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Autohydrolysis: A promising pretreatment for the improvement of acetone, butanol, and ethanol production from woody materials

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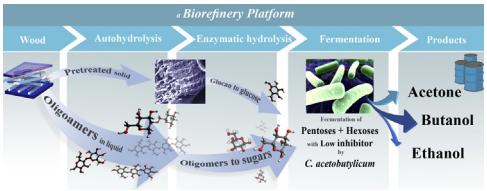
HIGHLIGHTS

- Acetone, butanol, and ethanol were efficiently produced from soft- and hardwoods.
- Autohydrolysis resulted in between 80 and 86% improvement in ABE production.
- From 1 kg of elm and pine, 117 and 104 g ABE was produced, respectively.
- Combination of auto- and enzymatic hydrolysis of pine improved ABE production.

GRAPHICAL ABSTRACT



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ABSTRACT

Acetone, butanol, and ethanol (ABE) were produced from softwood pine and hardwood elm using autohydrolysis pretreatment, enzymatic hydrolysis, and fermentation by *Clostridium acetobutylicum*. The solid residue obtained by autohydrolysis, "pretreated solids", was hydrolyzed using a mixture of two commercially available cellulases leading to production of 162 g sugar from each kg pine and 295 g sugar from each kg elm in the form of "cellulosic hydrolysates". The fermentation of cellulosic hydrolysates resulted in the production of 79.3 and 117.6 g ABE from each kg of pine and elm, respectively. Through the autohydrolysis, between 187 to 195 g soluble sugars and oligomers was also released from each kg of the materials into the liquid streams named "autohydrolysates". Enzymatic hydrolysis of pretreated solid residue in the autohydrolysate liquor resulted in hydrolysates with total sugar concentration of 21–23 g/l, named "overall hydrolysates". In this process, 51% of the oligomers of pine autohydrolysate were converted to monomeric sugars and subsequently used for ABE production. Therefore, the fermentation of the overall hydrolysates resulted in the production of 104.5 and 43.4 g ABE from each kg of pine and elm, respectively.

1. Introduction

Environmental and economic concerns caused by the over-consumption of depleting and non-renewable crude oil stimulated a large number of researchers to develop proper replacements for supplying demand for energy and chemicals. Lignocellulosic materials are probably the only renewable, nonedible, and widely available resource that can serve as a starting material for the production of fuel and chemicals to maintain social sustainable development (Carroll and Somerville, 2009; Menon and Rao, 2012).

The bioconversion of lignocellulosic materials to fermentable carbohydrates followed by the production of biofuels through

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fermentation is a promising approach for obtaining renewable energy in the form of liquid fuels (Jørgensen et al., 2007; Karimi and Chisti, 2015). In recent years, after introducing biobutanol as a biofuel with superior characteristics in comparison with bioethanol, reviving this process by the utilization of relatively low cost materials has gained renewed interest (Bankar et al., 2013; Kumar and Gayen, 2011; Wang et al., 2014).

Lignocellulosic materials typically contain 40–50% cellulose, 25–30% hemicellulose, and 15–20% lignin (Godin et al., 2013). Even though hemicellulose content of lignocelluloses is ignored in some typical processes for ethanol production from lignocelluloses, it can be used for acetone, butanol, and ethanol production, offering an opportunity to reduce the cost of ABE production from lignocelluloses (Saha, 2003). Unlike ethanol-producing microorganisms, *Clostridium* spp. are able to uptake hemicellulose-derived pentoses in addition to hexoses (Kumar and Gayen, 2011). However, the complex and recalcitrant structure of lignocellulosic materials makes their economic conversion to fermentable carbohydrates more challenging (García et al., 2013; Jørgensen et al., 2007; Shafiei et al., 2011).

After proper pretreatment, the cellulose content of lignocelluloses can efficiently be converted to glucose by enzymatic hydrolysis (Taherzadeh and Karimi, 2007). Most pretreatment technologies were developed for bioethanol production based mainly on the improvement of enzymatic hydrolysis (Taherzadeh and Karimi, 2007; Wyman et al., 2005). However, pretreatment may also facilitate ABE production by the hydrolysis of hemicellulose to fermentable sugars and oligomers (Saha, 2003; Sun and Liu, 2012). Therefore, pretreatment is a crucial step in ABE production from lignocellulosic materials (Karimi and Pandey, 2014). In addition, due to special features of ABE fermentation, e.g., high sensitivity to inhibitors, ABE production is highly affected by the type and conditions of the pretreatment (Jurgens et al., 2012). Pretreatment with concentrate alkaline and acids improved ABE production primarily from the cellulosic portion of rice straw (Moradi et al., 2013). However, between 81 and 86% of the hemicellulose content of rice straw was lost by these pretreatments, reducing the overall yield of ABE production (Moradi et al., 2013). Fractionation of lignocellulosic materials by ethanol organosolv pretreatment showed significant improvement in ABE production, but mainly from the cellulosic part of rice straw (Amiri et al., 2014). Even though hemicellulosic sugars and oligomers were separated and remained in aqueous liquor after the evaporation of ethanol, the fermentation of the liquor with hemicellulosic sugars was unsuccessful (Amiri et al., 2014).

Autohydrolysis is a relatively simple, environmentally friendly, and cost effective alternative pretreatment technology through which hemicellulosic oligomers can be recovered (Garrote et al., 1999b). This pretreatment allows a selective hydrolysis of hemicellulose at high temperature using water as the only reagent, leading to a "pretreated solid" and an extract or "autohydrolysate" containing sugars and oligomers with minor inhibitory compounds (Garrote et al., 1999a; Sun and Liu, 2012). Therefore, autohydrolysis can be used both as a pretreatment improving the following enzymatic hydrolysis and as a process for the hydrolysis of the hemicellulose portion of lignocelluloses. Sun and Liu (2012) used autohydrolysate of maple to produce acetone, butanol, and ethanol through a process containing a two-stage concentrating step and an additional acidic hydrolysis step to obtain a proper concentration of fermentable sugars. However, along with fermentable sugars, inhibitors were formed and concentrated into the autohydrolysate. As a result, the prepared hydrolysate completely inhibited the *Clostridium acetobutylicum* growth, and ABE production was achieved only after conducting a detoxification process (Sun and Liu, 2012). Some sugar loss, production of environmental pollutants, and extra chemical consumption accompanied the detoxification.

An alternative approach to obtain proper hydrolysates containing hemicellulosic sugars is to combine the pre-hydrolysis of hemicellulose with enzymatic hydrolysis. The combination of dilute acid hydrolysis with enzymatic hydrolysis has been extensively used for ABE production (Amiri et al., 2015). However, the overall hydrolysates obtained from combined dilute acid and enzymatic hydrolysis are not suitable for ABE production without an additional detoxification process (Ezeji et al., 2007; Sun and Liu, 2012). Therefore, the combination of the autohydrolysis as a less inhibitor-producing process with enzymatic hydrolysis may improve ABE production from lignocellulosic materials. To our knowledge, there is no publication on evaluating autohydrolysis both as a pretreatment and pre-hydrolysis in combination with enzymatic hydrolysis for ABE production.

The aim of this study was ABE production from two different types of wood, pine as a softwood and elm as a hardwood, using autohydrolysis, enzymatic hydrolysis, and fermentation with *C. acetobutylicum*. The lignocellulosic materials were subjected to autohydrolysis, leading to "autohydrolysates" and "pretreated solids". The enzymatic hydrolysis of the pretreated solids was conducted with two commercial hydrolytic enzymes resulting in "cellulosic hydrolysates". In addition, to obtain "overall hydrolysates" containing holocellulosic (both hemicellulosic and cellulosic) sugars and oligomers, the enzymatic hydrolysis of pretreated materials was conducted in the medium of autohydrolysate. The autohydrolysates, cellulosic hydrolysates, and overall hydrolysates were subjected to fermentation by *C. acetobutylicum*, evaluating autohydrolysis as a hydrolysis step, a pretreatment step, or both pre-hydrolysis and pretreatment steps for ABE production from woody materials.

2. Materials and methods

2.1. Raw materials and enzymes

Pinewood and elmwood, obtained from Isfahan University of Technology Forest (Isfahan, Iran, 32°43′N, 51°32′E), were used as substrates. The woods were debarked, cut into pieces with a size of less than 2 cm, and partially ball-milled. Next, the milled woods were screened to obtain powders with a size between 177 and 833 μm (20–80 mesh). The materials were placed in resealable plastic bags and stored at room temperature until use.

Two commercial enzymes, cellulase from *Trichoderma reesei* ATCC 26921 (Celluclast 1.5 L, Novozymes, Denmark) and β-glucosidase from *Aspergillus niger* (Novozyme 188, Novozymes, Denmark), were used for the enzymatic hydrolysis. Celluclast 1.5 L activity was determined as 65 FPU/ml (Adney and Baker, 1996), and the glucosidase activity was detected as 210 IU/ml (Ximenes et al., 1996).

2.2. Autohydrolysis

Forty grams of lignocellulosic materials (dry weight) were thoroughly mixed with 400 g water (solid-to-liquid ratio of 1:10) for 15 min. The mixture was heated in a high-pressure stainless steel reactor with 500 mL working volume at a rate of 3 °C/min (Amiri and Karimi, 2013). After reaching 180 °C, the treatment was conducted for 60 min by maintaining the temperature at 180 ± 1 °C, and the reactor was vigorously shaken every 5 min. The pretreatment conditions, i.e., resident time and temperature, were selected based on previous studies for recovery of sugars and oligomers from woody materials by autohydrolysis (Garrote et al., 1999b). At the end of the treatment, the reactor was cooled in an ice bath. The liquid fraction, referred to as autohydrolysate, was then separated from the pretreated solids by filtration through