Ecotoxicological characterization of photoelectrocatalytic process for degradation of pentachlorophenol on titania nanotubes electrode

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Abstract

Luminescence bioassay using *Vibrio fischeri* and *in vitro* ethoxyresorufin-*O*-deethylase (EROD) induction bioassay using H4-IIE rat hepatoma cell were employed to test toxicity changes during the photoelectrocatalysis process for the degradation of pentachlorophenol (PCP) on TiO₂ nanotubes electrode. Acute toxicity of the reaction solution reduced by 83% after 120 min of irradiation. Bioassay-derived 2,3,7,8-TCDD equivalents (TEQ_{bio}) increased from 0.72 to 6.72 ng l⁻¹ during the former reaction stage, decreasing rapidly to 1.25 ng l⁻¹ during the latter reaction stage. HRGC/HRMS analysis detected polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) congeners in the reaction mixtures. TEQ_{bio} and chemical-derived 2,3,7,8-TCDD equivalents (TEQ_{cal}) were about of the same order of magnitude. Both results illustrated the formation of dioxins and their removal by photoelectrocatalysis. Moreover, photoelectrocatalysis showed a significant priority over direct photolysis and photocatalysis depending on the reaction conditions, not only for PCP removal but also for reduction in toxicity.

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1. Introduction

The heterogeneous photocatalytic oxidation process using titanium dioxide (TiO₂) as photocatalyst has been studied as an emerging successful advanced oxidation process (AOP) to remove trace amounts of refractory organic contaminants in wastewater (Crittenden et al., 1997). When a biasing potential was introduced into the photocatalytic process, which is referred to photoelectrocatalysis (Vinodgopal et al., 1993; Kim and Anderson, 1994), the recombination of electron/hole pairs was reduced. Furthermore, TiO₂-based nanotubes began to attract wide attention because of the large surface area and corresponding potentials in many areas. Hence, when the supported TiO₂ nanotubes were used as working electrode

in the photoelectrocatalysis system, the oxidation efficiency will be further improved (Quan et al., 2005).

Pentachlorophenol (PCP) has many uses in agriculture and industry as an important component part of fungicides, bactericides, insecticides, and wood preservatives (Keith and Telliard, 1979). The wide use of PCP, as well as its improper disposal in landfills and in low-temperature combustion, contributes significantly to contamination of the environment (Ho and Bolton, 1998). Moreover, PCP is one of the simple and typical polychlorinated aromatic hydrocarbons, investigation on which is of important referential value for other polychlorinated compounds.

When classifying the efficacy of an advanced oxidation process, removal of the target compound or the total organic carbon (TOC) was used as the performance indicator in most studies. These parameters were not sufficient used on their own, since the formation of transient organic compounds, some of them more toxic than the starting one, should also be monitored (Köhler et al., 2006).

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For example, polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) have been detected in the direct photolysis process of PCP in both aqueous solution and solid matrices (Lamparski et al., 1980; Vollmuth et al., 1994), which are obviously more toxic than PCP. There were also some studies related to the toxicity evaluation for the direct photolysis of PCP (Jardim et al., 1997; Manilal et al., 1992). However, little is known hitherto about the ecotoxicological characteristics of the photoelectrocatalysis process for PCP removal. Moreover, acute toxicity was the only toxicity indicator in most related studies, which may be not sensitive enough to detect the low dose dangerous compounds. Hence, further investigation using more sensitive or specific toxicity indicator was warranted.

The bioluminescence inhibition assay with *Vibrio fischeri* is used worldwide to evaluate the toxicological effect of effluents and wastewaters due to its high sensitivity and perform simplicity (Pintar et al., 2004; Wang et al., 2002). The induction of ethoxyresorufin-*O*-deethylase (EROD) activity in the cell lines containing an inducible cytochrome P4501A (CYP1A) system, such as H4IIE rat hepatoma cell, fish liver cell line, and others, is the aryl hydrocarbon (Ah) receptor (AhR)-specific response that has been used extensively as a biomarker to characterize the existence of AhR agonists in the complex mixture system. These include PCDD/Fs, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) type of pollutants (Hilscherova et al., 2000).

Bioassays and chemical analysis were used in combination in this study to investigate the photoelectrocatalysis process of PCP. The purposes of this study are: (1) serve for the evaluation of the environmental safety of this process; (2) compare the efficiency of photoelectrocatalysis for the removal of PCP and its toxic products with direct photolysis and photocatalysis processes for the assessment of the feasibility of photoelectrocatalysis as an alternative method for the treatment of such recalcitrant compounds.

2. Materials and methods

2.1. Photoelectrocatalytic experiment

PCP is of analytical grade from Tianjin NanKai Chemical Company (China). The photoelectrocatalytic degradation was performed in a single photoelectrochemical compartment, mainly comprised of a quartz reactor (effective volume: 125 ml), an ultraviolet light source and a potentiostat. The potentiostat was connected with a counter electrode (Pt sheet), a working electrode (a supported TiO₂ nanotubes electrode) and a reference electrode (a saturated calomel electrode, SCE). Details for the preparation of TiO₂ nanotubes electrode and the reactor system were described elsewhere (Quan et al., 2005). The experiment was carried out under the following conditions: UV irradiation (a 300 W high-pressure mercury lamp from the factory of Shanghai YaMing Light with a maximum wavelength of 365 nm), vigorous stirring, 0.2 V (vs. SCE) of electric bias, 0.01 M sodium sulfate as electrolyte, and no airflow. In the case of direct photolysis, experiments were performed in the same reactor only with a UV irradiation. Photocatalytic degradation of PCP was also carried out in the same reactor with UV irradiation and the supported TiO₂ nanotubes as photocatalyst.

PCP concentration was analyzed by HPLC (PU-1580, UV-1575, Jasco Corporation, Japan) with a Kromasil ODS (5 μm, 4.6 mm × 250 mm) reverse-phase column. The mobile phase was 1.0 ml min⁻¹ of methanol and water (1% HAc (v/v)) (v/v = 85:15) and the detector wavelength was set as 254 nm.

2.2. Sample pretreatment procedure

The samples for bioassay were concentrated by solid-phase extraction using HLB Oasis cartridges (200 mg, Waters, USA). Before bioassay, the samples were eluted by 5 ml hexane:dichloromethane (v/v = 1:1) and 5 ml dichloromethane:methanol (v/v = 9:1) serially. Then the eluates were evaporated to dryness with gentle stream of nitrogen and dissolved in dimethyl sulphoxide (DMSO) (Sigma). Procedural blank using de-ionized water was run alongside the samples as an assay control. All the solvents were of HPLC grade from Dima Technology Inc.

2.3. Bioluminescence test

The luminescent bacteria *V. fischeri* were purchased as freeze-dried reagents. They were stored at -20 °C and hydrated prior to testing. The 15 min standard bioluminescence inhibition assay was carried out according to ISO 11348-2 (1994) and DIN 38412 L34/L341 (1997). Luminescence inhibition was calculated by a comparison of the light with control measured under the same condition. Bioluminescence was measured on a luminometer (Lumat LB9501, Berhold Co.). NaCl was used for osmotic adjustments. All bioassays were carried out at $T = 16 \pm 1$ °C, with a 15 min contact time with samples. For reaction product mixtures, the concentrations of samples were expressed as percent by volume, so did the median effective concentration (EC₅₀) value. All samples were tested in five concentrations and each concentration was tested in triplicate. We can define a relative toxicity index (RTI) from the toxic unit (TU) as

$$TU = \frac{1}{EC_{50}}, \quad (1)$$

$$RTI = \frac{TU_t}{TU_0}, \quad (2)$$

where TU₀ is the TU of the reaction solution at time $t = 0$, and TU_{*t*} is the TU of the reaction solution at time t .

2.4. In vitro EROD induction bioassay

The cell culture and EROD assay were carried out according to Donato et al. (1993) and Ma et al. (2005). Briefly, the H4-IIE rat hepatoma cell line was maintained in a humidified, 5% CO₂ atmosphere at 37 °C. Dulbecco's modified eagle medium (DMEM, Gibco, Germany) containing 3.7 g l⁻¹ NaHCO₃ was supplemented with 10% fetal bovine serum, 100 units ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin and used as cell culture medium. H4-IIE cells (1.5×10^4 cells ml⁻¹) were first seeded into individual wells of a 96-well tissue culture plate in 0.1 ml of medium, and the medium were refreshed with medium containing test samples after 24 h. Six concentration levels were chosen for test. The final concentration of DMSO was less than 0.5%, which showed no toxic effect on the cells. The same amount of the organic solvent was added to control cultures. 2,3,7,8-TCDD standard solutions were included on each plate as positive controls and for calibration purposes.

After incubation of 72 h, EROD enzyme activity was determined directly in intact rat hepatoma cell cultured on 96-well plates as in Donato's method (Donato et al., 1993). Assay was started by the addition of 100 μl well⁻¹ of culture medium containing 7-ethoxyresorufin (1 mM) as substrate and dicumarol (10 μM) to prevent further metabolism of the resorufin formed by the cytosolic enzyme diaphorase. The reaction was stopped by adding 130 μl methanol to each well after 60 min. A 100 μl aliquot of cell medium was withdrawn from each well and transferred to another 96-well plate. Fluorescence of the product resorufin was recorded

directly on Microplate Reader (TECAN-GENios, Australia) at 535 nm excitation and 590 nm emission wavelengths. The quantification of protein was performed according to the method of Bradford (1976). EROD activity was expressed as picomoles per minute per milligram of microsomal protein. A sigmoid nonlinear curve-fitting module was applied for the dose–response curves of 2,3,7,8-TCDD. The function is a four-parameter logistic equation described by Qiao et al. (2006). TEQ_{bio} was calculated by interpolating the EROD activity induced by the sample in the dose–response curve of 2,3,7,8-TCDD on the same plate.

2.5. Chemical analysis for PCDD/Fs

Samples photoelectrocatalyzed under the given conditions ($I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$) were assayed by HRMS/HRGC for PCDD/Fs congeners. Several representative samples were selected instead of a cost analysis for all the samples. Sample photoelectrocatalyzed for 40 min was chosen representative of samples with high potential risk as detected by bioassay. The starting PCP solution was analyzed because PCP can contain PCDD/Fs impurities (Pfeiffer et al., 1978). Sample photoelectrocatalyzed for 120 min was analyzed to ensure the removal of the PCDD/Fs. The sample extraction and PCDD/Fs analysis were carried out following US EPA Method 1613 protocols (1994). All the samples were spiked with ^{13}C -labeled PCDD/Fs standards before extraction and cleanup. The samples were extracted three times by dichloromethane. Then the extract was cleaned up by acid silica gel, basic alumina (ICN Medical, Germany) and florisil columns (Merck, Germany). An Autospec Ultima high-resolution mass spectrometer (Micromass, UK) interfaced with a Hewlett-Packard (Palo Alto, CA, USA) 6890 plus gas chromatograph was used. Details of the analysis were described by Ni et al. (2005). Quantitative determination was performed by the isotope dilution method based on the relative response factors. This study dealt with tetra- to octachlorinated dioxins and furans. Chemical-derived 2,3,7,8-TCDD equivalents (TEQ_{cal}) were obtained as the sum of TEQ for an individual compound by assuming additive responses of chemicals in the mixture. TEQ of individual PCDD/Fs chemical was the products of the measured concentrations and the corresponding toxic equivalence factors (I-TEFs). I-TEFs used in this study were according to NATO/CCMS (1988).

3. Results

3.1. Removal of PCP by photoelectrocatalysis, direct photolysis, and photocatalysis

Removal of PCP by photoelectrocatalysis, direct photolysis, and photocatalysis are compared in Fig. 1. Under the given conditions (irradiation intensity (I_0) = $600 \mu W cm^{-2}$, initial PCP concentration (C_0) = $20 mg l^{-1}$), the photoelectrocatalysis process achieved a PCP removal of 94% after 120 min of irradiation. PCP removal decreased as the irradiation intensity decreased ($I_0 = 300 \mu W cm^{-2}$) or the initial PCP concentration increased ($C_0 = 40 mg l^{-1}$), with removal of 76.7% and 73.0%, respectively.

Direct photolytic and photocatalytic process achieved rather lower removal than photoelectrocatalytic process under identical irradiation intensity and initial PCP concentration, in which the PCP removal was 65.2% and 79.1% after 120 min of irradiation, respectively.

3.2. Acute toxicity to *V. fischeri*

As Fig. 2 shows, after 120 min of photoelectrocatalysis under the given conditions ($I_0 = 600 \mu W cm^{-2}$,

Fig. 1. Changes in PCP concentration during photoelectrocatalysis (▲: $I_0 = 300 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$; ▼: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 40 mg l^{-1}$; ■: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$), direct photolysis (□: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$), and photocatalysis (△: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$) processes of PCP. Points represent mean value of triplicate samples at each time, bars represent standard errors.

Fig. 2. Changes in acute toxicity during photoelectrocatalysis (▲: $I_0 = 300 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$; ▼: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 40 mg l^{-1}$; ■: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$), direct photolysis (□: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$), and photocatalysis (△: $I_0 = 600 \mu W cm^{-2}$, $C_0 = 20 mg l^{-1}$) processes of PCP.

$C_0 = 20 mg l^{-1}$), relative toxicity index of the reaction solution determined by bioluminescence test reduced by 83%. When the irradiation intensity decreased ($I_0 = 300 \mu W cm^{-2}$) or the initial PCP concentration increased ($C_0 = 40 mg l^{-1}$), the acute toxicity removal decreased, reducing by 68.4% and 59.3%, respectively. The acute toxicity removal by direct photolytic and photocatalytic process were lower than photoelectrocatalytic process under identical irradiation intensity and initial PCP concentration, with relative toxicity index reducing by 55.9% and 77.4%, respectively.

3.3. In vitro EROD induction

During the photoelectrocatalytic process of PCP under the given conditions ($I_0 = 600 \mu\text{W cm}^{-2}$, $C_0 = 20 \text{ mg l}^{-1}$), TEQ_{bio} increased from 0.72 to 5.74 ng l^{-1} in the initial 15 min, and then remained nearly constant at 20–75 min, reaching maximum of 6.72 ng l^{-1} at 45 min, finally reduced to 1.25 ng l^{-1} after 120 min of irradiation (Fig. 3a).

Under identical irradiation intensity and initial PCP concentration, the TEQ_{bio} for the samples treated by direct photolytic and photocatalytic process for 120 min were 2.21 and 2.06 ng l^{-1} , respectively (Fig. 3b), higher than the samples treated by photoelectrocatalysis process (1.25 ng l^{-1}).

For the photoelectrocatalysis process, when the irradiation intensity decreased ($I_0 = 300 \mu\text{W cm}^{-2}$) or the initial PCP concentration increased ($C_0 = 40 \text{ mg l}^{-1}$), the EROD induction activity of the reaction solutions significantly

Fig. 3. Changes in TEQ_{bio} during photoelectrocatalysis (a) and comparisons of TEQ_{bio} in different processes (b). $I_0 = 600 \mu\text{W cm}^{-2}$, $C_0 = 20 \text{ mg l}^{-1}$.

Fig. 4. Changes in TEQ_{bio} during photoelectrocatalysis process under different reaction conditions. (a) $I_0 = 600 \mu\text{W cm}^{-2}$, $C_0 = 20 \text{ mg l}^{-1}$; (b) $I_0 = 300 \mu\text{W cm}^{-2}$, $C_0 = 20 \text{ mg l}^{-1}$; and (c) $I_0 = 600 \mu\text{W cm}^{-2}$, $C_0 = 40 \text{ mg l}^{-1}$.

increased, especially under the latter condition (Fig. 4). TEQ_{bio} increased to 2.51 and 26.95 ng l^{-1} , respectively, after 120 min of irradiation in those two processes.

3.4. PCDD/Fs concentration

The concentrations of 17 PCDD/Fs congeners that elicit 2,3,7,8-TCDD-like toxic effects during the photoelectrocatalysis process of PCP are presented in Table 1. Both PCDDs and PCDFs were detected in the samples. The starting PCP solution contained minor PCDD/Fs due to the impurities in the PCP chemical. A significant increase in PCDD/Fs concentrations (congener totals) was observed at 40 min. While concentrations of most of the PCDD/Fs chemicals decreased at 120 min, OCDD concentration was the highest among the congeners at 40 and 120 min in the reaction solutions. But OCDD contributed only about 1% to the overall TEQ_{cal} due to its low I-TEF. While 1,2,3,4,7,8-HxCDD kept at highest toxicity level, contributing about 30% to the overall TEQ_{cal} at 0, 40, and 120 min.

4. Discussion

4.1. Degradation efficiency of PCP by photoelectrocatalysis, direct photolysis, and photocatalysis

PCP could be effectively removed in the photoelectrocatalysis process and photoelectrocatalysis was more effective for the removal of PCP than direct photolysis and photocatalysis. This result was consistent with the observation of Quan et al. (2005) that there was a significant synergistic effect between the electrochemical process and the photocatalytic process. It is possible that

Table 1
PCDD/Fs concentration and TEQ_{cal} (ng l⁻¹) during the photoelectrocatalysis process of PCP

Chemical	I-TEF	0 min		40 min		120 min	
		Concentration	TEQ _{cal}	Concentration	TEQ _{cal}	Concentration	TEQ _{cal}
PCDD							
2,3,7,8-TCDD	1	0.140	0.140	0.095	0.095	0.029	0.029
1,2,3,7,8-PeCDD	0.5	0.207	0.103	0.348	0.174	9.351E−03	4.676E−03
1,2,3,4,7,8-HxCDD	0.1	4.964	0.496	2.836	0.284	1.117	0.112
1,2,3,6,7,8-HxCDD	0.1	0.168	0.017	1.603	0.160	0.145	0.015
1,2,3,7,8,9-HxCDD	0.1	0.049	4.894E−03	0.187	0.019	0.153	0.015
1,2,3,4,6,7,8-HpCDD	0.01	5.377	0.054	4.861	0.049	2.911	0.029
OCDD	0.001	1.420	1.420E−03	14.917	0.015	5.395	5.395E−03
PCDF							
2,3,7,8-TCDF	0.1	0.013	1.360E−03	0.015	1.500E−03	7.532E−03	7.532E−04
1,2,3,7,8-PeCDF	0.05	0.973	0.049	0.282	0.014	0.749	0.037
2,3,4,7,8-PeCDF	0.5	5.621E−03	2.810E−03	0.071	0.035	0.028	0.014
1,2,3,4,7,8-HxCDF	0.1	0.238	0.024	0.342	0.034	0.201	0.020
1,2,3,6,7,8-HxCDF	0.1	6.791E−03	6.791E−04	0.108	0.011	0.127	0.013
1,2,3,7,8,9-HxCDF	0.1	4.039	0.404	1.735E−03	1.735E−04	1.803E−03	1.803E−04
2,3,4,6,7,8-HxCDF	0.1	3.916E−04	3.916E−05	0.158	0.016	0.340	0.034
1,2,3,4,6,7,8-HpCDF	0.01	0.110	1.100E−03	0.958	9.580E−03	6.019	0.060
1,2,3,4,7,8,9-HpCDF	0.01	2.019E−03	2.019E−05	0.066	6.600E−04	0.063	6.301E−04
OCDF	0.001	0.191	1.914E−04	1.241	1.241E−03	0.061	6.086E−05
TEQ _{cal}			1.299		0.918		0.389

$I_0 = 600 \mu\text{W cm}^{-2}$, $C_0 = 20 \text{ mg l}^{-1}$.

upon driving away the photogenerated electron to the counter electrode by the external circuit, the survivability of photogenerated holes increased and thus the efficiency of the oxidation process improved.

4.2. Removal of acute toxicity by photoelectrocatalysis, direct photolysis, and photocatalysis

Comparison of the results of the bioluminescence test with those of the PCP residue analysis revealed that they exhibited quite similar trends. The acute toxicity decreased as the concentration of PCP decreased. Probably, PCP residue accounted for most of the acute toxicity of the samples. These results are not sufficient for making firm conclusions because a realistic ecotoxicological evaluation for chemicals necessitates more organism species and more toxicity indicators. Whereas luminescent bioassay results have been found showing good correlations with those of various other common bioassays with organisms of different trophic levels, including protozoa, fish, frogs, etc. (Kaiser and Palibrica, 1991). Thus, it seems that the photoelectrocatalysis process for the degradation of PCP did not generate significant levels of acutely toxic intermediates and could be used safely without concern for the final production of acutely toxic intermediates. Moreover, photoelectrocatalysis is safer than the direct photolysis and the photocatalysis process, since the reaction products from this process were less acutely toxic than those from the other two.

4.3. Formation of dioxin-like chemicals in the photoelectrocatalysis process of PCP

PCP was proved unable to induce EROD activity in this bioassay (data not shown); hence the EROD activity induction of the initial solution should have been caused by other EROD-active compounds. The obvious increase of EROD activity of the samples at the former reaction stage suggested the formation of dioxin-like chemicals in the photoelectrocatalysis reaction of PCP. Therefore, it should be noted that the photoelectrocatalysis process of PCP could pose risk to the ecosystem although a high removal of PCP has been achieved and an uncritical use of this method should be avoided. On the other hand, the decrease of EROD activity during the latter stage of the reaction illustrated that the photoelectrocatalysis process was capable of removing the EROD inducers formed and as long as reaction time is sufficient the dioxin-like chemicals could be completely removed.

Reaction solutions in direct photolytic and photocatalytic process were more potent to induce EROD activity under given reaction condition. That is to say, photoelectrocatalysis was more efficient in the control of dioxin-like chemicals formation under specific condition.

The dioxins at low dose level in this study were difficult to be detected in the acute toxicity test and routine instrumental analysis while the HRGC/HRMS analysis for dioxin congeners is always costly and time consuming. Therefore, the target compound residue analysis and the acute toxicity test were necessary but not sufficient for the

characterization of the treatment process. The EROD induction bioassay used in this study was a valuable method for indicating the presence of low dose dioxins since H4-IIIE rat hepatoma cell is sensitive to P4501A (CYP1A) induction, an Ah receptor-mediated response that is associated with many of biochemical and toxic effects of PCDD/Fs. Furthermore, cytochrome P450 (CYP) induction is considered to be one of the most early biochemical cellular responses and represents a sensitive indicator of the potentially chronic harmful effects of chemicals (Denison and Whitlock, 1995). Accordingly, this EROD induction bioassay can be used as a prescreening method for the toxic potential of complex mixture and be applied to the evaluation for the environmental safety of a treatment process.

4.4. Formation of PCDD/Fs in the photoelectrocatalysis process of PCP

PCDD/Fs identified by HRGC–MS in the reaction mixture could at least partly answer for the EROD induction activity of the reaction system. OCDD was in the highest concentration level among the PCDD/Fs congeners. This result was similar to several other studies in which OCDD was reported to be the main dioxin congener formed in the direct photolytic process of PCP (Liu et al., 2002; Vollmuth et al., 1994). So PCP seemed to form OCDD readily in both direct photolytic process and the photoelectrocatalytic process. But OCDD would not be the major problem when controlling the PCDD/Fs formation in this process despite of its high yield due to its low toxicity. Whereas 1,2,3,4,7,8-HxCDD was in the highest toxicity level among the congeners.

OCDD could be produced by the dimerization of the reaction intermediates derived from PCP. Then the dechlorination of OCDD could yield a variety of PCDDs of decreasing chlorine content, which was partly an explanation for the formation of lower-chlorinated PCDD/Fs and the loss of OCDD. Other reaction pathways may also be involved. For example, lower-chlorinated chlorophenol could be formed as the dechlorination products of PCP (Jardim et al., 1997), condensation of which could result to OCDD/F and lower-chlorinated PCDD/Fs.

The fact that PCDD/Fs could be removed efficiently by photoelectrocatalysis was exciting due to the extreme toxicity and persistence of dioxins. Photoelectrocatalysis process maybe an alternative method to remove these compounds in wastewater owing to its superior oxidation efficiency.

It can be seen that the PCDD/Fs could not completely answer for the EROD induction. The main reason may be that results from the bioassay integrated the biological effects of complex mixtures while calculating methods for TEQ assumed that the combined toxic effects of the components of a mixture are additive and it only deals with a limited number of compounds. In addition, it could not be excluded that other dioxin-like products besides PCDD/

Fs formed in the photoelectrocatalysis process of PCP. Thus, further investigation is needed to ensure the environmental safety of this process although the putative products may not be so dangerous as PCDD/Fs. Anyhow, TEQ_{bio} and TEQ_{cal} showed similar trend in the latter reaction stage when both of them significantly decreased which confirmed the effective removal of PCDD/Fs by photoelectrocatalysis.

4.5. Effect of reaction conditions on the removal of PCP and toxicity

Significant differences of PCP and toxicity removal were observed under different reaction conditions. Similar observations that degradation rate of target compound increased with increasing light intensity and decreased with increasing initial concentration within a certain range have been reported in the photocatalytic reaction of dyes and chlorophenols (Carneiro et al., 2004; Nogueira and Guimarães, 2000). However, little attention was paid on the effect of reaction conditions on the toxicity of the intermediates. Results in this study showed that lower oxidation efficiency under low light intensity or high initial PCP concentration resulted in the formation of more toxic intermediates that were able to induce EROD. It should be noted that TEQ_{bio} of the samples kept increasing under lower light intensity or higher initial PCP concentration and the products were even more potent to inducing EROD activity than products from direct photolysis and photocatalysis of PCP. That is to say, photoelectrocatalysis was superior to direct photolysis and photocatalysis depending on reaction conditions. Hence, the cautious and precise control on the reaction conditions is crucial for the application of photoelectrocatalysis. At the same time, it may be possible in the treatment system to exert control over reaction products toxicity by appropriate control over reaction conditions since reaction conditions affected the reaction products from a given parent compound.

5. Conclusion

The bioassay combined with chemical analysis employed in this study was time- and cost-effective and could be applied to the evaluation for the environmental safety of the photoelectrocatalysis process to remove organic contaminants in wastewater. The target compound PCP could be effectively removed in the photoelectrocatalysis process while it seemed that the process did not generate significant levels of acutely toxic intermediates. The *in vitro* EROD induction test disclosed the potential risk caused by the formation of dioxin-like chemicals. Chemical analysis identified OCDD as the main dioxin product of PCP, while many other lower-chlorinated PCDD/Fs were also detected. Photoelectrocatalysis exhibited potential applicability since dioxins could be efficiently removed in the system. Moreover, photoelectrocatalysis was superior to direct photolysis and photocatalysis for both PCP removal

and reduction in toxicity, although the reaction conditions should be under strict control.

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Assurance

Any studies involving humans or experimental animals were conducted in accordance with national and institutional guidelines for the *protection of human subjects and animal welfare*.

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