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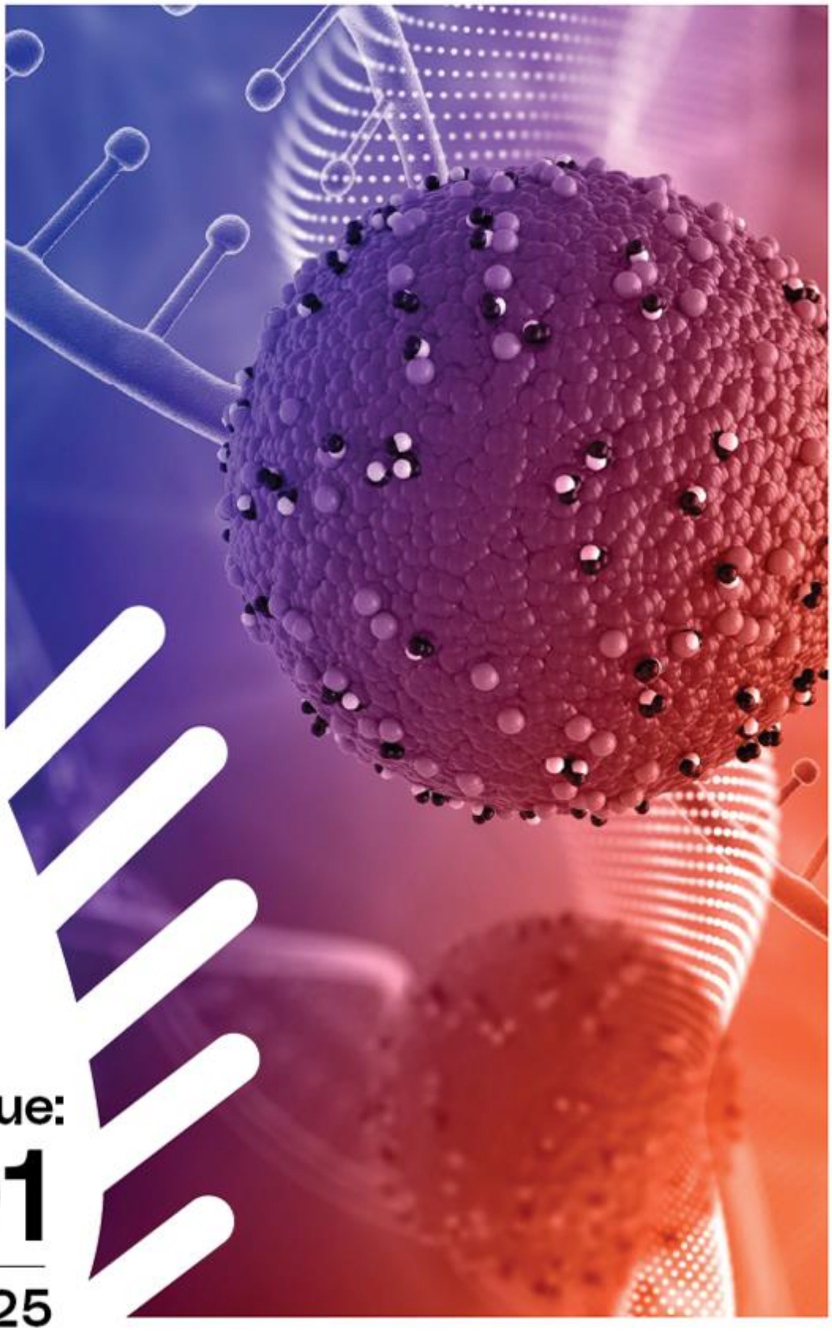

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REVIEW

Microbial fuel cell technology: Novelties for a clean future

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Abstract

The degree of civilization exhibited by a society is largely determined by its reliance on energy, and as traditional energy sources such as fossil fuels become scarcer, new technologies will be required to secure sustainable energy. Microbial fuel cell technology is one of the most creative ways to meet humanity's energy demands because it can generate electrical energy from carbon sources. The framework of the limitations limiting the dissemination of this technology has been used to explore in depth new designs and configurations that have been produced recently. Future developments and current applications of this technology in bioremediation investigations are explored. The use of microbial fuel cell technology as a microbial biosensor for the identification of environmental contaminants is particularly significant. However, for a clean and sustainable ecosystem, it is imperative to disclose the challenges associated with the future adoption of this technology.

Keywords: Electricity, microbial fuel cell, microbial electrochemistry, renewable energy

Introduction

Microbial fuel cells (MFCs) have attracted great attention in recent years as an important alternative technology for energy production from renewable sources (Bazina et al., 2023; Catal et al., 2024). These devices, utilize the power of current bioelectrochemical technologies via microorganisms to convert organic carbon resources found in waste materials into electricity (Ishaq et al., 2023). Thanks to this technology, which can be integrated into wastewater treatment facilities, clean energy can be produced and has potential for the future (Bhaduri and Behera 2024). Microbial fuel cells are actually similar to traditional batteries, but unlike batteries, they are open systems and contain biological objects. Although it has various configurations, there are basically two important parts: anode and cathode (Mohyudin et al., 2022). Biological reactions occur mostly in the anode part. Electrochemically active microorganisms break down organic substances as a result of their metabolic activities, and electrons and protons are formed in this process called oxidation (Thapa et al., 2022). Microbial fuel cells can produce electricity by capturing these released electrons (Vidhyeswari et al., 2022). The anode part of the microbial fuel cell attracts electrons from these microorganisms. These electrons pass through an external conductive outer wire, producing an electric current. Then, these electrons reaching the cathode react with oxygen (or another final electron acceptor) and form a water molecule (Zhuang et al., 2010). With these features, microbial fuel cells have the potential to solve two important problems: energy production and wastewater treatment (Gul et al., 2021). As exoelectrogenic microorganisms consume organic compounds, water can become cleaner while simultaneously producing electricity (Suresh et al., 2022). Additionally, microbial fuel cells are a sustainable and renewable energy source (Sorgato et al., 2023). Microbial fuel cells do not require fossil fuels to function and can use various organic substances found in nature as fuel (Apollon 2023). Although it is simple in terms of working principle, this technology is still in the development stage. One of the main challenges is the current levels of power production produced by this technology. Microbial fuel cells generally produce low power, which limits their areas of application. Therefore, research is being carried out on the development of new designs to increase power production (Priya et al., 2022). Additionally, there is a need to develop new electrode and membrane materials for the optimization of microbial fuel cells, as well as to identify the most efficient microbial communities to increase electron transfer and performance (Prathiba et al., 2022). Despite these challenges, the potential applications of this technology are diverse (Zhang et al., 2022a). Perhaps the most important of these application areas is wastewater treatment. Microbial fuel cells integrated into the wastewater treatment plant can both make the wastewater cleaner and contribute to the operating cost of the plant with the electrical energy it produces (Saran et al., 2023). Additionally, in areas where transportation is difficult, environmental sensors and small electronic devices can be powered by microbial fuel cells integrated into the facility (Khan et al., 2020). With extensive research to be carried out, this technology can become an important actor in the field of clean energy in the future. The reduction of fossil fuels already requires renewable energy technologies (Icaza-Alvarez et al., 2023), and as a more sustainable ecosystem element, microbial fuel cells can serve humanity by harnessing the power of microorganisms.

Although fossil fuels dominate the energy field today (Yildiz 2018), fuel cells are one of the alternative technologies (Chandran et al., 2022). However, microbial fuel cells have some advantages over traditional fuel cells such as operation at low temperatures (Roy et al., 2023). With these features, microbial fuel cells have the potential to revolutionize energy production techniques in the future. While traditional fuel cells require the use of various fuels such as hydrogen (Abdelkareem et al., 2021), microbial fuel cells can use a wide range of organic compounds as fuel, such as wastewater, organic wastes and lignocellulosic materials (Catal et al., 2008a; Catal et al., 2008b; Catal et al., 2011b; Bermek et al., 2014). In this way, they contribute to reducing our need for fossil fuels. They are also important in converting waste into a value-added product. In addition, microbial fuel cells are an environmentally friendly alternative due to their renewable and sustainable nature, as MFCs can significantly reduce methane and N_2O emissions (Wang et al., 2019). While traditional fuel cells focus only on electricity production, microbial fuel cells provide versatile use by performing both electricity production and wastewater treatment simultaneously. While traditional fuel cells require large-scale infrastructures for energy production (Kampker et al., 2020), microbial fuel cells also enable scale-up with their portable designs (Catal et al., 2018). Recently, researchers have been working to increase power output by combining multiple microbial fuel cells (Fan et al 2024). With these portable and scalable features, there are large-scale designs integrated into the wastewater treatment plant as well as small-scale designs for sensor use (Catal et al., 2019a). Although conventional fuel cells have special operational requirements, microbial fuel cells can operate in a wide variety of environmental conditions, including low temperatures and a wide range of substrates (Catal et al., 2011b; Catal et al., 2011c). Thanks to these adaptable features, microbial fuel cells provide the opportunity to operate various devices in regions where the use of traditional technologies is difficult. Ultimately, microbial fuel cells, as an alternative to traditional fuel cells, are promising in the field of clean energy production with features such as versatility, sustainability, scalability and adaptability. However, in order to advance this technology, there is a need to research and understand its working mechanisms, microbial metabolism principles and different configurations.

Essentials of MFCs

Microorganisms function in microbial fuel cells are electroactive (Catal et al., 2024). While these microorganisms produce energy for their own needs by breaking down carbon sources through oxidation reactions, they also release the electrons and protons produced. These released electrons are used to produce electricity (Aiyer 2020). The anode attracts electrons like a magnet. These electrons, transferred to the cathode through an external circuit, flow to the cathode and react with an electron acceptor such as oxygen to produce water (Arkatkar et al., 2021). A membrane is generally used in two-chamber microbial fuel cell configurations. These membranes separate the anode and cathode parts and allow only protons to pass (Kook et al., 2019). However, there is no need to use membranes in single-chamber configurations. Electric current occurs during the transfer of electrons through an external conductive wire.

To increase and optimize power production in microbial fuel cells, it is crucial to understand the energy metabolism, metabolic pathways, and substrate consumption of microbial cells (Thapa et al., 2022). Exoelectrogenic microorganisms are one of the most important components functioning in this technology. These microorganisms can transfer the electrons released during their metabolism to conductive surfaces (Chen et al., 2018). This is just one of the electron transfer mechanisms. These exoelectrogenic microorganisms can use different metabolic pathways while consuming the carbon source in organic substances as a substrate. Fermentation is a common metabolism for many exoelectrogenic microorganisms. In the absence of oxygen, they break down carbon sources and organic molecules and transfer the released electrons to the anode (Georg et al., 2020). Methane (CH_4) and electricity are the end products of ethanol fermentation, for example. First, ethanol is fermented to produce acetate and H_2, and then, on the anode, H_2 and acetate can be immediately oxidized to generate current (Georg et al., 2020). Additionally, some exoelectrogenic microorganisms support the function of the microbial fuel cell by using substances such as nitrate or sulfate as electron acceptors (Fu et al., 2013). Their ability to consume a wide range of substrates is a significant advantage for exoelectrogenic microorganisms. Generally, easily consumable substrates such as sugars are consumed first, followed by complex carbon sources (Catal et al., 2011b). This preference may vary depending on type, energy efficiency and metabolism of microorganisms. Investigating the metabolic pathways and substrate consumption preferences of these microorganisms is essential to increase the performance of microbial fuel cells. Understanding microbial communities, especially those that exhibit high electron transfer rates and efficient substrate consumption profiles, is necessary to develop strategies to increase energy efficiency (Kumru et al., 2012). In this way, optimizing the content of organic substances to be used as fuel seems to be beneficial for encouraging microorganisms that can produce electricity more efficiently. However, a thorough understanding of electron transfer mechanisms in microbial fuel cells is required to achieve efficient electricity production.

Electron transfer processes in MFCs

One of the most important steps in electricity production in microbial fuel cells is electron transfer to the anode. In general, these transfer mechanisms can be grouped under two main headings: direct and indirect electron transfer (Aiyer 2020). In the direct electron transfer mechanism, electroactive microorganisms make physical contact with the anode using structures such as pili or outer membrane cytoplasm (Li et al., 2021a). Electrons are transferred directly from the microorganism to the anode surface (Li et al., 2021a). Since there is no intermediary in this mechanism, electrons are transferred more efficiently. However, some microorganisms do not have the ability to transfer electrons directly and they transfer electrons through indirect electron transfer (Zhang et al., 2022b). In this mechanism, intermediary molecules generally take these electrons from electroactive microorganisms and act as intermediaries in their transfer to the anode surface. Substances such as various quinones, and methylene blue, methyl orange, methyl red can be used as electron mediators (Babanova et al., 2011; Freguia et al., 2009). Although there is a wide range of microorganisms involved in indirect electron transfer, it may be less efficient as electrons and energy may be lost during the shuttle process. The efficiency of electron transfer in microbial fuel cells depends on various factors (Zhao et al., 2009). Electrode materials with larger surface areas increase efficiency because they allow contact with electroactive microorganisms on a larger surface area. Research is being carried out to increase the anode surface with various nanotechnological approaches. By understanding the electron transfer mechanisms that occur with electroactive microorganisms, it may be possible to develop microbial fuel cells with the most appropriate design and configuration to increase power efficiency. New strains reported to be electroactive in microbial fuel cells continue to be identified. Electroactive microorganisms have also environmental protective effects through their interesting abilities (Dong et al., 2024; Sukkasem, 2024). Geobacter bacteria, known to form conductive batteries or adducts to electronically couple to extracellular electron acceptors such as uranium and iron oxide minerals, can be used in microbial fuel cells (Reguera, 2018). Application of magnetic field has been reported to increase the overall energy efficiency of *Geobacter sulfurreducens* and greatly enhance its ability to generate electricity (Zhou et al., 2023). Additionally, some studies have shown that *Geovibrio* species can also be found along with *Geobacter* species (Ait-Itto et al., 2024). Electron uptake from the cathode by *Lactiplantibacillus plantarum*, a predominantly fermentative bacterium found in fermented foods and in the intestines of mammals, has been reported (Tejedor-Sanz et al., 2023). Electricity production was reported with the *Shewanella chilikensis* MG22 strain discovered using 16S rRNA analysis in microbial fuel cells (Efraim et al., 2023). *Shewanella algae*-L3 was isolated from conditioned sludge of brewing wastewater and its role in electricity production was reported (Wu et al., 2024). When we consider how vast the microbial biomass is in our world, it is not surprising that new microorganisms are being discovered. Both the electricity production efficiency and biological remediation properties of microorganisms with this feature can be improved with genetic engineering approaches. However, there may also be microorganisms that are reported to be electroactive and are found in electroactive biofilms containing mixed microorganisms but do not have a direct or indirect function in electron transfer.

Different MFCs designs and configurations

Although the basic principle is similar in microbial fuel cells, there are designs with different types and configurations (Mohyudin et al., 2022). Depending on their electron transfer mechanism, microbial fuel cells can be divided into two main groups: mediated microbial fuel cells (i) and non-mediated microbial fuel cells (ii) (Moon et al., 2006; Babanova et al., 2011). In mediated microbial fuel cells, electrons are transferred through shuttle molecules that function as intermediaries, and these electrons are transferred to the analyte surface. Some of these intermediary molecules can be produced by electroactive microorganisms. Microorganisms capable of direct electron transfer are used in mediator-free microbial fuel cells (Moon et al., 2006; Catal et al., 2008a). Apart from these two groups, there are microbial electrolysis cells, which are a new type of microbial fuel cell (Kilinc et al., 2023). While microbial fuel cells can be used to produce electricity, microbial electrolysis cells enable the production of hydrogen gas (Cebecioglu et al., 2021). Thanks to this technology, hydrogen gas, which is a carbon-neutral source, is produced while requiring the use of energy as it needs application potential. In recent years, soil microbial fuel cells, in which soil is used in the anode part, have been investigated especially because they also have bioremediation potential (Kilinc and Catal 2023). Microbial fuel cell designs using algae or cyanobacteria capable of photosynthesis use light for electron production and are called phototrophic microbial fuel cells (Strik et al., 2010). Nanoporous membrane microbial fuel cells use nanoporous membranes that separate the anode and cathode parts (Biffinger et al., 2008). Similarly, durable and stable ceramic membranes that allow long-term operation can be used (Merino-Jimenez et al., 2019). The positioning of electrons and, if any, compartments in a microbial fuel cell also affects the configuration. According to their configuration, microbial fuel cells can be grouped as single chamber (i), double chamber (ii), or stacked type (iii) or air cathode (iv) (Santoro et al., 2015; Cebecioglu et al., 2022; Fan et al., 2024). Single-chamber microbial fuel cells have a very simple design (Akagunduz et al., 2022). Depending on its volume, it has advantages such as being portable, cost effective and easy to install. However, diffusion of oxygen into the system negatively affects microbial activity and electron transfer. Two-chamber microbial fuel cells are separated into two chambers using a membrane or salt bridge (Mirza et al., 2022). These components allow the transfer of protons but restrict the passage of other compounds. However, these membranes are mostly quite expensive materials. To increase power efficiency, multiple microbial fuel cells can be connected to each other in series or parallel in a stacked type (Fujimura et al., 2022). This allows for higher energy output. Air cathode microbial fuel cells eliminate the problem of air pumps or pure oxygen supply. In this type of microbial fuel cells, oxygen in the air is used to catalyze the spontaneous reaction and contributes to the operating cost by reducing complexity. The choice of configuration depends on the desired requirements and environmental conditions for the application, and significant advances have been made in this

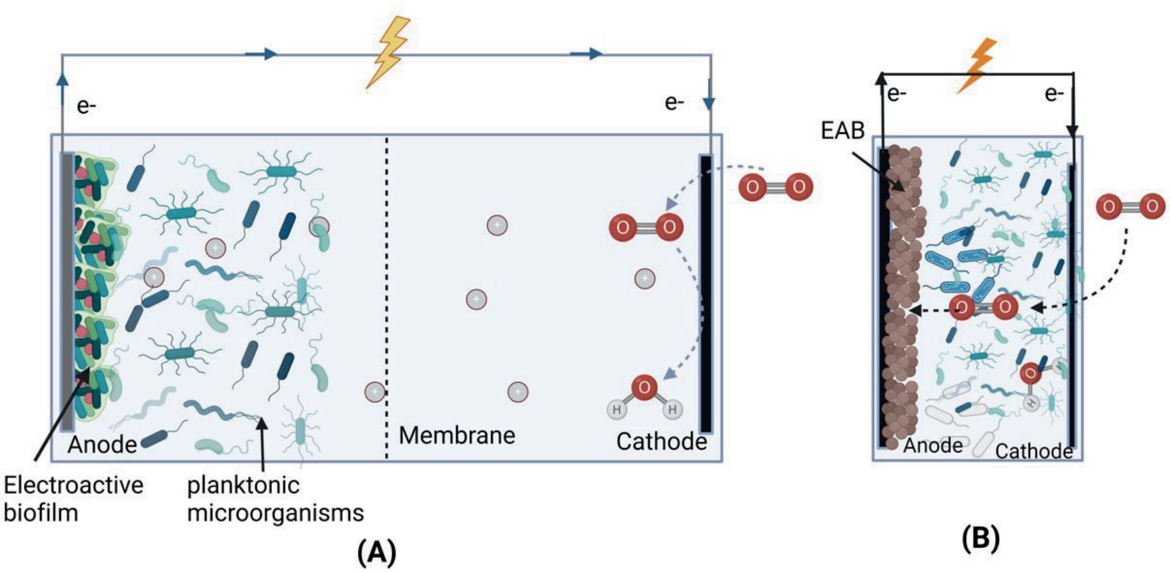

Figure 1. A dual chamber MFC (A) and a single chamber MFC configurations. EAB: electroactive biofilm (Created with BioRender.com).

Latest Advances in MFC Technology

One of the most important parameters affecting energy efficiency in microbial fuel cells is the anode material used. A suitable anode material must have various properties. A good anode electrode should easily receive electrons from microorganisms and transfer them effectively to the external circuit (Kang et al., 2015). In addition, it must provide a suitable environment for the growth of electroactive microorganisms and must be biocompatible (Tahir et al., 2021). In this way, it should allow microorganisms to form biofilm. Materials that provide a large surface area are especially preferred because they provide more effective electron transfer (Walter et al., 2020). It is important in terms of cost that the material to be used as the anode material be durable and stable for a long time and also be cheap (Zhu et al., 2022). So far, different materials have been researched and used with various advantages and disadvantages. One of the most used anode materials is carbon-based materials, which have a large surface area and high conductivity (Akul et al., 2021). Graphite felt, carbon fibers, activated carbon, carbon cloth are examples of commonly used anode materials (Wang and Feng 2017; Delord et al., 2017; Gajda et al., 2020; Sen et al., 2020). Some carbon materials can negatively affect energy production performance because they allow undesirable microorganisms to grow on the anode surface. However, metallic materials can also be used as anode due to their high conductivity and durability (Wang et al., 2016). However, they have disadvantages in terms of bioavailability. Recently, various composite materials that provide advantages in terms of biocompatibility and catalytic activity have also been investigated as anode materials (Roh and Woo 2015). Studies are being carried out on these composite materials, the composite of carbon materials, which have certain properties of different materials, with metal oxides. Conductive polymers such as polypyrrole and polyaniline have been proposed as anode materials because they have advantages in terms of bioavailability (Jia et al., 2020). Selection of a suitable anode material depends on the area of application, cost and desired properties. Extensive research is being carried out on anode materials with optimized properties, which can pave the way for cost-effective bioelectrochemical systems.

Cathode development

Although the anode has an important place in the performance of microbial fuel cells, the cathode is equally important. In order to produce electricity, electrons coming from the anode must react at the cathode with the help of cathode, usually in the presence of oxygen (Chen et al., 2023). The cathode material used significantly affects electricity generation performance. The most important element that a good cathode material should have is high oxygen reduction reaction activity. This reaction must be catalyzed efficiently (Zheng et al., 2022). When oxygen reduction reaction activity is slow, it causes performance loss. Additionally, its high electrical conductivity is important for efficient electron transport. It must be resistant to long-term operations and be able to maintain its function stably for a long time. Being cheap and competitive in terms of cost is also one of the reasons for choosing cathode materials (Behera et al., 2010). Air cathodes, one of the most commonly used cathode types, use the oxygen present in the air and do not require additional oxygen supply. These designs are generally simpler and can be said to be cost effective. In addition, since it requires oxygen in the air to operate, it may have a slower kinetic activity, resulting in lower power output. To increase catalytic activity, carbon materials treated with catalysts such as cobalt, iron and platinum are used (Mahmoud et al., 2011; Ozdemir et al., 2019; Yu et al., 2021). In recent years, biofuels that use microorganisms' own enzymes or biomimetic catalysts have come to the fore. Biocathodes can provide advantages in terms of high catalytic activity and compatibility (Qiu et al., 2021). Although metal-based cathodes are expensive, they are very advantageous in terms of high efficiency (Mahmoodzadeh et al., 2023). Composite cathodes also stand out as alternative materials in that they contain the desired properties of different materials (Antolini 2015). In selecting the most suitable cathode material, factors such as the area of application, desired efficiency and performance parameters, and cost are important. As microbial fuel cell technology develops, it will require the development of new and innovative cathode materials with high catalytic activity, cost-effectiveness and durability.

MFC configurations with improved efficiency

There are some limiting factors in the widespread use of microbial fuel cells. In order to overcome these limiting factors, innovative approaches have been demonstrated in recent years to increase power efficiency. Stacking approach has recently been used to increase the power efficiency of microbial fuel cells (Fan et al., 2024). This approach is based on the principle of stacking multiple microbial fuel cells in series or parallel (Estrada-Arriaga et al., 2018). When microbial fuel cells are connected in series, voltage production is increased. However, when connected in parallel, the current output increases. Various microbial fuel cells can be stacked in series or parallel depending on the purpose of the application. Modular microbial fuel cells allow the preparation of scale-up systems by assembling smaller units (Liang et al., 2018). This type of microbial fuel cells can be used especially for bioremediation purposes. Microfluidic microbial fuel cells have some advantages as they allow faster electron transfer (Ouyang et al., 2023). Particularly for biosensor applications, such microfluidic microbial fuel cells offer opportunities for integration. Bipolar plate-electrode assembly (BEA) design, which significantly increase the performance of microbial fuel cells, is also an innovation (An et al., 2014). New strategies are being developed within the scope of the development of electrode materials that encourage the growth of microorganisms and the formation of biofilms. By adding polyquaternium-7, the surface hydrophilicity was enhanced and the composite electrode's biocompatibility for bacterial attachment, colonization, and substrate diffusion was improved in a previous paper (Li et al., 2021b). Additionally, microbial fuel cells can be integrated into other systems. Table 1 shows recent advances in MFC performances. This technology can be integrated with other technologies for desalination (Aber et al., 2023). In addition, the salt water formed in this process can also serve as a food source for microorganisms. Bioremediation offers a wide range of research and application opportunities as an application area (Li et al., 2015). Thanks to their integration with microbial electrolysis cells, the microbial fuel cell produces electricity, while the microbial electrolysis cell can use this power to produce hydrogen gas.

Application areas of MFCs

MFCs in wastewater treatment and bioremediation

Microbial fuel cells have a wide range of applications in bioremediation and wastewater treatment. In this way, sustainable energy production can be obtained from renewable sources. Traditional water treatment technologies require high energy input. Microbial fuel cells offer an alternative paradigm as they use waste as an energy source. One of the most important parameters determining the effectiveness of microbial fuel cells in wastewater treatment is chemical oxygen demand (Yao et al., 2023). With this technology, a significant reduction in chemical oxygen demand, which is an important parameter of pollution, can be achieved. In addition, various heavy metals and pharmaceutical wastes found in wastewater can be bioremedied (Abourached et al., 2014; Catal et al.,, 2015; Akagunduz et al., 2022; Pugazhendi et al., 2022). In this regard, microbial fuel cell technology has an important potential for bioremediation. These environmental pollutants mixed into wastewater from various industries negatively affect not only humans but also other living elements of nature in our ecosystem (Ozdemir et al., 2019). In order to guarantee environmental health, these pollutants need to be removed, especially before releasing wastewater to nature. Exoelectrogenic microorganisms in microbial fuel cells can remove and detoxify these pollutants by various methods. Microbial fuel cells can function in environments with low oxygen levels. In addition, voltage data processed as signals during the electricity production process also has potential applications in the biosensor field (Adekunle et al., 2021). In addition, the energy produced can also be used to operate other devices used in a wastewater treatment plant. In the real field of application, there are still obstacles to be solved in terms of microbial fuel cells. Especially large-scale wastewater treatment and scale-up remain a significant obstacle. However, studies are continuing to adapt it to biosensor technologies, which are still very popular as a research field.

Table 1. Recent MFC performances.

MFC type	Electron donor (substrate)	Cathode	Power density	Reference
Multiple cloth electrode assemblies internally connected in series	Sodium acetate (5.9 g/L or 100mM)	Air cathode	3.5 W/m^2	Fan et al., 2024
Single chamber	Sodium acetate (1 g/L)	Air cathode	303 mW/m^2	Sonmez et al., 2024
Single chamber scaled-up (2 L)	Sodium acetate (850 mg/L)	Air cathode	7.87 ± 2.72 mW/m^2	Sorgato et al., 2023
Bio-anode microfluidic	Sodium acetate (4.65 g/L)	Potassium ferricyanide	1.05 W/m^2	Ouyang et al., 2023
A single-chamber with a ceramic membrane separator	Synthetic potato-process wastewater	Synthetic potato-process wastewater using submerged carbon cloth	130.2 ± 45.4 mW/m^2	Sato et al., 2023
Single chamber	Molasses	Air cathode	169.86 mW/m^2	Hu et al., 2023
Two chamber	Glucose (0.5 g/L)	Buffer using air pump	130.72 mW/m^2	Mahmoodzadeh et al., 2023
Soil-based	Sodium acetate (1.2 g/L)	Air cathode	227 mW/m^2	Kilinc and Catal 2023
Single chamber	Sodium acetate (30 mM)	Air cathode	6840 mW/m^2	Catal et al., 2024
Two chamber	LB + M9	Potassium ferricyanide (50 mM)	3366 ± 42 Mw/m^2.	Kirubaharan et al., 2023
duckweed composite constructed wetland	Domestic wastewater containing sucrose	Free water layer	42.93 mW/m^2	Jain et al., 2023
Dual-chamber MFC	Distillery wastewater and domestic wastewater	50 mM sodium phosphate solution using air pump	162.5 ± 2 mW/m^2	Jaswal et al., 2023

Biosensors and environmental monitoring

Microbial fuel cells have the potential to be used not only in electricity generation but also as biosensors. The voltage data resulting from the transfer of electrons released as a result of the metabolic activities of microorganisms to the anode surface and the production of electricity can function as a signal for detection of various compounds such as bisphenol A (Zhu et al., 2023). Microbial fuel cells can function as biosensors by exploiting the complex interaction between microorganisms and target compounds. However, there are some obstacles to overcome before microbial fuel cells can function as biosensors. The most important of these are sensitivity and selectivity (Zhu et al., 2023). The basic principle of using microbial fuel cells as biosensors is the interaction between the target molecule and the exoelectrogenic microbial community. The target molecule can often be a contaminant. This pollutant may be a heavy metal in the wastewater system or a waste drug molecule such as antibiotics (Catal et al., 2015). In the presence of the contaminant molecule, the metabolism of microorganisms is affected, which can cause measurable differences in voltage data that serve as signals. For the optimization of the system, researchers continue their research to correlate the chemical power with the concentration of the target molecule. However, one of the main problems here is that molecules other than the target molecule or physical or chemical factors may interfere with the interaction between the microorganism and the target molecule. Therefore, there is a need to investigate new approaches to increase specificity in the future. Various organic pollutants, heavy metals, biomolecules and pathogenic microorganisms can be detected with microbial fuel cells (Ma et al., 2022). While there are studies on the detection of organic pollutants such as pesticides and herbicides, heavy metals such as lead, zinc, mercury and various pathogenic microorganisms that are a threat to public health can also be detected by microbial fuel cells (Abourached et al., 2014; Chouler and Di Lorenzo 2019; Adekunle et al., 2023). In order to customize this microbial response, studies have been carried out to design microbial communities, and by using microorganisms known to sequester certain pollutants, it has become possible to develop more sensitive biosensors against these pollutants. There are still challenges to be overcome before they can be used as biosensors. Such application-oriented new designs and the development of easy-to-transport and inexpensive systems are approaches that can help microbial fuel cells become widespread. Despite all these difficulties, with the development of optimized microbial fuel cells in the future, molecules and compounds that pose a threat to environmental health can be monitored and even find a place in wide application areas such as food safety and biosecurity. Therefore, microbial fuel cells, which are a clean production technology, can also reach wide use in the field of biosensors in the future.

Challenges

Although microbial fuel cells can be used practically on a laboratory scale, there are some challenges when it comes to scale-up. The efficiency achieved with microbial fuel cells, which are mostly used at laboratory scale, decreases as the scale is increased (Selvasembian et al., 2022). In large-scale microbial fuel cells, the interaction between microorganisms and electrodes is negatively affected. This negatively affects microbial activity and productivity. Another problem is that as the scale increases, the internal resistance in microbial fuel cells also increases (Motos et al., 2017). Increasing internal resistance also causes ohmic losses to increase and power output to decrease. Pilot-scale microbial fuel cells studied before the development of microbial fuel cells suitable for wastewater treatment plants have shown promising results. Microbial fuel cells at laboratory and pilot scale also provide prototypes for larger-scale designs and provide preliminary data to understand the functioning of the system. Apart from laboratory applications, scale-up seems feasible, especially for use in remote areas. These scale-up studies require both cost and financial funding to sustain the research. Optimizing designs, investigating cost-effective materials, and creating proficient microbial fuel cell operation and support conventions are pivotal steps. In any case, the potential benefits of microbial fuel cells (wastewater treatment, bioremediation, and clean vitality generation) make overcoming these challenges beneficial. As investigate advances, microbial fuel cells can be anticipated to develop as a reasonable and economical innovation for a cleaner, more energy-independent future.

The type and concentration of organic matter found in wastewater significantly affects the power output of microbial fuel cells (Catal et al., 2008b). The abundance of substrates that can be degraded quickly and easily is also a disadvantage. It is also directly related to the metabolic activities of the microorganisms used in this technology. The efficiency of electron transfer is also related to the electrode materials used in the system. Increasing internal resistance due to increase in size also poses a problem in microbial fuel cells. It is especially important to implement new approaches that can positively affect the interaction between the electron surface and microorganisms with the developments in nanotechnology. Additionally, when it comes to wastewater, there are also factors that cause pollution and can accumulate or cause corrosion in microbial fuel cells. The fact that the cathode section requires additional ventilation to increase efficiency in most cases increases the cost and makes operations difficult.

Conclusion

In the future, it is very likely that wastewater treatment plants that can generate electricity on their own will be developed and microbial fuel cells that can work to detect targeted molecules remotely will be implemented. With this innovative and clean technology, converting organic waste materials into added value can be achieved with the help of exoelectrogenic microorganisms. Microbial fuel cells work like biological batteries, but unlike batteries, they are open systems. Since microorganisms are already living beings, they require the use of an open system and can break down the organic materials provided and convert them into electricity. This technology is versatile in many aspects. While it allows wastewater treatment, it can simultaneously produce clean energy. Since they work naturally with the help of microorganisms, they have many advantages in terms of renewable and sustainability. However, considering the research conducted and the field of application, it is safe to state that this technology is yet at the age of infantry. Efficiency values in power output are still a limiting factor for large-scale applications. There is also a need to develop appropriate optimized microbial fuel cell designs and discover suitable microbial communities. Achieving an increase in energy efficiency through research carried out despite all these difficulties can revolutionize power resources. With the widespread use of this innovative technology, the way can be paved for a cleaner and more environmentally friendly way that meets and supports humanity's energy needs.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics committee approval is not required for this article.

Authors' contribution statement

Tunc Catal and Hong Liu designed this article. Tunc Catal and Hong Liu wrote and revised the paper.

Use of Artificial Intelligence: No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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References

Abourached, C., Catal, T., & Liu, H. (2014). Efficacy of single-chamber microbial fuel cells for removal of cadmium and zinc with simultaneous electricity production. *Water Research 51*, 228-233. https://doi.org/10.1016/j.watres.2013.10.062

Abdelkareem, M. A., Elsaid, K., Wilberforce, T., Kamil, M., Sayed, E. T., & Olabi, A. (2021). Environmental aspects of fuel cells: A review. *The Science of the total environment*, *752*, 141803. https://doi.org/10.1016/j.scitotenv.2020.141803

Aber, S., Shi, Z., Xing, K., Rameezdeen, R., Chow, C. W. K., Hagare, D., & Jindal, T. (2023). Microbial Desalination Cell for Sustainable Water Treatment: A Critical Review. *Global challenges (Hoboken, NJ), 7*(10), 2300138. https://doi.org/10.1002/gch2.202300138

Adekunle, A., Gomez Vidales, A., Woodward, L., & Tartakovsky, B. (2021). Microbial fuel cell soft sensor for real-time toxicity detection and monitoring. *Environmental science and pollution research international*, 28(10), 12792–12802. https://doi.org/10.1007/s11356-020-11245-6

Adekunle, A., Bambace, S., Tanguay-Rioux, F., & Tartakovsky, B. (2023). Microbial Fuel Cell Biosensor with Capillary Carbon Source Delivery for Real-Time Toxicity Detection. *Sensors (Basel, Switzerland), 23*(16), 7065. https://doi.org/10.3390/s23167065

Aiyer, K. S. (2020). How does electron transfer occur in microbial fuel cells?. *World journal of microbiology & biotechnology, 36*(2), 19. https://doi.org/10.1007/s11274-020-2801-z

Ait-Itto, F. Z., Behan, J. A., Martinez, M., & Barrière, F. (2024). Development of bioanodes rich in exoelectrogenic bacteria using iron-rich palaeomarine sediment inoculum. *Bioelectrochemistry (Amsterdam, Netherlands), 156*, 108618. https://doi.org/10.1016/j.bioelechem.2023.108618

Akagunduz, D., Cebecioglu, R., Ozen, F., Ozdemir, M., Bermek, H., Tarhan, N., Arslan, A., Catal, T. 2022. Effects of Psychoactive Pharmaceuticals in Wastewater on Electricity Generation in Microbial Fuel Cells. *CLEAN–Soil, Air, Water*, 2100027. https://doi.org/10.1002/clen.202100027

Akul, N.B., Cebecioglu, R., Akagunduz, D., Bermek, H., Ozdemir, M., Catal, T. 2021. Effects of mevastatin on electricity generation in microbial fuel cells. *Polish Journal of Environmental Studies 30* (6), 5407. https://doi.org/10.15244/pjoes/133402

An, J., Kim, B., Jang, J. K., Lee, H. S., & Chang, I. S. (2014). New architecture for modulization of membraneless and single-chambered microbial fuel cell using a bipolar plate-electrode assembly (BEA). *Biosensors & bioelectronics, 59*, 28–34. https://doi.org/10.1016/j.bios.2014.02.063

Antolini, E. (2015). Composite materials for polymer electrolyte membrane microbial fuel cells. *Biosensors & bioelectronics, 69*, 54–70. https://doi.org/10.1016/j.bios.2015.02.013

Apollon, W. (2023). An Overview of Microbial Fuel Cell Technology for Sustainable Electricity Production. *Membranes*, *13*(11), 884. https://doi.org/10.3390/membranes13110884

Arkatkar, A., Mungray, A. K., & Sharma, P. (2021). Biological modification in air-cathode microbial fuel cell: Effect on oxygen diffusion, current generation and wastewater degradation. Chemosphere, 284, 131243. https://doi.org/10.1016/j.chemosphere.2021.131243

Babanova, S., Hubenova, Y., & Mitov, M. (2011). Influence of artificial mediators on yeast-based fuel cell performance. *Journal of bioscience and bioengineering, 112*(4), 379–387. https://doi.org/10.1016/j.jbiosc.2011.06.008

Bazina, N., Ahmed, T. G., Almdaaf, M., Jibia, S., & Sarker, M. (2023). Power generation from wastewater using microbial fuel cells: A review. J*ournal of biotechnology, 374*, 17–30. https://doi.org/10.1016/j.jbiotec.2023.07.006

Behera, M., Jana, P. S., & Ghangrekar, M. M. (2010). Performance evaluation of low cost microbial fuel cell fabricated using earthen pot with biotic and abiotic cathode. *Bioresource technology, 101*(4), 1183–1189. https://doi.org/10.1016/j.biortech.2009.07.089

Bermek, H., Catal, T., Akan, S. S., Ulutaş, M. S., Kumru, M., Özgüven, M., Liu, H., Özçelik, B., & Akarsubaşı, A. T. (2014). Olive mill wastewater treatment in single-chamber air-cathode microbial fuel cells. *World journal of microbiology & biotechnology, 30*(4), 1177–1185. https://doi.org/10.1007/s11274-013-1541-8

Bhaduri, S., & Behera, M. (2024). From single-chamber to multi-anodic microbial fuel cells: A review. *Journal of environmental management*, *355*, 120465. https://doi.org/10.1016/j.jenvman.2024.120465

Biffinger, J. C., Pietron, J., Bretschger, O., Nadeau, L. J., Johnson, G. R., Williams, C. C., Nealson, K. H., & Ringeisen, B. R. (2008). The influence of acidity on microbial fuel cells containing Shewanella oneidensis. *Biosensors & bioelectronics, 24*(4), 906–911. https://doi.org/10.1016/j.bios.2008.07.034

Catal, T., Xu, S., Li, K., Bermek, H., & Liu, H. (2008a). Electricity generation from polyalcohols in single-chamber microbial fuel cells. *Biosensors & bioelectronics, 24*(4), 855–860. https://doi.org/10.1016/j.bios.2008.07.015

Catal, T., Li, K., Bermek, H., Liu, H. (2008b). Electricity production from twelve monosaccharides using microbial fuel cells. J*ournal of Power Sources 175* (1), 196-200. https://doi.org/10.1016/j.jpowsour.2007.09.083

Catal, T., Fan, Y., Li, K., Bermek, H., Liu, H. (2011a). Utilization of mixed monosaccharides for power generation in microbial fuel cells. *Journal of Chemical Technology & Biotechnology 86 (4)*, 570-574. https://doi.org/10.1002/jctb.2554

Catal, T., Kavanagh, P., O'Flaherty, V., Leech, D. (2011b). Generation of electricity in microbial fuel cells at sub-ambient temperatures. *Journal of Power Sources 196 (5)*, 2676-2681. https://doi.org/10.1016/j.jpowsour.2010.11.031

Catal, T., Cysneiros, D., O'Flaherty, V., & Leech, D. (2011c). Electricity generation in single-chamber microbial fuel cells using a carbon source sampled from anaerobic reactors utilizing grass silage. *Bioresource technology, 102*(1), 404-410.. https://doi.org/10.1016/j.biortech.2010.07.006

Catal, T., Lesnik, K.L., & Liu, H. (2015). Suppression of methanogenesis for hydrogen production in single-chamber microbial electrolysis cells using various antibiotics. *Bioresource Technology, 187*, 77-83. https://doi.org/10.1016/j.biortech.2015.03.099.

Catal, T., Yavaser, S., Enisoglu-Atalay, V., Bermek, H., & Ozilhan, S. (2018). Monitoring of neomycin sulfate antibiotic in microbial fuel cells. Bioresource technology, 268, 116–120. https://doi.org/10.1016/j.biortech.2018.07.122

Catal, T., Kul, A., Atalay, V.E., Bermek, H., Ozilhan, S., & Tarhan, N. (2019a). Efficacy of microbial fuel cells for sensing of cocaine metabolites in urine-based wastewater. *Journal of Power Sources, 414*, 1-7. https://doi.org/10.1016/j.jpowsour.2018.12.078

Catal, T., Liu, H., Fan, Y., & Bermek, H. (2019b). A clean technology to convert sucrose and lignocellulose in microbial electrochemical cells into electricity and hydrogen. *Bioresource technology reports, 5*, 331-334.. https://doi.org/10.1016/j.biteb.2018.10.002

Catal, T., Liu, H., Kilinc, B., & Yilancioglu, K. (2024). Extracellular polymeric substances in electroactive biofilms play a crucial role in improving the efficiency of microbial fuel and electrolysis cells. *Letters in applied microbiology, 77*(3), ovae017. https://doi.org/10.1093/lambio/ovae017

Cebecioglu, R., Akagunduz, D., & Catal, T. (2021). Hydrogen production in single-chamber microbial electrolysis cells using Ponceau S dye. 3 Biotech, 11(1), 27. https://doi.org/10.1007/s13205-020-02563-0

Cebecioglu, R.E., Akagunduz, D., Bermek, H., Atalay, V.E., & Catal, T. (2022). Decolorization mechanisms of reactive yellow 145 and ponceau S in microbial fuel cells during simultaneous electricity production. *Main Group Chemistry, 21*(3), 851-863. https://doi.org/10.3233/MGC-210180

Chandran, M., Palanisamy, K., Benson, D., & Sundaram, S. (2022). A Review on Electric and Fuel Cell Vehicle Anatomy, Technology Evolution and Policy Drivers towards EVs and FCEVs Market Propagation. *Chem Rec, 22(*2), e202100235.. https://doi.org/10.1002/tcr.202100235.

Chen, L., Zhang, P., Shang, W., Zhang, H., Li, Y., Zhang, W., Zhang, Z., & Liu, F. (2018). Enrichment culture of electroactive microorganisms with high magnetic susceptibility enhances the performance of microbial fuel cells. *Bioelectrochemistry (Amsterdam, Netherlands), 121*, 65–73. https://doi.org/10.1016/j.bioelechem.2018.01.005

Chen, J., Zhao, K., Wu, Y., Liu, J., Wang, R., Yang, Y., & Liu, Y. (2023). Improved bioelectrochemical performance of MnO2 nanorods modified cathode in microbial fuel cell. *Environmental science and pollution research international, 30*(17), 49052–49059. https://doi.org/10.1007/s11356-023-25787-y

Chouler, J., & Di Lorenzo, M. (2019). Pesticide detection by a miniature microbial fuel cell under controlled operational disturbances. *Water science and technology: a journal of the International Association on Water Pollution Research, 79*(12), 2231–2241. https://doi.org/10.2166/wst.2019.207

Delord, B., Neri, W., Bertaux, K., Derre, A., Ly, I., Mano, N., & Poulin, P. (2017). Carbon nanotube fiber mats for microbial fuel cell electrodes. *Bioresource technology, 243*, 1227–1231. https://doi.org/10.1016/j.biortech.2017.06.170

Dong, M., Nielsen, L. P., Yang, S., Klausen, L. H., & Xu, M. (2024). Cable bacteria: widespread filamentous electroactive microorganisms protecting environments. *Trends in microbiology, 32*(7), 697–706. https://doi.org/10.1016/j.tim.2023.12.001

Efraim, A., Saeed, M., Elbaz, M. A., Alaa, M., Ahmed, N., Adel, R., Hazem, Y., Elshatoury, E., &

Gomaa, O. M. (2023). Shewanella chilikensis MG22 isolated from tannery site for malachite green decolorization in microbial fuel cell: a proposed solution for recirculating aquaculture system (RAS). *Microbial cell factories, 22*(1), 142. https://doi.org/10.1186/s12934-023-02152-9

Estrada-Arriaga, E. B., Hernández-Romano, J., García-Sánchez, L., Guillén Garcés, R. A., Bahena-Bahena, E. O., Guadarrama-Pérez, O., & Moeller Chavez, G. E. (2018). Domestic wastewater treatment and power generation in continuous flow air-cathode stacked microbial fuel cell: Effect of series and parallel configuration. *Journal of environmental management, 214*, 232–241. https://doi.org/10.1016/j.jenvman.2018.03.007

Fan, Y., Janicek, A., & Liu, H. (2024). Stable and high voltage and power output of CEA-MFCs internally connected in series (iCiS-MFC). *The European Chemistry and Biotechnology Journal, (1)*, 47–57. https://doi.org/10.62063/ecb-17

Freguia, S., Masuda, M., Tsujimura, S., & Kano, K. (2009). Lactococcus lactis catalyses electricity generation at microbial fuel cell anodes via excretion of a soluble quinone. *Bioelectrochemistry (Amsterdam, Netherlands), 76*(1-2), 14–18. https://doi.org/10.1016/j.bioelechem.2009.04.001

Fu, Q., Kobayashi, H., Kawaguchi, H., Wakayama, T., Maeda, H., & Sato, K. (2013). A thermophilic gram-negative nitrate-reducing bacterium, Calditerrivibrio nitroreducens, exhibiting electricity generation capability. *Environmental science & technology, 47*(21), 12583–12590. https://doi.org/10.1021/es402749f

Fujimura, S., Kamitori, K., Kamei, I., Nagamine, M., Miyoshi, K., & Inoue, K. (2022). Performance of stacked microbial fuel cells with barley-shochu waste. J*ournal of bioscience and bioengineering, 133*(5), 467–473. https://doi.org/10.1016/j.jbiosc.2022.02.004

Gajda, I., Greenman, J., & Ieropoulos, I. (2020). Microbial Fuel Cell stack performance enhancement through carbon veil anode modification with activated carbon powder. *Applied energy, 262*, 114475. https://doi.org/10.1016/j.apenergy.2019.114475

Georg, S., de Eguren Cordoba, I., Sleutels, T., Kuntke, P., Heijne, A. T., & Buisman, C. J. N. (2020). Competition of electrogens with methanogens for hydrogen in bioanodes. *Water research, 170*, 115292. https://doi.org/10.1016/j.watres.2019.115292

Gul, H., Raza, W., Lee, J., Azam, M., Ashraf, M., & Kim, K. H. (2021). Progress in microbial fuel cell technology for wastewater treatment and energy harvesting. *Chemosphere*, *281*, 130828. https://doi.org/10.1016/j.chemosphere.2021.130828

Jain, M., Sai Kiran, P., Ghosal, P. S., & Gupta, A. K. (2023). Development of microbial fuel cell integrated constructed wetland (CMFC) for removal of paracetamol and diclofenac in hospital wastewater. *Journal of environmental management*, *344*, 118686. https://doi.org/10.1016/j.jenvman.2023.118686

Jaswal, V., J, R. B., & N, Y. K. (2023). Synergistic effect of TiO2 nanostructured cathode in microbial fuel cell for bioelectricity enhancement. *Chemosphere, 330*, 138556. https://doi.org/10.1016/j.chemosphere.2023.138556

Jia, Y., Ma, D., & Wang, X. (2020). Electrochemical preparation and application of PANI/MWNT and PPy/MWNT composite anodes for anaerobic fluidized bed microbial fuel cell. *3 Biotech, 10*(1), 3. https://doi.org/10.1007/s13205-019-1950-y

Hu, X., Liu, J., Cheng, W., Li, X., Zhao, Y., Wang, F., Geng, Z., Wang, Q., & Dong, Y. (2023). Synergistic interactions of microbial fuel cell and microbially induced carbonate precipitation technology with molasses as the substrate. *Environmental research*, *228*, 115849. https://doi.org/10.1016/j.envres.2023.115849

Icaza-Alvarez, D., Jurado, F., Flores, C., Ortiz, G.R. (2023). Ecuadorian electrical system: Current status, renewable energy and projections. *Heliyon. 9*(5), e16010. https://doi.org/10.1016/j.heliyon.2023.e16010.

Ishaq, A., Said, M. I. M., Azman, S. B., Dandajeh, A. A., Lemar, G. S., & Jagun, Z. T. (2023). Utilization of microbial fuel cells as a dual approach for landfill leachate treatment and power production: a review. *Environmental science and pollution research international*, 10.1007/s11356-023-30841-w. Advance online publication. https://doi.org/10.1007/s11356-023-30841-w

Kampker, A., P. Ayvaz, C. Schön, J. Karstedt, R. Förstmann, F. Welker, (2020). Challenges towards large-scale fuel cell production: Results of an expert assessment study, International *Journal of Hydrogen Energy, 45*(53), 29288-29296. https://doi.org/10.1016/j.ijhydene.2020.07.180.

Kang, Y. L., Ibrahim, S., & Pichiah, S. (2015). Synergetic effect of conductive polymer poly(3,4-ethylenedioxythiophene) with different structural configuration of anode for microbial fuel cell application. *Bioresource technology, 189*, 364–369. https://doi.org/10.1016/j.biortech.2015.04.044

Khan, A., Salama, E.S., Chen, Z., Ni, H., Zhao, S., Zhou, T., Pei, Y., Sani, R.K., Ling, Z., Liu, P., Li, X. (2020). A novel biosensor for zinc detection based on microbial fuel cell system. *Biosensors and bioelectronics 147*, 111763. https://doi.org/10.1016/j.bios.2019.111763.

Kilinc, B., Akagunduz, D., Ozdemir, M., Kul, A., & Catal, T. (2023). Hydrogen production using cocaine metabolite in microbial electrolysis cells. *3 Biotech*, 13(11), 382. https://doi.org/10.1007/s13205-023-03805-7

Kilinc, B., & Catal, T. (2023). A Novel Microbial Fuel Cell for the Sensing of Sodium Acetate in Soil. Polish *Journal of Environmental Studies, 32*(5), 4931-4936. https://doi.org/10.15244/pjoes/168804

Kirubaharan, C. J., Wang, J. W., Abbas, S. Z., Shah, S. B., Zhang, Y., Wang, J. X., & Yong, Y. C. (2023). Self-assembly of cell-embedding reduced graphene oxide/ polypyrrole hydrogel as efficient anode for high-performance microbial fuel cell. *Chemosphere*, *326*, 138413. https://doi.org/10.1016/j.chemosphere.2023.138413

Koók, L., Quéméner, E. D., Bakonyi, P., Zitka, J., Trably, E., Tóth, G., Pavlovec, L., Pientka, Z., Bernet, N., Bélafi-Bakó, K., & Nemestóthy, N. (2019). Behavior of two-chamber microbial electrochemical systems started-up with different ion-exchange membrane separators. *Bioresource technology, 278*, 279–286. https://doi.org/10.1016/j.biortech.2019.01.097

Kumru, M., Eren, H., Catal, T., Bermek, H., & Akarsubaşı, A.T. (2012). Study of azo dye decolorization and determination of cathode microorganism profile in air-cathode microbial fuel cells. *Environmental technology, 33* (18), 2167-2175. https://doi.org/10.1080/09593330.2012.660655

Li, W. W., & Yu, H. Q. (2015). Stimulating sediment bioremediation with benthic microbial fuel cells. *Biotechnology advances, 33*(1), 1–12. https://doi.org/10.1016/j.biotechadv.2014.12.011

Li, H., Cheng, J., Dong, H., Fang, Z., Zhou, J., & Lin, R. (2021a). Zeolitic imidazolate framework-derived porous carbon enhances methanogenesis by facilitating interspecies electron transfer: Understanding fluorimetric and electrochemical responses of multi-layered extracellular polymeric substances. *The Science of the total environment, 781*, 146447. https://doi.org/10.1016/j.scitotenv.2021.146447

Li, Y., Liu, J., Chen, X., Wu, J., Li, N., He, W., & Feng, Y. (2021b). Tailoring Surface Properties of Electrodes for Synchronous Enhanced Extracellular Electron Transfer and Enriched Exoelectrogens in Microbial Fuel Cells. *ACS applied materials & interfaces*, 13(49), 58508–58521. https://doi.org/10.1021/acsami.1c16583

Liang, P., Duan, R., Jiang, Y., Zhang, X., Qiu, Y., & Huang, X. (2018). One-year operation of 1000-L modularized microbial fuel cell for municipal wastewater treatment. *Water research, 141*, 1–8. https://doi.org/10.1016/j.watres.2018.04.066

Ma, Z., Meliana, C., Munawaroh, H. S. H., Karaman, C., Karimi-Maleh, H., Low, S. S., & Show, P. L. (2022). Recent advances in the analytical strategies of microbial biosensor for detection of pollutants. *Chemosphere, 306*, 135515. https://doi.org/10.1016/j.chemosphere.2022.135515

Mahmoud, M., Gad-Allah, T. A., El-Khatib, K. M., & El-Gohary, F. (2011). Power generation using spinel manganese-cobalt oxide as a cathode catalyst for microbial fuel cell applications. *Bioresource technology, 102*(22), 10459–10464. https://doi.org/10.1016/j.biortech.2011.08.123

Mahmoodzadeh, F., Navidjouy, N., Alizadeh, S., & Rahimnejad, M. (2023). Investigation of microbial fuel cell performance based on the nickel thin film modified electrodes. *Scientific reports, 13*(1), 20755. https://doi.org/10.1038/s41598-023-48290-3

Mirza, S. S., Al-Ansari, M. M., Ali, M., Aslam, S., Akmal, M., Al-Humaid, L., & Hussain, A. (2022). Towards sustainable wastewater treatment: Influence of iron, zinc and aluminum as anode in combination with salt bridge on microbial fuel cell performance. *Environmental research, 209,* 112781. https://doi.org/10.1016/j.envres.2022.112781

Merino-Jimenez, I., Gonzalez-Juarez, F., Greenman, J., & Ieropoulos, I. (2019). Effect of the ceramic membrane properties on the microbial fuel cell power output and catholyte generation. *Journal of power sources, 429*, 30–37. https://doi.org/10.1016/j.jpowsour.2019.04.043

Mohyudin, S., Farooq, R., Jubeen, F., Rasheed, T., Fatima, M., & Sher, F. (2022). Microbial fuel cells a state-of-the-art technology for wastewater treatment and bioelectricity generation. *Environmental research*, *204*(Pt D), 112387. https://doi.org/10.1016/j.envres.2021.112387

Moon, H., Chang, I. S., & Kim, B. H. (2006). Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. *Bioresource technology, 97*(4), 621–627. https://doi.org/10.1016/j.biortech.2005.03.027

Motos, R.P., Molina, G., Ter Heijne, A., Sleutels, T., Saakes, M., & Buisman, C. (2017). Prototype of a scaled-up microbial fuel cell for copper recovery. J*ournal of chemical technology and biotechnology (Oxford, Oxfordshire: 1986), 92*(11), 2817–2824. https://doi.org/10.1002/jctb.5353

Ouyang, T., Liu, W., Shi, X., Li, Y., & Hu, X. (2023). Multi-criteria assessment and triple-objective optimization of a bio-anode microfluidic microbial fuel cell. *Bioresource technology, 382*, 129193. https://doi.org/10.1016/j.biortech.2023.129193

Ozdemir, M., Enisoglu-Atalay, V., Bermek, H., Ozilhan, S., Tarhan, N., & Catal, T. (2019). Removal of a cannabis metabolite from human urine in microbial fuel cells generating electricity. B*ioresource technology reports, 5*, 121-126. https://doi.org/10.1016/j.biteb.2019.01.003

Prathiba, S., Kumar, P. S., & Vo, D. N. (2022). Recent advancements in microbial fuel cells: A review on its electron transfer mechanisms, microbial community, types of substrates and design for bio-electrochemical treatment. *Chemosphere*, *286*(Pt 3), 131856. https://doi.org/10.1016/j.chemosphere.2021.131856

Priya, A. K., Subha, C., Kumar, P. S., Suresh, R., Rajendran, S., Vasseghian, Y., & Soto-Moscoso, M. (2022). Advancements on sustainable microbial fuel cells and their future prospects: A review. *Environmental research*, *210*, 112930. https://doi.org/10.1016/j.envres.2022.112930

Pugazhendi, A., Jamal, M. T., Al-Mur, B. A., & Jeyakumar, R. B. (2022). Bioaugmentation of electrogenic halophiles in the treatment of pharmaceutical industrial wastewater and energy production in microbial fuel cell under saline condition. *Chemosphere, 288*(Pt 2), 132515. https://doi.org/10.1016/j.chemosphere.2021.132515

Qiu, B., Hu, Y., Tang, C., Chen, Y., & Cheng, J. (2021). Simultaneous mineralization of 2-anilinophenylacetate and denitrification by Ru/Fe modified biocathode double-chamber microbial fuel cell. T*he Science of the total environment, 792*, 148446. https://doi.org/10.1016/j.scitotenv.2021.148446

Reguera G. (2018). Microbial nanowires and electroactive biofilms. *FEMS microbiology ecology, 94*(7), 10.1093/femsec/fiy086. https://doi.org/10.1093/femsec/fiy086

Roh, S. H., & Woo, H. G. (2015). Carbon Nanotube Composite Electrode Coated with Polypyrrole for Microbial Fuel Cell Application. *Journal of nanoscience and nanotechnology, 15*(1), 484–487. https://doi.org/10.1166/jnn.2015.8404

Roy, H., Rahman, T. U., Tasnim, N., Arju, J., Rafid, M. M., Islam, M. R., Pervez, M. N., Cai, Y., Naddeo, V., & Islam, M. S. (2023). Microbial Fuel Cell Construction Features and Application for Sustainable Wastewater Treatment. *Membranes, 13*(5), 490. https://doi.org/10.3390/membranes13050490

Santoro, C., Serov, A., Narvaez Villarrubia, C. W., Stariha, S., Babanova, S., Schuler, A. J., Artyushkova, K., & Atanassov, P. (2015). Double-chamber microbial fuel cell with a non-platinum-group metal Fe-N-C cathode catalyst. *ChemSusChem, 8*(5), 828–834. https://doi.org/10.1002/cssc.201402570

Saran, C., Purchase, D., Saratale, G.D., Saratale, R.G., Romanholo, Ferreira, L.F., Bilal, M., Iqbal, H.M.N., Hussain, C.M., Mulla, S.I., & Bharagava, R.N. (2023). Microbial fuel cell: A green ecofriendly agent for tannery wastewater treatment and simultaneous bioelectricity/power generation. *Chemosphere, 312*(Pt 1), 137072 https://doi.org/10.1016/j.chemosphere.2022.137072.

Sato, C., Apollon, W., Luna-Maldonado, A. I., Paucar, N. E., Hibbert, M., & Dudgeon, J. (2023). Integrating Microbial Fuel Cell and Hydroponic Technologies Using a Ceramic Membrane Separator to Develop an Energy-Water-Food Supply System. *Membranes*, *13*(9), 803. https://doi.org/10.3390/membranes13090803

Selvasembian, R., Mal, J., Rani, R., Sinha, R., Agrahari, R., Joshua, I., Santhiagu, A., & Pradhan, N. (2022). Recent progress in microbial fuel cells for industrial effluent treatment and energy generation: Fundamentals to scale-up application and challenges. *Bioresource technology*, 346, 126462. https://doi.org/10.1016/j.biortech.2021.126462

Sen, P., Akagunduz, D., Aghdam, A.S., Cebeci, F.Ç., Nyokong, T., & Catal, T. (2020). Synthesis of novel Schiff base cobalt (II) and iron (III) complexes as cathode catalysts for microbial fuel cell applications. *Journal of Inorganic and Organometallic Polymers and Materials, 30*, 1110-1120. https://doi.org/10.1007/s10904-019-01286-x

Sonmez, E., Avci, B., Mohamed, N., & Bermek, H. (2024). Investigation of performance losses in microbial fuel cells with low platinum loadings on air-cathodes. *The european chemistry and biotechnology journal, (1)*, 11–26. https://doi.org/10.62063/ecb-14

Sorgato, A. C., Jeremias, T. C., Lobo, F. L., & Lapolli, F. R. (2023). Microbial fuel cell: Interplay of energy production, wastewater treatment, toxicity assessment with hydraulic retention time. *Environmental research*, *231*(Pt 2), 116159. https://doi.org/10.1016/j.envres.2023.116159

Sukkasem, C. (2024) Exploring biofilm-forming bacteria for integration into BioCircuit wastewater treatment. *The european chemistry and biotechnology journal*, *2*, 96-109. https://doi.org/10.62063/ecb-28

Suresh, R., Rajendran, S., Kumar, P. S., Dutta, K., & Vo, D. N. (2022). Current advances in microbial fuel cell technology toward removal of organic contaminants - A review. *Chemosphere*, *287*(Pt 2), 132186. https://doi.org/10.1016/j.chemosphere.2021.132186

Strik, D. P., Hamelers, H. V., & Buisman, C. J. (2010). Solar energy powered microbial fuel cell with a reversible bioelectrode. *Environmental science & technology, 44*(1), 532–537. https://doi.org/10.1021/es902435v

Tahir, K., Miran, W., Jang, J., Maile, N., Shahzad, A., Moztahida, M., Ghani, A. A., Kim, B., & Lee, D. S. (2021). MnCo2O4 coated carbon felt anode for enhanced microbial fuel cell performance. *Chemosphere, 265*, 129098. https://doi.org/10.1016/j.chemosphere.2020.129098

Tejedor-Sanz, S., Li, S., Kundu, B. B., & Ajo-Franklin, C. M. (2023). Extracellular electron uptake from a cathode by the lactic acid bacterium Lactiplantibacillus plantarum. *Frontiers in microbiology, 14*, 1298023. https://doi.org/10.3389/fmicb.2023.1298023

Thapa, B. S., Kim, T., Pandit, S., Song, Y. E., Afsharian, Y. P., Rahimnejad, M., Kim, J. R., & Oh, S. E. (2022). Overview of electroactive microorganisms and electron transfer mechanisms in microbial electrochemistry. *Bioresource technology*, *347*, 126579. https://doi.org/10.1016/j.biortech.2021.126579

Walter, X. A., Santoro, C., Greenman, J., & Ieropoulos, I. (2020). Scaling up self-stratifying supercapacitive microbial fuel cell. *International journal of hydrogen energy, 45*(46), 25240–25248. https://doi.org/10.1016/j.ijhydene.2020.06.070

Wang, J., Song, X., Wang, Y., Abayneh, B., Ding, Y., Yan, D., & Bai, J. (2016). Microbial community structure of different electrode materials in constructed wetland incorporating microbial fuel cell. *Bioresource technology, 221*, 697–702. https://doi.org/10.1016/j.biortech.2016.09.116

Wang, G., & Feng, C. (2017). Electrochemical Polymerization of Hydroquinone on Graphite Felt as a Pseudocapacitive Material for Application in a Microbial Fuel Cell. *Polymers, 9*(6), 220. https://doi.org/10.3390/polym9060220

Wang, X., Tian, Y., Liu, H., Zhao, X., & Peng, S. (2019). The influence of incorporating microbial fuel cells on greenhouse gas emissions from constructed wetlands. T*he Science of the total environment, 656*, 270–279. https://doi.org/10.1016/j.scitotenv.2018.11.328

Wu, D., Zhang, B., Shi, S., Tang, R., Qiao, C., Li, T., Jia, J., Yang, M., Si, X., Wang, Y., Sun, X., Xiao, D., Li, F., & Song, H. (2024). Engineering extracellular electron transfer to promote simultaneous brewing wastewater treatment and chromium reduction. J*ournal of hazardous materials, 465*, 133171. https://doi.org/10.1016/j.jhazmat.2023.133171

Vidhyeswari, D., Surendhar, A., & Bhuvaneshwari, S. (2022). General aspects and novel PEMss in microbial fuel cell technology: A review. *Chemosphere*, *309*(Pt 1), 136454. https://doi.org/10.1016/j.chemosphere.2022.136454

Yao, H., Xiao, J., & Tang, X. (2023). Microbial Fuel Cell-Based Organic Matter Sensors: Principles, Structures and Applications. *Bioengineering (Basel, Switzerland), 10*(8), 886. https://doi.org/10.3390/bioengineering10080886

Yildiz, I. 1.12 Fossil Fuels, Editor(s): Ibrahim Dincer, Comprehensive Energy Systems, Elsevier, 2018, Pages 521-567, ISBN 9780128149256,

Yu, M., Yang, Q., Yuan, X., Li, Y., Chen, X., Feng, Y., & Liu, J. (2021). Boosting oxygen reduction and permeability properties of doped iron-porphyrin membrane cathode in microbial fuel cells. *Bioresource technology, 320*(Pt A), 124343. https://doi.org/10.1016/j.biortech.2020.124343

Zhang, J., Jiao, W., Huang, S., Wang, H., Cao, X., Li, X., Sakamaki, T. (2022). Application of microbial fuel cell technology to the remediation of compound heavy metal contamination in soil. J*ournal of Environment management 320*, 115670. https://doi.org/10.1016/j.jenvman.2022.115670.

Zhang, G., Liang, D., Zhao, Z., Qi, J., & Huang, L. (2022b). Enhanced performance of microbial fuel cell with electron mediators from tetracycline hydrochloride degradation. *Environmental research, 206*, 112605. https://doi.org/10.1016/j.envres.2021.112605

Zhao, F., Rahunen, N., Varcoe, J. R., Roberts, A. J., Avignone-Rossa, C., Thumser, A. E., & Slade, R. C. (2009). Factors affecting the performance of microbial fuel cells for sulfur pollutants removal. *Biosensors & bioelectronics, 24*(7), 1931–1936. https://doi.org/10.1016/j.bios.2008.09.030

Zheng, L., Lin, X., Liu, Y., Li, H., Sun, Y., & Li, C. (2022). Synergistically enhanced oxygen reduction reaction and oxytetracycline mineralization by FeCoO/GO modified cathode in microbial fuel cell. *The Science of the total environment, 808*, 151873. https://doi.org/10.1016/j.scitotenv.2021.151873

Zhou, H., Xuanyuan, X., Lv, X., Wang, J., Feng, K., Chen, C., Ma, J., & Xing, D. (2023). Mechanisms of magnetic sensing and regulating extracellular electron transfer of electroactive bacteria under magnetic fields. *The Science of the total environment, 895*, 165104. https://doi.org/10.1016/j.scitotenv.2023.165104

Zhu, K., Xu, Y., Yang, X., Fu, W., Dang, W., Yuan, J., & Wang, Z. (2022). Sludge Derived Carbon Modified Anode in Microbial Fuel Cell for Performance Improvement and Microbial Community Dynamics. *Membranes, 12*(2), 120. https://doi.org/10.3390/membranes12020120

Zhu, T. J., Lin, C. W., & Liu, S. H. (2023). Sensitivity and reusability of a simple microbial fuel cell-based sensor for detecting bisphenol A in wastewater. *Chemosphere, 320,* 138082. https://doi.org/10.1016/j.chemosphere.2023.138082

Zhuang, L., Zhou, S., Li, Y., & Yuan, Y. (2010). Enhanced performance of air-cathode two-chamber microbial fuel cells with high-pH anode and low-pH cathode. *Bioresource technology*, *101*(10), 3514–3519. https://doi.org/10.1016/j.biortech.2009.12.105

REVIEW

Unraveling epilepsy: Investigating stem cell approaches for innovative treatment and future cure

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Abstract

Epilepsy is a persistent neurological disorder characterized by repeated, spontaneous seizures that arise without a specific cause. These seizures result from abnormal electrical activity in the brain, leading to a range of symptoms, from brief periods of unconsciousness or minor sensory disturbances to severe convulsions. The management of epilepsy remains a significant challenge, as current treatment modalities, primarily involving antiepileptic drugs and surgical interventions to remove seizure foci, often provide adequate control for a substantial portion of patients. For this reason, stem cell therapies have become a hopeful approach because of their ability to potentially restore and renew impaired neural networks, which is particularly relevant for neurological disorders like epilepsy. This review investigates the present state of stem cell therapies in epilepsy, analyzing distinct types of stem cells, their mode of action, preclinical and clinical trials, as well as future research prospects.

Keywords: Epilepsy, treatment, neurological disorders, neural networks, seizures, stem cell

Introduction

Epilepsy, a prevalent brain condition affecting over 50 million people globally, is primarily prevalent in infants and older age groups (Miguel Sanz et al., 2023). The World Health Organization (WHO) considers epilepsy as a disorder if there are recurring seizures at least twice in 24 hours as a result of electrical imbalances between inhibitory and excitatory mechanisms of the brain (Klein et al., 2024), which necessitates a comprehensive strategy for successful treatment. Individuals afflicted with this neurological disorder experience significant adverse impacts that greatly diminish their quality of life in their social life, education, and employment. The seizures typically exert significant influence over people's lives, leaving them with little sense of living. However, there is still no efficient treatment targeting the root cause of seizures. Pharmaceutical treatments, surgeries, and certain diets are all common treatments for controlling seizures, and they are generally aimed to minimize the superficial side effects of the seizures or try to suppress them. Nevertheless, stem cell therapy emerges as a significant candidate that can target the core causes of seizures. The self-renewing and differentiating capacities of stem cells enable them to contribute to tissue repair and regeneration, potentially restoring and integrating dysfunctional neuronal circuits to a healthy state (Lybrand et al., 2020). This review discusses the present state of stem cell therapies in epilepsy as an innovative and promising approach for future treatment and cure (Wang et al., 2021).

Epilepsy is a complex and heterogeneous neurological disease with multiple underlying mechanisms. The molecular mechanism of this alteration is mainly based on the reduction of Gamma-Aminobutyric Acid (GABA) neurotransmission and the increase of glutamatergic neurotransmission (**Figure 1**). These alterations are typically due to modifications in the structure and synthesis of ion channels, the release and reabsorption of neurotransmitters, and issues with transporter and post-synaptic receptor activation. These changes are typically due to modifications in the structure and synthesis of ion channels, the release and reabsorption of neurotransmitters, and issues with transporter and post-synaptic receptor activation. Consequently, these alterations can disrupt the balance between excitatory and inhibitory neurons, destabilizing neuronal homeostasis and resulting in neuronal hyperexcitability (McNamara et al., 1999). Finally, these mechanisms generally cause one main problem, which is damaging the brain and subsequently causing changes in neuronal circuits (Patel et al., 2019). According to the underlying mechanisms, the etiology of epilepsy is divided into six groups: structural, genetic, infectious, metabolic, immune, and unknown epilepsies.

As a result of these underlying mechanisms, there are multiple epileptic seizure types that an individual with epilepsy may experience. The International League Against Epilepsy (ILAE) categorization divides epileptic seizures into three major categories: focal, generalized, and unknown (Sirven et al., 2015). Generalized seizures and focal seizures are the two types of seizures. A focal seizure occurs on one side of the brain, but can spread to both sides, causing mild or severe symptoms depending on how electrical discharges spread. Epilepsy cases categorized as focal seizures account for approximately 60% of all epilepsy cases. Additionally, generalized seizures occur simultaneously in both hemispheres of the brain. The primary cause of this type of seizure is an imbalance between the brain's excitatory and inhibitory pathways. On the other hand, around 23-35% of epilepsy cases are classified as generalized. Finally, the unknown seizures are those that do not fit into both classifications focal and generalized (Dubé et al., 2007). These seizures typically occur at random, although occasionally they can be triggered by stress, sleep deprivation, waking up, alcohol consumption, certain medications, flashing lights, or a woman's monthly period.

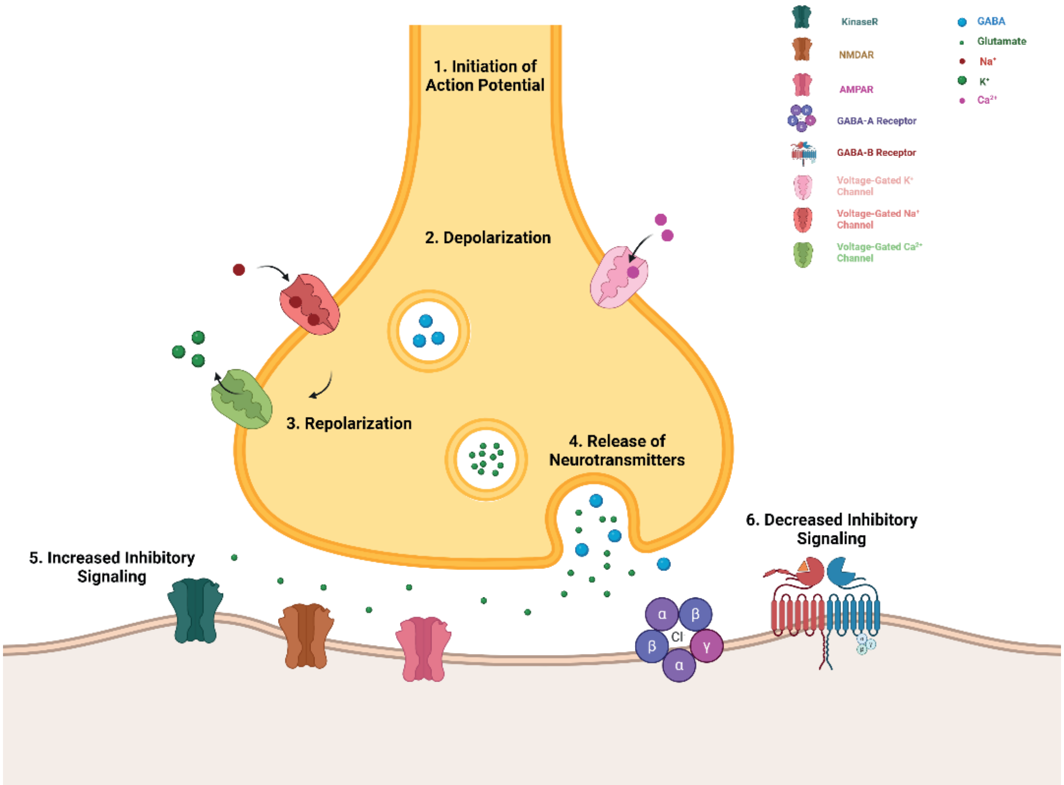

Figure 1. Mechanism of Epilepsy *(Created by BioRender)*.

Beyond seizure control, current treatments do not address the underlying neurobiological changes in the brain, such as neuronal loss and synaptic dysfunction, which contribute to the disease's progression. Stem cell therapy offers the potential to not only reduce seizures, but also to repair damaged neural circuits, promote neurogenesis, and restore normal brain function. This therapy aims to replace lost or damaged neurons, modulate abnormal neural networks, and even provide neuroprotective effects; making it a versatile option for addressing the multifaceted challenges of epilepsy (Chang & Chang, 2022). Furthermore, stem cells have the potential to release various trophic factors that support brain healing and reduce inflammation, offering a holistic approach to treatment that goes beyond symptom management (Das et al., 2019).

Current treatment methods

The World Health Organization states that epilepsy accounts for 0.3% of all deaths worldwide (Murray et al., 2012). According to current technological and scientific advancements, multiple treatment options aim to make patients seizure-free for those who try to live with severe life conditions like epilepsy. The most commonly used treatments in clinics include anti-seizure medications (ASMs) to control seizures, surgical procedures to remove the seizure focus, implantable devices like vagus nerve stimulators, and dietary therapies such as the ketogenic diet.

Pharmaceutical treatment

The first line of treatment method to treat epilepsy are pharmaceutical compounds called antiseizure medications (ASMs) or antiepileptic drugs to prevent or lessen the frequency and intensity of seizures by addressing different pathways and mechanisms in the brain that control neuronal excitability and transmission (Vezzani et al., 2019). A thorough understanding of the effects and mode of action of ASMs is important, especially to be able to apply them to individual patients according to their seizure type. However, ASMs can have severe side effects, including an increased risk of birth defects, allergic skin rashes, liver or bone marrow failure, and complications involving the liver or pancreas. Additionally, a significant decrease in white blood cell or platelet counts, though not common, can occur, particularly in polytherapy, and should not be ignored (Louis et al., 2009).

In addition to all these severe side effects, 30-50% of epilepsy patients are drug-resistant; non-responsive to the properly selected and administered antiepileptic drug regimens, either alone or combined with other treatments, according to the International League Against Epilepsy (Kwan et al., 2010). A major challenge in developing new treatments for drug-resistant epilepsy is the poor understanding of the biological basis of pharmaco-resistance (Voskuyl & Clinckers, 2009). This setback with pharmacological treatments highlights the need to explore alternative or supplementary treatment approaches.

Surgical treatment

Surgical treatment is the alternative option to ASMs, especially for the drug-resistant epilepsy patients. Surgical treatment is carried out via the removal of the specific area that originates the seizures (Kwan et al., 2011). The success and suitability of this treatment method varies according to the type of epilepsy, the affected brain area, and the patient's overall health. Brain mapping is the most essential part of surgical treatment, because it is crucial to know the functions of the affected area; such as language, motor, sensory, or vision, to understand how the patient's life will be affected after the surgery. Nevertheless, surgical treatment carries risks; including paralysis, memory issues, partial loss of peripheral vision, double vision, mood disturbances, diminished motor skills, and speech impediments (Guerreiro et al., 2016; Nair et al., 2016). Moreover, although surgery can be successful for certain types of epilepsy, most patients with drug-resistant epilepsy do not qualify for this option.

Other treatments

Alternative treatment modalities, especially for drug-resistant epilepsy, comprise vagus nerve stimulation and dietary interventions, such as ketogenic diet and modified Atkins diet. Surgically implanting a device to stimulate the vagus nerve aims to decrease or prevent seizures , while dietary therapies try to induce a state of ketosis, which is believed to have an anticonvulsant effect by altering metabolic processes (Johnson, 2019, Riva et al., 2021).

There are five main device types used for vagal stimulation, each with different mechanisms and characteristics for stimulating the vagus nerve; Deep Brain Stimulation (DBS), Responsive Neurostimulation (RNS), Vagus Nerve Stimulation (VNS) Therapy, External Trigeminal Nerve Stimulation, and Seizure Alert devices (Shaefi & Harkness, 2003). Unfortunately, if a patient has concurrent health conditions such as severe asthma, breathing difficulties, sleep apnea, or certain heart problems, vagal stimulation may lead to serious complications (Liu et al., 2017). In addition, it comes with risks that can reduce the quality of life, such as voice changes, shortness of breath, and pain in the throat or neck (Kwan et al., 2010).

Dietary therapies, often employed as adjuncts to seizure medications for seizure control, include the classic ketogenic diet, the low glycemic index treatment (LGIT), the medium-chain triglyceride (MCT) ketogenic diet, and the modified Atkins diet (MAD) (Lee et al., 2018). These diets require precise weighing and measurements. Maintaining moderate calorie, liquid, and protein intake illustrates the difficult and strict nature of these diets, particularly for children. Despite the benefits, these diets have been associated with an increased risk of serious acidosis and gastrointestinal issues, which can negatively impact a patient's daily life. Prolonged adherence to such dietary regimens can lead to severe consequences; including elevated blood cholesterol levels, kidney stones, constipation, inhibited growth, and bone fractures (Vezzani et al., 2019). Ultimately, these treatments do not cure the disease but provide only palliative care, highlighting the need for more effective and innovative solutions for epilepsy (Neal et al., 2008).

Against all recent progress, epilepsy still poses a significant challenge in terms of treatment and management (Alayli et al., 2023). Currently, epilepsy affects around 1-2% of the global population. Despite the presence of over thirty anti-epileptic medications in the market, 30% of patients continue to experience refractory epilepsy, indicating that medications alone are inadequate for seizure control (French et al., 2004). Moreover, alternative treatment modalities such as surgery and neurostimulator device implants are not universally applicable to every patient, as they offer relief to a limited subset of individuals. Even for those deemed suitable candidates, these options necessitate thorough patient evaluation and selection processes. Regarding dietary interventions, modified Atkins (MAD) and ketogenic (KD) diets are primarily utilized currently. However, neither of these options offers a comprehensive solution on its own, and they may not be suitable for every patient. Besides, these diets are not ideal for long-term use because of their strict regimen and side effects (Neal et al., 2008). Therefore, there is an urgent need for efficient treatment options and a prompt solution, as epilepsy is a severe disorder.

Stem cell-based therapy

In the treatment of epilepsy, stem cells have shown promise in treating a variety of neurological conditions. Stem cells are unspecialized cells that are capable of differentiating into different cell types. In stem cell therapy for epilepsy, the aim is to replace or repair damaged brain cells that play a role in the onset and progression of seizures. Prolonged or uncontrolled epileptic seizures can have wide-ranging adverse effects, including neuronal injury or death, mitochondrial dysfunction, increased reactive oxygen species, and astrocyte activation across the body. Because stem cells may regenerate, stem cell-based therapies present an appealing option for controlling long-term seizures, especially in drug-resistant epilepsy cases. This suggests that stem cell treatment holds potential as a new therapeutic strategy for treating this disease. Given their inherent capability to self-renew and differentiate into distinct cells, stem cells play a vital role in tissue regeneration. Additionally, this property offers the possibility of restoring and integrating disrupted neural circuits into a functioning condition. Stem cell therapy may play crucial roles in managing epilepsy, including inducing seizure remission, inhibiting epileptogenesis, averting the onset of chronic epilepsy, as well as enhancing cognition (Chang & Chang, 2022).

Different types of stem cells are primarily preferred by clinicians for use in treating epilepsy. Embryonic stem cells (ESCs) are undifferentiated cells obtained from embryos, and they can differentiate into numerous types of cells, notably neural cells, due to their pluripotency. Research has demonstrated their capacity to generate neurons and glial cells in order to repair the brain (Thompson et al., 2023). Nevertheless, ethical considerations and the possibility of tumor formation are substantial obstacles. Induced pluripotent stem cells (iPSCs), obtained from reprogrammed somatic cells, offer a solution to the ethical challenges faced with ESCs. Researchers are focusing on improving the differentiation efficiency of iPSCs to prevent tumorigenic properties, ensuring they mature fully before transplantation (Sayed et al., 2016). Furthermore, mesenchymal stem cells (MSCs), multipotent cells, give rise to several types of cells, including neurons. They contain anti-inflammatory and immunomodulatory effects, which can attenuate the epileptic brain environment (Huang et al., 2016). Neural stem cells (NSCs) are a promising treatment option for damaged neural networks, because they possess the ability to differentiate into both neurons and glial cells. Preclinical research NSCs have the ability to incorporate into the brain tissue of the host and decrease seizures (Xu et al., 2019). The limited availability of adult and fetal brain-derived NSCs and ethical concerns are among the issues that contribute to the scarcity of NSC treatments for epilepsy, despite their potential therapeutic advantages. Obtaining a sufficient quantity of adult brain-derived NSCs for therapeutic reasons is impossible from a living donor and difficult from the postmortem adult or fetal brain, since they are mostly found in certain locations like the subventricular zone and the hippocampus. Moreover, the act of collecting neural stem cells produced from fetal brains gives rise to ethical apprehensions about the destruction of human fetuses and the possibility of exploitation (Thodeson et al., 2018).

MSCs demonstrate the most therapeutic potential among these different stem cell types because of their beneficial characteristics, such as neuroprotection, immunomodulation, support for neurogenesis, and the ability to suppress oxidative stress damage and inflammation. MSCs exert many benefits, because they secrete diverse biologically active components like anti-inflammatory cytokines and neurotrophic factors (Milczarek et al., 2018; Vizoso et al., 2017). Research has demonstrated that these stem cells can traverse the blood-brain barrier (BBB) and target areas affected by the disease. Furthermore, MSCs have been shown to localize in the hippocampus of animal models with epilepsy; despite being given intravenously, they play a pivotal role in the treatment of the disease (Abdanipour et al., 2011). Studies have shown that administering MSCs can reduce seizure incidences (Hlebokazov et al., 2017, 2021; Milczarek et al., 2018; Szczepanik et al., 2020), enhance cognition (Fukumura et al., 2018; Milczarek et al., 2018; Wang et al., 2021) and motor control (Mohammed et al., 2014), raise the number of neurons (Abdanipour et al., 2011), and decrease oxidative stress (Salem et al., 2018). GABAergic interneurons have been shown to survive longer when MSCs were introduced (Fukumura et al., 2018; Mohammed et al., 2014).

Preclinical and clinical studies have demonstrated the advantages of stem cell therapy (**Table 1** and **Table 2**). In addition, MSCs have been found to enhance cognitive function and learning abilities, reduce or halt seizures, decrease neuroinflammation, and increase the number of neurons in patients diagnosed with epilepsy. For instance, clinical trials in phases I and II have demonstrated the safety of administering antiepileptic medications in conjunction with one or two doses of MSCs, either intravenously or intrathecally. Although stem cell therapy has not yet been integrated into standard clinical practice, its efficacy and safety have been validated by numerous clinical studies. The results have consistently shown that MSCs can offer substantial benefits without significant adverse effects, paving the way for future applications in clinical settings. The integration of stem cell treatment into standard epilepsy treatments could significantly enhance the quality of life for patients, offering hope for a more effective and comprehensive management of this disease.

Table 1: Preclinical studies of stem cell treatment for epilepsy.

Animal Model	Stem Cell Source	Results
Chemical induction: Glutamate	MSCs	Administration of MSCs led to reduced NMDA receptor activity and decreased calcium ion influx, protecting neurons from glutamate toxicity (Papazian et al., 2018).
Chemical induction: Lithium-pilocarpine	MSCs	Systemic infusion of stem cells in rats showed migration to the hippocampus, protecting neurons and reducing epileptogenesis and neurological impairments (Fukumura et al., 2018).
Transgenic	Neuroepithelial stem cells (NESs) derived from human embryonic stem cells	Genetically modified NESs lacking ADK gene increased adenosine production (Poppe et al., 2018).
Chemical Induction: Pilocarpine, pentylenetetrazole, and picrotoxin	NSCs	NSC infusion in rats exhibited antioxidant effects (de Gois da Silva et al., 2018).
Chemical induction: Kainate	Human induced pluripotent stem cell (hiPSC)-derived MEG-like precursor cells	Transplanted cells differentiated into inhibitory interneurons, reduced spontaneous recurrent seizures, and improved cognition and mood (Upadhya et al., 2019).
Chemical induction: Pilocarpine	NSCs and NSC-derived GABAergic neurons	Reduction of seizure frequency in the hippocampus (Xu et al., 2019).
Chemical induction: Pilocarpine	MSC-derived exosomes	Administration to hippocampal astrocytes reduced epilepsy-induced astrocyte alterations, neuroinflammation, and improved cognitive function (Xian et al., 2019).
Chemical induction: Kainate	hMSCs	Intranasal injection of hMSC-derived EVs resulted in neuron incorporation in hippocampal regions, providing neuroprotection and reducing chronic symptoms of epilepsy (Kodali et al., 2019).
Transgenic	Interneuron graft and periventricular endothelial cells	Enhanced migration of interneurons and reduced behavioral impairments in an experimental model (Datta et al., 2020).
Chemical induction: Kainic acid	Adipose-derived stem cell	Transplantation of stem cells enhanced learning and memory in a rate model (Wang et al., 2021).
Chemical induction: Scopolamine and pilocarpine	Olfactory mucosa MSC	Stem cell treatment ameliorated the neural network and inhibited inflammation in a mouse model (Liu et al., 2023).

Table 2: Clinical Studies of stem cell treatment for epilepsy.

Clinical Trial ID	Study Phase	Stem Cell Source	Route of administration	Results
NCT02497443	2	MSCs	Intravenous injection	Autologous MSC treatment in drug-resistant epilepsy patients reduced seizure frequency, with some patients becoming seizure-free or responsive to AEDs (Hlebokazov et al., 2017).
NCT00916266	1	Bone marrow mononuclear cells (BMMCs)	Intra-arterial injection	Intra-arterial delivery in MTLE patients led to seizure remission, improved cognition, and no adverse effects (DaCosta et al., 2018).
NCT03676569	1	Adipose-Derived Regenerative Cells (ADRCs)	Intrathecal infusion	Intrathecal delivery in autoimmune refractory epilepsy patients enhanced cognition and motor control (Szczepanik et al., 2020).

Mechanisms of action of stem cell treatment

Neuroprotection refers to the preservation and defense of the nervous system from damage or degeneration. Stem cells can secrete neurotrophic substances, which serve to safeguard neurons from harm. For instance, it has been shown that MSCs secrete neurotrophic factors that assists neuronal survival and adaptation like brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (Das et al., 2019). **Neurogenesis** refers to the process of generating new neurons. Research suggests that stem cells can differentiate into fully functional neurons, replacing the neurons that are destroyed due to epilepsy. ESCs, iPSCs, and NSCs are notable for their neurogenic potential, as they have the ability to generate new neurons (Bond et al., 2015; Hirose et al., 2020; Wesselschmidt et al., 2012). **Synaptic modulation** involves altering the strength or efficacy of synaptic connections between neurons. Transplanted stem cells have the ability to improve synaptic connection, hence repairing broken networks. NSCs, specifically, have demonstrated the ability to regulate synaptic activity, which reduces the seizures (Xu et al., 2019). **Immunomodulation** refers to the process of modifying or regulating the immune response in the body. Inflammation plays a key role in the epileptic brain, contributing to the onset and development of seizures. Stem cells, particularly MSCs, have the ability to alter the inflammatory environment, which may reduce the intensity of seizure activity (Jiang et al., 2020). MSCs also secrete neurotrophic substances, which serve to safeguard neurons from harm like BDNF and GDNF (Das et al., 2019). **Figure 2** shows the advantages of stem cells for the treatment of epilepsy.

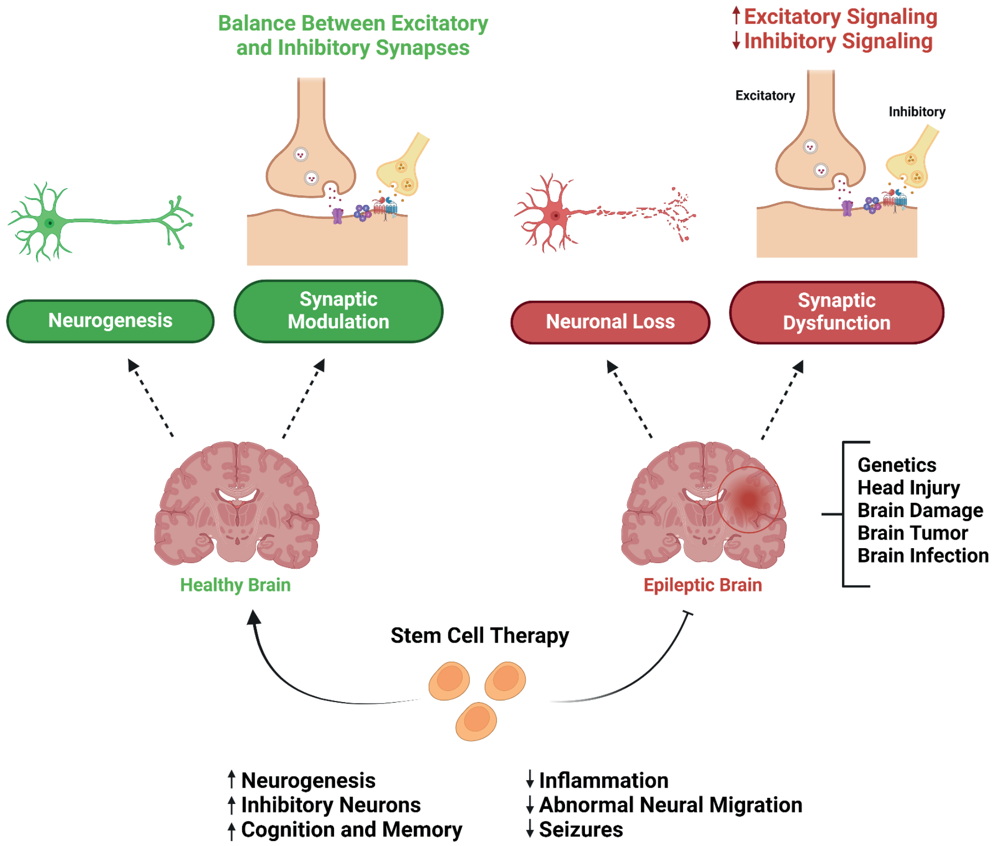

Figure 2. Mechanism of stem cell therapy in epilepsy *(Created by BioRender)*.

Conclusion

The application of stem cell therapy in clinical settings encounters various obstacles, such as enhancing the acquisition of cells, expanding production capacity, guaranteeing long-term safety, and resolving ethical dilemmas. Immunogenicity, cell viability, and incorporation into the host tissue are all supplementary challenges that require careful consideration.

This study is an important resource document for those working in the field, as it presents a comprehensive literature review on epilepsy; one of the most common neurological disorders today. In addition, since the approach presented is examined within the framework of stem cell technology, it also provides an important resource for today's regenerative medicine approach, because the point that today's stem cell science has reached is quite remarkable. Early clinical trials show that stem cell treatments can be safe and potentially beneficial, especially for individuals with epilepsy who do not respond to medications. The potential of these treatments to repair and rejuvenate damaged brain tissue represents an optimistic transition towards sustainable seizure management and improved quality of life, but there are still obstacles to overcome. Therefore, it is essential to focus on the long-term safety and effectiveness of stem cell treatments, considering potential risks such as immune rejection, tumor development, and unwanted differentiation. Addressing ethical and regulatory issues, as well as dealing with the high costs and technical intricacies of stem cell therapy, also requires attention.

Future studies should prioritize the refinement of treatment protocols, gaining insight into the lasting implications of integrating stem cells and devising economically viable approaches that can be widely implemented in clinical settings. As we delve deeper into refining these treatments, utilizing stem cell therapy has the chance to revolutionize epilepsy care and provide fresh optimism for individuals grappling with this persistent condition. By persisting in research and investigation, stem cell therapy may drastically change how epilepsy is treated, presenting new prospects and hope for millions of people.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics committee approval is not required for this study.

Authors' contribution statement

The authors acknowledge their contributions to this paper as follows: **Contributed to the conception and design of the study, conducted the literature review, and wrote the initial draft of the manuscript:** H.B.; **assisted in the literature review, contributed to the analysis and interpretation of data, and provided critical revisions to the manuscript for important intellectual content:** S.O.; **provided expertise and guidance on the subject matter, participated in the design of the study, and contributed to the final editing and approval of the manuscript:** S.I.; All authors reviewed the results and approved the final version of the manuscript.

Use of Artificial Intelligence: No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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References

Abdanipour, A., Tiraihi, T., & Mirnajafi-Zadeh, J. (2011). Improvement of the pilocarpine epilepsy model in rat using bone marrow stromal cell therapy. *Neurological research*, *33*(6), 625–632. https://doi.org/10.1179/1743132810Y.0000000018

Alayli, A., Lockard, G., Gordon, J., Connolly, J., Monsour, M., Schimmel, S., Dela Peña, I., & Borlongan, C. V. (2023). Stem Cells: Recent Developments Redefining Epilepsy Therapy. *Cell transplantation*, *32*, 9636897231158967. https://doi.org/10.1177/09636897231158967

Bond, A. M., Ming, G. L., & Song, H. (2015). Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. *Cell stem cell*, *17*(4), 385–395. https://doi.org/10.1016/j.stem.2015.09.003

Chang, B. L., & Chang, K. H. (2022). Stem Cell Therapy in Treating Epilepsy. *Frontiers in neuroscience*, *16*, 934507. https://doi.org/10.3389/fnins.2022.934507

DaCosta, J. C., Portuguez, M. W., Marinowic, D. R., Schilling, L. P., Torres, C. M., DaCosta, D. I., Carrion, M. J. M., Raupp, E. F., Machado, D. C., Soder, R. B., Lardi, S. L., & Garicochea, B. (2018). Safety and seizure control in patients with mesial temporal lobe epilepsy treated with regional superselective intra-arterial injection of autologous bone marrow mononuclear cells. *Journal of tissue engineering and regenerative medicine*, *12*(2), e648–e656. https://doi.org/10.1002/term.2334

Das, M., Mayilsamy, K., Mohapatra, S. S., & Mohapatra, S. (2019). Mesenchymal stem cell therapy for the treatment of traumatic brain injury: progress and prospects. *Reviews in the neurosciences*, *30*(8), 839–855. https://doi.org/10.1515/revneuro-2019-0002

Datta, D., Subburaju, S., Kaye, S., Baruah, J., Choi, Y. K., Nian, Y., Khalili, J. S., Chung, S., Elkhal, A., & Vasudevan, A. (2020). Human forebrain endothelial cell therapy for psychiatric disorders. *Molecular Psychiatry 2020 26:9*, *26*(9), 4864–4883. https://doi.org/10.1038/s41380-020-0839-9

de Gois da Silva, M. L., da Silva Oliveira, G. L., de Oliveira Bezerra, D., da Rocha Neto, H. J., Feitosa, M. L. T., Argôlo Neto, N. M., Rizzo, M. D. S., & de Carvalho, M. A. M. (2018). Neurochemical properties of neurospheres infusion in experimental-induced seizures. *Tissue & cell*, *54*, 47–54. https://doi.org/10.1016/j.tice.2018.08.002

Dubé, C. M., Brewster, A. L., Richichi, C., Zha, Q., & Baram, T. Z. (2007). Fever, febrile seizures and epilepsy. *Trends in neurosciences*, *30*(10), 490–496. https://doi.org/10.1016/J.TINS.2007.07.006

French, J. A., Kanner, A. M., Bautista, J., Abou-Khalil, B., Browne, T., Harden, C. L., Theodore, W. H., Bazil, C., Stern, J., Schachter, S. C., Bergen, D., Hirtz, D., Montouris, G. D., Nespeca, M., Gidal, B., Marks, W. J., Turk, W. R., Fischer, J. H., Bourgeois, B., … Glauser, T. A. (2004). Efficacy and Tolerability of the New Antiepileptic Drugs, I: Treatment of New-Onset Epilepsy: Report of the TTA and QSS Subcommittees of the American Academy of Neurology and the American Epilepsy Society. *Epilepsia*, *45*(5), 401–409. https://doi.org/10.1111/J.0013-9580.2004.06204.X

Fukumura, S., Sasaki, M., Kataoka-Sasaki, Y., Oka, S., Nakazaki, M., Nagahama, H., Morita, T., Sakai, T., Tsutsumi, H., Kocsis, J. D., & Honmou, O. (2018). Intravenous infusion of mesenchymal stem cells reduces epileptogenesis in a rat model of status epilepticus. *Epilepsy Research*, *141*, 56–63. https://doi.org/10.1016/J.EPLEPSYRES.2018.02.008

Guerreiro, C. A. M. (2016). Epilepsy: Is there hope? *The Indian Journal of Medical Research*, *144*(5), 657. https://doi.org/10.4103/IJMR.IJMR_1051_16

Hirose, S., Tanaka, Y., Shibata, M., Kimura, Y., Ishikawa, M., Higurashi, N., Yamamoto, T., Ichise, E., Chiyonobu, T., & Ishii, A. (2020). Application of induced pluripotent stem cells in epilepsy. *Molecular and Cellular Neuroscience*, *108*, 103535. https://doi.org/10.1016/J.MCN.2020.103535

Hlebokazov, F., Dakukina, T., Ihnatsenko, S., Kosmacheva, S., Potapnev, M., Shakhbazau, A., Goncharova, N., Makhrov, M., Korolevich, P., Misyuk, N., Dakukina, V., Shamruk, I., Slobina, E., & Marchuk, S. (2017). Treatment of refractory epilepsy patients with autologous mesenchymal stem cells reduces seizure frequency: An open label study. *Advances in Medical Sciences*, *62*(2), 273–279. https://doi.org/10.1016/J.ADVMS.2016.12.004

Hlebokazov, F., Dakukina, T., Potapnev, M., Kosmacheva, S., Moroz, L., Misiuk, N., Golubeva, T., Slobina, E., Krasko, O., Shakhbazau, A., Hlavinski, I., & Goncharova, N. (2021). Clinical benefits of single vs repeated courses of mesenchymal stem cell therapy in epilepsy patients. *Clinical Neurology and Neurosurgery*, *207*. https://doi.org/10.1016/J.CLINEURO.2021.106736

Huang, P. Y., Shih, Y. H., Tseng, Y. J., Ko, T. L., Fu, Y. S., & Lin, Y. Y. (2016). Xenograft of human umbilical mesenchymal stem cells from Wharton's jelly as a potential therapy for rat pilocarpine-induced epilepsy. *Brain, behavior, and immunity*, *54*, 45–58. https://doi.org/10.1016/j.bbi.2015.12.021

Jiang, M., Ye, J., Wang, X., Li, N., Wang, Y., & Shi, Y. (2020). Phosphatase SHP1 impedes mesenchymal stromal cell immunosuppressive capacity modulated by JAK1/STAT3 and P38 signals. *Cell and Bioscience*, *10*(1), 1–10. https://doi.org/10.1186/S13578-020-00428-W/FIGURES/4

Johnson, E. L. (2019). Seizures and Epilepsy. *The Medical Clinics of North America*, *103*(2), 309–324. https://doi.org/10.1016/J.MCNA.2018.10.002

Klein, P., Kaminski, R. M., Koepp, M., & Löscher, W. (2024). New epilepsy therapies in development. *Nature Reviews. Drug Discovery*. https://doi.org/10.1038/S41573-024-00981-W

Kodali, M., Castro, O. W., Kim, D. K., Thomas, A., Shuai, B., Attaluri, S., Upadhya, R., Gitai, D., Madhu, L. N., Prockop, D. J., & Shetty, A. K. (2019). Intranasally Administered Human MSC-Derived Extracellular Vesicles Pervasively Incorporate into Neurons and Microglia in both Intact and Status Epilepticus Injured Forebrain. *International Journal of Molecular Sciences 2020, Vol. 21, Page 181*, *21*(1), 181. https://doi.org/10.3390/IJMS21010181

Kwan, P., Arzimanoglou, A., Berg, A. T., Brodie, M. J., Hauser, W. A., Mathern, G., Moshé, S. L., Perucca, E., Wiebe, S., & French, J. (2010). Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia*, *51*(6), 1069–1077. https://doi.org/10.1111/J.1528-1167.2009.02397.X

Kwan, P., Schachter, S. C., & Brodie, M. J. (2011). Drug-resistant epilepsy. *New England Journal of Medicine*, *365*(10), 919–926. https://doi.org/https://doi.org/10.1056/nejmra1004418

Lee, V. L. L., Choo, B. K. M., Chung, Y. S., Kundap, U. P., Kumari, Y., & Shaikh, M. F. (2018). Treatment, Therapy and Management of Metabolic Epilepsy: A Systematic Review. *International Journal of Molecular Sciences*, *19*(3). https://doi.org/10.3390/IJMS19030871

Liu, G., Health, A., Slater, N., & Perkins, A. (2017). Epilepsy: Treatment Options. *American Family Physician*, *96*(2), 87–96. https://www.aafp.org/pubs/afp/issues/2017/0715/p87.html

Liu, Z. Z., Huang, Y., Hong, C. G., Wang, X., Duan, R., Liu, J. Y., He, J. L., Duan, D., Xie, H., & Lu, M. (2023). Autologous olfactory mucosa mesenchymal stem cells treatment improves the neural network in chronic refractory epilepsy. *Stem Cell Research and Therapy*, *14*(1), 1–16. https://doi.org/10.1186/S13287-023-03458-6/FIGURES/8

St Louis E. K. (2009). Truly "rational" polytherapy: maximizing efficacy and minimizing drug interactions, drug load, and adverse effects. *Current neuropharmacology*, *7*(2), 96–105. https://doi.org/10.2174/157015909788848929

Lybrand, Z. R., Goswami, S., & Hsieh, J. (2020). Stem cells: a path towards improved epilepsy therapies. *Neuropharmacology*, *168*, 107781. https://doi.org/10.1016/J.NEUROPHARM.2019.107781

McNamara, J. O. (1999). Emerging insights into the genesis of epilepsy. *Nature*, *399*(6738 Suppl). https://doi.org/10.1038/399A015

Miguel Sanz, C., Martinez Navarro, M., Caballero Diaz, D., Sanchez-Elexpuru, G., & di Donato, V.

(2023). Toward the use of novel alternative methods in epilepsy modeling and drug discovery. *Frontiers in Neurology*, *14*, 1213969. https://doi.org/10.3389/FNEUR.2023.1213969/BIBTEX

Milczarek, O., Jarocha, D., Starowicz–Filip, A., Kwiatkowski, S., Badyra, B., & Majka, M. (2018). Multiple Autologous Bone Marrow–Derived CD271+ Mesenchymal Stem Cell Transplantation Overcomes Drug–Resistant Epilepsy in Children. *Stem Cells Translational Medicine*, *7*(1), 20. https://doi.org/10.1002/SCTM.17-0041

Mohammed, A. S., Ewais, M. M., Tawfik, M. K., & Essawy, S. S. (2014). Effects of intravenous human umbilical cord blood mesenchymal stem cell therapy versus gabapentin in pentylenetetrazole-induced chronic epilepsy in rats. *Pharmacology*, *94*(1–2), 41–50. https://doi.org/10.1159/000365219

Murray, C. J. L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J. A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, S. Y., Ali, M. K., AlMazroa, M. A., Alvarado, M., Anderson, H. R., … Lopez, A. D. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet (London, England)*, *380*(9859), 2197–2223. https://doi.org/10.1016/S0140-6736(12)61689-4

Nair, D. R. (2016). Management of Drug-Resistant Epilepsy. *Continuum (Minneapolis, Minn.)*, *22*(1 Epilepsy), 157–172. https://doi.org/10.1212/CON.0000000000000297

Neal, E. G., Chaffe, H., Schwartz, R. H., Lawson, M. S., Edwards, N., Fitzsimmons, G., Whitney, A., & Cross, J. H. (2008). The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *The Lancet Neurology*, *7*(6), 500–506. https://doi.org/10.1016/S1474-4422(08)70092-9

Papazian, I., Kyrargyri, V., Evangelidou, M., Voulgari-Kokota, A., & Probert, L. (2018). Mesenchymal Stem Cell Protection of Neurons against Glutamate Excitotoxicity Involves Reduction of NMDA-Triggered Calcium Responses and Surface GluR1, and Is Partly Mediated by TNF. *International Journal of Molecular Sciences 2018, Vol. 19, Page 651*, *19*(3), 651. https://doi.org/10.3390/IJMS19030651

Patel, D. C., Tewari, B. P., Chaunsali, L., & Sontheimer, H. (2019). Neuron-glia interactions in the pathophysiology of epilepsy. *Nature Reviews. Neuroscience*, *20*(5), 282–297. https://doi.org/10.1038/S41583-019-0126-4

Poppe, D., Doerr, J., Schneider, M., Wilkens, R., Steinbeck, J. A., Ladewig, J., Tam, A., Paschon, D. E., Gregory, P. D., Reik, A., Müller, C. E., Koch, P., & Brüstle, O. (2018). Genome Editing in Neuroepithelial Stem Cells to Generate Human Neurons with High Adenosine-Releasing Capacity. *Stem Cells Translational Medicine*, *7*(6), 477–486. https://doi.org/10.1002/SCTM.16-0272

Riva, A., Golda, A., Balagura, G., Amadori, E., Vari, M. S., Piccolo, G., Iacomino, M., Lattanzi, S., Salpietro, V., Minetti, C., & Striano, P. (2021). New Trends and Most Promising Therapeutic Strategies for Epilepsy Treatment. *Frontiers in Neurology*, *12*, 753753. https://doi.org/10.3389/FNEUR.2021.753753

Salem, N. A., El-Shamarka, M., Khadrawy, Y., & El-Shebiney, S. (2018). New prospects of mesenchymal stem cells for ameliorating temporal lobe epilepsy. *Inflammopharmacology*, *26*(4), 963–972. https://doi.org/10.1007/S10787-018-0456-2

Sayed, N., Liu, C., & Wu, J. C. (2016). Translation of Human-Induced Pluripotent Stem Cells: From Clinical Trial in a Dish to Precision Medicine. *Journal of the American College of Cardiology*, *67*(18), 2161–2176. https://doi.org/10.1016/J.JACC.2016.01.083

Shaefi, S., & Harkness, W. (2003). Current Status of Surgery in the Management of Epilepsy. *Epilepsia*, *44*(SUPPL. 1), 43–47. https://doi.org/10.1046/J.1528-1157.44.S.1.15.X

Sirven J. I. (2015). Epilepsy: A Spectrum Disorder. *Cold Spring Harbor perspectives in medicine*, *5*(9), a022848. https://doi.org/10.1101/cshperspect.a022848

Szczepanik, E., Mierzewska, H., Antczak-Marach, D., Figiel-Dabrowska, A., Terczynska, I., Tryfon, J., Krzesniak, N., Noszczyk, B. H., Sawicka, E., Domanska-Janik, K., & Sarnowska, A. (2020). Intrathecal Infusion of Autologous Adipose-Derived Regenerative Cells in Autoimmune Refractory Epilepsy: Evaluation of Safety and Efficacy. *Stem Cells International*, *2020*. https://doi.org/10.1155/2020/7104243

Thodeson, D. M., Brulet, R., & Hsieh, J. (2018). Neural stem cells and epilepsy: functional roles and disease-in-a-dish models. *Cell and Tissue Research*, *371*(1), 47–54. https://doi.org/10.1007/S00441-017-2675-Z

Thompson, K., Kanemori, M., Kimes, E., Hobbs, E., Phan, J., Wilson, T., Wertheimer, A., Yoshida, T., Flores-Barnett, D., Kang, M., & Auyang, C. (2023). Transplantation of neurogenic-fusionogenic embryonic stem cells modified to overexpress GABA into a model of temporal lobe epilepsy: Promises and potential pitfalls. *Medical Research Archives*, *11*(10). https://doi.org/10.18103/MRA.V11I10.4510

Upadhya, D., Hattiangady, B., Castro, O. W., Shuai, B., Kodali, M., Attaluri, S., Bates, A., Dong, Y., Zhang, S. C., Prockop, D. J., & Shetty, A. K. (2019). Human induced pluripotent stem cell-derived MGE cell grafting after status epilepticus attenuates chronic epilepsy and comorbidities via synaptic integration. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(1), 287–296. https://doi.org/10.1073/PNAS.1814185115/-/DCSUPPLEMENTAL

Vezzani, A., Balosso, S., & Ravizza, T. (2019). Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nature Reviews. Neurology*, *15*(8), 459–472. https://doi.org/10.1038/S41582-019-0217-X

Vizoso, F. J., Eiro, N., Cid, S., Schneider, J., & Perez-Fernandez, R. (2017). Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. *International Journal of Molecular Sciences*, *18*(9). https://doi.org/10.3390/IJMS18091852

Voskuyl, R. A., & Clinckers, R. (2009). ANTIEPILEPTIC DRUGS | Pharmacological Approaches for the Assessment of Antiepileptic Drug Efficacy in Experimental Animal Models. *Encyclopedia of Basic Epilepsy Research*, 90–97. https://doi.org/10.1016/B978-012373961-2.00235-6

Wang, L., Zhao, Y., Pan, X., Zhang, Y., Lin, L., Wu, Y., Huang, Y., & He, H. (2021). Adipose-derived stem cell transplantation improves learning and memory via releasing neurotrophins in rat model of temporal lobe epilepsy. *Brain Research*, *1750*. https://doi.org/10.1016/J.BRAINRES.2020.147121

Wesselschmidt, R. L. (2012). Embryonic Stem Cells and Neurogenesis. *Neural Development and Stem Cells: Third Edition*, 31–59. https://doi.org/10.1007/978-1-4614-3801-4_2

Xian, P., Hei, Y., Wang, R., Wang, T., Yang, J., Li, J., Di, Z., Liu, Z., Baskys, A., Liu, W., Wu, S., & Long, Q. (2019). Mesenchymal stem cell-derived exosomes as a nanotherapeutic agent for amelioration of inflammation-induced astrocyte alterations in mice. *Theranostics*, *9*(20), 5956–5975. https://doi.org/10.7150/THNO.33872

Xu, K., Liu, F., Xu, W., Liu, J., Chen, S., & Wu, G. (2019). Transplanting GABAergic Neurons Differentiated from Neural Stem Cells into Hippocampus Inhibits Seizures and Epileptiform Discharges in Pilocarpine-Induced Temporal Lobe Epilepsy Model. *World Neurosurgery*, *128*, e1–e11. https://doi.org/10.1016/J.WNEU.2019.01.245

REVIEW

Biosensor applications in the monitoring of elderly patients

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Abstract

Nurse-based patient monitoring is prone to errors due to manual measurements and documentation, leading to potential inaccuracies in care. The use of biosensors offers a promising solution by enabling real-time and continuous monitoring of patient health. Categorizing patient care reports as critical or non-critical using mobile recording systems based on biosensor data can help prevent errors and improve care. The use of biosensors can significantly reduce morbidity and mortality, especially after emergencies and accidents. These devices improve the quality of care and increase the satisfaction of older people, their families and healthcare professionals. Wearable biosensors make it easier for older people to monitor their health, which can help reduce hospital admissions. Chronic diseases such as cardiovascular disease, cancer, diabetes, dementia and stroke pose challenges to healthcare delivery and interpretation of results. Integrating biosensors into health monitoring and measurement is an innovative approach to managing these chronic conditions more effectively. To improve self-management of chronic diseases in older people, it is essential to educate healthcare professionals and promote research in this area. As a result, the use of biosensors to monitor the daily activities and health parameters of elderly patients is expanding, highlighting the importance of multidisciplinary research in biotechnology, chemistry, engineering and nursing.

Keywords: Biosensor, geriatrics, wearable biosensor, nursing; innovative approaches

Introduction

The increasing elderly population in recent years has caused some problems to arise. The growth of health expenditures of the elderly struggling with aging and chronic diseases requires the adoption of effective measures worldwide. Traditional health monitoring methods often involve periodic assessments and manual documentation, which can be prone to delays and inaccuracies, but biosensors overcome these limitations by offering the potential for continuous and real-time monitoring (Kalid et al., 2018; Ledikwe et al., 2014). However, they have their own challenges, including issues related to battery life, user comfort and data security (Pateraki et al., 2020). Biotechnology has begun to be used to monitor these problems and to enable the elderly to live healthily in a comfortable, reliable environment. It is very important to develop and use biotechnology systems in daily life so that the elderly can continue their daily life activities and detect emergencies or accidents such as stroke, fall, syncope and myocardial infarction (Lin et al., 2007; Olmedo-Aguirre et al., 2022). Using biotechnology, chronic diseases can be prevented and adequately monitored, and health risks can be identified to improve the quality of life of the elderly (Olmedo-Aguirre et al., 2022).

The elderly population is expected to be 2.1 billion in 2050 (Keating, 2022). The increase in the elderly population increases the incidence of chronic diseases such as cardiovascular diseases, cancer, dementia and diabetes. In a study, it was found that 78.7% of the elderly had chronic diseases and the rate of those who stated that these diseases seriously limited the patient's daily life activities was 32.3%. It has been observed that 24% of the elderly have fallen inside or outside the home in the last year. 54.6% of the elderly stated that they would like to benefit from home care services in the future, and 41.3% stated that they would prefer a nursing home (TÜİK, 2023). In the world, the incidence of death due to cardiovascular diseases is 32%, the incidence of death due to cancer is 16.8% and the incidence of death due to diabetes is 2.5%. There are 422 million diabetics and more than 55 million dementia patients in the world (Hacker, 2024). It is imperative to monitor the health status of people who have these diseases and are at risk of contracting these diseases. Since sufficient time, attention, discipline and knowledge are required to monitor the patient's physiological changes, the use of biosensor technology in monitoring the changes occurring in the patient is extremely important. In the studies, patients' balance status changes, falls during work, physical activity and vital signs were monitored. In these studies, elderly people with cardiovascular disease, respiratory diseases, sleep problems, diabetes, osteoporosis, Parkinson's disease, alcohol addiction and seizures were followed (Olmedo-Aguirre et al., 2022).

Biosensors enable our body to communicate and react better with our environment, diagnose diseases early, and improve health by detecting diseases early. The body's physiological response transmits data to the control unit through wearable biosensors, the control unit analyzes the data, and health parameters are transmitted to the mobile device (Smith et al., 2023). Appropriate selection of biomarkers indicating health and disease states is vital for the diagnosis and treatment of the disease by detecting it before it occurs (Salek-Maghsoudi et al., 2018).

In intensive care, patients whose physiological parameters are monitored with a bedside monitor and alarm system can be monitored at any place and time using a lightweight, wearable, and wireless biosensor because of current developments. The biosensor processes digital data and transmits the necessary data to communicate. Transmission distance, frequency, and battery life are the most important problems in the application. In nursing homes, the elderly person carries a tiny biosensor. If he falls in an area such as a park or a toilet, it becomes easier for the nurse to detect the elderly person's condition early and find his location through a call containing information to the base station. To extend the battery life of the biosensor, it is recommended that it be turned on only for measurement and not monitored continuously. However, it is extremely important to have the sensors turned on in places where the elderly are alone, such as parks, neighborhoods, gardens, and toilets, to constantly monitor the elderly's vital signs such as temperature, pulse rate and number, respiratory rate, saturation, and heart rhythm, and to take immediate action for help by monitoring their location. In addition, the necessary health care can be provided because the parameters that the elderly follow themselves, such as blood pressure and blood sugar, can be monitored (Lin et al., 2007).

Through this system, we can track and record the elderly person's time to get up, go to the restroom, exercise, go to a restaurant, and perform daily living activities. We can follow the social life of the elderly and provide psychosocial support by detecting abnormal mental states at an early stage (Lin et al., 2007). Elderly people with chronic diseases can adjust their diets and daily routines in time according to system data and save money economically by reducing hospitalizations. It can enable patients to understand their health status in real time and ensure timely hospitalization (Smith et al., 2023).

Tracking physiological variables

With the development of technology, biosensors have been developed that can monitor the physiological variables seen in all chronic diseases. Heart rate and number, pulse rate and number, respiratory rate and number, blood oxygen saturation (SpO_2), blood pressure, and blood sugar of elderly people suffering from chronic diseases can be monitored through biosensors (Miller et al., 2021; Olmedo-Aguirre et al., 2022; Wang et al., 2017). Respiratory rate and rate can be used to detect, diagnose, and monitor patients affected by chronic diseases such as anxiety, pneumonia, heart failure, lung disease, and drug coma. In addition to smartwatches, intradermal sensors, portable sensors, rings, and insoles are used to monitor these variables. While it is easier to design sensors that can read parameters such as blood oxygen level and heart rate for monitoring cardiovascular diseases, device placement and maintenance becomes difficult because sensors must be implanted at the bone level for osteoporosis, which requires constant monitoring of the patient's bones. The size of the sensors used, the methods of placing the sensors on the human body, and their subsequent removal cause some difficulties. For these reasons, very few biosensors are still available for many chronic degenerative diseases such as osteoporosis, some types of cancer, and gastrointestinal diseases (Olmedo-Aguirre et al., 2022; Wang et al., 2017).

Blood glucose monitoring: Noninvasive intravascular blood glucose can be measured with ultra-thin skin-like biosensors on a flexible biocompatible paper battery. The battery connects to the skin and creates electrochemical channels in the subcutaneous tissue (Chen et al., 2017). Continuous blood sugar monitoring can be done using a contact lens with a photonic glucose sensor and smartphone camera reading (Elsherif et al., 2018). Electromagnetism sensors are a noninvasive, permanent, portable, patch-shaped system that mimics vascular anatomy. It is suitable for personalized monitoring according to the patient's characteristics (Hanna et al., 2020). It is also used in disposable patch-type devices that measure glucose levels in sweat and automatically apply the drug via a transdermal drug delivery device (Lee et al., 2017).

Pulse rate and respiration rate: Smart bracelets, watches, belts and armbands can be used to monitor health status and give timely warnings, displaying parameters such as pulse rate, electrocardiogram (ECG) and respiratory rate. Respiration rate is estimated from an arm-worn cuff ECG using a method based on changes in QRS slopes and the angle of the R wave. Predictions are compared with those obtained from the respiratory signal. The cuff contains a pair of dry electrodes that record the ECG and is designed for long-term monitoring (Lázaro et al., 2018). With IoT-based wearable devices, pulse rate and ECG findings can be transmitted to the computer or mobile phone and the data can be monitored continuously (Sani et al., 2019; Xiao et al., 2020). The leg belt is a portable ECG sensor system that captures the patient's vital data through the skin by detecting signals with patch electrodes. This system can collect 6 ECG signals (Hussein et al., 2017). In a study conducted with emergency room patients, it was observed that the respiratory rate measured using the Philips wearable biosensor was detected continuously and accurately without intervention. The fact that the Philips wearable sensor is a lightweight, wireless, battery-powered device and sticks to the skin makes it easier to use. It is attached to the patient's chest, allowing the patient's respiratory rate, pulse rate, gait and posture to be monitored (Li et al., 2019). Heart rate increases acutely with exertion and stress and decreases rapidly during relaxation and sleep. Heart rate is used to evaluate sleep quality. In addition, the slow decrease in the heart rate of dementia and Parkinson's disease over months allows the disease to be recognized in its early stages. Respiratory rate increases due to decompensation of stress, fever and lung diseases, and pulmonary edema in heart failure (Saner, 2018).

Blood pressure: Optical-based heart rate sensors can be attached to wristwatches, earbuds, behind-the-ears, and glasses. To measure blood pressure, the wristwatch is brought close to the chest and the micro-vibrations of the heartbeat in the chest are detected. As the pulse wave moves from the heart to the wrist, measurements are made by an optical sensor and an accelerometer in the watch (Carek et al., 2017). With the illuminated pulse sensor connected to an inflatable tube placed inside the ear, blood pressure measurements can be made easily during the daily activities of the elderly, maximizing the comfort level (Bui et al., 2019).

In a study conducted in the emergency department, continuous monitoring of vital signs with wearable biosensors enabled the detection of potential clinical problems 5.5 hours earlier compared to standard monitoring (Garbern et al., 2019). In a study conducted in a general ward, it was found that measurements made with wearable sensors gave up to 10 hours warnings earlier (Weenk et al., 2019).

The monitoring of balance disorders

Moderate or severe injuries occur in 30% of elderly people because of falls. In this case, it may cause disability in the elderly, restriction of physical activity, and earlier admission to a nursing home. For the elderly to maintain their independence and mobility, it is very important to determine the factors affecting postural stability and design special interventions. Balance control is a complex skill based on the interaction of sensorimotor processes. Accelerometers are used to evaluate the balance status of the elderly. Accelerometers are economical, mobile and lightweight inertial sensors used to perform post-urography. In case of imbalance, the rod on the accelerometer deflects and the springs detect their acceleration. Accelerometers monitor physical activity, that is, measure the duration of endurance in different activities, such as intense exercise or rest (sitting, lying). This device can encourage the elderly to do physical activity. The accelerometer can be carried by attaching to the elderly person's body, arm or any desired area with a fixed belt and can measure the elderly person's daily life activities (Leirós-Rodríguez et al., 2019). Studies have shown that placing more than one sensor on the patient is more reliable. It has been determined that if a single sensor is to be placed on the elderly, it should be placed on the waist, chest, head or pelvis instead of the ankle. Since the asymmetry in foot movements prevents the sensor from detecting the situation after the fall begins, it must be monitored by installing at least two sensors (Aziz et al., 2014; Howcroft et al., 2016). Additionally, the patient's data is transmitted to the mobile device via wearable sensors. If the patient falls, this system sends a message to the emergency room, family members or caregivers for timely intervention (Smith et al., 2023). Apart from this, the activity of the elderly can be detected, and risks can be evaluated by monitoring environmental conditions with environmental biosensors (Sun et al., 2022).

The monitoring of pressure sore

Pressure sore is a condition that can occur frequently in elderly patients. Approximately 9% of hospitalized patients develop pressure sores. As a result of impaired blood flow in areas exposed to constant pressure, tissue necrose and a pressure sore occur. To detect the onset of a pressure sore early, the electrochemical enzyme-based biosensor is attached to the patient's body and allows continuous and noninvasive monitoring of the lactate level in sweat. It is thought that pressure sores can be prevented by monitoring them with alarming biosensors. Studies have found that lactate level is directly related to pressure sore development (Tur García, 2014). Additionally, wound management is expensive as it requires several days of testing and reduces the patient's quality of life. Therefore, uric acid biosensors in the form of adhesive tape have been developed to prevent complications and to detect the progress of the wound in time. Uric acid concentration indicates the healing process in the wound area. The high uric acid concentration in chronic wounds decreases with treatment, and the healing process can be monitored by constantly measuring uric acid levels in the wound area. Apart from this, a biosensor that measures pH can also be used in wound monitoring. As the wound heals and the tissue regenerates, the pH of the skin passes from alkaline to acidic, in which case pH monitoring can be done for wound care (Arakawa et al., 2022).

Incontinent patient monitoring

Since it is difficult to collect the urine of elderly people who have urinary incontinence problems, it is important to use an economical and useful biosensor. In a study, a multi-parameter biosensor was placed on the diaper to detect urine biomarkers, and the nurse was able to monitor when the diaper needed to be changed by detecting it with detection systems. By placing a multi-parameter electrochemical biosensor in an ordinary cloth, on-site detection of glucose and uric acid in urine was achieved. With this sensor, doctors can access urine biomarker data to assess the health status of their patients. In this way, it can better monitor the elderly living in nursing homes. It is thought that thanks to the biosensor placed in the patient's diaper, patients will avoid embarrassment, and patients with incontinence will live with more dignity (Su et al., 2022).

Infection tracking

Microorganisms are not visible to the naked eye, but they are found everywhere and are the main causes of diseases. Microorganisms can be detected through biosensors. Some infectious diseases have no cure and are very dangerous. Therefore, preventing diseases by determining these infection biomarkers with biosensors can reduce the economic burden and deaths. Biosensors are used to detect many pathogens such as human papillomavirus, hepatitis B virus, mycobacterium tuberculosis, meningitis, toxoplasma gondii, and leprosy (Chatterjee et al., 2022).

Behavior tracking

Behavioral detection is a more objective approach than neuropsychiatric scales used to detect Alzheimer's disease at an early stage. It is safer and more comfortable than invasive methods. It is also more economical than neuroimaging tests. With the development of technology, behavioral detection biosensors such as motion sensors and sound sensors have begun to be used to measure various behavioral biomarkers of the elderly. Behavioral sensing sensors are used in society for entertainment, comfort, natural interaction, assisted living, security, etc. It attracts more attention because it supports many areas such as. For example, the problem of aging increases the demand for home healthcare services for the elderly. The continued use of behavior monitoring biosensors is urgently needed to ensure that elderly people, especially those living alone, live in a safer environment and to detect abnormal behavior. Additionally, behavioral detection biosensors can be used to detect Parkinson's disease, stroke, etc. Behavioral disorders of diseases are also determined. Behavioral biomarkers may be vital indicators in the early stages of Alzheimer's disease. In patients, symptoms such as changes in motor behavior occur before profound memory deficits. Therefore, since motor behavior changes usually occur with old age, behavioral detection biosensors are vital for both the early diagnosis of Alzheimer's disease and the ability of the elderly to perform daily living activities. With behavioral detection biosensors, body movement behavior, eye movement behavior, multimodal behavior, speech and language behavior applications are detected. Multimodal behavioral sensors enable the examination of multiple behavioral parameters such as physiological indicators, eye movement, voice and body movements, along with environmental factors. Body movement behavior sensors can detect walking speed, walking stride, balance, foot kicking, upper limb movement, and other activities in daily living. Eye movement behavior sensors are used to measure looking, blinking, fixation, saccades, and pupillary response. Speech and language behavior sensors serve to identify and record acoustic features. Among the biosensors used to detect behavior in the diagnosis of Alzheimer's disease, multimodal behavioral sensors are the method that best distinguishes normal and abnormal behaviors (Sun et al., 2022).

In a hospital or nursing home, nurse-based patient monitoring is prone to errors due to manual measurement and documentation. Therefore, categorizing patient care reports as critical or non-critical reports with mobile recording using network systems according to biosensor values can prevent problems. Important clinical parameters, such as heart rate, may be subject to measurement error and subsequently lead to inaccurate results in nursing care. Patient status alarms created using automation systems are triggered in abnormal situations that occur in the patient and can provide warnings at different levels depending on the severity of the situation. It also provides 24-hour support to the elderly, patients, and caregivers (Vithya & Vinayaga Sundaram, 2017).

With the use of biosensors, morbidity and mortality can be reduced after emergencies and accidents. The quality-of-care increases and the satisfaction of the elderly/family and the nurse increases (Lin et al., 2007). Chronic diseases such as cardiovascular diseases, cancer, diabetes, dementia and stroke make the delivery of health care and the interpretation of results difficult. The use of biosensors in health measurements and patient monitoring are innovative approaches to reduce the burden of chronic diseases. Informing healthcare professionals and conducting encouraging research on this issue is extremely important for the self-management of chronic diseases in elderly patients. However, biotechnology applications are costly, applications in the field of health are limited and difficult to access (Hacker, 2024). Advanced technologies are not accepted by the elderly, and they experience difficulties because they do not have the necessary skills to use them (Olmedo-Aguirre et al., 2022).

Conclusions

Biosensors offer significant advantages in monitoring the health of elderly patients by providing continuous data and enabling early detection of health issues. They are instrumental in real-time monitoring and intervention, potentially revolutionizing the management of chronic conditions and enhancing overall care. Various types of biosensors are currently under research worldwide, demonstrating their utility in diagnosing autoimmune diseases, cancer, neurodegenerative diseases, and cardiovascular conditions. While these advancements hold promise for improving early diagnosis and preventative care, challenges such as limited battery life, user discomfort, and concerns regarding data security and privacy remain. Ongoing research and technological improvements are essential to overcome these hurdles and fully realize the potential of biosensors. As the field progresses, biosensors are expected to play a crucial role in advancing healthcare and providing more effective and personalized care for elderly patients.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics committee approval is not required for this study.

Authors' contribution statement

Study conception and design: BK; **Data collection**: BK; **Manuscript draft preparation**: BK All authors reviewed the results and approved the final version of the manuscript.

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References

Arakawa, T., Dao, D. V., & Mitsubayashi, K. (2022). Biosensors and chemical sensors for healthcare monitoring: a review. *IEEJ Transactions on Electrical and Electronic Engineering, 17*(5), 626-636.

https://doi.org/10.1002/tee.23580

Aziz, O., Park, E. J., Mori, G., & Robinovitch, S. N. (2014). Distinguishing the causes of falls in humans using an array of wearable tri-axial accelerometers. *Gait & posture*, 39(1), 506-512. https://doi.org/10.1016/j.gaitpost.2013.08.034

Bui, N., Pham, N., Barnitz, J. J., Zou, Z., Nguyen, P., Truong, H., Kim, T., Farrow, N., Nguyen, A., & Xiao, J. (2019). ebp: A wearable system for frequent and comfortable blood pressure monitoring from user's ear. The 25th annual international conference on mobile computing and networking. https://doi.org/10.1145/3300061.3345454

Carek, A. M., Conant, J., Joshi, A., Kang, H., & Inan, O. T. (2017). SeismoWatch: wearable cuffless blood pressure monitoring using pulse transit time. *Proceedings of the ACM on interactive, mobile, wearable and ubiquitous technologies, 1*(3), 1-16. https://doi.org/10.1145/3130905

Chatterjee, N., Manna, K., Mukherjee, N., & Saha, K. D. (2022). Challenges and future prospects and commercial viability of biosensor-based devices for disease diagnosis. *Biosensor Based Advanced Cancer Diagnostics*, 333-352. https://doi.org/10.1016/B978-0-12-823424-2.00013-2

Chen, Y., Lu, S., Zhang, S., Li, Y., Qu, Z., Chen, Y., Lu, B., Wang, X., & Feng, X. (2017). Skin-like biosensor system via electrochemical channels for noninvasive blood glucose monitoring. *Science advances*, 3(12), e1701629. https://doi.org/10.1126/sciadv.1701629

Elsherif, M., Hassan, M. U., Yetisen, A. K., & Butt, H. (2018). Wearable contact lens biosensors for continuous glucose monitoring using smartphones. *ACS nano, 12*(6), 5452-5462. https://doi.org/10.1021/acsnano.8b00829

Garbern, S. C., Mbanjumucyo, G., Umuhoza, C., Sharma, V. K., Mackey, J., Tang, O., Martin, K. D., Twagirumukiza, F. R., Rosman, S. L., & McCall, N. (2019). Validation of a wearable biosensor device for vital sign monitoring in septic emergency department patients in Rwanda. *Digital health, 5*, 2055207619879349. https://doi.org/10.1177/2055207619879349

Hacker, K. (2024). The burden of chronic disease. *Mayo Clinic Proceedings: Innovations, Quality & Outcomes, 8*(1), 112-119. https://doi.org/10.1016/j.mayocpiqo.2023.08.005

Hanna, J., Bteich, M., Tawk, Y., Ramadan, A. H., Dia, B., Asadallah, F. A., Eid, A., Kanj, R., Costantine, J., & Eid, A. A. (2020). Noninvasive, wearable, and tunable electromagnetic multisensing system for continuous glucose monitoring, mimicking vasculature anatomy. *Science advances, 6*(24), eaba5320. https://doi.org/10.1126/sciadv.aba5320

Howcroft, J., Lemaire, E. D., & Kofman, J. (2016). Wearable-sensor-based classification models of faller status in older adults. *PLoS one, 11*(4), e0153240. https://doi.org/10.1371/journal.pone.0153240

Hussein, A. F., Hashim, S. J., Aziz, A. F. A., Rokhani, F. Z., & Adnan, W. A. W. (2017). A real time ECG data compression scheme for enhanced bluetooth low energy ECG system power consumption. *Journal of Ambient Intelligence and Humanized Computing*, 1-14. https://doi.org/10.1007/s12652-017-0560-y

Kalid, N., Zaidan, A., Zaidan, B., Salman, O. H., Hashim, M., & Muzammil, H. (2018). Based real time remote health monitoring systems: A review on patients prioritization and related" big data" using body sensors information and communication technology. *Journal of medical systems, 42*, 1-30.

https://doi.org/10.1007/s10916-017-0883-4

Keating, N. (2022). A research framework for the United Nations Decade of Healthy Ageing (2021–2030). *European Journal of Ageing, 19*(3), 775-787. https://doi.org/10.1007/s10433-021-00679-7

Lázaro, J., Bailón, R., Gil, E., Noh, Y., Laguna, P., & Chon, K. H. (2018). Pilot study on electrocardiogram derived respiratory rate using a wearable armband. Computing in Cardiology Conference (CinC). https://doi.org/10.22489/CinC.2018.054

Ledikwe, J. H., Grignon, J., Lebelonyane, R., Ludick, S., Matshediso, E., Sento, B. W., Sharma, A., & Semo, B.-w. (2014). Improving the quality of health information: a qualitative assessment of data management and reporting systems in Botswana. *Health research policy and systems, 12*, 1-10. https://doi.org/10.1186/1478-4505-12-7

Lee, H., Song, C., Hong, Y. S., Kim, M., Cho, H. R., Kang, T., Shin, K., Choi, S. H., Hyeon, T., & Kim, D.-H. (2017). Wearable/disposable sweat-based glucose monitoring device with multistage transdermal drug delivery module. *Science advances, 3*(3), e1601314. https://doi.org/10.1126/sciadv.1601314

Leirós-Rodríguez, R., García-Soidán, J. L., & Romo-Pérez, V. (2019). Analyzing the use of accelerometers as a method of early diagnosis of alterations in balance in elderly people: A systematic review. *Sensors, 19*(18), 3883. https://doi.org/10.3390/s19183883

Li, T., Divatia, S., McKittrick, J., Moss, J., Hijnen, N. M., & Becker, L. B. (2019). A pilot study of respiratory rate derived from a wearable biosensor compared with capnography in emergency department patients. *Open Access Emergency Medicine*, 103-108. https://doi.org/0.2147/OAEM.S198842

Lin, Y.-J., Su, M.-J., Chen, S.-J., Wang, S.-C., Lin, C.-I., & Chen, H.-S. (2007). A study of ubiquitous monitor with RFID in an elderly nursing home. International Conference on Multimedia and Ubiquitous Engineering (MUE'07). https://doi.org/10.1109/MUE.2007.55

Miller, K., Baugh, C. W., Chai, P. R., Hasdianda, M. A., Divatia, S., Jambaulikar, G. D., & Boyer, E. W. (2021). Deployment of a wearable biosensor system in the emergency department: a technical feasibility study. Proceedings of the... Annual Hawaii International Conference on System Sciences. Annual Hawaii International Conference on System Sciences. https://doi.org/10.24251/HICSS.2021.433

Olmedo-Aguirre, J. O., Reyes-Campos, J., Alor-Hernandez, G., Machorro-Cano, I., Rodriguez-Mazahua, L., & Sanchez-Cervantes, J. L. (2022). Remote healthcare for elderly people using wearables: A review. *Biosensors, 12*(2), 73. https://doi.org/10.3390/bios12020073

Pateraki, M., Fysarakis, K., Sakkalis, V., Spanoudakis, G., Varlamis, I., Maniadakis, M., Lourakis, M., Ioannidis, S., Cummins, N., & Schuller, B. (2020). Biosensors and Internet of Things in smart healthcare applications: Challenges and opportunities. *Wearable and Implantable Medical Devices*, 25-53. https://doi.org/10.1016/B978-0-12-815369-7.00002-1

Salek-Maghsoudi, A., Vakhshiteh, F., Torabi, R., Hassani, S., Ganjali, M. R., Norouzi, P., Hosseini, M., & Abdollahi, M. (2018). Recent advances in biosensor technology in assessment of early diabetes biomarkers. *Biosensors and Bioelectronics, 99*, 122-135. https://doi.org/10.1016/j.bios.2017.07.047

Saner, H. (2018). Wearable sensors for assisted living in elderly people. *Frontiers in ICT, 5,* 1. https://doi.org/10.3389/fict.2018.00001

Sani, M. I., Mutiara, G. A., & Putra, R. S. D. W. (2019). Fit-NES: Wearable bracelet for heart rate monitoring. *TELKOMNIKA (Telecommunication Computing Electronics and Control), 17*(1), 392-399. https://doi.org/10.12928/telkomnika.v17i1.11611

Smith, A. A., Li, R., & Tse, Z. T. H. (2023). Reshaping healthcare with wearable biosensors. *Scientific Reports, 13*(1), 4998. https://doi.org/10.1038/s41598-022-26951-z

Su, H., Sun, F., Lu, Z., Zhang, J., Zhang, W., & Liu, J. (2022). A wearable sensing system based on smartphone and diaper to detect urine in-situ for patients with urinary incontinence. *Sensors and Actuators B: Chemical, 357*, 131459. https://doi.org/10.1016/j.snb.2022.131459

Sun, X., Sun, X., Wang, Q., Wang, X., Feng, L., Yang, Y., Jing, Y., Yang, C., & Zhang, S. (2022). Biosensors toward behavior detection in diagnosis of alzheimer's disease. *Frontiers in Bioengineering and Biotechnology, 10*, 1031833. https://doi.org/10.3389/fbioe.2022.1031833

TÜİK. Türkiye Yaşlı Profili Araştırması, 2023. Retrieved June, 27 from https://data.tuik.gov.tr/Bulten/Index?p=Turkiye-Yasli-Profili-Arastirmasi-2023-53809.

Tur García, E. (2014). Development of a flexible biosensor for the monitoring of lactate in human sweat for its medical use in pressure ischemia. Cranfield University, UK.

Vithya, G., & Vinayaga Sundaram, B. (2017). Inpatient critical stage monitoring in smart hospitals by contextual Fuzzy based QoS routing for WBMS network nurse call system. *Wireless Personal Communications, 94*, 2065-2080. https://doi.org/10.1007/s11277-016-3361-2

Wang, Z., Yang, Z., & Dong, T. (2017). A review of wearable technologies for elderly care that can accurately track indoor position, recognize physical activities and monitor vital signs in real time. *Sensors, 17*(2), 341. https://doi.org/10.3390/s17020341

Weenk, M., Koeneman, M., van de Belt, T. H., Engelen, L. J., van Goor, H., & Bredie, S. J. (2019). Wireless and continuous monitoring of vital signs in patients at the general ward. *Resuscitation, 136*, 47-53. https://doi.org/10.1016/j.resuscitation.2019.01.017

Xiao, N., Yu, W., & Han, X. (2020). Wearable heart rate monitoring intelligent sports bracelet based on Internet of things. *Measurement, 164*, 108102. https://doi.org/10.1016/j.measurement.2020.108102

REVIEW

Microbial fuel cells: A potent and sustainable solution for heavy metal removal

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Abstract

The global water pollution problem is becoming increasingly crucial. One of the major contributors to water pollution is the presence of heavy metals. Heavy metals pose significant threat to both humans and all ecosystems. Various factors influence the removal of heavy metals from wastewater, including pH, temperature, natural organic matter (NOM), and ionic strength, which vary based on the chemical properties of the pollutants. More effective and modern approaches receive attention and extensively researched to substitute traditional methods such as adsorption, membrane filtration, and chemical-based separation. Among these methods, Microbial fuel cells (MFCs) are particularly intriguing. This review article focuses on MFCs and their potential applications in various fields, including clean water production. MFCs represent an innovative technology that not only generates electricity, but also demonstrates significant potential for heavy metal removal from wastewater. Cathodic chamber of MFCs effectively reduces heavy metals, while organic substrates act as carbon and electron donors in the anodic chamber. Through various mechanisms, including direct and indirect metal reduction, biofilm formation (metal sequestering), electron shuttling, and synergistic interactions among microbial communities, microorganisms exhibit remarkable efficiency in removing metals. Studies showed that dual- and single-chamber MFCs could efficiently remove a range of heavy metals, including chromium, cobalt, copper, vanadium, mercury, gold, selenium, lead, magnesium, manganese, zinc, and sodium, while simultaneously generating electricity, achieving high removal efficiencies ranging from 25% to 99.95%. This range of efficiency varies depending on the specific contaminant being targeted, the concentration of the contaminant, as well as the operating conditions such as pH and temperature. Moreover, MFCs demonstrated a wide range of power outputs, typically ranging from 0.15 W/m² to 6.58 W/m², depending on the specific configuration and conditions.

These findings underscore the potential of MFCs as a sustainable and efficient approach for both wastewater treatment and energy generation.

Keywords: Microbial fuel cell (MFC), heavy metal removal, wastewater treatment, sustainable energy generation, metal reduction mechanisms.

Introduction

Clean water is indispensable for life. Together with the demand for clean potable water, the pollution of clean water resources is also increasing (Xia et al., 2017). Along with the droughts that occur with climate change in progress, there is a rise in the toxicity of chemical pollutants (Xia et al., 2017; Noyes et al., 2009). Humanity is having to fight major health problems, not only due to scarcity of clean drinking water, but also for agricultural irrigation. Water-related health disorders are more common in developing countries (Lin et al., 2022). For example, about 1.6 million people die each year due to water-pollution-related diseases such as diarrhea and sepsis, and 90% of these deaths are in children under the age of 5. One of the greatest water pollution causes is heavy metal contamination. Heavy metals are substances that are difficult to dissolve in nature. These metals mix into water due to various industrial activities and agricultural runoff, and since they are difficult to dissolve in water resources, they can remain for a long time. This causes great harm to human health and the ecosystem (Pandit and Kumar, 2015).

Table 1. Characteristics of common heavy metals (EPA, 2024; WHO, 2017).

Heavy Metal	**Human Health Effects**	**Common Sources**	**Maximum Contaminant Level**	
			EPA	WHO
Arsenic (As)	Circulatory system issues Skin Damage	Electronics production Naturally occurring	0.010 mg L^{-1}	0.010 mg L^{-1}
Cadmium (Cd)	Carcinogenic Kidney damage	Chemical industries Naturally occurring	0.005 mg L^{-1}	0.003 mg L^{-1}
Chromium (Cr)	Diarrhea, vomiting, nausea Allergic dermatitis	Steel manufacturing Naturally occurring	0.1 mg L^{-1}	0.05 mg L^{-1}
Copper (Cu)	Liver and kidney damage Gastrointestinal issues	Household plumbing systems Naturally occurring	1.3 mg L^{-1}	2.0 mg L^{-1}
Mercury (Hg)	Nervous system damage Kidney damage	Electronics industries Fossil fuel combustion	0.002 mg L^{-1}	0.006 mg L^{-1}

Silver (Ag)	Breathing problems Stomach pain	Naturally occurring Naturally occurring	0.10 mg L^{-1}	100 µg L^{-1}
Selenium (Se)	Kidney damage Breath problems	Naturally occurring Chemical industries	0.05 mg L^{-1}	0.05 mg L^{-1}

In the developing world, industrial and urban activities are increasing in parallel with pollution caused by heavy metals. Various factors, such as polluted wastewater discharge from several industries, emissions from vehicles, and other urban activities are examples of such contributing factors (Joseph et al., 2019). According to the United Nations report, about 80% of all industrial and domestic wastewater in developing countries are released into the environment without any pretreatment (Xia et al., 2017). In addition, polluted urban stormwater runoff, rainwater contamination into potential drinking water sources, and agricultural runoff also increase pollution (Lye, 2009). Heavy metals and mixed organic substances mix with water bodies through direct discharge or domestic wastewater discharge. Textile, dye, leather and pharmaceutical industries produce most of such discharges. Heavy metals such as Cr, Cd, Pb, As and Hg are highly toxic and carcinogenic, and may create significant damage to vital organs (Balali-Mood et al., 2021). Organic pollutants pose additional toxicity and carcinogenicity, particulary upon mixing with heavy metals, and producing chemical complexes. Delayed maturation or complete growing inability of plants may be caused by the result of heavy metals-organic pollutants interactions ((Briffa et al., 2020). Likewise, the beneficial microbial ecosystems may be adversely affected (Ajiboye, 2021).

Treatment methods for drinking water, such as heat treatment, solar disinfection, and chlorination are mostly insufficient. Since wastewater removal efficiencies vary depending on energy, time, and environmental conditions, these techniques cannot completely remove chemical contaminants in drinking water and require additional technological equipment (Senanu et al., 2023). Some of the technologies and treatment methods that provide high removal efficiency for heavy metals, which are nowadays under investigation in developed countries, are as follows: microbial bioremediation, employing various modified adsorbents, activated carbon adsorption, membrane filtration, electrocoagulation, and carbon nanotechnology. On the other hand, since these technologies are not cost-effective, local employees should be trained to purify contaminated water in the developing world, and the cost should be low and access to the technologies used should be easy (Joseph et al., 2019). Therefore, this review article focuses on bioremediation by microbial fuel cells and the use of cheap, widely available materials that do not result in additional energy requirements for the removal of heavy metals from water supplies.

Impact on water quality and heavy metal removal

The main parameters influencing removal of heavy metals from water are NOM, pH, temperature, and ionic strength (Joseph et al., 2019).

Chemical properties of heavy metals

All living organisms require trace amounts of heavy metals such as copper, zinc, iron, and chromium for growth and development, but heavy metals taken in large amounts can cause major problems of toxicity (Tchobanoglus et al., 2003). Table 2 shows the chemical characteristics of some heavy metals in the environment.

Table 2. Some chemical characteristics of common heavy metals (Murray et al., 2004).

Heavy metal	MW(g/mol)	Oxidation state(s)	Electronegativity (Pauling Scale)
Arsenic	74.9	-3, + 3, + 5	2.18
Cadmium	112.4	+2	1.69
Cobalt	58.9	-1, 0, + 2, +3	1.88
Mercury	200.6	+1, +2	2.00
Zinc	65.4	+2	1.65

The effect of pH

pH plays a critical role in heavy metal accumulation in water bodies. Heavy metals are usually found in their cationic state and have high solubility at low or neutral pH values. Studies demonstrated that stability and mobility of copper increased when the pH value decreased (Violante et al., 2010). As the pH increases, heavy metals interact with hydroxide ions and cause oxidation, then precipitate in water. Lead is an example of this phenomenon (Olaniran et al., 2013). In case of chromium, increasing pH converts the more stable Cr(3) to more toxic Cr(4) via oxidation. Removal of heavy metal ions by adsorption can be done in lower levels at low pH (Pantsar-Kallio et al., 2001). With the increase in pH value, the concentration of H^+ ions decrease, thus the adsorption area increases, resulting in higher levels of heavy metal removal. Exceptions to this phenomenon, such as the removal of chromium from anionic species with increasing pH was also observed, where adsorption decreases as pH increases (Li et al., 2018). This exception arises from electrostatic repulsion, since negative surface charges on the adsorbent prevent the adsorption of anionic species. In conclusion, it can be stated that pH significantly affects the removal and behavior of heavy metals. Joseph et al., (2019) also showed that heavy metals decrease at low pH values (<4) in general, and this is more pronounced in between pH 5 and 7 (Joseph et al., 2019).

The effect of NOM

NOM usually comes from humic and fulvic acids, which are formed by the accumulation and decomposition of plant and animal substances. NOM has a wide range of organic acids and is highly reactive in conjunction with heavy metals, resulting in changes in their mobility and toxicity. Detecting the effects of NOM on heavy metals can be difficult due to a wide variety of additional factors, including pH, humification, and oxidation state (Kumpulainen et al., 2008). Heavy metals and NOM can interact via various mechanisms; the acidic nature of NOM is achieved through such mechanisms as surface adsorption, ion exchange and chelation. Metals such as zinc and copper can also form various complexes with NOM. Additionally, it has been shown that treating chromium in heavy metals that interact with NOM can reduce chromium from its toxic form to a more stable and less harmful form. (Yang et al., 2015; Joseph et al., 2019). In conclusion, there appears to be no clear relationship between NOM and heavy metal removal.

The effect of temperature

Another important parameter in removal of heavy metals is temperature. Removal of heavy metals and complexation reactions on the surface are carried out by increasing the temperature and continue to accelerate as the temperature increases. With increasing temperature, the adsorption zone expands and heavy metal removal increases (Chen et al., 2010). Study results showed that as temperature was increased from 5°C to 40°C, a large amount of Cr(4) in the peanut shell was removed.

Increase in adsorption surface was proportional with the increase in diffusion rate, consequently, the adsorption process proceeded faster at higher temperatures (Moussavi and Barikbin, 2010). Contrasting results were also reported. In a study with red algae, total chromium removal decreased from 90% to 78% as the temperature increased. Likewise, a decrease in the removal of heavy metals such as Ni(2) and Pb(2) was observed with increasing temperature. These results appeared to result from decreased surface activity (Sari and Tuzen, 2008; Joseph et al., 2019). Thus, when examining the effects of temperature on heavy metal removal, each adsorbent and its corresponding metal ion should be studied separately.

The effect of ionic strength

Ionic forces in the water source have a great impact on the removal of heavy metals. Chloride ion in water dissolves and forms metal-chloride complexes with uncharged or negatively charged heavy metals, which are difficult to remove. The heavy metal-chloride complex exhibits low-affinity adsorption and heavy metal-chloride accumulation occurs (Ferraz and Lourenco, 2000). For example, a decrease in the removal of Ni(2) and Cu(2) was detected as the ionic strength increased. This decline was caused by the increase in dissolved zinc and copper concentration with increasing salinity (Villaescusa et al., 2004; Wang et al., 2017). As a result, higher ionic strength causes a decrease in the adsorption of heavy metals by influencing the electrostatic interactions, Contradictory results were also reported in a study, in which As(3) and Ni(2) removal increased by about 25%, as the ionic strength of a solution increased from 0.01 to 1.0 M Cl. In a research with crab shell pieces, it was observed that when ions such as K^+ and Na^+ were added for the removal of copper and cobalt, the heavy metal removal increased by 2-5% (Yang et al., 2016; Joseph et al., 2019).

Techniques for eliminating heavy metals

Adsorption-based separation

Because of its low cost, high removal capacity, ease of use, and capability to regenerate the adsorbed heavy metal ions, the adsorption method has been described as a straightforward technique (Yang et al., 2019). Carbon-based nanoporous, chitosan, mineral and magnetic adsorbents are prominent in this separation method.

Due to their exceptional surface areas (500-1500 m^2/g), carbon-based nanoporous adsorbents such as activated carbons (ACs), carbon nanotubes (CNTs) and graphene (GN) are widely used in heavy metal removal applications (Karnib et al., 2014). Oxidation, sulfuration and nitrogenation are the most widely applied techniques in carbon adsorption. According to Kumar et al. (2015), these techniques are used to improve pore structure, specific surface area, adsorption capacity, thermal stability and mechanical strength. Carbon-based adsorbents have become more expensive due to their various procedures, therefore researchers are working to create more effective and economical surface modification methods. One of the naturally occurring adsorptive polymers used to remove heavy metals is chitosan. However, some properties such as poor chemical and mechanical stability and biodegradability may affect how widely chitosan-based adsorbents can be used. Microorganisms can access oxidizable or hydrolyzable bonds in chitosan, which can lead to its biodegradation (Stafiej, 2007).

Mineral adsorbents such as clay, zeolite and silica are known to be frequently used and considered suitable for the cost-effective removal of heavy metals (Gu et al., 2018). Clay, in particular, is desirable because it significantly reduces water pollution and improves water quality. Studies have shown that scientists often use ion exchange processes to remove heavy metals and physical or chemical adsorption with mineral adsorbents due to their low cost (Qasem et al., 2021) Typically, magnetic adsorbents are made of magnetic materials with magnetic properties, such as iron oxide nanoparticles. After the adsorption process, they are easily removed from the solution by applying an external magnetic field (Mehtaa, 2015). As a result, the adsorption method provides effective heavy metal removal from wastewater, reducing their concentrations to safe levels (Tamjidi et al., 2019).

Membrane-based filtration and separation

The high efficiency, applicability, and simplicity of membrane separation techniques make them viable strategies for the removal of heavy metals. A permeable membrane is used in ultrafiltration; a membrane filtration technique to separate heavy metals according to gradients in concentration or pressure. In this method, ultrafiltration, nanofiltration, microfiltration, semi-permeable spiral-wound membranes, ion-exchange membranes will be examined.

In ultrafiltration, bigger particles, such as heavy metal ions, are retained while solvent molecules and tiny particles are selectively allowed to pass through a membrane with a particular pore size. The membrane acts as a barrier to movement, preventing particles larger than a given size threshold from passing through (Xiang et al., 2022).

Membranes for nanofiltration (NF) are essential to the treatment of wastewater. Because of their exceptional separation capabilities, they are frequently used in a variety of wastewater treatment-related operations (Abdel-Fatah, 2018). Regarding pore size range and separation capabilities, nanofiltration (NF) is regarded as a technology that falls between reverse osmosis (RO) and ultrafiltration (UF) (Bellona, 2015). Compared to RO membranes, NF membranes have larger pores, but UF membranes have the largest pores (UF: 5–20 nm, NF: >1 nm, RO: 0–1–1 nm) (Xiang et al., 2022). Because NF membranes have a specified molecular weight cutoff (MWCO), solutes can be separated according to their molecular weight and size. The MWCO of NF membranes is higher than that of RO membranes, but lower than that of UF membranes. Water can have ions, dissolved particles, and chemical substances selectively removed using NF membranes (Mondal and Wickramasinghe, 2008).

Microfiltration is an adaptable and popular separation method that provides effective microbial control and particle removal in a range of applications. It offers a practical and affordable way to get rid of bacteria and particles smaller than microns from fluids, which enhances process effectiveness, protects the environment, and produces better-quality products. Membranes with pore sizes between 0.05 and 10 microns are thought to be ideal for microfiltration. Numerous materials, including silica, zirconia, ceramics, polyamides, polypropylene, and composite materials, can be used to create MF membranes (Hakami Het al., 2020).

Semi-permeable spiral wound membranes are used to filter heavy metals and other impurities from water in the reverse osmosis process. Reverse osmosis membranes can effectively limit the passage of suspended solids, ions and other unwanted substances due to their small pore size (typically between 0.1 and 1 nm) (Garud et al., 2011). Clean water molecules can flow through these membranes when water is pushed by high pressure, but contaminants are left behind and washed away as a trash stream (Lee et al., 2011).

Ion exchange membranes are alternately positioned in a direct current field in electrodialysis (ED). Ionic solutes are separated by ED using anion exchange membranes (AEM) and cation exchange membranes (CEM) (Xu and Huang, 2008). These two membrane types are alternately aligned in a membrane stack to form repeating units. These units consist of compartments with cation exchange membranes on the right and anion exchange membranes on the left. The membrane stack is powered by providing a difference in electrical potential using electrodes at either end (Van der Bruggen, 2015; Gurreri et al., 2020).

Chemical-based separation

Chemical precipitation is a commonly employed technique for eliminating dissolved metals from solutions, particularly in the treatment of process wastewaters containing hazardous metals. Particles are formed when soluble metal compounds undergo a chemical interaction with particular precipitating agents that turns them into insoluble forms. Then, using techniques like settling or filtering, these particles can be extracted from the mixture (Dahman, 2017). The kind and concentration of metals in the solution, the precipitating agent selected, the reaction conditions (particularly pH), and the possibility of other substances that could impede the precipitation reaction affect the efficiency of the chemical precipitation process (Ramakrishnaiah and Prathima, 2012). Coagulation and flocculation procedures are widely used in drinking water treatment due to their high effectiveness in reducing turbidity. The main objective of coagulation and flocculation processes is to collect colloids and other small particles present in water and form larger particles called flocs (Muruganandam et al., 2017). In the first stage, called coagulation, a coagulant is added to the water to reduce repulsive interactions between colloids, which eventually causes the particles to become unstable. The destabilized particles come together to form flocs in the subsequent flocculation stage due to attractive factors, such as van der Waals interactions (Ho et al., 2020).

Gas bubbles are used as transporters in the flotation separation process. During this process, suspended particulate matter adheres to the bubbles and rises to the surface of the aqueous solution, regardless of whether it is hydrophobic by nature or may become so through conditioning. It's important to keep in mind that this upward motion defies gravity (Gharai and Venugopal, 2016).

Microbial fuel cell technology

Interest in ecological and sustainable renewable energy sources has increased in recent years. Among these sources, bioenergy is the world's fourth-largest energy source and can be derived from various forms of biomass (Kilinc and Catal, 2023, Arslan et al., 2020; Dahiya, 2020). A promising technology to address environmental pollution and energy needs is the MFCs (Sonmez et al., 2024). They are especially beneficial, because they produce electricity, while yielding byproducts like methane (CH_4), hydrogen (H_2), and hydrogen peroxide (H_2O_2) from the chemical energy found in wastewater. Because of their ability to perform two tasks at once, MFCs can clean wastewater and recover energy, which makes them a viable option for both pollution reduction and energy recovery. MFCs have several advantages over conventional wastewater treatment techniques, such as lower energy consumption and less sludge production than aerobic treatment. Additionally, they exhibit adaptability in difficult circumstances like low substrate concentrations and temperatures (less than 20°C), which can restrict the use of other treatment technologies. On the other hand, some of the drawbacks of MFCs are high cost, low power output, and short operational lifetimes of the technology. These difficulties are exacerbated by problems like membrane fouling, catalyst instability, and the difficulty of maintaining microbe-based systems. Long-term dependability and efficiency of MFCs are impacted by cathode catalyst and membrane deterioration, which frequently limits the device's lifespan. Furthermore, even though MFCs have a lower environmental impact than some other technologies, they still need to be carefully managed to avoid any unfavorable outcomes, such as the formation of byproducts and unstable systems. All things considered, MFCs have a lot of potential for producing energy and treating wastewater sustainably, although more research is required to get past the obstacles and improve their functionality (Naha et al., 2023; Guo et., 2020).

The MFCs utilize exoelectrogenic microorganisms, including bacteria, algae, and fungi present in the anode compartment made of carbon; a sturdy conductor, to create a biofilm structure on the anode. This process converts chemical energy derived from biomass into electricity or other chemical products in an electrochemical manner (Sukkasem, 2024; Fan et al., 2024; Kilinc and Catal, 2023; Akagündüz et al., 2022; Cebecioğlu et al. 2022). They are composed of two compartments separated by a proton exchange membrane: an anode chamber and a cathode chamber (Figure 1).

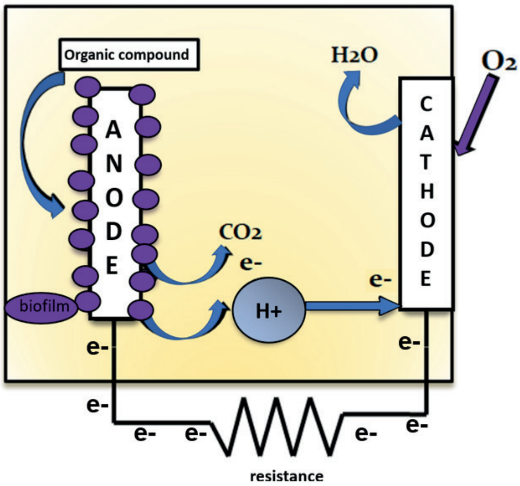

Figure 1. The operating principle of the single chamber microbial fuel cell has been modified from Akagündüz et al., (2022).

In the anode chamber, electroactive microorganisms adhere to a conductive electrode. The microorganisms form a biofilm in the anode chamber, which oxidizes organic compounds, releasing electrons and protons. Protons and electrons released from the anode chamber flow through an external circuit to the cathode chamber where they combine with a terminal electron acceptor (usually oxygen) to form water or other reduced products. This process produces an electric current that can be harvested for power generation.

The anodic and cathodic reactions are presented below:

Organic compound → CO_2 + H^+ (cathode) + e^- (external circuit)

O_2 + H^+ (from cathode) + e^- (external circuit) → H_2O

These two half-reactions lead to an overall reaction:

Organic compound + O_2 → CO_2 + H_2O + external power (Catal et al., 2018a; Lee et al., 2015; Pant et al., 2010).

The scaling-up process is one of the major obstacles in bringing MFCs to commercial applications, even with the technology's successful demonstration in lab settings. It is not always possible to transfer lab-scale reactor performance directly to field applications. For example, expanding the anodic chamber's size can actually result in a decrease in overall performance, rather than a proportionate increase in power output. Moreover, increased internal resistances and cathode kinetics constraints mean that larger electrode surface areas do not always translate into higher power harvesting rates. Modular MFC units with sophisticated power management systems have been created to address these problems, but mass transport constraints and voltage reversal continue to be major roadblocks to the widespread adoption of this technology (Jadhav et al., 2021).

Notwithstanding these difficulties, studies on low voltage generation and the treatment of wastewater contaminated by pharmaceuticals are the two real-world applications where microbial fuel cells have proven effective (Akagündüz et al., 2022; Catal et al., 2018b). In addition, MFC technology has successful applications in the recovery of ammonium and nitrogen from urine (Sharma and Mutnuri, 2019), drug recovery (Akagunduz et al., 2022; Akul et al., 2022), biosensor studies used in water quality assessment (Adekunle et al., 2021), removal of toxic dyes from the environment (Cebecioğlu et al., 2022) and in removal of various pollutants from environment (Catal et al., 2019; Ozdemir et al., 2019). Microbial fuel cell is a promising technology for simultaneous removal of heavy metals from wastewater and electricity generation. In MFCs, heavy metals such as chromium, cobalt, copper, silver, mercury, vanadium, gold and selenium are effectively reduced in the cathode chamber, while organic substrates serve as carbon and electron donors in the anode chamber. Numerous studies have investigated the presence of electrochemically active bacterial species in MFCs, with over 20 distinct species being identified. Notable examples include *Aeromonas, Ochrobactrum, Arcobacter, Pseudomonas, Desulfuromonas, Desulfobulbus, Thermincola, Geothrix, Comamonas, Geopsychrobacter, Propionibacterium, Shewanella, Klebsiella, Enterobacter, Acidiphilium, Citrobacter, Rhodoferax, Rhodopseudomonas, Geobacter and Clostridium.* These microorganisms have been widely observed in alum sludge or activated sludge (Catal et al., 2024; Wang et al., 2016). *Aeromonas* species are resistant to acid and are gram-negative bacteria that can be found in a variety of settings, including food, water, and soil. They are used in biotechnology, including in MFCs, despite being pathogenic. For instance, it has been demonstrated that *A. hydrophila* breaks down chitin to produce metabolites that improve MFCs' ability to produce energy (Li et al., 2017). *Ochrobactrum* species are well-known for their capacity to produce electricity in MFCs and eliminate heavy metals like lead. In a two-chamber MFC, *Ochrobactrum* greatly enhanced power generation and eliminated 84.86% of Pb^{2+} (et al., 2023). *Arcobacter* species can reduce Fe and Mn in low oxygen environments and are associated with diseases in humans and animals. In MFCs, *Arcobacter butzleri* demonstrated encouraging outcomes in the oxidation of acetic acid and the production of electricity (Fedorovich et al., 2009). A significant factor in MFCs and industrial applications are the exoelectrogenic qualities of *Pseudomonas* species. Using oily wastewater, *P. citronellolis* showed a significant reduction in COD and production of electricity in MFCs (Varnava et al., 2024). Sulfur and metals like iron and manganese are reduced by species of *Desulfuromonas*. They help MFCs form an anodic biofilm, which increases power density (Xu et al., 2023). According to Sun et al. (2009), *Desulfobulbus* species are essential for sulfate reduction and sulfide oxidation, which sustain continuous current production in MFCs. A thermophilic Gram-positive bacterium called *Thermincola ferriacetica* is well-known for its ability to transfer electrons directly and produce stable electricity at high temperatures in MFCs (Marshall and May, 2009). *Geothrix fermentans* is useful in MFCs for energy production, because it can oxidize organic acids and transfer electrons directly to electrodes (Bond and Lovley, 2005). It has been discovered that *Comamonas* species, which are significant in bioremediation, are essential for the production of electricity in MFCs contaminated with wastewater from fish markets (Padmanabhan et al., 2023). According to Holmes et al. (2004), *Geopsychrobacter* species are psychrotolerant bacteria that can transfer electrons to electrodes and grow in low temperatures, enabling the production of electricity in MFCs. *Propionibacterium* species are classified into two groups: cutaneous and classical. The former is employed in a variety of industries. High power density MFCs have demonstrated successful performance from *P. freudenreichii ssp. shermannii* (Reiche et al., 2015). The extracellular electron transfer capabilities of *Shewanella* species are noteworthy, because they improve MFC performance without the need for mediators. It has been demonstrated that *S. oneidensis MR-1* can generate electricity from a variety of carbon sources (Dai et al., 2020). Significant pathogens, *Klebsiella* species are also investigated for their potential to produce energy in MFCs. *Shewanella oneidensis* and *Klebsiella pneumoniae* worked together to process glycerol in MFCs to efficiently produce energy (Li et al., 2017). *Enterobacter* species play a key role in the generation of biohydrogen and bioremediation. In MFCs, *Enterobacter sp.* was employed as a sensor to track the production of hydrogen and the health of the microbiota (Lim et al., 2022). Certain acidophilic bacteria that can reduce iron are called *Acidiphilium* species. Borole et al. (2008) reported that *A. cryptum* was effectively employed as an anodic biocatalyst in MFCs, thereby augmenting power output in low pH conditions. Because of their capacity to decrease iron, *Citrobacter* species have demonstrated potential in MFC applications. *Citrobacter sp. LAR-1* produced electricity in MFCs with success (Liu et al., 2016). With the capacity to produce a sizable current density in MFCs using sugars as fuel, *Rhodoferax ferrireducens* is essential in anaerobic conditions (Liu et al., 2006). In microbial electrochemical cells fed with acetate, *Rhodopseudomonas palustris strain RP2* is well known for its capacity to break down hydrocarbons and produce energy (Venkidusamy and Megharaj, 2016). In MFCs, *Geobacter* species are renowned for having a high potential for electrical output. When nanofluid proteins were overexpressed, *G. sulfurreducens* generated more electricity, demonstrating their importance in bioremediation and energy production (Wang et al., 2023). *Firmicute* bacteria, such as *Clostridium* species, have thick cell walls that allow them to survive in challenging conditions. In MFCs*, Clostridium butyricum* has been utilized, and after 10 hours of inoculation, it produced a maximum current of 0.22 mA (Cao et al.,2019). These microbes are important for the biological and electrochemical processes that are necessary for the removal of metal from MFCs, in addition to help generate electricity.

Metal removal in MFCs involves a variety of biological and electrochemical processes. Direct metal reduction is where certain microorganisms have the ability to directly convert metal ions from high to low oxidation states through their electron transfer capacity. Bacteria such as *Shewanella* and *Geobacter* are notable for their use of extracellular electron transfer mechanisms to accomplish this reduction. Additionally, metal removal can occur indirectly via metabolic pathways. Microorganisms utilize organic substrates, such as wastewater contaminants or electron donors, as a source of carbon and energy. As they metabolize the organic matter, metabolic byproducts like organic acids or hydrogen sulfide are produced, which can react with metal ions to form less soluble precipitates such as metal sulfides or metal hydroxides. These precipitates are more easily separated from the soluble fraction, resulting in metal removal from the system. Biofilm formation on electrode surfaces within MFCs plays a significant role in metal removal. In MFCs, biofilm growth on the electrode surfaces is crucial to the removal of metal. Complex bacterial colonies that stick to surfaces are called biofilms. Biofilms let cells communicate with each other and regulate the admission of heavy metals and other hazardous materials from the outside. To protect themselves, bacteria in biofilms create metal-binding proteins such metallothionein (MT) and extracellular polymeric substances (EPS). When heavy metals enter the cell, these proteins respond by sequestering the metals within the cell. As a result, they offer stable complexes and lessen cellular toxicity. Zinc removal has been found to be significantly aided by the MT SmtA protein generated by the cyanobacterium *Synechococcus elongates* (strain PCC7942). According to a different study, MTs produced by the *Mycobacterium tuberculosis* (strain H37Rv) strain are crucial for the elimination of copper. Certain microorganisms produce redox-active compounds known as electron shuttles or mediators. These compounds indirectly transfer electrons from microorganisms to metal ions, enhancing the reduction process. This mediated electron transfer mechanism allows for the utilization of microorganisms that may not directly interact with the metal ions , but still contribute to metal removal through shuttle-mediated electron transfer. In MFCs, a diverse microbial community with varied metabolic capabilities is often present. Synergistic interactions among microorganisms occur, where one group of microorganisms provides reducing equivalents or metabolites that are utilized by other microorganisms involved in metal removal processes. These cooperative interactions contribute to the overall efficiency of metal removal in MFCs (Roy et al., 2023; Noori et al., 2022; Abbas et al., 2017; Rabaey et al., 2007; Rabaey et al., 2004).

Methods

This review paper primarily focuses on the application of MFCs for the removal of heavy metals. We searched a number of databases, including PubMed, Web of Science, and Academia, adhering to PRISMA principles. In light of papers published by researchers in the previous 30 years, we searched the aforementioned databases using the following keywords: microbial fuel cell, heavy metal removal, wastewater treatment, sustainable energy generation, and metal reduction processes. Using the PRISMA checklist, reviewers independently chose reviews, extracted data, and evaluated the included reviews' methodological quality. In order to settle differences and come to a consensus, the researchers presented and debated these results.

The literature presents numerous studies on metal removal using microbial fuel cells. Presents studies reveal the potential of MFCs as innovative technologies that provide dual benefits in both energy production and wastewater treatment (Table 3).

Table 3. Summary of various studies conducted on heavy metal removal using microbial fuel cells.

Study	Metal	Microbial Species	MFC Configuration	Electron Donor	Metal Concentration	pH Range	Maximum Removal Efficiency	Maximum Power Output
Zhang et al. (2021)	Chromium Cr(VI)	Anaerobic sludge	Double-chamber MFC	Sodium acetate	6-15-40-100 mg.L^{-1}	7	66.5%	35.3 mWm^{-2}
Wang et al. (2008)	Chromium Cr(VI)	Domestic wastewater	Double-chamber MFC	Sodium acetate	100 mg.L^{-1}	2-6	100%	0.150 Wm^{-2}
Huang et al. (2013)	Cobalt (Co(III))	Domestic wastewater	MFC with $LiCoO_2$ electrodes	Sodium acetate	50 mg.L^{-1}	1-3	99.1% (conversion ratio to soluble Co(II))	298 mWm^{-3}
Heijne et al. (2010)	Copper (Cu)	Mixed microbial culture from operating MFCs	Double-chamber MFC	Sodium acetate	1000 mg.L^{-1}	3	99.88% (anaerobic), 99.95% (aerobic)	0.43 Wm^{-2} (anaerobic), 0.80 Wm^{-2} (aerobic)
Tao et al. (2011)	Copper (Cu(II))	Mixed microbial culture from operating MFCs	Membrane-free bio electrochemical system	Sodium acetate	600-2000 mg	2	91.95% and, 47.54% respectively.	0.585 mW
Zhang et al. (2009)	Vanadium (V(V))	Anaerobic granular sludge	Double-chamber MFC	Glucose	500 mg.L^{-1}	2	25%	0.572 Wm^{-2}
Wang et al. (2011)	Mercury (Hg(II))	Domestic wastewater	Double-chamber MFC	Sodium acetate	25-100 mg.L^{-1}	2	98.2% and, 99.5% respectively.	0.433 Wm^{-2}
Choi and Hu (2013)	Gold (Au(III))	Mixture sludge with artificial wastewater	Double-chamber MFC	Tetrachloroaurate	100-2000 mg.L^{-1}	1-6.5	99.8%	6.58 Wm^{-2}
Catal et al. (2009)	Selenium (Se(IV))	Domestic wastewater	Single-chamber MFC	Acetate or glucose	50-200 mg.L^{-1}	7	99%	2.90 Wm^{-2}

Yang et al. (2021)	Copper (Cu)	Anaerobic sludge and, *Chlorella*	Three-chamber MFC	Sodium acetate	30 mg.L^{-1}	not specified	99%	0.42 Wm^{-2}
Zhang et al. (2020)	Copper (Cu)	Anaerobic sludge	Three-chamber MFC	Glucose	500 mg.kg^{-1}	2	94.78% (with 1 mol.L^{-1} HCL)	363.04 mWh^{-1}(with 1 mol.L^{-1} HCL)
Zhang et al. (2023)	Lead (Pb2+)	*Ochrobactrum*-related strain CD-1	Double-chamber MFC	Sodium acetate, sodium citrate and, glucose respectively	2500 mg.L^{-1}	not specified	52.3%,37.5% and, 19.2% respectively	371.0 mWm^{-2}(with sodium acetate)
Munoz-Cupa and Bassi, (2023)	Cu(II), Mg(II), Mn(II), Zn(II) and, Na (I) re-spectively	*Shewanella oneidensis* MR-1	Double-chamber MFC	Sodium lactate	13.5, 7.5, 415, 10.5 and, 2410 mg.L-1 respec-tively	7	93%, 85%, 93%, 88% and, 36% respec-tively (for anode chamber)	517.6 mV

A study by Zhang et al. (2021), focused on the removal of chromium (Cr(VI)) in a dual-chamber MFC system with electrochemically active bacteria and an abiotic cathode and its effect on power generation. The findings revealed that the MFC system with an abiotic cathode exhibited high efficiency in removing heavy metals, particularly Cr(VI), achieving a maximum removal rate of 66.5%. Furthermore, the MFC system simultaneously generated electricity, with the highest power density of 35.3 mW m^{-2} observed at an initial concentration of 100 mg L^{-1} Cr(VI). Similarly, Wang et al. (2008), investigated the removal of Cr(VI) using a dual-chamber MFC with graphite plates for both cathode and anode. The electron donor used was sodium acetate at a concentration of 2.64 g L^{-1}. The study used four different initial concentrations of $K_2Cr_2O_7$, and the pH range was 2-6. The study found that the system removed Cr(VI) with 100% efficiency at a concentration of 100 mg L^{-1} in 150 hours. The maximum power output was 0.150 W m^{-2} at a concentration of 200 mg L^{-1} Cr(VI) and pH 2.

In another study, Huang et al. (2013), investigated the removal potential of cobalt (Co(III)) from contaminated water using $LiCoO_2$ particles as electrode material and sodium acetate as electron donor in dual chamber MFC. The conversion of insoluble Co(III) with a solid/liquid ratio of 50 mg L^{-1} to soluble Co(II) was investigated using a resistor of 2000 Ω in the pH range of 1-3. The researchers reported that 99.1% of insoluble Co(III) was converted from soluble CO(II) at the end of 48 hours. They also reported that the power generation was 298 mW m^{-3} at a resistor of 2000 Ω.

Heijne et al. (2010), investigated copper removal and maximum power generation in a dual chamber MFC using sodium acetate as electron donor. The researchers used two different systems, anaerobic and aerobic cathode. They reported that 99.88% removal of copper with a concentration of 1000 mg L^{-1} was achieved in 6 days in the anaerobic cathode. They reported that 99.95% removal of copper with a concentration of 1000 mg L^{-1} was achieved in 7 days in the aerobic cathode. They also reported that the maximum power generation was 0.43 W m^{-2} in the anaerobic cathode and 0.80 W m^{-2} in the aerobic cathode.

Tao et al. (2011), conducted a study on copper (Cu(II)) removal and power generation in a 16 mL volume bioelectrochemical system (BES) without a membrane. They used anaerobic microorganisms and $CuSO_4$ as a catholyte solution in the system. The researchers worked on two concentrations, 600 and 2000 mg, and operated for 480 hours and 672 hours, respectively. As a result, they reported that the removal efficiency of 600 mg Cu(II) was 91.95% and 2000 mg Cu(II) was 47.54%. They reported that the maximum power generation in the system was 0.585 mW at 2000 mg Cu(II) concentration and pH 2.

Zhang et al. (2009), investigated vanadium(V(V)) removal and maximum power generation in a dual chamber MFC system (500 mL volume, pH 2) with the anode chamber (250 mL) containing 100 mg L^{-1} sulfur and the cathode chamber containing 500 mg L^{-1} V(V). As a result, the researchers reported that the removal of sulfur and organics was 84.7% and the reduction ratio of V(V) was 25.3% within 72 hours. They also reported that the maximum power generation was 572.4 mW m^{-2}.

In another study, Wang et al. (2011) investigated the removal of mercury (Hg^{2+}) from artificial wastewater using a double chamber MFC system (120 mL anode chamber, 120 mL cathode chamber), and reported that the removal efficiency of Hg^{2+} in the concentration range of 25 -100 mg L^{-1} was 98.2-99.5% in 10 hours. They also reported that the maximum power output was determined as 0.433 W m^{-2} at a concentration of 100 mg L^{-1} Hg^{2+}.

Choi and Hu (2013), investigated the removal of gold (Au(III)) and maximum power output using double chamber MFC. Tetrachloroaurate was used as electron acceptor in the study. They investigated the removal of Au(III) ranging from 100 to 2000 mg L^{-1} at pH 2. As a result, they determined that the removal efficiency of 99.8% could be achieved at 2000 mg L^{-1} concentration of Au(III) in five days. They also reported that the maximum power generation at 2000 mg L^{-1} Au(III) concentration was 6.58 W m^{-2}

The study by Catal et al. (2009), investigated the removal of selenium (Se(IV)) from water using a single-chamber MFC with a carbon cloth anode and a coated carbon cloth cathode. Acetate or glucose was used as the electron donor, and SeO32- was used as the metal salt at concentrations ranging from 50 to 200 mg L^{-1} Se(IV) at a pH of 7. The results showed that using an acetate-fed microbial fuel cell, 99% of 75 mg L^{-1} Se(IV) could be removed within 48 hours. The maximum power output was 2.90 W m^{-2} at a Se(IV) concentration of 25 mg L^{-1}.

Yang et al. (2021) established a three-chamber MFC supported by algae (*Chlorella)* to assess the removal efficiency of copper (Cu^{2+}) and electricity production. With a starting concentration of 30 mg L^{-1} Cu^{2+}, the first cathodic chamber removed about 86.2% of the Cu^{2+}, with the remaining amount being absorbed by algae. This resulted in a 99.9% removal efficiency for the entire system. The researchers reported that the anode and cathode potentials were affected by the Cu^{2}□ concentration, with the uptake of Cu^{2+} by algae being a significant factor. The maximum power density was measured to be approximately 0.42 W/m².

A three-chamber microbial fuel cell (TC-MFC) was employed in Zhang et al. (2020) study to examine the removal of copper and the production of electricity. In the investigation, auxiliary reagents such as 1 mol L^{-1} HCl, citric acid, and acetic acid at varying concentrations (0.05–1.0 mol L^{-1}) were used to test TC-MFC, which included anodic, cathodic, and soil chambers. The initial preparation of the soil sample included 500 mg kg^{-1} of copper contamination. The application of 1 mol L^{-1} HCl produced the greatest electricity generation (363.04 mW h) and copper removal efficiency (94.78%) over the course of the 74-day experiment. According to the study, the most effective auxiliary reagent for removing copper from the TC-MFC system was 1 mol L^{-1} HCl, which also produced excellent efficiency.

Zhang et al. (2023) used *Ochrobactrum*-related strain CD-1 to investigate the effects of various carbon sources (sodium acetate, sodium citrate, and glucose) on power density and their efficacy on Pb^{2+} removal in a dual-chamber MFC system. Using sodium acetate allowed for the study's highest power density of 371.0 mW m^{-2} to be achieved in less than 6 hours. When glucose and sodium citrate were used, lower potentials and power densities were noted, with the claim that glucose in particular significantly slowed down the production of power. Furthermore, Pb^{2+} removal efficiency with sodium acetate was reported to be 52.3%, with sodium citrate to be 37.5%, and with glucose to be 19.2%.

Munoz-Cupa and Bassi (2023), used a dual-compartment microbial fuel cell inoculated with *Shewanella oneidensis* MR-1 in the anode compartment to investigate heavy metal removal and simultaneous energy production. The study employed synthetic wastewater that included phenol, Cu (II), Mg (II), Mn (II), Zn (II), and Na from refinery processes. In the anode compartment, the maximum open circuit voltage at a concentration of 5 (13.5, 7.5, 415, 10.5 and, 2410 mg L^{-1} respectively) was 517.6 mV, whereas in the cathode compartment, it was observed to be 27.7 mV. At the anode, the metal removal efficiencies for Cu (II), Mg (II), Mn (II), Zn (II), and Na were 93%, 85%, 93%, and 36%, respectively. 98% of Cu (II), 49% of Mg (II), 57% of Mn (II), 59% of Zn (II), and 36% of Na were removed in the cathode compartment.

Because different techniques and system configurations are employed in different studies on metal removal in MFCs, notable discrepancies are observed. Research focusing specifically on the elimination of copper and chromium metals has demonstrated these variations. The evaluations conducted for chromium include noteworthy examples from Zhang et al. (2021) and Wang et al. (2008). Zhang et al. (2021) removed chromium with 66.5% efficiency in a dual-chamber MFC system. Wang et al. (2008), on the other hand, attained 100% efficiency in a dual-chamber MFC system at a lower metal concentration. This could be attributed to various factors, including the system's pH range and the electrode materials employed (Saravanan et al., 2022).

Heijne et al. (2010), Tao et al. (2011), Yang et al. (2021), Zhang et al. (2020), and Munoz-Cupa and Bassi (2023) are a few examples of studies on copper removal in MFCs. Heijne et al. (2010) used both aerobic and anaerobic cathodes to remove copper, and they were successful in both systems with high efficiencies: anaerobic cathodes produced 99.88% efficiency, while aerobic cathodes produced 99.95% efficiency. By contrast, Tao et al. (2011) found that when using a membraneless system at high copper concentrations (2000 mg L^{-1}), the efficiency was 47.54%. Long-term studies and the absence of a membrane in the system could be the cause of this low efficiency (Du et al., 2007; Saravanan et al., 2022). Using various MFC configurations and carbon sources, Yang et al. (2021) and Zhang et al. (2020) studies also achieved successful results in copper removal. Zhang et al. (2020) used 1 mol L^{-1} HCl to achieve 94.78% removal, while Yang et al. (2021) used a three-chamber MFC to achieve 99% efficiency. Munoz-Cupa and Bassi (2023) worked on copper with different metals, achieving 93% efficiency. The variations in MFC configurations, carbon sources, and metal concentrations employed could be the cause of these removal efficiencies discrepancies (Du et al., 2007; Jatoi et al., 2020). Additionally, the variety of microorganism sources used may have an impact on the removal efficiency. Other researchers used different microbial mixtures, but Munoz-Cupa and Bassi (2023) used the *Shewanella oneidensis* MR-1 strain for copper removal. Utilizing a mixed microbial consortium has been shown in earlier research to improve metabolic cooperation and aid in the development of a biofilm layer, thereby improving MFC performance (Nevin et al., 2021).

Research on other metal species yields results that are likewise quite variable. For instance, Huang et al. (2013) found that cobalt had a high conversion rate. Working with gold, Choi and Hu (2013) produced a high-power output (6.58 W/m^2) and removal efficiency (99.8%). Selenium removal efficiency of 99% was attained by Catal et al. (2009); however, the efficiency of the single-chamber system may have differed based on the carbon source and pH levels (Saravanan et al., 2022; Jatoi et al., 2020). These variations rely on how different metal removal and energy production systems are designed and used. A number of variables affect efficiency and power output, including the types of microorganisms used, pH range, metal concentration, and materials used in MFC systems. Therefore, each study's unique conditions and methodology should be considered when evaluating the results.

Conclusions

In the developing world, the need for water is increasing along with water pollution. One of the main causes of water pollution is heavy metal pollution from various sources. Heavy metals must be eliminated from wastewater because of their toxic and cancer-causing properties, which endanger ecosystems and humans alike. The removal of heavy metals is influenced by numerous variables and techniques. These factors are pH, ionic strength, temperature and organic matter. Enhancing these parameters can lead to better metal removal performance from MFC. The interaction between heavy metal properties and these factors should be considered separately. Evaluating the strengths and weaknesses of each technique highlights the need for innovative and economically viable alternatives such as MFCs. The MFCs are a cutting-edge technology that extracts heavy metals from wastewater in addition to producing electricity. In MFCs, organic substrates act as electron donor in the anodic chamber and reduce heavy metals in the cathodic chamber. Metals are metabolized by microorganisms via a variety of pathways, such as electron shuttling aided by redox-active substances, biofilm formation on electrode surfaces, direct metal reduction, and synergistic interactions among microbial communities. Research revealed that a variety of heavy metals, including chromium, cobalt, copper, vanadium, mercury, gold, selenium, lead, magnesium, manganese, zinc, and sodium could be effectively removed by dual- and single-chamber MFCs, while also producing electricity. These MFCs were able to achieve high removal efficiencies, which ranged from 25% to 99.95%. This efficiency range is dependent on the particular contaminant being targeted, the contaminant's concentration, and the operational parameters (pH, temperature, etc.). Additionally, MFCs showed a broad range of power outputs, typically ranged from 0.15 W/m^2 to 6.58 W/m^2, depending on the particular configuration and conditions. Even under difficult circumstances, MFCs have benefits like lower energy consumption and less sludge production. However, obstacles like exorbitant expenses, low power output, problems with catalyst degradation and membrane fouling compromise their long-term viability. The scalability issue with MFCs is an additional drawback. The performance of laboratory-scale reactors cannot always be directly applied to field settings. When scaling, expanding the anode chamber's size could result in leaks in the anode chamber or a drop in overall performance instead of a proportionate increase in power output. To solve these issues, scalability studies require more research. Combining MFCs with other techniques for treating wastewater could be an additional option.

Future perspectives

The field of heavy metal removal in MFCs holds promising future prospects. Advancements in MFC technology, along with ongoing research efforts, are expected to further enhance the efficiency and effectiveness of heavy metal removal from wastewater. Future studies may focus on optimizing MFC configurations by exploring novel electrode materials, improving electron transfer kinetics, and enhancing the catalytic activity of biocatalysts. Additionally, the development of tailored microbial consortia with enhanced metal-reducing capabilities could improve the removal efficiency for specific heavy metals. Further investigation into the impacts of factors such as pH, metal concentrations and, temperature on MFC performance will enable a better understanding of their influence and facilitate the design of optimized MFC systems. Moreover, exploring sustainable and renewable electron donors, such as organic waste streams or renewable energy sources, could enhance the environmental and economic viability of MFC-based heavy metal removal. Overall, with continued research and technological advancements, microbial fuel cells have a promising future for efficient and sustainable heavy metal removal, contributing to the development of environmentally friendly wastewater treatment strategies.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics committee approval is not required for this study.

Authors' contribution statement

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References

Abbas, S. Z., Rafatullah, M., Ismail, N., & Syakir, M. I. (2017). A review on sediment microbial fuel cells as a new source of sustainable energy and heavy metal remediation: Mechanisms and future prospective. *International Journal of Energy Research, 41*(9), 1242–1264. https://doi.org/10.1002/er.3706

Abdel-Fatah, M. A. (2018). Nanofiltration systems and applications in wastewater treatment. *Ain Shams Engineering Journal*, *9*(4), 3077-3092. https://doi.org/10.1007/s10646-021-02409-2

Adekunle, A., Rickwood, C., Tartakovsky, B. (2021). On-line monitoring of water quality with a floating microbial fuel cell biosensor: field test results. *Ecotoxicology, 30(5), 851–862.* https://doi.org/10.1007/s10646-021-02409-2

Ajiboye, T. O., Oyewo, O. A., & Onwudiwe, D. C. (2021). Simultaneous removal of organics and heavy metals from industrial wastewater: A review. *Chemosphere, 262*, 128379. https://doi.org/10.1016/j.chemosphere.2020.128379

Akagündüz, D., Cebecioğlu, R., Özdemir, M., & Catal, T. (2021). Removal of psychoactive pharmaceuticals from wastewaters using microbial electrolysis cells producing hydrogen, *Water Science & Technology, 84*(4), 931-940. https://doi.org/10.2166/wst.2021.269

Akagündüz, D., Cebecioğlu, R., Ozen, F., Ozdemir, M., Bermek, H., Tarhan, N., Arslan, A., Catal, T. (2022). Effects of Psychoactive Pharmaceuticals in Wastewater on Electricity Generation in Microbial Fuel Cells. *Clean - Soil, Air, Water, 50*(3), 2100027. https://doi.org/10.1002/clen.202100027

Akul, N.B., Cebecioglu, R., Akagunduz, D., Bermek, H., Ozdemir, M., & Catal, T. (2021). Effects of mevastatin on electricity generation in microbial fuel cells. Polish Journal of Environmental Studies, 30(6), 5407-5412. https://doi.org/10.15244/pjoes/133402

Arkatkar, A., Mungray, A. K., & Sharma, P. (2021). Study of electrochemical activity zone of Pseudomonas aeruginosa in microbial fuel cell. Process Biochemistry, 101, 213–217. https://doi.org/10.1016/j.procbio.2021.01.005

Arslan, B. A., Akdoğan, E., Cebeci, F. Ç., & Catal, T. (2020). Bioelectricity generation using human neuronal-like cells in single chamber biofuel cells. Journal of Cleaner Production, (271), 122505. https://doi.org/10.1016/j.jclepro.2020.122505

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. Frontiers in pharmacology, 12, 643972. https://doi.org/10.3389/fphar.2021.643972

Bellona, A. S. (2015). Nanofiltration technology in water treatment and reuse: applications and costs. *Water Science & Technology,* 309-319. https://doi.org/10.2166/wst.2015.015

Bond, D. R., & Lovley, D. R. (2005). Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. *Applied and Environmental Microbiology, 71*(4), 2186–2189. https://doi.org/10.1128/aem.71.4.2186-2189.2005

Borole, A. P., O'Neill, H., Tsouris, C., & Cesar, S. (2008). A microbial fuel cell operating at low pH using the acidophile Acidiphilium cryptum. Biotechnology Letters, 30(8), 1367-1372. https://doi.org/10.1007/s10529-008-9700-y

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon, 6(9).* https://doi.org/10.1016/j.heliyon.2020.e04691

Cao, Y., Mu, H., Liu, W., Zhang, R., Guo, J., Xian, M., & Liu, H. (2019). Electricigens in the anode of microbial fuel cells: pure cultures versus mixed communities. *Microbial Cell Factories*, 18(1). https://doi.org/10.1186/s12934-019-1087-z

Catal, T., Bermek, H., & Liu, H. (2009). Removal of selenite from wastewater using microbial fuel cells. *Biotechnology Letters, 31*(8), 1211–1216. https://doi.org/10.1007/s10529-009-9990-8

Catal, T., Kul, A., Atalay, V. E., Bermek, H., Ozilhan, S., & Tarhan, N. (2019). Efficacy of microbial fuel cells for sensing of cocaine metabolites in urine-based wastewater. Journal of Power Sources, 414, 1–7. https://doi.org/10.1016/j.jpowsour.2018.12.078

Catal, T., Liu, H., Fan, Y., & Bermek, H. (2018b). A clean technology to convert sucrose and lignocellulose in microbial electrochemical cells into electricity and hydrogen. Bioresource Technology Reports, 5, 331-334. https://doi.org/10.1016/j.biteb.2018.10.002

Catal, T., Liu, H., Kilinc, B., & Yilancioglu K. (2024). Extracellular polymeric substances in electroactive biofilms play a crucial role in improving the efficiency of microbial fuel and electrolysis cells. *Letters in Applied Microbiology, 1*;77(3). https://doi.org/10.1093/lambio/ovae017

Catal, T., Yavaser, S., Enisoglu-Atalay, V., Bermek, H., & Ozilhan, S. (2018a). Monitoring of neomycin sulfate antibiotic in microbial fuel cells. *Bioresource Technology, (268)*, 116-120. https://doi.org/10.1016/j.biortech.2018.07.122

Cebecioğlu R. E., Akagündüz, D., Bermek, H., Atalay, V. E., Catal, T. (2022). Decolorization mechanisms of reactive yellow 145 and ponceau S in microbial fuel cells during simultaneous electricity production, *Main Group Chemistry,* 1-13. https://doi.org/10.3233/MGC-210180

Chen, H., Zhao, J., Dai, G., Wu, J., & Yan, H., (2010). Adsorption characteristics of Pb(II) from aqueous solution onto a natural biosorbent, fallen Cinnamomum camphora leaves. *Desalination 262*, 174e182. https://doi.org/10.1016/j.desal.2010.06.006

Choi, C., & Hu, N. (2013). The modeling of gold recovery from tetrachloroaurate wastewater using a microbial fuel cell. *Bioresource Technology, 133*, 589-598. https://doi.org/10.1016/j.biortech.2013.01.143

Corbella, C., Hartl, M., Fernandez Gatell, M., & Puigagut, J. (2019). MFC-based biosensor for domestic wastewater COD assessment in constructed wetlands. *Science Total Environment, 10(660),* 218-226. https://doi.org/10.1016/j.scitotenv.2018.12.347

Dahiya, A. (2020). Part 1 Bioenergy-biomass to biofuels: an overview. *Bioenergy,* 1-4. https://doi.org/10.1016/B978-0-12-815497-7.02001-7

Dahman, Y. (2017). Nanopolymers. Nanotechnology and Functional Materials for Engineers. *Elsevier* 121-144. https://doi.org/10.1016/B978-0-323-51256-5.00006-X

Dai, H. N., Nguyen, T.-A. D., Le, L.-P. M., Tran, M. V., Lan, T.-H., & Wang, C.T. (2021). Power generation of Shewanella oneidensis MR-1 microbial fuel cells in bamboo fermentation effluent. International Journal of Hydrogen Energy, 46(31), 16612-16621. https://doi.org/10.1016/j.ijhydene.2020.09.264

Du, Z., Li, H., & Gu, T. (2007). A state-of-the-art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. Biotechnology Advances, 25(5), 464–482. https://doi.org/10.1016/j.biotechadv.2007.05.004

Environmental Protection Agency (EPA) (2024). ''National Primary Drinking Water Regulations'', https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations , Last access date: 21 August 2024.

Fan, Y., Janicek, A., & Liu, H. (2024). Stable and high voltage and power output of CEA-MFCs internally connected in series (iCiS-MFC). *The European Chemistry and Biotechnology Journal, 1,* 47–57. https://doi.org/10.62063/ecb-17

Fedorovich, V., Knighton, M. C., Pagaling, E., Ward, F. B., Free, A., & Goryanin, I. (2009). Novel electrochemically active bacterium phylogenetically related to Arcobacter butzleri, isolated from a microbial fuel cell. *Applied and Environmental Microbiology*, 75(23), 7326–7334. https://doi.

org/10.1128/AEM.01345-09

Ferraz, M., & Lourenco, J., (2000). The influence of organic matter content of contaminated soils on the leaching rate of heavy metals. Environ. Prog. 19 (1), 53e58. https://doi.org/10.1002/ep.670190118

Garud, R. M., Kore, S. V., Kore, V. S., & Kulkarni, G. S. (2011). A Short Review on Process and Applications of Reverse Osmosis. *Universal Journal of Environmental Research & Technology*, *1*(3).

Gharai, M., & Venugopal, R. (2016). Modeling of flotation process—An overview of different approaches. *Mineral Processing and Extractive Metallurgy Review*, *37*(2), 120-133. https://doi.org/10.1080/08827508.2015.1115991

Gu, S., Kang, X., Wang, L., Lichtfouse, E., & Wang, C. (2018). Clay mineral adsorbents for heavy metal removal from wastewater: A Review. *Environmental Chemistry Letters, 17(2),* 629–654. https://doi.org/10.1007/s10311-018-0813-9

Guo, Y., Wang, J., Shinde, S., Wang, X., Li, Y., Dai, Y., Ren, J., Zhang P., & Liu, X. (2020). Simultaneous wastewater treatment and energy harvesting in microbial fuel cells: an update on the biocatalysts. *RSC Advances*, 10(43), 25874–25887. https://10.1039/d0ra05234e

Gurreri, L., Tamburini, A., Cipollina, A., & Micale, G. (2020). Electrodialysis applications in wastewater treatment for environmental protection and resources recovery: A systematic review on progress and perspectives. *Membranes*, *10*(7), 146. https://doi.org/10.3390/membranes10070146

Hakami, M. W., Alkhudhiri, A., Al-Batty, S., Zacharof, M.-P., Maddy, J., & Hilal, N. (2020). Ceramic microfiltration membranes in wastewater treatment: Filtration behavior, fouling and prevention. *Membranes, 10(9)*, 248. https://doi.org/10.3390/membranes10090248

Heijne, A. T., Liu, F., Weijden, R. van, Weijma, J., Buisman, C. J. N., & Hamelers, H. V. M. (2010). Copper recovery combined with electricity production in a microbial fuel cell. *Environmental Science Technology*, *44(11),* 4376–4381. https://doi.org/10.1021/es100526g

Ho, Y. C., Chua, S. C., & Chong, F. K. (2020). Coagulation-flocculation technology in water and wastewater treatment. *Handbook of Research on Resource Management for Pollution and Waste Treatment.* 432-457. https://doi.org/10.4018/978-1-7998-0369-0.ch018

Holmes, D. E., Nicoll, J. S., Bond, D. R., & Lovley, D. R. (2004). Potential role of a novel psychrotolerant member of the family Geobacteraceae, Geopsychrobacter electrodiphilus gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. Applied and Environmental Microbiology, 70(10), 6023–6030. https://doi.org/10.1128/AEM.70.10.6023-6030.2004

Huang, L., Li, T., Liu, C., Quan, X., Chen, L., Wang, A., & Chen, G. (2013). Synergetic interactions improve cobalt leaching from lithium cobalt oxide in microbial fuel cells. *Bioresource Technology, 128,* 539-546. https://doi.org/10.1016/j.biortech.2012.11.011

Jadhav, D. A., Mungray, A. K., Arkatkar, A., & Kumar, S. S. (2021). Recent advancement in scaling-up applications of microbial fuel cells: From reality to practicability. *Sustainable Energy Technologies and Assessments*, *45,* 101226. https://10.1016/j.seta.2021.101226

Jatoi, A. S., Akhter, F., Mazari, S. A., Sabzoi, N., Aziz, S., Soomro, S. A., Mujawar Mubarak, N., Baloch, H., Memon, A. Q., & Ahmed, S. (2020). Advanced microbial fuel cell for wastewater treatment—a review. **Environmental Science and Pollution Research, 28**, 5005–5019. https://doi.

org/10.1007/s11356-020-11691-2

Joseph, L., Jun, B. M., Flora, J. R., Park, C. M., & Yoon, Y. (2019). Removal of heavy metals from water sources in the developing world using low-cost materials: A review. *Chemosphere, 229*, 142-159. https://doi.org/10.1016/j.chemosphere.2019.04.198

Karnib, M., Kabbani, A., Holail, H., & Olama, Z. (2014). Heavy metals removal using activated carbon, silica and silica activated carbon composite. *Energy Procedia, 50,* 113–120. https://doi.org/10.1016/j.egypro.2014.06.014

Kilinc, B. & Catal T. (2023). A Novel Microbial Fuel Cell for the Sensing of Sodium Acetate in Soil *Polish Journal of Environmental Studies, 32* (5), 4931-4936. https://doi.org/10.15244/pjoes/168804

Kumar, A. S. K., Jiang, S. J., & Tseng, W. L. (2015). Effective adsorption of chromium (VI)/Cr (III) from aqueous solution using ionic liquid functionalized multiwalled carbon nanotubes as a super sorbent. *Journal of Materials Chemistry A*, *3*(13), 7044-7057. https://doi.org/10.1039/C4TA06948J

Kumpulainen, S., von der Kammer, F., & Hofmann, T. (2008). Humic acid adsorption and surface charge effects on schwertmannite and goethite in acid sulphate waters. *Water Research, 42(8-9),* 2051-2060. https://doi.org/10.1016/j.watres.2007.12.015

Kuntke, P., Zamora, P., Saakes, M., Buisman, C., & Hamelers, H. (2016). Gas-permeable hydrophobic tubular membranes for ammonia recovery in bio-electrochemical systems. *Environmental Science: Water Research & Technology, 2(2),* 261-265. https://doi.org/10.1039/C5EW00299K

Lee, D. J., Chang, J. S., & Lai, J. Y. (2015). Microalgae-microbial fuel cell: A mini review. *Bioresource Technology, (198),* 891-895. https://doi.org/10.1016/j.biortech.2015.09.061

Lee, K. P., Arnot, T. C., & Mattia, D. (2011). A review of reverse osmosis membrane materials for desalination—development to date and future potential. *Journal of Membrane Science, 370(1–2),* 1–22. https://doi.org/10.1016/j.memsci.2010.12.036

Li, F., Yin, C., Sun, L., Li, Y., Guo, X., & Song, H. (2017). Synthetic Klebsiella pneumoniae-Shewanella oneidensis consortium enables glycerol-fed high-performance microbial fuel cells. Biotechnology Journal, 13(5), 1700491. https://doi.org/10.1002/biot.201700491

Li, S.-W., He, H., Zeng, R. J., & Sheng, G.-P. (2017). Chitin degradation and electricity generation by Aeromonas hydrophila in microbial fuel cells. Chemosphere, 168, 293–299. https://doi.org/10.1016/j.chemosphere.2016.10

Li, X., Zhang, D., Sheng, F., & Qing, H. (2018). Adsorption characteristics of Copper (II), Zinc (II) and Mercury (II) by four kinds of immobilized fungi residues. *Ecotoxicology and environmental safety*, *147*, 357-366.https://doi.org/10.1016/j.ecoenv.2017.08.058

Lin, L., Yang, H., & Xu, X. (2022). Effects of water pollution on human health and disease heterogeneity: a review. *Frontiers in Environmental Science, 10*:880246. https://doi.org/10.3389/fenvs.2022.880246

Liu, H., Yang, G., Jia, H., & Sun, B. (2022). Crude oil degradation by a novel strain Pseudomonas aeruginosa AQNU-1 isolated from an oil-contaminated lake wetland. Processes, 10(2), 307. https://doi.org/10.3390/pr10020307

Liu, L., Lee, D.J., Wang, A., Ren, N., Su, A., & Lai, J.Y. (2016). Isolation of Fe(III)-reducing bacterium, Citrobacter sp. LAR-1, for startup of microbial fuel cell. International Journal of Hydrogen Energy, 41(7), 4498-4503. https://doi.org/10.1016/j.ijhydene.2015.07.072

Liu, Z., Lian, J., Du, Z., & Li, H. (2006). Construction of sugar-based microbial fuel cells by dissimilatory metal reduction bacteria. Chinese Journal of Biotechnology, 22(1), 131–137. https://doi.org/10.1016/s1872-2075(06)60010-1

Lovley, D. R., Ueki, T., Zhang, T., Malvankar, N. S., Shrestha, P. M., Flanagan, K. A., Aklujkar, M., Butler, J. E., Giloteaux, L., Rotaru, A.-E., Holmes, D. E., Franks, A. E., Orellana, R., Risso, C., & Nevin, K. P. (2011). Geobacter: The microbe electric's physiology, ecology, and practical applications. Advances in Microbial Physiology, 59, 1-100. https://doi.org/10.1016/B978-0-12-387661-4.00004-5

Lye, D., (2009). Rooftop runoff as a source of contamination: a review. *Science of The Total Environment, 407(21),* 5429e5434. https://doi.org/10.1016/j.scitotenv.2009.07.011

Marshall, C. W., & May, H. D. (2009). Electrochemical evidence of direct electrode reduction by a thermophilic Gram-positive bacterium, Thermincola ferriacetica. Energy & Environmental Science, 2(7), 699-705. https://doi.org/10.1039/B823237G

Mehtaa, D. S. M. (2015). Magnetic adsorbents for the treatment of water/wastewater-A review. *Journal of Water Process Engineering*, 244-265. https://doi.org/10.1016/j.jwpe.2015.07.001

Mondal, S., & Wickramasinghe, S. R. (2008). Produced water treatment by nanofiltration and reverse osmosis membranes. *Journal of membrane science*, *322*(1), 162-170. https://doi.org/10.1016/j.memsci.2008.05.039

Moussavi, G., & Barikbin, B. (2010). Biosorption of chromium(vi) from industrial wastewater onto pistachio hull waste biomass. *Chemical Engineering Journal, 162(3)*, 893–900. https://doi.org/10.1016/j.cej.2010.06.032

Munoz-Cupa, C., & Bassi, A. (2024). Investigation of heavy metal removal from salty wastewater and voltage production using Shewanella oneidensis MR-1 nanowires in a dual-chamber microbial fuel cell. *Environmental Progress & Sustainable Energy*, 43(1), e14237. https://doi.org/10.1002/ep.14237

Murray, K. S., Rogers, D. T., & Kaufman, M. M. (2004). Heavy metals in an urban watershed in southeastern Michigan. *Journal of Environmental Quality, 33(1),* 163–172. https://doi.org/10.2134/jeq2004.1630

Muruganandam, L., Saravana Kumar, M. P., Jena, A., Gulla, S., & Godhwani, B. (2017). Treatment of waste water by coagulation and flocculation using biomaterials. *IOP Conference Series:Materials Science and Engineering, 263,* 032006. https://doi.org/10.1088/1757-899x/263/3/032006

Naha, A., Debroy, R., Sharma, D., Shah, M. P., & Nath, S. (2023). Microbial fuel cell: A state-of-the-art and revolutionizing technology for efficient energy recovery. *Cleaner and Circular Bioeconomy*, *5,* 100050. https://doi.org/10.1016/j.clcb.2023.100050

Nascimento, M., Rodrigues, J., Matias, R., & Jordao, L. (2024). Aeromonas spp. in freshwater bodies: Antimicrobial resistance and biofilm assembly. Antibiotics, 13(2), 166. https://doi.org/10.3390/antibiotics13020166

Nevin, K. P., Richter, H., Covalla, S. F., Johnson, J. P., Woodard, T. L., Orloff, A. L., Jia, M., Zhang, M., & Lovley, D. R. (2008). Power output and columbic efficiencies from biofilms of Geobacter sulfurreducens comparable to mixed community microbial fuel cells. *Environmental Microbiology,* *10*(10), 2505–2514. https://doi.org/10.1111/j.1462-2920.2008.01656.x

Noori, M. T., Thatikayala, D., Pant, D., & Min, B. (2022). A critical review on microbe-electrode interactions towards heavy metal ion detection using microbial fuel cell technology. *Bioresource Technology, 347*:126589. https://doi.org/10.1016/j.biortech.2021.126589

Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., & Levin, E.D. (2009). The toxicology of climate change: environmental contaminants in a warming world. *Environment International, 35(6)*, 971e986. https://doi.org/10.1016/j.envint.2009.02.006

Olaniran, A. O., Balgobind, A., & Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International Journal of Molecular* Sciences*, 14(5),* 10197-10228. https://doi.org/10.3390/ijms140510197

Ozdemir, M., Enisoglu-Atalay, V., Bermek, H., Ozilhan, S., Tarhan, N., & Catal, T. (2019). Removal of a cannabis metabolite from human urine in microbial fuel cells generating electricity. Bioresource Technology Reports, 5, 121-126. https://doi.org/10.1016/j.biteb.2019.01.003

Padmanabhan, S., Yadavar, K. R., Muthaiah, M., & Muthukumar, A. (2023). Identification of predominant bacterial species involved in microbial fuel cell assisted electricity generation using fish market wastewater. Cleaner and Circular Bioeconomy, 6, 100051. https://doi.org/10.1016/j.clcb.2023.100051

Pandit, A. B., & Kumar, J. K. (2015). Clean Water for Developing Countries. *Annual Review of Chemical and Biomolecular Engineering, 6(1),* 217–246. https://doi.org/10.1146/annurev-chembioeng-061114-123432

Pant, D., Van Bogaert, G., Diels, L., & Vanbroekhoven, K. (2010). A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresource Technology, 101(6),* 1533-1543. https://doi.org/10.1016/j.biortech.2009.10.017

Pantsar-Kallio, M., Reinikainen, S.P., & Oksanen, M. (2001). Interactions of soil components and their effects on speciation of chromium in soils. *Analytica Chimica Acta, 439(1),* 9–17. https://doi.org/10.1016/S0003-2670(01)00840-6

Park, Y. H., Park, E., & Smith, G. (2019). Energy harvesting using exoelectrogenic *Shewanella oneidensis* bacteria. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 45*(2), 3879-3886. https://doi.org/10.1080/15567036.2018.1557856

Piwowarek, K., Lipińska, E., Hać-Szymańczuk, E., Kieliszek, M., & Ścibisz, I. (2018). Propionibacterium spp.—source of propionic acid, vitamin B12, and other metabolites important for the industry. Applied Microbiology and Biotechnology, 102(2), 515-538. https://doi.org/10.1007/s00253-017-8616-7

Qasem, N. A., Mohammed, R. H., & Lawal, D. U. (2021). Removal of heavy metal ions from wastewater: A comprehensive and critical review. *Npj Clean Water, 4(1),* 1-15. https://doi.org/10.1038/s41545-021-00127-0

Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M., & Verstraete, W. (2004). Biofuel Cells Select for Microbial Consortia That Self-Mediate Electron Transfer. *Applied and Environmental Microbiology, 70(9),* 5373–5382. https://doi.org/10.1128/aem.70.9.5373-5382.2004

Rabaey, K., Rodríguez, J., Blackall, L. L., Keller, J., Gross, P., Batstone, D., Verstraete, W., & Nealson, K. H. (2007). Microbial Ecology meets electrochemistry: Electricity-driven and driving communities. *The ISME Journal, 1(1*), 9–18. https://doi.org/10.1038/ismej.2007.4

Ramakrishnaiah, C. R., & Prathima, B. (2012). Hexavalent chromium removal from industrial wastewater by chemical precipitation method. *International Journal of Engineering Research and Applications, 2*(2), 599-603.

Reiche, A., Sivell, J., & Kirkwood, K. M. (2015). Electricity generation by Propionibacterium freudenreichii in a mediatorless microbial fuel cell. *Biotechnology Letters*, 38(1), 51–55. https://doi.org/10.1007/s10529-015-1944-8

Roy, B., Maitra, D., Sarkar, S., Podder, R., Das, T., Ghosh, J., & Mitra, A. K. (2023). Biofilm and metallothioneins: A dual approach to bioremediate the heavy metal menace. *Environmental Quality Management*, *33(4),* 659–676. https://doi.org/10.1002/tqem.22139

Ryan, M. P., Sevjahova, L., Gorman, R., & White, S. (2022). The emergence of the genus Comamonas as important opportunistic pathogens. *Pathogens, 11*(9), 1032. https://doi.org/10.3390/pathogens11091032

Saravanan, A., Senthil Kumar, P., Srinivasan, S., Jeevanantham, S., Kamalesh, R., & Karishma, S. (2022). Sustainable strategy on microbial fuel cell to treat the wastewater for the production of green energy. *Chemosphere, 290*, 133295. https://doi.org/10.1016/j.chemosphere.2021.133295

Sari, A., & Tuzen, M. (2008). Biosorption of total chromium from aqueous solution by red algae (Ceramium virgatum): Equilibrium, kinetic and thermodynamic studies. *Journal of Hazardous Materials*, *160*(2-3), 349-355. https://doi.org/10.1016/j.jhazmat.2008.03.005

Senanu, L. D., Kranjac-Berisavljevic, G., & Cobbina, S. J. (2023). The use of local materials to remove heavy metals for household-scale drinking water treatment: A review. *Environmental Technology & Innovation*, 103005. https://doi.org/10.1016/j.eti.2023.103005

Sharma, P. & Mutnuri, S. (2019). Nutrient recovery and microbial diversity in human urine fed microbial fuel cell. *Water Science and Technology, 79*(4), 718-730. https://doi.org/10.2166/wst.2019.089

Sonmez, E., Avci, B., Mohamed, N., & Bermek, H. (2024). Investigation of performance losses in microbial fuel cells with low platinum loadings on air-cathodes. *The European Chemistry and Biotechnology Journal*, 1, 11-26. https://doi.org/10.62063/ecb-14

Stafiej, K. P. (2007). Adsorption of heavy metal ions with carbon nanotubes. *Separation and Purification Technology,* 49-52. https://doi.org/10.1016/j.seppur.2007.07.008

Sukkasem, C. (2024). Exploring biofilm-forming bacteria for integration into BioCircuit wastewater treatment. T*he European Chemistry and Biotechnology Journal, 2, 39*–52. https://doi.org/10.62063/ecb-28

Sun, M., Mu, Z.-X., Chen, Y.-P., Sheng, G.-P., Liu, X.-W., Chen, Y.-Z., Zhao, Y., Wang, H.-L., Yu, H.-Q., Wei, L., & Ma, F. (2009). Microbe-assisted sulfide oxidation in the anode of a microbial fuel cell. *Environmental Science & Technology*, 43(9), 2941-2947

Tamjidi, S., Esmaeili, H., & Kamyab Moghadas, B. (2019). Application of magnetic adsorbents for removal of heavy metals from wastewater: A review study. *Materials Research Express, 6(10),* 102004. https://doi.org/10.1088/2053-1591/ab3ffb

Tao, H. C., Zhang, L. J., Gao, Z. Y., & Wu, W. M. (2011). Copper reduction in a pilot-scale membrane-free bioelectrochemical reactor. *Bioresource Technology, 102(22),* 10334-10339. https://doi.org/10.1128/AEM.70.9.5373-5382.2004

Tchobanoglus, G., Burton, F., & Stensel, H. D. (2003). Wastewater engineering: treatment and reuse. *American Water Works Association. Journal, 95(5),* 201.

Vakili, M., Deng, S., Cagnetta, G., Wang, W., Meng, P., Liu, D., & Yu, G. (2019). Regeneration of chitosan-based adsorbents used in heavy metal adsorption: A Review. *Separation and Purification Technology, 224*, 373–387. https://doi.org/10.1016/j.seppur.2019.05.040

Van der Bruggen, B. (2015). Advances in electrodialysis for water treatment. In Advances in membrane technologies for water treatment. *Woodhead Publishing.* 185-203. https://doi.org/10.1016/B978-1-78242-121-4.00006-X

Varnava, C. K., Persianis, P., Ieropoulos, I., & Tsipa, A. (2024). Electricity generation and real oily wastewater treatment by Pseudomonas citronellolis 620C in a microbial fuel cell: Pyocyanin production as electron shuttle. *Bioprocess and Biosystems Engineering, 47*(4), 903–917. https://doi.org/10.1007/s00449-024-02903-7

Venkidusamy, K., & Megharaj, M. (2016). A novel electrophototrophic bacterium Rhodopseudomonas palustris strain RP2 exhibits hydrocarbonoclastic potential in anaerobic environments. *Frontiers in Microbiology*, 7, 1071. https://doi.org/10.3389/fmicb.2016.01071

Villaescusa, I., Fiol, N., Martinez, M., Miralles, N., Poch, J., & Serarols, J. (2004). Removal of copper and nickel ions from aqueous solutions by grape stalks wastes. *Water Research, 38 (4),* 992-1002. https://doi.org/10.1016/j.watres.2003.10.040

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., & Pigna, M. (2010). Mobility and bioavailability of heavy metals and metalloids in Soil Environments. *Journal of Soil Science and Plant Nutrition, 10(3).* https://doi.org/10.4067/s0718-95162010000100005

Wang, G., Huang, L., & Zhang, Y. (2008). Microbial fuel cells for cathodic reduction of hexavalent chromium [Cr (VI)] and simultaneous electricity generation. *Biotechnology Letters, 30*, 1959-1966. https://doi.org/10.1007/s10529-008-9792-4

Wang, J., & Chen, C. (2009). Biosorbents for heavy metals removal and their future. *Biotechnology Advances, 27(2),* 195–226. https://doi.org/10.1016/j.biotechadv.2008.11.002

Wang, J., Song, X., Wang, Y., Abayneh, B., Ding, Y., Yan, D., & Bai, J. (2016). Microbial community structure of different electrode materials in constructed wetland incorporating microbial fuel cell. *Bioresource Technology, 221*, 697-702. https://doi.org/10.1016/j.biortech.2016.09.116

Wang, W., Chen, M., Guo, L., & Wang, W.X. (2017). Size partitioning and mixing behavior of trace metals and dissolved organic matter in a South China estuary. *Science of The Total Environment, 603-604,* 434–444. https://doi.org/10.1016/j.scitotenv.2017.06.121

Wang, Z., Hu, Y., Dong, Y., Shi, L., & Jiang, Y. (2023). Enhancing electrical outputs of the fuel cells with Geobacter sulfurreducens by overexpressing nanowire proteins. *Microbial Biotechnology, 16*(3), 534–545. https://doi.org/10.1111/1751-7915.14128

Wang, Z., Lim, B., & Choi, C. (2011). Removal of Hg2+ as an electron acceptor coupled with power generation using a microbial fuel cell. *Bioresource Technology, 102,* 6304-6307. https://doi.org/10.1016/j.biortech.2011.02.027

WHO. (2017). Guidelines for drinking-water quality fourth edition incorporating the first addendum. Who chronicle, 38(4), 104-108.

Xia, J., Duan, Q.Y., Luo, Y., Xie, Z. H., Liu, Z.Y., & Mo, X.G. (2017). Climate change and water resources: Case study of Eastern Monsoon Region of China. *Advances in Climate Change Research, 8(2),* 63–67. https://doi.org/10.1016/j.accre.2017.03.007

Xiang, H., Min, X., Tang, C. J., Sillanpää, M., & Zhao, F. (2022). Recent advances in membrane filtration for heavy metal removal from wastewater: A mini review. *Journal of Water Process Engineering*, *49*, 103023. https://doi.org/10.1016/j.jwpe.2022.103023

Xu, H., Chen, Y., Wen, Q., Lin, C., Gao, H., Qiu, Z., Yang, L., & Pan, X. (2023). The role of binary transition metal Cobalt-Nickel sulfide as an anode catalyst in specifically selection of Desulfuromonas and improved performance of microbial fuel cell. *Chemical Engineering Journal, 470*, 144163. https://doi.org/10.1016/j.cej.2023.144163

Xu, T., & Huang, C. (2008). Electrodialysis-based Separation Technologies: A critical review. *AIChE Journal, 54(12),* 3147–3159. https://doi.org/10.1002/aic.11643

Yang, K., Miao, G., Wu, W., Lin, D., Pan, B., Wu, F., & Xing, B. (2015). Sorption of Cu2+ on humic acids sequentially extracted from a sediment. *Chemosphere, 138,* 657-663. https://doi.org/10.1016/j.chemosphere.2015.07.061

Yang, S., Wu, Y., Aierken, A., Zhang, M., Fang, P., Fan, Y., & Ming, Z. (2016). Mono/ competitive adsorption of arsenic(III) and nickel(II) using modified green tea waste. *Journal of the Taiwan Institute of Chemical Engineers, 60,* 213-221. https://doi.org/10.1016/j.jtice.2015.07.007

Yang, X., Wan, Y., Zheng, Y., He, F., Yu, Z., Huang, J., Wang, H., Ok, Y. S., Jiang, Y., & Gao, B. (2019). Surface functional groups of carbon-based adsorbents and their roles in the removal of heavy metals from Aqueous Solutions: A critical review. *Chemical Engineering Journal, 366,* 608–621. https://doi.org/10.1016/j.cej.2019.02.119

Yanuka-Golub, K., Reshef, L., Rishpon, J., & Gophna, U. (2020). Specific Desulfuromonas strains can determine startup times of microbial fuel cells. *Applied Sciences*, 10(23), 8570. https://doi.org/10.3390/app10238570

Zhang, B., Zhao, H., Shi, C., Zhou, S., & Ni, J. (2009). Simultaneous removal of sulfide and organics with vanadium(V) reduction in microbial fuel cells. *Journal of Chemical Technology & Biotechnology, 84,* 1780-1786. https://doi.org/10.1002/jctb.2244

Zhang, C., Lu, H., & Hu, Z. (2023). Electricity production by Ochrobactrum-related strain CD-1 and Pb2+ removal in dual-chamber microbial fuel cell. *Global NEST Journal*, 25(7), 18-24. https://doi.org/10.30955/gnj.005083

Zhang, J., Cao, X., Wang, H., Long, X., & Li, X. (2020). Simultaneous enhancement of heavy metal removal and electricity generation in soil microbial fuel cell. *Ecotoxicology and Environmental Safety*, 192, 110314. https://doi.org/10.1016/j.ecoenv.2020.110314

Zhang, P., Liu, Y., Fu, M., Wang, B., Ding, S., Ma, X., Zhang, X., & Shen, Z. (2024). Meta-analysis on the global prevalence of Arcobacter in food-producing animals and humans. *One Health Advances*, 2, 13. https://doi.org/10.1016/j.onehad.2024.100013

Zhang, X., Liu, Y., Li, C. (2021).Influence of Cr (VI) concentration on Cr (VI) reduction and electricity production in microbial fuel cell, *Environmental Science and Pollution Research, 28(38),* 54170-54176. https://doi.org/10.1007/s11356-021-15889-w

REVIEW

Application of salt-assisted liquid-liquid extraction in bioanalytical methods

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Abstract

This review provides a comprehensive analysis of bioanalytical methods employed for the quantification of drug molecules in various biological matrices, including human plasma, urine, breast milk, and mouse plasma. The study not only examines traditional sample preparation techniques such as protein precipitation (PP), liquid-liquid extraction (LLE), and solid-phase extraction (SPE), but also delves into the relatively new and innovative salting-assisted liquid-liquid extraction (SALLE). It offers a thorough comparison of analytical methods utilizing SALLE, focusing on key parameters such as analysis time, calibration range, and the type and quantity of salts and organic solvents used. This review aims to serve as an essential resource for researchers and practitioners in selecting the most suitable bioanalytical methods for pharmacokinetic studies and drug monitoring, ultimately enhancing data quality and analytical efficiency in both clinical and research settings.

Keywords: bioanalytical methods, chromatography, mass spectrometry, SALLE

Introduction

Bioanalytical methods are analytical methods that have the purpose of establishing the appropriate dosing schedule by considering the pharmacokinetic variability between individuals, performing routine drug analysis, detecting the abuse of drugs, and evaluating pharmacokinetics in new drug discovery. Since analyte concentrations are very low, the method must meet guidelines for reproducibility, accuracy, selectivity, and precision (Nováková & Vlčková, 2009). A bioanalytical method consists of 3 parts. These are the collection of the sample (serum, hair, saliva, blood, organ tissue, plasma, urine, feces), sample preparation, which is a crucial step to improve the performance of the method and minimize the matrix effect, and sample analysis (Moein et al., 2017). Bioanalyses are considered quite complex and difficult, because biological matrices are highly complex and the amount of analyte is quite low (Nazario et al., 2017). Sample preparation is an essential step to isolate the analyte from the matrix, minimize the matrix effect, and enrich the analytes if necessary (Li et al., 2019). It has a significant impact on data quality, analysis throughput, and employee satisfaction (Chang et al., 2007). This step should have the following goals: minimize or eliminate the consumption of hazardous and toxic organic solvents, reduce sample volume, reduce extraction time and increase yield, and should be easily implementable (Kabir et al., 2017). It is widely recognized that converting a sample into a format compatible with analytical instrumentation is the most time-consuming, labor-intensive, and error-prone step. This conversion can be as simple as dilution or filtration or can involve multi-step procedures (Clark et al., 2016; Theodoridis & Papadoyannis, 2006).

Sample Collection

Sample collection in bioanalyses has a critical role in maintaining the stability of the analyte in the sample matrix. This process involves the collection, processing, storage and transport of samples and often samples are collected from different facilities or laboratories and shipped worldwide (Pawula et al., 2013). Therefore, the handling and transport of samples needs to be meticulously planned and implemented. In bioanalysis, it is important that analyte concentrations in samples accurately reflect the concentrations at the time of collection. For this purpose, it may be necessary to use stabilizers to prevent degradation or non-specific binding of analytes (Hilhorst et al., 2015). In recent years, clinical laboratories and pharmaceutical manufacturers have shown a marked increase in interest for drug analysis in biological fluids. An increasing focus in clinical chemistry and forensic toxicology is the use of alternative samples. Sweat, hair and oral fluids are some of the non-traditional samples that have attracted attention. The collection of oral fluids from these samples does not require the same level of expertise as blood sampling, but physiological factors can still lead to fluid deficiencies(Fura et al., 2003). Moreover, the intake of certain foods and the use of techniques that stimulate oral fluid production can influence drug concentrations. The clinical acceptance of monitoring drug levels in oral fluids is limited to a few drugs, as the correlation between plasma and oral fluid levels is not sufficiently strong (Elmongy & Abdel-Rehim, 2016). Pharmacokinetic and metabolism studies typically involve monitoring drug and metabolite concentrations in samples such as plasma, urine, or bile, which are stored, processed and analyzed after collection (Fura et al., 2003).

Conventional sample preparation techniques

Techniques such as liquid-liquid extraction (LLE), protein precipitation (PP), and solid phase extraction (SPE) were historically prevalent in sample preparation, but are now regarded as traditional methods. In recent years, there has been a swift advancement in innovative sample preparation techniques within the realm of bioanalysis (Kole et al., 2011).

Protein precipitation is a technique that uses water-miscible organic solvents to denature and agglutinate proteins, thereby removing analytes from plasma samples. In this process, proteins are usually precipitated by disrupting their hydrogen bonds with solvents such as acetonitrile or methanol, which helps to eliminate interferences from large molecules (Ma et al., 2008). The protein precipitation method is widely preferred due to its low cost, rapid results and allows rapid development of analyses, especially in preclinical pharmacokinetic studies of drug discovery (Ping et al., 2022). Furthermore, it provides sufficient purity for LC-MS analyses and, when the number of samples is large, the process can be more efficient and automated, with the use of 96-well plate structure (Zhang et al., 2022). However, this method also has some negative aspects. Steps requiring manual handling may prevent full automation of the process and limit the capacity of the operator to perform other tasks in parallel (Ping et al., 2022). Furthermore, due to the sensitivity of proteins to different denaturation conditions, complete precipitation of some proteins may be difficult, which may prevent complete separation of analytes with unwanted proteins (Nfor et al., 2011). Therefore, although the protein precipitation method is practical in the laboratory environment, it has some limitations, especially in large-scale and high-sensitivity studies.

LLE is a commonly utilized clean-up method for extracting compounds from aqueous samples. This technique facilitates the transfer of target compounds between phases by bringing immiscible or only partially soluble liquids into contact. Moreover, LLE is recognized as one of the most traditional and extensively applied methods in sample preparation for both qualitative and quantitative analysis, making it a preferred choice for sample pretreatment in various analytical methods (Cantwell & Losier, 2002; Lorenzo-Parodi et al., 2023). The main advantages of this method include its simplicity, low costs, and ease of scale-up (Mazzola et al., 2008). LLE also exhibits several limitations. The process demands significant quantities of organic solvents, which often possess toxic characteristics and present environmental and health hazards. Moreover, it is recognized as a process that requires considerable time and effort from the analyst, thereby increasing the likelihood of human error (Lorenzo-Parodi et al., 2023). To overcome these disadvantages, different strategies have been proposed and utilized. These strategies aim to minimize the occurrence of errors by reducing solution consumption, minimizing waste generation, and limiting operator intervention and exposure (Silvestre et al., 2009). In conclusion, although liquid-liquid extraction is a common and effective cleaning technique, more sustainable alternatives need to be developed, considering the environmental and operational challenges.

SPE is a common method employed for sample preparation aimed at isolating specific analytes, typically from a mobile phase. During this process, analytes are captured in a solid phase and stay there for the duration of the sampling. These analytes are then retrieved from the solid phase through thermal desorption into a liquid phase. The primary objectives of SPE include enhancing the concentration, purifying the sample, and moving analytes from the original sample matrix to an alternative solvent phase (Poole, 2003). SPE has become a popular sample preparation method of choice in many applications, because it offers many advantages over other conventional methods. The technique has been developed as an alternative that eliminates the use of large quantities of solvents, long processing times and procedural steps, as well as potential sources of error. Furthermore, SPE can be used in combination with other analytical methods and sample preparation techniques (Ötles & Kartal, 2016). However, SPE also has some disadvantages, including the difficulty of mastering the technique, the perceived complexity of the method due to the large number of options for changing solvent and pH conditions, the need for additional time due to the need for several steps, and the generally higher cost compared to simple LLE (Płotka-Wasylka et al., 2015).

Salting-assisted liquid-liquid extraction

Salting consists of incorporating an electrolyte into an aqueous solution to improve the solute's distribution (Sazali et al., 2019). Salting-assisted liquid-liquid extraction (SALLE) utilizes the principle of separating water-miscible organic solvents from aqueous biological fluids, such as plasma, to facilitate the extraction of a diverse array of drugs and metabolites, including various hydrophilic compounds. The resulting organic phase can be employed directly for bioanalysis or may require minimal dilution. SALLE offers a more straightforward approach compared to protein precipitation and yields cleaner extracts due to effective phase separation. Moreover, SALLE is noted for being quicker, more eco-friendly, and cost effective compared to traditional liquid-liquid extraction (LLE) and solid-phase extraction (SPE). In the SALLE process, incorporating inorganic salts like sodium chloride, sodium sulfate, or magnesium sulfate into the mixture induces the separation of organic solvents such as methanol, isopropanol, acetone, ethanol, and acetonitrile, creating a two-phase system (Martins et al., 2024; Tang & Weng, 2013). When these salts are used, the salting-out effect boosts the solution's ionic strength, reducing the solubility of weak electrolytes in water. This process enhances the extraction of target analytes into the organic phase, achieving high efficiency for extracting polar or moderately polar compounds from aqueous samples. Additionally, SALLE methods ensure compatibility of extraction solvents with most analytical instruments, particularly chromatographic systems, allowing direct injection of the extract for subsequent analysis (Bekele et al., 2023).

Bioanalysis

Bioanalytical techniques have undergone significant advancements over the years. Previously, chromatography HPLC-UV detection was regarded as the gold standard, but recently it has been largely replaced by tandem mass spectrometry (MS/MS).(Nováková, 2013). Today, mass spectrometry is considered the gold standard for bioanalytical studies, as it is a highly sensitive and specific method (Douxfils et al., 2016). Although LC-MS/MS has been recognized as a powerful method of analyses, it has disadvantages such as matrix effect, ion suppression or enhancement, and interferences from metabolites (Matuszewski et al., 2003). GC-MS is one of the most widely used analytical techniques for identifying and quantifying substances in complex matrices. It has many applications in environmental science, forensics, healthcare, medical and biological research (Sparkman et al., 2011). Volatile and low molecular weight analytes can be analyzed directly in GC-MS, but if the analyte contains polar functional groups oris not thermally stable at the temperatures required for separation, or non-volatile, derivatization must be performed before analysis (Koek et al., 2011).

Table 1. The methods presented in the literature.

References	Instrument	Matrix	Calibration Range	LLOQ	Analysis Time	Salt type and amount	Organic solvent type and amount
(Gupta et al., 2010)	HPLC-UV	Plasma	amoxapine: 0.0025–15 mg/L nortriptyline: 0.0025–15 mg/L	0.0025 mg/L	12 min	2.5 g of ammonium sulphate	0.5 mL acetonitrile

(Kul & Sagirli, 2023b)	GC-MS	Plasma	Biperiden: 0.5 and 15 ng/mL	0.5 ng/mL	8 min	200 mg NaCl	300 µl acetone
(Pourhossein & Alizadeh, 2018)	HPLC-UV	Plasma	Carvedilol: 5-500 µg/L	3.3 µg/L	12 min	1.5 gr NaCl	1.5 ml acetonitrile
(Al et al., 2024)	LC-MS/MS	Plasma	Haloperidol: 1-15 ng/ml	1 ng/ml	4 min	200 mg NaCl	300 µl acetonitrile
(Hammad et al., 2021)	HPLC-UV	Plasma	Alogliptin: 0.1–50 µg/mL	0.06 µg/mL	8 min	250 mg NaCl	500 µL acetonitrile
(Manousi et al., 2022)	HPLC-DAD	Urine	Piroxicam-Meloxicam: 0.1–4.0 µg/mL	0.1 µg/mL	15 min	480 µL (2.5 M) of sodium sulfate	600 µL acetonitrile
(Myasein et al., 2009)	LC-MS/MS	Plasma	Lopinavir: 19.2-16 ng/mL ritonavir: 9.73-8110 ng/mL	Lopinavir: 20 ng/mL ritonavir: 10ng/mL	0.40 min	100 L of 3 M zinc sulfate	200 µL of acetonitrile
(Zhang et al., 2010)	LC-MS/MS	Plasma	“simvastatin simvastatin acid”0.097-51.1 ng/mL	0.094 ng/mL	2 min	50 µL o5 M ammonium formate buffer	200 µL of acetonitrile
(Xiong & Yang, 2015)	LC-MS/MS	rat plasma	trimetazidine 0.1–100 ng/mL	0.1 ng/mL	2.5 min	25 µL of 5 M ammonium formate	100 µL of acetonitrile
(Kvamsøe et al., 2020)	LC-MS/MS	blood	Tacrolimus: 0.4–85 ng/mL Sirolimus: 1.4–84 ng/mL Everolimus: 0.06–83 ng/mL Cyclosporine: 0.4–959 ng/mL	Tacrolimus:0,4 ng/mL Sirolimus:1,4 ng/mL Everolimus:0,06 ng/mL Cyclosporine:0.4 ng/mL	1.1 min	100 µL of 5M NaCl	350 µL acetonitrile
(Alshishani et al., 2017)	HPLC-UV	plasma urine	metformin: 20–2000 µg/L buformin: 20–2000 µg/L phenformin: 20–2000 µg/L	metformin: 13 µg/L buformin: 12 µg/L phenformin: 17 µg/L	16 min	0.48 mg NaOH	400 µL acetonitrile
(Hajkova et al., 2016)	LC-MS	tissue samples	methoxetamine 2.5–250 ng/g	2.5- 5 ng/g	8 min	200 µL 10 mmol/L NH4HCO3	100 µL acetonitrile
(Sparidans et al., 2016)	LC-MS/MS	mouse plasma	afatinib 0.5–500 ng/ml	0.5 ng/ml	1.2 min	5 µl of 3 M magnesium chloride	25 µL acetonitrile
(Kul & Sagirli, 2023a)	LC–MS/MS	breast milk	cefuroxime 25-1000ng/ml	25 ng/ml	8 min	200 mg zinc sulfate	300 µl acetonitrile

(Yang et al., 2015)	LC–MS/MS	plasma	atorvastatin 0.0200–15.0 ng/mL	0.0200 ng/ml	2.2 min	100 μL of 6 M ammonium acetate	400 μL acetonitrile
(Stratigou et al., 2020)	HPLC-FL	Urine	doxorubicin 100-2000 ng/ml	100 ng/ml	15 min	1 mL of Na2SO4 solution 2.35 mol/L	2.35 mL acetonitrile
(Zhao et al., 2020)	LC-MS	plasma	entecavir 0.05-20 ng/mL	0.05 ng/mL	11 min	500 μl of MgSO4 solution (37.5%)	1 mL acetonitrile

Conclusions

SALLE offers many advantages over conventional methods. SALLE uses the principle of separation of water-immiscible organic solvents to extract drugs and metabolites from biological fluids with high yields. This method offers clean extracts and considered an eco-friendly approach compared to PP and LLE. In addition, SALLE is faster and cost effective, making analytical processes more efficient. The results obtained from the studies show that the use of the SALLE technique in combination with different analytical methods provides high performance in biological samples. In particular, LC-MS/MS studies using salts such as ammonium sulphate, magnesium sulphate and zinc sulphate provided faster analysis times and lower LLOQ values. This demonstrates that the integration of the SALLE technique with various analyzers increases the capacity for fast and sensitive measurements. However, the applicability and efficiency of each method varies depending on factors, such as the compound being analyzed, biological matrix, instrument and analysis time. Shorter analysis times may be preferable, especially for laboratories working with high sample numbers, while longer analysis times generally offer higher precision and accuracy. In conclusion, the flexibility and versatility of the SALLE method increases its adaptability to different analytical needs, making it a promising alternative for various bioanalytical applications.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics committee approval is not required for this study.

Authors' contribution statement

Study conception and design: SA, OS; **Data collection**: SA; **Manuscript draft preparation**: SA **Supervisor:** OS. All authors reviewed the results and approved the final version of the manuscript.

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References

Al, S., Kul, A., & Sagirli, O. (2024). Salt-assisted liquid–liquid microextraction for determination of haloperidol in human plasma by LC-MS/MS. *The European Chemistry and Biotechnology Journal(1).,* 39-46. https://doi.org/10.62063/ecb-16

Alshishani, A. A., Saad, B., Semail, N. F., Mohamad Salhimi, S., & Talib, M. K. M. (2017). Salting-out assisted liquid-liquid extraction method coupled to gas chromatography for the simultaneous determination of thujones and pulegone in beverages. *International journal of food properties., 20(sup3),* S2776-S2785. https://doi.org/10.1080/10942912.2017.1373665

Bekele, H., Yohannes, W., & Megersa, N. (2023). A Highly Selective Analytical Method Based on Salt-Assisted Liquid-Liquid Extraction for Trace-Level Enrichment of Multiclass Pesticide Residues in Cow Milk for Quantitative Liquid Chromatographic Analysis. *International journal of analytical chemistry., 2023(1),* 1754956. https://doi.org/10.1155/2023/1754956

Cantwell, F. F., & Losier, M. (2002). Liquid—liquid extraction. *In Comprehensive analytical chemistry.,37*, 297-340*).* https://doi.org/10.1016/S0166-526X(02)80048-4

Chang, M. S., Ji, Q., Zhang, J., & El-Shourbagy, T. A. (2007). Historical review of sample preparation for chromatographic bioanalysis: pros and cons. *Drug Development Research., 68(3),* 107-133. https://doi.org/10.1002/ddr.20173

Clark, K. D., Zhang, C., & Anderson, J. L. (2016). Sample preparation for bioanalytical and pharmaceutical analysis. *ACS Publications, 88(23),* 11262-11270. https://doi.org/10.1021/acs.analchem.6b02935

Gupta, M., Jain, A., & Verma, K. K. (2009). Salt-assisted liquid–liquid microextraction with water-miscible organic solvents for the determination of carbonyl compounds by high-performance liquid chromatography. *Talanta., 80(2),* 526-531. https://doi.org/10.1016/j.talanta.2009.07.021

Hajkova, K., Jurasek, B., Sykora, D., Palenicek, T., Miksatkova, P., & Kuchar, M. (2016). Salting-out-assisted liquid–liquid extraction as a suitable approach for determination of methoxetamine in large sets of tissue samples. *Analytical and bioanalytical chemistry., 408,* 1171-1181. https://doi.org/10.1007/s00216-015-9221-1

Hammad, S. F., Abdallah, I. A., Bedair, A., & Mansour, F. R. (2021). Salting-out induced liquid–liquid microextraction for alogliptin benzoate determination in human plasma by HPLC/UV. *BMC chemistry., 15,* 1-10. https://doi.org/10.1186/s13065-020-00729-8

Kabir, A., Locatelli, M., & Ulusoy, H. I. (2017). Recent trends in microextraction techniques employed in analytical and bioanalytical sample preparation. *Separations., 4(4),* 36. https://doi.org/10.3390/separations4040036

Kul, A., & Sagirli, O. (2023). A novel method for the therapeutic drug monitoring of biperiden in plasma by GC-MS using salt-assisted liquid–liquid microextraction. *Clinica chimica acta., 543,* 117322. https://doi.org/10.1016/j.cca.2023.117322

Kul, A., & Sagirli, O. (2023). Determination of cefuroxime in breast milk by LC–MS/MS using SALLME technique. *Biomedical Chromatography., 37(11),* e5744.https://doi.org/10.1002/bmc.5744

Kole, P. L., Venkatesh, G., Kotecha, J., & Sheshala, R. (2011). Recent advances in sample preparation techniques for effective bioanalytical methods. *Biomedical Chromatography., 25(1-2),* 199-217. https://doi.org/10.1002/bmc.1560

Kvamsøe, M. M., Hansen, K. R., Skadberg, Ø., Vethe, N. T., & Brede, C. (2020). Salting Out-Assisted Liquid–Liquid Extraction for Liquid Chromatography–Tandem Mass Spectrometry Measurement of Tacrolimus, Sirolimus, Everolimus, and Cyclosporine a in Whole Blood. *Therapeutic drug monitoring., 42(5),* 695-701. https://doi.org/10.1097/FTD.0000000000000794

Li, W., Jian, W., & Fu, Y. (2019). Basic sample preparation techniques in LC-MS bioanalysis: protein precipitation, liquid–liquid extraction, and solid-phase extraction. *Sample preparation in LC-MS bioanalysis.,* 1-30. https://doi.org/10.1002/9781119274315.ch1

Lorenzo-Parodi, N., Kaziur-Cegla, W., Gjelstad, A., & Schmidt, T. C. (2023). Liquid-phase microextraction of aromatic amines: hollow fiber–liquid-phase microextraction and parallel artificial liquid membrane extraction comparison. *Analytical and bioanalytical chemistry., 415(9),* 1765-1776. https://doi.org/10.1007/s00216-023-04579-w

Ma, J., Shi, J., Le, H., Cho, R., Huang, J. C.-j., Miao, S., & Wong, B. K. (2008). A fully automated plasma protein precipitation sample preparation method for LC–MS/MS bioanalysis. *Journal of chromatography B., 862(1-2),* 219-226. https://doi.org/10.1016/j.jchromb.2007.12.012

Manousi, N., Tsiona, S. V., & Zacharis, C. K. (2022). Salt-induced homogeneous liquid–liquid microextraction of piroxicam and meloxicam from human urine prior to their determination by HPLC-DAD. *Applied Sciences., 12(13),* 6658. https://doi.org/10.3390/app12136658

Martins, L. C., Acevedo, M. S. M., Gama, M. R., & Rocha, F. R. (2024). Salt-assisted liquid-liquid extraction and on-column concentration for chromatographic determination of phenolic compounds in beer. *Advances in Sample Preparation., 9,* 100107. https://doi.org/10.1016/j.sampre.2024.100107

Mazzola, P. G., Lopes, A. M., Hasmann, F. A., Jozala, A. F., Penna, T. C., Magalhaes, P. O., Rangel-Yagui, C. O., & Pessoa Jr, A. (2008). Liquid–liquid extraction of biomolecules: an overview and update of the main techniques. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology., 83(2),* 143-157. https://doi.org/10.1002/jctb.1794

Moein, M. M., El Beqqali, A., & Abdel-Rehim, M. (2017). Bioanalytical method development and validation: Critical concepts and strategies. *Journal of chromatography B., 1043,* 3-11. https://doi.org/10.1016/j.jchromb.2016.09.028

Myasein, F., Kim, E., Zhang, J., Wu, H., & El-Shourbagy, T. A. (2009). Rapid, simultaneous determination of lopinavir and ritonavir in human plasma by stacking protein precipitations and salting-out assisted liquid/liquid extraction, and ultrafast LC–MS/MS. *Analytica Chimica Acta., 651(1),* 112-116. https://doi.org/10.1016/j.aca.2009.08.010

Nazario, C. E. D., Fumes, B. H., da Silva, M. R., & Lanças, F. M. (2017). New materials for sample preparation techniques in bioanalysis. *Journal of chromatography B., 1043,* 81-95. https://doi.org/10.1016/j.jchromb.2016.10.041

Nfor, B. K., Hylkema, N. N., Wiedhaup, K. R., Verhaert, P. D., Van der Wielen, L. A., & Ottens, M. (2011). High-throughput protein precipitation and hydrophobic interaction chromatography: salt effects and thermodynamic interrelation. *Journal of Chromatography A., 1218(49),* 8958-8973. https://doi.org/10.1016/j.chroma.2011.08.016

Nováková, L., & Vlčková, H. (2009). A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation. *Analytica Chimica Acta., 656(1-2),* 8-35. https://doi.org/10.1016/j.aca.2009.10.004

Ötles, S., & Kartal, C. (2016). Solid-Phase Extraction (SPE): Principles and applications in food samples. *Acta Scientiarum Polonorum Technologia Alimentaria., 15(1),* 5-15. https://doi.org/10.17306/J.AFS.2016.1.1

Pan, T.-Y., Tsai, W.-C., Tan, C.-H., Cheng, C.-M., Chen, W., Soundappan, T., Arasu, M. V., Al-Dhabi, N. A., Wu, C.-F., & Ponnusamy, V. K. (2021). Rapid simultaneous clinical monitoring of five oral anti-coagulant drugs in human urine using green microextraction technique coupled with LC–MS/MS. *Journal of King Saud University-Science., 33(8),* 101602. https://doi.org/10.1016/j.jksus.2021.101602

Ping, T., Zheng, M., Zhang, P., Yan, T., Miao, X., Wang, K., & Lian, K. (2022). Determination of 12 anti-obesity drugs in human plasma by a 96-well protein precipitation plate using HPLC-MS. *RSC advances., 12(40),* 26016-26022. https://doi.org/10.1039/D2RA03423A

Płotka-Wasylka, J., Szczepańska, N., de La Guardia, M., & Namieśnik, J. (2015). Miniaturized solid-phase extraction techniques. *TrAC Trends in Analytical Chemistry., 73,* 19-38. https://doi.org/10.1016/j.trac.2015.04.026

Poole, C. F. (2003). New trends in solid-phase extraction. *TrAC Trends in Analytical Chemistry., 22(6),* 362-373. https://doi.org/10.1016/S0165-9936(03)00605-8

Pourhossein, A., & Alizadeh, K. (2018). Salt-assisted liquid-liquid extraction followed by high performance liquid chromatography for determination of carvedilol in human plasma. *Journal of Reports in Pharmaceutical Sciences., 7(1),* 79-87. https://doi.org/10.4103/2322-1232.256324

Retmana, I. A., Wang, J., Schinkel, A. H., Schellens, J. H., Beijnen, J. H., & Sparidans, R. W. (2017). Liquid chromatography-tandem mass spectrometric assay for the quantitative determination of the tyrosine kinase inhibitor quizartinib in mouse plasma using salting-out liquid-liquid extraction. *Journal of chromatography B., 1061,* 300-305. https://doi.org/10.1016/j.jchromb.2017.07.034

Sazali, N. H., Alshishani, A., Saad, B., Chew, K. Y., Chong, M. M., & Miskam, M. (2019). Salting-out assisted liquid–liquid extraction coupled with high-performance liquid chromatography for the determination of vitamin D3 in milk samples. *Royal Society open science., 6(8),* 190952. https://doi.org/10.1098/rsos.190952

Sparidans, R. W., van Hoppe, S., Rood, J. J., Schinkel, A. H., Schellens, J. H., & Beijnen, J. H. (2016). Liquid chromatography–tandem mass spectrometric assay for the tyrosine kinase inhibitor afatinib in mouse plasma using salting-out liquid–liquid extraction. *Journal of chromatography B., 1012,* 118-123. https://doi.org/10.1016/j.jchromb.2016.01.025

Stratigou, I.-C., Tsiasioti, A., Tzanavaras, P. D., Markopoulou, C. K., Fytianos, K., & Zacharis, C. K. (2020). Homogeneous liquid liquid extraction using salt as mass separating agent for the ultra high pressure liquid chromatographic determination of doxorubicin in human urine. *Microchemical Journal., 158,* 105260. https://doi.org/10.1016/j.microc.2020.105260

Silvestre, C. I., Santos, J. L., Lima, J. L., & Zagatto, E. A. (2009). Liquid–liquid extraction in flow analysis: A critical review. *Analytica Chimica Acta., 652(1-2),* 54-65. https://doi.org/10.1016/j.aca.2009.05.042

Tang, Y. Q., & Weng, N. (2013). Salting-out assisted liquid–liquid extraction for bioanalysis. *Bioanalysis., 5(12),* 1583-1598. https://doi.org/10.4155/bio.13.117

Theodoridis, G. A., & Papadoyannis, I. N. (2006). Novel advanced approaches in sample preparation and analyte detection for bioanalysis. *Current Pharmaceutical Analysis., 2(4),* 385-404. https://doi.org/10.2174/157341206778699591

Xiong, X., & Yang, L. (2015). Salting-out-assisted liquid–liquid extraction with acetonitrile for the determination of trimetazidine in rat plasma using liquid chromatography–mass spectrometry. *Biomedical Chromatography., 29(2),* 268-274. https://doi.org/10.1002/bmc.3271

Yang, Y., Xu, Q., Zhou, L., Zhong, D., & Chen, X. (2015). High-throughput salting-out-assisted liquid–liquid extraction for the simultaneous determination of atorvastatin, ortho-hydroxyatorvastatin, and para-hydroxyatorvastatin in human plasma using ultrafast liquid chromatography with tandem mass spectrometry. *Journal of separation science., 38(6),* 1026-1034. https://doi.org/10.1002/jssc.201401227

Zhang, J., Rodila, R., Gage, E., Hautman, M., Fan, L., King, L. L., Wu, H., & El-Shourbagy, T. A. (2010). High-throughput salting-out assisted liquid/liquid extraction with acetonitrile for the simultaneous determination of simvastatin and simvastatin acid in human plasma with liquid chromatography. *Analytica Chimica Acta., 661(2),* 167-172. https://doi.org/10.1016/j.aca.2009.12.023

Zhang, J., Wu, H., Kim, E., & El-Shourbagy, T. A. (2009). Salting-out assisted liquid/liquid extraction with acetonitrile: a new high throughput sample preparation technique for good laboratory practice bioanalysis using liquid chromatography–mass spectrometry. *Biomedical Chromatography., 23(4),* 419-425. https://doi.org/10.1002/bmc.1135

Zhang, X., Wang, K., Wu, S., Ruan, C., Li, K., Wang, Y., Zhu, H., Liu, X., Liu, Z., & Li, G. (2022). Highly effective identification of drug targets at the proteome level by pH-dependent protein precipitation. *Chemical Science., 13(42),* 12403-12418. https://doi.org/10.1039/D2SC03326G

Zhao, F.-J., Tang, H., Zhang, Q.-H., Yang, J., Davey, A. K., & Wang, J.-p. (2012). Salting-out homogeneous liquid–liquid extraction approach applied in sample pre-processing for the quantitative determination of entecavir in human plasma by LC–MS. *Journal of chromatography B., 881,* 119-125. https://doi.org/10.1016/j.jchromb.2011.12.003

REVIEW

Hippocampal contributions to biological, behavioral, and cognitive deficits in autism: An updated review

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Abstract

The most characteristic symptoms for the diagnosis of autism spectrum disorder (ASD) and the future life of the individual are deterioration in social communication and stereotyped or repetitive behaviors. ASD is associated with diverse atypical difficulties, including memory, learning, language, emotion, and cognitive impairment. Consequently, the hippocampus is important for memory, learning, language ability, emotional regulation, and cognitive mapping. Thus, the hippocampus plays an influential role in the pathophysiological mechanisms of ASD. Here, we provide an updated review of hippocampal structural and functional abnormalities and highlight the hippocampus as an important area for future research.

Keywords: autism spectrum disorder (ASD), behavior, biologic, cognitive, brain-derived neurotrophic factor (BDNF), hippocampus, neuroinflammation, synaptic plasticity

Introduction

Autism spectrum disorder (ASD) is a group of complex neurodevelopmental disorders that include impairments in behavioral skills and stereotypic movements that affect social communication and interaction (Solmi et al, 2022). According to current data, 1 in every 36 children is diagnosed with ASD. The risk is four times higher in boys than in girls and the combined prevalence per 1000 Children is 27.6 (23.1-44.9). Additional information on ASD is available from the CDC at https://www.cdc.gov/autism/data-research/data-table.html. The pathogenesis of ASD remains to be elucidated , causing a significant limitation in the development of new therapeutic or preventive techniques. Hippocampal research is important in ASD, which is characterized by learning and cognitive impairment. Hippocampal neurogenesis is at the forefront of this research (Gage, 2019) because atypical hippocampal anatomy and neuroplasticity have been observed mostly in individuals with autism (Li et al. 2019). Individuals with ASD present clinical symptoms with impairments in faces, working, and social memory (Wang et al. 2017). ASD in particular is characterized by learning disabilities due to various cognitive dysfunctions. Previous estimates suggest that 75% of individuals with ASD have impaired learning abilities, including learning skills (Georgiou and Spanoudis, 2021; Girolama et al. 2024). Considering the important role of the hippocampus in memory, learning, verbal ability, emotional behavior, and cognitive attitude, it is a brain region that is regarded in the investigation of the pathophysiological mechanisms and therapeutic approaches of ASD (Long et al. 2024). Previous longitudinal research reported that accelerated hippocampal volume loss in ASD results in declines in verbal and short-term memory (Pagni et al. 2022).

The pathogenesis of ASD remains a matter of great curiosity, and it is the most important factor limiting the development of therapeutic and preventive strategies. Since the hippocampus is one of the important regions where neurogenesis occurs, it plays an active role in many learning and memory processes. Therefore, the biological causes of learning difficulties, memory impairments, and behavioral disorders that occur in neurodevelopmental disorders such as ASD are being investigated (Li et al. 2019). Although many brain regions other than the hippocampus have been studied for ASD, thousands of risk genes, proteins, and molecular pathway deficits have been identified, resulting from the many heterogeneous etiologies, phenotypes, and pathophysiologies of ASD in general (Wan et al. 2024).

In this updated review, we highlight the hippocampus as a brain region of interest for investigating the biological, behavioral, and cognitive status of ASD. Here, we will first review biological abnormalities in the hippocampus of individuals with ASD and discuss discrepancies in the results of existing studies. In the next section, we will discuss the hippocampus's known behavioral and cognitive contribution to ASD, mostly in post-mortem studies, mice and rats, that exhibit hippocampal synaptic plasticity impairments and hippocampus-dependent deficits.

Biologic changes in the hippocampus

More than 10,000 cells per person, an average of more than 1860 genes per cell, and 26 primary cortical cell types—including glial cells—have all been linked to ASD. With the wide-use of genomic sequencing, more genomic data on ASD have been obtained through rapid developments in imaging technologies. Studies of different brain regions and their many specific genetic mechanisms associated with increased risk for ASD diagnosis continue to pave the way for a variety of approaches that can help diagnose ASD at an early stage.

Findings from experimental studies indicate that the association with ASD-related impairment of social recognition memory is specifically supported by the CA2 region of the hippocampus (Hitti and Siegelbaum, 2014). ASD-related social memory deficits emerged due to decreased neurotransmitter release because of decreased axon diameter in the Hippocampo-fusiform pathways associated with face recognition (Trontel et al. 2013). Genome-wide association studies (GWAS), as well as gene expression profiling techniques such as RNAseq identified 2851 differentially expressed genes in the hippocampus of children with ASD, including genes implicated in genetic analysis studies (Coley and Gao, 2018).

Different studies have reported variability in brain metabolite concentrations in ASD compared to neurotypical participants (Ajram et al. 2024, Kurochkin et al. 2019). It has been reported that N-acetyl aspartate concentrations and N-acetyl aspartate/creatine ratios are reduced in the hippocampus of children with ASD (Libero et al. 2016, Thomson et al. 2024). In the ASD group, N-acetyl-aspartate levels in the hippocampal region were positively correlated with IQ in a multivoxel proton magnetic resonance spectroscopy (1H-MRS) study (Dionísio et al. 2024). These metabolites were not associated with the hippocampus. For instance, Graham et al. (2016) used LC-MS to find changes in the concentrations of 37 metabolites in the cerebellum between 11 people with ASD and 11 controls. However, more research is required to understand the biological pathways' activity. Additional research is needed to understand the genetic basis of the metabolic alterations in various hippocampal areas associated with ASD.

ASD is associated with genetic and environmental factors that develop in the brain. Regarding the preclinical aspect of ASD, many animal models and clinical studies show that methods such as neuroimaging focus on areas such as the hippocampus, amygdala, frontal region, and cerebellum (Leisman et al. 2023). The neurogenesis of the perinatal and adult hippocampus involves fundamental activities linked to spatial processing, pattern discrimination, functional integration, cognitive flexibility, and learning. Brain-derived neurotrophic factor (BDNF) is a crucial regulatory marker for long-term potentiation (LTP), learning, and memory. It also has a major function in synaptic transmission and plasticity in the hippocampus (Ilchibaeva et al. 2023).

Common neuroplastic disorders are seen in ASD. These changes in neuroplasticity may result from disturbances in synaptic function (Zhao et al. 2021). In the studies conducted, peripheral BDNF levels in serum or plasma were evaluated differently. However, some research has argued the reverse findings (Meng et al. 2016, Segura et al. 2015). Meta-analysis studies have revealed a positive connection between high blood BDNF levels and autism (Barbosa et al. 2020, Elhamid et al. 2024, Liu et al. 2021). However, hippocampal BDNF levels have generally been studied in animal models of ASD, and this number was quite small. Valproate-induced autism-like rats exhibited an autism-like behavioral profile characterized by deficits in social interaction, anxiety-like behavior, and repetitive behaviors. Valproate induction decreases BDNF levels in the dentate gyrus (DG) and CA3 regions of the rats examined (Camuso et al. 2022, Fuentealba et al. 2019). However, clinical and preclinical studies on hippocampal BDNF levels were still insufficient.

Some postmortem studies are interesting in clinical research. Rexrode et al. recently reported decreased expression of the synaptic proteins PSD95 and SYN1, increased expression of the extracellular matrix (ECM) protease MMP9, and reduced expression of MEF2C on postmortem hippocampus samples from male children with ASD (n=7) (Rexrode et al. 2024). Additionally, postmortem studies have reported that although the concentration of neural cells in the hippocampus in ASD is dense, the cells are abnormally small (Fetit et al. 2021).

Bove et al. 2022 reported that ketamine administration helped mimic the typical symptoms in adult mice at postnatal days 7, 9, and 11 with behavioral aspects. They found decreased BDNF and enhanced glial fibrillary acidic protein (GFAP) expression levels with increased glutamate and reduced GABA levels in the amygdala and hippocampus. In another study, the BDNF protein amount is decreased both in the hippocampus and frontal cortex in the BTBR Mouse Model of Autism (Jasien et al. 2014). As a small dimeric protein, BDNF is structurally homologous to Nerve Growth Factor (NGF), with 50% amino acid identity with NGF, neurotrophin-3 (NT-3), and NT-4/5 (Bathina and Undurti, 2015).

Neurotrophic factors are highly effective in cell proliferation and differentiation, neuroto- and synaptogenesis, synaptic function, and synaptic plasticity. There have not been many studies on NT-3, NT4/5, and the insulin-like growth factors IGF-1 and IGF-2. It has been reported that VPA-induced significantly increased levels of pro-inflammatory markers (IL-1β, TNF-α, IL-6, IFN-γ, IL-17, TGF-β) and decreased anti-inflammatory (IL-10) levels in the hippocampus of experimental animals (Barzegari et al. 2023, Eissa et al. 2019). However, these results are controversial depending on age. Hippocampal neuroinflammation occurs in VPA-induced rats during adolescence and may also occur with microglial and astrocyte activation in the postnatal period, possibly with VPA exposure. Improvements in the expression of neuroglial markers in the hippocampus of adult rats exposed to VPA may be a finding of improvement in the neuroinflammatory phenotype (Gifford et al. 2022). In particular, hippocampal research is limited when the status of neurotrophic factors in ASD is evaluated.

To date, many transgenic animal models that mimic ASD have been studied. The genetic cause has been identified in cases of syndromic ASD, which often co-occurs with ASD-related behavioral phenotypes (Li et al. 2021). Duarte-Campos et al. 2024 discovered elevated levels of interferon-gamma (IFN-γ) and monocyte chemoattractant protein 1 (MCP-1) in the hippocampus, suggesting increased inflammation, alongside a reduction in the anti-inflammatory enzyme arginase 1 (ARG1) in the hippocampus of adult male C58/J mice. Animal models, such as the C58/J-inbred mouse strain, are used to study the biological properties of ASD. This type is considered a model of idiopathic autism because of reduced social preferences and repetitive behaviors. Therefore, neuroinflammatory markers identified in the hippocampal region of an animal model are of great importance to understanding biological effects in the hippocampus for ASD.

According to Fuchs et al. (2018), Cdkl5 +/− mice had respiratory issues, significant difficulties with motor coordination and memory, and showed autistic-like behavior. These defects are associated with neuroanatomical changes, such as decreased dendritic arborization and decreased spine density in hippocampal neurons. Decreased pyramidal neurons in the CA1 region, reduced dendritic maturation, and reduced dentate gyrus were reported in Mecp2 mutant mice (Sun et al. 2019). Interestingly, this was potentially associated with inadequate BDNF expression in hippocampal neurons (Bertoldi et al. 2019).

Current research on behavioral and cognitive changes in the hippocampus

ASD is typified by focused, repetitive actions and difficulties in social communication. Alongside these behavioral symptoms are sensory and cognitive issues, including episodic memory, working memory, spatial reasoning, and executive function deficiencies (Bangerter et al. 2017). Although the relationship between brain activity to social integration in ASD has been investigated in neuroimaging studies, symptoms, and preclinical studies, memory, orientation, and spatial deficits are also important in the etiology of this disorder. Individuals with ASD have also consistently reported deficits in episodic memory, a major hub of sensory-perceptual-conceptual-affective processing (Cooper and Simons, 2019). In ASD participants, more activity was found in the occipital region (hippocampus, premotor region, and ventral (occipitotemporal) areas in the left hemisphere compared to the right hemisphere. Functional impairment in episodic memory has been highlighted by an fMRI study, which has highlighted neuronal connectivity deficits in the hippocampus (Desaunay et al. 2023). Atypical memory processes in ASD include the dominance of verbal information over spatial information, disruption of working memory, and impaired processing in episodic memory. It is believed that these cognitive and behavioral effects in the hippocampus may be due to biological causes in the hippocampus (Figure 1).

Recently, a meta-analysis on declarative memory in ASD was conducted, highlighting areas of working memory (especially verbal), visual recognition, and episodic long-term memory. As a result, visual-spatial memory appears to be more impaired than verbal memory, making it more difficult to recognize faces than verbal behavior (Griffin et al. 2021). Individuals with ASD experience more problems with working memory than with episodic long-term memory. Recent studies have linked ASD to impaired social memory capacity and neuronal connectivity in hippocampal CA2, suggesting that the role of CA2 in social memory encompasses both short-term and long-term social memories (Cum et al. 2024).

Increased hippocampal activation was detected in individuals with ASD associated with over-reactivity to auditory and visual stimuli (Long et al. 2024). Allsop et al. (2018) reported that the anterior cingulate cortex-basolateral amygdala integration plays an important role in the acquisition of fear conditioning to various stimuli in individuals with ASD and impaired social activity and that hippocampus-related 5-7 Hz oscillations are effective in social behaviors such as lack of empathy. Social recognition impairment was associated with inadequate oxytocin receptor expression in the medial amygdala and hippocampus (Raam et al. 2017).

Changes in hippocampal structure in ASD have been the subject of further research. In particular, recent clinical studies have reported the atypical hippocampal volume of children with ASD compared to normal children (Li et al. 2023, Rexrode et al. 2024). Task-based fMRI studies on ASD have shown impairment in hippocampal region-related learning and memory and decreased perception of social emotions (Hashimoto et al. 2021). Disturbance of hippocampal and caudate nucleus connections during a task has been associated with social performance in individuals with ASD (Solomon et al. 2015).

ASD-like experimental models have reported a critical role of the hippocampus and reduced neuroplasticity in social memory impairment (Sato et al. 2023). Considering that ASD is a neurodevelopmental disorder, neurodevelopmental and neuroimmunological abnormalities occurring in the prenatal period have shed light on the neurobiological features of ASD and trigger hippocampal dysfunction (Carlezon et al. 2019; Hanamsagar et al. 2022; Tsilioni et al. 2019).

Cognitive and behavioral reflection in ASD

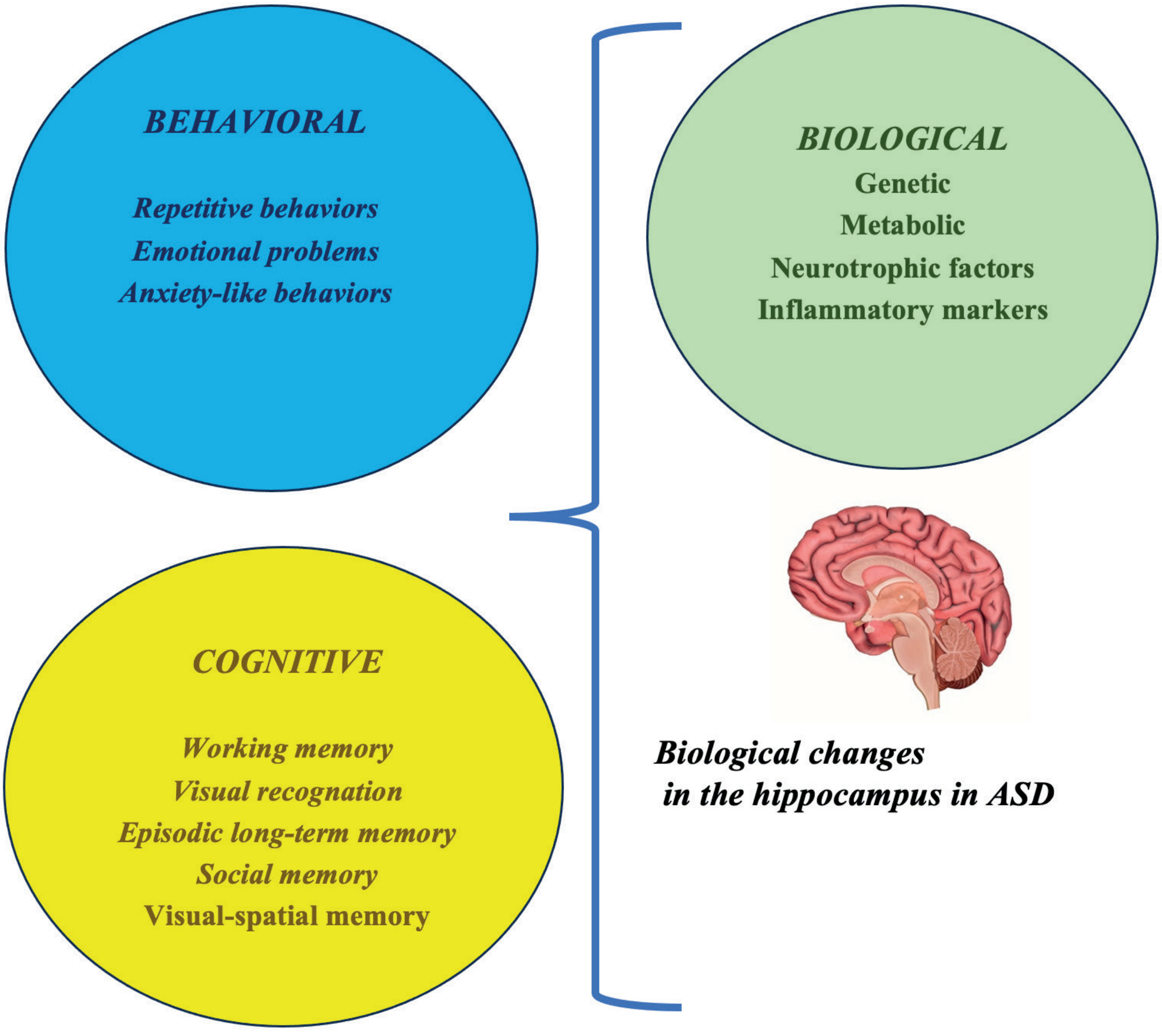

Figure 1. Summary of the cognitive and behavioral implications of hippocampal biological influences on ASD development according to current literature.

Human hippocampal formation performs grid-like mapping of the visual field, and previously learned structures are projected onto newly lettered information, generating the repetitive behaviors associated with ASD, according to a small number of studies using magnetoencephalography (MEG) and electroencephalography (EEG) (Staudigl et al. 2018). These results suggest that the hippocampus impacts the formation of cognitive maps. Complex functions, such as memory, spatial reasoning, and socialization all require a hippocampal neurogenesis system that allows for flexible planning and decision-making (Barón-Mendoza et al. 2024).

In a study by Fuchs et al. (2018), Cdkl5 −/− female mice showed cognitive impairment, and hippocampus-dependent learning and memory were evaluated using the Morris water maze (MWM). Additionally, Fmr1-KO mice exhibited impaired hippocampus-dependent fear memory; these deficits were suggested by low levels of freezing behavior response to fear conditioning. Metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD), which is excessively increased in the CA1 region, and N-methyl-D-aspartate receptors (NMDA) in the dentate gyrus, have been reported to negatively affect LTP (Chen et al. 2022). In a novel object recognition test study, Fmr1-mutant mice were reported to be indifferent to the new object and spent significantly more time sniffing the old object. This finding highlighted visual recognition memory deficits in mice (Jeon et al. 2022).

Neural deficits in the hippocampus also cause emotional problems, including anxiety-like behaviors. Updated reports from open field tests and elevated plus maze showed that Fmr1-KO mice had high anxiety behaviors (Chen et al. 2022). Rett syndrome-like animal models have provided important scientific data on hippocampus-related impairments in memory and learning. For example, Mecp2-mutant mice exhibit impaired spatial memory and learning in the Morris water maze task (Hao et al. 2015).

The current literature mostly consists of known genetic, environmentally mediated, and idiopathic ASD animal models in mice and rats that exhibit hippocampal synaptic plasticity disorders and hippocampus-related behavioral deficits. This situation causes translational limitations in the obtained data. Additionally, the limited investigation into hippocampal-focused functions, such as memory and spatial reasoning has been devoted to ASD research because impairments in these functions have not been recognized as core aspects of the ASD phenotype.

Conclusions

The current literature is quite conflicting regarding ASD pathophysiology, and studies have shown few brain regions with consistent or descriptive functional changes in ASD. Considering this biological infrastructure, the etiology of ASD remains a matter of research and curiosity, as it includes less than 1% of clinical cases. However, most behavioral symptoms are similar and common across cases. The hippocampus has been the focus of very few clinically relevant post-mortems and GWAS studies in ASD, perhaps because the current literature lacks evidence of hippocampal involvement in social behavior that is also included in clinical diagnostic criteria. In summary, recent research has demonstrated that hippocampus abnormalities in ASD frequently result in cognitive learning and memory issues, which may have a variety of biological causes. These biological effects are mostly understood through genetic, metabolic, neurotrophic, and inflammatory markers. Because this overall assessment was obtained on very few postmortem and many different animal models, it seems that futher studies are needed for ASD. Post-mortem studies are limited due to legal permissions, budget issues, and etiology. Very few studies have focused on the hippocampus. In future studies, it is recommended to conduct more behavioral and hippocampus-focused biological studies in ASD-like animal models in the current literature. In particular, it is recommended to increase the numbers of neuroinflammatory responses, neurotrophic factors, ASD-related gene expressions, proteomic analyses, or volumetric studies in the hippocampal region of different types of animal models and compare them. Because ASD has a multifactorial etiology from a clinical perspective, clinical research should focus on GWAS studies related to disease subtypes or studies that conduct correlative and risk association studies of cognitive aspects.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

Data sharing was not applied to this review article as no datasets were generated or analyzed during the current study.

Ethics committee approval

Ethics Committee approval was not required for this study.

Authors' contributions

Use of Artificial Intelligence: No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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References

Ajram, L.A., Pereira, A.C., Durieux, A.M.S., Velthius, H.E., Petrinovic, M.M., & McAlonan, G.M. (2019). The contribution of [1H] magnetic resonance spectroscopy to the study of excitation-inhibition in autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 89,* 236-244. https://doi.org/10.1016/j.pnpbp.2018.09.010

Allsop, S.A., Wichmann, R., Mills, F., Burgos-Robles, A., Chang, C.J., Felix-Ortiz, A.C., Vienne, A., Beyeler, A., Izadmehr, E.M., Glober, G., Cum, M.I., Stergiadou, J., Anandalingam, K.K., Farris, K., Namburi, P., Leppla, C.A., Weddington, J.C., Nieh, E.H., Smith, A.C., Ba, D., Brown, E.N., % Tye, K.M. (2018). Corticoamygdala Transfer of Socially Derived Information Gates Observational Learning. *Cell, 173(6),* 1329-1342.e18. https://doi.org/10.1016/j.cell.2018.04.004

Bangerter, A., Ness, S., Aman, M.G., Esbensen, A.J., Goodwin, M.S., Dawson, G., Hendren, R., Leventhal, B., Khan, A., Opler, M., Harris, A., & Pandina, G. (2017). Autism Behavior Inventory: A Novel Tool for Assessing the Core and Associated Symptoms of Autism Spectrum Disorder. *Journal of Child and Adolescent Psychopharmacology, 27(9),* 814-822. https://doi.org/10.1089/cap.2017.0018

Barbosa, A.G., Pratesi, R., Paz, G.S.C., Dos Santos, M.A.A.L., Uenishi, R.H., Nakano, E.Y., Gandolfi, L., % Pratesi, C.B. (2020). Assessment of serum BDNF levels as a diagnostic marker in children with autism spectrum disorder. *Scientific Reports,10,* 17348. https://doi.org/10.1038/s41598-020-74239-x

Barón-Mendoza, I., Mejía-Hernández, M., Hernández-Mercado, K., Guzmán-Condado, J., Zepeda, A., & González-Arenas, A. (2024). Altered hippocampal neurogenesis in a mouse model of autism revealed by genetic polymorphisms and atypical development of newborn neurons. *Scientific Reports*, *14,* 4608. https://doi.org/10.1038/s41598-024-53614-y

Bathina, S., & Das, U.N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science, 11(6),* 1164-78. https://doi.org/10.5114/aoms.2015.56342

Bertoldi, M.L., Zalosnik, M.I., Fabio, M.C., Aja, S., Roth, G.A., Ronnett, G.V., & Degano, A.L. (2019). MeCP2 Deficiency Disrupts Kainate-Induced Presynaptic Plasticity in the mosaic fiber projections in the Hippocampus. *Frontiers in Cellular Neuroscience, 13,* 286. https://doi.org/10.3389/fncel.2019.00286

Barzegari, A., Amouzad Mahdirejei, H., Hanani, M., Esmaeili, M.H., & Salari, A.A. (2023). Adolescent swimming exercise following maternal valproic acid treatment improves cognition and reduces stress-related symptoms in offspring mice: Role of sex and brain cytokines. *Physiology & Behavior, 269,* 114264. https://doi.org/10.1016/j.physbeh.2023.114264

Camuso, S., La Rosa, P., Fiorenza, MT., & Canterini, S. (2022). Pleiotropic effects of BDNF on the cerebellum and hippocampus: Implications for neurodevelopmental disorders. *Neurobiology of Disease, 163,* 105606. https://doi.org/10.1016/j.nbd.2021.105606

Carlezon, W.A.Jr, Kim, W., Missig, G., Finger, B.C., Landino, S.M., Alexander, A.J., Mokler, E.L., Robbins, J.O., Li, Y., Bolshakov, V.Y., McDougle, C.J., & Kim, K.S. (2019). Maternal and early postnatal immune activation produce sex-specific effects on autism-like behaviors and neuroimmune function in mice. *Scientific Reports, 9,* 16928. https://doi.org/10.1038/s41598-019-53294-z

Chen, Y.S., Zhang, S.M., Yue, C.X., Xiang, P., Li, J.Q., Wei, Z., Xu, L., & Zeng, Y. (2022). Early environmental enrichment for autism spectrum disorder Fmr1 mice models has positive behavioral and molecular effects. *Experimental Neurology, 352*, 114033. https://doi.org/10.1016/j.expneurol.2022.114033

Coley, A.A., & Gao, W.J. (2018). PSD95: A synaptic protein implicated in schizophrenia or autism? Prog Neuropsychopharmacol *Biological Psychiatry, 82*, 187-194. https://doi.org/10.1016/j.pnpbp.2017.11.016

Cooper, R.A., & Simons, J.S. (2019). Exploring the neurocognitive basis of episodic recollection in autism. *Psychonomic Bulletin & Review, 26,* 163-181. https://doi.org/10.3758/s13423-018-1504-z

Cum, M., Santiago Pérez, J.A., Wangia, E., Lopez, N., Wright, E.S., Iwata, R.L., Li, A., Chambers, A.R., & Padilla-Coreano, N. (2024). A systematic review and meta-analysis of how social memory is studied. *Scientific Reports, 14,* 2221. https://doi.org/10.1038/s41598-024-52277-z

Desaunay, P., Guillery, B., Moussaoui, E., Eustache, F., Bowler, D.M., & Guénolé, F. (2023). Brain correlates of declarative memory atypicality in autism: a systematic review of functional neuroimaging findings. *Molecular Autism, 14*, 2. https://doi.org/10.1186/s13229-022-00525-2

Dionísio, A., Espírito, A., Pereira, A.C., Mouga, S., d'Almeida, O.C., Oliveira, G., & Castelo-Branco, M. (2024). Neurochemical differences in core regions of the autistic brain: a multivoxel 1H-MRS study in children. *Scientific Reports,14,* 2374. https://doi.org/10.1038/s41598-024-52279-x

Duarte-Campos, J.F., Vázquez-Moreno, C.N., Martínez-Marcial, M., Chavarría, A., Ramírez-Carreto, R.J., Velasco Velázquez, M.A., De La Fuente-Granada, M., & González-Arenas, A. (2024).

Changes in neuroinflammatory markers and microglial density in the hippocampus and prefrontal cortex of the C58/J mouse model of autism. *European Journal of Neuroscience*, *59(1),* 154-173. https://doi.org/10.1111/ejn.16204

Eissa, N., Azimullah, S, Jayaprakash P, Jayaraj RL, Reiner D, Ojha SK, Beiram R, Stark H, Łażewska D, Kieć-Kononowicz K, & Sadek B. (2019). The dual-active histamine H3 receptor antagonist and acetylcholine esterase inhibitor E100 ameliorates stereotyped repetitive behavior and neuroinflammation in sodium valproate-induced autism in mice. *Chemico-Biological Interactions, 312*, 108775. https://doi.org/10.1016/j.cbi.2019.108775

Elhamid, S.A.A., Alkherkhisy, M.M. & Kasem, R.E. (2024). Assessment of brain-derived neurotrophic factor levels in serum of children with autism spectrum disorders. *Middle East Current Psychiatry, 31*, 18. https://doi.org/10.1186/s43045-024-00403-y

Fetit, R., Hillary, R.F., Price, D.J., & Lawrie, S.M. (2021). The neuropathology of autism: A systematic review of post-mortem studies of autism and related disorders. *Neuroscience & Biobehavioral Reviews, 129*, 35-62. https://doi.org/10.1016/j.neubiorev.2021.07.014

Fuchs, C., Gennaccaro, L., Trazzi, S., Bastianini, S., Bettini, S., Lo Martire, V., Ren, E., Medici, G., Zoccoli, G., & Rimondini, R., Ciani, E. (2018). Heterozygous CDKL5 Knockout Female Mice Are a Valuable Animal Model for CDKL5 Disorder. *Neural Plasticity,* 9726950. https://doi.org/10.1155/2018/9726950

Fuentealba, C.R., Fiedler, J.L., Peralta, F.A., Avalos, A.M., Aguayo, F.I., Morgado-Gallardo, K.P., & Aliaga, E.E. (2019). Region-Specific Reduction of BDNF Protein and Transcripts in the Hippocampus of Juvenile Rats Prenatally Treated with Sodium Valproate. *Frontiers in Molecular Neuroscience, 12*, 261. https://doi.org/10.3389%2Ffnmol.2019.00261

Gage, F.H. (2019). Adult neurogenesis in mammals. *Science, 364(6443),* 827-828. https://doi.org/10.1126/science.aav6885

Georgiou, N., & Spanoudis, G. (2021). Developmental Language Disorder and Autism: Commonalities and Differences on Language. *Brain Sciences, 11(5),* 589. https://doi.org/10.3390/brainsci11050589

Girolamo, T., Shen, L., Monroe Gulick, A., Rice, M.L., & Eigsti, I.M. (2024). Studies assessing domains about structural language in autism vary in reporting practices and approaches to assessment: A systematic review. *Autism, 28(7),* 1602-1621. https://doi.org/10.1177/13623613231216155

Graham, S. F., Chevallier, O. P., Kumar, P., Trkolu, O. & Bahado-Singh, R. O. (2016). High-resolution metabolomic analysis of ASD human brain uncovers novel biomarkers of disease. *Metabolomics, 12*, 1-10.https://doi.org/10.1007/s11306-016-0986-9

Griffin, J.W., Bauer, R., & Scherf, K.S. (2021). A quantitative meta-analysis of face recognition deficits in autism: 40 years of research. *Psychological Bulletin, 147(3)*, 268-292. https://doi.org/10.1037/bul0000310

Gifford, J.J., Deshpande, P., Mehta, P., Wagner, G.C., & Kusnecov, A.W. (2022). The Effect of Valproic Acid Exposure throughout Development on Microglia Number in the Prefrontal Cortex, Hippocampus and Cerebellum. *Neuroscience, 481*, 166-177. https://doi.org/10.1016/j.neuroscience.2021.11.012

Hanamsagar, R., Alter, M.D., Block, C.S., Sullivan, H., Bolton, J.L., & Bilbo, S.D. (2017). Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia, 65(9),* 1504-1520. https://doi.org/10.1002/glia.23176

Hao, S., Tang, B., Wu, Z., Ure, K., Sun, Y., Tao, H., Gao, Y., Patel, A.J., Curry, D.J., Samaco, R.C., Zoghbi, H.Y., & Tang, J. (2015). Forniceal deep brain stimulation rescues hippocampal memory in Rett syndrome mice. *Nature, 526,* 430-4. https://doi.org/10.1038/nature15694

Hashimoto, T., Yokota, S., Matsuzaki, Y., & Kawashima, R. (2021). Intrinsic hippocampal functional connectivity underlying rigid memory in children and adolescents with autism spectrum disorder: A case-control study. *Autism, 25(7),* 1901-1912. https://doi.org/10.1177/13623613211004058

Hitti, F.L., & Siegelbaum, S.A. (2014). The hippocampal CA2 region is essential for social memory. *Nature, 508,* 88-92. https://doi.org/10.1038/nature13028

Ilchibaeva, T., Tsybko, A., Lipnitskaya, M., Eremin, D., Milutinovich, K., Naumenko, V., & Popova, N. (2023). Brain-Derived Neurotrophic Factor (BDNF) in Mechanisms of Autistic-like Behavior in BTBR Mice: Crosstalk with the Dopaminergic Brain System. *Biomedicines, 11(5),* 1482. https://doi.org/10.3390/biomedicines11051482

Jasien, J.M., Daimon, C.M., Wang, R., Shapiro, B.K., Martin, B., & Maudsley, S. (2014). The effects of aging on the BTBR mouse model of autism spectrum disorder. *Frontiers in Aging Neuroscience, 6*, 225. https://doi.org/10.3389/fnagi.2014.00225

Jeon, S.J., Kwon, H., Bae, H.J., Gonzales, E.L., Kim, J., Chung, H.J., Kim, D.H., Ryu, J.H., & Shin, C.Y. (2022). Agmatine relieves behavioral impairments in Fragile X mice model. *Neuropharmacology, 219*, 109234. https://doi.org/10.1016/j.neuropharm.2022.109234

Kurochkin, I., Khrameeva, E., Tkachev, A., Stepanova, V., Vanyushkina, A., Stekolshchikova, E., Li, Q., Zubkov, D., Shichkova, P., Halene, T., Willmitzer, L., Giavalisco, P., Akbarian, S., & Khaitovich, P. (2019). Metabolome signature of autism in the human prefrontal cortex. *Communications Biology, 2*, 234. https://doi.org/10.1038/s42003-019-0485-4

Leisman, G., Melillo, R., & Melillo, T. (2023). Prefrontal functional connectivities in autism spectrum disorders: A connectopathic disorder affecting movement, interoception, and cognition. *Brain Research Bulletin, 198*, 65-76. https://doi.org/10.1016/j.brainresbull.2023.04.004

Li, G., Chen, M.H., Li, G., Wu, D., Lian, C., Sun, Q., Rushmore, R.J., % Wang, L. (2023). Volumetric Analysis of Amygdala and Hippocampal Subfields for Infants with Autism. *Journal of Autism and Developmental Disorders, 53(6),* 2475-2489. https://doi.org/10.1007/s10803-022-05535-w

Li, Y., Shen, M., Stockton, M.E., & Zhao, X. (2019). Hippocampal deficits in neurodevelopmental disorders. *Neurobiology of Learning and Memory, 165*, 106945. https://doi.org/10.1016/j.nlm.2018.10.001

Li, Z., Zhu, Y.X., Gu, LJ, & Cheng Y. (2021). Understanding autism spectrum disorders with animal models: applications, insights, and perspectives. *Zoological Research, 42(6),* 800-824. https://doi.org/10.24272/j.issn.2095-8137.2021.251

Libero, L.E., Reid, M.A., White, D.M., Salibi, N., Lahti, A.C., & Kana, R.K. (2016). Biochemistry of the cingulate cortex in autism: An MR spectroscopy study. *Autism Research, 9(6),* 643-57. https://doi.org/10.1002/aur.1562

Liu, S.H., Shi, X.J., Fan, F.C., & Cheng, Y. (2021). Peripheral blood neurotrophic factor levels in children with autism spectrum disorder: a meta-analysis. *Scientific Reports, 11,* 15. https://doi.org/10.1038/s41598-020-79080-w

Long, J., Li, H., Liu, Y., Liao, X., Tang, Z., Han, K., Chen, J., & Zhang, H. (2024). Insights into the structure and function of the hippocampus: implications for the pathophysiology and treatment of autism spectrum disorder. *Frontiers in Psychiatry, 23,15*, 1364858. https://doi.org/10.3389/fpsyt.2024.1364858

Meng, W. D., Sun, S. J., Yang, J., Chu, R. X., Tu, W., & Liu, Q. (2016). Elevated serum brain-derived neurotrophic factor (BDNF) but not BDNF gene Val66Met polymorphism is associated with autism spectrum disorders. *Molecular Neurobiology, 54, 1167-1172.* https://doi.org/10.1007/s12035-016-9721-9

Pagni, B.A., Walsh, M.J.M., Ofori, E., Chen, K., Sullivan, G., Alvar, J., Monahan, L., Guerithault, N., Delaney, S., & Braden, B.B. (2022). Effects of age on the hippocampus and verbal memory in adults with autism spectrum disorder: Longitudinal versus cross-sectional findings. *Autism Research, 10,* 1810-1823. https://doi.org/10.1002/aur.2797

Raam, T., McAvoy, K.M., Besnard, A., Veenema, A.H., & Sahay, A. (2018). Author Correction: Hippocampal oxytocin receptors are necessary for discrimination of social stimuli. *Nature Communications, 9(1),* 552. https://doi.org/10.1038/s41467-018-02965-y

Rexrode, L.E., Hartley, J., Showmaker, K.C., Challagundla, L., Vandewege, M.W., Martin, B.E., Blair, E., Bollavarapu, R., Antonyraj, R.B., Hilton, K., Gardiner, A., Valeri, J., Gisabella, B., Garrett, M.R., Theoharides, T.C., & Pantazopoulos, H. (2024). Molecular profiling of the hippocampus of children with autism spectrum disorder. *Molecular Psychiatry, 29*, 1968–1979. https://doi.org/10.1038/s41380-024-02441-8

Sato, M., Nakai, N., Fujima, S., Choe, K.Y., & Takumi, T. (2023). Social circuits and their dysfunction in autism spectrum disorder. *Molecular Psychiatry, 28,* 3194–3206. https://doi.org/10.1038/s41380-023-02201-0

Segura, M., Pedreno, C., Obiols, J., Taurines, R., Pamias, M., Grunblatt, E., & Gella, A. (2015). Neurotrophin blood-based gene expression and social cognition analysis in patients with autism spectrum disorder. *Neurogenetics, 16,* 123–131. https://doi.org/10.1007/s10048-014-0434-9

Solmi, M., Song, M., Yon, D.K., Lee, S.W., Fombonne, E., Kim, M.S., Park, S., Lee, M.H., Hwang, J., Keller, R., Koyanagi, A., Jacob, L., Dragioti, E., Smith, L., Correll, C.U., Fusar-Poli, P., Croatto, G., Carvalho, A.F., Oh, J.W., Lee, S., Gosling, C.J., Cheon, K.A., Mavridis, D., Chu, C.S., Liang, C.S., Radua, J., Boyer, L., Fond, G., Shin, J.I., & Cortese, S. (2022). Incidence, prevalence, and global burden of autism spectrum disorder from 1990 to 2019 across 204 countries. *Molecular Psychiatry, 27,* 4172-4180. https://doi.org/10.1038/s41380-022-01630-7

Solomon, M., Ragland, J.D., Niendam, T.A., Lesh, T.A., Beck, J.S., Matter, J.C., Frank, M.J., & Carter, C.S. (2015). Atypical Learning in Autism Spectrum Disorders: A Functional Magnetic Resonance Imaging Study of Transitive Inference. Journal of the *American Academy of Child & Adolescent Psychiatry, 54(11),* 947-55. https://doi.org/10.1016/j.jaac.2015.08.010

Staudigl, T., Leszczynski, M., Jacobs, J., Sheth, S.A., Schroeder, C.E., Jensen O., & Doeller, C.F. (2018). Hexadirectional Modulation of High-Frequency Electrophysiological Activity in the Human

Anterior Medial Temporal Lobe Maps Visual Space. *Current Biology, 28(20),* 3325-3329.e4. https://doi.org/10.1016/j.cub.2018.09.035

Sun, Y., Gao, Y., Tidei, J.J., Shen, M., Hoang, J.T., Wagner, D.F., & Zhao, X. (2019). Loss of MeCP2 in immature neurons leads to impaired network integration. *Human Molecular Genetics, 28(2),* 245-257. https://doi.org/10.1093/hmg/ddy338

Thomson, A.R., Pasanta, D., Arichi, T., & Puts, N.A. (2024). Neurometabolic differences in Autism as assessed with Magnetic Resonance Spectroscopy: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews, 62*, 105728. https://doi.org/10.1016/j.neubiorev.2024.105728

Trontel, H.G., Duffield, T.C., Bigler, E.D., Froehlich, A., Prigge, M.B., Nielsen, J.A., Cooperrider, J.R., Cariello, A.N., Travers, B.G., Anderson, J.S., Zielinski, B.A., Alexander, A., Lange, N., & Lainhart, J.E. (2013). Fusiform correlates of facial memory in autism. *Behavioral sciences (Basel, Switzerland), 3(3),* 348-71. https://doi.org/10.3390/bs3030348

Tsilioni, I., Patel, A.B., Pantazopoulos, H., Berretta, S., Conti, P., Leeman, S.E., & Theoharides, T.C. (2019). IL-37 is increased in the brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. *Proceedings of the National Academy of Sciences (PNAS), 116(43),* 21659-21665. https://doi.org/10.1073/pnas.1906817116

Wan, L., Yang, G., & Yan, Z. (2024). Identification of a molecular network regulated by multiple ASD high-risk genes. *Human Molecular Genetics, 33(13),* 1176-1185. https://doi.org/10.1093/hmg/ddae058

Wang, Y., Zhang, Y.B., Liu, L.L., Cui, J.F., Wang, J., Shum, D.H, van Amelsvoort, T., & Chan, R.C. (2017). A Meta-Analysis of Working Memory Impairments in Autism Spectrum Disorders. *Neuropsychology Review, 27,* 46-61. https://doi.org/10.1007/s11065-016-9336-y

Zhao, H., Mao, X., Zhu, C., Zou, X., Peng, F., Yang, W., Li, B., Li, G., Ge, T., & Cui, R. (2022). GABAergic System Dysfunction in Autism Spectrum Disorders. *Frontiers in Cell and Developmental Biology, 9*, 781327. https://doi.org/10.3389/fcell.2021.781327