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Emerging investigator series: Emerging biotechnologies in wastewater treatment: from biomolecular engineering to multiscale integration

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Water Impact Statement

The frontier review discusses the recent progress in biocatalyst and bioreactor engineering to improve the efficiency and specificity of existing bioremediation technology, which is expected to open up new opportunities for many relevant applications from hazardous pollutant removal to valuable materials recovery from waste water.

Emerging investigator series: Emerging biotechnologies in wastewater treatment: from biomolecular engineering to multiscale integration

Yixin Zhang, ⊥ Huan-Hsuan Hsu, ⊥ John J. Wheeler, Sishuang Tang, Xiaocheng Jiang*

Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA

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ABSTRACT: Biological wastewater treatment is the process in which toxic chemicals can be degraded into small, environmentally friendly molecules by various microorganisms. Given the fact that traditional physical and chemical purification methods are high-cost, unsustainable and unspecific, biotreatment is playing an increasingly important role in the wastewater treatment field. The effective implementation of biotreatment strategy relies strongly on the intrinsic degradation capability of the microorganisms as well as their interaction with pollutants. In this review, we will focus on recent technological advances in engineering and improving biotreatment at both biocatalyst and bioreactor levels. Specifically, we will discuss the progress in synthetic biology for enhancing biosorption and biotransformation, and the challenges in applying engineered microorganisms on contaminated sites. We will further review the latest development in bioreactor design, particularly the prospects of additive manufacturing/bioprinting to further optimize the mass transport inside bioreactors through complex 3-D structures and flexible material selections. These research efforts are redefining the frontier of biotreatment, and opening up new opportunities for cost-effective, efficient, and sustainable wastewater treatment.

1.Introduction

The generation of considerable amounts of wastewater owing to increased human activities, unsustainable agricultural practices and rapid industrialization exacerbates the water shortage and pollution problem in modern society.[1] Among various strategies used for restoring wastewaters such as chemical methods (oxidation, reduction) and mechanical methods (filtration, solidification and reverse osmosis),[2-4] biological treatment methods, which involve utilization of microorganisms to remediate pollutants in wastewater, have become one of the major approaches.[5, 6] After billions of years of evolution, living microorganisms have developed various detoxifying mechanisms to maintain homeostasis and resistance to the contaminated environment by transforming organic or inorganic wastes into biologically degraded or less toxic forms.[7] Therefore, biotreatment strategies are considered cost-effective and environmentally-friendly as they do not add additional toxic chemicals or secondary pollutants during purification.[8]

Biological treatments can be realized either independent of microbial metabolism, which is known as "biosorption" or "passive uptake", or through metabolic activities, which is referred to as "biotransformation" or "active uptake".[9] Based on physical/chemical interactions, microorganisms such as bacteria, algae and fungi have shown tremendous potential for remediating a wide range of pollutants, such as heavy metal ions (e.g. arsenic, cadmium, chromium, lead etc.), organic micropollutants (e.g. Ibuprofen, Carbamazepine Metoprolol, etc.) and industrial wastes (e.g. azo dye).[10-12] Although a broad range of treatment has been achieved based on biological activities, the biological process is usually slow, which could lead to accumulation of pollutants and failure to efficiently degrade some complex contaminants.[13] And for some specific anthropogenic chemicals, including persistent organic contaminants, such as dichlorodiphenyltrichloroethane (DDT), 1,2,3-trichloropropane (TCP), and some polychlorinated biphenyls (PCB), natural strains have not evolved efficient catabolic traits to degrade these pollutants.[14, 15] As a result, there is an ongoing interest to construct engineered strains that are capable of degrading various wastes in a fast and efficient manner in contaminated environments. Advantages such as small genome size, relative simplicity of the cell, short replication time, rapid evolution and adaption to the new environments make microorganisms especially favorable candidates to meet genetic engineering purposes.[14] Meanwhile, the development of multi-omics technologies including genomics, transcriptomics, proteomics, metabolomics and fluxomics has enabled researchers to better understand and reprogram biological systems.[16] So far, there are many successful examples of engineering microbes to enhance treatment efficiency which can be summarized in these aspects: expressing degrading genes for different targets in one single stain, modifying proteins/enzymes to increase affinity and specificity, constructing stable and efficient metabolic pathways, and regulating communications in coordinated bacterial networks.[8, 17-19]

Effective operation of biological treatment systems not only relies on highly active microorganisms but is also contingent upon microbial cell-contaminant interactions that happen inside the bioreactor. The efficient mass transfer inside the bioreactor, by providing fresh gas and nutrients and exhausting purified water timely, will allow microbes to work in the most appropriate environment and ensure optimal treatment efficiencies. Conventionally, mass transfers inside bioreactors are facilitated through optimizing flow mechanics such as continuously agitating microorganism/wastewater mixtures or choosing porous material to allow bacteria to anchor and grow. Nowadays, breakthroughs in materials and mechanical engineering offer extensive opportunities for engineering flow dynamics beyond reactor level and up to millimeter to sub-millimeter scale. Specifically, 3D printing technology stands out as it offers a unique platform to customize bioreactors by designing complex 3D structures with flexible material. Through rational design of the biotic-abiotic interface and spatial modulation of bio-physical and bio-chemical microenvironments, the microbial loading density can be maximized while maintaining the mass transfer/bioactivity in bioreactors. These superior characteristics open up new possibilities in engineering microbial communities for wastewater treatment.

Considering abundant progress in engineered biosystems for wastewater treatments, in this review, we will provide an overview of recent advances on engineering biological systems for higher treatment efficiency in two parts. In the first part, synthetic biology tools used to engineer two basic treatment functions of microorganisms- physical adsorption and catalytic transformation- will be introduced. The second part will focus on engineering bioreactors by exploring 3D printing technology. Two key parameters that have a large impact on the interactions between microbes and pollutants -- --structure and materials--will be analyzed. Lastly, a sustainable approach -- Microbial Fuel Cells (MFCs) will also briefly covered. Especially, we identify municipal wastewater as the primary focus of this review, which is considered as the major point-source that can cause severe damages without proper treatments.[20] As municipal wastewater is rich in phosphorus and nitrogen related compounds (the general target of biological wastewater treatment), biotechnology is one of the most important strategies in the treatment of municipal wastewater.[21] Furthermore, advanced biotechnologies also provide new possibility in removing emerging containments (e.g. metals, pharmaceuticals, synthetic organic pesticides, microplastic etc.)[22, 23] that continuously challenging the conventional treatment technologies.

2. Biological machineries: overview, microbial consortia, and practical challenges

2.1 Biosorption – surface binding engineering

Microorganisms exhibit high, non-specific metal adsorption abilities, which are enabled by their high surface area per unit weight and the prevalence of electronegative functional groups on cell surfaces such as hydroxyl groups, sulfhydryl groups, carboxyl in anionic groups, phosphate groups and nitrogen containing groups like the amino groups.[24, 25] Good adsorption performances have been identified in gram positive bacteria (*Bacillus, Corynebacterium, Streptomyces, Staphylococcus* sp., etc.), gram negative bacteria (*Pseudomonas, Enterobacter, Aeromonassp,* etc.) and cyanobacterium (*Anabaena* sp., etc.). Numerous papers have summarized recent progress by using natural strains as sorbent to treat polluted water which can be found elsewhere.[26, 27] Specifically, by expressing metal-binding proteins/peptides in the cytoplasm (intracellularly) or on the cell surface (extracellularly), followed by cell lysis to release metal ions or direct desorption, respectively (Fig. 1a),[28] some types of microorganisms have shown specific metal removal capability with superior selectivity and binding efficiency.[29, 30] However, expression in limited types of strains, limited binding sites on these expressed proteins/peptides, as well as low selectivity of specific ions in the presence of other chemicals, compromise the applicability of using microbial cells as biosorbents.[31] Therefore, there is a great need to engineer these metal binding proteins/peptides to be displayed on different cells with both enhanced affinity and specificity towards metal ions. Currently, metallothioneins (MTs) and phytochelatins (PCs) are widely used to engineer microbial biosorbents for metal removal.[32-35] By using microbial cell surface display technology, these foreign proteins/peptides can be immobilized on different host cells by fusing with anchoring proteins (Fig. 1b). Among widely used host cells, *E. coli*, *P. putida*, and the yeast *S. cerevisiae* stand out for their well-studied genetic engineering paradigms. These microorganisms have been engineered to display MTs or PCs for binding a wide range of heavy metal ions, such as Cu^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and Ni^{2+}.[36-40] When high specificity for a target metal ion is required, metalloregulatory proteins can be also expressed due to the "metal sensing" capability by translating binding events into conformational changes.[41] For example, by co-expressing Hg^{2+} transport system and MTs in E. coli, Deng et al. demonstrated efficient and specific removal of mercury (> 90%) in the presence of other metal ions.[42] Similarly, high and specific uptake for Ni^{2+} was also realized by using recombinant *E. coli* where both nickel transmembrane proteins and MTs were expressed.[43]

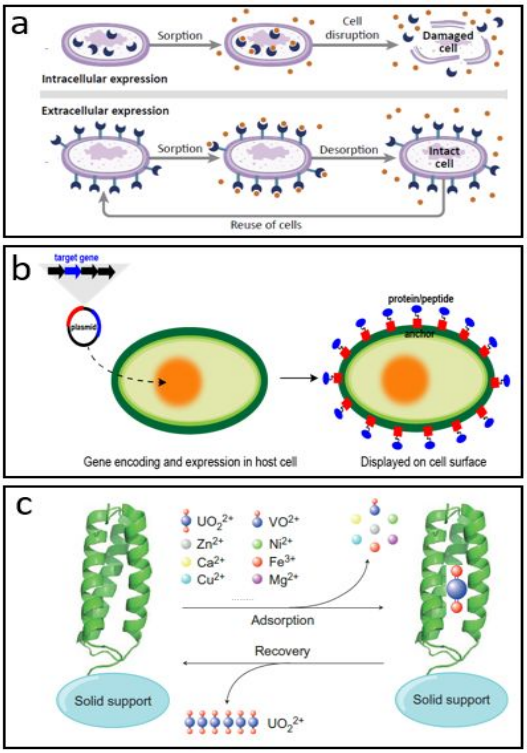

Fig. 1 Natural or artificial microbial metal adsorption ability. (a) Natural microbial adsorption ability enabled by intracellular or extracellular expressed metal binding proteins/peptides. Reproduced with permission from ref. 28. Copyright © 2018 Elsevier Ltd. (b) Cell-surface display allows foreign proteins expressed in various host cells by synthetic biology tools. Both stability and functionality of the expressed proteins could be well maintained. (c) *De novo* designed protein could effectively sequester uranyl from sea water or uranyl-containing groundwater. Reproduced with permission from ref. 44. Copyright © 2014 Springer Nature.

In addition to displaying existing proteins/peptides, protein engineering that enables novel functions and improved performances by random design or directed evolution could also enrich metal-binding abilities of known proteins/peptides. Recently, Zhou et al. developed an engineered uranyl-binding protein that is thermally stable and offers superb affinity and selectivity for uranyl (Fig. 1c).[44] Incorporating multiple binding sites within one protein/peptide is also promising to enhance binding affinity that could target several different metal ions simultaneously. For example, Mauro et al. constructed multidomain polypeptides and expressed them in *E. coli*.[45] A 65-fold increased Cd^{2+} uptake ability was achieved with recombinant cells, showing the possibility of developing novel multifunctional peptides/proteins to detoxify a wide range of compounds.

Because the biosorption process is metabolism-independent, non-living biomass can be also used as sorbents for pollutants removal. Particularly, extracellular polymeric substance (EPS) secreted by microbial cells during biofilm formation has attracted considerable attention owing to the abundance of charged functional groups, such as carboxylic and phosphoryl groups on the EPS matrix.[46, 47] These charged moieties could serve as natural binding sites to adsorb other

charged molecules including heavy metal ions. Considering its high surface area and sustainability, EPS may provide more efficient and cost-effective adsorption capabilities compared with the cell surface display strategy. By now, there has been numerous examples of using EPS as biosorbents for removing metal ions, such as Zn^{2+}, Cu^{2+}, Cr^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+}.[48-51] Instead of using the whole EPS matrix, which consists of polysaccharides, proteins, lipids and DNA, adsorption only requires single components to be extracted directly and immobilized onto a supporting substrate to act as "living filter" versus traditional non-living filtration technologies, such as reverse osmosis and nanofiltration. One example of using a single component is illustrated by Singh et al. used alginate gel beads, extracted from seaweed and brown algae, to effectively remove divalent metal ions Cu^{2+} from aqueous media. Another important EPS component frequently used is amyloid protein nanofibers produced by E. coli bacteria, which could be easily genetically engineered to endow a variety of functions including specific binding to metal ions (Fig. 2a).[52] Recently, Courchesne et al. developed a filtration method to quickly purify engineered fibrous proteins from the *E. coli* biofilm matrix, which then self-aggregated onto membranes to form free-

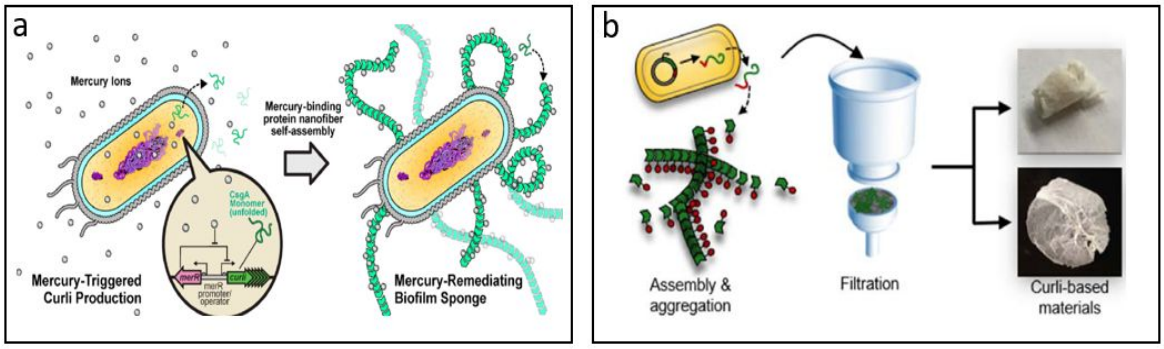

Fig. 2 EPS component-amyloid protein nanofibers secreted by E. coli could exhibit metal binding capabilities after being genetically engineered. (a) E. coli is engineered to produce mercury-absorbing self-assembling extracellular protein nanofiber by integration ofintegrating a mercury-responsive promoter and an operon into bacteria gene. Engineered bacteria can detect and sequester toxic Hg^{2+} ions from the environment. Reproduced with permission from ref. 52. Copyright © 2017 American Chemical Society. (b) Scalable production of genetically engineered nanofibrous via vacuum filtration procedure. Reproduced with permission from ref. 53. Copyright © 2017 American Chemical Society.

standing films by removing anchoring protein CsgB (Fig. 2b).[53] This scalable approach further facilitates applicability of recombinant amyloid proteins to treat various pollutants.

2.2 Biotransformation – metabolic engineering

2.2.1 Degradative metabolism overview

In addition to the physiochemical adsorption of wastes using either living organism or derived non-living biocomponents, microorganisms also possess astonishing metabolic pathways to utilize various toxic compounds as a source of energy for growth and development, through respiration, fermentation, and co-metabolism (Fig. 3). In contrast to the biosorption process that is dependent on the contaminant concentration and kinetic equilibrium of microbial binding sites, biotransformation is metabolism-driven and therefore, may be superior when high targeting sensitivity is required under the circumstance of low concentration of pollutants. In the presence of oxygen, aerobic bacteria could oxidize organic contaminants into non-toxic counterparts, usually carbon dioxide through respiration. Based on this principal, a wide range of organic contaminants such as aromatic hydrocarbons and pesticides have been remediated.[54, 55] While in the anaerobic

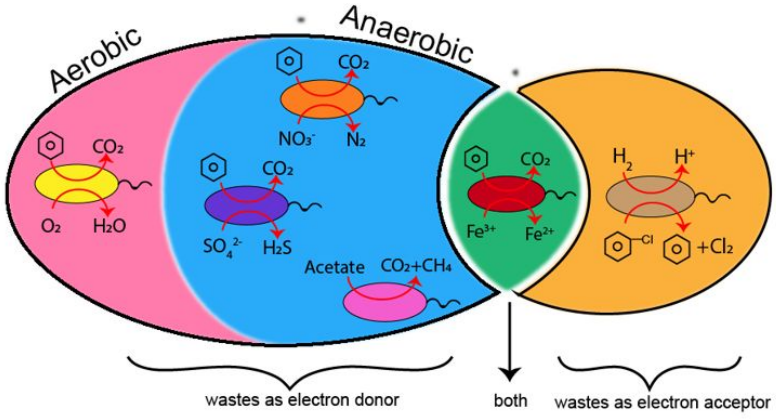

Fig. 3 Pollutants treatment through microbial metabolism. For most types of bacteria, organic compounds are removed by acting as electron donors, while the electron acceptors could be oxygen (aerobic, pink region),) or nitrate, or sulfate (anaerobic, blue region). For chlorinated contaminants, which are not easily oxidized, undergo reductive dichlorination (yellow region). For electrochemically active bacteria (EAB)), they could consume both organic wastes and oxidized metal ions (green region).

environment, microorganisms could instead utilize different electron acceptors such as nitrate, sulphate and acetic acid to oxidize organic contaminates through denitrification, sulfidogenesis or methanogenesis reactions.[56, 57] Not only can they serve as electron donors to be removed, some contaminants can also work as electron acceptors in the reduction process. For example, by utilizing reductive dehalogenation metabolism, chlorine present in the contaminants can be degraded by some anaerobic microorganisms. [58-60]

The aforementioned metabolic activities taking place inside the cells do not represent all possibilities. Some electrochemically active bacteria (EAB), such as *S. oneidensis*, *G. sulfurreducens*, and *P. aeruginosa*, have developed extracellular electron transfer (EET) pathway to "dump" metabolically-generated electrons out of the cell membrane, which can then be captured by external electron acceptors or electrodes.[61-63] Therefore, when toxic metal ions are present in their growth environment, these soluble contaminants can be transformed into non-soluble forms by acting as electron acceptors, thereby realizing the purification purpose.[64] For example, *S. loihica* has been used to reduce toxic vanadium (V), chromium (VI) to less-toxic, insoluble V (III) and Cr (III) simultaneously.[65] Similarly, Geobacter species have also shown the ability of in situ biotreatment of uranium-contaminated groundwater by reducing soluble U(VI) to insoluble U(IV).[66] In terms of electron donor, although in most scenarios EAB consumes simple substrates such as acetate, lactate or glucose, research has found that it is possible to utilize more complex substrates such as industrial or domestic water,[67, 68] which will further expand potential of EABs-based wastewater treatment. When bacteria grow on the electrode, which has been utilized in Microbial Fuel Cells (MFCs), electricity could be generated by using solid electrodes as electron acceptors and recovering chemical energy stored in wastewater.[69, 70] Therefore, EAB-based treatment holds a lot of promise as it simultaneously deals with water availability and energy shortage, two major issues society is facing today. Moreover, MFCs are known as an energy-saving technology. This is due to it being able to work well at ambient temperature, thus requiring less energy for temperature maintenance than common anaerobic digestion (AD) reactors, which also enable energy recovery in the form of methane or hydrogen.[71] And more details about MFCs will be discussed in the last section.

In order to maximize the pollutants treatment efficiency, physiology manipulation can be adopted to stimulate activities of microorganisms related to the degradation of contaminants by optimizing environmental conditions, such as pH and temperature, and adding nutrients required for their metabolic reaction. For example, a bacterial community varies significantly in sludge of different pH.[72] The acidification of media was thought of as harmful for most types of bacteria due to biofilm cracking and low growth rate.[73] For EAB, Young et.al found that EET ability of *S. oneidensis* MR-1 is closely related to the pH level of the electrolyte, where electricity generation increases in the pH range of 6-9 and reaches peak value at pH=9.[74] This is attributed to the improvement of riboflavin (electron mediator) biosynthesis by Shewanella at alkaline pH. Thus, optimizing pH and other environmental factors provides an easy and efficient way to enhance biotreatment ability by influencing growth and metabolism of microorganisms.

2.2.2 Metabolism rewriting

For some intrinsically slow and inefficient metabolic activities, engineering biological pathways of microorganisms provides an alternative avenue to solve the above limitations by designing and constructing new catabolic pathways with improved pollutants treatment ability. The development of whole-genome sequencing and high-throughput screening in genetic engineering assists the global view of gene expression, enzymes, and biosynthetic pathways in microbes under stress condition caused by pollutants.[75] Based on acquired biological information, metabolic engineering can then be exploited to modify already existed metabolic pathways or introduce new catabolic pathways into different host cells to achieve an enhanced biotreatment capability by increasing enzyme activity, extending targeted pollutants range or enhancing biofilm formation. [76] Enzymes, the building blocks for powering the biotransformation process, plays a quite an important role in microbial metabolic pathways. However, the expression levels in an enzyme's native host may be low in natural conditions, which will compromise its stability and activity in extreme environmental conditions. On the other hand, genetic engineering provides a way to enhance the production of these enzymes by isolating and transferring the coding genes into another expression host. Moreover, when combined with directed evolution or rational design technology,[77] desired enzyme properties like substrate utilization, stress tolerance (pH, temperature, solvents) and even the reaction mechanism can be also tailored.[78] For example, Coconi-Linares et al. realized heterologous co-expression of recombinant enzymes peroxidases and laccases in *P. chrysosporium* strains, which show a broad spectrum of phenolic/non-phenolic biotransformation and a high percentage in synthetic dye decolorization compared with the wild strain.[79] Harford-Cross et al. expressed a mutant cytochrome P450 in *P. putida*, and the two directed mutations in the enzyme active site bring an enhanced treatment activity against different polycyclic aromatic hydrocarbons, including phenanthrene, fluoranthene, pyrene and benzo[*a*]pyrene.[80]

For some complex organic pollutants, however, degradation relies on coordination among multiple enzymes. Therefore, the heterologous expression of complete catabolic pathways is necessary. However, engineering entire pathways is challenging because the expression of different enzymes in a recombinant host organism often consumes a significant amount of the host cell's resources, such as energy molecules (ATP, NADPH) and carbon source, thus placing a metabolic burden on the host.[81] As a consequence of the imposed metabolic load, the biochemistry and physiology of the host will be

dramatically altered. To deal with this obstacle, different computational models such as metabolic flux analysis and machine-learning approaches can be used to weigh, standardize and predict metabolic costs during engineering.[82] A successful example is illustrated by Nagendra et al., who assembled a synthetic route for conversion of highly toxic and recalcitrant 1,2,3-trichloropropane (TCP) to glycerol in *E. coli* via a five-step catabolic pathway (Fig. 4a).[83] Specifically, by using a mathematical model, they optimized the enzyme ratios by adjusting copy number of plasmids to obtain maximal production of glycerol as well as minimal toxicity of metabolites. A mineralization pathway for a highly toxic organophosphorus pesticide, paraoxon, was also functionally assembled in *P. putida* to allow complete mineralization within 142 h and use it as the sole carbon and phosphorus source.[84]

For the exoelectrogenic bacteria, their metabolically-driven EET pathway is mainly composed of five modules--substrate oxidation, NADH recycling, quinone recycling, shuttle redox reaction, and transmembrane electron transport (Fig. 4b).[85] Therefore, engineering any of the above highly coordinated networks is possible to facilitate EET and enhance treatment performance. For example, the TCA cycle (a.k.a. citric acid cycle) involved in substrate oxidation is the central part of metabolism where ATP and NAD(P)H are generated to support downstream quinone reduction. Adjusting TCA cycle activity is therefore feasible to increase EET rate. For example, Izallalen et al. artificially constructed an ATP drain in G. sulfurreducens to decrease the ATP content of the cell and contribute to the higher respiration rates of engineered cells.[86] Attempts have also been made to introduce riboflavin synthesis pathways from Bacillus subtilis into S. oneidensis MR-1 to increase secreted electron shuttles, which in turn enhances biomineralization-based metal treatment (Fig. 4c).[87]

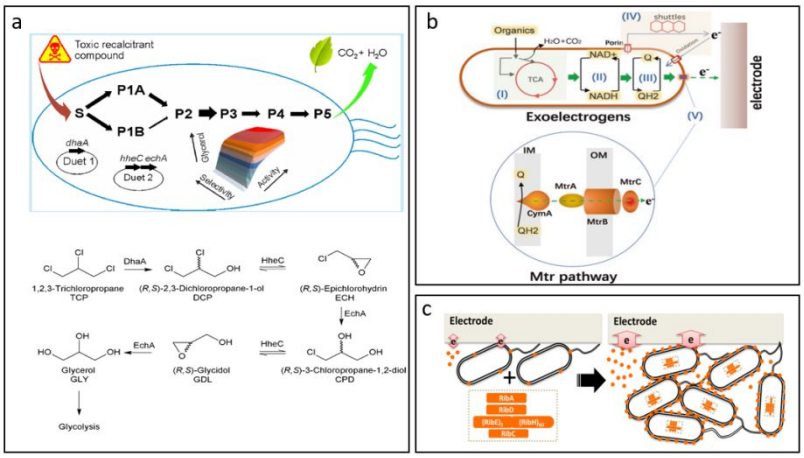

Fig. 4 EngineeringEngineer biological pathways to enhance metabolism-driven pollutants removal. (a) Top: Schematic illustration of toxic compound is degraded into nontoxic, clean product through pre-designed metabolic pathway. Bottom: The synthetic pathway for the biodegradation of TCP assembled in *E. coli* into glycol. Reproduced with permission from ref. 83. Copyright © 2014, American Chemical Society. (b) Five modules in electroactive bacteria for EET. (*I*) The oxidation of organics (initial electron donor) and TCA cycle; (*II*) the redox of NADH; (*III*) the redox of quinone pool; (*IV*) electron transfer to extracellular electrode by shuttles through porin complex; and (*V*) the representative *Shewanella* metal-reducing (Mtr) pathway for EET. Reproduced with permission from ref. 85. Copyright © 2019, Springer Nature Singapore Pte Ltd. (c) Synthetic flavin biosynthesis pathway from Bacillus subtilis was heterologously expressed in *S. oneidensis* MR-1, resulting in ~25.7 times' increase in secreted flavin concentration and enhanced EET performance. Reproduced with permission from ref. 87. Copyright © 2015, American Chemical Society.

2.3 Engineering consortia

Whereas previous discussions are focused on monocultures, microbial consortia where multiple microorganisms work coordinately have unique advantages, such as performing complex tasks that individual populations are otherwise incapable of doing, as well as having higher tolerance to changing environments.[88, 89] Wastewater usually contains a variety of pollutants or certain pollutants with complicated chemical structures. As a result, it is hard to use a single strain to remove all the wastes simultaneously. Additionally, more efficient and effective treatment can be anticipated when co-metabolic activities within microbial consortia complement each other. Currently, by developing specific consortia, researchers have successfully degraded various wastes, such as phenol,[90] organic acid,[91] nitrate and phosphate [92, 93] and cellulose.[19] One example is consortia between cyanobacteria/microalgae and bacteria.[94, 95] Specifically, photosynthetic microorganisms provide oxygen, which is indispensable for pollutant-degrading heterotrophic bacteria. In return, carbon dioxide from bacterial mineralization completes the photosynthetic cycle. This symbiotic interaction can be regarded as an ideal self-sustainable system which is better than conventional engineering designs of adding oxygen. Researchers also found when biomass degrader-anaerobic fungi are co-cultured with methanogenic archaea, effectiveness of waste breakdown can be enhanced by boosting synergistic relationships between them.[96] For example, co-cultivation of fungi *Neocallimastix* strain N1 with *Methanobacterium formicicum* strains cause cellulose digestion rate increased and at the same time, fermentation products are also shifted from less-valued chemicals, lactate and ethanol to more valued fuel energy--methane.[97] Being inspired by the natural symbiotic strains, rationally choosing and controlling desired cell-cell communications among engineering microorganisms to form coordinated cellular network may provide a

way to mitigate metabolic burden in microbial cells or degrade complex compounds that are hard to construct entire degradation pathways in one strain.

2.4 Challenges in the real world

Although genetically-engineered biological machineries have demonstrated promising treatment outcomes attributable to their extraordinary capabilities in adsorption and/or catalysis, their operations are still limited to ideal laboratory environments.[98] There are mainly two challenges remained to be solved before engineered microorganism can function in real-world scenario. The first issue is the robustness of engineered cells in complex polluted environments where pH, salinity, temperature, dissolved oxygen, redox potential, radioactivity, and overall cleanliness show large deviations compared with laboratory parameters. Furthermore, overexpression of genes in the plasmids of engineered bacteria can cause unnecessary burdens for the cells and slow down their growth and reproduction.[81, 99] As a result, it's still difficult for engineered bacteria to compete against natural strains in real environments as they are vulnerable and easily degraded. Therefore, future researchers shall identify key physiological parameters that have large influence on in-site biotreatment effect and explore more advanced synthetic biology technologies to minimize side-effects after gene editing. A simple strategy is to use low, rather than high, copy number plasmid vectors.[100, 101] Alternatively, avoiding the use of vectors by directly integrating foreign DNA into chromosomal DNA of host microorganisms could also eliminate unnecessary antibiotic resistance marker gene products.

Concerns of genetically engineered microbes lie with their potential threats to the ecological system and long-term impact to human-beings. These concerns could be addressed by exploring biological containment strategies to eliminate gene leakage to the environment. One approach is to introduce suicide systems into engineered bacteria so that it dies after completing required tasks.[102] More advanced genome-free gene editing technologies, such as CRISPR-Cas9, are also capable of creating marker-free engineered cells.[103, 104] Furthermore, physical encapsulation strategies by rationally designing bioreactors as discussed below also deserve special attention as they are more accessible compared with complex biological isolation methods.

3. Bioreactors: overview, upgrades, and self-sustaining operation

3.1 Overview of Bioreactors

Central to biological wastewater treatments are the physical (e.g. absorption) and chemical (e.g. nitrification, transformation etc.) interactions between microorganisms and contaminants, which determine the treatment efficiency of each bioreactor.[105] Hence, accelerating these interactions through promoting mass transfers inside the bioreactors can significantly improve the treatment efficiencies. Since microbial communities in the bioreactors are developed through different growth conditions from suspended-growth (i.e. activated sludge; with high microbial activity) to attached-growth (i.e. biofilm; with high microbial loading density) based on diverse treatment conditions (e.g. reactor size, contaminate concentration, etc.),[106] enhancing mass transfers in these bioreactors requires customized engineering approaches.

Conventionally, mass transfers inside these bioreactors are facilitated through optimizing their flow dynamics. In suspended-growth microbial communities, stirred tank bioreactors are the earliest and still the most common approach to promote mass transfer by continuously agitating microorganism/wastewater mixtures.[107] Besides, agitation can also improve the gas phase mass transfer in wastewater to increase oxygen content, which is favorable to the metabolism of aerobic microorganisms and leads to higher treatment efficiency.[107] Furthermore, in attached-growth microbial communities, mass transfer is commonly enhanced through increasing the interface area between wastewater and biofilm. For example, packed bed biofilm reactors have demonstrated outstanding treatment outcomes by utilizing particular support biocarriers such as silica granules, polymer beads, activated carbon particles, etc. to construct porous matrix for microorganisms to anchor and develop high surface area biofilms.[107-109] Due to their high biomass content, the treatment efficiency of packed bed reactors are usually superior to the stirring tank reactors. Nevertheless, the concentration gradients of waste contained in the packed bed reactors (waste concentration continuously decrease along wastewater flow as a result of biotreatment) within the packed bed reactors can lead to hydraulic instability owing to uneven biofilm distribution and consequently, increasing the complexities in operations and maintenances. The developments and optimizations of these bioreactors have been systematically summarized in several review articles.[107, 110]

Combining the advantages of suspended-growth and attached-growth bioreactors, the moving bed biofilm reactor (MBBR) was developed in Norway during the late 80s to early 90s. In MBBR, biofilms are grown on small biocarriers that can be suspended and flow within the water streams inside the reactors where agitation is applied to ensure uniform mass transfer. MBBR can advance the volumetric treatment capability in stirred tank bioreactors as the biofilm-covered biocarriers can largely increase in biomass loading. These "floating" biofilm can also eliminate the common drawback of packed bed

reactors in complex operations caused by the uneven biofilm distributions and backwash requirements.[111, 112] The designs of biocarriers are always essential because they determine the efficiencies of MBBRs. There are two key criteria in creating high performance biocarriers, namely: (i) high specific surface area; and (ii) strong bacterial-surface interactions since high specific surface area can increase the volumetric loads of biomass while strong bacterial-surface interactions can avoid the detachment of biofilms induced by external hydraulic shearing forces. For instance, Wang et al. utilized porous polyurethane particles as floating biocarriers to construct a bioreactor that is capable of treating organic contents with high loading rates.[113] Additionally, biocarriers consisting of different materials such as zeolite,[114] nylon,[115] ceramics,[116] porous glass,[117] PVA,[118] and polyacrylamide[119, 120] were also explored by various research groups. These developments in synthetic biocarriers have been comprehensively reviewed by Bouabidi et al[121] and Biase et al.[111]

Emerging developments in addictive manufacturing/3-D printing offer extensive opportunities to further advance the designs of biocarriers beyond the conventional approaches (e.g. molding, machining, etc.) by customizing biocarriers with complex 3-D structures with flexible material selections to maximize both surface area and bacterial-surface interactions. Moreover, water-based polymerization technology allows immobilizing bacteria inside polymer matrix to limit the mobility while maintaining their catalytic activity. These immobilized bacteria have shown their potential in the degradation of various types of wastewater contaminants, especially in nutrients, with enhanced efficiency.[121] Based on these achievements in bacterial immobilization, bioprinting tools are recently applied to assemble these immobilized bacteria into hierarchical filters with modulated mass transfer, bacterial concentration, and polymer binding strength, which provide a novel approach for effective water treatment strategies with minimum environmental impacts. Other than these conventional biological treatments that rely on chemical-to-chemical transformation, microbial fuel cell is proposed as an attractive solution by using microbial communities to directly convert organic waste inside wastewater into electrical energy for sustainable wastewater treatments. In the following sections, the two frontier developments will be systematically summarized.

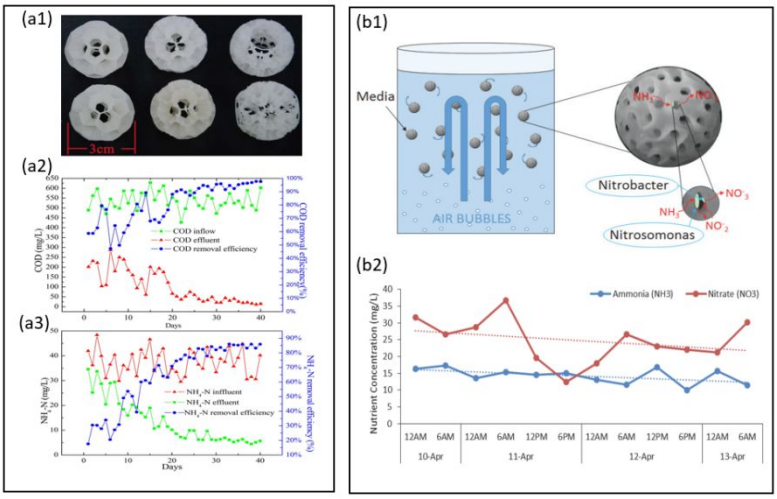

Fig. 5 3D-printedpritinted biocarriers with various structures. (a) 3-D printed honeycomb spherical biocarriers consisted of pentahedrons and hexahedrons: (a1) Images and treatment efficiencies of (a2) COD & (a3) Ammonia; Reproduced with permission from ref. 122. Copyright © 2015 Springer Nature. (b) Biocarriers made of 3-D printed acrylate polymer with spherical gyroid structures: (b1) images and schemes of bioreactor and (b2) treatment results of simulated wastewater that contained ammonia and nitrate. Reproduced with permission from ref. 123. Copyright © 2017 John Wiley and Sons.

3.2 Emerging designs in functionally enhanced biocarriers

The unique abilities of 3-D printing technologies in engineering the morphologies and mass transfer dynamics of biocarriers have demonstrated boosted efficiencies in MBBR. For example, Dong et al. created a series of 3-D honeycomb spherical biocarriers consisting of pentahedrons and hexahedrons for COD and NH_3 removal (Fig. 5a).[122] These customized hollow designs enable an extra mass transfer pathway from inside the biocarriers toward biofilm. Combining with the mass transfer at wastewater/biofilm interfaces, biofilms on the 3-D printed biocarriers present enhanced bioactivity, whereas their improvements in treatment efficiency were not clearly observed. Exploiting a similar strategy, Elliott et al. manufactured spherical biocarriers with gyroid-based hollow interspacing (Fig. 5b), which provides up to 4.5 times larger specific surface area (2309 m^2/m^3) than many commercially available biocarriers (~ 500 m^2/m^3). This increase in specific surface area can directly benefit the waste (NH_3) removal rate 1.620 ppm/day as compared to commercially available biocarriers (0.710 ppm/day).[123] In summary, current studies provide promising results which demonstrate the unique advantages of 3-D printing in fabricating biocarriers to produce biofilms with high biomass loading and/or high bioactivities. However, future research and optimizations in these 3-D printed biocarriers are necessary to achieve the elevation in MBBR efficiencies. From this standpoint, future research is suggested to devote into two key parameters other than the current focus on surface area, which can also dominate the performance of biocarriers: (i) structure and hydrodynamics; and (ii) materials and surface modifications.

In terms of encapsulated biocarriers, current development in natural and synthetic polymers such as Polyvinyl alcohol (PVA),[124]Polyurethane,[125] alginate,[119, 120, 126] chitosan,[127] agar,[119, 120] and carrageenan[128] provide another material selection of biocarriers. Different from attached-growth versions where the biofilms cover on their surfaces, polymers allow microorganisms to be fully embedded "inside" the biocarriers, which offers a biologically relevant environment and effective mass transfer to preserve the normal functions of microorganisms. Additionally, the polymer matrix provides a physical boundary to minimize the microorganisms' exposure to toxic contents in wastewater. Generally, there are two types of polymer carriers, natural polymers and synthetic polymers. Comparing two materials, natural polymers provide good biologically relevant environments (e.g. suitable mechanical strength, sufficient mass transfer etc.), which can well preserve the viabilities of immobilized bacteria, whereas synthetic polymers have high mechanical strength and are more stable in wastewater compared with natural polymers. The selection between natural and synthetic materials should be determined by specific wastewater conditions. Among all materials, alginate is proposed to be one of the most used carriers for microorganism immobilization due to its water-based gelation process, mechanical & chemical stabilities, high porosity and superior biocompatibility, which can well preserve the activity of microorganism at both during and after immobilization.[121, 129] For example, Ozer et al. synthesized *P. boryanum* contained alginate-based biocarriers for Chromium (VI) (Cr^{6+}) treatments, which are able to remove 97% of Cr^{6+} at 90 minutes after introduced into 100 mg/L Cr^{6+} solutions.[130] Furthermore, attributed to the hydrogel boundary, environmental stresses such as toxicity of Cr^{6+} (up to 400 mg/L) and pH show negligible influence on bioactivities of embedded *P. boryanum*. PVA is another widely used material in bacteria immobilization owing to its low cost, simple handling process, low-toxicity properties, as well as its proper pore size for oxygen/waste organic matters diffusion.[131] For instance, El-Naas et al. have utilized PVA immobilized *P. putida* for designing the treatment of contaminations from petroleum refineries in both lab and pilot plant scales.[132, 133] The result indicated that these biocarriers can ensure certain level of activities even under highly toxic environments (i.e. high phenol concentration), which can achieve 96% reduction of COD and 100% reduction of phenol and cresols in the treatment of real petroleum refinery wastewater. In addition to alginate and PVA, various polymers are also fabricated for toxic contaminants (e.g. phenol, trichloroethane) removals such as *B. cereus* @ alginate,[126] methanogenic consortium @ agar,[134] and *P. putida* @ Chitosan[127]. These developments of polymer immobilized bacteria in wastewater treatment are reviewed by Bouabidi et al in details.[121]

While tremendous progress has been made through 3-D printing and hydrogel encapsulations, further enhancement of MBBR performance demands advanced structural and material designs of biocarriers that could transform the bio-integration to achieve higher treatment efficiency and longer-term stability. Potential opportunities include: (1) engineering the structures and materials of biocarriers to accelerate the interfacial mass transport; (2) effective immobilization/encapsulation of microorganisms to extend the lifetime; Key parameters from biomaterials engineering perspective will be summarized in the next few sections.

3.3 Prospects of 3-D printed biocarriers

3.3.1 Structure and hydrodynamics

During biofilm formation, shear stress is an essential parameter which can dominate the attachment, morphology, and detachment of bacterial communities. Chang et al. demonstrated that the biomass density of biofilm in liquid fluidized beds increases while the biofilm thickness decreases when growing under high shear stress.[135] As high-density biofilms usually demonstrate fewer sloughing phenomena, which are considered more stable than thick yet fluffy biofilms, high shear stress on biocarriers is desired during MBBR operations. Nevertheless, biofilm detachment and deformation occur when shear stress overcome the adhesive force between biofilm and substrates/biocarriers.[136] Besides, hydrodynamics can crucially impact biofilm developments by controlling the mass transfer of oxygen and wastes/nutrients.[137] 3-D printing is specialized in fabricating hierarchical structures with spatially controlled physical/chemical properties.[138] This unique capability can offer extensive possibilities to create biocarriers with optimal structural integrity and hydrodynamic patterns to enhance MBBR performance through precise control and engineering of following parameters:

First, various research indicates that sizes of biocarriers can have significant influence on MBBR efficiency. Similar to the 3-D printed biocarriers, carriers with small sizes and great specific surface area have high capability in biomass attachment, which can increase biomass quantity and result in better performance than a big carrier of small specific surface area.[139] However, studies by Muhammad et al. on the texture and topography of biofilms on carriers of various sizes suggested otherwise.[140] In this study, cube-shape biocarriers with six different diameters (5, 10, 15, 20, 30, and 50 mm in diameter) were introduced into separated cylindrical bioreactors that were operated continuously under limited aerobic conditions. Characterizations of developed biofilms on each carrier indicated that the biofilms on carriers with 15 mm demonstrate the highest roughness and strongest bonding between both bacteria-carrier and the bacterial communities. Further analysis in mass transfer and shear stress of each carrier demonstrated that this 15mm diameter can balance the cohesive and shear forces introduced from the agitation in bioreactor while generating suitable aerobic, anoxic and anaerobic zones on their surface for the optimum growth of bacterial communities. Consequently, these 15 mm biocarriers result in

the best wastewater treatment efficiency, while both increasing and reducing the carrier size can lead to adverse impacts. Nevertheless, Ødegaard et al. studied the treatment efficiencies of 4 biocarriers with different sizes/surface areas (490; 1910; 1500; and 7700 mm^2), which suggested that size of biocarrier shows minimum effect in removal rates as long as the organic loading rate of specific surface area (g $COD/m^2 \cdot$ time) is similar.[141] Dias et al. also suggested that the rates of biofilm formation and ammonia removal in their pilot MBBR showed minor correlations with carrier size, but were strongly correlated with a combination of the media physical factors such as voidage and hydraulic efficiency.[142] Based on these studies, we conclude that the relationships between carrier sizes and treatment efficiencies remain unclear and may associate with different factors (e.g. wastewater contains, bacteria species, bioreactor shape & volume etc.).Future designs of 3D-printed biocarriers could be optimized by computational fluid dynamic tools to ensure the treatment outcomes.

Pore size is another important parameter that can affect MBBR performance. Under similar morphology, biocarriers with smaller pore size can possess higher specific surface areas for cell attachment and biofilm development, which can enhance the treatment efficiency of MBBRs through increasing the loads of biomass.[143] However, carriers with small pore size (high surface to volume ratio) can also demonstrate a strong tendency for clogging. Clogged pore spaces reduce the mass transfer, which can significantly reduce the long-term treatment efficiency of MBBR.[144] Generally, the selection of pore size of carriers can be determined by the nature of the treatment process. Carriers with big pore size are suitable for treatments requiring fast-growing aerobic biofilm in order to avoid loss of mass transfer caused by biofilm induced clogging; whereas, carriers with small pore size can be applied in treatments based on slow-growing autotrophic biofilm (e.g. nitrification) to increase the loads of biomass.[111]

It is widely believed that surface roughness can promote the development of stable biofilms in both growth and stationary phases by (i) providing a larger surface area for attachment and (ii) providing anchors to protect the biofilm from detachments due to fluid shearing and collision.[145] However, based on fundamental hydrodynamics, surface roughness may also induce local turbulence and lead to the detachment of biofilm. Since MBBRs can involve diverse hydrodynamic setups and microbial communities based on individual treatment needs, 3D-printed biocarriers are considered as superior to conventional batch fabricated counterparts, which allow rapid customization of biocarriers with desired surface roughness to adapt to various conditions in MBBRs.

3.3.2 Materials and surface modifications

High density polyethylene (HDPE) (AnoxKaldnes™), Polypropylene (PP) (FLOCOR-Henderson) and polyethylene (PE) (Seimens (USA)) are three commonly used materials in biocarrier fabrication for full size MBBRs owing to their stability, plasticity, and proper density. Based on these carriers, the desired treatments of MBBRs have been widely demonstrated in many studies.[146] As filaments of these materials for extrude-type 3-D printer are all commercially available, future research is suggested to also explore HDPE, PP and PE based biocarriers, which can better adapt to existing MBBR setups in the context of hydrodynamic setting and filling fractions as compared with recent developed acrylate polymer[123] and Nylon[122] based 3-D printed biocarriers.

In addition, surface modifications can improve the biological affinity to enhance the biomass load of biocarriers, which has shown positive influence in treatment outcomes. For example, Zhang et al. coated hexadecyl trimethyl ammonium chloride (CTAC) on basalt rock based biocarriers. Biofilms on the modified carriers show increased biomass loads and microorganism diversity with shorter biofilm formation time.[147] Similar phenomena are also found by Chen et al. who studied both ferric ion covered- and gelatin grafted- polyethylene carriers,[148] of which the modified carriers presented biocarriers 8.64 to 10.63 % increases in COD removal efficiencies. Furthermore, the HDPE-based biocarriers are also modified by Klaus et al. through either ozone or potassium permanganate oxidations,[149] which result in significant acceleration in ammonia removals. Other surface modifications of biocarriers are also summarized in the review articles of Biase et al.[111] Based on these studies, we identify that the reduction of both hydrophobic and electrostatic repulsions at bacteria-carrier interfaces through coating/grafting/growing the hydrophilic yet positively-charged materials/functional groups on carrier surface can favor the bacteria attachments and eventually lead to a boost in treatment efficiency. This principle can serve as general guidance for future developments in the surface modifications of 3-D printed biocarriers.

3.3.3 Bioprinting

Recently, rapid developments in bioprinting provide opportunities in assembling these polymer immobilized microorganisms into integrated communities.[150] Compared with conventional particular microbe-polymer matrix, bioprinting allows rationally programming the assembling process in terms of the morphologies, compositions, cellular interactions and microenvironments. These engineered microbial communities have demonstrated enhanced performance over naturally-derived biosystems. These bioprinted microbial matrixes have demonstrated potentials in various applications such as material synthesis[151] and biocatalysis.[152] For example, Qian et al. exploited freeze-dried cells as both biological modules and structural supports, which significantly increased cell loading density, thereby increasing the biocatalytic activity in their yeast-based hierarchical 3-D biocarriers.[152] Specifically, the mass transport within the living 3-D matrixes

can be optimized by creating a highly porous lattice structure with a substantially expanded liquid-solid interface. The productivity of the yeast-contained 3-D biocarriers increases threefold compared with its bulk counterpart, which contains a similar amount of cells but with much lower surface area.[152]

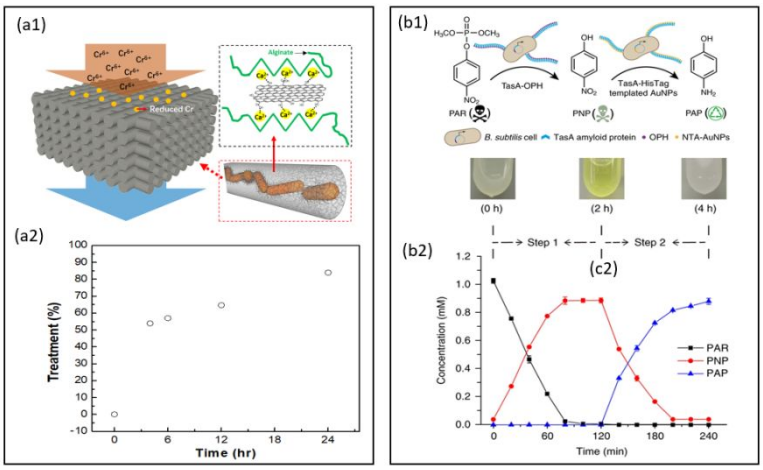

Fig.6 (a) Bioprinted living filter for Cr^{6+} treatment (a1) scheme of the living filter consisted of PV-4 (biocatalysts), bio-reduced rGO (additional electron transfer pathways) and alginate (structural supports) and (a2) Cr^{6+} treatment efficiency. The results indicate that this living filter can achieve around 90% removal of Cr^{6+} after a 24 hour of treatment. Reproduced with permission from ref. 153. (b) Genetic engineered *B. subtilis*-based living material for pesticide treatments (b1) reaction cascades of biocatalytic organophosphate pesticide degradations using assembled TasA-OPH and TasA-HisTag biofilms (b2) concentrations of PAR (pesticide), PNP (intermediate product), and PAP (non-toxic product) at different stages and their correspondent images. Reproduced with permission from ref. 164. Copyright © 2018 Springer Nature.

Beyond these biosynthesis applications, the bioprinting strategy in creating living materials also opens up possibilities in engineering microbial communities for wastewater treatments. Pu et al. seamlessly integrated the nanoscale mediators (i.e. reduced graphene oxide) into the microbial matrix to maximize treatment efficiency of chromium ions (Cr(VI)) (Fig. 6a).[153, 154] Specifically, *S. loihica* PV-4 is known to be able to reduce GO by its unique extracellular electron transfer (EET) process.[155] As the EET of PV-4 is mainly accomplished through the conductive protein matrix on the bacterial outer membranes within the electron tunneling distance (few nm), this as-synthesized reduced graphene oxide (rGO) is seamlessly coupled with the protein matrix and greatly extends the electrically active surface area of PV-4 communities. Combined with a bioprinting platform, this PV-4/rGO hybrid matrix can be hierarchically assembled into a "living filter" which shows improved treatment efficiency and chronic stability over naturally derived biosystems. Although our bioprinted carriers have demonstrated promising results in lab-scale, optimizations are inevitable before their applications in full scale MBBRs. In addition to the material selection that have been discussed in previous sections, we suggest three aspects that could be the focus of consecutive research, namely, (i) bioprinting mechanism, (ii) porosity and interconnectivity and (iii) bacterial interactions.

Taking the advantages of the recent developments in biofabrication for tissue engineering, many bioprinting mechanisms were comprehensively investigated using various mammalian cells as models. These studies are reviewed in many book chapters and journal articles that focus on different prospects of bioprinting mechanisms such as designs,[156] terminology,[157] printability,[158] and materials.[159] Based on these articles, solution- and semi-solution based assembling processes (e.g. stereolithography, liquid-phase gelation 3-D prints, extrusion etc.) are considered as a superior approach for microbial bioprinting due to their biocompatibility, fabrication speed, and ability in scaling up; whereas the solid-phase technologies such as electrospinning and laser sintering are usually challenged by insufficient cell viability.

Porosity and interconnectivity of bioprinted carriers can also play important roles in their performance. Interconnected pores with sufficient openings (pore sizes) can effectively exchange supply (e.g. oxygen, organic matters, etc.) and metabolic waste to support the growth and functions in the immobilized microbial communities, which are usually desired in bioprinted structures.[160] However, one should expect increasing porosity to also significantly reduce the biomass loads due to a large pore (vacant) space, which can eventually lead to a negative impact in overall treatment results. Hence, the balance between porosity and biomass load is essential, which requires further in-depth investigations.

Bioprinting allows rational assembly of heterogeneous materials into complex structures (e.g. multi-layer assemblies,[161] multi-material blocks,[162] and core-shell fibers[163] which provide unique potential in engineering the bacterial interactions that can never be explored by traditional particular biocarriers. For example, aerobic and anaerobic bacteria can be immobilized in a core-shell block by encapsulating anaerobic bacteria at core and aerobic bacteria at shell. This structure allows spontaneous programming of the oxygen content (low at core and high at shell) to favor the growth of all species; therefore, both aerobic and anaerobic treatments can be achieved in this biocarrier. Moving forward, various microbial

consortia can be accommodated to advanced biocarriers through exploring emerging bioprinting tools; hence, more multi-functional, high performance biocarriers are expected to be discovered in the near future.

Additionally, bioprinting has been also combined with powerful genetic engineering tools. Such a strategy allows for creating customized microbial systems with desired and/or advanced functionalities for removal of toxic contaminants. Huang et al. integrated B. subtilis into a tunable living material based on the genetically programmable TasA amyloid machinery of that microorganism (Fig. 6b).[164] Through genetically modifying the TasA, the functionalities of either organophosphate hydrolase (OPH) or HisTag-immobilized gold nanoparticles (AuNPs) can be expressed in the B. subtilis bacterial matrices. OPH can catalyze the degradation of a pesticide, paraoxon, into paranitrophenol (PNP), while AuNPs can further degrade PNP into harmless p-aminophenol. By rationally assembling the TasA-OPH - and TasA-HisTag-AuNPs - bacterial communities with programmed biocatalytic cascades, Huang et al. created a living 3-D material for paraoxon treatment.[164]

3.4 Sustainable approach - microbial fuel cell (MFC)

In biological wastewater treatment, energy consumption is always one of the major concerns that adversely impact the environment. In this regard, microbial fuel cell (MFC) is proposed as an attractive solution by directly converting organic waste inside wastewater into electricity.[165, 166] In MFC, bacteria on the anode decompose organic matter and free H^+ ions and electrons by utilizing their unique EET capacities. Electrons produced by the bacteria from these substrates are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) to produce electricity. The H^+ ions flow through the semi- permeable membrane to the cathode, then combine with dissolved oxygen by either bio- or abio-catalyst-triggered oxygen reduction reactions (ORR) to form water. However, most studies indicated that electricity generation due to low EET efficiency remains the major challenge to be solved before MFCs could recover sufficient energy and truly reduce the environmental impacts of wastewater treatments.[62, 167] To overcome this bottleneck, different designs of MFCs along with their working principles, electrodes and bacteria species have been extensively investigated in the last decades. Conventionally, energy density in MFC is enhanced through increasing the surface area of anode to maximize the bacterial coverage. For this approach, carbon-based materials have been extensively studied to serve as the high performance MFC anode due to their high surface area. Additionally, their biocompatibility and conductivity can also facilitate the interactions between bacteria and the electrode. Recently, various carbon-based materials such as carbon cloth,[168] carbon brush,[169] carbon mesh,[170] carbon veil,[171] carbon paper,[172] graphite felt/rod/foam,[173] granular graphite,[174] etc. have been tested in MFC. The achievements of these works are well-summarized by Zhou et al.[175] and Santoro et al.[176] On the cathode of MFC, various synthetic ORR catalysts can be applied to elevate the overall energy efficiency. Platinum (Pt) remains the most effective catalyst of ORR and is widely used in MFC for high energy productions.[177] However, the complex compositions in wastewater commonly challenge the long-term stability of Pt catalysts. Recently, many non-Pt catalysts such as gold,[178] palladium[179] and porphyrin-related compounds[180-182] have also been used to build high performance yet long-lasting MFCs. The current progress is reviewed by Wang et al.[183]

Furthermore, nanomaterials that possess superb electrical properties and tunability present great potential to facilitate the EET at both bio-bio and bio-electrode interfaces to boost the energy generations inside MFC. State-of-the-art development in nanomaterial assisted MFC have been reviewed and discussed by Hsu et al.[62] Lastly, by exploiting bioprinting (to accelerate the mass transfer) and nanomaterials (to facilitate the EET) to program the assembly of an S. oneidensis MR-1 community, Freyman et al. created a bio-abiotic integrated MFC anode that demonstrate over two times improvement in energy density as compared to its solid-state counterpart. This work provides significant insights in advancing energy generation of bioanode through engineering microbial communities, which can open up many new possibilities apart from conventional approaches that mainly focus on the materials of electrodes.[184]

4. Conclusion

Microorganisms have shown tremendous potential in restoring contaminated wastewater inexpensively and sustainably. Recent progress in synthetic biology has provided reliable platforms to precisely engineer their structure and function, enhance adsorption and catalytic capability, and improve the efficiency and specificity for biotreatment. The advances in 3-D printing technology further promote microbial loading, mass transport and bioactivity through the development of novel biocarriers. Together these efforts are opening up new opportunities for many relevant applications from hazardous pollutant removal to valuable materials recovery and will benefit future research in minimizing potential environmental risks caused by genetically engineered microorganisms and their scale-up production in the real word.

AUTHOR INFORMATION

Corresponding Author

* Xiaocheng. Jiang@tufts.edu

Author Contributions

[⊥]Y.Z. and H.H. contributed equally to this work.

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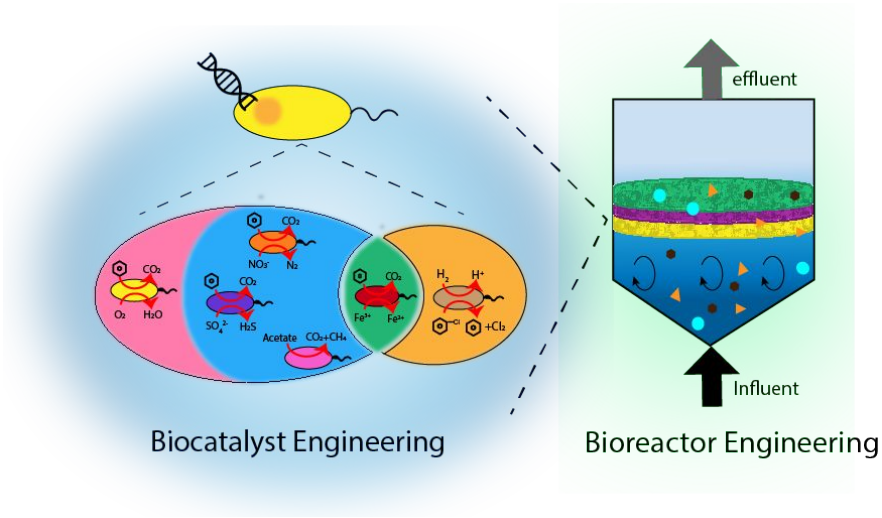
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