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Highly Efficient Degradation of 2-Methylisoborneol by Laccase Assisted by a Micro-Electric Field

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Abstract: Taste and odor (T&O) compounds have emerged as crucial parameters for assessing water quality. Therefore, identifying effective methodologies for the removal of these compounds is imperative. In this study, an effective approach utilizing laccase assisted by a micro-electric field was developed for the degradation of 2-methylisoborneol (2-MIB). For this purpose, the optimal conditions for the laccase-catalyzed degradation of 2-MIB were determined, and they were pH 4.0, 25 °C, 150 rpm, 0.1 U/mL of laccase, and 200 ng/L of 2-MIB. Under these specified conditions, the degradation efficiency of 2-MIB was approximately 78% after a 4 h reaction period. Subsequently, the introduction of an electric field yielded a synergistic effect with the enzyme for 2-MIB degradation. At an electric current intensity of 0.04 A over a 4 h duration, the degradation efficiency increased to 90.78%. An analysis using SPME-GC/MS provided information on the degradation intermediates of 2-MIB resulting from laccase-catalyzed degradation, electrocatalytic degradation, and micro-electric-assisted laccase degradation. The potential degradation pathways of 2-MIB illustrated that these three methods result in common degradation products, such as capric aldehyde, nonylaldehyde, and 2-ethylhexanol, and their final products include 3-pentanone, acetone, and 2-butanone. This study provides an enzyme–electrochemical method for the efficient and rapid degradation and removal of 2-MIB. The strategy of laccase catalysis assisted by a micro-electric field has good potential for the removal of pollutants from the natural environment.

Keywords: 2-methylisoborneol; laccase; micro-electric field; oxidation degradation; possible degradation pathway

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1. Introduction

Taste and odor (T&O) compounds not only jeopardize the safety of drinking water but also increase the operational costs of water treatment processes. These compounds can significantly impact the palatability of water, prompting consumer dissatisfaction and necessitating additional measures to ensure water quality and safety [1]. T&O compounds originate from two primary sources. One source is the improper disposal of water resources by individuals, which can introduce odoriferous substances into natural water bodies, including chemicals, mineral salts, inorganic nitrides, and sulfides. The other source is excretions from algae, actinomycetes, fungi, and organic materials such as humus, which contribute diverse odors to water [2,3]. Various T&O compounds are present in natural water, such as geosmin (GSM), 2-methylisoborneol (2-MIB), 2-isopropyl-3-methoxypyrazine (IPMP), 2-isobutyl-3-methoxypyrazine (IBMP), and 2,4,6-trichloroanisole (TCA) [2–4]. Among these

compounds, 2-MIB is particularly noteworthy as a significant odorant in drinking water sources, characterized by a musty, earthy odor primarily originating from blue-green algae and actinomycetes [5].

2-MIB is considered a typical T&O compound due to its exceedingly low odor detection threshold in the ng/L range [6], which poses significant challenges to its effective removal using conventional water treatment methods [3,7]. While T&O substances in drinking water typically do not pose a direct health threat to humans, their presence can lead individuals to reject water due to the undesirable taste, potentially resulting in refusal to consume or utilize it. Consequently, the removal of 2-MIB from drinking water has garnered significant attention in recent years. The removal of 2-MIB from water involves a combination of physical, chemical, and biological methods [3,8,9]. Traditional physical water treatment techniques such as coagulation, precipitation, sand filtration, and activated carbon adsorption have exhibited minimal efficacy and notable instability in their capacity to eliminate these compounds [10]. Likewise, conventional chemical oxidation processes such as chlorination and permanganate oxidation have not only demonstrated low efficiency but also possess a limited capability to oxidize odorous compounds, potentially yielding hazardous by-products [11,12]. Biological methods have shown greater promise for the removal of 2-MIB, with studies indicating that specific aquatic and soil bacteria can effectively biodegrade this compound [13,14]. Bacteria are crucial in the degradation of 2-MIB, breaking down, co-metabolizing, and enzymatically degrading the compound through diverse metabolic processes [15]. Additionally, enzymes isolated from 2-MIB degraders have been shown to effectively reduce 2-MIB concentrations [16].Nevertheless, research into enzymatic degradation methods for 2-MIB remains limited, underscoring the significant potential of enzymatic approaches in tackling the challenge of 2-MIB removal.

Laccase, a widely distributed multicopper glycoprotein enzyme, is renowned for its capability to oxidize a diverse range of both phenolic and non-phenolic compounds. Enzymatic degradation utilizing laccase has garnered significant attention due to its high removal efficiency and environmentally friendly characteristics. This enzyme has demonstrated the ability to oxidize a broad spectrum of substrates, encompassing environmental contaminants such as pharmaceuticals, dyes, pesticides, phenols, and components commonly found in wastewater [17–19]. Enhancing laccase catalytic efficiency through the use of mediators and immobilization techniques and exploring laccase from diverse sources are promising strategies to improve the bioremediation of environmental pollutants [20]. These strategies can enhance the performance of laccase in degrading various pollutants, positioning it as a promising tool for environmental remediation efforts.

Electrochemical water treatment technology utilizes an applied potential or current to facilitate electron transfer and multi-proton-coupled electron transfer processes [21]. This process leads to the oxidation, reduction, adsorption, and migration of pollutants [22]. Electrocatalysis has sparked significant interest in the field of electrochemical oxidation [23]. On the one hand, it follows non-radical pathways, involving direct electron transfer processes, where pollutants act as electron donors or acceptors, leading to direct oxidation or reduction [24]. On the other hand, it can generate powerful oxidants, such as hydroxyl radicals, at the catalytic electrode surface through radical pathways. These oxidants effectively oxidize and decompose pollutants, leading to efficient water purification. Most reviews on electrocatalysis technology have focused on specific areas, with some studies exploring the co-catalytic oxidation of various oxidants and peroxides [25].

This study aimed to explore how different reaction conditions affect the free laccase-catalyzed degradation of 2-MIB, optimize these conditions, and investigate the mechanism under optimal settings. To enhance electron transfer during the laccase-catalyzed oxidation process, this research examined the influence of an external micro-electric field on 2-MIB degradation by laccase. This study compared the efficiency of 2-MIB degradation catalyzed by laccase alone, a micro-electric field alone, and micro-electric-field-assisted laccase. Additionally, the potential degradation pathways of 2-MIB were also analyzed.

2. Results and Discussion

2.1. Effects of Reaction Parameters on Degradation of 2-MIB Catalyzed by Laccase

2.1.1. Effect of pH on Catalytic Degradation of 2-MIB by Laccase

At pH 4.0, the degradation efficiency of 2-MIB was optimal, achieving a degradation efficiency of 77.80% (Figure S1). Deviations from this optimal pH significantly reduced the degradation efficiency of 2-MIB, particularly under alkaline conditions. For instance, at pH 8.0, laccase catalyzed the degradation of 2-MIB at only 27.46% of the rate observed at pH 4.0. The efficacy of the biocatalysts was notably compromised under both acidic and alkaline conditions, with a more pronounced effect observed in alkaline environments. This was because pH influenced not only the substrate's ionization but also the spatial arrangement of the enzyme molecules and the ionization state of their surface-active groups [26]. Under alkaline conditions, the diminished degradation efficiency of 2-MIB could be attributed to the strong electrostatic repulsion between the oppositely charged groups at the enzyme's active site, leading to structural alterations that impacted its functionality [27].

According to the results in Figure 1a, linear fitting of the degradation efficiency plot was performed (Figure 1a), and the apparent rate constant for 2-MIB was calculated (Figure 1b). As shown in Figure 1b, the rate constant (k) at pH 4.0 was 0.0091 min^{-1}, representing the maximum reaction rate. Deviations from pH 4.0, either lower or higher, resulted in a decreased reaction rate. Laccase exhibited higher biological activity at the optimal pH, as different pH levels affected the charged states of both the substrate and the enzyme, influencing their binding interaction [28]. In this reaction, laccase primarily served as a catalyst, and changes in pH impacted its activity, thereby affecting the overall reaction rate. Similarly, Onaizi and Alshabib also found a non-linear trend in the conversion rate of bisphenol A catalyzed by laccase, where the rate first increased and then decreased with rising pH levels, indicating an optimal pH value [29].

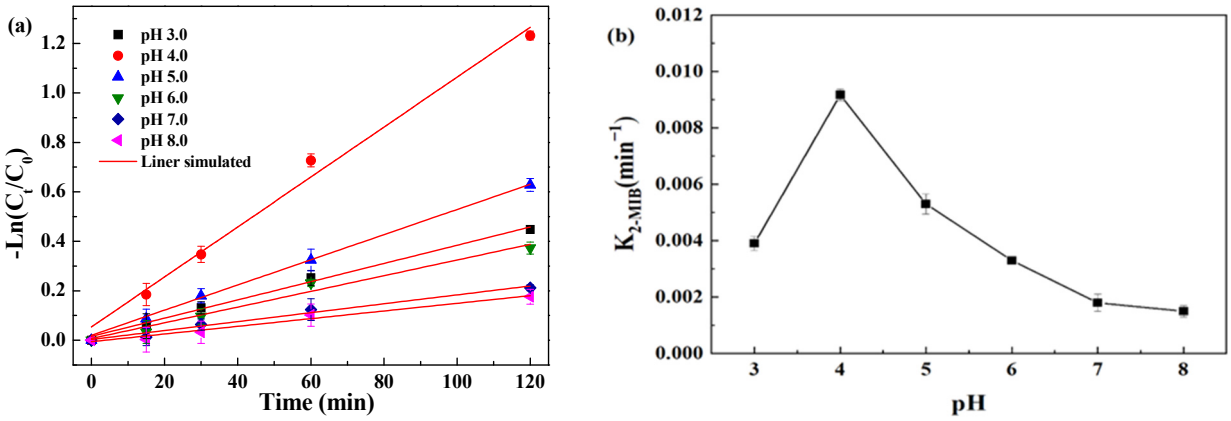

Figure 1. Fitting linear plots (**a**) and apparent rate constants (**b**) of 2-MIB degradation efficiency at different pH values (laccase dosage: 0.1 U/mL, 2-MIB concentration: 200 ng/L, 25 °C, 150 r/min).

2.1.2. Effect of Temperature on Catalytic Degradation of 2-MIB by Laccase

As shown in Figure S2, the degradation efficiency of 2-MIB reached 78.24% at 25 °C, indicating optimal efficiency. When the temperature exceeded 25 °C, the degradation efficiency of 2-MIB significantly decreased, dropping to 37.84% at 40 °C. Temperature affected the degradation of 2-MIB in two main ways: Firstly, the degradation efficiency of 2-MIB catalyzed by laccase increased with respect to the temperature. This was because the enzymes had an optimal temperature for their best activity, and the enzyme activity was suppressed at lower temperatures [30,31]. Secondly, the degradation efficiency of 2-MIB decreased when the temperature increased to more than 25 °C; this could be due to the heat-induced conformational changes in the enzyme at higher temperatures, leading to enzyme inactivation [29]. Based on the results in Figure S2, linear fitting of the degradation efficiency plot was performed (Figure 2a), and the apparent rate constant of 2-MIB was

calculated (Figure 2b). At 25 °C, the k value was 0.0089 min^{-1}, reaching the maximum reaction rate. In the ascending region below the optimal temperature, the increase in the reaction rate constant due to the rising temperature was moderate. Conversely, in the descending region above the optimal temperature, the rate of enzyme deactivation due to heat became more significant, leading to a substantial decrease in the efficiency of 2-MIB degradation. It was reported that the reaction rate for the degradation of polycyclic aromatic hydrocarbons catalyzed by laccase exhibited an initial incline followed by a decline as the temperature increased, consistent with the results of this study [32].

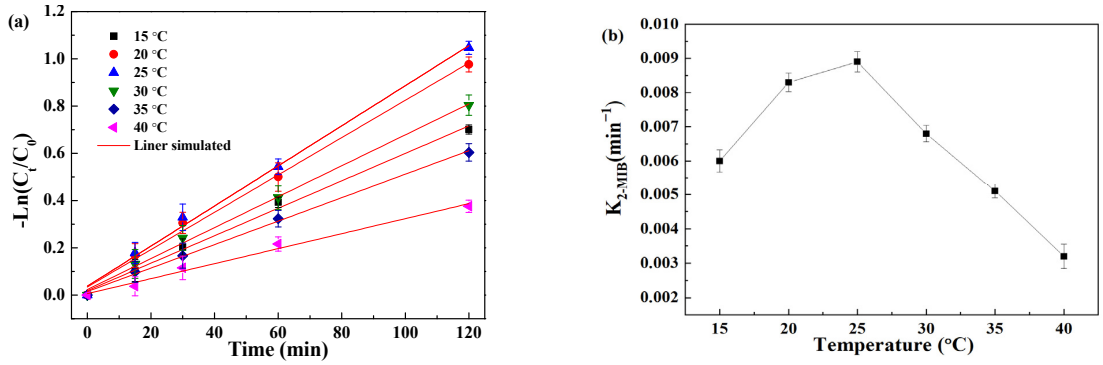

Figure 2. Fitting linear plots (**a**) and apparent rate constants (**b**) of 2-MIB degradation efficiency at different temperatures (laccase dosage: 0.1 U/mL, 2-MIB concentration: 200 ng/L, pH 4.0, 150 r/min).

2.1.3. Effect of Stirring Speed on Catalytic Degradation of 2-MIB by Laccase

Upon increasing the agitation speed from 75 r/min to 150 r/min, the degradation efficiency of 2-MIB increased from 40.72% to 78.28% (Figure 3a). Agitation served to improve the mixing of the substrate and enzyme in the reaction system, enhancing their interaction frequency and thereby facilitating the reaction [33]. At lower rotational speeds, the rate of the entire process is governed by mass transfer, whereas increasing the agitation speed consistently enhances the mass transfer rates, thereby boosting degradation efficiency [34]. However, further escalation of the agitation speed to 200 r/min resulted in a decline in the degradation efficiency of 2-MIB. In the degradation of ciprofloxacin hydrochloride catalyzed by laccase assisted by ultrasound, a higher stirring rate resulted in worse degradation, which is similar to the trend observed in this study [35]. Therefore, an agitation speed of 150 r/min was chosen for the optimal degradation of 2-MIB. Based on the results in Figure S3, linear fitting of the degradation efficiency plot was performed (Figure 3a), and the apparent rate constant of 2-MIB was calculated (Figure 3b). In Figure 3b, it is evident that, with an increasing agitation speed, the reaction rate initially increased and then decreased, peaking at 150 r/min with a k value of 0.0081 min^{-1}.

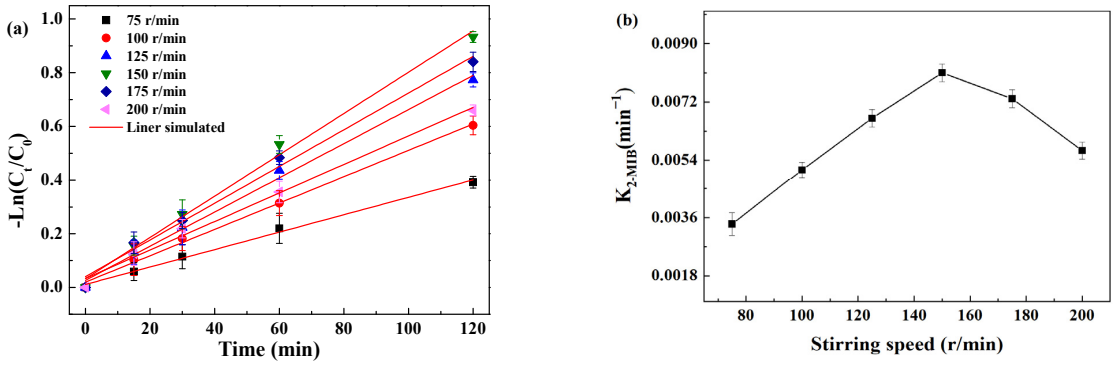

Figure 3. Fitting linear plots (**a**) and apparent rate constants (**b**) of 2-MIB degradation efficiency at different stirring speeds (laccase dosage: 0.1 U/mL, 2-MIB concentration: 200 ng/L, 25 °C, pH 4.0).

2.1.4. Effect of Enzyme Dosage on Catalytic Degradation of 2-MIB by Laccase

As shown in Figure S4, when the concentration of laccase was 0.01 U/mL, the degradation efficiency of 2-MIB after 4 h was only 25.69%. An insufficient enzyme dosage likely reduced the frequency of substrate–enzyme interactions, thereby hindering the degradation of 2-MIB [36]. Increasing the laccase concentration to 0.1 U/mL significantly boosted the degradation efficiency to the maximum of 78.24%. The optimal enzyme concentration enhanced the reaction rate by maximizing the frequency of substrate–enzyme interactions, thereby facilitating the degradation of 2-MIB. In the case of 0.1 U/mL, the concentration of laccase was 1.3 nM, and the concentration of 2-MIB was 1.2 nM. However, further increases in the laccase concentration to 0.15 U/mL and 0.2 U/mL did not significantly enhance the degradation efficiency beyond that achieved at 0.1 U/mL. At a higher enzyme concentration, the binding of the substrate with the enzymes was saturated, and the enzyme was redundant [37,38]. Therefore, a laccase concentration of 0.1 U/mL appeared to be optimal for efficiently degrading 200 ng/L of 2-MIB.

Based on the results in Figure S4, linear fitting of the degradation efficiency plot was performed (Figure 4a), and the apparent rate constant of 2-MIB was calculated (Figure 4b). It is evident in Figure 4a that increasing the enzyme dosage from 0.01 U/mL to 0.1 U/mL resulted in a significant increase in the k value to 0.0081 min^{-1}, thereby substantially enhancing the reaction rate of 2-MIB. However, further increasing the enzyme dosage to 0.2 U/mL only slightly raised the k value from 0.0083 min^{-1} to 0.0097 min^{-1}, representing a minor increase of 0.0014 min^{-1}, with no significant improvement in the reaction rate of 2-MIB. Therefore, the enzymatic dosage of 0.1 U/mL was selected as the appropriate condition for 2-MIB degradation.

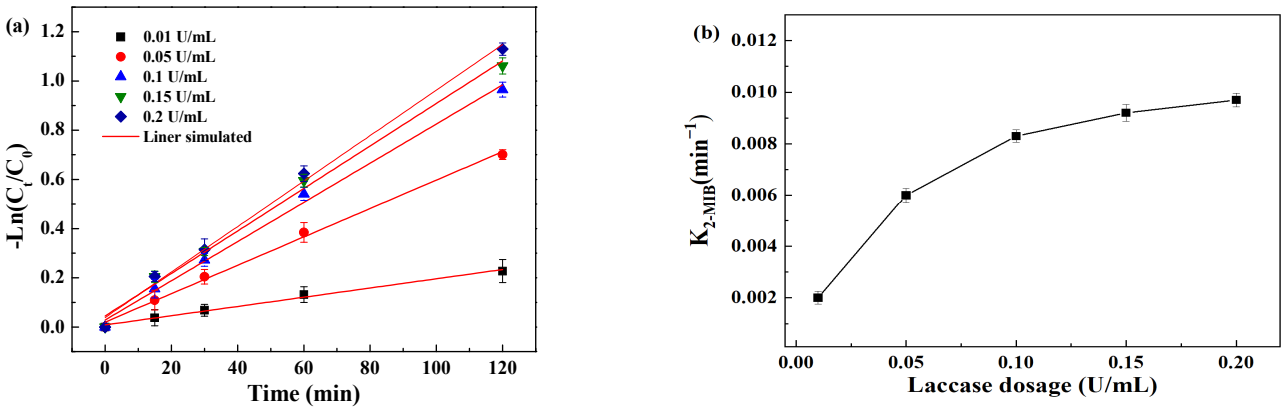

Figure 4. Fitting linear plots (**a**) and apparent rate constants (**b**) of 2-MIB degradation efficiency at different laccase dosages (2-MIB concentration: 200 ng/L, 25 °C, pH 4.0, 150 r/min).

2.1.5. Effect of Initial 2-MIB Concentration on Its Degradation Catalyzed by Laccase

In Figure S5, as the initial concentration of 2-MIB increased from 50 ng/L to 200 ng/L, the degradation efficiency rose from 51.49% to 78.34%. This was due to the enhanced opportunities for laccase–2-MIB interactions at higher concentrations, thereby augmenting degradation. However, when the initial concentration was further elevated to 500 ng/L, the degradation efficiency declined to 69.14%. Although the degradation efficiency was the highest at 200 ng/L, the actual degradation amount of 2-MIB increased with respect to the substrate concentration. These results indicate that, within a certain range, increasing the 2-MIB concentration could enhance the reaction rate, but high concentrations may negatively impact degradation efficiency [39,40]. This decrease was attributed to the limited availability of active sites on laccase at higher 2-MIB concentrations. Additionally, the higher initial 2-MIB concentration resulted in an increased production of degradation by-products, which may compete with the substrate for the binding sites of laccase or inhibit enzyme activity, thus reducing the overall degradation efficiency [41]. In the case of 500 ng/L, the concentration of laccase was 1.3 nM, and the concentration of 2-MIB was

3.0 nM. Based on the results in Figure S5, linear fitting of the degradation efficiency was performed (Figure 5a), and the apparent rate constant of 2-MIB was calculated (Figure 5b). It is evident in Figure 5b that, with an increase in the initial 2-MIB concentration, the reaction rate initially increased and then decreased. The peak reaction rate was observed at an initial 2-MIB concentration of 200 ng/L, with a rate constant (k) of 0.0089 min^{-1}. At the same time, under our reaction conditions, we found that there was no loss of enzyme activity after 4 h. In order to better understand the data, the kinetic parameters of laccase in the catalysis of 2-MIB were determined (Figure S6), where K_m was 3.75 mmol/L, and K_{cat} was 1.27 × 10^3 (Table S1).

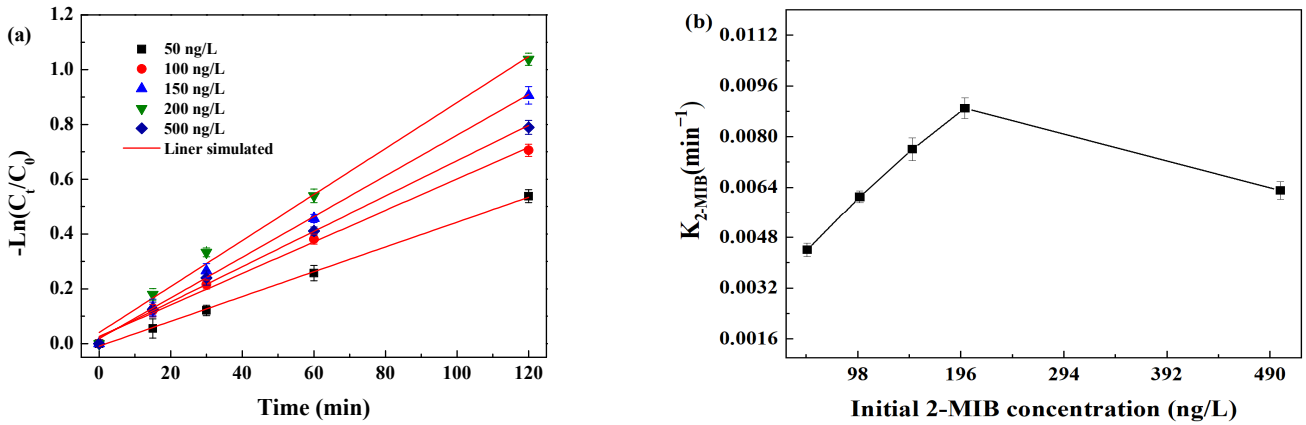

Figure 5. Fitting linear plots (**a**) and apparent rate constants (**b**) of 2-MIB degradation efficiency at different initial 2-MIB concentrations (laccase dosage: 0.1 U/mL, 25 °C, pH 4.0, 150 r/min).

2.1.6. Effect of Electric Field Intensity on 2-MIB Degradation Catalyzed by Laccase

The catalytic mechanism of laccase involved sequential stages: substrate binding, electron transfer resulting in the formation of intermediates and reduction of the intermediates [40]. Research has highlighted that the rate-limiting step in the oxidation of various substrates by laccases is the transfer of substrate electrons to the type I copper (Cu) center of the enzyme's native intermediate [42,43]. The impact of a direct current electric field on laccase activity in aqueous systems was investigated, and an enhancement in laccase activity was observed near the positive electrode, resulting in the acceleration of the catalytic reaction rate [44]. This study's results showed that the application of an external electric field increased the degradation efficiency of 2-MIB catalyzed by laccase. As the applied current increased from 0.01 A to 0.04 A, the degradation efficiency of 2-MIB increased to 90.78% (Figure 6). This was likely due to the enhanced electron transfer between the electrode, solution, and laccase with the increased current. The higher current generated a large number of highly oxidative free radicals, thereby promoting the degradation of 2-MIB [45]. However, an incremental gain in degradation efficiency occurred when the current increased from 0.04 A to 0.08 A. In fact, the application of an external electric field induced a gradual rise in pH in the cathode region (pH 4.2) and a decline in pH in the anode region (pH 3.8). Thus, the anode region became marginally acidic, while the cathode region became more alkaline with a higher current, influencing laccase activity. As depicted in Figure 7, the application of a micro-electric field alone in the degradation of 2-MIB provided a degradation efficiency of only 8.15% after a 4 h reaction. In contrast, the degradation catalyzed by laccase resulted in a degradation efficiency of 77.65% for 2-MIB. These results indicate that the degradation efficiency of 2-MIB catalyzed by laccase assisted by a micro-electric field was higher than the sum of the degradation efficiencies obtained using a micro-electric field and laccase. Therefore, the catalytic reaction by laccase assisted by a micro-electric field exhibited a synergistic effect, which could be due to the electric field's acceleration of electron transfer [44,46], facilitating the laccase-catalyzed reaction. The combination of an electric field and bioremediation was used to degrade aliphatic

hydrocarbons and achieved good degradation efficiency [47]. The application of an appropriate direct current can stimulate microbial activity and accelerate the biodegradation of petroleum pollutants [48]. A large-scale electro-biological method was utilized to repair soil contaminated by PAHs, where the degradation degree of PAHs was significantly enhanced compared with that achieved by bioremediation alone [49]. These results indicate that the combined strategy of an electric field and biological treatment has good potential for the removal of pollutants.

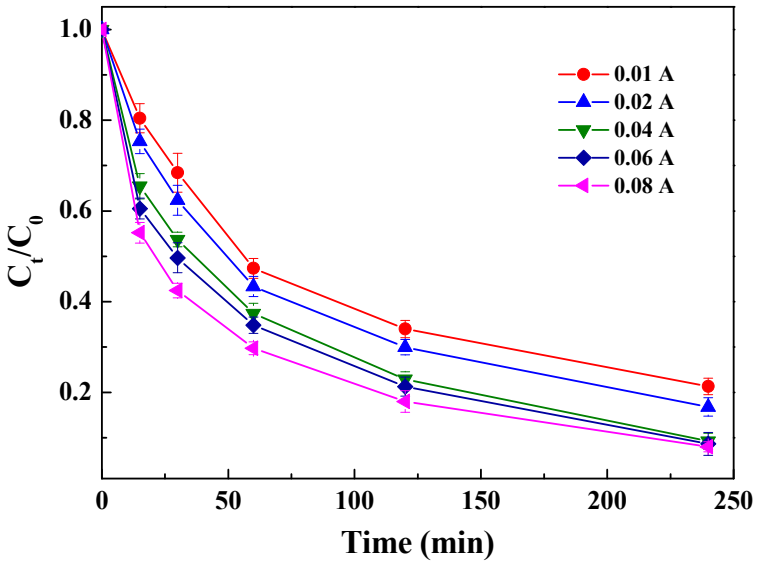

Figure 6. Time profile of 2-MIB degradation by laccase at different electric field intensities.

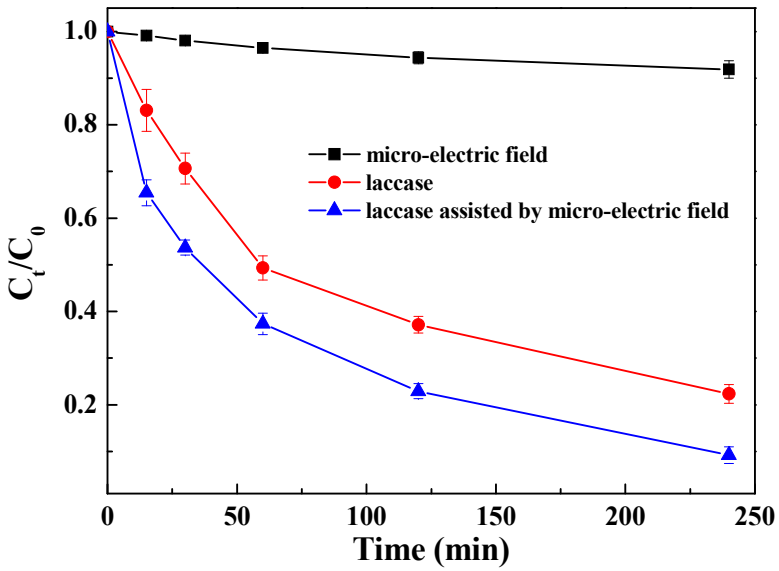

Figure 7. Time profile of 2-MIB degradation by micro-electric field, laccase, and laccase assisted by a micro-electric field (current intensity: 0.04 A, laccase dosage: 0.1 U/mL, 2-MIB concentration: 200 ng/L).

2.2. Analysis for the Intermediates of 2-MIB Degradation

Through an SPME-GC/MS analysis, the degradation intermediates of 2-MIB resulting from different reaction processes were identified and are listed in the Supplementary Materials (Tables S1–S3). Based on the information, the potential degradation pathways of 2-MIB catalyzed using different strategies were deduced and are illustrated in Figures 8–10.

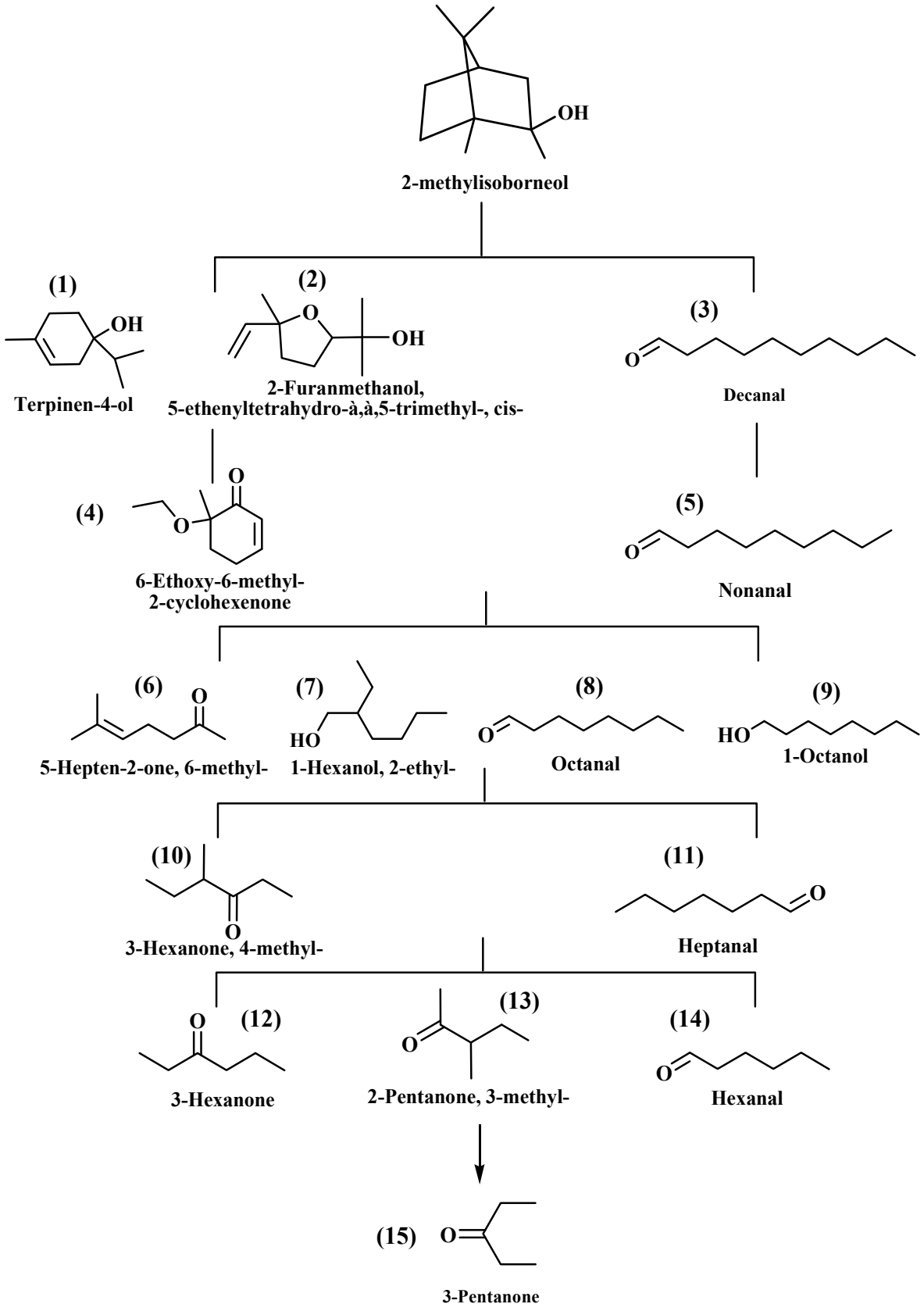

Figure 8. Possible degradation pathways of 2-MIB catalyzed by laccase.

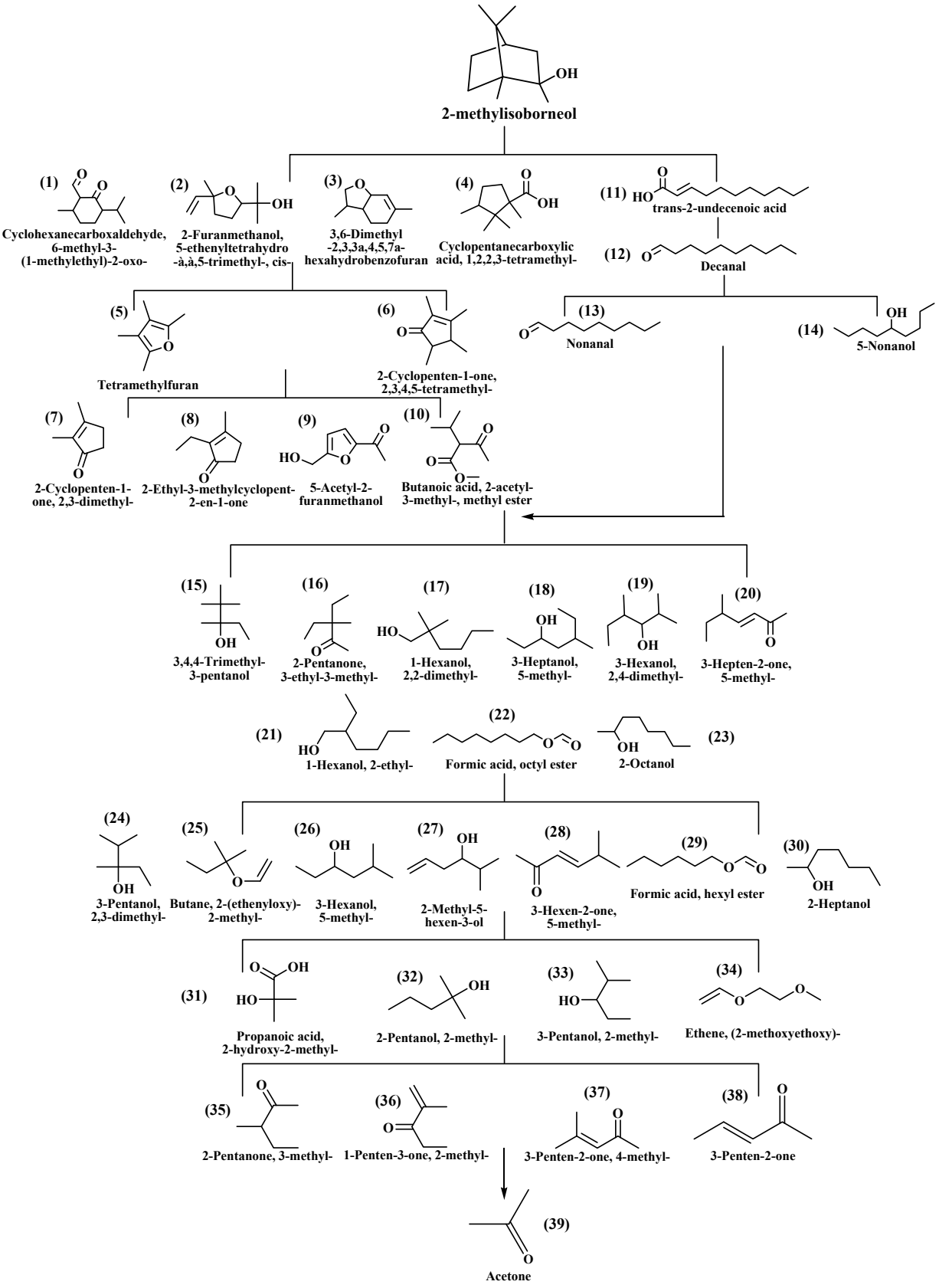

Figure 9. Possible degradation pathways of 2-MIB catalyzed by a micro-electric field.

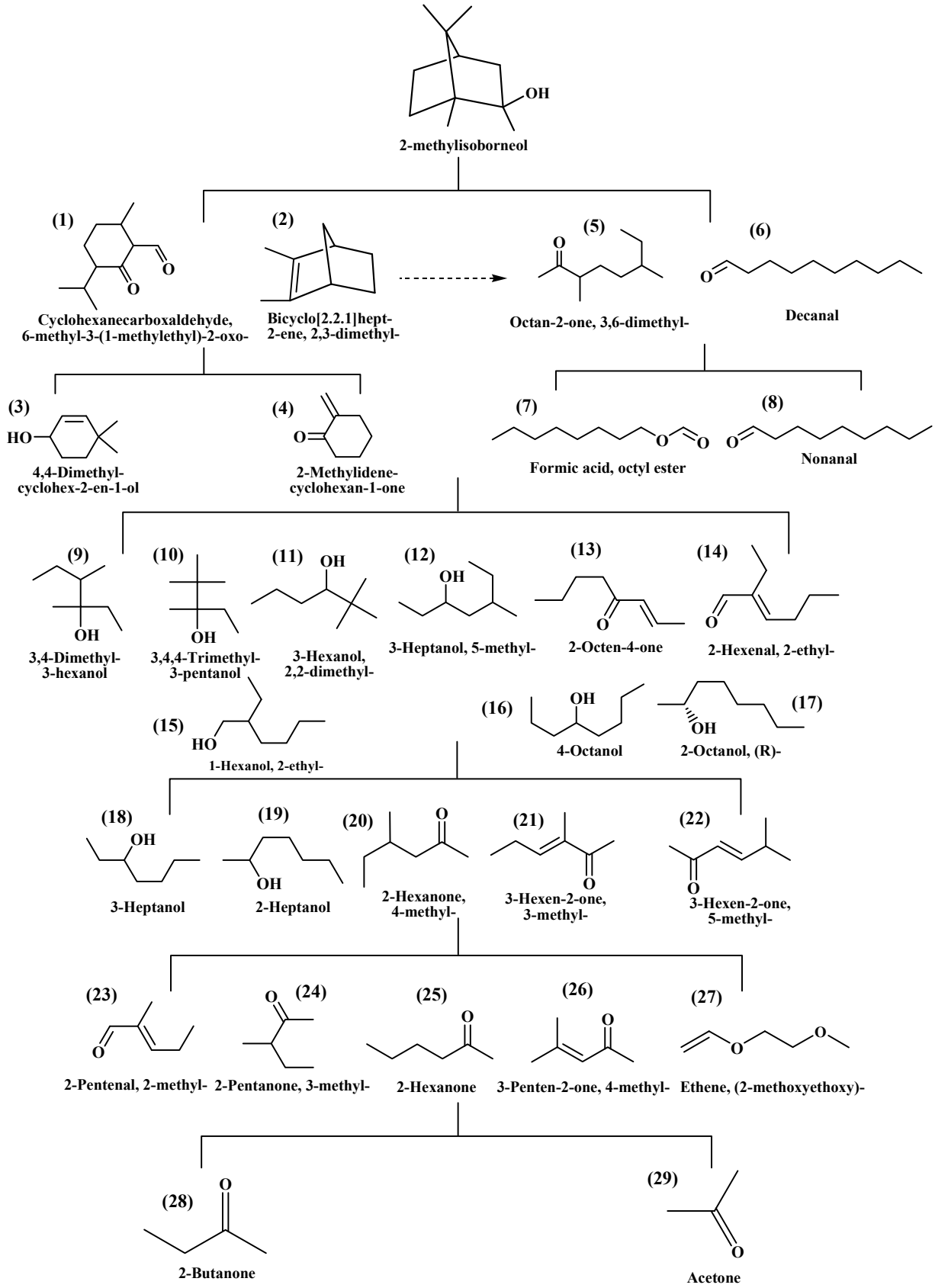

Figure 10. Possible degradation pathways of 2-MIB catalyzed by laccase assisted by a micro-electric field.

2.2.1. Possible Degradation Pathway of 2-MIB Catalyzed by Laccase

Figure 8 depicts the potential degradation pathways of 2-MIB catalyzed by laccase under previously optimized conditions. Products (**1**) and (**2**), comprising ten carbons, were likely formed through the oxidation of 2-MIB. Further oxidation by laccase may have led to the formation of the nine-carbon monocyclic compound 6-ethoxy-6-methyl-2-cyclohexenone (Product **4**). Alternatively, 2-MIB may have undergone ring-opening and recombination reactions, directly yielding the ten-carbon linear compound decanal (Product **3**). Due to the instability of linear chains relative to branched structures, further C-C bond cleavage resulted in the formation of the nine-carbon linear compound nonanal (Product **5**). Products (**4**) and (**5**), each containing nine carbons, underwent additional oxidation or monocyclic cleavage to yield four linear compounds with eight carbons: 6-methyl-5-hepten-2-one, 2-ethyl-1-hexanol, octanal, and 1-octanol (Products **6**–**9**). This degradation pathway involved oxidation, ring-opening, and recombination reactions, ultimately yielding a shorter-chain compound with fewer carbons (Product **15**), namely, 3-pentanone. Enzymes from *Pseudomonas mandelii* were used for 2-MIB degradation, and the final degradation product of 2-methyl-2-bornene was detected [16]. In the catalytic degradation process of cyclic compounds (bisphenol A) using laccase as a catalyst, similar reactions occurred, including oxidation and ring-opening reactions [50].

2.2.2. Possible Degradation Pathways of 2-MIB by Electrocatalysis

Figure 9 shows the possible degradation pathways of 2-MIB under a current of 0.04 A. Initially, 2-MIB underwent oxidation processes generating a spectrum of five-membered rings, six-membered rings, and bicyclic compounds (**1**–**6**). These intermediates subsequently underwent further oxidation or demethylation to yield three five-membered rings (**7**–**9**) and ring-opening compounds (**10**). Further oxidation or C-C bond cleavage resulted in the formation of diverse alcohols, ketones, esters, and other intricate branched-chain compounds. This degradation pathway encompassed reactions such as oxidation, ring-opening, and cleavage reactions, ultimately resulting in the production of the small molecule acetone. In contrast, previous investigations into the electrocatalytic degradation of 2-MIB have observed its ultimate transformation into carbon dioxide and water [50]. Notably, this distinction may be attributed to the elevated current density employed in those studies (2.5 mA cm^{-2}), markedly differing from the lower current density of 0.08 mA cm^{-2} utilized in this study, thereby yielding different final degradation products [51].

The chemical transformations that occurred in the presence of an electric field were notably complex. Typically, the degradation of pollutants under electrocatalytic conditions primarily proceeded through anodic oxidation, which encompassed both direct oxidation reactions and indirect oxidation mediated by hydroxyl radicals [52]. Direct oxidation entailed the potential adsorption of pollutants onto the electrode surface, where they interacted with active oxygen species adsorbed on the electrode [52,53]. Indirect oxidation, facilitated by hydroxyl radicals, involves generating these radicals through the oxidation of water on the anode surface under the influence of the electric field. These hydroxyl radicals subsequently participated in the oxidation of pollutants [52]. Guided by the principles of electrocatalysis, the degradation of 2-MIB involved the cleavage of chemical bonds and electron transfer processes, leading to the gradual transformation of the compound into lower-molecular-weight intermediates.

2.2.3. Possible Degradation Pathways of 2-MIB Catalyzed by Laccase Assisted by a Micro-Electric Field

Figure 10 integrates the potential degradation pathways of 2-MIB via laccase-catalyzed degradation assisted by a micro-electric field. Initially, 2-MIB underwent oxidation to form cyclic compounds (**1**) or dehydrogenation to yield compounds (**2**). These intermediates could be further oxidized to two cyclic compounds, namely, 4,4-Dimethyl-cyclohex-2-en-1-ol (**3**) and 2-Methylidene-cyclohexan-1-one (**4**), both comprising two cyclic units. Alternatively, ring-opening reactions may have generated branched ketone compounds containing

10 carbons, such as Octan-2-one, 3,6-dimethyl- (**5**). Subsequently, C-C bond cleavage and successive oxidation led to the formation of shorter-chain compounds. Moreover, 2-MIB underwent ring-opening and recombination reactions, directly forming long-chain compounds with 10 carbons (**5**, **6**). These long-chain compounds, unstable in structure, further underwent C-C bond cleavage to generate ketones, alcohols, and esters with nine or eight carbons. This degradation process involved oxidation, dehydrogenation, and ring-opening reactions, ultimately resulting in the formation of the small-molecule compounds 2-butanone (**28**) and acetone (**29**).

By comparing these three degradation pathways, it was observed that 2-MIB degradation generated some common degradation products, such as decanal, nonanal, and 2-ethylhexanol. Additionally, while electro-assisted enzyme catalysis accelerated the degradation of 2-MIB by promoting enzyme-catalyzed reactions, the enhancement effects were more complex, resulting in a greater variety of degradation intermediates than enzymatic degradation alone.

3. Materials and Methods

3.1. Chemicals

The 2-MIB standard (98% purity) was procured from Wako Pure Chemicals, Ltd. (Osaka, Japan). It was dissolved in methanol to prepare as a stock solution with a concentration of 20 mg/L. Laccase derived from *Trametes versicolor*, with an enzymatic activity of $\geq$0.5 U/mg, was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). All other materials were of analytical grade and were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

3.2. Experimental Procedure for 2-MIB Degradation

3.2.1. Preparation of Laccase Stock Solution

A specific amount of laccase was dissolved in 0.1 mol/L pH 3.5 acetic acid–sodium acetate buffer solution. After stirring for 30 min, the solution was filtrated through a 0.22 μm filter and stored at 4 °C until further experimentation.

3.2.2. Degradation of 2-MIB Catalyzed by Laccase

The degradation experiment was carried out in a 2-MIB (200 ng/L) solution with 0.1 U/mL of laccase. The experiment was conducted in a shaker at 25 °C and 150 r/min. Samples were taken during the reaction, and a certain amount of 6 mol/L NaOH solution was added to the samples to stop the reaction. Finally, headspace solid-phase microextraction gas chromatography–mass spectrometry (SPME-GC/MS) was used to determine the residual 2-MIB in the samples.

The effects of pH on the degradation efficiency and kinetics of 2-MIB were studied by adjusting the initial pH to different levels (3.0, 4.0, 5.0, 6.0, 7.0, 8.0). To determine the influence of temperature and shaking speed on 2-MIB degradation, different temperatures (15, 20, 25, 30, 35, 40 °C) and various shaking speeds (75, 100, 125, 150, 175, 200 r/min) were tested. The effects of laccase dosage (0.01, 0.05, 0.1, 0.15, 0.2 U/mL) and initial 2-MIB concentration (50, 100, 150, 200, 500 ng/L) on degradation were also investigated.

3.2.3. Degradation of 2-MIB Catalyzed by Micro-Electric Field

The degradation of 2-MIB catalyzed by a micro-electric field with/without laccase was carried out in the electrocatalysis device described in our previous research with some modifications [54]. For this test, graphite–graphite plate electrodes were used, and a magnetic agitator was applied to ensure mixing during catalysis. For the degradation of 2-MIB, the optimized conditions obtained in Section 3.2.2 were used.

3.3. Analysis

3.3.1. Determination of Laccase Activity

An appropriate amount of laccase was added to 3 mL of 0.1% (*w*/*v*) catechol solution prepared in 0.1 mol/L pH 3.0 tartaric acid buffer. The reaction was carried out at 25 °C for 5 min, and the change in absorbance at 450 nm was measured. The amount of laccase required to oxidize one micromole of catechol per minute was defined as one enzyme activity unit (U).

3.3.2. Analysis of 2-MIB and Its Intermediate Degradation Products

The analysis and identification of 2-MIB and its potential degradation products were carried out using SPME-GC/MS. The headspace extraction time was 50 min, and the injection was performed manually. The chromatographic column used was HP-5MS (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injection temperature was set to 270 °C. The column temperature was initially maintained at 60 °C for 1 min, then increased to 150 °C at a rate of 5 °C/min, and finally increased to 270 °C at a rate of 20 °C/min and maintained for 5 min. The mass spectrometry conditions were set as follows: the ion source temperature was 280 °C, the quadrupole temperature was 300 °C, the ionization mode was Electron Impact (EI), and the mass range was 50 *m*/*z* to 300 *m*/*z*. The mass spectra of all detected compounds were compared with those in NIST spectral library 2.0.

3.3.3. Calculation of 2-MIB Degradation Rate

The degradation rate of 2-MIB was calculated according to the following formula:

$$\text{Degradation Rate} = (1 - C_0/C_t) \times 100\%. \quad (1)$$

In Formula (1), C_t represents the residual concentration of 2-MIB (ng/L), and C_0 represents the initial concentration of 2-MIB (ng/L).

The degradation reaction of 2-MIB followed first-order reaction kinetics:

$$\text{Ln}(C_0/C_t) = kt. \quad (2)$$

In Formula (2), t is the reaction time, and k is the reaction rate constant.

4. Conclusions

In this study, the conditions for the laccase-catalyzed degradation of 2-MIB were successfully optimized, achieving a degradation efficiency of approximately 78% under the optimal conditions of pH 4.0, 25 °C, 150 r/min, and 0.1 U/mL after 4 h. Compared with the degradation processes of laccase catalysis and electrochemical oxidation alone, laccase catalysis assisted by a micro-electric field exhibited a synergetic effect for 2-MIB degradation and improved the degradation efficiency to 90.78%. The application of an external micro-electric field may have accelerated electron transfer during oxidation. The degradation pathways of these three catalytic strategies were suggested based on the SPME-GC/MS results. Some degradation intermediate products appeared in all three pathways, and their final degradation products were ketone compounds. This study provides new insights into the degradation of T&O substances catalyzed by laccase, highlighting the innovative approach of combining enzymatic catalysis with electrocatalysis for enhanced degradation efficiency and reduced odor intensity. Future research will focus on developing new methods or optimizing conditions for the complete degradation of 2-MIB.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal14090649/s1, Figure S1: Time profile of 2-MIB degra-dation rate at different pH; Figure S2: Time profile of 2-MIB degradation by laccase at different temperature; Figure S3: Time profile of 2-MIB degradation by laccase at different stirring rate; Figure S4: Time profile of 2-MIB degradation by laccase at different laccase dosage; Figure S5: Time profile of 2-

MIB degradation by laccase at different initial 2-MIB concentration; Figure S6: Lineweaver-Burk plots of free laccase. Table S1: Kinetic parameters of laccase; Table S2: Summary of intermediates determined by SPME-GC/MS in 2-MIB degradation catalyzed by laccase; Table S3: Summary of intermediates determined by SPME-GC/MS in 2-MIB degradation catalyzed by laccase assisted with micro-electric field.

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