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Engineering microbial communities using thermodynamic principles and electrical interfaces

Christian Zerfaß^{1,2}, Jing Chen² and Orkun S Soyer^{1,2}



Microbial communities present the next research frontier. We argue here that understanding and engineering microbial communities requires a holistic view that considers not only species–species, but also species–environment interactions, and feedbacks between ecological and evolutionary dynamics (eco-evo feedbacks). Due this multi-level nature of interactions, we predict that approaches aimed solely at altering specific species populations in a community (through strain enrichment or inhibition), would only have a transient impact, and species–environment and eco-evo feedbacks would eventually drive the microbial community to its original state. We propose a higher-level engineering approach that is based on thermodynamics of microbial growth, and that considers specifically microbial redox biochemistry. Within this approach, the emphasis is on enforcing specific environmental conditions onto the community. These are expected to generate higher-level thermodynamic bounds onto the system, which the community structure and function can then adapt to. We believe that the resulting end-state can be ecologically and evolutionarily stable, mimicking the natural states of complex communities. Toward designing the exact nature of the environmental enforcement, thermodynamics and redox biochemistry can act as coarse-grained principles, while the use of electrodes — as electron providing or accepting redox agents — can provide implementation with spatiotemporal control.

Addresses

¹ Warwick Integrative Synthetic Biology Center (WISB), University of Warwick, United Kingdom

² School of Life Sciences, University of Warwick, United Kingdom

Corresponding author: Soyer, Orkun S (o.soyer@warwick.ac.uk)

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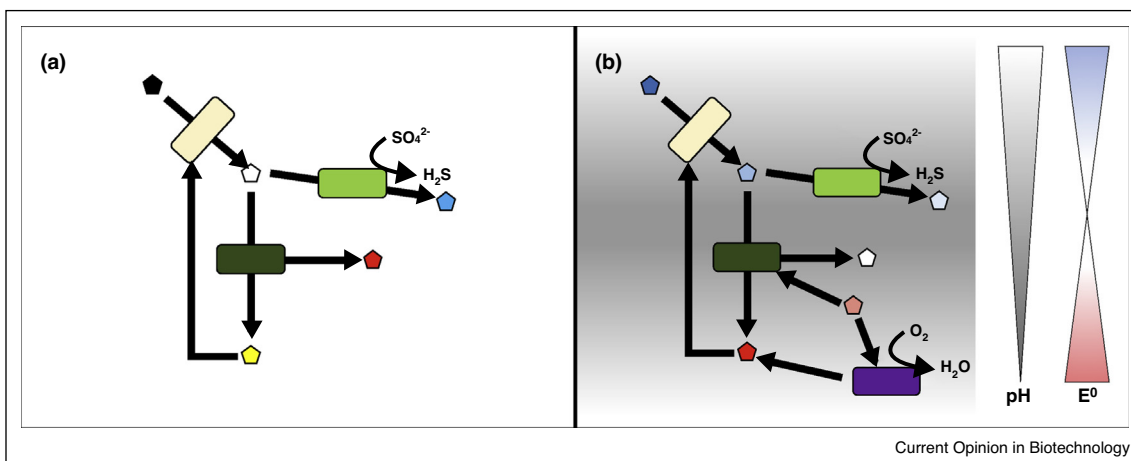
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Introduction

Microbial communities perform key biochemical transformations of organic and inorganic matter, underpinning the biogeochemical cycles on Earth [1,2] and playing a crucial part in the nutrition and health of higher organisms including humans, animals, and plants [3,4]. Thus, it is not surprising that there is increasing interest in understanding and engineering microbial communities for environmental, medical, and biotechnological applications [5*,6–9,10*,11]. Engineering of microbial communities has been proposed both as a top-down approach, controlling metabolic processes for stabilizing complex, natural communities [5*,6,7] and as a bottom-up approach, for designing defined, synthetic communities with desired functionality [8,9,10*,11]. In the former direction, most focus has been on gut communities for impacting human and animal health [6,7], and on anaerobic digestion (AD) communities for improving industrial methane production from organic wastes [11]. In the latter direction, early studies focused on implementing defined communities for degradation of organic matter using existing species (e.g. [12–14]), while more recent studies focused on creating synthetic communities with defined (and sometimes synthetically engineered) interactions that give rise to specific biotechnological applications, population dynamics, and community control (e.g. [15–17,18*,19*]). In the future, these top-down and bottom-up approaches could merge, with defined, synthetic communities being used to impact and engineer the behavior of complex, natural communities.

Irrespective of their specific aims and level of focus, any engineering approach to microbial communities requires predictive principles for describing community structure and function relationships, and practical tools for shaping these. A simplistic view (that could be considered as a guiding principle in the engineering sense) is to consider complex microbial communities as being composed of different functional groups performing key tasks. This viewpoint suggests that the overall behavior of a complex community can then be modulated in a desired way by including the necessary functional groups or by altering the population fractions of such groups (Figure 1a). We believe that this simplistic view is, however, unlikely to be fruitful as an engineering approach to microbial communities, as it ignores secondary interactions between species and the environment, and the ensuing feedback dynamics.

Figure 1



Microbial communities are affected by species–species and species–environment interactions. **(a)** Classically, microbial communities are thought as functionally distinct groups of microbes (sketched as rectangles) connected through species–species interactions involving metabolite (pentagons) conversions. These can include for example cross-feeding, competition, or auxotrophic interactions (involving the production of metabolites affecting the growth of other species, e.g. yellow pentagon). **(b)** We call for a more holistic view of microbial communities that explicitly takes into account species–environment interactions (indicated with the shaded background), and the feedbacks and intertwined ecological and evolutionary dynamics arising from these. For instance, the impact of metabolic activities of microbes (primarily driven by redox conversions) can directly lead to changes in redox potentials of other reactions (indicated by the standard potential E^0 , bar on the right) and the environmental conditions (such as pH, shown on the right). These changes would then exert a feedback on the whole microbial community, selecting for or against certain groups.

Species–species and species–environment interactions, as well as evolutionary dynamics present significant challenges to complex community engineering. To illustrate the above point, consider for example, increasing the population fraction of a species involved in the fermentation of a particular organic compound. Such an intervention is expected to impact other species in a community directly through creation of substrate-competition (e.g. for carbohydrates), but also indirectly through environmental pH-changes (e.g. acidification through formation of organic acids) [20] and emergence of new cross-feeding interactions (e.g. through organic acids acting as new substrates) [21]. Thus, altering the population of one functional group might present unexpected impacts, or alternatively no impact at all. Indeed, several recent studies find that functional improvements to a community emerge from large-scale community ‘implantation’ or mixing of multiple communities [22,23^{*}], supporting the notion that community function is the result of a community as a whole, inclusive of its myriad species–species and species–environment interactions.

Given the short generation times of some microbes, it is also possible that long-term species–species interactions can result in the evolution of additional genetic interactions. Such evolutionary adaptation is implicated for example by findings of abundant auxotrophic interactions (emerging from the inability of one species to synthesize a compound required for its growth) in communities enriched for degradation of specific compounds [24^{*}].

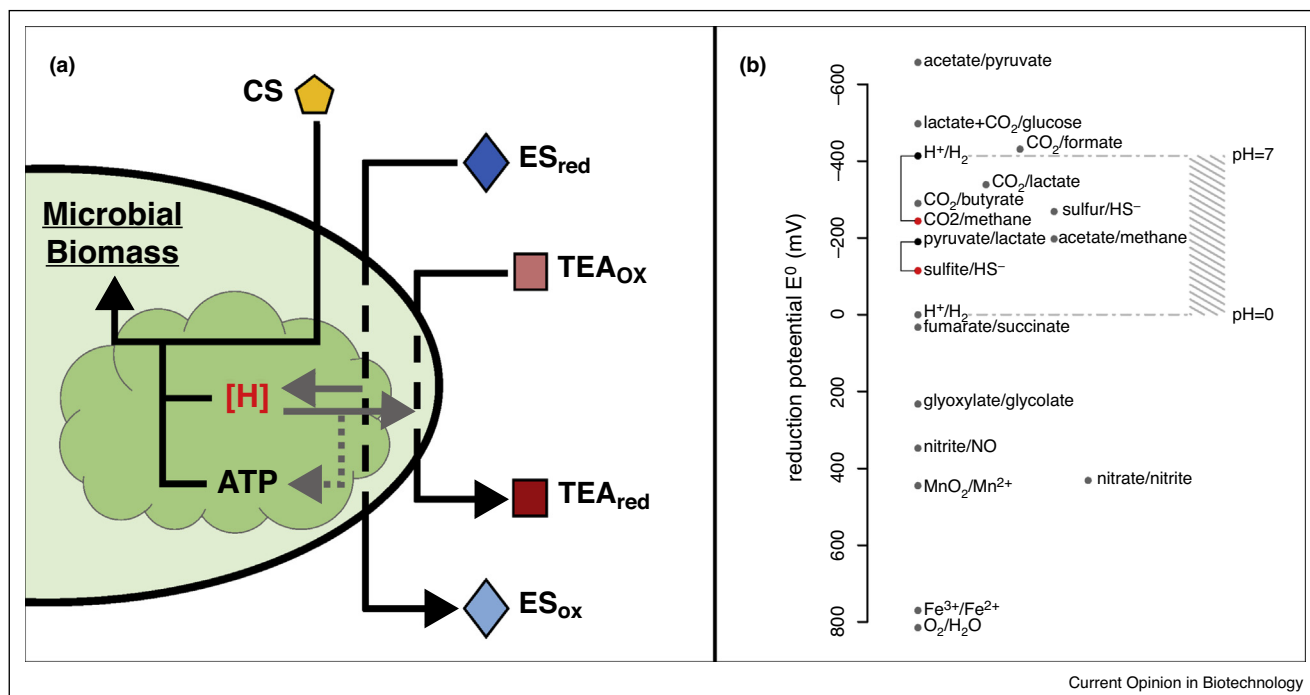
Evolutionary dynamics can also be driven by species–environment interactions resulting in so-called eco-evo feedbacks [25]. These feedbacks are shown to impact the population dynamics of cooperative traits in a population [26,27,28^{*}], and are proposed as a potential driving force beyond physiological specialization [29,30]. The latter possibility has been demonstrated theoretically in the context of monocultures of *Escherichia coli*, where it is shown that metabolic activities altering the environment can result in a feedback that drives the evolution of different metabolic strategies within this organism [21].

To develop applications of microbial communities, engineering approaches hence need to deal not only with species–species, but also with species–environment interactions and with the ensuing eco-evo feedbacks (Figure 1b).

Bottom-up engineering needs to consider species–environment and eco-evo feedbacks

For different species to co-exist and achieve a common functional goal, their environment needs to be designed in a way to support (or even enforce) their growth and interactions. This has been achieved for synthetic auxotrophic interactions within one species [17], and cross-feeding and syntrophic interactions among different species [15,16,31]. A key example in the latter direction involves a methanogen and a sulfate reducer, which co-exist in an environment that lacks sulfate (sulfate reducers’ natural choice as an electron acceptor) [31]. This model system is achieved by enforcing a specific environment,

Figure 2



Microbial metabolism can be seen as composed of intra-molecular and inter-molecular redox reactions with associated energy harvesting. **(a)** Microbes couple oxidation of reduced energy sources (ES_{red}) with the reduction of terminal electron acceptors (TEAs), thereby harvesting reductive energy ([H]). This energy is invested in the building of biomass by reducing external carbon sources (CS), or in the production of other energy equivalents (e.g. ATP). Note that CS and ES can be the same compound, and that in fermentation ES essentially equals TEA, but after intramolecular redox conversion. Further biomass components (e.g. nitrogen, phosphorous) are ignored in this scheme for simplicity. **(b)** Standard reduction potentials at biological conditions (i.e. all solute concentrations = 1 M (except for [H⁺] = 10⁻⁷), pressure = 1 bar, temperature = 25 °C) of biologically relevant organic and inorganic compounds (note that proton reduction potential at [H⁺] = 1 M, i.e. pH = 0, is shown separately, corresponding to standard hydrogen electrode potential). Each gray dot indicates a reduction reaction, with the substrate and product shown in the labels. Microbial growth involves combining one such reduction reaction with another that is run in the reverse (i.e. oxidation direction), so to form a redox couple. Two examples for such microbial growth-supporting redox couples are highlighted; the overall process in methanogenesis, where H₂ oxidation (to H⁺) is coupled to reduction of CO₂ (to methane); and a key part of the sulfate reduction, where lactate oxidation (to pyruvate) is coupled to reduction of sulfite (from activated sulfate). For these couplings, the oxidation and reduction reactions are shown in black and red respectively (adapting the cathode and anode color-coding). Note that individual reactions' reduction potentials would shift as participating compounds concentrations deviate from standard conditions, and where protons are involved, with environmental pH (e.g. see shifting reduction potential for H⁺/H₂ pair with changing pH, shown as dashed area on Figure 2b). Reduction potentials are calculated from stoichiometrically balanced reduction reactions (using water, protons, and electrons), and using tabulated standard Gibbs free energy of formation values for each of the involved compounds [58].

namely the lack of sulfate, that then naturally drives the emergence of the syntrophic interaction. Indeed, it is interesting to note that the syntrophic interaction in this model system is impacted by evolutionary dynamics; a maintained polymorphism in the sulfate reducer is found to be required for the initiation of syntrophy [32^{*}], and a range of potentially stabilizing mutations are found to arise over long-term co-culturing [33,34^{**}]. Similarly, microbial communities enriched for degradation of specific organic compounds are found to display multiple syntrophic interactions, as well as secondary dependencies such as auxotrophic interactions [24^{*}]. These examples suggest that microbial communities can naturally adapt their structure and specific metabolic interactions to the given environmental conditions and to achieve stability and productivity. In other words, it might be possible that enforcing

specific environmental constraints can directly facilitate the engineering of community structure and function toward an evolutionarily stable state.

Thermodynamics and redox processes as 'design principles' for community engineering

In the above example of syntrophic interactions, the enforcement of the environment (through sulfate depletion) relates directly to the thermodynamic basis of microbial growth. Microbial (catabolic) metabolism can, at a coarse level, be understood as a collection of pathways, each implementing a different redox reaction utilizing a different terminal electron acceptor (TEA) (Figure 2) [35,36^{*},37,38^{*},39^{*}]. Shortage of strong TEAs is common in many micro- and macro-environments, including soil, AD reactors, gut, and lakes, while oxygen

depletion can even happen in sealed cultures or biofilms of fast-growing facultative organisms [40*,41]. Under the absence of strong TEAs, the community-members need to adapt to redox processes through weak TEAs (such as H^+ , CO_2 , and SO_4^{2-}), and fermentative pathways which mitigate intracellular reductive energy overflow. This, in turn, leads to the possibility of thermodynamic inhibition, whereby microbes cease growth due to accumulation of their own metabolic end-products [42].

Avoiding thermodynamic inhibition is only possible by switching to alternative redox processes with new chemical products or actively engaging in syntrophic interactions [38*,42]. Indeed, thermodynamic inhibition is suggested to lead to maintenance of microbial and metabolic diversity [38*], and to strongly affect community structure and dynamics [1,2]. In anaerobic communities, for example, depletion of strong TEAs leads to accumulation of acetate and hydrogen, which can be consumed by methanogens. At the same time, however, acetate accumulation can decrease environmental pH and inhibit methanogens [43]. The resulting delicate balance can spiral out to a feedback dynamic, with increased acidification leading to more inhibition of methanogenesis, and therefore to more acidification, and finally to whole-community inhibition. Differential TEA availability can also cause more direct alterations in community structure. A drastic example is the finding that gut host cell responses to inflammation can lead to formation of nitrate, which can act as TEA for selective groups of microbes and thereby give rise to host-mediated changes in community dynamics [44*]. Similarly, several redox active compounds such as Azo dyes and humic substances (i.e. polyaromatic lignin-degradation fragments) are found to alter overall methanogenesis rates in AD communities [45–47,48*].

Electrical interferences as dynamic and controllable means for community engineering

The above findings highlight TEA availability as a key driver of both environmental conditions and microbial interactions, giving rise to the possibility that community structure and function could be manipulated through TEAs provision or removal, guided by thermodynamic considerations of different microbial respiration processes. This approach has already been used successfully to achieve enrichment of specific microbial processes, predicted by thermodynamics (e.g. [49,50**]), and is suggested as a route to design anaerobic production strategies in biotechnology [36*,51,52*]. The TEA-based redox interventions can be achieved both at cellular- and community-levels, and to date were successfully implemented using different approaches including the provision of gases (i.e. oxygen, hydrogen), chemical supplementation of media (chemical electron donors/acceptors) [45,47,48*], genetic modification (rate-control on NADH-synthesis/depletion) (e.g. [53]), and electrode-based intervention [54,55*,56].

Chemical interventions are established particularly in the context of AD, however, they do not readily allow temporal control. Similarly, genetic interventions cannot be triggered in a temporal fashion and only target a specific species, which might not be stable in the context of a community. Electrode-based intervention, where electrons can be supplied or retrieved from the system at set reduction potentials, provide a more direct and temporal control over redox processes, provided that some key community members are able to interface with the electrode by direct or indirect electron exchange.

Promising results of electrode-based intervention with community dynamics are already being obtained. In the context of AD, electrons were successfully supplied to a complex anaerobic community via electrodes poised at different potentials and currents [57**]. This study found that at constant potential, a high current favored acetogenesis with some methane production through acetotrophic methanogens. This suggests that the rate of electron supply can to some extent control the electrons' destiny, highlighting the interlinked effect of thermodynamic and kinetic reaction control in cell metabolism [36*,38*,59]. A similar use of electrodes to control community dynamics in bottom-up engineering is still to be attempted, but several studies have shown that many different bacteria including *Clostridia* species [59] and methanogens [60,61] are capable of electron transfer from and to electrodes, opening the route to implement separate redox reactions such as organics degradation and methanogenesis across compartments coupled with electrodes.

Future outlook

We argued here for a microbial community engineering approach that takes a holistic view and that considers not only species–species, but also species–environment interactions and eco–evo feedbacks. Shifting the emphasis away from individual species (or functional groups) to the system as a whole, this approach is similar to those advocated for modeling connected biotic and abiotic geochemical processes [62*], where thermodynamics and considerations of entropy maximization are brought to the fore as fundamental guiding principles. Holistic engineering of microbial communities with such high-level guiding principles will require us to better understand the thermodynamic basis of microbial growth, and in particular the energetics and dynamics of respiratory and fermentative metabolic pathways. A crucial gap in this understanding, amongst others, is the energetics of cellular growth within micro-environments, such as biofilms and microbial granules. Besides improved measurement techniques of micro- and macro-environments, the filling of this gap will also require increased interaction between research communities from geochemistry, physics, electrochemistry, and biology. Increased understanding at the micro level can subsequently allow better use of thermodynamic principles at the microbial community

level, and to design specific electrode-based intervention strategies. In this direction, more research is needed to better understand electrode-microbe interactions, as well as to develop cost effective electrical manipulations. Together, these developments can allow electrical engineering of complex microbial communities found in the soil, gut, and the anaerobic digestion reactors, and bottom-up design of functional, defined communities.

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