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Research article

Assessment of wasteland derived biomass for bioethanol production

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ABSTRACT

Background: The bioethanol produced from biomass is a promising alternative fuel. The lignocellulose from marginal areas or wasteland could be a promising raw material for bioethanol production because it is present in large quantities, is cheap, renewable and has favorable environmental properties. Despite these advantages, lignocellulosic biomass is much more difficult to process than cereal grains, due to the need for intensive pretreatment and relatively large amounts of cellulases for efficient hydrolysis. Therefore, there is a need to develop an efficient and cost-effective method for the degradation and fermentation of lignocellulosic biomass to ethanol.
Results: The usefulness of lignocellulosic biomass from wasteland for the production of bioethanol using pretreatment with the aid of ionic liquids of 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium chloride was evaluated in this study. The pretreatment process, enzymatic hydrolysis and alcoholic fermentation lasted a total of 10 d. The largest amounts of bioethanol were obtained from biomass originating from agricultural wasteland, in which the dominant plant was fireweed (*Chamaenerion angustifolium*) and from the field where the common broom (*Cytisus scoparius*) was the dominant.
Conclusions: The plants such as fireweed, common broom, hay and goldenrod may be useful for the production of liquid biofuels and it would be necessary in the further stage of research to establish and optimize the conditions for the technology of ethyl alcohol producing from these plant species. Enzymatic hydrolysis of biomass from agricultural wastelands results in a large increase in fermentable sugars, comparable to the enzymatic hydrolysis of rye, wheat, rice or maize straw.
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1. Introduction

Lignocellulose is a very good renewable source for the production of biofuels and chemicals [1,2]. The importance of biorefineries as places for biomass converting into liquid fuels can significantly increase in the future compared to the existing oil refineries. Lignocellulosic raw materials include residues from agriculture, forestry, energy crops and residues from the food and pulp industry [3]. In the energy sector, ethanol is currently the most important liquid biofuel. Bioalcohols are produced by the fermentative transformation of simple sugars through the use of yeast, enzymes or bacteria as biocatalysts. The use of ethanol biofuel on a large scale will require production from lignocellulosic raw materials, which are not intended for food production [4]. The production process of bioethanol from lignocellulosic raw materials consists of 4 main stages: pretreatment of raw material, enzymatic hydrolysis, alcoholic fermentation and ethyl alcohol distillation. Both the conditions as well as the manner in which ethanol production process is conducted largely depend on the raw material used. The present study has focused on the search for new sources of lignocellulosic biomass from areas excluded from agricultural production. Agricultural wastelands are overgrown by highly diverse vegetation, therefore the fields selected for bioethanol production are easy to access, are not wetlands, set aside for at least 5 years, with an area of not less than 2 ha, and overgrown by grass and shrub vegetation, the collection of which will not require specialized equipment. Processes that may enhance the efficiency of bioethanol production from lignocellulose include: biomass pre-treatment, enzymatic hydrolysis and alcoholic fermentation. Obtaining fermenting sugars from cellulose is associated with the use of a series of physical, physicochemical, chemical and biological methods. Thus far, no universal lignocellulose pretreatment method has been developed, which could be applied for all types of biomass or subject to scaling. The majority of the proposed biomass

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purification methods were introduced at a laboratory scale. Those methods utilizing environment friendly solvents or preparations gain advantages among purification methods. Such methods include pretreatment with the use of ionic liquids [5]. Ionic liquids, which as green solvents seem to be the most prospective type of biomass purification, have been chosen for lignocellulose pretreatment. This type of biomass pretreatment is undoubtedly environmentally friendly, due to the low toxicity of ionic liquids and the process that affects the reduction of CO_2 emissions during ethyl alcohol production. Ionic liquids can also be recovered, but existing methods for ionic liquids recycling are not satisfactory to be able to use them on an industrial scale. Particularly good cellulose dissolving properties characterize liquids containing imidazolium cation and chloride, acetate and formic anions [6]. Two ionic liquids, 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium chloride, were used for the present study, and their usefulness in the process of ethanol obtaining was evaluated [7,8,9]. Thus, pre-treatment of biomass with ILs offers advantages over conventional methods allowing alteration of physicochemical properties of the biomass macromolecular components, such as reduction of the cellulose crystallinity, extraction of specific macromolecules, such as lignin and hemicellulose and execution of different fractionation approaches after biomass dissolution in ILs [10,11]. Cellulose conversion to glucose may be catalyzed by enzymes known as cellulases. Cellulases have been separated primarily from *Trichoderma viride*, *Trichoderma reesei* fungi and *Clostridium thermocellum* bacteria. These microorganisms produce three types of cellulolytic enzymes: endocellulases (EC 3.2.1.4), catalyzing decomposition of random glycoside bonds of the cellulosic chain and exocellulases (cellobiohydrolases) (EC 3.2.1.91) responsible for separation of cellobiosic molecules from reducing (cellobiohydrolase I) or non-reducing (cellobiohydrolase II) end of cellulose chain and β-glucosidase (EC 3.2.1.21) decomposing cellobiose to two molecules of glucose [12]. Cellulose hydrolysis is a micellar-permutoidal process catalyzed enzymatically, in which the most important element is for the enzyme to penetrate into the cellulosic material and damage the glycoside bonds. Even with preceding delignification of biomass, penetration of cellulolytic enzymes inside the cellulose structure is inhibited due to average 2 times smaller distance between cellulose chains in the crystalline areas than the dimensions of enzyme molecules [13]. The rate, efficiency and cost of enzymatic hydrolysis process depend not only on the chemical composition and structure of lignocellulosic raw materials, but also on their pretreatment. From the economic standpoint, the use of biomass derived from wasteland for ethanol production may be of significance as it consists of vegetation overgrowing areas excluded from agricultural production, thus no competition exists here with energy crops or food crops. According to Klein-Marcuschamer et al. [14] up to 73% of all bioethanol production costs come from the costs of biomass obtaining. On the other hand, the profitability of bioethanol production from lignocellulose largely depends also on the applied technology (type of pretreatment — type and amount of ionic liquid, used cellulolytic enzymes) and on the possibility of recovering ionic liquid and its reuse. Thus, further research on bioethanol production from wasteland will concern optimization of the pretreatment and biomass enzymatic hydrolysis stages, so that the amounts of expensive reagents will be as low as possible, and the yield of reducing sugars as high as possible.

The aim of the present publication was to examine the possibility of obtaining ethyl alcohol from lignocellulosic biomass from wasteland using pretreatment with the use of ionic liquids and cellulase, a commercial enzymatic agent. This study commences the search of plants which thus far have never been used for bioethanol production, overgrowing areas excluded from agricultural activities and that could become a new source of biomass for liquid biofuel production. In addition, the applicability of selected ionic liquids for lignocellulose pretreatment has been determined.

2. Material and methods

The research material consisted of 4 samples of lignocellulosic biomass collected from fields excluded from agricultural activity from 2, 5, 8 and 12 years, respectively. From each field, general biomass sample was collected, which consisted of plants collected from at least 5 sites. A general sample is approx. 1 kg of biomass, which was used for further analyses. Its botanical composition was determined, and then it was ground. Only aboveground parts of plants were used for the tests, which were dried to a water content below 10% and then subjected to grinding. From thus prepared samples, 50 g of lignocellulose was collected for pretreatment. The biomass samples were pretreated using ionic liquids 1-ethyl-3-methylimidazolium acetate (EMIMOAc) and 1-ethyl-3-methylimidazolium chloride (EMIMCl) from IoLiTec Company. Both ionic liquids remained in liquid state in room temperature. Enzyme CellicCTec2 (Sigma Aldrich) was used for enzymatic hydrolysis and *Saccharomyces cerevisiae* type II yeast for alcoholic fermentation (Sigma Aldrich). This product contains cellulases, β-glucosidases, and hemicellulase, for the application of degrading cellulose to fermentable sugars. This enzyme is effective on a wide variety of pre-treated lignocellulosic biomass materials, for converting the carbohydrates in these materials into simple sugars prior to fermentation, for application in biofuels research. The enzyme activity was expressed as follows: 1 FPU — dose of enzyme releasing 1 μmol glucose from the Whatman no. 1 filter paper in 1 min. *S. cerevisiae* type II yeast was use for alcoholic fermentation (optimum temperature: 35–37°C; Sigma Aldrich, Poland). Liquid YPG medium with the following composition per 1 L was used for yeast cultures: 20 g glucose, 20 g peptone, and its acidity was set at pH 5.1–5.3 using concentrated sulfuric acid. Following medium sterilization at 121°C for 20 min, the medium was supplemented with 1.0 g *S. cerevisiae* type II yeast and it was incubated at 35°C for 24 h, in a water bath with shaking at 250 rpm.

Table 1
Chemical composition of the substrates after pretreatment with ionic liquids.

Number of sample	Pretreatment of ionic liquid	Cellulose [%]	Hemicellulose [%]	Lignin [%]
1	Untreated	34,64 ± 0,14	8,49 ± 0,50	17,05 ± 0,20
	EmimOAc	40,88 ± 0,22	20,97 ± 0,07	16,48 ± 0,08
	EmimCl	31,63 ± 0,17	25 ± 0,22	16,81 ± 0,13
2	Untreated	33,03 ± 0,34	13,93 ± 0,12	13,22 ± 0,32
	EmimOAc	36,37 ± 0,29	23,51 ± 0,41	13,13 ± 0,14
	EmimCl	23,29 ± 0,07	17,57 ± 0,05	26,7 ± 0,22
3	Untreated	32,95 ± 0,11	10,9 ± 0,30	12,01 ± 0,09
	EmimOAc	37,63 ± 0,10	25,72 ± 0,42	11,82 ± 0,57
	EmimCl	29,01 ± 0,09	19,01 ± 0,22	7,92 ± 0,20
4	Untreated	32,54 ± 0,37	11,05 ± 0,51	10,59 ± 0,43
	EmimOAc	42,7 ± 0,16	20,34 ± 0,29	13,56 ± 0,10
	EmimCl	34,26 ± 0,12	28,86 ± 0,30	11,67 ± 0,34

Table 2
Characteristics of samples used for the production of bioethanol 2nd generation.

Number of sample	1	2	3	4
Place of biomass samples collection				
Time of exclusion from agricultural production	15 years	5 years	8 years	12 years
Botanical composition	Fireweed, meadowsweet, nettle, couch grass	Common broom, black bent, common tansy, European Michaelmas-daisy	Mixture of grasses: orchard grass, false oat-grass, **bentgrass**, yarrow, tufted vetch, cow parsley, meadow fescue, common tansy	European goldenrod, cow parsley European Michaelmas-daisy, clover, common rush
Dominant	Fireweed	Common broom	Mixture of grasses: orchard grass, false oat-grass	European goldenrod

2.1. Ionic liquid pretreatment

After grinding, biomass samples were subjected to purification (pretreatment) with the use of ionic liquids. This procedure aimed at increasing the efficiency of enzymatic hydrolysis of polysaccharides contained in raw materials through disturbance of hemicellulose structures and loosening of lignin and cellulose complex, as well as increasing the amorphous fraction of cellulose. Two ionic liquids have been used in the experiment: 1-ethyl-3-methylimidazolium acetate and chloride were used to compare an efficiency of the pretreatment and the influence of the type of ionic liquid application on the content of reducing sugars and bioethanol. Each of 4 biomass samples in an amount of 5 g dry matter was dissolved in 50 mL ionic liquid and homogenized. The samples were then incubated at 120°C for 2 h, after that time the samples were cooled to room temperature and the material was precipitated using deionized water. The material was thoroughly rinsed with deionized water and then dried at 105°C for 1 h.

2.2. Enzymatic hydrolysis

The lignocellulosic biomass after and without pretreatment was subject to enzymatic hydrolysis with the use of Cellic CTec2. The 0.5 g of biomass previously purified with ionic liquids was weighed into round bottom flasks and then dissolved in 0.05 M acetate buffer pH 4.8 (50 mL). CellicCTec2 enzyme (25 FPU/g substrate) was added to the biomass solutions and incubated at 50.5°C for 72 h agent in water bath with shaking at 250 rpm. In order to examine the progress of hydrolysis process, 50 μL samples were collected every 24 h and the content of reducing sugars was determined.

2.3. Alcoholic fermentation

Hydrolysate solutions, previously filtered to separate the lignocellulose residue, were subjected to alcoholic fermentation. The pH of the fermentation broth was measured at each sampling and adjusted to 5.0 by an addition of either 10 wt.% H_2SO_4 (POCH, Poland) or 20 wt.% NaOH (POCH, Poland). Fermentation was started by an addition of freeze-dried distiller's yeast *S. cerevisiae* type II (Sigma-Aldrich) (5% v/v). Ethanol fermentation was conducted for 4 d in anaerobic conditions. Samples were taken and analyzed for ethanol concentrations after fermentation.

2.4. Analytical techniques

The content of lignin, cellulose and hemicellulose was determined in the collected biomass using the method with an application of filter bags and AnkomA200 apparatus. Determination of the content of neutral detergent fiber (NDF) was carried out using the Van Soest method, and determination of acidic detergent fiber (ADF) and acidic detergent lignin (ADL) was carried out according to the standard [15]. The content of cellulose was determined based on the difference between the share of ADF and ADL fractions, whereas the content of hemicellulose — from the difference of NDF and ADF fraction shares. The total content of reducing sugars after enzymatic hydrolysis was determined by the colorimetric method based on the properties of reducing saccharides which reduce the nitro groups of 3,5-dinitrosalicylic acid (Sigma Aldrich) to amino groups and cause a change of color to orange in an alkaline environment. The concentration of reducing sugars was determined based on the color intensity of the reactant mixture. The concentrations of the stained compound were measured in the Helios spectrometer at 540 nm wavelength. In the analyzed samples, glucose levels could be determined quantitatively due to the non-specificity of the applied method where DNS reduction (3,5-dinitrosalicylic acid reduction) was a measure of the sample's general reducing ability. Glucose concentrations were determined by comparing absorbance results with the absorbance profiles of reference solutions [16]. The amount of ethanol produced during decomposition of cellulose was determined with the use of the ROCHE40 kit (Enzymatic BioAnalysis/Food Analysis) that relies on UV radiation to measure ethanol concentrations in food products. Following this principle, the alcohol (ethanol), with the use of alcohol dehydrogenase and NAD is transformed enzymatically into acetaldehyde and NADH. Thus produced NADH was determined photometrically at 340 nm wavelength. Measurements of reducing sugars and ethanol content were performed in three repetitions.

2.5. Statistical methods

The process of obtaining ethyl alcohol from biomass obtained from 4 wasteland sites was repeated three times, similarly to repetitions of reducing sugars, ethyl alcohol content and biomass composition determination. Values presented in Table 1 are average values with standard deviation for individual variants. The PCA (Principal Component Analysis) technique is a very useful tool used for analysis of multidimensional result sets. It constitutes a set of statistical methods and procedures enabling i.e. reduction the number of variables, detection of the structure and general regularities in the relationships between the variables, verification of the detected regularities and relationships as well as a description and classification of the studied objects in new orthogonal spaces defined by new variables (components). The present study assessed the applicability of lignocellulosic biomass originating from wasteland to produce bioethanol with pretreatment by means of ionic liquids, 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium chloride. In order to examine the possibility for determination of reducing sugar content in samples of lignocellulosic biomass with similar botanical composition with samples used in the described study, a PLS (Partial Least Squares) regression analysis was carried out. Validation of the obtained calibration model was performed using values of Mean Squared Error (MSE) and values of

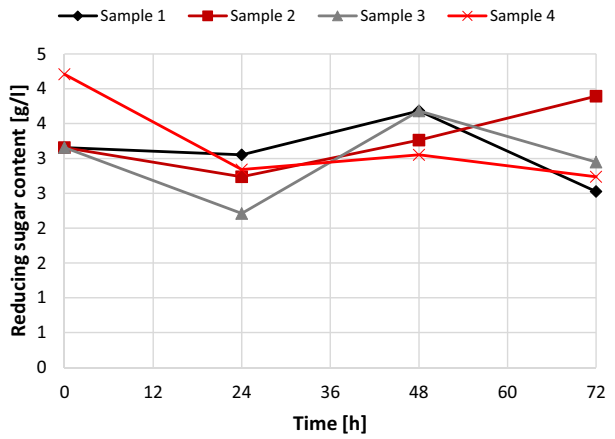

Fig. 1. The content of reducing sugars in the untreated samples. Sample 1: biomass with dominant — fireweed; Sample 2: biomass with dominant — common broom; Sample 3: mixture of grasses; Sample 4: biomass with European goldenrod.

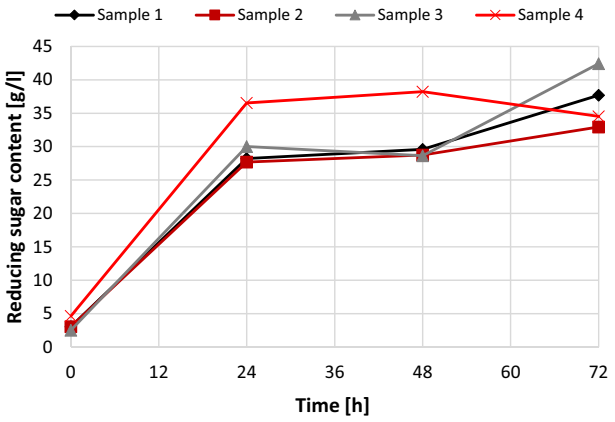

Fig. 3. The content of reducing sugars in the biomass after pretreatment with EMIMOAc. Sample 1: biomass with dominant — fireweed; Sample 2: biomass with dominant — common broom; Sample 3: mixture of grasses; Sample 4: biomass with European goldenrod.

Root Mean Squared Error (RMSE). The lower the values of both indices the better the match of the model. This model may be applied for preliminary estimation of reducing sugar content based on the composition of individual lignocellulose fractions and without the need to conduct a long and expensive process of enzymatic hydrolysis. Thanks to this method, samples originating from various wasteland areas can be initially classified, which will accelerate determination of their final usability for ethanol production. The XLSTAT version 10 software was used for analysis of the results presented in the study.

3. Results and discussion

In the biological conversion of biomass, pretreatment is an essential step for effective conversion of biomass. It reduces and/or removes the biomass recalcitrance, and results in an increase of cellulose accessibility to enzymes. The 1-ethyl-3-methylimidazolium acetate ([Emim]OAc) and many other ILs have been applied as pretreatment solvents to various feedstocks including agricultural, herbaceous and woody biomass [17,18]. Lignocellulosic biomass is rich in cellulose and easily available raw material which resources are unlimited. One of the places where a cheap resource is harvested may be agricultural wastelands, i.e., fields, meadows, and boundary strips removed from agricultural production, which percentage in Poland is about 10% in relation to all agricultural land. This is one of many sources of lignocellulose, which can be used to produce ethanol in agricultural biorefineries and as an alternative to plants originating from energy crops [5]. The biomass that was collected for the study came from 4 different agricultural wastelands, which were most often excluded from agricultural production due to anthropogenic reasons or poor soil quality. Table 2 presents the botanical composition of individual samples taken from the fields, the time of soils excluding from agricultural production and the dominant plant — about 50% in relation to the whole sample weight. It is recognized that the resistance of lignocellulose to dissolution and enzymatic saccharification is strongly related to the crystallinity of the polysaccharide, the degree of polymerization (DP), the content of lignin and surface availability (porosity) [18,19,20,21,22,23]. Hashmi et al. [24] evaluated the efficiency of 1-butyl-3-methylimidazolium acetate pretreatment on sugarcane bagasse and reported an improved digestibility and hydrolysis rates as compared to high severity autohydrolysis pretreatment. All biomass samples had a comparable content of cellulose, lignin and hemicellulose. Cellulose content was determined in native biomass and after pretreatment with individual ionic liquids to be able to check the influence of ionic liquids on the basic of lignocellulose composition, including possible changes in

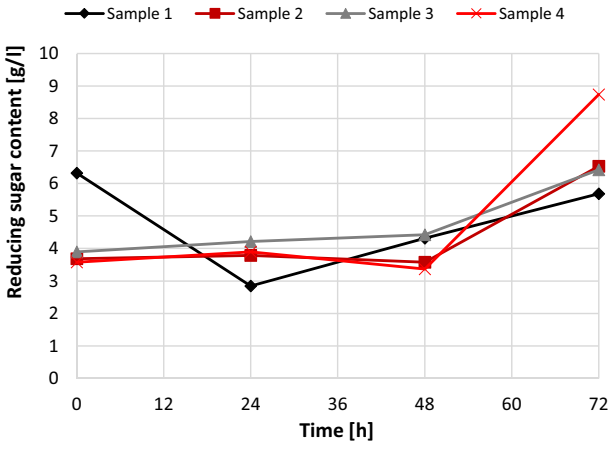

Fig. 2. The content of reducing sugars after pretreatment with EMIMCl. Sample 1: biomass with dominant — fireweed; Sample 2: biomass with dominant — common broom; Sample 3: mixture of grasses; Sample 4: biomass with European goldenrod.

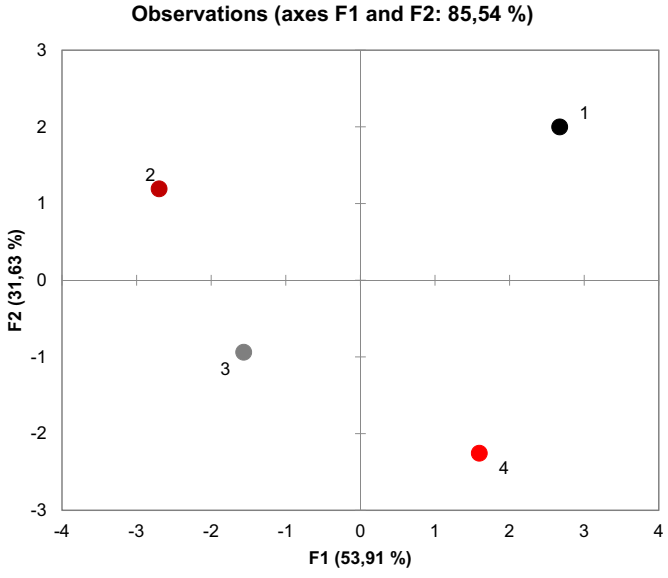

Fig. 4. Observation chart Projection of lignocellulosic biomass onto the space defined by F1 and F2 primary components. Numbers indicate the number of sample.

lignin content in the samples (Table 1). Two ionic liquids were used for biomass pretreatment, 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium chloride. The highest amount of cellulose was observed in the biomass sample from wasteland No. 4, in which the dominant plant was goldenrod and in No. 1, where the dominant was fireweed, and the samples were subjected to purification using ionic liquid — 1-ethyl-3-methylimidazolium acetate. The use of ionic liquid causes the development of cellulose fibers and the rearrangement of hydrogen bonds between cellobiose molecules in cellulose. Such loosening of the lignocellulosic complex contributed to better determination of cellulose content by the Filter Bag method in the AnkomA200 apparatus, where the biomass samples were subjected to acid bathing.

The cellulose content changed on average by 5% in favor of samples treated with 1-ethyl-3-methylimidazolium acetate. This confirmed the fact that pretreatment with ionic liquids allows increasing cellulose availability as a substrate for enzymatic hydrolysis. In the case of 1-ethyl-3-methylimidazolium chloride being used for biomass purification, no increase in the cellulose content was observed, but a decrease in the lignin content in relation to the untreated biomass samples. In biomass from field No.3, which included various grass species, a decrease in lignin from 12% to 8% after purification with 1-ethyl-3-methylimidazolium chloride was recorded. This may be due to the dissolution of lignin in the ionic liquid and its extraction. This phenomenon did not occur in the remaining samples of biomass, which included woody plants such as common broom or fireweed. Fort et al. [25] investigated the dissolution of wood chips in BMIMCl. The dissolution process of wood in [Bmim]Cl was observed through NMR analysis and they found that the weight ratio of the dissolved cellulosic material to lignin across the whole dissolution profiles was largely constant at 2:1, which is consistent with the original composition of the biomass, demonstrating that the dissolution of cellulose and lignin occurs simultaneously without obvious selectivity. The content of hemicellulose also significantly increased in all samples. The use of ionic liquids caused the expansion of hemicellulose surface, thus the degree of its determination increased even by 15% in relation to the content of this compound in native biomass samples. Hemicelluloses comprise the most varied group of polysaccharides. Different types of hemicelluloses dominate cell walls of various plant groups, and even in cell walls of the given plant at a different stage of growth. These are typically long, linear polymers of sugars bound with β bonds (1 → 4), to which short side chains are attached. This branched structure results in the fact that hemicelluloses do not interact as strongly as cellulose chains in microfibrils. In cell walls, hemicelluloses are bound with hydrogen bonds with microfibrils, creating a cellulose–hemicellulose network, maintaining the wall stability [26]. Hemicellulose is a physical barrier that covers cellulose fibers and protects it against enzymatic hydrolysis. It was demonstrated that hemicellulose removal increases the average pore size of the substrate, and thus increases the availability and probability of cellulose hydrolysis [27,28,29]. Chandra et al. [27] showed in their study a correlation between the available surface of cellulose and hemicellulose (pore size) and the enzymatic digestibility of lignocellulosic materials. The main advantage of this correlation is the improvement of enzymatic hydrolysis by lignin removal. Lignocellulose biomass has two types of surface: external and internal. The external surface is associated with the size and shape of the particles; whereas the internal surface depends on the capillary structure of cellulose fibers [27]. Sun et al. [30] observed the changes of the wall morphology and chemical compositions of wood during IL pretreatment at the cell level via hyperspectral confocal fluorescence microscopy and Raman microscopy. They found that the dissolution of lignin by EMIM OAc occurs rapidly in the secondary cell walls, while the dissolution of cellulose occurs with no preference in different layers or regions of cell walls. It was also observed that the dissolution rate of cellulose is higher than that of lignin despite the crystallinity of cellulose.

Table 4
Example results of determinations of reducing sugars content in lignocellulosic biomass samples based on the obtained PLS calibration model.

Reducing sugars content [g/L]	PLS	
	Predicted reducing sugars content [g/L]	Standard deviation
2.5263	2.5404	0.1002
3.8947	3.9032	0.1009
2.9474	2.8664	0.0607
2.7368	2.7953	0.0826

In the present study, an increase in the amount of available cellulose fibers is visible, however, lignin content decreased only in the case of 1-ethyl-3-methylimidazolium chloride purification. Low amounts of lignin extraction in EMIMOAc may stem from the solution heating temperature, which was at the level of 120°C. It has been proven that the degree of lignin extraction, apart from the use of chloride ionic liquids, is also influenced by the temperature of over 170°C and the processing time [31,32]. Nevertheless, the treatment with ionic liquids has a significant impact on improving the efficiency of enzymatic hydrolysis.

3.1. Enzymatic hydrolysis of biomass from wasteland

An effect of ionic liquid type was also visible during the enzymatic hydrolysis with an application of Celluclast enzyme preparation, a mixture of cellulolytic enzymes. The content of reducing sugars was determined in all samples every 24 h for 3 d. In the samples without pretreatment, the highest concentration of reducing sugars recorded after 72 h was at 3.89 g/L for biomass from field No. 2 (common broom was the dominant) (Fig. 1).

Pretreatment with 1-ethyl-3-methylimidazolium chloride did not improve the enzymatic hydrolysis. The content of reducing sugars after 72 h increased in all samples compared to the samples without pretreatment, but in the best case, no more than 8.73 g/L was recorded (in sample No. 4, where goldenrod was the dominant) (Fig. 2). The best results were obtained using 1-ethyl-3-methylimidazolium acetate for the initial treatment. Due to the purification, the content of reducing sugars after enzymatic hydrolysis increased to 42.42 g/L after 72 h for sample No. 3. In other examined biomass samples, the content of reducing sugars was also high, i.e., above 32 g/L (Fig. 3). The present study assessed the applicability of lignocellulosic biomass originating from wasteland to produce bioethanol with pretreatment by means of ionic liquids, 1-ethyl-3-methylimidazolium acetate and 1-ethyl-3-methylimidazolium chloride. The major target of the PCA method used was an attempt to classify in terms of possible similarities 4 samples of the obtained lignocellulosic biomass originating from wasteland for bioethanol production. The active variables were, as follows: mean cellulose value in the zero sample (CEL[O]), mean value of cellulose in sample subject to pretreatment with 1-ethyl-3-methylimidazolium acetate (CEL[OAc])

Table 3
Results of PLS regression analysis for mode determining percentage content of reducing sugars.

Model	Number of cases	Number of explaining variables	Number of explaining variables	R^2	MSE	RMSE
PLS	40	3	1	0.9806	0.0026	0.0506

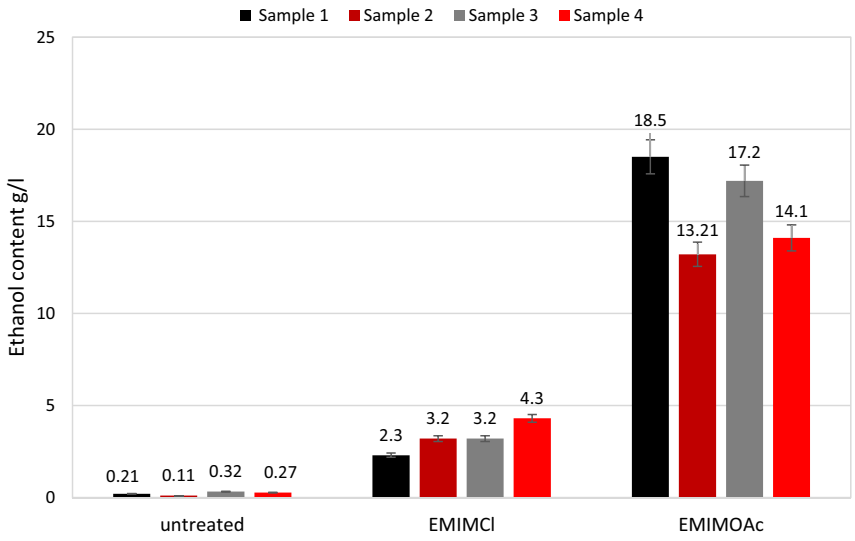

Fig. 5. Ethanol concentration in biomass samples originating from agricultural wasteland with pretreatment using ionic liquids.

ionic liquid, mean value of cellulose in sample subject to pretreatment with 1-ethyl-3-methylimidazolium chloride (CEL[Cl]) ionic liquid, mean hemicellulose value in the zero sample (Hemi[O]), mean value of hemicellulose in sample subject to pretreatment with 1-ethyl-3-methylimidazolium acetate (Hemi[OAc]) ionic liquid, mean value of hemicellulose in sample subject to pretreatment with 1-ethyl-3-methylimidazolium chloride (Hemi[Cl]) ionic liquid, mean lignin value in the zero sample (Lign[O]), mean value of lignin in sample subject to pretreatment with 1-ethyl-3-methylimidazolium acetate (Lign[OAc]) ionic liquid, and mean value of lignin in sample subject to pretreatment with 1-ethyl-3-methylimidazolium chloride (Lign[Cl]) ionic liquid.

The PCA analysis was performed on the basis of correlation matrixes. The number of primary components characterizing the set of data used in the analyses was determined using the Keiser criterion, according to which only factors with own values higher than 1 should be retained. According to this criterion, first two factors (F1 and F2) were selected and they explained over 85% of the total variability (factors F1 and F2 constitute 53.9 and 31.6% of variance, respectively). Computer data have revealed that all variables provide important data and may greatly contribute to the subsequent classification of objects. In order to determine the possible similarities between biomass samples, an observation chart was prepared (Fig. 4), which demonstrates the location of variables grouping in a new coordinate system defined by the F1 and F2 components determined in the analysis. The measure of likelihood during graphic interpretation of results was the Euclidean distance. Analysis of the chart has demonstrated existence of four fully separated points. The obtained result suggests that these samples principally differed not only by the botanical composition but also by chemical composition (different contents of cellulose, hemicellulose and lignin). The next step was to examine the possibility for determination of reducing sugar content in samples of lignocellulosic biomass with similar botanical composition with samples used in the described study. To this end, PLS regression analysis was conducted. The explaining variables consisted of cellulose, hemicellulose and lignin values obtained through measurements in individual biomass samples. The results of the regression analysis are presented in Table 3.

Analysis of the obtained model corresponding to the quantitative content of reducing sugars in cellulose biomass allows determining that the model possesses low matching degree ($R^2 = 0.98$). Low values of MSE and RMSE obtained in the analysis also indicate good prediction capability in the model. The developed calibration model was used to predict quantitative content of sugars in lignocellulosic biomass originating from wasteland with specified botanical composition. Example results from estimation are presented in Table 4. The obtained results indicate good accuracy of the obtained model. Within the present study, the content of reducing sugars was associated with the type of ionic liquid used for pretreatment as well as the type of biomass sample. The highest concentration of reducing sugars after 72 h of enzymatic hydrolysis was observed in a sample of soft biomass (no. 3 — grass mix) whereas in the samples where over 50% of biomass consisted of woody plants (hard biomass) the content of reducing sugars was lower by approx. 10 g/L. The EMIMOAC ionic liquid produced considerable increase of reducing sugars during hydrolysis in all biomass samples. The results above show that [Emim][OAc] demonstrated a high ability to convert the highly crystalline structure of cellulose to amorphous cellulose. This could have been because EMIM OAc has a low deactivation effect for cellulase compared with other ILs (EMIM Cl). Similarly, Xiao et al. [33] reported that 200 mM of EMIMOAc had no effect on commercial cellulase activity in previous studies. Yamada et al. [34] obtained ethanol from hard biomass (eucalyptus and cedar) using EMIMOAc for pretreatment. They obtained good saccharification results in samples purified with EMIMOAc, but the ethanol content remained at low level. This was because hard biomass such as eucalyptus and cedar is less degradable than a soft biomass such as bagasse. It is important to improve pretreatment efficiency and cellulase activity for efficient ethanol production from hard biomass in future work.

3.2. Alcoholic fermentation

Ethanol fermentation is a subsequent stage of bioethanol production. The bioprocess takes place with the participation of microorganisms. Typically, *S. cerevisiae* yeast is used, which converts sugars to ethanol under anaerobic conditions in temperature approximately 30–37°C. Application of this group of microorganisms enables obtaining ethanol at 12.0–17.0% w/v concentration, 90% theoretical efficiency [35,36,37,38]. The issue is the fact that yeasts only metabolize hexoses to ethanol. Research is conducted on isolation of new and enhancement of the currently available microorganisms to obtain a strain capable of pentose and hexose fermentation [39]. However, it is still one of the most important problems in bioprocesses using lignocellulose as low ethanol content in relation to the costs of preparation of pretreatment, hydrolysis and ethanol recovery, tolerance to multifunctional environmental stress generated by among others the presence of inhibitors in the form are still evident in many of the projects demonstrated [40]. After enzymatic hydrolysis, the samples were decanted, and the

supernatant fluid that was inoculated with *S. cerevisiae* yeast was used for alcoholic fermentation. The ethanol content was determined after 96 h in all examined samples. The best results were obtained after the fermentation of lignocellulosic biomass, which was pretreated using 1-ethyl-3-methylimidazolium acetate. In samples without pretreatment, the ethanol concentration was below 0.5 g/L, and after treatment with 1 ethyl-3-methylimidazolium chloride it was maximum 4.3 g/L for biomass from field No. 4, where goldenrod was the dominant (Fig. 5). The best-fermenting type of lignocellulosic biomass was the one pretreated using 1-ethyl-3-methylimidazolium acetate. The concentration of ethanol in the sample from the first field where the dominant was fireweed was 18.5 g/L. In the other materials purified with EMIMOAc, the fermentation course was significantly better than in the case of samples with raw material or after EMIMCl treatment. High ethanol concentration was also recorded in biomass samples from field No. 3 (grass mix).

The study allowed the emergence of new areas excluded from agricultural production, which vegetation may be a promising raw material for bioethanol production from lignocellulose. Undoubtedly, plants such as fireweed, common broom, hay and goldenrod may be useful for the production of liquid biofuels and it would be necessary in the further stage of research to establish and optimize the conditions for the technology of ethyl alcohol producing from these plant species. Enzymatic hydrolysis of biomass from agricultural wastelands results in a large increase in fermentable sugars, comparable to the enzymatic hydrolysis of rye, wheat, rice or maize straw. On the other hand, alcoholic fermentation of these materials is inferior in comparison to wheat, rye, rice or miscanthus straw. This may be due to the presence of numerous inhibitors derived from biomass as well as products of lignocellulose degradation [7]. The results of such samples fermentation can also be improved by using microorganisms capable of converting lignocellulose into ethyl alcohol such as *Pichia stipitis*, *Kluyveromyces marxianus* or *Clostridium thermophilum* [40,41].

4. Conclusion

The use of 1-ethyl-3-methylimidazolium acetate allows obtaining bioethanol by hydrolysis and alcoholic fermentation of lignocellulosic biomass from agricultural wastelands. The concentration of bioethanol depended on the botanical composition of biomass and the ionic liquid used for the treatment of lignocellulose. The highest content of reducing sugars was obtained in the sample from grass mixture (42.42 g/L) and from fireweed (37.68 g/L). The highest concentration of bioethanol (18.5 g/L) was noted in the sample of lignocellulose from fireweed after the treatment with 1-ethyl-3-methylimidazolium acetate.

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Conflict of interest

The authors declare that they have no conflict of interest.

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