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Hybrid Enzymatic and Organic Electrocatalytic Cascade for the Complete Oxidation of Glycerol

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Supporting Information

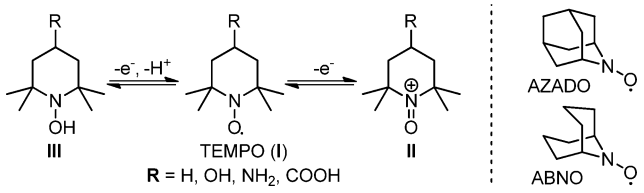
ABSTRACT: We demonstrate the complete electrochemical oxidation of the biofuel glycerol to CO_2 using a hybrid enzymatic and small-molecule catalytic system. Combining an enzyme, oxalate oxidase, and an organic oxidation catalyst, 4-amino-TEMPO, we are able to electrochemically oxidize glycerol at a carbon electrode, while collecting up to as many as 16 electrons per molecule of fuel. Additionally, we investigate the anomalous electrocatalytic properties that allow 4-amino-TEMPO to be active under the acidic conditions that are required for oxalate oxidase to function.

Enzymatic biofuel cells utilize an enzyme to electrochemically catalyze the anodic oxidation of a fuel or the cathodic reduction of an oxidant. While enzymes are often limited in the extent to which they can oxidize a fuel due to high substrate specificity, they typically exhibit significantly higher catalytic rates per active site than their precious metal counterparts. In addition, enzymes are capable of operating under ambient temperatures and in mild aqueous environments. Recent research efforts have focused on the use of multi-enzyme cascades to facilitate deep oxidation of a biofuel at a bioanode.[1] This strategy maximizes the energy density extracted per molecule of fuel; however, there are several oxidative transformations for which a suitable biocatalyst is not yet known. Thus, there remains a need to design green anodic systems capable of complete oxidation of a fuel. Consequently, we considered the possibility of merging the advantages of enzymatic oxidation with a small-molecule catalyst to achieve complete oxidation of a biofuel. Herein, we present a study illustrating the potential of this approach by combining a simple oxidation catalyst (TEMPO) with the enzyme oxalate oxidase to completely convert glycerol to CO_2, while collecting up to 16 electrons per glycerol molecule.

Oxalate oxidase (OxO) has previously been shown to catalyze the oxidation of simple carboxylic acids such as mesoxalic acid and oxalic acid.[2] However, this enzyme is not capable of recognizing and effectively oxidizing a high-energy density and simple alcoholic biofuel such as glycerol. Thus, to achieve complete oxidation of glycerol, another enzyme or a small molecule catalyst would need to be utilized. In considering this multicatalytic cascade strategy in a biofuel cell setting, a promiscuous catalyst with the ability to oxidize various alcohol starting materials and intermediates formed throughout the conversion under electrocatalytic conditions is desired. Additionally, the catalyst would need to be compatible with both the conditions of enzymatic oxidation as well as the enzyme itself. In view of the numerous examples of alcohol oxidation catalysts, we hypothesized TEMPO as a possible solution in that it has an extensive history as an effective alcohol oxidation catalyst and does not have the substrate specificity limitations enzymes face.[3] Furthermore, unlike many enzymatic catalysts, TEMPO is capable of catalyzing the oxidation of multiple oxygen-containing functional groups, such as those encountered in biofuel oxidation processes.

The mechanism of a TEMPO catalyzed oxidation can proceed through initial oxidation of the native form of TEMPO, which contains a stable nitroxyl radical (I) to form the catalytically active oxoammonium ion (II), as shown in Scheme 1.[4] This can be accomplished either electrochemically

Scheme 1. Redox Cycle Highlighting the Nitroxyl Radical (I), Oxoammonium Ion (II), and Hydroxylamine (III) Oxidation States of TEMPO



or using a stoichiometric chemical oxidant. Recently, Liebminger et al. reported such a system wherein TEMPO catalyzes the oxidation of glycerol to mesoxalic acid using a copper-containing oxidase to enzymatically regenerate the active TEMPO catalyst with molecular oxygen as the stoichiometric oxidant.[5] However, there are no examples to the best of our knowledge of coupling the redox capability of both TEMPO and an enzyme in the oxidative processing of a biofuel.

Success using this hybrid approach would require TEMPO catalyzing the first five oxidative steps in the proposed glycerol cascade (**1** → **6**, Scheme 2). The initial step in the cascade results in the oxidation of glycerol (**1**) to glyceraldehyde (**2**). While multiple subsequent oxidative pathways are possible, the net result is the formation of mesoxalic acid (**6**). A combination of OxO and TEMPO can then transform mesoxalic acid to glyoxalic acid (**7**), oxalic acid (**8**), and finally CO_2. Clearly the main issue is compatibility in terms of catalytic activity of both systems as TEMPO has not been explored extensively at both

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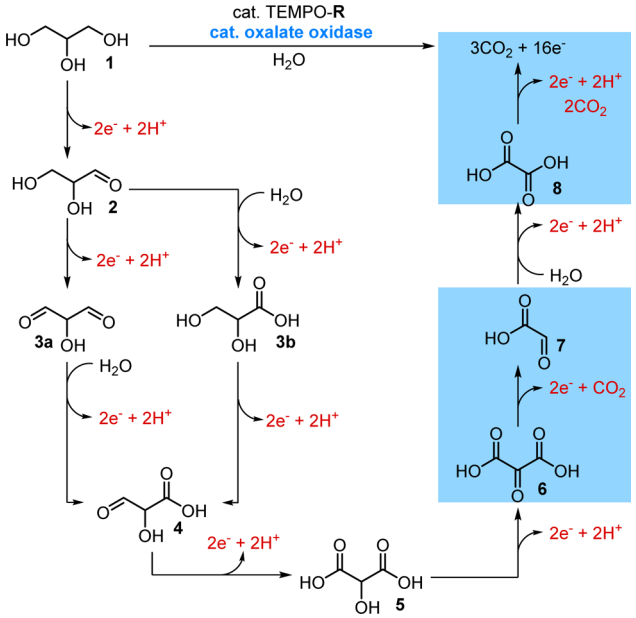
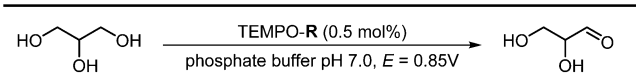
Scheme 2. Proposed Electrocatalytic Oxidation Cascade of Glycerol by TEMPO-R and Oxalate Oxidase



the pH and aqueous conditions required for effective oxidation using OxO.

To initiate our investigation several commercially available derivatives of TEMPO were evaluated using cyclic voltammetry (CV) at pH 7.0 to determine their electrocatalytic activity toward glycerol. The results from the catalytic screening are summarized in Figure 1. Both 9-azabicyclo[3.3.1]nonane-*N*-oxyl (ABNO) and 2-azaadamantane-*N*-oxyl (AZADO) exhibit a 5-fold catalytic rate increase over unmodified TEMPO.[6] This is likely the result of reduced steric hindrance from a more rigid structure surrounding the nitroxyl radical within ABNO and AZADO as has been observed previously.[3a] The highest catalytic rate among the TEMPO compounds evaluated was achieved by 4-amino-TEMPO (TEMPO-NH_2), which exhibited an 8-fold increase in catalytic rate over unmodified TEMPO. To the best of our knowledge, the enhanced electrocatalytic activity of TEMPO-NH_2 over unmodified TEMPO in aqueous environments has not previously been reported. Similar experiments performed using unmodified TEMPO with 2 equiv of triethylamine did not result in any significant increase in catalytic current density (J_{max}) over experiments performed without triethylamine (Figure S3). The absence of an increase in J_{max} suggests that the increased rate of catalysis for TEMPO-NH_2 is due to the presence of the adjacent amine functional group, although its exact role in the oxidation is unclear at this time.



TEMPO-**R**	$E_{1/2}$ / V vs SCE	J_{max} / μA cm^{-2}	v / μmol min^{-1}	$D \times 10^{-6}$ / cm^2 sec^{-1}
TEMPO	0.499	282	0.005	12.34
TEMPO-OH	0.583	507	0.012	8.06
TEMPO-NH_2	0.623	1453	0.039	4.94
TEMPO-COOH	0.548	224	0.004	6.62
ABNO	0.485	855	0.025	4.16
AZADO	0.466	801	0.026	5.13

Figure 1. Electrocatalytic screening of TEMPO compounds with glycerol. Catalytic activity was determined by comparison of cyclic voltammograms in the presence and absence of 0.1 M glycerol using 40 mM Robinson buffer, pH = 7.0, at 25 °C.

Much of the previous work detailing the electrochemical oxidation of alcohols using TEMPO was either performed under alkaline conditions or required a stoichiometric amount of base to facilitate regeneration of the TEMPO nitroxyl radical species.[7] This presents a challenge as the basic conditions required for catalytic TEMPO oxidation are mutually exclusive to the acidic functional pH range of OxO. The Mn^{2+}/Mn^{3+} active site of OxO is accessible through a narrow channel containing a negative surface charge near the opening at pH 7, which limits substrate access through a proposed gating mechanism.[8] In Figure 2, we depict the overlaid pH profiles versus catalytic activity of OxO with TEMPO for oxalic acid and glycerol, respectively.

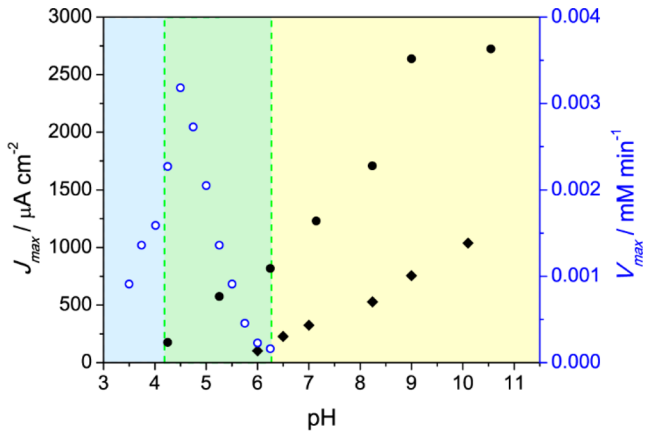


Figure 2. Overlaid pH profiles of oxalate oxidase (○), TEMPO (◆), and TEMPO-NH_2 (●). Dotted lines highlight the overlapping pH range between OxO and TEMPO-NH_2.

While UV–vis assays found that the activity of OxO was unaffected by the presence of TEMPO (Figure S6), the incompatibility of these two catalysts in a single system is clear from the lack of overlap in their respective pH profiles. Based on the intriguing properties of TEMPO-NH_2 described above, we also evaluated its pH profile. Unmodified TEMPO is electrocatalytically active from alkaline conditions to pH 6.0, while TEMPO-NH_2 maintains measurable catalytic activity as low as pH 4.0. This result identifies the expanded pH tolerance of TEMPO-NH_2, which allows for electrocatalytic activity under acidic conditions that are otherwise required for OxO catalysis.

The electrocatalytic performance of unmodified TEMPO, shown in Figure 2, correlates to the pK_a of the hydroxylamine intermediate (formed during the TEMPO oxidation sequence).[9] The reduced activity at low pH suggests that the coupled deprotonation/oxidation of TEMPO hydroxylamine (III, shown in Scheme 1) can no longer be facilitated and prevents completion of the catalytic cycle. The ability of TEMPO-NH_2 to catalyze oxidation processes at a pH lower than its pK_a indicates that the amine functional group is presumably capable of lowering the energy required for deprotonation/oxidation of the TEMPO-NH_2 hydroxylamine intermediate. A comparison of the effects of pH on oxidation

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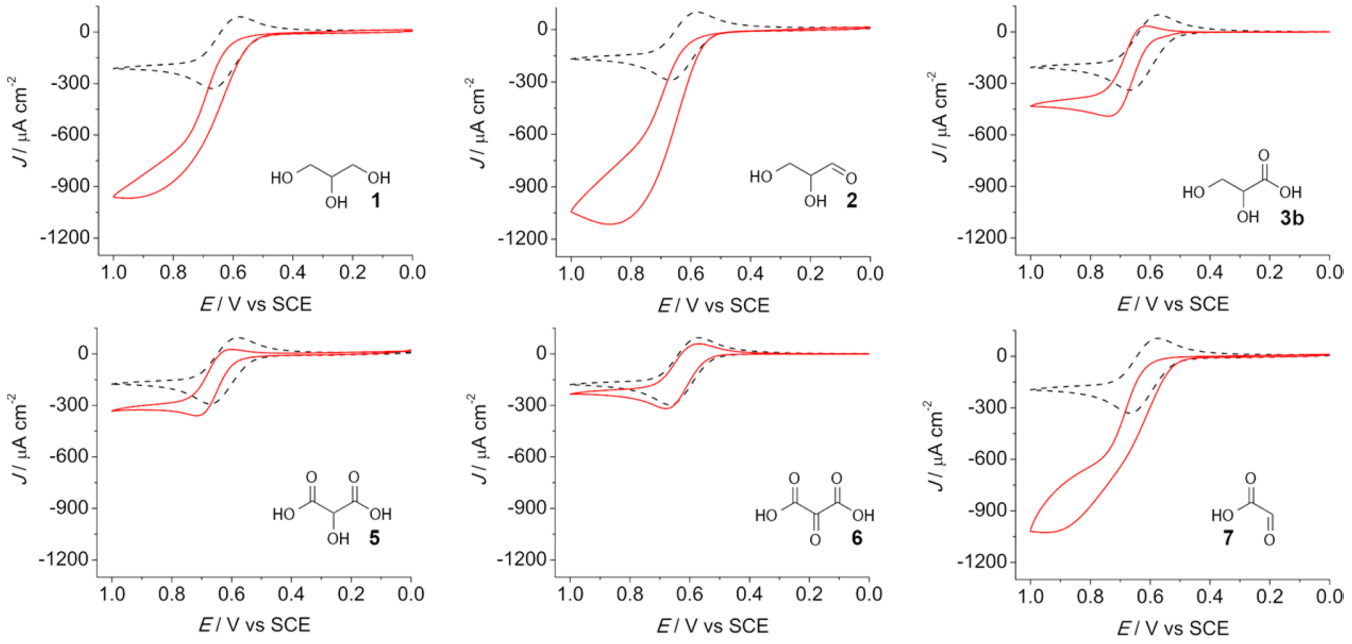

Figure 3. Catalytic CVs of 5 mM TEMPO-NH_2 in the absence (dashed) and presence (solid) of 100 mM substrate: (left to right, top row) glycerol, glyceraldehyde, glyceric acid, (second row) tartronic acid, mesoxalic acid, glyoxalic acid. Experiments were performed using a 3 mm glassy carbon electrode with 50 mM phosphate buffer, pH 5.2, at 5 mV s^{-1} and 25 °C.

potential shows that the protonation state of the amine on TEMPO-NH_2 significantly impacts its oxidation potential from 0.73 V at pH 4.0 to 0.45 V at pH 10.5 (Figure S4). The oxidation potential of unmodified TEMPO is virtually unaffected by pH. This shift in oxidation potential observed for TEMPO-NH_2 with pH could suggest a conformational component in which the amine/ammonium species is in close enough proximity to the nitroxyl radical to alter its electronic properties through Coulombic repulsion.[10] The exact nature of this interaction as it relates to the expanded catalytic pH range of TEMPO-NH_2 is under further investigation.

The electrocatalytic properties of TEMPO-NH_2 with several intermediates of the proposed glycerol cascade were characterized using CV to ensure that each step of the cascade could be accomplished. The resulting CVs confirm the ability of TEMPO-NH_2 to catalyze the oxidation of glycerol (**1**), glyceraldehyde (**2**), glyceric acid (**3b**), tartronic acid (**5**), and glyoxalic acid (**7**) (Figure 3). Solutions of TEMPO-NH_2 were capable of generating >800 μA cm^{-2} in the presence of either 100 mM glycerol, glyceraldehyde, or glyoxalic acid at pH 5.2 and 25 °C. Not surprisingly, the highest rates of electrocatalytic oxidation were observed with substrates containing a primary alcohol or an aldehyde. Conversely, the lowest catalytic rates were observed for substrates in which only a secondary alcohol was available for oxidation, such as tartronic acid. This is consistent with previously reported results suggesting that the hindered nature of a secondary alcohol causes a decreased oxidation rate using TEMPO.[8a,11] Despite the modest rate of tartronic acid oxidation with TEMPO-NH_2, the observed catalytic current density of 200 μA cm^{-2} is significantly higher than that of tartronic acid with unmodified TEMPO. The catalytic CV of TEMPO in the presence of 100 mM tartronic acid indicates that TEMPO is virtually unreactive even at pH 7.0 (Figure S5).

The complete electrochemical oxidation of glycerol by TEMPO-NH_2/OxO was carried out by bulk electrolysis of a solution containing both the enzymatic and organic catalysts at pH 5.2. The oxidation of glycerol proceeded with TEMPO-NH_2 and OxO while generating current densities as high as 1.2 mA cm^{-2}. The progress of the 22 h glycerol oxidation cascade was monitored by HPLC, and the resulting analysis provided evidence for the conversion of glycerol (**1**) to glyceric acid (**3**), tartronic acid (**5**), mesoxalic acid (**6**), and glyoxylic acid (7) (Figure S8). Product analysis also indicated that neither glyceraldehyde nor oxalic acid is formed within detectable concentrations after 22 h. These results suggest that the steady-state concentration of both intermediates is below the detectable limit due to the relatively high activity of both TEMPO-NH_2 for glyceraldehyde (0.02 μmol min^{-1}, see Figure 3) and OxO for oxalic acid (0.90 μmol min^{-1}, see Figure 2). In addition to HPLC analysis, an isotopic labeling study was carried out using ^{13}C-labeled glycerol. In order to capture the $^{13}CO_2$ generated by the reaction cascade, a small canister of NaOH was suspended above the bulk electrolysis solution. At completion, the contents of the canister were dissolved in D_2O and analyzed by ^{13}C NMR. From the resulting ^{13}C NMR spectrum shown in Figure 4, a significant peak corresponding to ^{13}C-enriched CO_3^{2-} at ca. 165 ppm was observed. While it is difficult to quantify the catalytic turnover frequency for this system due to diffusional kinetics, we do observe a Coulombic yield of 90.6 C. This strongly suggests that TEMPO-NH_2 and OxO are operating as catalysts in the complete oxidation of glycerol.

In summary, we have shown that TEMPO-NH_2 and OxO can be combined in a single electrochemical cell to catalyze the complete oxidation of glycerol to CO_2. This process completes within 22 h when performed at 25 °C, while collecting a charge of 90.6 C. Furthermore, catalytic current densities as high as 875 μA cm^{-2} were observed for individual steps of the oxidative glycerol cascade. Ongoing research is focused on characterizing the precise nature of the anomalous electrocatalytic activity of TEMPO-NH_2 as well as the immobilization of TEMPO-NH_2 onto the surface of an electrode in the presence of OxO to improve the performance of this hybrid system.

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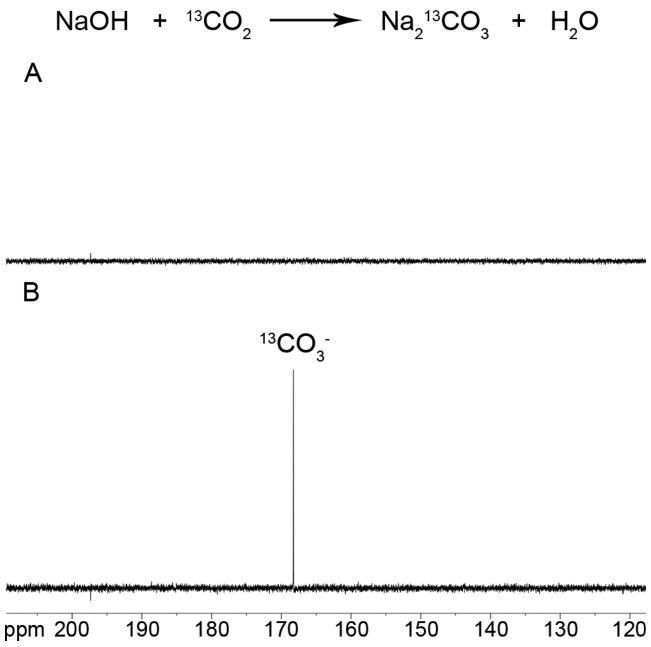

Figure 4. ^{13}C NMR spectrum of (a) a control sample of NaOH exposed to ambient conditions for 22 h, and (b) $^{13}CO_2$ trapped in the form of $Na_2{}^{13}CO_3$ from ^{13}C-labled glycerol that was completely oxidized from a solution of TEMPO-NH_2 and OxO.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental conditions, electrochemical controls, catalytic CVs for both various TEMPO catalysts and substrates, HPLC chromatograms, and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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