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Environ. Sci. Technol., Just Accepted Manuscript • DOI: 10.1021/acs.est.8b06044 • Publication Date (Web): 07 Jan 2019

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Deterministic assembly and diversity gradient altered the biofilm community performances of bioreactors

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ABSTRACT ART

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ABSTRACT

Community assembly process (determinism vs. stochasticity) determines the composition and diversity of microbial community, and then shapes its functions. Understanding this complex process and its relationship to the community functions becomes a very important task for the applications of microbial biotechnology. In this study, we applied microbial electrolysis cells (MECs) with moderate species numbers and easily tractable functions as a model ecosystem, and constructed a series of biofilm communities with gradient biodiversity to examine the roles of community assembly in determining microbial community structure and functions. After stable biofilms formed, the best MEC reactor performances (e.g., gas productivity, total energy efficiency) were achieved in the group which biofilms had the second highest $\alpha$-diversity, and biofilms with even lower diversity showed declining performance. Null model analyses indicated that both deterministic and stochastic assembly played roles in the formation of biofilm communities. When deterministic assembly dominates this formation, the higher diversity of biofilm community would generally show better reactor performance. However, when the stochasticity dominates the assembly process, the bioreactor performance would decline. This study provides novel evidence that the assembly mechanism could be one of the key processes to shift the functions, and proposes an important guidance for selecting the most efficient microorganisms for environmental biotechnologies.
INTRODUCTION

Microorganisms, regulating all major biogeochemical cycles, serve essential roles in environmental biotechnology.\textsuperscript{1,2} With the attempts linking microbial communities with environmental processes, researchers believe that understanding the ecological mechanisms of community assembly controlling community diversity and its relationships to community functions becomes a very important task for the application of microbial biotechnology.\textsuperscript{3,4} Knowledge about the process and factors controlling community assembly is critical to our understanding of the patterns of species composition and diversity. Traditional niche-based theory hypothesizes that deterministic factors such as species traits, interspecies interactions (\textit{e.g.}, competition, mutualisms, and predation), and environmental conditions (\textit{e.g.}, pH, temperature, moisture) govern community structure.\textsuperscript{5,6} Consequently, the deterministic process can directly determine the microbial communities under specific environmental conditions.\textsuperscript{7} In contrast, neutral theory assumes that all species are ecologically equivalent and community structures are governed by stochastic processes, which typically include random birth-death events, colonization, extinction and speciation.\textsuperscript{8} Microbial communities assembled via stochastic processes may exhibit less of a direct link between the environment and processes.\textsuperscript{9} Currently it is recognized that community assembly is simultaneously influenced by both deterministic and stochastic processes.\textsuperscript{10-12} Although the relative importance of each process in controlling community structure, succession, and biogeography, has been intensively studied recently,\textsuperscript{13-15} its association with the community functions is still elusive.
The relationship between biodiversity and community functions is also important.\textsuperscript{16} Biodiversity is the most important point in community ecology, and considered to be closely related to the ecosystem functions.\textsuperscript{17-19} For macro-ecology, studies have shown that loss of biodiversity can have significant consequences for ecosystem process, for example, the productivity and stability of ecosystems.\textsuperscript{20,21} However, this relationship is poorly understood in microbial ecosystem due to its intrinsic traits, such as large number of species, the difficulty in controlling them, and high complexity of their interactions.\textsuperscript{22} Since even the simplest microbial communities from natural environment could contain tens to more than thousands of species, it is almost impossible to experimentally verify which species in a habitat are actively part of the community, or are performing key functions.\textsuperscript{23} The diversity and assembly of natural microbial communities are also very difficult to control or characterize.\textsuperscript{24} Therefore, simplified model ecosystems that retain the key features of natural communities are desperately needed to assess the role of key ecological, structural and functional features of communities in a feasible way.\textsuperscript{22,25} However, those model ecosystems are quite rare.

Microbial electrolysis cell (MEC), as a type of bioelectrochemical system, is a promising technology for efficient and sustainable hydrogen production from biodegradable organic matter with little energy input.\textsuperscript{26,27} In the initial start-up stage, the microorganisms from an original inoculation source (\textit{i.e.}, activated sludge, wastewater) are strictly selected to attach the anode of a MEC. After a stable biofilm has been successfully formed, the exoelectrogenic microorganisms, as major components, would generate electrons through the oxidation of organic matter.\textsuperscript{13} The released protons and
electrons flow to the cathode to produce hydrogen on the cathodic side through the addition of a small voltage to the circuit.\textsuperscript{28,29} The gas productivity and relevant bioelectrochemical efficiency could be regarded as measurable functions for biofilm microbial community in macroscopic performance.\textsuperscript{30,31} Meanwhile, the whole MEC is a closed microbial reactor with moderate richness of bacterial species, and their taxonomy and functional genes can be easily detected with novel metagenomic tools.\textsuperscript{32} This closed ecosystem with relatively simple microbial community and easily measurable performance is quite appropriate for microbial ecological studies.\textsuperscript{33} Our previous study found that microbial communities in MEC bioreactors were mainly shaped by stochastic processes.\textsuperscript{13} However, it is still not clear whether, and how, their relative importance varies within a diversity gradient.

In this study, we used a microbial electrolysis cell (MEC) as a model system to acquire a better understanding of the role of the diversity in microbial community assembly at both taxonomic and functional aspects. Experimentally, we constructed a series of biofilm communities with a diversity gradient by diluting the input initial community at 1, 10\textsuperscript{2}, 10\textsuperscript{4}, 10\textsuperscript{6} times (6 replicate bioreactors in each concentration), and applied 16S rRNA gene sequencing to assess their diversity. The performances of those reactors have been simultaneously measured. We hypothesized that (i) the higher biodiversity may have the best performances following the general ecological rule; (ii) stochastic assembly is critical to form the MEC microbial communities with higher inoculated concentration, but the roles of deterministic assembly (\textit{i.e.}, selection) could increase along with the decrease in the inoculation concentration.
MATERIALS AND METHODS

Reactor construction and operation. The activated sludge (AS) was collected from Beixiaohe Reclaimed Water Plant (Beijing, China) as the initial inoculation solution. Microbial electrolysis cells (MECs) used in this study were single chamber with membrane-less reactor (Supplementary Figure S1). Four groups of single chamber reactors, with six replicates per group, were set up with gradient diluted inoculations (A, 1:1; B, 1:10^2; C, 1:10^4; D, 1:10^6, V/V) to obtain different biofilms with a diversity gradient. All reactors were run under identical conditions and followed previous research under neutral condition in an effort to minimize technical variations. The continuously recorded current could reflect reactor status timely and directly relate to anodic biofilm community performance, MEC reactors can be considered to be successfully started once current is higher than 1mA, recording as the start-up time. Five parameters of MEC performance with different meanings, including hydrogen productivity rate, hydrogen recovery, electrical energy recovery, substrate energy recovery, and total energy efficiency, were further evaluated. Details for reactor operation and calculative processes of performance parameters were provided in Supporting Information.

DNA extraction and microbial community analysis. At the end of MEC operation, all anodic biofilm samples were collected. Microbial genomic DNA of the anodic biofilms was extracted following the protocol of the FastDNA SPIN Kit for Soil (MP-Biomedicals). Thereafter the V4 region of 16S rRNA was amplified using the primers 515F (5’-GTGCCAGCMGCGGTAA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) for high-throughput sequencing. Sequencing
run was conducted on MiSeq (Illumina) for 2 × 250 bp paired-end sequencing in Central South University (Changsha, China).

Raw data was processed using an in-house pipeline (http://mem.reees.ac.cn:8080) integrated with bioinformatics tools. UParse\textsuperscript{36} was used to remove chimeras and classify the sequences into operational taxonomy units (OTUs) at 97% similarity level. Random resampling was performed with the lowest number of sequences (> 20,000 sequences) among samples. This resampled OTUs summary table was used for further statistical analyses. Representative sequences of each OTU were then aligned using PyNAST\textsuperscript{37} against the GreenGenes database, and the maximum-likelihood tree was constructed with FastTree\textsuperscript{38} for further phylogenetic analysis. Raw sequences were submitted to the NCBI Sequence Read Archive (SRA) under the accession number SRP127703.

**Ecological and Statistical analysis.** In this study, we calculated α-diversity with five indexes, including Shannon and Inverse Simpson indexes, Richness, Chao1, and Phylogenetic diversity (PD) to measure the biodiversity of microbial community in MEC. Phylogenetic diversity (PD) was measured based on Faith’s approach,\textsuperscript{39} which is the sum of total phylogenetic length of OTUs in each sample, and calculated using Picante package in R (v.3.2.5). To determine the site-to-site variability in microbial community composition of these reactors, two different metrics for measuring β-diversity were evaluated, including Bray-Curties and Jaccard indexes, using vegan package (v.2.3-5) in R (v.3.2.5). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance was used for representing the microbial community structure changes. Spearman correlation test was applied to correlate the MEC performance with α-diversity and the
relative abundance of genera *Geobacter* and *Methanobrevibacter*. The significance between two groups was determined by two-tailed Student’s *t* test, and significance comparing the four groups was obtained using one-way analysis of variance (ANOVA). Mantel test were used to test the correlation between the MEC performance and β-diversity indexes. Other statistical methods were provided in *Supporting Information*.

**Null Model Analysis.** To investigate the relative importance of deterministic and stochastic processes underlying community assembly, we performed the null model analysis using abundance-based similarity metrics as previously reported.\textsuperscript{40-42} Here, we used the Bray-Curtis distance as the dissimilarity metric (*D*\textsubscript{obs}) across all communities, ranging from 0 to 1. The observed similarity (*S*\textsubscript{obs}) across the actual communities was complementary to the dissimilarity, that was, \( S_{\text{obs}} = 1 - D_{\text{obs}} \). Then the randomly expected similarity (*E*\textsubscript{exp}) of null expected communities could be generated using null model algorithm keeping richness the same as observed and species occurrence frequencies proportional to observed ones.\textsuperscript{40,41} This procedure was repeated 1,000 times. An average null expected similarity (*E*\textsubscript{exp}) and its SD could be estimated based on 1,000 drawings. The nonparametric permutation test, permutational multivariate analysis of variance (PERMANOVA), was used to test the significance of the difference of the biofilm communities between the observed similarity matrices with the null model expectation. If community assembly is primarily shaped by deterministic processes, the similarity observed across the actual communities will be significantly higher than the random null expectation.\textsuperscript{14,43} On the contrary, if ecological drift (e.g., stochastic colonization and extinction) and possibly priority effects leading to multiple stable
equilibria play predominant roles in determining community composition, the actual similarity observed will be statistically indistinguishable from the random null expectation.\(^\text{14}\)

Theoretically, deterministic processes can drive ecological communities more similar or more dissimilarity than null expectation.\(^\text{44}\) The difference between the observed and average null expectation can be used to quantitatively estimate the strength of determinism acting against otherwise stochastic forces in shaping community composition and structure,\(^\text{7}\) which is also termed as deterministic ratio (DR).\(^\text{14}\) The DR was measured as a proportion of the differences between the observed total similarity and the null expected similarity divided by the total similarity (DR = \((S_{\text{obs}} - E_{\text{exp}})/S_{\text{obs}}\)) and their average across all pairwise comparisons was used as a quantitative index for measuring the importance of determinism vs. stochasticity in shaping biodiversity.\(^\text{14}\) The relevant R code was available on [http://mem.rcees.ac.cn/download/](http://mem.rcees.ac.cn/download/).

**Ecological Processes Govern the Community Assembly.** To quantify the contributions of various ecological processes (e.g., selection and dispersal) to microbial community structure, we used a null-model-based statistical framework developed by Stegen *et al.*\(^\text{41}\) Firstly, we quantified βNTI (β nearest-taxa index) for all pairwise community comparisons using an in-house pipeline ([http://mem.rcees.ac.cn:8080](http://mem.rcees.ac.cn:8080)). The βNTI is based on a null model test of the phylogenetic βMNTD (β mean nearest-taxon distance), which was used to characterize the turnover in phylogenetic community composition. A value of |βNTI| > 2 indicates that observed turnover between a pair of communities is governed primarily by selection, which could be divided into
homogeneous selection ($\beta_{\text{NTI}} < -2$) and heterogeneous selection ($\beta_{\text{NTI}} > +2$). Details for
calculative processes were provided in Supporting Information.

As part of the second major step in this procedure, pairwise comparisons with
nonsignificant $\beta_{\text{NTI}}$ values were further evaluated by comparing observed Bray-Curtis
($BC_{\text{obs}}$) to Bray-Curtis expected under the randomization ($BC_{\text{null}}$). The value of
Bray-Curtis-based Raup-Crick ($RC_{\text{bary}}$) characterizes the magnitude of deviation between
$BC_{\text{obs}}$ and $BC_{\text{null}}$; the value of $|RC_{\text{bary}}| > 0.95$ was considered significant by dispersal,
containing homogenizing dispersal ($RC_{\text{bary}} < -0.95$) and dispersal limitation ($RC_{\text{bary}} >
+0.95$). However, if $|\beta_{\text{NTI}}| < 2$ and $|RC_{\text{bary}}| < 0.95$, then drift (referred to as
'undominated' processes) drives compositional turnover processes. Drift was used to
estimate the fraction that neither selection nor dispersal is the primary cause of
between-community compositional differences.

**RESULTS**

**Performance of MEC reactors with gradient diluted inoculation.** Four different
concentrations of activated sludge were inoculated into 24 MEC reactors. Once the
electric current in the circuit was higher than 1 mA, the MEC reactor was considered to
be successfully started and recorded as startup time (Table 1, Supplementary Figure S2).
Group A with highest inoculated concentration took nearly 5 days for initial startup,
while Group D with $10^6$ times diluted inoculation took more than one month for start-up
(Table 1), indicating that higher gradient diluted inoculation needed more time to
generate stable performance. In the end of MEC operation with diluted inoculation, the
total biomass attached on the anodic biofilms were quantified according to two different
analytic methods, quantitative PCR (qPCR) and volatile suspended solids (VSS) (Supplementary Table S1). The copy numbers of 16S rRNA genes in the biofilms from those four groups, ranged from $1.31 \times 10^{12} \pm 7.78 \times 10^{11}$ to $2.01 \times 10^{12} \pm 2.21 \times 10^{11}$ per g graphite brush. Meanwhile, the total cell numbers of biofilm communities derived from the amount of VSS showed a range from $1.65 \times 10^{12} \pm 2.81 \times 10^{11}$ to $1.84 \times 10^{12} \pm 1.81 \times 10^{11}$ per g graphite brush. Both the ANOVA analysis for the four groups and Student’s $t$ test for each two groups showed no obvious differences in the microbial biomass of biofilms operated with diluted inoculations (Supplementary Table S2).

Table 1. MEC performance response over a gradient of diversity.

<table>
<thead>
<tr>
<th></th>
<th>H$_2$ production rate (mL/mL reactor cycle)</th>
<th>Hydrogen recovery (%)</th>
<th>Electrical energy recovery (%)</th>
<th>Substrate energy recovery (%)</th>
<th>Total energy efficiency (%)</th>
<th>Start-up time (day) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.156 ± 0.127</td>
<td>72.8 ± 7.4</td>
<td>145.7 ± 15.3</td>
<td>83.7 ± 9.1</td>
<td>53.1 ± 5.7</td>
<td>5.00 ± 0.00</td>
</tr>
<tr>
<td>Group B</td>
<td>1.242 ± 0.094</td>
<td>80.7 ± 5.5</td>
<td>162.5 ± 11.1</td>
<td>89.7 ± 6.8</td>
<td>57.8 ± 4.2</td>
<td>7.33 ± 0.82</td>
</tr>
<tr>
<td>Group C</td>
<td>0.670 ± 0.117</td>
<td>42.0 ± 6.7</td>
<td>83.6 ± 13.9</td>
<td>46.1 ± 7.4</td>
<td>29.7 ± 4.8</td>
<td>25.60 ± 2.41</td>
</tr>
<tr>
<td>Group D</td>
<td>0.609 ± 0.050</td>
<td>39.6 ± 4.1</td>
<td>79.5 ± 8.6</td>
<td>45.1 ± 5.4</td>
<td>28.7 ± 3.2</td>
<td>35.4 ± 4.33</td>
</tr>
</tbody>
</table>

$^a$ Once the electric current in the circuit is higher than 1 mA, the MEC reactor is considered to be successfully started.

The other five performance parameters (hydrogen production rate, hydrogen
recovery, electrical energy recovery, substrate energy recovery and total energy efficiency) were measured after all bioreactors were running stably (Table 1). The hydrogen production rates of MEC groups with diluted inoculation were further visualized in figure 1. Surprisingly, Group B had performed the highest hydrogen yields compared with the other three groups (Student’s $t$ test, $P < 0.05$), while Group C and D obtained similar hydrogen yield with less difference ($P > 0.05$). Other performance indexes (hydrogen recovery, electrical energy recovery, substrate energy recovery and total energy efficiency) exhibited similar pattern with hydrogen production rate (Supplementary Figure S3). Thus, it seems that the gradient diluted inoculation resulted in a decrease of reactor performance in terms of the effectiveness production, but distinct from, the initial inoculation with the highest concentration (Group A).
Figure 1. Hydrogen production rates of MEC groups responding to inoculation concentrations, subsidiary with one-way ANOVA analysis and Student’s t test results, t value (P-value). Bold font means the significance at P < 0.05 level.

Microbial compositions of anodic biofilms in MECs. To determine the biodiversity of MEC biofilm communities, 16S ribosomal RNA (rRNA) gene was amplified and sequenced using high-throughput sequencing. After quality control of original reads, total 2,570,638 sequences were classified into 24 biofilm samples under 4 inoculated concentrations. An average of 85,688 ± 59,045 sequences per sample was obtained and the rarefaction curves indicated these numbers of sequences were enough to reach the saturation (Supplementary Figure S4). All samples were randomly resampled at 29,220 reads per each.

To further elucidate the major players in these bioreactors, relative abundance of microorganisms was investigated at phylum/class and genus levels (Figure 2, Supplementary Figure S5). Generally, the most abundant phyla/classes of anodic biofilms were Deltaproteobacteria, Betaproteobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Epsilonproteobacteria, and Gammaproteobacteria, accounting for over 90% total abundance across all four groups of biofilms, which were significantly distinct with the composition from the initial AS (Supplementary Figure S5). This was especially evident with the relative abundance of class Deltaproteobacteria, which was lower than 4% in the initial AS, but significantly increased to more than 70% in the biofilm communities from group B.
More specifically, the genus *Geobacter* was dominant in all MEC biofilms (Figure 2). Almost concordant with Deltaproteobacteria, *Geobacter* (accounted for more than half percentage in relative abundance) predominated in those four groups, but was among the rare species in the initial AS. For another dominant genus, *Methanobrevibacter*, belonging to phylum Euryarchaeota, which had higher percentage in Groups C and D (11.40% and 9.65%), was rare in the other two groups (0.17% in group A; 0.31% in group B), exhibiting completely opposite trend with *Geobacter*.

**Effects of diluted inoculation on biodiversity of anodic biofilms in MECs.** To investigate the microbial community in different groups, the shared and unique OTUs
were distinguished through a Venn diagram (Supplementary Figure S6). There were a total of 5,908 OTUs observed in the initial AS and anodic biofilms with diluted inoculation. The number of OTUs in the initial AS and different diluted groups, varied from 5,085 to 515, with 121 of these OTUs observed in all groups (Supplementary Figure S6A). In general, most initial OTUs were not observed in the anodic biofilms (65.25%, 3,318 of 5,085 total OTUs in initial AS). Besides, 4591 OTUs (77.71%) were identified as rare taxa (only containing 2.24% of total sequences), while the abundant taxa included 36 OTUs (0.61%) containing 70.93% of the total sequences (Supplementary Figure S6B and S6C). The distribution of rare and abundant OTUs across the initial AS and four groups of anodic biofilms showed that the abundant OTUs shared by multiple groups containing much higher relative abundance than those in initial AS, while the number of rare OTUs decreased more sharply in the anodic biofilms operated with diluted inoculations, which might contribute to the biodiversity gradient in the final biofilms (Supplementary Figure S6B and S6C, Supplemental Table S4).

The Faith’s phylogenetic diversity (PD) of microbial communities showed that the \( \alpha \)-diversity of all 24 anode biofilms (from 56.90 ± 6.10 to 14.17 ± 2.35) were significantly lower than those of initial AS (138.34 ± 2.78, Figure 3, Supplementary Table S3), indicating a strong selection on microbial species in MEC applying voltage and substrate. The highest PD values were obtained from Group A, significantly higher than the other three groups (Student’s \( t \) test, \( P < 0.05 \), Figure 3). Meanwhile, the PD values of Group B were also significantly higher than those of Group C and D (Student’s \( t \) test, \( P < 0.05 \)), but that of Group C, D showed less difference (Figure 3). The other four
diversity characteristics (Shannon index, Inverse Simpson index, Chao1 estimated richness and observed richness) performed similar pattern as the PD values (Supplementary Table S3, Supplementary Figure S7). These results indicated that α-diversities of biofilm communities were significantly decreased with the gradient decreasing concentrations of inoculations for Group A, B, C and D.

![Figure 3. Phylogenetic diversity (PD) of the microbial communities in MEC anodic biofilms.](image)

One-way ANOVA test was calculated among Group A, B, C, and D.

In terms of β-diversity, the non-metric multidimensional scaling analysis (NMDS) based on Bray-Curtis distance matrix exhibited that the microbial communities of the four groups were distinctly separated from other groups (Supplementary Figure S8). Further
three dissimilarity tests, multiresponse permutation procedure (MRPP), analysis of similarity (ANOSIM), and Permutational multivariate analysis of variances (PERMANOVA), also revealed that substantial variations in biofilm community structure were observed among these biofilm communities (Supplementary Table S5). Additionally, PERMANOVA also showed significant differences ($P < 0.05$) between each pair of groups (Supplementary Table S6). Both the $\alpha$-diversity and $\beta$-diversity analyses indicated that lower sludge inoculation concentrations could lead to lower species richness, and also changed the community structures in anode biofilms.
To investigate the associations between biofilm biodiversity and reactor performances, MEC performance parameters were correlated to five α-diversity indexes (Table 2) and β-diversities (Supplementary Table S7) by using Spearman correlation test and Mantel test, respectively. Three of the five α-diversity indexes (exceptions being Shannon and Inverse Simpson indexes), showed significantly positive correlations with hydrogen yield, hydrogen recovery, energy recovery, substrate recovery, and total energy efficiency ($P < 0.05$, Table 2). The polynomial fit method was the best fitting model between the hydrogen production rates and PD, indicating that diversity and function of MEC matched the binomial distribution (Figure 4). These results revealed that the biofilm diversity and function were positively correlated with reactor performance.
communities with the highest α-diversity from MEC reactors, may not achieve the best performance. Mantel test demonstrated that all MEC performances showed significant correlations with β-diversity in anode biofilm community (Supplementary Table S7), indicating the dissimilarity rates of microbial communities are highly associated with the differences among all bioreactors.

![Phylogenetic diversity vs. hydrogen production rate](image)

**Figure 4.** Fitted curve between the hydrogen production rates with the phylogenetic diversity (PD) of the biofilm communities with gradient diluted inoculation. The 95% confidence interval was shown by dotted lines.

**Ecological processes in the community assembly of anodic biofilms in MEC.**
The null model analysis was adapted to disentangle the importance of deterministic mechanisms from stochastic mechanisms underlying community assembly. Here, the regional species pool is defined as the total number of all OTUs found in all 24 bioreactors with diluted inoculation and 6 samples of initial AS. The permutational multivariate analysis of variances (PERMANOVA) test revealed that the observed similarity of actual communities was not significantly distinguishable ($P = 0.152$) from that of the null expectation for microbial communities in Group A, indicating that the stochasticity of community assembly was more important than the determinism in Group A (Table3). Whereas the significant difference ($P < 0.05$) between the observed similarities with the null expectation indicated the dominant position of deterministic community assembly in Group B, C and D (Table3).

To further quantify the relative importance of deterministic and stochastic processes in shaping the biofilm community structures, the relative ratio of determinism (DR) was calculated. Meanwhile, the stochastic ratio (SR), serving as the complement of the deterministic ratio (DR), was also calculated. Significant differences in DR was observed for the communities across different dilution groups (PERMANOVA: $F_{5,30} = 10.156, P < 0.001$). The embedded graph in Supplementary Figure S9 showed that the relative importance of deterministic processes would be significantly correlated ($P < 0.001$) with the magnitudes of the dilution ratio. The stochastic process contributed an average of 76.9% of the community variations at the initial AS, subsequently increased to 86.7% in Group A after MEC startup. However, the role of deterministic processes in microbial community composition increased substantially with the dilution ratio increasing, ranging
from 13.3% (Group A, non-dilution) to 51.0% (Group B, 10²-time dilution) and 54.4% (Group C, 10⁴-time dilution, Supplementary Figure S9). Interesting, the highest deterministic ratio (77.7%) was observed in the Group D with 10⁶-time dilution (Supplementary Figure S9), suggesting that the deterministic assembly might dictate the changes of the biofilm communities in the MEC anodes responding to the increasing gradient biodiversity.

Following the quantitative framework of nearest-taxa index (NTI), the βNTI values for all biofilm communities from MEC groups were quantified (Table 3). After diluted inoculation, the diversities of biofilm communities in Group B, C, and D, were shaped by homogeneous selection of deterministic processes (βNTI value < -2). However, the |βNTI| value < 2 and RC_{Bray} values of Group A < 0.95 indicated that the undominated stochastic processes mainly shape the biofilm community diversity with non-diluted inoculation.
Table 3. Significance test of the similarity between the biofilm microbial communities and null model simulations across different dilution ratio of inoculations, and $\beta$NTI and $\text{RC}_{\text{Bray}}$ values based on weighted Bray-Curtis distances.

<table>
<thead>
<tr>
<th>Bray-Curtis Mean of observed</th>
<th>Mean of null expected</th>
<th>F</th>
<th>$P^a$</th>
<th>$\beta$NTI $^b$</th>
<th>$\text{RC}_{\text{Bray}}$ $^c$</th>
<th>Ecological processes shaping biodiversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Similarity</td>
<td>Similarity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GroupA</td>
<td>0.590</td>
<td>0.536</td>
<td>2.586</td>
<td>0.152</td>
<td>-1.861</td>
<td>-0.204</td>
</tr>
<tr>
<td>GroupB</td>
<td>0.498</td>
<td>0.214</td>
<td>21.615</td>
<td><strong>0.001</strong></td>
<td>-3.653</td>
<td>-0.752</td>
</tr>
<tr>
<td>GroupC</td>
<td>0.329</td>
<td>0.136</td>
<td>14.135</td>
<td><strong>0.014</strong></td>
<td>-2.848</td>
<td>-0.736</td>
</tr>
<tr>
<td>GroupD</td>
<td>0.583</td>
<td>0.122</td>
<td>152.835</td>
<td><strong>0.001</strong></td>
<td>-2.399</td>
<td>-0.933</td>
</tr>
</tbody>
</table>

$^a$ Permutational multivariate analysis of variance (PERMANOVA) was conducted. $P$ values < 0.05 were in bold.

$^b$ $\beta$NTI ($\beta$nearest-taxon index) is based on a null model test of the phylogenetic $\beta$-diversity index $\beta$MNTD ($\beta$ mean nearest-taxon distance).

$^c$ $\text{RC}_{\text{Bray}}$ (modified Raup-Crick index) is based on a null model test of the Bray-Curtis taxonomic $\beta$-diversity index.
DISCUSSION

Ecological mechanisms (determinism vs. stochasticity) of community assembly play important roles in shaping microbial community composition and diversity, and thus its relationship to the community functions is a very important task for the application of microbial biotechnology, but still remains elusive. In this study, we applied MEC reactors as a model ecosystem, to investigate the changes of community performance (i.e., gas production) responding to the decreased biodiversity. In general, we found that the performance of MEC reactors would decline as biofilm diversity decreased, however the best performance was not achieved in the biofilms with the highest $\alpha$-diversity. Further null model tests revealed the biofilm community with highest diversity was formed under stochastic assembly process. According to these results, we propose a conceptual scheme to better understand the relationship between the biodiversity as shaped by different assembly processes and the performance responding to the inoculant’s gradient dilution (Figure 5).
Figure 5. Conceptual scheme for modeling the relationship between the micro-diversity with the performance responding to the gradient diluted inoculation. We conceptualize multiple species within different colors. Blue organisms in this figure represent the species that are well-adapted to and major functional groups in the given environment, while the red and grey ones represent the unexpected species for the community functions. Other organisms represent all species that are less adapted to the environment than the blue organisms, and might have positive or negative interactions with the blue organisms. In the present study, the environmental selection was the selection pressure from the environmental and operational conditions, which should be same for all reactors since we used identical nutrients and operation condition. The gray from shallow to deep indicates intensity of deterministic strength, and the width of arrow indicates the relative number of species that suitable for environmental selection. Gradient dilution of the initial community will sharply reduce the OTU numbers (Richness), indicating the decrease in $\alpha$-diversity of the inoculation. After the MEC startup, the decreased concentrations of the MEC inoculation will substantially increase the deterministic strength (Determinism) for the biofilm community formation, although which might be determined by the initial colonization.
(Stochasticity); following that, the distinguished biofilm communities with different abundance distribution of the dominant species, were obtained after a steady operation for several cycles. As a consequence, the MEC performance increase with the biofilm α-diversity increasing when the determinism dominant; while it is reversed when the stochasticity dominant.

The establishment of desired microbial diversity is still a major restriction in microbial ecological studies. The biggest obstacle is lack of sound experimental approaches to establish directed and predictable changes in the diversity of microbial communities. An approach that is often used to assess the effects of diversity is the so-called dilution method. The rare species in an initial inoculation could be sharply decreased when diluted thousands to millions times, leading to a biodiversity gradient in the experimental ecosystem. A number of studies have used the dilution method to artificially change microbial diversity in complex experimental ecosystems such as soils and sewages. In this study, we followed this approach at $1, 10^2, 10^4$ and $10^6$ dilutions to generate a gradient biodiversity in this model ecosystem (Figure 5). All reactors were inoculated with gradient dilutions from initial AS, which could inevitably reduce the biomass of the inoculums, including the biomass and species richness. Afterward, all experimental procedures were conducted under sterile conditions; in other words, the environmental filtering could result in decrease of the observed species, rather than increase. Therefore, the observed species in all biofilms should be derived from the initial activated sludge, where the unique OTUs in the anodic biofilms might the uncommonly rare species without sequenced (Supplementary Figure S6).

After the diluted inoculations were injected into the bioreactors, the microbial species attached the anodes following certain ecological assembly rules. Even though the diluted
inoculation induced some unavoidable compositional differences across the final biofilm communities, the dissimilarity between the each two groups of the biofilm communities was much lower than that between the biofilms with the initial inoculation communities (Supplementary Table S6, Supplementary Figure S8), indicating that the ecological assembly selections in anodes had stronger impacts on the biofilm communities than the diluted inoculation. It is well recognized that community assembly could be shaped by both deterministic and stochastic processes, however, the relative importance of each in controlling community structure is still controversial. Null model-based methods have been widely used to investigate the relative importance of deterministic and stochastic mechanisms underlying community assembly. Previously, Chase et al. proposed a null model analysis based on Jaccard distance without accounting for the species’ relative abundance, however that might ignore some useful information for understanding community assembly processes. In the present study, we performed the null model analyses based on Bray-Curtis distance with taking the species’ relative abundance into account, which might be able to reflect the assembly processes more clearly. Our previous study indicated that the stochastic assembly could impact the initial colonization of the biofilm communities in the MEC reactors to a greater degree. Based on the neutral models, several other related studies also indicated that the microbial communities in wastewater treatment plants and bioreactors were primarily controlled by stochastic process as well, though deterministic processes were also important. Generally, the deterministic process described the ecological process involved in non-random and niche-based mechanisms, including environmental filtering and various biological interactions. Here in MEC bioreactors, the selection pressure, from substrate (acetate) and external voltage, could be considered as a represent of
deterministic factors governing community assembly. Our results showed that the deterministic strength (determinism) significantly increased from 13.3% to 77.7% responding to the gradient diluted inoculation (Supplementary Figure S9), suggesting that the main assembly processes of the biofilm community structure will change from stochasticity to determinism during the increase of the dilution ratio. Overall, the gradient dilution of the initial inoculation could decrease the biodiversity and stochasticity, while increase the relative importance of determinism (Figure 5).

After the biofilm community assembled on the anode, gas yields from the MEC bioreactor were consistent and community composition reached a saturation stage. The phylogenetic diversity (PD) of the biofilm communities was significantly negative correlated with the magnitude of the dilution ratio, suggesting the dilution of the inoculation could generate a gradient of α-diversity in MEC (Figure 3). The results according to the Venn diagram (Supplementary Figure S6) indicated that the diluted inoculation sharply decreased the rare species in the anodic microbial communities, which might contribute to the biodiversity gradient in the final biofilms. Moreover, we also measured the total biomass of all biofilms in the end of MEC operation using two methods, quantitative PCR (qPCR) and volatile suspended solids (VSS) (Supplementary Table S1). Both the ANOVA analysis and student’s t test \( P > 0.05 \) showed that there were no obvious differences in the microbial biomass of biofilms operated with diluted inoculations (Supplementary Table S2), suggesting that the relative abundance of the major microorganisms could be compared as the actual abundance. As a well-known functional bacterial genus in MEC related to electron delivery, \textit{Geobacter} could contribute to the functional performance in biofilm community. Kiely \textit{et al.} inferred that the decreased presence of \textit{Geobacter} in the bacterial community could result in the poor
In the present study, the genus *Geobacter*, which was very rare (< 0.01%) in the initial community, became the most dominant species (> 50%) among all of the biofilm communities after the MEC operation (Figure 2), but there is non-significant correlation ($P > 0.05$) between the relative abundance of genus *Geobacter* with the MEC performance (Table 2). Meanwhile, *Methanobrevibacter* as unexpected and detrimental microorganisms for hydrogen production in MECs, was the predominant genus in Group C and D following *Geobacter*, and showed significantly negatively correlated (Table 2, $P < 0.05$) with the MEC performance. Another exoelectrogen, *Arcobacter*, which could oxidize various organic acids and use electrode as an electron acceptor directly, also occupied a higher proportion in Group D. All these suggest the dilution inoculation with decreased biodiversity and increased deterministic strength could alter the dominant species in biofilms, including the major functional groups and detrimental groups (Figure 5).

Diversity is a most fundamental concept in community ecology and often implicated as a cause of success or failure of a microbial community. There has often been an assumption that high diversity is implicitly a good or desirable outcome for communities, and that higher diversity is also somehow more meritorious ecologically. However, as the outcome of the ecological processes, higher diversity is not necessarily better. The relationships between diversity and emergent properties of a community, such as stability, productivity, or invisibility, are much more nuanced. Turnbaugh *et al.* demonstrated that the $\alpha$-diversity indexes in both of high-fat and low-fat diets were comparable in humanized mouse models. A comparison of two sets of replicated methanogenic bioreactors responded differently to a pulse glucose shock, suggested that communities with higher diversity were not necessarily more functionally stable in
the face of disturbance. On the contrary, our previous study showed that the biofilm community with higher biodiversity exhibited better resilience ability (short recovery time) in response to environmental disturbance.\textsuperscript{33}

Recent experimental research has proposed the hypothesis that it is the community assembly mechanisms that drives the relationships between microbial community structure and function.\textsuperscript{66} Both positive and negative associations between stochasticity and community function have been hypothesized.\textsuperscript{24} From one side of the debate, high levels of stochasticity could enhance community functioning, proposing that high diversity communities are more likely to contain more beneficial species properties on average than lower diversity communities. On the contrary, high rates of stochasticity can add organisms to a microbial community that are not expected to local environmental conditions, which may decrease community function. Grahan \textit{et al.} demonstrated that the microbial assembly processes exert greater influence over the community function when there is variation in the relative contributions of stochasticity and determinism among communities.\textsuperscript{15} Here, in this study, our results suggested that in general the higher diversity of biofilm community would gain better performance in MEC reactor, but the best performance could not be achieved when the biofilm community was formed under stochastic process (Figure 4; Figure 5). High level of stochasticity decreased the reactor performance by increasing the proportion of non-functional taxa in the anodic biofilms, which contributed more for the higher diversity. Meanwhile, the increasing of deterministic strength caused by the diluted inoculation, could also decrease the reactor performance, due to the enrichment of those unexpected and detrimental microorganisms, such as \textit{Methanobrevibacter}. This result proved high diversity might not be a desirable outcome for community, especially in microbiology. The assembly mechanism could be
one of the key processes to shift the functions of a community when we desire a better performance. Our study also has an important implication to microbial engineering (i.e., MEC) that highly complex community in initial inoculation will not always achieve high performance outcomes. Increasing the selection stress or appropriately diluting the inoculation concentration that brings the community assembly to determinism stage will better select most efficient microorganisms from natural resources.

In conclusion, we investigated the relationship between the microbial diversity and functions by using MEC reactors with gradient dilution ratio of inoculations under identical environmental and operational conditions. A gradient diversity in biofilm communities was generated. Null model analysis indicated that both deterministic and stochastic processes played important roles in controlling the assembly of the anodic biofilm communities in MECs, but the most important ecological process in shaping the microbial diversity changed from stochasticity to determinism with the increasing dilution ratio of inoculations. As a consequence, the highest MEC performance was obtained by the biofilm community with the next-highest biodiversity, but not the highest biodiversity biofilm resulting from the stochastic factors. The results presented in this study imply that improving the biodiversity may enhance the functions of microbial biofilm communities, only when the deterministic assembly becomes dominant. This study provides new insights into our understanding of the relationships between community assembly and biodiversity with ecosystem functions.

ASSOCIATED CONTENT

Supporting Information

Description of supplementary tests; additional figures showing schematic diagram of...
MEC operation, current production schematic and performance parameters of MEC reactors, rarefaction curve, community composition and distribution, biodiversity indexes and deterministic ratio of microbial communities in MEC anodic biofilms; and tables listing quantitative results of the microbial biomass in anodic biofilms, and the ANOVA analysis and T test results of the quantitative results in the anodic biofilms with diluted inoculation, α-diversity indexes of microbial communities in MEC anodic biofilms, difference of rare and abundant species across all groups of biofilm communities, dissimilarity of microbial communities among reactor groups, and correlation between microbial β-diversities and reactor performances.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGEMENTS

This study was supported by Key Research Program of Frontier Sciences, CAS (QYZDB-SSW-DQC026), the Key Research Program of the Chinese Academy of Sciences (KFZD-SW-219-3), CAS 100 talent program and the Open Project Program of State Key Laboratory of Applied Microbiology Southern China (SKLAM001-2015). The authors are very grateful to Dr. James Walter Voordeckers for careful edition on the final
version.

REFERENCES


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