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Performance evaluation and multidisciplinary analysis of catalytic fixation reactions by material–microbe hybrids

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Abstract

Hybrid systems that integrate synthetic materials with biological machinery offer opportunities for sustainable and efficient catalysis. However, the multidisciplinary and unique nature of the materials–biology interface requires researchers to draw insights from different fields. In this Perspective, using examples from the area of N_2 and CO_2 fixation, we provide a unified discussion of critical aspects of the material–microbe interface, simultaneously considering the requirements of physical and biological sciences that have a tangible impact on the performance of biohybrids. We first discuss the figures of merit and caveats for the evaluation of catalytic performance. Then, we reflect on the interactions and potential synergies at the materials–biology interface, as well as the challenges and opportunities for a deepened fundamental understanding of abiotic–biotic catalysis.

Advanced catalysis is pivotal for today's society. With a rapid increase in the world's population, new approaches are needed for both the utilization of sustainable energy for chemical production[1] and the remediation of environmental pollutants[2]. Despite the long history of catalysis based on synthetic materials or molecules, it remains challenging for synthetic catalysts to achieve sustainable and efficient conversion for the targeted production of complex molecules with multi-carbon frameworks and stereochemical attributes through multi-step cascade reactions, such as the synthesis of sugar from carbon dioxide. One potential approach to address such challenges is the integration of different systems from various subdisciplines in catalysis.

The fledging integration of synthetic materials with microbial components, so-called material–microbe hybrids[2–7] (Fig. 1a), offers exciting opportunities to address the aforementioned challenges. These material–microbe hybrids are proposed to offer advanced

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reactivities that are difficult to achieve with either materials or biological catalysts alone[3], due to the unique combined properties from both abiotic and biotic components. Whereas synthetic materials excel in energy conversion/generation including the use of solar energy, biological systems are particularly efficient in the synthesis of complex molecules owing to their enzymatic cascades[8]. Solar- and electricity-driven chemical syntheses have been demonstrated by these abiotic–biotic systems with promising future applications (Fig. 1b), including the fixation of CO_2 or N_2 into single chemicals (for example, acetic acid, ammonia and so on)[5], the simultaneous fixation of CO_2 and N_2 into composite mixtures (for example, biomass) for use as fertilizers[5], as well as the harvesting of electric energy from wastewater treatment[2,6,7].

However, the multidisciplinary nature of such catalytic hybrids can lead to knowledge disparities among researchers with different backgrounds, and understanding the properties of the integrated system is a priority to advance the field as a whole. For example, when incorporating the electrochemical generation of H_2 with acetogenic bacteria (the microorganisms that utilize H_2 as the electron donor for CO_2 fixation) for acetate production, the overall formation rate of acetate is determined by the rate-limiting step of the integrated material–microbe hybrid system, which is not necessarily the same as that of the individual components in the hybrid. Therefore, the development of integrated biohybrids demands a systemic and interdisciplinary understanding of materials, biology and their synergies. Tremendous opportunities, although plentiful challenges, await in the study of material–microbe hybrids, both physically and intellectually.

In this Perspective we aim to provide a multidisciplinary assessment of the figures of merit and ensuing caveats in evaluating the catalytic performance of material–microbe hybrid systems; moreover, the methodologies and approaches toward a mechanistic understanding of abiotic–biotic interfaces in the context of designing advanced hybrid catalysis will be discussed. One particular challenge is to remain specific while covering the wide range of potential applications that hybrid systems are capable of (Fig. 1b). On the basis of our expertise and past experience[9–12], we choose two specific catalytic reactions as examples: the acetogenic fixation of CO_2 to form acetic acid by *Sporomusa ovata* through the Wood–Ljungdahl pathway; and the ambient co-fixation of CO_2 and N_2 by the diazotrophic bacterium *Xanthobacter autotrophicus*. The catalysis of these material–microbe hybrids can be driven by electrons from the electrode (for example, as in the cobalt–phosphorus|cobalt phosphate ($CoP|CoP_i$) electrochemical system) and/or photons from quantum dots (for example, CdTe and CdS) as direct energy providers. With such examples, perspectives from both physical and biological science backgrounds are proposed, to offer a unified discussion on the system-level design of material–microbe hybrids for diverse applications. We ultimately hope to mitigate some of the field-dependent information gaps from materials chemistry to biology and therefore promote interdisciplinary collaboration among researchers from different communities.

Evaluating catalysis in material–microbe hybrids

As the interdisciplinary nature of the material–microbe hybrids leads to different methods of evaluating catalytic performance, we discuss here some major figures of merit and important caveats in the context of assessing the activity of material–microbe hybrids (Fig. 2).

Proof and quantification of catalytic reactions

The first step of assessing material–microbe hybrids is to determine the stoichiometric catalytic reactions with sufficient experimental evidence. Such requirements can be challenging due to the complexity of biology, especially with the complicated network of possible biochemical processes. From the perspective of chemical catalysis, explicit statements of the proposed reactant(s) and product(s) (R and P, respectively, in Fig. 2), as well as the corresponding stoichiometry, are needed. Conventional techniques such as liquid chromatography and NMR spectroscopy will be applicable when the yielded products themselves are straightforward to quantify using these methods, for example, as with acetic acid from acetogenic CO_2 fixation and ammonia from N_2-fixing diazotrophic organisms. However, experimental demonstration of the proposed reaction in a material–microbe hybrid system can be masked by the complicated composition of the microbial culturing medium, which sometimes contains nutrient-rich components such as yeast extract that is added as a common substrate, as well as by the presence of sacrificial reagents (termed as hole/electron scavengers) that are commonly added in photocatalytic systems[13]. Therefore, additional experiments such as isotope labelling are needed to corroborate the catalytic reaction[14], and high-resolution mass spectrometry should be applied simultaneously to quantify the absolute concentration of related complex biomolecules[15]. Of particular interest are the metabolites that are fundamental in bioenergetics—such as adenosine mono-, di- or tri-phosphate and the respective oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD^+/NADH) and nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH)–and/or critical to central metabolism (for example, pyruvate, acetyl coenzyme A and so on) or unique to the studied reactions and corresponding regulatory pathways (for example, glutamate/glutamine/2-oxoglutarate in nitrogen regulation, among others). Meanwhile, the biochemical pathways in the microorganisms should be characterized via suitable omics techniques[16], including transcriptomics, proteomics, metabolomics and/or fluxomics, that measure RNA transcript abundance (transcriptomics), protein expression levels (proteomics), metabolite concentrations (metabolomics) and the metabolic flux (fluxomics) within the biochemical reaction network. These omics methods[16] not only affirm the proposed metabolic processes in the biohybrid but also unveil the possible existence of any unexpected biochemical pathways that may inadvertently generate the purported products, which would lead to an overestimation of the catalytic activity or false-positive results[13]. In the case of acetogenic CO_2 fixation, those multi-omics analyses will help in confirming the activity of the Wood–Ljungdahl pathway as well as illustrating the limiting intermediates and/or the bottleneck steps of the microbial metabolic activities, which may vary substantially under different electron donors and solution compositions.

Additional considerations should be taken into account when the final product includes biomass, the most general microbial product from the catalytic fixation of CO_2 and N_2 in

autotrophic organisms. Whereas the measurement of optical density (OD) is a conventional and accessible method of determining biomass accumulation, the quantitative relationship between the OD value and the measurement of biological products, such as the amount of dry biomass, must be calibrated. Such a relationship needs to be obtained under the same or similar growth conditions as the biomass composition can vary greatly under different nutrient mediums and growth conditions. The same precaution should be taken when the elemental composition of dry biomass is being determined to establish a detailed mass balance in catalysis. Furthermore, OD measurement can suffer notable interference when nanoparticles or nanoclusters are involved in the microbial culture because of the absorption or scattering effect of the materials in the visible spectrum. This interference can further complicate the catalytic evaluation and the calculation of quantum yields in dispersion-based photocatalysis as optical scattering will alter the light path in the material–microbe suspension, therefore changing the number of absorbed photons (see ‘Calculation of efficiency’). Additional analyses that include the quantification of DNA abundance and protein amount, which serve as other surrogates of dry biomass, are recommended for cross-validation.

Calculation of efficiency

One argument in favour of material–microbe hybrids is their capability of harvesting light or electricity and conducting chemical transformations with high efficiency. However, as highlighted recently by others[17], there are two different efficiency metrics to be considered: the energy efficiency (EE) (equation (1)), which is defined as the ratio of the Gibbs free energy accumulated in the products to the energy input that is offered to drive the system; and the Faradaic efficiency (FE) (equation (2)) or equivalently the internal quantum yield (IQY) of photocatalysis, which are defined as the ratio between the electrons/photons required for product generation based on the chemical stoichiometry and the total number of electrons/photons taken in by the system.

$$\mathrm{EE} = \frac{\Delta_r G^0 \text{ gain by product synthesis}}{\text{energy input}} \tag{1}$$

$$\mathrm{FE/IQY} = \frac{\text{electrons or photons utilized for product synthesis}}{\text{input of electrons or absorbed photons}} \tag{2}$$

The different definitions suggest that there are several approaches to evaluating the efficiency of material–microbe hybrids. Biological research tends to report the efficiency as the weight ratio between the product and the input of organic substrate (in the units of grams per gram), yet it is necessary to translate this ratio into EE values based on reaction thermodynamics and stoichiometry. A high EE value describes the overall energy of the catalytic process and is desired for the ultimate practical application of the hybrid. A high FE/IQY value is necessary but not sufficient for a high EE value, because a high EE value not only demands efficient utilization of electrons or photons

but also requires minimal energy loss due to the reaction thermodynamics. By contrast, from a mechanistic perspective, the FE/IQY provides more insight into the fundamentals of biohybrids as it represents the effectiveness of the charge transfer between the abiotic and biotic moieties[9,18,19].

Caveats exist when calculating experimental EE and FE/IQY values. Whereas biochemistry commonly uses the reaction enthalpy ($\Delta_r H^0$) to describe the reaction thermochemistry, it is the Gibbs free energy of the reaction ($\Delta_r G^0$) that should be used for the EE calculations (see the numerator of equation (1)), particularly when gaseous species such as CO_2 are involved in the catalysis and bring appreciable entropy changes. When mixtures of products—such as amino acids, biopolymers or biomass–are among the reaction products, it is important to determine the product distribution and the reaction selectivity as well as the corresponding $\Delta_r G^0$ value for a specific product. For example, the Gibbs free energy of formation ($\Delta_r G^0$) of dry bacterial biomass is reported to range widely from −0.02 to −3.04 kJ g^{-1} (ref. 20), indicating the different compositions of bacteria. Therefore, experimental determinations of $\Delta_r G^0$ using bomb calorimetry are needed for biomass and ill-defined products for the accurate calculation of EE values.

The input energy and electrons (see the denominators of equations (1) and (2)) of a material–microbe hybrid system for chemical synthesis can be calculated using information about the energy source (for example, photons and electrons supplied by the light source or potentiostat). However, it is noteworthy to evaluate the possible energy and electron contribution from any added chemicals that also contribute to the input energy and reducing equivalents. Such chemicals include but are not limited to common nutrients in biological buffers (for example, yeast extract), residual carbohydrates from the washed cells, inducible promoters in chemical biology, such as isopropyl β-D-1-thiogalactopyranoside, and sacrificial reagents added to photocatalytic reactions. If possible, such additives should be removed before experiments. Alternatively, the consumption of the additives' during catalysis should be quantified via analytical techniques, such as liquid/gas chromatography, to establish a detailed catalytic mass balance by subtracting the energy/electrons that are contributed from those additives. The aforementioned practice of isotope-labelling experiments will also help to determine the product quantity from the designated reactant sources. For dispersion-based photocatalysis, quantification of the IQY specifically requires the accurate counting of absorbed photons (see the denominator of equation (2)), which could be substantially influenced by strong light scattering from the microorganisms; therefore, the deployment of an integration sphere is advised.

For proper data interpretation it is also important to calculate the theoretical limits of the determined efficiencies that material–microbe hybrids can achieve[21]. Such limits not only guide judicious choices of biochemical pathways for the proposed catalysis[22,23] but also help to identify and evaluate synergies at the materials–biology interface[9,18,19]. Yet the evaluation of the theoretical maximum performance can sometimes be burdensome for two reasons: (1) the challenge of identifying the catalytic process and its stoichiometry and (2) the complexity and multiplicity of competing biochemical pathways. Researchers are advised to evaluate the upper bounds of efficiencies on a case-by-case basis following the

literature-reported practices for a general assessment of catalytic material–microbe hybrid systems[9,22,23].

The selectivity and reaction rate of catalysis

An important feature of the material–microbe hybrid is its unique reactivity and selectivity for challenging chemical reactions, which distinguishes biocatalysts from purely materials-based catalysts[24]. Indeed, the FE/IQY value for a specific desired product confirms the reaction selectivity of these hybrids. As whole-cell biocatalysis proceeds via a biochemical network, the experimentally observed reaction rate and selectivity of the hybrid system indeed result from the whole biochemical network, from a system biology perspective. This suggests that only a metabolic flux analysis under various regulatory signalling/controls can offer fundamental insights towards the measured reaction rate and selectivity. Nonetheless, some specific reaction steps will be presumed to be rate-limiting in the first-order approximation under many scenarios. For example, the challenging reaction of ambient N_2 fixation proceeds through and is commonly presumed to be limited by the fascinating nitrogenase enzyme. Although such an assumption seems fair initially, it is important to recognize the intense energy demand of N_2 fixation. Moreover, the strict regulations of nitrogenases at transcriptional and post-translational levels may lead other bio-machinery to be the limiting factor, suggesting different constraints of biocatalytic N_2 fixation under different reaction conditions.

Another important metric in catalysis is the turnover frequency (TOF), which measures the intrinsic reactivity of the catalytic active sites for a specific product and is determined by normalizing the reaction rate against the number of active sites. Specific to material–microbe hybrids, issues arise given the ambiguity regarding the definition of active sites in biohybrids. From one perspective, a single microbial cell can be deemed to be one catalytic site, in which case the TOF values are normalized against the number of viable cells quantified using techniques such as flow cytometry. For instance, the TOFs of N_2 fixation by a microbial-based hybrid system have been reported by normalizing the number of moles of fixed N_2 against the average number of viable cells throughout the experimental duration[11]. However, such an estimation neglects the variability in cellular enzyme copy numbers among different microbial cells in different environments. The same issues apply to a similar TOF definition (commonly used in biocatalysis) that normalizes the reactivity against the dry cell weight (common units: grams per gram dry cell weight per hour). Alternatively, it is of interest to calculate TOF values based on the total number of active enzymes involved in the turnover-limiting biochemical step. This practice, however, demands a deep understanding of microbial metabolism and a reasonable designation of the turnover-limiting step, in addition to the fact that experimental quantification of the enzyme copy number is non-trivial in proteomics. In the same aforementioned reports of N_2-fixing biohybrids[11], TOF values of N_2 fixation were also estimated by normalizing the number of moles of fixed N_2 against the estimated copy number of active nitrogenases, with the explicit assumption that the nitrogenase-catalysed biochemical step is turnover-limiting. We also note that TOF values should be reported under the same experimental conditions as the reported EE and/or FE/IQY values, to avoid an undesired mismatch of performance metrics under different conditions, reminiscent of recent discussions in molecular electrocatalysis[25].

Industrial-scale applications of the material–microbe hybrid demand the evaluation of the net volumetric rate r_{vol} in the reactor, which in biocatalysis requires the concentrations of desired products (as a titre in the units of grams per litre) within a given reaction time to be reported. Whereas this practice is applicable with photocatalytic dispersions of material–microbe hybrids, titre reporting seems uncommon in electrochemically driven systems, due partly to the different reaction designs. A modified reporting of r_{vol} that accounts for different reactor designs is advocated.

Stability and resilience towards intermittent energy sources

The stability and resilience of material–microbe hybrids against environmental perturbations and an intermittent energy supply are crucial for practical applications. We suggest that testing the biohybrids' long-term catalytic stability is carried out for a period at least longer than the microbial doubling time—the timescale that is characteristic of metabolic activities and which can vary greatly, up to days, for the specific prokaryotic organisms deployed. Additional testing of microbial viability is welcomed with aliquoted samples measured using either flow cytometry[26] or the plate counting of colony-forming units[27]. Performance evaluation under on–off cycles mimicking an intermittent energy supply is particularly recommended for organisms with relatively short doubling times, which has been demonstrated for many photocatalytic hybrid systems with 12 h light and 12 h dark cycles.

Mechanistic study of materials–biology interfaces

One design consideration of material–microbe hybrids is whether to establish a materials–biology interface in a single reactor, as, alternatively, a tandem cascade process that combines materials and biological catalysts in separate reactors can potentially be equally viable[28]. The answer to such a design consideration begins with a systematic mechanistic enquiry of whether or not there are any potentially beneficial and synergistic features that result from the interplay between the microbial and material components at the abiotic–biotic interfaces in the context of chemical catalysis, for example, new reactivities. In addition, the experimental design should include suitable characterization techniques across multiple disciplines to elucidate the possible synergies. Here we discuss how the deployment of multidisciplinary characterization techniques will mechanistically probe the materials–biology interface in search of potential synergies (Fig. 3).

Abiotic–biotic physical contacts

One prerequisite of a materials–biology interface is the establishment of physical contact between the abiotic and biotic components in a hybrid system (Fig. 3a). Such physical contact is needed to facilitate charge transfer and the exchange of reaction intermediates between the materials and biological moieties, which could be potentially more efficient than separated cascade processes. Typical methods to encourage the attachment of microbes to materials include: surface functionalization, such as attaching different functional groups to the electrode surface[29], to promote bacterial adhesion based on the EDLVO (extended Derjaguin–Landau–Verwey–Overbeek) theory that accounts for the van der Waals, Lewis acid–base and electrostatic double-layer interactions between spherical particles and the

surface; and increasing the volumetric surface area, that is, deploying nanowires as the inorganic part in an array that provides electrons[30] and increases the attachment between the abiotic and biotic components. Interestingly, the microbial–material interaction can also be adjusted by changing the salt concentration of the buffer[31]. In contrast to the surfaces of antimicrobial materials that are commonly designed for biomedical applications, a biocompatible material interface is desirable to preserve the cell viability.

There are two questions that need to be answered in the study of such physical contacts. Are there actually any microbial–material physical contacts? And if yes, how strong is the binding affinity between the abiotic and biotic components? Optical microscopy is capable of spatially co-localizing microbes and (nano)materials in vivo. However, an intimate material–microbial interaction with nanometre-scale resolution cannot be clearly characterized using conventional optical microscopy due to the diffraction-limited resolution of the technique (~200–350 nm) in the visible-light region. Therefore, it will be intriguing to deploy super-resolution microscopy techniques to optically observe the materials–biology interfaces[32]. An alternative ex situ strategy is electron microscopy[18], including scanning electron microscopy and transmission electron microscopy, with nanoscopic resolution to characterize the interface. However, one caveat of electron microscopy is that biological samples need to be at least fixed with stabilized structures and further dehydrated before characterization, which may mask the authentic material–biology interfaces under catalytically functioning conditions. Flow cytometry, which measures the physical and chemical properties of a large population of particles can instead offer an alternative. For instance, this technique has been applied to determine the binding affinity and the maximal binding numbers between microbes and nanomaterials, under the assumption of the Langmuir adsorption model[26]. Atomic force microscopy can also be applied to measure the hydrodynamic force of particle attachment on microbial surfaces under physiological conditions[33,34]. Coupled with the zeta potential and dynamic light scattering for characterization of the surface charge and size distribution on biotic/abiotic moieties, it is feasible to obtain a comprehensive picture of the physical interactions at the materials–biology interface.

Interfacial electron transfer and mass exchange

The charge transfer and delivery of electrons at the materials–biology interface is a key prerequisite for efficient catalysis by material–microbe hybrids (Fig. 3b). Extensive studies in microbial fuel cells, with the model organisms *Shewanella oneidensis* and *Geobacter sulfurreducens*[33,35], have indicated two charge-transfer mechanisms: the direct/unmediated pathway and the indirect/mediated pathway[7,36]. It is generally considered that the direct charge transfer proceeds via electron hopping or tunnelling[37] from the material to the transmembrane redox proteins or conductive microbial multi-haem protein extensions (or vice versa), which requires physical contact between the material and microbe[38]; however, the mediated pathway includes redox shuttling between the microbe and material with exogenous or endogenous redox mediators that include dihydrogen (H_2), cytochromes and derivatives of quinones, phenazines and flavins. Theoretically, quantitative evaluations suggest that the direct mechanism has a higher upper bound of EE, especially with the existence of electron bifurcation that optimizes energy utilization[21]; the direct charge-

transfer mechanism is proposed to theoretically afford a larger intrinsic charge-transfer rate without mass-transport constraints[39] and is amenable to flow-reactor design[37]. Practically, the required high concentrations of exogenous or secreted redox mediators for appreciable net rates of charge transfer are constrained by diffusion, the solubility limits of the mediators and cost considerations, particularly in a flow reactor with the potential loss of electron shuttles[39]. It can be desirable to establish a materials–biology interface for either direct charge transfer, with the prospect of a high EE and a fast charge-transfer rate, or a hybrid with suitable microenvironments to facilitate mediated charge transfer or engender additional beneficial effects such as new chemical activities. Below we will discuss the strategies for deciphering the charge-transfer mechanism, designing the abiotic–biotic interface and designing the microenvironments in abiotic–biotic systems.

As the observation of intimate microbial–material interfaces is necessary but not sufficient for unambiguously proving the mechanism of direct charge transfer, additional experimental evidence is needed to corroborate a proposed direct charge-transfer mechanism. In studies of microbial fuel cells, sophisticated electronic devices have been designed to temporally correlate electrical signals from *S. oneidensis* and *G. sulfurreducens* in vivo with the microbial attachment on electrodes[33,37,40]. However, the limited spatial resolution of conventional optical microscopy leaves ambiguity about the nanoscopic interactions of such perceived microbial impacts on electrodes, potentially contributing to various mechanistic interpretations[41]. The observed electrical conductance of protein extensions such as bacteria nanowires[33,34] suggests direct charge transfer via those conductive moieties, although it does not exclude the presence of any indirect/mediated pathways. In photocatalytic biohybrids of light-absorbing materials, photophysical transient spectroscopy is used to measures the lifetimes of the light-absorbing materials[9,42] and probe the charge transfer at the material–microbial interfaces. It is advised to combine multiple, comprehensive characterization methods to support or disprove a proposed charge-transfer mechanism.

The possible existence of endogenous redox mediators secreted from microorganisms[43] complicates the mechanistic analysis at the microbial–material interface and calls for a broadened scope of characterization. When specific membrane proteins are hypothesized to be responsible for direct charge transfer, comparative studies that correlate charge-transfer rates with a variety of genetically engineered mutant strains offer convincing evidence of the role of the proteins in question during charge transfer[34,43]. In the case of *G. sulfurreducens*, a mutant that is deficient of pili, which are composed of protein assemblies, is reported to be incapable of transferring electrons to Fe(III) oxides, highlighting the need for pili in the process of extracellular electron transfer. Moreover, the characterization of charge transfer at the single-cell or even subcell resolution can benefit from recent developments in single-entity electrochemistry[44], scanning electrochemical cell microscopy[45] and single-molecule super-resolution microscopy in catalysis[46]. Notably, there is little information regarding the subpopulation distribution of microbial charge-transfer mechanisms that can vary greatly even within the same genotype. The information obtained at the single-cell level will not only yield population-level averaged charge-transfer kinetics but also inform the subpopulation distribution and competition among different charge-transfer pathways as a function of the experimental conditions.

Generally, as electrons can scarcely be labelled directly or traced, comprehensive characterization that spans from materials to chemical biology is needed for a more definitive conclusion about the charge-transfer mechanism at the microbial–material interface. It is of note that a dichotomy between direct and indirect charge transfer may not exist: the *S. oneidensis* strain MR-1 has been reported to simultaneously possess direct and indirect pathways, and the exact charge-transfer mechanism can be quite situational[41]. Careful characterization and discussions are needed to support a proposed mechanism for each specific material–microbe hybrid system.

Design of abiotic–biotic interfaces and microbial microenvironments

A well-designed materials–biology interface could be potentially beneficial in creating biocompatible microenvironments for maximal cell/enzyme viability/activity and accelerating charge transfer for boosted performance (Fig. 3c). As the electrochemistry and/or photochemistry of synthetic catalysts may inadvertently yield toxins such as reactive oxygen species and transition metal ions, it is critical to design biocompatible interfaces that minimize them. For example, O_2 molecules can be detrimental to anaerobic microbes/enzymes such as N_2-fixing nitrogenase and microorganisms that fix CO_2 via the Wood–Ljungdahl pathway. Hence, a locally O_2-depleted microenvironment generated by synthetic materials can preserve O_2-sensitive biochemical activities in aerobic environments[14,30]. However, it remains challenging to characterize the biotic moieties under such a materials-enabled microenvironment, due mostly to the limited amount of biological sample. The use of fluorescence microscopy could benefit the in vivo monitoring of metabolites, enzymes and even transcriptions of protein expression[47] for microorganisms under such microenvironments enabled by the designed materials–biology interface with appreciable spatiotemporal resolution.

Another potential benefit of well-designed materials–biology interfaces resides in the prospect of boosted charge transfer and net reaction rates. Multiple design considerations have been reported to facilitate a faster rate of charge transfer at biotic–abiotic interfaces: designing a suitable biocompatible buffer for a reduced electrochemical overpotential[48]; deploying high-surface-area electrodes with a sufficiently high buffer capacity[49]; introducing metallic nanoparticles into the microbial periplasmic spaces for faster direct charge transfer[18,50]; and decorating microbes with nanoemulsions of high gas solubilities for accelerated gas uptake[10,26]. There exists a wealth of opportunities in controlling and manipulating the materials–biology interfaces for synergistic benefits.

Metabolomic responses and potential catalytic synergies

As microorganisms are highly regulated bio-machines, how will microbial physiology and metabolism be perturbed and altered by the materials–biology interface for chemical catalysis (Fig. 3d)? It is well known that microbe–materials interactions lead to modified microbial physiology at both the transcriptional and post-translational levels and will potentially affect microbial motility and adhesion. Indeed, multiple omics techniques (see ‘Interfacial electron transfer and mass exchange’) have been applied to biohybrids and have unveiled microbial metabolic responses/regulations[9,13,51], whereas pinpointing how materials regulate a specific metabolic pathway exactly remains challenging.

One technique that is envisioned to be particularly handy is fluxomics, an omics analysis that determines the rates of individual steps within a metabolic reaction network[52]. By quantifying the absolute metabolic flux via isotope labelling of biochemical substrates, an integrated framework of metabolic flux analysis will help to determine the limiting steps of the overall biocatalysis[53], which will provide mechanistic insight on the reaction rate and selectivity in the hybrid system for future improvement.

Nonetheless, from a system-level perspective and combining the aforementioned discussion of materials design, the abiotic and biotic moieties in the hybrid system are both subject to the mutual interactions and regulations that combine systems biology[54] and systems materials engineering[55]. Specifically, a system-level study of a material–biology hybrid requires more than just understanding any individual part in the hybrid and instead emphasizes the interactions between the two parts. We envision that the material part of the hybrid could function similarly to the signal molecules in regulating metabolism, by passing a regulating signal through the materials–biology interface[9]. Moreover, the current design of material–microbe hybrids assumes a one-directional and irreversible procession of reaction steps from material to microbe or vice versa. Yet it is foreseeable to engender unique catalytic activities and substrate-channelling behaviours[8] through intertwined reaction steps and a two-directional exchange of mass at the materials–biology interface. The materials–biology interface renders the biohybrids inseparable entities; therefore, a holistic understanding and design of the whole biohybrid should emerge in the future.

Outlook

Looking forward in the field of material–microbe catalytic hybrids, two general research strategies are perceived. First, a fundamental understanding of materials–biology interfaces must be further advanced. The spatiotemporal correlation, dynamics and electron/mass transfer between abiotic and biotic moieties could be studied with greater detail via a palette of multidisciplinary techniques such as super-resolution microscopy, transient spectroscopy and multi-omics methods combined with synthetic biology capabilities. By taking advantage of these in vivo super-resolution microscopy/spectroscopy techniques, the transport or activity of enzymes/molecules of interest that are tagged with particular signals (for example, fluorescent markers) using bioorthogonal chemistry could be characterized and tracked, therefore enabling us to depict the detailed picture of mass and charge transfer in material–microbial hybrids. Second, the catalytic biohybrids could be developed further towards practical applications that benefit from the deepened mechanistic understanding at a system level. Endowed with the multidisciplinary tools available from materials and biological sciences, a system-level design of material–microbe hybrids may enable their potential to be realized through matching the reaction fluxes in both materials and biological components, thus facilitating the rate-limiting step of the whole catalytic cycle.

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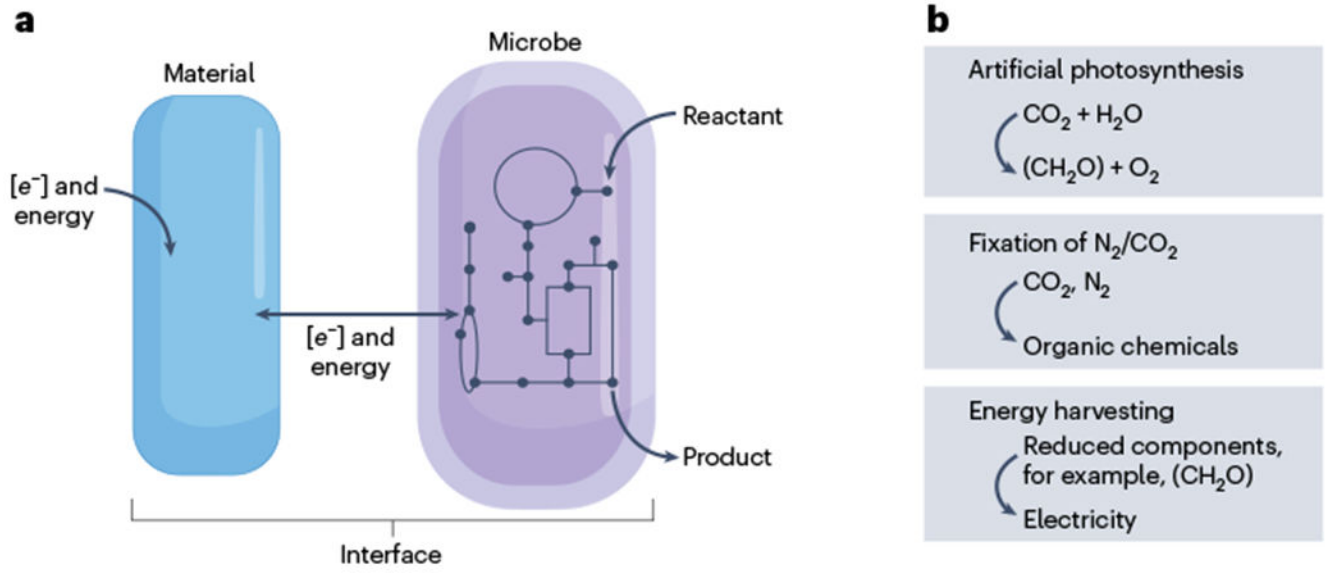

Fig. 1|. The material–microbe hybrid system.

a,**b**, Schematic (**a**) and applications (**b**) of the material–microbe hybrid system. Reducing equivalents ([e^-]) and energy produced on the material are exchanged at the abiotic–biotic interface for chemical reactions catalysed by microbial metabolism. The formula (CH_2O) represents the generated carbohydrate.

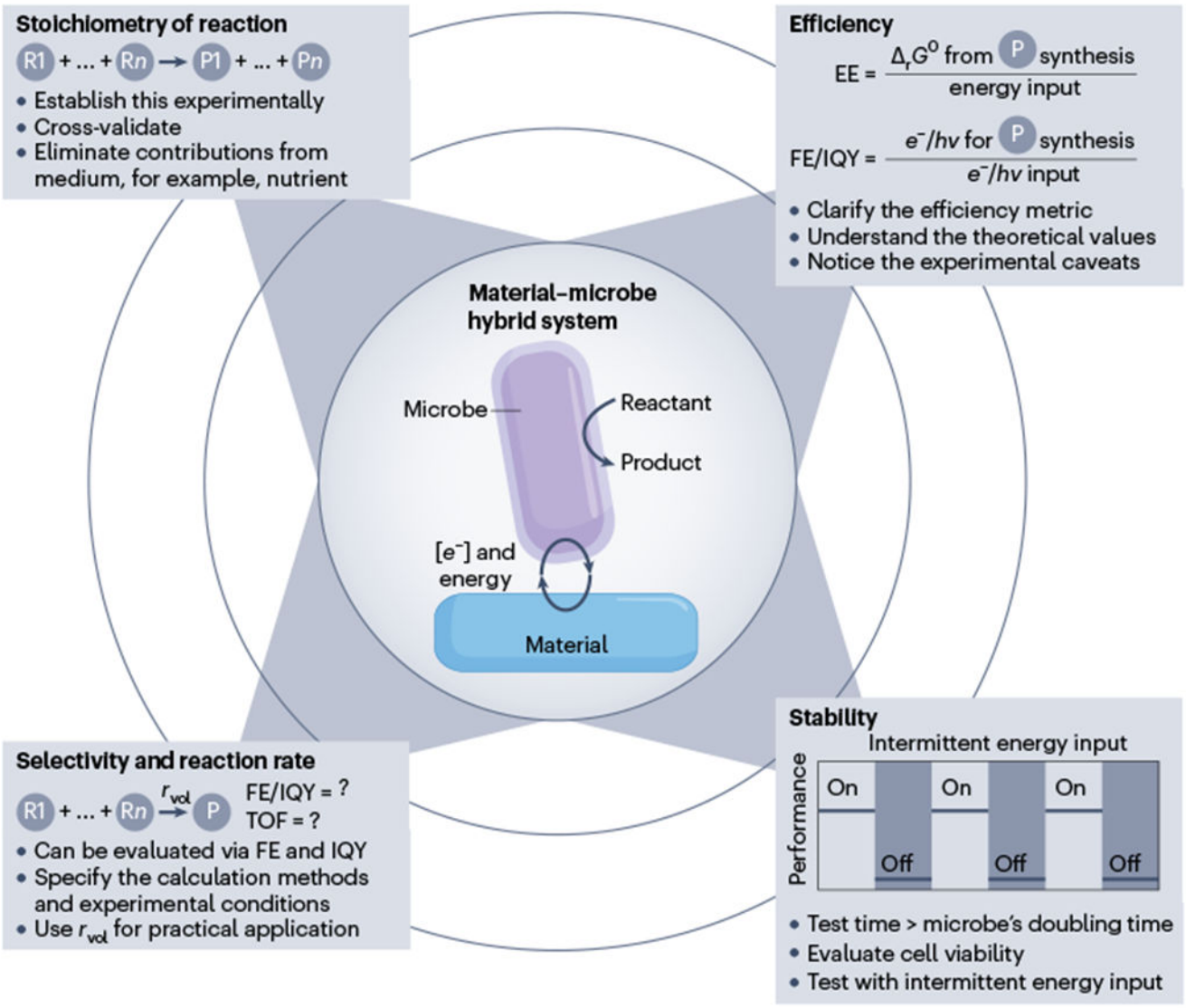

Fig. 2|. Figures of merit for assessing the catalysis of material–microbe hybrids on a radar plot.

Schematic representation of the quaternary evaluation criteria for material–microbe hybrid systems, including the reaction stoichiometry, efficiency, selectivity and reaction rate, as well as stability. [e^-] reducing equivalent transferred at material–microbe interfaces; R/P, reactant/product of the chemical reaction catalysed by the hybrid system; r_{vol}, volumetric reaction rate; FE, Faradaic efficiency; IQY, internal quantum yield; TOF, turnover frequency; EE, energy efficiency; $\Delta_r G^0$, Gibbs free energy of the reaction; e^-, electron; hv, photon.

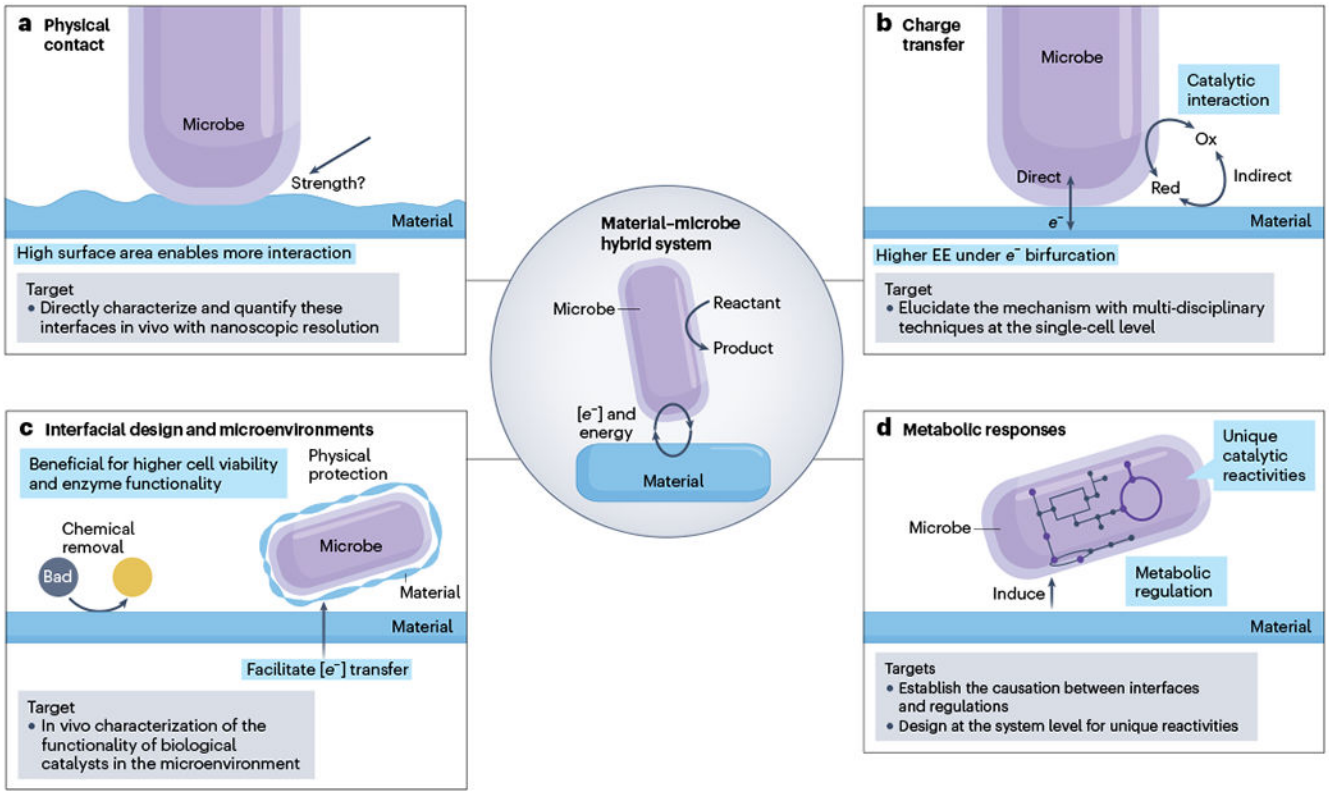

Fig. 3|. Fundamental questions regarding material–microbe interfaces.

a, Physical contact between the material and microbial components. **b**, Charge transfer between materials and microbes through direct/indirect charge-transfer mechanisms. **c**, Microenvironments with improved biocompatibility or enhanced charge transfer enabled by the materials through chemical reaction or physical protection, **d**, Regulation of the microbial metabolism induced by the materials. Red/Ox, reduced/oxidized form of electron shuttles for electron transfer; Bad, species incompatible with the biological parts in the hybrids. The text in blue boxes denotes the possible benefits of the materials–biology interfaces, and the targets identify future challenges.