Rapid Degradation of Atrazine by Hydroxyl Radical Induced from Montmorillonite Templated Subnano-sized Zero-Valent Copper

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Abstract

In this study, subnano-sized zero-valent copper (ZVC) was synthesized using montmorillonite clay mineral as the template. The discrete distribution of surface charge on montmorillonite effectively separates the formed ZVC particles and inhibits their aggregation. X-ray diffraction result indicates that the size of ZVC particles on montmorillonite is $\sim 6 \text{ \AA}$, which is much smaller than nano-ZVC prepared by conventional method. The montmorillonite templated ZVC (ZVCMMT) shows superior reactivity as indicated by the degradation of atrazine, over 90% atrazine ($15 \text{ }\mu\text{M}$) could be degraded in a few min. Hydroxyl radical is confirmed as the reactive species, which is produced from the activation of oxygen by ZVC. It was also shown that the degradation process is strongly dependent on the hydration status of synthesized ZVCMMT. The freeze dried ZVCMMT exhibits higher reactivity compared to freshly prepared ZVCMMT, which can be explained by the higher adsorption of atrazine and oxygen residue on freeze dried ZVCMMT surface. In addition, the toxicity of atrazine is significantly decreased after the reaction with ZVCMMT, indicating that ZVCMMT could be used as a promising material for rapid remediation of persistent organic contaminants.

Keywords: zero-valent copper; molecular oxygen activation; in-situ H_2O_2 generation; montmorillonite; atrazine

1. Introduction

Due to the strong reductive activity, zero-valent metals (ZVMs) have been extensively studied and widely used for environmental remediation of various heavy metals and organic contaminants (Ponder et al., 2000; Dries et al., 2005; Jacqueline et al., 2005). It has been shown that ZVMs can effectively reduce chromate (VI) (Liu et al., 2008), nitro-aromatic compounds (Gu et al., 2010), and chlorinated organic chemicals (Pearson et al., 2005) under anaerobic conditions. However, recent studies reveal that ZVMs could also induce Fenton-like reactions to eliminate organic pollutants by activating the molecular oxygen (Joo et al., 2004; Englehardt et al., 2007; Katsoyiannis et al., 2008; Keenan and Sedlak, 2008a; b). For instance, in the case of zero-valent iron (ZVI), the reaction is initiated by a two-electron transfer from ZVI to the oxygen adsorbed on its surface and forms a reduced oxygen anion (O_2^{2-}), which could subsequently protonate and finally generate hydrogen peroxide (H_2O_2) in the presence of free protons (Keenan and Sedlak, 2008a). It was also reported that H_2O_2 could be produced through a one-electron transfer process by reaction between ferrous iron and oxygen (Huang et al., 2002; Kim et al., 2015). The formed H_2O_2 can be further decomposed into hydroxyl radical ($\cdot OH$) catalyzed by Fe^{2+} , and $\cdot OH$ is considered as one of the most reactive species to oxidize many organic contaminants (Buxton et al., 1988).

Among the ZVMs, ZVI is most studied. Due to the strong reduction potential,

especially under acidic conditions, ZVI can be consumed by free protons to form H_2 directly (Wang et al., 2014), which might limit the usage of ZVI at low pH. However, as protons are necessary to dismutate $\cdot O_2^-$, low pH would be essential for the production of H_2O_2 (Keenan and Sedlak, 2008a). Therefore, it is desirable to find other ZVMs to overcome the drawbacks of ZVI. Copper has a standard reduction potential of 0.34 V (Cu^{2+}/Cu) (Gang et al., 2014), which would prevent the reaction with protons via displacement reaction to produce H_2 gas. Thus this renders zero-valent copper (ZVC) as an alternative for ZVI.

Currently, only a few researches have been conducted to investigate the reactivity of ZVC. A prior study showed that ZVC could effectively degrade azo dyes by activation of molecular oxygen to produce Cu^+ and $\cdot OH$, leading to the cleavage of $N=N$ bonds of the azo dyes through a *Sandmeyer* reaction under neutral pH and aerobic conditions (Dong et al., 2014). The results from Gang and his co-workers (Gang et al., 2014) indicated that ZVC exhibited higher persistent oxidation capacity compared to ZVI, zero-valent aluminum and zero-valent zinc as indicated by the oxidative degradation of diethyl phthalate.

Due to the high surface area and reactivity, nano-sized ZVC (nZVC) was synthesized by various methods. For example, nZVC can be formed by addition of $NaBH_4$ to reduce Cu^{2+} to Cu^0 , and the average diameter is about 50 nm (Huang et al., 2012). A solvothermal method was also developed to synthesize nZVC with the

diameter of ~30 nm (Dong et al., 2014). It is well known that nanoparticles tend to agglomerate, which diminishes their reactivity. Hence, different strategies have been employed to stabilize the formed nanoparticles. Stabilizers such as carboxylic acids and polyvinyl alcohol (Khanna et al., 2007), oleylamine (Dadgostar et al., 2010), and poly amidoamine dendrimers (Balogh and Tomalia, 1998) have been used to disperse copper nanoparticles. Whereas, these stabilizers are all organic chemicals, which might interfere with the reactive oxygen species (ROS) generated from the activation of oxygen by ZVC.

Montmorillonite is a common clay mineral widely distributed in soils, subsoils and sediments (Gu et al., 2008). As a natural nanomaterial, the size of elementary platelet of montmorillonite is a few tens to hundreds of nanometers wide and 1-1.8 nanometers thick (Pinnavaia, 1983; Laszlo, 1987). Our previous studies showed that montmorillonite could be used as a template to synthesize subnano-sized ZVI (Gu et al., 2010; Zhang et al., 2015). With the unique structure of montmorillonite, ZVI was formed in the constrained interlayer region of montmorillonite with average size of 0.5 nm. The montmorillonite template ZVI showed higher reactivity and efficiency compared to other forms of ZVIs (Gu et al., 2010). Therefore, it is expected that highly reactive nZVC could also be formed with montmorillonite as the supporting matrix.

Atrazine was used worldwide as the herbicide since 1958 and has been banned in

the European Union in 2004 due to its highly persistent nature in the environment. It has been reported that the concentration of atrazine could reach $0.068 \mu\text{g L}^{-1}$ in groundwater even after 21 years since its last application (Vonberg et al., 2014). Atrazine can cause chronic prostatitis (Stanko et al., 2010) and mammary gland development problems in Long-Evans rats after a prenatal exposure (Enoch et al., 2007). It also can cause algae death after an acute exposure (Ralston-Hooper et al., 2009). Recently, numerous methods have been developed to remove atrazine through oxidation (Guan et al., 2013; Hu and Cheng, 2014), reduction (Stephen et al., 1998) and biological degradation (Ghosh and Philip, 2004).

In this study, a new method to synthesize nZVC was developed using natural montmorillonite as the support and the degradation process of atrazine by this montmorillonite templated nZVC (ZVCMMT) through a Fenton-like reaction was investigated without addition of H_2O_2 . The results clearly demonstrate that the isolated distribution of exchangeable Cu^{2+} effectively separates the nZVC nanoparticles and prevents their aggregation upon the reduction by NaBH_4 . With the smaller particle size ($6 \text{ \AA}$), ZVCMMT exhibits exceptional reactivity for the decomposition of atrazine under acidic conditions, as the initial degradation rate increases 27 and 13 times compared to commercial available ZVC ($\sim 20 \mu\text{m}$) and nZVC ($\sim 25 \text{ nm}$) prepared by conventional method, respectively. Our results also indicated that the degradation process is strongly affected by the hydration status of

ZVCMMT. Freeze dried ZVCMMT showed higher reactivity than the freshly prepared, which may be explained by the higher adsorption of atrazine and residual oxygen on the surface of dried clay minerals.

2. Materials and Methods

2.1. Chemicals

Atrazine (99.1%) and its metabolites (atrazine-desethyl (DEA), 99.5%; atrazine-desisopropyl (DIA), 97.5%; atrazine-desethyl-desisopropyl (DEDIA), 96.3%), and atrazine standard solution (101 ng μL^{-1} , dissolved in methanol) were obtained from Fluka (Ronkonkoma, NY, USA). Xanthine, xanthine oxidase, sodium borohydride, copper chloride dihydrate, zero-valent copper powder (spheroidal) with the average diameter size of 20 μm and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) were all purchased from Sigma-Aldrich. Methanol and acetonitrile were of HPLC grade and from Merk (Darmstadt, Germany). All the chemicals were used as received without any further purification.

2.2. Preparation of ZVCMMTs and nZVC

Montmorillonite clay mineral used in this study was obtained from Fenghong Chemical Co. (Zhejiang Province, China) with the measured cation exchange capacity (CEC) of 77 cmol kg^{-1} . The preparation of ZVCMMT followed the method of Gu and his co-workers (Gu et al., 2010). Briefly, Na^+ -montmorillonite was initially exchanged with cupric ion (Cu^{2+}) by simply saturating the clay mineral using 0.1 M CuCl_2

solution. Copper content in clay mineral was determined by a flame atomic absorption spectrometry (Thermo fisher, M6, USA) after digestion with a mixture of hydrofluoric and hydrochloric acid. Then, the pH of the Cu^{2+} -montmorillonite slurry was adjusted to ~2 using 1 M HClO_4 before addition of NaBH_4 with the molar ratio of $\text{NaBH}_4/\text{Cu}^{2+} = 30$. Finally, the clay suspension was washed with oxygen free water to remove the excess of NaBH_4 . For each experiment, ZVCMMT was freshly prepared. In addition, a portion of ZVCMMT was freeze dried to investigate the effect of hydration status on the degradation of atrazine, which was then stored in a desiccator with nitrogen gas atmosphere. Montmorillonite templated zero-valent iron (ZVIMMT) was also prepared following the same procedure as ZVCMMT.

Nano-sized ZVC was synthesized with the modified method from Huang and his co-workers (Huang et al., 2011). CuCl_2 (0.05 M) and NaBH_4 (0.15 M) solutions were mixed with the volume ratio of 1:1, and then washed by oxygen free water to removal excessive NaBH_4 until the pH of the supernatant was in the range between 6 and 7 after centrifugation. The synthesized nZVC was then freeze dried and stored under anaerobic condition.

2.3. Characterization of ZVCMMTs

The powder diffraction pattern of the freeze dried ZVCMMT was collected between 2° to 14° at a scanning rate of 1° min^{-1} by X-ray diffractometer (XRD) (X'TRA, ARL, Switzerland) with graphite monochromated $\text{CuK}\alpha$ radiation ($\lambda=1.5406$

Å), operating at 40 kV and 40 mA. To confirm the reduction of Cu^{2+} to Cu^0 , the freeze dried ZVCMMT sample was analyzed by X-ray photoelectron spectroscopy (XPS) (PHI 5000 VersaProbe, UIVAC-PHI, Japan) with a monochromatic $\text{AlK}\alpha$ source. Transmission electron microscope (TEM) (Tecnai G²F20 200-kV field emission microscope) was used to obtain the image of synthesized material and to measure the particle size. X-ray energy dispersive spectroscopy (EDS) was also equipped with TEM and employed to identify the element composition and distribution on ZVCMMT and nZVC before and after the reactions. BET surface areas of commercially available ZVC, nZVC, ZVCMMT and Cu^{2+} -montmorillonite were evaluated using a surface area and porosity analyzer (Micromeritics, ASAP 2020, USA).

2.4. Degradation of atrazine by ZVCMMTs, ZVIMMT, nZVC and commercial available ZVC

The degradation experiment was conducted at room temperature (23°C) in a 250 mL conical flask with a magnetic stir at 600 rpm to ensure sufficient oxygen supply. The initial concentrations for atrazine and ZVCMMTs were 15 μM and 0.5 g L^{-1} , respectively. In comparison studies, the concentrations for nZVC, commercially available ZVC and ZVIMMT were also at 0.5 g L^{-1} . No organic solvent was used to avoid any possible quenching effect for radicals produced during the reaction. The initial pH of the solution was adjusted to 3.0, 4.5 and 6.0 by addition of 1 M HClO_4 or

1 M NaOH. The pH of the system before and after reaction was monitored with a calibrated pH meter (Thermo Scientific, ORION 5 STAR, USA). At predetermined time intervals, aliquot of 0.5 mL reaction solution was withdrawn and transferred to a glass tube containing 1 mL methanol, then vortexed for 15 min. Methanol was used as both quenching and extraction agent. After the extraction, sample was filtered through a 0.22 μm polytetrafluoroethylene syringe filter and stored at 4 $^{\circ}\text{C}$, then analyzed within 24 h. Our preliminary experiments showed that the recovery for atrazine was higher than 95%. Similar experiment was also conducted inside the anaerobic chamber.

Atrazine concentration was analyzed by a HPLC system (Waters Alliance 2695, Milford, MA, USA) with a C18 column (5 μm , 4.6 $\times$ 250 mm, Waters SunFire) monitored at the wavelength of 220 nm by a photodiode array detector (Waters Alliance 2998, Milford, MA, USA) with the injection volume of 25 μL . The reaction products were identified by comparing the retention times of the intermediates with the standards we obtained. The same HPLC system was used, and the mobile phase consisted of acetonitrile-water gradient elution with a constant flow rate of 1 mL min^{-1} . The gradient started with 5% acetonitrile and 95% water, then linearly increased to 35% acetonitrile within 50 min, finally returned to the initial condition in 2 min and held for another 8 min for equilibration. The reaction products were also analyzed by GC-MS (Thermo Scientific, trace1030 ISQ, USA) after the sample was concentrated

with solid phase extraction (SPE) method. Details of GC-MS and SPE methods were described in text S1 and text S2.

Concentration of H_2O_2 produced in the reaction system was determined by the photometric method (Gang et al., 2014), in which N,N-diethyl-*p*-phenylenediamine was oxidized by a peroxidase catalyzed reaction. To determine the active ROS for oxidation of atrazine, methanol was utilized as a probe molecule to quench $\cdot\text{OH}$ due to its fast reaction with $\cdot\text{OH}$ ($9.8 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$) (Buxton et al., 1988). To test the possibility for the reaction of atrazine with $\cdot\text{O}_2^-$, xanthine (1 mM) and xanthine oxidase (0.2 unit mL^{-1}) were used to generate $\cdot\text{O}_2^-$ (Dong et al., 2014).

The radicals produced in the system were monitored by electron paramagnetic resonance (EPR) spectrometer (EMX-10/12, Bruker, Germany) at room temperature. DMPO was used as the effective radical scavenger. For the EPR measurement, clay and DMPO concentrations were 5 g L^{-1} and 40 mM, respectively. The instrumental parameters for EPR analysis were: microwave frequency and power at 9.85 GHz and 20 mW, modulation frequency and modulation amplitude at 100 kHz and 1 G, receiver gain at 1.0×10^3 , sweep width at 200 G and center field at 3510 G.

The concentration of copper in solution after reaction was measured with a flame atomic absorption spectrometry (Thermo Fisher, M6, USA) after filtration. The generation of H_2 during the reaction in the ZVIMMT and ZVCMMT systems was evaluated in a 40 mL gas-sealed bottle with 10 mL headspace volume. The amount of

hydrogen gas in the headspace was measured using a gas chromatography (GC, BeiFen3420A, China) coupled with a thermal conductivity detector.

2.5. Effect of hydration status of ZVCMMT on the degradation of atrazine

To investigate the effect of hydration status of synthesized ZVCMMT on the degradation process, experiments for both freshly prepared and freeze dried ZVCMMT were conducted following the same procedure as described above. During the reduction of Cu^{2+} -montmorillonite by NaBH_4 , the negative charges on clay mineral originally balanced by Cu^{2+} ion would be compensated by Na^+ (Gu et al., 2010). Therefore, the adsorption of atrazine on freshly prepared and freeze dried Na^+ -montmorillonite was also studied. The concentrations for Na^+ -montmorillonite and atrazine used in adsorption experiment were 12.5 mg mL^{-1} and in the range between 1.42 and $44.31 \text{ }\mu\text{M}$, respectively.

2.6. Computational methods

Molecular orbital calculation of atrazine was carried out using B3LYP/6-311G** method with Gaussian 09W program (Frisch et al.). The frontier electron densities (FEDs) of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were determined from Gaussian output files. The values of $2\text{FED}_{\text{HOMO}}^2$ and $(\text{FED}_{\text{HOMO}}^2 + \text{FED}_{\text{LUMO}}^2)$ were used to predict the reaction sites of atrazine.

2.7. Toxicity assay

The change of acute toxicity before and after reaction with ZVCMMT was assessed by luminescent bacteria test (*Vibrio fischeri*) according to the standard method of ISO11348-3-2007. Before the test, pH was adjusted to 7.0 with HClO₄ (1 M) or NaOH (1 M) and the residual particles were removed through centrifugation.

3. Results and discussion

3.1. Characterization of ZVCMMT

As shown in table S1, negligible amount of copper was detected in reference montmorillonite (Na⁺-montmorillonite), indicating that almost no copper is present in clay crystal structure. The measured copper content in Cu²⁺-montmorillonite was ~4.3% (w/w) after clay mineral was saturated by Cu²⁺ ion (Tab. S1). It was worth noting that based on the CEC of montmorillonite (77 cmol kg⁻¹), the calculated copper content is ~2.5%, the higher amount of copper (4.3%) in Cu²⁺-montmorillonite might be explained by the formation of copper oxyhydroxide oligomers, which could lower the charge of copper ions (Tanaka et al., 2007). The copper content in synthesized ZVCMMT decreased to 3.3% (w/w) (Tab. S1), probably due to the loss of copper in the washing steps during preparation, this phenomenon was also observed for preparing subnano-sized ZVI in montmorillonite (Gu et al., 2010).

With the addition of NaBH₄, clay suspension turned into black immediately indicating the formation of ZVC. As shown in the XRD measurements (Fig. 1), the basal spacing of montmorillonite increases from 13.89 Å (Cu²⁺-montmorillonite) to

14.72 Å after reduction at $\text{NaBH}_4/\text{Cu}^{2+}$ molar ratio of 15, which further increases to 15.71 Å with the increase of $\text{NaBH}_4/\text{Cu}^{2+}$ ratio of 30. Similar to our previous observation (Gu et al., 2010), the increase of basal spacing of clay mineral is attributed to the formation of nZVC clusters in clay interlayer. Considering that the thickness of aluminosilicate layer of montmorillonite is 9.6 Å (Malla et al., 1991), the synthesized particle size in the vertical dimension is ~6 Å, which is much smaller than other forms of nZVCs reported in the literatures (Liou et al., 2007; Huang et al., 2012; Dong et al., 2014). The TEM image of synthesized ZVCMMT, and copper/silicon mapping in the same area are shown in Figure S1, it is indicated that the distribution of Cu follows the same pattern as Si, suggesting that the copper particles are formed homogeneously on the surface of montmorillonite (Yu et al., 2012).

Based on the XPS analysis (Fig. 2), the copper species in the original Cu^{2+} -montmorillonite is Cu^{2+} (935.5 eV) (Koppelman and Dillard, 1977). A strong peak corresponding to Cu^+ (933.2 eV) was also observed, which is consistent with the results of Cai and Xue (Cai and Xue, 2008), and can be explained by the photo-reduction process induced with the X-ray irradiation. After NaBH_4 reduction, most of copper turns into ZVC (932.2 eV), with a small shift compared to the standard binding energy of Cu^0 (932.4 eV) due to the small particle size effect (Oberli et al., 1981). The metal surface is slightly oxidized by oxygen, as evidenced by the cupric hydroxide shoulder at 934.4 eV (Jiang et al., 2013).

3.2. Degradation of atrazine by ZVCMMTs, nZVC and commercial available

ZVC

Control experiments show that the degradation of atrazine in the absence of ZVC and ZVCMMTs is negligible under the experimental conditions, suggesting that atrazine is resistant to photo-degradation and volatilization (Fig. S2). According to our results, the degradation of atrazine by ZVCMMT is strongly pH dependent (Fig. S3). For the initial pH range between 3 and 6, the initial removal rate of atrazine for freshly prepared ZVCMMT decreases from $0.549 \mu\text{M min}^{-1}$ at pH 3 to $0.001 \mu\text{M min}^{-1}$ at pH 6 (Tab. 1). Previous studies had demonstrated that acidic condition would facilitate the formation of H_2O_2 and subsequent hydroxyl radical, favoring the Fenton-like reactions (Masarwa et al., 2005; Gang et al., 2014). Concomitantly, as shown in EPR results (Fig. 3), when the pH increases from 3 to 6, the detected EPR signal changes from $\text{DMPO}\cdot\text{OH}$ to the carbon center radical, which is generated from the reaction between DMPO and $\cdot\text{O}_2^-$ (Wang et al., 2013; Dong et al., 2014). Our experimental results further indicate that atrazine could not be degraded by $\cdot\text{O}_2^-$ radicals (Fig. S4), and when the reaction is quenched with methanol, the degradation process is significantly suppressed (Fig. S2), which confirms that $\cdot\text{OH}$ is the predominant ROS for degradation of atrazine.

The subsequent studies were all performed at initial pH of 3 to investigate the underlying mechanisms for the extraordinary reactivity of natural clay templated ZVC.

As shown in Figure 4, the initial hydration status of ZVCMMT substantially affects the degradation of atrazine. At pH 3, more than 90% of atrazine can be quickly degraded by the freeze dried ZVCMMT within 2 min (Fig. 4), which was also supported by the high H_2O_2 concentration detected in the beginning of the reaction in the presence of the freeze dried ZVCMMT (Fig. S5). Whereas, for the reaction system with freshly prepared ZVCMMT, the initial degradation rate decreases nearly 12 times compared to freeze dried ZVCMMT (Tab. 1). The final pHs for freeze dried and freshly prepared ZVCMMT systems are 3.65 and 3.63, respectively. It has been reported that the hydration and swelling situation of clay mineral could substantially affect the adsorption of atrazine onto clay surfaces (Chappell et al., 2005). As indicated in Figure S6, the freeze dried montmorillonite has much greater affinity for atrazine than fresh Na^+ -montmorillonite with ~ 2 order of magnitude higher for the adsorption coefficient (Tab. S2), which could be explained that since dried montmorillonite is less hydrated and atrazine can form direct interaction with the opposing basal surface of the clay mineral (Chappell et al., 2005). The enhanced adsorption of atrazine on montmorillonite would increase the contact possibility between atrazine and ZVC, hence increasing the degradation rate, similar phenomenon has also been observed in our previous studies (Gu et al., 2010; Yu et al., 2012; Tian et al., 2015). In addition, other factors may also contribute to the higher reactivity for freeze dried ZVCMMT. As we have mentioned, oxygen is essential to

produce ROS. For freeze dried ZVCMMT, due to the oxygen residue retained on the surface of mineral, the reaction can be quickly initiated. However, for freshly prepared ZVCMMT, during the reduction by NaBH_4 , the oxygen on the mineral surface has been quickly consumed by the strong reductant. Therefore, the degradation reaction is slowed down due to the slow diffusion of oxygen into the system. To further support this hypothesis, the degradation experiments were conducted inside an anaerobic chamber. There was negligible degradation of atrazine (less than 3.3%) observed in the freshly prepared ZVCMMT system, whereas, for freeze dried ZVCMMT, a quick degradation occurred in the first few min, then the reaction leveled out, indicating that there is some oxygen to initiate the reaction and no further oxygen input to sustain the degradation (Fig. S2).

For comparison, the degradation experiments by commercial available ZVC, nZVC were also conducted. Even the copper concentrations for both ZVC and nZVC (0.5 g L^{-1}) are much higher than the copper content in ZVCMMT ($\sim 0.0175 \text{ g L}^{-1}$) system used in this study, only 20% and 60% of atrazine with the initial concentration of $15 \text{ }\mu\text{M}$ can be degraded in 3 h for ZVC and nZVC. On the other hand, atrazine can be fully degraded within 2 and 120 min for freeze dried and freshly prepared ZVCMMT, respectively (Fig. 4). The high reactivity of ZVCMMT is attributable to the small particle size of ZVC with high surface area. Due to the constrained clay interlayer space, the size of formed ZVC in the vertical dimension is $\sim 0.6 \text{ nm}$ based

on the XRD results (Fig. 1). However, for nZVC, the average diameter of ZVC particles is ~25 nm, which could then form aggregates with the size even larger than several micrometers (Fig. S7). In addition, the final pHs for nZVC and commercially available ZVC systems with initial pH of 3 are 5.79 and 6.57, respectively. When $\cdot\text{OH}$ radical or O_2 reacts with nZVC or ZVC, the layer of copper hydroxide might be formed on the surface at higher pH, preventing further reactions with the copper core, which could explain why the reaction stops for commercial available ZVC and nZVC even there is sufficient copper available. It is also supported from the dark-field TEM and EDS images of nZVC, as shown in Figure S8, after the reaction, the copper core is surrounded with amorphous copper oxide. In the dark-field image, the bright and dark areas represent metallic and oxidized copper, respectively (Fig. S8), which is in agreement with the results of LaGrow and his co-workers (LaGrow et al., 2016). In addition, as indicated in Table S4, ZVCMMT has the highest BET surface area ($120.43 \text{ m}^2 \text{ g}^{-1}$) among the materials tested, suggesting that ZVCMMT could provide more reactive sites for the degradation of atrazine.

The release of copper ions was observed in our ZVCMMT systems, after 3h, the measured copper concentration in solution is 7.85 mg L^{-1} . While for nZVC and commercially available ZVC, the released copper concentrations are 47.6, 50.2 mg L^{-1} , respectively. So lower release of copper is another benefit for ZVCMMT, which can be explained that the negatively charged surface of MMT could retain the released

copper ions by electron static adsorption. The elevated copper level could be further reduced by adjusting solution pH to neutral before discharge, our results also showed that the copper was undetectable at pH 7 in our system. Stability of ZVCMMT was also evaluated after this material was stored for 7 d and 14 d in a desiccator, no significant decrease of reactivity was observed (Fig S14).

Compared to ZVCMMT, only limited degradation (17.48%) of atrazine was observed in the presence of ZVIMMT. At pH 3, ZVI can be consumed by protons, the production of hydrogen gas was also detected (Fig. S13). However, in the system of ZVCMMT, no H₂ was produced (Fig. S13).

3.3. Proposed degradation mechanism of atrazine

As shown in the HPLC chromatogram (Fig. S9), according to the retention time of the available standards, the peaks at 8.131, 15.805 and 21.036 min correspond to DEDIA, DIA and DEA, respectively. Furthermore, the peak at 29.55 min is assigned to 2-chloro-4-ethylimino-6-isopropylamino-s-triazine (atrazine-imine) according to the results reported by Acero and his co-workers (Acero et al., 2000). In addition, 4-acetamido-2-chloro-6-isopropylamino-s-triazine (CDIT) and 4-acetamido-6-amino-2-chloro-s-triazine (CDAT) were also detected by GC-MS analysis (Fig. S10). Based on the reaction products identified in our experiment and the structures of intermediates reported in the prior studies, the degradation pathway of atrazine by ZVCMMT is proposed in Figure 5. Initially, $\cdot\text{OH}$ is produced via the

activation of oxygen molecule by ZVC, then the radical would attack the aliphatic side chains of atrazine. With the cleavage of N-C bond, DIA and DEA are produced, and subsequently forming DEDIA. Similar products were observed in the previous studies (Hu et al., 2012; Marchetti et al., 2013). On the other hand, a carbon centered radical can also be formed via a H-abstraction process (von Sonntag and Schuchmann, 1991; Acero et al., 2000). When the carbon radical reacts with oxygen, an organic peroxy radical is produced, then yielding C=N and C=O bonds at the α carbon via a mono or bimolecular reaction to form atrazine-imine, CDIT, and CDIA, respectively.

Theoretical calculations were used to help interpret the degradation mechanism of atrazine. The electron density was visualized by exhibition of isosurfaces with LOMO and HOMO frontier orbitals (Fig. S11). It was reported that the values of $2FED^2_{HOMO}$ and $FED^2_{HOMO}+FED^2_{LUMO}$ can characterize the potential of the reactive sites for electron extraction and $\cdot OH$ addition, respectively (Lee et al., 2001; Qu et al., 2015), and the degradation of atrazine mainly occurs on the side chain of the molecule (Yang et al., 2016). Nitrogen 3 and nitrogen 11 have higher $2FED^2_{HOMO}$ and $FED^2_{HOMO}+FED^2_{LUMO}$ values compared to other atoms on the side chain, indicating that these two sites would be involved in the degradation process, which is consistent with our experimental results.

3.4. Toxicity assay

The toxicity of the system was evaluated by the inhibition of light emission of

Vibrio fischeri. Before the reaction, the inhibition value for 15 μM atrazine is 24.95%, whereas, after the 180 min reaction with the freeze dried ZVCMMT, the inhibition becomes negligible (-0.53%). The significant decrease of the toxicity can be explained by the fast decomposition of atrazine and formation of less toxic intermediates, e.g., DIA and DEA. Similar results were also observed by Ralston-Hooper and his co-workers (Ralston-Hooper et al., 2009), the degradation products show lower toxicity compared to atrazine when algae was used as the indicator.

4. Conclusions

In this study, natural montmorillonite templated ZVC was synthesized by intercalating Cu^0 into clay interlayer, the unique structure of clay mineral could effectively prevent the aggregation of ZVC particles and inhibit particle growth. The particle size of ZVCMMT is 1 or 2 order of magnitude smaller than nZVC prepared by the conventional method. The ZVCMMT exhibits high reactivity for degradation of organic contaminant, i.e., atrazine by activation of oxygen to form reactive hydroxyl radical.

This material can be considered as a promising technique for water treatment, a burst of hydroxyl radical can be produced on the surface of ZVCMMT in a short time without addition of any hydrogen peroxide. It provides a convenient and efficient alternative for rapid degradation of persistent contaminants. Since montmorillonite has been widely used as the clay barrier for waste disposal, the ZVCMMT can be

incorporated as the reactive cap for *in situ* remediation of many organic pollutants. Further studies are needed to investigate the effect of other environmental factors, e.g., the presence of humic substances and competing ions on the degradation process for field application of this novel material.

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Appendix A. Supplementary data

GC-MS Method; SPE Method; Total copper contents of montmorillonite saturated with $\text{Na}^+/\text{Cu}^{2+}$ and the freeze dried ZVCMMT; Fitted linear adsorption coefficients for atrazine adsorption on Na^+ -montmorillonite with different hydration status; TEM image of ZVCMMT and elemental distribution mapping of Si and Cu of the same region; Degradation kinetics of atrazine under different control conditions; Degradation kinetics of atrazine with freshly prepared ZVCMMT at various pHs; Concentrations of atrazine before and after reaction with superoxide radicals; Hydrogen peroxide generation vs time by ZVCMMTs, nZVC, and commercially available ZVC; Atrazine adsorption on Na^+ -montmorillonite with different hydration status; TEM image of synthesized nZVC; TEM image of nZVC after reaction and copper distribution in the same region; HPLC chromatograms of atrazine and its metabolites after reaction; GC-MS chromatograms of atrazine standard and its metabolites after reaction; Frontier electron densities of atrazine. Supplementary data related to this article can be found at <https://www.journals.elsevier.com/chemosphere/>.

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Table 1. Initial removal rate of atrazine with different forms of ZVC at different pH

<i>pH</i>	Initial removal rate ($\mu\text{M min}^{-1}$)		
	<i>3.0</i>	<i>4.5</i>	<i>6.0</i>
Freshly prepared ZVCMMT	0.549	0.222	0.001
Freeze dried ZVCMMT	6.909	2.143	0.608
nZVC	3.255	0.441	0.038
Commercially available ZVC	0.096	0.271	0.021

Initial rate was defined as the change of the concentration of atrazine in the first two min of the reactions. Experimental conditions: the initial concentration of atrazine and the solid materials were $15 \mu\text{M}$ and 0.5 g L^{-1} , respectively; the initial pH was adjusted to 3, 4.5, and 6 with HClO_4 (1 M) and NaOH (1 M).

Figure Legends

Figure 1. X-ray diffraction patterns of Cu^{2+} -montmorillonite and Cu^0 -montmorillonite after treatment by NaBH_4 at $\text{NaBH}_4/\text{Cu}^{2+}$ ratio of 15 and 30, respectively.

Figure 2. Fitted curve of narrow XPS scans for Cu ($2p_{3/2}$) of Cu^{2+} -montmorillonite and the freeze dried ZVCMMT. Solid black lines are the fitted curves. Wavy-like lines are the observed photoelectron curves.

Figure 3. EPR spectra of freeze dried ZVCMMT at pH 3.0 (top) and pH 6.2 (bottom), DMPO was used as the effective radical scavenger. Experimental conditions: pH was adjusted to 3.0 or 6.2 with HClO_4 (1 M) or NaOH (1 M); the concentration of DMPO and the freeze dried ZVCMMT were 40 mM and 0.5 g L^{-1} , respectively.

Figure 4. Degradation kinetics of atrazine by different forms of ZVC. Experimental conditions: the initial concentrations of atrazine and different materials were $15 \mu\text{M}$ and 0.5 g L^{-1} . The initial pH was adjusted to 3.0 with HClO_4 (1 M). Three reactors were sampled for each time point, and the data were averaged. Error bars represent the standard deviations.

Figure 5. Proposed degradation pathway of atrazine in the reaction system.

Fig. 1

Fig. 2

Fig.3

Fig. 4

Fig. 5

tetrahedral repeat unit

octahedral repeat unit

magnesium atom