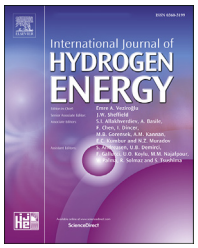
Available online at www.sciencedirect.com

ScienceDirect

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



Short Communication

Assessment via the modified gompertz-model reveals new insights concerning the effects of ionic liquids on biohydrogen production

Nándor Nemestóthy [a], *Péter Bakonyi* [a,*], *Tamás Rózsenberszki* [a], *Gopalakrishnan Kumar* [b], *László Koók* [a], *Gábor Kelemen* [a], *Sang-Hyoun Kim* [c], *Katalin Bélafi-Bakó* [a]

[a] *Research Institute on Bioengineering, Membrane Technology and Energetics, University of Pannonia, Egyetem Ut 10, 8200 Veszprém, Hungary*

[b] *Institute of Chemistry, Bioscience and Environmental Engineering, Faculty of Science and Technology, University of Stavanger, Box 8600 Forus, 4036 Stavanger, Norway*

[c] *School of Civil and Environmental Engineering, Yonsei University, Seoul 38722, Republic of Korea*

ARTICLE INFO

Article history:
Received 4 June 2018
Received in revised form
24 July 2018
Accepted 26 August 2018
Available online 19 September 2018

Keywords:
Biohydrogen
Ionic liquid
Lignocellulose
Inhibition
Modified gompertz-model

ABSTRACT

Lignocellulosic biofuel, in particular hydrogen gas production is governed by successful feedstock pretreatment, hydrolysis and fermentation. In these days, remarkable attention is paid to the use of ionic liquids to make the fermentable regions of lignocellulose biomass more accessible to the biocatalysts. Although these compounds have great potential for this purpose, their presence during the consecutive fermentation stage may pose a threat on process stability due to certain toxic effects. This, however, has not been specifically elaborated for dark fermentative biohydrogen generation. Hence, in this work, two common imidazolium-type ionic liquids (1-butyl-3-methylimidazolium acetate, ([bmim][Ac]) and 1-butyl-3-methylimidazolium chloride, ([bmim][Cl])) were employed in mixed culture biohydrogen fermentation to investigate the possible impacts related to their presence and concentrations. The batch assays were evaluated comparatively via the modified Gompertz-model based on the important parameters characterizing the process, namely the biohydrogen production potential, maximum biohydrogen production rate and lag-phase time.

Introduction

The improvement of gaseous biofuel, in particular biohydrogen production from lignocellulosic resources has been a definitive target of recent scientific activities [1–3]. On that matter, the wide consent throughout the literature studies reveals the importance of several consecutive steps, including: (i) feedstock pretreatment, (ii) carbohydrate hydrolysis and (iii) fermentation [4]. Attributed to the recalcitrant composition of lignocellulose

* *Corresponding author.*
E-mail address: bakonyip@almos.uni-pannon.hu (P. Bakonyi).
https://doi.org/10.1016/j.ijhydene.2018.08.174

biomass – comprising mainly of cellulose, hemicellulose and lignin – the objective of pretreatment is to enhance the accessibility to the carbohydrate (cellulose, hemicellulose) regions, to be considered as the primary source of substrates for the fermentation [5]. Because of the fact that most C_6 as well as C_5 sugars are bound in these complex fractions (which are not directly convertible by the microorganisms), an efficient hydrolysis is needed to achieve depolymerization and concurrent liberation of monomeric components such as glucose and xylose. Hereafter, these simple compounds can be consumed and undergo a biocatalytic transformation [6].

In the line of the methods available for the pretreatment of lignocelluosic organic matter, those relying on ionic liquids (ILs) as novel green solvent have received notable attention [7], including the field of biological hydrogen production [8]. Actually, it has been demonstrated that ILs can not only open up the lignocellulose structure but at the same time, solubilize the carbohydrate parts, i.e. cellulose [9] assisting the subsequent enzymatic hydrolysis. In this aspect, the concept of the so-called one-pot/single-pot process has emerged [10], representing an integrated scheme to accomplish in-situ saccharification.

In such a system, however, one significant challenge to be considered is the compatibility of ILs with the biocatalysts. As it was reported, certain ILs may inhibit or even deactivate enzymes contributing to cellulose decomposition [11]. On these grounds, if the conversion of sugars is intended in a bioreactor where ILs (even in lower quantities) are present, a similarly adverse impact on the whole cell strains as microbiological catalysts may be encountered [12]. Overall, it can be concluded that ILs potentially affect the performance of biotechnological processes ascribed to their impact on cell growth as well as activity/stability of enzymes.

However, this subject in the field of biological hydrogen production is quite underdeveloped and needs therefore further exploration. In fact, as concluded in the review of Nissila et al. [13], the application of ILs in the sequence of hydrogen fermentation technology has not expanded yet and consequently, not much relevant feedback is available. In the frame of lignocellulose to biohydrogen conversion, the influence of various inhibitors such as phenolic and furanic compounds has been evaluated by various researchers. Normally, the components to be studied are chosen based on expected by-product release during lignocellulose pretreatment. As a result, substances such as furfural, hydroxymethylfurfural (HMF), acetic acid, vanillin, syringaldehyde, 4-hydroxybenzoic acid, etc. have been tested in the biohydrogen process [14–16]. However, again, it is hard to find any feedback related to the effect of ionic liquids on biohydrogen production, although as mentioned, it could be of importance due to its possible occurrence when this material is applied to purposes e.g. lignocellulose pretreatment.

Hence, in this work, biohydrogen fermentation from glucose (as the model substrate of cellulose hydrolysis) was investigated in the presence of imidazolium-type ILs (to be described by the general formula of $[C_nmim]^+[X]^-$ [17], such as 1-butyl-3-methylimidazolium acetate ([bmim][Ac]) and 1-butyl-3-methylimidazolium chloride ([bmim][Cl]). The ILs consisting of imidazolium cation have been among the most frequently employed ones for cellulose processing [18] and here, the main emphasis was set on the possible impact of anion attached (either $[Ac]^-$ or $[Cl]^-$). In the course of the experiments, the effect of IL concentration was evaluated via the modified Gompertz-model. This is a well-accepted model to assess batch biohydrogen fermentation and can deliver useful parameters to characterize the process, in particular the biohydrogen production potential, maximal biohydrogen production rate and lag-phase time [19].

Material and methods

Inoculum and ionic liquids

For the inoculation of biohydrogen assays, mesophilic anaerobic sludge collected from municipal wastewater treatment plant was used, with main physico-chemical and microbiological features (i.e. relative abundance of species) reported in our recent publication [20]. Prior to application, the biohydrogen-producing bacteria in the sludge were enriched by conventional heat-shock pretreatment (80 °C, 30 min), as documented in our previous article [21]. Although in this study, the shift of bacterial community as a result of heat pretreatment was not followed, literature studies [22–24] confirmed the appropriateness of this method to enrich *Clostridia*, which are considered as efficient biohydrogen-producing strains and were present in the inoculum according to our previous work [20].

Ionic liquids, namely [bmim][Ac] (> 98% purity) and [bmim][Cl] (> 99% purity) were purchased from IoLiTec, Germany and employed as received.

Batch biohydrogen assays

The measurements were performed in manometric vessels in accordance with our earlier paper [25]. Briefly, 60 mL heat-pretreated sludge was loaded to 500 mL reactors, along with 0.3 g glucose (Sigma-Aldrich, USA), resulting in 5 g/L initial substrate concentration for all cases. The concentration range of ILs (mg/mL) in the broth was chosen based on feedback from our preliminary experiments with biocatalytic systems employing ionic liquids i.e. Nemestóthy et al. [11,26] and varied subsequently according to Table 1. Besides ILs, no other components were supplied externally to the heat-pretreated sludge (serving also as the fermentation medium). The prepared reaction mixtures (with pH adjusted to 5.5) were initially flushed with high purity nitrogen (> 99.9%) to establish anaerobic condition, which was confirmed by the successful H_2 production tests. The increasing pressure inside the closed bottles as a function of time (attributed to gas production) was registered (in the unit of hPa) by manometric heads (WTW OXITOP®) for 65 h at 37 °C temperature, ensuring 220 rpm stirring rate in all vessels by means of magnetic bars. The H_2, CO_2 and CH_4 contents of the gaseous phase were determined at the end of the tests by gas chromatography, as detailed elsewhere [25]. Taken into account the pressure and composition of the evolved gas (as quantitative and qualitative information, respectively), biohydrogen generation (in the unit of mmol) was estimated by the ideal gas law.