Bioresource Technology 172 (2014) 283–289

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Ionic liquid pretreatment to increase succinic acid production from lignocellulosic biomass

Caixia Wang [a,b], Daojiang Yan [c], Qiang Li [b], Wei Sun [a], Jianmin Xing [b,*]

[a] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, No. 16, Nanxiaojie, Dongzhimennei, Beijing 100700, China
[b] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[c] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

HIGHLIGHTS

- AmimCl pretreated biomass were explored for succinic acid production.
- Bacterial growth and succinic acid production was inhibited by AmimCl.
- Cellulose content and enzymatic hydrolysis rate increased after AmimCl pretreatment.
- 20.7 g/L succinic acid was produced from IL pretreated pine wood.

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 9 September 2014
Accepted 10 September 2014
Available online 21 September 2014

Keywords:
Ionic liquid
Lignocellulosic biomass
Inhibition
Succinic acid
Fermentation

ABSTRACT

In this study, pinewood and corn stover pretreated with the ionic liquid (IL) 1-allyl-3-methylimidazolium chloride (AmimCl) were used as a feedstock for succinic acid production. Results reveal that 5% (v/v) AmimCl inhibited bacterial growth, whereas 0.01% (v/v) AmimCl inhibited succinic acid production. AmimCl was effective in extracting cellulose from pinewood and in degrading pinewood into a uniform pulp, as revealed by scanning electron microscopy (SEM). The rate of enzymatic hydrolysis of pinewood extract reached 72.16%. The combinations of AmimCl pretreatment with steam explosion or with hot compressed water were effective in treating corn stover, whereas AmimCl treatment alone did not result in a significant improvement. Pinewood extract produced 20.7 g/L succinic acid with an average yield of 0.37 g per gram of biomass. Workflow calculations indicated pine wood pretreated with IL has a theoretical yield of succinic acid of 57.1%. IL pretreatment led to increase in succinic acid yields.

1. Introduction

Because of the increasing concern for the sustainability of fossil fuels and global warming, much effort has been dedicated to the application of biotechnology to produce fuels and chemicals (Willke and Vorlop, 2004). Among bio-chemicals, succinic acid is one of the most important C4 building-block chemicals, which can be used for the synthesis of many high-value derivatives (Bozell and Petersen, 2010). Species that naturally produce succinate include *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, and *Mannheimia succiniciproducens* (Guettler et al., 1999; Hong et al., 2004). Besides these, genetically engineered *Escherichia coli* and *Saccharomyces cerevisiae* have been constructed for use in succinic acid production (Yan et al., 2014; Thakker et al., 2013). Optimization of fermentation and its downstream processes have been intensively studied to design economically feasible production of biologically synthesized succinic acid (bio-succinic acid). However, the production cost of bio-succinic acid is higher than that of petroleum-based succinic acid. One key problem lies in the high cost of substrates (McKinlay et al., 2007). To develop industrial production of bio-succinic acid, its process sustainability should be studied and the cost of production must be reduced.

To decrease the cost of bio-succinic acid, researchers have attempted to replace fermentation media with cheaper sources such as sugarcane molasses, waste yeast, and biomass. Similar to sugarcane molasses and other sources, lignocellulosic biomass, which consists mainly of cellulose, hemicellulose, and lignin, has attracted increasing interest because of its utility as an inexpensive, abundant, and inedible feedstock for biofuel and biochemical production. The

* Corresponding author at: P. O. Box 353, Zhongguancun Bei-er-tiao 1, Haidian District, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China. Tel./fax: +86 10 62550913.
E-mail address: jmxing@home.ipe.ac.cn (J. Xing).

http://dx.doi.org/10.1016/j.biortech.2014.09.045
0960-8524/

key point affecting the utility of biomass in fermentation is the release of sugars from the recalcitrant polysaccharides in lignocellulosic biomass. Several pretreatments have been developed to render the biomass more accessible to enzyme hydrolysis. Among these pretreatments, those involving steam explosion (SE) technology, dilute acid, or hot compressed water (HCW) have been most widely studied, and great progress has been made in the development of pretreatments that weaken the lignocellulosic structure and release sugars from lignocellulosic biomass (Chen and Liu, 2007; Saha et al., 2005). Succinic acid production from biomass employing such methods has been investigated.

Recently, ionic liquids (ILs) have been found to effectively dissolve cellulose and have been used in biomass pretreatment technology. ILs are able to effectively dissolve cellulose and destroy the link between cellulose and hemicelluloses or other components under relatively mild conditions. In addition, IL pretreatment reduces cellulose crystallinity, thereby rendering the biomass more susceptible to enzymatic hydrolysis. Among ILs, 1-allyl-3-methylimidazolium chloride (AmimCl) can completely dissolve most types of wood, including hardwood and softwood. However, AmimCl is highly viscous (Shill et al., 2011), which creates serious difficulties during biomass pretreatment. Various solvents have been mixed with AmimCl in attempts to decrease its viscosity (Li et al., 2008; Brandt et al., 2011). Xu et al. (2008) found that dimethylsulfoxide (DMSO) is an effective cosolvent, as it destroys the hydrogen bonds in the biomass and significantly decreases the surface tension and viscosity of ILs.

Because of the advantages of IL pretreatment, many researchers have focused on production of biofuel and biochemicals from IL-pretreated biomass. To investigate the prospects of succinic acid production from IL-pretreated biomass, assessing the impact of IL pretreatment on downstream processes such as enzymatic hydrolysis, bacterial growth, and succinic acid fermentation is important. Previous studies showed that most ILs have no negative effect on enzymatic hydrolysis (Bose et al., 2012). However, growth and ethanol production of *S. cerevisiae* were found to be inhibited by [C2mim][OAc] in the hydrolysates (Ouellet et al., 2011). Thus, research is required to determine whether ILs have an inhibitory effect on succinic acid production by certain strains. Furthermore, different kinds of biomass pretreated with ILs should be evaluated for their potential use in succinic acid production. To our knowledge, production of succinic acid from IL pretreated biomass is not described in the current literature.

In this study, two typical biomasses, corn stover and pinewood, were pretreated with IL. Because of its strong capacity to dissolve lignocellulosic biomass, IL AmimCl was used. Influences of AmimCl on bacterial growth and succinic acid fermentation were detected. Chemical and structural changes in biomass after AmimCl pretreatment under moderate conditions were investigated, and succinic acid was successfully produced from the pretreated biomass materials. This study provides new knowledge on succinic acid production from biomass pretreated with IL.

2. Methods

2.1. Bacterial strains, media, and cultivation

A. succinogenes 130Z and *E. coli* MG1655 were used in the study. Luria Bertani (LB) medium (10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter) was used to cultivate the bacteria. The fermentation medium contained 30 g glucose, 10 g yeast extract, 20 g $MgSO_4$, 1.37 g K_2HPO_4, 1.53 g KH_2PO_4, 1.5 g NaCl, 0.05 g $MnCl_2$, 0.38 g $CaCl_2$, and 5 g $MgCO_3$ per liter. Media containing enzymatic hydrolysates were similar in composition to the fermentation media, except that they contained enzymatic hydrolysates instead of glucose. All media were sterilized at 115 °C for 30 min, and glucose was sterilized separately. A 250 mL flask with 100 mL of fresh fermentation medium was used for succinic acid production, and the size of inoculum was 5%.

To explore the influence of AmimCl on bacterial growth, 0%, 1%, 5%, 10%, 20%, or 40% (v/v) AmimCl solution was added to the LB medium. The strains were cultivated aerobically at 220 rpm and 37 °C for 12 h. To explore the influence of AmimCl on succinic acid production, AmimCl (0–10%, v/v; Table 1) of various concentrations was added to the fermentation media. Experiments were conducted in triplicate.

A high-density stock culture (optical density (OD) of 12) for succinic acid production was obtained by 12 h aerobic cultivation in LB medium at 37 °C. The culture was subsequently centrifuged at 5000 rpm for 10 min, and then the pellet was resuspended in water. It was added to each fermentation flask to an OD of 4 to start anaerobic fermentation. Succinic acid production using either fermentation media or enzymatic hydrolysate media was conducted for 30 h. AmimCl with 99% purity was purchased from Chengjie Chemical Reagent Shanghai Co., Ltd. (China). Reagents used in the study were of analytical grade and were from Oxoid (England) or from Sinopharm Chemical Reagent Beijing Co., Ltd. (China) unless otherwise indicated.

2.2. Pretreatment and enzymatic hydrolysis of lignocellulosic biomass

2.2.1. SE and HCW treatment

Corn stover and pinewood were obtained from Shandong Province, China. They were first dried in sunlight and then cut into small pieces. They were then milled in a vegetation disintegrator and then separated by passing through an 80-mesh screen to obtain particle sizes of 0.18–0.25 mm. Before use, the raw material was dried at 80 °C to constant weight (~5%water content).

Experimental conditions for SE and HCW pretreatments were selected according to regular procedures in our laboratory (data not shown). Operating conditions for SE of corn stover were established in our previous study (Wang et al., 2013). In the HCW pretreatment, milled corn stover was loaded into a tube reactor fabricated from 316 stainless-steel tubes with a working volume of 100 mL each. The loading of the milled corn stover was 8%. An oil-conducting heating pipe and a WHF-7 temperature control system (WEIBA Automatic Control Reaction Kettle Co., China) were respectively used for heat-up and temperature control of the tubes. The corn stover was pretreated at 180 °C with 3 °C intervals for 40 min. After pretreatment, the tube was cooled to room temperature. Subsequently, the HCW-pretreated corn stover was obtained by filtering the sample slurry. The SE–corn stover and HCW–corn stover were dried at 80 °C to constant weight and then collected. The water content of the SE–corn stover and HCW–corn stover was ~5%.

Table 1
Influence of AmimCl concentration on succinic acid production by *A. succinogenes*.

Percentage of ILs (%)	Succinic acid concentration (g/L)	Glucose depletion (g)	Yield (mol/mol)
0	16.09 ± 0.17	3.21 ± 0.03	1.23 ± 0.03
0.01	14.65 ± 0.44	2.88 ± 0.29	0.94 ± 0.08
0.05	15.05 ± 0.81	2.95 ± 0.17	0.93 ± 0.04
0.1	16.00 ± 1.52	3.04 ± 0.38	0.96 ± 0.04
0.5	13.40 ± 0.55	2.38 ± 0.72	1.11 ± 0.38
1	12.41 ± 0.04	2.16 ± 0.65	1.43 ± 0.38
3	–	0.39 ± 0.09	–
5	–	0.25 ± 0.43	–
10	–	0.11 ± 0.08	–