

# Bibliometric analysis of global research on white rot fungi biotechnology for environmental application

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## Research Article

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## Abstract

In recent years, white rot fungi (WRF) have received tremendous attention as a biotechnological tool for environmental pollution control. Although there are a large number of research articles and reviews concerning WRF biotechnology in environmental application, to our knowledge no bibliometric research of this topic has been published. This study used bibliometric method to evaluated 3962 related publications collected from Web of Science Core Collection database published between 2003 and 2020. The results indicated that China was the most productive country, while the USA is the most active country in international cooperation. The closest international collaborative networks were evidenced between the USA and China. Most authors tend to cooperate within a small group, which leads to the lack of effective cooperation among different groups. The most prominent journals in this field were "International Biodeterioration & Biodegradation" and "Bioresource Technology", and "Biotechnology Applied Microbiology" was the most popular subject category. The analysis of high-frequency keywords revealed that "laccase", "biodegradation", "decolorization" and "*Phanerochaete chrysosporium*" were the most cited terms among all publications, and that the hottest topics focus on pretreatment of biomass waste by WRF, decolorization of dye wastewater by WRF, and bioremediation of polluted environment by WRF, etc. Finally, the frontier topics and active authors in this research field were identified using burst detection. This bibliometric study promoted the future cooperative research and knowledge exchange by visualizing the recent developments and future trends in WRF biotechnology for environmental applications.

## Introduction

The rapid development of industrial activities has greatly increased the release of toxic and harmful substances into the environment, and the contamination of soil, water, and air by toxic and harmful chemicals has become one of the major environmental problems in the world today. These dangerous compounds have carcinogenic and/or mutagenic effects, persistent in the environment and may have negative effects on human health and ecology (Almetwally et al. 2020). Due to the magnitude of this problem, there is an urgent need for a rapid, cost-effective, ecologically responsible method to remove pollutants. Most research in the field of bioremediation has focused on bacteria, and fungal remediation attracting much attention just in the past three decades (Singh et al. 2015). Mycoremediation is a promising technology using their metabolic potential to remove xenobiotics. White rot fungus (WRF) is an eco-physiological group mainly composed of basidiomycetes and litter-decomposing fungi. It is well known that WRF can degrade lignin in the way that the mycelium of organism penetrates the cell cavity and releases ligninolytic enzymes (LEM) to decompose the substance into white sponge-like mass (Cabana et al. 2007). These extracellular LEM have very low substrate specificity so they are able to degrade a wide range of highly organopollutants that are structurally similar to lignin. Their capacities to remove xenobiotic substances into less or non-toxic substances, make them a useful and powerful tool for bioremediation of polluted environment (Lebkowska and Zaleska-Radziwill 2014; Xiao and Kondo 2019). In fact, WRF are robust, ubiquitous organisms that can survive in the presence of high concentrations of various pollutants, even with a low bioavailability (Xiao and Kondo 2020a). In addition, WRF have an advantage over bacteria in terms of the diversity of compounds that can be oxidize.

In recent years, researchers have done a lot of work on the development of WRF biotechnology, focusing on the intracellular and extracellular enzymes involved in the biodegradation of pollutants. Rodríguez-Rodríguez et al. (2013) described the ability of WRF to biodegrade pesticides and summarized the potential role of WRF play in on-farm biopurification systems. The report by Tortella et al. (2015) summarized the evidence about the potential of WRF to degrade various types of organic pollutants, such as antibiotics, PAHs and dyes, as well as the limitations of WRF bioremediation in contaminated sites. Asif et al. (2017a) reviewed the performance of WRF and its LEM to degrade trace organic pollutants from synthetic and real wastewater. In another review by the same author, the removal mechanism, degradation pathway and the formation of intermediate byproducts in bioconversion of personal care products and pharmaceuticals using whole cell WRF and its LEM were discussed in detail (Asif et al. 2017b). A recent report from Mir-Tutusaus et al. (2018) reviewed the WRF-based technologies for micropollutant removal from wastewater, and proposed a series of solutions to overcome several limitations of this technology. Another review by Huang et al. (2018) introduced the trends in the application of WRF biocatalysis and its related combined technology such as adsorption of nanomaterials, for biotransformation of endocrine-disrupting compounds. He et al. (2017) proposed that in the process of wastewater treatment, nanoparticles as supports or synergist can improve the biodegradation performance and stability of WRF. Furthermore, Voberkova et al. (2018) summarize current progress in the use of immobilized LEM form WRF for treatment of wastewater, decolorization of dyes, or elimination of pharmaceutical active compound.

Although there have been a large number of research and review articles on the application of WRF biotechnology for environmental pollution control, it is difficult for traditional review articles to organize, summarize, and quantify the development of a certain field effectively on a large time scale, and there is still a lack of research on this topic by using bibliometric methods to our knowledge. Bibliometric analysis is a common tool for statistical and quantitative analysis of publications in a certain research field, which can assess countries, institutions, authors, and keywords related to publications (Li et al. 2017). Moreover, bibliometric analysis can reveal the current situation and development trend, helps scholars grasp the developmental characteristics of a certain research field, and better guide their future work (Gao et al. 2019).

Bibliometric methods have been more and more widely applied in the field of environmental pollution control. For example, Singh and Borthakur (2018) conducted a review of the biodegradation and photocatalytic degradation of organic pollutants with bibliometric and comparative analysis. Li et al. (2019) discussed the research hotspots and trends in the field of phytoremediation in heavy metal pollution by using the bibliometric analysis. Based on bibliometric method, Qi et al. (2019) analyzed the research status and development trend of algae-bacteria symbiotic wastewater treatment technology between 1998 and 2017. Usman and Ho (2020) investigated the publications on the application of Fenton oxidation in soil remediation and wastewater treatment using bibliometric methods, and further pointed out the future development trend. In addition, a number of studies have carried

out systematic bibliometric analysis on municipal solid waste (Chen et al. 2015), groundwater remediation (Zhang et al. 2017), and soil remediation (Vanzetto and Thomé 2019). There have been few efforts on systematically review the related researches on WRF biotechnology for environmental application, and none of them has been visualized.

Therefore, the systematic review of global research on the application of WRF biotechnology to environmental pollution control by bibliometric analysis is beneficial to strengthening knowledge accumulation and the capabilities of theoretical innovation. Through these analysis, the core technology and research direction of WRF biotechnology in environmental application can be revealed, thereby promoting the further utilization of WRF biological resources in global research. Considering these reasons, a comprehensive and systematic perspective on the environmental application research of WRF biotechnology was motivated to provide in this study, based on the bibliometric method. The purpose of this study is to: 1) identify the most productive and influential countries, institutions, journals and authors; 2) analyze the cooperation relationships at the level of countries, institutions and authors; 3) understand the knowledge domains and research topics with keyword analysis; and 4) propose research frontiers in the future within this research field.

## Materials And Methods

### Data Sources

Scientific output data was extracted from the Web of Science Core Collection (WoSCC), which is one of the most widely used databases in academic and bibliometric studies and allows the download of full citation records with new records being updated every day (AlRyalat et al. 2019). WoSCC can provide comprehensive information for bibliometric analysis, including the Science Citation Index-Expanded (SCI-E) and the Social Sciences Citation Index (SSCI). This database was used to retrieve the related research of WRF biotechnology in environmental application for the period from 2003 to 2020. To find different variations among the words in the databases, a wildcard (\*) was used. Data retrieval strategy of this study is as follows: TS = ((“white rot fungi” or “white rot fungus” or “white rot fungal” or “white rot basidiomycete”) and (“\*degrad\*” or “treatment” or “remov\*” or “\*remediation” or “\*conversion” or “\*transformation” or “decolorization” or “elimination” or “detoxification”)). The language is limited to English. As a result, a total of 3962 publications that consist of articles, reviews, proceedings papers, early access, meeting abstract and book chapter, were obtained on December 28, 2020 for further analysis. These data, which includes full records, were downloaded and exported in text format for further analysis. All data are exported within the same day to avoid deviations caused by the daily updates of this database.

### Analysis Methods

The publication characteristics (publishing year, counties, authors and subject categories) were analyzed using Microsoft Office Excel 2007. Some conventional methods in bibliometrics, including citation analysis, social network analysis, co-word clustering analysis and burst detection, are employed in this study. The total citations, average citations per article and h-index obtained from Citation Reports of WoSCC were used for citation analysis. The impact factor (IF) of journals was collected from the Journal Citation Report in 2019. VOSviewer software has text mining capabilities to extract important terms from a large number of scientific literatures for construction and visualization of co-occurrence networks (van Eck and Waltman 2010). In this study, VOSviewer 1.6.15 was used to generate social network maps for cooperative relationship analysis and co-occurring keywords analysis in related research fields. In addition, the software is also used to extract the high-frequency keywords published in this field in different countries and time periods. The network maps are composed of nodes and links, where nodes represent elements such as counties, institutions, authors, and keywords. The nodes represent the number or frequency, and the lines between the nodes denote associations. The thicker the line is, the greater the relationship. In the parameter settings of VOSviewer, the counting method adopt full counting, and the maximum number of authors per document is set to 25. Furthermore, Citespace V is a free software package for scientific visualization based on Java, which is utilized to identify research trends and active authors through burst detection (Yang et al. 2018). For instance, in the burst analysis with keywords, the appearance of a keyword burst in a period of time, which means that a related field represented by this burst keyword has attracted extensive attention of researchers, and may become a research hotspot in the future. It should be pointed out that some repetitive and unrelated words are excluded from the results of keyword analysis and burst detection.

## Results And Discussion

### Publication outputs

Figure 1 shows the yearly numbers of related publications from 2003 to 2020. Specifically, the number of publications on WRF biotechnology for environmental application increased significantly from 133 in 2003 to 253 in 2009, and the number of publications has almost doubled, which indicating the growing interest in scientific community to this research field. This is mainly due to the great attention paid by China, India and Spain in this period. However, it is interesting to note that after 2009, the number of publications around the world shows a regular fluctuation. In particular, in 2014, the number of publications dropped to 221 at the bottom, and then increased gradually, reaching the highest of 273 in 2018, and then falling to 230 again in 2019. It can be seen that since 2009, the number of publications has not shown a straight-line growing trend, and has always fluctuated within a certain number range. It is expected that the research will enter a stable period in the future. In 2020, only 224 publications have been published in this field worldwide, which may be related to the impact of COVID-19 on research.

### Countries and Collaborations

In 2003, only 41 countries/regions published articles in this field, while 94 countries/regions carried out related research by 2020, indicating that there have been an increasing number of countries/regions participating in this research each year. Among these countries, 15 countries have published more than 100 articles. As shown in Table S1, China is the country producing maximum research outputs on this period with 613 publications, followed by the USA, India, Japan and Spain, with more than 300 publications. The high output of publications in these countries can be explained by their strong economic strength and the huge investment in research, development, and innovation. It has to be noted that both developed countries (such as USA, Japan, Germany and Italy) and developing countries (such as China, Brazil, India and Turkey) were found to be developing research in environmental pollution control using WRF. However, in terms of the number of publications in this research field, there is a relative heterogeneity among these countries. For example, the number of publications by the first ranked China is more than 200 than that of the second ranked USA, while Brazil and Germany as the 6th and 7th ranked countries have published 175 and 169 articles respectively, accounting for only a third of China's publications on the topic. The USA, Italy and Spain were significantly ahead of other countries in terms of publication output in the early years, but after 2007, China gradually narrowed the gap with leading countries and became the leader (Fig. 1). On the other hand, in terms of total citations, citations per article and h-index, the USA surpassed China and ranked first. Although China has the largest number of publications, the average citation frequency of publications is only 21.23, less than half of that of the USA (44.97), more efforts still should be engaged for improving academic influence.

Academic cooperation between different countries or research institutions plays a guiding role in promoting dissemination of knowledge and academic exchange among scholars (Chen et al. 2020). The academic cooperation relationship among the top 30 countries of publications from 2003 to 2020 was shown in Fig. 2a. The nodes in the figure represent different countries, the lines connecting the nodes indicate international cooperation between countries, and thickness of the line represents the closeness of cooperation. It can be seen that the USA is the most active country in global cooperation on research of WRF biotechnology for environmental application, and is located at the center of the collaborative network. USA has cooperated with 52 countries to produce publications in relevant research, followed by Germany (42), Sweden (41), Spain (40), France (40), British (33), Japan (32) and China (31). In terms of the number of cooperative publications, the USA and China had the closest collaboration, followed by USA-Canada, Brazil-Chile, USA-Spain and USA-Germany. As the country with the largest number of publications, China has not cooperated closely with other countries except the USA, thus should greatly strengthen its academic cooperation with other countries. Additionally, there is still much room for the development in cooperation and exchange in this research field between countries. For example, the USA and China, the two major countries with the largest publications, have never collaborated with Russia and Argentina.

### **Institutions and Collaborations**

More than 2400 institutions have contributed publications on the field of WRF biotechnology for environmental application. Table S2 showed top 10 productive institutions and their relevant indicators. The top 10 institutions completed 813 publications totally, accounting for 20.5% of total publications. The top 10 institutions belong to eight countries, with Spain, China and Japan each having two, and Czech Republic, Finland, Pakistan and the USA each having one. It is worth noting that there are no institutions from India, Brazil or Germany in the top 10 most productive institutions. The Consejo Superior de Investigaciones Científicas (CSIC) from Spain has the most publications with a total of 150 articles, followed by Czech Academy of Sciences (101) and Chinese Academy of Sciences (CAS, 83). The CSIC also has the highest total citation (6388) and h-index (43), followed by Czech Academy of Sciences. A bias could appear here because these research institutions have branches in different cities, and publications classified according to their branches may lead to differences in rankings. In addition, although the USA Department of Agriculture ranked only tenth in terms of the number of publications, their average citation frequency was the first with 74.17, indicating that the high average quality of their publications. Of note, one author can belong to more than one institution and this fact can be a bias risk for institution analysis.

Figure 2b showed the academic collaborations among institutions in the field of WRF biotechnology for environmental application. In terms of the number of cooperation institutions, CSIC cooperates with 112 institutions in this research field, and indicating that it has the closest cooperation relationship with other institutions. The top 10 cooperative organizations with the largest number of publications were CSIC—University of Autonoma de Barcelona, University of Autonoma de Barcelona—Universitat de Girona, Kyushu University—Miyazaki University, University of Helsinki—Utrecht University, Seoul National University—Korea Forest Research Institute, University of Wollongong—RMIT University, Hunan University—Hunan Agricultural University, CSIC—Universitat de Girona, Institut National de la Recherche Agronomique (INRA)—Aix Marseille University, and Charles University—Czech Academy of Sciences. Obviously, cooperation between different institutions often occurs in the same country, such as Spain, Japan, Korea, Australia, China, France and Czech Republic, only the University of Helsinki—Utrecht University comes from different countries among the top 10 cooperative institutions. The results show that the CAS has cooperated with 46 other institutions, but most of them are domestic institutions. In recent years, the publications number from CAS in cooperation with institutions from Italy, UK, USA, Netherlands, Denmark and Canada has continued to increase, indicating that CAS has made efforts to promote international cooperation. Cooperation between institutions from different countries is conducive to the development and dissemination of knowledge, thus international cooperation in this research fields needs to be closer.

### **Authors and collaborations**

In general, more than 10000 authors participated in the researches of WRF biotechnology for environmental application from 2003 to 2020. Figure 3 showed the top 20 productive authors in the number of articles and total citations. Of the top 20 authors, 6 authors were from Spain, 5 from Japan, 4 from China, 2 from Pakistan, and 1 from Malaysia, Czech Republic and Costa Rica, respectively. Surprisingly, no authors from the USA and India, which ranked second or third in the number of articles published, entered the top 20 author list. Zhang Xiaoyu, from Huazhong University of Science and Technology, is the most productive author with 53 articles, followed by Asgher Muhammad from University of Agriculture Faisalabad (48). The number of articles published by the third to eighth authors was between 31 and 39. With regards to total citation of articles, two Spanish authors, Vicent Teresa (1664), and Caminal Gloria (1565) can be regarded as the most concerned scholars for this research. In contrast, the total citations of several scholars

from Japan are relatively low, among the top 20 authors. Another Spanish scholar, Marco-Urrea Ernest, ranked first among the top 20 authors with an average citation of 57.58. By tracking the top authors, it is useful for other scholars to find classic and excellent publications in this research field. These results indicate that the publications of Spanish scholars in this field have the highest academic influence, and their work could have a deep impact on the future research of other scholars.

Figure 4 displayed the collaboration network of 69 most productive authors. This cooperation network includes a large cooperation network and several small cooperation networks which are not connected with each other. The large cooperative network has a cluster of many core authors, such as Caminal Gloria, Vicent Teresa, Martinez Angel T., Marco-Urrea E. from Spain, Rodriguez-Rodriguez Carlos E. from Costa Rica and Cajthaml Tomas from Czech Republic, which can be regarded as the active and representative research team, providing highly personalized scientific reference for other related scholars. However, most authors tend to cooperate within a small group, such as those from Japan, China and Pakistan, which leads to the lack of efficient cooperation among various groups. Co-publishing among authors, especially those from different industries or countries, needs to be valued, which can greatly promote the exchanges and innovation of academic ideas.

#### Journals and subject categories

Journals have a prominent role as the main disseminators of knowledge. A total of 628 journals published relevant researches from 2003 to 2020. Among these journals, 42.7% (268) have published only one publication on WRF research for environmental application. Another 16.88% (106) of the journals have published two publications, 9.1% (57) have published three, 4.3% (27) four, and 27.1% of the journals (170) have published five or more publications. The top 10 journals with the most productive published a total of 1062 articles in the research fields (Table S3). Except for "Chemosphere" and "Journal of Hazardous Materials", the other eight journals are the journals that mainly publish biological research articles. The most productive journal was "International Biodeterioration & Biodegradation" with 195 articles covering 4.92% of the total publications, indicating that the research on the biodegradation ability of WRF and their enzymes has attracted much attention. The followed major journals were "Bioresource Technology" (182, 4.59%), "Applied Microbiology and Biotechnology" (114, 2.88%) and "Chemosphere" (111, 2.80%). The impact factors of these 10 journals ranged from 1.409 to 9.038, and "Journal of Hazardous Materials" had the highest impact factor. "Bioresource Technology" not only received the highest number of total citations (11533) and average citations per article (63.37), but also has the highest h index (55). The number of total citations (5365) and average citations per article (60.28) ranked second was "Enzyme and Microbial Technology".

All publications were divided into 90 Web of Science subject categories, and Fig. 5a shows the top 10 most productive categories. It should be mentioned that the sum of percentages in all categories is greater than 100%, because the same article can be classified into multiple categories in Web of Science. Obviously, the category of "Biotechnology Applied Microbiology" contributed the largest number of 1611 articles (40.7%), followed by "Environmental Sciences" (990, 25.0%). However, there are no more than 500 publications in other categories such as "Microbiology" (428), "Biochemistry Molecular Biology" (353), "Engineering Chemical" (316), etc. Monitoring the temporal evolution of major categories (Fig. 5b), it can be observed that that the number of articles published in the category of "Biotechnology Applied Microbiology" shows a fluctuating trend in 2003–2020, peaked at 127 in 2011, and then decreased gradually. The number of articles published in category of "Environmental Sciences" increased significantly after 2008 and decreased slowly after experiencing two peaks in 2010 (73) and 2017 (80). Interestingly, the number of publications in category of "Environmental Sciences" exceeded the number of publications in category of "Biotechnology Applied Microbiology" in 2020, and the ranking rises to the first place. From 2003 to 2020, there is no significant fluctuation in the number of articles published in other categories.

#### Most cited articles

Citation analysis has been regarded as an important indicator to measure the quality of articles in bibliometric research, and it represents the influence and attention of a research in the academic circles (He et al. 2019). Table 1 lists the 10 most frequently cited articles from 2003–2020 in the research field of WRF biotechnology for environmental application with relevant information including title, authors, journal, the country, total citations, and publication years. Eight research articles and two review articles entered the list of top 10 cited articles. Among the first authors of the top 10 cited articles, three are from the USA, and the other seven are from different countries. Surprisingly, in the top 10 citation list, there are no articles from the top 10 most productive countries except the USA, which shows that there is no close relationship between the highly cited publications and most productive countries.

Table 1  
Top 10 most frequently cited articles from 2003 to 2020

Rank	Title	Paper type	First author	Country	Journal	Total citations	Year
1	Non-conventional low-cost adsorbents for dye removal: A review	Review	Crini G	France	Bioresource Technology	2804	2006
2	Removal of synthetic dyes from wastewaters: a review	Review	Forgace E	Hungary	Environmental International	2023	2004
3	Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production	Review	Kumar P	USA	Industrial & Engineering Chemistry Research	2005	2009
4	Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review	Review	Taherzadeh M J	Sweden	International Journal of Molecular Sciences	1350	2008
5	Fungal laccases - occurrence and properties	Review	Baldrian P	Czech	FEMS Microbiology Reviews	1317	2006
6	The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes	Article	Floudas D	USA	Science	836	2012
7	White-rot fungi and their enzymes for the treatment of industrial dye effluents	Review	Wesenberg D	Belgium	Biotechnology Advances	722	2003
8	Lignocellulosic residues: Biodegradation and bioconversion by fungi	Review	Sanchez C	Mexico	Biotechnology Advances	716	2009
9	Principles of microbial PAH-degradation in soil	Review	Johnsen A R	Switzerland	Environmental Pollution	706	2005
10	Genome sequence of the lignocellulose degrading fungus <i>Phanerochaete chrysosporium</i> strain RP78	Article	Martinez D	USA	Nature Biotechnology	579	2004

The most highly cited article is titled “Non-conventional low-cost adsorbents for dye removal: A review”, authored by Crini (2006), in Bioresource Technology in 2006, with total 2804 times. The author introduces the application and feasibility of various low-cost adsorbents to remove dyes from wastewater, among which biosorption is a promising method to alternative traditional adsorption for dye removal. As a kind of biosorbent, the decolorization and/or biosorption ability of WRF to dye wastewater has become a research topic. In WRF decolorization, living biomass was often used to adsorb and degrade dyes, while dead biomass was applied to adsorb dyes. The article with the second-highest number of citations (2023) is titled “Removal of synthetic dyes from wastewaters: a review”, as authored by Forgace et al. (2004) and published in Environmental International. This article described the various removal methods of synthetic dyes in wastewater by adsorption, oxidation, photodegradation, and microbiological decoloration. Using WRF to degrade synthetic dyes is a convenient, environmentally friendly and attractive method. Some strains, such as *Phanerochaete chrysosporium* (Kiran et al. 2019), *Phlebia brevispora* (Harry-Asobara and Kamei 2019), *Irpef lacteus* (Malachova et al. 2013), *Trametes versicolor* (Legerska et al. 2018), *Pleurotus ostreatus* (Dogan et al. 2018), and *Pleurotus sajor caju* (Yehia and Rodriguez-Couto 2017) have been demonstrated to be able to degrade synthetic dyes due to their high enzyme production. The remaining eight most frequently cited articles primarily focus on pretreatment and bioconversion of lignocellulosic wastes by WRF, treatment of industrial dye effluents by WRF and their enzymes, occurrence and properties of laccases, fungal degradation of PAH in soil, and genome sequence analysis of WRF.

#### High-frequency keywords analysis

Keywords clearly reflect the core content of the article in a concise form, which can be regarded as the soul of the article. By analyzing the high-frequency keywords, the research hotspots and overall development trends in the field are accurately revealed (Qi et al. 2019). Using VOSviewer software to analyze the frequency of the author keywords, and then a total of 6605 keywords were collected, among which 4831 (73.1%) keywords were used only once, 790 keywords (12.0%) were used twice, and 224 keywords (4.6%) appeared more than 10 times. The use of a large number of low-frequently keywords indicated a wide range of research content and different research focuses in the field. The 20 most frequently used keywords during the period of 2003–2020 are shown in Table 2. Similar keywords with different writings, such as “white rot fungi”, “white rot fungus”, “white-rot fungi” and “white-rot fungus”, “decolourisation” and “decolourization”; and, “polycyclic aromatic hydrocarbons”, “PAHs” and “PAH” were merged. The “white rot fungi”, “laccase”, “biodegradation”, “decolorization” and “*Phanerochaete chrysosporium*” are the most frequently used five keywords, which shows that these keywords are favored by scholars all over the world. Except for “white rot fungi”, “biodegradation”, “bioremediation”, “fungi”, and “degradation” (the searching words in the present research), most of these keywords are mainly related to the following topics: WRF strains (*Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus*), degrading enzyme (laccase, manganese peroxidase, ligninolytic enzymes, and lignin peroxidase), treatment of dyes (decolorization and dye decolorization), degradation substrate (PAHs, lignin and lignin peroxidase), and treatment and disposal methods (biosorption and immobilization).

Table 2  
Top 20 author keywords and frequency in different periods

Rank	2003–2020		2003–2008		2009–2014		2015–2020	
	Keywords	Frequency	Keywords	Frequency	Keywords	Frequency	Keywords	Frequency
1	White rot fungi	822	White rot fungi	234	White rot fungi	313	White rot fungi	275
2	Laccase	606	Laccase	181	Laccase	235	Laccase	190
3	Biodegradation	362	Decolorization	132	Biodegradation	158	Biodegradation	116
4	Decolorization	210	Biodegradation	88	Decolorization	108	Lignin	77
5	<i>Phanerochaete chrysosporium</i>	210	Manganese peroxidase	74	<i>Phanerochaete chrysosporium</i>	76	Decolorization	70
6	Bioremediation	200	<i>Phanerochaete chrysosporium</i>	73	Bioremediation	73	Ligninolytic enzymes	70
7	Manganese peroxidase	196	<i>Trametes versicolor</i>	63	Ligninolytic enzymes	68	Fungi	67
8	<i>Trametes versicolor</i>	170	Bioremediation	60	<i>Trametes versicolor</i>	67	Bioremediation	67
9	Lignin	168	Lignin	42	Manganese peroxidase	67	<i>Phanerochaete chrysosporium</i>	61
10	Ligninolytic enzymes	141	PAHs	40	Lignin	49	Manganese peroxidase	55
11	Fungi	141	Basidiomycete	35	Degradation	42	Lignocellulose	41
12	Degradation	112	Fungi	33	Fungi	41	Degradation	40
13	<i>Pleurotus ostreatus</i>	103	Textile dyes	32	Lignin peroxidase	38	<i>Trametes versicolor</i>	40
14	PAHs	101	Ligninolytic enzymes	31	<i>Pleurotus ostreatus</i>	38	<i>Pleurotus ostreatus</i>	39
15	Lignin peroxidase	88	Degradation	30	PAHs	35	Pretreatment	32
16	Lignocellulose	76	Lignin peroxidase	26	Dye decolorization	32	Lignin degradation	31
17	Lignin degradation	72	<i>Pleurotus ostreatus</i>	26	Dyes	30	Delignification	30
18	Dyes decolorization	70	<i>Ceriporiopsis subvermispora</i>	24	Biosorption	29	Lignocellulose biomass	26
19	Biosorption	66	Azo dyes	23	Basidiomycetes	27	PAHs	26
20	Immobilization	63	Biopulping	22	Immobilization	27	Biosorption	24

By observing the changes of the ranking of most frequently used keywords in different periods, the changes of hotspots in related research can be directly understood. The evolution of top 20 author keywords and frequency in different periods was also listed in Table 2. The growth rates of author keywords “lignocellulose”, “pretreatment”, “lignin”, “lignin degradation”, “delignification”, and “lignocellulose biomass” is higher than other keywords, and it has been used frequently in recent years. In particular, “pretreatment” and “ligocellulose biomass” ranked more than 150 before 2008, and increased rapidly to 15th and 18th in 2015–2020, respectively. Another notable change is the keyword “delignification,” which ranked 78th in 2003–2008, then increased to 27st in 2009–2004, and 17th in 2015–2020. In addition, “lignocellulose” also increased from 48th in 2003–2008 to 11th in 2015–2020. The microbial degradation of lignocellulosic biomass is considered to be an efficient and economical way for the utilization of biomass waste. However, there is a major obstacle to energy production because lignocellulosic biomass contains lignin which is difficult to be transformed (Rouches et al. 2016a). In this case, WRF is considered to be the most capable microorganisms for delignification pretreatment of waste biomass resources (Isroi et al. 2011). In contrast, the author keywords “textile dyes,” “biopulping”, and “*Ceriporiopsis subvermispora*” lost attention in the research period, because their ranking in the list of frequently used keywords dropped significantly. Among them, “textile dyes” and “biopulping” ranked 13th and 20th in 2003–2008 respectively, and then dropped sharply to less than 200th place in 2015–2020.

In order to compare the research focus of different countries, Table S4 lists the top 20 author keywords in the five countries with the most contributions to this research. It was apparent that *Phanerochaete chrysosporium* was the most commonly used WRF strain in China, USA, India and Japan, strain A, while *Trametes versicolor* was the most widely used strain in Spain. The “PAHs”, “biosorption” and “corn stover” only appears in the top 20 keywords of

China, while “pretreatment”, “wood decay”, “enzymatic hydrolysis” and “ethanol” only appears in the top 20 keywords of USA. In the study of India, the author keywords “delignification”, “azo dyes”, “wheat straw”, “response surface methodology” and “optimization” have a higher ranking than other countries. During the retrieval period, “cytochrome P450”, “*Phanerochaete sordida*”, “hydroxylation”, “*Lentinula edodes*”, “estrogenic activity” and “dioxin” entered the top 20 keywords of Japan, indicating these were full of research interests. Moreover, Spain pays more attention to “versatile peroxidase”, “*Trametes hirsute*”, “bioethanol”, “phytotoxicity”, “pharmaceuticals” and “anthracene”.

#### Visualization of Co-occurrence keywords

This study carried out cluster analysis of author keywords that appeared more than 16 times to reflect the close relationship between keywords, and determine the mainstream research direction in this field. In general, keywords with strong correlation are close to each other. The co-occurrence frequency of keywords in the same cluster is high, which jointly represents a similar research field. According to the result of cluster analysis as shown in Fig. 6a, 130 author keywords were clustered into seven categories depicted in different colors, of which clusters 1, 2 and 3 are the main clusters with more author keywords. Cluster 1, the largest cluster with 30 author keywords, was marked in red, focused on the pretreatment of biomass waste by WRF for biopulping, energy production or lignin modification, etc., mainly including “lignin”, “lignin degradation”, “delignification”, “lignocellulose”, “wheat straw”, “biopulping”, “biological pretreatment”, “enzymatic hydrolysis”, “*Ceriporiopsis subvermispora*”, “lignocellulosic biomass”, etc. Cluster 2 has 26 author keywords, with green items, focused on the application of WRF and its enzymes in decolorization of dye wastewater, with keywords of “laccase”, “decolorization”, “decolourization”, “*Trametes versicolor*”, “biosorption”, “kinetics”, “immobilization”, “azo dyes”, “dye”, “textile dyes”, etc. Cluster 3 has 25 author keywords, with blue items, focused on the ability of WRF to remove environmental pollutants, including the main keywords of “biodegradation”, “bioremediation”, “*Phanerochaete chrysosporium*”, “degradation”, “fungi”, “*Pleurotus ostreatus*”, “ligninolytic enzymes”, “PAHs”, “biotransformation”, “mycoremediation”, “pesticides”, etc.

Furthermore, the density visualization map of author keywords and hotspots intensity with color spectrum was shown in Fig. 6b, where warm colors represents hot research fields and cool colors represents cool research fields. The author keywords with the dark red are “laccase”, “white rot fungi”, “*Phanerochaete chrysosporium*”, “biodegradation”, “*Trametes versicolor*”, “bioremediation”, “decolorization”, “ligninolytic enzymes”, and “manganese peroxidase”, which indicated that these research contents represented by these keywords are important research hotspot from 2003 to 2020 all over the world.

#### Burst detection analysis

Burst keywords refer to words whose appearance frequency suddenly increased in some period. In general, the stronger the burst strength of keywords, the more likely the topic will become emerging research frontiers (Gao et al. 2019). In this study, CiteSpace V was used to identify the burst keywords, and the top 50 keywords with the strongest bursts is shown in Fig. 7, where the length of the entire line (green and red) in the last column represents the research period from 2003 to 2020, and the red line represents the burst period. On the whole, the high frequency keywords with low burst strength were classical terms that have been stably cited during the whole research period, while the low frequency keywords with high burst strength have attracted more attention in a certain research period, which may represent a emerging research direction.

Based on the results of burst keywords detection, three development stages in application of WRF biotechnology in environmental field in the past 20 years was identified. In the first stage before 2004, “*Bjerkandera adusta*”, “*Pleurotus eryngii*”, “mineralization”, “*Coriolus versicolor*”, “*Phanerochaete chrysosporium*”, “metabolism”, “oxidation”, “oxidase” and “culture” had higher burst strength ( $> 10.0$ ), while “mineralization”, “metabolism” and “culture” showed longer burst period, indicating that they were the frontier technologies in this period. The burst keywords in the second stage from 2005 to 2012 include “effluent”, “wastewater”, “optimization”, “decolourization”, “reactive dye”, “waste”, “contaminated soil”, “phenol”, etc. At this stage, researchers have realized the advantages of the application of fungal biotechnology in the environmental pollution control, and the value of WRF in bioremediation has been continuously discovered. Many studies have successfully applied WRF to the remediation of contaminated soil and water. The third stage was from 2013 to 2020, the major keywords include “lignocellulosic biomass” (33.83), “enzymatic hydrolysis” (22.11), “pretreatment” (21.67), “chemical composition” (20.98), “lignocelluloses” (13.80), “biological pretreatment” (12.88), “toxicity” (12.03) and “wood decay” (11.66), according to their burst strength. Moreover, the above keywords and “pesticide”, “brown rot”, “solid state fermentation” have a long burst period, which has extended to 2020. These emerging burst keywords can provide inspiration sources for researchers to propose new research topics and perspectives for future research (Qi et al. 2019).

To determine which authors are the active in a specific period of time, burst detection of authors was performed. Fig. S1 shows the burst authors in the research field of WRF biotechnology for environmental application from 2012 to 2020. Among the 28 authors, 6 are from China, 4 from Spain and the Netherlands, 2 from Italy, Malaysia and USA, and 8 from other different countries. The burst period of 9 authors extends to 2020, in which the burst period of Asif MB and Ma FY started in 2017, and that of Ma Q, Helm CV, van Erven G, Yang ST, Nayan N, Kable MA and Peng M started in 2018. Tracking the research of these burst authors is instrumental for us to grasp the latest development trend of specific research field. Ma FY mainly carried out the research on the application of WRF such as *Pleurotus ostreatus* and *Irpea lacteus* and their lignocellulolytic enzymes in dye decolorization and delignification (Ma et al. 2017). In the recent report of Asif MB, some high-efficiency enzymatic membrane bioreactors were constructed to remove trace organic contaminants (Asif et al. 2018). Ma Q and Yang ST are from the same team of Southwest Minzu University in China, and they mainly studied the toxicity of carbon-based nano-materials such as nanodiamonds, carbon nanotubes and graphene to WRF, and fungal transformation of these nano-materials by WRF (Ma et al. 2019; Ming et al. 2018). The research of Helm CV mainly focuses on the decolorization of textile industry wastewater by *Oudemansiella canarii* and *Fanoderma lucidum* on solid state fermentation with sugarcane bagasse-wheat bran mixture or peach-palm residue as substrates (Chicatto et al. 2018). Nayan N, van Erven G and Kabel MA are all from Wageningen University & Research in Netherlands, and their main

work is to screen WRF capable of pretreatment of wheat straw to improve their nutritional value or ruminal digestibility for ruminant feeding (Nayan et al. 2019).

#### **Research hotspots and future trends**

As a result of human activities, the accumulation of lignocellulosic wastes gradually has evolved into an environmental problem that cannot be ignored. The pretreatment of lignocellulose is a critical step in production of value-added products such as biofuel and enzymes from woody materials (Sharma et al. 2019). Among various pretreatments of lignocellulose, WRF are gaining popularity due to its financial and environmental benefits (Ding et al. 2019). Several studies have found that the combination of WRF pretreatment with other physicochemical methods can not only reduce the time necessary for the whole process, but also introduce cost-effectiveness (Shirkavand et al. 2016; Xie et al. 2017). Anaerobic digestion of lignocellulosic biomass has been considered as an efficient way to produce energy (Rouches et al. 2016b). However, application of WRF in the pretreatment of substrates for anaerobic digestion started late, and further exploration is necessary.

In recent studies, whole-cell WRF or its extracellular extracts have been successfully applied to remove trace organic contaminants (TrOC) in aqueous phase (Asif et al. 2017a). Whole-cell WRF or their extracellular lignin-modifying enzymes (LMEs) have also been reported to degrade pesticide, nicotine, pharmaceuticals and personal care products that are persistent to the environment (Su et al 2016; Asif et al. 2017b; Xiao and Kondo 2020b). Meanwhile, the use of cross-linked enzyme aggregates (CLEAs) such as porous-, combi-, or magnetic CLEAs to immobilize LMEs is a promising technology to overcome the stability and non-reusability of free LMEs from WRF in wastewater treatment (Vrsanska et al. 2018; Voberkova et al. 2018). Another recent focus is use of WRF as low-cost and efficient adsorbent for the removal of heavy metal from wastewater (Srivastava et al. 2015; Noormohamadi et al. 2019). On the other hand, WRF also shows excellent application potential in remediation of industrial contaminated soils (Stella et al. 2017).

Furthermore, the combination of WRF and nanomaterials for pollutant removal is regarded as a highly competitive technology, which is also called nano-biotechnology. Some researchers have pointed out that the stability and treatment performance of WRF can be greatly improved by adding nanoparticles as carriers or synergists to the wastewater treatment systems (He et al. 2017; Huang et al. 2018). At the same time, it is necessary to investigate the environmental bio-effects of nano-materials on WRF to ensure the safe applications (Ma et al. 2020). Recent research have confirmed that WRF can produce HO<sup>•</sup> with strong oxidation through a Fenton-like process, and then improve the degradation effect of pollutants in wastewater(Vasiliadou et al. 2019). Therefore, the combining WRF biotechnology with advanced oxidation processes and maximizing their respective advantages in wastewater treatment is also one the research frontiers in the future.

At present, studies on degradation performance of WRF are mainly focused on sterile batch tests, while few studies have been conducted on the bioreactor scale under non-sterile conditions and in continuous mode. Since the performance of WRF will be seriously affected by bacterial contamination, the large-scale application of WRF in bioremediation remains a technical challenge, and more exploration is needed to improve this limitation (Asif et al. 2017b; Svobodova and Novotny 2018). In addition, although the practical use of WRF biotechnology in environmental remediation shows its unique charm, it is necessary to make breakthroughs in process optimization and cost reduction before this goal can be achieved (Rodríguez-Couto. 2017).

## **Strengths And Limitations**

As far as the author knows, this is the first time to conduct a relatively objective and comprehensive bibliometric analysis on the researches of WRF biotechnology for environmental application in a global scale based on the publications data collected from WoSCC. The two kinds of visualization software was used for cooperation network analysis, cluster analysis and bursts analysis, which clearly reveals the current status and development trends of WRF biotechnology applied to environmental pollution control. However, it must be pointed out that there are still some unavoidable limitations in this study, just like any studies. Due to the incomplete search items, some relevant articles may have been missed, while some irrelevant articles may be collected. Also, the citation analysis does not exclude self-citation, which may lead to the fact that the results of citation analysis cannot fully and accurately reflect the academic influence represented. Furthermore, some high-quality non-English publications were not included in this study, which will lead to incomplete analysis. These deficiencies will be corrected in future research, in order to draw obtain more detailed and accurate research conclusions.

## **Conclusions**

Based on 3962 documents retrieved from the WoSCC database, a bibliometric analysis on the evolution and development of WRF biotechnology for environmental applications was carried out in order to fully understand the publications, the most productive countries, institutions, authors, journals, and research categories, as well as research hotspots and future research directions. These results are helpful for researchers to analyze the existing publications, thereby helping them improve their research direction and keep up with the research frontier. This is an important contribution because it is the first time that bibliometric analysis has been carried out in the research field of WRF biotechnology for environmental application from author's knowledge.

## **Declarations**

### **Ethics approval and consent to participate**

Not applicable

#### Consent for publication

Not applicable

#### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Competing interests

The authors declare that they have no competing interests

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#### Authors' contributions

Pengfei Xiao: data collection and analysis, funding acquisition, investigation, methodology, visualization, writing—original draft; Dedong Wu: writing—review & editing; Jianqiao Wang: validation. All authors read and approved the final manuscript.

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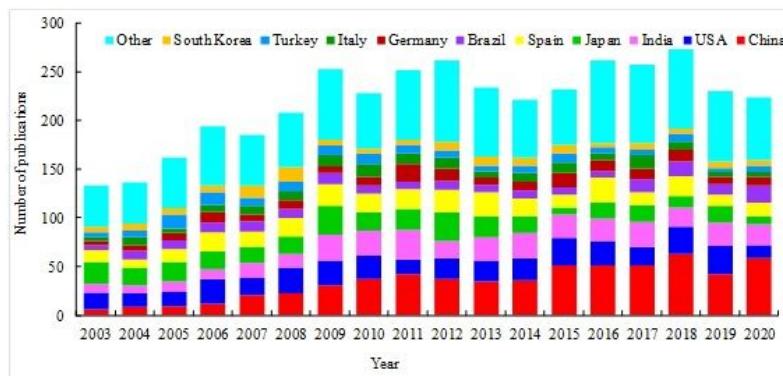
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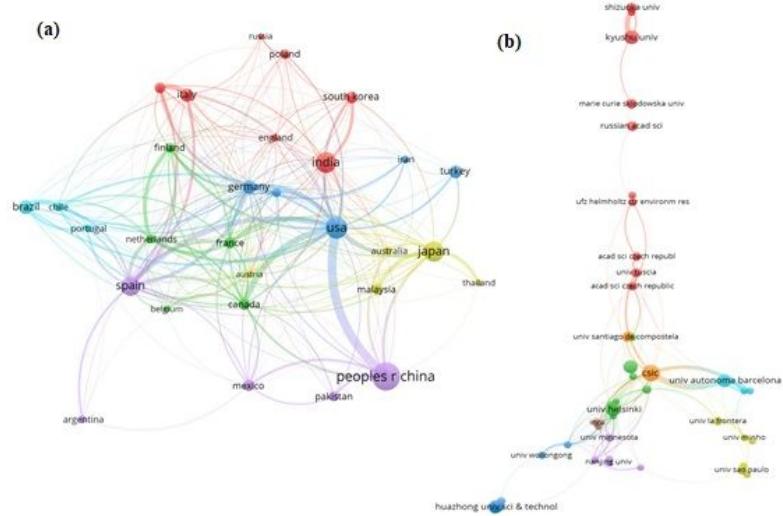
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## Figures



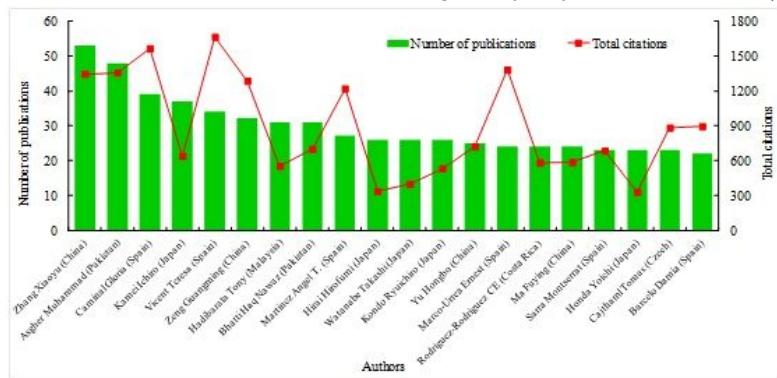
**Figure 1**

The number of publication and main productive countries in the researches on WRF biotechnology for environmental application by year.



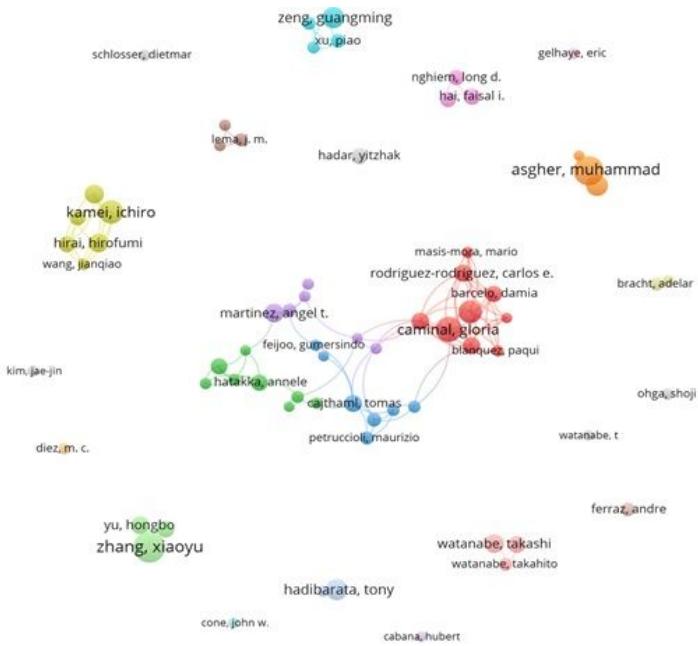
**Figure 2**

The academic collaboration networks among the top 30 productive countries (a) and the top 40 productive institutions (b).



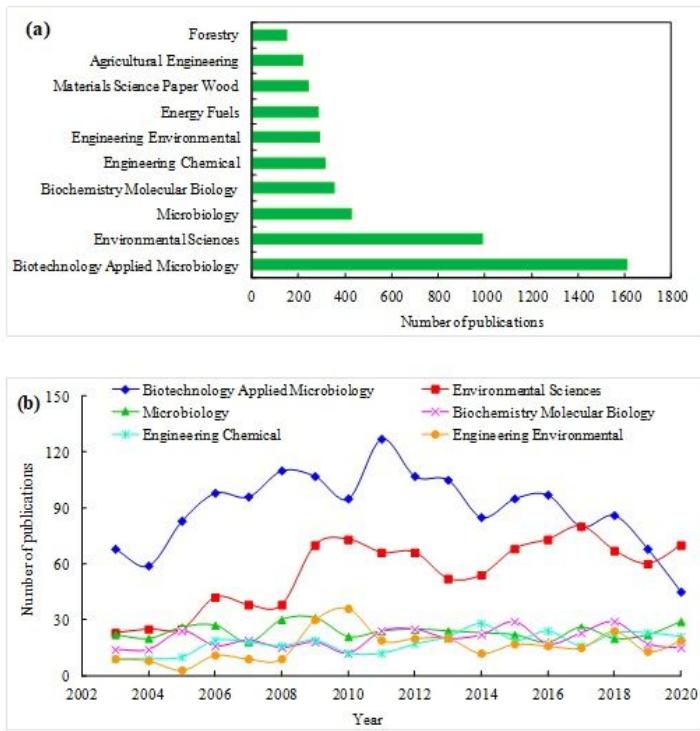
**Figure 3**

The top 20 productive authors according to the number of articles and total citations.



**Figure 4**

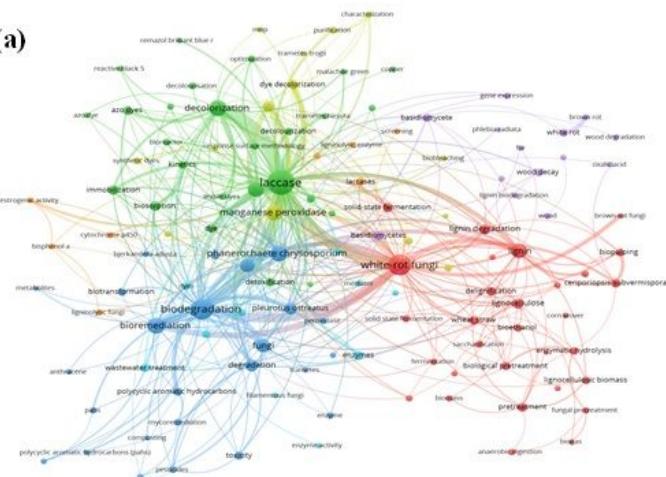
The academic collaboration network among the top 69 productive authors.



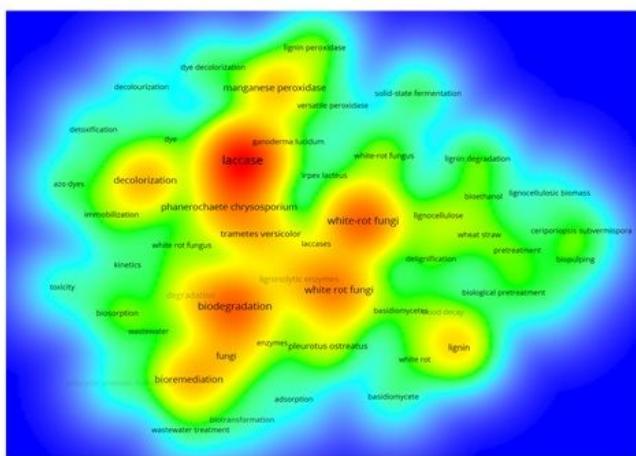
**Figure 5**

The total output (a) and temporal evolution (b) of publications in different subject categories in the researches on WRF biotechnology for environmental application.

(a)



(b)

**Figure 6**

The network visualization map (a) and density visualization map (b) of co-occurrence keywords in the researches on WRF biotechnology for environmental application.

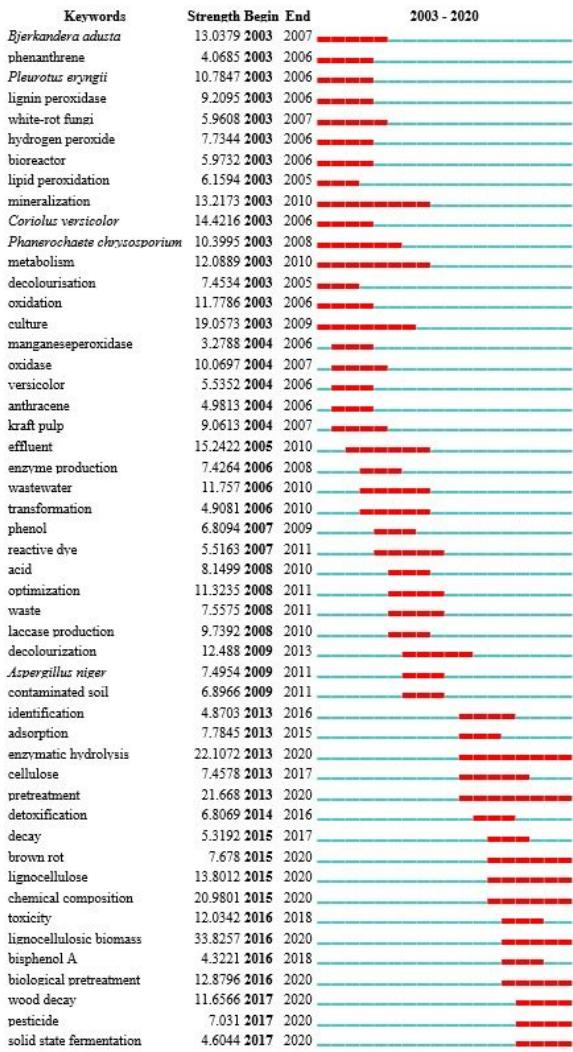


Figure 7

Top 50 Keywords with the strongest citation bursts in the researches on WRF biotechnology for environmental application.

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