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Degradation of high concentration 2,4-dichlorophenol by simultaneous photocatalytic–enzymatic process using TiO_2/UV and laccase

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ABSTRACT

Removal of 2,4-dichlorophenol (2,4-DCP) by TiO_2/UV photocatalytic, laccase, and simultaneous photocatalytic–enzymatic treatments were investigated. Coupling of native laccase with TiO_2/UV showed a negative synergetic effect due to the rapid inactivation of laccase. Immobilizing laccase covalently to controlled porous glass (CPG) effectively enhanced the stability of laccase against TiO_2/UV induced inactivation. By coupling CPG–laccase with the TiO_2/UV the degradation efficiency of 2,4-DCP was significantly increased as compared with the results obtained when immobilized laccase or TiO_2/UV were separately used. Moreover, the enhancement was more remarkable for the degradation of 2,4-DCP with high concentration, such that for the degradation of 5 mM 2,4-DCP, 90% removal percentage was achieved within 2 h with the coupled degradation process. While for the TiO_2/UV and CPG–laccase process, the removal percentage of 2,4-DCP at 2 h were only 26.5% and 78.1%, respectively. The degradation kinetics were analyzed using a intermediate model by taking into account of the intermediates formed during the degradation of 2,4-DCP. The high efficiency of the coupled degradation process therefore provided a novel strategy for degradation of concentrated 2,4-DCP. Furthermore, a thermometric biosensor using the immobilized laccase as biorecognition element was constructed for monitoring the degradation of 2,4-DCP, the result indicated that the biosensor was precise and sensitive.

1. Introduction

Chlorophenols are extensively used as fungicide, herbicide, wood preservative and pesticide intermediate. Because of their carcinogenicity, toxicity and persistence, chlorophenols are listed among top priority control pollutants by the US EPA [1] and the EU [2]. Photochemical method with TiO_2 as photocatalysts and biochemical method with microorganism or enzymes have been widely applied for chlorophenols removal. However, the application of photochemical method is limited by low photonic efficiency that makes it difficult to treat highly loaded chemical industry wastewaters or wastewater less transparent [3–5], while the biological degradation methods are related to long treatment period and strong inhibitory effect from higher concentration of chlorophenols such that 20–30 mg/L chlorophenol was reported to lead to a severe inhibition to activated sludge bacteria [6].

In recent years, a variety of hybrid methods by coupling photochemical and biochemical treatment have been highly focused on and proven to be more efficient for degradation of chlorophenols [7–9] and other biorecalcitrant pollutants [10–12]. In most cases, hybrid methods were operated in a sequential manner, that is to say, the photocatalytic pretreatment was followed with biological process. The chemical oxidation breaks down toxic and poorly biodegradable pollutants into less toxic and more biodegradable molecules that can then be easily biodegraded [4,8,9,11,13–15]. Above sequential process will, however, largely increase the cost on reactor construction and operation, furthermore, the whole treatment process will take rather long time due to the slow biological transformation.

Enzymatic methods may provide a good alternative to current biological post-treatment step in the sequential photochemical–biological process for chlorophenols degradation. Generally, the efficiency of the isolated enzymes is higher, and its storage and handling are easier than microorganism [16–19]. Furthermore, tolerance of enzyme for concentrated chlorophenols [20] makes it possible to couple the photochemical and enzymatic process in a simultaneous way, so that the efficiency may be further improved. To our best knowledge, however, simultaneous photochemical–enzymatic process has not been reported. The rapid inactivation of enzyme by UV exposure in the presence of photocatalyst [21] will be the biggest difficulty to overcome. Here in the present study, we attempted to degrade 2,4-dichlorophenol (2,4-DCP) by a simultaneous photochemical–enzymatic process with TiO_2 nanoparticles as photocatalyst and laccase as biocatalyst.

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We showed that after immobilization, laccase exhibited improved stability against inactivation induced by TiO_2–UV, therefore such simultaneous strategy became feasible and a higher 2,4-DCP degradation rate was achieved compared to the process with photochemical or enzymatic methods was applied separately. Furthermore, the immobilized laccase was also used as biorecognition element to construct an enzyme thermistor biosensor for monitoring the degradation process of 2,4-DCP.

2. Materials and methods

2.1. Chemicals

TiO_2 (Degussa P25) was obtained from Degussa (Essen, Germany). Laccase (from *Trametes versicolor*, EC 1.10.3.2) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Fluka. Trisoperl controlled-pore glass beads (CPG; particle diameter, 125–140 μm; pore diameter, 54.3 nm; specific area, 73.28 m^2/g) with free amino groups were purchased from Schuller GmbH (Steinach, Germany). 2,4-DCP was obtained from Beijing Chemical Reagents Company (Beijing, China). All other chemicals were of analytically grade.

2.2. Immobilization of laccase on CPG

Coupling of laccase to alkylamino CPG was conducted following the procedure described by Dey et al. [22]. In a typical immobilization experiment, 0.7 g CPG particles was firstly activated by 17.5 mL 2.5% (v/v) glutaraldehyde for 30 min under vacuum and 30 min under atmospheric pressure, then washed with distilled water and drained on frit before mixed with 7 mL 5 mg/mL laccase solution prepared with phosphate buffer (0.1 M, pH 7.0). The immobilization of laccase to CPG was carried out for 24 h at 4 °C with gentle shaking. The immobilized laccase was then washed with phosphate buffer solution (0.1 M, pH 8.0) till no protein could be detected in the supernatant. The unreacted functional groups on the activated CPG blocked by mixing the immobilized enzyme with 5 mL of 0.05 M ethanolamine in phosphate buffer (pH adjusted to 8.0) for 2 h, followed by thoroughly washing with phosphate buffer solution (0.1 M, pH 8.0). Protein concentration in the supernatant was measured by Bradford method [23]. Based on mass balance, the laccase coupled to CPG was calculated to be 48.8 mg-laccase/g-CPG. The immobilized laccase was stored in 0.1 M pH 7.0 phosphate buffer prior to use. The laccase coupled to CPG was referred as CPG–Lac in the following text.

2.3. Photocatalytic and enzymatic degradation of 2,4-DCP

Definite amount of TiO_2 was added to 0.1 M, pH 4.5 sodium acetate buffer, and then sonicated for 5 min to get uniformly dispersed TiO_2 stock solution of 50 mg/mL. 2,4-DCP stock solution with the concentration of 10 mM was also prepared with the same buffer. 2,4-DCP degradation experiments were conducted under three conditions as follows: (1) TiO_2/UV photochemical degradation, the 2,4-DCP solution was irradiated under UV in the presence of different amount of TiO_2 as the catalyst; (2) enzymatic degradation, the 2,4-DCP solution was incubated with definite amount of free or immobilized laccase; (3) simultaneous photocatalytic–enzymatic degradation, the 2,4-DCP solution was irradiated under UV in the presence of both TiO_2/UV and laccase. All the above reactions were carried out in 0.1 M, pH 4.5 sodium acetate buffer at 25 ± 2 °C. The light source for photochemical process consists of six tubular mercury vapor UV lamps (UVP-CPQ-7871) which emits its maxima radiation at 365 nm.

2.4. Analytical methods

Activity of free and immobilized laccase was determined using ABTS as substrate. The oxidation of ABTS was measured by monitoring the absorbance increase at 420 nm (ε_{420} = 36,000 M^{-1} cm^{-1}) with USB2000 UV-Vis Spectrophotometer (Ocean Optics, USA). The assay mixture (1 mL) contained 200 μL free enzyme solution or definite amount of immobilized laccase, 640 μL 0.1 M pH 4.5 sodium acetate buffer and 160 μL 0.5 mM ABTS solution. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol ABTS per minute at 25 °C.

The degradation of 2,4-DCP was monitored by Agilent 1200 HPLC system (USA) equipped with a Supelcol C-18 RP-column (4.6 mm × 250 mm). The mobile phase consisting of methanol, pure water and acetic acid (60:38:2, v/v/v) was applied at the flow rate of 0.75 mL/min. The 2,4-DCP concentration was detected using the UV–vis detector at 280 nm.

The degradation of 2,4-DCP was also monitored with thermometric flow injection biosensor [24] with CPG–Lac as biorecognition element. To construct the enzyme thermistor (ET), CPG–Lac prepared as above were transferred into a column (5 mm in diameter and 20 mm in length), and assembled with the thermometric system. The concentration of 2,4-DCP was tested under following conditions: (1) sodium acetate buffer (0.1 M, pH 4.5) was used as running buffer at a constant flow rate of 0.5 mL/min; (2) samples were introduced via a chromatographic valve and each time 0.5 mL was injected into the ET. The concentration of 2,4-DCP was determined from the peak height representing temperature change caused by heat produced during the enzymatic conversion of 2,4-DCP.

3. Results and discussion

3.1. Photochemical degradation of 2,4-DCP by TiO_2–UV

Because the TiO_2 powder called Degussa P-25 shows high activity for many kinds of photocatalytic reactions, it has been used in many studies as a standard material in the field of photocatalytic reactions [13,25]. The P-25 powder contains 75% anatase and 25% rutile phases, average sizes of the anatase and rutile elementary particles are 85 and 25 nm, respectively. The apparent BET surface area of P-25 is about 49.2 m^2/g [26].

The degradation kinetics of 2,4-DCP under UV irradiation in presence of different amount of TiO_2 is shown in Fig. 1. In the

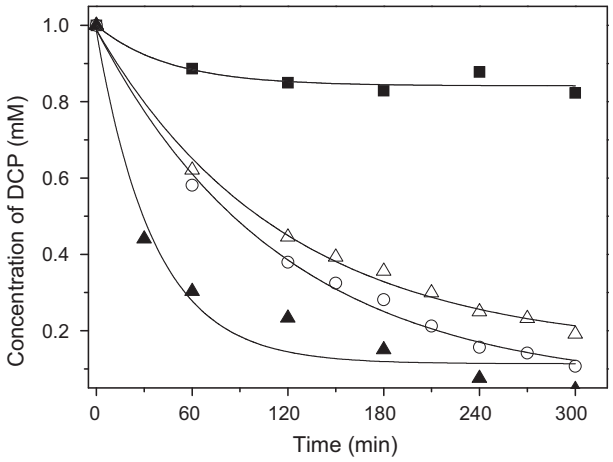

Fig. 1. Degradation kinetic curves of 2,4-DCP by TiO_2 under UV irradiation. TiO_2 concentrations (g/L): (■) 0, (△) 0.2, (○) 0.4, and (▲) 0.6. Solid lines present the results predicted by intermediate kinetic model.

absence of TiO_2, although the 2,4-DCP concentration reduced from 1 mM to 0.85 mM within 60 min by direct UV irradiation, the 2,4-DCP concentration did not decrease further by extending the irradiation time. The introduction of TiO_2 significantly accelerated the degradation of 2,4-DCP and the removal percentage increased with the increase of TiO_2 concentration within the tested range.

First-order kinetic expression has been widely used for the photochemical degradation of 2,4-DCP [7,27–29] due to its simplicity with good agreement for the initial reaction times. However, for photocatalytic degradation of a variety of pollutants, when experimental continues, presence of intermediates that compete with the initial pollutant usually led to the decrease of degradation rate and kinetic data do not fit well the first-order kinetics. A new intermediate kinetic model proposed by Bayarri et al. [3] with the consideration of the influence of intermediate on the reaction rate has been proven to describe the photocatalytic degradation of 2,4-DCP by TiO_2/UV more accurately. Thus, here in this work, the intermediate model was adopted; the kinetic equation is as following:

$$r = \frac{dc_t}{dt} = k_1 c_t - k_2(c_0 - c_t) \quad (1)$$

where r is the reaction rate, c_0 and c_t are the initial concentration of 2,4-DCP and DCP concentration at time t, respectively, k_1 the kinetic constant related to degradation of 2,4-DCP, and k_2 the kinetic constant associated to the intermediates degradation. The boundary conditions: $c_t = c_0$ when $t = 0$ and $c_t = c_t$ when $t = t$.

By integrating Eq. (1), the function describing the relationship between 2,4-DCP concentration, c_t, with time t is presented:

$$c_t = \frac{k_2 c_0 + k_1 c_0 \exp(-(k_1 + k_2)t)}{k_1 + k_2} \quad (2)$$

By fitting the data for degradation of 2,4-DCP in presence of different amount of TiO_2 to Eq. (2), the kinetic constants, k_1 and k_2, were estimated and summarized in Table 1. The model predicted degradation curves of 2,4-DCP were presented in Fig. 1 as solid lines. It can be seen that the model predicted results are in good agreement with the experimental results in all the cases. When 0.6 g/L TiO_2 was applied, a removal percentage of 95% was achieved in 300 min. By considering the cost of TiO_2 nanoparticles and reaction time, 0.6 g/L TiO_2 was adopted in the following photocatalytic experiments.

3.2. Enzymatic degradation of 2,4-DCP with laccase

Laccase (EC 1.10.3.2) is a multi-copper oxidase that can catalyze the oxidation of phenolics compounds by generating phenoxy radicals that react themselves or other phenolics to form dimmers, and eventually convert the substrate to higher oligomers and polymers of low solubility that can be easily removed by sedimentation or filtration [30]. Unlike widely used peroxidases, the oxidation catalyzed by laccase does not require hydrogen peroxide which is harmful to the environment. Therefore laccase is considered to be a more promising enzyme for the removal of phenol-polluted system [15,31]. Here in this work, the degradation of 2,4-DCP by native laccase was studied, and the kinetic data were fitted to the intermediate model. As shown in Fig. 2, the enzymatic oxidation process of 2,4-DCP can also be well described by the intermediate model. The estimated reaction rate constants were compiled in Table 1. The kinetic constant related to the degradation of 2,4-DCP, k_1, are 0.0046, 0.013 and 0.038 min^{-1}, for laccase concentration of 0.05, 0.1, and 0.2 mg/L, respectively. The removal percentage of 2,4-DCP could reach 94% within 120 min with 0.2 mg/L laccase. So in the following enzymatic experiments the amount of laccase was set as 0.2 mg/L.

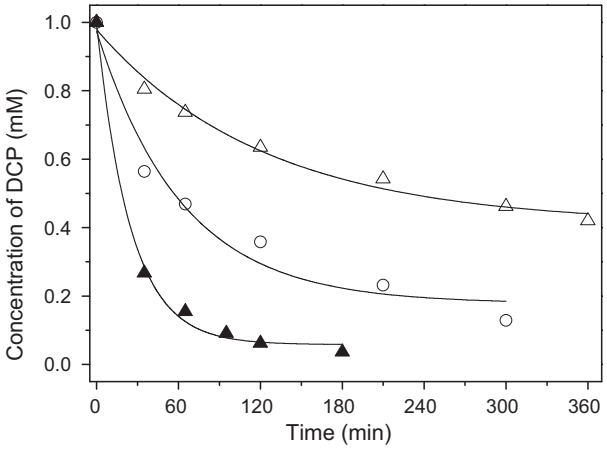

Fig. 2. Degradation kinetic curves of 2,4-DCP by native laccase. Laccase concentrations (g/L): (△) 0.05, (○) 0.1, and (▲) 0.2. Solid lines present the results predicted by intermediate kinetic model.

3.3. Degradation of 2,4-DCP with simultaneous CPG–Lac and TiO_2–UV

Coupling of photocatalytic and enzymatic degradation of 2,4-DCP with TiO_2 and laccase is our focus, while the results shown in Fig. 3 were not as what expected. The kinetic constant, k_1, for degradation of 2,4-DCP in the presence of 0.6 mg/L TiO_2 and 0.2 mg/L native laccase with UV-irradiation is 0.015 min^{-1} (Table 1), lower than what obtained when TiO_2/UV ($k_1 = 0.024\ min^{-1}$) or laccase ($k_1 = 0.038\ min^{-1}$) was separately applied, indicating a negative synergetic effects by combining TiO_2/UV and free laccase. This result was speculated mainly caused by rapid inactivation of laccase upon exposure to TiO_2/UV. Fig. 4 indicates that 2 h UV-irradiation led to about 94% loss in activity of laccase, while in the presence of 0.6 mg/L TiO_2, laccase was completely deactivated within 30 min. The rapid inactivation of laccase was caused by the hydroxyl and superoxide radicals produced on the surface of photoexcited TiO_2 under UV irradiation [32,33]. The absorbing of hydroxyl and superoxide radicals by laccase may in turn lead to decrease in the photo-degradation efficiency of 2,4-DCP.

To achieve high efficiency for the coupled photocatalytic–enzymatic degradation process of 2,4-DCP, the key problem need to be solved is how to enhance laccase stability against TiO_2/UV induced inactivation. Conjugating enzyme with

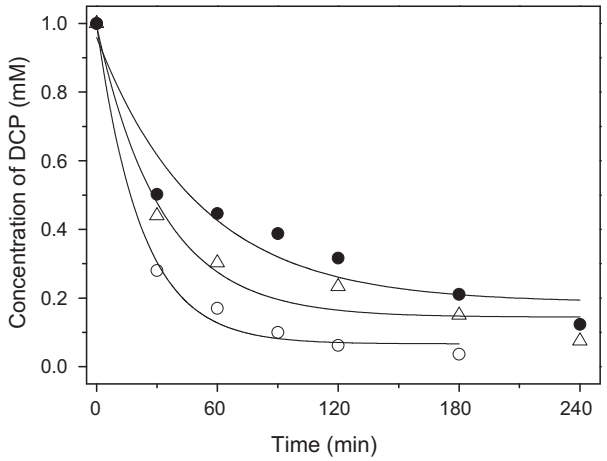

Fig. 3. Degradation kinetic curves of 1 mM 2,4-DCP by (△) 0.6 g/L TiO_2/UV, (○) 0.2 g/L native laccase, and (●) combination of TiO_2/UV and native laccase. Solid lines present the results predicted by intermediate kinetic model.

Table 1
Intermediate kinetic model parameters of 2,4-DCP degradation.

Model parameters	TiO_2/UV (g-TiO_2/L)				Free laccase (g-laccase/L)			TiO_2/UV + free laccase
	0	0.2	0.4	0.6	0.05	0.1	0.2	0.6 g/L TiO_2 + 0.2 g/L laccase
k_1 (min^{-1})	0.0035	0.0072	0.0081	0.024	0.0046	0.013	0.038	0.015
k_2 (min^{-1})	0.019	0.0013	0.00043	0.0031	0.0033	0.0028	0.0023	0.0036
r^2	0.85	0.99	0.99	0.96	0.98	0.95	0.99	0.91

UV-absorbing molecules has been reported to enhance stability of chymotrypsin against photooxidation [32,33]. However, the synthesis of UV-absorber and the conjugating process are complicated, and the enhancement in stability is still rather limited. Immobilizing enzymes to solid carrier by multipoint-covalent binding allows improving enzyme stability effectively [34]. Here in the present work, laccase was immobilized to alkylamino CPG via glutaraldehyde crosslinking, and the stability of the immobilized laccase, referred as CPG–laccase, was examined. Fig. 4 shows that after immobilization, the stability of laccase was significantly improved that about 40% of the original activity could be retained after 6 h UV irradiation, and more importantly, about same stability was attained even in the presence of UV/TiO_2. The operational stability of CPG–laccase was also investigated. When 0.2 mg-enzyme/mL immobilized laccase was applied to 2,4-DCP solution with initial concentration of 5 mM, removal percentage at 2 h could reach 80% (Fig. 5). After 9 repeated usages, the removal percentage of 2,4-DCP was about 60%, indicating that more than 70% of the original activity of CPG–laccase was retained. The significant increased stability of CPG–laccase against UV/TiO_2 and good operational stability makes it feasible to combine the photooxidation and enzymatic process for degradation of 2,4-DCP.

The efficacy of the simultaneous photocatalytic–enzymatic process with UV/TiO_2 and CPG–laccase was examined by measuring the degradation of 2,4-DCP. Fig. 6(a) and (b) presents the degradation curves of 2,4-DCP with initial concentration of 1 mM and 5 mM, respectively. The results show that the coupled degradation process is remarkably faster than the uncoupled process with TiO_2/UV or CPG–Lac separately used, and the enhancement in the degradation rate of 5 mM 2,4-DCP is more significant than that of 1 mM 2,4-DCP. For the degradation of 5 mM 2,4-DCP with TiO_2/UV or CPG–Lac separately used, the removal percentage at 2 h was only 26.5% and 78.1%, respectively. By coupling the photocatalytic and enzymatic degradation process, more than 90% of the initial 2,4-DCP could be removed within 2 h.

Fig. 7 compares the kinetic constants, k_1 and k_2, derived from intermediate model. For the TiO_2/UV treatment, both k_1 and k_2 decreased when the DCP concentration was increased to 5 mM from

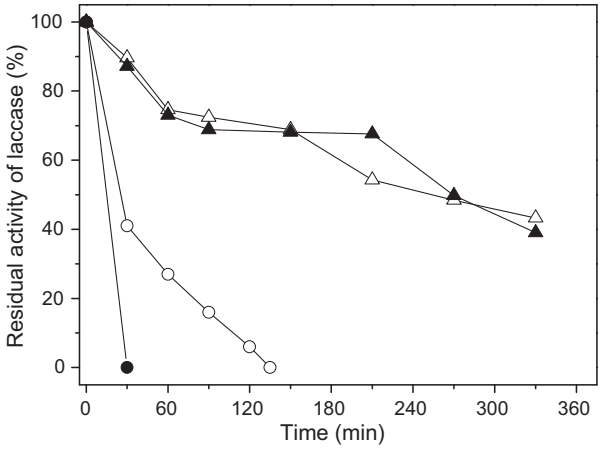

Fig. 4. Inactivation of 0.2 g/L native (circle) and immobilized (triangle) laccase (CPG–Lac) upon exposure to UV (○, △) and 0.6 g/L TiO_2/UV (●, ▲).

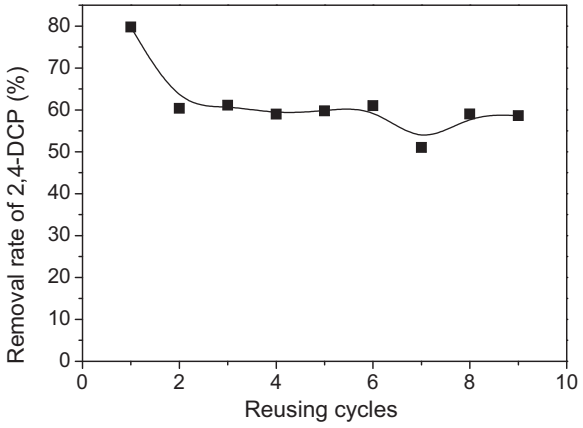

Fig. 5. Multi-use of CPG–Lac for degradation of 5 mM 2,4-DCP. The enzyme dosage is 4.3 g-CPG/L (corresponding to 0.2 g-enzyme/L), each cycle was lasted for 2 h.

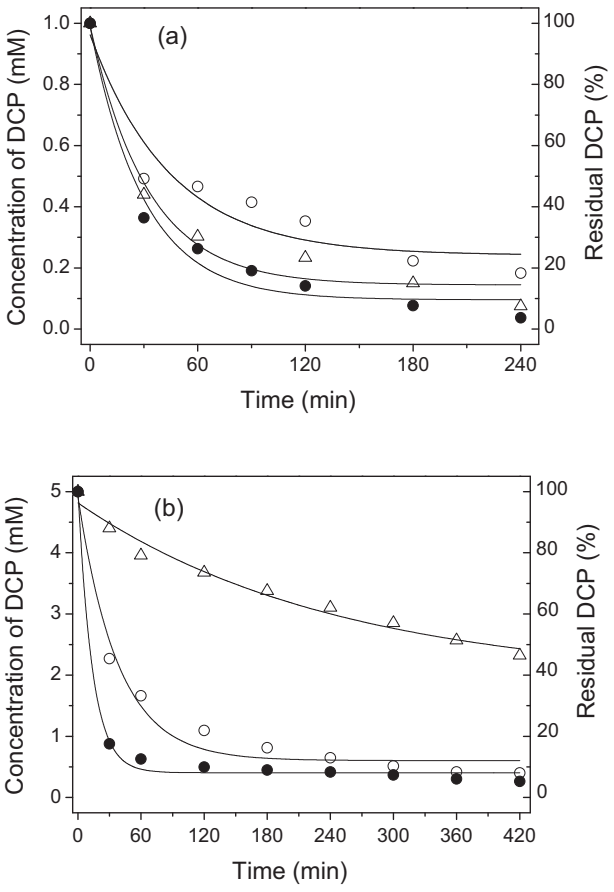

Fig. 6. Degradation kinetic curves of 2,4-DCP by (△) 0.6 g/L TiO_2/UV, (○) 0.2 g/L CPG–Lac, and (●) combination of TiO_2/UV and CPG–Lac. Initial concentration of 2,4-DCP was 1 mM (a) and 5 mM (b), respectively. Solid lines present the results predicted by intermediate kinetic model.

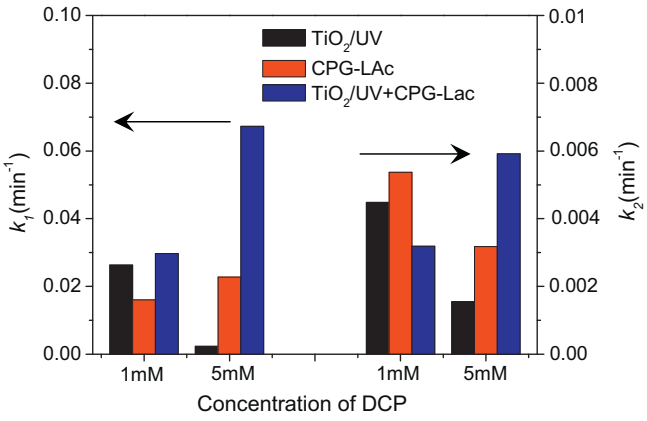

Fig. 7. Comparison of intermediate kinetic model parameters, k_1 and k_2, of degradation of 2,4-DCP by TiO_2/UV, CPG–Lac, and combination of TiO_2/UV and CPG–Lac.

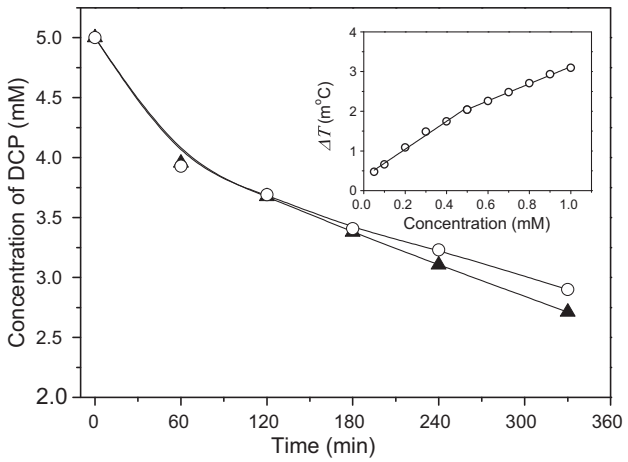

Fig. 8. Degradation curve of 5 mM 2,4-DCP with 0.6 g/L TiO_2/UV monitored with (▲) HPLC and (○) CPG–Lac-based ET. Inset: Calibration curve of 2,4-DCP determined with ET.

1 mM, indicating the photocatalytic degradation process is inefficient for high concentration 2,4-DCP. The combination of TiO_2/UV and CPG–Lac led to significant increase in k_1 and k_2, particularly for the degradation of 5 mM 2,4-DCP. The high efficiency of the coupled degradation process therefore provides a novel strategy for degradation of concentrated 2,4-DCP.

The mechanisms of laccase-catalyzed degradation and TiO_2/UV photocatalytic degradation of 2,4-DCP have been widely studied [5,27,29,35,36]. The main reaction pathway of laccase catalysis includes the generation of phenoxy radicals and the subsequent oxidative coupling reaction to form oligomer products with molecular large enough to precipitate from solution. During this polymerization reaction, chloride ions will be released, and the dechlorination of chlorinated phenols is considered contribute to the overall detoxification effect which results from enzymatic polymerization [35,36]. The polymerization reaction was even visible in our work, as the laccase-catalyzed degradation of 2,4-DCP proceed, the solution turned red with the formation of precipitation.

As regards the photocatalytic degradation of chlorinated phenols, most of the research work suggested that the reductive decomposition by light-generated negative electron is the major reaction pathway since the release of chloride ion was detected, whereas no polymerization product was identified [5,7,37]. In the current study, unlike what was found for the laccase-catalyzed degradation of 2,4-DCP, no formation of reddish precipitation was observed during the TiO_2–UV treatment process.

Based on the above discussions, as for the simultaneous degradation of 2,4-DCP investigated in the current study, the reaction pathway might be similar to the mechanisms firstly proposed by Yin et al. [4,5] for a sequential photocatalysis and laccase-catalysis degradation of pentachlorophenol. Briefly, attributed to electron reduction, 2,4-DCP was photocatalytically dechclorinated stepwise into cyclohexanol and cyclohexanone, which might be further decomposed into low alcohols, acid and ketones. At the same time, the 2,4-DCP underwent laccase-catalyzed dechclorination and polymerization. The intermediate radical products formed from photocatalysis might also enter the pathway of laccase-catalysis to form insoluble polymer. Therefore, the overall reaction will be accelerated. It is noteworthy that, the degradation kinetic data of TiO_2/UV and laccase process could be generally described by the most widely adopted first-order kinetic model (data are not shown). While for the simultaneous degradation, the data could not be fitted to the first order kinetic model at all, indicating that the competition of the intermediates against the initial pollutant, 2,4-DCP, plays an important role. Particularly for the degradation of high concentration 2,4-DCP, the coupling of photocatalysis and laccase-catalysis afforded a significant positive synergetic effect on both the degradation of 2,4-DCP and the intermediate product, as confirmed by the increasing kinetic constants, k_1 and k_2.

3.4. Monitoring of 2,4-DCPdegradation with CPG–Lac-based enzyme thermistor

Laccase has been widely used as biorecognition elements of electrochemical biosensor for determination of phenolic compounds [38–41]. Compared with electrochemical biosensor, thermistor-based biosensor, also known as enzyme thermistor (ET), has many distinguished advantages such as high operational stability, no electrochemical or optical interference, high specificity, short response times, and easy automated working for continuous flow injection analysis [24]. Above advantages make the ET particularly suitable for on-line monitoring of pollutant. However, laccase-based thermometric biosensor for DCP and other phenolic compounds has not been reported. In the present work, CPG–Lac was constructed into an enzyme thermistor for flow-injection analysis of 2,4-DCP concentration during the degradation. Fig. 8 (inset) shows the calibration plots for 2,4-DCP obtained with ET. A good linear relationship between signal (converted to temperature change) and 2,4-DCP concentration up to 1 mM was observed. The slope of calibration curve is 3.514 (r^2 = 0.991) for concentration ranging from 0 to 0.5 mM, and 2.151 (r^2 = 0.998) for concentration ranging from 0.5 to 1 mM. The limit of detection is as low as 0.05 mM. The degradation of 5 mM 2,4-DCP with TiO_2–UV was monitored separately by HPLC or CPG–Lac-based ET during the photochemical degradation. The result shown in Fig. 8 indicates that ET is a highly precise and sensitive method. This results point to a potential use of the immobilized laccase as a 2,4-DCP biosensor for quick and sensitive on-line analysis of DCP with wide measuring range.

4. Conclusion

In the present work, a simultaneous photocatalytic–enzymatic process for removal 2,4-DCP was investigated, and an enzyme thermistor based on immobilized laccase was constructed for monitoring the degradation process of 2,4-DCP. The stability of laccase against the inactivation caused by TiO_2–UV was found decisive for the efficiency of the coupled process. With the immobilized laccase, the coupled method was proved more efficient especially for the degradation of 2,4-DCP with high concentration. With the coupled degradation process, 90% removal percentage was achieved within 2 h for the degradation of 5 mM 2,4-DCP. While for the degradation

process with TiO_2–UV or CPG–Lac separately used, the removal percentage of 2,4-DCP at 2 h were only 26.5% and 78.1%, respectively. The high efficiency of the coupled degradation process therefore provide a promising novel strategy for degradation of concentrated 2,4-DCP. With the immobilized laccase as biorecognition element of thermometric biosensor, the degradation of 2,4-DCP could be precisely monitored.

Acknowledgements

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