*, *180*, 1-6.

Mahdy, A., Mendez, L., Ballesteros, M., & González-Fernández, C., (2014). Autohydrolysis and alkaline pretreatment effect on Chlorella vulgaris and Scenedesmus sp. methane production. *Energy*, *78*, 48-52.

Martín, C., Wei, M., Xiong, S., & Jönsson, L. J. (2017). Enhancing saccharification of cassava stems by starch hydrolysis prior to pretreatment. *Industrial Crops and Products, 97*, 21-31.

Mattila, H., Kuuskeri, J., & Lundell, T. (2017). Single-step, single-organism bioethanol production and bioconversion of lignocellulose waste materials by phlebioid fungal species. *Bioresource Technology*, *225*, 254-261.

McKendry, P. (2002). Energy production from biomass (part 1): overview of biomass. *Bioresource Technology*, *83*(1), 37-46.

Mirmohamadsadeghi, S., Karimi, K., & Horváth, I. S. (2016). Improvement of Solid-State Biogas Production from Wood by Concentrated Phosphoric Acid Pretreatment. *BioResources*, *11*(2), 3230-3243.

Monlau, F., Kaparaju, P., Trably, E., Steyer, J. P., & Carrere, H., (2015). Alkaline pretreatment to enhance one-stage CH 4 and two-stage H 2/CH 4 production from sunflower stalks: mass, energy and economical balances. *Chemical Engineering Journal*, *260*, 377-385.

Montgomery, L. F., & Bochmann, G. (2014). Pretreatment of feedstock for enhanced biogas production. *IEA Bioenergy*, 1-20.

Mora-Pale, M., Meli, L., Doherty, T. V., Linhardt, R. J., & Dordick, J. S. (2011). Room temperature ILs as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnology and Bioengineering*, *108*(6), 1229-1245.

Nguyen, T. A. D., Kim, K. R., Han, S. J., Cho, H. Y., Kim, J. W., Park, S. M., & Sim, S. J. (2010). Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. *Bioresource Technology*, *101*(19), 7432-7438.

Ostovareh, S., Karimi, K., & Zamani, A. (2015). Efficient conversion of sweet sorghum stalks to biogas and ethanol using organosolv pretreatment. *Industrial Crops and Products*, *66*, 170-177.

Pan, S. Y., Du, M. A., Huang, I. T., Liu, I. H., Chang, E. E., & Chiang, P. C. (2015). Strategies on implementation of waste-to-energy (WTE) supply chain for circular economy system: a review. *Journal of Cleaner Production*, *108*, 409-421.

Park, G. W., Kim, I., Jung, K., Seo, C., Han, J. I., Chang, H. N., & Kim, Y. C. (2015). Enhancement of volatile fatty acids production from rice straw via anaerobic digestion with chemical pretreatment. *Bioprocess and Biosystems Engineering*, *38*(8), 1623-1627.

Parthasarathi, R., Sun, J., Dutta, T., Sun, N., Pattathil, S., Konda, N. M., Peralta, A. G., Simmons, B. A., & Singh, S. (2016). Activation of lignocellulosic biomass for higher sugar yields using aqueous ionic liquid at low severity process conditions. *Biotechnology for Biofuels*, *9*(1), 160.

Passos, F., Felix, L., Rocha, H., de Oliveira Pereira, J., & de Aquino, S. (2016). Reuse of microalgae grown in full-scale wastewater treatment ponds: Thermochemical pretreatment and biogas production. *Bioresource Technology*, *209*, 305-312.

Pérez-Pimienta, J. A., Vargas-Tah, A., López-Ortega, K. M., Medina-López, Y. N., Mendoza-Pérez, J. A., Avila, S., Singh, S., Simmons, B.A., Loaces, I., & Martinez, A. (2017). Sequential enzymatic saccharification and fermentation of ionic liquid and organosolv pretreated agave bagasse for ethanol production. *Bioresource Technology*, *225*, 191-198.

Petersson, A., Thomsen, M. H., Hauggaard-Nielsen, H., & Thomsen, A. B. (2007). Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. *Biomass and Bioenergy*, *31*(11), 812-819.

Pingali, S. V., Urban, V. S., Heller, W. T., McGaughey, J., O'Neill, H., Foston, M Myles, D.A, Ragauskas, A., & Evans, B. R. (2010). Breakdown of cell wall nanostructure in dilute acid pretreated biomass. *Biomacromolecules, 11*(9), 2329-2335.

Qing, Q., Zhou, L., Guo, Q., Huang, M., He, Y., Wang, L., & Zhang, Y. (2016). A combined sodium phosphate and sodium sulphide pretreatment for enhanced enzymatic digestibility and delignification of corn stover. *Bioresource Technology*, *218*, 209-216.

Rafique, R., Poulsen, T. G., Nizami, A. S., Murphy, J. D., & Kiely, G. (2010). Effect of thermal, chemical and thermo-chemical pre-treatments to enhance methane production. *Energy*, *35*(12), 4556-4561.

Rasit, N., Idris, A., Harun, R., & Ghani, W. A. W. A. K. (2015). Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludge: An overview. *Renewable and Sustainable Energy Reviews*, *45*, 351-358.

Reina, L., Botto, E., Mantero, C., Moyna, P., & Menéndez, P. (2016). Production of second generation ethanol using Eucalyptus dunnii bark residues and ionic liquid pretreatment. *Biomass and Bioenergy*, *93*, 116-121.

Rodríguez-Zúñiga, U. F., Cannella, D., de Campos Giordano, R., Giordano, R. D. L. C., Jørgensen, H., & Felby, C. (2015). Lignocellulose pretreatment technologies affect the level of enzymatic cellulose oxidation by LPMO. *Green Chemistry*, *17*(5), 2896-2903.

Sangian, H. F., Kristian, J., Rahma, S., Dewi, H. K., Puspasari, D. A., Agnesty, S. Y., Gunawan, S., & Widjaja, A. (2015). Preparation of Reducing Sugar Hydrolyzed from High-Lignin Coconut Coir Dust Pretreated by the Recycled Ionic Liquid [mmim] [dmp] and Combination with Alkaline. *Bulletin of Chemical Reaction Engineering & Catalysis*, *10*(1), 8-22.

Sannigrahi, P., Kim, D. H., Jung, S., & Ragauskas, A. (2011). Pseudo-lignin and pretreatment chemistry. *Energy & Environmental Science*, *4*(4), 1306-1310.

Sathitsuksanoh, N., Sawant, M., Truong, Q., Tan, J., Canlas, C. G., Sun, N. Zhang, W., Renneckar, S. Prasomsri, T., Shi, J. Çetinkol, Ö., Singh, S., Simmons, B.A., & Çetinkol, Ö. (2015). How Alkyl Chain Length of Alcohols Affects Lignin Fractionation and Ionic Liquid Recycle During Lignocellulose Pretreatment. *BioEnergy Research*, *8*(3), 973-981.

Schroyen, M., Vervaeren, H., Van Hulle, S. W., & Raes, K. (2014). Impact of enzymatic pretreatment on corn stover degradation and biogas production. *Bioresource technology*, *173*, 59-66.

Simmons, B (2013) Ionic Liquid Pretreatment. Join BioEnergy Institute. EERE Webinar. At: https://www.google.com.ng/url?sa=t&rct=j&q=&esrc=s&source=web&cd=6&cad=rja&uact=8&ved=0ahUKEwiIz9_9wpnRAhXlCcAKHdWqCL0QFghEMAU&url=http%3A%2F%2Fenergy.gov%2Fsites%2Fprod%2Ffiles%2F2014%2F03%2Ff14%2Fjune2013_snl_webinar.pdf&usg=AFQjCNGs03ckc7RPpzVMPOLCKQOGw-GOzw&sig2=Riof2hN2Ty8O-u6buJnUFg. (Accessed on 24th December 2016).

Singh, J., Suhag, M., & Dhaka, A. (2015). Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: a review. *Carbohydrate Polymers*, *117*, 624-631.

Singh, S., Simmons, B. A., & Vogel, K. P. (2009). Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass. *Biotechnology and Bioengineering*, *104*(1), 68-75.

Smeets, E. (2015). An assessment of the global land use change and food security effects of the use of agricultural residues for bioenergy. Paper prepared for presentation at the International Conference "Food in the Bio-based Economy; Sustainable Provision and Access" Wageningen (The Netherlands), May 27-29, 2015.

Stamatelatou, K., Antonopoulou, G., Ntaikou, I., & Lyberatos, G. (2012). The effect of physical, chemical, and biological pretreatments of biomass on its anaerobic digestibility and biogas production. Biogas production: pretreatment methods in anaerobic digestion, 55-90. Scrivener Publishing LLC, Massachusetts.

Stuckey, D. C., & McCarty, P. L. (1984). The effect of thermal pretreatment on the anaerobic biodegradability and toxicity of waste activated sludge. *Water Research*, *18*(11), 1343-1353.

Su, Y., Du, R., Guo, H., Cao, M., Wu, Q., Su, R., Qi, W., & He, Z. (2015). Fractional pretreatment of lignocellulose by alkaline hydrogen peroxide: characterization of its major components. *Food and Bioproducts Processing*, *94*, 322-330.

Suanon, F., Sun, Q., Li, M., Cai, X., Zhang, Y., Yan, Y., & Yu, C. P. (2017). Application of nanoscale zero valent iron and iron powder during sludge anaerobic digestion: Impact on methane yield and pharmaceutical and personal care products degradation. *Journal of Hazardous Materials*, *321*, 47-53.

Sun, F. F., Zhao, X., Hong, J., Tang, Y., Wang, L., Sun, H., Li, X., & Hu, J. (2016). Industrially relevant hydrolyzability and fermentability of sugarcane bagasse improved effectively by glycerol organosolv pretreatment. *Biotechnology for Biofuels*, *9*(1), 1.

Sun, N., Rodríguez, H., Rahman, M., & Rogers, R. D. (2011) Where are IL strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? *Chemical Communications*, *47*(5), 1405-1421.

Tadesse, H., & Luque, R. (2011). Advances on biomass pretreatment using ILs: an overview. *Energy & Environmental Science*, *4*(10), 3913-3929.

Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences*, *9*(9), 1621-1651.

Teramura, H., Sasaki, K., Oshima, T., Matsuda, F., Okamoto, M., Shirai, T., Kawaguchi, H., Ogino, C., Hirano, K., Sazuka, T., Kitano, H., Kikuchi, J., & Kitano, H. (2016). Organosolv pretreatment of sorghum bagasse using a low concentration of hydrophobic solvents such as 1-butanol or 1-pentanol. *Biotechnology for Biofuels, 9*(1), 1.

Tsapekos, P., Kougias, P. G., Frison, A., Raga, R., & Angelidaki, I. (2016). Improving methane production from digested manure biofibers by mechanical and thermal alkaline pretreatment. *Bioresource Technology*, *216*, 545-552.

Van Acker, R., Vanholme, R., Piens, K., & Boerjan, W. (2016). Saccharification protocol for small-scale lignocellulosic biomass samples to test processing of cellulose into glucose. *BIO-PROTOCOL*, *6*(1).

Vancov, T., Alston, A. S., Brown, T., & McIntosh, S. (2012). Use of ionic liquids in converting lignocellulosic material to biofuels. *Renewable Energy*, *45*, 1-6.

Vargas, F., Domínguez, E., Vila, C., Rodríguez, A., & Garrote, G. (2016). Biorefinery Scheme for Residual Biomass Using Autohydrolysis and Organosolv Stages for Oligomers and Bioethanol Production. *Energy & Fuels*, *30*(10), 8236-8245.

Wang, G. S., Lee, J. W., Zhu, J. Y., & Jeffries, T. W. (2011). Dilute acid pretreatment of corncob for efficient sugar production. *Applied Biochemistry and Biotechnology*, 163(5), 658-668.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*, *99*(17), 7928-7940.

Weerachanchai, P., Leong, S. S. J., Chang, M. W., Ching, C. B., & Lee, J. M. (2012) Improvement of biomass properties by pretreatment with ILs for bioconversion process. *Bioresource Technology*, *111*, 453-459.

Widjaja, A., Agnesty, S. Y., Sangian, H. F., & Gunawan, S. (2015). Application of Ionic Liquid [DMIM] DMP Pretreatment in the Hydrolysis of Sugarcane Bagasse for Biofuel Production. *Bulletin of Chemical Reaction Engineering & Catalysis*, *10*(1), 70-77.

Wu, L. J., Kobayashi, T., Li, Y. Y., & Xu, K. Q. (2015). Comparison of single-stage and temperature-phased two-stage anaerobic digestion of oily food waste. *Energy Conversion and Management*, *106*, 1174-1182.

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G., & Zhan, X. (2011). Effects of thermo-chemical pretreatment of grass silage on methane production by anaerobic digestion. *Bioresource Technology*, *102*(19), 8748-8755.

Xu, F., Sun, J., Konda, N. M., Shi, J., Dutta, T., Scown, C. D., Simmons, B.A., & Singh, S. (2016). Transforming biomass conversion with ionic liquids: process intensification and the development of a high-gravity, one-pot process for the production of cellulosic ethanol. *Energy & Environmental Science*, *9*(3), 1042-1049.

Yaman, S. (2004). Pyrolysis of biomass to produce fuels and chemical feedstocks. *Energy Conversion and Management*, *45*(5), 651-671.

Yang, L., Xu, F., Ge, X., & Li, Y. (2015). Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, *44*, 824-834.

Yejun, H., &Hongzhang, C. (2007). Plant cell wall proteins & enzymatic hydrolysis of lignocellulose.

You, T. T., Zhang, L. M., & Xu, F. (2016). Progressive deconstruction of Arundodonax Linn. to fermentable sugars by acid catalysed ionic liquid pretreatment. *Bioresource Technology*, *199*, 271-274.

Zhang, K., Pei, Z., & Wang, D. (2016). Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review.ac *Bioresource Technology*, *199*, 21-33.

Zhao, X., & Liu, D. (2012). Fractionating pretreatment of sugarcane bagasse by aqueous formic acid with direct recycle of spent liquor to increase cellulose digestibility–the Formiline process. *Bioresource Technology*, *117*, 25-32.

Zhao, X., Zhang, L., & Liu, D. (2012a). Biomass recalcitrance. Part II: Fundamentals of different pre-treatments to increase the enzymatic digestibility of lignocellulose. *Biofuels, Bioproducts and Biorefining*, *6*(5), 561-579.

Zhao, X., Zhang, L., & Liu, D. (2012b). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels, Bioproducts and Biorefining*, 6(4), 465-482.

Zhen, G., Lu, X., Kato, H., Zhao, Y., & Li, Y. Y. (2017). Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: Current advances, full-scale application and future perspectives. *Renewable and Sustainable Energy Reviews, 69*, 559-577.

Zheng, Y., Pan, Z. & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol production. *International journal of Agricultural and Biological Engineering*, *2*(3), 51-68.

Zheng, Y., Zhao, J., Xu, F. & Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science*. *42*(2014), 35-53.

Zhu, J. Y., Pan, X., & Zalesny Jr, R. S. (2010). Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. *Applied Microbiology and Biotechnology*, *87*(3), 847-857.