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Credit Author Statement

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Near real-time analysis of *para*-cresol in wastewater via a laccase-carbon nanotube-based biosensor

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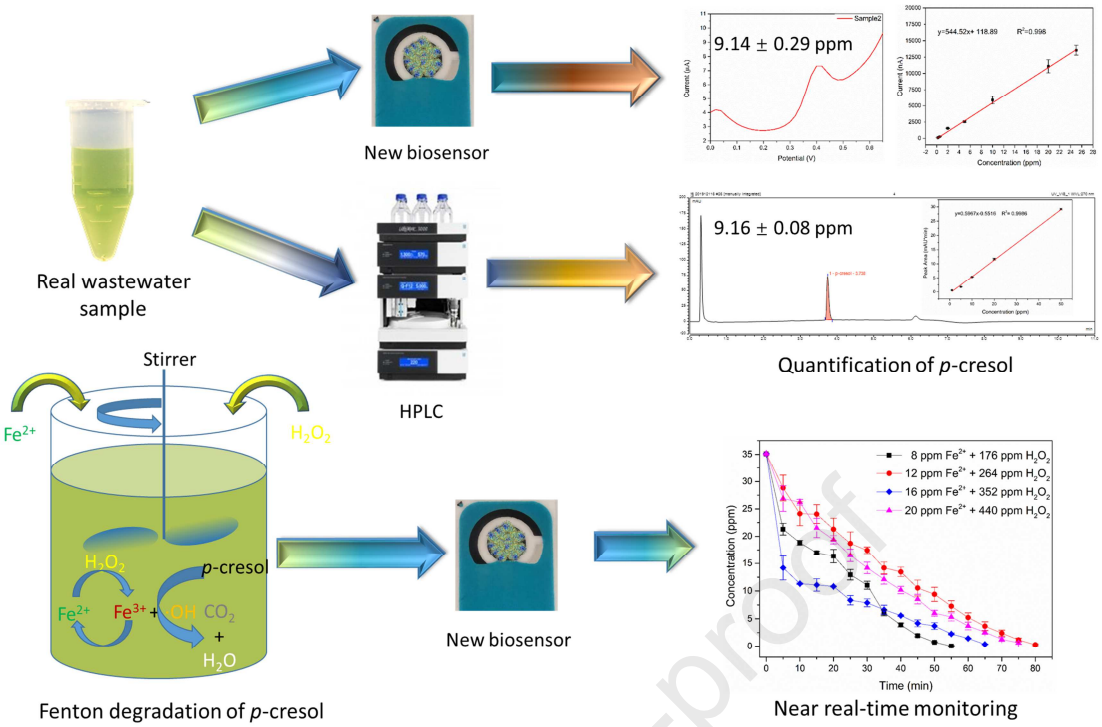


Abstract

Para-Cresol is a water-soluble organic pollutant, which is harmful to organisms even at low concentrations. Therefore, it is important to rapidly detect the *p*-cresol in wastewater as well as natural water. In this work, a new, simple and stable biosensor was developed for on-site quantitatively determination and near real-time monitoring *p*-cresol in wastewater. The new biosensor was designed and fabricated using a screen-printed carbon electrode (SPCE) modified by waste-derived carbon nanotubes (CNTs) immobilized with laccase (LAC). The fabrication processes and performance of the biosensors were systematically characterized and optimized by Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscope (FESEM), transmission electron microscopy (TEM) and electrochemical methods. With improved conductivity, the proposed biosensor could provide the direct quantitation of *p*-cresol. The linear range of the biosensor is 0.2-25 ppm of *p*-cresol with a detection limit of 0.05 ppm. Additionally, the biosensor exhibited high reproducibility, stability and reusability during the validation. More importantly, the biosensor was successfully applied for the rapid detection of *p*-cresol in environmental lab wastewater under the interference of metal ions and other organics, and the results were consistent with high-performance liquid chromatography (HPLC). Finally, the biosensor with a portable potentiostat was approved as an easy-to-use, sensitive and inexpensive platform that could provide near real-time monitoring of *p*-cresol concentration in wastewater during Fenton oxidation treatment process.

Keywords: Biosensor; laccase; waste-derived carbon nanotubes; screen-printed carbon electrode; *p*-cresol; near real-time monitoring

1. Introduction

The 4-methylphenol (*p*-cresol), as a representative of phenolic compounds, is a common contaminant in industrial wastewater, especially in sectors associated with oil refinery, metallurgy, petrochemical, steelworks, dyes, ceramic plants, pesticides and phenolic resin manufacturing (Michałowicz and Duda, 2007; Singh et al., 2008). Thus, if the wastewater is discharged without proper treatment, it would pose a threat to the environment and ecosystem. In nature, bacteria can metabolize tyrosine to *p*-cresol under anaerobic conditions, following its accumulation in the environment (Dari and Barker, 1985; Sridharan et al., 2014). It was found that *p*-cresol even at low concentrations can cause skin problems via the disruption of keratinization (Miyazaki et al., 2014), damage the kidneys and blood vessels through the effects on endothelial cells (Ketteler, 2006; Gonzalez-Parra et al., 2013; Chao and Chiang, 2015), aggravate autism severity and gut dysfunction (Persico and Napolioni, 2013), cause stomach tumors and colorectal cancer (Singh et al., 2008; Diether and Willing, 2019). In addition, it was also found that *p*-cresol in urine can be used as a biomarker for the diagnosis of autism in children aged 2-7 years (Altieri et al., 2011).

As the impact of phenolic compounds on the environment had become more and more severe, they were listed by the US Environmental Protection Agency (EPA) and the European Union (EU) as their priority pollutants (Montero et al., 2005). According to EPA, the regulatory level of *p*-cresol in both hazardous wastes from non-specific sources and the leachates after toxicity characteristic leaching

procedure (TCLP) is 200 ppm. The requirement from the National Environment Agency (NEA) of Singapore for the total phenolic compounds in is below 0.5 ppm. Consequently, a method for the rapid detection of *p*-cresol in the environment/wastewater is urgently demanded. Furthermore, the establishment of analytical methods for *p*-cresol detection is also important for the subsequent treatment of *p*-cresol contamination.

At present, the detection methods for *p*-cresol, as well as other phenolic compounds mainly included ultraviolet-visible (Tchaikovskaya et al., 2007), Folin–Ciocalteu method (Ballus et al., 2015), liquid/gas chromatography (Khoschsorur and Petek, 2000; Korytowska et al., 2019) and electrochemical detections (Sudhan et al., 2017). Among these methods, ultraviolet-visible and Folin–Ciocalteu methods were applied as nonspecific conventional techniques for evaluating the levels of phenolic compounds in different samples. Liquid/gas chromatography could provide more accurate and sensitive analysis, but required bulky and expensive laboratory instruments, labor-intensive sample preparation, and specialized operators. Compared with those laboratory scale methods, electrochemical detection methods, especially electrochemical biosensors attracted widespread attention, because of their simple protocols, fast detections, no requirement for bulky instrumentation, low limit of detection (LOD) and high accuracy (Talarico et al., 2015).

For the electrochemical biosensors sensitive to phenolic compounds, the three most commonly used enzymes are laccase (LAC), tyrosinase and horseradish

peroxidase (Rodríguez-Delgado et al., 2015). However, among these three enzymes, tyrosinase has low stability and can be easily inhibited by reaction products, while horseradish peroxidase cannot oxidize phenolic compounds without hydrogen peroxide (Freire et al., 2002). In contrast, LAC is a much more stable enzyme for biosensors, which could effectively catalyze the oxidation of phenolic compounds by molecular oxygen dissolved in the solution (Xu and Yang, 2013). Even though the LAC has high solubility, it was considered to be poorly conductive as a weak electrolyte (Saoudi et al., 2017). Therefore, it was necessary to apply a highly conductive material to enhance electron transfer for improving the performance of the biosensor.

The commonly used conductive nanomaterials for electrode surface modification mainly included metal-based nanomaterials (such as nanometals and nano metal oxides) and carbon-based nanomaterials (such as nano graphene, carbon quantum dots and carbon nanotubes) (Dervisevic et al., 2019; Wang et al., 2019; Shrivas et al., 2020). Since metal-based nanomaterials might release toxic heavy metal ions during application, carbon-based nanomaterials were chosen to enhance the conductivity. As a nanomaterial with high conductivity and high specific surface area, carbon nanotubes (CNTs) were widely used in different electrochemical sensors (Dhara and Mahapatra, 2019; Eguílaz et al., 2019). Plastic is one of the most commonly used materials in daily life, it was reported that eight million tons of plastic waste entered the ocean each year, which had seriously

affected the environment (Taufik et al., 2020). Nevertheless, the plastic packaging waste could also be used as a raw material for the synthesis of CNTs, which would be helpful to solve the environmental problems caused by waste plastics (Ahamed et al., 2019; Veksha et al., 2019).

By far, the major challenge for biosensors was the application for complex samples. Compared with other environmental samples, the environmental laboratory wastewaters were considered to be more difficult to test and treat, because they were of great diversity, and contained various endogenous components and complex sample matrices. For treating phenol-containing wastewater, Fenton oxidation method was considered as an effective advanced oxidation process owing to the excellent degradation efficiency for organics (Pliego et al., 2015). However, in practical applications, due to the complexity of actual wastewaters, the specific wastewater treatment conditions should be optimized case by case. Therefore, it was necessary to monitor *p*-cresol concentration during the Fenton reaction to achieve the best treatment efficiency (Jain et al., 2018).

In this study, a new electrochemical biosensor was developed for rapid, accurate, low-cost and one-step detection of *p*-cresol in laboratory wastewater samples by selecting LAC as a catalyst for the oxidation of *p*-cresol and functionalized carbon nanotubes (FCNTs) as an electron carrier. Furthermore, the performance of biosensor for on-site detecting *p*-cresol in environmental laboratory wastewater was validated by high-performance liquid chromatography (HPLC)

technique. Finally, the biosensor with optimized conditions was applied to monitor the Fenton degradation of *p*-cresol in near real-time.

2. Materials and Methods

2.1. Chemicals and reagents

Analytical grade reagents and high purity solvents were used throughout the study. Nickel(II) nitrate hexahydrate, ($Ni(NO_3)_2{\cdot}6H_2O$, purum p.a., crystallized, > 97%), ammonium molybdate tetrahydrate, ($H_{24}Mo_7N_6O_{24}{\cdot}4H_2O$, ACS reagent, 81-83% MoO_3 basis), potassium dihydrogen phosphate (KH_2PO_4), *p*-cresol, di-potassium hydrogen phosphate (K_2HPO_4), *N*-hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), laccase from *Rhus vernicifera* (≥50 units mg^{-1}), glutaraldehyde (GA) solution, sulfuric acid (H_2SO_4), nitric acid (HNO_3), iron (II) sulfate heptahydrate ($FeSO_4{\cdot}7\ H_2O$), hydrogen peroxide solution (H_2O_2, 30%) and methanol (HPLC grade) were purchased from Merck Pte. Ltd. (Singapore). Polypropylene and polyethylene were purchased from Lotte Chemical. Calcium carbonate ($CaCO_3$, ACS grade) was purchased from Sinopharm Chemical Reagent Co. Commercial carbon nanotubes (CCNTs, 95%) was from Nanografi Nano Technology. Deionized (DI) water from a Millipore Milli-Q purification system was used in all the experiments. The 0.2 M phosphate buffers with different pH were obtained by adjusting the different ratios of 0.2 M K_2HPO_4 and 0.2 M KH_2PO_4, which was monitored by a pH meter.

133 *2.2. Synthesis of plastic packaging waste derived CNTs and functionalized CNTs*

134 The synthesis of plastic packaging waste derived CNTs was carried out using
135 Ni-Mo catalyst loaded on $CaCO_3$ support and prepared using the reported method
136 (Veksha et al., 2019). Nickel (II) nitrate hexahydrate and ammonium molybdate
137 tetrahydrate were used as catalytically active components. Briefly, 4.2 g
138 ammonium molybdate followed by 4.0 g nickel nitrate were dissolved in 60 mL
139 deionized water, and 20 g $CaCO_3$ was added to the solution. Water was evaporated
140 in a rotary evaporator (Hei-Vap Precision, Heidolph Instruments) and the solid
141 material was transferred to an oven for drying at 100 °C overnight and then to a
142 muffle furnace for calcination in air at 750 °C for 3 h (heating rate 2 °C min^{-1}). The
143 calcined catalyst was crushed and sieved to obtain the particles with sizes smaller
144 than 100 μm. The synthesis of CNTs was carried out in a vertical quartz reactor at
145 625 °C. The reactor was loaded with 2.0 g of catalyst and heated to the synthesis
146 temperature in a flow of N_2 (100 mL min^{-1}). Once the desired temperature was
147 reached, the catalyst was contacted for 4 h with a mixture of N_2 (50 mL min^{-1}) and
148 non-condensable pyrolysis gas (50 mL min^{-1}) produced by pyrolysis of a plastic
149 packaging waste mixture (70% polypropylene and 30% polyethylene) at 600 °C.
150 The prepared CNTs were purified by acid and water washing. The yield calculated
151 as the ratio of masses of purified CNTs (dried overnight at 105 °C) and catalyst
152 used for the synthesis was 110%.

153 Both plastic packaging waste derived and commercial CNTs were later
154 functionalized with carboxyl groups, which were labeled as FCNTs and FCCNTs,

respectively. The method was followed to the practice of reported work (Milani Hosseini and Motaharian, 2015). Generally, 0.7 g of CNTs were added into a 200 mL mixture of H_2SO_4/HNO_3= 3/1 (v/v), sonicated for 4 hours, and then stirred at room temperature for 8 hours. After that, the solid was washed with deionized water until pH reached 7.0, and then filtered and vacuum dried at 60 °C for 24 hours to obtain FCNTs.

2.3. Modification of working electrode

The process of electrode modification is shown in Scheme S1. The LAC (1 mg) and different amounts of the FCNTs (0, 2, 3, 4, 5, 6, 7 and 8 mg) were added into 20 mL aqueous solution with 5 mM NHS and 5 mM EDC, the suspension solutions were sonicated for 5 min and then stored overnight at 4 °C (labeled as LAC-FCNTs). Subsequently, the suspension solutions were sonicated for 5 min, then 10 μL of each solution was dropped on the working electrode of screen-printed carbon electrode (SPCE, DRP-C110, Metrohm, with a conventional three-electrode system, in which the working and reference electrode were printed with carbon paste and as the counter electrode was printed with silver paste). After drying at room temperature, the LAC was cross-linked by immersing the electrodes in a 2.5% GA solution overnight at 4 °C. These enzyme-modified electrodes were stored in 0.2 M phosphate buffer (pH= 7) when not in use (labeled as LAC-FCNTs-SPCE). For comparison, the electrodes modified with only CNTs/FCNTs were also prepared by dropping 10 μL of sonicated CNTs/FCNTs suspension solution (0.25 mg mL^{-1}) on the working electrode, then dried at room

177 temperature. They were labeled as CNTs-SPCE and FCNTs-SPCE. LAC-FCCNTs
178 was obtained by the same method as LAC-FCNTs, except that FCCNTs instead of
179 FCNTs was used.

180 *2.4. Electrochemical measurement*

181 Each of the new enzyme-modified electrodes was activated and stabilized by
182 cyclic voltammetry (CV) by scanning the potentials between + 0.65 to – 0.65 V at a
183 scan rate of 50 mVs^{-1} and step potential of 2 mV for 20 times. The electrochemical
184 detection of *p*-cresol was carried out as follows: 80 μL of *p*-cresol standard
185 solutions (with different concentrations dissolved in 0.2 M phosphate buffer of
186 different pH) was dropped on the electrodes, then the samples were tested by SWV
187 with the potential from 0 to + 0.65 V at a step potential of 20 mV, amplitude
188 potential of 20 mV and frequency of 10 Hz. CV and SWV were performed by a
189 portable multi-channel potentiostat (μstat8000p, Metrohm). The data of
190 electrochemical measurements were recorded and analyzed by DropView 8400
191 software. After each test, the surface of the sensor was rinsed with DI water for 30
192 seconds, and then dried in the air for next usage.

193 *2.5. HPLC analysis*

194 To verify the practical application performance of the developed biosensor, a
195 high-performance liquid chromatography equipped with a diode-array detector
196 (HPLC, Ultimate 3000, Thermo Fisher Scientific) was used to detect the
197 concentration of *p*-cresol in actual samples and compared with the concentration
198 obtained using the biosensor.

Different environmental laboratory wastewater samples that contained a variety of inorganic ions and organic compounds were used as real samples. After filtration using a 0.45 μm filter membrane, the samples (5 μL) were injected into the HPLC system. An Agilent ZORBAX SB-C18 column (2.1× 50 mm, 1.8 μm) was adopted for analytical separation, and the mobile phases were A: methanol and B: 0.1% (v/v) acetic acid in water with a flow rate of 0.4 ml min^{-1}. The gradient elution cycle was 11 min in length with initial setting as 90% B, a 2-min linear gradient to 50% B, a 3-min hold with 50% B, an 1-min linear gradient to 90% B, and finally a 5-min isocratic segment (90% solvent B). The column effluents were monitored at 270 nm with the diode-array detector.

2.6. Near real-time monitoring of Fenton oxidation process

For the treatment of *p*-cresol with Fenton oxidation, the recommended molar ratio of H_2O_2/Fe^{2+} was 35:1, which could convert into a mass ratio of about 22:1 (Kavitha and Palanivelu, 2005). Herein, different amounts of H_2O_2/Fe^{2+} with fixed the mass ratio of 22:1 was added to 50 mL of environmental laboratory wastewater (pH≈ 3.5) with a *p*-cresol content of about 35 ppm. After the addition of H_2O_2/Fe^{2+} reagents, 50 μL of the reaction solution was taken at 5 min intervals and then mixed with 50 μL of 0.2 M phosphate buffer. Finally, the concentration of *p*-cresol in the mixed solution was on-site quantified by the biosensor until the concentration was below the detection range.

3. Results and Discussion

3.1. LAC immobilization on the electrode

The Fourier transform infrared spectroscopy (FTIR, Tensor 27, Bruker) analysis was performed to obtain information about the immobilization of LAC enzyme onto FCNTs (the ratio of LAC and FCNTs was 1/5, m/m). The characteristic peaks to carbon nanotubes could be clearly observed in Fig. 1, namely ν (O-H) at 3695 cm^{-1} (Mansor et al., 2012), ν (O-H)/ν (N-H) at 3427 cm^{-1} (Baykal et al., 2013), ν (C-H) at 2958, 2918 cm^{-1} and 2848 cm^{-1} (Liao and Pan, 2011), ν (C=O) at 1797 and 1639 cm^{-1} (Chen et al., 2012), ν (C=C) at 1575 and 1542 cm^{-1} (Mansor et al., 2012), ν (C-N) at 1427, 1261 and 800 cm^{-1} (Baykal et al., 2013), ν (C-O) at 1159,1093 and 1029 cm^{-1} (Barrios et al., 2012), δ/γ (C-H) at 875 cm^{-1} and ν (C-S) at 715 cm^{-1} (Teng and Tang, 2008; Mansor et al., 2012) (Fig. 1a). After functionalization, a new peak appeared at 1710 cm^{-1} (red dot frame), which indicated the formation of carboxyl groups (-COOH) at the CNTs (Steffen et al., 2019) (Fig. 1b). The FTIR spectrum of LAC immobilized FCNTs was found very similar to that of FCNTs, the characteristic peak of the amide bond (CO-NH, magenta dash frame) at about 1639 cm^{-1} was not detected, however, this might be due to the fact of peak overlap from the raw CNTs, which had a peak at that position while the amount of LAC modification was considered to be relatively small (Tavares et al., 2015) (Fig. 1c).

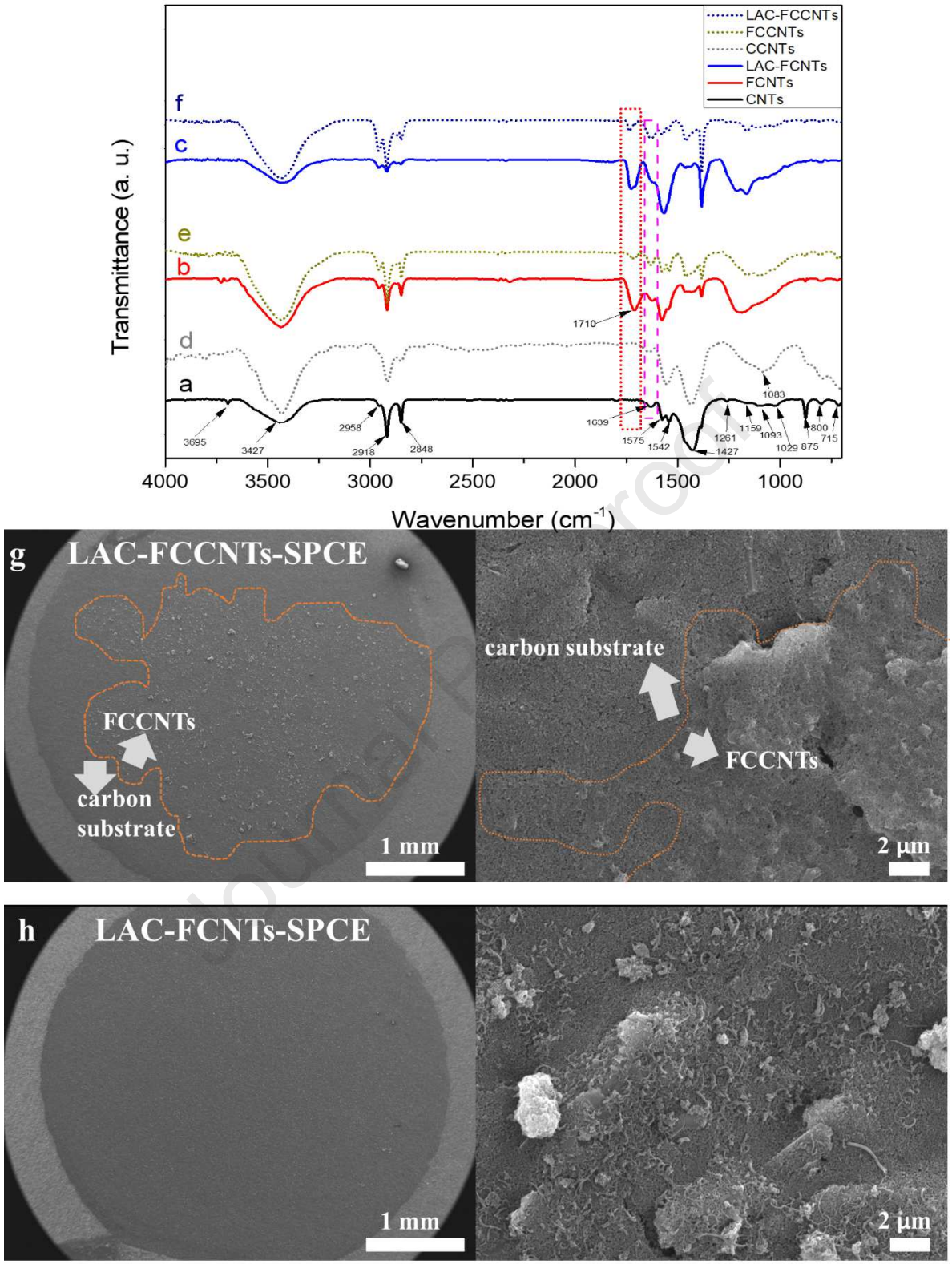


Figure 1. FTIR spectra of electrodes (a: unmodified plastic packaging waste derived CNTs, b: carboxyl functionalized plastic packaging waste-derived CNTs, c: laccase immobilized carboxyl functionalized plastic packaging waste derived CNTs, d: unmodified commercial CNTs, e: carboxyl functionalized commercial CNTs, f: laccase immobilized carboxyl functionalized commercial CNTs) and FESEM

245 images of surfaces for the working electrodes (g: LAC-FCCNTs and h:
246 LAC-FCNTS modified electrodes).

247 The FTIR spectra of CCNTs, FCCNTs and LAC-FCCNTs are shown in Fig. 1d~f.
248 It was found that the prepared FCNTs contained more carboxyl groups than
249 FCCNTs under the same functionalization conditions, which was due to the
250 different initial constituent groups of CCNTs and CNTs synthesized from waste
251 materials. As the carboxyl group could serve as not only a binding site for enzymes
252 but also a hydrophilic group, it enabled FCNTs to bind more enzymes and
253 disperse better in water. As shown in the field emission scanning electron
254 microscope (FESEM, JSM-7200F, Jeol) images (Fig. 1g and h), after modification
255 and drying, the LAC-FCCNTs aggregated on the electrode surface and had a clear
256 boundary with the carbon substrate; while LAC-FCNTs could be more uniformly
257 dispersed, which was consistent with our previous expectations. The aggregation
258 and inhomogeneity of the electrode material could lead to the shielding of active
259 catalytic sites and the reduction of effective area., which further resulted in low and
260 unstable response signals of the sensor. Therefore, only LAC-FCNTs were used in
261 the following experiments.

262 As shown in Fig. 2, the working electrode of commercial SPCE had been
263 covered by carbon particles, while working electrode of the CNTs/FCNTs modified
264 SPCEs were covered by CNTs/FCNTs. There was no obvious difference between
265 the morphology of CNTs and FCNTs, except for the elemental analysis (Fig. S1),
266 the content of oxygen in FCNTs was significantly higher than that of CNTs,

267 indicating the surface had been oxidized (Shanmugharaj et al., 2007). Due to the
268 presence of LAC, the surface of LAC modified SPCE became smoother and
269 contained more potassium, chlorine and oxygen compared with bare SPCE. The
270 consistent results could be observed in Fig. 2 by transmission electron microscopy
271 (TEM, JEM-1400Plus, Jeol), in which the LAC-FCNTs appeared more agglomerated
272 and denser. Furthermore, more oxygen was found on the surface of the FCNTs and
273 the potassium and chlorine from the LAC were also detected (Fig. S1), which can
274 be the direct supporting evidence that the LAC was actually immobilized onto the
275 FCNTs.

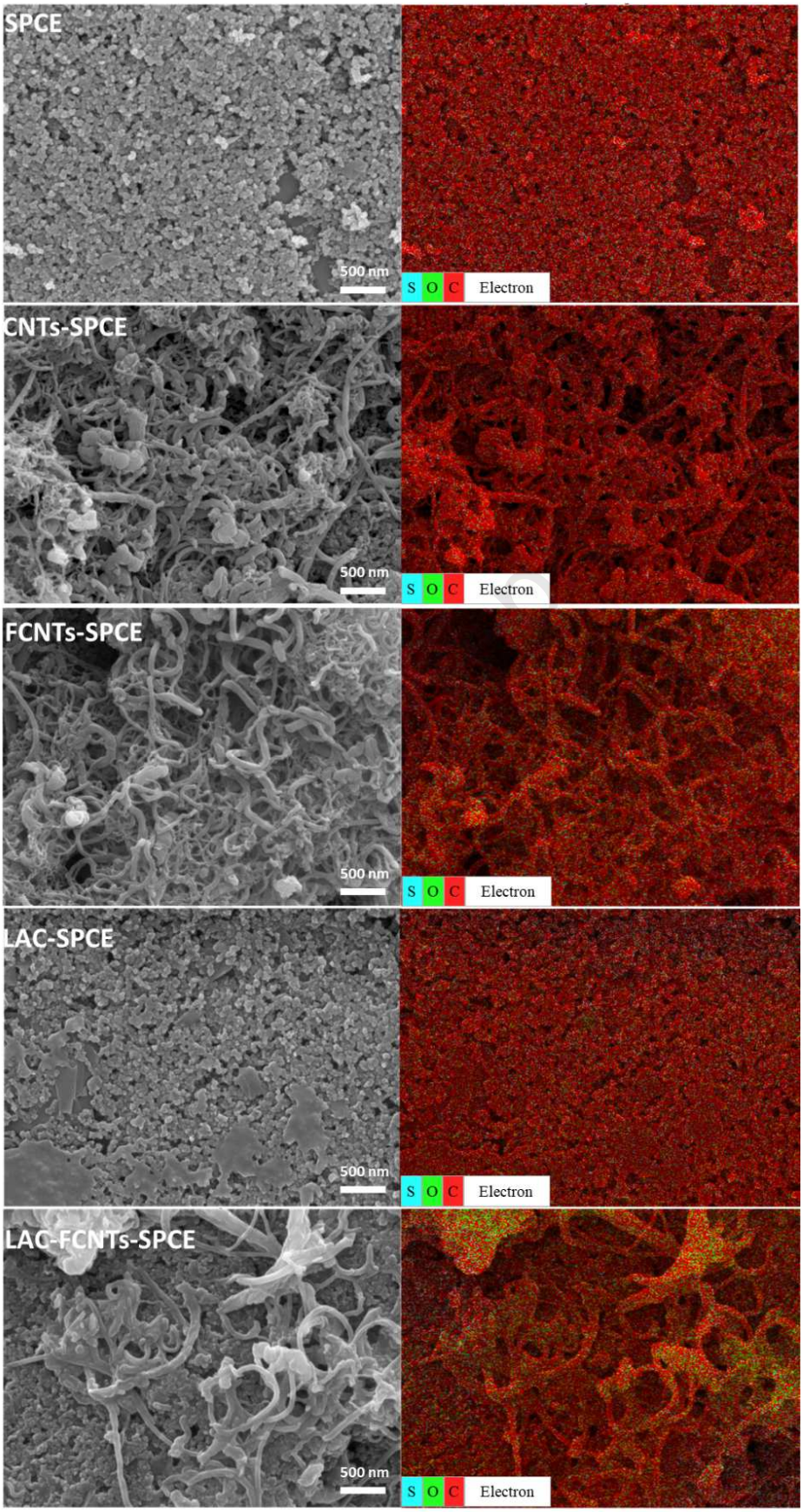


Figure 2. FESEM images and elemental mapping of the bare and modified SPCEs.

3.2. Optimization of voltammetric response for developed biosensors

Different analytical conditions for detecting *p*-cresol, including different electrodes, testing pH levels and the ratios of LAC to CNTs were applied. As shown in Fig. 3a, the detection signal was reduced by modifying the SPCE with only LAC or FCNTs, which probably was due to the fact that LAC has the poor conductivity and FCNTs are insensitive to *p*-cresol without LAC. Another phenomenon observed is that when only carbon-based electrodes (SPCE and FCNTs-SPCE) were used, the position of the oxidation peak was at 0.34 ~ 0.36 V, while the oxidation peak position shifted to 0.42 ~ 0.46 V by using enzyme-modified electrodes (LAC-SPCE and LAC-FCNTs-SPCE). This indicated that the enzyme was involved in the electrocatalytic oxidation process, and the possible mechanism of this process is shown in Fig. S3.

The effect of pH was also studied. According to Fig. 3b, it was found that the suitable pH range for LAC electrocatalytic oxidation of *p*-cresol was approximately at 7~8, while the suitable pH range for the LAC to oxidize phenols spontaneously with dissolved oxygen is 5~7 (Rodríguez-Delgado et al., 2015). Thus, with the increase of the pH, the position of oxidation peak shifted from 0.5 V to 0.4 V, and the activity of the LAC first increased and then decreased. Therefore, it was believed that a higher pH (7) could prevent spontaneous oxidation of *p*-cresol by LAC (Shleev et al., 2005). The balance of the electrode's sensitivity to *p*-cresol and its conductivity requirements were to be controlled by adjusting the ratio of LAC

and FCNTs. From Fig. 3. (c), the optimal ratio of the LAC and the FCNTs was determined to be 1/5.

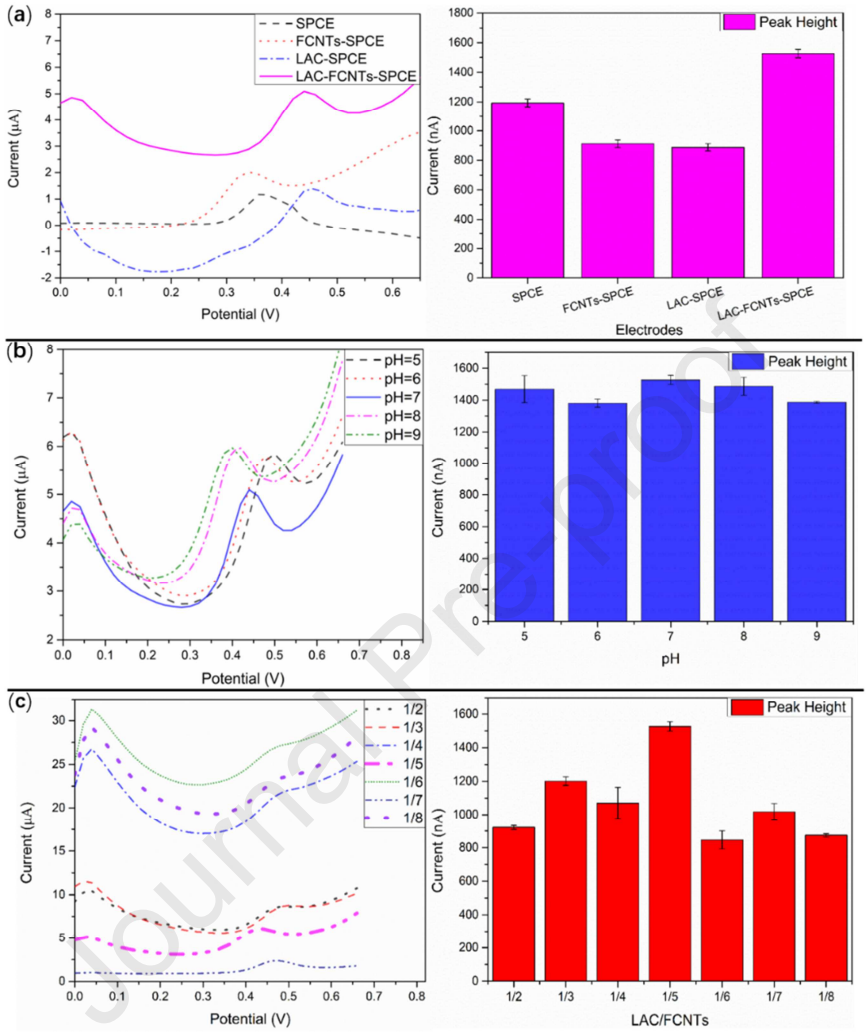


Figure 3. SWV measurements with different protocols and their corresponding peak intensities (a: Response of 2 ppm *p*-cresol (in 0.2 M phosphate buffer, pH=7.0) with bare/modified electrodes (m_{LAC}/m_{FCNTs}=1/5); b: Response of 2 ppm *p*-cresol (in 0.2 M phosphate buffer, pH=5.0~9.0) with modified electrodes (m_{LAC}/m_{FCNTs}=1/5); c: Response of 2 ppm *p*-cresol (in 0.2 M phosphate buffer, pH=7.0) with modified electrodes (m_{LAC}/m_{FCNTs} =1/2~1/8).

3.3. Analytical response evaluation of LAC-FCNTs-SPCE

After determining the optimal test conditions, the voltammetric response of the biosensor to various concentrations of *p*-cresol solutions were tested. Fig. 4 shows the SWV measurements and analytical calibration curve of *p*-cresol obtained by the

LAC-FCNTs-SPCE under the optimized experimental conditions. The linear range is 0.2 ppm to 25 ppm, which could meet the requirements of EPA and NEA standards. The LOD estimated by three times of signal-to-noise ratio was 0.05 ppm.

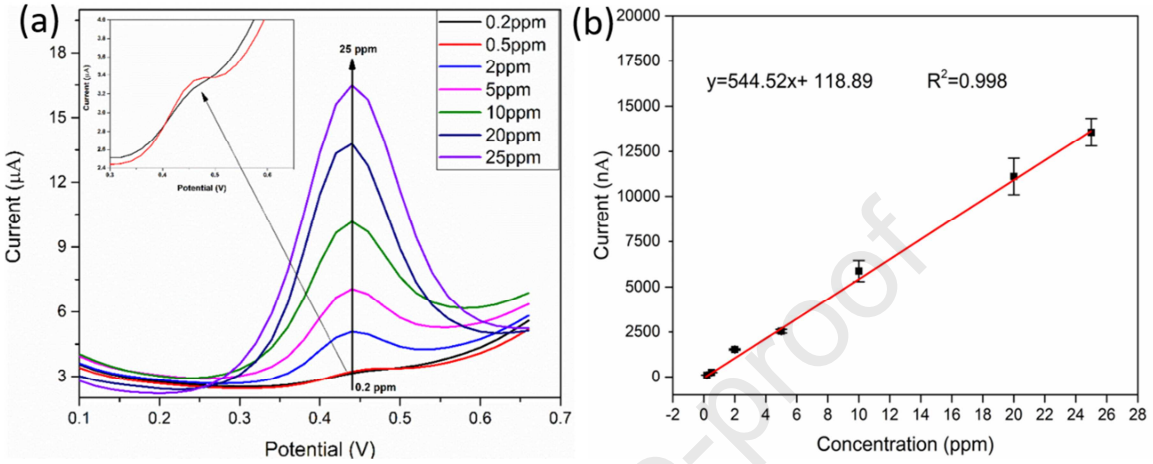


Figure 4. a: SWV measurements of different concentrations of *p*-cresol (0.2~ 25 ppm) in 0.2 M phosphate buffer, pH= 7.0 with LAC-FCNTs-SPCE and b: the resulting analytical calibration curve of *p*-cresol.

Since stability and reusability are extremely important for establishing reliable and practical assays, independent experiments were tested and response signals were recorded. The signal intensities of the LAC-FCNTs-SPCE could remain at 97.30%, 96.13%, 96.06%, 93.09%, 91.43% and 86.04%, after storage at the room temperature for 1, 2, 3, 4, 5 and 6 h, respectively (Fig. S4).

Furthermore, when combined with a portable electrochemical device, it could facilitate rapid detection of *p*-cresol *in-situ*, without the analytical laboratory. In addition, the activity of the LAC-FCNTs-SPCE after long-term storage at 4 °C was also tested. The signal intensities could be maintained at 96.40%, 95.28%, 94.17%, 90.11% and 86.05% at the 5^{th}, 10^{th}, 20^{th}, 30^{th} and 40^{th} day, respectively (Fig. S5).

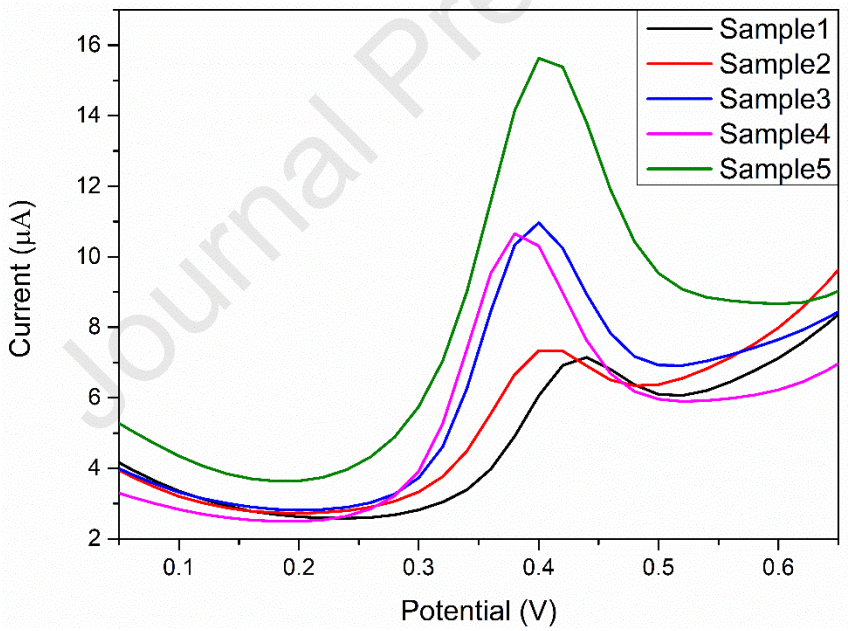
Besides, the LAC-FCNTs-SPCE could be reused 20 times without a significant change in signal (RSD of 1.21%).

There are a number of reported works on the LAC modified biosensors for detecting phenolic compounds (Rodríguez-Delgado et al., 2015). However, most of them required to test the reduction signal of the oxidation product after the pre-reaction of phenol compounds solution with LAC (Roy et al., 2005; Santhiago and Vieira, 2007; Fernandes et al., 2008; Chen et al., 2015). Compared with reported biosensors, LAC-FCNTs-SPCE could directly test of *p*-cresol, which led to shorter measurement time and less complex experimental control.

3.4. Application of LAC-FCNTs-SPCE for determination of p-cresol in real samples

Phenolic compounds and heavy metal ions could coexist in many industrial wastewaters, as well as laboratory wastewater (Huang, 2009). In this work, 5 different laboratory wastewater samples that contained heavy metals, such as Cd, Pb, Mg, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Se, Mo, Ag, Hg, Sb and Ba (Tab. S1), different concentrations of *p*-cresol and some other unknown endogenous organics (Fig. S6) were used as the actual samples to verify the accuracy and interference-free performance of LAC-FCNTs-SPCE in the complex matrices. Before the tests, all the real samples were diluted with an equal volume of 0.2 M phosphate buffer (pH=7). Fig. 5 shows that the interferences may cause baseline changes and affect the peak position of *p*-cresol. Nevertheless, there are no apparent peaks that interfere with the signal, and the peak shape was still sharp,

352 which was conducive to the calculation of peak height. The quantitative results
353 were further validated by HPLC technique. The results for quantifying *p*-cresol in
354 laboratory wastewater with external calibration curves by LAC-FCNTs-SPCE (Fig.
355 4b) and HPLC (Fig. S7) were listed in Tab. 1, which were found comparable to each
356 other. The *t*-test was performed to show there were no significant differences ($p >$
357 0.05) between two groups of data. These results indicated that LAC-FCNTs-SPCE
358 is reliable for rapid detection of *p*-cresol in real samples. Furthermore,
359 LAC-FCNTs-SPCE has the advantages of being faster, more convenient and lower
360 cost for on-site analysis, compared with conventional HPLC methods.



361
362 Figure 5. SWV measurements of real samples by LAC-FCNTs-SPCE.
363
364 Table 1. Comparison of *p*-cresol content in real samples measured by
365 LAC-FCNTs-SPCE and HPLC.

Sample	Biosensor	HPLC	*p* value*
1	8.51 ± 0.07 ppm	8.47 ± 0.19 ppm	0.77
2	9.14 ± 0.29 ppm	9.16 ± 0.08 ppm	0.23
3	21.74 ± 0.32 ppm	21.47 ± 0.06 ppm	0.76
4	23.02 ± 0.71 ppm	22.76 ± 0.07 ppm	0.94
5	34.98 ± 0.87 ppm	35.14 ± 0.12 ppm	0.56

366 * The *p* value was calculated by 3 pairs of tests for each sample.
367

3.5. *Application of* LAC-FCNTs-SPCE *for monitoring p-cresol in Fenton treatment process*

In the process of Fenton oxidation treatment of wastewater containing *p*-cresol, this biosensor was used to monitor the concentration of *p*-cresol in near real-time. The results of Fenton oxidation with 4 different concentrations of H_2O_2/Fe^{2+} are shown in Fig. 6, which indicated that the LAC-FCNTs-SPCE could provide a precise control of the effect of reagents along reaction time. Compared with HPLC or other lab scale testing methods, this biosensor offered the advantages of simple operation and fast response speed, which would be beneficial for the study of reaction kinetics.

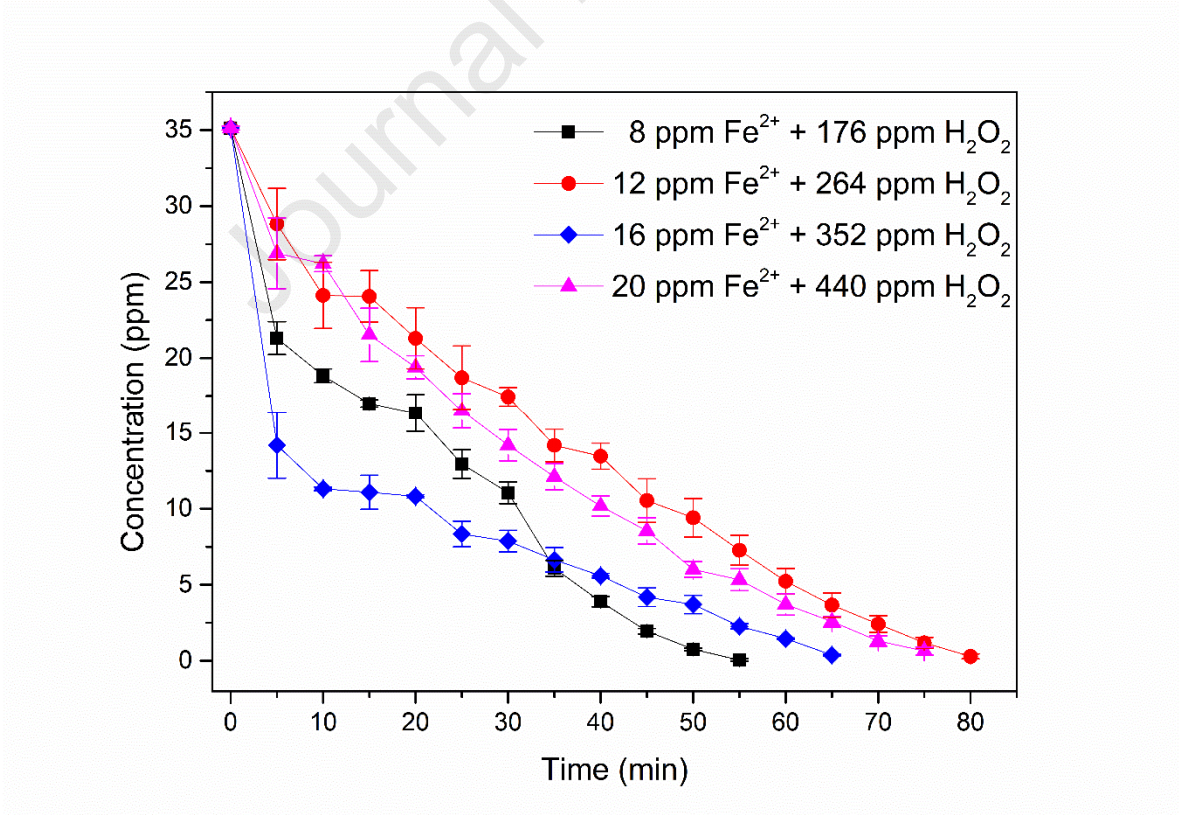


Figure 6. Near real-time monitoring of *p*-cresol concentration by LAC-FCNTs-SPCE during the Fenton oxidation process with different concentrations of H_2O_2/Fe^{2+}.

4. Conclusions

In this paper, the development of a new electrochemical biosensor for the direct detection of *p*-cresol was reported. The LAC was successfully immobilized onto carboxyl-functionalized plastic packaging waste derived CNTs, which was later modified on the surface of SPCEs. The fabrication processes and performance of the biosensors were systematically characterized and optimized by FTIR, FESEM, TEM and electrochemical methods. The experimental results showed that the fabricated biosensor has high sensitivity, wide linear range, low LOD, short response time, and good reproducibility. Furthermore, the short-term stability in room temperature, long-term stability in low temperature and reusability of LAC-FCNTs-SPCE was found to be sufficient for its practical application and commercial development.

Most importantly, LAC-FCNTs-SPCE could quickly and accurately detect the concentration of the *p*-cresol in laboratory wastewater under the interference of metal ions and other organic substances, and the quantitative results of *p*-cresol determined by LAC-FCNTs-SPCE were consistent with the results obtained by the HPLC. Finally, the biosensor was successfully applied for near real-time monitoring of *p*-cresol in wastewater during Fenton oxidation treatment processes, which could provide a precise control of the reagent dosages along reaction time. It was anticipated that the LAC-FCNTs-SPCE in this work would find great potentials for *in situ* detection of *p*-cresol in the environmental and wastewater samples, and near

real-time monitoring of *p*-cresol degradation to optimize the advanced oxidation treatment processes.

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Highlights

- Laccase was successfully immobilized on the plastic packaging waste derived CNTs.
- A biosensor was fabricated with SPCE modified by waste derived CNTs and laccase.
- The biosensor could be used for the direct detection of *p*-cresol in a single step.
- The quantitative results of the biosensor were in good agreement with HPLC.
- The biosensor was successfully applied to monitor *p*-cresol during Fenton processes.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: