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Life Cycle Assessment of Pilot-Scale Bio-Refining of Invasive Japanese Knotweed Alien Plant towards Bio-Based Bioactive Compounds

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Abstract: Japanese knotweed is an invasive alien plant species with characteristic rapid expansion in Europe and North America and resistance to extermination. It displaces autochthonous biodiversity and causes major damage to infrastructure, thus causing global ecological and economic damage. The Japanese knotweed plant is usually eradicated using various chemical, biological, or mechanical techniques, which at a large scale include heavy equipment, usually followed by incineration. Therefore, excavation is preferred to eradication techniques, and as a biomass waste recovery method due to the extraction of high-value biocompounds. This is supported by the fact that the Japanese knotweed possesses various bioactive compounds with beneficial effects on human health. Its rhizome bark extract produces strong and stable antioxidant activity over time, as well as apoptotic, antibacterial, and other beneficial activities. In this work, an environmental impact assessment, including greenhouse gas footprint, acidification, eutrophication, and ecotoxicity for extraction route of the Japanese knotweed rhizome bark, is performed. A comparative case study between the lab-based and proposed pilot-scale production of active added-value extract was evaluated. The results show the pilot-scale production exhibits lower environmental burdens, mainly due to greater electricity requirements for the lab-scale alternative.

Keywords: Japanese knotweed rhizome bark extract; invasive alien plant species; bioactive compounds; lab-scale; pilot-scale; life cycle assessment (LCA); environmental burden assessment

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1. Introduction

In the 19th century, the plant species Japanese knotweed (*Fallopia japonica* Houtt. or *Polygonum cuspidatum* Siebold & Zucc.) was introduced to Europe from East Asia as an ornamental plant. Since then, it has shown rapid expansion and resistance to extermination in Europe and North America and was therefore classified in the group of invasive alien plant species. Japanese knotweed is currently considered to be one of the worst invasive species in the world [1], displacing autochthonous plant species and causing major damage to building and transport infrastructure, thus leading to global ecological and economic harm [2].

Eradication of Japanese knotweed's abundant biomass produces excessive amounts of waste biomass, of which 2/3 correspond to the underground plant parts. Moreover, only a small fragment of its rhizome or a stem is needed for a new individual plant to grow. Several techniques, such as mechanical (incineration, excavation), chemical (herbicides), and biological (nematodes, mites, fungi), can be used for its eradication. These procedures cannot be considered either fully environmentally or economically justifiable [2].

Sustainability consciousness needs to be the crux of process, product, and service delivery [3]. This means technology should be developed with values that support better use of resources and bioremediate the environmental damage. These include technologies for renewable energy utilization, sustainable waste disposal, water and general resource management and treatment, improved material processing, retrofitting and whole system redesign to increase efficiency, green chemistry, nanotechnology, and bioengineering [4]. The concept of sustainability involves a balanced biogeochemical cycle that essentially entails waste minimization and recycling, sustainable energy and food production (energy and food security), emission reduction, climate-resilient designs, social justice, better understanding and utilization of science, and more.

The above is supported by the fact that the Japanese knotweed extracts and compounds possess various bioactive compounds, with beneficial effects on human health (e.g., apoptotic, anti-inflammatory, antiviral, antibacterial, antioxidant, and anticancer activities) [5]. The rhizome is, however, considered to be the richest part of phytochemicals. Among the bioactive secondary metabolites, anthraquinones [6], proanthocyanidins [7], stilbenes [8], phenolic acids [9], flavan-3-ols [10], triterpenic acids [11], naphthalenes [12], monoterpenes [13], etc. have been identified.

Japanese knotweed extracts' antioxidant activity was proven by comparing their antioxidant activity to six common dietary spices and herbs [14]. In a recent study by Jug et al. (2021) [15] it was shown that the 70% ethanolic (aq) extract of the Japanese knotweed rhizome bark (JKRB) has potent antioxidant activity (half-maximal inhibitory concentration—IC_{50}~3.7 mg mL^{-1}), which was in the range of the antioxidant activity of ascorbic acid (vitamin C). Moreover, the extracts' antioxidant activity remained stable for at least 14 days, which was not the case with vitamin C (which showed a decrease in antioxidant activity over time (IC_{50}: 3.115–62.787 μg mL^{-1}).

The extraction procedure was further optimized (double extraction) and the JKRB was incorporated into an active (antioxidant and antimicrobial) chitosan-based biofoil for potential applications in food packaging and other industries [16]. The confirmed migration of the JKRB antioxidants from the biopolymer into food simulants makes the JKRB extract an excellent source of antioxidant/s for its incorporation into the various materials to protect packed goods or packaging materials against oxidation and bacteria. (−)-Epicatechin was determined to be a marker of the extract's antioxidant activity [15]. The upscaled production of the biofoil would support the fight against the invasiveness of Japanese knotweed and plastic waste, thus contributing to the reduction of greenhouse gas (GHG) emissions and supporting the so-called "zero-waste" strategy [16].

The potential uses of JKRB-enriched (bio)polymers were thus predicted as follows: (a) for active packaging (e.g., packaging for food, pharmaceuticals, food supplements, and cosmetics) [17], (b) for rapidly oxidizing materials protection (e.g., industrial fluids) [16], (c) for skin-contact products (e.g., patches, face masks, etc.) [18], (d) for food (e.g., edible gummy bears) [16]. A Japanese knotweed rhizome extract has also been used as a natural dye for formulating screen-printing inks, due to its orange color [19]. In a recent study [20] JKRB proved to inhibit live SARS-CoV-2 in vitro, encouraging future formulation of JKRB food supplements (for prevention or complementary treatment of COVID-19), JKRB biofoils (for potential prevention of its spread), and for hygiene products such as JKRB-enriched soaps and disinfectant solutions (for an enhanced disinfectant effect). Market research showed the presence of various Japanese knotweed root and rhizome products in the form of powders and food supplements marketed as a source of natural antioxidants and tinctures against Lyme disease.

To identify the environmental hotspots of the extract production process and the associated environmental burdens and unburdens of the process, life cycle assessment (LCA) can be used to quantify the environmental impact of the products and services life cycle [21]. The LCA method is defined by the international standards ISO 14040 and 14044 for analyzing the environmental aspects and impacts of product systems [22]. The potential environmental impacts of the product are studied throughout its life from raw material acquisition through production (cradle-to-gate), use, and disposal (cradle-to-grave) [23]. The usual categories of environmental impacts considered include resource use, human health, and ecological consequences [24].

JKRB production is a process with a potential upscaling opportunity, which will enable utilization of larger quantities of Japanese knotweed rhizomes. Upscaling of the lab-scale processes influences their environmental burdens due to the different equipment used, recycling methods integrated into the process, and increased heat and power efficiency [25]. Highlighting the differences in the environmental burdens using LCA can influence future decisions regarding the industrialization of the production routes. In this sense, this work aims to compare the environmental footprints of the lab-based and proposed pilot-scale JKRB extract production for the first time while utilizing this invasive species. Lab-scale and proposed pilot-scale extraction routes are considered and compared in this study. Comparison is made considering the GHG footprint and various potential environmental impacts, such as abiotic depletion (minerals and fossil fuels), acidification, eutrophication, human toxicity, freshwater and marine ecotoxicity, ozone layer depletion, and photochemical oxidation. As electricity contributes the most to environmental burdens, a sensitivity analysis is conducted considering different electricity sources. Finally, the electricity demand of the main equipment is analyzed at both lab and proposed pilot scales.

2. Materials and Methods—Environmental Impact Assessment

The JKRB extract production was considered for LCA analysis at both lab- and proposed pilot scales from cradle to gate. The system boundary, starting with the extraction of natural resource Japanese knotweed (JK) rhizomes and ending with the produced JKRB extract, is presented in Figure 1. For the system, a functional unit has been defined as 1 kg of produced extract at the lab and proposed pilot scales.

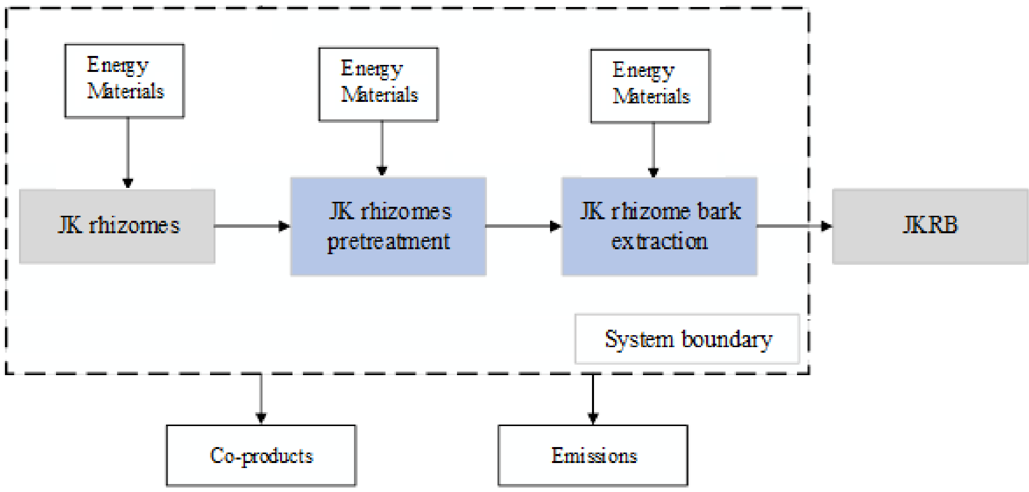

Figure 1. System boundary for cradle-to-gate JKRB extract production.

The production pathway of the process at both scales is presented in Figure 2. The main components of the processes are presented in rectangles where the inputs are blue; outputs cyan; key processes gray; the main resource, JK, light green; and final produced JKRB extract green. The extraction phase consists of six individual steps, which are encircled with a black dashed line. The key difference between the proposed pilot and lab scales is in the last evaporation stage, where the lab alternative is presented on the left and the pilot on the right, both encircled with a black line.

2.1. Lab-Scale Extract Production

Rhizomes of Japanese knotweed were harvested in Slovenia (N 46°02′33.9″; E 14°27′00.9″) and a voucher specimen was placed in the Herbarium LJU (LJU10143477). The rhizomes were transported to the National Institute of Chemistry. At this location, the rhizomes were washed with tap water and dried on paper tissues. The rhizome bark was peeled before further processing. Peeled bark was then lyophilized at −50 °C for 24 h (Micro Modulyo, IMAEdwards, Bologna, Italy). The lyophilized material was further frozen (under liquid N_2) and pulverized (by a Mikro-Dismembrator S, Sartorius, Göttingen, Germany) for 1 min at a frequency of 1700 min^{-1}. The final material (2 g) was extracted in plastic centrifuge vials with 70% ethanol (aq), followed by 5 min vortexing, 15 min ultrasonication, and 5 min centrifugation at 6700× *g*. The solid residue was re-extracted with 20 mL of 70% ethanol (aq). The following steps were the same as described for the first extraction. Finally, both supernatants were pooled together and transferred into pre-weighted glass storage vials where the solvent was evaporated under N_2 flow. The extracted mass of JKRB was calculated by weighting the full vials and calculating the difference between the full and empty vials. The extraction yield was 51%.

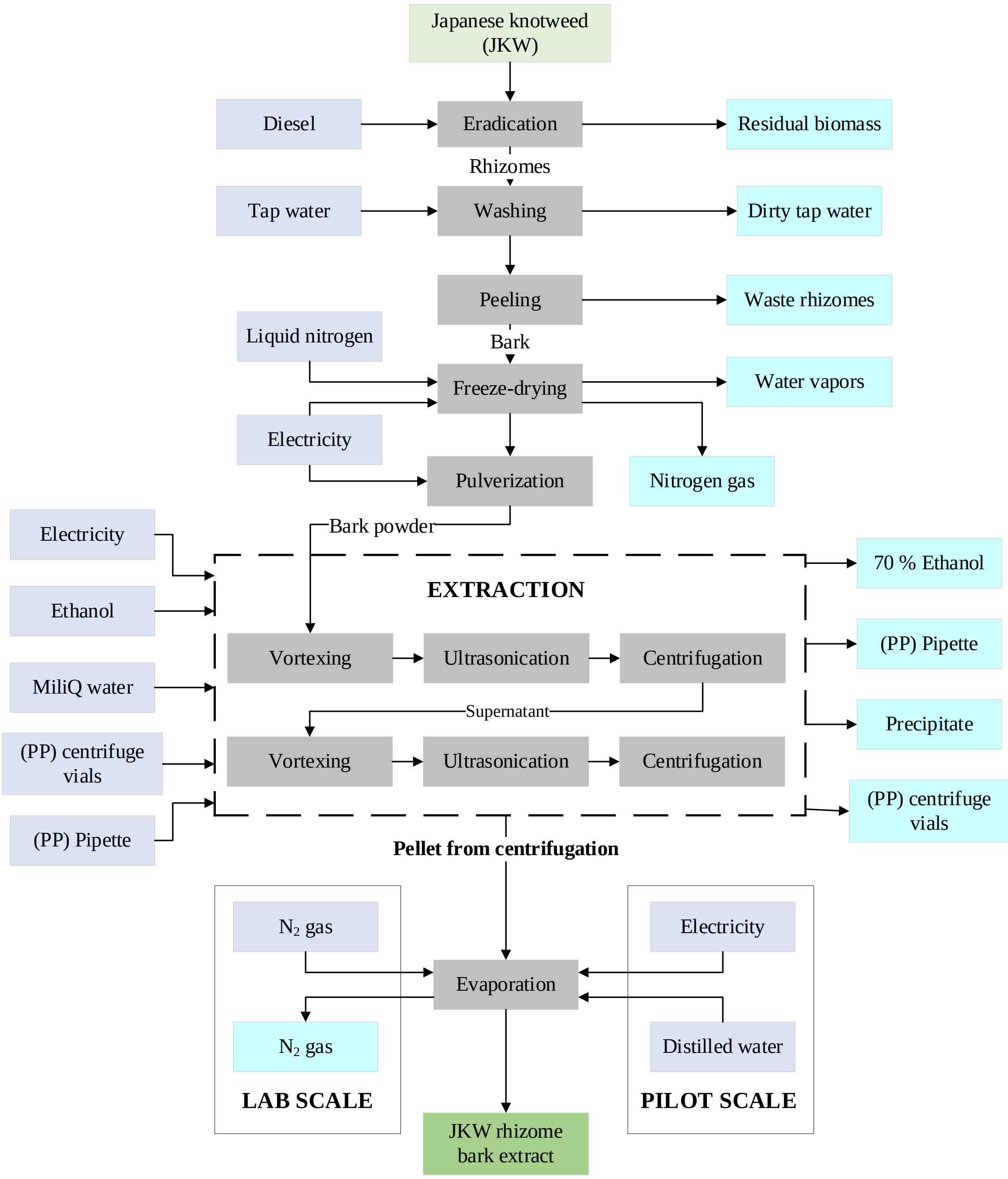

Figure 2. Japanese knotweed rhizome bark extract (JKRB) production at lab and pilot scales.

2.2. Proposed Pilot-Scale Extract Production

The same procedure as for the lab scale was assumed for a proposed pilot-scale scenario, but with a few modifications as explained below. Rhizomes at a pilot scale were eradicated using a mini excavator and transported by a light-medium track. In addition to larger-capacity equipment, multi-use vials were predicted for extraction instead of disposable vials. The solid residue was re-extracted with recycled 70% ethanol(aq). For the evaporation, a rotary evaporator was used at a pilot scale instead of N_2 flow used at a lab scale. The equipment used at lab and pilot scales is presented in Table 1. At the lab scale, the equipment used for the experiments was considered, while at the pilot scale, the equipment was assumed with the help of online catalogues and brochures.

2.3. Environmental Impact Assessment

Environmental analysis was performed based on the experimental data for the lab-scale process and based on assumed modifications for the pilot-scale process. Cradle-to-gate analysis was performed, with the use of openLCA software [26] and the ecoinvent 3.7 [27] and Agribalyse 3.0 [28] databases. Based on the functional unit of 1 kg of produced extract, both scale processes were evaluated for their GHG footprint, abiotic depletion, abiotic depletion (fossil fuels), acidification, eutrophication, freshwater ecotoxicity, human toxicity, marine aquatic ecotoxicity, ozone layer depletion, and photochemical oxidation.

Table 1. Equipment used for lab-scale (1 g) vs. proposed pilot-scale (1 kg) production of JKRB.

Unit Process	Equipment	
	Lab Scale	**Pilot Scale**
Eradication	Manual work	Mini excavator with rated power of 42 kW and engine tier IV and engine displacement of 2.83 L [29]
Transport	Manual handling	Light-medium track with EU certification diesel fuel (Euro 5 or Euro 6) [30]
Washing	Manual work	Manual work
Peeling	Manual work	Manual work
Freeze-drying	Freeze-dryer with electricity consumption of 310 W	Freeze-dryer with capacity of 10 kg and electricity consumption of 9 kW [31]
Pulverization	Dismembrator with electricity consumption of 100 W	Roll ball mill with capacity of 5 L and electricity consumption of 0.75 kW [32]
Extraction	• Shaker with electricity consumption of 60 W; • Ultrasound with electricity consumption of 300 W; • Centrifuge with electricity consumption of 550 W.	• Large shaking incubator with capacity of 115 L and electricity consumption of 450 W [33]; • Ultrasound with tank labor capacity of 133 L and electricity consumption of 2000 W [34]; • Centrifuge with capacity of 8 × 2 L and electricity consumption of 5.4 kW [35]
Evaporation	(Evaporation under N_2)	Rotary evaporator (100 L capacity) with: • Electricity consumption of 2 kW [36]; • Predicted water consumption: 1.2 L/min.

The GHG footprint refers to global warming caused by human activities and with the release of gases such as CO_2, N_2O, CH_4, and halogen carbohydrates. For the GHG footprint, the impact assessment method IPCC 2013 in openLCA was used and is expressed in kg CO_2 eq./kg of product [37]. Potential environmental impacts were evaluated using the CML-baseline method in openLCA. Abiotic depletion refers to the consumption of non-biological resources such as fossil fuels, minerals, etc. The value is expressed in kg Sb eq./kg of product and for the fossil depletion in MJ of depleted fossil fuel. Acidification refers to compounds that are predecessors of acid rain, namely SO_2, NO_x, NO, and N_2O expressed in kg SO_2 eq./kg of product [38]. Eutrophication is the pollution of water ecosystems with nutrients inducing algae blooming, causing the death of other organisms in the ecosystem, and is expressed in kg PO_4 eq./kg of product [39]. Ecotoxicity is measured separately regarding freshwater, marine, and land environments. It is based on the maximum tolerable concentration of toxic substances in environments and is measured in kg of 1,4-dichlorobenzene (DCB) equivalent [40]. Human toxicity potential is also expressed in kg 1.4-DCB eq./kg product, and is caused by chemicals released into the environment that can cause numerous diseases, for instance cancer in humans [41]. Ozone layer depletion presents ozone-depleting gases, damaging the stratospheric ozone. Most of these gases are chlorinated or brominated gases such as chlorofluorocarbons (CFCs), halons, and hydro chlorofluorocarbons (HCFCs). This potential is expressed in kg of CFC-11 eq./kg of product [38]. Photochemical oxidation refers to ground-level ozone that is formed by the reaction of volatile organic compounds (VOC) and nitrogen oxides in the presence of heat and sunlight. This impact category is dependent on the amounts of CO, SO_2, NO, ammonium, and non-metal VOC, and is expressed in kg of ethylene eq./kg product [38].

Inputs and outputs for lab-scale (1 g) and proposed pilot scale (1 kg) production of the extract are presented in Table 2. Table 3 presents a detailed Life Cycle Inventory (LCI) table of the needed resources and formed products for 1 kg of extract production. The diesel was used for the excavation and transportation of JK at the pilot scale, while at the lab scale this was performed manually. At the pilot scale, 15 L more tap water was used due to cleaning re-usable centrifuge tubes and vials, while disposable polypropylene (PP) counterparts were used in the lab scenario. The pilot scenario also used more distilled water and ethanol, as they were used for cleaning the rotary evaporator flask, but it did not consume any nitrogen. The biggest difference between the two processes is their electricity consumption. The lab scale comparably used smaller equipment with lower feed amounts, resulting in greater electricity consumption per mass of product.

Table 2. Inputs and outputs for lab-scale (1 g) and pilot-scale (1 kg) production of the Japanese knotweed rhizome bark extract.

		Lab Scale	**Pilot Scale**
Eradication	Input	Manual work—no input	Mini excavator: cca. 5 L/h (3 h of work) → Total 15 L
	Output	Cca. 15 g of biomass (above-ground parts: stems, leaves, etc.)—to be incinerated (Japanese knotweed is classed as "controlled waste" and needs to be cut down carefully and either burnt on site or taken away to a licensed landfill site or incineration facility.)	cca. 15 kg of biomass (above-ground parts: stems, leaves, etc.)—to be incinerated (Japanese knotweed is classed as "controlled waste" and needs to be cut down carefully and either burnt on site or taken away to a licensed landfill site or incineration facility.)
Transport	Input	/	Light-medium truck: cca. 25 L/100 km (10 km back and forth) → Total 2.5 L
	Output	/	/
Washing	Input	• Cca. 30 mL of tap water • Paper tissues (100% cellulose) for drying at room temperature: 0.25 g	• Cca. 1 L of tap water/kg of rhizome → Total: 30 L tap water • Paper tissues (100% cellulose) for drying at room temperature: 247.4 g
	Output	• Cca. 30 mL of dirty tap water • Paper tissues (100% cellulose) for drying at room temperature: 0.25 g.	• Cca. 1 L of tap water/kg of rhizome → Total: 30 L dirty tap water • Paper tissues (100% cellulose) for drying at room temperature: 247.4 g.
Peeling	Input	Manually: no environmental impact	Manually: no environmental impact
	Output	From cca. 30 g rhizomes we obtain cca. 10 g of bark (for further work) and 20 g of waste biomass (possible further incineration or formulation of other products for human use).	From cca. 30 kg rhizomes we obtain cca. 10 kg of bark (for further work) and 20 kg of waste biomass (possible further incineration or formulation of other products for human use).
Freeze-drying	Input	• Electricity: 310 W ∗ 24 h → Total 7.440 kW • Liquid nitrogen: 20 mL	• Electricity: 9 kW ∗ 24 h → Total 216 kW • Liquid nitrogen: 20 L
	Output	• Water vapor: 6.81 mL • Nitrogen gas: 16.16 g	• Water vapor: 6.81 L • Nitrogen gas: 16.16 kg
Pulverization	Input	• Electricity: 100 W ∗ 5 min → Total 8.33 W	• Electricity: 0.75 kW ∗ 2 h → Total 1.5 kW
	Output	• Water consumption for cleaning: cca. 15 mL • Ethanol consumption for cleaning: cca. 5 mL	• Water consumption for cleaning: cca. 15 L • Ethanol consumption for cleaning: cca. 5 L

Table 2. *Cont.*

		Lab Scale	Pilot Scale
Extraction and evaporation	Input	• Ethanol: 66.99 mL • MiliQ water: 28.71 mL • Shaker: 60 W ∗ 5 min → 5 W • Ultrasound: 300 W ∗ 15 min → Total 75 W • Centrifuge: 550 W ∗ 5 min → Total 45.8 W • N_2 gas: 1.9 L/min ∗ 2 vial ∗ 24 h → Total: 5472 L • 2 Disposable 50 mL plastic centrifuge vials (PP)—(2 pieces = 27.6 g) • 1 plastic Pasteur pipette (PP)—(1 piece = 1.5 g)	• Ethanol: 66.99 L • MiliQ water: 28.71 L • Shaker: 450 W ∗ 5 min → 37.5 W = 0.0375 kW • Ultrasound: 2000 kW ∗ 15 min → Total 500 kW • Centrifuge: 5.4 kW/h ∗ 31 min → Total 2.8 kW • Rotary evaporator: 2 kW ∗ 800 h → Total 1600 kW; 1.2 L/min water ∗ 60 min ∗ 16 h → Total 1152 L water
	Output	• 70% Ethanol: 95.7 mL • Waste biomass produced (precipitate): 1.55 g • N_2 gas: 1.9 L/min ∗ 2 vial ∗ 24 h→ Total: 5472 L • 2 Disposable 50 mL plastic centrifuge vials (PP)—(2 pieces = 27.6 g) • 1 plastic Pasteur pipette (PP)—(1 piece = 1.5 g)	• 70% Ethanol: 95.7 L (if rotary evaporator is used instead of N_2 flow, the solvents can be recycled; recommended for pilot- and large-scale applications) • Water consumption for cleaning Rotavapor flasks: cca. 0.5 L • Ethanol consumption for cleaning Rotavapor flasks: cca. 0.1 L • Waste biomass produced (precipitate): 1.55 kg • Tap water for cleaning centrifuge bottles: 15 L • Ethanol for cleaning centrifuge bottles: 4 L

Table 3. LCI table for 1 kg of Japanese knotweed rhizome bark extract production.

	Lab Scale	Pilot Scale	Unit	Description
Inputs				
Diesel	/	17.50	L	Excavation/Transport
Distilled water	15.00	15.50	L	Cleaning
Electricity	7574.13	2320.34	kWh	Power equipment
Ethanol	71.99	75.99	L	Extraction
Japanese knotweed	45.00	45.00	kg	From Ljubljana region
Liquid nitrogen	20.00	20.00	L	Freezing
MiliQ water	28.71	28.71	L	Extraction
Nitrogen gas	5472.00	/	m^3	Evaporation
Paper tissue	0.25	0.25	kg	Drying of rhizomes
Tap water	30.00	45.00	L	Washing/Cleaning
Outputs				
Bio-waste	21.55	21.55	kg	Rhizome and precipitate
Ethanol	71.99	75.99	L	
JKRB	1.00	1.00	kg	Main product
Nitrogen gas	5472.00	/	m^3	Emitted into air
Paper tissue	0.25	0.25	kg	Landfilled
PP * waste	29.1	/	kg	Vials and pipettes
Residual biomass	15.00	15.00	kg	For incineration
Wastewater	73.71	89.21	L	
Water vapors	6.81	6.81	L	Emitted into air

* PP—polypropylene.

3. Results and Discussion

This section presents the results of the environmental impact assessment and the results of the sensitivity analysis that focused on three different types of electricity mix.

3.1. Environmental Impact Assessment

The main normalized results of the lab- and pilot-scale process for the footprint assessment are presented in Figure 3. For clarity, the values of some environmental impact categories were multiplied or divided by a certain number that is presented on the left side of Figure 3. For example, the values for terrestrial ecotoxicity should be multiplied by 3.5 at both lab and proposed pilot scales to determine the actual value of terrestrial ecotoxicity.

The results indicate that the lab-scale production of JKRB mainly exhibits significantly greater environmental burdens than the pilot-scale production. In most cases, the major difference is the electricity used for the process, which is more significant at the lab scale due to smaller equipment and smaller amounts of produced products. Electricity usage especially affects GHG footprint, fossil abiotic depletion, and marine ecotoxicity, where it contributes more than 90%. A high electricity contribution is also seen in all other categories, except terrestrial ecotoxicity. The second-largest contributor to environmental burdens is excavation/transport (in the case of proposed pilot scale), especially in the case of abiotic and ozone layer depletion, due to heavy machinery that is used for this purpose. As for ethanol production, it contributes greatly to terrestrial ecotoxicity and photochemical oxidation due to the cultivation and processing of crops used for its manufacture. A greater impact is seen at the pilot scale, since more ethanol is used for cleaning of the reusable centrifuge vials and the cleaning of Rotavapor flasks that are not used in the lab-scale process.

3.2. Electricity Demand Distribution and Sensitivity Analysis

Identifying electricity as the main hotspot of the JKRB production, a comparison of the electricity demand distribution has been made for the lab- and pilot-scale processes. It should be noted that the pie charts present only the equipment that consumed more than 0.5% of total electricity, excluding the pulverization and shaking equipment. The results are presented in Figure 4A for the lab-scale process and in Figure 4B for the proposed pilot-scale process.

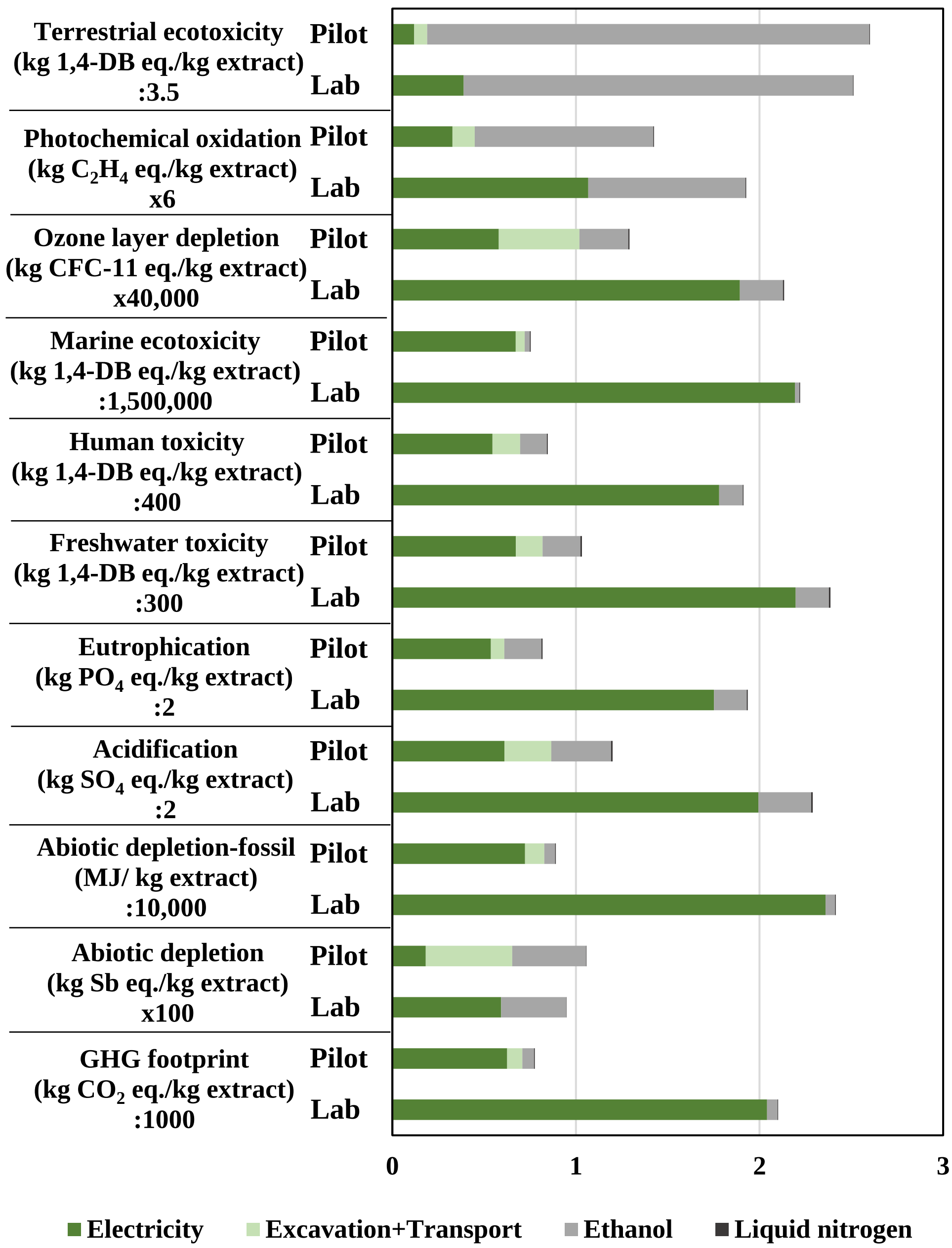

Figure 3. Environmental impact assessment of Japanese knotweed rhizome bark extract production.

The results indicate that for the lab scale, lyophilization consumes most of the electricity (more than 98%), as seen in Figure 4A. At the pilot scale, the evaporator for solvent evaporation becomes the biggest electricity consumer, accounting for almost 70% of all the electricity used. At a larger scale, a larger-capacity lyophilizer is used, decreasing the amount of electricity needed per unit of product. Other equipment is also replaced, such as the ultrasound, which has the second-largest electricity demand at the pilot scale.

Finally, a sensitivity analysis was conducted considering different electricity sources, shown in Table 4. The chosen alternatives were the current Slovenian electricity mix, the European electricity mix, and the COP26 electricity mix, which is based on the guidelines of the Glasgow climate conference 2021 [42]. For the analysis, only the pilot-scale JKRB production was compared with different electricity sources.

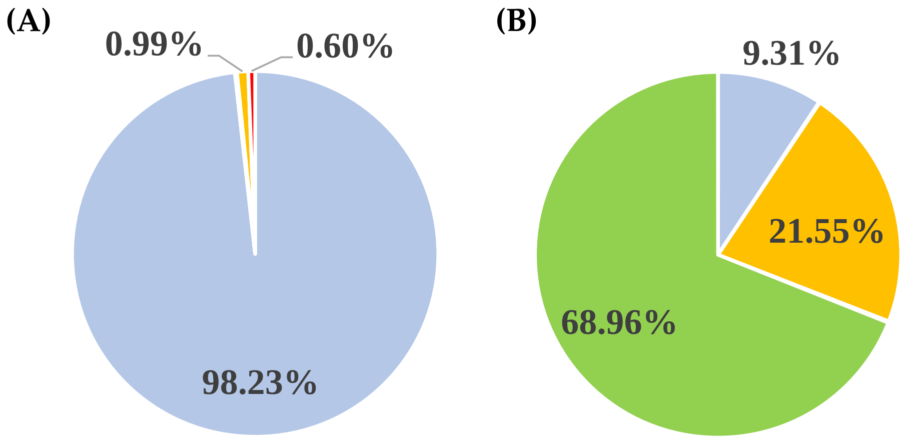

Figure 4. Electricity demand distribution for JKRB production: (**A**) Lab-scale process; (**B**) pilot-scale process.

Table 4. Electricity alternatives with their contributions in %.

Electricity Source	Slovenian [a]	European [b]	COP26 [c]
Hydro energy	32.16	12.00	21.00
Thermal energy (coal)	24.18	23.00	/
Thermal energy (oil)	0.01	/	/
Thermal energy (natural gas)	2.73	21.00	/
Thermal energy (syngas)	/	/	5.00
Thermal energy (renewable fuels)	1.27	0.20	3.00
Thermal energy (industrial waste)	0.05	/	/
Nuclear energy	37.76	26.20	31.00
Solar energy	1.80	4.50	36.00
Wind energy	0.04	13.00	4.00
Geothermal energy	/	0.20	/

[a] The Slovenian electricity mix used in the year 2020 [43]. [b] The European electricity mix in 2019 including 27 countries of the European union, with Germany being the largest producer, accounting for 20.8% [44]. [c] Electricity source alternative according to the guidelines of the Glasgow climate conference 2021 based on the Slovenian nuclear plan of building a new nuclear plant, excluding coal and natural gas electricity production, having at least 40% electricity from renewable sources, and using CO_2 neutral syngas for electricity production [42,45].

The results are presented in Figure 5, where for better representation, the values were again multiplied or divided by a certain number (the same number as in Figure 3). The greatest environmental burden is mostly obtained (except for the abiotic depletion category) in the case of the European electricity mix alternative where 43.6% of electricity is produced from fossil fuels: either coal or natural gas. The main cause of environmental burdens is namely the use of coal. Reducing the use of fossil fuels for electricity production can lower the environmental footprints significantly, as can be seen when using the COP26 alternative. The only category where the COP26 alternative has comparably the greatest environmental burden is abiotic depletion, due to the production and installation of photovoltaic panels, wind turbine production, and fuel cell production that consume large amounts of precious resources.

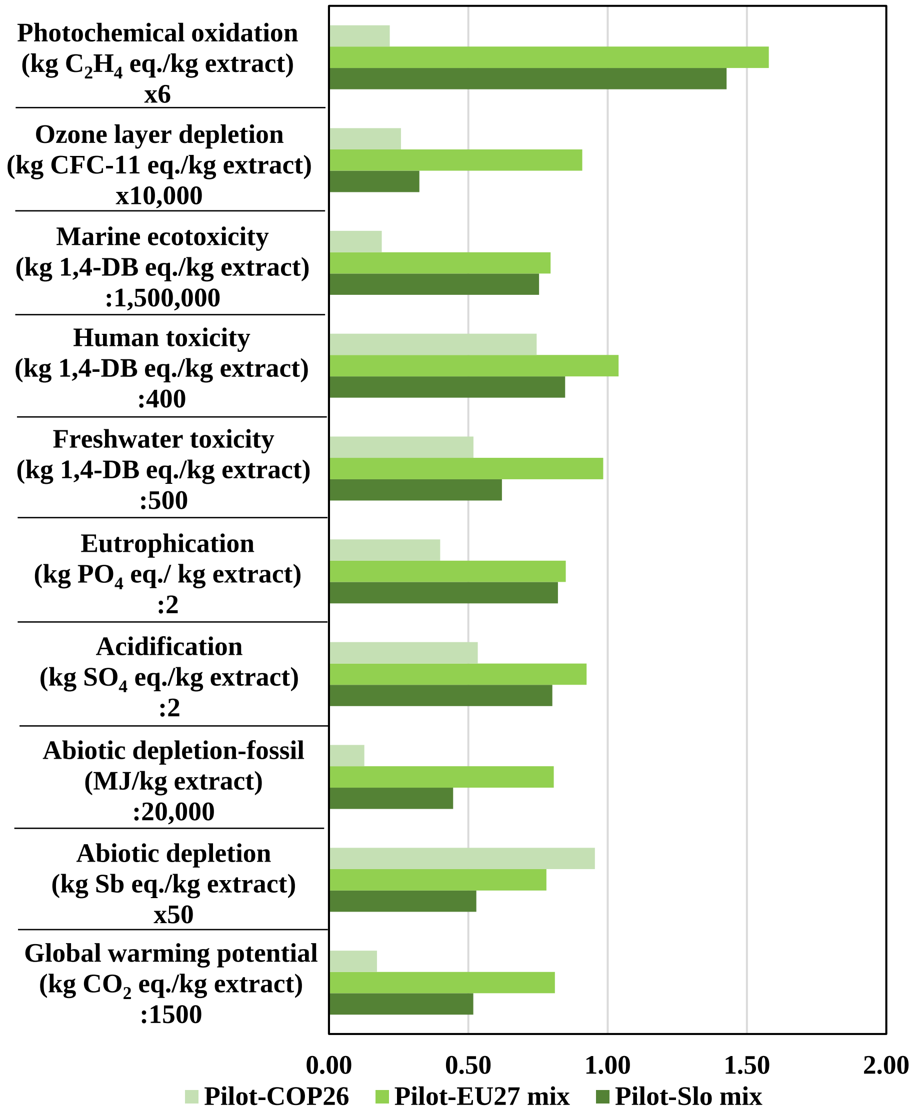

Figure 5. Sensitivity analysis of different electricity sources on the environmental impact potentials of pilot-scale JKRB production.

4. Conclusions

This study analyzed the environmental burdens related to production of JKRB at lab-scale and in a proposed pilot-scale procedure. As expected, it was found that the pilot-scale alternative exhibits lower environmental burdens compared to the lab-scale production of JKRB. The main contributor to all the evaluated categories is the production of electricity, especially in the lab-scale alternative where comparably greater amounts per unit of product are required. At the lab scale, the greatest amount of electricity is consumed by lyophilization, while evaporation is the most electricity-demanding step at the pilot scale.

Based on the obtained results, it is proven that the processes at larger scales can reduce the environmental burdens. Sensitivity analysis confirmed that the use of renewable energy sources for electricity production helps to reduce environmental burdens. In the future, the process should be investigated further with the goal of further environmental-burden reduction through equipment optimization, recycling of chemicals, or even proposing a different process route.

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References

1. Lowe, S.; Browne, M.; Boudjelas, S.; De Poorter, M. *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database*; Invasive Species Specialist Group: Auckland, New Zealand, 2000; Volume 12.
2. Dommanget, F.; Cavaillé, P.; Evette, A.; Martin, F. Asian knotweeds-an example of a raising threat. In *Introduced Tree Species in European Forests: Opportunities and Challenges?* European Forest Institute: Joensuu, Finland, 2016.
3. Seebode, D.; Jeanrenaud, S.; Bessant, J. Managing innovation for sustainability. *RD Manag.* **2012**, *42*, 195–206.
4. Mulvihill, M.J.; Beach, E.S.; Zimmerman, J.B.; Anastas, P.T. Green chemistry and green engineering: A framework for sustainable technology development. *Annu. Rev. Environ. Resour.* **2011**, *36*, 271–293. [CrossRef]
5. Cucu, A.-A.; Baci, G.-M.; Dezsi, Ş.; Nap, M.-E.; Beteg, F.I.; Bonta, V.; Bobiş, O.; Caprio, E.; Dezmirean, D.S. New Approaches on Japanese Knotweed (*Fallopia japonica*) Bioactive Compounds and Their Potential of Pharmacological and Beekeeping Activities: Challenges and Future Directions. *Plants* **2021**, *10*, 2621. [CrossRef]
6. Glavnik, V.; Vovk, I. Extraction of anthraquinones from Japanese knotweed rhizomes and their analyses by high performance thin-layer chromatography and mass spectrometry. *Plants* **2020**, *9*, 1753. [CrossRef]
7. Glavnik, V.; Vovk, I. High performance thin-layer chromatography–mass spectrometry methods on diol stationary phase for the analyses of flavan-3-ols and proanthocyanidins in invasive Japanese knotweed. *J. Chromatogr. A* **2019**, *1598*, 196–208. [CrossRef]
8. Nawrot-Hadzik, I.; Ślusarczyk, S.; Granica, S.; Hadzik, J.; Matkowski, A. Phytochemical diversity in rhizomes of three Reynoutria species and their antioxidant activity correlations elucidated by LC-ESI-MS/MS analysis. *Molecules* **2019**, *24*, 1136. [CrossRef]
9. Lachowicz, S.; Oszmiański, J. Profile of bioactive compounds in the morphological parts of wild *Fallopia japonica* (Houtt) and *Fallopia sachalinensis* (F. Schmidt) and their antioxidative activity. *Molecules* **2019**, *24*, 1436. [CrossRef]
10. Glavnik, V.; Vovk, I.; Albreht, A. High performance thin-layer chromatography–mass spectrometry of Japanese knotweed flavan-3-ols and proanthocyanidins on silica gel plates. *J. Chromatogr. A* **2017**, *1482*, 97–108. [CrossRef]
11. Lachowicz, S.; Oszmiański, J.; Wojdyło, A.; Cebulak, T.; Hirnle, L.; Siewiński, M. UPLC-PDA-Q/TOF-MS identification of bioactive compounds and on-line UPLC-ABTS assay in Fallopia japonica Houtt and *Fallopia sachalinensis* (F. Schmidt) leaves and rhizomes grown in Poland. *Eur. Food Res. Technol.* **2019**, *245*, 691–706. [CrossRef]
12. Nawrot-Hadzik, I.; Granica, S.; Domaradzki, K.; Pecio, Ł.; Matkowski, A. Isolation and determination of phenolic glycosides and anthraquinones from rhizomes of various *Reynoutria* species. *Planta Med.* **2018**, *84*, 1118–1126. [CrossRef]
13. Fan, P.; Hay, A.-E.; Marston, A.; Lou, H.; Hostettmann, K. Chemical variability of the invasive neophytes *Polygonum cuspidatum* Sieb. and Zucc. and *Polygonum sachalinensis* F. Schmidt ex Maxim. *Biochem. Syst. Ecol.* **2009**, *37*, 24–34. [CrossRef]
14. Chan, C.L.; Gan, R.Y.; Corke, H. The phenolic composition and antioxidant capacity of soluble and bound extracts in selected dietary spices and medicinal herbs. *Int. J. Food Sci. Technol.* **2016**, *51*, 565–573. [CrossRef]
15. Jug, U.; Naumoska, K.; Vovk, I. (−)-Epicatechin—An Important Contributor to the Antioxidant Activity of Japanese Knotweed Rhizome Bark Extract as Determined by Antioxidant Activity-Guided Fractionation. *Antioxidants* **2021**, *10*, 133. [CrossRef]
16. Naumoska, K.; Jug, U.; Kõrge, K.; Oberlintner, A.; Golob, M.; Novak, U.; Vovk, I.; Likozar, B. Antioxidant and antimicrobial biofoil based on chitosan and Japanese knotweed (*Fallopia japonica*, Houtt.) rhizome bark extract. *Antioxidants* **2022**, *11*, 1200. [CrossRef]
17. Drago, E.; Campardelli, R.; Pettinato, M.; Perego, P. Innovations in smart packaging concepts for food: An extensive review. *Foods* **2020**, *9*, 1628. [CrossRef]
18. Zhou, Y.; Yang, D.; Chen, X.; Xu, Q.; Lu, F.; Nie, J. Electrospun water-soluble carboxyethyl chitosan/poly (vinyl alcohol) nanofibrous membrane as potential wound dressing for skin regeneration. *Biomacromolecules* **2008**, *9*, 349–354. [CrossRef]
19. Klančnik, M. Screen printing with natural dye extract from Japanese knotweed rhizome. *Fibers Polym.* **2021**, *22*, 2498–2506. [CrossRef]
20. Jug, U.; Naumoska, K.; Malovrh, T. Japanese Knotweed Rhizome Bark Extract Inhibits Live SARS-CoV-2 In Vitro. *Bioengineering* **2022**, *9*, 429. [CrossRef]

21. van der Giesen, C.; Cucurachi, S.; Guinée, J.; Kramer, G.J.; Tukker, A. A critical view on the current application of LCA for new technologies and recommendations for improved practice. *J. Clean. Prod.* **2020**, *259*, 120904. [CrossRef]
22. Finkbeiner, M.; Inaba, A.; Tan, R.; Christiansen, K.; Klüppel, H.-J. The new international standards for life cycle assessment: ISO 14040 and ISO 14044. *Int. J. Life Cycle Assess.* **2006**, *11*, 80–85. [CrossRef]
23. Gordon, J. Cradle to Grave—Explained. Available online: thebusinessprofessor.com/en_US/mgmt-operations/cradle-to-grave-definition (accessed on 20 April 2022).
24. Klöpffer, W. Life cycle sustainability assessment of products. *Int. J. Life Cycle Assess.* **2008**, *13*, 89–95. [CrossRef]
25. Tsoy, N.; Steubing, B.; van der Giesen, C.; Guinée, J. Upscaling methods used in ex ante life cycle assessment of emerging technologies: A review. *Int. J. Life Cycle Assess.* **2020**, *25*, 1680–1692. [CrossRef]
26. openLCA. Green Delta. Available online: openlca.org (accessed on 9 April 2022).
27. ecoinvent. ecoinvent Database. Available online: ecoinvent.org/the-ecoinvent-database/ (accessed on 10 April 2022).
28. Nexus, O. Agribalyse. Available online: nexus.openlca.org/database/Agribalyse (accessed on 10 April 2022).
29. Heidari, B.; Marr, L.C. Real-time emissions from construction equipment compared with model predictions. *J. Air Waste Manag. Assoc.* **2015**, *65*, 115–125. [CrossRef]
30. Volvo. Emissions from Volvo's Trucks. Available online: volvotrucks.com/content/dam/volvo-trucks/markets/global/our-values/environmental-care/our-trucks/Emis_eng_10110_14001.pdf (accessed on 3 March 2021).
31. Lyomac. LG1.0 Lyophilization Equipment for Flowers Pilot Mini Freeze Drying Lyophilizer with 10 kg Loading Capacity. Available online: lyomac.en.china.cn (accessed on 27 November 2022).
32. Yonglekang. Roll Ball Mill. Available online: ylkeqpt.com/show-7-55.html (accessed on 27 November 2022).
33. Stuart. SI600 Large Shaking Incubator. Available online: tequipment.net/Stuart/SI600/Incubators/#description (accessed on 27 November 2020).
34. Elma. Elmasonic Xtra ST. Available online: elma-ultrasonic.com (accessed on 27 November 2020).
35. Scientific, T.F. Centrifuges Designed for Reliable, High-Throughput Bioprocessing. Available online: assets.thermofisher.com/TFS-Assets/LED/brochures/BRCFGSORVALLBIOS%201220.pdf (accessed on 27 November 2020).
36. HAWACH. Rotary Evaporator. Available online: hawach.com (accessed on 27 November 2020).
37. Hauschild, H. *Life Cycle Impact Assessment*; Springer: Dordrecht, The Netherlands, 2015.
38. Aitor, A.; Christina, R.; Andreas, C. LCIA Methods. Available online: openlca.org/wp-content/uploads/2016/08/LCIA-METHODS-v.1.5.5.pdf (accessed on 25 December 2021).
39. Glibert, P.M.; Seitzinger, S.; Heil, C.A.; Burkholder, J.M.; Parrow, M.W.; Codispoti, L.A.; Kelly, V. Eutrophication. *Oceanography* **2005**, *18*, 198. [CrossRef]
40. Rosenbaum, R.K.; Bachmann, T.M.; Gold, L.S.; Huijbregts, M.A.; Jolliet, O.; Juraske, R.; Koehler, A.; Larsen, H.F.; MacLeod, M.; Margni, M. USEtox—The UNEP-SETAC toxicity model: Recommended characterisation factors for human toxicity and freshwater ecotoxicity in life cycle impact assessment. *Int. J. Life Cycle Assess.* **2008**, *13*, 532–546. [CrossRef]
41. BRE Group. Human Toxicity. Available online: bregroup.com/greenguide/page.jsp?id=2098 (accessed on 30 August 2021).
42. UNCCC. Glasgow Climate Pact. Available online: unfccc.int/documents/310475 (accessed on 14 November 2021).
43. Republika Slovenija Statistični urad SiStat. Električna Energija (GWh), Slovenija, Mesečno. Available online: pxweb.stat.si/SiStatData/pxweb/sl/Data/Data/1817601S.px/table/tableViewLayout2/ (accessed on 29 May 2021).
44. Eurostat, S.E. Electricity Production, Consumption and Market Overview. Available online: ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_production,_consumption_and_market_overview#Electricity_generation (accessed on 6 October 2021).
45. Urbančič, A.Č.M.; Stegnar, G.; Janša, T.; Merše, M.; Sučić, B.; Đorić, M.; Košnjek, Z.; Majcen, B.; Pretnar, G.; Verbič, J.; et al. Končno Poročilo C3.2: Povzetek Analize Scenarijev za Odločanje o Dolgoročni Podnebni Strategiji Slovenije do Leta 2050. Available online: gov.si/assets/ministrstva/MOP/Javne-objave/Javne-obravnave/podnebna_strategija_2050/dolgorocna_podnebna_strategija_2050_strokovne_podlage.pdf (accessed on 6 October 2021).