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## SECCIÓN II: INDUSTRIAL AND ENVIRONMENTAL BIOTECHNOLOGY MINI REVIEW

### Biotecnología ambiental: desafíos y perspectivas en la aplicación de tecnologías combinadas para mejorar la remediación y la generación

#### Environmental Biotechnology: Challenges and perspectives in applying combined technologies to enhance remediation and renewable energy generation

##### Abstract

The various industrial sectors, as well as livestock and agricultural activities, are increasing the production of inputs to meet the demand of the worldwide demographic explosion, making a challenge the clean maintenance of water, soil, and air. Therefore, the search for solutions for a pollutant-free environment without compromising economic development has become extremely important. Thereby, biotechnological studies in order to solve environmental issues have been gaining extensive attention through the coupling of technology procedures to biological systems as sustainable solutions to remediate contaminated areas. In this sense, this review covers topics such as the role of Omics era in microbial environmental biotechnology for pollution control as well as the microbial fuel cell use in energy production. Moreover, phytoremediation and the perspective of applying chemical methods are approached as environmentally friendly tools for the pollutant control to improve remediation processes.

##### Resumen

Los diversos sectores industriales, así como las actividades ganaderas y agrícolas, están aumentando la producción de insumos para satisfacer la demanda de la explosión demográfica mundial, lo cual dificulta el mantenimiento limpio del agua, el suelo y el aire. Por lo tanto, la búsqueda de soluciones para un medio ambiente libre de contaminantes sin comprometer el desarrollo económico se ha vuelto extremadamente importante. De este modo, los estudios biotecnológicos para resolver problemas ambientales han recibido una gran atención a través del acoplamiento de procedimientos tecnológicos a sistemas biológicos como soluciones sostenibles para remediar áreas contaminadas. En este sentido, esta revisión cubre temas como el papel de la era Ómica en la biotecnología ambiental microbiana para el control de la contaminación, así como el uso de celdas de combustible microbianas en la producción de energía. Además, la fitorremediación y la perspectiva de aplicar métodos químicos se abordan como herramientas ecológicas para el control de contaminantes y mejorar los procesos de remediación.

##### Palabras clave:

Biotecnología ambiental; Biorremediación; Procesos de oxidación avanzados; Electroquímica.

##### Keywords:

Environmental Biotechnology; Bioremediation; Advanced oxidation processes; Electrochemistry.

## Introduction

Environmental biotechnology depicts the biological system application (e.g. microorganisms, plant, algae) to improve environmental quality by removing pollutants (Vallero 2016). Overall, biological processes can be used to biotreat solid, liquid, and gaseous wastes to generate renewable energy and bioremediate polluted environments (Petsas & Vagi 2019).

Microorganisms and their metabolites play a significant role in environmental bioremediation process and are reported for their ability

to degrade hydrocarbon pollutants (Lustosa et al. 2018), heavy metals (Verma & Kuila, 2019), and pesticides (Jariyal et al. 2018). The pollutant biodegradation involves several steps, using different enzymes produced by an individual microorganism strain or a microbial consortium (Abbasian et al. 2015). Regarding bacteria, enzymes involved in biodegradation are mostly encoded in plasmids, constituting an oxidase system. On the other hand, Fungi and other eukaryotic organisms oxidize aromatic compounds through mono-oxygenases, forming a trans-diol intermediate (Varjani 2017).

In this context, the access to omics datasets (e.g. metagenomics, transcriptomics, proteomics, metabolomics) is revolutionizing the biology, enabling approaches to understand biological processes and apply them in the field of environmental biotechnology. These tools provided molecular studies of microbial enzyme characterization as a biotechnological approach to develop biological agents to solve environmental problems to recover contaminated water or soil (Padey et al. 2019).

Besides microorganisms, green plants are also used to remove hazardous compounds, through a process called phytoremediation. This green technology is based on the interaction between plants and soil microbiota to reduce the concentration or toxic effect of pollutants, considered as a cost-effective and effective and sustainable environmental recovery technology (Jeevanantham et al. 2019). Several hazardous compounds can be degraded by phytoremediation, including heavy metals (Pb, Zn, Cd, Cu, Ni, Hg), radioactive elements (U, Cs, Sr), petroleum hydrocarbons, pesticides and herbicides (atrazine, bentazone, chlorinated and nitroaromatic compounds), explosives (TNT, DNT), as well as industrial organic wastes (PCPs, PAHs), metalloids (As, Sb) and inorganic compounds ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ) (Favas et al. 2019).

Just as pollutants from intense industrial activity accumulate in soil and water, fossil fuels as energy sources have raised atmospheric  $\text{CO}_2$  to critical levels. Therefore, there is an urgent need for alternative renewable energy sources in order to minimize environmental impacts. Biomass as an alternative energy source can be harnessed and transformed into ethanol, biodiesel, hydrogen cells, and also under microbial fuel cells (MFCs) (Bajwa et al. 2018).

A microbial fuel cell is a promising technology of applying microorganisms as biocatalysts to oxidize organic substrates and transfer their electrons to an anodic surface to produce bioelectricity (Santoro et al. 2017). Several pollutant chemical waste, such as phenol, *p*-nitrophenol, nitrobenzene, polycyclic aromatic hydrocarbons, indole, ethanolamine and sulphide, have been used as MFCs oxidizable substrates (Li et al. 2017). Thereby, MFC may provide an effective, sustainable and environmentally friendly route to energy production.

Although microorganisms and plants display potential to remove several pollutants, many compounds exhibit low degradability by applying only biological systems. Thereafter, other procedures can be coupled with the biotechnological process in order to achieve

complete pollutant degradation. The electrochemistry is highlighted in the pollutant degradation process, evidencing the interdisciplinary importance in the environmental biotechnology context.

Electrochemistry is based on chemical reactions involving the electric charge transfer across an electrified interface between electronic and ionic conductors (Strasser & Ogasawara 2008). This process has been applied in order to improve the biodegradability of persistent compounds from industrial effluents (e. g. dairy waste, pyrolysis wastewater, vinasse) and sewage treatment, aiming at organic waste mineralization (Markou et al. 2017, Silva et al. 2017, Vilar et al. 2018, Tang et al. 2019).

Overall, this report summarizes plant and conventional microbial procedures applied in environmental biotechnology, as well as its interdisciplinary by coupling methodologies to assist pollutant degradation processes in order to generate clean energy.

#### Application of omics tools in environmental biotechnology

Approaches applying metagenomics, transcriptomics, proteomics, and metabolomics tools, summarized under the name omics, have contributed to the advancement in environmental biotechnology research. Based on the data high-throughput, omics tools are a key point due to their analytical contribution to determining biodiversity, understanding the effects of toxic chemicals (pollutants) on health and environment by assessing their effects on living organisms and the resulting changes in metabolic, protein, and gene levels (Misra et al. 2018).

In this regard, metagenomics and proteomics studies of microbial systems have been performed to investigate functional genes and protein expression profiles from activated sludge (Zhao et al. 2018), exposure of freshwater and soil samples to heavy metals (Gang et al. 2019), polycyclic aromatic hydrocarbons (PAH) (Nzila et al. 2018), pesticides (Sineli et al. 2018) and cyanide (Luque-Almagro et al. 2016).

Another relevant point in the metagenomics application is to determine biodiversity in sample-based on environmental DNA (eDNA) to identify prokaryotic and eukaryotic organism species. On the other hand, metatranscriptomics provides data related to the real physiological activity of organisms in the environmental samples by RNA extraction from a microbial community, whose mRNA or cDNA, after sequencing, indicates the protein encoded by a gene, which can express a real or future quantitative or qualitative activity. This tool allows determining potential genes in microorganisms to apply in the bioremediation of environmentally hazardous compounds (Thakur et al. 2018).

Metabolomics in environmental studies aims to characterize an organism's metabolic response to natural or anthropogenic stressors in its environment (Bedia et al. 2018). This tool can be applied to the study of microbial communities in order to discover new metabolites to expand the knowledge on metabolic pathways regarding

the microbial consortium application to promote pollutant degradation. Furthermore, metabolomics methods facilitate a better understanding of the toxicant effects on organisms such as plants, animals, and humans by providing a toxicological data concerning living organisms (Kozłowska et al. 2019, Zhou et al. 2019).

#### Phytoremediation as strategies in the environmental biotechnology

Besides microorganisms, plants are used in contaminated environment remediation processes, called phytoremediation. This method is efficient in remediating a range of environmental pollutants, comprising six different strategies: 1) Phytoextraction, involving the plant root used to absorb soil contaminants with contaminant accumulation in plant aerial parts, and subsequent safe; 2) Phytovolatilization, conversion of absorbed soil contaminants in less toxic contaminant vapour; 3) Phytofiltration, plant biomass used to filter pollutants from contaminated water systems; 4) Phytostabilization plants use to stabilize pollutants and reduce their mobility and bioavailability in the surrounding environments and food chain; 5) Phytodegradation, organic xenobiotic absorption by plants, and their degradation by plant enzymes; 6) Rhizodegradation, pollutant degradation in the rhizosphere through microbial activity (Favas et al. 2014).

Phytoremediation displays a high efficiency and cost-effective method to remove contaminants compared to other methods. Although pollutant removal time is longer, phytoremediation is a permanent and efficient solution to remove environmental pollutants compared to other techniques, including heavy metals (Midhat et al. 2019), organic contaminants such as PAHs (Sivaram et al. 2019) and radionuclides (Lee et al. 2019). Despite clear evidence of the phytoremediation effectiveness under many environmental conditions, this biological method for pollutant remediation is still commercially underutilized in the environmental biotechnology field.

#### Microbial fuel cells and energy

Beyond pollutant remediation, renewable energy generation is another relevant aspect concerning environmental biotechnology, since fossil fuel use as an energy source promotes drastic climate change, altering the earth's habitat. In this context, microbial fuel cell (MFC) may provide an effective, sustainable and environmentally friendly route for energy generation, due to the viable microorganism bio-catalytic capacities to transform the energy stored in the chemical bonds of wastewater compounds to generate electrical current (Logan & Regan 2006).

In an MFC system, exoelectrogenic microorganisms display the ability to facilitate direct and indirect electron transfer. The direct electron transfer requires a physical connection between the bacterial cell and electrode surface by nanowires and/or redox-active proteins. Regarding indirect electron transfers, no physical connection is required, since this mechanism relies on electron shuttling molecules as nanowires, membrane-bound cytochromes and electron mediators (Slate et al. 2019).

Some exoelectrogens bacteria such as *Geobacter* sp., *Shewanella*, *Pseudomonas* and *Rhodospirillum rubrum* have been widely studied (Li et al. 2017), while fungal species *Debaryomyces hansenii*, *Aspergillus awamori*, *Hansenula anomala* and *Mortierella polycephala* (Li et al. 2019) have been used for both contaminants remediation and electricity production. Pollutants waste from pulp, food, brewery/distillery industrial effluents as well as metal-contaminated and swine wastewaters, marine sediments and pesticides have also been successfully used at laboratory level to generate bioelectricity (Li et al. 2017, Li et al. 2019). In addition, toxic chemical waste such as phenol, *p*-nitrophenol, nitrobenzene, PAHs, indole, ethanolamine, and sulfide have been used as oxidizable substrates for MFCs (Li et al. 2017).

Although MFCs are considered as a potential technology for renewable energy, some disadvantages are reported regarding high costs, low energy production, and limited system life. Therefore, advances in Omics techniques, synthetic biology, as well as further studies with electrogenic and metabolically complementary microbiomes could enable MFCs to become a viable technology in the future.

#### Coupled electrochemical and biological technologies as a perspective to enhance remediation

Wastewater from diverse sources such as agriculture, industry, hospital and domestic uses could be a potential water resource if appropriate treatment technologies could be developed. The presence of organic micropollutants is one of the barriers to obtaining high-quality water from wastewater arises. Most of the conventional wastewater treatment plants (WWTPs) have inadequate equipment to entirely remove organic micropollutants at low concentrations, making the treatment processes one of the sources of such pollution (Tijani et al. 2013). Micropollutant concentrations in water range from a few nanograms/liter to several milligrams/liter, and impair the water quality (Kanaujiya et al. 2019).

Furthermore, industrial wastewaters usually present a high concentration of chemical oxygen demand (COD), sometimes with the inability to biodegrade due to its toxicity or inhibitory effect on bacterial metabolism. Many of these compounds are refractory and are not removed in the WWTPs, requiring more complex, advanced, and innovative treatment technologies are needed (Minière et al. 2019). In this context, advanced oxidation processes (AOPs) are reported to be able to efficiently degrade micropollutants and some refractory compounds. Among them, electrochemical advanced oxidation processes have several advantages such as environmental compatibility, versatility, high energy efficiency, amenability to automation, and cost-effectiveness (Martínez-Huitle et al. 2015).

Electrochemical technologies can be applied as an advanced treatment method further to reduce COD or color in the water to achieve relevant effluent standards. However, in order to improve the treatment efficiency, hybrid systems by using electrochemical technologies

combined with the biological process have been reported. Thus, the effluent from a biological treatment system can be subjected by electrochemical technologies in order to eliminate all the toxic by-products secreting from the biological system. At this point, industrial effluents, synthetic wastewater, olive washing water, textile effluent, and vinasse, after biological pretreatment were also treated by electro-oxidation and photo-assisted electro-oxidation to mineralize remnant organic compounds (Aravind et al. 2016, Tatoulis et al. 2017, Trelu et al. 2016, Vilar et al. 2018).

Alternatively, electrochemical technologies can be used as a pretreatment step to increase the biodegradability of a pollutant and, consequently, the treatment efficiency of a biological treatment system. In this sense, electro-oxidation pretreatment has been reported to enhance the organic compounds biodegradability (e.g. dyes, pharmaceutical residues) from industrial, pyrolysis wastewaters as well as synthetic wastewaters (He et al. 2017, Silva et al. 2017, Yahiaoui et al. 2016). Coupling electrochemical oxidation to biological treatments to remove persistent residual molecules, such as pre- or post-treatments, in order to mineralize organic compounds as target pollutants and synthetic solution, as well as industrial effluents, provides a high-efficiency rate.

Other electrochemical processes have also been successfully applied as strategies for the treatment of pollutant residues. Therefore, electrochemical technologies have found a niche, in which these processes tend to become dominant in the near future as environmental tools to decrease the accumulation of refractory molecules. Within this framework, several methods can be highlighted, such as electro/ $\text{Fe}^{3+}$ /peroxodisulfate (Ledjeri et al. 2016), electrochemical dechlorination in an EC-COCEL reactor (Arellano-González et al. 2016), electro-oxidation and oxidation induced by sunlight (Santhanam et al. 2017), direct and indirect electrochemical reduction (Zaghdoudi et al. 2017) and electro-Fenton (Pęziak-Kowalska et al. 2016; Aboudalle et al. 2018a and 2018b).

Further studies focused only on increasing the biodegradability measured as BOD (Pęziak-Kowalska et al. 2016, Aboudalle et al. 2018a), application of activated sludge cultures (Zaghdoudi et al. 2017, Aboudalle et al. 2018b, Pęziak-Kowalska et al. 2019), as well as the use of specific microorganisms (Rajeswari et al. 2016, Silva et al. 2017, Santhanam et al. 2017).

Although some electrochemical procedures have been used in combination with biological processes, several electrochemical techniques were not still used. Thus, this situation opens the opportunity to further development of coupled systems employing electrocoagulation, electroflotation, electrodialysis, and photoassisted systems like photoelectro-Fenton and photoelectrocatalysis. Since the electrochemical advanced oxidation processes are highly efficient for the removal of micropollutants and refractory compounds, their use after biological systems needs to be a future perspective studied intensely.

## Conclusion

This review describes some biotechnological tools applied in polluted environments remediation processes, based on the urgency and efforts to implement chemical control measures and their environmental impact. In this context, the importance of molecular biology within the broad omics technology field is addressed, considering the analysis of metabolites, proteins as well as genes of living organisms with potential contribution to bioremediation. Also, some aspects of the phytoremediation mechanism are described as a technology, whose potential must be further studied in order to implement its application. Beside the bioremediation, an MFC overview has been reported as a sustainable and environmentally friendly method to generate renewable energy. Although further studies are required to establish its technological feasibility since its implementation is currently a challenge. Despite the use of biological systems in environmental biotechnology, it is concluded that there is a need for research technology integration from different scientific fields in order to overcome environmental conservation challenges.

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