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Current Opinion in Biotechnology

A methodological framework for linking bioreactor function to microbial communities and environmental conditions

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In the continuing quest to relate microbial communities in bioreactors to function and environmental and operational conditions, engineers and biotechnologists have adopted the latest molecular and 'omic methods. Despite the large amounts of data generated, gaining mechanistic insights and using the data for predictive and practical purposes is still a huge challenge. We present a methodological framework that can guide experimental design, and discuss specific issues that can affect how researchers generate and use data to elucidate the relationships. We also identify, in general terms, bioreactor research opportunities that appear promising.

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Current Opinion in Biotechnology 2015, **33**:112–118

This review comes from a themed issue on **Environmental biotechnology**

Edited by **Spiros N Agathos** and **Nico Boon**

For a complete overview see the Issue and the Editorial

Available online 21st February 2015

http://dx.doi.org/10.1016/j.copbio.2015.02.002

Introduction

Environmental engineers and biotechnologists who work on engineered systems have benefitted from and been challenged by the ongoing 'omics revolution in microbiology. Molecular biological tools and genomic, transcriptomic, and proteomic approaches have generated copious amounts of data about various ecosystems, and are now being applied to the complex microbial communities in bioreactors that convert wastestreams. The challenge is how to organize, analyze, gain insight from, and use the data for predictive, design, and operational purposes, such as improving the function of specific engineered bioprocesses. This is not an easy challenge, given the complexity of microbial communities in multispecies, interacting, and dynamic systems such as activated sludge, anaerobic reactors, landfills, and other biological reactor systems. Furthermore, there is a certain allure to continually using a variety of 'newer' techniques to answer the question of 'who's there' in one's favored system. The other tendency is to become overly reliant upon a favored technique that becomes Maslow's hammer, subsequently viewing every problem as the same 'nail' without exploring potentially better approaches. Such observation-based studies may have some usefulness, as when they are used to generate or test hypotheses, but few of these studies lead to deeper mechanistic understanding [1].

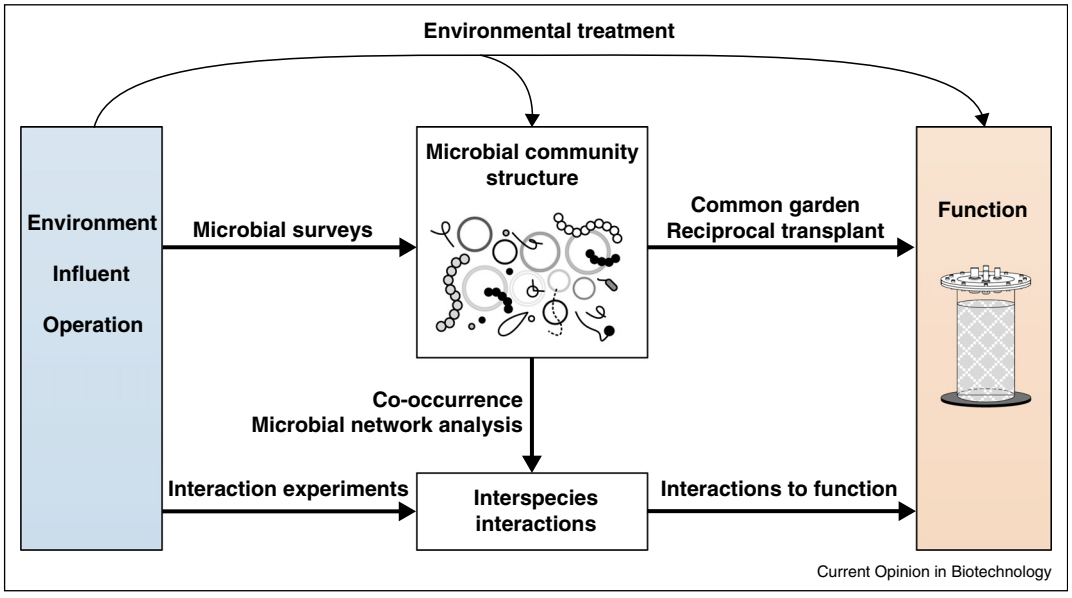
There is a need for a clearer methodological framework for relating bioreactor function to microbial communities and environmental conditions. Here 'function' can be defined specifically for the system. Example functions include the ability to remove specific contaminants (e.g. nitrogen and phosphorus removal in activated sludge, degradation of low levels of pharmaceuticals), the ability to convert wastes to energy or valuable products (e.g. to methane, hydrogen, organic acids), and the ability to be resilient, resistant, or stable in the face of stressors such as toxic shocks or overloads. Microbial community analysis includes elucidating structure, interactions, and dynamics. For our purposes, 'microbial community structure' can be defined as the numbers and kinds of ecological units in the specific system, and the ecological units can be phylogenetic 'species', OTUs, traits-based, or based on marker and functional genes, as appropriate [2–4]. 'Interactions' encompasses both classic ecological symbiosis and microbial phenomena such as cometabolism and horizontal gene transfer. The time dependence and often unstable nature of these living systems falls under the category of 'dynamics'. An advantage of bioreactors over natural ecosystems is that the system boundaries are well defined; large-scale spatial effects that occur in open environments are less of a concern.

We suggest the methodological framework shown in Figure 1 as a way of summarizing research approaches that can elucidate mechanistic relationships between the different variables: those that can be influenced (left portion of the diagram), microbial communities, and reactor function. The types of studies that can reveal the connections between variables/phenomena are shown along the respective arrows.

Environmental treatment

We label as 'environmental treatment' the traditional studies that look at the effects of environmental factors (e.g. pH, temperature), influent (e.g. substrate type, substrate

Figure 1



Methodological framework for linking bioreactor function to microbial communities and influencing conditions. The labels indicate the possible types of research approaches that can be used to understand individual linkages. Darker arrows represent approaches that have not been historically used in bioreactor studies, and that we suggest could lead to more fundamental insights.

loading), and operational factors (e.g. retention times, reactor configuration) on bioreactor function. Typical environmental treatment studies would compare the performances of a control reactor and a treatment reactor, and correlate changes in variables to changes in function (as in [5]). The hypothesis in such studies is that 'environmental treatment' changes the microbial community, which then leads to changes in function. While 'black box' approaches that do not measure changes in microbial communities serve to provide operational guidance, they may not suffice for elucidating mechanisms. Environmental treatment studies can also incorporate microbial community analysis, and these studies are more powerful and can potentially provide a more fundamental basis for improving biological process engineering.

Microbial surveys

Culture independent molecular fingerprinting techniques and DNA-targeted and RNA-targeted assays [6] have allowed researchers to begin answering that most basic of ecological questions: 'Who's there?' Despite lacking the coverage and depth of high throughput sequencing (HTS) methods, first generation molecular biological tools (MBTs) remain popular, partially due to their simplicity, availability, and established use. This is likely to change as HTS technologies, reviewed elsewhere [3,7], continue to become cheaper while providing ever-increasing throughput.

When performing bioreactor studies that include some form of microbial survey elucidating community structure, it is useful to remember the role of well thought out experimentation with testable hypotheses and that current limitations of metagenomics (e.g. with respect to annotation of specific gene sequences, challenges with associating phylogeny to function) may lead to noise generation [3]. Many studies fall in the category of 'differential diagnosis' [8], where differences in the microbial communities between control and treatment reactors are regarded as explanatory or even causal. This can potentially be misleading, given the issues of limited experimental replication, effects of functional redundancy, unacknowledged assumptions behind methods, and known reproducibility issues, especially under low sampling [9].

Microbial surveys are justified in the same way as all other data collection: when the data produced either generates or tests hypotheses. Justified surveys enable bioreactors to serve as model systems for the study of larger ecological questions. Ofiteru [10], for instance, did not simply describe the relative abundances of heterotrophs and ammonia oxidizing bacteria (AOB) in an aerated basin, but used Terminal Restriction Fragment Length Polymorphism (T-RFLP) data with the specific intention of determining the suitability of neutral community models for describing open, complex systems. Likewise, Wells [11] used T-RFLP to determine if taxa-time relationships can be used to quantify community shifts in activated