**Influence of ionic liquids and seawater on the catalytic activity and stability of cellulases from *Penicillium verruculosum*.**

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**Highlights**

* Endoglucanase (EG) possesses higher stability in presence of effectors in compare with cellobiohydrolase (CBH).
* Cellulases stability well correlates with the results of saccharification of aspen wood (AW).

**1. Introduction**

The main obstacle in the bioconversion of wood is its resistance to enzymatic hydrolysis. Existing pretreatment methods aimed at increasing the reactivity of wood (reducing the degree of crystallinity and lignin content, increasing the available surface area, etc.) remain imperfect in terms of “green chemistry” [1]. The use of ionic liquids (ILs) does not lead to the formation of toxic products; in addition, they are easily regenerated, practically non-volatile, non-combustible, and dissolve many inorganic and organic compounds and gases well [2]. However, the best ILs are extremely expensive for use in industry. In recent years, seawater and its concentrates have been considered an affordable and cheap alternative to ILs [3].

The use of any pretreatment of cellulosic raw materials with its subsequent saccharification should imply the presence of an enzyme complex of cellulases, including cellobiohydrolase (CBH), endoglucanase (EG) and -glucosidase (BG) that are intact to chemical residue and capable of deep destruction of cellulose. We have previously shown that CBH from the *Tricoderma viride* was more sensitive to the presence of hydrophobic ILs with a long alkyl substituent (1-octyl-3-methylimidazolinium chloride), and EG activity was sensitive to short ([bmim]Cl) while *Aspergillus niger* β-glucosidase was the most stable of the enzymes in the presence of ILs in concentrations of 1, 5 and 10 g/l [4]. In this work, we studied the effect of [Bmim]Br, [Choline]Cl and seawater on the catalytic activity of the cellulase complex from fungus *Penicillium verruculosum*. We simulated the technological process of saccharification of pretreated aspen wood (AW) under conditions of varying degrees of washing from the corresponding reagent.

**2. Method**

Saccharification of AW (80 g/l in the reaction mixture) was performed under the action of cellulase enzymatic preparation (CEP, 10 mg/g or 0.8 mg/ml of the reaction mixture) with an excess of β-glucosidase activity (0.6 mg/g or 0.048 mg/ml of the reaction mixture ) in 2 ml test tubes in a temperature-controlled shaker. The hydrolysis process was carried out in the presence of 0.1 g/l of ampicillin in 0.1 M Na-acetate buffer pH 5.0 and 40 ° C. To study the effect of effectors, [Bmim] Br, [choline] Cl, (20, 10, 5, 2 and 1% in the reaction mixture) or seawater (200, 100, 50, 20 and 10%) were added to the reaction mixture. During the process, aliquots were taken, in which the glucose concentration was determined.

**3. Results and discussion**

The presence of 1% [Bmim] Br, 1-2% [Choline] Cl in the reaction mixture did not affect the stability of the EG and CBH. The effect of ILs on cellulases at high concentrations was different: after 48 hours, the EG activity decreased to 75% and 15% in the presence of [Choline]Cl and [Bmim]Br, respectively. The activity of CBH in the presence of [Choline]Cl decreased 2 times, and was not detected after 3 h of incubation in 20% [Bmim]Br. Seawater taken in studied concentartions did not affect the stability of the enzymes (data are not shown). The presence of effectors had little effect on the results of hydrolysis using AW as a substrate (Fig.1). The yield of glucose after 48 hours of hydrolysis in presence of 20% [Bmim] Br or 20% [Choline]Cl was 47 and 67% of the control, with 10% ILs - 72 and 76%, with 5% ILs - 77 and 80%, with 2% ILs - 85 and 91%, with 1% IL - 94 and 100%, respectively. In the presence of 200% seawater, the glucose yield was 74% of the control, at 100% - 87%, in more dilute solutions of seawater, the glucose yield values close to the control were obtained.

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**Figure 1.** The yield of glucose (g/l) under enzymatic hydrolysis of milled AW (80 g/l) using CEP (10 mg of protein/1 g of substrate) and -glucosidase (0.6 mg of protein / 1g of substrate) in the presence of ILs and seawater of various concentrations; 50 ° C, 0.1 M Na-acetate buffer with pH 5.0.

**4. Conclusions**

Pretreatment of aspen wood using ILs or seawater can adversely affect the reactivity and stability of the enzyme complex as a whole or its individual components, therefore the creation of new enzyme preparations that are tolerant to IL exposure is a promising task of industrial biotechnology. *This research was funded by Ministry of Science and High Education (MON) of Russia, project identification number: RFMEFI61617X0081.*

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