

Tracing the Evolution of Enzymatic Bioremediation Using Scientometric Analysis

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ABSTRACT

Enzymes are responsible for any biochemical reactions including the transformation of toxic pollutants by microbes. Identification, isolation and application of enzymes for biodegradation of environmental pollutants have become one of the research hotspots. Whether it is due to the constant seeking of a better enzyme, advances in techniques, or increases in some pollutants, there have been exponential increases in the number of research on enzymatic bioremediation. A Scientometrics study was undertaken to capture the holistic view of enzymatic bioremediation. 14,952 research papers were identified from a topic search covering the period 1983 to 2023 from the 'web-of-science' database. The social network analysis approach was used for understanding the structure and dynamics of the research. The study identifies that among the enzymes, research activity is most intense in laccase followed by peroxidase and dioxygenase whereas, in microbes, *Pseudomonas* sp., *Bacillus* sp. and *Phanerochaete* sp. are areas show maximum research. In contrast to fungus and fungal enzymes, there has been gradual increases in research on bacteria and bacterial enzymes. Dye, Polyaromatic hydrocarbons (PAHs) along with other emerging pollutants like plastics and pesticides are the most studied substrates. The study also draws attention to research increasingly being undertaken to identify, isolate and manipulate new enzymes using various emerging techniques such as metagenomics, genetic engineering, molecular docking and simulations. The results and implications of the study are discussed.

Keywords: Enzymes, Biodegradation, Scientometric, Laccases, Dye, Polycyclic aromatic hydrocarbons (PAHs).

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INTRODUCTION

Enzymes are central to any biochemical processes of life. Enzymatic bioremediation involves the use of enzymes from living organisms (bacteria, fungi, or plants) to degrade or reduce toxic environmental contaminants into their less toxic metabolites. Despite its huge potential, microbial bioremediation is a slow process and has certain limitations in the field or actual natural ecosystem. These limitations can be attributed to an inadequate understanding of microbial behavior in the field, the bioavailability of the pollutants, the availability of other easily accessible alternative compounds as well as difficulty maintaining active microbial population owing to various environmental stresses including pH, temperature, moisture and biotic interactions (intra- and interspecific interactions).^[1-4] Owing to its specificity, enzymatic bioremediation is considered swifter and more effective than microbial bioremediation.

Compared to microbes, enzymes can operate under a wider range of conditions of temperature and ionic strength and catalyze reactions without producing any toxic by-products.^[5] It also removes the possibility of biomass buildup in the environment which could be of serious concern.^[6] Further, genetic engineering can easily manipulate the enzymes to increase their stability and potential. This will eliminate the risk associated with the release of a genetically engineered organism into the environment.^[7] Therefore, the focus of bioremediation research has been shifted from traditional microbial approaches towards more advanced enzymatic bioremediation.

Following the discovery of the first enzyme, diastase, by French chemist Anselme Payen in 1833, there has been a gradual increase in the number of known enzymes.^[8] In 1964, the International Union of Biochemistry and Molecular Biology (IUBMB) classified 712 enzymes based on their reactions into 6 classes-oxidoreductase, transferase, hydrolase, lyase, isomerase and ligase. A seventh class, translocase, was added in 2018 to include transport proteins.^[9] At present, there are 6711 total enzyme counts in the IUBMB ExplorEnz-The Enzyme Database.^[10]



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Enzymatic bioremediation does have its share of limitations including the need for proper coenzymes, maintenance of suitable conditions-pH, temperature, concentration, contact time, etc., and being unable to self-propagate it requires large-scale production to maintain suitable concentration. Stability, higher cost of production, recyclability and reusability of the enzymes remain the biggest obstacles in enzymatic bioremediation technology.^[11] Despite these limitations, enzymatic bioremediation has advanced considerably in the last two decades. Enzymes isolated from various sources like bacteria, fungi, algae and plants have been used for bioremediation of many pollutants. Other than the discovery of new enzymes with different functions, there has been considerable research to enhance enzyme stability and increase the catalytic efficiency and specificity of the enzymes using various enzyme engineering and immobilization techniques.

Scientometric or bibliometric studies are quantitative studies of science through which one examines scientific and technological advancements and makes comparisons between activity, productivity and innovation in science.^[12] It helps to analyse the development of science as an informational process which can provide an unrealistic notion of the scientific relevance of the analyzed elements. Like all other studies, the field of enzymatic biodegradation research developed and evolved in tandem with the progress of molecular biology tools and changes in pollutant chemistry. The last 20 years have seen a considerable increase in the quantity and variety of both microorganisms and enzymes, which has enhanced our knowledge of enzymatic biodegradation processes. In the present study, we aimed to trace the evolutionary trends of enzymatic biodegradation studies using a scientometric approach. The current research provides a condensed database of knowledge which helps to access the significant information on trends and remaining gaps for future research that could increase the applicability of the enzymatic biodegradation.

LITERATURE REVIEW

Now a days, there are many factors concerning stability, specificity, high cost of production and scale-up challenges which hamper the application of enzymes for bioremediation in the field conditions. However, continuous efforts have been made to identify, isolate and manipulate new enzymes using various emerging techniques such as metagenomics, genetic engineering, molecular docking and simulations. Additionally, a lot of advancements have been made to well-established methods including immobilization, fermentation and bioreactors and their application is still common today. Ozcan Konur,^[13] analyses research trends on algal bioremediation by using scientometric analysis. Baskaran *et al.*,^[14] mapping the bioremediation research output in India. They analyse that in India most of the scientometric analyses have been done by using HistCite and VOSviewer software. In India, the Indian Institute of Technology, Baba Atomic Research Centre and CSIR are the major producers

of research output in the area of bioremediation. Song *et al.*,^[15] did a bibliometric analysis of the current status of bioremediation of petroleum-contaminated soils during 2000-2019. They showed that in the present scenario, mainstream research is centered on biostimulation, bioaugmentation and biosurfactant application. Combined pollution of petroleum hydrocarbons and heavy metals, microbial diversity monitoring, biosurfactant application and biological combined remediation technology are considered future research hotspots. Roslee *et al.*,^[16] analyze the causes and potential of bioremediation approaches for diesel pollution in Antarctic Territories by using scientometric analysis. They found that the hydrocarbonoclastic microbes studied in the past, known and proposed metabolic pathways and genes related to hydrocarbon biodegradation as well as bacterial adaptations to low-temperature conditions. Rodrigues *et al.*,^[17] analyze the research trends in the design of laccase biocatalysts for the decolorization of synthetic dyes and present the fact that innovative technologies have been developed to render enzyme production more sustainable and economically viable. This paper presents the first-ever scientometric study of the research in the field of enzymatic bioremediation using a wide sample of nearly 14,952 papers and represents scientometric analysis as an analytical tool. It has a great potential to gain valuable insights into the evolution of the research in this field as in the case of new emerging technologies.

METHODOLOGY

Data Collection

WoS is one of the most reliable and comprehensive databases for bibliometric investigations. Hence this database was taken for accessing the research activity in this field. Data from the Web of Science (WoS) core collection was retrieved using the search string TS (Topic Search)=(“enzyme*” or “enzymatic*” and “biodegradation*” or “bioremediation*”). A total of $n=15,244$ results were obtained. The search results were refined by excluding all the documents except articles, review articles and proceeding papers, which yielded 14,952 documents. The period of 35 years from 1989 to 2023 was subdivided into three periods, 1989-2003, 2004-2013 and 2014-2023 having 2121, 4158 and 8673 results respectively. The complete data set downloaded from the Web of Science is in the English language from the core collection category of WoS.

Data Preparation and Analysis

The plain text data exported from Web of Science includes all the Bibliometric information including title, abstract, authors, keywords, references and citation data which are required for analysis. Based on the citation study, we analyzed prominent countries, journals, authors, institutions, corresponding authors and key research areas in the field of enzymatic biodegradation. This gives an idea about the most influential work based on respective parameters. We prefer local citation analysis in our

work since highly cited publications are those that acquired more citations in the same field. The subdivision of the entire study period into three different phases enables us to study the main area of research viz. enzymes, microbes, substrates, techniques, etc. in the respective period. The data was processed and mined with the help of Bibexcel, followed by network map preparation and visualization in VOSviewer. Tables and pivot charts were created using MS Excel using the data from BibExcel. Some parameters were also analyzed in R using RStudio.

Objective and Research Question

The key purpose of this study is to use scientometric analysis to study and compare the key research areas of enzymatic biodegradation at different time intervals and understand the underlying evolutionary trends. We measure the scientific impact of publications over time using various methodologies to identify the emergence of novel concepts and potential directions for future research. We investigate the research trend on enzymatic biodegradation to identify the document-wise distribution of publications and analyze the pattern of authorship and collaboration among them. We evaluate the research concentration of each institution, determine the journal-wise distribution of publications and rank the authors according to their publications.

The present study attempts to address the following research questions:

What is the global trend of research in the field of enzymatic biodegradation?

Which countries, institutions, authors and sources (journals) are prominent in this area?

How do various countries, institutions and authors collaborate in the field?

What are the key areas of research in the field of enzymatic biodegradation and how it changes over time?

RESULTS

Key Information about Scientific Data

A total of 14952 documents (12556 articles, 411 proceedings papers and 1558 review articles) were published in 1538 sources by 40987 authors. Of this, 364 documents were single-authored by 313 authors, with an overall average of 5.04 authors per document. The average number of citations per document is 34.95 with a total of 406617 references. The international co-authorship percentage of 22.42 indicates significant research collaborations among the authors. The annual growth rate is 22.17% with the average document age 9.58 years. The average citation per year per document is 3.08 with the highest mean citation per year of 5.28 during 2020 followed by 4.85 and 4.75 during 2018 and 2019 respectively (data not shown).

Figure 1 summarizes the number of peer-reviewed articles that used the terms "enzyme*" or "enzymatic*" and "bioremediation*" or "biodegradation*" in the Web of Science database till 31st August 2023 and their average total citations per year. There is a continuous, almost exponential rise in the number of publications, indicating the growing interest in the topic. With 1301 documents, 2021 has the maximum number of publications followed by 2022

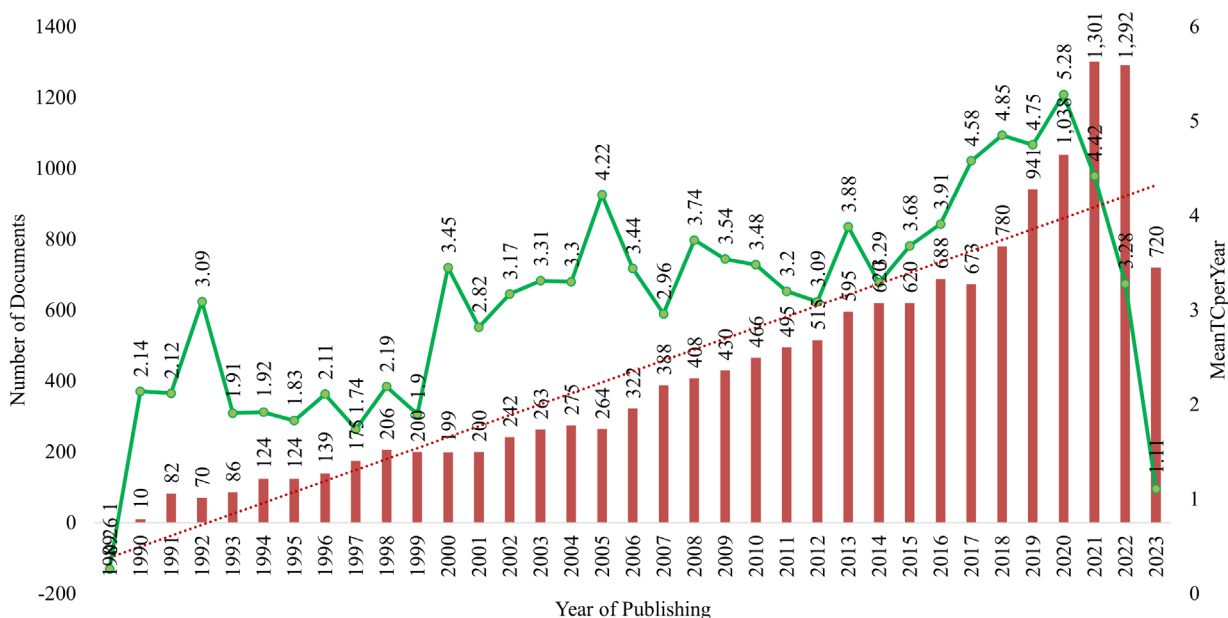


Figure 1: Publication trend and MeanTCper year on enzymatic biodegradation.

(1292) and 2020 (1038). However, the documents published in the year 2020 have the highest average total citations per year of 5.28 followed by 2018 (4.85) and 2019 (4.75).

Influential journal on Enzymatic Biodegradation Studies

Of the 1538 sources, the top ten most relevant sources based on several documents are tabulated in Table 1. Applied and Environmental Microbiology of American Society for Microbiology ("ASM") with 420 documents top the overall list of most relevant sources followed by two Elsevier journals, Chemosphere (382) and Journal of Hazardous Materials (366), respectively. Applied and Environmental Microbiology with 169 documents topped the list during 1989-2003, Bioresource Technology and International Biodeterioration and Biodegradation with 131 documents each was the joint most relevant sources during 2004-2013, while Journal of Hazardous Materials topped the list during 2014-2023 with 268 (Table S1, Supplementary data). Local citation is the number of times a document from the source is cited by the current document set and therefore, represents the importance of the journal in the field. Applied and Environmental Microbiology also topped the list of most locally cited sources with 35569 citations followed by Bioresource Technology (20089) and Applied Microbiology and Biotechnology (16328).

Documents Information

Though the number of citations indicates the quality and impact of the article in the respective field, it is well well-known fact that review articles, because of their wider coverage, generally receive more citations than the original research articles. This trend can be seen in the field of enzymatic bioremediation too. Despite its huge number, more than eight times the number of review articles, only seventeen research articles could make it to the

top fifty most locally cited documents in the field of enzymatic bioremediation (Table S2; Supplementary data). Since local citation only considers the citations received by the document from the analyzed set of documents, it measures the true impact of the document in the analyzed field. Of the top 10 articles, the top 5 are review articles and the rest are research articles. Two review articles describe white-rot fungi and their enzymes, two describe about biodegradation of plastics and the remaining one focuses on the degradation of PAHs (Table 2). On the other hand, three of the most locally cited research articles are on plastic (including PET) biodegradation while the other two are on the mechanism of manganese peroxidase enzyme and PAH biodegradation by white-rot fungi respectively.

Prominent Authors in Enzymatic Biodegradation

The h-index measures the cumulative impact of an author's scholarly output and performance. Table 3 shows the top 10 authors having the best h-index in the field. Govindwar SP tops the list with an h-index of 41 with 82 publications and 5672 total citations. Santerre JP and Zeng GM with h-index of 37 and 32 are the second and third most impactful authors respectively. Interestingly, Wang Y who has the highest number of publications ($n=103$) is placed 5th in the list of authors with the highest scientific impact. The g-index which gives more weightage to the highly-cited articles of the authors is also highest for Govindwar SP (74) but the m-index which is calculated as the h-index per year since the first publication is highest for Bilal M (3.125) whose first publication year is 2016 followed by Govindwar SP (2.278) and Zeng GM (1.684).

Co-Author Network

The co-author network analysis of the top 50 authors with a minimum publication of 32 shows 9 clusters (Figure 2). Cluster 1 (red) has 15 authors followed by Cluster 2 (green) with 9 authors,

Table 1: Profile of the Top ten most relevant sources.

Sources	No. of Documents	Publication Start Year*	Average Publication per Year (Rank)	Local citation (Rank)
Applied and Environmental Microbiology.	420	1976	12.35 (2)	35569 (1)
Chemosphere.	382	1972	11.24 (4)	15730 (4)
Journal of Hazardous Materials.	366	1975	10.76 (5)	12821 (7)
International Biodeterioration and Biodegradation.	353	1992	11.39 (3)	9539 (9)
Applied Microbiology and Biotechnology.	336	1984	9.88 (6)	16328 (3)
Bioresource Technology.	314	1991	9.81 (7)	20089 (2)
Environmental Science and Pollution Research.	231	1994	7.97 (8)	4950 (19)
Frontiers in Microbiology.	227	2010	17.46 (1)	4564 (24)
Science of the Total Environment	218	1972	6.41 (9)	9604 (8)
Polymer Degradation and Stability	213	1979	6.26 (10)	6017 (13)

*Source: NLM Catalog, National Center for Biotechnology Information (NCBI) <https://www.ncbi.nlm.nih.gov/nlmcatalog>

Table 2: List of top most locally cited Review and Research Articles.

Document	LC	GC	LC/GC Ratio (%)	Nor. LC	Nor. GC	References
Haritash AK, 2009, ^[17] Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. <i>J HAZARD MATER.</i>	270	1981	13.63	33.86	37.26	18
Wesenberg D, 2003, ^[18] White-rot fungi and their enzymes for the treatment of industrial dye effluents. <i>BIOTECHNOL ADV.</i>	238	800	29.75	27.92	11.49	19
Pointing SB, 2001, ^[19] Feasibility of bioremediation by white-rot fungi. <i>APPL MICROBIOL BIOT.</i>	205	626	32.75	26.87	9.65	20
Shah AA, 2008, ^[20] Biological degradation of plastics: A comprehensive review. <i>BIOTECHNOL ADV.</i>	182	1398	13.02	25.4	23.36	21
Tokiwa Y, 2009, ^[21] Biodegradability of Plastics. <i>INT J MOL SCI.</i>	150	845	17.75	18.81	15.89	22
Yoshida S, 2016, ^[22] A bacterium that degrades and assimilates poly (ethylene terephthalate). <i>SCIENCE.</i> 10.1126/science.aad6359	278	1181	23.54	58.49	37.78	23
Wariishi H, 1992, ^[23] Manganese (II) oxidation by manganese peroxidase from the basidiomycete <i>Phanerochaete chrysosporium</i> . Kinetic mechanism and role of chelators. <i>J BIOL CHEM.</i>	244	892	27.35	16.11	9.02	24
Austin HP, 2018, ^[24] Characterization and engineering of a plastic-degrading aromatic polyesterase. <i>P NATL ACAD SCI USA.</i>	146	448	32.59	32.39	15.39	25
Muller RJ, 2005, ^[25] Enzymatic Degradation of Poly (ethylene terephthalate): Rapid Hydrolyse using a Hydrolase from <i>T. fusca</i> . <i>MACROMOL RAPID COMM.</i>	112	298	37.58	14.02	3.71	26
Field JA, 1992, ^[26] Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. <i>APPL ENVIRON MICROB</i>	91	300	30.33	6.01	3.03	27
LC=Local citations; GC=Global citations; Nor.=Normalized.						

Table 3: Top ten authors with highest impact on enzymatic bioremediation studies.

Authors	<i>h</i> -index	<i>g</i> -index	<i>m</i> -index	TC	NP	PY_start
Govindwar SP	41	74	2.278	5672	82	2006
Santerre JP	37	63	1.194	4078	65	1993
Zeng GM	32	48	1.684	2365	50	2005
Spain JC	30	56	0.909	3350	56	1991
Wang Y	30	47	1	2552	103	1994
Jadhav JP	28	43	1.556	2389	43	2006
Liu Y	27	45	1.174	2329	89	2001
Zhang J	27	39	1.35	1664	77	2004
Labow RS	26	39	0.839	2232	39	1993
Bilal M	25	46	3.125	2230	60	2016
TC=Total citations; NP=Number of Publications; PY=Publication year.						

cluster 3 (blue) and 4 (yellow) has 6 authors each, cluster 5 (purple) and 6 (turquoise) have 4 authors each while the remaining cluster 7 (coral), 8 (brown) and 9 (pink) have 2 authors each (Table S3, Supplementary data). Chen X and Li F of Cluster 3, Zhang Q of Cluster 5, Li J and Wang Z of Cluster 6 and Hi J of Cluster 9 have a maximum number of links i.e. 44. Li Y (233) of cluster 1 has the highest overall total link strength followed by Wang Y (223) and Wang X (206). Wang Y (23) has the largest user-defined weight followed by Wang Y (22), Zhang Y (22) and Li S (22).

Productive Institutions

Chinese Academy of Sciences with 828 documents is the most relevant affiliation in terms of publication followed by the University of Minnesota System and Council of Scientific and Industrial Research (CSIR)-India with 360 and 356 documents respectively (Table 4). Though the number of publications increases with time for all the affiliations, the most significant increases were seen with the Chinese Academy of Sciences, Council of Scientific and Industrial Research (CSIR)-India and Indian Institute of Technology System (IIT System). Chinese

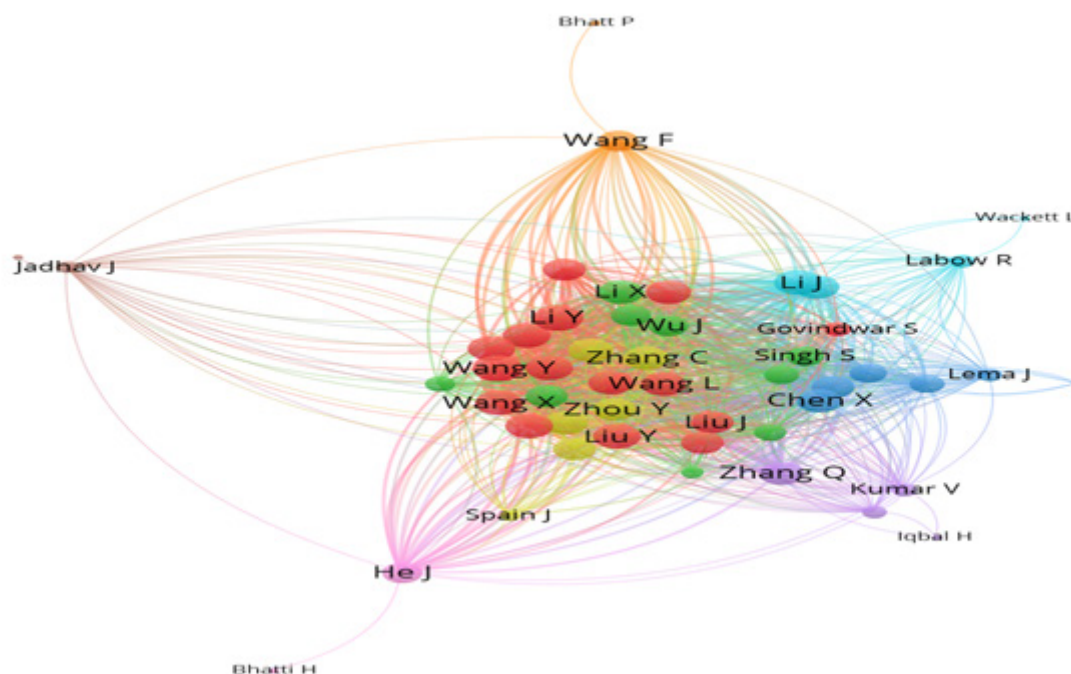


Figure 2: Research collaboration among prominent authors on enzymatic biodegradation.

Table 4: Most relevant affiliations in terms of publication.

Affiliation	1989-2003		2004-2013		2014-2023		Overall	
	n	Rank	n	Rank	n	Rank	n	Rank
Chinese Academy of Sciences.	34	16	214	1	580	1	828	1
University of Minnesota System.	119	1	76	13	165	6	360	2
Council of Scientific and Industrial Research (CSIR) -India.	38	11	100	9	218	3	356	3
Centre National De La Recherche Scientifique (CNRS).	43	8	103	7	186	5	332	4
Helmholtz Association.	51	4	127	3	149	7	327	5
UDICE-French Research Universities.	48	7	109	5	164	6	321	6
Consejo Superior De Investigaciones Cientificas (CSIC).	28	23	128	2	145	10	301	7
University of California System.	59	2	104	6	128	15	291	8
United States Department of Energy (DOE).	37	13	102	8	148	9	287	9
Indian Institute of Technology System (IIT System).	14	57	77	12	187	4	278	10

Table 5: Top ten most relevant countries by corresponding authors.

Country	1989-2003			2004-2013			2014-2023			Overall	
	n	Rank	n	Rank	n	Rank	n	SCP	MCP	Freq	MCP_R
China	64	9	650	1	2715	1	3429	2818	611	0.229	0.178
India	71	7	457	3	1100	2	1628	1374	254	0.109	0.156
USA	462	1	510	2	445	3	1417	1159	258	0.095	0.182
Japan	243	2	256	4	154	13	653	572	81	0.044	0.123
Germany	99	3	166	6	227	8	492	352	140	0.033	0.283
Brazil	25	14	144	8	307	4	476	396	80	0.032	0.168
Spain	53	11	178	5	229	7	460	324	136	0.031	0.296
Korea	60	10	127	10	232	6	419	308	111	0.028	0.264
Italy	68	8	149	7	198	9	415	305	110	0.028	0.265
Poland	25	15	95	11	270	5	390	340	50	0.026	0.128
SCP = Single Country Publications; MCP = Multiple Country Publications; MCP_R = MCP Ratio											

SCP = Single Country Publications; MCP = Multiple Country Publications; MCP_R = MCP Ratio

Table 6: Profile of the top 10 countries with the highest number of citations.

Country	1989-2003			2004-2013			2014-2023			Overall		
	n	AAC	Rk	n	AAC	Rk	n	AAC	Rk	n	AAC	Rk
China	4108	64.20	9	27684	42.6	3	48930	18	1	80530	23.5	1
USA	31300	67.70	1	32874	64.5	1	9416	21.2	3	73498	52	2
India	5285	74.40	7	28208	61.7	2	23381	21.3	2	56722	34.8	3
Japan	13875	57.10	2	10193	39.8	7	3653	23.7	13	27688	42.5	4
Germany	7061	71.30	3	10215	61.5	6	6066	26.7	4	23302	47.5	5
Canada	6949	76.40	4	10757	77.4	5	5405	34.2	7	23081	59.5	6
Spain	3072	58.00	10	12280	69	4	5694	24.9	5	21031	45.8	7
Italy	4256	62.60	8	7991	53.6	8	3888	19.5	11	16111	38.8	8
United Kingdom	5850	77.00	5	4941	79.7	11	3552	31.4	14	14324	57.1	9
France	5396	76.00	6	4822	51.3	12	3663	31.6	12	13863	49.5	10

n=Number of Citations; AAC=Average Article Citations; Rk=Rank.

Academy of Sciences was ranked 16th during 1989-2003 with just 34 publications which soar to 214 in 2004-2013 and 580 during 2014-2023. Similarly, the Council of Scientific and Industrial Research (CSIR)-India rose from 11th rank during 1989-2003 with 38 publications to 9th and 3rd rank with 100 and 218 publications during 2004-2013 and 2014-2023, respectively. On the other hand, the Indian Institute of Technology System (IIT System) jumped to 4th position during 2014-2023 with 187 publications from 57th rank with just 14 publications during 1989-2003 and 12th position with 77 publications during 2004-2013.

Highly productive Countries of Corresponding Authors

Among the top ten highly productive countries of corresponding author in the field of enzymatic bioremediation, China attains first position with 3429 publications followed by India and USA with 1628 and 1417 publications respectively (Table 5). The

publications of China, India, and Brazil increases significantly over time while that of USA, Japan and Germany decreases. China increases from 9th rank during 1989 – 2003 with 64 publications to 1st rank during 2004 – 2013 and 2014 – 2023 with 650 and 2715 publications respectively. The average multiple country publication (MCP) ratio of the top ten country is 0.2043 i.e. at least two publications out of 10 has at least one co-author from different countries. Spain with the MCP ratio of 0.296 has the highest international collaboration among the top ten countries followed by Germany (0.283) and Italy (0.265) respectively. Japan (0.123), Poland (0.128) and India (0.156) has the least international collaborations.

Total Citation of Country

We identify the most prominent countries based on their citations over the years. China with 80530 citations tops the list of countries with the highest number of citations followed by the

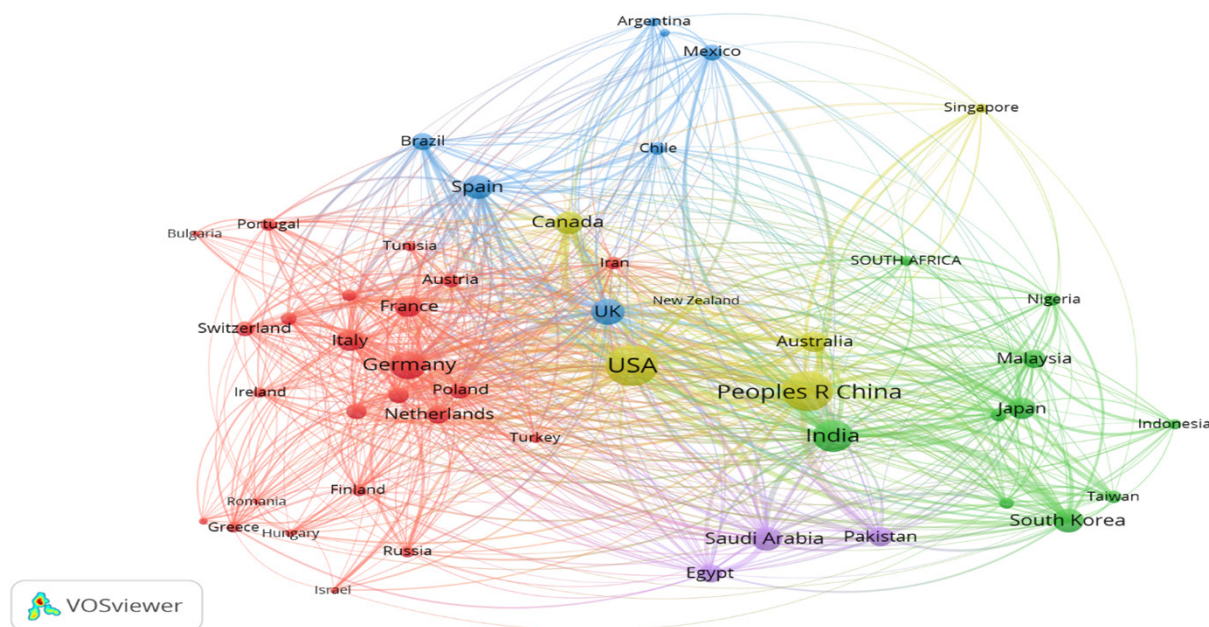


Figure 3: Research collaboration among countries on enzymatic biodegradation.

Table 7: Top ten most studied microbes in the research area of enzymatic biodegradation.

Microbes	1989-2003		2004-2013		2014-2023		Total	
	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank
<i>Pseudomonas</i> sp.	69 (3.25)	1	146 (3.50)	1	250 (3.04)	1	465 (3.20)	1
<i>Bacillus</i> sp.	13 (0.61)	8	80 (1.92)	2	244 (2.97)	2	337 (2.32)	2
<i>Phanerochaete</i> sp.	59 (2.78)	2	57 (1.36)	3	43 (0.52)	8	159 (1.09)	3
<i>Aspergillus</i> sp.	7 (0.33)	14	50 (1.20)	5	99 (1.20)	3	156 (1.07)	4
<i>Pleurotus</i> sp.	21 (0.99)	3	44 (1.05)	7	80 (0.97)	5	145 (1.00)	5
<i>Rhodococcus</i> sp.	8 (0.38)	10	46 (1.10)	6	89 (1.08)	4	143 (0.98)	6
<i>Trametes</i> sp.	20 (0.94)	4	56 (1.34)	4	59 (0.72)	6	135 (0.93)	7
<i>Streptomyces</i> sp.	13 (0.61)	7	32 (0.77)	8	38 (0.46)	10	83 (0.57)	8
<i>Trichoderma</i> sp.	5 (0.24)	18	20 (0.48)	11	48 (0.58)	7	73 (0.50)	9
<i>Penicillium</i> sp.	2 (0.09)	34	28 (0.67)	9	41 (0.50)	9	71 (0.49)	10

USA (73498) and India (56722) (Table 6). Until 2013, the USA was the most cited country with 31300 and 32874 citations during 1989-2003 and 2004-2013 respectively but fell to 3rd position with just 9416 citations during 2014-2023. On the other hand, India rises from 7th position during 1989-2003 with 5285 citations to 2nd position during 2004-2013 and 2014-2023 with 28208 and 23381 citations respectively. The number of citations for Japan, Italy, the United Kingdom and France gradually decreased while Spain considerably gained its position with time. Germany and Canada maintained more or less same positions throughout the study period. However, Canada with 59.5 has the maximum overall average article citations followed by the United Kingdom (57.1), the USA (52), France (49.5) and Germany (47.5). On the other hand, China (23.5) has the lowest number of average article

citations followed by India (34.8), Italy (38.8), Japan (42.5) and Spain (45.8).

Country Collaborations

The top 50 countries were screened to study collaborations among countries. The 50 countries form 5 clusters with cluster 1 having 24 countries while the remaining clusters 2, 3, 4 and 5 have 10, 7, 6 and 3 countries respectively (Figure 3). The USA (48) has the maximum number of links followed by France, Italy and India with 45 links each. The USA also has the highest total link strength (1103) followed by China (1048) and India (691). The top three countries with user-defined weight are China, the USA and India with values of 3505, 2047 and 1837 respectively (Table S4; Supplementary data).

Prominent research area of enzymatic biodegradation

Keywords are the index term that sums up a document's subject matter in its entirety and search engines use them to locate the article of our interest. As such, it becomes an essential part of a research paper and functions to define the research issue, topic field, subfield and technique of publications, thereby summarizing their content.^[28] The total number of publications almost doubled during each phase from 2121 during 1989- 2003 to 4158 during 2004- 2013 and 8673 during 2014-2023 (till

31st August 2023). As a result, comparing the actual number of occurrences of keywords would be unfair because it would favor recent times with a higher number of documents. Therefore, we compare the keyword occurrence in terms of the percentage of the total publication assuming that the keyword is cited only once per document and is ranked accordingly.

Microbes

Pseudomonas sp., topped the list of most studied microbe followed by *Bacillus* sp. and *Phanerochaete* sp., (Table 7). It also topped in all three time periods with 3.25, 3.50 and 3.04 of the documents

Table 8: Top ten most studied enzymes in the research area of enzymatic biodegradation.

Enzymes	1989-2003		2004-2013		2014-2023		Total	
	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank
Laccase	75 (3.54)	2	274 (6.56)	1	473 (5.75)	1	822 (5.66)	1
Peroxidase	160 (7.54)	1	222 (5.32)	2	311 (3.78)	2	693 (4.77)	2
Dioxygenase	45 (2.12)	5	124 (2.97)	3	139 (1.69)	4	308 (2.12)	3
Oxidoreductase	27 (1.27)	6	80 (1.92)	5	180 (2.19)	3	287 (1.98)	4
Manganese peroxidase	61 (2.88)	3	86 (2.06)	4	81 (0.98)	9	228 (1.57)	5
Hydrolase	20 (0.94)	11	59 (1.41)	7	137 (1.67)	5	216 (1.49)	6
Lipase	23 (1.08)	8	61 (1.46)	6	102 (1.24)	7	186 (1.28)	7
Lignin peroxidase	57 (2.69)	4	47 (1.13)	9	51 (0.62)	17	155 (1.07)	8
Monooxygenase	21 (0.99)	10	37 (0.89)	13	94 (1.14)	8	152 (1.05)	9
Esterase	16 (0.75)	15	36 (0.86)	15	77 (0.94)	10	129 (0.89)	10

Table 9: Top ten most studied substrates in the research area of enzymatic biodegradation.

Substrate	1989-2003 (%)		2004-2013 (%)		2014-2023 (%)		Total (%)	
	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank
Dye	32 (1.51)	4	187 (4.48)	1	233 (2.83)	1	452 (3.11)	1
Polycyclic aromatic hydrocarbons (PAHs)	19 (0.90)	11	123 (2.95)	2	216 (2.63)	2	358 (2.46)	2
Cellulose	33 (1.56)	2	50 (1.20)	8	169 (2.05)	4	252 (1.73)	3
Plastics	6 (0.28)	34	26 (0.62)	20	198 (2.41)	3	230 (1.58)	4
Phenol	32 (1.51)	5	74 (1.77)	4	115 (1.40)	7	221 (1.52)	5
Lignin	41 (1.93)	1	55 (1.32)	6	121 (1.47)	6	217 (1.49)	6
Pesticide	9 (0.42)	26	45 (1.08)	10	161 (1.96)	5	215 (1.48)	7
Polyesters	33 (1.56)	3	81 (1.94)	3	92 (1.12)	9	206 (1.42)	8
Polyurethane	25 (1.18)	9	63 (1.51)	5	77 (0.94)	12	165 (1.14)	9
Polyethylene (PE)	10 (0.47)	24	20 (0.48)	30	112 (1.36)	8	142 (0.98)	10

citing it during 1989-2003, 2004-2013 and 2014-2023 respectively. The study on *Bacillus* sp. increases significantly from 0.62% (8th rank) during 1989-2003 to 2nd rank with 1.92% and 2.97% during 2004-2013 and 2014-2023 respectively. Similarly, the study on *Trichoderma* sp. increased from 18th rank during 1989-2003 to 11th and 7th during 2004-2013 and 2014-2023 respectively. On the other hand, the study of *Phanerochaete* sp. decreases gradually from 2nd rank with 2.78% during 1989-2003 to 3rd with 1.36% and 8th with 0.52% during 2004-2013 and 2014-2023 respectively. The study on *Aspergillus* sp., *Rhodococcus* sp. and *Penicillium* sp., significantly increased during 2004-2013 while that of *Pleurotus* sp., *Trametes* sp. and *Streptomyces* sp., essentially stayed the same throughout all three phases.

Enzymes

Laccase is the most studied enzyme with a total citation of 822 or 5.66% of total publications. The study increased significantly from

3.54% (2nd rank) during 1989-2003 to 6.56% in 2004-2013 and 5.75% in 2014-2023 (Table 8). Though peroxidase (includes all peroxidases viz. manganese peroxidase, lignin peroxidases, etc.) was the second most studied enzyme with a total citation of 693 (4.77% of total publications), its study decreased gradually from 7.54% citations in 1989-2003 to 5.32% in 2004-2013 and 3.78% in 2014-2023. Similarly, the study on dioxygenase, manganese peroxidase and lignin peroxidase decreased gradually over time. On the other hand, studies on lipase, hydrolase, oxidoreductase, monooxygenase and esterase, either remain the same or are increasing.

Substrates

Dye, including azo dye, is the most studied substrate in the field of enzymatic bioremediation with a total of 452 documents (Table 9). Though the number of documents increases with time, the frequency was highest during 2004-2013 (4.48%) followed by

Table 10: Top ten most studied techniques in the research area of enzymatic biodegradation.

Words	1989-2003		2004-2013		2014-2023		Total	
	n (%)	Rank	n (%)	Rank	n (%)	Rank	n (%)	Rank
Immobilization (all types)	20 (0.94)	1	92 (2.20)	1	339 (4.12)	1	451 (3.10)	1
Engineering (tissue, protein, metabolic, genetic, etc.)	11 (0.52)	6	36 (0.86)	8	150 (1.82)	2	197 (1.36)	2
Fermentation (all types)	11 (0.52)	7	57 (1.36)	3	121 (1.47)	3	189 (1.30)	3
Bioreactor (all types)	20 (0.94)	2	65 (1.56)	2	97 (1.18)	6	182 (1.25)	4
Mass spectrometry (all types)	10 (0.47)	8	42 (1.01)	5	120 (1.46)	4	172 (1.18)	5
Modeling (all types)	12 (0.56)	5	36 (0.86)	7	82 (0.99)	7	130 (0.89)	6
Purification (all types)	16 (0.75)	4	47 (1.12)	4	58 (0.70)	15	121 (0.83)	7
Immobilized cells/enzymes/microbes	17 (0.80)	3	39 (0.93)	6	63 (0.76)	12	119 (0.82)	8
Metagenomics	0 (0)	--	12 (0.28)	27	106 (1.23)	5	118 (0.81)	9
PCR techniques	9 (0.42)	9	30 (0.72)	11	62 (0.75)	14	101 (0.69)	10

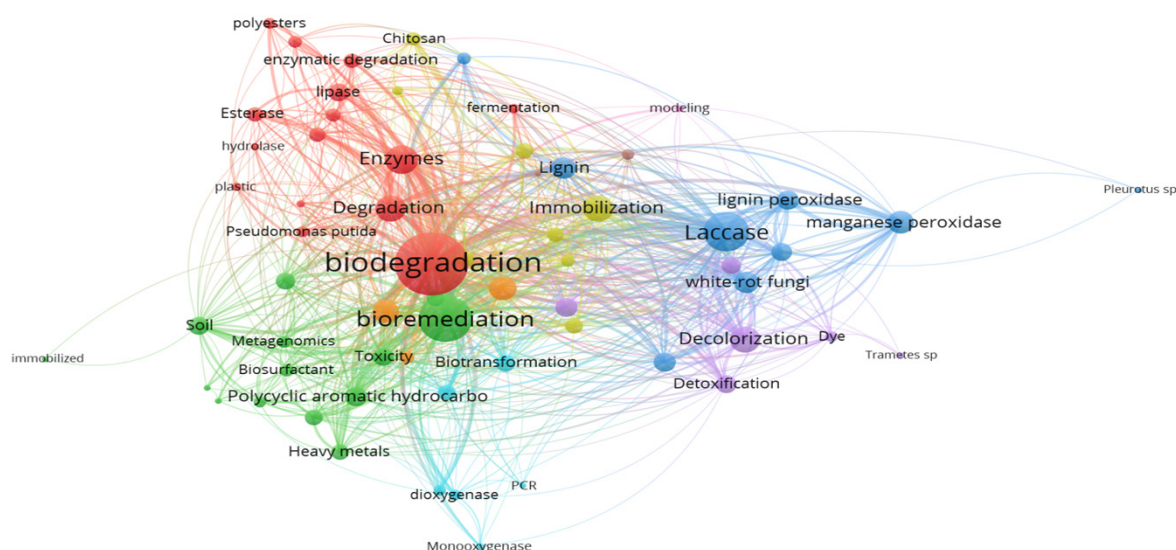


Figure 4: Co-occurrence network of author keywords showing the prominent research area of enzymatic biodegradation.

2014-2023 (2.83) and 1989-2003 (1.51%) respectively. Similarly, the study on Polycyclic aromatic hydrocarbons (PAHs), Phenol, Polyesters and Polyurethane were all highest during the 2004-2013 period. On the other hand, the studies on Plastics (including bioplastics, microplastics, thermoplastics, etc.,) Pesticides and Polyethylene (PE) significantly increased with time and occupied the 3rd, 5th and 8th place among the most studied substrates during 2014-2023 from their respective 34th, 26th and 24th place during 1989-2003. Lignin and cellulose were the most studied substrates during the first phase of enzymatic biodegradation studies and were ranked first and second respectively. While lignin remained the 6th most studied substrate in the later phases, cellulose rose from 8th rank during 2014-2013 to 4th rank in 2014-2023.

Techniques

Compared to 1989-2003, there has been a notable surge in the application of all techniques during 2004-2013, with immobilization techniques being the most extensively researched approach throughout all study periods (Table 10). Other techniques related to engineering, mass spectrometry and metagenomics have been used much more frequently over the past decades, while bioreactor, purification and immobilized cells/enzymes/microbes have become less common. The study on the remaining three techniques viz. fermentation, modelling and PCR techniques remain roughly the same.

Keywords co-occurrence

Keywords with a minimum occurrence of 100 were selected for the analysis of co-occurrence (Figure 4). The size of a keyword indicates the frequency of occurrence and the distance between two keywords gives an idea about the relationship between them. A total of 67 keywords qualify the cut-off of which 64 meet the threshold resulting in 9 clusters. Biodegradation has the maximum link (62), total link strength (1806) and user-defined weight (3206) followed by bioremediation with 59 links, 1014 total link strength and 1440 user-defined weight (Table S5, Supplementary data). Laccase is the most studied enzyme with 49 links, 727 total link strength and 818 user-defined weights followed by Peroxidase (30 links, 116 total link strength and 709 user-defined weight) and Manganese peroxidase (29 links, 230 total link strength and 228 user-defined weight). *Phanerochaete chrysosporium* topped the list among microbial species with 27 links and 149 total link strength followed by *Pseudomonas* sp. and *Bacillus* sp., Among substrates, lignin has the maximum number of links (33) and total link strength (207) followed by Polycyclic aromatic hydrocarbons (32 and 165) and Wastewater (31 and 172). Immobilization leads the techniques with 41 links and 331 total link strength followed by Bioreactor with 27 links and 70 total link strength.

DISCUSSION

Enzymes are central to any biotransformation process and thus play an important role in the biodegradation of any toxic pollutants in the environment. Over the past two decades, the focus of bioremediation research has gradually shifted from traditional microbial methods to more specific and effective enzymatic bioremediation. This is supported by the fact that the number of documents about enzymatic biodegradation and bioremediation increases exponentially during these periods (Figure 1). Other factors like a better understanding of enzyme biochemistry, the development of various techniques and a rise in the number of environmental pollutants might also contribute to the increased number of studies. The data also indicates that one in every five documents has international co-authorship suggesting significant research collaborations among the authors. This is good for the research as it facilitates efficient resource exchanges among authors in different countries who are all working toward the same objective. Environmental pollution was never or will ever be the problem of a single nation. The best examples the global warming and climate change.

Every journal has its specific aims and scopes which govern the field of its publications. To be published in the journal, a document must adequately fit within its subject scope or contribute to its editorial goals. All the journals in the top 10 most relevant sources (Table 1) have common scopes in bioremediation, microbial technology, enzymology, etc. Most of these journals started their publication well before 1989 which could account for their higher number of documents. For instance, Applied and Environmental Microbiology which topped the list has only 12.35 average publications per year lower than 17.46 of Frontiers in Microbiology which started its publication in 2010. In addition, Applied and Environmental Microbiology topped the list only in the first phase i.e. during 1989-2003 and were placed 4th and 9th during 2004-2013 and 2014-2023, respectively (Table S1, Supplementary data). Applied and Environmental Microbiology also have the most local citations (35569), which might be because it has the highest total number of publications both overall and in the early stages of the study. On the other hand, despite having fewer documents, Bioresource Technology (20089) and Applied Microbiology and Biotechnology (16328) are among the top cited sources in the field indicating that the aim and scopes of these sources are more pertinent to the topic of enzymatic bioremediation.

The number of citations received by the documents indicates its impact in the field of research and the number of citations tends to increase with the number of citable years. Out of the top 50 most cited documents (Table S2; Supplementary data), 17 belong to the early phase of the study i.e. 1989-2003, while 22 belong to 2004-2013 and the remaining 11 are from 2014-2023. However, the most cited document is a research article published in 2016 about a novel bacterium, *Ideonella sakaiensis* 201-F6 and two

enzymes involved in hydrolysis of Poly (Ethylene Terephthalate) (PET) to terephthalic acid and ethylene glycol.^[29] Even though studies on plastic biodegradation started only during the last two decades, five of the top ten most cited documents are on plastic biodegradation (Table 2). This emphasizes the seriousness of plastic pollution and the importance of studying the biodegradation of plastic. The discovery of the PETase enzyme from *Ideonella sakaiensis* strain 201-F6 in 2016 led to substantial increases in the enzymatic biodegradation study of plastics.^[27-29] PAHs, one of the most studied pollutants and white-rot fungi are other widely cited topics in the field of enzymatic biodegradation. White-rot fungi which include *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor* and their extracellular oxidative enzymes-laccases, manganese peroxidases and lignin peroxidases are among the most studied microbes and enzymes for the biodegradation of many xenobiotic compounds including aromatic amines, phenolic compounds such as chlorophenols, secondary aliphatic polyalcohols, Polycyclic Aromatic Hydrocarbons (PAHs), herbicides, pesticides, etc.,^[30-32]

The most influential author in the field of enzymatic bioremediation is Sanjay P. Govindwar, who has an h-index of 41 from 82 publications and 5672 total citations (Table 3). He has worked in the area of bioremediation/phytoremediation of dyes and textile effluent. His work primarily focuses on the mechanism of degradation of textile dyes using bacteria, fungi and plants and their interactions, as well as the elucidation of enzymatic pathways using purified oxidoreductases, such as lignin peroxidase, polyphenol oxidase and laccase.^[33-35] J. Paul Santerre, the second most prominent author, studied how the body breaks

down polymers-polyurethanes, polymethyl methacrylate and polyethylene-using enzymes to develop new materials for tissue engineering, implants and medical equipment.^[36-38] Guangming Zeng's (Zeng GM) research includes the development of biosensors and bioimaging for environmental pollutants such as PAHs, heavy metals, lignin, wastewater, carbon nanotubes, etc. He has purified and studied various enzymes like laccases, Lignin peroxidase (Lip), Manganese Peroxidase (MnP), Laccase-like Multicopper Oxidases (LMCOs), etc. from different organisms viz. *Phanerochaete chrysosporium*, *Trichoderma viride*, *Aspergillus niger*, *Streptomyces* sp., etc.,^[39-41] Spain, Jim C., the fourth most influential author works on environmental biotechnology to eliminate environmental pollutants with an emphasis on the discovery and characterization of bacteria and novel enzymes that catalyze the degradation of organic pollutants.^[42,43]

Co-authorship network analysis shows that the number of links and total link strength of most authors are above the average of the whole network i.e. 36 and 123, respectively. This indicates that these authors have significant research collaboration. Wang Y with 43 links and 223 total link strength, ranked second in the overall list (Table S3, Supplementary data) and is linked with almost all the authors in Table 3 viz. Govindwar SP, Zeng G, Spain JC, Jadhav JP, Liu Y, Zhang J and Labow RS and thus, act as connecting links between these authors (Figure 5). The software's limitations, which prevented it from differentiating between authors with the same initials and surname, such as Yue Wang (University of Amsterdam), Yu-Zhong Wang (Sichuan University), Ya-Wu Wang (Tsinghua University), Yingying Wang

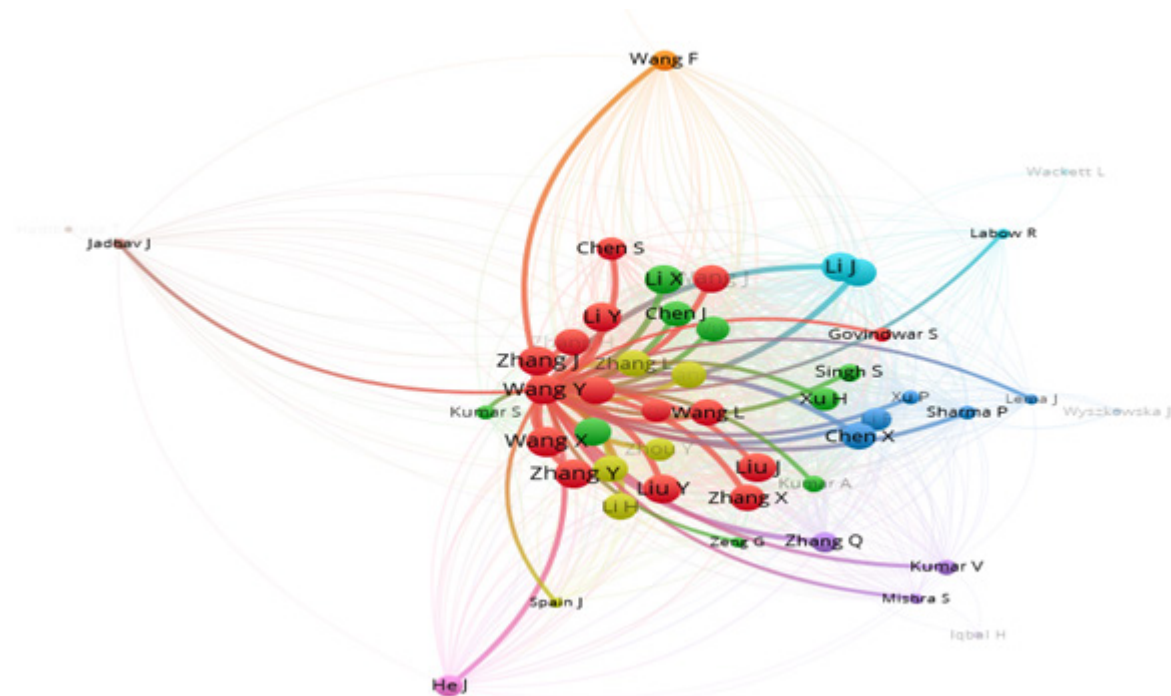


Figure 5: Author collaboration network of Wang Y.

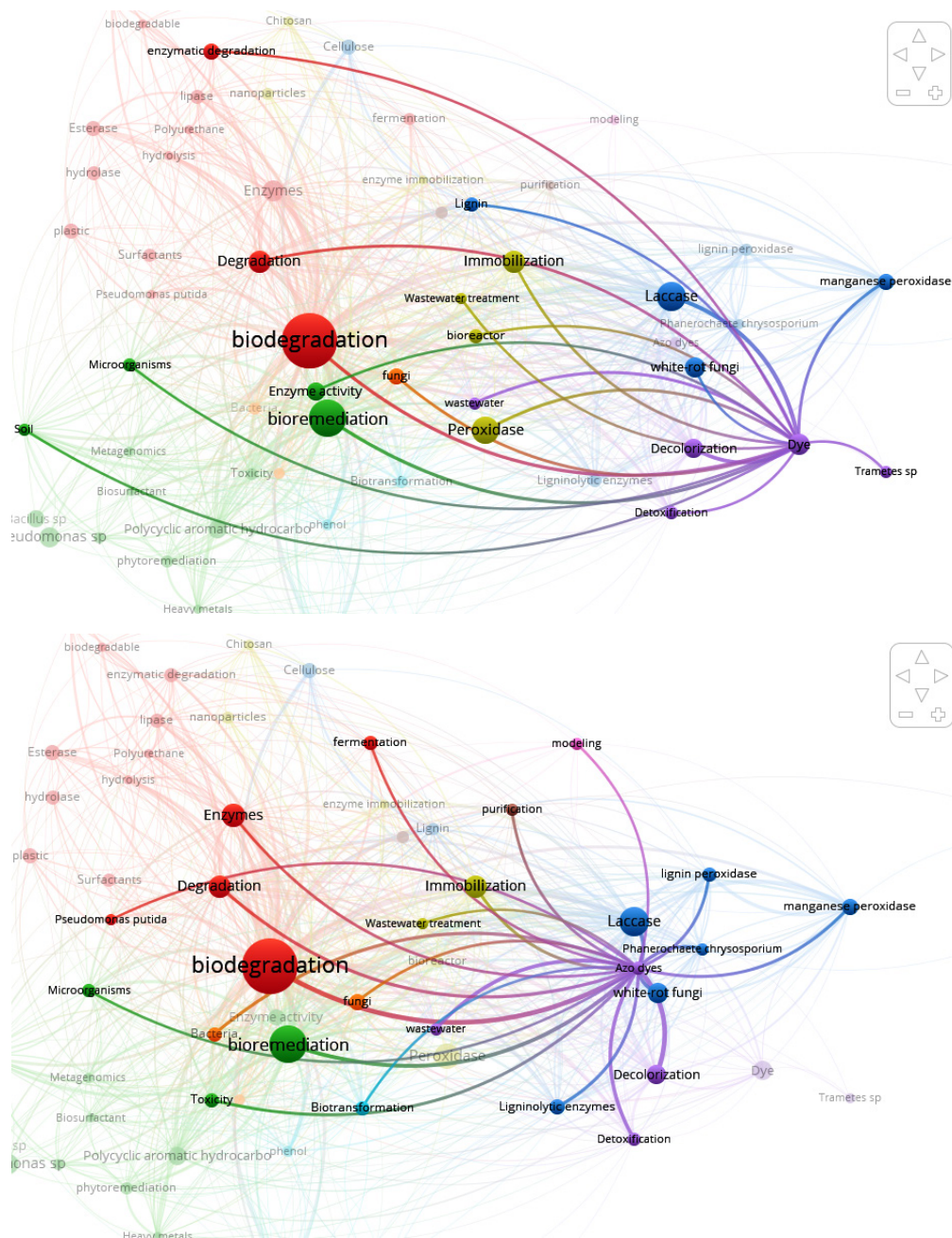


Figure 7: Keyword co-occurrence network of A. Dye and B. Azo dye.

decreases in several pollution-related problems in these countries resulting in a focus shift to some other research topics.^[45] On the other hand, being developing countries, India and Brazil continuously face pollution problems.^[46]

Number of citations indicates the overall influence and impact of the publication in the research field. China topped the list of countries with the highest number of citations followed by the USA and India (Table 6). However, China has the lowest average article citations followed by India and Italy. Therefore, the higher number of citations for China could be attributed to their higher number of publications and does not indicate the impact of the

publications in the research field. In contrast, the USA, Germany and Spain have published a greater number of influential studies on the subject of enzymatic bioremediation despite their relatively smaller publication counts. This is corroborated by the fact that, of the 50 most cited articles, eight of the corresponding authors are affiliated with India; six are from the United States; five are from Canada and Japan; four are from Germany and Spain; two are from China (including Hong Kong SAR); and one each from Austria, Belgium, Brazil, Czech Republic, Finland, France, Italy, Netherlands, Poland and the United Kingdom (Table S2, Supplementary data).

Keyword Co-occurrence analysis can be used to identify current topics of the research area. It also gives a direction to detect and carry out the advances in scientific research.^[47] White-rot fungi-*Phanerochaete* sp., *Pleurotus* sp. and *Trametes* sp., are among the top 10 most studied microbes in the field of enzymatic bioremediation along with three other fungal species-*Aspergillus* sp., *Trichoderma* sp. and *Penicillium* sp., Similarly, fungal enzymes viz. laccases, peroxidase, oxidoreductase, manganese peroxidase and lignin peroxidases are also among the top 10 studied enzymes. Laccase, a multicopper enzyme, found in both white-rot fungi and bacteria, is the most studied enzyme. Due to its ability to oxidize phenolic and non-phenolic substances, it has been used in the fields of biotechnology to synthesize anti-cancer drugs and cosmetic ingredients and detoxify industrial effluents from the pulp, paper, textile and petrochemical industries. Due to its low specificity, it has been used in the bleaching and finishing of jeans, delignification of paper, color enhancement in tea, oxidants in bakery products, as well as in biosensors, cosmetics, organic synthesis and bioremediation.^[48,49] Except for *Trichoderma* sp., which is the most prevalent culturable fungi, the frequency of study on other fungal species either decreases or remains more or less constant with time.^[50] On the other hand, a study on two bacterial species viz. *Bacillus* sp. and *Rhodococcus* sp., significantly increased during the last two decades, while the frequency of study on *Pseudomonas*, which remain the most studied microbes throughout the study period and *Streptomyces* sp. remains relatively constant (Table 7). Among the microbes, *Phanerochaete chrysosporium* has the highest number of links (27) and is strongly linked to ligninolytic enzymes, manganese peroxidase, lignin peroxidase, peroxidase and laccase. On the other hand, *Pseudomonas putida* has been linked to hydrolase, lipase and laccase (Figure 6). In general, research on bacterial enzymes such as lipase, hydrolase, oxidoreductase, monooxygenase and esterase has significantly increased over time, while research on fungal enzymes such as lignin peroxidase, manganese peroxidase and peroxidase gradually decreased. Therefore, there are strong signals that the focus of enzymatic bioremediation research is gradually moving from fungal enzymes to bacterial enzymes. This could be because bacteria being prokaryotes are easier to culture and study compared to fungus. Moreover, bacterial enzymes are more convenient for isolation and manipulation using genetic engineering tools. Besides, most of the fungal enzymes belong to the oxidoreductase family and require co-factors which limits their applicability in the bioremediation techniques. On the other hand, bacterial enzymes include hydrolases such as esterase and lipase which increase their applicability making them economically favorable.

The frequency of the substrate can be directly correlated to the importance of the substrates during the study period. Dye, including azo dye, is the overall most studied substrates which rose from 4th rank during 1989-2003 to first from 2004 onwards (Table 9). Industrialization has led to massive growth in the usage

of dyes in a variety of industries, including food, paper, cosmetics, textiles and leather. Since the dyes in industrial effluent are often less reactive to oxygen, acids and bases, bioremediation is the most effective way to address its pollution.^[51,52] The keyword "dye" is linked to 20 other keywords and is primarily associated with fungus and their enzymes, whereas "azo dye," which has 24 total links, is associated with both bacteria and fungi (Figure 7). These suggest that both microbes and their enzymes have been investigated for the biodegradation of the compound.

Similarly, Polycyclic Aromatic Hydrocarbons (PAHs), which were ranked 11th during 1989-2003, moved up to the second spot from 2004 onwards. Emerging pollutants which though realized much later, are extremely harmful to the environment like plastics and pesticides also rose from 34th and 26th rank during 1989-2003 to 3rd and 5th during 2014-2023 respectively. These substrates are also linked with bacteria and fungi, as well as their enzymes, suggesting their involvement in the biodegradation of the contaminants.

Immobilization remained the most used technique throughout the study period, however, the use of immobilized cells/enzymes/microbes decreased gradually over time. An immobilized enzyme is more stable, reusable, controlled quickly, easily separated from the result and enables the creation of a multi-enzyme reaction system. Since its discovery in 1916, the techniques of enzyme immobilization have evolved from simple absorption on a solid surface to various covalent methods, physical entrapment, encapsulation, cross-linking, etc.,^[53] Other techniques viz. engineering, mass spectrometry and metagenomics also increases significantly over the last decades indicating the advent of genetic engineering to modify and optimize enzymes for increased efficiency and specificity. Enzyme engineering approaches increase a wide range of enzyme properties, such as the interactions between enzymes and their substrates, the robustness of enzymes under non-physiological conditions and the introduction of new chemical reactivities.^[54] Advances in metagenomics and functional genomics allowed scientists to explore entire microbial communities for novel enzymes involved in bioremediation. This holistic approach expanded the range of target pollutants and increased the overall efficiency of enzymatic bioremediation.^[55] More recent techniques like metagenomics, are linked to bacteria and their enzymes including esterase, laccase, dioxygenase, etc., while more established techniques like immobilization and bioreactor are linked to both bacteria and fungi (Figure 3). This also supports the fact that the focus of enzymatic bioremediation is gradually shifting towards bacterial enzymes.

Keyword cluster analysis is a way to group related keywords into smaller clusters with similar meanings.^[56] In the keyword co-occurrence visualization map, each node represents a keyword and the size of the node denotes the frequency of its occurrence i.e., the larger the size of the node, the higher the frequency of

occurrence and vice versa. Nine distinct clusters based on cluster analysis were found, indicating how a variety of different methods and enzymes collaborate in the field of biodegradation (Table S5; Supplementary data). Cluster 1 and 3 explore various types of enzymes used in the research area of enzymatic biodegradation. This cluster gives information about the microorganism used for the isolation of the degrading enzymes. As we know, substrates are essential to all enzyme-catalyzed processes; this cluster also includes a summary of the essential substrates for enzymatic bioremediation research. Cluster 2 indicates that the field of enzymatic biodegradation is not limited to the isolation of enzymes from microbes; we can also explore enzymatic biodegradation using metagenomics techniques. These days, there is a lot of research being done on heavy metal pollution in water and soil environments. As of yet, no long-term fix for this issue has been found. Enzymatic biodegradation, which has recently been used in heavy metal bioremediation studies, offers fresh promise for solving the issue.^[57] Enzymatic biodegradation is also linked with phytoremediation research for faster results.

Clusters 4 and 6 explore various techniques widely used in the field of enzymatic bioremediation. Bioreactor, Enzyme immobilization, Nanoparticles and PCR, are the keywords that represent different techniques helpful in the enzymatic biodegradation of various harmful and xenobiotic compounds. Clusters 5 and 7 give an idea about various sectors in which this technique has been used i.e. pesticide degradation, catalyst in decolorization of an azo dye, wastewater treatment, etc. Cluster 8 and 9 are the new research areas that are currently flourishing in enzymatic biodegradation studies. Recently, modeling and simulation have gained popularity as an alternative to labor-intensive and expensive lab investigations. These in-silico bioinformatics techniques are used to gain insight into the relationship between enzymes and substrates, their binding affinity, reaction mechanism, rates, pathways and final product, etc.,^[47,58,59]

CONCLUSION

Due to its huge potential for environmental clean-up, enzymatic bioremediation receives huge attention. This is evident from the fact that considerable research has been conducted globally. However, many factors including the stability of enzymes under harsh environmental conditions, high specificity towards particular substrates, high cost of production and scale-up challenges hinder its application. Therefore, there have been continuous efforts to identify, isolate and manipulate new enzymes capable of degrading many pollutants. The most studied enzyme is laccase, a multicopper oxidase that is found in many plants, fungi and bacteria. Peroxidases which include manganese peroxidase, lignin peroxidase, etc. and bacterial dioxxygenase enzymes are also among the most studied enzymes. *Pseudomonas* sp., *Bacillus*

sp. and the white rot fungi, *Phanerochaete chrysosporium* are the most studied microbes in the field of enzymatic bioremediation. The majority of fungal enzymes, which are members of the oxidoreductase family and need co-factors, are less suited for bioremediation than bacterial enzymes like hydrolases and esterases. This could be the reason why there is a clear shift in the focus of enzymatic bioremediation from fungal to bacterial enzymes.

Emerging pollutants such as plastics and pesticides are gradually receiving increased attention in enzymatic bioremediation. This is corroborated by the fact that a research article on the biodegradation of Poly (ethylene terephthalate) by Yoshida S.^[23] is the most locally cited document while Santerre JP whose work is on the biodegradation of polymers including polyurethane and polyethylene is the second most locally impactful authors. Other pollutants like dye, PAHs, heavy metals and pesticides, also received significant attention in the field. The emergence of new techniques such as metagenomics, genetic engineering, molecular docking, simulations, etc. has facilitated the discovery of novel enzymes and modified the enzymes to increase their stability, specificity and applicability. Also, there has been considerable enhancement in the already established techniques such as immobilization, bioreactors and fermentations and their usage is still prevalent at present.

Among the countries, China has emerged as a top-performing country during the last two decades. However, it still lacks quality in research evident from the fact that it has the least average article citations. The USA on the other hand remains the most impactful country in the research and has a maximum number of collaborations. Research quality is directly correlated with a nation's level of development; countries such as Brazil and India still struggle to do high-quality, effective research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Supplementary Tables

Table S1: List of top 10 most relevant sources during three different phases of study.

1989-2003	2004-2013	2014-2023
Applied and Environmental Microbiology (<i>n</i> =169).	Bioresource Technology (<i>n</i> =131).	Journal of Hazardous Materials (<i>n</i> =268).
Applied Microbiology and Biotechnology (<i>n</i> =71).	International Biodeterioration and Biodegradation (<i>n</i> =131).	Chemosphere (<i>n</i> =267).
Biotechnology and Bioengineering (<i>n</i> =47).	Applied Microbiology and Biotechnology (<i>n</i> =129).	Frontiers in Microbiology (<i>n</i> =221).
Enzyme and Microbial Technology (<i>n</i> =43).	Applied and Environmental Microbiology (<i>n</i> =111).	Environmental Science and Pollution Research (<i>n</i> =207).
Polymer Degradation and Stability (<i>n</i> =39).	Biodegradation (<i>n</i> =104).	Science of the Total Environment (<i>n</i> =201).
Applied Biochemistry and Biotechnology (<i>n</i> =36).	Journal of Hazardous Materials (<i>n</i> =95).	International Biodeterioration and Biodegradation (<i>n</i> =197).
Biodegradation (<i>n</i> =36).	Chemosphere (<i>n</i> =90).	Bioresource Technology (<i>n</i> =173).
Journal of Applied Polymer Science (<i>n</i> =31).	Polymer Degradation and Stability (<i>n</i> =77).	Ecotoxicology and Environmental Safety (<i>n</i> =150).
FEMS Microbiology Letters (<i>n</i> =30).	World Journal of Microbiology and Biotechnology (<i>n</i> =75).	Applied and Environmental Microbiology (<i>n</i> =140).
Biomaterials (<i>n</i> =29).	Enzyme and Microbial Technology (<i>n</i> =64).	Applied Microbiology and Biotechnology (<i>n</i> =136).

Table S2: List of top 50 most locally cited documents.

Document	C	LC	GC	LC/GC Ratio (%)	Nor. LC	Nor. GC
YOSHIDA S, 2016, SCIENCE; 10.1126/science.aad6359	Japan	278	1181	24	58	38
HARITASH AK, 2009, J HAZARD MATER; 10.1016/j.jhazmat.2009.03.137	India	270	1981	14	34	37
WARIISHI H, 1992, J BIOL CHEM	USA	244	892	27	16	9
WESENBERG D, 2003, BIOTECHNOL ADV; 10.1016/j.biotechadv.2003.08.011	Belgium	238	800	30	28	11
POINTING SB, 2001, APPL MICROBIOL BIOT	Hong Kong SAR	205	626	33	27	10
SHAH AA, 2008, BIOTECHNOL ADV; 10.1016/j.biotechadv.2007.12.005	Pakistan	182	1398	13	25	23
TOKIWA Y, 2009, INT J MOL SCI; 10.3390/ijms10093722	Japan	150	845	18	19	16
AUSTIN HP, 2018, P NATL ACAD SCI USA; 10.1073/pnas.1718804115	USA	146	448	33	32	15
MARTINEZ AT, 2005, INT MICROBIOL	Spain	138	782	18	17	10
DURAN N, 2000, APPL CATAL B-ENVIRON; 10.1016/S0926-3373(00)00168-5	Brazil	133	661	20	15	8
SARATALE RG, 2011, J TAIWAN INST CHEM E; 10.1016/j.jtice.2010.06.006	India	132	980	13	23	24
SINGH BK, 2006, FEMS MICROBIOL REV; 10.1111/j.1574-6976.2006.00018.x	United Kingdom	131	716	18	16	12

Document	C	LC	GC	LC/GC Ratio (%)	Nor. LC	Nor. GC
WEI R, 2017, MICROB BIOTECHNOL; 10.1111/1751-7915.12710	Germany	130	363	36	26	11
MAYER AM, 2002, PHYTOCHEMISTRY; 10.1016/S0031-9422(02)00171-1	Israel	124	1013	12	16	15
HARMS H, 2011, NAT REV MICROBIOL; 10.1038/nrmicro2519	Germany	124	575	22	22	14
LUCAS N, 2008, CHEMOSPHERE; 10.1016/j.chemosphere.2008.06.064	France	122	700	17	17	12
RIVA S, 2006, TRENDS BIOTECHNOL; 10.1016/j.tibtech.2006.03.006	Italy	120	921	13	14	15
MULLER RJ, 2005, MACROMOL RAPID COMM; 10.1002/marc.200500410	Germany	112	298	38	14	4
ASGHER M, 2008, BIODEGRADATION; 10.1007/s10532-008-9185-3	Pakistan	110	324	34	15	5
GHOSAL D, 2016, FRONT MICROBIOL; 10.3389/fmicb.2016.01369	South Korea	105	665	16	22	21
CANAS AI, 2010, BIOTECHNOL ADV; 10.1016/j.biotechadv.2010.05.002	Spain	101	499	20	16	10
MAJEAU JA, 2010, BIORESOURCE TECHNOL; 10.1016/j.biortech.2009.10.087	Canada	93	330	28	15	7
FERNANDEZ-FERNANDEZ M, 2013, BIOTECHNOL ADV; 10.1016/j.biotechadv.2012.02.013	Spain	92	462	20	16	11
FIELD JA, 1992, APPL ENVIRON MICROB; 10.1128/AEM.58.7.2219-2226.1992	Netherlands	91	300	30	6	3
PANDEY A, 2007, INT BIODETER BIODEGR; 10.1016/j.ibiod.2006.08.006	India	90	812	11	14	16
KAUSHIK P, 2009, ENVIRON INT; 10.1016/j.envint.2008.05.010	India	90	428	21	11	8
SPAIN JC, 1995, ANNU REV MICROBIOL; 10.1146/annurev.mi.49.100195.002515	USA	89	574	16	12	11
SANTO M, 2013, INT BIODETER BIODEGR; 10.1016/j.ibiod.2012.03.001	Israel	89	194	46	16	5
NOVOTNY C, 2004, SOIL BIOL BIOCHEM; 10.1016/j.soilbio.2004.07.019	Czech Republic	88	197	45	10	3
KADRI T, 2017, J ENVIRON SCI; 10.1016/j.jes.2016.08.023	Canada	88	223	39	18	7
KALYANI DC, 2008, BIORESOURCE TECHNOL; 10.1016/j.biortech.2007.06.058	India	85	232	37	12	4
TANIGUCHI I, 2019, ACS CATAL; 10.1021/acscatal.8b05171	Japan	85	242	35	22	10
OLLIKKA P, 1993, APPL ENVIRON MICROB; 10.1128/AEM.59.12.4010-4016.1993	Finland	83	265	31	11	4
AKUTSU Y, 1998, APPL ENVIRON MICROB	Japan	82	146	56	11	3
LEONOWICZ A, 1999, FUNGAL GENET BIOL; 10.1006/fgbi.1999.1150	Poland	82	313	26	11	7
MARGESIN R, 2000, CHEMOSPHERE; 10.1016/S0045-6535(99)00218-0	Austria	82	344	24	9	4

Document	C	LC	GC	LC/GC Ratio (%)	Nor. LC	Nor. GC
SON HF, 2019, ACS CATAL; 10.1021/acscatal.9b00568	Republic of Korea	82	188	44	22	8
SHIMAO M, 2001, CURR OPIN BIOTECH; 10.1016/S0958-1669(00)00206-8	Japan	80	327	24	10	5
MARTEN E, 2005, POLYM DEGRAD STABIL; 10.1016/j.polymdegradstab.2004.12.001	Germany	80	193	41	10	2
RESTREPO-FLOREZ JM, 2014, INT BIODETER BIODEGR; 10.1016/j.ibiod.2013.12.014	Canada	80	333	24	17	10
KALME SD, 2007, BIORESOURCE TECHNOL; 10.1016/j.biortech.2006.05.023	India	78	249	31	12	5
SPAIN JC, 1991, APPL ENVIRON MICROB; 10.1128/AEM.57.3.812-819.1991	USA	77	315	24	10	5
ARCHIBALD FS, 1992, APPL ENVIRON MICROB; 10.1128/AEM.58.9.3110-3116.1992	Canada	77	230	33	5	2
HOWARD GT, 2002, INT BIODETER BIODEGR; 10.1016/S0964-8305(02)00051-3	USA	77	454	17	10	7
ALCALDE M, 2006, TRENDS BIOTECHNOL; 10.1016/j.tibtech.2006.04.002	Spain	77	276	28	9	4
SINGH RL, 2015, INT BIODETER BIODEGR; 10.1016/j.ibiod.2015.04.027	India	77	344	22	17	10
SHARMA B, 2018, J ENVIRON MANAGE; 10.1016/j.jenvman.2017.12.075	India	77	238	32	17	8
MOHANAN N, 2020, FRONT MICROBIOL; 10.3389/fmicb.2020.580709	Canada	77	235	33	23	11
EGGERT C, 1996, FEBS LETT; 10.1016/0014-5793(96)00719-3	USA	76	342	22	11	6
GU JD, 2003, INT BIODETER BIODEGR; 10.1016/S0964-8305(02)00177-4	China	75	436	17	9	6
C=Country; LC=Local citations; GC=Global citations; Nor.=Normalized; Shaded=Research Articles.						

Table S3: Number of links, total link strength and user-defined weight of 50 top authors in the Co-Authorship network.

Clusters	Authors	Link	Total link strength	User-defined weight
1	Chen S	40	149	13
	Govindwar S	38	50	4
	Li Y	43	233	21
	Liu J	42	156	21
	Liu Y	43	201	21
	Wang H	43	201	19
	Wang J	42	183	19
	Wang L	43	188	17
	Wang W	42	170	13
	Wang X	43	206	23
	Wang Y	43	223	22
	Zhang H	43	193	18
	Zhang J	43	199	21
	Zhang X	42	161	18
	Zhang Y	43	201	22
2	Chen J	42	138	16
	Kumar A	42	76	6
	Kumar S	38	67	4
	Li S	42	186	22
	Singh S	41	110	7
	Wu J	42	139	15
	Xu H	41	91	13
	Zeng G	36	37	2
	Zhang W	43	162	19
3	Chen X	44	182	17
	Lema J	39	43	2
	Li F	44	168	12
	Sharma P	42	104	4
	Wyszkowska J	5	5	1
	Xu P	43	109	4
4	Li H	43	152	17
	Spain J	25	26	2
	Yang Y	43	173	18
	Zhang C	43	169	17
	Zhang L	43	172	17
	Zhou Y	43	164	13
5	Iqbal H	3	3	1
	Kumar V	40	48	5
	Mishra S	38	39	2
	Zhang Q	44	177	9

Clusters	Authors	Link	Total link strength	User-defined weight
6	Labow R	39	41	2
	Li J	44	206	21
	Wackett L	3	3	1
	Wang Z	44	186	19
7	Bhatt P	1	1	1
	Wang F	43	130	10
8	Hadibarata T	1	1	1
	Jadhav J	31	31	2
9	Bhatti H	1	1	1
	Hi J	44	129	11
Average		36.66	123.66	11.72

Table S4: Number of links, total link strength and user-defined weight of 50 top countries in the country-collaboration network.

Cluster	Country	Link	Total link strength	User-defined weight
1	Austria	34	123	123
	Belgium	35	92	97
	Bulgaria	13	29	67
	Czech Republic	30	115	226
	Denmark	36	171	142
	Finland	30	84	107
	France	45	306	438
	Germany	44	555	761
	Greece	25	80	102
	Hungary	24	50	55
	Iran	31	116	275
	Ireland	27	76	68
	Israel	16	35	71
	Italy	45	321	533
	Netherlands	39	219	187
	Poland	42	179	457
	Portugal	28	112	170
	Romania	15	28	90
	Russia	21	98	261
	Serbia	13	32	72
	Sweden	36	166	148
	Switzerland	30	150	166
	Tunisia	22	75	79
	Turkey	25	60	178

Cluster	Country	Link	Total link strength	User-defined weight
2	India	45	691	1837
	Indonesia	18	76	57
	Japan	40	304	809
	Malaysia	33	237	255
	Nigeria	23	105	105
	South Africa	27	78	152
	South Korea	35	366	553
	Taiwan	21	112	180
	Thailand	24	108	150
	Vietnam	23	84	53
3	Argentina	17	60	151
	Brazil	31	188	533
	Chile	31	113	137
	Colombia	17	42	58
	Mexico	29	167	274
	Spain	43	397	612
	UK	44	466	504
4	Australia	40	276	303
	Canada	42	358	549
	New Zealand	18	38	48
	Peoples R China	44	1048	3505
	Singapore	18	53	80
	USA	48	1103	2047
5	Egypt	34	227	222
	Pakistan	34	248	323
	Saudi Arabia	35	367	212

Table S5: Number of links, total link strength and user-defined weight of 64 top authors keywords in the Keyword co-occurrence network.

Cluster	Keyword	Link	Total link strength	User-defined weight
1	Biodegradable	14	64	107
	Biodegradation	62	1806	3206
	Degradation	51	319	450
	Enzymatic degradation	26	102	231
	Enzymes	47	392	510
	Esterase	19	104	281
	Fermentation	19	43	183
	Hydrolase	11	26	251
	Hydrolysis	25	103	100
	Lipase	27	147	231
	Plastic	12	37	279
	Polyesters	9	55	148
	Polyurethane	22	79	107
	Pseudomonas putida	18	55	102
	Surfactants	16	31	206

Cluster	Keyword	Link	Total link strength	User-defined weight
2	<i>Bacillus</i> sp	7	15	347
	Bioremediation	59	1014	1440
	Biosurfactants	23	70	101
	Enzyme activity	34	121	312
	Heavy metals	22	115	107
	Immobilized	2	2	148
	Metagenomics	24	74	116
	Microbial community	19	61	140
	Microorganisms	26	134	139
	Phytoremediation	25	123	174
	Polycyclic aromatic hydrocarbons	32	165	364
	<i>Pseudomonas</i> sp.	10	20	478
	Soil	32	149	126
	Toxicity	36	153	166
3	Cellulose	20	68	266
	Laccase	49	727	818
	Lignin	33	207	173
	Lignin peroxidase	27	183	163
	Ligninolytic enzymes	25	177	197
	Manganese peroxidase	29	230	228
	Phanerochaete chrysosporium	27	149	133
	<i>Pleurotus</i> sp.	3	3	143
	White-rot fungi	31	215	396
4	Biocatalysis	29	111	112
	Bioreactor	27	70	189
	Chitosan	20	86	127
	Enzyme immobilization	22	111	106
	Immobilization	41	331	457
	Nanoparticles	17	42	162
	Peroxidase	30	116	709
	Wastewater treatment	34	105	106
5	Azo dyes	24	125	153
	Decolorization	35	323	335
	Detoxification	26	162	140
	Dye	20	57	443
	<i>Trametes</i> sp.	4	4	131
	Wastewater	31	172	128

Cluster	Keyword	Link	Total link strength	User-defined weight
6	Biotransformation	37	138	168
	Dioxygenase	17	62	314
	Monooxygenase	9	28	164
	PCR	9	12	101
	Phenol	27	114	142
	Rhodococcus	18	56	146
7	Bacteria	40	237	175
	Fungi	41	269	223
	Pesticides	22	76	153
8	Mass spectrometry	8	13	172
	Purification	20	57	120
9	Modeling	11	26	128