Intensification of monosaccharide usage in the process of second-generation bioethanol production

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Abstract

The need for energy security and reduction of greenhouse gas emissions, resulting from the implementation of the EU climate and energy policy objectives, obliges member states to increase energy production from renewable sources, including the development of biofuels from biomass. Liquid biofuels, used as fuel additives, play a special role in the transport sector. Until now, these were mainly first-generation biofuels, the production of which uses high-starch, sugar, and oil plants. However, currently, higher-generation biofuels are attracting more and more attention due to the common will to ensure food security, the risk of an increase in the prices of food and feed products, as well as the need to protect biodiversity, which is in line with the adopted criteria of sustainable development. The cereal straw appears to be particularly useful material for biofuel preparation, with an estimated surplus production of more than 13 million tons in Poland in 2020. Nevertheless, the conversion of lignocellulosic raw materials to monosaccharides by biochemical means is a more difficult process than the standard conversion of starchy feedstocks and usually requires one or more pretreatment steps due to the crystalline structure of cellulose and the presence of lignin, both of which inhibit enzymatic hydrolysis. Another problem associated with the production of second-generation bioethanol is the simultaneous fermentation of pentoses and hexoses available in lignocellulosic hydrolysates.

The study aimed to intensify the use of monosaccharides (glucose, xylose, and arabinose) in the production of second-generation bioethanol, obtained during alcoholic fermentation of media based on rye straw hydrolysates. In this work, the optimization of three main stages of the lignocellulosic bioethanol production process, namely pretreatment, enzymatic hydrolysis, and alcoholic fermentation, was carried out.

In the first phase of the research, the chemical composition of rye straw, both native and pretreated with selected chemical reagents (i.e., sodium base, sulfuric acid (VI), hydrogen peroxide, and ionic liquids) was determined. Native rye straw contained more than 65% of total structural polysaccharides, i.e. cellulose and hemicellulose, which made it eligible for further testing. Among the analyzed types of feedstock pretreatment, the most effective was the one with 5% w/v sodium base, after which the highest efficiency of enzymatic hydrolysis of glucan per native raw material was obtained (48.9% of theoretical yield), which resulted from more than 98% removal of lignin.

In the next stage of the research, enzymatic hydrolysis of the pretreated raw material was optimized using five commercial enzyme preparations (i.e., Cellic CTec2, Flashzyme Plus 200, Celluclast 1.5 L, Ultraflo Max, Viscoferm) and five surfactants (i.e., PPG 400, PPG 4000, Tween 20, Tween 80, PEG 6000). It was shown that in most cases increasing the enzyme dosage, extending the process time, as well as surfactant application, improved the efficiency of enzymatic hydrolysis. The use of preparation Cellic CTec2 at a dose of 20 FPU/(g glucan) and surfactant Tween 20 at a dose of 2.0 g/l, enabled the highest concentrations of glucose (26.35 g/l), xylose (5.45 g/l), and arabinose (0.55 g/l) in the hydrolysate after 72 h of hydrolysis at 50°C. The synergistic action of the enzyme preparation and surfactant effectively prevented irreversible adsorption of the enzyme to lignin, which led to an intensification of the sugar release.

In the last phase of the research, the obtained cellulosic hydrolysates were fermented via seventeen yeast cultures including: reference industrial strain Ethanol Red (*S. cerevisiae*), nine native strains (*S. stipitis* 1541, *S. stipitis* 0047, *S. stipitis* 0048, *K. marxianus* 0024, *K. marxianus* 0025, *K. marxianus* 0027, *P. tannophilus* 0037, *P. tannophilus* 0042, *P. tannophilus* 0043), and seven genetically engineered strains (UUU, SUU, SSS, S2A3K, S1A3, SXA-R2P-E, SXA-R2P). A key aspect of this part of the doctoral dissertation was to select the most active yeasts in terms of ethanol concentration and yield, as well as utilization of five-carbon sugars. Among samples fermented with native strains, the highest ethanol concentration of 11.05 g/l was determined in a sample fermented with yeast *P. tannophilus* 0043, with yields reaching 75.5% of the total sugars used. The sugar consumption was as follows: glucose – 99.8%, xylose – 46.7%, arabinose – 9.4%. Among recombinant yeasts, the highest ethanol concentration (10.48 g/l with 74.5% theoretical yield of the total sugars used) was produced by the strain SSS. During fermentation with its participation, glucose was utilized in 99.7%, xylose in 19.8%, and arabinose in 69.4%. Further studies have shown that the concentration of ethanol in fermentation media can be increased by using mixed yeast cultures applied by simultaneous inoculation, with respect to at least one of the constituting monocultures (e.g., Ethanol Red + *S. stipitis* 0047 compared to *S. stipitis* 0047).

The supplementation of the mineral salts KH_2PO_4, $MgSO_4 \cdot 7H_2O$, and $CaCl_2 \cdot 2H_2O$ to rye straw hydrolysate-based media, regardless of the yeast used for fermentation, resulted in no increase in ethanol concentration after 72 h of the process compared to control samples ($p > 0.05$). In contrast, the addition of yeast extract, a source of free amino acids, to the

fermentation media at a dose of 1.0 g/l had a significant ($p < 0.05$) effect on the enhancement in ethanol concentration and yield. The highest values of these parameters after 72 h of the process were obtained in the sample fermented with yeast *P. tannophilus* 0043, i.e., 11.74 g/l of the ethanol and 79.8% of the theoretical yield of the total sugars used. When evaluating the effect of biomass loading on the ethanol fermentation of rye straw hydrolysates, it was observed that in most cases with increasing loading (from 3% to 12% w/v dry substance) the concentration and productivity of ethanol augmented, but its yield declined in relation to the theoretical yield. The fermented sample with strain *P. tannophilus* 0043 achieved the highest ethanol concentration of 44.42 g/l at 12% w/v biomass loading. It was also observed that compared to the reference yeast Ethanol Red and the recombinant yeast SSS, the native strain *P. tannophilus* 0043 took longer to adapt to the elevated concentration of sugars at the beginning of fermentation, resulting in a more extended time required to reach the maximum ethanol concentration.

The results presented in this dissertation demonstrate the feasibility of using native yeast *P. tannophilus* 0043 and genetically engineered strain SSS for the alcoholic fermentation of rye straw-based hydrolysates. After 72 h of hydrolysate fermentation with a 3% w/v dry substance biomass loading supplemented with 1.0 g/l yeast extract, there were both increased ethanol concentrations (by 10% and 8%) and improved xylose utilization (by 28% and 19%) with these strains compared to the reference yeast Ethanol Red. The conducted research may constitute the basis for further improvement of second-generation bioethanol production via the biochemical method, including application studies.