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Controlling Radical Polymerization with Biocatalysts

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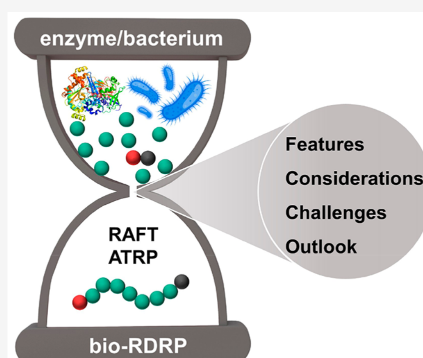
ABSTRACT: Reversible deactivation radical polymerization (RDRP) is a set of powerful and versatile methods for the synthesis of well-defined polymers. Over the past two decades, the engagement of biocatalysts, namely, enzymes and bacteria, has granted distinctive features to RDRP and propelled RDRP toward a more sustainable future. In this Perspective, we highlight the green conditions, oxygen tolerance, versatile function, and the ability to access difficult polymers in RDRP conducted by biocatalysis (bio-RDRP), discuss major considerations when conducting bio-RDRP, and point out the drawbacks and bottlenecks that limit its further development. The future of bio-RDRP may benefit from expanding the biocatalyst library, improving the redox potential of bacteria, enhancing the biocatalyst robustness, and in-depth mechanistic studies.



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INTRODUCTION

An important object of modern polymer chemistry is to precisely control polymer structural parameters under mild synthetic conditions. Reversible deactivation radical polymerization (RDRP) is such a powerful and user-friendly synthetic tool that has been widely recognized with the ability to realize this goal.[1−3] Ever since its advent, RDRP has undergone explosive growth because it allows convenient control of many facets of a resulting polymer including molecular weight, dispersity, topology, and sequence, thanks to the rapid establishment of an equilibrium between active and dormant radical species.[4−7] With no doubt, its popularity has been greatly augmented by its tolerance to impurities, operational ease, and applicability to a wide range of conditions.

In recent years, growing interest in green chemistry has driven the development of RDRP toward more sustainable pathways. Notably, various room-temperature methods, instead of traditional heating, have been developed to implement a RDRP. Clean, low-energy consumption methods have embraced the use of visible light, electricity, and mechanical force to externally manipulate a RDRP, which permits spatiotemporal regulation of polymerization kinetics, broadens the scope of control parameters, and eliminates or minimizes the use of metal catalysts.[8−13] These novel RDRP methods have been greatly appreciated by the polymer and materials community, and some with a high degree of biocompatibility have been quickly adopted to prepare biohybrid materials from the surface or even inside of living cells. In this context, the use of biocatalysts would render a greener and more naturally biocompatible RDRP process. Indeed, over the past decade, biocatalysis has emerged as a notable strategy for conducting RDRP (bio-RDRP) under mild and biocompatible conditions.[14−16]

Biocatalysis has long been studied in the field of organic synthesis on account of its well-recognized mildness, efficiency, and selectivity, but bio-RDRP has only been studied for a relatively short period of time.[16−20] Research on bio-RDRP commenced in 2011 when metalloproteins were used to replace the metal catalysts used in atom transfer radical polymerization (ATRP), aiming to mitigate the toxicity.[21−23] Interestingly, di Lena and co-workers added a dithioester chain transfer agent (CTA) to the "ATRP" process in an attempt to reduce the dispersity of the resulting polymer by the mechanism of reversible addition−fragmentation chain transfer (RAFT).[22] Subsequently, enzymes were used in RAFT polymerization, where glucose oxidase (GOx) was used for *in situ* deoxygenation in 2014 and horseradish peroxidase (HRP) was used to initiate polymerization in 2015.[24,25] Around the same time, living bacteria were explored to initiate ATRP for the synthesis of bacteria-templated binding polymers.[26] These bio-RDRP pathways have attracted increasing attention over the past decade because they have opened a new avenue to the synthesis of well-defined polymers in aqueous solution and under ambient conditions.

In this Perspective, we highlight recent developments in bio-RDRP, focusing on the characteristics of enzymatic and

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bacterial bio-RDRP. Unlike chemical catalysts, biocatalysts are more fragile and require special attention when performing RDRP synthesis; therefore, we also summarize some key points that should be considered when implementing bio-RDRP, which may serve as guidelines for nonexperts or newcomers who are interested in exploiting bio-RDRP for their own purposes. Finally, we offer a personal view of the challenges and future directions of this thriving field.

FEATURES OF bio-RDRP

Green Polymerization Conditions. In nature, most biocatalytic processes occur under mild conditions. For an efficient bio-RDRP to take place, it should be ensured that the enzymes or bacteria have a high biological activity; thus, most bio-RDRP is conducted at low temperatures (10−45 °C), under ordinary pressure, in water, buffer solution, or culture media (Figure 1). This mildness avoids the use of heating devices and saves energy. Water, a widely accepted green solvent, is cheap, readily available, and safe to use and will facilitate biological applications of the resulting polymers.

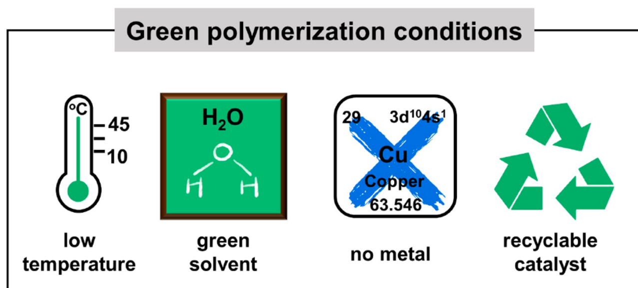

Figure 1. Bio-RDRP features green polymerization conditions.

Metal catalysts are detrimental to biological, electronic, and optical applications.[27,28] While tremendous efforts have been dedicated to the development of ATRP with minimal use of metal catalysts, alternative strategies that can preempt the use of metal catalysts are highly desirable.[29−31] This motivated the initial studies of metalloproteins being used to replace traditional metal catalysts in ATRP.[21−23,32] In contrast to traditional ATRP catalysts, the metal complexes are tightly bound to the proteins, resulting in little or no metal leaching to the solution during the polymerization. These enzymes have been demonstrated to be removed more easily than traditional molecular metal catalysts. For example, Bruns et al. used laccase to catalyze the aqueous ATRP of *N*-vinylimidazole (NVIm), and pure polymers free of metal ions could be obtained by simple filtration and precipitation after the polymerization.[33] While metalloproteins need to be removed after polymerization, this operation can be evaded for metal-free enzymes, especially for polymerizations that employ only negligible amounts of biocatalysts. Therefore, it is reasonably safe to say that metal-free polymers can be obtained by enzyme-catalyzed RDRP synthesis, and this less stringent requirement for removal of biocatalysts could at least compensate some cost associated with the high price of enzymes.

Catalyst recycle and reuse has been well perceived as a cost-effective process in catalysis. As mentioned above, enzymes can be removed by precipitation, ultrafiltration, or chromatography and can therefore be reused for polymerization. With a much larger size than enzymes, bacteria can be removed from a polymerization solution with significantly less effort, for example, simply by centrifugation. In this regard, Keitz et al. used viable, spent bacteria after a short recovery period (~6 h) and supplied fresh reagents for additional polymerizations, which showed comparable polymerization kinetics to those using freshly cultured bacteria, and the resulting polymer had a similar molecular weight and dispersity.[34] Gurnani et al. found that the initial bacterial culture could be reused at least three times without supplementing with growth media or nutrients.[35] These studies demonstrated that bacteria can be effectively recycled and reused, one of the advantages of using living bacteria as catalysts for RDRP.

Oxygen Tolerance. Radical polymerization is sensitive to oxygen because it can deactivate a carbon-centered radical by forming a peroxy radical and prevent polymer propagation.[36,37] As such, oxygen needs to be removed by nitrogen/argon bubbling or a freeze−pump−thaw procedure prior to polymerization, or polymerization needs to be conducted in a glovebox. These deoxygenation procedures are laborious and time-consuming and require special equipment, presenting hurdles for widespread adoption of RDRP. To address this limitation, several strategies have been successfully developed, including chemical deoxygenation, photoinduced electron/energy transfer catalysis, and biodeoxygenation (Figure 2).[24,34,38,39]

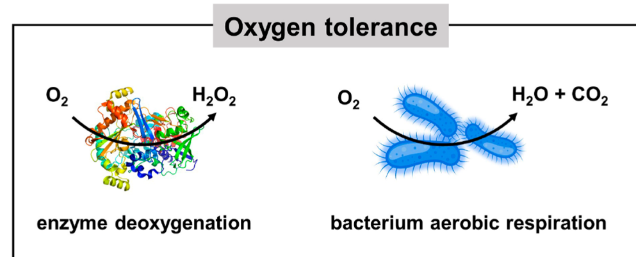

Figure 2. Enzymes and bacteria can be used to deoxygenate RDRP.

Although GOx was used by biochemists for deoxygenation a long time ago due to its effective degassing ability and easy availability, it was not until 1991 that GOx degassing was adapted by chemists to the field of radical polymerization.[40,41] In 2014, Stevens et al. reported the use of GOx for deoxygenation of RAFT polymerization, where 1−4 μM GOx was sufficient to fully degas the solution in an open vessel.[24] GOx deoxygenation for ATRP is more complicated than for RAFT. This is because the H_2O_2 produced by GOx deoxygenation reacts with Cu(I) catalyst in a Fenton-like manner, which produces hydroxy radicals, triggers the formation of new chains, and finally results in an uncontrollable polymerization. Matyjaszewski et al. solved this dilemma by consuming the produced H_2O_2 with sodium pyruvate, a reaction inspired by the cell respiration process.[42] It can be imagined that many oxidases can be used for the same purpose and, indeed, now the enzymes used for radical polymerization deoxygenation expand to embrace pyranose oxidase (P2Ox) and formate oxidase (FOx).[43,44] Oxygen can also be consumed efficiently by aerobic respiration of living facultative anaerobic bacteria, such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Enterococcus faecalis*, providing anaerobic conditions for radical polymerization.[34,45]

The Stevens' group demonstrated the excellent oxygen removal capability of GOx by modeling the kinetics of GOx deoxygenation (open system).[24,46] Due to its robustness, GOx

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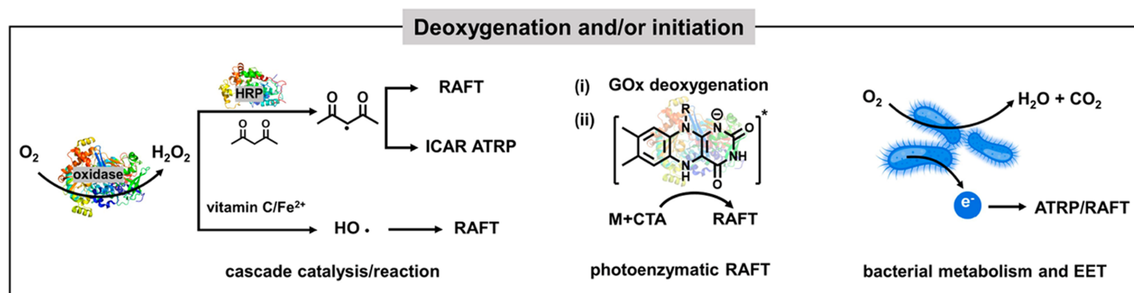

Figure 3. Coupling biodeoxygenation and initiation in a polymerization makes bio-RDRP oxygen tolerant.

has also been used to deoxygenate complex solutions including organic/aqueous mixtures and even liquors.[24,47,48] By using an oxygen sensor to online monitor the oxygen concentration in polymerization solution, our group found that GOx or FOx can reduce the concentration of oxygen to a similarly low level to that of degassing with N_2 (sealed system).[44,47] In addition to degassing in open and sealed vials, oxidase has also been used to deoxygenate other systems with a higher surface/volume ratio or more complex geometries: (i) High-throughput, low-volume (down to 40 μL) RAFT polymerization in microtiter well plates, a system characterized by a large air contact area/solution volume ratio that is highly susceptible to oxygen.[44,49,50] (ii) Surface-initiated ATRP for preparation of polymer brushes from an open-to-air initiator-modified surface.[51] (iii) Large-scale preparation of polymer nanoparticles with different morphologies via RAFT polymerization-induced self-assembly (PISA) under continuous flow conditions.[52−54] The quick expansion of enzyme deoxygenation to different application scenarios testifies that this process is indeed very mild, highly efficient and versatile, and adaptable to various conditions. We expect that bacterial deoxygenation will find a similar magnitude of application breadth considering bacteria are more easily recycled and reused.

Simultaneous Deoxygenation and Initiation. In some systems that use an enzyme to deoxygenate the solution, the enzyme acts only as a deoxygenator but not a catalyst to initiate or control a polymerization; these systems should be more accurately referred to as "enzyme-assisted" RDRP.[24,42,49,52] In metalloenzyme-catalyzed ATRP, the dormant polymer chain needs to be activated in the enzyme cavity. As the molecular weight of the polymer increases in the later stage of polymerization, it becomes increasingly difficult for the enzyme cavity to accommodate the polymer, and thus, a poor control of polymerization may arise. In contrast, some enzymatic redox reactions generate free radicals, which can be released out of the enzyme to initiate polymerization in solution. For example, HRP catalyzes the oxidation of acetylacetone (ACAC) by H_2O_2 to produce ACAC radicals, a catalytic process that was used previously to initiate free-radical polymerization.[20,55,56] In 2015, our group first used the HRP/H_2O_2/ACAC ternary initiation system for RAFT polymerization.[25] Konkolewicz and co-workers later termed HRP as a RAFT initiase, and they studied the effect of different substances (CTA, ACAC, H_2O_2, and monomer) on HRP activity and polymerization rate.[57,58] As can be seen from the above examples, in early studies, enzyme deoxygenation and initiation were decoupled processes.

Coupling enzyme deoxygenation and initiation in a single polymerization is highly desirable, which can endow an enzyme-catalyzed RDRP with oxygen tolerance (Figure 3). This simultaneous deoxygenation and initiation comes to reality through judicious construction of enzymatic cascade catalysis. GOx-HRP was the first reported cascade for controlling RAFT polymerization with oxygen tolerance.[25,46] Our group has also developed P2Ox-HRP and FOx-HRP cascade catalytic systems by combining HRP with P2Ox and FOx, respectively, both of which can yield well-controlled ultrahigh molecular weight (UHMW) polymers, defined as MW $\geq 1 \times 10^6$ g/mol.[43,44] The use of GOx-HRP cascade catalysis in ATRP is interesting because the H_2O_2 produced by GOx deoxygenation can be consumed by HRP, avoiding the Fenton-like reaction, while the ACAC radical generated by HRP catalysis can reduce Cu(II) to Cu(I) to achieve ICAR (initiators for continuous activator regeneration) ATRP.[59] The H_2O_2 produced by GOx deoxygenation can also be cascaded with chemical reactions. For example, H_2O_2 can react with vitamin C or Fe^{2+} to produce hydroxyl radical that subsequently initiates a RAFT polymerization.[60−62] It should be noted that, in the above bienzyme cascade and enzyme/chemical reaction cascade systems, oxygen is used as an essential reactant and polymerization cannot occur if oxygen is removed prior to polymerization.

The cascade reactions can be enriched by introducing additional reactions to the redox cycles to accelerate radical generation, minimize catalyst loading, and ultimately achieve a fast polymerization but still with an excellent polymerization control. For instance, Tang and co-workers found that adding ascorbic acid to the GOx deoxygenation-Fenton reaction cascade system increased the RAFT polymerization rate, in part due to increased hemin Fe(II) regeneration via hemin Fe(III) reduction by ascorbic acid, in addition to the claimed oxygen activation by hemin.[63] In another study also involving the GOx deoxygenation-Fenton reaction cascade, Gurnani and co-workers demonstrated that Fe^{3+} can be reduced by *Cupriavidus metallidurans* CH34 to generate Fe^{2+} *in situ*, thus accelerating the generation of hydroxyl radical and hence the RAFT polymerization rate.[35] These studies are very inspiring because they illustrate that, on top of the cascade reactions consisting of two interchained reactions, additional channels can be geared to the catalytic system, ideally forming a cascade consisting of three reactions in a chain. Such complex but elegant catalytic cycles allow for further manipulation of the radical generation and polymerization processes, which may provide new opportunities for on-demand initiation or well-controlled polymerizations with enhanced rate and livingness.

Surely, bienzymatic cascade catalysis represents a delicate strategy to realize dual functions of deoxygenation and initiation, and its potential has been illustrated by successful synthesis of challenging polymers (*vide infra*). However, it would be truly intriguing to realize these functions using just

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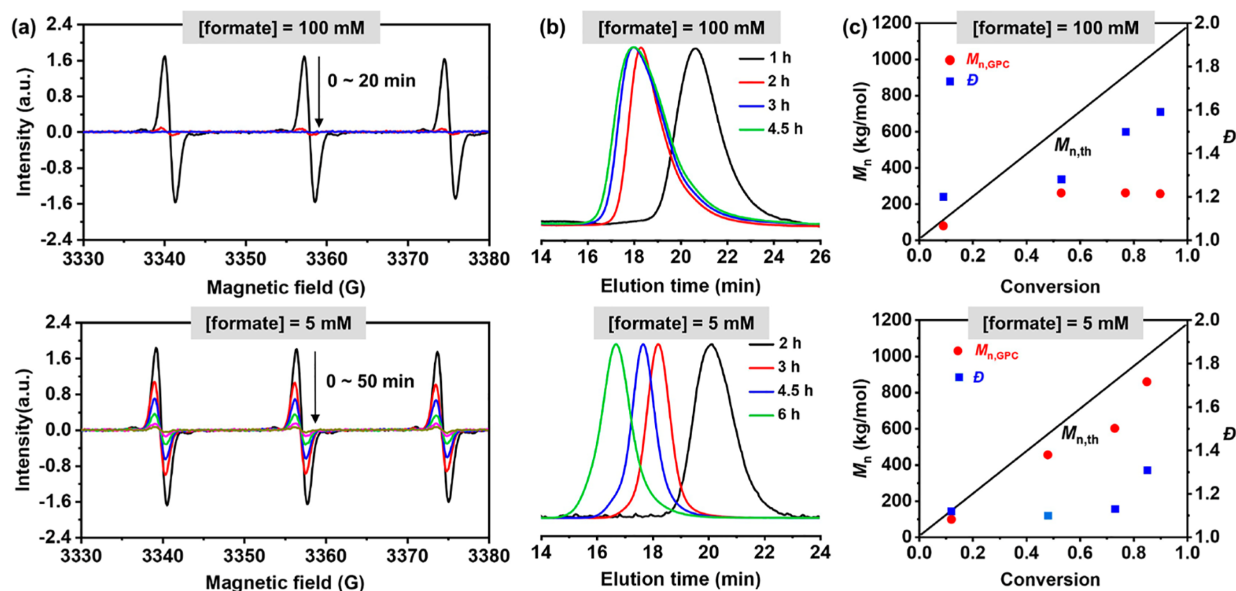

Figure 4. Concentration of substrate (formate) affects the rate of ACAC radical production in FOx-HRP cascade catalysis and, consequently, the acquisition of UHMW polymers via RAFT. (a) Decay of TEMPO EPR signal due to ACAC radical generation. (b) GPC traces of the obtained polymers at different times. (c) Plots of MW and dispersity versus conversion. Reproduced from ref 44 with permission. Copyright 2022 John Wiley and Sons.

one single enzyme, and the advantage of that is obvious: minimizing the number and loading of enzymes used in a polymerization. This challenge has been tackled by the discovery of photoenzymes that catalyze visible-light controlled RAFT polymerization with oxygen tolerance.[64] Taking GOx as an example, its natural catalytic function is deoxygenation. When the limited oxygen in the polymerization system (usually a closed vial) is consumed, its cofactor FAD can still be reduced to $FADH^-$ by excess glucose, and the resulting $FADH^-$ can be excited by light to the highly reducing $FADH^{-*}$, which then transfers an electron to the monomer or RAFT agent, thus inducing efficient RAFT polymerization. The beauty of photoenzymatic RAFT polymerization is that it seamlessly fuses two green catalytic processes, enzymatic and visible-light catalysis, into a functional one, which allows unprecedented spatiotemporal control of biocatalytic RAFT with oxygen tolerance.

Interestingly, by integrating its metabolic process with polymerization, bacteria can also play dual roles of deoxygenation and initiation. Keitz et al. utilized aerobic respiration of facultative electrogen *Shewanella oneidensis* to consume dissolved oxygen prior to initiation of radical polymerization, and *Shewanella oneidensis* subsequently directed metabolic electron flux via its extracellular electron transfer (EET) mechanism to Cu-based catalysts to drive ATRP under anaerobic conditions.[65]

Continuous Generation of a Low Concentration of Radicals. Among the features that bio-RDRP has demonstrated, the way through which radicals are produced is particularly noteworthy and has important implications for the successful synthesis of some arduous polymers. Enzyme produces radicals via redox catalysis of its substrate(s), where only a trace amount of enzyme is used and the concentration of substrate(s) is generally saturated with respect to that of the enzyme. According to the Michaelis–Menten equation, in this case, the enzymatic reaction is zero-order with respect to the substrate(s) and the rate of radical generation is constant.[66] This radical generation profile is in stark contrast to that of traditional radical production via thermal decomposition of radical initiators, whose concentration undergoes an exponential decay. To simulate the H_2O_2 generation process by GOx, Qiao and co-workers injected H_2O_2 via a syringe pump into the polymerization solution containing Fe(II) to continuously produce hydroxy radicals via Fenton reaction. They concluded that continuous generation of a low concentration of radicals is crucial for producing UHMW polymers.[62] Very recently, our group used radical trapping agent 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) to monitor the rate of radical generation in the FOx-HRP cascade by electron paramagnetic resonance (EPR) and UV–vis spectroscopies and correlated the radical generation process with the control of RAFT polymerization.[44] These results showed that a high radical generation rate led to more pronounced bimolecular termination at the early stage of polymerization, while at the late stage of polymerization, the experimental molecular weights were much lower than the theoretical values, and as such, UHMW polymers could not be produced. In contrast, a slow radical generation rate led to a well-controlled RAFT polymerization and UHMW polymers with low dispersities could be achieved (Figure 4).

Although the radical generation process has not be investigated in detail for bacteria, it is expected that bacteria should be able to similarly sustain a continuous radical influx relying on its metabolic balance. Qiao et al. found that the polymers formed in the presence of heat-killed cells and lysates of *E. coli* had lower molecular weight and higher dispersity than those formed when catalyzed with living bacteria.[67] This observation suggests that active cellular metabolism may help mediate radical production by continuously delivering redox mediators into the extracellular space and keep the radical flux within the proximity where RAFT can effectively control the polymerization. Despite the success that bacterium-RDRP has been demonstrated, it should be emphasized that this exciting

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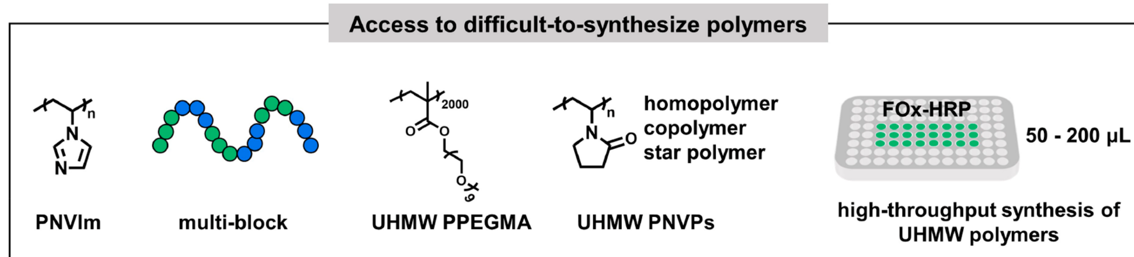

Figure 5. Bio-RDRP allows access to difficult-to-synthesize polymers.

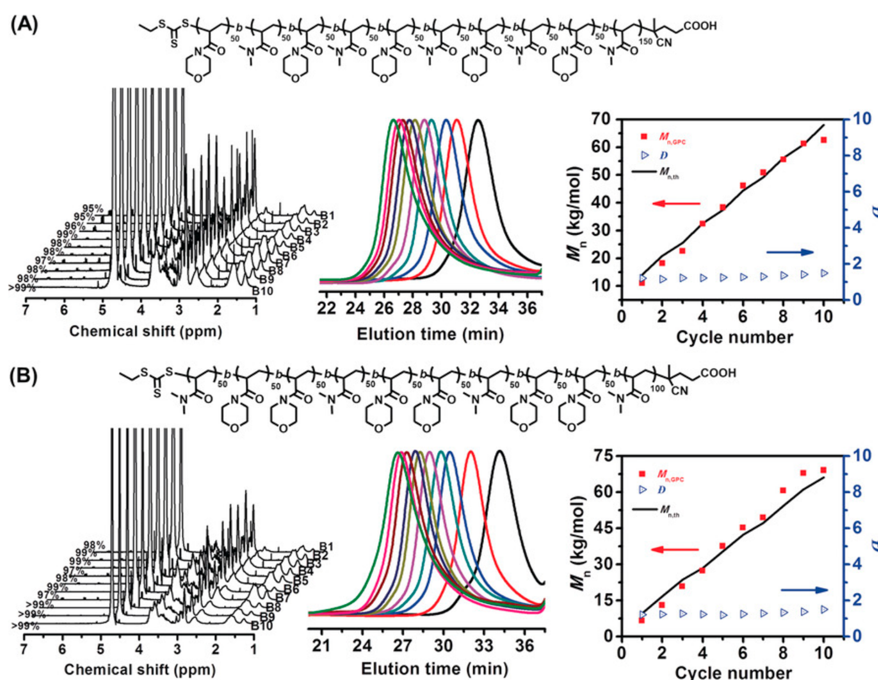

Figure 6. Synthesis of decablock copolymers of DMA and AML by P2Ox-HRP cascade catalysis. Adapted with permission from ref 43. Copyright 2017 John Wiley and Sons.

area is still in its infancy and more insights are yet to be revealed.

Access to Difficult-to-Synthesize Polymers. The rapid development of RDRP has facilitated the acquisition of sophisticated polymers, but challenges still remain. For example, some vinyl monomers cannot be polymerized with conventional RDRP yet, and in some cases, the polymerization efficiency and livingness are not exceptional enough to obtain multiblock copolymers and UHMW polymers. Thanks to the development of bio-RDRP, particularly enzyme-RDRP, the synthesis of some previously inaccessible or difficult-to-synthesize polymers has now become possible (Figure 5).

Poly(*N*-vinylimidazole) (PNVIm) is one of the most important imidazole-based polymers that has attracted long-lasting interest for numerous applications in gene and drug delivery, fuel cell production, and personal toiletries.[68] However, the synthesis of PNVIm by ATRP has rarely been reported due to the high reactivity of the propagating radicals and, moreover, because both NVIm and PNVIm bind tightly to metal ions, leading to metal stripping from the ATRP catalyst. Early views even assumed that PNVIm could not be synthesized by ATRP.[69] Bruns et al. achieved polymerization of NVIm with laccase-catalyzed ATRP by taking advantage of the tight binding of Cu to the protein.[33] This example illustrates metalloproteins are promising catalysts for the synthesis of polymers with ligating motifs and can expand the monomer families that are previously considered unsuitable for ATRP.

RDRP is an effective method to obtain multiblock copolymers and UHMW polymers, but achieving a similar level of control under easily operational benchtop conditions is a major challenge.[70−72] Synthesis of both types of polymers requires high polymerization efficiency and high livingness as well as an effective deoxygenation process to enable oxygen tolerance. Delightfully, enzyme catalysis has shown great potential to meet these challenges owing to its *in situ* deoxygenation and its ability to sustain a suitably low concentration of radicals throughout the polymerization process. For example, leveraging P2Ox-HRP cascade catalysis in open vessels, decablock copolymers of *N*,*N*-dimethylacrylamide (DMA), and 4-acryloylmorpholine (AML) were successfully achieved with relatively large molecular weights (~80 kg/mol) and reasonably low dispersities ($Đ < 1.5$) (Figure 6).[43] Using the same P2Ox-HRP cascade catalysis in closed vessels without prior degassing, low-dispersity ($Đ$ = 1.35) UHMW PDMAs with molecular weights up to 2.3×10^6 g/mol were obtained.[43]

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Photoenzymatic RAFT is very attractive because it uses only one single enzyme to realize deoxygenation and initiation of a polymerization. This excellent method has also been employed to synthesize UHMW polymers from poly(ethylene glycol) methyl ether methacrylate (PEGMA) (M_n = 500 g/mol) and unconjugated monomer *N*-vinylpyrrolidone (NVP).[47,64] The successful synthesis of low-dispersity linear, block, and star-shaped PNVPs is particularly remarkable because these molecular weights (MW > 1.0×10^6 g/mol, $Đ$ < 1.40) are at least 1 order of magnitude higher than what previously can be achieved. Except homogeneous polymerization, photoenzymatic RAFT emulsion polymerization was also carried out without prior deoxygenation to obtain PDMA-*b*-PMA (MA: methyl acrylate) UHMW block copolymers.[73]

It is even more challenging to synthesize UHMW polymers in a high-throughput manner at low volumes (such as in a well-plate). Recently, we have developed a new FOx-HRP cascade catalysis.[44] FOx catalyzes the generation of CO_2 from the C1 substrate formate while reducing oxygen to H_2O_2. CO_2 can be easily expelled from the solution, while H_2O_2 can be used to oxidize ACAC to produce ACAC radicals in the presence of HRP. UHMW PDMAs using RAFT agents of different structures have been synthesized in both small vials and 96-well plates by this atom-economic enzyme cascade catalysis. UHMW PDMAs (MW = 1.3×10^6 g/mol, $Đ$ = 1.37) can be obtained at an extremely low volume of 50 μL, which is unprecedented in the synthesis of UHMW polymers and convincingly proves the extraordinary ability of enzyme catalysis for the synthesis of challenging polymers under mild conditions.

KEY CONSIDERATIONS WHEN CONDUCTING bio-RDRP

With distinct features of mild conditions, no toxicity, oxygen tolerance, a multitude of polymerization control, and the ability to synthesize complex and challenging polymers, bio-RDRP has quickly evolved into a green, efficient, and versatile method and has attracted increasing attention from a wider community. To better make bio-RDRP a more easily accessible and more generally acceptable technique, in this section, we point out some important aspects that need to be considered when conducting bio-RDRP (Figure 7). In addition to some basic considerations of traditional RDRP (monomer, initiator, catalyst, ligand, etc.), special care needs to be paid to the conditions relating to the biocatalyst used.

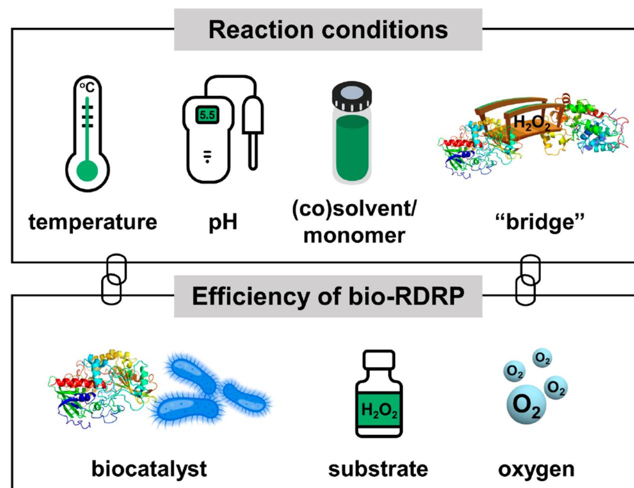

Figure 7. Key points that need to be considered when conducting bio-RDRP.

Since the polymerization solution is different than the native environment of biocatalysts, it is of paramount importance to maximize the activity of biocatalysts under polymerization conditions in order to achieve an efficient bio-RDRP. Temperature, pH, and the polymerization components, such as monomer type and concentration, RAFT agent, ATRP initiator, metal catalyst, and ligand, all can affect biocatalyst activity. Ideally, bio-RDRP should be carried out under conditions of maximum activity of biocatalyst; however, this consideration needs to be modified in concert with polymerization requirements. Taking the effect of pH as an example, the optimal pH for GOx is 5.0−5.5, but pH 7.0 was used instead when conducting photoenzymatic RAFT polymerization of NVP.[47] This is because NVP is prone to hydrolysis in acidic aqueous solution.[74] In order to prevent NVP from hydrolysis, some GOx activity had to be sacrificed. In a bienzymatic cascade catalytic system, the individual optimal conditions for each of the two enzymes can be different. In this case, screening conditions to achieve a maximum polymerization efficiency and excellent polymerization control are thus necessary. For example, FOx is most active at pH 4.0, and the optimal pH for HRP is 6.0−6.5; however, polymerization was found to be most efficient at pH 5.0−6.0 using FOx-HRP cascade catalysis, since both a high efficiency of deoxygenation and an effective production of ACAC radicals were ensured at the polymerization pH.[44,55,75] If other polymerization conditions are found to exert a big influence on the biocatalyst activity, conducting a similar screening process is encouraged.

Bacterium culture media are more complex than simple buffer solutions used in enzyme catalysis, with additional ingredients including carbon source, amino acids, nucleotides, growth factors, etc. Some components may interfere with the radical polymerization process through radical chain transfer or radical scavenging.[76] To avoid potential interference from the culture media, Rawson group and Gurnani group both conducted polymerization in PBS buffer after culturing bacteria in the appropriate media.[35,77] However, the lack of growth supplements inevitably reduced bacterial activity; about 20% of the bacteria died after 24 h of polymerization. Qiao et al. performed polymerization in M9 minimal media, which contained only the minimal, essential supplements, in order to maximize bacterial activity while avoid interference with polymerization.[67] As polymerization progresses, the metabolism of bacteria depletes nutrients in the culture media and produces metabolic wastes, which lowers the metabolic activity of bacteria and thus may retard the polymerization process. This unfavorable effect may be alleviated by improving the catalytic efficiency of the bacteria such that polymerization can be completed within the shortest time possible.

In constructing an enzymatic cascade for RDRP, it is crucial to consider the fate of the species that bridges these two interconnected enzymatic reactions, especially when this very species can participate in side reactions and thus adversely affect the polymerization. Thus far, the most frequently encountered bridging species is H_2O_2, and it is a known participant in several reactions, which may directly affect the outcome of a polymerization. Therefore, understanding its reactivity and accordingly devising rational pathways to instruct its destiny is necessary. Interesting examples of this are the use of the same GOx-HRP cascade for both RAFT and

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ATRP and their different ways of treating H_2O_2. In RAFT polymerization, H_2O_2 is considered innocuous due to its low concentration (no oxidation of CTA is detected), and thus, no action on H_2O_2 is necessary, whereas in ATRP, H_2O_2 is considered detrimental to polymerization control because it not only deactivates Cu catalyst but also participates in a Fenton reaction with the Cu catalyst to generate hydroxyl radical and consequently invokes background polymerization.[25,42] As such, H_2O_2 must be annihilated in ATRP, and this is done by introducing sodium pyruvate, which clears H_2O_2 away via a reaction inspired by cell respiration.

The thiol group of cysteines can act as chain transfer agents in both free-radical polymerization and RDRP. This can be used as an advantage, as demonstrated by the di Lena group where they added cysteines as chain transfer agents to reduce the molecular weight of the polymers that formed.[22] However, problems arise when the chosen enzyme has surface-exposed cysteines. Unwanted chain transfer reactions not only make the molecular weight of the obtained polymer uncontrollable but also result in the formation of protein−polymer conjugates. Thus, if the enzymes of choice feature surface-exposed cysteines, they should be blocked with a thiol-reactive reagent before the polymerization.[78] Obviously, this is not a problem with enzymes that do not display cysteines on their surface. However, it might become a major problem in more complex biological media and in the presence of bacteria. Similarly, if the enzymes used, such as HRP, contain amine groups on its surface, consideration should be given to whether the amines will disrupt the structure of the RAFT agent. This effect may be negligible when the amount of enzyme is relatively small, but special attention should be given when the amount of enzyme increases.

Apparently, aqueous solution, buffer or culture medium, is the most suitable solution to carry out bio-RDRP. This is an obvious advantage because water is a green solvent and radical polymerization in water shows a high rate. However, the downside is that some reagents necessary for RDRP have limited solubility in aqueous solution. This solubility problem can be satisfactorily addressed for ATRP initiators or RAFT agents by attaching solubilizing groups.[64] Another approach involving less synthetic effort is to use a carefully chosen cosolvent.[24,47] Together with the monomer, cosolvent can be very effective to improve the solubility of the reactants, especially for synthesis targeting high DPs. It has been shown that enzyme catalysis is reasonably tolerant to monomers and cosolvents and the solid content can generally reach 10% w/v, but bacteria are considerably less tolerant of monomers and cosolvents. For instance, *E. coli* and *S. typhimurium* can maintain their viability with up to 5% w/v (104 mM) of PEGMA (M_n = 480 g/mol), a monomer with a low toxicity, while *Cupriavidus metallidurans* can tolerate only 14 mM PEGMA (M_n = 300 g/mol).[67,77] Most of the monomers currently used in bio-RDRP are water-soluble monomers. Emulsion polymerization has been demonstrated to be effective in bio-RDRP for hydrophobic oily monomers since the di Lena group reported the first example of enzyme-catalyzed emulsion polymerization.[22,73,79,80] If the monomer is soluble in water but the resulting polymer is insoluble, dispersion polymerization needs to be implanted.[25,50,64] No homogeneous polymerization in purely organic solvents has been reported for bio-RDRP, and this may seem to be easily understandable because organic solvents are known to be harmful to biocatalysts, but future advances along this line are highly desirable, which would help realize the full potential of bio-RDRP.

After the basic conditions ensuring optimal activity of biocatalysts are met, the components relating to the catalytic system itself should be optimized since its performance determines the efficiency and control of a RDRP. Ideally, less biocatalyst loading is preferred if a satisfactory catalytic efficiency can be provided. This saves both the cost and effort of biocatalyst removal. Substrates are generally much cheaper than the biocatalyst itself, but they cannot be increased indefinitely to increase the catalytic efficiency because some enzymes show substrate inhibition and some substrates such as H_2O_2 may also deactivate the enzyme (HRP).[58]

The degassing capability of biocatalysts is a fantastic function because it brings about a paradigm shift in RDRP operation, allowing polymerizations to be conducted in a simple and enjoyable mix-and-go style on the benchtop under ambient conditions. When deoxygenation is the only function desired, depending on the geometry of the reactor, which defines the surface/volume ratio and oxygen diffusion rate, the amount of biocatalyst can be simply adjusted to satisfy the creation of an oxygen-free condition. In cases where the biocatalyst plays multiple roles rather than sole deoxygenation, as in enzymatic cascade catalysis, photoenzymatic catalysis, and bacterium catalysis, the rates of deoxygenation and polymerization must be balanced. A faster deoxygenation usually requires a higher concentration of biocatalyst, which also produces a higher flux of radicals in photoenzymatic and bacterium catalysis and may risk a higher level of biomolecular termination and accordingly a poorer polymerization control. A faster deoxygenation also produces a higher instantaneous concentration of H_2O_2, which may again lead to a higher flux of radicals in enzymatic cascade catalysis, though in this specific case the radical flux can also be alleviated by adjusting the dosage of the second enzyme (e.g., HRP), which reflects the power of enzymatic cascade catalysis for multifaceted manipulation of a polymerization.

■ CHALLENGES AND OUTLOOK

In the last two decades, extensive research has been carried out to improve the environmental benignity of RDRP.[2,8,29] In this context, the recent progress in bio-RDRP is particularly compelling. The distinct features of bio-RDRP have enabled access to sophisticated polymers under mild conditions. Despite the great success, significant bottlenecks remain to be resolved in order to realize its full potential for various applications (Figure 8).

To date, there are only a handful of biocatalysts that have been studied in bio-RDRP, but it is very encouraging that multiple functions of deoxygenation, initiation, and polymerization control have already been realized. Expanding the biocatalyst family would certainly diversify the toolbox and allow broader conditions to be used for bio-RDRP. For instance, in most reports, the enzyme used for deoxygenation is GOx because of its commercial availability and cheap price, as well as efficiency. However, GOx deoxygenation produces gluconic acid, which can lower the solution pH unless a high buffer concentration is used. This problem is evaded by replacing GOx with P2Ox; the latter produces a hydrolytically stable 2-dehydro-D-glucose.[43] Moving further, FOx deoxygenation is a more atom-economic and greener catalysis because it uses a C1 substrate (formate) and produces CO_2 that fleets away from the solution.[44] This progress in searching suitable

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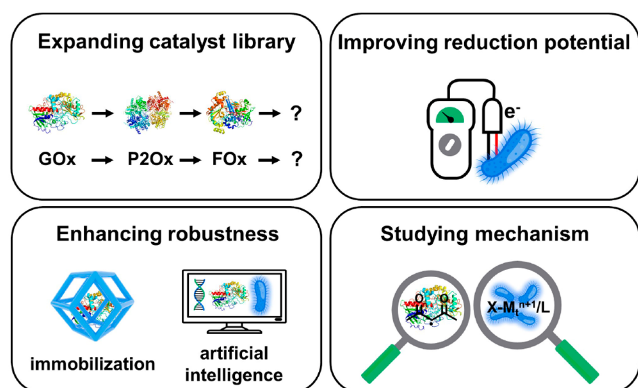

Figure 8. Challenges of bio-RDRP and future directions.

oxidases for deoxygenation illustrates how identification and incorporation of more enzymes can enrich biocatalyst selection and better serve the purpose of green bio-RDRP. Along a similar line, bacterial reduction is one of the main functions employed in RDRP and most of the bacteria have a mild reduction potential (up to −350 mV vs standard hydrogen electrode).[65,81,82] Existing methods to improve the efficiency of bacterial electron transfer include introducing transmembrane and outer-membrane silver nanoparticles into bacteria or coating bacteria with conductive polymers, which may potentially be applied to bacterial-catalyzed RDRP.[83,84] Furthermore, identifying or engineering better reductive bacteria will be beneficial, which could potentially eliminate the use of redox mediators as in RAFT or enhance the activation rate of metal catalysts as in ATRP.

Bio-RDRP is typically conducted in aqueous solution at room temperature, which is a favorable strength but may also be subject to criticism as a weakness due to the fragile nature of biocatalysts. It should be emphasized that a broad range of monomers including hydrophilic and hydrophobic ones have been successful polymerized via bio-RDRP at low temperatures, but we also admit that high polymerization temperatures and the use of organic solvents would benefit the polymerization of low-k_p and solid hydrophobic monomers. At least two approaches can be taken to address the temperature and solvent tolerance issue for enzymes. One well perceived approach is enzyme immobilization, for which a few works have been reported. Tang and co-workers explored the use of zeolitic imidazolate framework-8 (ZIF-8) to coencapsulate GOx and iron porphyrin and conducted RAFT polymerization at 80 °C.[85] In another interesting system, the temperature tolerance of GOx could be increased to 70 °C after coadsorption of chitosan and GOx to lignin.[79] Immobilized enzymes not only increase the resistance to high temperatures and organic solvents but also bring the benefit of easy separation. The other approach to enhance enzyme stability is through protein engineering where artificial intelligence can play an important role.[86] Bacterial catalysts may be improved via similar strategies but are expected to be more challenging.

Various interesting biocatalytic systems have been successfully developed for the polymerization of several monomer families, but mechanistic studies have generally lagged behind. For enzymes whose known catalysis reactions are involved in a RDRP, e.g., in FOx-HRP cascade catalysis, mechanistic studies have focused on how the cascade reactions are effectively coupled, how the radical generation rate is manipulated, and how these processes affect polymerization. These mechanistic studies have provided insights on the exceptional ability of enzymatic cascade catalysis for UHMW achievement.[44] However, a comprehensive understanding of new enzyme activities in RDRP is currently lacking, and these include ATRPase and photoenzyme activities.[22,23,47,64] Rapid progress has been achieved in exploiting photoenzymes and metalloproteins to effect enantioselective radical reactions,[87−90] whereas stereoselective radical polymerization catalyzed by enzymes remains elusive. Even more challenging is to decipher the mechanism of bacterial catalysis due to the complexity and dynamic metabolic process. Although it is accepted that the bacterial EET machinery modulates the redox state of metal ions to facilitate ATRP or reduces a mediator to facilitate RAFT, it remains unclear how exactly the extracellular electron transfer of different bacteria catalyzes RDRP.[34,67,77] This field would greatly benefit from a more detailed mechanistic understanding, and we expect that new discoveries would arise from such a fundamental investigation.

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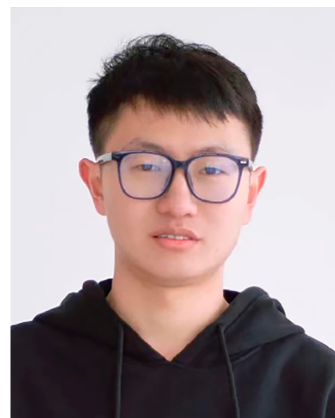
Notes

The authors declare no competing financial interest.

Biographies



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