Bioresource Technology 130 (2013) 339–344

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Effects of selected ionic liquids on lipid production by the oleaginous yeast *Rhodosporidium toruloides*

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HIGHLIGHTS

- Oleaginous yeast *R. toruloides* tolerated ionic liquids used for biomass pretreatment.
- Assimilation of the anion of [Emim][OAc] led to a rapid alkaline-pH shift.
- Ionic liquids tended to increase saturated fatty acids contents of lipid samples.

ARTICLE INFO

Article history:
Received 16 August 2012
Received in revised form 1 December 2012
Accepted 5 December 2012
Available online 14 December 2012

Keywords:
Microbial lipid
Rhodosporidium toruloides
Ionic liquids
Biofuel
Lipid production

ABSTRACT

Lignocellulosic biomass pretreatment with ionic liquids (ILs) has been emerged as a new technology, but the effects of residual ILs on the downstream biotransformation remain largely unknown. Here, three typical ILs were tested for their effects on lipid production by the oleaginous yeast *Rhodosporidium toruloides* AS 2.1389. When cultures were maintained at pH 6.0 in the presence of 30 mM ILs, [Emim]Cl, [Emim][DEP], or [Emim][OAc], minor inhibition effects were observed. When cultures were performed in the presence of 60 mM ILs or without pH control, inhibition was largely dependent on ILs. Detailed analysis indicated that the anion of [Emim][OAc] was assimilated, leading to a rapid alkaline-pH shift and enhanced inhibition on cell growth and lipid production. Our results demonstrated that *R. toruloides* is a robust lipid producer tolerating ILs at low concentrations, and that care should be taken in bioprocess control and data analysis when ILs are involved.

1. Introduction

Increasing energy demand, together with rapid depletion of fossil fuels, necessitated searching for renewable fuels (Padmanabhan et al., 2011). Lignocellulosic biomass has received much attention as a potential feedstock for biofuel production (Lynd et al., 2008). However, lignocellulose is a highly ordered and rigid polymer, and the crystalline structure limits biodegradation by cellulolytic enzymes (Nakashima et al., 2011). To ensure the enzymatic hydrolysis process practical, lignocellulose is typically pretreated by using various procedures (Ouellet et al., 2011). The most widely used method is dilute acid pretreatment, which can depolymerize hemicellulose, remove some lignin, and make the cellulose more amenable to enzymatic hydrolysis (Wyman et al., 2005). However, this method is known to rust equipment and produce inhibitors detrimental to downstream microbial transformations (Palmqvist and Hahn-Hagerdal, 2000a; Palmqvist and Hahn-Hagerdal, 2000b; Pienkos and Zhang, 2009). Hence, alternative pretreatment methods have been emerging. Recently, pretreatment with ionic liquids (ILs) has been received intensive study (Klein-Marcuschamer et al., 2011), and more biocompatible ILs have been developed. Regardless of detailed procedures (Li et al., 2010; Ninomiya et al., 2012; Wang et al., 2011), however, ILs are carried over and present at various concentrations in the stream used for fermentation (Zhao, et al., 2009). This may cause unacceptable effects. For example, the presence of [Emim][OAc] at 33–52 mM in the corn stover and switchgrass hydrolysates showed significant inhibition on cell growth and ethanol production by *Saccharomyces cerevisiae* (Ouellet et al., 2011). Hexyl- and octyl-imidazolium and pyridinium bromides at concentrations as low as 1000 ppm had significant antimicrobial activity to *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *S. cerevisiae*

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http://dx.doi.org/10.1016/j.biortech.2012.12.022