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Potential of Pine Needle Biomass for Bioethanol Production

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Abstract: Currently, fossil fuels are used to produce fuels and electricity, which are finite sources and have a negative impact on the natural environment. An excellent alternative to these fuels is biofuels, such as bioethanol from waste forest biomass. Pine needles are one of the most important available forest biomass materials with s significant impact on local understory vegetation. Forest waste biomass, which is a rich source of lignocellulose, can be used in various ways, such as for the eco-economical production of bioethanol. The aim of this study was to analyze the possibilities of bioethanol production from pine needle biomass obtained from forest land following different soil preparations and logging residue management. The pine needle dry matter yield, chemical components of pine needle biomass (cellulose, hemicellulose, lignin), and the amount of ethanol yield per hectare were evaluated. The highest average yield pine needle equal to 6.17 Mg·ha^{-1} was observed. Bioethanol yield per hectare from this biomass was the highest for plowing with the LPZ-75 plow and was 1.08 m^3·ha^{-1}. The discussed results were confirmed by detailed statistical analysis. To sum up, the researched pine needle biomass turned out to be an interesting raw material with the potential for bioethanol production.

Keywords: lignocellulosic biomass; soil preparation; logging residues management; ethanol; ethanol yield

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1. Introduction

In the last decades, the global production and use of biofuels have grown significantly. Increased interest in the production of biofuels is the result of a significant reduction in greenhouse gas emissions that are generated by fossil fuels. It is assumed that about 60% of the global production of bioethanol is based on sugar cane, corn, and other food raw materials. However, it does contribute to fluctuations in food prices and may have negative effects on access to fields [1]. Therefore, it is necessary to develop new technologies for obtaining advanced biofuels that do not compromise food safety.

Increasingly, national and international policy, through legal regulations as well as various types of subsidies, strongly encourage the production of biofuels. The latest regulations on advanced biofuels in the European Union (including the Renewable Energy Directive 2009/28/EC) and in the United States (i.e., the Energy Policy Act) indicate an increase in the percentage share of renewable biofuels in gasoline, with an emphasis on the use of cellulose and lignocellulosic raw materials. In the EU, by 2030, the share of these biofuels is to be at least 3.5%, while in the USA, by the end of 2022, it is planned to produce 60 billion liters of second-generation biofuels, i.e., about 20% of liquid transport fuels [2–4].

Second-generation biofuels including bioethanol do not compete against food supplies as they are based on non-food raw materials. This bioethanol is produced from lignocellulosic biomass. Such biomass is usually relatively inexpensive as well as locally available. Lignocellulose is considered a renewable and sustainable carbon source and is a component of the cell wall of many plants. Lignocellulose resources can be classified into three main groups including dedicated energy crops which grow on low-quality soil (e.g., herbaceous crops and perennial grasses or switchgrass). The other group is agricultural wastes, such as wheat straw, cereal straw, rice husk, corn cob, and bagasse from processing sugar cane. The last group is forest-based wastes which are, among others, bark, branches, leaves, needles, sawdust, shavings, and biomass fractions from industries based on forestry.

The progress of bioethanol production from lignocellulosic biomass is treated more as a social responsibility, and the issue of economic profitability is still an ambiguous and often highly debatable issue.

Pine trees are one of the most popular and widely available sources of biomass worldwide, and this biomass in various forms is widely used as a renewable energy source.

In Poland, the share of Scots pine (*Pinus sylvestris* L.) in all forests is the largest and it is the main species used for economic purposes. Pine, according to the Large-area Inventory of the State of the Forest, covers 58.6% of the forest area of all ownership forms in Poland, which in terms of volume translates into the value of 56.0% of wood resources (Report on the condition of forests in Poland 2021). Timber harvesting in Poland in 2021 slightly exceeded 40 million m^3, with the share of coniferous wood accounting for about 75% of the total harvesting. Assuming that at maturity, the share of needles in dry pine biomass is about 4%, this gives a significant amount of material for potential use. Pine forest biomass as an object of scientific research has been of interest for many years [5–7]. The main aspect, however, was the study of its amount in terms of its use in the production of various products, as a source of energy, as an important element of the environment in absorbing carbon dioxide [8], or as a reservoir of elements that are accumulated in it and removed from the forest environment in the process of forest use. Elements of arbomass, including primarily wood, are a source of energy mainly in the process of its combustion. However, there are other possibilities to use this raw material. One of them is the production of bioethanol from various parts of trees, including needles [9]. Although the share of needles is not the largest in the entire mass of the tree, it cannot be omitted, especially in younger stands. The interest in obtaining the entire mass of trees is so great that it is worth researching the possibility of using needles as a raw material for bioethanol production. In commercial stands, breeding activities, including those related to the removal of redundant trees, are carried out from an early age, up to the age of felling (cleaning, thinning). In younger stands, however, biomass is not of interest to the wood industry. However, stands that are about 20 years old produce significant amounts of biomass, which is left in the forest after breeding operations and therefore can be of use as a source of raw material for liquid biofuel production. The amount of biomass produced is influenced by many factors [10], including features of the stand or preparatory activities for forest regeneration [11]. These include the management of logging residues and soil preparation, and these activities can be carried out in various ways.

Pine needle biomass contains about 75% (by weight) of polysaccharides (cellulose, hemicellulose) which can be hydrolyzed into simple sugars that may be exploited as raw materials to produce biofuel [12].

Pine needle biomass as a lignocellulosic raw material contains a complex polymeric matrix known as lignocellulose which, due to its complicated structure, is hardly biodegradable. The lignocellulosic complex occurs in the cell walls of biomass and contains three basic components—cellulose, hemicellulose, and lignin [13]. Cellulose and hemicellulose are potential substrates in the fermentation process, while lignin is not a desirable element and destabilizes the process of biomass hydrolysis. The biomass conversion process to bioethanol consists of three main stages. The inaccessibility of the structure of lignocellulosic biomass makes it necessary to apply the first step, pretreatment, the purpose of which is to purify, break up the solid phase, and loosen the compact structure of lignocellulose, which allows for the reduction of the particle size and facilitates the hydrolysis of the cellulose [12,14]. Several types of lignocellulosic biomass pretreatment can be distinguished, such as physical, chemical, or biological methods. Physical pre-treatment methods include milling, extrusion processing, or ultrasonic treatment. Another type of pretreatment is chemical treatment, the purpose of which is to loosen the compact structure of lignocellulose. Chemical processes include acidic, alkaline, and neutral treatment, organosolv processing, SO_2 vapor explosion, ammonia, and ozonolysis. Depending on the method used, various transformations take place within the lignocellulose complex. The second important stage to produce bioethanol from lignocellulosic biomass is enzymatic hydrolysis, which determines the number of simple sugars metabolized by yeast in the fermentation process. The third and final stage is the ethanol fermentation of hydrolysates obtained from forest biomass using distillery yeast *Saccharomyces cerevisiae* [15]. Currently, one of the main goals is to increase the efficiency of bioconversion processes for lignocellulosic raw materials, as well as to look for alternative solutions that affect the quantity and quality of the final product.

Based on previous studies on the production of bioethanol from lignocellulosic biomass and the authors' experience in this scope, it was concluded that forest biomass in the form of pine needles described in this article may be a valuable energy resource. Moreover, no literature has been published before in relation to the possibility of bioethanol production from pine needle biomass obtained from forest land following different logging residue management methods and different ways of soil preparation.

Therefore, the aim of this presented study is the determination of the bioethanol yield produced from pine needles obtained from forest land following different soil preparation methods and logging residue management methods.

2. Materials and Methods

2.1. Raw Material

The raw material for bioethanol production in the form of pine needles was obtained from the experimental area established in the administrative area of the Kalisz Pomorski Forest District, in the Grzybów Forestry Range, subcompartment 321a. The needle was obtained from a 20-year-old Scots pine stand (*Pinus sylvestris* L.) growing on the site type of the fresh coniferous forest. Before the establishment of forest cultivation in 2000, the area to be regenerated was prepared in 9 different variants with the use of mechanical devices. Three methods of logging residues management were used: 1-complete removal of the logging residues; 2-leaving residues on the clear-cut; 3-comminution of all residues, and three ways of soil preparation: A-plowing furrows with the LPZ−75 plow; B-plowing furrows with an active U-162 plow; C-plowing up ridges using a plow-miller. The entire area of 1.68 ha was divided into 27 plots where 9 different experimental variants were applied, each in 3 replications. The trees were selected using the Hartig method [16,17]. After felling each of the sample trees, a 2-kg sample of needled branches from the crown was collected, and then the ratio of needle to shoots was determined [18]. In laboratory conditions, the dry weight of the pine needle was determined and converted into individual variants of logging residue management and soil preparation.

2.2. Bioethanol Production

Dried pine needle biomass was then disintegrated on a knife mill (Retsch SM-200, Germany) with sieves of 2 mm mesh.

Then, the research material prepared in this way was subjected to the action of 1% NaOH solution. The alkaline pretreatment process was carried out at 90 °C by 5 h incubation. After this time, filtration of the tested biomass suspension under reduced pressure was started to stop the process in progress. To neutralize the filtered biomass, it was washed with portions of distilled water until the desired pH equal to 7.0 was reached. This filtrate was used as a substrate in the next stage of the bioethanol production process.

The enzymatic hydrolysis and ethanol fermentation process (SSF) was carried out in 100 mL flasks and the total volume of prepared pine needle biomass hydrolysate was 40 mL. pH regulation to the desired value equal to 4.8 was made by application, using 1 M sulfuric acid (pure p.a.; POCH, Gliwice, Poland) and 1 M sodium hydroxide. The enzyme

Flashzyme Plus 200 (AB Enzyme, Darmstadt, Germany) was added to the flask with treated biomass in the amount of 20 FPU·g^{-1}. Subsequently, 0.5 g·L^{-1} of non-hydrated lyophilized *S. cerevisiae* yeast was added to the same flask (cell concentration after inoculation cell concentration was approximately 1×10^7 cfu·mL^{-1}). Flasks were incubated at 37 °C for 72 h on a laboratory shaker at a speed of 200 rpm (GLS Aqua 18 Plus, GRANT, Swindon, UK). All tests were performed in triplicate.

2.3. Analytical Methods

The chemical composition of the pine needle biomass was determined according to standard methods.

- Extractive contents according to the Soxhlet method were determined using 96% EtOH (pure p.a.; POCH, Poland) (TAPPI–T204 cm-07) [19].
- Cellulose content was determined by the Seifert method with a mixture of acetylacetone (pure p.a.; POCH, Poland) and dioxane (pure p.a.; POCH, Poland) [20].
- Lignin content was determined by the Tappi method (Technical Association of the Pulp and Paper Industry) (T-222 om-06) with concentrated H_2SO_4 (pure p.a.; POCH, Poland) [21].
- Pentosans were determined using 1–13% HCl (pure p.a.; POCH, Poland) and phloroglucinol (pure p.a., Sigma-Aldrich, Sofia, Bulgaria), according to Tollen's method [22].
- Theoretical hemicellulose content was arithmetically calculated as the difference between holocellulose and cellulose [23].

All results of this analysis are an average of three measurements and are calculated from the dry matter of the raw material.

Determination of the ethanol concentration from forest biomass was performed using an Elite LaChrom liquid chromatograph from VWR-Hitachi and an RI L-2490 detector, using a Rezex ROA 300 × 7.80 mm column from Phenomenex, at 40 °C and at a flow rate of 0.6 mL/min. The samples were loaded onto the column at 10 µL. The quantitative identification was performed by the external standard method (standard- EtOH pure p.a., Sigma-Aldrich) using the peak area (measurement and computer integration using the Ez-Chrom Elite program).

2.4. Bioethanol Yield Calculation

Ethanol yield from 100 g of raw material (Ys) [24] was calculated according to the equation:

$$Y_S = \frac{Et}{M} \times 100\ [g/100\ g\ \text{ of raw material}]$$

where: Et—amount of ethanol in 1000 mL of the tested sample (g) M—mass of material in 1000 mL of fermentation sample (g).

Based on the ethanol yield from 100 g of raw material, the yield of ethanol in L per ton of straw dry matter (L·Mg^{-1}) and then the ethanol yield per hectare (m^3·ha^{-1}) was calculated.

2.5. Statistical Analysis

Two-way ANOVA was carried out to test the effect of the soil preparation technique (Factor I) and logging residue management method (Factor II) on average pine needle yield and on average ethanol yield in two variants, per 100 g and per yield according to the observation model:

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$$

where μ—general mean,

τ_i—effect of factor I (soil preparation method), i = 1, 2, 3

β_j—effect of factor II (logging residues management method), j =1, 2, 3

$(\tau_i\beta_j)_{ij}$—interaction effect,

ε_{ij_k}—random error.

An experiment was carried out in $k = 3$ independent replications. If the null hypothesis for main effects or interactions was rejected at the significance level $\alpha = 0.05$, the post hoc Tukey HSD test was used to compare the significance of the differences among the means. The analyses were preceded by the Shapiro–Wilk Normality Test and the Fligner-Killeen test of homogeneity of variances. All carried out analyses were performed in statistical software R, version 4.2.2 [25,26].

3. Results and Discussion

3.1. Pine Needle Biomass Yield

Variance analysis showed no significant differences in pine needle dry matter yield resulting from the use of different soil preparation methods and different ways of logging residue management. There was also no interaction between these factors. The highest average pine needle dry matter yields, 6.17 Mg·ha^{-1} and 5.71 Mg·ha^{-1}, were found in the case of plowing with the LPZ-75 plow and, respectively, complete removal and leaving residues on the clear-cut (ways of managing logging residues), while the lowest, 3.99 Mg·ha^{-1}, was also with the use of the LPZ-75 plow and comminution of all residues (Table 1). Among the soil preparation methods, the highest average pine needle dry matter yield at 5.29 Mg·ha^{-1} was obtained using the LPZ-75 plow, and the lowest, 4.86 Mg·ha^{-1},using the U-162 plow. For ways of logging residue management, complete removal was the most effective, giving a result like the method of leaving residues on the clear-cut.

Table 1. Statistical analysis of pine needle dry matter yield (Mg·ha^{-1}).

Kolumna1	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
Soil preparation	2	0.86	0.429	0.073	0.929
Logging residues management	2	4.09	2.045	0.350	0.710
Soil preparation * logging residues management	4	4.55	1.136	0.194	0.938
Residuals	18	105.26	5.848		

	1	2	3	A,B,C * 1,2,3		GENERAL MEAN
A	6.173	5.706	3.993	5.29	a	
B	4.696	5.133	4.743	4.86	a	5.06
C	5.30	4.98	4.80	5.03	a	
	5.39	***5.27***	***4.51***			

*,1,2,3—logging residues management; A,B,C—soil preparation; a-means followed by a common letter are not significantly different as per the Tukey post-hoc test at the 5% level of significance.

In turn, in the same area, in the earlier years of cultivation, a statistically significant effect of both soil preparation methods and logging residue management on the biomass yield of the aboveground part of *Pinus sylvestris* L. (including needles) [27]. This research was carried out in the 17th year of the stand's life when the impact is slowly fading away. This may be because in the second decade of the stand's life (II age class), stand maintenance treatments were already carried out, which consisted of removing weaker pine trees, giving space for the growth of the remaining trees.

The results of pine needle yield biomass obtained in this study are comparable to those of other authors. For the plantation of the 15-year-old *Pinus taeda* stand, 6.06 to 8.60 Mg·ha^{-1} was obtained, depending on the planting spacing used [28]. In turn, Watzlawick et al. [29], also examined *Pinus taeda*, and showed the following values of needle biomass: for a 14- and 16-year-old plantation 5.62 Mg·ha^{-1}; for a 19-year-old plantation 3.68 Mg·ha^{-1}, and for a 21-year-old plantation 8.06 Mg·ha^{-1}.

Different methods of soil preparation may affect the amount of biomass obtained, which results from various factors, e.g., from the degree of interference with the soil environment. When plowing, especially in poor habitats (fresh coniferous forest), the LPZ-75 plow removes most of the organic and mineral layers of the soil and sets it aside, which results in a lower survival rate and poorer growth of seedlings in the early years of cultivation. In turn, after using the active plow U162, due to reduced invasiveness and maintaining the soil accumulation level, better results were obtained for the growth of pine seedlings [30]. However, other studies have shown the superiority of the LPZ-75 plowing method over other methods (active plow, forest mulcher, disc plow, disc harrow) in terms of the most favorable conditions for the growth of pine seedlings [31,32].

Typically, the impact of logging residue management methods on pine growth parameters (including biomass) is smaller than the impact of soil preparation methods. In this study, the results, despite the observed tendencies, were not statistically confirmed (no significant differences). However, in other studies, especially those concerning the first years of pine cultivation growth, significant correlations were found between the methods of logging residue utilization. Most often, the fragmentation of logging residues resulted in better growth and success of pine cultivation, compared to other methods, e.g., clearing or leaving residues on the clear-cut [33,34]. On the other hand, the removal of logging residues usually had a negative impact on the condition of nutrients in the soil and litter, which in turn translated into poorer tree growth [11].

3.2. Chemical Composition of Pine Needle Biomass

The analysis of the chemical composition of the pine needle biomass before and after NaOH pretreatment was an important step in this research, in order to confirm the efficiency of the alkaline treatment. The contents of extractive substances, pentosans, cellulose, hemicellulose, and lignin were determined (Table 2).

Table 2. Chemical composition of pine needle biomass (% of dry matter); BP: before pretreatment; AP: after pretreatment.

Pine Needle	Extractive Substances	Pentosans	Cellulose	Hemicellulose	Lignin
BP	27.67 ± 0.41	8.82 ± 0.12	38.49 ± 0.24	19.85 ± 0.98	24.81 ± 0.18
AP	2.23 ± 0.03	12.98 ± 0.14	51.90 ± 0.07	24.37 ± 0.82	20.63 ± 0.14

As is known, the purpose of alkali pretreatment is to remove lignin from materials with lignocellulose and to increase the accessibility of the biomass structure. Properly selected parameters of the initial preparation of the raw material determine the effectiveness of enzymatic hydrolysis of polysaccharides and ethanol fermentation of released sugars. For chemical treatment, 1% of NaOH was used, which is the most popular reagent used in alkali pretreatment. This reagent effectively loosens the structure of lignocellulose and increases the efficiency of subsequent stages of bioethanol production, which results from the literature reports and our research experience.

Pine wood not pretreated with NaOH contained approx. 32% cellulose, approx. 14% hemicellulose, and approx. 30% lignin [35]. On the other hand, research by Sjulander and Kikas [36] showed cellulose content in pine wood at the level of 40.4%, lignin content at the level of 24.3%, hemicellulose content at the level of 13.2%, and the content of extractive substances at the level of 21.4%. A similar chemical composition of pine needle biomass in our study was observed, i.e., approx. 38% cellulose, approx. 20% hemicellulose, and approx. 25% lignin. The analysis of the chemical composition of the pine needle biomass before and after pretreatment showed that the chemical treatment process was carried out effectively and resulted in a visible increase in the cellulose content (by approx. 13%) and the partial degradation of hemicellulose (by approx. 5%). Very significantly, a decrease in the amount of lignin was also observed (by approx. 4%) (see Table 2). The pentosan content in Polish softwood is usually as high as 25%, but their content after application of 1% NaOH solution ranges from 13 to 16% [16,22,37]. Similar results for the content of pentosans were obtained in our results. Extractive substances include phenols, terpenes, waxes, fats, resin acids, fatty acids, steroids, etc. [22]. The content of the extractive substance in the examined biomass of pine needles was very high and amounted to over 27% before treatment, but after treatment this amount was significantly reduced to 2.23%.

Similar observations regarding the chemical composition of pine needle biomass were presented by Tandon and Sharma [14], who showed that untreated pine wood biomass contains 57% of the total content of cellulose and hemicellulose, 23% of lignin, and 20% of extractives. After the initial alkaline treatment, they found that the content of cellulose and hemicellulose increased by 30%, while the contents of lignin and extractive substances were reduced by several percent.

The obtained results allow us to conclude that the initial alkaline treatment led to a decrease in the degree of cellulose crystallinity and to the violation of the lignin structure, which is of particular importance for the further stages of bioethanol production, as lignin is a strong obstacle in the pine needle biomass conversion process. Reducing the amount of lignin can contribute to improved bioethanol yields from pine needle biomass.

3.3. Bioethanol Production

3.3.1. Bioethanol Yield per 100 g of Raw Material

Based on the analysis of variance (Table 3), significant differences were found in the average ethanol yield per 100 g of raw material when using different preparation methods.

Table 3. Statistical analysis of ethanol yield from 100 g of pine needle biomass.

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
Soil preparation	2	5.631	2.8156	5.336	0.0151 *
Logging residues management	2	1.689	0.8447	1.601	0.2291
Soil preparation * logging residues management	4	2.528	0.632	1.198	0.3457
Residuals	18	9.497	0.5276		

	1	2	3	A,B,C * 1,2,3		GENERAL MEAN
A	16.56	15.373	16.193	16.04	a	
B	15.54	15.67	14.913	15.37	ab	15.45
C	15.227	14.46	15.107	14.93	b	
	15.78	***15.17***	***15.40***			

*,1,2,3 logging residues management; A,B,C—soil preparation; a, ab, b—means followed by a common letter are not significantly different as per the Tukey post-hoc test at the 5% level of significance.

The highest yield was found for method A, i.e., 16.04 $g \cdot 100\ g^{-1}$ of pine needles which differed significantly from the average ethanol yield for the C soil preparation method, which was 14.93 $g \cdot 100\ g^{-1}$ of pine needles. The average yield of ethanol per 100 g of pine needles for all methods of logging residue management was comparable, being the highest for the first method, i.e., complete removal (15.78) and the lowest for the second method, i.e., leaving residues on the clear-cut (15.17) (see Table 3). The average ethanol yield per 100 g of raw pine needles is high compared to similar studies. For example, the yield of ethanol obtained from hemp biomass under similar conditions of treatment, hydrolysis, and fermentation reached a similar level, i.e., 13–15 $g \cdot 100\ g^{-1}$ [38]. In turn, Cotana et al. [35] conducted research on obtaining bioethanol from pine wood chips. After carrying out the hydrolysis and fermentation process, they obtained an ethanol yield of 7.21–10.60 $g \cdot 100\ g^{-1}$ of raw material. Research on obtaining bioethanol from waste wood biomass was also conducted by Victor et al. [39]. After pretreatment with NaOH, microwave irradiation, and the fermentation process, they obtained an ethanol yield of 5.7 $g \cdot 100\ g^{-1}$ of pinecones.

3.3.2. Bioethanol Yield per Hectare

Table 4 show bioethanol yield from pine needle biomass per hectare.

Table 4. Statistical analysis of ethanol yield per hectare of pine needle ($m^3 \cdot ha^{-1}$).

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
Soil preparation	2	0.101	0.05074	0.215	0.809
Logging residues management	2	0.196	0.09808	0.415	0.666
Soil preparation * logging residues management	4	0.205	0.5134	0.217	0.925
Residuals	18	4.249	0.23608		

	1	2	3	A,B,C * 1,2,3		GENERAL MEAN
A	1.293	1.123	0.810	1.80	a	
B	0.926	1.006	0.896	0.94	a	0.99
C	1.016	0.910	0.916	0.95	a	
	6.46	***5.93***	***5.23***			

*,1,2,3—logging residues management; A,B,C—soil preparation; a—means followed by a common letter are not significantly different as per the Tukey post-hoc test at the 5% level of significance.

As with the pine needle dry matter yield, variance analyses showed no significant differences in ethanol yield per hectare of pine needle [$m^3 \cdot ha^{-1}$] resulting from the use of different soil preparation methods and different ways of logging residue management (Table 4). The average ethanol yield per hectare of pine needles in the whole experiment was 0.99 $m^3 \cdot ha^{-1}$. It was the highest for the first soil preparation method (LPZ-75 plow) at 1.08 $m^3 \cdot ha^{-1}$. The other two methods gave average yields at a very similar level, respectively 0.95 $m^3 \cdot ha^{-1}$ and 0.94 $m^3 \cdot ha^{-1}$, for plowing up ridges using a plow-miller and plowing with an active U-162 plow. The most effective combination in terms of ethanol yield per hectare of pine needle was plowing with the LPZ-75 plow and complete removal, giving an average yield of 1.29 $m^3 \cdot ha^{-1}$.

Bonifacino et al. [40] conducted research on obtaining bioethanol using wood from Eucalyptus and after pre-hydrolysis, simultaneous saccharification, and the fermentation process, they obtained ethanol yield at a level equal to 2.5 $m^3 \cdot ha^{-1}$. In turn, in the research conducted on obtaining bioethanol from the biomass of invasive plants (*Reynoutria* × *bohemica*), the highest yield of ethanol was 2.6 $m^3 \cdot ha^{-1}$ [41]. Research on the production of bioethanol from pine biomass was also conducted by Daud et al. [42] and after pretreatment and the SSF process, they obtained an ethanol yield of 1.5 $m^3 \cdot ha^{-1}$. It should be noted that despite the satisfactory content of ethanol in 100 g of raw material, the yield of ethanol is not very high, which is due to the lower biomass yield of pine needles per hectare.

4. Conclusions

Pine needle biomass with nine different variants was tested. Chemical composition studies have shown that an effective alkaline pretreatment allows the lignocellulosic complex to be broken down, separating lignin from polysaccharides. The presented results confirm that there was a decrease in the lignin content and an increase in the cellulose content, which may indirectly lead to an improvement in the efficiency of the bioethanol production process.

The results of the study showed that the highest average yield of pine needles and the bioethanol yield per hectare of pine needle was obtained by plowing with the LPZ-75 plow (soil preparation methods) and complete removal (logging residue management methods). After the statistical analysis, no significant differences between the amount of pine needle biomass from forest land following different logging residue management methods and different ways of soil preparation were observed.

The conducted research confirms that the biomass of pine needles has an energy potential and can be a raw material to produce bioethanol, which will contribute, among other things, to the production of useful industrial products. It should be added that the production of biofuels from waste forest biomass is innovative and contributes to solving a key issue in the production of biocomponents for transport biofuels.

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References

1. Malode, S.J.; Prabhu, K.K.; Mascarenhas, R.J.; Shetti, N.P.; Aminabhavi, T.M. Recent Advances and Viability in Biofuel Production. *Energy Convers. Manag. X* **2021**, *10*, 100070. [CrossRef]
2. Cadillo-Benalcazar, J.J.; Bukkens, S.G.F.; Ripa, M.; Giampietro, M. Why Does the European Union Produce Biofuels? Examining Consistency and Plausibility in Prevailing Narratives with Quantitative Storytelling. *Energy Res. Soc. Sci.* **2021**, *71*, 101810. [CrossRef]
3. Vallejos, M.E.; Kruyeniski, J.; Area, M.C. Second-generation Bioethanol from Industrial Wood Waste of South American Species. *Biofuel Res. J.* **2017**, *15*, 654–667. [CrossRef]
4. Prussi, M.; Panoutsou, C.; Chiaramonti, D. Assessment of the Feedstock Availability for Covering EU Alternative Fuels Demand. *Appl. Sci.* **2022**, *12*, 740. [CrossRef]
5. Kubiak, M.; Grodecki, J.; Różański, H. An Attempt at a Quantitative Assessment of Biomass in Mature Pine Stands in Relation to the Forest Site Type. *Sylwan* **1985**, *6*, 21–33. (In Polish)
6. Gornowicz, R.; Pilarek, Z. Biomass of Scots Pine (*Pinus silvestris* L.) in a 23-year old Stand. *PTPN* **1993**, *76*, 53–57. (In Polish)
7. Oleksyn, J.; Reich, P.B.; Chałupka, W.; Tjoelker, M.G. Differential Above- and Below-Ground Biomass Accumulation of European *Pinus sylvestris* Populations in a 12-year-old Provenance Experiment. *Scand. J. For. Res.* **1999**, *14*, 7–17. [CrossRef]
8. Uri, V.; Kukumägi, M.; Aosaar, J.; Varik, M.; Becker, H.; Aun, K.; Lõhmus, K.; Soosaar, K.; Astover, A.; Uri, M.; et al. The Dynamics of the Carbon Storage and Fluxes in Scots Pine (*Pinus sylvestris*) Chronosequence. *Sci. Total Environ.* **2022**, *817*, 152973. [CrossRef]
9. Dwivedi, D.; Rathour, R.K.; Sharma, V.; Rana, N.; Bhatt, A.K.; Bhatia, R.K. Co-fermentation of Forest Pine Needle Waste Biomass Hydrolysate into Bioethanol. *Biomass Convers. Biorefin.* **2022**, *187*, 1–13. [CrossRef]
10. Jagodziński, A.M.; Dyderski, M.K.; Gęsikiewicz, K.; Horodecki, P. Effects of Stand Features on Aboveground Biomass and Biomass Conversion and Expansion Factors Based on a *Pinus sylvestris* L. Chronosequence in Western Poland. *Eur. J. For. Res.* **2019**, *138*, 673–683. [CrossRef]
11. Węgiel, A.; Jakubowski, J.; Molińska-Glura, M.; Polowy, K.; Węgiel, J.; Gornowicz, R. Effect of Logging Residue Removal and Mechanical Site Preparation on Productivity of the Subsequent Scots Pine (*Pinus sylvestris* L.) Stands. *Ann. For. Sci.* **2023**, *80*, 5. [CrossRef]
12. Singh, S.; Anu Vaid, S.; Singh, P.; Bajaj, B.K. Physicochemical Pretreatment of Pine Needle Biomass by Design of Experiments Approach for Efficient Enzymatic Saccharification. *J. Mater. Environ. Sci.* **2016**, *7*, 2034–2041.
13. Mahajan, R.; Chandel, S.; Puniya, A.K.; Goel, G. Effect of Pretreatments on Cellulosic Composition and Morphology of Pine Needle for Possible Utilization as Substrate for Anaerobic Digestion. *Biomass Bioenergy* **2020**, *141*, 105705. [CrossRef]
14. Tandon, D.; Sharma, N. Evaluation of Different Pretreatments for Enhanced Saccharification of *Pinus roxburghii* Biomass by Using Mixture of Polymerizing Enzymes and Bioreactor Studies for Its Bioconversion into Ethanol. *J. Pharmacogn. Phytochem.* **2018**, *7*, 3423–3432.
15. Inyang, V.; Laseinde, O.T.; Kanakana, G.M. Techniques and Applications of Lignocellulose Biomass Sources as Transport Fuels and Other Bioproducts. *Int. J. Low Carbon Technol.* **2022**, *17*, 900–909. [CrossRef]
16. Lachowicz, H.; Wróblewska, H.; Wojtan, R.; Sajdak, M. The Effect of Tree Age on the Chemical Composition of the Wood of Silver Birch (*Betula pendula* Roth). *Wood Sci Technol.* **2019**, *53*, 1135–1155. (In Polish) [CrossRef]
17. Grochowski, J. *Dendrometria*; PWRiL: Warsaw, Poland, 1973. (In Polish)
18. Lemke, J. Wielkość i Wydajność Aparatu Asymilacyjnego Sosny Wejmutki w Produkcji Drewna Strzały. *Sylwan* **1979**, *123*, 21–31. (In Polish)

19. *TAPPI T 204 cm-07*; Solvent Extractives of Wood and Pulp. Technical Association of the Pulp and Paper Industry: Atlanta, GA, USA, 2007.
20. Seifert, K. Zur Frage der Cellulose-Schnellbestimmung nach der Acetylaceton-Methode. *Das Pap.* **1960**, *14*, 104–106. (In German)
21. *TAPPI T 222 om-06*; Acid-Insoluble Lignin in Wood and Pulp. Technical Association of the Pulp and Paper Industry (TAPPI): Atlanta, GA, USA, 2006.
22. Prosiński, S. *Wood Chemistry*; PWRiL: Warsaw, Poland, 1984. (In Polish)
23. *TAPPI T9 wd-75*; Holocellulose in Wood. Technical Association of the Pulp and Paper Industry (TAPPI): Atlanta, GA, USA, 2015.
24. Kawa-Rygielska, J.; Pietrzak, W. Bread waste management for the production of bioethanol. *Żywność Nauka Technol. Jakość* **2011**, *79*, 105–118. (In Polish)
25. Batog, J.; Frankowski, J.; Wawro, A.; Łacka, A. Bioethanol Production from Biomass of Selected Sorghum Varieties Cultivated as Main and Second Crop. *Energies* **2020**, *13*, 6291. [CrossRef]
26. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022.
27. Gornowicz, R.; Pilarek, Z.; Kwaśna, H.; Łakomy, P.; Kuźmiński, R.; Jakubowski, J.; Stempski, W. Biomasa Upraw Sosnowych na Siedlisku Boru Świeżego w Zależności Od Metod Zagospodarowania Pozostałości Zrębowych i Sposobu Przygotowania Gleby. *Sylwan* **2021**, *165*, 21–29. (In Polish)
28. Vale, A.B.; Filho, A.F.; Retslaff Guimarães, F.A.; Karasinski, M.A. *Influence of Initial Density on Biomass Production in a Pinus taeda L. Planatation in the Central-South Region of Parana, Brazil*; Floresta: Curitiba, Brazil, 2022; Volume 52, pp. 377–383.
29. Watzlawick, L.F.; Winckler Caldeira, M.V.; de Oliveira Godinho, T.; Balbinot, R.; Trautenmüller, J.W. *Aboveground Stock of Biomass and Organic Carbon in Stand of Pinus taeda L.*; Cerne: Lavras, Brazil, 2013; Volume 19, pp. 509–515.
30. Andrzejczyk, T.; Augustyniak, G. Wpływ Przygotowania Gleby na Wzrost Sosny Zwyczajnej w Pierwszych Latach Uprawy. *Sylwan* **2007**, *151*, 3–8. (In Polish)
31. Aleksandrowicz-Trzcińska, M.; Drozdowski, S.; Brzeziecki, B.; Rutkowska, P.; Jabłońska, B. Effect of Different Methods of Site Preparation on Natural Regeneration of *Pinus sylvestris* in Eastern Poland. *Dendrobiology* **2018**, *71*, 73–81. [CrossRef]
32. Drozdowski, S. Wpływ różnych sposobów przygotowania gleby na wyniki naturalnego odnowienia sosny zwyczajnej. *Acta Sci. Pol. Silv. Colendar. Rat. Ind. Lignar.* **2002**, *1*, 27–34.
33. Gałęzia, T. Economic Methods for the Utlilisation of Logging Resiudes. *For. Res. Pap.* **2016**, *77*, 50–55.
34. Gomez-Rey, M.X.; Madeira, M.; Vasconcelos, E. Effects of Organic Residue Management and Legume Cover on Growth of Pine Seedlings, Nutrient Leaching and Soil Properties. *Ann. For. Sci.* **2008**, *65*, 1. [CrossRef]
35. Cotana, F.; Cavalaglio, G.; Gelosia, M.; Nicolini, A.; Coccia, V.; Petrozzi, A. Production of Bioethanol in a Second-Generation Prototype from Pine Wood Chips. *Energy Procedia* **2014**, *45*, 42–51. [CrossRef]
36. Sjulander, N.; Kikas, T. Two-Step Pretreatment of Lignocellulosic Biomass for High-Sugar Recovery from the Structural Plant Polymers Cellulose and Hemicellulose. *Energies* **2022**, *15*, 8898. [CrossRef]
37. Rowell, R.M.; Han, J.S.; Rowell, J.S. Characterization and Factors Effecting Fiber Properties. In *Natural Polymers and Agrofibers Composites*; Frollini, E., Leao, A.L., Mattoso, L.H.C., Eds.; Embrapa Instrumentacao Agropecurária: San Carlos, Brasil, 2000; pp. 115–134.
38. Wawro, A.; Batog, J.; Gieparda, W. Polish Varieties of Industrial Hemp and Their Utilisation in the Efficient Production of Lignocellulosic Ethanol. *Molecules* **2021**, *26*, 6467. [CrossRef] [PubMed]
39. Victor, A.; Pulidindi, I.N.; Gedanken, A. Assessment of Holocellulose for the Production of Bioethanol by Conserving *Pinus radiata* Cones as Renewable Feedstock. *J. Environ. Manag.* **2015**, *162*, 215–220. [CrossRef] [PubMed]
40. Bonifacino, S.; Resquín, F.; Lopretti, M.; Buxedas, L.; Vazquez, S.; Gonzalez, M.; Sapolinski, A.; Hirigoyen, A.; Doldan, J.; Rachid, C.; et al. Bioethanol Production using High Density Eucalyptus Crops in Uruguay. *Heliyon* **2021**, *7*, e06031. [CrossRef] [PubMed]
41. Wiatrowska, B.M.; Wawro, A.; Gieparda, W.; Waliszewska, B. Bioethanol Production Potential and Other Biomass Energy Properties of Invasive Reynoutria, Solidago, and Spiraea Plants. *Forests* **2022**, *13*, 1582. [CrossRef]
42. Daud, M.; Syafii, W.; Syamsu, K. Bioethanol Production from Several Tropical Wood Species using Simultaneous Saccharification and Fermentation Processes. *Wood Res.* **2012**, *3*, 106–116. [CrossRef]