Influence of Ionic Liquids on growth of Escherichia coli strain possessing β-galactosidase activity

Magdalena Stobińska, Dawid Urbański, Artur Bartkowiak

1 Center of Bioimmoblization and Innovative Packaging Materials, West Pomeranian University of Technology in Szczecin, ul. Klemensa Janickiego 35, 71-270 Szczecin, Poland, email: mstobinska@zut.edu.pl

Ionic liquids (ILs) have gained a significant attention as alternative solvents in many chemical and biological reactions. ILs have evolved as a new type of non-aqueous solvents for biocatalysis, because they can stabilize and activate enzymes. Ionic liquids to be considered as so called "green solvents" should possess only very low or no toxicity to living organism and the environment. Currently, very little is known about the biotoxicity of ionic liquids. Within this paper a relative toxicity of two ionic liquids 1,3 dimethylimidazolium methyl sulfate [MMIM][$MeSO_4$] and 1-hexyl-3-methylimidazolium chloride [HMIM][Cl]) on the growth of *Escherichia coli (E.coli)* was evaluated. In this study we focused on ionic liquids, which potentially could increase activity of β-galactosidase produced by selected strain of *E. coli*. The inhibition of cell growth in the presence of various ionic liquids was determined using both, solid and liquid cultures. Liquid culture was incubated in culture medium with different concentrations of ionic liquids (0.1, 1, 5, 10% (v/v)). Determination of the minimal inhibitory concentration (MIC50) of ionic liquids was based on monitoring of *E. coli* growth by optical density measured at 600 nm using densitometer. In the second method the solid culture plate with addition of various amount of ILs has been used. The number of colonies formed on the solid culture plate was converted to colony forming units (CFUs). The obtained results have confirmed, that prescreening based on cytotoxicity tests is a key element in selection procedure of ILs for any biotechnological processes based on application of microorganisms.

Keywords: ionic liquids, β-galactosidase, toxicity.

Introduction

Ionic liquids (ILs) are organic salts composed solely of ions. Ionic liquids are low-melting (<100°C) salts which represent a new class of nonmolecular, ionic solvents. Many are liquid at room temperature. ILs have gained a significant attention as alternative solvents in many chemical and biological reactions [1]. Ionic liquids contain organic cations that are relatively large and inorganic anions. ILs have unique properties such as nonvolatility, low flammability, high ionic conductivity, good dissolution power towards many substrates and high thermal and chemical stability. Because of these unique properties, ionic liquids have been widely recognized as solvents, co-solvents or co-catalysts in variety of applications, including organic catalysis, inorganic synthesis, biocatalysis, polymerization and engineering fluids. ILs have evolved as a new type of non-aqueous polar solvents for biocatalysis, because they can stabilize and activate enzymes [2-3]. Ionic liquids to be considered as so called "green solvents" should be nontoxic or possess only low toxicity to living organisms and final neutral impact on the environment. The green character of ILs usually has been related to their negligible vapour pressure [3-4]. However, the number of studies on toxicity, biodegradation, safety, health and environmental impact data of the conventional ionic liquids has been limited until now. Ionic liquids potentially could increase activity of various enzymes including β-galactosidase [4-5] produced by *E.coli*. Some of the most commonly ILs which increase activity of β-galactosidase like [BMIM][BF_4] and [BMIM][PF_6] were already tested for toxicity [6-7]. These papers showed that key factors are the type of ILs anions, water-miscibility, concentration and obviously type of microorganism applied in the study. Despite that both 1-hexyl-3-methylimidazolium chloride [HMIM][Cl] and 1,3 dimethylimidazolium methyl sulfate [MMIM] [$MeSO_4$] are known as ionic liquids, which increase activity of β-galactosidase, so far there is no scientific report on their potential toxicity towards microorganisms [1,2,9].

Materials and methods

Two ionic liquids 1,3 dimethylimidazolium methyl sulfate [MMIM] [$MeSO_4$] and 1-hexyl-3-methylimidazolium chloride [HMIM][Cl] were obtained from Ionic Liquids Technologies (IoLiTec GmbH, Germany). All other chemicals were purchased from Sigma (Steinheim, Germany), Merck (Darmstadt, Germany) and Chempur (Poland).

The *Escherichia coli* (DSMZ, Germany) strain was grown in MacConkey liquid media based on beef bile, pepton G, lactose and bromocresol purple (BIOCORP, Poland). The solid media MacConkey consisted of peptone, lactose, bile salts, neutral red, crystal violet, agar, sodium chloride (Merck, Germany).

Cultivation conditions

Liquid cultures

Cultures were grown overnight using MacConkey solid media at 37°C. After 24h incubation the suspension of bacteria in 0.9 % NaCl was prepared so that the optical density (measured at 600 nm) of cultures reached 0.125 value, corresponding to 1.5 x 10^8 CFU/mL. 100 µL of the cultures was then transferred to 5 mL of fresh media in a new sterile tube containing varying amounts of the ionic liquids [MMIM][$MeSO_4$] or [HMIM][Cl] at concentration of 0.1, 1, 5, 10 % (v/v). As a control the culture without any additive was used.

Determination of the minimal inhibitory concentration (MIC50) of ionic liquids was based on monitoring of *E. coli* growth by optical density measured at 600 nm using a WPA CO8000 Cell densitometer (Biochrom, UK).

Solid cultures

The solid MacConkey-agar plates were used for colony counting to determine the number of viable cells. The plates of 60 mm in diameter with final volume of 5 ml of media have contained varying amounts of both ionic liquids [MMIM][$MeSO_4$] and [HMIM][Cl] at concentration of 0.1, 1, 5, 10 % (v/v). Each number of colonies in solid plate was counted after 24h incubation at 37°C. Finally, the number of colonies formed on the solid culture plates were converted into the colony forming units (CFUs).

Results and discussion

Both selected ionic liquids could increase the activity of the enzyme β-galactosidase produced by *E. coli* [1,2]. However, there is no information of their direct cytotoxicity against such bacteria. The present study has shown that the growth of *Escherichia coli* bacteria is inhibited in aqueous media at various concentrations of two selected ionic liquids (Fig. 1). The optical density liquid media with *E. coli* after incubation in [MMIM][$MeSO_4$] and [HMIM][Cl] was determined at 600nm using a densitometer. The OD for control – cultures without any ionic liquids were 0.9 and 1.14 and after incubation of cultures with [MMIM][$MeSO_4$] was 0.88 for 0.1% (v/v) of IL, 0.76 for 1% (v/v) of IL, 0.6 for 5% (v/v) of IL and 0.01 (v/v) of IL (Fig. 1a). This result has shown that with increasing amount of ionic liquid value OD is decreasing.

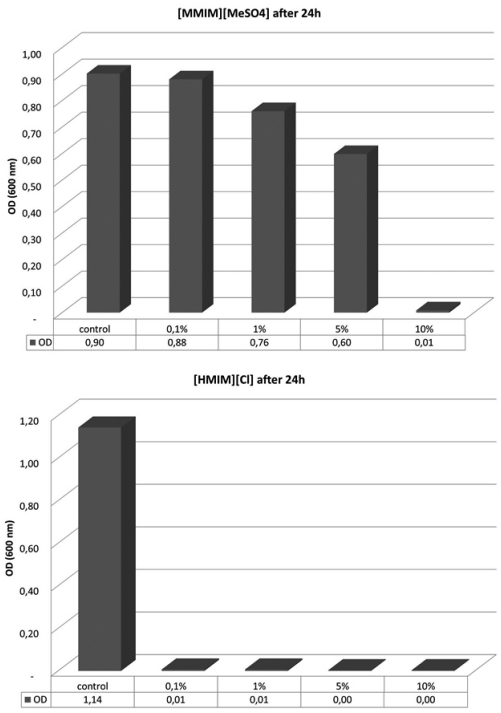

Fig. 1. Optical density (OD) measured at 600 nm of control solution and after 24h incubation of *E.coli* bacteria with 0.1%, 1%, 5% and 10% (v/v) of [MMIM][MeSO4] (a) and [HMIM][Cl] (b) in liquids medium

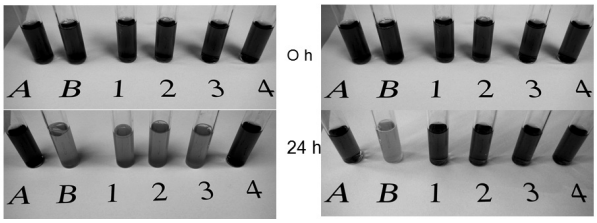

Fig. 2. Influence of [MMIM][MeSO4] (left picture) and [HMIM][Cl] (right picture) on growth of *E.coli* in liquid medium. A – medium, B – control and numbers 1-5 corresponds to 0.1, 1, 5 and 10% (v/v) of IL, respectively

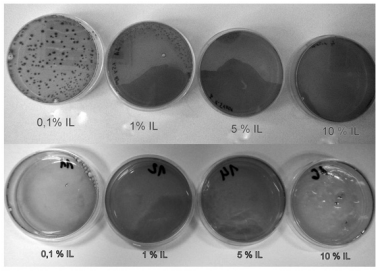

Fig. 3. The culture of E.coli solid agar – [MMIM][$MeSO_4$] media (upper picture) and agar – [HMIM][Cl] (lower picture) for 0.1%, 1%, 5% and 10% of IL

OD after incubation cultures with [HMIM][Cl] was 0.01 for 0.1% and 1% (v/v) of IL, respectively and, 0.00 for 5 % and 10% (v/v) of IL, respectively (Fig. 1b). This outcome have shown that [HMIM][Cl] even in the lowest concentrations, i.e. 0.1% (v/v) is very toxic, no allowing the culture growth. Comparing both results it can be stated that the ionic liquid [MMIM][$MeSO_4$] is less toxic than [HMIM][Cl]. The minimal inhibitory concentration (MIC50) for [MMIM][$MeSO_4$] is above 5% (v/v), where for [HMIM][Cl] is less than 0.1% (v/v).

As evidenced on the images on Fig.2 (0 h) all the fresh media were purple color, i.e., dark gray on the figure. After 24 h the bacteria incubation in a medium containing [MMIM][$MeSO_4$] evidenced its growth by change of color to light yellow, i.e., light gray on Fig. 2 (lower left image; 24 h). No change of color in the samples containing [HMIM][Cl] in the medium confirms the absence of active bacteria (lower right image Fig. 2; 24 h).

The toxicity of ILs on solid agar plate was evaluated as CFU/mL for the bacteria grown on [MMIM] [$MeSO_4$] and [HMIM][Cl]- based medium (Fig. 3). The appearance of small dots on upper picture of Fig. 3 evidenced a growth of solid cultures with [MMIM][$MeSO_4$]. The colony forming units for these samples were of $273x10^{13}$ CFU/mL, $273x10^{13}$ CFU/mL and $34x10^{11}$ CFU/mL for the samples containing 0.1%, 1% and 5% of [MMIM] [$MeSO_4$], respectively. For the sample containing 10% of [MMIM][$MeSO_4$] as for all the samples with the [HMIM][Cl] (lower picture on Fig. 3) no bacterial growth was observed.

Analysing the data obtained for the liquid and solid cultures it can be stated that an increasing in the concentration of the [MMIM][$MeSO_4$] above 5% (v/v) leads to a delay in growth of *E.coli* bacteria. Contrary to these results the ionic liquid [HMIM][Cl] have been shown to be extremely toxic for bacteria, already at very low concentration, i.e., at 0,1% (v/v).

Finally it should be stated that both Ils have, either different anions or cations, so more detailed study to evaluate the influence of them is in progress.

Conclusions

In this study it was shown, that ionic liquids [MMIM] [$MeSO_4$] and [HMIM][Cl] despite their positive effect on β-galactosidase activity can have a considerable distinct effect on the growth of *Escherichia coli* strain. [MMIM][$MeSO_4$] in both, liquid and solid cultures at concentrations to 5% (v/v) show only small inhibition on *E.coli* bacteria growth indicating that can be applied in biocatalysis reactions to stabilize and increase the activity of β-galactosidase. However, the [HMIM] [Cl] was considered toxic for *E.coli* bacteria in all concentration in the same conditions. More studies should be done aiming to determine the toxic role of either anion or cation on the *E.coli* bacteria and other microorganism, however it is already known that ionic liquids are promising molecule as green solvents for at least general extraction system.

References

[1] Kragl U., Eckstein M., Kaftzik N., Enzyme catalysis in ionic liquids, Current Opinion in Biotechnology, 2002, 13:565-571

[2] van Rantwijk F., Sheldon R. A., Biocatalysis in ionic Liquids, Chem. Rev,. 2007, 107, 2757-2785

[3] Yang Z., Pan W., Ionic liquids: Green solvents for nonaqueous biocatalysis, Elsevier Enzyme and microbial technology, 2005, 37:19-28

[4] Kaftzik N., Wasserscheid P., Kragl U., Use of Ionic Liquids to Increase the Yield and Enzyme Stability in the β-Galactosidase Catalysed Synthesis of *N*-Acetyllactosamine, Organic Process Research&Development, 2002, 6:553-557

[5] Lee J. K., Kim M-J., Ionic Liquid-Coated Enzyme for Biocatalysis in Organic Solvent, J. Org. Chem., 2002, 67:6845-6847

[6] Ganske F., Bornscheuer U. T., Growth of *Escherichia coli*, *Pichia pastoris* and *Bacillus cereus* in the presence of the ionic liquids [BMIM][BF_4] and [BMIM][PF_6] and organic solvents, Biotechnology Letters, 2006, 28:465-469

[7] Lee Sang-Mok, Chang Woo-Jin, Choi Ah-Rom, Koo Yoon-Mo, Influence of Ionic Liquids on Growth of *Escherichia coli*, Korean J. Chem. Eng., 2005, 22(5):687-690,

[8] Brendan F. Gilmore, Antimicrobial Ionic Liquids, Ionic Liquids: Applications and Perspecties, Alexander Kokorin (Ed.), InTech, 2011

[9] Pinto P., Costa S., Lima J., Saraiva M., β-Galactosidase activity in mixed micelles of imidazolium ionic liquids and sodium dodecylsulfate: A sequential injection kinetic study, Elsevier Talanta, 2012