Converting lignin into long-chain fatty acids with the electro-Fenton reaction

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Abstract

The lack of effective lignin valorization technology remains a major barrier to utilize lignocellulose for producing biofuels and biochemicals. In this work, a new technology via the three-phase three-dimensional electro-Fenton reaction was employed for the first time to convert lignin or its derived aromatics. The operating parameters were optimized by central composite design, which was controlled to prompt the synthesis of long-chain fatty acids from lignin instead of degrading lignin to CO2. Under the optimized condition, the yields of palmitic acid and octadecanoic acid from lignin reached 138.41 and 112.31 mg/g, respectively. Key intermediators were identified during the electrolysis of lignin model compound using gas chromatography–quantitative time of flight mass spectrometry. The mechanism for the production of long-chain fatty acids from lignin was proposed. Lignin successively underwent degradation, opening ring, and couple reactions and produced free radicals. This study is the first to report the conversion of lignin into the precursor of biodiesel or advanced biofuels through electro-Fenton electrolysis at room temperature and atmosphere pressure. This study provides an environment-friendly route for direct conversion of lignin into high-value added products as a new strategy to valorize this undervalued component in lignocellulosic biomass.

Keywords

advanced biofuels, electro-Fenton reaction, fatty acid, Kolbe reaction, lignin, lignocellulosic biomass
1 | INTRODUCTION

Lignocellulose is an abundant source of feedstock for biofuel production that cannot only contribute to meet the energy demands but also address climate change concerns (Ghosh & Singh, 1993; Kishore et al., 2004). Sugars derived from cellulose and hemicellulose in lignocellulosic biomass can be converted through fermentation to various liquid fuels, such as ethanol, or fuel precursors, such as lipids (Demirbas, 2009; Gong et al., 2012; Zhu & Pan, 2010). However, the high production cost of cellulosic biofuels is currently limiting its wide application at industry scale. A commonly recognized approach for overcoming this barrier is to produce high-value co-products in addition to fuel. A major opportunity in this approach is the alternative use of lignin. Lignin, which accounts for 10%–25% dry weight of lignocellulose (Hamelinck et al., 2005), is usually burned as a low-grade energy source after separation from cellulose and hemicellulose (Bu, Lei, Ren, et al., 2012; Bu, Lei, Zacher, et al., 2012). Numerous efforts have been devoted to valorize lignin by converting it to biofuels and high-value bioproducts.

Many reports showed that lignin and its derived aromatics can be converted to biofuels through physicochemical or biochemical techniques. Runnebaum et al. discovered that lignin-derived aromatics can be converted into hydrocarbon by Pt/γ-Al2O3 in the presence of H2 (Runnebaum, 2011). Wang et al. confirmed that alkali-extracted corn stover lignin can be employed to produce cyclic hydrocarbons with Ru/Al2O3 and acidic zeolite (Wang et al., 2015). Cheng et al. further increased the conversion yield of alkali lignin to biofuel (27.5%) using NiO/HZSM-5 as catalyst in a two-stage reactor (Cheng et al., 2017). Shimanskaya et al. directly obtained 68% biofuel yield from softwood sawdust with 5% loading of Pt/MN-270 catalyst (Shimanskaya et al., 2018). However, the technical application of physicochemical techniques is hampered by harsh operating conditions, such as high temperature and pressure, as well as high purification cost, catalyst thermal instability and deactivation, and safety considerations (Ragauskas et al., 2014; Werhan et al., 2013). Biological conversion of lignin using microbes and enzymes can be accomplished under milder conditions. In a typical biological conversion process, lignin-derived aromatics are first metabolized into 3-carboxymuconate through the “funneling pathway” (Linger et al., 2014); the 3-carboxymuconate enters the TCA cycle and fatty acid synthesis pathway to produce lipid accumulating in cells (Vardon, 2015). Lignin-derived aromatics are utilized by oleaginous Rhodococcus to reach a lipid content of 4.08% for 9 days (Kosa & Ragauskas, 2013). Wang et al. further reported that R. opacus NRRL B-3311 could consume 20% AFEX-lignin and accumulate up to 32 mg/L lipids in 72 h (Wang et al., 2019). However, a major drawback of biological conversion is its long fermentation time, which makes it less effective in industrial application.

Several new technologies, including microwave pyrolysis (Bu, Lei, Ren, et al., 2012; Bu, Lei, Zacher, et al., 2012), electrochemical conversion (Li et al., 2012), photocatalyzed conversion (Ibrahim et al., 2014; Walsh et al., 2014), have been tested to convert lignin and its derived aromatics to biofuels. Electrochemical conversion is recognized as one of the most promising environment-friendly methods (Brillas et al., 2009; Tian et al., 2010). This technique can decrease chemical oxygen demand in industrial effluent. Aromatics in waste water are directly mineralized to CO2 through electro-oxidation reactions, including anode oxidation (Aden et al., 2002; Lopes et al., 2004), three-phase-three-dimensional oxidation (Ugurlu et al., 2006; Zhang et al., 2013), and advanced oxidation process (AOP), such as electro-Fenton reaction (Oturan et al., 2000; Stasinakis, 2008). Oturan et al. employed a carbon felt electrode to generate HO· in situ by the electro-Fenton reaction to mineralize 2,4-dichlorophenol acetate into CO2 and H2O (Oturan, 2000). Shao et al. and Permentel et al. confirmed that aromatic ring-opening products are composed of volatile fatty acids (VFAs), which include short-chain carboxylic acids (C1–C6), such as muconic acids, maleic acid, oxalic acid, etc. (Pimentel et al., 2008; Shao et al., 2014). Lignin valorization to biodiesel requires long-chain carboxylic acids (C12–C22), whereas lignin mineralization or short-chain carboxylic acids (C1–C6) do not meet the quality requirements. In the present study, the three-phase-three-dimensional electro-Fenton reaction was used to oxidize lignin or its derived aromatics. The operating parameters were controlled to promote the synthesis of long-chain fatty acids from VFA intermediates instead of degrading VFAs into CO2. This study is the first to report the conversion of lignin into fatty acids through electro-Fenton electrolysis and provides new insights in developing a green pathway to convert lignin and its derivatives from bio-refinery process and pulp industry to fatty-acid-based biofuels and bioproducts.

2 | MATERIALS AND METHODS

2.1 | Materials

Alkali-extracted lignin was obtained from Longlive Company. Sulfonate lignin was acquired from Bohui Paper Company. All regents were of analytical grade.

2.2 | Experimental design

Central composite design (CCD) was used to optimize the process parameters of the electro-Fenton reaction for
lignin/lignin derivative conversion. The parameters for optimization included reaction time, current intensity, oxygen flow rate, catalyst loading, and NaCl and Fe$^{2+}$ concentrations (Tables 1 and 2). The process variables in the preliminary experiments were estimated through single-factor experiments. Triplicate runs were conducted for each combination: reaction time (10–20 min for lignin; 1–7 min for lignin derivative), current intensity (0.4–1A), oxygen flow rate (1–4 L/min), catalyst loading (1%–3 w/w), NaCl concentration (50–150 mM), and Fe$^{2+}$ concentration (0.52 mM). The results from the experimental work were statistically analyzed using “Statistica 6.0” (StatSoft Inc.) and “Minitab” (Minitab Inc.). The effect of each parameter was evaluated to determine the optimal parameters at which the highest yield of palmitic acid as product representative can be obtained.

### 2.3 | Electrochemical oxidation of lignin and lignin-derived aromatics

Electrochemical oxidation was carried out in the undivided three-phase three-dimensional electrode reactor (Figure 1) through Uğurlu’s method with modification (Uğurlu et al., 2006). Graphite electrodes were employed as cathode and anode. The whole electrolysis volume was 40 ml, and the distance between electrodes (3 cm$^2$ effective area) was 2 cm. The reaction system was stirred at 350–400 rpm to enhance mass transfer. Compressed oxygen was supplied to the reactor from the bottom of the cathode through a PVC tube. Galvanostatic analysis was performed by a DC power supply (APS3005S-3D, Nanjing Guorui Atten Technologies Industrial Corporation). Lignin and lignin-derived aromatics (Guaiacol, salicylic acid, phthalic acid, and ferulic acid) were chosen as the substrates.

| Reaction time (min) | Current intensity (A) | Oxygen flow rate (L/min) | Catalyst loading (%) | NaCl concentration (M) | Fe$^{2+}$ concentration (mM) | Palmitic acid (mg/g) |
|---------------------|-----------------------|--------------------------|----------------------|------------------------|----------------------------|---------------------|
| 10                  | 0.4                   | 1                        | 1                    | 0.05                   | 0.5                        | 115.87              |
| 10                  | 0.4                   | 1                        | 1                    | 0.1                    | 1                           | 118.98              |
| 10                  | 0.4                   | 1                        | 1                    | 0.2                    | 2                           | 117.87              |
| 10                  | 0.7                   | 2                        | 3                    | 0.05                   | 0.5                        | 120.41              |
| 10                  | 0.7                   | 2                        | 3                    | 0.1                    | 1                           | 123.54              |
| 10                  | 0.7                   | 2                        | 3                    | 0.2                    | 2                           | 120.87              |
| 10                  | 1                     | 4                        | 5                    | 0.05                   | 0.5                        | 116.41              |
| 10                  | 1                     | 4                        | 5                    | 0.1                    | 1                           | 121.11              |
| 10                  | 1                     | 4                        | 5                    | 0.2                    | 2                           | 119.21              |
| 16                  | 0.4                   | 2                        | 5                    | 0.05                   | 1                           | 120.87              |
| 16                  | 0.4                   | 2                        | 5                    | 0.1                    | 2                           | 129.78              |
| 16                  | 0.4                   | 2                        | 5                    | 0.2                    | 0.5                         | 126.87              |
| 16                  | 0.7                   | 4                        | 1                    | 0.05                   | 2                           | 124.41              |
| 16                  | 0.7                   | 4                        | 1                    | 0.1                    | 2                           | 131.41              |
| 16                  | 0.7                   | 4                        | 1                    | 0.2                    | 0.5                         | 126.32              |
| 16                  | 1                     | 1                        | 3                    | 0.05                   | 0.5                         | 117.48              |
| 16                  | 1                     | 1                        | 3                    | 0.1                    | 1                           | 126.61              |
| 16                  | 1                     | 1                        | 3                    | 0.2                    | 2                           | 119.24              |
| 20                  | 0.4                   | 4                        | 3                    | 0.05                   | 2                           | 108.78              |
| 20                  | 0.4                   | 4                        | 3                    | 0.1                    | 0.5                         | 110.69              |
| 20                  | 0.4                   | 4                        | 3                    | 0.2                    | 1                           | 109.87              |
| 20                  | 0.7                   | 1                        | 5                    | 0.05                   | 0.5                         | 111.21              |
| 20                  | 0.7                   | 1                        | 5                    | 0.1                    | 1                           | 114.33              |
| 20                  | 0.7                   | 1                        | 5                    | 0.2                    | 2                           | 109.61              |
| 20                  | 1                     | 2                        | 1                    | 0.05                   | 2                           | 91.21               |
| 20                  | 1                     | 2                        | 1                    | 0.1                    | 1                           | 98.34               |
| 20                  | 1                     | 2                        | 1                    | 0.2                    | 0.5                         | 95.36               |
Initial electrolysis was performed in 400 ml of NaCl solution (and pH 3) containing 0.1% (wt/v) lignin or its derived aromatics and right amount of activated carbon-based catalyst (wt/v) at right current and 350–400 rpm magnetic stirring. After electrolysis, the resulting electrolyte was extracted three times with 50 ml of ethyl acetate, which was used for fatty acid analysis.

2.4 Fatty acid analysis and quantification

The composition and amounts of fatty acids were analyzed by GC-FID according to the reported method with a little modification (Zhang et al., 2016). The extracts (5 ml) were dried in a glass tube at 70°C for 1 h at a N$_2$ flow rate of 10 ml min$^{-1}$, and further derivatized to fatty acid methyl esters (FAMEs) before GC-FID analysis. Briefly, 3 ml 0.5 M KOH–methanol solution was added into the glass tube containing the dried extract from the previous step. The tube was incubated in a water bath at 60°C for 15 min. After that, the tube was naturally cooled to room temperature followed by the addition of 6 ml of 14% (w/w) BF$_3$/methanol solution. The mixture was again heated in a water bath at 60°C for 2 min. To obtain fatty acid methyl esters (FAMEs) from the mixture, 2 ml of hexane and 1 ml of saturated brine were added into the cooled tube (room temperature) followed by vertexing for 20 s. After that, the upper phase was collected and dried with anhydrous Na$_2$SO$_4$ overnight.

| Reaction time (min) | Current intensity (A) | Oxygen flow rate (L/min) | Catalyst loading (w/w) | NaCl concentration (M) | Fe$^{2+}$ concentration (mM) | Palmitic acid (mg/g) |
|-------------------|----------------------|--------------------------|------------------------|------------------------|-----------------------------|----------------------|
| 2                 | 0.4                  | 1                        | 1                      | 0.05                   | 0.5                         | 56.21                |
| 2                 | 0.4                  | 1                        | 1                      | 0.1                    | 1                           | 57.35                |
| 2                 | 0.4                  | 1                        | 1                      | 0.2                    | 2                           | 56.01                |
| 2                 | 0.7                  | 2                        | 3                      | 0.05                   | 0.5                         | 59.27                |
| 2                 | 0.7                  | 2                        | 3                      | 0.1                    | 1                           | 59.98                |
| 2                 | 0.7                  | 2                        | 3                      | 0.2                    | 2                           | 59.47                |
| 2                 | 1                    | 4                        | 5                      | 0.05                   | 0.5                         | 58.01                |
| 2                 | 1                    | 4                        | 5                      | 0.1                    | 1                           | 59.31                |
| 2                 | 1                    | 4                        | 5                      | 0.2                    | 2                           | 58.21                |
| 4                 | 0.4                  | 2                        | 5                      | 0.05                   | 1                           | 60.01                |
| 4                 | 0.4                  | 2                        | 5                      | 0.1                    | 2                           | 60.14                |
| 4                 | 0.4                  | 2                        | 5                      | 0.2                    | 0.5                         | 60.01                |
| 4                 | 0.7                  | 4                        | 1                      | 0.05                   | 1                           | 60.78                |
| 4                 | 0.7                  | 4                        | 1                      | 0.1                    | 2                           | 61.11                |
| 4                 | 0.7                  | 4                        | 1                      | 0.2                    | 0.5                         | 60.87                |
| 4                 | 1                    | 1                        | 3                      | 0.05                   | 2                           | 56.21                |
| 4                 | 1                    | 1                        | 3                      | 0.1                    | 1                           | 59.87                |
| 6                 | 0.4                  | 4                        | 3                      | 0.05                   | 2                           | 57.33                |
| 6                 | 0.4                  | 4                        | 3                      | 0.1                    | 0.5                         | 57.89                |
| 6                 | 0.4                  | 4                        | 3                      | 0.2                    | 1                           | 56.21                |
| 6                 | 0.7                  | 1                        | 5                      | 0.05                   | 2                           | 58.71                |
| 6                 | 0.7                  | 1                        | 5                      | 0.1                    | 0.5                         | 59.98                |
| 6                 | 0.7                  | 1                        | 5                      | 0.2                    | 1                           | 59.87                |
| 6                 | 1                    | 2                        | 1                      | 0.05                   | 2                           | 50.71                |
| 6                 | 1                    | 2                        | 1                      | 0.1                    | 0.5                         | 53.41                |
| 6                 | 1                    | 2                        | 1                      | 0.2                    | 1                           | 49.24                |
2.5 | Intermediators identified by GC-QTOF

The important intermediates obtained from the electro-Fenton treatment of guaiacol were detected and identified by gas chromatography-quadrupole time-of-flight mass spectrometry (GC-QTOF). Prior to the analysis, the as-obtained intermediates in the dried extract were silylated based on Zhou et al. (2007). The resulting products were analyzed using a 7890A GC/7200 Q-TOF MS (Agilent Technologies) equipped with a 30 m × 250 μm i.d. (0.25 μm film thickness) Agilent 19091S-433 HP-5MS capillary column. Helium was used as the carrier gas with a flow rate of 1.2 ml min⁻¹. NIST 8.0 database was used to interpret the MS results.

2.6 | Hydroxyl radical detection

The amount of HO· formed in the electro-Fenton reaction was monitored by the modified salicylic acid method (Jen et al., 1998). The experimental apparatus was the same as that in Section 2.3. Based on the optimized parameters of the electro-Fenton reaction, 5 ml salicylic acid–ethanol solution (90 mM) was added into the electro-Fenton reactor to capture HO·. Samples were collected at 0, 3, 5, 10, and 16 min and filled through a 0.22 μm luer syringe filter before analysis using an HPLC (Shimadzu, LC-20AD). HPLC analysis was performed on ODS-BD C-18 column (Sinochrom, 4.6–260 mm, 5.0 μm) at 35°C. In the mobile phase, an acetic acid–water–methanol solution (1:79:20) was passed through the column with a flow rate of 1 ml min⁻¹. The concentrations of salicylic acid and its derivatives were measured using a UV detector at 296 nm. The injection volume was 10 μl each time (Jen et al., 1998).

2.7 | Elemental carbon analysis

The elemental carbon was analyzed according to the method recommended by Han et al. (2009). The product yields on a carbon basis were calculated based on the following equation:

\[ EC = \left( \frac{n_{C \text{ in product}}}{n_{C \text{ in reactant}}} \right) \times 100\% \]

where EC represents the conversion efficiency of carbon atoms from different raw materials to products during the electro-Fenton process.

3 | RESULTS AND DISCUSSION

3.1 | Effect of different factors on electro-conversion rate of lignin-derived aromatics for long-chain fatty acid production

Oturan et al. (2000) suggested that the mechanism of the electro-Fenton reaction includes the following: oxygen is transferred to the cathode to produce H₂O₂ in the presence of electricity, and H₂O₂ is converted into HO· catalyzed by Fe²⁺; these processes are described in Reactions (1), (2), and (3). Organic compounds are initially activated by HO· and then attacked by oxidative species (O₂ and HO·) to generate CO₂ and H₂O as final products (Reactions 4 and 5; Jen et al., 1998; Vilota et al., 2014):

\[ \text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}_2, \]  
\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{HO}, \]  
\[ \text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}, \]  
\[ \text{R} - \text{H} + \text{HO} \cdot \rightarrow \text{R} \cdot + \text{H}_2\text{O}, \]  
\[ \text{R} \cdot + \text{O}_2 \rightarrow \text{ROO} \cdot \rightarrow \text{degradation production} \rightarrow \text{CO}_2 + \text{H}_2\text{O}, \]
design for lignin/lignin derivatives conversion using palmitic acid yield as representative. The optimal conditions for lignin electro-conversion were 0.1 M NaCl, 1 mM Fe$^{2+}$, 3% (wt/v) activated carbon-based catalyst loading, 0.7 A current intensity, 2 L min$^{-1}$ oxygen flow rate, and 16 min of reaction, which led to the highest concentrations of palmitic and octadecanoic acids (138.41 and 112.31 mg/g, respectively) from alkali-extracted lignin (Figure 2). The optimal condition was 0.1 M NaCl with 1 mM Fe$^{2+}$, 3% (wt/v) activated carbon-based catalyst loading, 0.7 A current intensity, 2 L min$^{-1}$ oxygen flow rate, and 4 min of electrolysis, which led to the highest concentrations of palmitic and octadecanoic acids (62 and 43 mg/g, respectively) from guaiacol as lignin derivatives (Supplemental data). Lignin derivatives need shorter reaction time due to their smaller molecular weight and higher solubility. Any value of these factors that were either higher or lower than the optimal conditions would decrease the yield of fatty acids produced (Figure 2).

Oxygen was employed as the reactant and captured electrons on the cathode to produce H$_2$O$_2$ (Brillase et al., 2009). Low oxygen flow rate (<2 L min$^{-1}$) was unsuitable for H$_2$O$_2$ production, but higher rate caused mass transfer problems in the electro-Fenton system, thereby inhibiting oxygen to obtain electrons from the cathode. Graphite electrode could not supply sufficient reaction surface. In this regard, activated carbon catalyst was polarized and became the third electrode in the high current density and voltage environment. The voltage should be higher than 10 V to effectively polarize the activated carbon, and 3% catalyst loading was used to ensure sufficient electro-chemical reaction sites (Ugurlu et al., 2006). In our experiment, the average voltage in the 0.4 A and 0.1 M NaCl environment was higher than 15 V, which resulted in satisfactory function of the third electrode (activated carbon-based catalyst). The resultant H$_2$O$_2$ was sustainably catalyzed by Fe$^{2+}$ to generate HO, which promoted lignin oxidation and long-chain fatty acid synthesis (Vilota et al., 2014). Higher current density benefited this process, which was proven by Schäfer (2012) and Naber (1980). On the other hand, HO· could attack the long-chain fatty acid molecules and completely mineralize them; consequently, the manipulation of electrolysis time was quite important for the synthesis of long-chain fatty acids from guaiacol. Thus, the method of HO· production in the electro-Fenton process should be strictly controlled.

3.2 | Production of long-chain fatty acids from different lignin-derived model aromatics and different lignin compounds

Table 3 displays all the products identified by QC-TOF after lignin electro-oxidation under the optimum reaction conditions.
condition. Lignin was degraded to be single aromatic ring derivatives, such as 4-hydroxybenzoic acid, acetovanillone, 3-vanilpropanol, etc., through ether bond cleavage and side chain oxidation, which resulted in the formation of polyaromatic rings, such as 4H-1-Benzopyran-4-one, 3-(3,4-dimethoxyphenyl)-6,7-dimethoxy, isopimaric acid, abietic acid, etc., through the oxidative coupling reaction. Long-chain fatty acids, such as oleic acid, myristic acid, etc., were discovered in the product, with palmitic acid and octadecanoic acid as the main products. These fatty acids were considered as important products in lignin valorization due to their possible use in biofuel production.

This work confirmed whether palmitic and octadecanoic acids could be produced from lignin or lignin derivatives from different sources. Lignin (alkali-extracted lignin; sulfonate lignin) and lignin-derived aromatics (guaiacol, salicylic acid, ferulic acid, and phthalic acid) were chosen for electrolysis treatment. Under the optimum condition, palmitic and octadecanoic acids were produced as two major products from different lignin or lignin-derived aromatics. However, the yields of fatty acids between lignin and lignin derivatives varied greatly. The highest yields of palmitic and octadecanoic acids from alkali-extracted lignin were 138.41 and 112.31 mg/g, respectively, whereas those from salicylic acid were 2.55 and 1.54 mg/g, respectively (Table 4). The conversion efficiency of carbon atoms from alkali-extracted lignin to palmitic and octadecanoic acids was 33.47% and 27.41%, respectively. Lignin had copious structure and could be formed as intermediator, which is beneficial to promote the electro-Fenton reaction (Brebu & vasile, 2010; Liu et al., 2008). Among lignin derivatives, guaiacol was easier to realize the electrochemical conversion. The yield of palmitic and octadecanoic acids was more than 15 folds than that from salicylic acid and ferulic acid. Furthermore, comparison of different strategies toward conversion of lignin and its derivatives to biofuels in the representative literature with that in this work is summarized in Table S1. To date, the major approaches can be divided into three types, involving fermentation, thermal treatment (e.g., hydrogenation and pyrolysis), and electrocatalytic treatment. It is noted that electrocatalytic

| No. | RT (min) | Compound | Molecular formula |
|-----|----------|----------|------------------|
| 1   | 5.780    | Butanedioic acid | C₉H₆O₄        |
| 2   | 6.973    | Hydroquinone | C₆H₆O₂        |
| 3   | 8.683    | 4-Hydroxybenzoic acid | C₇H₆O₃    |
| 4   | 11.800   | Acetovanillone | C₉H₁₀O₃   |
| 5   | 12.219   | 1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone | C₁₀H₁₂O₄ |
| 6   | 12.925   | 2,6-Dimethoxyhydroquinone | C₈H₁₀O₄ |
| 7   | 14.915   | Vanillic Acid | C₈H₀O₄       |
| 8   | 16.091   | Dihydroconiferyl alcohol | C₁₀H₁₄O₃ |
| 9   | 16.841   | Myristic acid | C₁₄H₂₅O₂     |
| 10  | 18.693   | 4-Coumaric acid | C₈H₆O₄     |
| 11  | 20.863   | Palmitic acid | C₁₆H₃₂O₂     |
| 12  | 23.828   | Linolelaidic acid | C₁₆H₃₂O₂ |
| 13  | 23.949   | cis-Oleic acid | C₁₈H₃₄O₂     |
| 14  | 24.452   | Stearic acid | C₁₈H₃₆O₂     |
| 15  | 25.220   | 1,1’-(1,2-ethyenediy) bis[2,4-dimethoxy]-Benzene | C₁₈H₁₈O₄ |
| 16  | 25.693   | cis-11,14-Eicosadienoic acid | C₂₀H₃₆O₂ |
| 17  | 25.898   | Isopimaric acid | C₂₀H₃₆O₂     |
| 18  | 26.190   | Abietic acid | C₂₀H₃₆O₂     |
| 19  | 26.721   | Callitrisic acid | C₂₀H₃₆O₂ |
| 20  | 27.256   | 4H-1-Benzopyran-4-one, 3-(3,4-dimethoxyphenyl)-6,7-dimethoxy- | C₁₉H₁₅O₆ |
| 21  | 27.703   | Vanillylmandelic acid | C₉H₄O₅  |
| 22  | 28.476   | 4,4’-Methylenebis(2-tert-butyl-6-methylphenol) | C₂₂H₂₂O₂ |
| 23  | 30.376   | 2-Hydroxyestrone | C₁₈H₂₂O₃ |

TABLE 3  Identification of lignin-degraded compounds through the electro-Fenton reaction
treatment seems to be a promising way to combine the attributes of the formers, such as mild reaction conditions, short reaction time, and high yield. However, the feedstock of this approach needs to be lignin-derived monomers or oligomers due to their good solubility or dispersity in the electrolyte. To make lignin as a suitable feedstock, an additional lignin electro-degradation process (e.g., electro-oxidation) has been investigated (Wijaya et al., 2020). To address this issue, we developed a novel strategy to combine lignin degradation and valorization to fatty acid via one step electro-Fenton reaction with activated carbon catalyst in this work. Compared with all the existing approaches, electro-Fenton treatment displays an improved production rate (10–20 min) and comparable yield (~25%) in the mild conditions.

3.3 Mechanism for production of long-chain fatty acids from lignin-derived aromatics

1. Hydroxyl radical generation in the electro-Fenton reaction
   The concentration of HO· was calculated according to this theory that one equivalent of HO· drives one equivalent of hydroxyl group added to salicylic acid. Figure 3 presents the line plots for salicylic acid-captured HO· products against electrolysis time using guaiacol as substrate. The production rates of 2,5-dHBA and 2,3-dHBA were initially kept at 0.74 and 0.65 mM min⁻¹, respectively. At the optimal time (4 min), 5.74 mM HO· was formed in the electro-Fenton process. This result confirmed that HO· was formed in the electro-Fenton process. During electrolysis, the yield of free radicals increased linearly. HO· may participate in lignin oxidization and hydroxylation to generate catechol and hydroquinone, which were further oxidized into ring-opening products (Lee, 2002).

2. Key intermediator identified by GC-QTOF
   Nine intermediators were identified by GC-QTOF, and the key intermediators were classified into five reactions (Table 5). The qualitative and quantitative analyses of key intermediators indicated that guaiacol underwent ring-opening oxidation during initial degradation. The resulting dicarboxylic acids participated in the Kolbe reaction for synthesis of long-chain fatty acids in the electro-Fenton treatment. During the electro-Fenton process, the concentrations of intermediates present in the prophase decreased gradually, whereas the concentration of the final product increased gradually (Figure 4).

3. Mechanism derivation
   The possible route of guaiacol electrochemical degradation and fatty acid synthesis in the electro-Fenton process is shown in Figure 5. Initially, guaiacol (compound 1) was oxidized by Cl₂ and HO· to undergo demethoxylation and hydroxylation, forming catechol (compound 2), where Cl₂ and HO· were generated from NaCl electrolysis and electro-Fenton process. Catechol was oxidized by O₂ with Fe²⁺ as catalyst under the acidic condition to generate o-quinone (compound 3). Given that quinone was easily degraded by HO, o-quinone was converted into muconic acid (compound 4), which was further reduced into adipic acid (compound 5). Muconic acid was also degraded into 2-butenedioic acid (compound 6), which was further reduced to succinic acid (compound 7). Adipic acid generated succinic acid through

| Substrate                  | Palmitic acid | Octadecanoic acid |
|---------------------------|---------------|-------------------|
|                           | Yield (mg/g)  | CE (%)            |
|                           |               |                   |
| Salicylic acid            | 2.55 ± 0.19   | 0.314 ± 0.001     |
| Ferulic acid              | 2.88 ± 0.11   | 0.349 ± 0.001     |
| Phthalic acid             | 6.21 ± 0.78   | 0.805 ± 0.001     |
| Guaiacol                  | 62.40 ± 5.23  | 6.906 ± 0.006     |
| Alkali extracted lignin   | 138.41 ± 1.27 | 33.47 ± 0.51      |
| Sulfonate lignin          | 127.38 ± 1.10 | 30.21 ± 0.67      |
|                           | 1.54 ± 0.08   | 2.92 ± 0.001      |
|                           | 1.88 ± 0.10   | 0.231 ± 0.001     |
|                           | 4.49 ± 0.38   | 0.590 ± 0.001     |
|                           | 43.34 ± 3.68  | 4.863 ± 0.001     |
|                           | 112.31 ± 1.09 | 27.41 ± 0.54      |
|                           | 103.14 ± 1.03 | 24.78 ± 0.51      |
electro-oxidation. Compounds 4, 5, 6, and 7 were named as volatile fatty acids (VFAs). Two kinds of oxidation reaction occurred for VFAs: first, VFAs were completely mineralized to produce H₂O and CO₂ by HO; second, HO· drove VFAs to polymerize each other and synthesize fatty acids through oxidation coupling reaction (Kolbe reaction; Naber, 1980). Consequently, two pathways were involved in fatty acid synthesis. First, in octadecanoic acid synthesis, two equivalents of adipic acids were coupled with each other, generating one equivalent of decanoic acid (Compound 8). One equivalent of decanoic acid condensed with equal adipic acid to produce one equivalent of n-pentadecanoic acid (Compound 9). One equivalent of n-pentadecanoic acid was coupled with one equivalent of succinic acid to form one equivalent of octadecanoic acid (Compound 10). Second, in palmitic acid synthesis, two equivalents of adipic acids polymerized each other, generating one equivalent of decanoic acid (Compound 8). One equivalent of decanoic acid was coupled with equivalent of succinic acid to produce one equivalent of n-tridecanoic acid (Compound 11). One equivalent of n-tridecanoic acid was coupled with one equivalent of succinic acid to generate one equivalent of palmitic acid (Compound 12).

A similar strategy is reported in natural degradation process of lignin. Vardon (2015) discovered that lignin subunits, for example, p-coumarate and ferulate, could not be directly used as carbon source by Pseudomonas putida. These subunits typically occurred during aromatic ring oxidation and

| Reaction                       | No. | RTa (min) | Compound                  | Molecular formula |
|-------------------------------|-----|-----------|---------------------------|-------------------|
| Chlorination                  | 4   | 7.213     | 4-Chloro-2-methoxyphenol  | C₇H₇ClO₂          |
| Hydroxylation                 | 1   | 5.457     | 2-Methoxyhydroquinone     | C₇H₈O₃            |
| Hydroxylation and demethoxylation | 2   | 6.084     | Catechol                 | C₆H₆O₂            |
|                               | 5   | 7.711     | Resorcin                  | C₆H₇O₂            |
| Ring opening                  | 3   | 6.884     | trans, trans-Muconic acid | C₆H₆O₄            |
|                               | 10  | 6.481     | Fumaric acid             | C₆H₄O₄            |
|                               | 11  | 9.295     | Adipic acid              | C₆H₁₀O₄           |
| Coupling reaction             | 6   | 8.264     | Dicanoic acid            | C₁₀H₂₀O₂           |
|                               | 7   | 17.203    | Tridecanoic acid         | C₁₃H₂₆O₂           |
|                               | 8   | 21.191    | Palmitic acid            | C₁₆H₃₂O₂           |
|                               | 9   | 24.811    | Stearic acid             | C₁₈H₃₆O₂           |

aRT represents the retention time during GC-QTOF analysis.

FIGURE 4 Changes in the intermediate concentration during the electro-Fenton process
opening for β-ketoadipate generation before being consumed in TCA recycle and fatty acid biosynthesis. The β-ketoadipate pathway from aromatics to fatty acid was also reported in *Pseudomonas, Acinetobacter*, and *Rhodococcus* (Bugg et al., 2011). The proposed lignin electro-conversion technique in this work is a bio-mimic process. The speculated route from lignin to long-chain fatty acids is shown in Figure 6. Lignin was initially degraded to small fragments, such as phenolic compounds, which underwent ring-opening reactions to form dicarboxylic acids (e.g., adipic acid) that were converted into long-chain fatty acids through Kolbe synthesis.

4 CONCLUSION

A three-dimensional electro-Fenton reaction, a kind of AOP treatment, was used for the first time to valorize lignin into long-chain fatty acids. The reaction was successfully controlled by optimizing six different parameters in electrolysis to balance lignin electro-oxidation and dicarboxylic acid coupling reaction. The highest yields of palmitic and octadecanoic acids from lignin under the optimal conditions reached 138.41 and 112.31 mg/g, respectively, and those from guaiacol as a representative of lignin derivatives reached 62.40
and 43.34 mg/g, respectively. Based on the identification of key intermediators, the mechanism deduced stated that lignin successively experienced degradation, opening ring, and couple reactions along with the production of free radicals. This study provides a new pathway for direct conversion of lignin into high-value added products at room temperature and atmosphere pressure. This strategy could also be used to valorize this undervalued component in lignocellulosic biomass.

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CONFLICT OF INTEREST
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT
A new technology via the three-phase three-dimensional electro-Fenton reaction was employed for the first time to convert lignin or its derived aromatics. The yields of palmitic acid and octadecanoic acid from lignin reached 138.41 and 112.31 mg/g, respectively. Lignin successively underwent degradation, opening ring, and couple reactions and produced free radicals. This study is the first to report the conversion of lignin into the precursor of biodiesel or advanced biofuels at room temperature and atmosphere pressure. This study provides an environment-friendly route for direct conversion of lignin into high-value added products to valorize this undervalued component in lignocellulosic biomass.

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