Evaluation of the potential of fireweed (*Epilobium angustifolium* L.), European goldenrod (*Solidago virgaurea* L.), and common broom (*Cytisus scoparius* L.) stems in bioethanol production

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**Abstract**

One of the main goals of industrial biotechnology is to develop an effective method for ethanol production for fuel purposes using lignocellulosic biomass. Variability of lignocellulosic raw materials, selection of an effective method for the pretreatment of raw material, and selection of microorganisms with the ability to ferment not only hexoses but also pentoses and are moreover resistant to environmental stress generated by the products of lignocellulosic complex decomposition, are the challenges encountered in ethanol production. The use of agricultural wastelands and overgrowing plants that have little possibility of application in processes other than energy production seem to be an interesting alternative to conventional, but very often rather cultivation demanding energy crops. The aim of this study was to evaluate the possibility of using the stems of fireweed (*Epilobium angustifolium* L.), European goldenrod (*Solidago virgaurea* L.), and common broom (*Cytisus scoparius* L.) for ethanol production. The key elements studied were characteristics of the lignocellulosic complex structure, influence of the selected ionic liquids on the structural changes in biomass, and efficiency of enzymatic hydrolysis and ethanol fermentation processes. The results showed that under the assumed conditions the best effect was observed with the fireweed materials subjected to pretreatment with 1-ethyl-3-methylimidazolium acetate and enzymatic hydrolysis with Viscozyme® preparation. The final concentration of ethanol obtained was 2.509 g L$^{-1}$ with a yield of 92.3%. This was due to the highest share of cellulose (40.9%) in the whole lignocellulosic complex compared to other raw materials, which in combination with the selection of an appropriate ionic liquid and an enzymatic preparation, led to high bioprocess efficiency.

**KEYWORDS**

bioethanol, enzymatic hydrolysis, ionic liquids, plant stems
1 | INTRODUCTION

The production of bioenergy and the use of renewable raw materials in farms are among the most important objectives of the European Commission. In practice, these are met by artificially established energy crops on areas previously used for food production or the ones which are degraded or unused. Contrary to popular belief, starting of the cultivation of any energy crops often requires large financial outlays, including the purchase of good-quality seedlings and plant-protective products (especially during the first year of cultivation), which involve high costs in addition to those resulting from the necessity to fertilize plantations. Moreover, important prerequisites for such a cultivation are accurate and reliable estimates from fast-growing plantations regarding both current and potential yield of biomass, profitability, climate etc. An alternative to plantations exhibiting a targeted and strictly planned cultivation is the plants growing on set-aside land. Biomass obtained from an uncultivated land often consists of a mixture of grassland and woody plants growing in marginal areas for more than 5 years. The botanical composition of such biomass depends mainly on the geographical location and the time of land exclusion from agricultural production. In Poland, agricultural wastelands occupy about 10% of the total agricultural area. In addition, the country also has many green areas such as baulks, forest clearings, and fallow land overgrown with perennial vegetation and a mixture of grasses. Among the perennial plants, fireweed (Epilobium angustifolium L.) and European goldenrod (Solidago virgaurea L.) can be distinguished. Moreover, from the family of shrub plants one can distinguish common broom shrubs (Cytisus scoparius L.). A common feature of all three plants is the structure and composition of their stems, which contain about 20% lignin, 40% cellulose, and 25% hemicellulose. All three species require very modest soil quality and therefore can be an interesting source of biomass. Moreover, these plants behave like pioneer species and grow very well on the recultivation lands, such as coal combustion waste deposits or postmining soils (eg after sulfur exploitation), and are suitable for the protection of set-aside land. In addition, goldenrod and fireweed are melliferous species, what expands their usage in biorefineries. Usually, they form compact floristic groups or clusters, which together with their large size (over 1 m) favors their application as an attractive biomass source. Furthermore, considering the fact that 5%-8% of worldwide lignocellulose production per year would be sufficient to meet the annual demand of fossil oil, the use of common weeds for energy purposes seems justified. However, it should be noted that the production of bioethanol from lignocellulose is a multistage process, which success depends on the effective delignification of the material and change of the cellulose structure from crystalline to amorphous, as well as on efficient enzymatic hydrolysis and alcoholic fermentation. One of the methods of lignocellulose pretreatment is the use of imidazolium ionic liquids, the purpose of which is to dissolve cellulose fibers and facilitate effective enzymatic hydrolysis via increasing of the porosity of the material and dissolution of lignin. The interest in using ionic liquids and enzymatic hydrolysis for the production of bioethanol is growing rapidly mainly due to the benefits of this method. Some of the known already ionic liquids may be a great alternative to conventional lignin and cellulose solvents such as sulfuric acid and sodium hydroxide. Ionic liquids dissolve cellulose, change its structure by increasing the number and size of pores between fibers, and improve the efficiency of cellulolytic enzymes. They can also be recycled after the process and reused. Several methods for recovery of ionic liquids including distillation, extraction, adsorption, membrane separation, aqueous two-phase extraction, crystallization, and external force field separation are considered to be most valuable when it comes to IL solutions. The methods used for their recovery are distillation, adsorption, and membrane separation.

In addition to the typical energy-related aspects of use, the importance of high-yielding crops and rural development should be mentioned, including the benefits of using areas not exploited as farmlands due to their poor quality. In this view, the aim of this study was to evaluate the possibility of using the stems of fireweed, European goldenrod, and common broom in the production of second-generation ethanol.

2 | EXPERIMENTAL

2.1 | Materials

Fireweed (E angustifolium L.), European goldenrod (S virgaurea L.), common broom (C scoparius L.) used in the study were obtained from agricultural wastelands located in Zachodniopomorskie Voivodeship in Poland. The period of land exclusion from agricultural production was in the range from 5 to 15 years. The obtained biomass consisted of aboveground parts of the plants, which were harvested in September 2017 and ground and dried to a water content below 5%.

The stems were pretreated with two imidazolium ionic liquids: 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]; purum 95%, Iolitec) and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]; purum 98%, Iolitec). Simultaneously, studies were carried out on the use of biomass in its native form to compare the course of the process. The following cellulolytic preparations were used for enzymatic hydrolysis: cellulase from Aspergillus sp (≥1000 units g⁻¹), cellulase from Trichoderma reesei (≥700 units g⁻¹), Viscozyme®
(13.4 FBG (fungal beta-glucanase unit) mL\(^{-1}\)), and Cellic® CTec2 (115.6 FPU (filter paper unit) mL\(^{-1}\)) (Merck).

2.2 | Methods

2.2.1 | Pretreatment with ionic liquids

Each biomass sample was purified with two imidazolium ionic liquids: [EMIM][OAc] and [BMIM][OAc]. For this purpose, 5 g of ground material was mixed with 50 mL of the ionic liquid. The samples were then homogenized for 2 minutes and incubated at 120°C for 2 hours. After the incubation, the samples were cooled to room temperature and then the cellulose fibers were separated with deionized water, through rinsing the sample with water at least three times until the ionic liquid was removed. The solid fraction obtained was resuspended in 100 mL of 50 mmol/L acetate buffer (pH 5.0) and then subjected to enzymatic hydrolysis.

2.2.2 | Enzymatic hydrolysis and alcoholic fermentation

Four enzymatic preparations (cellulase from *Aspergillus* sp, cellulase from *T. reesei*, Cellic® CTec2, and Viscozyme® (Sigma-Merck)) were used for enzymatic hydrolysis at an amount of 20 FPU g\(^{-1}\) of cellulose in the material. Biomass fractions mixed with *Aspergillus* sp cellulase and *T. reesei* cellulase were incubated at 47°C for 72 hours, while those treated with Cellic®CTec2 and Viscozyme® were incubated at 50°C for 72 hours.

Hydrolysate solutions (50 mL), after filtration in order to remove any lignocellulose residues, were subjected to alcoholic fermentation. The pH of the fermentation broth was measured at each sampling point and adjusted to 5.0 by adding either 10 wt.% H\(_2\)SO\(_4\) or 20 wt.% NaOH. Ethanol fermentation was initiated by adding freeze-dried distiller's yeast *Saccharomyces cerevisiae* type II (Sigma-Aldrich) (5%, w/v). The samples were placed in fermentation flasks (volume of 100 cm\(^3\)) in a 100 rpm shaking incubator. The fermentation process took 96 hours at the temperature of 37°C in anaerobic conditions. Samples of a volume of 2 cm\(^3\) were taken for HPLC analysis, and 70 cm\(^3\) for distillation and pycnometric measurements.

2.2.3 | Analysis of raw materials structure

The content of lignin, cellulose, and hemicellulose was determined in the collected biomass by using filter bags and the AnkomA200 apparatus. The content of neutral detergent fiber (NDF) was determined using the Van Soest method, while that of acidic detergent fiber (ADF) and acidic detergent lignin (ADL) was measured according to the standard. The content of cellulose was determined based on the difference between the shares of ADF and ADL fractions, whereas the content of hemicellulose was determined from the difference between the shares of NDF and ADF fractions.

Changes in the crystalline structure of the raw material were evaluated by analyzing the obtained scanning electron microscopy (SEM) images. The morphology of cellulose fibers in the samples before and after pretreatment with ionic liquids was observed using a scanning electron microscope Quanta 200 Mark II produced by FEI Company. All images were taken at the magnification of 500×, at the acceleration voltage 25 kV. Prior to placing the sample in a high vacuum environment, they were dried at elevated temperatures and placed on a conductive foil.

2.2.4 | High-performance liquid chromatography

The content of glucose and ethanol was determined by high-performance liquid chromatography (HPLC). For chemical analysis, the samples were first centrifuged at 4000 g for 10 minutes at 4°C (Multifuge 3SR), filtered through a 0.22-µm membrane filter (Millex-GS, Millipore), and then analyzed on an HPLC system (Merck Hitachi). The fractions of glucose, ethanol, acetic acid, lactic acid, and glycerol were separated using an Aminex HPX-87P system (Bio-Rad) at 30°C using a 5 mmol/L H\(_2\)SO\(_4\) solution as the mobile phase at a flow rate of 0.6 mL min\(^{-1}\), and then detected with a refractive index detector (Model L-7490, Merck Hitachi).

2.2.5 | Efficiency of enzymatic hydrolysis and ethanol production

The lignocellulose to ethanol conversion rate (%) was calculated according to the formula\(^{10}\):

\[
Y = \frac{C_e \times V \times 100}{M \times C \times 1.1 \times 0.51} \times 100 \text{ (%)};
\]

where \(C_e\) – ethanol concentration (g L\(^{-1}\)); \(V\) – sample volume (L); \(M\) – total amount of substrate in the sample (g s.s.); \(C\) – cellulose and hemicellulose concentration in the material (%); 1.1 – cellulose to glucose conversion factor; 0.51 – glucose to ethanol conversion factor.
3 | RESULTS

3.1 Chemical characteristics of raw materials before and after the treatment with ionic liquids

In the first stage of this work, three raw materials selected for the study were evaluated for the content of cellulose, hemicellulose, and lignin, which are the key elements of the lignocellulosic complex. The analysis of the composition showed that the content of cellulose was similar in common broom and European goldenrod and was equal to 30.7% and 30.4%, respectively. The highest amount of cellulose was found in fireweed, constituting 41.0% of the lignocellulosic complex. Hemicellulose content was lower amounting to 21.9% in fireweed, 28.8% in common broom, and 29.2% in European goldenrod. Lignin, which is not a source of fermenting sugars but acts rather as a specific binder of cellulose and hemicellulose, constituted a large share in the studied raw materials—20.4% in fireweed, 16.0% in common broom, and 19.2% in European goldenrod.

The raw materials were subjected to pretreatment with ionic liquids [BMIM][OAc] and [EMIM][OAc], which resulted in a change in the proportion of individual components of the lignocellulosic complex. This change was more obvious in the variant in which [EMIM][OAc] was applied. In the samples of fireweed, the content of lignin was reduced from 20.4% to 17.9%, and in European goldenrod, the content reduced from 19.2% to 12.8%. There were also differences observed in the content of hemicellulose after pretreatment with ionic liquids with a decrease by about 2% on average. On the other hand, the percentage of cellulose increased by as much as 10% in the samples of fireweed and European goldenrod after pretreatment. To determine the changes in the structure of the examined raw materials, their images were taken with a scanning electron microscope (Figures 1-3). It was observed that in each plant material, after the application of imidazoliumionic liquids, the fibers were untangled and the structural integrity of the material was permanently lost. On comparing the plant materials with each other, it was found that both European goldenrod and fireweed were more susceptible to ionic liquids than common broom.

3.2 Enzymatic hydrolysis

The next stage of the study involved enzymatic hydrolysis for the release of fermenting sugars. The effectiveness of enzymatic hydrolysis carried out using the four commercial enzyme preparations was verified in the study. The raw materials from all three species were subjected to enzymatic hydrolysis. Three experimental variants were prepared—raw material not processed with ionic liquids, raw material pretreated with BMIM Ac, and raw material pretreated with [EMIM][OAc]. All the prepared variants were saccharified using Cellic® CTec2, Viscozyme®, T reesei cellulase, and Aspergillus sp cellulase.

**FIGURE 1** SEM images of a European goldenrod structure before and after the treatment with ionic liquids (magnification 500x, scale bar 400 µm). (A) Untreated, (B) pretreatment with [BMIM][OAc], and (C) pretreatment with [EMIM][OAc].
The process was carried out for 72 hours at temperatures and pH that were appropriate for the enzymes, following which the glucose content was determined (Figure 4).

Based on the results observed after enzymatic hydrolysis, it can be concluded that the glucose content was more influenced by the type of ionic liquid used for pretreatment.
and as the type of enzymatic preparation in comparison to the species of the plant. The best results were obtained in the experiment in which the pretreatment was carried out with [EMIM][OAc] and enzymatic hydrolysis with Viscozyme® preparation. The final glucose concentration observed in the hydrolysates of fireweed, common broom, and European goldenrod of this variant was 4.82, 4.9 g L⁻¹, and 5.13 g L⁻¹, respectively. Significant differences were also noted in the effectiveness of saccharification by individual enzymatic preparations (Figure 4). The cellulase from T. reesei was found as the least effective enzyme. The glucose concentration in the hydrolysate of European goldenrod after enzymatic hydrolysis did not exceed 3.5 g L⁻¹. A significant interaction between the effectiveness of the enzymatic preparation and the applied pretreatment was also observed. The glucose concentration in the samples of goldenrod, fireweed, and broom treated with [BMIM][OAc] was higher than that of the samples purified with [EMIM][OAc] (Figure 4).

A comparable glucose concentration of about 4.5 g L⁻¹ was noted in the samples of European goldenrod and common broom hydrolyzed using Cellic® CTe2 preparation, with no visible difference resulting from the type of ionic liquid used. The study showed that pretreatment with ionic liquid was crucial for the effective hydrolysis of the raw materials studied. In the experimental variants in which the native material was hydrolyzed using Cellic® CTe2, the glucose concentration in the hydrolysates of fireweed, common broom, and European goldenrod after 72 hours of hydrolysis was 0.555, 0.541, and 0.671 g L⁻¹, respectively. After pretreatment with [EMIM][OAc] or [BMIM][OAc], a significantly higher concentration of glucose was observed in comparison to no pretreatment, with an increase on average by 4 g L⁻¹.

The highest hydrolysis efficiency was observed in the samples in which the Cellic® CTe2 enzymatic preparation was applied and in the material treated with Viscozyme®. The effectiveness of cellulase from Aspergillus sp and
Viscozyme® depended on the ionic liquid used for pretreatment. With respect to these enzyme preparations, higher efficiency of hydrolysis was observed in the samples of biomass purified with [EMIM][OAc].

3.3 | Ethanol fermentation of lignocellulosic hydrolysates

The last stage of the work involved the assessment of the influence of pretreatment with ionic liquid and enzymatic hydrolysis on ethanol concentration after fermentation. The results showed that the fermentation efficiency was directly related to the success of pretreatment and enzymatic hydrolysis, as presented at the Figure 5. Alcoholic fermentation was carried out under conditions suitable for microorganisms, using the same procedure for all the hydrolysates. This allowed comparing the efficiency of ethanol production depending on the type of raw material and the ionic liquid used for pretreatment. The concentration of ethanol after 96 hours of fermentation was the highest in the samples of materials that were purified with [EMIM][OAc] and treated with Viscozyme® (European goldenrod) and Aspergillus sp (common broom) for enzymatic hydrolysis—which was equal to 2.86 and 2.65 g L⁻¹, respectively. In the case of fireweed, the highest concentration of ethanol (2.51 g L⁻¹) was obtained in the sample purified with [EMIM][OAc] and hydrolyzed with Viscozyme®. The Viscozyme® preparation contains thermostable xylanases, so it can be concluded that in the material hydrolyzed with this enzyme, the content of monosaccharides was higher which originated from the decomposition of cellulose and hemicellulose. Only a smaller difference was observed in ethanol content between the samples purified with [BMIM][OAc] and [EMIM][OAc] before treatment with Cellic® CTe2. However, in the remaining samples, where other enzymes were used for hydrolysis, the influence of the type of ionic liquid used was clearly observed.

Figure 6 presents the efficiency of the ethanol fermentation process depending on the ionic liquid used for pretreatment and the enzymatic preparation used for hydrolysis. The efficiency of the ethanol fermentation process ranged from 1.5% as observed in the native fireweed material hydrolyzed with Viscozyme® to 81.4% as observed in the European goldenrod material pretreated with [EMIM][OAc] and hydrolyzed with Viscozyme® preparation. It should be noted that, similar to enzymatic hydrolysis, the pretreatment of raw materials with [EMIM][OAc] was of importance in ethanol fermentation.

4 | DISCUSSION

The production of second-generation biofuels has been a challenge for biotechnologists, chemists, botanists, physicists, and gardeners for many years. The structural diversity of the raw materials and the difficulty involved in the hydrolysis of lignocellulosic complex make the production of second-generation bioethanol unprofitable. In addition, the biomass preparation for the fermentation process is complex and involves multiple stage action. Therefore, any attempts to reduce the costs are important to reach competitive production costs. Taking all of this into account, the selection of an effective and safe method for biomass pretreatment, selection, and optimization of the dose of enzymatic preparations, isolation, and screening of microorganisms resistant to toxic products of biomass decomposition, along with the selection of plants with high energy potential and that are not used for other purposes are considered as the most important objectives in the current research.11,12

In our study, we especially focused on the last objective, that is, the selection and evaluation of the usefulness of common weeds (fireweed, European goldenrod, and common broom). All three species are high-yielding perennial and shrubby plants, which grow in marginal areas and do
not require cultivation or fertilization. The stems of goldenrod, broom, and fireweed are easy to collect, dry, and grind. Although important, the abovementioned technological values of these plants remain in the shade as the qualitative and quantitative structure of the lignocellulosic complex of their stems is unfavorable. Generally, plant biomass is composed mainly of cellulose, hemicellulose, lignin, pectin, and proteins. Lignin is one of the most problematic structural elements hindering the decomposition of cell wall and subsequently preventing effective hydrolysis. Therefore, it is important to first understand the basic composition of the lignocellulosic complex, such as cellulose, hemicellulose, and lignin, in order to be able to estimate the efficiency of the bioprocess from the outset. It is worth noting here that lignin is not the only barrier hindering the hydrolysis of the polymers composing the lignocellulose, and xylan is also an example.13-16 Thus, our analyses of the native material allowed for the basic characterization of the selected raw materials (Table 1).

The next step in the production of second-generation bioethanol from lignocellulosis feedstock is the selection of appropriate biomass pretreatment. Many methods of biomass pretreatment are known, and each one of them has his advantages but also drawbacks.17 It is crucial that the pretreatment method should be so effective that the lignin fraction from the cellulose pulp is completely removed. One of the methods available for pretreatment is the use of ionic liquids, which has already been applied in many studies.18-24 The main purpose of the pretreatment with ionic liquids was to loosen the complex and create larger spaces between the cellulose and hemicellulose fibers so that the enzymes can penetrate the deeper layers of biomass during hydrolysis. The imidazolium ionic liquids selected in the present study effectively dissolved lignin-rich biomass and even caused its partial separation from cellulose fibers. In addition, they had a low viscosity and mixed well with biomass.

The results presented in the study showed a change in the biomass structure of the examined plants and also that the amount of lignin extracted after pretreatment was low, which in the further stages of the bioprocess may result in the inhibition of enzymatic hydrolysis and consequently lower ethanol production (Figure 1). The interaction of cellulose with hemicellulose, with the latter fraction being dominant, may also hinder enzymatic hydrolysis. Tavares et al25 claimed that the system of supramolecular structure in plants, including the size of polysaccharides in cells and tissues, is determined by the glycomic code, which provides information on how polymeric bonds are formed. The other aspects that determine the availability of polymers for enzymes are the size of pores and the gaps between the cellulose and hemicellulose fibers, and therefore, the size of enzyme molecules is also an important factor affecting their effective penetration into the plant material. It is worth pointing out that biomass pretreatment with ionic liquids is more effective when the water content in the raw material is lower. Our study showed that the ionic liquids [BMIM][OAc] and [EMIM][OAc] undoubtedly affected the structure of the plants studied (Figures 1-3). The ability of ionic liquids to dissolve lignocellulosic biomass under gentle conditions and with little or no by-product formation makes them highly interesting alternatives for pretreatment in the processes where high product yields are of critical importance.26

Enzymatic hydrolysis is a safe and effective alternative to acidic or alkaline hydrolysis because it takes place under milder conditions and provides a high yield of hydrolysate without the need for neutralization/purification.27 This process may be compatible with ionic pretreatment as ionic liquids do not adversely affect the activity of enzymatic proteins. Enzymatic hydrolysis of cellulose, which leads to full depolymerization, requires the use of several enzymes that can act synergistically, such as cellulases, hemicellulases, and pectinases. Cellulose-hydrolyzing cellulases include

| Sample                        | Pretreatment | Cellulose [%] | Hemicellulose [%] | Lignin [%] |
|-------------------------------|--------------|---------------|-------------------|------------|
| Epilobium angustifolium L.     | Untreated    | 41.0a         | 21.9c             | 20.4c      |
|                               | [BMIM][OAc] | 46.4b         | 20.7a             | 19.6a      |
|                               | [EMIM][OAc] | 42.9a         | 20.9b             | 17.9d      |
| Cytisus scoparius L.           | Untreated    | 30.7c         | 28.8a             | 16.0a      |
|                               | [BMIM][OAc] | 43.7a         | 24.7              | 14.9d      |
|                               | [EMIM][OAc] | 38.6c         | 20.8d             | 16.1d      |
| Solidago virgaurea L.          | Untreated    | 30.4d         | 29.2a             | 19.2a      |
|                               | [BMIM][OAc] | 38.0a         | 22.0d             | 13.3b      |
|                               | [EMIM][OAc] | 40.6a         | 22.0d             | 12.8d      |

Note: Means of three replications based on the least significant difference procedure at α = 0.05 level. Means with the same letter in the same column are not significantly different.
endoglucanases (EG I, EG III) and cellulobiohydrolases (CBH I, CBH II), which degrade crystalline cellulose to soluble cellulose and amorphous cellulose in the first stage, and β-glucosidase (BG), which hydrolyses cellulose to glucose.\textsuperscript{28,29} Commercial enzyme preparations that can be used for saccharification are available in the form of mixtures of several enzymes (“enzyme cocktails”) containing, for example, cellulases, hemicellulases, and pectinases.\textsuperscript{30} In this study, four commercial enzymatic preparations were used and their effectiveness in the context of hydrolysis efficiency and the final concentration of glucose, which is the basic source of carbon for the \textit{S. cerevisiae} yeast used by us, was evaluated. The preparation which allowed the most effective hydrolysis was Viscozyme® (Figures 5 and 6). It is a product containing a range of carbohydrases including arabinase, cellulase, β-glucanase, hemicellulase, and xylanase. It also breaks down the branched pectin-like substances found in plant cell walls.

Other enzymatic preparations used, such as cellulase from \textit{T. reesei} or from \textit{Aspergillus} sp, did not allow for the achievement of better results, despite the fact that they were used in the hydrolysis of biomass from plants tested by us.\textsuperscript{31-35} For example, Bombeck et al\textsuperscript{35} showed that the use of the enzymes from \textit{Aspergillus} sp for hydrolysis caused a significant decrease in the proportion of amorphous cellulose on the substrate/tree surfaces, leaving the portions of mannan and xylan relatively intact. This mixture of enzymes also hydrolyzed chemical-thermo-mechanical pulps more effectively than cellulose pulp. In a study on raw material obtained from \textit{Miscanthus giganteus}, which was initially treated with 5\% NaOH at 121°C and hydrolyzed with Celluclast 1.5 L, a saccharification efficiency of 52\% was noted, while almost all hemicellulose (94.6\%) was degraded during the pretreatment stage.\textsuperscript{33} In another study on pretreatment with dilute acid and hydrophilic ionic liquids, the maximum glucose yield obtained from the reaction catalyzed by the cellulase from \textit{T. reesei} did not exceed 75\%.\textsuperscript{32} Dąbkowska et al\textsuperscript{36} subjected \textit{Miscanthus} stems to saccharification before and after pretreatment with organosolv method (80\% (w/w) of glycerol, 1.25\% of H2SO4) using various enzymatic preparations for hydrolysis. The most effective enzyme mixture that was composed of Cellic®CTec2 (10\%, w/w), β-glucanase (5\%, w/w), and Cellic®HTec2 (1\%, w/w) resulted in high yields of glucose (93.1\%) and xylose (69.2\%) after glycerol-based pretreatment. In another study, \textit{Miscanthus} was pretreated with gaseous ammonia (temperature 150°C) and a hydrolysis efficiency of almost 100\% was achieved after 72 hours using Cellic®CTec2 cellulases.

Ethanol fermentation is the last stage of the bioprocess aimed to obtain alcohol, the final concentration of which is required to be as high as possible and often determines the profitability of the production. In our study, we obtained the maximum ethanol concentration of 2.86 g L\(^{-1}\) with a yield of up to 81.43\% from the biomass of European goldenrod, which was subjected to treatment with [EMIM][OAc] ionic liquid and Viscozyme® enzyme preparation. The lowest ethanol concentration of 0.05 g L\(^{-1}\), with a yield of 1.5\%, was obtained for the native material from fireweed, treated with Viscozyme®. For comparison, Ferreira et al (2010) fermented \textit{Pterospartum tridentatum} samples after the previous pretreatment with sulfuric acid. The authors obtained the maximum ethanol concentration of 0.26 g/g total sugars, without previous detoxification.\textsuperscript{37} In turn, Razmovski et al\textsuperscript{38} obtained an ethanol yield of 0.48 g/g (94\% theoretical yield) from \textit{Jerusalem artichoke} stems by treatment with dilute acid and hydrolysis. Goshadrou et al\textsuperscript{39} used [EMIM][OAc] to pretreat Aspen wood (\textit{Populus tremula}) and obtained 224 g of ethanol from 1 kg of biomass. A similar method of pretreatment with [EMIM][OAc] (5 hours pretreatment in 120°C) was used by Poornejad et al,\textsuperscript{40} who purified the rice straw obtaining 2.4 g ethanol from 100 g of straw. Idi et al\textsuperscript{41} purified cocoa waste with [EMIM][MeSO4]. After fermentation, the authors obtained 7.85 g L\(^{-1}\) of ethanol from the treated material and 5.12 g L\(^{-1}\) from the untreated material. Yamada et al\textsuperscript{42} obtained the ethanol production and yield from [Bmim][OAc]-pretreated bagasse on the level of 0.81 g L\(^{-1}\) after fermentation for 96 hours. In case of triticale straw, we have previously reported, that the same [EMIM][OAc] ionic liquids biomass pretreatment, and further ethanol fermentation process conducted in a very similar way, leads to 10.64 g L\(^{-1}\) of ethanol.\textsuperscript{43} The presented studies cover the various lignocellulosic raw materials from which bioethanol has been obtained using the methodology described in this publication. Several reviews on the pretreatment, hydrolysis, and ethanol fermentation have been published lately.\textsuperscript{17,28,44,45} Elgharbawy et al\textsuperscript{28} pointed out that the use of ionic liquids is a good solution that can benefit the production of bioethanol on a larger scale, combining pretreatment and enzymatic hydrolysis in one step. It should be noted, however, that the design of the production process with separate pretreatment and hydrolysis steps is equally promising. It seems crucial to design a specific process for each source of raw material, as the ethanol yield depends mainly on a well-performed pretreatment (including lignocellulose conversion and hydrolysis to monosaccharides and pretreatment specific conditions: temperature, time, or biomass loading).

5 | SUMMARY

The production of bioethanol is a multidisciplinary issue, requiring knowledge of the structure of biomass and the phenomena occurring during conversion, development of more new effective enzymes, and optimization of individual steps of the process to minimize energy costs. Moreover, the successful development of bio refineries based on the production of bioethanol from biomass depends not only on the
technology used for fuel production but also on the use of by-products produced, which would allow reducing the costs of bioethanol making it more competitive.

The present paper proposes the use of three plants growing on agricultural wastelands for the production of bioethanol, which allows obtaining a high yield of biomass and easy harvesting and storage. The highest content of ethyl alcohol was obtained for European goldenrod: 2.86 g L\(^{-1}\), next for common broom: 2.65 g L\(^{-1}\), and for fireweed 2.51 g L\(^{-1}\), all samples purified by [EMIM][OAc] and hydrolyzed using Viscozyme\(^\circ\). The efficiency of enzymatic hydrolysis depends on the type of pretreatment applied and the enzymes used, and to a less extent on the very species of the three plants investigated.

Therefore, in subsequent studies focusing on increasing the scale of the production, plants from agricultural wastelands, such as European goldenrod, common broom, and fireweed, which are a good source of cellulose and often occur in the same area (adjacent to each other), can be used as raw materials. As a future prospect, we believe that a mixture of these plants can be used for biofuel production because of the similar yield of bioethanol obtained during the process.

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