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Bioethanol Production Potential and Other Biomass Energy Properties of Invasive *Reynoutria*, *Solidago*, and *Spiraea* Plants

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Abstract: Due to the threat posed by the spread of invasive plant species, there is an urgent need to develop effective methods of eradicating and managing their biomass. The aim of the study was to examine selected invasive plants in terms of their use for energy purposes and to find out whether they can be a raw material for the production of second-generation biofuels. First, their chemical compositions were determined. The higher heating value (HHV) and lower heating value (LHV) were also calculated. High values of the higher heating value, ranging from 18.490 MJ·kg^{-1} to 19.900 MJ·kg^{-1}, indicate the possibility of using the biomass of invasive plants for energy purposes (combustion). All investigated invasive plant species were also subjected to the process of obtaining ethanol. This included an alkaline pretreatment with 1% sodium hydroxide, followed by a simultaneous saccharification and fermentation (SSF) process. The highest ethanol yield per ha of plants was obtained at 2.6 m^3·ha^{-1} for the *Reynoutria* × *bohemica* biomass. The remaining species showed an ethanol yield below 2 m^3·ha^{-1}. The conducted research allows for the conclusion that the studied invasive plants can be a promising raw material for the production of bioethanol.

Keywords: invasive plants' biomass; SSF process; bioethanol; biomass' chemical composition; higher heating value; lower heating value

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1. Introduction

Biological invasions are considered as a global problem and a major challenge for environmental management and conservation [1–3]. Plant invasions disrupt ecological processes and induce changes in land use patterns They are one of the main causes of biodiversity loss worldwide [4–7] and cause disruptions of many ecosystem services [8–12]. It is likely that no ecosystem in the world is resistant to these migrations [13], and invasive alien species (IAS) affect humans in a number of ways including economic and social impacts (e.g., negative impact on human health) [14–17]. The economic costs of IAS incurred only in protected areas worldwide alone between 1975 and 2020 reached USD 22.24 billion [17].

As a result, many institutions such as the European Union, which is part of the Convention on Biological Diversity, are involved in preventing the introduction, control, and eradication of alien species that threaten ecosystems, habitats, or species. A plan to protect 30% of its land and sea territory by 2030 [18] was also put in place. Hence, there is an urgent need to fund effective methods to control invasive species. When trying to eradicate an invasive species, there is a need to manage the resulting biomass. IAS biomass is most often considered to be bio-waste, but there may be ways to make use of it (e.g., as a raw material or product that can be sold [19]).

With the great energy crisis and alarmingly rapid climate change, there is a growing awareness of the search for more sustainable and distributed methods of energy production, waste minimization, air pollution reduction, the protection of native forests, and a reduction in greenhouse gas (GHG) emissions. The aforementioned goals can be achieved i.a. by bioenergy production [20].

The characteristics of invasive plants such as rapid growth have been shown to be identical to those of bioenergy crops [21]. The biomass of some invasive alien species has already been tested for its suitability for biofuel production (e.g., biooil, biogas, biodiesel [22,23] or solid biofuels such as briquettes [24] or pellets [25]). However, to date, there have been few studies on the use of invasive plants for bioethanol production [26,27]. As some plants show high bioethanol production efficiency after carbohydrate hydrolysis, detoxification, and fermentation, this work provides the basis for developing a bioprocess using lignocellulosic material from invasive species as a substrate and an alternative to using their biomass as a fuel energy source.

The main components of plant derived lignocellulosic raw materials are cellulose, lignin, and hemicellulose. Cellulose (40%–55% in wood, and up to 90% in cotton) [28] is a carbohydrate component composed of β-D-glucose units and is an important structural component. During hydrolysis, it breaks down into simple sugars. Lignin (18%–25%), composed of phenylpropane systems [29], strengthens plant tissue. Its amount in the plant influences the higher heating value. Hemicellulose, mainly pentosans and hexosans (15%–25%), have a structural and nutritional function in plants [30]. They are easily hydrolyzed and subject to other destructive factors, breaking down to simple sugars. Knowledge of the chemical composition of lignocellulosic raw materials allows one to plan their effective use, which is especially important in the case of invasive plants.

The conversion of plant biomass to second generation bioethanol consists of three main stages: effective pretreatment of plant material, enzymatic hydrolysis including the selection of suitable enzymes, and ethanol fermentation including the selection of efficient microorganisms. The production of biofuels from lignocellulosic raw material involves the deconstruction of cell walls into individual polymers and the hydrolysis of carbohydrates into simple sugars [31–33]. Plant biomass contains a complex polymeric structure that is relatively resistant to biodegradation. It is found in the plant cell walls and consists of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are potential substrates for fermentation processes, while the aromatic nature and complex structure of lignin adversely affects the hydrolysis of plant biomass. Effective pretreatment processes and simultaneous saccharification and fermentation processes depend on the biomass chemical composition and its structural properties [34]. The efficiency of ethanol production from plant biomass is directly related to the carbohydrate content of the biomass [35]. Therefore, the chemical composition of biomass is an important factor determining the ethanol productivity [36].

Another way of using biomass for energy purposes is the well-known combustion of biomass. The heating value (or calorific value) including the higher heating value (HHV) or lower heating value (LHV) defines the energy content of biomass fuels and is one of the most important fuel properties [37,38]. Higher heating value, also known as the gross calorific value or gross energy, refers to the heat released due to the complete combustion of the fuel, assuming that the water originally present in the fuel and any water generated are present in the condensed state. Lower heating value, also known as the net heating value, assumes that water is present in the vapor state at the end of combustion and is determined by subtracting the latent heat of water vaporization from the higher heating value. Therefore, the higher heating value and lower heating value were used in this study to determine the biomass energy potential of invasive plants.

The use of invasive species biomass for energy purposes fits in perfectly with the EU energy policy. According to the EU RED II directive (European Union Renewable Energy Directive II), the share of advanced biofuels and biogas produced from raw materials listed in Annex IX, part A of this directive including lignocellulosic raw material as a share of

final energy consumption in the transport sector, is expected to be at least 0.2% in 2022, at least 1% in 2025 and at least 3.5% in 2030 [39].

The authors concluded, based on previous studies on the process of obtaining bioethanol from plant biomass and the experience drawn from invasive species control, that the biomass of invasive species can be a valuable energy resource. Although the possibility of using some invasive species for energy purposes has already been studied, there is no literature on the possibility of the effective use of selected invasive species for bioethanol production. Therefore, the aim of this paper was to study selected invasive plants forming large-area monodominant stands and producing large amounts of relatively easy to obtain biomass. The focus was on their use for energy purposes (determination of chemical composition and higher heating value) and to identify which invasive plants show the greatest energy potential, and may be a promising resource for bioethanol production.

2. Materials and Methods

The research involved selecting invasive plants that produce sufficiently large and uniform populations, harvesting biomass and its appropriate preparation for further processing, followed by its fragmentation and laboratory analyzes. The general scheme of material collection, biomass preparation and analyzes are presented in the block diagrams (Figure 1) and described in detail in Sections 2.1–2.4.

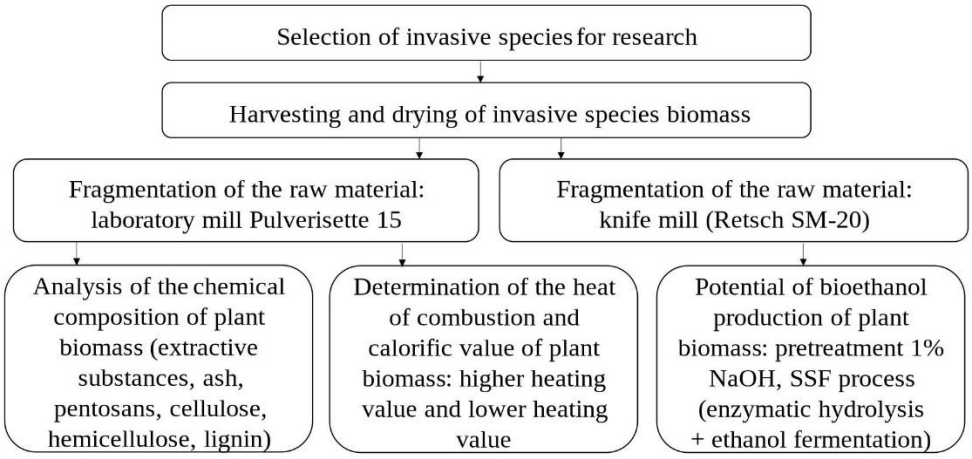

Figure 1. The general block diagram showing the work steps.

2.1. Invasive Plant Species and Preparation of Biomass

For this study, we selected invasive in Europe taxa from the genera *Reynoutria* (*R. japonica* Houtt., *R. sachalinensis* (F. Schmidt) Nakai and *R.* × *bohemica* Chrtek & Chrtkova), *Solidago* (*S. canadensis* L. and *S. gigantea* Aiton) and *Spiraea* (*S. tomentosa* L.), which are considered to produce a lot of aboveground biomass and form large-area monodominant stands that can be cut down relatively easily and cheaply.

Reynoutria japonica and *R. sachalinensis* (Polygonaceae) are perennials native to the Asian temperate zone [40]. These were imported to Europe in the 19th century [41–46] and their hybrid (*R.* × *bohemica*) was first described in the Czech Republic in the 1980s [47]. Currently, all three taxa are considered to be among the most aggressive invasive weeds in temperate terrestrial ecosystems [41,48–52]. The plants reach 2–4 m in height, form monodominant stands, and in terms of yield, up to 27.67 Mg DM ha^{-1} yr^{-1} for *R. sachalinensis* [53] and 24.2 Mg DM ha^{-1} yr^{-1} for *R.* × *bohemica* [54], are among the most luxuriant herbaceous crops in Central Europe [55] (Figure 2a–c).

Figure 2. (**a**–**f**) Invasive plant species studied for energy purposes: *Reynoutria japonica* (**a**), *R. sachalinensis* (**b**), *R.* × *bohemica* (**c**), *Solidago canadensis* (**d**), *S. gigantea* (**e**), and *Spiraea tomentosa* (**f**) (photo: B. Wiatrowska).

Solidago canadensis and *S. gigantea* (Asteraceae) are perennials native to North America [56,57]. They have been recorded in the flora of Europe since the mid-1700s [58], and their cultivation and spontaneous spread has led to a secondary range of these plants in almost all of Europe [59,60], where both species are considered invasive [59–62]. The plants reach an average height of approx. 1.5 m, quickly establish monodominant covers [63,64], and have a yield of 8.64–15.9 Mg DM ha^{-1} yr^{-1} [65–67] (Figure 2d,e).

Spiraea tomentosa (Rosaceae) is a shrub native to North America [68]. On the European continent, the shrub has been cultivated as an ornamental plant since the 18th century [69,70]. It is currently considered as a fully acclimated invasive neophyte in five countries: Belgium, Denmark, Germany, Sweden, and Poland [71,72]. The shrub grows up to 1.5 m [70], develops compact, single-species fields [73], and produces 38.87 Mg DM ha^{-1} of dry weight of the total terrestrial biomass. It is not the same as a yield, but is an estimate of the shrub's cumulative biomass that can be obtained (e.g., during the first control treatment (bush cutting) [74]) (Figure 2f).

Biomass of the selected invasive plants was collected in 2021 from large areas dominated by these species within their secondary range in Western Poland (Appendix A). Plants were harvested in the full growing season, when the mineral element allocation patterns are stable [75] and when the areas occupied by the invasive species in Central Europe are most often mown. The fresh aboveground biomass of each of the tested species (approx. 5 kg) was collected and dried at 50–55 °C for 24 h.

2.2. Analysis of the Chemical Composition of Plant Biomass

The collected biomass was seasoned in a climatic room at approx. 20 °C to a constant humidity of approx. 12%. It was then manually crushed into smaller pieces with horticultural shears and ground in a Pulverisette 15 laboratory mill. The analytical fraction of 0.1–0.4 mm was separated on sieves.

The chemical composition of the main biomass components was determined according to standard methods.

- Extractive contents were determined using 96% ethanol according to Soxhlet (TAPPI-T204 cm-07) [76].
- Cellulose content was determined by the Seifert method using a mixture of acetylacetone and dioxane [77].
- Lignin content was determined by the Tappi (Technical Association of the Pulp and Paper Industry) method (T-222 om-06) using concentrated sulfuric acid [78].
- Pentosans were determined using phloroglucinol [28].
- The theoretical hemicellulose content was arithmetically calculated as the difference in holocellulose and cellulose [28].
- The ash content, important for the combustion of biomass, was determined according to the DIN 51731 standards [79].

All chemical composition results were an average of three measurements and were calculated from the dry weight of the raw material.

2.3. Determination of the Heat of Combustion and Calorific Value

The biomass was manually crushed into smaller pieces as in Section 2.2. The higher heating value of milled biomass was determined according to the PN-81/G-04513 standard in the ZKL-4 calorimeter, designed to determine the heat of combustion of solid fuels [79]. An analytical sample of 1 g (dust, fraction < 0.1 mm) of raw material was completely combusted in an oxygen atmosphere and 3 MPa pressure. The higher heating values were calculated according to the formula:

$$Q_s^a = \frac{(C(D_t - k) - c)}{m} \ [\mathrm{kJ \cdot kg^{-1}}] \quad (1)$$

where

C is the heat capacity of the calorimeter, 12,783.69 ($\mathrm{J \cdot °C^{-1}}$);
D_t is the temperature rise in the main period (°C);
k is the correction for heat exchange with the surroundings (°C);
c is the sum of corrections for additional thermal effects (J);
m is the mass of the fuel sample (g).

To provide a more comprehensive characterization of the analyzed raw material, the lower heating value of the investigated grasses was also calculated. It is defined as the higher heating value reduced by the heat of vaporization of water released during combustion [79].

The lower heating values were calculated according to the following formula:

$$Q_i^a = Q_s^a - 24.42(W^a - 8.94H^a) \ \left[\mathrm{kJ \cdot kg^{-1}}\right] \quad (2)$$

where

Q_s^a is the average gross calorific value of solid fuel in the analytical state ($\mathrm{J \cdot g^{-1}}$);
The heat of vaporization of water at 25 °C is 24.42, corresponding to 1% of water in the fuel (J/g);
W^a is the moisture content in the analytical sample of fuel (%);
H^a is the hydrogen content in the analytical sample of fuel.

2.4. The Process of Obtaining Bioethanol

The invasive plant biomass was preliminary crushed into particles of 20–40 mm. The material was disintegrated on a knife mill (SM-200, Retsch GmbH, Haan, Germany) with sieves of 2 mm mesh.

The pretreatment of the disintegrated plant biomass was carried out with 1% sodium hydroxide at 90 °C. After a 5 h incubation, the process was ended by filtering the biomass suspension under reduced pressure. The filtered biomass was washed with portions of distilled water until the pH was neutral. The filtrate thus prepared was used as a substrate in the SSF process.

The SSF process was carried out in 100 mL flasks and the total volume of the prepared plant biomass hydrolysate was 40 mL. The resulting hydrolysate was subjected to pH adjustment to the desired value (pH 4.8) using 10% sulfuric acid and 10% sodium hydroxide. Then, the enzyme Flashzyme Plus 200 (AB Enzyme) was added in the amount of 20 FPU·g^{-1}. After thorough mixing of the above-mentioned components, non-hydrated lyophilized yeast (*Saccharomyces cerevisiae*) was added to the hydrolysate at a rate of 0.5 g·L^{-1}, which corresponded to a post-inoculation cell concentration of about 1×10^7 cfu·mL^{-1}. The flasks (plugged with stoppers with fermentation tubes) were incubated at 37 °C on a shaker (200 rpm). All tests were performed in triplicate.

The HPLC method allows one to determine the ethanol content (amount of ethanol in 1000 mL of the tested sample (g)) in the tested biomass after the SSF. It uses the flow of an appropriately selected mobile phase through a column with a narrow cross-section, filled with a bed of small diameter grains as the stationary phase. A distinctive aspect of the HPLC method is that the process takes place under high pressure. Ethanol concentration from the plant biomass was determined by HPLC on an Elite LaChrom liquid chromatograph from VWR-Hitachi using an RI L-2490 detector, Rezex ROA 300 × 7.80 mm column from Phenomenex, at a flow rate of 0.6 mL/min, at 40 °C. The samples were loaded onto the column at 10 µL. The quantitative identification was performed by the external standard method using the peak area (measurement and computer integration using the Ez-Chrom Elite software, Agilent Technologies, Santa Clara, CA, USA).

Ethanol yield from 100 g of raw material (Y_s) [80] was calculated according to the equation:

$$Y_S = \frac{Et}{M}\, x\ 100\ [\text{g}\cdot(100\ \text{g of raw material})^{-1}] \quad (3)$$

where Et is the amount of ethanol in 1000 mL of the tested sample (g); M is the weight of material weighed in 1000 mL of fermentation sample (g).

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

All experiments were carried out in triplicate. Standard deviation, mean, and median were calculated using ANOVA analysis of variance, Statistica 13.0 software from StatSoft Polska Sp. z o.o., Krakow, Poland ($p < 0.05$). The p-value in the ANOVA analysis determines whether the differences between some of the means are statistically significant [81,82].

3. Results and Discussion

3.1. Analysis of Chemical Composition of Invasive Plants

The first step of the research was to determine the chemical composition of the plant biomass. The content of extractive substances, ash, pentosans, cellulose, hemicellulose, and lignin were determined (Table 1).

Table 1. The chemical composition of invasive plant species biomass (% of dry matter).

Plant Species	Extractive Substances	Ash	Pentosans	Cellulose	Hemicellulose	Lignin
Reynoutria japonica	16.14 ± 0.33	9.49 ± 0.01	15.98 ± 0.15	31.94 ± 0.77	20.87 ± 0.64	20.18 ± 0.17
Reynoutria sachalinensis	21.16 ± 0.55	5.77 ± 0.01	20.09 ± 0.52	29.57 ± 0.49	29.80 ± 0.72	19.17 ± 0.11
Reynoutria × bohemica	19.72 ± 0.21	6.58 ± 0.09	20.51 ± 0.42	31.71 ± 0.95	34.48 ± 0.57	19.41 ± 0.40
Solidago canadensis	14.42 ± 0.52	2.39 ± 0.01	20.07 ± 0.46	38.95 ± 0.67	23.78 ± 1.17	28.68 ± 0.13
Solidago gigantea	13.27 ± 0.26	4.93 ± 0.01	18.54 ± 0.3	38.50 ± 0.18	29.59 ± 0.21	24.79 ± 0.22
Spiraea tomentosa	14.19 ± 0.32	6.95 ± 0.01	16.70 ± 0.16	32.32 ± 0.09	22.46 ± 0.82	23.63 ± 0.12

Table 1 shows the content of each biomass component. The cellulose content for the studied species ranged from 29.57% in *Reynoutria sachalinensis* to 38.95% in *Solidago canadensis*. *Solidago gigantea* also had a high cellulose content of 38.50%. The remaining three species showed similar amounts of cellulose (31.7%–32.3%). Similar amounts of cellulose were found in the esparto species (33%–38%) [83] and *Miscanthus* (38.38%) [84]. The lignin content of the invasive plant samples ranged from 19.17% in *Reynoutria sachalinensis* to 28.68% for *Solidago canadensis* (Table 1). These amounts of lignin were significantly higher than those determined in grasses (12%–21%) and fiber plants (9%–21%) [83]. Smaller amounts of lignin were also found in *Miscanthus* (17.6%) [84]. On the other hand, *Miscanthus* × *giganteus* contained a similar amount of lignin (21%) compared to *R. japonica*, *R. sachalinensis*, and *R.* × *bohemica* with 20.18%, 12.17%, and 19.41%, respectively [85]. The amount of hemicellulose in the tested invasive plant species ranged from 20.87% in *R. japonica* to 34.48% in *R.* × *bohemica*. *R. sachalinensis* and *S. gigantea* also had a high content of these compounds with 29.80% and 29.59%, respectively. Similar amounts of hemicellulose were determined in different miscanthus varieties, ranging from 24.83% to 33.98% [86]. A comparably high amount of hemicellulose (30.84%−34.31%) was found in grasses from Polish meadows [86]. The pentosane content in plants usually ranges between 18 and 25% [28,83,87]. *Solidago canadensis* (20.07%), *S. gigantea* (18.54%), *Reynoutria sachalinensis* (20.09%), and *R.* × *bohemica* (20.51%) were within this range. *Reynoutria japonica* (15.98%) and *Spiraea tomentosa* (16.70%) contained much smaller amounts of pentosans. The extractive substances included phenols, resin acids, waxes, fats, fatty acids, terpenes, steroids etc. [28,83]. The content of extractive substances in the tested invasive plant species was very high, ranging from 13.27% for *S. gigantea* to 21.16% for *R. sachalinensis*. Such amounts could be due to the high content of chlorophyll and phenolic substances. Ash in plants is classified as a by-product and represents a small fraction of a percentage of the plant dry weight. Only grasses (2%–5%) and grains (2%–20%) contained slightly more ash [83]. The tested invasive plant species contained a fairly high amount of ash, ranging from 2.39% for *S. canadensis* to 9.49% for *R. japonica*. It is vital to know the ash content of the biomass being burned, as a large amount of ash can cause slag formation and obstruction of the grate [88].

3.2. Higher Heating Value and Lower Heating Value

In the next stage of the research, the higher heating value (1) and lower heating value (2) of plants were determined [79] (Table 2).

Table 2. The higher heating value and lower heating value of the invasive plant species.

Plant Species	Higher Heating Value [MJ·kg^{-1}]	Lower Heating Value [MJ·kg^{-1}]
Reynoutria japonica	18.485	16.965
Reynoutria sachalinensis	19.927	18.405
Reynoutria × *bohemica*	19.210	17.683
Solidago canadensis	19.894	18.369
Solidago gigantea	19.403	17.868
Spiraea tomentosa	18.892	17.353

Table 2 shows the higher heating value and the lower heating value of the invasive plant species under study. The higher heating value was quite high, ranging from approx. 18.490 MJ·kg^{-1} for *R. japonica* to approx. 19.900 MJ·kg^{-1} for *R. sachalinensis*. The higher heating value of the remaining species was approx. 19.000 MJ·kg^{-1}. Such high values of higher heating value are comparable to the values obtained for some energy willow species (19.36–19.58 MJ·kg^{-1}) [89] and *Miscanthus*, from 17 to 20 MJ·kg^{-1} [85]. A higher heating value of biomass is related to the lignin content [90–93]. This is true for *Solidago canadensis* and *S. gigantea*, which showed high lignin contents of 28.68% and 24.79%, and had high values of the higher heating value of 19.894 MJ·kg^{-1} and 19.403 MJ·kg^{-1} respectively. Unfortunately, this is not applicable to other invasive plant species (e.g., *R. sachalinensis* and

R. × *bohemica*), which have a high higher heating value, while the lignin content remained low. This may be due to the unique characteristics of these species.

3.3. Potential of Bioethanol Production

The invasive plant species biomass was converted to bioethanol to determine its energy potential. Pretreatment was performed, followed by the SSF process (enzymatic hydrolysis and fermentation). Both processes were performed in accordance with the conditions described in the Section 2.4. The physical pretreatment process involved cutting the biomass into fragments up to 1 cm in size, followed by shredding in a knife mill to a mesh size of 2 mm. For effective chemical treatment, 1% of sodium hydroxide was used, which is the most common reagent used in this process. According to the experience of other researchers, it effectively loosens the structure of lignocellulose and thus increases the availability of biomass and its susceptibility to the subsequent action of enzymes [31,94].

The subsequent SSF process was carried out using the Flashzyme Plus 200 enzyme and non-hydrated lyophilized yeast. It is a process in which enzymatic hydrolysis and alcoholic fermentation occur simultaneously. It is more effective than the separate hydrolysis and fermentation (SHF) process [95], which consists of two separate steps: enzymatic hydrolysis, and then fermentation. Many researchers have dealt with the comparison between SHF and SSF. The results of their experiments clearly indicate that the SSF process was more efficient in bioethanol production than the SHF process, despite using a lower reaction temperature that was not optimal for the enzymatic hydrolysis reaction [96–98]. In the SSF process, it is very important to select such reaction conditions in which both enzymes and yeast can function effectively. These parameters were selected on the basis of previous experiments, also taking into account the sensitive nature of plant material and its susceptibility to enzymes.

After the processes, the amount of bioethanol obtained was tested using the high performance liquid chromatography (HPLC) method (Figure 3).

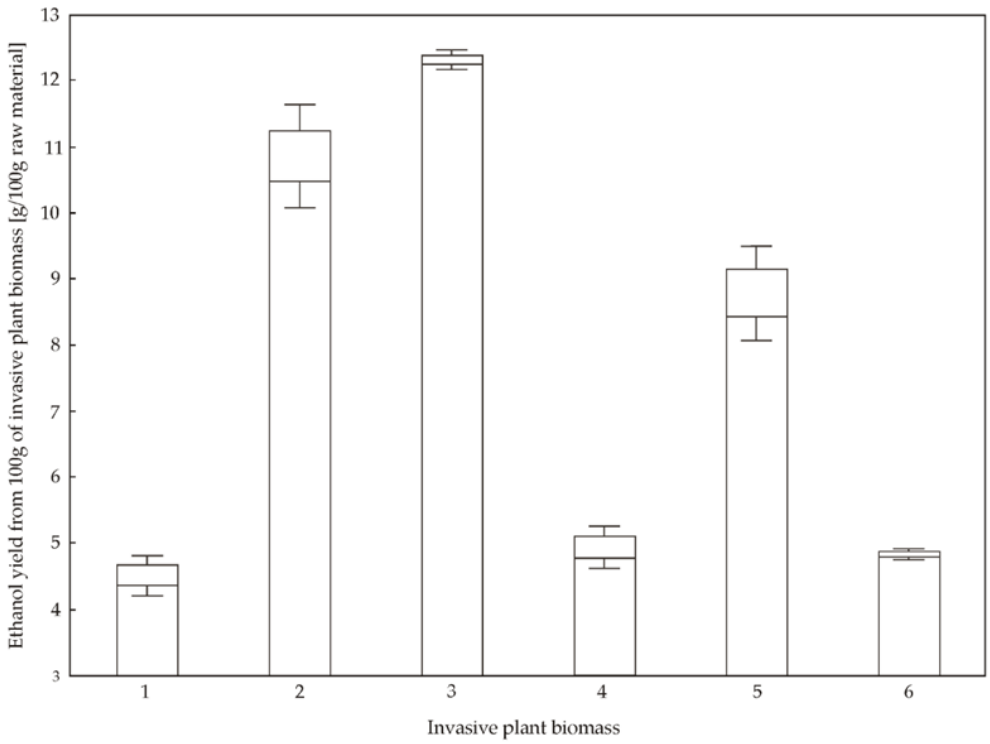

Figure 3. Ethanol yield from 100 g of invasive plant species biomass, where: 1—*Reynoutria japonica*; 2—*Reynoutria sachalinensis*; 3—*Reynoutria* × *bohemica;* 4—*Solidago Canadensis*; 5—*Solidago gigantea*; 6—*Spiraea tomentosa*. Mean, mean ± standard error—box, mean ± 1.96 standard error—whiskers.

The highest ethanol content was obtained in the *Reynoutria* × *bohemica* biomass (12.24 g·100 g^{-1} of biomass) and in the *Reynoutria sachalinensis* biomass (10.46 g·100 g^{-1} of biomass). The third in this respect was *Solidago gigantea* with 8.42 g·100 g^{-1} of biomass. Considering the results of the ethanol content in terms of the chemical composition of invasive plant species, it can be seen that the highest ethanol content was obtained from the biomass of plants with the highest concentration of hemicellulose. This may also have been influenced by the relatively low lignin content in these plants compared to the other tested plant species (see Table 1). In the remaining three plant species, *Solidago canadensis*, *Spiraea tomentosa*, *Reynoutria japonica*, the ethanol content was about 5 g·100 g^{-1} of biomass.

On the other hand, when calculating the ethanol yield in $m^3 \cdot ha^{-1}$, these relationships differed slightly. The highest value of bioethanol yield was found for *Reynoutria* × *bohemica* (2.6 $m^3 \cdot ha^{-1}$). This is due to both the highest ethanol content (see Figure 2) and the highest dry matter annual yield of this plant, which amounted to approx. 17 Mg·ha^{-1}. More than half of the lower bioethanol efficiency was achieved for two invasive plant species cultivars, *Solidago canadensis* and *Solidago gigantea*. Ethanol productivity per annual dry matter yield was 1.02 and 0.92 $m^3 \cdot ha^{-1}$, respectively. In this case, the ethanol content, determined in g·L^{-1} (Figure 2) as well as the annual dry matter yield, which ranged from 9–15 Mg·ha^{-1}, were also significantly affected. In contrast, for the other three remaining invasive plant species, the annual bioethanol yield ranged from 0.1 to 0.6 $m^3 \cdot ha^{-1}$. For the *Reynoutria sachalinensis* biomass, the ethanol content was over 10 g·L^{-1}, but a very low annual yield of this species, less than 4 Mg·ha^{-1}, had a negative impact on the final yield of bioethanol produced. For the *Spiraea tomentosa* biomass, the ethanol yield calculated on the basis of total terrestrial biomass, in other words, 38.87 Mg·ha^{-1} (not annual, see Section 2.1) was 2.36 $m^3 \cdot ha^{-1}$, which was high compared to the ethanol yield for other species. However, it should be taken into account that the ethanol concentration in this case was only 4.8 g·100 g^{-1} of biomass.

The bioethanol yield from the *Reynoutria* × *bohemica* biomass reached a similar value to the values from the Polish variety of hemp Rajan, which was also over 2 $m^3 \cdot ha^{-1}$. The ethanol concentration was also at a similar level (i.e., 13–15 g·100 g^{-1} biomass for Rajan biomass; in order to express the concentration in units of g·100 g^{-1} of biomass, the concentration in g·L^{-1} given in the article was converted, and 12.24 g·100 g^{-1} biomass for the *Reynoutria* × *bohemica* biomass, respectively [95]. The process of obtaining bioethanol from the hemp biomass was carried out under very similar conditions using the same enzyme and yeast strain. The only difference was the concentration of sodium hydroxide during the pretreatment (1% in the case of invasive plant species). Cotana et al. conducted a study on the use of *Cynara cardunculus* L. biomass for the production of lignocellulosic ethanol. For this purpose, they performed two different processes, separate hydrolysis and fermentation and simultaneous hydrolysis and fermentation, using cellulolytic enzymes and *S. cerevisiae* distillery yeast. They observed that the separate hydrolysis and fermentation process was more beneficial, as the ethanol yield was 13.64 g·100 g^{-1} of raw material [99]. In turn, during a study on the bioethanol production from *Miscanthus*, it was observed that an ethanol yield of 14.72 g·100 g^{-1} of raw material could be obtained after the pretreatment and SSF process [100]. López-Sandin et al. conducted a study on obtaining bioethanol from the biomass of an annual plant, sorghum [101]. They also calculated the ethanol yield and obtained a value of 2.1 $m^3 \cdot ha^{-1}$ at the highest annual biomass yield. Roozeboom et al. conducted research on the effect of the annual and perennial harvesting of lignocellulosic biomass on the ethanol yield [102]. They concluded that from the miscanthus (*Miscanthus sacchariflorus*) biomass of 14 Mg·ha^{-1}, bioethanol production was 3.6 $m^3 \cdot ha^{-1}$, half as much as from several annual crops with similar biomass yields.

It should be taken into account that it is necessary to optimize the bioethanol production process in order to increase the production efficiency as well as consider the economic issues. Choosing the right pretreatment will reduce the cost of energy consumption, reduce the risk of inhibitor formation, and enable the recycling of reagents. On the other hand, adjusting the conditions for carrying out the hydrolysis and fermentation process may bring

additional benefits in terms of reducing the time consumption or minimizing the quantity of enzymes and microorganisms used by applying higher yielding products and strains. An example of this is running the SSF process, which combines enzymatic hydrolysis and ethanol fermentation into one. This approach reduces the time and energy.

Based on the ethanol yields of the tested plants and the optimized process of bioethanol production at a laboratory scale, it will be possible to develop technological assumptions for a larger scale process. It is then necessary to compile data for the preparation of the material and energy assessments for each of the devices of the bioethanol production plant (type, dimensions and work schedule). It is also important to prepare the characteristics of technological utilities, a technological scheme, and how to minimize the environmental emissions. This will provide insights into all of the economic and environmental costs of production and evaluate its profitability in commercial production.

4. Conclusions

The primary goal was to compare all six species of invasive plants in terms of the energy potential including, above all, the possibility of obtaining bioethanol from these plants. The chemical composition studies showed that the content of individual biomass components in each plant was at a comparable level. The amount of cellulose (approx. 30%–40%) and hemicellulose (approx. 20%–35%) allowed for the conclusion that the biomass of these plants had a significant energy potential. Moreover, the combustion test showed that the higher heating value and the lower heating value were high for all of the tested invasive plant species. The highest values of these parameters were obtained for *Reynoutria sachalinensis* of 19.927 and 18.405 $MJ \cdot kg^{-1}$, respectively. In turn, in the conversion of biomass to bioethanol, three species showed a high ethanol efficiency (i.e., *Reynoutria* × *bohemica*, *Reynoutria sachalinensis*, and *Solidago gigantea* at 8.42–12.24 $g \cdot 100\ g^{-1}$ of biomass. Furthermore, the highest ethanol yield per hectare was obtained at 2.6 $m^3 \cdot ha^{-1}$ for the *R.* × *bohemica* biomass. The remaining species showed an ethanol yield below 2 $m^3 \cdot ha^{-1}$. It can certainly be stated that the tested invasive plants, especially *R.* × *bohemica*, proved to be a potential energy material. They also exhibited susceptibility to second-generation bioethanol production as an alternative to petroleum-based fossil fuels. It is important to note that bioethanol production from these plants is a process that still needs to be optimized economically. Therefore, in future research on bioenergy production from invasive plants, we plan to optimize the biomass conversion process to maximize the bioethanol yield. In addition, in order to use invasive plants for energy purposes, which can bring both environmental and economic benefits, it is important to conduct research on the coexisting environmental risks, mainly related to the problem of transporting this biomass. If this process can be optimized and safe methods for transport can be developed (during transport there will be no accidental spread of alien species), obtaining bioethanol from the studied taxa can be considered as one of the biomass management methods.

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Appendix A

Table A1. Plant stands from which the samples for research were collected.

Taxon	Co-Ordinates		Harvest Date
	N	E	
Reynoutria japonica	52°28′49.45″	17°17′00.77″	13 June 2021
Reynoutria sachalinensis	52°29′12.88″	17°50′31.35″	13 June 2021
Reynoutria × *bohemica*	52°25′12.98″	16°53′36.75″	14 June 2021
Solidago canadensis	51°23′47.48″	15°10′11.98″	24 July 2021
Solidago gigantea	51°23′31.19″	15°90′58.23″	28 July 2021
Spiraea tomentosa	51°24′44.24″	15°40′40.44″	25 July 2021

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