

Degradation of Metronidazole by carboxymethyl cellulose stabilized iron sulfide nanoparticles in water and soil phases

by

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Abstract

The removal of Metronidazole (MNZ) from water and soil phases by iron sulfide (FeS) nanoparticles was investigated. From the results, for the batch test, 10 mg/L MNZ in the solution can be fully removed by the 350 mg/L FeS at pH = 7.5 in 2 hours then the FeS dosage effect, carboxymethyl cellulose (CMC) stabilization effect, pH effect, humic acid (HA) effect, CMC to FeS molar ratio were investigated to find out the best degradation conditions. The removal of MNZ by FeS followed a pseudo-first order kinetic model. The removal efficiency is increasing while the FeS concentration increasing, pH increasing, HA decreasing and a suitable CMC to FeS molar ratio from 0.0005 to 0.00225. The CMC stabilized FeS was compared with the bare FeS, CMC stabilized FeS showed faster removal rate and removal efficiency. In the soil phase experiment, adsorption, desorption, degradation and column tests were conducted, the result showed the MNZ can be adsorbed and degraded in the soil with lower removal efficiency, low removal rate and longer treatment time compared to the batch tests.

Key words: Metronidazole (MNZ); Nanoparticle; Iron sulfide; Degradation;

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List of Abbreviations

MNZ	Metronidazole
CMC	Carboxymethyl cellulose
FeS	Iron sulfide
HA	Humic acid
FeCl ₂	Iron chloride
NaS	Sodium sulfide
LC-MS	Liquid chromatography–mass spectrometry
HPLC	High performance liquid chromatography

1. Introduction

1.1. Overview

In 1800s, synthetic compounds were firstly used as an anesthetic drug then the medical science developed quickly (Sneader et al, 2005). After that, synthetic therapeutic compounds were widely investigated. Thousands of Pharmaceuticals have been developed and keep increasing. The increasing number of Pharmaceuticals led to the accumulation in the environment because a large part of Pharmaceuticals cannot be degraded by human body, which will finally be released to wastewater. As a result, it is necessary to treat the Pharmaceutical wastewater.

Metronidazole (MNZ) is an antibiotic, which can be used to treat pelvic inflammatory disease, endocarditis, bacterial vaginosis, Dracunculiasis, giardiasis, trichomoniasis, and amebiasis. It can be taken orally. MNZ is also widely used to treat animals such as dogs, cats and fishes for any potentially susceptible anaerobic infection. It can be used to treat the giardia and chronic inflammatory bowel diseases in dogs, cats and other animals. It is also commonly used to treat bacterial and protozoan infections in reptiles and amphibians (Barr et al, 1994). So MNZ accumulated in the animals, water and the related meat industries (Kümmerer et al, 2001).

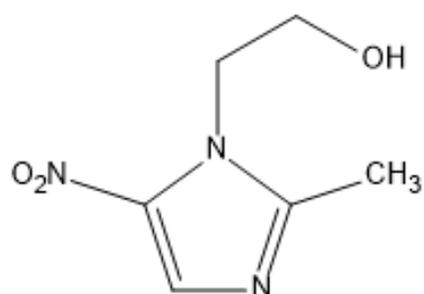
MNZ began to be used in 1960 in France (Li et al, 2013), it is widely used because of its effectiveness, low price and safety. MNZ can be found in most of places in the world (Gülmezoglu et al, 1998). It has been listed on the World Health Organization's list of essential medicines.

Metronidazole is a Nitroimidazole Antimicrobial.

Table 1. Chemical and physical properties of MNZ

MW	171.156 g/mole
molecular formula	C ₆ H ₉ N ₃ O ₃
water solubility	9.5 g/L
pKa	2.38
Vp	3.1*10 ⁻⁷ Hg at 25 °C
KH	5.92*10 ⁷

2D Molecular structure



3D conformer

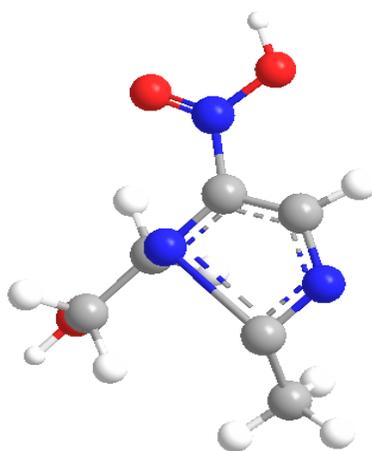


Table 1 shows the physical and chemical properties of Metronidazole. MNZ cannot be biodegraded by the water so MNZ will keep accumulating in the aquatic environment.

Many methods have been investigated to remove MNZ from the wastewater and soil. All the methods can be classified into three types: adsorption, biodegradation and photocatalytic degradation.

Rivera-Utrilla et al (2009) studied the adsorption and bio adsorption of MNZ by activated carbon with different chemical and texture properties. The results showed that the adsorption of MNZ is non- electrostatic process and electron-activating groups can favor the adsorption. The microorganisms used in the biological stage of a wastewater treatment plant cannot degrade MNZ, so the whole process is only adsorption related. The result also showed that this method would be more effective to the underground and surface water than the urban water.

Farzadkia et al (2015) studied the photocatalytic degradation of MNZ by illuminated TiO₂ nanoparticles which showed that maximum MNZ removal can be obtained near the neutral pH by UV/TiO₂. Degradation of MNZ via UV, Fenton and photo-Fenton processes were also reported by Shemer et al (2006), UV, UV/H₂O₂, H₂O₂/Fe²⁺ and UV/H₂O₂/Fe²⁺ were tested, the result showed that degradation efficiency of MNZ by the UV can be enhanced by adding more ferrous iron and UV/H₂O₂ oxidation are more effective than the photolysis. UV light can increase the removal efficiency during degradation. Boxi et al (2014), also studied photocatalytic degradation of metronidazole under visible light by silver doping on TiO₂, CdS and ZnS, it showed that silver can improve removal efficiency significantly. ZnSnO₃ (Dong et al, 2014), BiVO₄ (Yu et al, 2014), BiVO₄/FeVO₄ (Li et al, 2015), K₆Nb_{10.8}O₃₀ (Wang et al, 2010) were also reported that they can be used in photocatalytic degradation. However, the photocatalytic degradation process has some

limitations such as high cost. The usage of radiation sources in the large scale is unrealistic in some ways.

ZVI is another useful method used to degrade the MNZ in recent years. Fang et al (2011) studied the removal of MNZ by ZVI. It showed that MNZ could be fully degraded by the ZVI with a suitable concentration in a short time (5min). The pH, ZVI dosage and MNZ dosage can affect the removal of MNZ. The use of ZVI showed better removal efficiency than commercial iron powder under the same experiment condition, which gives a potential way to the removal of MNZ. Wang et al (2016), further studied the carbon spheres-supported nanoscale zero valent iron which can get higher stability, dispersion, longtime efficiency and higher activity for degrading MNZ in wastewater. Carbon spheres effectively improve the reduction of the ZVI. Rectorite- supported nanoscale zero-valent iron was investigated by Yuan et al (2016), which showed the similar results.

In recent years, iron-sulfide nanoparticles have been used in degrading a large variety of pollutants because of its high efficiency and easy preparation. Pollutants including heavy metals (Jeong et al, 2007), chlorinated organics (Butler et al, 1998), nitroaromatic compounds (Oh et al, 2011), polybrominated diphenyl ethers, nitrates, pesticide, dyes etc. Iron sulfide is an acid volatile sulfides and nanoscale iron sulfide significantly improve the particle surface area, which will create more active sites and increase its reactivity. Many papers investigated the use of iron sulfide nanoparticles in degrading the pollutants. Based on this background information, iron sulfide nanoparticles could be an alternative way to treat the pharmaceutical wastewater.

Yuan et al (2015), investigated the removal of p-chloroaniline (PCA) by iron sulfide. Iron sulfide was found to be an application to decompose PCA compared with ZVI or Fe^{2+} . The p-chloroaniline is a pharmaceutical, iron sulfide was used as an alternative electron donor. Iron sulfide was found to be a role, which can release the Fe^{2+} continuously and showed better removal

efficiency than Fe^{2+} . In Comparison to Fe^0 , recycling of Fe^{3+} to Fe^{2+} can prevent the accumulation of Fe^{3+} .

1.2. Goals and objectives

The overall goal of this research is to find a suitable FeS solution, which can be used to degrade MNZ in the water and soil and find the factors, which can affect the particle size and effectiveness. The aim of my research was preparing nano-size FeS solution by using Carboxymethyl cellulose (CMC). The objectives are:

Bare FeS solution and CMC stabilized FeS solution were prepared. Precipitation and effectiveness in degrading MNZ in water phase were investigated. CMC stabilized FeS was prepared by adding CMC solution during the FeS preparation.

1. Test the effectiveness of the bare FeS and CMC stabilized FeS. FeS dosage effect, pH effect and Humic acid dosage effect were investigated by batch tests.
2. Test the effectiveness of CMC stabilized FeS for different CMC molar ratio.
3. Test the effectiveness of CMC stabilized FeS in treating MNZ in the soil.

The findings can provide the basis for the treatment of pharmaceutical wastewater by FeS nanoparticles.

2. Materials and methods

2.1. Materials

Soil from the E.V. smith research center of Auburn University, AL was used in the soil phase experiment. Before use, the soil has been washed twice a day for a week (14 times) and air-dried for another week to remove all the water-soluble particles. Then the soil was filtered by a 2 mm sieve. The soil was stored at the room temperature (20 ± 1 °C). The quality of the soil (pH, texture,

compositions and cation exchange capacity) has been tested by the Auburn university soil-testing laboratory (Table.2).

All the chemicals used in the experiment are analytical or higher grade. FeCl₂·4H₂O was purchased from Alfa Aesar. (Ward Hill, MA, USA) Sodium sulfide nona-hydrate, ACS, 98.0% min crystalline (Na₂S· 9H₂O) was purchased from Alfa Aesar. (30 Bond Street, Ward Hill, MA 01835) Metronidazole, 99% was purchased from BTC. (9 Sagamore Park Rd, Hudson, NH 03051) Tris (hydroxymethyl) aminomethane 99.8%, ACS reagent was purchased from ACROS Organics (Janssen Pharmaceuticaaan 3aB-2440 Geel, Belgium). CMC (M.W. = 90000 in sodium form) was purchased from ACROS Organics company. (Morris Plains, NJ, USA) Acetonitrile was of HPLC grade.

Table 2. Physical and Chemical Properties of the Soil Used in This Work.

Table S1. – Physical and Chemical Properties of the Soil Used in This Work													
Sample		Taxonomya			CECb	OMc,	Sc,	PZSEd	Cae	K	Mg	P	
					meq/100g	%	%		mg/L	mg/L	mg/L	mg/L	
Smith Research													
Center soil		Sandy Loam			3.2	0.4	0.02	5.20	234	51	71	11	
Alf	Cu	Fe	Mn	Zn	Na	As	Ba	Cd	Cr	Mo	Ni	Pb	B
mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
72	3	25	22	2	4	<0.1	5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

a. Soil textural analysis was conducted following the Bouyoucos hydrometer method

(Bouyoucos, 1962).

b. CEC: Cation exchange capacity, measured per EPA Method 9081.

c. Organic carbon and sulfur were determined following the combustion method (Kirsten, 1979).

- d. The pH at point of zero salt effect (PZSE) of the soil was measured following the potentiometric titration method (Marcano-Martinez and McBride, 1989).
- e. Soil potassium, calcium, magnesium and sodium were extracted following Melich I procedure.
- f. Soil metal contents were acid digested following EPA method 3230.

2.2. Preparation of CMC-stabilized FeS particles

CMC-stabilized FeS particles were prepared using Gong's method (Gong et al, 2012). For example, when a 300 mg/L FeS with 0.001 wt% CMC was prepared, 76mL DI water, 61.36mL 1g/L CMC solution with 13.55 mg FeCl₂ and 40 mL 4.091 g/L Na₂S were prepared separately and purged with 99.9% N₂ for 20 min in order to remove all the oxygen. Then Na₂S solution and DI water was added into the CMC - FeCl₂ solution under N₂ environment. The reactor was shaken at 200 rpm during this process for 20 min in order to make the solution fully mixed, FeS particles can be prepared under the same situation, and the growth time is same. After preparation, the particles will grow for 24 h at room temperature (20 ± 1 °C). Bare FeS particles will be prepared using the same procedure but the CMC solution will be replaced by DI water. In the experiment, 200 mg/L, 350 mg/L and 500mg/L FeS particles were prepared. Zeta potential and particle size were tested by the dynamic light scattering (DLS) system using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, U.K.).



2.3. Degradation of MNZ in water phase

To test the bare FeS dosage effect, 200 mg/L, 350 mg/L and 500 mg/L bare FeS solution were prepared, the experiment was duplicate and carried out in a 250 mL flask sealed by the rubber stoppers under N₂ condition. 20 mL 0.1mol/L Tris buffer solution was added into 176 mL 200

mg/L of FeS at pH = 7.0. To test the MNZ, 4 mL 500 mg/L MNZ solution was added into the reactor and the reactor was sealed and shaken during the process. The final solution was 200mL containing 200 mg/L of FeS and 10 mg/L MNZ at pH = 7.0. The reactor was shaken at 200rpm at the room temperature (20 ± 1 °C). At the selected time, samples were taken by a glass pipe from the batch and centrifuged at 2000 rpm for 5 min. Then the samples were taken from the supernatant and filtered by a 0.22 μ m membrane to stop the test by removing all the FeS and make the samples ready for the HPLC test. The removal of MNZ by the membrane can be ignored. Continue the test until MNZ in the sample was stable or zero.

To test the CMC stabilization effect on the removal of MNZ, the procedure is the same. But DI water was replaced by the CMC solution. It is important that we should use a 50 nm membrane to filter the sample because the size of CMC stabilized FeS is much smaller than the bare particles. If the 20.22 μ m membrane was used, CMC stabilized FeS can easily pass through and cause blocking in HPLC. In this experiment, the filtration is very slow which might cause the large error in the first few points.

To test the pH effect on the removal of MNZ by CMC stabilized FeS, the same batch tests were conducted in the pH = 6, 7 and 8 buffered by Tris solution (pH = 7 and 8) and sodium acetate buffer solution (pH = 6), control test showed that buffer itself has negligible effect to the MNZ degradation.

To test the HA dosage effect, different concentrations of HA were added into the reactor. The system had the same initial volume, initial FeS dosage and MNZ dosage but different HA dosages which are 1 mg/L, 5 mg/L and 10 mg/L. The control test was also conducted with 0 mg/L FeS. The control test showed that the HA itself has negligible effect to the MNZ degradation.

To test the CMC molar ratio effect on the removal of MNZ, The FeS concentration used in this experiment was 200 mg/L because MNZ was not completely degraded under this dosage according to the experiment before. 61.36 mL 1 g/L CMC solution will be replaced by 2.05 mL, 2.625 mL, 5.125 mL, 7.69 mL, 10.25 mL, 20.5 mL, 30.75 mL and 41 mL 1 g/L CMC solution, the volume of the DI water added will change accordingly to adjust the volume of the final solution was 200 mL.

2.4. Degradation of MNZ in soil phase

2.4.1 Adsorption and desorption test

Adsorption and desorption tests were conducted in a 50 mL glass bottles and sealed by a lid. The test was designed to find a suitable soil sample to conduct the following tests. The adsorption effectiveness should be high while desorption effectiveness should be low then the treatment will be meaningful. The 4 g soil samples were placed in each bottle, 38 mL DI water and 2 mL 500 mg/L MNZ were added. The bottles were sealed and shaken at 200 rpm. At the selected time, two duplicate samples would be taken out from two different bottles, samples were taken by a glass pipe from the bottle and centrifuged at 2000 rpm for 5 min. Then the samples were taken from the supernatant. These samples showed the MNZ concentration in the water phase, then the bottles were centrifuged at 2000 rpm for 5 min, supernatant was taken out by a pipe (about 39 mL) and 39 mL methanol was added. The bottle was sealed by a lid and put in the water bath at 70 °C for 12 h to fully desorb the MNZ from the soil to the methanol then use the same way above to test the MNZ concentration in the methanol to get the MNZ concentration in the soil. The total MNZ from soil and liquid phase should reach 95% of the initial MNZ added to the reactor to make sure the soil did not degrade the MNZ directly.

2.4.2 Degradation test

Degradation test was conducted to test the degradation of MNZ in the soil by FeS in the liquid phase. 500 mg/L FeS was prepared before the experiment. Degradation test was conducted in a 50mL glass bottles and sealed by a cover. The test was designed to show the effectiveness of FeS in degrading the MNZ in the soil. The 4 g soil samples were placed in each bottle, 38 mL DI water and 2 mL 200 mg/L MNZ were added. The bottles were sealed and shaken at 200 rpm. After 24 hrs, the bottles were centrifuged at 2000 rpm for 5 min. Supernatant was taken out by a pipe (about 39 mL) and 39 mL 500 mg/L FeS solution was added. The bottle was sealed by a lid and shaken on a shaken bed. At the selected time, two bottles were centrifuged at 2000 rpm for 5 min. Then, the samples were taken from the supernatant in both bottles as duplicate.

Then supernatant was taken out by a pipe (about 39 mL) and 39 mL methanol was added. The bottle was sealed by a lid and put in the water bath at 70 °C for 12 h to fully desorb the MNZ from the soil to the methanol, and then use the same way above to test the MNZ concentration in the methanol to get the MNZ concentration in the soil.

The total MNZ in the system should be equal to the combination of MNZ in the FeS solution and the MNZ desorbed from the soil by methanol. Different FeS concentrations were tested to show the effectiveness of FeS in degrading MNZ in the soil.

2.4.3 Column test

Column test was conducted to test the soil-sorbed MNZ degradation. Soil was taken from the E.V. smith research center of Auburn University, AL. Before use, the soil has been washed twice a day for a week (14 times) and air-dried for another week to remove all the water-soluble particles, then use a 2 mm sieve filter the soil to remove all the large colloids. The soil was fully

mixed when being used and stored at the room temperature (20 ± 1 °C). In the column test, the soil was firstly tested by DI water to see the breakthrough curve.

The 40 g air-dried soil was added to a glass bottle (500 mL) and 400 mL 10 mg/L MNZ solution was added. The bottle was put on a shaker for 2 days and then air dried to get the MNZ-sorbed soil. In each test, 12 g of MNZ sorbed sandy loam was dry-packed in the column. About 6 pore volume of a glass flasks was added in the column to avoid the air in the column. Then the FeS solution prepared before was pumped through the column. At the selected times, samples were taken from the column, the sample will be neutralized by the HCl solution to make it ready for the HPLC test. Fe was tested by ICP to get the Fe particle breakthrough curve and MNZ was tested by HPLC to get the MNZ leaking breakthrough curve. All the columns lasted for about a week until the MNZ concentration in the samples became stable.

2.5. Chemical analysis

MNZ was analyzed using an HP Agilent 1100 high performance liquid chromatography (HPLC) system equipped with a ZORBAX SB-C18 column (4.6 150 mm, 3.5 mm) and the analytical method was from Zhangqiang et al (2011). The mobile phase was a mixture of 20:80(v/v) of acetonitrile and water and flow rate was 1mL/min. The wavelength of 210nm was used as UV detector (G1314A VWD). Fe ion was analyzed using an inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista-MPX Axial Spectrometer, Varian, Walnut Creek, CA, USA) per EPA method 3050B. The potentiometric titration method was used to determine the soil point of zero charge (PZC) (Vanraij and Peech, 1972). The particle hydraulic size and zeta Potential was analyzed by using a Nano-ZS90 Zetasizer (Malvern Instruments, UK). An Oakton pH meter (pH 510 Benchtop Meter, Oakton Instruments, Vernon Hills, IL, USA) was used to test the solution pH.

3. Results and discussion

3.1 Effect of FeS dosage

FeS dosage effect on the removal of MNZ was investigated in a series of batch experiments. From Figure.1, 10 mg/L MNZ was degraded by FeS with different concentration. The initial pH is 7.5 and the final pH is 6.8. It shows that the MNZ concentration decreases significantly over time. The removal efficiency is 31.6%, 100%, 100% in the presence of 200, 350 and 500 mg/L FeS within the same reaction volume. Higher FeS concentration will lead to higher removal rate, from the figure.1, removal efficiency is much lower than the other two lines when FeS concentration is 200 mg/L, the removal efficiency was found to be 31.6% for the FeS dosage of 200 mg/L. After 120 minutes, the removal rate was almost zero (C_t/C_0 did not change over the time) and MNZ concentration achieved the steady state. The MNZ was fully removed when the FeS dosages are 350 mg/L and 500 mg/L and the removal rate for dosage of 500 mg/L is higher than the dosage of 350 mg/L, indicates that increasing the FeS dosages will increase the total particle surface areas and the adsorption on active sites. With the stirring, when the concentration of FeS was increased, the surface area of activated sites on FeS surface increased, so the degradation rate of the MNZ in the solution increased accordingly.

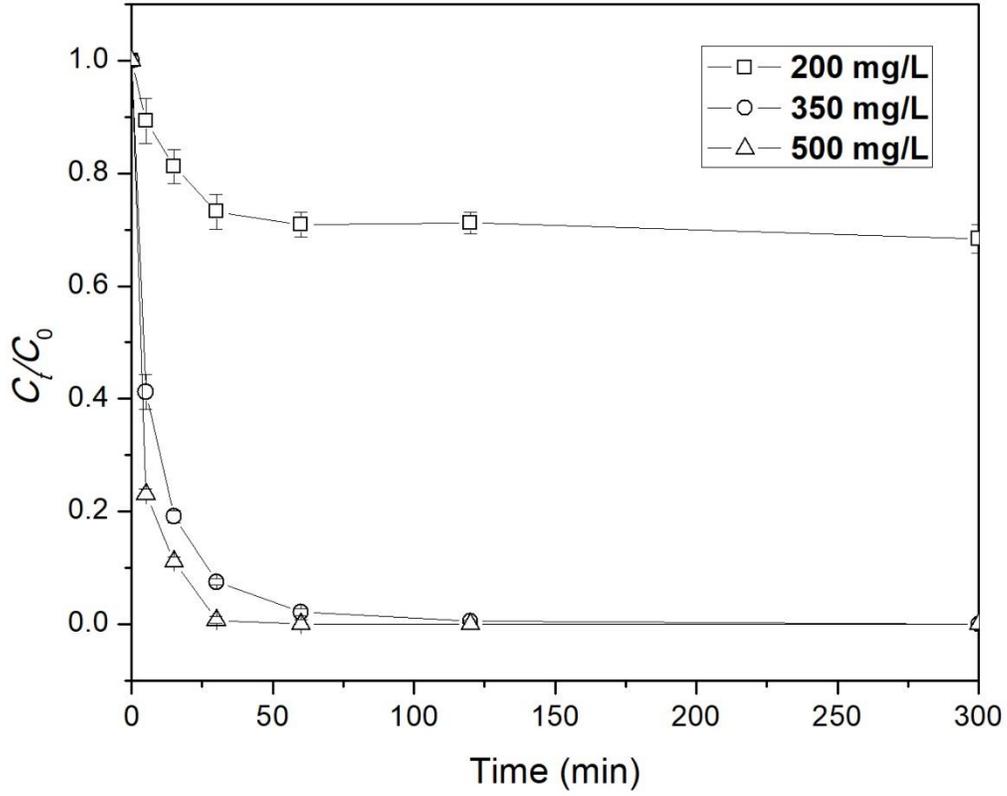


Fig. 1. FeS dosage effect on the removal of MNZ. Date points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration = 10 mg/L, initial pH = 7.5, reaction volume = 200 mL)

Kinetic model used for fitting the data was pseudo first order,

$$\frac{dc}{dt} = -kC \quad (2)$$

$$C = C_0 * e^{-kt} \quad (3)$$

$$\ln\left(\frac{C}{C_0}\right) = -kt \quad (4)$$

Where C_0 is the initial MNZ concentration, k is the first rate constant and C is the MNZ concentration. The first order rate constant (k) and corresponding correlation coefficients (R^2) for the removal of MNZ are listed in table 3.1. The value of R^2 is larger than 0.70 means the data fits

the pseudo first order well. The equations above can be used to calculate the MNZ degradation by FeS.

Table 3. First order rate constant (k) and corresponding correlation coefficient (R²)

FeS	MNZ	pH	k	R ²
mg/L	mg/L		1/min	
200	10	7.5	0.0018	0.749
350	10	7.5	0.1482	0.9811
500	10	7.5	0.2734	0.9961

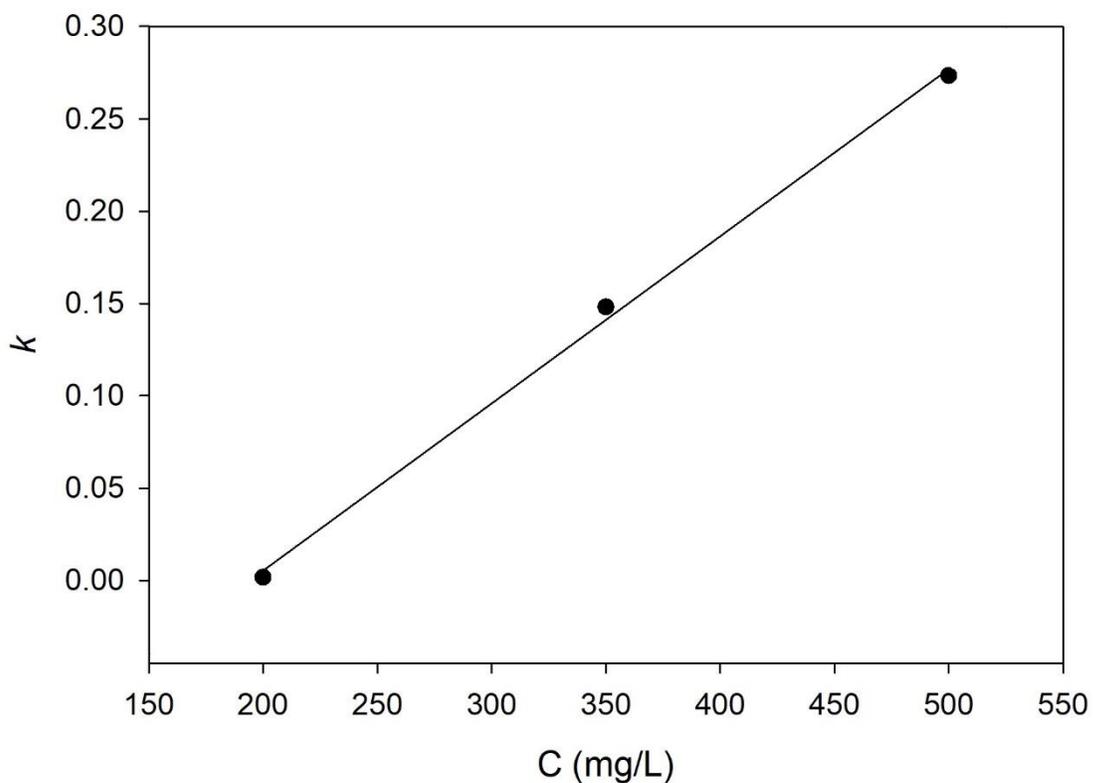


Fig. 2. Relationship between the concentration of FeS and first order rate constant.

The figure.2 shows the relationship between the concentration of FeS and first order rate constant. The FeS concentration was proportional to the removal rate constant which means that we can improve the k in Eqn.2 by increasing the FeS concentration. If k increase proportionally, dC/dt (reaction rate) will increase proportionally.

3.2. CMC Stabilization Effect

3.2.1 CMC effect

The effect of CMC stabilization on the removal of MNZ was investigated in a series of batch tests. Figure.3 shows the degradation of MNZ in the presence of bare particle or CMC-FeS. The MNZ concentration decreases significantly over time. Within the same reaction volume, CMC stabilized FeS will lead to higher removal rate, from the figure.3, removal efficiency for CMC stabilized particle is lower than the bare particle. At 120 minutes, the removal rate was almost zero, The removal efficiency is 31.6% while using the bare FeS and The removal efficiency is 79.2% while using CMC-FeS which indicating that CMC stabilization can lead smaller particle size and large particle surface area, active sites on the particle surface increased accordingly, as a result, reaction rate increased.

CMC was used during the process of FeS preparation, which can effectively control the particle size by controlling the particle's nucleation and growth. CMC covered the FeS particle and form the electrostatic repulsion and steric hindrance from particle to particle, which can prevent the FeS particles from aggregating (Gong et al, 2012). In the experiment, bare FeS was found to be easily oxidized while CMC stabilized FeS could stay for longer time even exposed in the air, indicating the CMC on the FeS surface can also protect the FeS from being oxidized which increase the effectiveness of the FeS after one day growth. CMC-FeS was also found suspended

in the solution while bare FeS precipitated indicating that the size of CMC-FeS is smaller than the bare FeS, the gravity can be ignored.

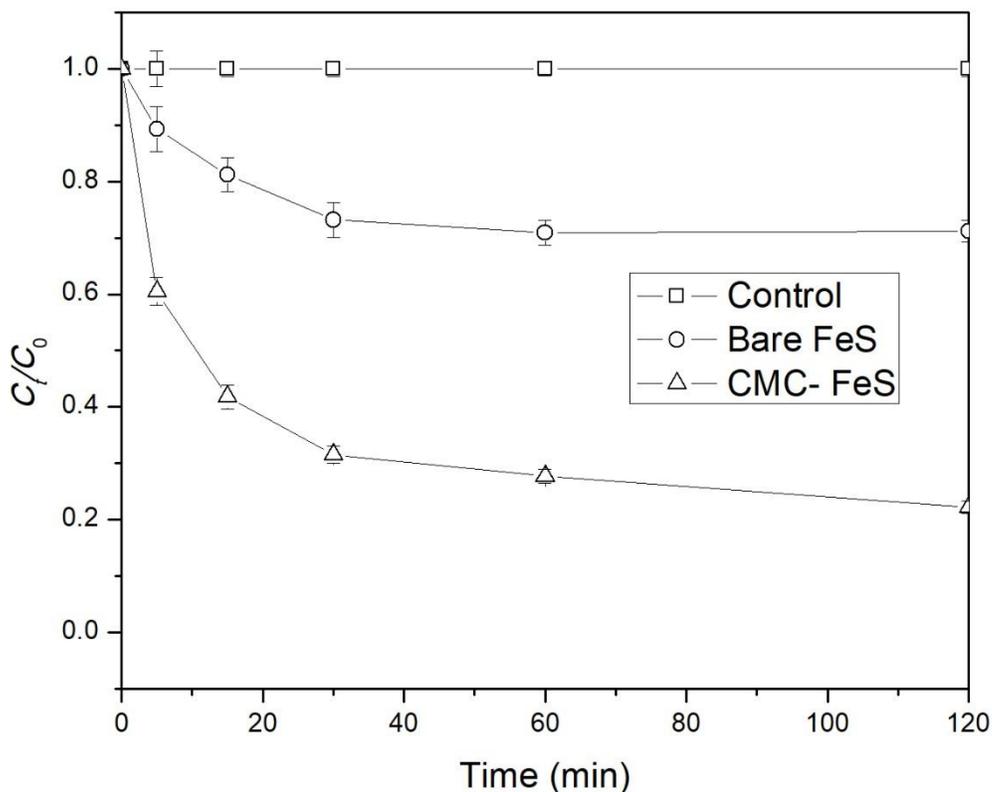


Fig. 3. Effects of CMC stabilization on the removal of MNZ. Date points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration = 10 mg/L, initial pH = 7 ± 0.2 , CMC: FeS = 0.001:1, reaction volume= 200 mL, FeS concentration = 350 mg/L)

3.2.2 CMC to FeS molar ratio

The effect of variations in CMC to FeS molar weight on the removal of MNZ was investigated in a series of batch tests. Figure.4 shows the removal efficiency in the presence of CMC-FeS with different CMC to FeS molar ratio after 24 hrs. Within the same reaction condition, the removal efficiency is low when the CMC to FeS molar weight is too low or too high, The

removal efficiency is 25.3%, 43.2%, 36.8%, 43.0%, 45.6%, 35.2%, 13.3% and 8.2% when the CMC to FeS molar ratio is 0.000375, 0.0005, 0.00075, 0.0015, 0.00225, 0.003, 0.006 and 0.0075. From the figure.4, removal efficiency is higher when CMC to FeS molar weight is between 0.0005 and 0.003. The removal efficiency decreased significantly when the removal efficiency was lower than 0.0005 and higher than 0.003. After 24h, the removal efficiency was only 8.2% when CMC to FeS molar weight was 0.00025 while the removal efficiency was 45.6% and 43.2% when CMC to FeS molar weight is 0.0005 and 0.00225, which indicates that CMC to FeS molar weight have a suitable value. With this value, the CMC stabilized FeS can get the best effectiveness. The following experiment, we used 0.00225 CMC to FeS molar weight in all situations and 24 hours will be spent to let the particle grow.

Based on the previous work, CMC molecular can be used as a stabilizer to the iron-based nanoparticles such as ZVI and FeS by being adsorbed to nanoparticle's surface (He et al., 2007), FeS (Xiong et al., 2009), Fe₃O₄ (An et al., 2011),

CMC was adsorbed by the particles, it can be used to stabilize the particles through producing steric, and electrostatic repulsions, which can prevent the nanoparticles from aggregation thus the particle size, will be smaller because of the lack of aggregation. CMC concentration would be different based on the type of the particles. We can control the particle size by controlling the CMC concentration. From the figure.4, we could see that when CMC to FeS molar weight was 0.00225, the FeS particle was fully stabilized. The particle size will become larger either increasing or decreasing the CMC to FeS molar ratio. In addition, the best removal efficiency is achieved at this point. The suitable CMC to FeS molar ratio will lead to smaller particle size by controlling the particle growth, thus more surface area was achieved and removal efficiency will increase accordingly. However, with higher CMC to FeS molar ratio, CMC will

cover the surface of the FeS which will block the active sites and contact of the MNZ and FeS, thus, the removal efficiency decreases when the CMC to FeS molar ratio is too high.

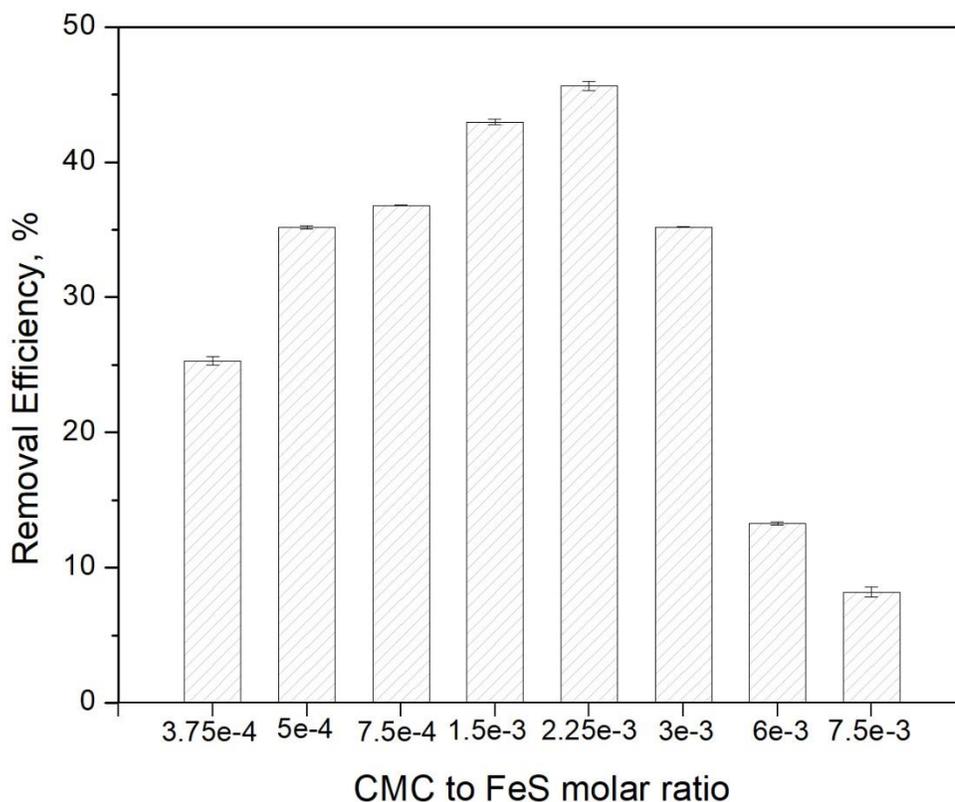


Fig. 4. Effects of variations in CMC to FeS molar weight on the removal of MNZ. Data points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration = 10 mg/L, reaction volume = 200 mL, FeS concentration = 200 mg/L, pH = 7 ± 0.2)

From Table 4, the mean hydrodynamic size of CMC- FeS was 225.6 nm and the mean hydrodynamic size of bare FeS was 825.35 nm. The stabilized particles can be stable in the sealed flask for 2 days (black). Lower CMC to FeS molar ratio will significantly increase the particle hydrodynamic size to 825.35 nm and particle will precipitate in 6 hours to the bottom of the flask and easily be oxidized to Fe³⁺ (yellow). More CMC concentration will also lead to uniform

hydrodynamic size, i.e. the hydrodynamic size is unstable when bare FeS particle is prepared. From the Table.4, Zeta potential of the CMC-FeS was more negative, which means more electrostatic repulsion. The 0.00225 CMC to FeS molar ratio was used in the following experiment. From Gong et al (2012), research where we can get the similar result.

Table 4. Zeta potential and hydraulic size of CMC-FeS and bare FeS

	zeta potential	hydrodynamic size
CMC-FeS	-40.3 mV	225nm
	-39.6 mV	226.2nm
bare FeS	-29.1mV	912.3nm
	-29.7mV	738.4nm

3.3. Effects of pH

From Figure. 5, the effect of variations in pH on the removal of MNZ was investigated. Figure shows that the MNZ concentration decreases over time. Within the same reaction volume, Lower pH will lead to higher removal rate. From the figure.5, removal efficiency is higher when the pH = 7 and 8. At 120 minutes, the removal rate was very low and MNZ was almost removed when the pH = 7 and 8, however, MNZ left about 35% when the pH = 6. The possible reason may be: (1) at the lower pH, iron oxidization could be higher, which will lead to the loss of the Fe²⁺ in the solution based on the Eqn. 5 because of the small amount of oxygen in the reaction system (Zhang et al., 2006). (2) The pH is low means more H⁺ in the water which will lead to more positive zeta potential. Smaller electrostatic repulsion can get by less negative zeta potential, the aggregation of particles will be easier and lead to larger particle size, i.e. smaller particle surface area and fewer active size. This might cause lower reaction rate and efficiency. (3)The much lower

uptake at pH = 6 is because of the partial dissolution of FeS nanoparticles and loss of binding sites. I.e. ion exchange and surface complexation caused the loss of FeS (Gong et al, 2012).

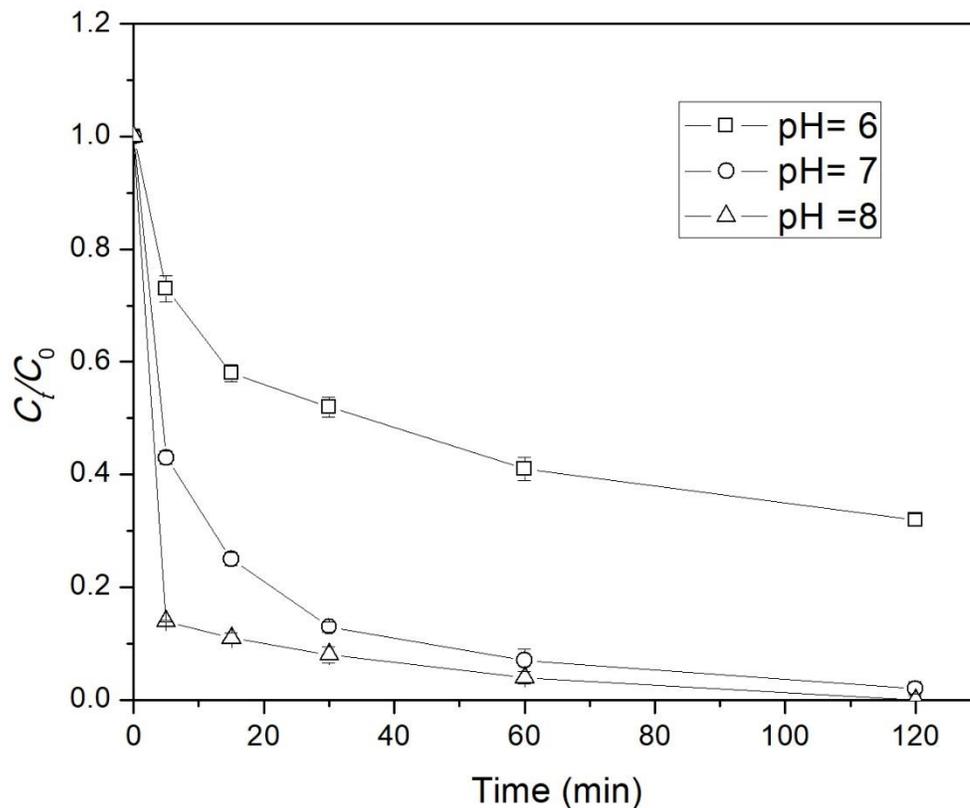


Fig. 5. Effects of variations in pH on the removal of MNZ. Data points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration = 10 mg/L, reaction volume = 200 mL, FeS concentration = 200 mg/L, Buffer: Tris for pH = 7 and 8, Sodium acetate buffer solution for pH = 6)

3.4. Effect of humic acid

The effect of variations in HA concentration on the removal of MNZ was investigated in a series of batch tests. The pH was controlled by tris solution. Figure.6 shows 10 mg/L FeS was degraded by 350 mg/L FeS in the presence of humic acid. MNZ concentration decreased over time. Within the same reaction volume, Lower HA concentration will lead to higher removal rate. From the figure.6, the removal efficiency is 100%, 77.9%, 72.1% and 75.6% in the presence of 0, 1, 5 and 10 mg/L humic acid. Removal efficiency is higher when HA did not exist in the system. At 120 minutes, the removal rate was low and MNZ was only highly removed when HA did not exist. MNZ left about 35% when HA was added.

HA was reported to be a major problem for the organic degradation in wastewater (Chaplin et al, 2006). Atomic hydrogen can be shared by both MNZ and HA, compared with no HA situation, MNZ used all atomic hydrogen (Zhu et al, 2008). In addition, the HA may also be adsorbed at the FeS surface, blocking the active sites on the FeS which will affect the removal rate and efficiency (Han et al, 2017).

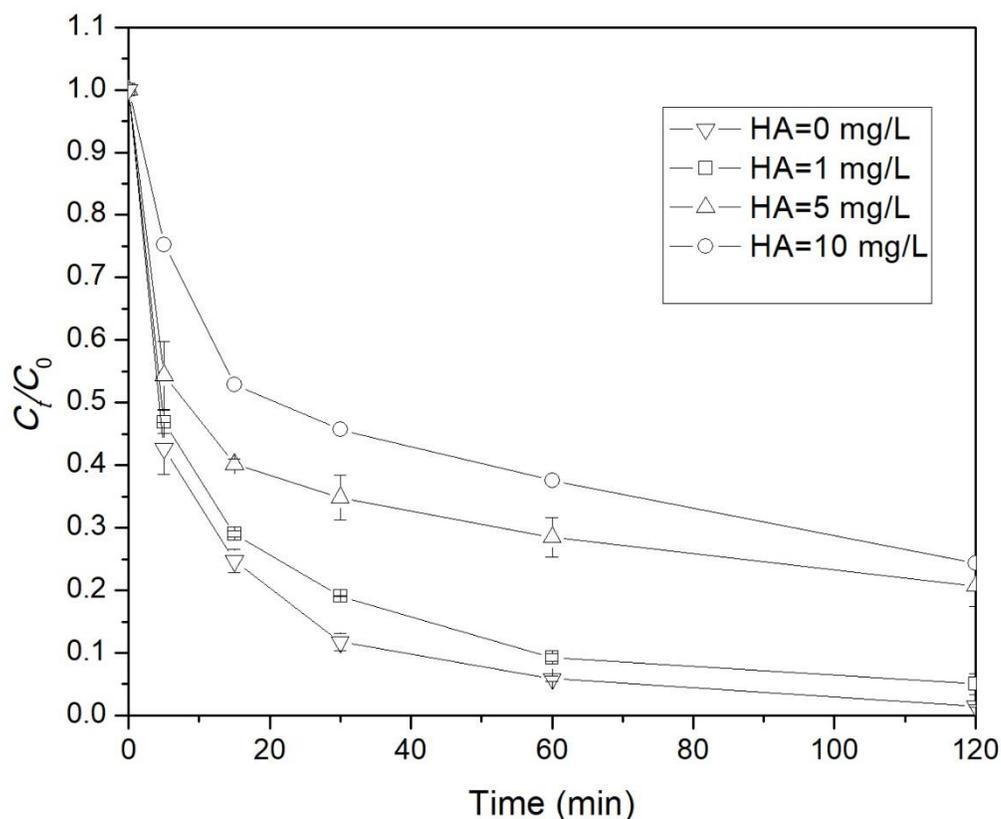


Fig. 6. Effects of variations in HA concentration on the removal of MNZ. Data points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration = 10 mg/L, reaction volume = 200 mL, FeS concentration = 350 mg/L, pH= 7 ± 0.4 , CMC: FeS = 0.001: 1, HA: IHSS leonardite HA (NaOH dissolved))

3.5. Soil test

3.5.1 Adsorption and desorption test

From two figures, figure.7 showed that the MNZ left in the solution over time when MNZ was adsorbed by the soil from the solution. Figure.7 showed that 61.4% MNZ was left in the solution after 2 days and the concentration of MNZ was almost stable after one day. The adsorption rate decreased over time. The final concentration of the FeS left in the solution is 4.12 mg/L, and

MNZ in the soil phase was 26.2 mg MNZ/kg soil, which was based on calculation and proven by the methanol desorbed test. The total amount of MNZ left in the solution and MNZ desorbed by the methanol was more than 95% of original MNZ.

Figure.8 showed that desorption of MNZ from soil to the solution, the soil in this experiment was from the adsorption test after 2 days. MNZ in the solution increased over time. Figure.8 showed that 78.1% MNZ was desorbed to the solution after 8 days and the concentration of MNZ was almost stable after 4 days. The removal rate decreased over time. The final concentration of the FeS left in the solution is 8.03 mg/L, and MNZ in the soil phase was 5.1614 mg MNZ/kg soil, which was based on calculation and proven by the methanol desorbed test. The total amount of MNZ left in the solution and MNZ desorbed by the methanol was more than 95% of original MNZ.

The adsorption and desorption rate and desorption efficiency is suitable for the degradation test in the batch test and column test. The adsorption test is fast enough and the desorption rate is low enough to make sure the test is meaningful, i.e. MNZ will not be fully desorbed by the water directly.

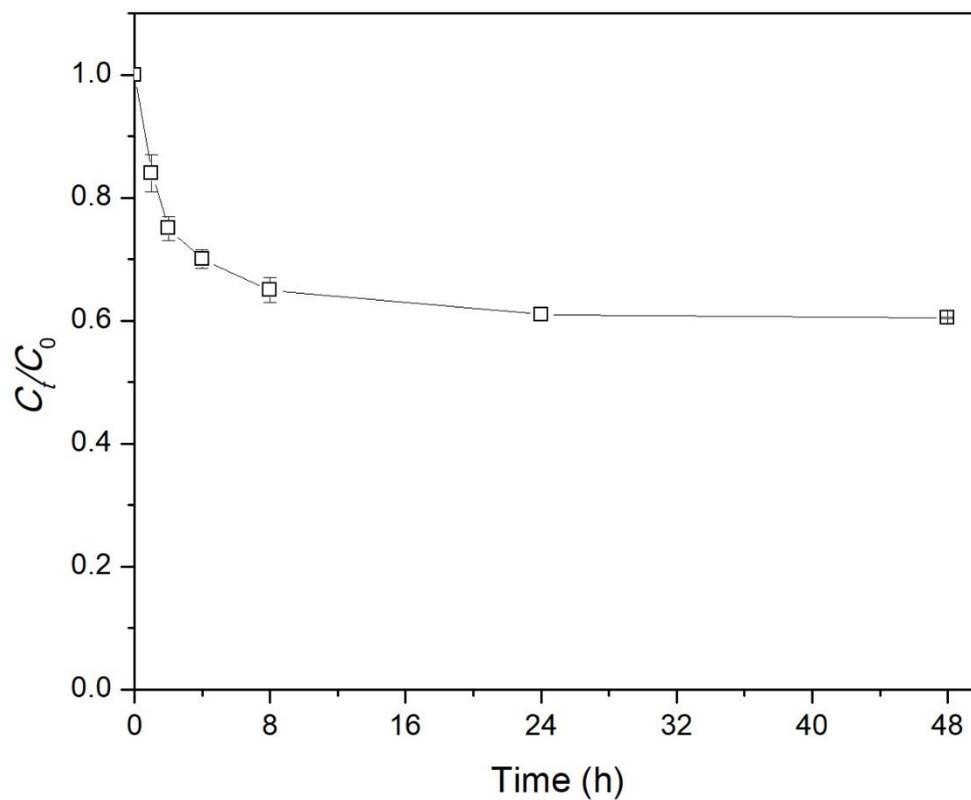


Fig. 7. The adsorption of MNZ by the soil (sandy loam). Data points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration in liquid phase = 10 mg/L, liquid phase volume = 40 mL, solid phase weight = 4 g, pH = 5.5 ± 0.5)

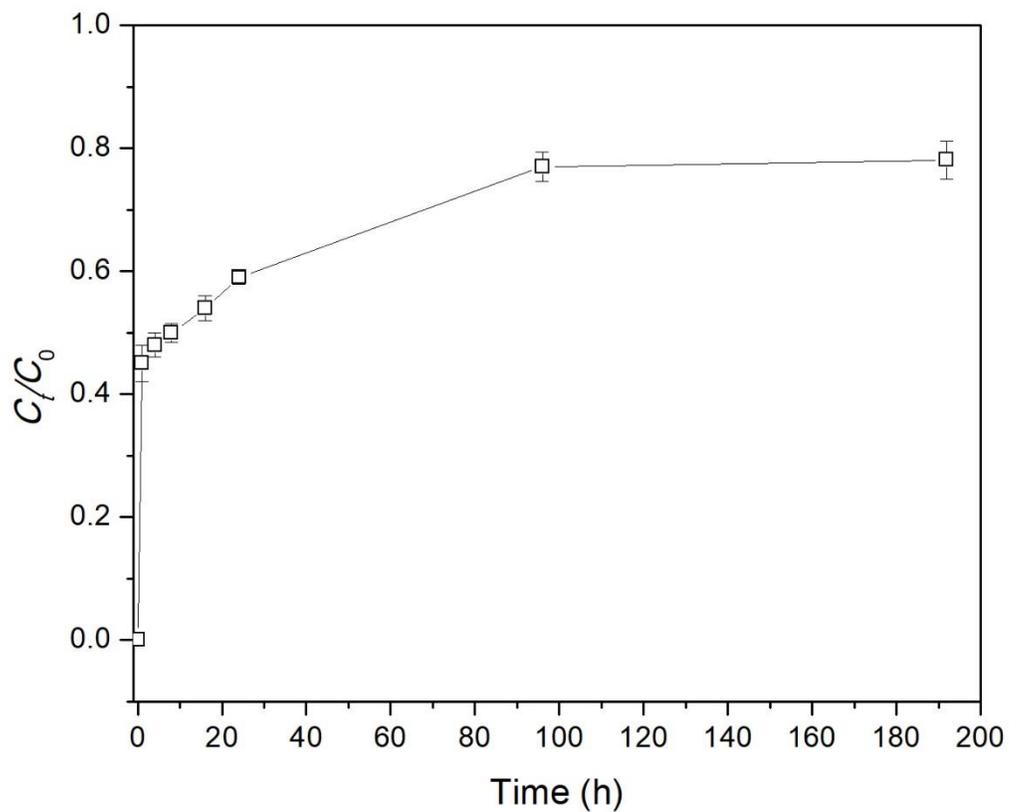


Fig. 8. The desorption of MNZ from the soil (sandy loam). Date points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration in soil = 26.2 mg MNZ/kg soil, liquid phase volume = 40 mL, solid phase weight = 4 g, pH = 5.5 ± 0.5)

3.5.2 Degradation test

The effect of variations in FeS concentration on the removal of MNZ was investigated. 500 mg/L FeS on the removal of MNZ in soil was investigated first. Figure.9 shows that the MNZ concentration decrease significantly over time. The removal rate decreased over time. From the figure.9, removal efficiency is much lower than the degradation of MNZ in solution, the removal efficiency was found to be 95.8% for the FeS dosage of 500 mg/L. After 4 days, the removal rate was almost zero. The MNZ was 4.2% left for the FeS dosage of 500 mg/L,

When FeS concentration was changed to 350 mg/L, the removal rate was found to be 67.3% for the FeS dosage of 350 mg/L the MNZ was 32.7% left for the FeS dosage of 350 mg/L, indicating that increasing the FeS dosages increased the total particle surface areas and the adsorption on active sites. This not only works in solution but also soil phase.

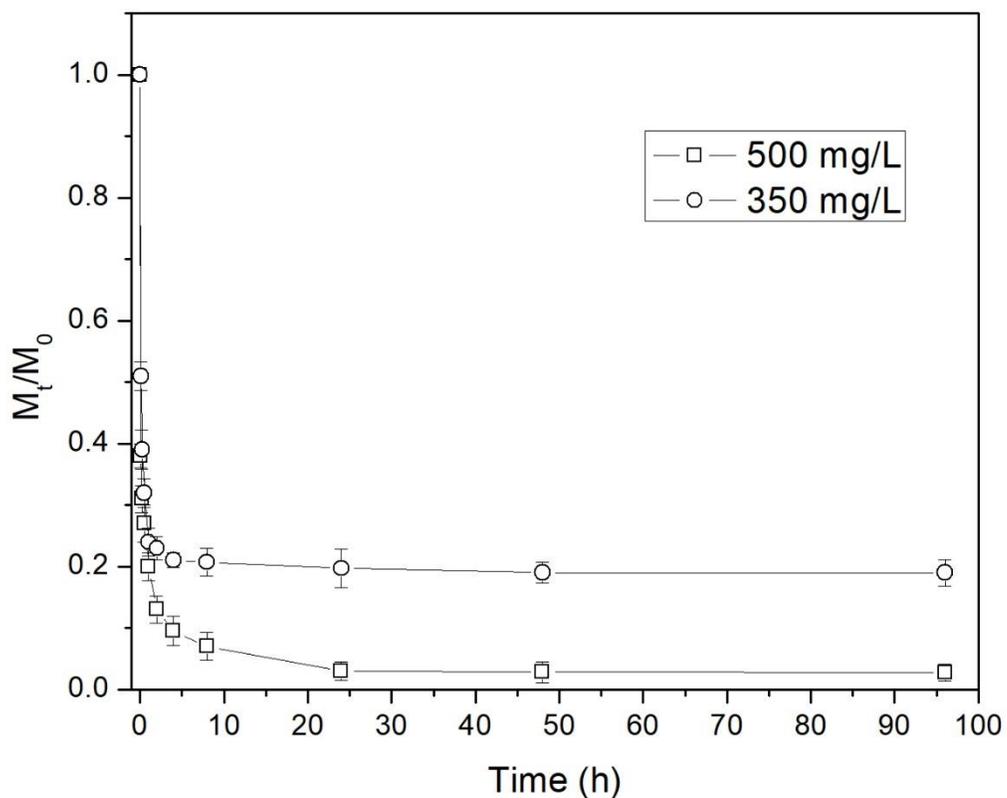


Fig. 9. M_t : mass in the soil; M_0 : initial mass. The degradation of MNZ from the soil by CMC-FeS. Date points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration in soil = 26.2 mg MNZ/kg soil, liquid phase volume = 40 mL, CMC-FeS concentration in the liquid phase = 4 g, pH = 5.5 ± 0.5)

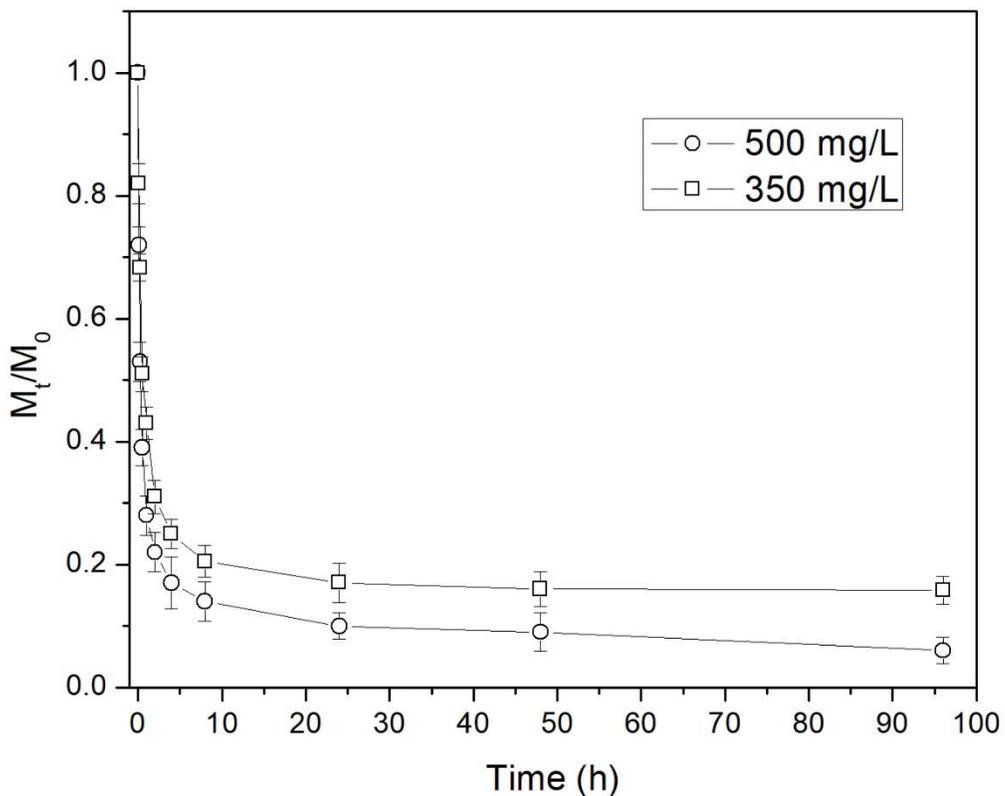


Fig. 10. M_t : mass in the whole system; M_0 : initial mass. The degradation of MNZ from the soil by CMC-FeS. Data points and error bars represent the average of duplicate samples and standard deviation. Respectively. (Experimental conditions: initial concentration in soil = 26.2 mg MNZ/kg soil, liquid phase volume = 40 mL, CMC-FeS concentration in the liquid phase = 4 g, pH = 5.5 ± 0.5)

3.5.3 Column test

The soil deliverability and efficiency of the nanoparticles was tested by measuring breakthrough curve. Figure.11 shows the breakthrough curve of FeS and MNZ through the soil bed. The FeS breakthrough occurred quickly at 1.2 PV and reach the full breakthrough at 11 PV, and kept at a constant C/C_0 over 0.90 after the fully breakthrough point. This high breakthrough plateau at the low inject pressure indicates that CMC- FeS is deliverable through the soil which means FeS have a potential to be used in remediation uses. On the other hand, ~41.3% of the injected nanoparticles stayed in the soil phase during the whole process calculated by breakthrough data. This indicates that FeS can be transferred to soil phase and used to degrade the MNZ in the soil. In the practical application point. CMC-FeS can be transferred to certain places by controlling the injection pressure and stopping the transport by change the injection pressure to 0 (He et al., 2009). In contrast, it is difficult to transfer the bare FeS through the soil because of its large particle size. From figure. 12, MNZ elution history in the soil was investigated, MNZ elution concentration keep increasing until 6 PV and reach the highest point at 6 pV then MNZ eluted concentration went down. This indicates that MNZ was transferred from the soil to thermodynamically more stable particle and then it can be degraded by the CMC-FeS nanoparticles.

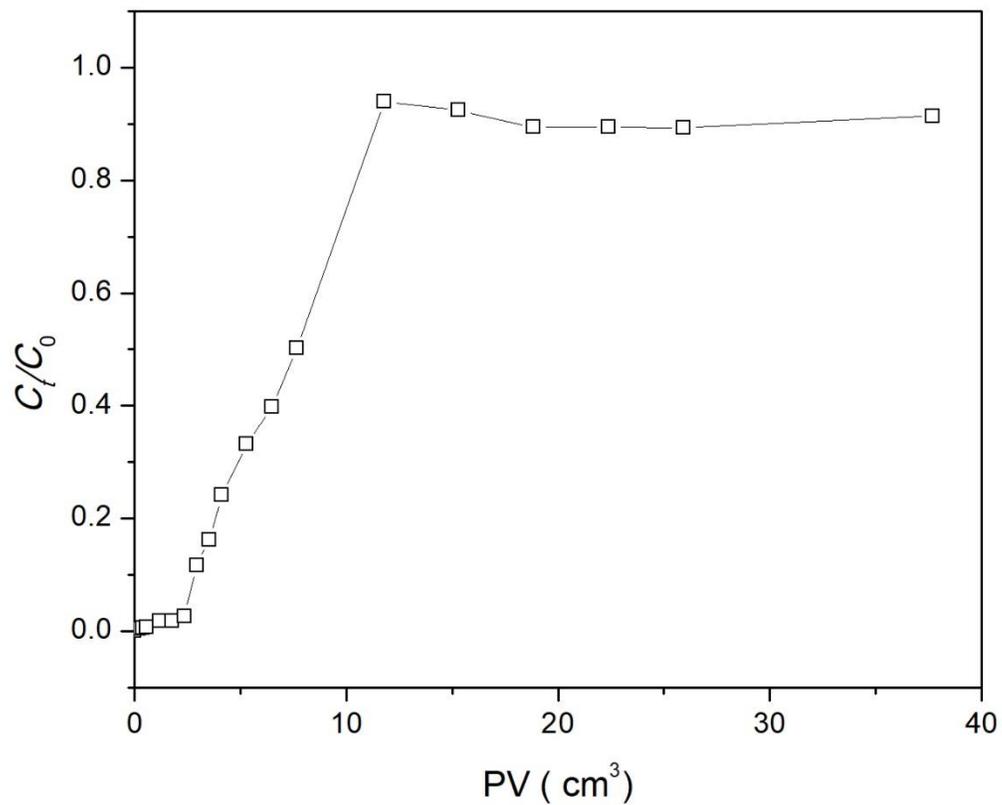


Fig. 11. Breakthrough curve of CMC- stabilized FeS through a sandy loam soil. FeS = 500 mg/L, soil in the column = 12g (air- dried), inlet flow = 0.05 mL/min, pore velocity = 0.167 cm/min, C and C_0 are influent and effluent concentrations.

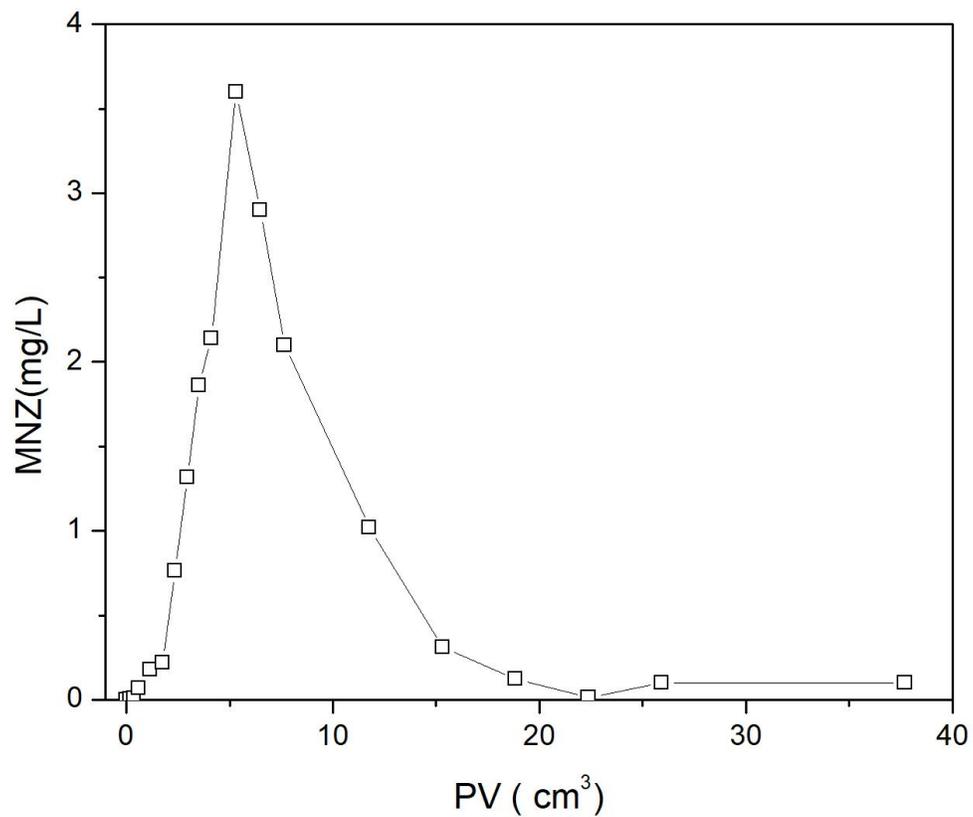


Fig. 12. MNZ elution history from soil when subjected to 500mg/L CMC-FeS nanoparticles (CMC: FeS = 0.001: 1, soil in the column = 12g (air- dried), inlet flow = 0.05 mL/min, pore velocity = 0.167 cm/min)

3.6. Reaction mechanism

The LC –MS figure.13 of the MNZ degradation at different reaction time was investigated. When the bare FeS was used, from the figure.13a, 13b and 13c, Metronidazole peak (No.2 peak) at retention time of 2.4 min decreased over the whole reaction time while the intermediate products and final products appeared. One major product and four byproducts were observed from the figure.13c at retention time 0.46 min, 5.35 min, 6.64 min, 7.73 min and 8.6 min. Obvious, at 0 min, no peaks appeared before the MNZ retention time. Then, from the reaction time of 1min, the No.1 peak keep increased. No.1 Peak indicates the formation of 1-(2-hydroxyethyl)-2-methyl-5-aminoimidazole which produced from the nitro-degradation process of MNZ, the possible reaction process is shown in Eqn. 6. No. 4, 5 and 6 peaks appeared since 1 min and No. 3 peak appeared since 30 min. These peaks are possibly the by-product of the reaction between Na^+ and the organic groups or the combination of the organic groups, which will form larger organic compounds. Before 5 min, No. 6 peak showed increasing trend but it decreased at 10 min, at 15 min, it increased again until 30 min. The change of the No. 6 peak indicated that this might stood for an intermediate product. Other peaks all slowly increased which indicates the final products. The LC- MS results showed the No. 3 peak stood for $\text{C}_{16}\text{H}_{35}\text{N}_3\text{O}_3\text{Na}$, $\text{C}_{18}\text{H}_{34}\text{N}_3\text{O}_3$ or $\text{C}_{21}\text{H}_{35}\text{NONa}$; No. 4 peak stood for $\text{C}_{30}\text{H}_{45}\text{O}_3$; No. 5 stood for $\text{C}_{33}\text{H}_{57}\text{N}_3\text{O}_3\text{Na}$, $\text{C}_{35}\text{H}_{56}\text{N}_3\text{O}_3$ or $\text{C}_{38}\text{H}_{57}\text{NONa}$; No. 6 peak stood for an unknown compound, which should be, discussed in the future research.

When the CMC- FeS was used, From the figure.13a 13d and 13e, The peak standing for metronidazole decreases to 0, and the peak standing for 1-(2-hydroxyethyl)-2-methyl-5-aminoimidazole is the only product in the system. Which means that the use of CMC can prevent the formation of by-products.

Compare the figure. 13c and 13d, after 1h, MNZ peak was 0 in the figure. 13d, which means MNZ was fully removed by CMC- FeS in 1 hr. However, MNZ peak was still high in 13c which means MNZ cannot be fully degraded by bare FeS in 1hr. CMC can improve the removal efficiency and rate.

From the changes of the LC-MS figure.13, Nitro- reduction process might be the main process during the MNZ degradation, and the main product is 1-(2-hydroxyethyl)-2-methyl-5-aminoimidazole.

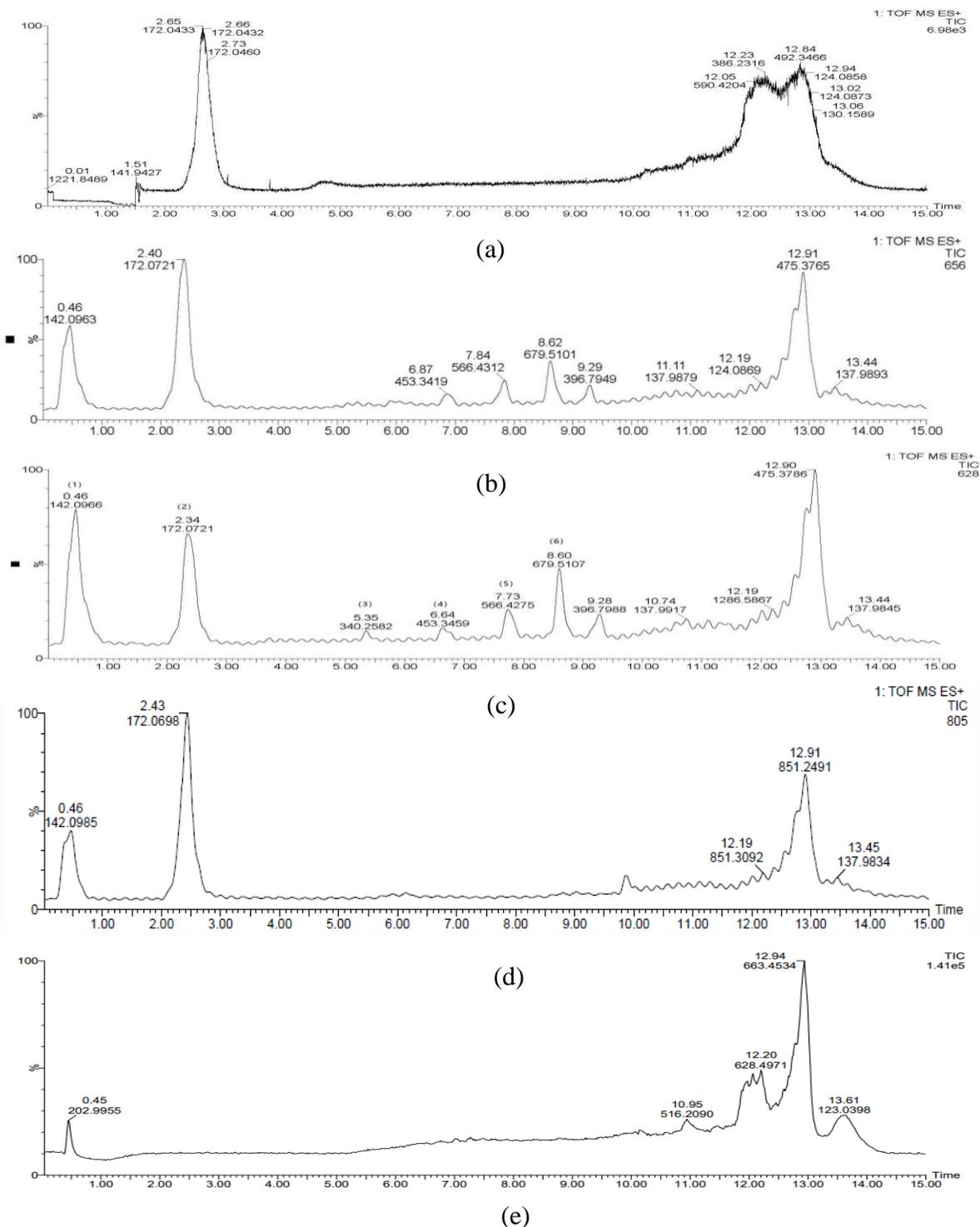
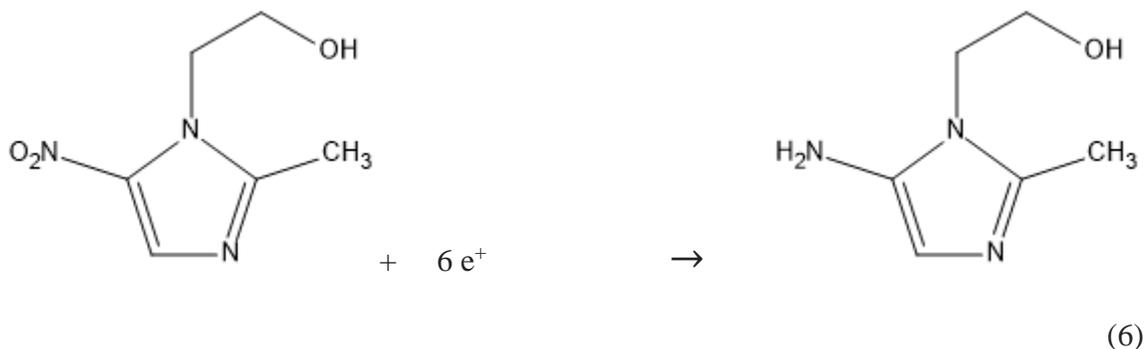


Fig. 13. (a) LC-MS spectra of MNZ in the water (MNZ = 40 mg/L); (b) LC-MS spectra of MNZ degradation in the water by bare FeS at 15 min (MNZ = 40 mg/L, bare FeS = 350 mg/L); (c) LC-MS spectra of MNZ degradation in the water by bare FeS at 60 min (MNZ = 40 mg/L, bare FeS = 350 mg/L); (d) LC-MS spectra of MNZ degradation in the water by CMC-FeS at 1 min (MNZ = 40 mg/L, FeS = 350 mg/L CMC: FeS = 0.001: 1) LC-MS spectra of MNZ degradation in the water by CMC-FeS at 60 min (MNZ = 40 mg/L, FeS = 350 mg/L CMC: FeS = 0.001: 1)



(Fang et al, 2011)

4. Conclusions

From the batch test, bare FeS solution and CMC stabilized FeS solution are prepared, both two kinds of particles are tested for the effectiveness of degrading MNZ under different conditions (Different FeS concentration, pH, HA concentration and CMC to FeS molar weight). The major findings are listed as followed:

Within the same reaction volume, higher FeS concentration will lead to higher removal rate. The removal rate was found to be 69.4% for the FeS dosage of 200 mg/L. After 120 minutes, the removal rate was almost zero. The MNZ was fully removed for the FeS dosage of 350 mg/L and 500 mg/L. The data fits the pseudo first kinetic model.

1. Within the same reaction volume and reaction condition, CMC stabilized FeS will lead to higher removal rate due to large surface area.
2. Within the same reaction volume, higher pH will lead to higher removal rate. Removal efficiency is higher when the pH = 7 and 8 and the MNZ in both two conditions are almost removed.

3. Within the same reaction volume and reaction condition, Lower HA concentration will lead to higher removal rate. MNZ left about 35% when HA was added as 10 mg/L. The removal rate was also lower when the HA existed.

4. Within the same reaction condition, the removal efficiency is low when the CMC to FeS molar weight is too low or too high. Removal efficiency is higher when CMC to FeS molar weight is between 0.0001 and 0.0004. From the experiment, when CMC to FeS molar weight was 0.0001, the FeS particle was fully stabilized.

From the soil test, CMC stabilized FeS solution are prepared, particles are tested for the effectiveness of degrading MNZ in the soil in batch test and column test. The major findings are listed as followed:

5. The adsorption and desorption rate both decreased over time. 61.4% MNZ was left in the solution after 2days and the concentration of MNZ was almost stable after one day. 78.1% MNZ was desorbed to the solution after 8days and the concentration of MNZ was almost stable after 4 days.

6. Removal efficiency of MNZ degradation in soil is much lower than the degradation of MNZ in solution.

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