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# Anodic microbial community diversity as a predictor of the power output of microbial fuel cells

James P. Stratford [a,1,2], Nelli J. Beecroft [a,2], Robert C.T. Slade [b], André Grüning [c], Claudio Avignone-Rossa [a,*]

[a] Department of Microbial and Cellular Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK
[b] Department of Chemistry, University of Surrey, Guildford, Surrey GU2 7XH, UK
[c] Department of Computing, University of Surrey, Guildford, Surrey GU2 7XH, UK

## HIGHLIGHTS

- There is a correlation between microbial consortia diversity and electrical output.
- Diversity indices correlate with power in bivariate and multiple linear regression.
- Shannon index correlation with power is stronger than richness or Simpson index.
- Shannon diversity and DNA content predict power output in microbial communities.

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## ABSTRACT

The relationship between the diversity of mixed-species microbial consortia and their electrogenic potential in the anodes of microbial fuel cells was examined using different diversity measures as predictors. Identical microbial fuel cells were sampled at multiple time-points. Biofilm and suspension communities were analysed by denaturing gradient gel electrophoresis to calculate the number and relative abundance of species. Shannon and Simpson indices and richness were examined for association with power using bivariate and multiple linear regression, with biofilm DNA as an additional variable. In simple bivariate regressions, the correlation of Shannon diversity of the biofilm and power is stronger ($r = 0.65$, $p = 0.001$) than between power and richness ($r = 0.39$, $p = 0.076$), or between power and the Simpson index ($r = 0.5$, $p = 0.018$). Using Shannon diversity and biofilm DNA as predictors of power, a regression model can be constructed ($r = 0.73$, $p < 0.001$). Ecological parameters such as the Shannon index are predictive of the electrogenic potential of microbial communities.



## 1. Introduction

Microbial fuel cells (MFCs) are a promising technology for the generation of energy and treatment of wastewaters (Sun et al., 2010; Rahimnejad et al., 2012). These devices are fed substrates which are processed by the metabolism of the microorganisms present in the anode (either single species or multi-species consortia). As the system is kept under anaerobic conditions, organisms will use the anode of the MFC as their terminal electron acceptor (Logan et al., 2006). A number of microbial species has been observed to be electrogenic, and representatives of most classes of bacteria have been reported to be present in the microbial communities used in MFCs (Logan et al., 2006; Nimje et al., 2012). Many different parameters affect the power output and performance of MFCs. In addition to internal resistance, cathode performance and proton transfer, the power output of an MFC will also depend on the efficiency with which the community (usually attached to the anode as a biofilm) transfers electrons to the anode, and the potential link between community structure and functioning in MFCs has been recently shown for the first time (Wrighton et al., 2010). While a great variety of organisms have been shown to be electrogenic, the taxonomic structure of communities is highly variable with no established taxonomic rules as to what constitutes an electrogenic organism. It has also been observed that there is no ideal electrogenic consortium, and observed trends include a tendency for enriched mixed electrogenic communities to contain a larger proportion of β-proteobacteria (Chae et al., 2009). While communities have been characterised in previous work, generally studies focus on variation in substrate, reactor design, or monocultures of novel organisms (Di Lorenzo et al., 2010; Kiely et al., 2011;

* Corresponding author. Tel.: +44 1483 686457; fax: +44 1483 686401.
*E-mail address:* c.avignone-rossa@surrey.ac.uk (C. Avignone-Rossa).
[1] Current address: School of Biology, University of Nottingham, Nottingham NG7 2RD, UK.
[2] JPS and NJB contributed equally to this manuscript.

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Wang et al., 2011). Interestingly, ecological models to discover predictive relationships between fundamental community ecology and power output in replicate systems have not been used to a large extent in MFC research. Diversity is a logical starting point for this type of analysis, as is an assessment of the value of different methods for its measurement. It has been observed that diversity varies significantly with design, and links have been made between more powerful fuel cell architecture and increased diversity. The systems that have been compared were not identical in design and operating parameters, lending the situation to interpretation (Kim et al., 2011; Sun et al., 2010).

The diversity and abundance in microbial communities can be assessed by a number of methods commonly used in ecological studies. The Shannon and Simpson indices of diversity are two of the most widely used measures for biodiversity in macro ecology (Keylock and Lane, 2005), while richness, a measure of species number, is also usually employed (Grunewald, 2006). The Shannon index has been used in the analysis of microbial communities (Gafan et al., 2005; Steen et al., 2010). Interestingly, no examples could be found in microbial ecology of biodiversity being examined as a predictive independent variable for a performance measure rather than a dependent measure in its own right. This is likely to be because most ecological analyses assume biodiversity is an inherently desirable outcome, rendering it the dependent variable in analysis. Despite the important role of microbial communities associated with the anode, there has not been a systematic quantitative analysis of the predictive power of different measures of diversity on the output of microbial fuel cells.

The objective of this study is to analyse the predictive power of the Shannon index, the Simpson index and the richness of the anodic microbial communities with regard to the power output of replicate MFCs over the course of 91 days. As different measures of diversity weigh low and high abundance components differently, it is interesting to analyse which notion of diversity best captures those structural aspects that support community function. Richness, the inverse Simpson index and the Shannon index are members of one family of indices ${}^{q}D$ (Hill, 1973; Jost, 2006; Tuomisto, 2010), with the parameter $q$ determining how a particular index weighs relative differences in the component abundances. Lower values of $q$ emphasise the contribution of low abundance components to diversity, while higher $q$ values highlight the contribution of predominant components. The case of $q = 0$ corresponds to species abundance being ignored completely and only their presence or not is taken into account: ${}^{0}D$ is the species richness. The case of $q = 1$ gives all component abundances their "natural" weight; an example of ${}^{1}D$ is the Shannon index. Finally ${}^{2}D$ is the (inverse) Simpson index, a measure of the diversity of the system. The numerical value of all indices ${}^{q}D$ is interpreted as the "effective species number", ESN (Tuomisto, 2010), and all ${}^{q}D$ yield value $N$ if $N$ species are present with equal abundance. Hence, it can be seen as the number of evenly distributed species required to produce the observed index value. The ESN is considered to represent a measure of "true diversity", and the different ${}^{q}D$ allow comparisons between variables to be carried out on a scale using the same units.

Richness is the simplest measure of community diversity, and is defined as the number of distinct species present within a given community. Richness does not take account of the relative abundances of the species present or any other quantifiable properties of the community. Hence the effective number of species for this measure is the actual number of species. Of the three measures considered here, richness gives the least information about the distribution of species.

The Shannon index is a measure of information entropy (Cover and Thomas, 1991), and describes not only the presence and absence of species but also the information contained in their relative abundances. The sum of the product of the probabilities with their natural logarithms gives the value of the Shannon index for a community.

$$H' = -\sum_{i=1}^{R} P_i \ln P_i$$

To convert the Shannon index into the effective species number the exponential is taken.

$$ESN_H = {}^{1}D = \exp H'$$

The Simpson index $\lambda$ is the sum of the squares of the fractional species abundances, and is used to assess diversity.

$$\lambda = \sum_{i=1}^{R} P_i^2$$

where $P_i$ is the fractional abundance of the $i$th species.

The Simpson index value represents the probability that two organisms chosen at random from the community will be of the same species. Throughout this work, the inverse has been used

$$ESN_G := {}^{2}D = \frac{1}{\lambda}$$

## 2. Methods

### 2.1. MFC setup and operation

Four replicate continuously sucrose-fed microbial fuel cells (MFC1, MFC2, MFC3, and MFC4) were used. The single-chamber MFCs consisted of anode chambers (9 $cm^3$) and cover plates made of Perspex, with stainless steel metal plates serving as a contact between the cathode and the electrical circuit. The anode electrode contained a carbon fibre veil (PRF Composite Materials, UK) with polyvinyl alcohol binder, with a geometric area of 32 $cm^2$, which was placed inside the anode chamber and connected to an electrical circuit with an insulated Ni/Cr wire (Advent Research Materials, UK) knitted across the multi-layered anode. The air-breathing cathode consisted of type A carbon cloth (9 $cm^2$, E-TEK) coated with 4 mg $cm^{-2}$ of Pt black catalyst with polytetrafluoroethylene binder. The platinum side of the cathode was painted with 0.5–1.0 mg $cm^{-2}$ of Nafion perfluorinated ion-exchange ionomer (5% w/v dispersion in lower aliphatic alcohols and $H_2O$, Aldrich). A Nafion-115 proton-exchange membrane (20 $cm^2$, DuPont) separated anode chamber from the cathode.

MFCs were started up by suspending anaerobic digester sludge (sieved through 0.6-mm mesh) in sucrose-containing medium (per litre: $NH_4Cl$, 0.31 g; $NaH_2PO_4$. $H_2O$, 5.38 g; $Na_2HPO_4$, 8.66 g; KCl, 0.13 g (pH 7.0) (Kim et al., 2007), supplemented with trace element (12.5 mL) and vitamin (12.5 mL) solutions (Lovley et al., 1984) at a 10% volume ratio. The concentration of sucrose in the medium was 5 $gL^{-1}$ in batch operation and 0.1 $gL^{-1}$ in continuously fed MFCs. The MFCs were operated in batch-mode during the initial enrichment period (approximately 2 weeks). During that time, the anodic suspension was repetitively replaced (five times) initially by mixing (1:9) anodic suspension with fresh $N_2$-purged sucrose-containing medium and then after 1 week by replacing the entire volume of the anodic suspension with fresh medium. MFCs were operated in batch-mode until repeatable cycles of voltage generation were observed. In continuous-mode, medium was supplied to MFCs at a flow rate of 0.18 mL $min^{-1}$ and purged with $N_2$ gas. The MFCs were operated at room temperature (21–22 °C). MFC voltage was monitored using an Arbin BT2000 battery tester (Arbin Instruments, USA) controlled with MITS Pro software (Arbin Instruments) across a fixed external resistance of 40 kΩ. Polarisation curves were recorded with decreasing external resistance (700 kΩ–500 Ω) and measuring the decrease in voltage.