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Factors Affecting the Effectiveness of Bioelectrochemical System Applications: Data Synthesis and Meta-Analysis

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Abstract: Microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) are promising bioelectrochemical systems (BESs) for simultaneous wastewater treatment and energy/resource recovery. Unlike conventional fuel cells that are based on stable chemical reactions, these BESs are sensitive to environmental and operating conditions, such as temperature, pH, external resistance, etc. Substrate type, electrode material, and reactor configuration are also important factors affecting power generation in MFCs and hydrogen production in MECs. In order to discuss the influence of these above factors on the performance of MFCs and MECs, this study analyzes published data via data synthesis and meta-analysis. The results revealed that domestic wastewater would be more suitable for treatment using MFCs or MECs, due to their lower toxicity for anode biofilms compared to swine wastewater and landfill leachate. The optimal temperature was 25–35 °C, optimal pH was 6–7, and optimal external resistance was 100–1000 Ω. Although systems using carbon cloth as the electrodes demonstrated better performance (due to carbon cloth's large surface area for microbial growth), the high prices of this material and other existing carbonaceous materials make it inappropriate for practical applications. To scale up and commercialize MFCs and MECs in the future, enhanced system performance and stability are needed, and could be possibly achieved with improved system designs.

Keywords: bioelectrochemical system (BES); microbial fuel cell (MFC); microbial electrolysis cell (MEC); maximum power density; hydrogen production rate; COD removal; data synthesis; meta-analysis

1. Introduction

Bioelectrochemical systems (BESs) are emerging hybrid systems of biotechnology and electrochemistry that are able to sustainably generate chemical or electrical energy through redox reactions catalyzed by microorganisms [1]. Depending on the mode of applications (Figure 1), BESs can be divided into microbial fuel cells (MFCs), microbial electrolysis cells (MECs), microbial electrosynthesis (MESs), microbial desalination cells (MDCs), and microbial solar cells (MSCs), among which MFCs and MECs are the most studied in the recent years [2].

Like other types of electrochemical cells (e.g., batteries), both MFCs and MECs consist of two electrodes (i.e., an anode and a cathode) that are connected with a conductive wire to form a closed electrical circuit (Figure 1). When the redox potential of the cathode reduction half-reaction (E_{cat}) is higher than that of the anode oxidation half-reaction (E_{an}), electricity is generated, due to a positive cell potential ($E_{cell} = E_{cat} - E_{an}$). Otherwise, external power (i.e., electrolysis) is needed to propel the redox reactions. The biological and electrochemical processes in both types of BESs are uniquely

linked by the electron exchange between the microorganisms and electrodes [1]. The electrons can be transferred (1) directly, via the outer membrane proteins (e.g., cytochromes) or conductive nanowires of the microorganisms; or (2) indirectly, through electron shuttles (e.g., flavins), when there is no physical interaction between the electrodes and microorganisms [3]. On the cathode side, the transferred electrons can be utilized to accomplish any reduction reactions, which lead to diverse application possibilities of BESs [2].

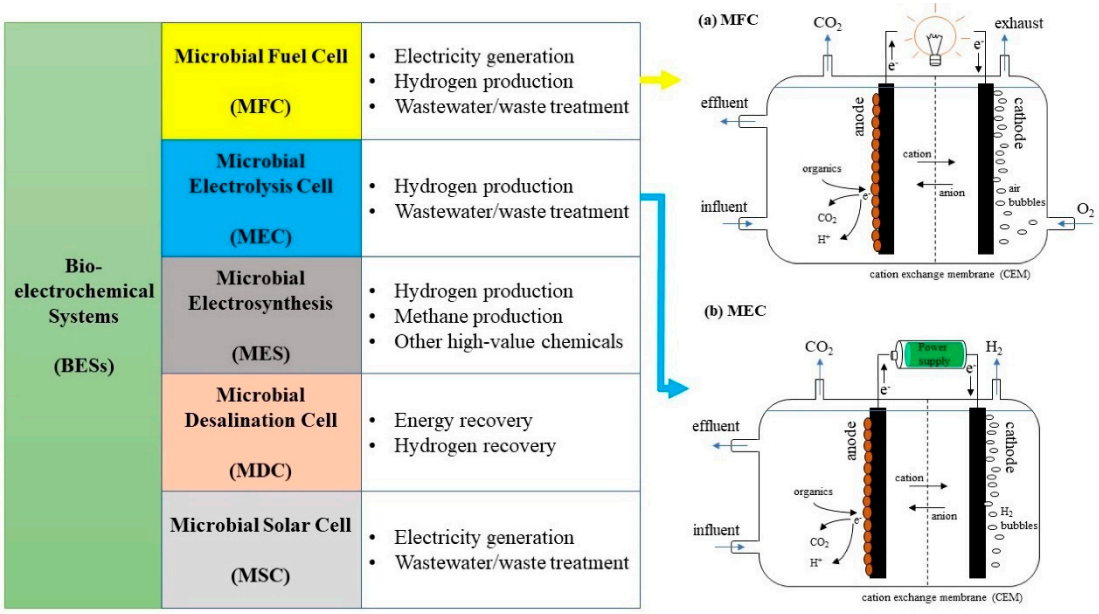

Figure 1. Overview of various types of bioelectrochemical systems (BESs), and schematics of the two most common BESs: (**a**) two-chamber microbial fuel cell (MFC); (**b**) two-chamber microbial electrolysis cell (MEC) for H_2 production.

MFCs are capable of converting the chemical energy directly from a wide range of organic matter (e.g., wastewater and lignocellulosic biomass) to electrical energy, using exoelectrogens (e.g., *Geobacter sulfurreducens* and *Shewanelle putrefaciens*) at the anode [4]. The electrons collected at the anode flow through the external circuit to the cathode, where they combine with the protons and O_2 to produce water. Thus, an electric current is formed, due to the flow of electrons, and the generated electricity can be directly utilized or stored. The most common designs of MFCs are two-chamber and single-chamber. Unlike the two-chamber MFC shown in Figure 1, a single-chamber MFC has only the anode chamber, which is wrapped by a chamber wall consisting of a waterproof cation exchange membrane and cathode material (e.g., carbon cloth) on the outer surface.

Inversely, MECs are able to generate chemical energy (e.g., H_2, CH_4, and other value-added chemicals) at the cathode, when an external input of electricity is applied to the circuit in order to overcome the thermodynamic barrier, and drive the flow of electrons released by the microorganisms at the anode [2]. Unlike an MFC, whose cathode chamber is generally oxygen-enriched, the cathode chamber of an MEC must be maintained as anaerobic (without O_2) or anoxic (low O_2), in order to facilitate the formation of desired products. For example, with high concentrations of H_2 under anoxic conditions, CH_4 can be produced with MECs at the presence of CO_2 and methanogens. Single-chamber MECs can also function properly [5]. However, unlike single-chamber MFCs, in a single-chamber MEC, the anode and cathode are both inside a closed/semi-closed O_2-limiting chamber. Notably, regardless of the reactor configurations, an MEC can be coupled with an MFC, which serves as an in-situ power supply for biohydrogen production from organic substrates (e.g., acetate) [6].

BESs have been investigated for their potential applications in various areas, especially wastewater treatment with simultaneous production of bioenergy and biopolymers [7,8]. Considerable removal of

chemical oxygen demand (COD) using MFCs or MECs has been reported for the biological treatment of domestic wastewater [9], swine wastewater [10], brewery wastewater [11], landfill leachate [4], etc., along with the simultaneous benefits of energy generation and biohydrogen production that greatly help cut down the high costs of energy. However, the performance of these complex BESs, in terms of COD removal and energy/chemical production, is mutually affected by many factors, including the wastewater (substrate) type [12], electrode material [13], separator material [14], inoculum type [14], reactor configuration [15], mode of operation [16], etc. Although many laboratory experiments have shown the significant potentials of BESs, only a few pilot studies have been successfully operated in real-world conditions [17]. Identifying the affecting factors and understanding the impacts of these factors on the effectiveness of BESs are of essential importance for the optimization of these systems.

This work aimed to summarize and compare the effects of the major factors that have been reported to influence the performance of the major two types of BESs (i.e., MFCs and MECs). Under the influences of these factors, the effectiveness of the systems was evaluated in terms of both treatment and energy/biohydrogen production. Specifically, the treatment level was represented by the COD removal in an MFC or MEC, the energy production of an MFC was characterized by its maximum power density (i.e., maximum power per unit surface area of the anode, mW/m^2), while the biohydrogen generation of an MEC was assessed based on the hydrogen production rate (HPR; i.e., H_2 produced per unit working volume per day, $m^3H_2/m^3/d$). A meta-analysis using statistical techniques to combine and analyze findings from multiple independent studies was performed in order to improve estimates of the sizes of the effects. The current barriers and future directions for commercial installations of the two types of BESs are also discussed at the end.

2. Results and Discussion

2.1. Effects of Temperature on the Performance of Microbial Fuel Cells and Microbial Electrolysis Cells

Like many other anaerobic reactors, MFCs and MECs are sensitive to temperature (Figure 2). The power generation in MFCs and the hydrogen production in MECs, as well as the COD removal in both types of reactors, varies drastically as the working temperature changes. In general, temperatures that are either too high (e.g., >45 °C) or too low (e.g., <15 °C) significantly hinder power generation in MFCs and hydrogen production in MECs, while temperatures between 30 and 40 °C seems to favor the performance of these reactors. At the same time, the highest COD removal in both MFCs and MECs has been found within the temperature range from 25 to 35 °C. In terms of the principle of economy, Tang et al. suggested the optimal operating temperature of MFC be 30 °C, even though the performance at 35 °C slightly improved [18]. According to the fitted results, the optimal operating temperature of MECs was very close to that of MFCs, indicating the feasibility of integrating these two types of reactors under the same operating temperature.

The impacts of temperature are attributed to the different resulting microbial activities [15,19–21]. Studies have revealed the dominance of electrogenic bacteria (e.g., *Geobacter sulfurreducens*) [22] and better growth and activity of electrochemically active biofilms [23] at the optimal temperatures near 30 °C. The overall microbial activity decreases as the operating temperature becomes more extreme, which in turn slows down the microbial generation of electrons and protons [15]. One encouraging fact is that the changes of power generation in MFCs and hydrogen production in MECs are usually smaller than 10% when the operating temperature is between 20 to 35 °C [15,24,25], indicating that these reactors could tolerate temperature changes over a wide range of temperatures in real-world applications for wastewater treatment. Heidrich et al. speculated that the biofilms attached to the electrodes possessed self-heating effects that allowed them to adapt to sub-optimal temperatures [24]. It should be noted that the optimal temperatures for power generation and hydrogen production are different from the optimal temperatures for COD removal, possibly because the microbial species most effective in utilizing the biodegradable organics are oftentimes different from electrochemically active species [3].

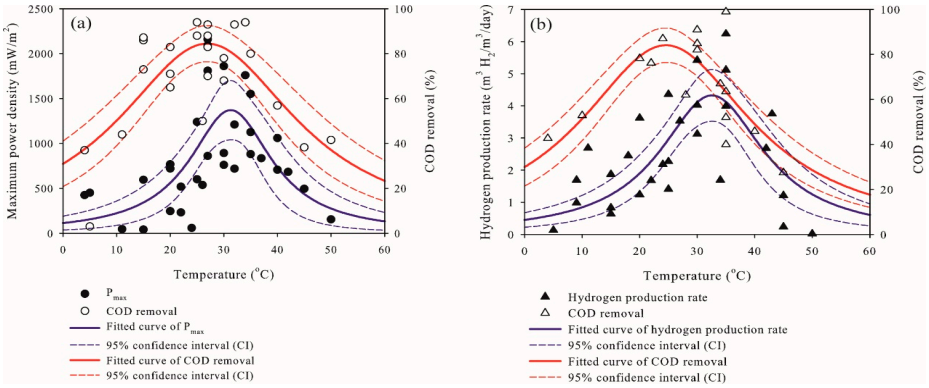

Figure 2. Influences of temperature on (**a**) MFCs' power generation and chemical oxygen demand (COD) removal and (**b**) MECs' hydrogen production and COD removal.

2.2. *Effects of pH on the Performance of Microbial Fuel Cells and Microbial Electrolysis Cells*

The anode pH also greatly affects the performance of MFCs and MECs (Figure 3). A pH between 6.5 and 7.5 correlates with relatively higher power densities, up to over 1200 mW/m^2, whereas a pH between 5 and 6 derives a more significant hydrogen production rate, as high as about 8 m^3 $H_2/m^3/d$. The impacts of pH on the COD removal in both types of reactors are also pronounced. The COD removal at the optimal pH ranges is nearly three times higher than that at extremely low or high pH ranges. These results reflect that the microbial activities in MFCs, and MECs are slower at a sub-optimal pH than at the optimal pH. The low values for both power generation in MFCs and hydrogen production in MECs at high pH (>10) might be due to the poor proton transfer, as the proton concentration gradient across the proton exchange membrane (PEM) decreased [26]. Proton transfer is known as a rate-limiting factor for both types of reactors [27]. At a relatively low pH, the proton concentration gradient accelerates the proton transfer, which increases the proton availability in the cathode chamber for biohydrogen production. This would explain why the relatively lower pH is preferred for hydrogen production in MECs. However, the reactor performance deteriorates as the pH decreases to below 3, as the acidic environment becomes no longer suitable for the growth of most electrogenic microbes [28]. Nevertheless, certain species have been reported to function well in MFCs even at such severe low-pH conditions, generating power densities ranging from 20 to 55 mW/m^2 using single-chamber, air-cathode, tubular MFCs [29]. Comparing to MFCs, the performance of MECs are more vulnerable to the increase of pH, because the production of biohydrogen would not occur without the presence of a proton in the cathode chamber.

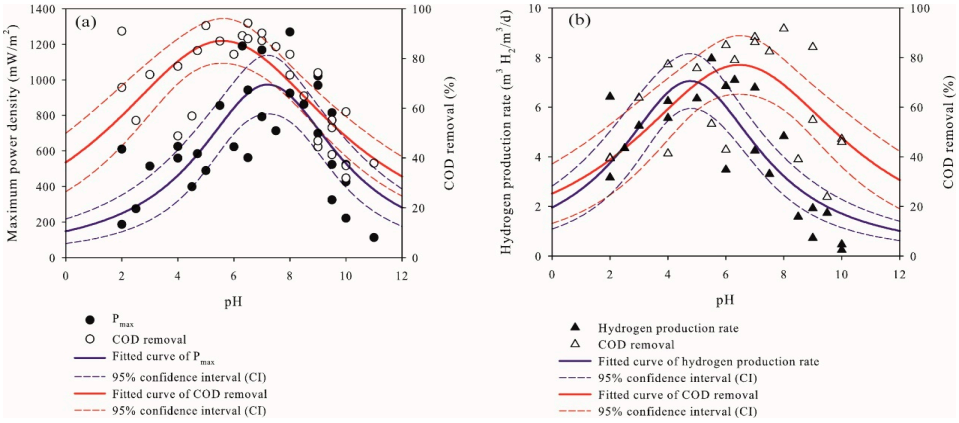

Figure 3. Influences of pH on (**a**) MFCs' power generation and COD removal and (**b**) MECs' hydrogen production and COD removal.

2.3. Effects of External Resistance on the Performance of Microbial Fuel Cells and Microbial Electrolysis Cells

External resistance (R_{ext}) affects the performance of MFCs and MECs, by limiting the flow of electrons form the anode to the cathode [30]. According to Ohm's Law ($V = I R_{ext}$), both the potential (V) and the current (I) outputs would be influenced. Consequently, the power output (W) is also affected as shown by $W = I^2 R_{ext}$. In MFCs and MECs, increased external resistance decreases the power density, as well as the treatment efficiency (Figure 4). Generally, the anode potential, which directly determines the anode availability as electron acceptors, is regulated by the external resistance [31]. As a result, the growth competition between the electrogenic and non-electrogenic microbial communities is influenced when different external resistances are applied to the circuits. Likewise, the growth competition among different electrogenic bacteria is also impacted, directly through the utilization of an anode or indirectly through the alteration of the microenvironmental conditions [30]. Eventually, the microbial community structures established under different external resistance would differ, thus affecting the consumption of organic substrates and the associated production of protons in the anode biofilm [32]. The accumulation of protons in the anode biofilm would lower the pH, which again would affect the biofilm environment. The microbial community in the anode biofilm is most susceptible to the change of external resistance when the anode potential is low. Below anode potentials of approximately −1.5 V, the impacts on most identified *Geobacter* strains have been reported to be pronounced [32].

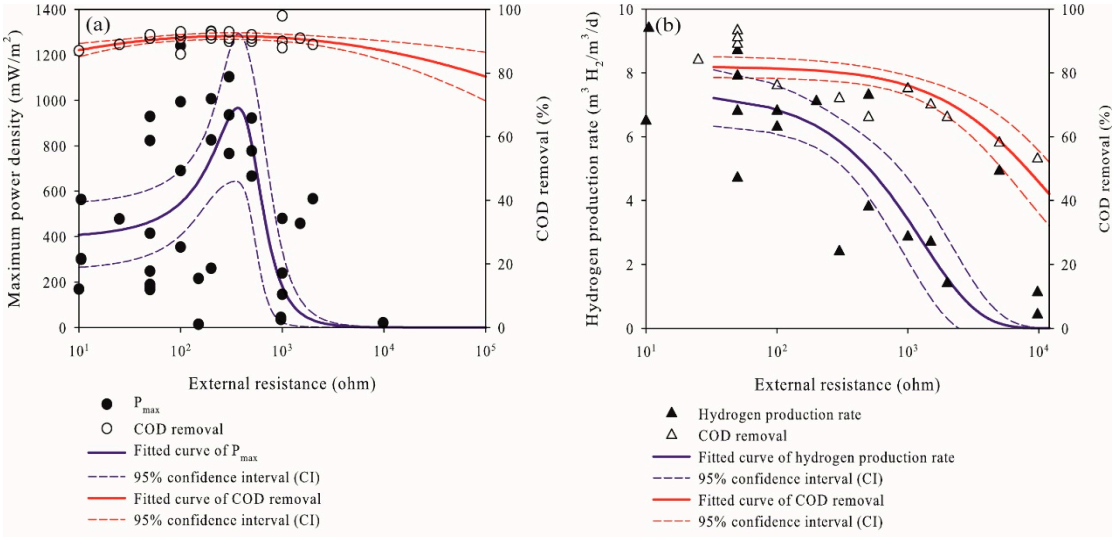

Figure 4. Influences of external resistance on (**a**) MFCs' power generation and COD removal and (**b**) MECs' hydrogen production and COD removal.

When the external resistance was either too low (e.g., 10 Ω) or too high (e.g., 10 kΩ), power generation in MFCs became weak. At low resistance, the low redox potential at the anode probably made it unfavorable as an electron acceptor for the microbes; at high resistance, the resistance was maybe too high (almost mimics an open circuit) for the microbes to transfer their electrons. MFCs operating at optimal external resistance have been reported to have enhanced power generation, reduced methane production, and increased Coulombic efficiency [33]. However, low resistance would not hinder the electron transfer in an MEC, because the electrons are driven by the external power provided to the system.

2.4. Effects of Substrate Type on the Performance of Microbial Fuel Cells and Microbial Electrolysis Cells

Three major types of wastewater (i.e., domestic wastewater, swine wastewater, and landfill leachate) have often been used to feed MFCs and MECs while being treated or pretreated. The power

generation in MFCs, the hydrogen production in MECs, and the treatment efficiency in both types of reactors were statistically analyzed and compared, as shown in Figure 5. From the comparison, it is apparent that domestic wastewater is the most suitable in terms of power generation in MFCs and hydrogen production in MECs, while the lowest power densities and hydrogen production rates have been found with MFCs and MECs using landfill leachate. Interestingly, as reflected by the COD removal, higher treatment efficiency has been shown in those reactors treating swine wastewater.

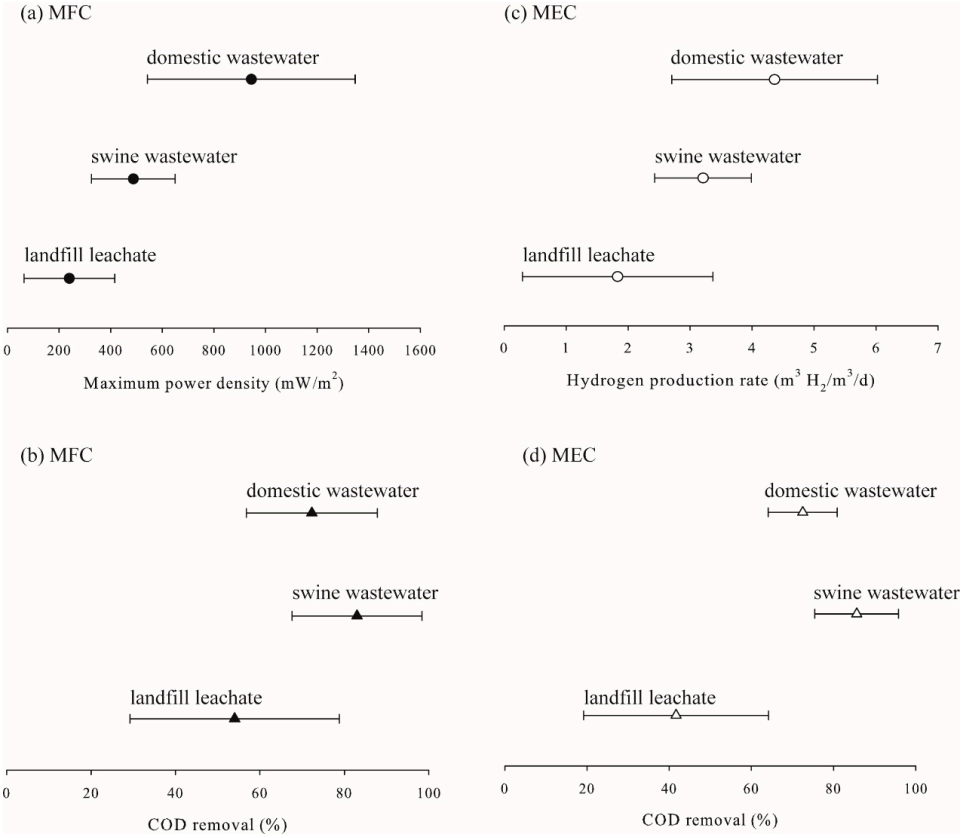

Figure 5. Influences of substrate type on (**a**) an MFCs' power generation; (**b**) an MFCs' COD removal; (**c**) an MECs' hydrogen production; and (**d**) MECs' COD removal.

The suitability of wastewater sources for MFCs and MECs mainly depends on the biodegradability of the containing substrates [24], but other characteristics of the wastewater, such as pH, ionic strength, and toxicity, would also make a difference.

The influences of pH have been discussed earlier in this paper. A high ionic strength usually reduces the internal resistance, and results in a decrease of anode potential and an increase of cathode potential, giving a larger overall circuit voltage. Although swine wastewater and landfill leachate are rich in biodegradable organics and usually have high ionic strengths, the relatively high concentrations of toxic compounds like ammonium [34] and heavy metals [35] would negatively impact the microbial growth and eventually the reactor performance when these types of wastewater were used to feed MFCs and MECs.

2.5. Effects of Electrode Type on the Performance of Microbial Fuel Cells and Microbial Electrolysis Cells

Various types of electrode materials have been investigated and developed in the recent years to improve the performance of MFCs and MECs while reducing the cost of the reactors. In these BESs, the electrodes are not only conductors, but also habitats for electrogenic microbes. Therefore, good electrode materials should demonstrate some special surface characteristics, such as good

biocompatibility, high specific surface area, considerable surface roughness, and the ability to efficiently transfer electrons between microbes and electrodes [36]. Considering these above characteristics, carbonaceous materials are often preferred as anode materials.

In the study, the three most commonly used carbonaceous electrode materials (i.e., carbon paper, carbon cloth, and graphite brush) were compared (Figure 6). Carbon paper is thin and firm yet slightly brittle, and is characterized by a relatively smooth surface. On the contrary, carbon cloth is more flexible and porous. Graphite brush is a fiber fabric, much thicker than the above two materials, and the brush configuration allows for more available surface area (although less than carbon cloth) for the attached growth of microbes. The statistical results demonstrate that electrodes made of carbon cloth are the best for both power generation in MFCs and hydrogen production in MECs. The performance of reactors using carbon paper as the electrodes is slightly better than that of reactors using graphite brush. The high specific surface areas of carbon cloth and carbon paper are believed to be the key to their greater power generation and COD removal. However, the high prices of these carbonaceous materials, especially carbon cloth (approximately \$1000/m^2) [37], are prohibitive for practical applications of these BESs. Another concern is the high electrical resistivity of these carbonaceous materials, which would lead to large electrode ohmic losses in large-scale systems [36].

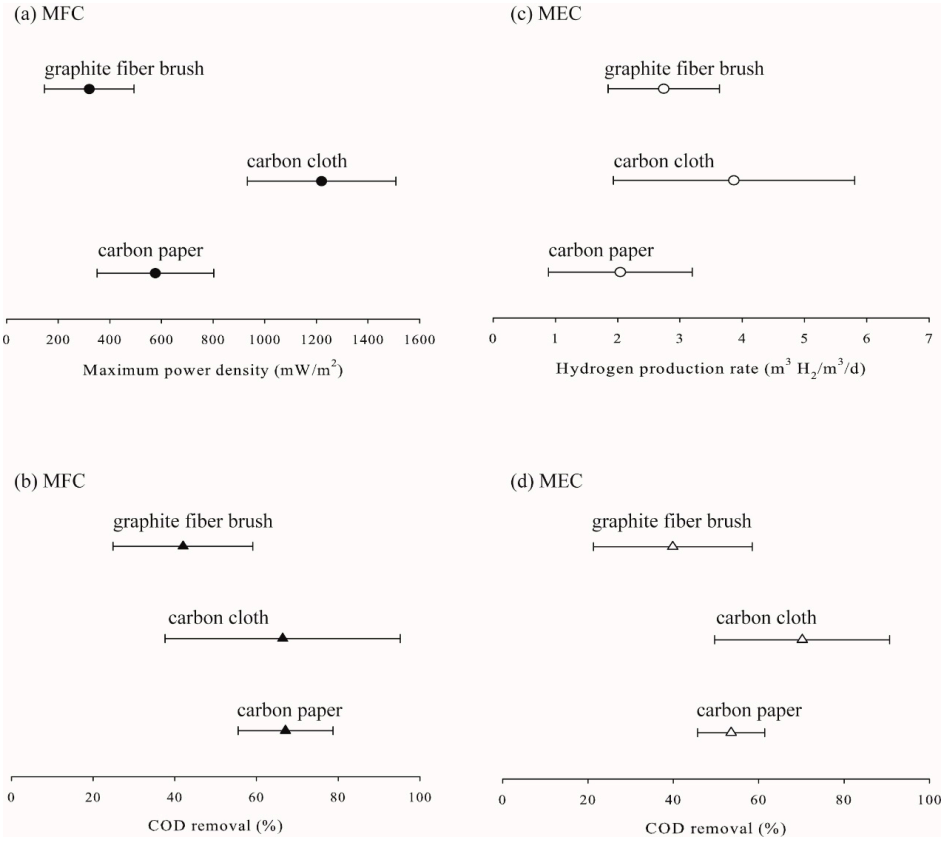

Figure 6. Influences of electrode material on (**a**) MFCs' power generation; (**b**) MFCs' COD removal; (**c**) MECs' hydrogen production; and (**d**) MECs' COD removal.

2.6. Effects of Reactor Configuration on the Performance of Microbial Fuel Cells and Microbial Electrolysis Cells

In order to optimize the performance of MFCs and MECs, a wide range of configurations have been designed and developed. The single-chamber design and the two-chamber design were compared in this study, while the performance of the scaled-up systems (>1 L) [17] was also discussed (Figure 7). The two-chamber systems are as shown in Figure 1, in which the proton would travel through the membrane and arrive at the cathode chamber to join further bioelectrochemical reactions.

The single-chamber systems have only the anode chamber as the electron acceptor of the cathode, which is typically the oxygen in the air (a.k.a., an air-cathode). The results show that the two-chamber MFCs generally generate higher power, while the one-chamber MECs could produce more biohydrogen. However, the two-chamber systems are more problematic with their high electrical resistivity, which should result in relatively lower power generation in MFCs than the single-chamber designs [15]. In fact, two-chamber systems were more often used in existing studies because of their simplicity and stability. A great variety of substrates and operating conditions have been investigated using two-chamber systems, and oftentimes the substrates were readily biodegradable (e.g., acetate) and the conditions were optimal [38–41]. The preference of two-chamber systems in previous studies might have introduced biased results for the comparisons.

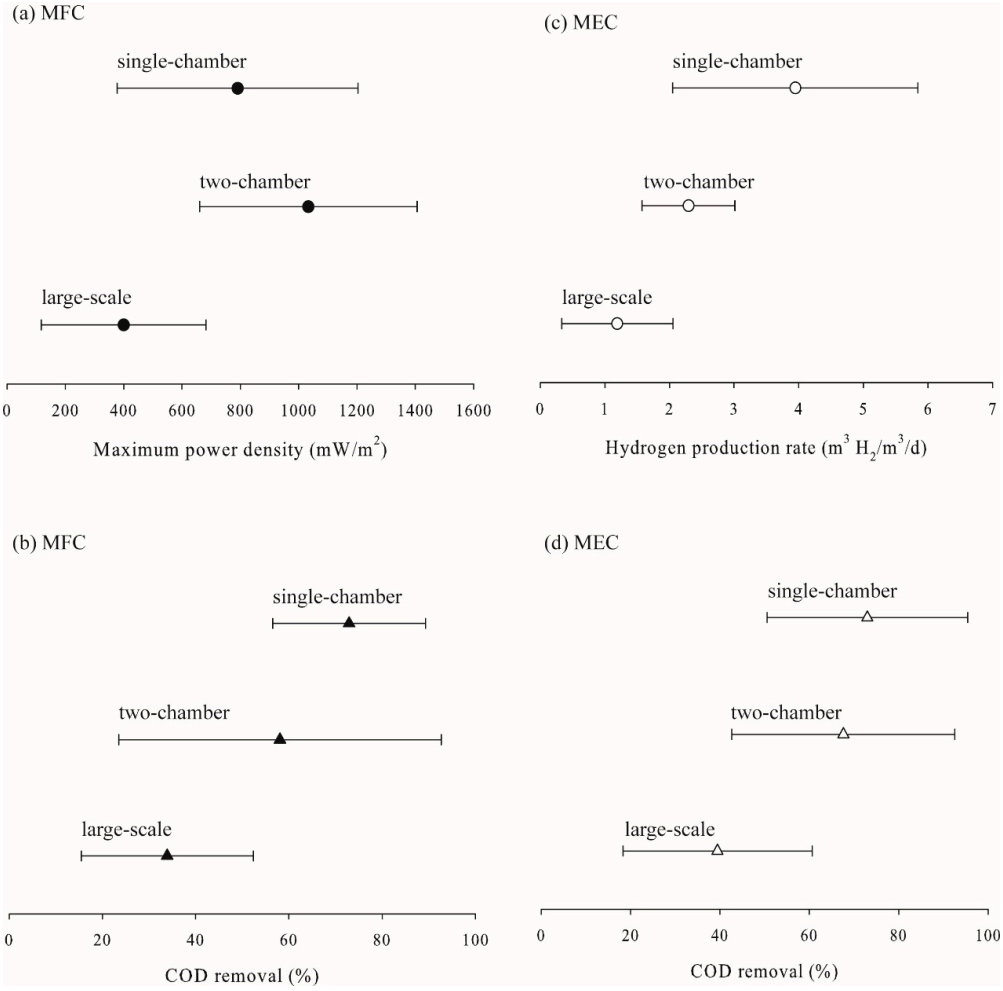

Figure 7. Influences of reactor configuration on (**a**) MFCs' power generation; (**b**) MFCs' COD removal; (**c**) MECs' hydrogen production; and (**d**) MECs' COD removal.

Notably, power generation in MFCs, hydrogen production in MECs, and COD removal in both systems were significantly lower when the reactors were scaled up. Larger systems are faced with more complex challenges, such as higher electrical resistivity and instability [42]. Although there were a few successful examples of pilot systems, the high cost is another issue that obstructs the real-world applications of these BESs. Novel configurations and cheaper electrode materials with larger surface areas must be further investigated, in order to cut down the expenses.

3. Implications for Commercial-Scale Applications

MFCs and MECs are promising technologies for wastewater treatment and energy supply. The electricity generated in MFCs and the biohydrogen recovered in MECs could significantly reduce the economic burden to treat domestic and industrial wastewater, while providing safe water to the public. The real-world applications of these BESs must be scaled up and commercially profitable. Therefore, the two major challenges are (1) to optimize the system performance and (2) to lower the material costs.

The results presented in this study suggested that the optimal operating conditions are as follows: a temperature of 25–35 °C, a pH of 6–7, and an external resistance of 100–1000 Ω. Single-chamber systems would be more attractive, especially for MECs. Although it is possible to treat almost all types of organic-containing wastewater, some complex wastewater, such as landfill leachate, should be pretreated for detoxification. The electrode materials that are currently used are problematic, with either their high prices or low surface areas. Novel biocompatible electrodes should be the focus in future studies.

The performance and stability of the systems can also be improved with enhanced configurations. For wastewater treatment, multiple MFC or MEC units should be connected as stacks, in order to further increase their power generation, biohydrogen recovery, and treatment efficiency. However, the MFC and MEC stacks are plagued by low efficiency and instability, due to the nonlinear nature of the unit systems [43]. Unlike conventional fuel cell stacks, which are based on stable chemical reactions in each unit, the MFC and MEC units depends on relatively instable microbial activities for power and hydrogen outputs. The performance of each individual unit could be very sensitive to the environmental and operating conditions, which would result in even more pronounced fluctuation of the system outputs. In addition, the overall system efficiency is usually reduced because of the worst performing unit(s). To solve this issue, the output from each unit could be individually collected, and then combined in the desired ways with the use of an energy/resource-harvesting station. Recently, a multi-anode approach using segmented anode arrays has also been developed, in order to improve the performance of each MFC and MEC unit [44].

4. Methods

4.1. Data Sources

The Scopus database (Elsevier, New York, NY, United States) was used for an extensive literature search with the search terms "microbial fuel cell AND temperature", "microbial fuel cell AND pH", and "microbial fuel cell AND scale up". Similarly, the same search was performed for "microbial electrolysis cell". However, the search results needed to be carefully selected, because many of the studies were either unable to provide sufficient information or irrelevant. The same searches were also performed using Google Scholar and Web of Science, but no additional studies were found that were suitable for the data synthesis and meta-analysis in this paper. Studies with no quantitative results or measures of variance were excluded. The cut-off date for publications that were included in this meta-analysis was 15 May 2018. Consequently, a total of 108 studies on MFCs or MECs were used, as summarized in Table 1. The relevant data were extracted directly or indirectly (DataThief III was used for the extraction when the data was presented graphically) from each study, and subsequently inserted into Excel for further processing.

Although independent studies were selected randomly for the data synthesis and meta-analysis, the results might have been affected due to the intrinsic publication bias, as studies with no significant effects were less likely to be considered for publication [45].

Table 1. Matrix of studies providing data for the data synthesis and meta-analysis.

Groups	Subgroups	Number of Studies	References
Temperature (Temp.)	Low (<25 °C)	13	[15,19–21,24,46–53]
	Moderate (25–35 °C)	12	[15,19,21,50,51,54–60]
	High (>35 °C)	9	[19,60–67]
pH	Low (<6)	5	[68–72]
	Moderate (6–8)	6	[5,61,73–76]
	High (>8)	5	[73,77–80]
External resistance (R_{ext})	Low (<100 Ω)	7	[30,50,81–85]
	Moderate (100–1000 Ω)	9	[30,50,82–84,86–89]
	High (>1000 Ω)	5	[50,83,88,90,91]
Substrate (S)	Domestic wastewater	10	[20,21,49,79,92–97]
	Swine wastewater	7	[10,41,98–102]
	Landfill leachate	8	[4,103–109]
Electrode material (Electro.)	Graphite fiber brush	7	[110–116]
	Carbon cloth	7	[15,117–122]
	Carbon paper	5	[123–127]
Reactor configuration (Config.)	Single-chamber	10	[48,87,123,128–134]
	Two-chamber	9	[20,52,122,135–140]
	Large-scale	7	[49,56,141–145]

4.2. Data Grouping and Processing

The units of data (whichever applicable) reported in different studies were converted in order to be consistent and comparable. For example, the units "mW/m^2" (power per anode surface area) and "m^3 $H^2/m^3/d$" (hydrogen per reactor volume per day) were used for power density and hydrogen production rate, respectively. Due to the continuous nature of the reported data of temperature, pH, and external resistance, data synthesis was performed for these factors to show the system responses to the changes of each factor, and to locate the range for the optimal performance (if applicable). For grouped data, such as substrate type, electrode material, and configuration, a meta-analysis was conducted for each factor to discuss the size of its effect.

For the meta-analysis, the Excel data file was exported to MetaWin (Version 2.1) for further processing. A categorical random effects model was used for the calculation of grouped effect sizes—i.e., a random effects model was used for groups of different categorical predictors, while a fixed effects model was used for those with negative estimated pooled variances [146]. All results with a p-value smaller than 0.05 were considered to be statistically significant. The calculated effect sizes were used to generate forest plots reflecting the impacts on the reactor performance. Each centered point stood for the mean effect size for each grouping, while the extended lines represented its 95% confidence interval (CI). Because the sets of data in this study were relatively small (mostly less than 30), the t-distribution (instead of the normal distribution) was utilized to construct the confidence interval following Equation (1).

$$95\%\ \mathrm{CI} = \overline{X} \pm t\frac{S}{\sqrt{N}} \quad (1)$$

where $\overline{X}$ is the mean of the reported results; S is the standard deviation; N is the number of data inputs; and t is the t-value, which measures the size of difference relative to the variation in the sample data.

4.3. Curve Fitting

The experimental results were fitted using the nonlinear least squares regression, thus deriving the data for generating the fitted curves presented in Figures 2–4. The data were processed in MATLAB (R2018a, MathWorks, Natick, MA, USA).

5. Conclusions

The analysis of published data revealed the importance of operating temperature, pH, external resistance, substrate, electrode material, and reactor configuration for the performance of MFCs and MECs. Domestic wastewater, in comparison with swine wastewater and landfill leachate, was the most suitable for these two BESs because of the higher power generation in MFCs, greater biohydrogen generation in MECs, and better treatment efficiency in terms of COD removal. The optimal temperature was found to be 25–35 °C, while optimal pH was 6–7 and optimal external resistance was 100–1000 Ω. Although systems using carbon cloth as the electrodes demonstrated better performance, the high price of this material and other existing carbonaceous materials requires future investigations into novel materials that would be economic and porous. In order to scale up and commercialize MFCs and MECs, enhanced system performance and stability are needed, and could be possibly achieved with improved system designs.

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