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Meta-analysis of Microbial Fuel Cells Using Waste Substrates

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Abstract Microbial fuel cell experimentation using waste streams is an increasingly popular field of study. One obstacle to comparing studies has been the lack of consistent conventions for reporting results such that meta-analysis can be used for large groups of experiments. Here, 134 unique microbial fuel cell experiments using waste substrates were compiled for analysis. Findings include that coulombic efficiency correlates positively with volumetric power density ($p < 0.001$), negatively with working volume ($p < 0.05$), and positively with percentage removal of chemical oxygen demand ($p < 0.005$). Power density in mW/m^2 correlates positively with chemical oxygen demand loading ($p < 0.005$), and positively with maximum open-circuit voltage ($p < 0.05$). Finally, single-chamber versus double-chamber reactor configurations differ significantly in maximum open-circuit voltage ($p < 0.005$). Multiple linear regression to predict either power density or maximum open-circuit voltage produced no significant models due to the amount of multicollinearity between predictor variables. Results indicate that statistically relevant conclusions can be drawn from large microbial fuel cell datasets. Recommendations for future consistency in reporting results following a MIAMFCE convention (Minimum Information About a Microbial Fuel Cell Experiment) are included.

Keywords Microbial fuel cell · Meta-analysis · Renewable energy · Bioelectrochemical systems · Food waste · Agricultural waste

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Introduction

Microbial fuel cells (MFCs) are a bioelectrochemical technology that can transform organic waste materials into renewable energy. MFCs use bacteria that are exoelectrogenic; they are able to generate an electrical current by oxidizing organic compounds and passing electrons to a conductive anode attached to a circuit [14]. Since solid wastes and processing wastewaters are a readily available source of organic content for energy harvesting [7], this technology can broadly impact waste management by generating electrical power via bioconversion of waste organic compounds.

Despite the quantity of MFC research, results between experiments are often hard to compare due to differences in fuel cell construction (i.e., reactor configuration), microbial community structure, and substrate type. Authors in the field have noted the lack of sufficient data collection for statistical analysis and the necessity of reporting findings in a way that allows broad cross comparisons of datasets [8, 11, 13, 15]. That being said, current reviews compile results [3, 6, 10, 12, 16] but do not yet engage in broad multivariate analyses across experiments. A meta-analysis approach is required to quantitatively gauge how MFC performance is related to the physical characteristics of device construction and feedstock choice. Additionally, this approach can use exploratory data analysis to formulate new hypotheses for MFC technology improvement and future research that are still unexplored, as well as being able to build statistical models that describe how MFC design, operating, and substrate variables interact with each other. However, it is difficult to broadly compare all MFC experiments using waste substrates due to the differences in data collected and the methods of reporting in reviews; for this reason, a minimum set of MFC performance metrics is needed across both single-experiment scientific articles and review papers that aggregate those experiments to create a large dataset that engenders MFC performance modeling. Additionally, the scientific community needs a statistical analysis of these power metrics described in MFCs as a function of these different MFC design factors to understand how to improve power production. This enables identification and bioengineering of unique MFCs and operating conditions that optimize power output.

This meta-analysis gathers and curates a large MFC dataset to achieve meaningful statistical analyses across multiple experiments. Our scope includes recent review articles published after 2010 that aggregated MFC experimental data, and focuses specifically on reviews of MFCs that use waste streams as substrates. MFC variables and parameters of interest included waste substrate type, reactor design (single, double, or stacked), working volume, maximum power density, and coulombic efficiency. Rather than describe previously published results, this meta-analysis performs original research across multiple MFC experimental conditions and results. These results were used to identify significant interactions between MFC process variables that could inform future research to improve MFC performance.

Materials and Methods

Data Aggregation

Data was gathered from four main review articles that have each done extensive work in compiling MFC experimental data for waste streams [3, 6, 10, 16]. These reviews were selected because their tabular format of data reporting was necessary for aggregating a data table amenable to meta-analysis. A fifth review was excluded due to data being reported in current density rather than power density, unlike the other reviews, as well as a lack of data categories that corresponded with the other reviews used [12]. Power density was chosen for analysis rather than current density due to its predominant use in the previously mentioned reviews found to match inclusion criteria. PDFs for the reviews included in the meta-analysis were inspected and all relevant data on MFC research with respect to waste streams was extracted. This was done by using Nitro Pro 9 PDF software (Nitro Software Inc., San Francisco, CA, USA), which contains PDF to Excel table functionality when PDFs contain information presented in a tabular format. The data was then opened in Excel and validated. This step included verifying and curating the data such that statistical analyses could be performed. Individual steps within data validation included first reformatting column names to match database names, adding a metadata column of which review this specific result came from, and separating power density normalized by area from power density normalized by volume. Units were then converted to be common across cells (e.g., mW/m^3 to W/m^3), and text units were removed from data cells (e.g., "2 W/m^3" to "2"). Tildes ("~") and plus/minus ("±") signs were removed. Empty cells where reference, substrate, or reactor configuration was implied from the preceding line were filled in. Ranges of values were replaced with maximum values and cells with subscripts denoting absence of data were deleted.

After the data table was curated to enable data aggregation, a database in MS Access was used to collect all the different data tables from the different reviews. MS Access was utilized because of its ability to combine sets of records with different fields in different orders, automatically mapping the correct information from each record to the correct field. These import steps were repeated for each table in each review article found. After import into MS Access, 178 records existed. To check for duplicates, records were filtered which contained the same author and year, and then adjoining cells were inspected for duplicate values. If the records were true duplicates, both records were copied to another table and one of the duplicates was deleted from the main data table. After de-duplicating, 134 individual records of MFC experiments remained.

At this point, the categorical data was inspected and grouped into umbrella terms to facilitate data exploration and analysis. Feedstock varieties were grouped into larger descriptors (e.g., "hydrolyzed corn stover" changed to "corn") according to the waste product used. Reactor configurations (variants of "single chamber" and "double chamber" all reformatted as "single," "double," or other simplified terms). Double-chamber formats included two-chambered, dual-chambered, twin compartment, and two-chambered loop configuration MFCs. Single-chamber formats included one chamber, single chamber, upflow membrane-less, granular-activated carbon-based single chamber, annular single chamber, and membrane-less cross-linked. All "membrane-less" reactor formats were re-categorized as "single" reactor configuration types. After final data curation, the data table resulted in 134 individual records with 18 columns (fields) of information. Despite some gaps, 1132 individual pieces of data were collected. This large quantity of data enabled statistical conclusions to be drawn. After all the data had been reformatted and condensed into a single table in Excel, the data was saved and imported into JMP Pro 12 statistical software (SAS Institute Inc., Cary, NC, USA). JMP allowed exploration of data and determination of significant differences between experimental groups.

Data Manipulation

Problems with collecting and curating the data from the original reviews were numerous. After aggregation into a single table and during deduplication, duplicate entries often named two different results for the same experiment. This mandated returning to the original literature for determination of true results. Additionally, the data within a single review often contained numbers with different units for a given response; for example, chemical oxygen demand (COD) in mg/L and kg/m^3, which required conversion for consistency by changing the units used in the original review. Reactor volumes for stacked units were often reported as "Entire volume (# units * unit volume)". These data were replaced with the entire volume to generate accurate working volume to power output correlations. Power densities were also formatted differently depending on their unit of normalization (power per cathode area vs power per working volume of unit). Coulombic efficiencies and COD removal percentages were expressed as an integer from 0 to 100 to ease data gathering and conversion from source documents, since most source documents labeled these numbers in this format.

In terms of individual review articles, several discrepancies were found. In their book chapter, Li et al. [6] quote Fornero et al. [4] twice with the same coulombic efficiency several rows apart, indicating that these may be duplicate entries within a single review. The COD load for these two rows, however, was different. Both data points were kept.

ElMekawy et al. [3] reported anode volume rather than working volume of the entire reactor. For data analysis, the anode volume was changed from the original review and was doubled to calculate working volume for a common volume metric between all experiments. This assumed that anode volume and cathode volume were equal, which is true for many established MFC designs [2, 5, 9]. Additionally, commas were sometimes used for periods, such that cross-checking with original data tables was needed. Some of the COD loading rates were organic loading rates in kg COD/(m^3 day) but were included in the same data column with a superscript next to the data. These cells were excluded—in the future another data column may be created for these data. Finally, the authors split food and agricultural waste between two tables. Agricultural wastes in MFCs did not have accompanying working volume or COD load data, unlike the food wastes data.

Sun et al. [16] reported data such that two rows contained two different experimental conditions for the same reference. Not all data was carried between rows, such that reactor size, reactor configuration, and referenced literature had to be inferred. It was unclear whether the missing data was unavailable in the original experimental literature or if it was meant for the reader to infer the missing data from the preceding row. Corresponding with the author led to clarification of empty data fields. Additionally, the phrase "insignificant" replaced a datum for maximum power density; this involved changing data from the original review and labeling the data as "0". Finally, COD removal efficiencies were not reported nor were COD loadings.

Pandey et al. [10] collected the most data from individual MFC experiments, including OCV max, anode type, and cathode type. As will be discussed later, this format is more conducive to generating statistical results from large MFC experimental datasets.

Data Analysis

Data were analyzed statistically by first describing the categorical variables. The 134 individual records from the four different reviews were grouped by reviewer to examine how many references each reviewer included in their paper. Individual references were then grouped by each of the categorical variables. This method answered the question of how many MFC experiments used specific feedstocks and how many used specific reactor configurations.

After examination of the two categorical variables, seven continuous variables used in literature to describe the operating conditions and performance of MFCs fed with wastes were analyzed. These numerical variables included coulombic efficiency, power density normalized to surface area, power density normalized to reactor volume, reactor working volume, COD load of substrate, COD removal efficiency, and OCV max. Numerical variables were analyzed with multivariate analysis. Spearman's rho analysis was used to measure the strength of rank correlation between numerical variables when a direct linear relationship was not present. One-way ANOVA tests were performed on each of the seven numerical variables matched to the two categorical variables to discern differences in mean values between feedstock and reactor configuration groups. One-way ANOVA of mean reactor working volume by feedstock type was ignored since one is not a response to the other.

Results and Discussion

Univariate Data Analysis

The meta-analysis revealed trends not previously reported in MFC literature. Grouping by feedstocks showed that 35 different feedstock groups had been examined in waste-fed MFC literature, with the most prominent feedstocks being tested including brewery, dairy, manure, domestic, and wine wastewaters (see Fig. 1). Records were then grouped by reactor configuration (see Fig. 2). Overall, 13 different reactor configuration groupings were used to describe the dataset, with the most prominent being single-chamber configurations ($n = 47$) and double-chamber ($n = 45$). The emphasis on single- and double-chamber configurations in the literature was understandable due to their simplicity in construction and function.

Summary histograms of each of the numerical variables were generated (see Fig. 3). These histograms indicated that four of the numerical variables showed a log-normal distribution, presumably due to results often being orders of magnitude apart. COD removal efficiencies, however, skewed left in their distribution, indicative of the maximum value of 100% with

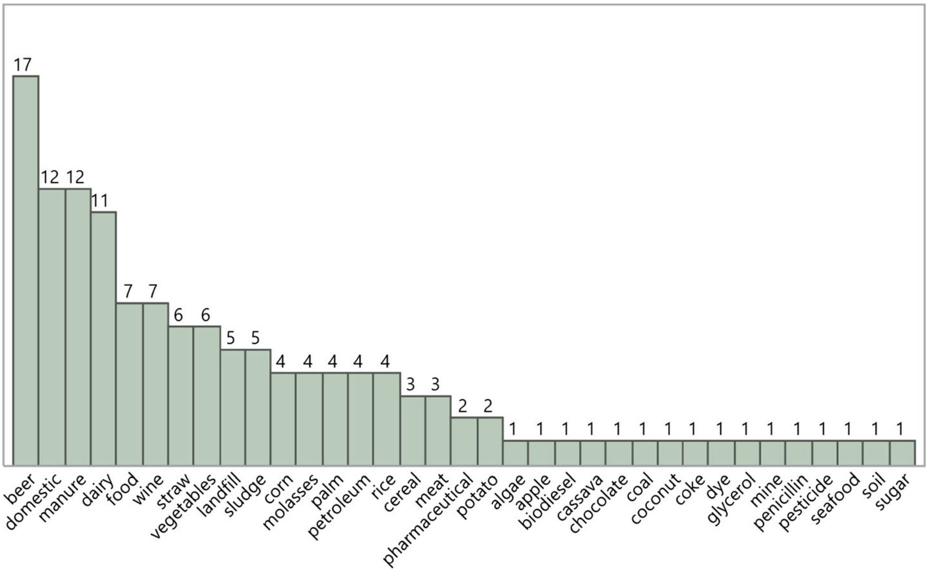

Fig. 1 Waste feedstock groups used in published MFC research and considered for meta-analysis

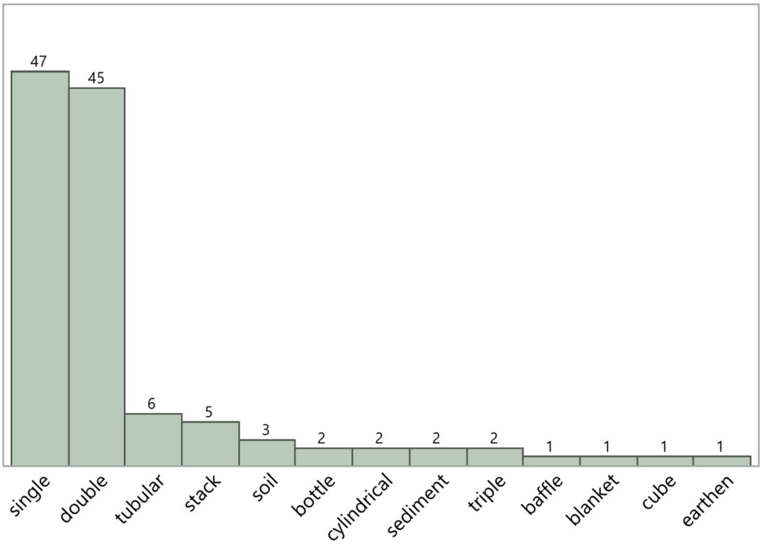

Fig. 2 MFC reactor configuration groups employed in published MFC studies using waste substrates

many data points showing high COD removal efficiencies. This skew left behavior may be due to the tendency of experimenters to operate units at long hydraulic retention times to increase COD removal efficiencies. Coulombic efficiencies displayed a skewed right distribution, indicating that high coulombic efficiencies are rare in waste-fed MFCs. Additionally, OCV max displayed a more normal distribution than other numerical variables, indicating that OCV max may be a normally distributed random variable that depends randomly on multiple experimental factors.

Multivariate Data Analysis

Unlike previous MFC literature, this meta-analysis enabled simultaneous multivariate analysis of 134 unique MFC experiments to indicate significant findings across all MFC experimentation using waste substrates. A scatterplot matrix was created for multivariate analysis, showing a regression for each of the seven log-transformed numerical variables compared against the other six (see Fig. 4). Correlations were estimated using the restricted maximum likelihood (REML) method. Statistical significance was analyzed with hypothesis tests for the population correlation coefficient. Pearson correlation coefficients (r) were generally poor despite statistical significance, indicating low predictive power of one variable against another but high probability of the existence of linear relationships. p values were examined with Benjamini-Hochberg correction using a chosen false discovery rate of 0.1. Control of false discovery rate did not change the significance of findings. Power density in mW/m^2 correlated positively with OCV max ($p < 0.05$), which fits into previous research on how increasing OCV max values increase the total power generated by MFCs. Power density in mW/m^2 also correlated negatively with COD removal % ($p < 0.05$), indicating there may be a connection between high COD removal but low power conversion with an optimal value being lower. This may be due to long periods of low power output that occurs when MFCs are operated to exhaust as much COD as possible during a long incubation. In addition to the previous findings, power density in mW/m^2 correlated positively with COD load ($p < 0.005$), indicating

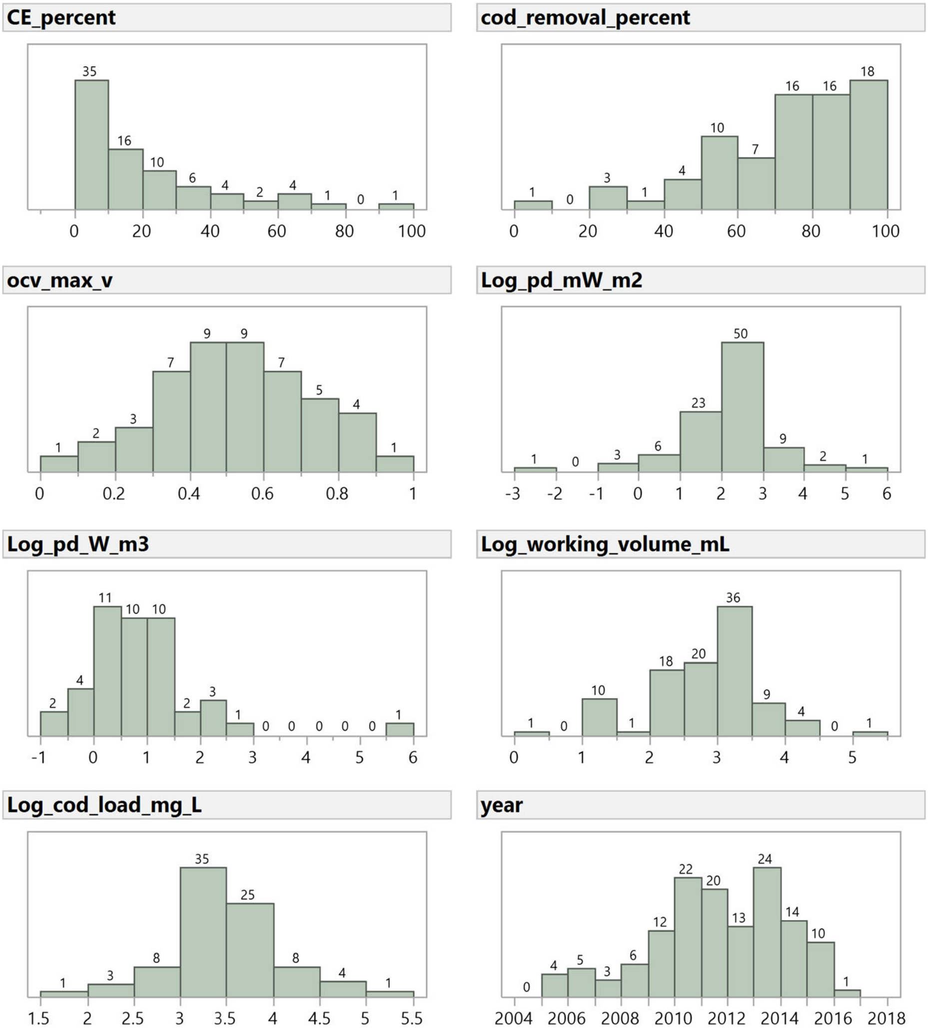

Fig. 3 Summary histograms of numerical variables for collected MFC experiments. The variables (left to right, top to bottom) are % coulombic efficiency, COD removal as % of load, OCV (open-circuit voltage) max in volts, log transform of the power density in mW/m^2, log transform of the power density in W/m^3, log transform of working volume in mL, COD (chemical oxygen demand) load in mg/L, and year the MFC experiment was published

that increasing feedstock concentrations to a certain extent generally yield increasing power densities.

Coulombic efficiencies yielded surprising data on MFC performance. Coulombic efficiencies correlated with both normalized power density measurements, but correlated better with volumetric power density measured in W/m^3 ($p < 0.0005$) than with area-normalized power density in mW/m^2 ($p = 0.0622$). Coulombic efficiency correlated negatively with working volume ($p < 0.05$), indicating that larger working volumes may limit coulombic efficiency through mass transfer and diffusion limitations. This finding is singularly important in that it could guide research on optimizing MFCs for minimal mass transfer restrictions. Coulombic

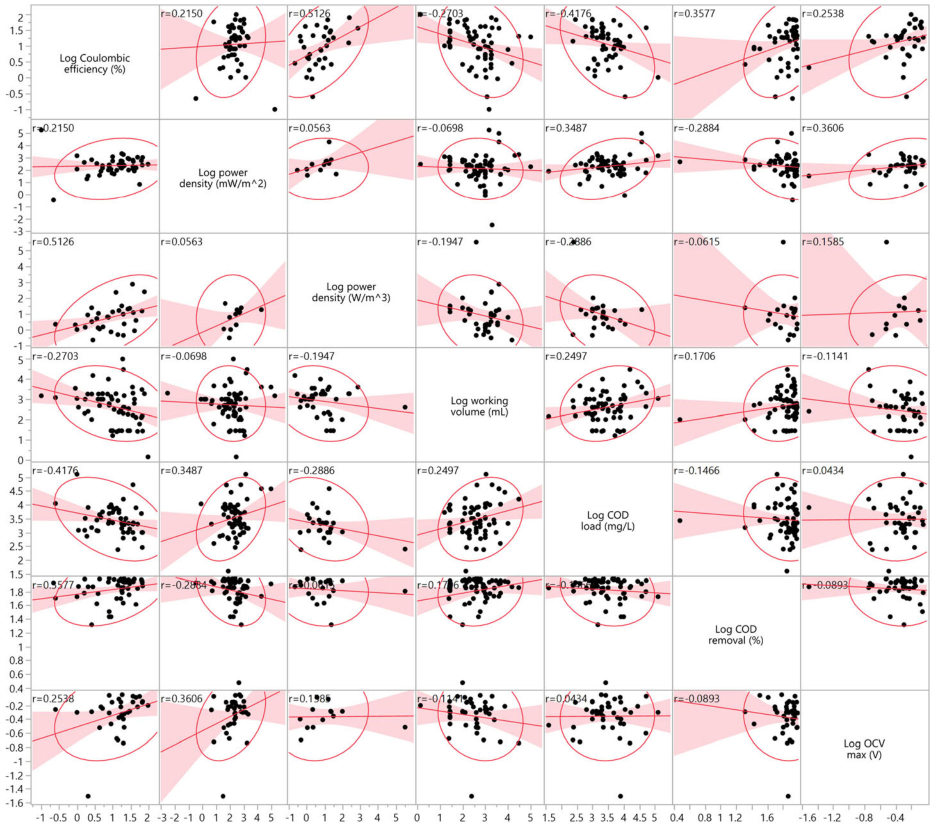

Fig. 4 Scatterplot matrix of the seven numerical MFC data fields. Pearson's correlation coefficient and resulting tests for significance of correlation were conducted without removal of outliers and with Benjamini-Hochberg correction for multiple comparisons. Correlations were estimated with restricted maximum likelihood (REML) method. Significant results are signified with one asterisk ($p < 0.05$) or two ($p < 0.01$) next to the Pearson correlation coefficient values. Straight lines indicate least-squares regression. Shaded regions define the 95% level confidence curves for the fitted regression line. A 95% bivariate normal density ellipse is shown in each scatterplot

efficiency correlated negatively with COD load ($p < 0.01$), indicating that there may be an optimal loading rate for waste-fed MFCs beneath their maximum COD load. Coulombic efficiency correlated positively, on the other hand, with COD removal percent ($p < 0.01$), indicating that biomass-to-electricity conversion efficiency is related to biomass consumption efficiency. Finally, working volume and COD loading rates correlated positively ($p < 0.05$), indicating that larger MFC working volumes may be used for scale-up experiments with higher COD-loading rates. This may be an artifact of how researchers set up experiments rather than a reflection of MFC operation since both values are independently chosen by the experimenter. Additionally, as this was an exploratory data analysis, these findings should be considered hypothesis-generating and studied further.

ANOVA tested the effect of single and double reactor configurations, the most commonly reported, on each of the numerical variables. Data was analyzed with a two-tailed t test. Double-chamber reactors showed significantly higher OCVs than single-chamber reactors ($p < 0.005$) (see Fig. 5). This effect may be due to the exclusion of oxygen from the anode chamber in double-chamber reactors. These findings indicate the usefulness of including OCV max results in MFC experiments and subsequent review articles.

Multiple linear regression was performed for both power density terms and OCV max as a function of coulombic efficiency, working volume, COD load, and COD removal. No significant models were found due to the amount of multicollinearity between model effects. Variance inflation factors > 10 were observed for all model effects.

Pant et al. [12] reported maximum current density at maximum power rather than the maximum power density itself, which was different from other reviewers and resulted in the exclusion of their data from the meta-analysis. COD reduction percentages and coulombic efficiencies were not reported which limited the data analysis as well.

Overall, the implications of this meta-analysis that have not been gleaned from past reviews is that large datasets allow for modeling MFC performance (power density, coulombic efficiency, etc.) as a function of operational parameters (substrate, architecture, COD loading, etc.). For example, building a dataset of 134 unique MFC experiments allows for multiple research questions on optimizing MFC operation to be asked at the same time in a way that is impractical for a single researcher to do. Collating MFC performance and metadata can thus facilitate testing of hypotheses that were not targeted by the individual studies. For example, this study tested the hypothesis that single- and double-chamber MFCs exhibit differing performance across all waste substrates utilized for MFCs in literature, a question that cannot be answered by an individual study. This method of questioning will lead to better models of how MFC performance changes as a function of operational characteristics, and lends itself to optimization modeling that can answer the questions of the best COD load or working volume to achieve a maximum power density, for example. This analysis lends itself particularly well to response surface methodology once a large, complete data set is built. In the future, clear reporting and noting all relevant metrics will lead to better modeling. Finally, it is worthwhile to note that the MFC microbiome is potentially different in each of these 134 different experiments, but these data are not available in the publications or online. The growing accessibility of high-throughput 16S rRNA gene sequencing, however, can support this goal. Building a database that allows for the inclusion of microbiome data will allow for tools to be utilized that ask even better questions about the relevancy of bioengineering to MFC

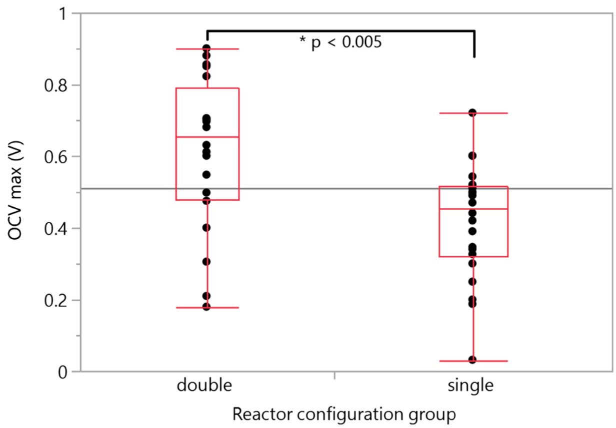

Fig. 5 Box plot of double-chamber MFCs versus single-chamber MFCs in terms of maximum open-circuit voltage (V). The p value corresponds to t testing of mean open-circuit voltages (OCV) factored by the two most studied reactor configuration groups

performance. In this way, MFC functional data will be coupled with community data such that conclusions can be drawn about which specific microbes and which communities of microorganisms function best as exoelectrogens.

To resolve the issue of inconsistency in data reporting and for MFC informatics to move forward, we propose that individual papers standardize reported information in a table within the document in a format that is conducive to export and data analysis. This table may contain the two categorical variables and seven numerical variables presented here, as well as the anode and cathode type information reported by Pandey et al. In the future, we propose an online database be used to crowdsource this information. Each record may be paired with a DOI (digital object identifier) to function as a unique record key for the literature reference. This will enable comparison between experiments in the field of MFC research, similar to the MIAME convention in microarray experimentation (the Minimum Information About a Microarray Experiment) [1]. We similarly propose a MIAMFCE convention—Minimum Information About a Microbial Fuel Cell Experiment—to attain statistically relevant results across multiple experiments for the expanding field of MFC research. The groundwork for these standards has been laid by Logan et al. [8] in first defining what metrics should be measured and Sharma et al. [15] in defining the best denominator for normalizing power readings such as electrochemically active surface area; in contrast to these standards which enumerate all possible MFC metrics, we propose defining explicit conventions for minimum data collected and specific units for reporting in both MFC literature and reviews.

Data fields suggested for inclusion (see Table 1) are feedstock type, feedstock group, reactor type, reactor configuration group, coulombic efficiency (%), power density (mW/m^2), power density (W/m^3), working volume (mL), COD load (mg/L), COD removal (%), OCV max (V), anode type, and cathode type. These specific data fields were chosen because (a) this minimum set of fields was found across multiple reviews that aggregated MFC data, indicating these data fields are already in common usage, and (b) these metrics provide a framework for response-modeling the performance of MFCs in the future because it includes both independent variables (feedstock type, reactor type, working volume, COD load, anode type, cathode type) and dependent variables (coulombic efficiency, power densities, COD removal, OCV max).

Additionally, future creation of an online database that aggregates the data contained herein and that of studies which appear in the future as well as crowdsourcing the data entry could enable the global research community to upload individual MFC results for statistical analysis, not only for MFCs fed with wastes. This database could be modified by the research community and further analyzed by MFC researchers. A large collection of data of this type enables model building, imputing missing data, and determining optimal avenues for future research.

Conclusions

To the authors' knowledge, this is the first effort to produce an MFC meta-analysis that catalogues research findings to engender straightforward data analysis and statistical regressions. Multiple linear regression of power density and OCV max produced no significant statistical models due to the amount of multicollinearity between model effects. However, our results show that statistically significant effects can be detected regarding MFC configurations, operating conditions, and response interactions. Findings such as the negative correlation between Coulombic efficiency and working volume will guide future MFC research towards

Table 1 Variables suggested for inclusion in the MIAMFCE convention—Minimum Information About a Microbial Fuel Cell Experiment—to attain statistically relevant results across multiple experiments. These variables were also included in this meta-analysis. Feedstock group is the umbrella grouping for specific type of feedstock, e.g., "dairy" is the feedstock group for "milk processing wastewater." Similarly, reactor configuration group is the umbrella term for specific reactor types

Variable	Units	Data type
Feedstock type	N/A	Categorical
Feedstock group	N/A	Categorical
Reactor type	N/A	Categorical
Reactor configuration group	N/A	Categorical
Coulombic efficiency	%	Numerical
Power density (by area)	mW/m^2	Numerical
Power density (by volume)	W/m^3	Numerical
Working volume	mL	Numerical
COD load	mg/L	Numerical
COD removal	%	Numerical
OCV max	V	Numerical
Anode type	N/A	Categorical
Cathode type	N/A	Categorical

N/A not applicable

designing units with minimal mass transfer limitations. These findings will inform future research to improve MFC performance and understand exoelectrogenic microbial activity in MFCs. The emerging field of MFC informatics can draw conclusions from multiple MFC experiments.

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