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Short Communication

Degradation and Toxicity Assay During Electrocatalysis of Chlorobenzene in Aqueous Solution

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The degradation and toxicity assay on a three-dimensional electrocatalytic oxidation (ECO) of organic pollutants were described in this paper. Aqueous chlorobenzene (CB, 50 mg/L) was chosen as model pollutant and *C. vulgaris* was applied to assess the toxicity evolution during electrocatalytic oxidation for this study. The degradation and mineralization rate of CB, and the toxicity of wastewater sample during the electro-oxidation were determined meantime under suitable experimental conditions. The experimental results indicated that CB, oxidized incompletely to CO_2 and H_2O, was removed prior to total organic carbon (TOC), and the bio-toxicity was relevant to the intermediate products of CB degradation. The major intermediate products included phenol, biphenyl, 1,4-benzoquinone and other small molecular organic acids, unequivocally identified by GC/MS and IC. It was essential to maintain enough mineralization for toxicity reduction during electro-oxidation of organics in water.

Keywords: Electrocatalytic oxidation chlorobenzene intermediate products toxicity assay

1. INTRODUCTION

Organic chlorides are widely applied in pharmaceutical industry, petrochemical engineering and pesticide et al as an organic solvent and raw material, which have demonstrated good chemical stability, heat endurance and low biodegradability and thus will stay in the nature longer polluting surface water and ground water [1, 2]. Chlorobenzenes as persistent toxic substances (PTS) are listed as priority pollutants by the US EPA [3] due to their toxicity and persistence. The decomposition of organic chlorides is a hot spot of research on water contamination control [4, 5].

Researches showed that effective control techniques for organic chloride removal included chemical oxidation, biological oxidation. Advanced oxidation processes (AOPs), defined in 1987 by Glaze [6], which are popular to the generation of hydroxyl radicals with high oxidizing power that can react on the resistance compounds in water in an unselective way, are considered as one of the environment friendly wastewater treatment technologies [7]. Electrocatalytic oxidation (ECO), an effective process of AOPs, has been successfully used to the decomposition of many organic contaminants, for its high performance, no selecting for pollutants and being friendly to environment [8, 9].

Organic chlorides can be decomposed in two paths by ECO and other AOPs technologies: one is incomplete oxidation to some other organic or inorganic pollutants, the other is complete oxidation to CO_2, H_2O and other small inorganic pollutants through degradation. Numerous studies have showed that organic chlorides were incompletely oxidized, only parts of which were completely mineralized to CO_2 and H_2O [10-12]. This means that ECO and other AOPs technologies are often used to improve the biodegradability of refractory organic materials. Thus it is necessary and important to investigate the biotoxicity evolution of wastewater during pretreatment with AOPs technologies and make sure the biotoxicity reduction before treated with biological process.

However, the biocompatibility and biotoxicity of these intermediates from organic chlorides degradation incompletely were not fully determined, and the viewpoints that all AOPs technologies are favorable for enhancing the biodegradability of refractory organic materials need to be justified. In fact, most AOPs produced various toxic intermediates at different operation stages, the species and concentration of these intermediates are affected by the processes and operating conditions [13-15]. BOD, COD, TOC are inadequate parameters for determining the efficiency of AOPs methods, and bio-toxic assessment is highly needed for evaluating AOPs [16-18]. Fernández-Alba and co-workers [19] found that TOC and toxicity were irrelevant during P-TiO_2 and P-Fenton to treat methomyl, and different AOPs methods might form the different metabolites. P-TiO_2 reduced the toxicity to below 50% after 125 min at a TOC of 10 mg/L, but P-Fenton needed 350 min at a TOC of 5 mg/L. So using TOC to evaluate AOPs was inadequate.

In a previous study [20], chlorobenzene (CB) was taken as a model compound for its widely used and low biodegradability and persistent toxicity, to evaluate a novel three-dimensional ECO process. The variables, such as current density, pH and electrolyte concentration that influence CB degradation in aqueous solution by ECO, were investigated. What were the changes in toxicity of aqueous solution? Increase or lower? Is it suitable for biological treatment followed-up? These must be answered unequivocally before applied widely. This study aimed at a comparing the organics degradation and the bio-toxicity evolution of aqueous CB during ECO. Algae was very importance in the primary production of the aquatic ecosystem [21-23], which have been considered as indicators of the bioactivity of industrial wastewater according to its response sensitively to toxicity (e.g. pesticides) [24, 25], was performed to assess the toxicity evolution during electro-catalysis of organic chlorides in aqueous solution.

2. MATERIAL AND METHODS

2.1 EC Treatment

The three-dimensional ECO reactor was a glass trough vessel (effective volume of 1.5 L). Ti/β-PbO_2 (150 mm×50 mm, thickness of 1.5mm) was selected as the anode and stainless steel plate (with the same specifications) was selected as the cathode [20]. Mn-Sn-Sb/γ-Al_2O_3 honeycomb catalysts were packed between two electrodes. The support electrolyte was Na_2SO_4 (0~0.15 g/L), and the solution temperature was maintained at 25 ±1℃ by circulating water in a double jacket cooling array.

2.2 CB and its intermediates Analysis

Chlorobenzene (CB) was analysed by GC-2014 with an AT-FFAP column (30 m×0.32 mm×0.33 μm) and fitted to an FID detector. CB solution was in a 0~50mg/L concentration range.

Formation of intermediate products were followed by Purge-and-Trap chromatography-mass spectrometry gas (P&T/GC/MS), an Angilent 6890 N gas chromatograph coupled to an Angilent 5975 mass detector. Chromatographic separations were carried out on a HP-5MS (30 m×250 μm×0.25 μm).

The Cl^- and small organic acid were detected by ion chromatography Dionex ICS-2000.

Total organic carbon (TOC) was analyzed by direct injection of the filtered samples into the TOC-V_{CPH}.

2.3 Hydroxyl radicals Determination

The hydroxyl radicals (•OH) produced from the electrolysis process had very short life-time, low content in solution and were not easy to be determined directly. p-hydroxybenzoic acid (4-HBA) could react with •OH quickly and scavenge the latter, was selected as scavenger to reflect the •OH concentration[26, 27]. The proper dose of 4-HBA was 2.0 g/L according to pre-experiment, 4-HBA and its hydroxylated derivatives of 3,4-dihydroxybenzoic acid (3,4-DHBA) in solution were analysed by the high performance liquid chromatography (HPLC) using an Agilent Technologies 1200Series liquid chromatograph, and the production rate of •OH was calculated indirectly from the following formula.

$$\mathrm{HBA} + \cdot\mathrm{OH} \xrightarrow{k_1} \mathrm{DHBA}$$

generating rate of •OH， $r_{\cdot\mathrm{OH}} \approx \frac{d[\mathrm{DHBA}]}{dt}$

2.4 Toxicity Measurement on Alga

The Alga *C. vulgaris* was obtained from Institute of Hydrobiology, Chinese Academy of Sciences and maintained in Shuisheng-4 medium [28]. Alga cells were cultured at 25±0.5℃ in flasks (100 mL) containing 50 mL of medium and illuminated with fluorescent lights (4000 lux) for daily cycles of 16-h lighting period and 8-h dark period. Each time, the amounts of wastewater samples from the ECO reactor added to the flasks were 0.25, 0.50, 0.75, 1mL and got toxicity measurement samples with four concentration gradients. All the batch experiments were prepared in triplicate. The cell density of culture was monitored spectrophotometrically at 685 nm (OD685). The regression equation between the density of algal cells (Y´105 /mL) and OD685 (X) was established as Y=162.1X+1.3463 (R^2=99.34%). The growth inhibition parameter (EC_{50}) was calculated according to OECD guideline 201[29].

3. RESULTS AND DISCUSSION

3.1 Mechanism of electro-oxidation

Electrocatalysis is defined as the heterogeneous catalytic reaction, which occurs at the electrode-electrolyte interface, and where the electrode plays both the role of electron donor/acceptor and of catalyst. It is essential to understand the relationship between electrochemical reaction mechanisms and catalytic properties of electrodes by studying the structure and composition of the electrode surfaces and the adsorbed layers on the electrode surfaces. According to Comninellis [30], Fockedey [31] and Zhu [32], the decomposition of organic compounds by electrolysis includes two pathways: direct electro-redox, indirect electro-redox. With the electro-redox occurring at anode and cathode, organic compounds are mineralized to inorganic constituents or transformed into other organic compounds through electrode oxidation-reduction reaction. Of course, some side reaction (oxygen and hydrogen evolution) also occurred oftentimes meanwhile, which made a negative effect on electrolysis efficiency.

For electro-redox, oxidants generation is the key step in the mechanism of electrochemical reaction. The properties of electrodes and electrolyte, the input of electric energy produce impacts on the oxidant generation and direct-redox rate [33]. The three-dimensional electrodes, one of conventional structures, proposed by Backhurst and co-workers in 1960s [34], have higher surface-to-volume ratio, better current efficiency and get focused in wastewater treatment region by many scientists and engineers [31, 35]. In this case, Ti/β-PbO_2 electrode and Mn-Sn-Sb/γ Al_2O_3 honeycomb catalyst are selected as anode and particle electrode, to constitute a three-dimensional electrochemical reactor. The surfaces structures of anode and particle electrode analyzed by the scanning electron microscope (SEM), are rough and porous, which are benefit for oxidants (e.g. hydroxyl radicals, H_2O_2, HClO) and organic compounds adsorption [36]. The anode Ti/β-PbO_2 was found to have oxygen overpotential of 1.23V and chlorine overpotential of +1.40V, which shows good catalytic activity for the oxidation of organic compounds [37].

The rate of •OH produced from the electrolysis process was affected by many factors, such as input power, electrolyte, pH and electrolysis time. p-hydroxybenzoic acid (4-HBA) was selected as the scavenger to determine indirectly •OH concentration. As shown in Table1, with the electrolyte concentration of 0.1mol/L, pH10.0 and the electrolysis time of 15 min, the input power enhanced improved the initial production rate of •OH. However, high input power might lower the current efficiency, which was expressed by the generation ratio of hydroxyl radicals proportional to the input power. With the input power of 3.35W, the mixmum initial rate of •OH production was $0.109 \times 10^{-}$

^{4}mmol/(L•s•W), which was higher than the values of high-voltage pulse discharge liquid and ultrasound-assisted electrocatalysis (listed in Table 2). Mn-Sn-Sb/γ Al_2O_3 catalyst has a honeycomb structure (not granular), avoided current or voltage fluctuations caused by particle moving under the function of electrolyte fluid. The honeycomb structure also provides a numerous active surface area of 80~100 cm^2/g, which is benefit for hydroxyl radical and other oxidants generation and higher current efficiency. With the same experimental conditions, the current efficiency of three-dimensional electrochemical reactor was twice that of two-dimensional electrochemical reactor (The two-dimensional reactor was similar with three-dimensional electrochemical reactor except honeycomb structure catalyst).

Table 1. Production rate of ·OH under different input powers

Power$_{in}$, W	3.35	9.60	28.8
$r_{\cdot OH}$,mmol/(L·s)	0.365×10^{-4}	0.406×10^{-4}	0.430×10^{-4}
$k_{\cdot OH}$, mmol/(L·s·W)	0.109×10^{-4}	0.042×10^{-4}	0.015×10^{-4}

Table 2. Comparison of free radical production rate per energy

Experimental methods	free radical production rate per energy mmol/(L•s•W) ×10^{-4}	**Cited references**
ECO in this paper	0.109	
high-voltage pulse discharge	0.039	[38]
ultrasound-assisted electrocatalysis	0.0104	[36]

3.2 Degradation of chlorobenzene

The previous work [20] has studied about the electro-catalysis degradation of CB. The effects of current density, pH and electrolyte concentration on CB degradation were determined. The degradation efficiency of CB was almost 100% with an initial chlorobenzene concentration of 50 mg/L, current density 15 mA/cm^2, initial pH 10.0, electrolyte concentration 0.1 mol/L and temperature 25℃ at 90 min.

Figure 1 showed that the removal rate of TOC was slower than that of CB. After 60 min of degradation, the removal efficiency of CB was 60~70%, but TOC only 20~30%. After 90 min, the removal efficiency of CB was almost 100%, but TOC only 82%. The maximum TOC removal efficiency of 98% was detected under the same conditions at 120min. This means that only parts of CB were completely mineralized to CO_2 and H_2O and some intermediates occur during CB degradation before 90min. It was impossible to mineralize CB completely by electrocatalytic oxidation in a shorter time (e.g. 60 min), some other steps oxidizing organics to CO_2 (e.g. bio-oxidation) might be applied after electro-oxidation, but this was not demonstrated here. Therefore, it was necessary to identify the toxicity of these intermediates produced by electrolysis.

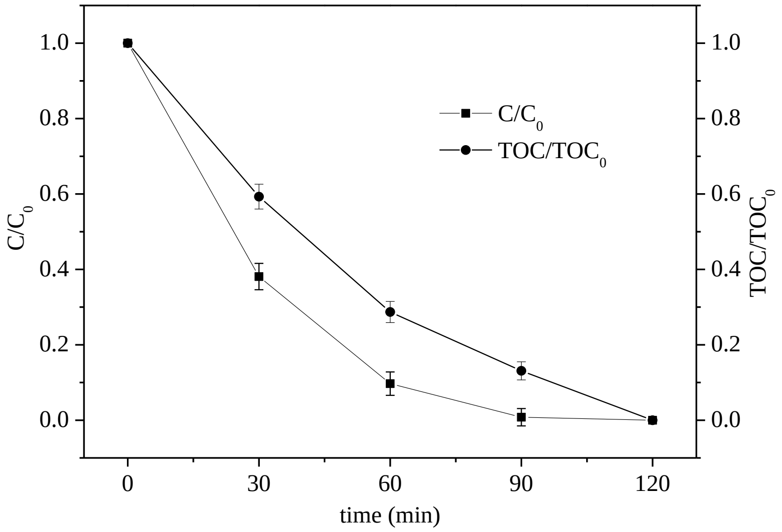

Figure 1. Degradation of chlorobenzene.

3.3 Toxicity evolution during chlorobenzene degradation

To assess the acute toxicity of the samples collected during the degradation process, the *Alga C. vulgaris* growth inhibition test caused by the presence of toxic compounds was investigated. As shown in Figure 2, the algae growth inhibition rate was in linear with the gradient concentration. According to the OECD test guideline [27] and Fig.3, the EC_{50} at 0, 15, 30, 60, 90, 120 min were calculated as 0.60, 1.94, 1.82, 2.65, 3.20, 3.37 mL/100mL.

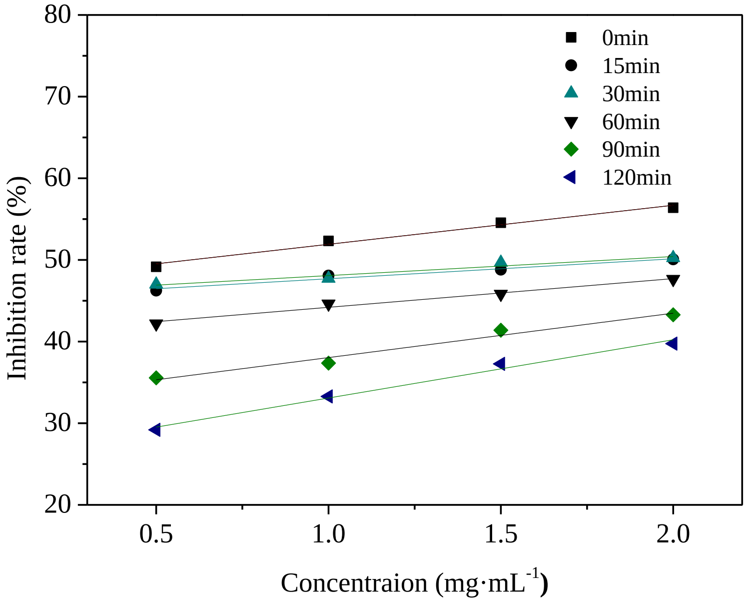

Figure 2. The algae growth inhibition rate

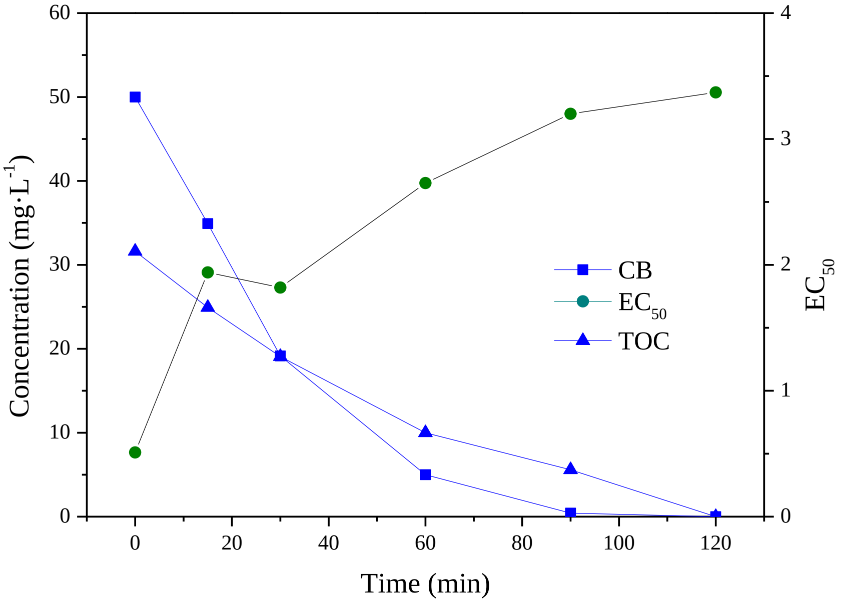

Figure 3. Toxicity evolution during chlorobenzene degradation.

Figure 3 showed the evolution of toxicity in the bioassays applied as a function of electro-oxidation time during CB degradation tests. However, CB in aqueous solution was removed prior to toxicity by ECO. From Figure 3, CB concentration and TOC values decreased all the time, EC_{50} values on growth of C. vulgari increased within initial 15 minutes, decreased slightly from 15~30 min and increased again from 30~120 min. After 120min, EC_{50} value for C.*vulgari* was 3.4 mL/100mL as CB almost disappeared, about 5.6 times of pure CB in solution (0.60 mL/100mL). This incongruence in stage of 15~30min, could be due to the presence of some intermediate compounds produced during electro oxidation that affect toxicity.

At the stage of 15 minute oxidation time from15 min to 30min, EC_{50} value decreased from 1.94 mL/100mL to 1.82 mL/100mL at TOC from 25.26 mg/L to 18.90 mg/L and CB from 34.60 mg/L to 19.98 mg/L, meaning that the toxicity of solution increased slightly. Toxicity increase indicated that some more toxic intermediates occurred at this stage of CB degradation with ECO, meaning wastewater at this stage not suitable for bio-oxidation. After 45 minutes later, the toxicity began to decline and get a stable EC_{50} value of 3.4 mL/100mL with a longer oxidation time of 120 minutes, which showed that the toxicity was closely related with the degradation products generated during electro-oxidation and the toxicity of CB solution might change because of the intermediate products as CB degraded. In the ECO experiments performed, the intermediates formed could be degraded when it underwent enough electro-oxidation time.

3.4 Intermediates Analysis

The major intermediates of CB degradation by ECO were unequivocally identified by GC/MS and IC. Phenol, biphenyl, 1,4-benzoquinone and other small molecular organic acids, reported elsewhere [20] as CB degradation metabolites were listed in table 3, and the concentration evolution of some intermediates were showed in Figure 4. 1,4-benzoquinone, one of major intermediates, reached a maximum concentration value at 10 minutes, disappeared after 45 minutes. Formate, another intermediate, had a maximum concentration of 6.4 mg/L at 30 minutes, reduced slowly for a longer time and still got a value of 1.7 mg/L after 120 minutes.

For the impact mechanism discussion of intermediates on the toxicity evolution, the toxicology data of some intermediates were showed in table 3. LD_{50}, represented the Lethal Dose of a material causing the death of 50% of a group of test animals, is one way to measure the acute toxicity of a material. From table 4, LD_{50} values of phenol and 1,4-benzoquinone, two intermediates of CB degradation, are 317 mg/kg and 103 mg/kg (acute oral, rat), lower than that of CB (2290 mg/kg).The LD_{50} value of biphenyl is 3280 mg/kg (acute oral, rat), higher than that of CB. These toxicology data indicated that the toxicity increase of wastewater samples was relative to the concentration of phenol and1,4-benzoquinone at the stage of 15-minutes from 15 min to 30 min. With the decomposition of these higher toxic intermediates after 30 minutes later, lots of small molecular organic acids formed, which had lower toxic and easier biodegradable, and the toxicity decreased accordingly. It is very conducive to biological treatment follow-up.

Table 3. Intermediates identified by GC/MS and IC

Compounds	Structural formula	Analytical methods		Sample time(min)		
		GC/MS	IC	30	60	90
Phenol		√		√		
Biphenyl		√		√		
1,4-benzoquinone		√		√	√	
butenedioic			√	√	√	
Oxalate			√	√	√	
Acetate			√	√	√	√
Formate			√	√	√	√
Chloride			√	√	√	√

Table 4. Toxicity of some organic compounds

Organic compounds	toxicity		
chlorobenzene	acute oral, rat LD_{50}:2290 mg/kg		
Phenol	rat per os LD_{50}:317mg/kg	mouse per os LD_{50}:270mg/kg	rabbit per os LD_{50}:630mg/kg
Biphenyl	rat per os LD_{50}:3280mg/kg	person can inhale <1mg/m^3	
1,4-benzoquinone	rat per os LD_{50}:103mg/kg	mouse per os LD_{50}:2000mg/kg	hunman per os <2%

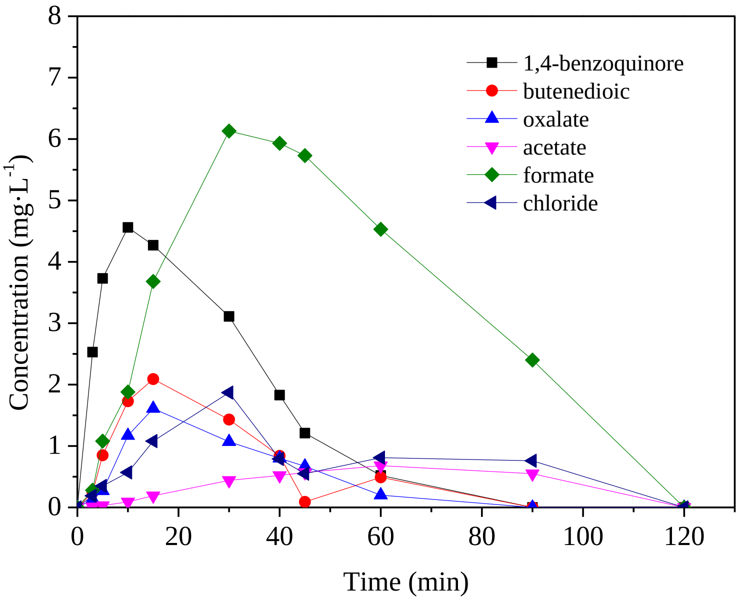

Figure 4. Concentration evolution of some degradation products during electro- oxidation of CB in aqueous solution.

4. CONCLUSIONS

The electrochemical method, based on electrocatalytic generation of hydroxyl radicals and other oxidants, is very efficient for the degradation of organic pollutants. Due to some intermediate products, the removal rate of TOC is slower that CB. The concentration of intermediate products changed along the time of electrocatalytic oxidation and the toxicity of wastewater sample changed accordingly. Sometimes the bio-toxicity would increase as some more toxic products occurred, but decline finally as these products degraded. Therefore, the electro-oxidation did not always reduce the toxicity of organics especially at the earlier stage, like other AOPs. It is necessary to design enough treating time for electrolysis process as pretreatment method, which could avoid some negative effect on followed bio-process causing by some intermediate products occurring during the electrolysis.

As commented above, the toxicity assay for *C. vulgaris* demonstrated that ECO could be considered as a suitable technique to degrade CB and reduce its toxicity in aqueous solution after enough treating time.

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