

Review

The Use of Plants from the Lemnaceae Family for Biofuel Production—A Bibliometric and In-Depth Content Analysis

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Abstract: Plants of the *Lemnaceae* family are becoming increasingly popular among researchers. The goal of the study was to characterize trends in scientific research related to the use of aquatic plants from the *Lemnaceae* family for energy purposes, especially for the production of biogas, bioethanol, and other biofuels. These plants fit perfectly into the concept of a circular economy. This study performed a bibliometric and in-depth content analysis to review the use of plants from the *Lemnaceae* family for biofuel production. A set of 666 articles published from 2008 to 2022 was identified from the Scopus and Web of Science databases. Different analytical scientometric tools (topic mapping and overlay visualization networks) were used to analyze 141 articles; the most influential countries, institutions, authors, journals, and articles were identified. Depth content analysis reveals five research areas: (i) development of duckweed growth and starch accumulation; (ii) development of the pretreatment techniques; (iii) development of ethanol fermentation; (iv) hydrothermal liquefaction and bio-oil production; and (v) anaerobic digestion and biogas production.

Keywords: duckweed; Lemnaceae; biofuel; circular economy; bioethanol; bio-oil; biogas; pyrolysis



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1. Introduction

Lemnaceae are small, monocotyledonous, free-floating aquatic plants of the angiosperm class [1]. Worldwide, the five genera of the *Lemnaceae* family (*Lemna*, *Spirodela*, *Wolffia*, *Wolffiella*, *Landolita*) include 37 described species [2]. Plants belonging to this family are small and leafless. Their stems are green and flattened and float on the surface of the water (nymphs) or are submerged (elodeites) [3]. Lemnaceae are characterized by the development of a single root (genus *Lemna*) or a bundle of roots (genus *Spirodela*) on the lower surface of the plant. In some cases, these plants do not have a root at all (genus *Wolffia* and *Wolffiella*) [4]. Flowers can be occasionally observed and are monoecious, and pollination usually occurs through snails, small insects, or water [4]. In various Asian countries, plants of the *Lemnaceae* family have been used as human food [5] due to their high digestible protein content [6,7].

As numerous studies have shown, duckweed has also proven to be a beneficial food for livestock, e.g., cattle, sheep, horses, rabbits [7,8] poultry and waterfowl ([9,10], fish [11,12], and swine [13]. In addition to being used as food and fodder, watercress can be processed into biomass, which, when grown under the right conditions, has a high starch content [14–16] and can also be used as a feedstock for biofuel and biogas production [17–19]. In summary,

plants of the *Lemnaceae* family show high potential as innovative crops. Table 1 shows the classification of selected plant species from the *Lemnaceae* family.

Table 1. Selected plant species of the *Lemnaceae* family (only *Lemna* and *Spirodela*).

Lemna	Spirodela
<i>L. gibba</i>	<i>S. biperforata</i>
<i>L. disperma</i>	<i>S. intermedia</i>
<i>L. japonica</i>	<i>S. oligorrhiza</i>
<i>L. minima</i>	<i>S. polyrrhiza</i>
<i>L. minor</i>	<i>S. punctata</i>
<i>L. minuscula</i>	
<i>L. paucicostata</i>	
<i>L. perpusilla</i>	
<i>L. polyrrhiza</i>	
<i>L. turionifera</i>	
<i>L. trisulca</i>	
<i>L. valdiviana</i>	

Source: [3].

1.1. General Characteristics of *Spirodela Polyrrhiza*

Spirodela polyrrhiza is a common aquatic plant that is small in size and native all over the world except in Antarctica [20]. In Poland, it is most common in inland water bodies in lowland areas.

Spirodela tissues have specialized cells (idioblasts) with calcium oxalate crystals in the form of raphides and druses. These cells are considered a defense against small organisms [21]. When plant tissue is damaged, calcium oxalate crystals are ejected from it and act as irritants. The vegetative shoots of *Spirodela* die back as the growing season terminates. Beginning in late summer and ending in late autumn, the plant produces spore forms (turions) that, after detaching from the parent plant, fall to the bottom of water bodies [20,22–24].

1.2. Characteristics of *Lemna minor* (L.)

Lemna minor (L.) is a small aquatic plant belonging to the *Lemnaceae* family. It comprises 13 species, 5 of which are present in Poland. The name *lemna* is derived from the Greek word *limni*, which means lake. *Lemna* are the smallest vascular plants; their length is between 1 and 5 mm. The structure resembles thallus plants. The only organs (based on which they can be distinguished) are heavily reduced flowers and roots. Each organism is a loose shoot segment. Duckweed floats either singly- or in colony-like groups of several (usually two to eight) plants connected by a stem. They are often numerous, forming a dense covering of reservoirs [25].

Lemna minor (L.) is composed of elliptical, green, egg-shaped, bilaterally flattened shoot segments. It has a single root. It is a proliferative organism. It reproduces vegetatively by growing new adventitious shoots. During winter, it falls to the bottom of the water body and regrows with buds in spring. *Lemna minor* (L.) is found in bodies of stagnant water [8].

1.3. Aquatic Plants of the Family *Lemnaceae* as a Source of Biomass for Energy Production

One of the latest and intensively developing directions in renewable energy production is the use of biomass extracted from aquatic plants. Dynamic research into the production of biofuels such as bioethanol and biodiesel seeks to make the fuel industry independent of fossil fuels and to reduce the emission of carbon into the environment [26].

The production of traditional crops used for energy purposes poses many problems, such as availability of soils with the right amount of nutrients, reduction of crop biodiversity, and, most importantly, high water consumption. Water is a very important natural resource. Its value continues to rise due to increasing environmental pollution. Therefore, it is important to treat it properly and to be able to reuse it [27].

Another problem with traditional crop production is fertilization and the use of pesticides, which have a negative impact on the environment. Using biomass from aquatic plants does not require pesticides and allows water to be kept clean and to be reused [28].

Aquatic plants from the *Lemnaceae* family can also be used for the phytoremediation of water bodies, as they are characterized by rapid growth and a high potential for bioaccumulation, biotransformation, and biodegradation. Compared to other aquatic plants, Lemnaceae can accumulate heavy metals and radionuclides to a greater extent, as they are hyperaccumulators.

The biomass of plants of *Lemna* sp. and *Spirodela* sp. can also be used as food for livestock. It contains high levels of lysine and methionine, amino acids that are present in higher amounts in animals than in plants [29,30]. The uses of Lemnaceae plants can be multidirectional. Their biomass can be used as a feedstock for biogas, biofuels, biorefineries, in phytoremediation, and as animal feed [3].

Future energy biomass alternatives may include both aquatic plants (*Lemna* sp. and *Spirodela* sp.) from the Lemnaceae family [3]. *Spirodela polyrrhiza* is one of the novel feedstocks that can be used for large-scale bioethanol production [31]. It is a plant that grows quickly and has a high starch accumulation capacity and a low cellulose content. *Spirodela*, under proper culture conditions, can double its biomass every 16–24 h [3,32].

Cheng et al. [33] reported that the growth rate of duckweed reared on swine manure leachate can reach as much as 29 g of dry matter per 1 m³ for one day, which can be converted to 106 t from 1 ha for one year. This yield far exceeds the production capacity of most starch crops, e.g., corn (7.84 t from 1 ha/year), wheat (3.15 t from 1 ha/year), or barley (3.70 t from 1 ha/year), confirming the fact that duckweed has excellent potential for starch synthesis and, thus, use in bioethanol production. Lemnaceae can also be used in anaerobic digestion to produce biogas (an equally significant biofuel).

Among the energy plants that produce biomass for renewable energy sources and conform to the European Union's energy policy, *Spirodela polyrrhiza* and *Lemna* sp. (Lemnaceae) can also be used [20]. It is characterized by high starch storage capacity (up to 70% dry weight) and relatively low cellulose content (about 10%) [3]. An additional advantage of *Spirodela polyrrhiza* is its ability to absorb many elements and compounds as well as its high tolerance to the diverse constituents found in inland waters [34]. This characteristic may be particularly important in managing the digestate from biogas production in agricultural biogas plants.

The use of *Spirodela polyrrhiza* and *Lemna minor* L. for energy purposes is still minimal; however, research is being conducted to implement these macrophytes into biogas production. According to Ma et al. [35], *Spirodela polyrrhiza* is considered a promising feedstock for bioethanol production due to its high biomass production and starch content. Under optimal thermal and light conditions and with the right substrate concentration of macro- and micronutrients, *Spirodela polyrrhiza* doubles its mass in two days [1]. The average annual biomass yield of the macrophyte in tropical and subtropical climates is estimated at 10–30 t of dry matter/ha [36].

There are reviews on the processing of duckweed for biofuel production. For example, [37] compared conventional fuel and aquatic biofuel properties; [38] discussed producing bio-oil, bioethanol, biogas, and other kinds of bioenergy from duckweed through hydrothermal liquefaction, pyrolysis, anaerobic digestion, and fermentation processes; and [39,40] focuses on pyrolysis and hydrothermal liquefaction processes used for the bio-oil production from duckweed. Researchers also discussed how photosynthesis and metabolism-related processes affect the accumulation of starch in duckweed biomass [19]. Appenroth et al. [41] studied the accumulation of starch in duckweeds, which might be used

as feedstock for biofuel production. Pena et al. [42] proposed an integrated approach of biomass production and valorization, nutrient recovery from wastewater, and production of a renewable energy. However, there is a research gap: a lack of combined bibliometric and content analysis studies on biofuel production using duckweed as a substrate in the context of a circular economy. The use of bibliometrics makes it possible to analyze the impact, visibility, and influence of the literature in the scientific community in quantitative terms. A study using bibliometrics can be helpful for policymakers and institutions providing financial support, assessing the productivity and quality of researchers' work, and understanding the latest advances in research. Researchers can track trends and, through mapping, can identify major competitors or collaborators. It also allows for the analysis of research trends, productivity and scientific connections, and representation of different fields [43]. This knowledge facilitates the establishment of scientific cooperation between research units from different countries and the integration of the scientific community at the global level. The present study aims to present both quantitative and qualitative analyses of articles concerning biofuel production using duckweed as feedstock.

The following research questions were formulated:

RQ1: What are the bibliometric characteristics of the articles related to duckweed (*Lemnaceae*, *Lemna*, or *Spirodela*) and biofuel production?

RQ2: What are the research areas related to biofuels and duckweed?

RQ3: What are the main findings in the studies of biofuel production using duckweed as a feedstock?

RQ4: What are the future research directions in biofuel production using duckweed as a feedstock?

2. Materials and Methods

The authors adopted the research protocol of bibliometric review described by [44] and [45,46]. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis), as suggested by [47,48], was followed and is presented in Figure 1.

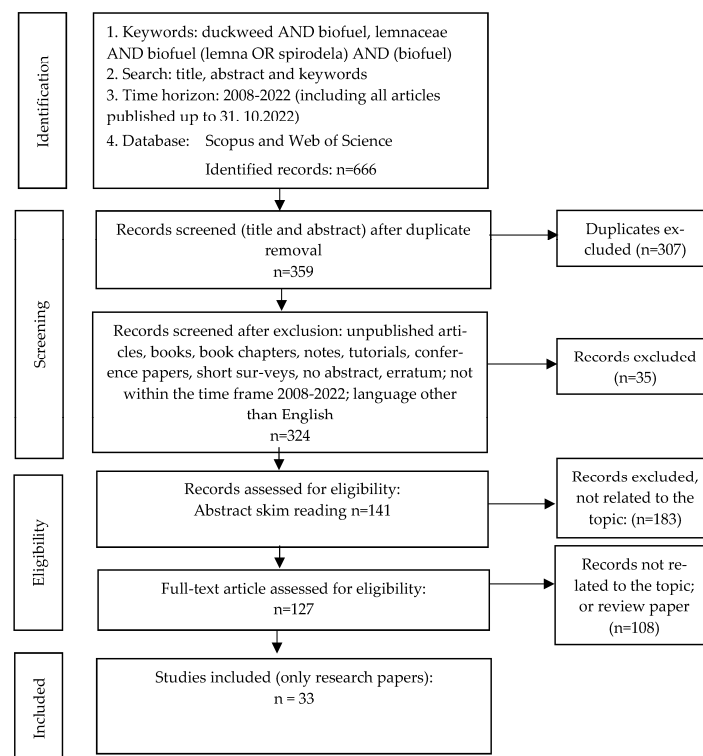


Figure 1. The methodology of the literature review.

A bibliometric and in-depth content analysis was used to review the use of Lemnaceae: *Lemna* sp. or *Spirodela* sp. for biofuel production. Web of Science and Scopus databases were selected, as suggested [49]. The datasets generated by Scopus and WOS used in bibliometric analysis are provided as Supplementary Materials.

The Scopus database was used for template data according to the recommendations of [50]. Topic mapping and overlay visualization networks [50,51] were performed using VOSviewer (ver. 1.6.14.) software (<http://www.vosviewer.com/>) (released 27 January 2020); Center for Science and Technology Studies, Leiden University, Leiden, The Netherlands) [51,52].

3. Results of the Bibliometric Analyses

3.1. Descriptive Analysis

The first mentions of using duckweed as a feedstock for biofuel production were in documents published in 2008 (Figure 2). From 2014 to 2018, a clear upward trend was observed, with a slight decrease in 2016. Since 2018, the number of publications has remained at a similar level (approx. 50 documents per year). This increment proves the increased interest in duckweed and its use in the production of biofuels.

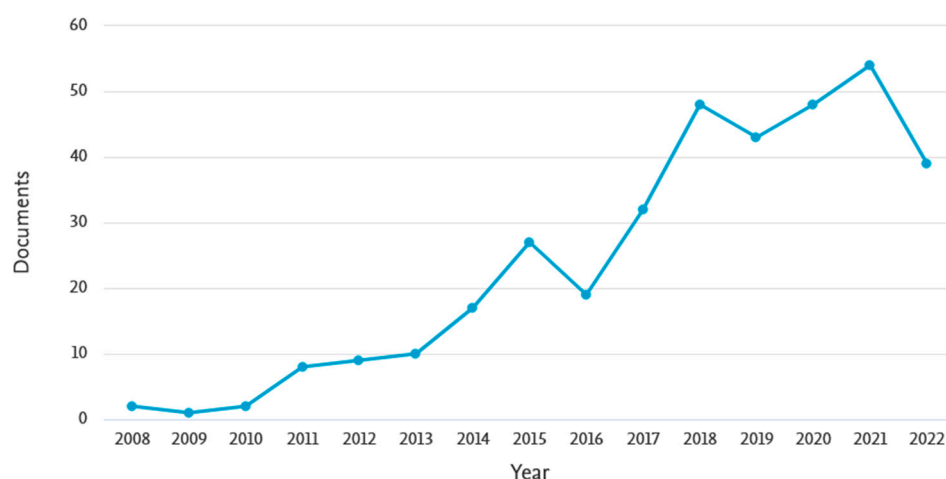


Figure 2. All published articles from 2008 to 2022 in an analyzed sample of 324 articles (2022 is not a full year).

Research articles constituted 83.3% of the total documents published, and reviews accounted for 9.2%; the rest are conference papers, book chapters, and editorials (Figure 3).

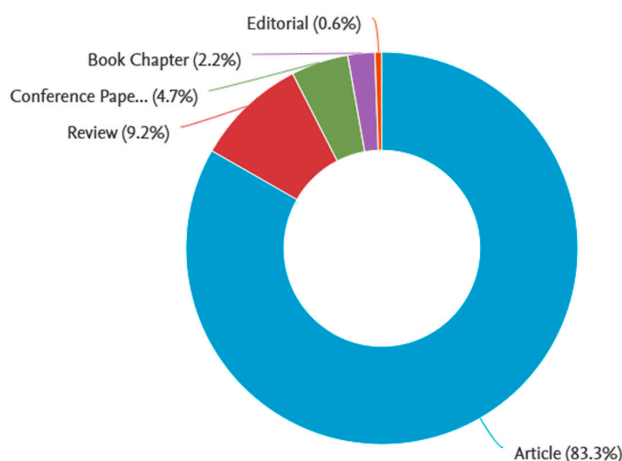


Figure 3. Distribution of papers.

Figure 4 shows documents published by five top journals. The most active was *Bioresource Technology*, with a high number of published articles per year from 2018 to 2020. From 2015 to 2017, *Biotechnology for Biofuels* was popular among researchers. From 2018 *Chemosphere*, *Plants* and *Environmental Science and Pollution Research* was popular. The fact that more articles were published in *Plants* and *Chemosphere* than in *Bioresource Technology* and *Biotechnology for Biofuels* might have been caused by changing trends during the last years and a greater focus on physiology or genetics than on practical application.

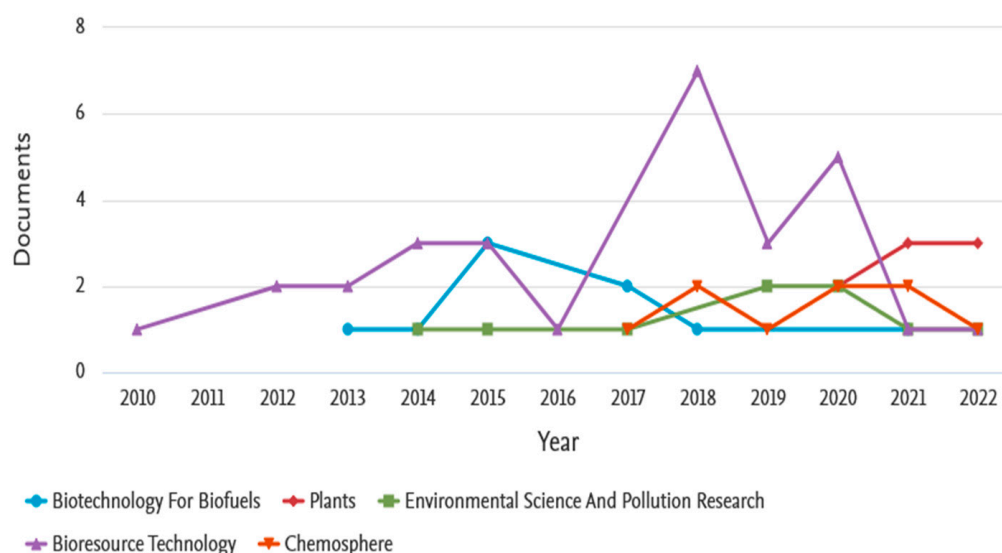


Figure 4. The most productive journals within the analyzed time frame (2022 is not a full year).

The most productive was Zhao H, who published 33 papers; the second most productive was Fang Y, with 28 papers. Appenroth K. J. was third with 20 published articles (Figure 5).

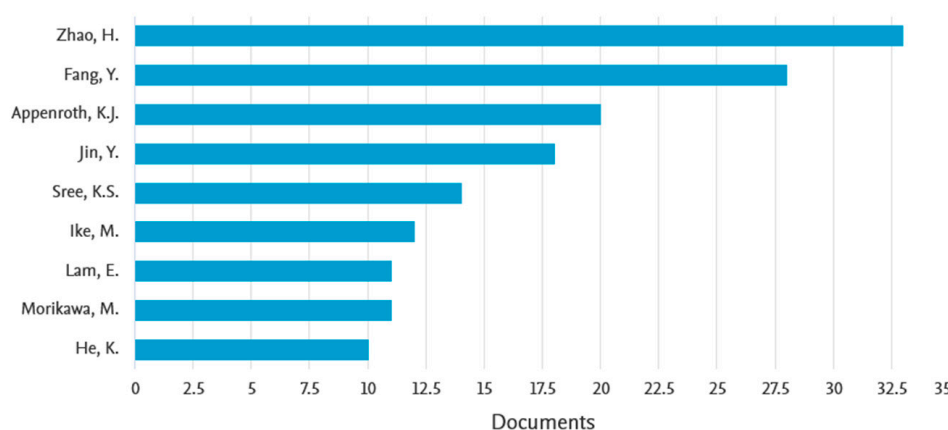


Figure 5. The most productive authors within the studied research domain.

The most active among affiliated universities is the *Chinese Academy of Sciences*, with more than 60 published articles; the second and third are also Chinese Universities (*University of Chinese Academy of Sciences* and *Chengdu Institute of Biology Chinese Academy of Sciences*) (more than 30 articles per organization) (Figure 6).

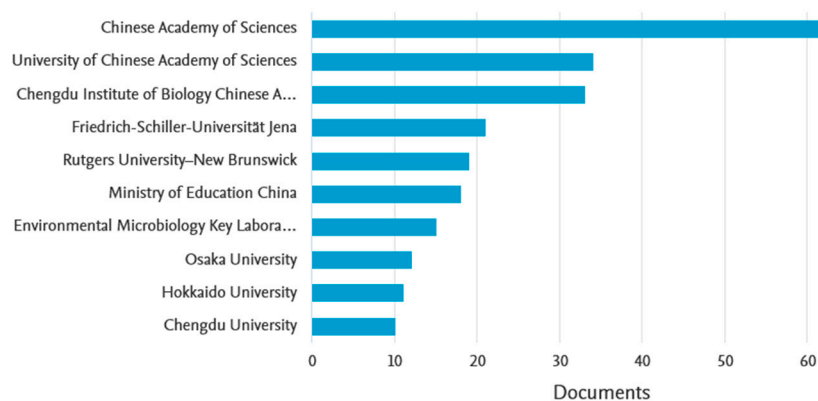


Figure 6. The most productive institutes/universities within the studied research domain.

In total, the authors affiliated with China published 132 articles; those affiliated with the USA were in the second position (69); and those affiliated with India were in the third position (42) (Figure 7).

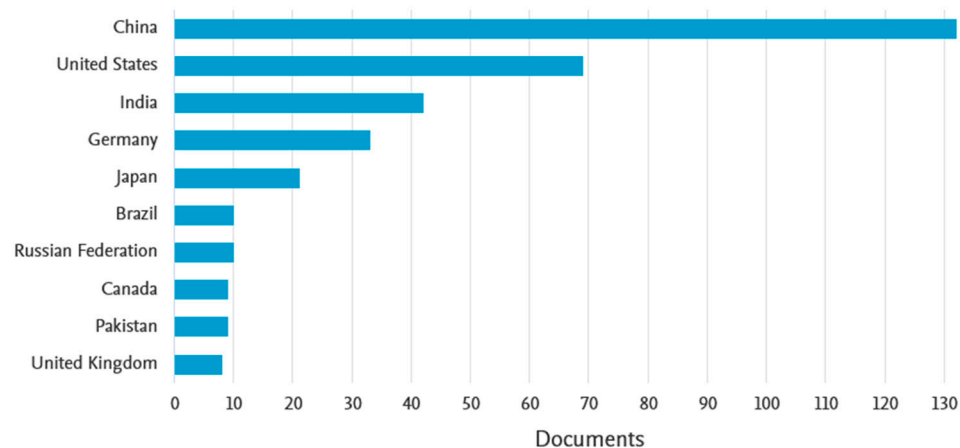


Figure 7. Distribution by region/country among published articles.

3.2. Scientometric Analysis

A scientometric analysis was performed to analyze the authors' impact. Out of a total of 482 authors having published documents in the field under study, 23 authors had published at least five documents, which were cited at least five times (Table 2).

Table 2. Documents with high impacts based on normalized citations.

No.	Author	Documents	Citations	Total Link Strength	Normalized Citations	Average Year of Publication	Average Citations	Average Normalized Citations
1	Appenroth K.-J.	6	119	159	5.56	2017.33	18.83	0.93
2	Brennan R.A.	5	67	12	4.96	2020	13.4	0.99
3	Duan P.	5	189	45	6.29	2016.4	37.8	1.26
4	Fang Y.	18	458	513	13.64	2016.94	25.44	0.76
5	He K.	7	195	224	5.63	2016.57	27.86	0.8
6	Huang M.	10	254	259	7.77	2016.2	25.4	0.78
7	Jin Y.	9	285	288	7.3	2015.67	21.67	0.81

Table 2. Cont.

No.	Author	Documents	Citations	Total Link Strength	Normalized Citations	Average Year of Publication	Average Citations	Average Normalized Citations
8	Lam E.	5	86	47	4.01	2019	17.2	0.8
9	Li Q.	6	151	160	4.35	2017.5	25.17	0.73
10	Liu Y.	9	229	232	7.99	2017.71	25.44	0.89
11	Sree K.S.	7	130	159	6.86	2017.71	18.57	0.98
12	Sun J.	5	153	159	4.79	2016	30.6	0.96
13	Tao X.	6	242	241	6.08	2014.83	40.33	1.01
14	Waldron K.W.	5	136	32	3.89	2015.4	27.2	0.78
15	Wang F.	5	256	46	7.86	2015.4	51.2	1.57
16	Wang W.	6	160	71	5.85	2015.33	26.67	0.97
17	Wang X.	5	56	120	2.41	2018.4	11.2	0.48
18	Xiao Y.	5	206	215	4.49	2013.8	41.2	0.9
19	Xu Y.	8	265	141	7.52	2016.25	33.12	0.94
20	Zhang Y.	5	93	101	5.06	2019.2	18.6	1.01
21	Zhao H.	22	593	575	16.85	2016.41	26.95	0.77
22	Zhao X.	7	160	93	5.17	2016.71	22.86	0.74
23	Zhao Y.	7	263	262	6.13	2014.57	37.57	0.88

The average publication years of the published papers seem quite old (Table 2). This might represent diminishing research; however, they are shown in relation to the citations. It also might have been caused by changing trends during the last years and a greater focus on physiology or genetics than practical applications. There is still a place for improvement of the plants related to applications such as biofuel production. Another reason is that researchers might be in the process of scaling up/or have already completed implementation research (industrial scale), and the results may not be available before they are patented.

The visualization of the authors' co-citations is presented in Figure 8. There are two clusters, which means there are two sets of closely related nodes that the VOSviewer software assigns to a network. Authors in one cluster are closely related and visualized on the map (Figure 8). The node size indicates the number of citations obtained by an author. The larger the node, the more citations the author has received.

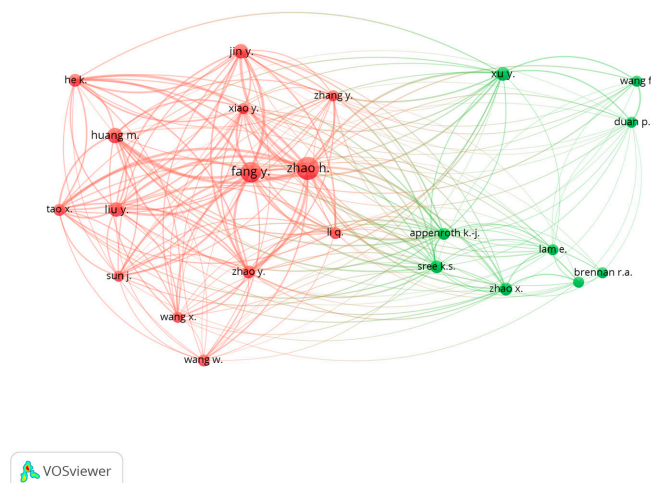


Figure 8. Co-citations of the authors.

A scientometric analysis was also performed to analyze the published documents' impact (Table 3). Out of 141 documents published in the research area under study, 87 were cited at least 10 times, and 20 were cited at least 46 times. Table 3 lists highly cited and the most influential articles based on the normalized number of citations.

Table 3. Documents with high impacts based on normalized citations.

No.	Document	Ref.	Citations	Links	Normalized Citations
1	Xiong J.-Q. (2018)	[53]	245	0	7.21
2	Cui W. (2015)	[19]	125	20	3.22
3	Muradov N. (2010)	[54]	103	11	1
4	Duan P. (2013)	[55]	83	7	1.44
5	Wu K. (2014)	[56]	80	1	2
6	Khoo H.H. (2013)	[57]	79	2	1.37
7	Muradov N. (2014)	[58]	74	9	1.85
8	Yin Y. (2015)	[59]	73	18	1.88
9	Chouhan A.P.S. (2013)	[60]	71	0	1.23
10	Tao X. (2013)	[61]	70	19	1.22
11	Kaur M. (2018)	[62]	68	11	2
12	Xiao Y. (2013)	[63]	67	24	1.16
13	Campanella A. (2012)	[64]	64	7	1.39
14	Van Hoeck A. (2015)	[65]	59	5	1.52
15	Xu J. (2012)	[1]	58	12	1.26
16	Yan W.-H. (2016)	[66]	57	5	2.55
17	Toyama T. (2018)	[67]	54	4	1.59
18	Liu Y. (2015)	[68]	53	14	1.37
19	Gaur R.Z. (2017)	[69]	49	6	2.22
20	Zhao x. (2014)	[70]	46	9	0

The top three most cited documents were Xiong J.-Q. (2018), Cui W. (2015), and Muradov N. (2010) published in *Trends in Biotechnology*, *Plant Biology*, and *Bioresource Technology*, respectively. The high number of citations might be related to the fact that each article is of the review type, not the research type. Another reason might be that the sources in which they were published are interdisciplinary. However, some of the most cited documents (Xiong J.-Q. (2018) and Chouhan A.P.S. (2013)) are not related to others (0 links). Among 87 documents, 82 are in the network and constitute 9 clusters (Figure 9).

Out of a total of 78 sources, 11 sources published a minimum of 3 documents that were cited a minimum of 3 times. Table 4 lists the most popular and highly cited sources. The top two are *Bioresource Technology* and *Biotechnology for Biofuels*. The next most popular and highly cited sources are *Biomass and Bioenergy* and *Industrial Crops and Products*. Those sources with six published papers each are in the third position. However, the number of total citations placed *Plant Biology* with 154 citations in the third place among highly cited sources.

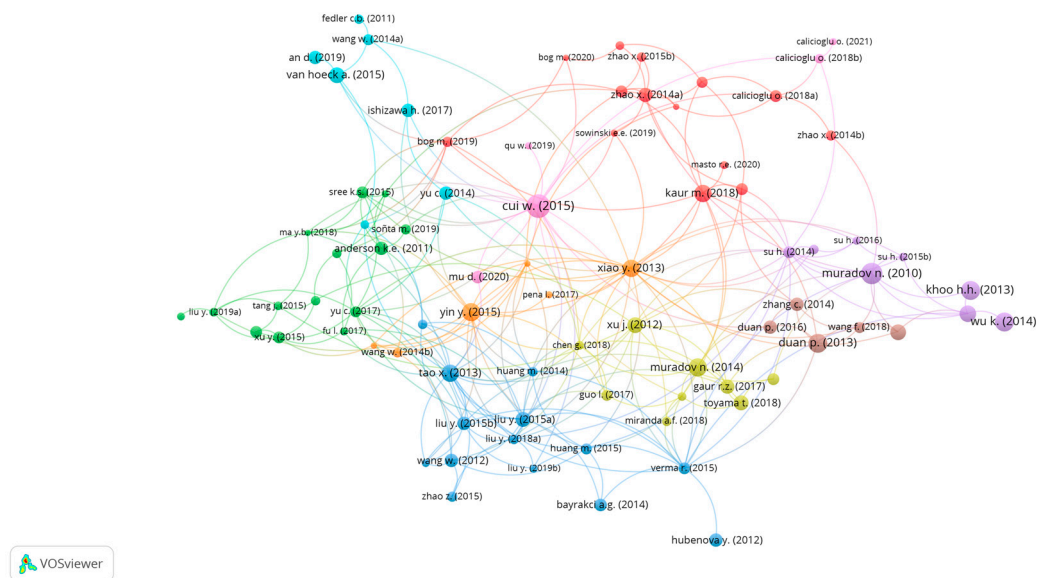


Figure 9. Co-citations of the documents.

Table 4. Sources with high impacts based on normalized citations.

No.	Source	Documents	Citations	Total link Strength	Average Citation	Average Year of Publication	Normalized Citation	Average Normalized Citations
1	Bioresource Technology	14	764	51	54.57	2015.71	21.77	1.55
2	Biotechnology for Biofuels	9	394	44	43.78	2016.11	11.8	1.31
3	Biomass And Bioenergy	6	143	18	23.83	2017.17	4.81	0.8
4	Industrial Crops and Products	6	132	35	22	2017.33	3.38	0.56
5	Science of the Total Environment	5	46	15	9.2	2020	4.47	0.89
6	Bioenergy Research	4	40	23	10	2018.75	2.2	0.55
7	Environmental Science and Pollution Research	4	47	12	11.75	2017.5	1.72	1.72
8	Plants	4	18	13	4.5	2020.75	2.25	0.56
9	Plant Biology	3	154	24	51.33	2016	4.01	1.34
10	Planta	3	59	7	16.67	2016	1.64	0.55
11	Scientific Reports	3	45	10	15	2017	1.79	0.6

In Figure 10, two clusters are presented based on co-citation visualization of the sources where analyzed documents were published in the studied research field.

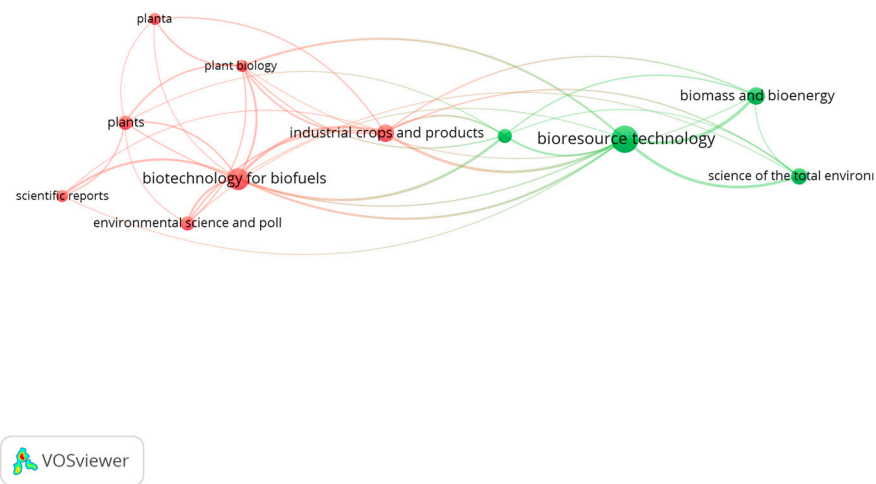


Figure 10. Co-citation map of the sources.

With regard to analyzing organizations (337 in total), eight were in the network and had published a minimum of three documents cited at least three times. The *Chinese Academy of Sciences (Beijing, China)* was the most productive. This organization was also the most cited in the analyzed sample. However, the average year of the published documents affiliated with this organization is 2017.55, which means that currently, researchers from this university might not be highly active in the study field (Table 5.)

Table 5. Organizations with high impacts based on normalized citations.

No.	Organization	Documents	Citations	Total Link Strength	Normalized Citations	Average Year of Publication	Average Citations	Average Normalized Citations
1	University of Chinese Academy of Sciences, Beijing, China	11	270	31	9.62	2017.55	24.55	0.87
2	Indian Oil Corporation Limited, R&D Centre, Faridabad, Haryana India	3	113	3	5.16	2018.67	37.67	1.72
3	Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu, China	6	140	21	4.72	2017	23.33	0.79
4	Key Laboratory of Environmental and Applied Microbiology, Chengdu Institute Of Biology, Chinese Academy of Sciences, Chengdu, China	4	67	16	2.84	2018	16.75	0.71
5	Institute of Food Research, Norwich Research Park, Colney, Norwich, United Kingdom	3	80	6	2.04	2014.67	26.67	0.68
6	School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, United Kingdom	3	80	6	2.04	2014.67	26.67	0.68
7	Chongqing Key Laboratory of Environmental Materials & Remediation Technologies, Chongqing University of Arts and Sciences, Chongqing, China	3	37	11	1.91	2019.33	12.33	0.64
8	School of Water Resources and Environment, China University of Geosciences, Beijing, China	3	27	4	0.85	2017	9.0	0.28

A visualization of the most influential organizations is presented in Figure 11.

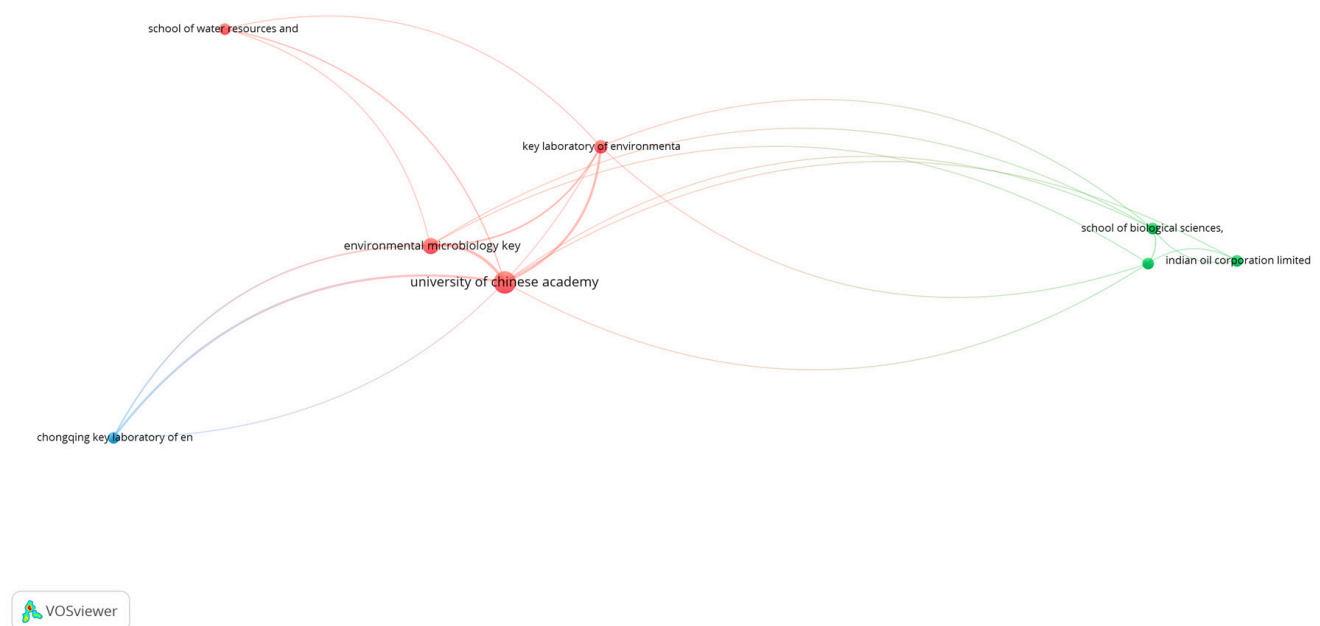


Figure 11. Co-citations of the organizations.

Out of thirty countries/regions involved in the research area under study, assuming that at least three documents were published, and each document was cited at least three times, nine meet the threshold (Table 6). China was the most productive and cited 63 and 1572, respectively, which means that China is the most influential country in this field of study. However, the average year of publication is quite old (2014). The newest documents published (average year 2020.14) are affiliated with Brazil.

Table 6. Countries with high impacts based on normalized citations.

Country	Documents	Citations	Total Link Strength	Normalized Citations	Average Year of Publication	Average Citations	Average Normalized Citations
China	63	1572	334	56.75	2014	24.95	0.9
United States	30	822	218	28.18	2016.67	27.4	0.94
India	23	557	190	28.9	2018.04	24.22	1.26
Germany	12	212	138	10.86	2018.58	17.67	0.91
United Kingdom	7	153	40	5.04	2016.57	21.86	0.72
Australia	4	118	29	4.82	2017.75	29.5	1.2
Japan	4	102	22	6.13	2019.5	25.5	1.53
Brazil	7	38	53	3.53	2020.14	5.43	0.5
Poland	3	27	6	1.79	2017	9	0.6

The visualization of influencing countries is presented in Figure 12.

The full counting method in VOSviewer was used to create the network and visualization map of the most popular keywords. The minimum occurrence in each term in the analyzed documents was five. The terms with the same meaning, for example, "bio-energy" and "bioenergy", as well as words in singular or plural ("biofuel" and "biofuels") were unified and replaced. Moreover, words without a connection, such as "article" and "priority journal", were removed from the analyzed database. Out of a total of 1795 keywords, 114 terms were in relation to one another. As a result, visualization of the terms' occurrence with six clusters was obtained (Figure 13).

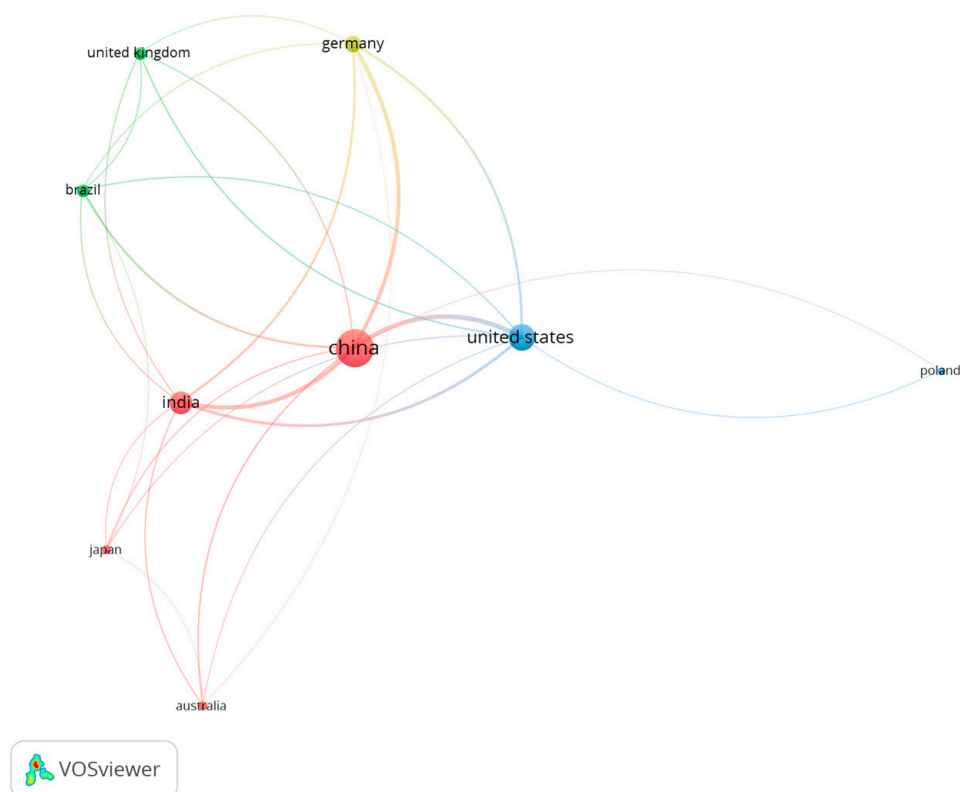


Figure 12. Co-citations of the regions/countries.

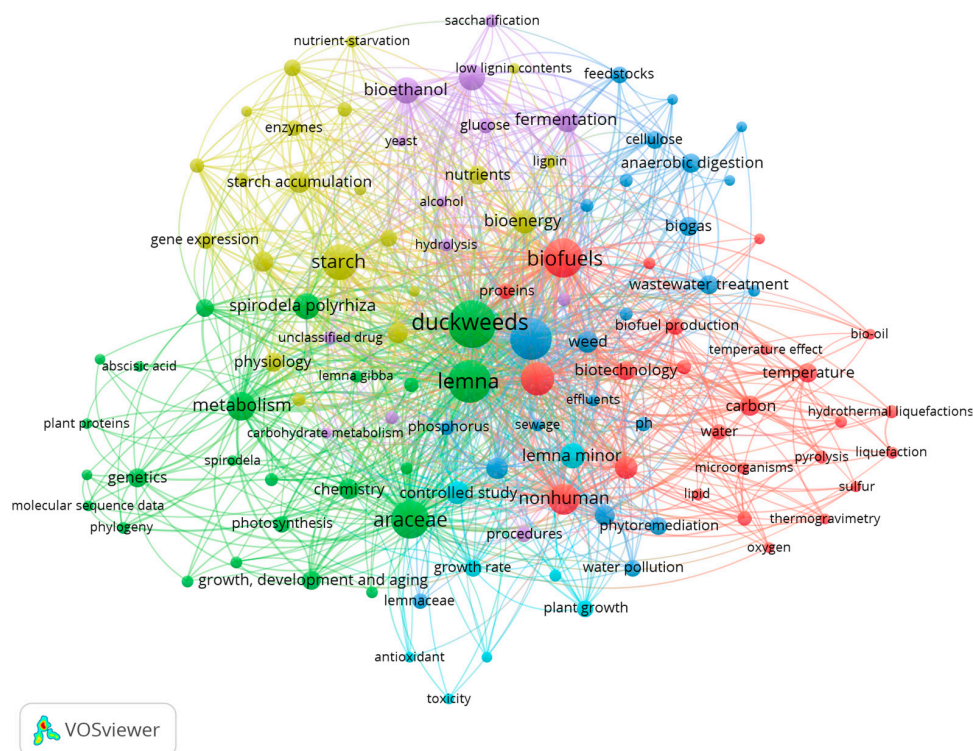


Figure 13. Co-occurrence of author's keywords-network.

The first cluster (red) refers to hydrothermal liquefaction, pyrolysis, and bio-oil production. The second one (green) reflects the researchers' focus on growth development

using genetics, and the third one (blue) refers to anaerobic digestion and biogas, wastewater treatment, bioremediation, and phytoremediation. The fourth cluster (yellow) shows an interest of scientists in examining duckweed as an energy crop cultivation in the context of starch accumulation and production. The fifth cluster (purple) reflects studies on ethanol, fermentation, enzyme activity, saccharification, hydrolysis, and carbohydrate metabolism. The last cluster (turquoise) focuses on controlled studies on the toxicity of pollutants and their effect on plant growth.

Figure 14 presents a network map with the trend topics according to the keywords used in analyzed documents. The most recent keywords used in publications are indicated in yellow, and the oldest are indicated in purple. More recently published studies focused on wastewater treatment, anaerobic digestion, biogas production, feedstock, and phytoremediation. The appearance frequency of keywords is represented by circle size, and their correlation is represented by the distance between two circles.

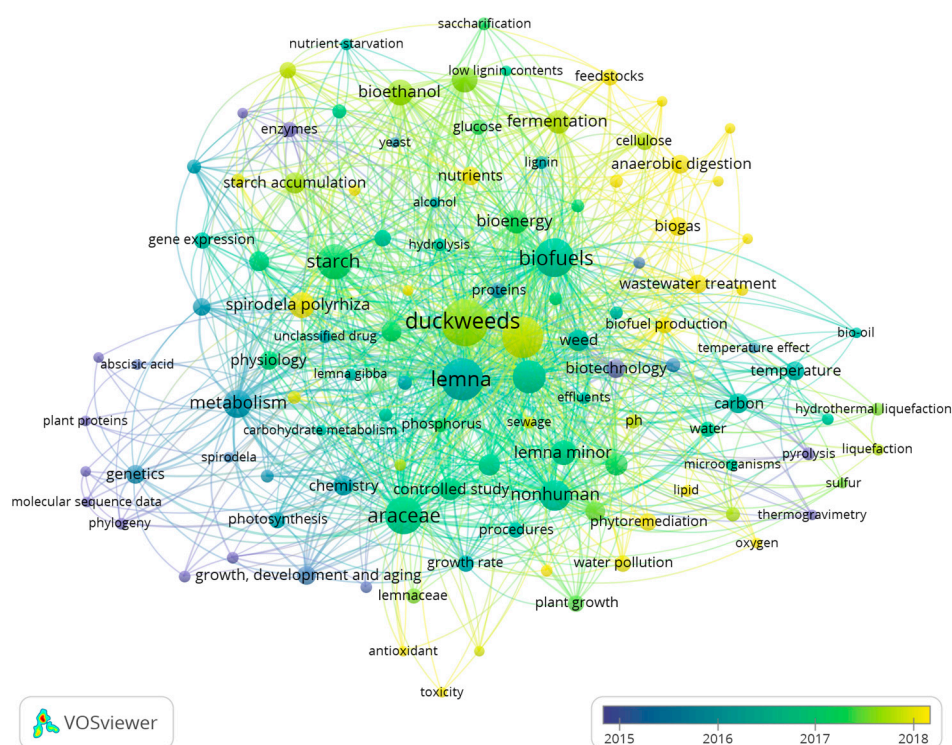


Figure 14. Co-occurrence of author's keywords—overlay visualization.

3.3. Content Analysis

In-depth content analysis defined five research areas related to biofuels and duckweed:

- Starch accumulation and duckweed growth development.
- Development of the pretreatment techniques (e.g., enzymatic and acid hydrolysis).
- Ethanol fermentation, enzyme activity, saccharification, and carbohydrate metabolism.
- Hydrothermal liquefaction of duckweed biomass and bio-oil production.
- Anaerobic digestion and biogas production of biomass used for wastewater treatment, bioremediation, and phytoremediation.

Duckweed is the smallest and fastest-growing aquatic plant on earth, which can double its biomass in 16 h to 2 days and accumulate starch. Under optimal growth conditions, the dry weight of starch content can reach 75% [68]. However, increased starch production in plants can be caused by stress related to nutrient deficiency; for example, the *Spirodela* plants under nutrient stress enhanced their starch content from 21 to 80% [71]. Tao et al. [61], after a comprehensive gene expression profiling of *Landoltia punctata*, exposed a bioengineered strain to nutrient deficiency. They suggested that the final starch accumulation was caused

by the continuous intake of carbon, hydrogen, oxygen, and light combined with the suppression of many metabolic pathways and redirected metabolic flux. They revealed a decrease in the expression of key lignin biosynthesizing enzymes and an increase in the expression of transcripts involved with the synthesis of flavonoids [61]. The light effect on *L. aequinoctialis* 6000 biomass production was studied by Yin et al. [59]. High light induction was responsible for starch accumulation in duckweed. They suggested that from an economical perspective, 110 $\mu\text{mol}/\text{m}^2/\text{s}$ is the best light condition to obtain a high content of starch.

Starch accumulation by *Landoltia punctata* might also be caused by heavy metals such as Co^{2+} and Ni^{2+} . With the increase in the concentration of heavy metals, the activity of starch biosynthesis enzymes rises, and its accumulation grows in a very short time [72]. The ability of duckweed to produce high biomass with a high starch content and a low lignin content renders it a raw material with high energy potential [61].

Another factor that caused the rapid accumulation of high levels of starch in *Landoltia punctata* is the application of plant growth retardant (uniconazole) [73]. Starch accumulation resulted from the regulated expression of enzymes in the relevant pathways [74].

Xu et al. [75] studied the potential of different ecotypes in the turion of *S. polyrrhiza* strains and starch production. They obtained the highest starch productivity of 2.90 $\text{g}/\text{m}^2/\text{d}$. The authors demonstrated that after the two-step enzymatic hydrolysis, turion could be efficiently converted into ethanol (50.5 g/L) with a theoretical conversion rate of 91.67%. Moreover, they obtained an ethanol yield based on the initial turion biomass and starch at 0.34 g/g and 0.468 g/g , respectively. It is worth noting that a turion cultivation biomass yield of 13.8 t/hectare can be achieved. This yield can give an annual ethanol yield of 4.69 t/hectare, which is higher than the potential bioethanol yield of wheat and corn.

Some duckweed starch consists of 35.7% amylose and 64.3% amylopectin [76], as well as the cell wall of cellulose (43.7%), pectin (20%), hemicellulose (3.5%), and lignin (about 3% for *Lemna minor*) [70]. This means biomass must be pretreated into simple sugars to be used in fermentation processes. For example, Su et al. [77] tested five different methods of the enzymatic hydrolysis of *Landoltia punctata* biomass for biobutanol production via fermentation. They tested different enzyme amounts and mixes, additives, temperatures and lengths of hydrolysis, and process pH values.

Duckweed biomass might be hydrolyzed using acidic treatment [31,78]. For example, according to Rana et al. [31] very efficient hydrolysis of *Spirodela polyrrhiza* biomass by 0.1% sulfuric acid showed a conversion yield of starch to glucose of 99.4%. Hydrolyzed glucose was fermented with *Saccharomyces cerevisiae* QG1 MK788210, and after 72 h fermentation under optimized conditions, authors obtained the highest amount of ethanol (1.21 g/L) from 2.2 g/L of glucose with 100% theoretical ethanol yield [31]. Acid hydrolysis (1% H_2SO_4) was used to pretreat duckweed biomass for biohydrogen production through dark fermentation and simultaneously using the fermentative waste to produce microalgal lipids [78].

Moreover, in the study performed by Rana et al. [71], plant biomass of *Landoltia punctata* with a high starch content was pretreated with diluted acid for conversion of starch to glucose and was next processed via fermentation with an indigenously isolated and optimized yeast strain. The process resulted in 99.8% of the theoretical ethanol yield. Interestingly, the authors proposed a closed loop and digested fermentation vinasse with a yield of 0.88 NL/g VS of biogas in anaerobic conditions. Analyses carried out on *Spirodela* showed that the most energy is generated in producing biomethane and bioethanol, and in terms of energy efficiency, duckweed has a greater potential for biogas production. Pretreatment methods in the form of lyophilization and steam explosion were tested [71].

Various methods for biomass pretreatment, such as the freeze-drying method and steam explosion, were also tested. Obtained hydrolysates were used to study the effect of pretreatment on the process of simultaneous saccharification using two commercial enzyme mixtures (Celluclast and CTec2) supplemented with additional β -glucosidase and fermentation carried out using the *Saccharomyces cerevisiae* NCYC 2826 [79]. Similar

results of approximately 78% were obtained after 24 h incubation. However, doubling the concentration of enzymes did not provide any improvement to the yield. No β -glucosidase supplementation resulted in a decrease in yields of approximately 60%.

Hydrolysate from plants belonging to the *Lemnaceae* family was the subject of the study to serve as a medium not only for ethanol but also for higher alcohol fermentation, for example, *Landoltia punctata* hydrolysate. However, Su et al. [80] used a bioengineered strain of *Corynebacterium crenatum* with several inserted *Saccharomyces cerevisiae* genes and a gene originating from *Lactococcus lactis cremoris* via electroporation. Various modified bioengineered strains were able to produce higher alcohols. The yield of higher alcohols from the duckweed is extremely low (especially C5 alcohols such as 2-methyl-1-butanol and 3-methyl-1-butanol). According to the authors, further research will address the development of an optimal host for cell proliferation to best match the fermentation substrate of the duckweed and obtain the highest possible amounts of alcohols [80].

Chen et al. [81] indicated that the duckweed biomass (*Spirodela polyrrhiza*, *Lemna minor*, *Landoltia punctata*) from the sewage treatment plant could not only effectively remove nitrogen and phosphorus from water but also effectively produce starch, which renders it as suitable feedstock for the production of biofuel [81].

Secondary effluents of municipal and swine wastewater, as well as effluent from anaerobic digestion diluted with tap water, were used by Toyama et al. [67] for the grown biomass of *Spirodela polyrrhiza*, *Lemna minor*, *Lemna gibba*, and *Landoltia punctata* to determine their starch production capabilities, caloric values for bioethanol, and biogas production. From one gram of dry biomass of *S. polyrrhiza* and *L. punctata* cited, a range from 0.165 to 0.191 g of ethanol was achieved, and these results were higher than for *L. minor* and *L. gibba*. In the case of biogas production, potential varies between the biomasses of different species of *Lemnaceae*.

To improve bioethanol yield, Ma et al. [35] suggested that the selection of duckweed strains with high starch-producing ability is required. They tested 20 duckweed geographically isolated strains for biomass production, starch content, and starch production, and as a result, it was found that the best strain was *Lemna aequinoctialis* 6000, with a biomass production of 15.38 ± 1.47 g/m², a starch content of $28.68 \pm 1.10\%$, and starch production of 4.39 ± 0.25 g/m² [35]. Biomass (containing 34% of starch) from the aforementioned strain grown on sewage was used by Yu et al. [82] in a one-step enzymatic saccharification process with an over 94% recovery yield. They obtained 0.44 g ethanol per g of glucose with a common yeast strain (Angel yeast).

Gusain and Suthar [83] grew locally found strains of *Lemna gibba*, *Lemna minor*, *Pistia stratiotes*, and *Eichhornia* sp. in 500 L containers in tap water with wastewater and a piece of cow manure as a nutrient source. The obtained biomass was tested for enzymatic saccharification of powdered dry biomass for bioethanol fermentation efficiency. All tested duckweed species have a similar yield of ethanol per unit of biomass from 0.189 g/g (*Eichhornia* sp.) to 0.218 g/g for *L. minor* [83].

When four species of duckweeds (*Landoltia punctata*, *Lemna aequinoctialis*, *Spirodela polyrrhiza*, and *Wolffia arrhiza*) after enzymatic pretreatment (α -amylase and amyloglucosidase (NovozymesTM)) were used in the test of the fermentation process with yeast *S. cerevisiae*, the final ethanol concentration was from 0.17 to 0.19 g ethanol/g dry biomass [76].

The ethanol yields for untreated, freeze-dried, and steam-exploded were 31.4%, 61.3%, and 78.5%, respectively, of the calculated theoretical maximum. Although the ethanol yield from the exploded biomass was high, the final concentration in the medium was 0.25% (v/v), whereas concentrations generally considered viable for distillation must exceed 4%. Moreover, increased stirring and addition of different amounts of yeast that was preconditioned for natural inhibitors resulted in an increase in yields of up to nearly 70% of the theoretical maximum (13.5% g/g dry matter) [79].

Pretreated *Lemnaceae* was fermented to butanol and isopentanol by *Saccharomyces cerevisiae* strains, *Clostridium acetobutylicum*, and bioengineered *Escherichia coli* [18]. The yields obtained for ethanol and isopentanol from acid hydrolysate were 15 times higher

than what could be obtained through the fermentation of the yeast mutant. The authors confirmed that it is possible to obtain butanol, isopentanol, and pentanol from the acid-hydrolyzed biomass via fermentation by the bioengineered strains of *E. coli*. [18].

Hydrothermal pretreatment was the subject of the study by [69–72]. Kaur et al. [84] combined hydrothermal pretreatment and anaerobic digestion to improve the enzymatic digestibility of biomass by facilitating the maximal removal of hemicellulose (68.5–73.5%). As a result, glucose production was 36.5–44.2 g/biomass, ethanol yield 0.167–0.231 g/g biomass, and methane yield 32.9–52.5 m³/ton. The authors showed that the integration of both processes, i.e., anaerobic fermentation and ethanol fermentation in a biorefinery, will allow for the achievement of higher energy efficiency [84].

A combination of freeze milling and microwave hydrothermal pretreatment (130 °C to 210 °C, 10–40 min) was applied for the preprocessing of *Landoltia punctata* biomass, and its effects on the process of bioethanol production were studied by Souto et al. [85]. As a result, insoluble materials in the biomass were significantly reduced. After the higher severity treatment (210 °C for 40 min), biomass contained 48.8% of insoluble material, whereas biomass pretreated in lower temperatures (130 °C for 10 min) contained about 69.2%. A decrease in starch and an increase in monosaccharide concentration for the treated biomass were also observed. It is also reported that approximately 67% of hemicellulose was solubilized at the highest severity. Pretreated biomass was used in a simultaneous saccharification and fermentation process with CellicTM CTec2 cellulase and the *Saccharomyces cerevisiae* NCYC 2826 strain, and the maximal yields of ethanol were achieved at 88.81 wt.% for biomass pretreated at 200 °C for 10 min [85].

Gaur et al. [69] pointed out that the thermal pretreatment caused a positive effect on chemical dynamics and CH₄ production in a digester. They demonstrated that *Lemna minor* is a promising feedstock for biomethanation if mixed in the appropriate proportion (50–60%) in sludge. The maximum CH₄ yield was 468 mL CH₄/g VS in DW. Thermally treated setups showed higher CH₄ than non-treated setups.

Researchers also studied the hydrothermal liquefaction of duckweed [86,87]. For example, Chen et al. [86] explored potential catalysts for upgrading duckweed (*Lemna minor*) biocrude in subcritical water. The most active catalyst was Ru/C, which obtained liquid fuel with properties similar to those of hydrocarbon fuels derived from fossil fuel resources. The upgraded oil was characterized by low viscosity and high energy density, making it suitable for the co-feedstock in a conventional refinery to produce transportation fuels.

Hydrothermal liquefaction at temperatures of (250 °C–370 °C) and times of (15–60 min) was tested by Chen et al. [87] for bio-oil production from duckweed biomass after phytoremediation. They obtained the highest bio-oil yield of 35.6 wt.% at 370 °C after 45 min pretreatment. Moreover, the higher heating value of bio-oil was 40.85 MJ/kg, and the H/C ratio (1.72–1.98) was similar to that of petroleum (1.84).

Lemna minor was assessed for its potential as a feedstock for gaseous fuel production (bio-hythane-hydrogen and methane) in an integrated strategy. Kaur, Srikanth, et al. [88] applied three approaches: acidogenic fermentation, electrohydrogenesis, and methanogenesis, which were evaluated in a single stage and in different combinations of two and three stages to tap the maximum feasible energy. The single-stage processes were insufficient in substrate degradation and its energy conversion. The authors decided that to increase energy conversion efficiency, bioprocesses should be integrated with acidogenic fermentation as an initial stage. The acidogenesis causes a significantly increased content of VFA and H₂, which can be an additional source of biofuels [88].

When *L. minor* was used in the feed mixture, there was a clear improvement in the gas production rate (40%) and methane-specific production (41%) compared to mono-substrate digestion [42]. The proposed co-digestion is in line with the most recent trends regarding resources and waste valorization, which aim to promote a circular economy, recovering energy, water, and nutrients from swine wastewater [42].

Anaerobic co-digestion processes using *Lemna minor* biomass with manure and food waste were also studied by Chusov et al. [89] to determine biogas potential (biogas volume,

methane content). The obtained results confirm the possibility of using this type of waste for biogas/biomethane production.

Aquatic plants, including *Azolla filiculoides* and *Landoltia punctata*, are high in carbohydrates and can also be used for the phytoremediation of industrial wastewater. Their biomass has been used as an alternative carbon source for biodiesel production. The aforementioned biomass might also serve as a raw material for bio-hydrogen fermentation using *Enterobacter cloacae* [90].

The dried biomass of *Lemna gibba*, which was cultivated on urban wastewater, showed a high content of total sugar (38.0%), starch (24.5%), and lipid (9.3%). The extracted lipid showed high contents of C16:0-palmitic acid (37.68%), C18:2-linoleic acid (18.11%), and C18:3-linolenic acid (33.76%). The heating value ranged between 15.07 and 18.58 MJ/Kg, and it was in higher ranges as per standards [91].

Duckweed biomass has also been considered feedstock for producing advanced biofuel precursors. Calicioglu et al. [92] studied the effect of operating conditions (i.e., mesophilic (35 °C) or thermophilic (55 °C) conditions; an acidic (5.3) or basic (9.2) pH) on the yield and composition of the end products from wastewater-derived duckweed during acidogenic digestion. Operating conditions significantly affect the end product resulting from the acidogenic digestion of duckweed.

There have also been studies on dark fermentation for biohydrogen production using an acid hydrolysate of duckweed biomass. Mu et al. [78] achieved a maximum hydrogen production of 169.30 mL/g DW at 35 °C and an initial pH of 7.0.

The production of biofuels highly depends on costs. As Yu et al. [82] pointed out, high medium costs and ingredients might render cultivation not economical. Furthermore, the cost of the biorefining process might increase due to the high moisture content of fresh duckweed biomass [70], which results in additional energy consumption for drying [93]. During biorefinery operations, it is important to maintain an uninterrupted supply chain. Duckweed is considered an inexpensive, sustainable source of plant biomass for biofuels [18].

Furthermore, duckweed is cheaper than straws of cultivated plants [93]. However, there are additional costs, such as transport costs, the risk of biomass spoilage, and energy for drying. Moreover, the capital and operating costs of a biorefinery depend on the area (rural, urban), the price and availability of land for duckweed cultivation, and differences in the concentrations of inflowing sewage [93]. Another economic barrier hindering the commercialization of lignocellulose-based biorefinery is extensive water consumption during the cultivation of feedstock and biofuel processing [84].

Patel and Bhatt [94] provided a circular economic model for the utilization of duckweed for sustainable feedstock for biofuel and a variety of natural products described, such as pigments, lipids, and nanocatalysts in an integrated manner from fresh biomass *S. polyrrhiza*, with a small amount of generated waste. One ton of *S. polyrrhiza* biomass gave 0.8–1.2 kg of R-phycoerythrin, 0.7–0.9 kg of R-phycocyanin, 2.7–4.3 kg of lipids, 5.3–6.1 kg of zerovalent Iron, 79.7–80.4 kg of starch. The starch was fermented to ethanol with a yield of 38.8–40.8 L. The waste generated in each step produced 2.23 L biogas equivalent and 8.51 GJ energy. The residues were reduced by 79–85% in chemicals and energy usage during starch extraction.

4. Discussion

The bibliometric analysis provided information on the scientific structure of the research field of using duckweed for biofuel production (RQ1).

The first mentions of using duckweed as a feedstock for biofuel production were in documents published in 2008. Since 2018, approx. 50 documents per year have been published. This value proves the steady interest in duckweed and its use in the production of biofuels. Although the most productive journal is *Bioresource Technology*, changing trends have been observed during the last few years, and more documents have been published on physiology or genetics than the practical application of duckweed. The most productive

and cited author was Zhao H. What is more, the analyzed documents were mostly affiliated with the *Chinese Academy of Sciences*, and China was the most productive and cited region, which means that China is the most influential country in this field of study.

Many different networks (related to, e.g., co-citations and cooperation between universities and countries) were revealed in this paper. Based on the results of the network analysis of the keywords, there are six clusters in the analyzed research field. More recently published studies focused on wastewater treatment, anaerobic digestion, biogas production, feedstock, and phytoremediation.

The in-depth content analysis defined five research areas related to biofuels and duckweed (RQ2): (i) development of duckweed growth and starch accumulation; (ii) development of the pretreatment techniques; (iii) development of ethanol fermentation; (iv) hydrothermal liquefaction and bio-oil production; (v) anaerobic digestion and biogas production.

With regard to the answer to RQ3, it is worth emphasizing that the presented in-depth content analyses revealed that most of the studies were still on the laboratory scale, which is in agreement with findings provided by Femeena et al. [95], who studied circular nitrogen bioeconomy using the terms of scale of technologies and stated that the duckweed-based studies in 58% were conducted at a laboratory scale.

Using duckweed as an alternative bioenergy feedstock has great potential. Researchers are still struggling with pretreatment processes before the next step and developing bio-engineering of microorganisms used for fermentation and pretreatment techniques.

It is feasible to use turion from duckweed for the feedstock for bioethanol production as a second-generation biofuel, with an annual yield estimated at 4.69 t/hectare [75]; however, the major economic barriers in the development of biofuels are still high substrate costs and expensive biomass pretreatment [88]. Simultaneous biohydrogen production and waste utilization caused a reduction in the costs of microalgal cultivation and wastewater treatment [78].

Duckweed can also be a good substrate for biomethanization. *L. gibba* may be a suitable substrate for biogas production after thermal pretreatment and optimization of the substrate ratio in a co-fermenter. Using pretreatment will accelerate hydrolysis, thanks to which high biogas production can be obtained [69]. What is interesting is that weed biomass can be used in a sequential process to produce the two biofuel variants using the same batch of feedstock. Regarding energy output, duckweed has more potential for biogas production than does ethanol, although many researchers have focused on the production of ethanol [71]. However, it is necessary to perform a full technical and economic study, including modeling and simulation, in order to precisely determine the overall economics of the proposed process and predict its scale [88].

This study presents future research directions in biofuel production using duckweed as a feedstock (RQ4). Although the molecular mechanism of starch accumulation has not yet been described in detail, it is a very promising direction of research that allows simultaneous bioremediation of heavy metals and obtaining starch-rich biomass [72].

Moreover, as Su et al. [80] pointed out, further research should address the development of an optimal host such as *S. cerevisiae* for cell proliferation to best match the fermentation substrate of the duckweed and obtain the highest possible amounts of alcohols, especially C5 alcohols such as 2-methyl-1-butanol and 3-methyl-1-butanol. A full techno-economic study with detailed modeling and simulation is necessary to accurately determine the overall economics of the proposed process to predict its scale [88]. Researchers should also consider the significant amount of generated waste when duckweed biomass is produced and utilized [89].

As indicated by Calicioglu et al. [93], the highly detrimental impact on land use when duckweed is grown in ponds suggests that vertical farming options should be investigated, especially for urban areas. Life cycle analysis inventory and a higher resolution analysis are needed for a down-scale biorefinery model due to the possibility of the presented model not being sufficient to assess much smaller-scale process impacts.

Further studies of other duckweed species as well could determine the culture conditions leading to the optimization of their biomass growth and, at the same time, the preferred chemical composition of the cell wall. This aspect of the research is very important because the chemical profile of the cell wall structure plays a significant role in the enzymatic saccharification and fermentation of biomass into ethanol [70].

Further research should address the development of an optimal host for cell proliferation to best match the fermentation substrate of the duckweed and obtain the highest possible amounts of alcohols [80].

Policies are needed to support supply chain development, market penetration, and acceptance of these technologies by consumers, who need to see the socio-economic and environmental benefits of treating agricultural wastewater with microalgae and duckweed [95]. A full techno-economic study with detailed modeling and simulation is necessary to accurately determine the overall economics of the proposed process to predict its scale [88]. However, reported tests were carried out mainly on a laboratory scale and should be scaled up with appropriate technical-economic and lifecycle analysis. The energy efficiency and costs of this large-scale process should be assessed [71].

The present study analyzes academic papers to identify a link between duckweed and biofuels, focusing on plants from the genera of the *Lemnaceae* family. It contributes to the field by providing a comprehensive and objective picture (including structure, dynamics, and lines of research) of the analyzed research area and the objectivity results using bibliometric methods. Bibliometric data presented in this study may help researchers choose journals. Moreover, this paper demonstrated many research gaps that academics should fill.

This study has some limitations which should be acknowledged. First, the keywords used in searching references may limit documents not included in this study. The second limitation is that the reviewed databases were limited to Scopus and Web of Science, and this literature review was not performed on comprehensive databases such as Google Scholar. Moreover, in this study, the language of the reviewed documents was limited to English. However, the above-presented limitations may be overcome in future studies to provide a comprehensive picture of the biofuels from plants belonging to the *Lemnaceae* family research field.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/en16042058/s1>, The datasets generated by Scopus and WOS used in bibliometric analysis.

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