Microbial Biosensors for Wastewater Monitoring

Subjects: Environmental Sciences

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Research on the use of microbial biosensors for monitoring wastewater contaminants is a topic that covers few publications compared to their applicability in other fields, such as biomedical research. It was possible to demonstrate the usefulness of microorganisms as components of biosensors to monitor biochemical oxygen demand (BOD), heavy metals, and inorganic contaminants in wastewater that also had a high sensitivity. Additionally, recombinant DNA techniques were shown to improve the performance of this type of biosensor and can finally be coupled to other emerging technologies, such as microbial fuel cells (MFCs). In conclusion, it was established that microbial biosensors have high acceptability and monitoring characteristics that make them a useful tool to detect low concentrations of pollutants in wastewater that can also provide results in real-time, thus generating forms of ecological safety and social responsibility in companies where wastewater is generated.

Keywords: microbial biosensors ; monitoring ; wastewater

1. Introduction

The current water demand exceeds the amount of fresh water on the planet due to rapid urbanization, accelerated growth of populations, industry, etc., which release contaminants that are distributed into aqueous systems [1][2][3]. These activities discharge a large number of harmful contaminants, which become part of the wastewater, where its composition and concentration of microorganisms, inorganic chemical products, and organic contaminants varies according to the origin of the pollutants [4]. The rapid growth of the world population endangers the water balance of ecosystems and generates a significant amount of wastewater [5]. The wastewater reaches the treatment plants (WWTP), where it is subjected to conventional mechanical and biological methods, however, the efficiency is not adequate to eliminate all of the contaminants before it is released back into the biotic and abiotic environments of the ecosystem [6][7]. For this reason, it is important to identify and monitor the constituents of these released waters since they vary over time and location, which is why new low-cost and real-time monitoring technologies are required. The ability to monitor pollutants in this way allows for the environmental impact to be minimized and the good ecological status of water bodies to be ensured [5][6]. For this reason, WWTPs play an important role in the purification of polluted waters and in monitoring how the treated waters leave the facility [8][9]. Given this, monitoring alternatives have emerged for the efficient detection of contaminants, such as biosensors.

Biosensors are devices that integrate a receiver and a transducer, through which biological or chemical reactions are measured when a signal proportional to the concentration of an analyte is generated [8][10][11]. The biological part of the biosensor can be microorganisms, antibodies, enzymes, DNA, etc., while the transducers can be electrochemical, colorimetric, optical, piezoelectric, acoustic, etc., to obtain a signal output [12][13]. Biosensors traditionally use a bioreceptor (bioelement), which is a biological molecule that binds to a transducer and generates a signal, and it is the bioreceptor that provides the specified sensitivity of the biosensor [14]. However, while having good specificity, they also have low detection limits, and research is being carried out to improve their sensitivity, such as the development of bioreceptor-free biosensors and the application of nanotechnology [14][15][16]. The increasing attention toward biosensors is due to their usefulness in different areas of science [17][18][19][20][21], in such a way that different specialized journals are dedicated to this subject [8]. Likewise, during the pandemic caused by the SARS-CoV-2 coronavirus in 2020, interest was aroused in using biosensors to detect the coronavirus in wastewater, as displayed in the Scopus database, where nine articles were published between 2020–2022 [22][23][24][25][26][27][28][29][30]. Another possible application that has gained interest is the use of biosensors for detecting minimum levels of contaminants in complex matrices such as wastewater [4].

Microbial biosensors detect a target substrate and evidence it by emitting a signal that can be quantified physiologically, electrically, or biochemically [31]. This type of biosensor has advantages in terms of low cost, unlike other methods. In addition, microorganisms can be large quantities produced in culture media, some can withstand wide ranges of pH and temperature [31], and, thanks to molecular techniques, microorganisms can be genetically manipulated via gene insertion to help determine the toxicity of heavy metals in water [32]. Bose et al. (2021) reported that microbial biosensors are more efficient and have a wider detection range compared to other conventional biosensors [32].

2. Microbial Biosensors for Wastewater Monitoring

Figure 1 shows that some databases had more articles related to the topic than others because some databases specialize in biomedical literature such as the PubMed platform [33][34]. A disadvantage of this database is that articles on biosensors mostly consider medical applications, which limits finding articles on biosensors with applicability in other fields, unlike the Web of Science and Scopus [35] databases. On the other hand, the ScienceDirect database provided the highest number of publications (*n* = 1129) found between 2012–September 2022, followed, in order, by SpringerLink (*n* =816), Scopus (*n* = 108), and PubMed (*n* = 41). The greater number of publications is possibly because non-specialized databases cover more multidisciplinary literature, with Scopus being one of the three most important sources in the last 15 years [36]. However, when the inclusion and exclusion criteria were applied, the number of publications was reduced (*n* = 45), and the number of publications related to the topic varied for each database. In another sense, the databases that had the most articles found were ScienceDirect and SpringerLink, which was possibly because the search engine of these databases has shown better precision compared to other databases (PubMed and Google Scholar) [37][38].

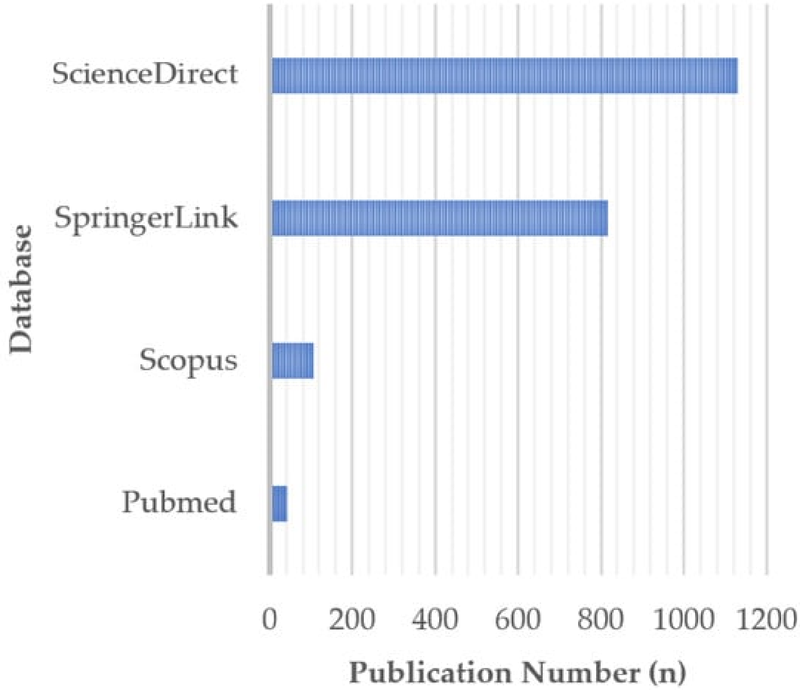

Figure 1. Publications in different databases between 2012–September 2022 (*n* = 2094).

Figure 2 shows a comparison between the graph obtained with the number of publications selected and related to the use of microbial biosensors in wastewater monitoring and the graph obtained in Scopus when using the search formula: “Biosensor” AND “Bacteria” AND “wastewater”. **Figure 2** was obtained after applying the inclusion and exclusion criteria, which show the same tendency to increase over the last 10 years (from 2012 to September 2022). This comparison was possible thanks to the fact that the Scopus database allows for bibliographic analysis. The similarity in the increase of research related to the topic also shows the importance that microbial biosensors have gained in the last decade in relation to their application in the monitoring of different pollutants present in different types of wastewater.

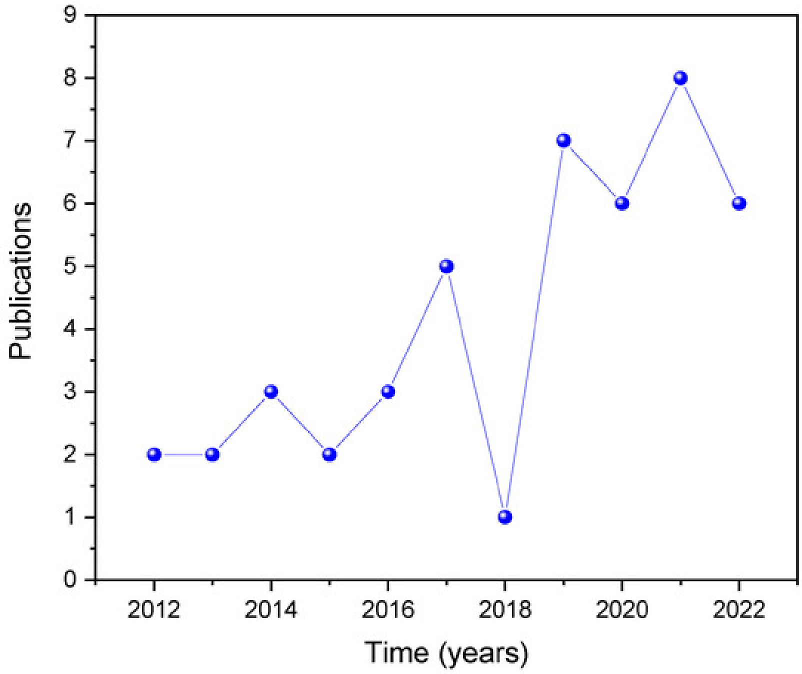

Figure 2. Comparison of graphs between the number of publications obtained in this mini-review and the Scopus database during the period 2012 to September 2022. Number of documents obtained in Scopus (*n* = 82), with the search formula: "Biosensor" AND "Bacteria" AND "wastewater".

The reduction in the total number of publications after applying the exclusion and inclusion criteria was due to the fact that during the analysis, duplicate publications were detected between the different databases. In addition, publications that were not specifically related to wastewater monitoring using microbial biosensors were excluded. The duplicity of publications was due to the fact that the databases share the following characteristics as search subcategories other bibliography search engines such as Medline, PubMed [38], and SpringerLink.

Figure 2 shows that, although in the last decade there has been an increasing trend in research, this has not been significant, as can be compared with the total number of articles related to the use of microbial biosensors from 1981 to 2017, where a total of 2323 publications were registered in the databases [39]. However, there was no exact data on the number of publications about wastewater monitoring using these microorganism-based devices. The monitoring of this type of water possibly began due to the concerns about transferring pathogens and contaminants that put human health at risk [23][28]. However, the use of microbial biosensors in wastewater monitoring continues to attract the attention of the scientific community due to the need for new monitoring alternatives capable of detecting minimum concentrations of pollutants in real-time and thus being able to guarantee public and environmental health [4][40]. Another problem that may have delayed research in this area of biomonitoring was the COVID-19 pandemic [41][42]. According to Riccaboni and Verginer (2022), subsidies from other areas unrelated to COVID-19 were displaced during the pandemic [43], while Gao et al. (2021) reported that the average number of hours dedicated to research decreased in the first year of the pandemic [44].

Another important aspect that stands out is the recent use of genetically manipulated microorganisms [45][46][47][48][49][50][51][52][53][54], which improves the biosensor's specification and sensitivity [55]. This is possible thanks to recombinant DNA technology that allows genes encoding transcriptional regulators to be integrated into biorecognition genes. Some of the most widely used genes are *lux*/*luc*, *lacZ*, and *gfp*, which code for the enzyme firefly/bacterial luciferase, β-galactosidase, and green fluorescent protein, respectively [56][57]. On the other hand, the microorganisms most used in biosensors for the monitoring of environmental contaminants are bacteria because they are easy to reproduce in cheap media, resistant to stress, detect specific signals, and also provide online analysis, in vivo, and dose-response. Of these bacteria, the *E. coli* species is the most widely used due to its easy handling [56]. In this way, this species can be used with the respective genes for the detection of environmental contaminants such as heavy metals and various inorganic contaminants [45][46][50][51][54][56][58][59][60][61][62] present in wastewater, as described and demonstrated in the analyzed articles. However, other bacterial species can also be used as components of biosensors with good detection limits, such as species of *Pseudomonas*, *Bacillus*, *Burkholderia*, *Vibrio*, etc. [63].

It can also be seen that emerging technologies such as microbial fuel cells (MFCs) have been used as electrochemical microbial sensors, they have been very useful in monitoring BOD [64][65][66][67][68][69][70][71][72][73][74][75] and other pollutants such as Zn^{2+} [59] and linear alkyl benzene sulfonate [76]. The latter is one of the most dangerous contaminants in wastewater and comes from the detergent industry, which is extremely important to detect early [76]. This utility as a microbial biosensor is possible due to its operation. Chu et al. (2021) [77] explained how these electrochemical devices function as biosensors, where the anode biofilms fulfill the role of component detection, monitoring toxic compounds by monitoring the extracellular transfer of electrons (ETE) by electroactive microorganisms and the anode. However, the cathode can also function as a biosensor, which is based on the electrochemical reduction of the analytes to be detected or through the inhibition of the oxygen reduction reaction. Likewise, the same author emphasized that through this type of biosensor, an early warning of the toxic compounds present in a body of water can be generated. Another novelty of these devices was presented by Emaminejad et al. (2022), who evaluated for the first time in the long term, the quantification of the sensitivity to variations in the organic load in a channel of primary effluents for 247 days, yielding encouraging results. However, it is also necessary to appreciate the environmental factors such as pH, the concentration of volatile fatty acids, and temperature that influenced the accuracy of the electrochemical biosensor [78].

The versatility of MFCs provides potential to monitor heavy metals in wastewater and, at the same time, generate bioelectricity, as shown in Zhang et al. (2022) [79] and Do et al. (2022) [80]. Likewise, Hui et al. (2022) highlighted the advantages of these electrochemical biosensors to detect toxic compounds in polluted water bodies since they are easy to operate, provide fast results in real-time [81], and can be built on small scales, which adds to their portability, making them very useful tools for in-situ tests. On the other hand, Tucci (2020), in his academic work, showed that electrochemical biosensors are useful for the detection of pollutants related to agriculture, such as herbicides, since for their detection there are classical analytical techniques (HPLC, GC-MS, etc.,), which are expensive and take a long time to issue results [82]. Although the low output potential and the scaling of MFCs indeed represent a challenge for electricity generation, that is different from its potential as a biosensor, of which it is a very practical monitoring system [83]. On the other hand, the sensitivity and specificity of these biosensors are lower than that of a subcomponent-based electrochemical biosensor (for example, an electrochemical enzyme biosensor) and an electrochemical sensor equipped with a chemically modified electrode, respectively. However, enzyme purification techniques make these other biosensors expensive and laborious, representing an economic disadvantage for researchers and companies [77].

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