國立臺灣大學工學院環境工程學研究所

碩士論文

Graduate Institute of Environmental Engineering

College of Engineering

National Taiwan University

Master Thesis

自身光敏藥物氨甲蝶呤對錳的光氧化還原機制之研究

Photoredox Mechanism of Manganese via Self-Photosensitized Compound Methotrexate

蘇怡瑄

Yi-Hsuan Su

指導教授：林郁真 博士

Advisor: Angela Yu-Chen Lin, Ph.D.

中華民國 108 年 7 月

July, 2019

doi:10.6342/NTU201901808

國立臺灣大學碩士學位論文

口試委員會審定書

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本論文係蘇怡瑄君(學號 R06541113)在國立臺灣大學環境工程學研究所完成之碩士學位論文，於民國 108 年 7 月 11 日承下列考試委員審查通過及口試及格，特此證明

論文審查委員：

林郁真博士
國立台灣大學環境工程系教授

林逸彬博士
國立台灣大學環境工程系副教授

康佩群博士
Professor of Department of Civil & Environmental Engineering, University of Utah

指導教授：

所　　長：

誌謝

首先要感謝的是指導老師——林郁真教授，在最後這兩年的求學階段讓我學到很多教課書不會講的事，例如資料該如何引用才不會變成抄襲、搜集文獻資料的時候要注意什麼資訊、學術簡報如何製作才顯得脈絡清晰等等，每次 meeting 都悉心指正我們應該注意的地方，所以在研究所期間真的獲益良多。尤其是實驗假說的部分多虧老師的引導才順利建立，論文的完成度因而大大提升，而且口試簡報的製作在老師的提點之下也更加引人注意並獲得口試委員們的讚賞，真的非常謝謝老師這兩年的指導。

也感謝實驗室的成員們，Webber、明皓、明季、政憲及宥翔等博士班學長以他們豐富的經驗給予我各方面的建議，在儀器或是實驗操作上有問題時也都十分熱心的教導指正，特別是 Webber 及明皓學長在我的論文撰寫與修改上花費了許多心思；還有碩士班學長姐坤霖、昆圃及昱蓉，從他們身上更深刻的了解如何在碩士短短兩年期間盡其所能的完整一個碖文的架構；患難與共的同屆夥伴松娟及冠宇更是在我徬徨無助時給我許多鼓勵與建議；而學弟妹俊宇、姿伃、至演及欣瑜則是在我們畢業典禮還有準備口的時候非常有義氣的情義相挺。很喜歡我們實驗室的氛圍與風格，多虧整個實驗室團隊在實驗架構及操作上的互相切磋討論、報告時的指正建議還有實驗室運作的分工，這些都使我這兩年在學術研究跟待人處事方面有許多成長。除此之外也感謝家人及親友的支持，陪我度過實驗不順利的低潮期，讓我再次打起精神，最後順利完成碩士論文。

最後也要感謝學校提供良好的資源以及師資，讓我們有機會得以專心地進行學術研究。

doi:10.6342/NTU201901808

摘要

許多藥物無法經由傳統污水處理程序有效去除，導致其普遍殘留於環境水體並經歷各種自然淨化途徑；其中，太陽光降解反應及自然界豐富存在的二氧化錳礦物所造成的氧化還原作用為藥物於水環境內的關鍵自然淨化機制。許多藥物吸收太陽光能量後將誘發一系列的光化學反應；而有些藥物具自身光敏能力(self-photosensitization)，其照光後生成的激發態物質將會與二氧化錳產生交互作用，進而影響藥物及二氧化錳於水環境內的降解轉化。因此本研究選用一具自身光敏能力的藥物－氨甲蝶呤(methotrexate)為目標污染物，研究其與二氧化錳於太陽光照射下的氧化還原機制。

存在 methotrexate 與二氧化錳的溶液於模擬太陽光照射下，在溶液內可以測得 29.5 μM 的錳離子生成($[methotrexate]_0$ = 20 μM, $[MnO2]_0$ = 200 μM, pH_0 = 7 and t = 8 hrs)；且越高的 methotrexate 初始濃度(10–40 μM)將導致越多的錳離子生成(0–82.0 μM)。然而，其它不具自身光敏能力的藥物(7-aminocephalosporanic acid 及 acetaminophen)與二氧化錳溶液照射模擬太陽光 12 至 14 小時後仍無法測得錳離子。此結果顯示具自身光敏能力的藥物受陽光照射後會使二氧化錳加速還原成錳離子；本研究亦進一步藉由抑制劑及曝氣試驗以釐清細部反應機制並瞭解關鍵反應物質(例如三重激發態物質或活性氧物質)。首先，藉由添加異丙醇(抑制•OH)的結果發現，•OH 不會影響 methotrexate 降解及錳離子生成速率；然而添加山梨酸(抑制三

doi:10.6342/NTU201901808

重激發態物質)將會對於 methotrexate 降解及錳離子生成有明顯抑制效果。此外，於溶液中分別曝氮氣及氧氣的實驗結果得知，水中溶氧將會搶奪三重激發態物質的能量並進而抑制錳離子的生成。故由上述結果闡明，methotrexate 經由光照激發所產生的三重激發態物質是使二氧化錳加速還原成錳離子的主因。

本研究亦推測當溶氧存在時，methotrexate 於光反應過程產生的三重激發態物質會與溶氧反應並生成超氧離子($O_2^{\bullet-}$)，而$O_2^{\bullet-}$會將二價錳離子(由二氧化錳經還原反應後所產生)氧化為三價錳;此具氧化能力的三價錳將進一步使得 methotrexate 降解。本機制是經由以下實驗所驗證：methotrexate 於純光解作用下只降解 60%，然而額外添加二價錳離子並同時照光則可使 methotrexate 降解率大幅提升至 100% ([methotrexate]0 = 20 μM, [Mn2+]0 = 200 μM, pH0 = 3 and t = 4 hrs)；但若於溶液內再添加對苯醌以抑制$O_2^{\bullet-}$後，methotrexate 的降解趨勢又與其純光解反應類似。本篇研究結果顯示，具自身光敏能力的藥物會影響二氧化錳礦物於陽光照射下的光氧化還原機制；而除本研究中的目標藥物 methotrexate 外，水體中存在其它同樣具自身光敏能力的藥物及有機物，因此本成果將有助於全盤瞭解此類化合物於自然水體內的環境宿命。

關鍵字：自身光敏藥物；二氧化錳；模擬太陽光；氨甲蝶呤；三重激發態物質；超氧離子

doi:10.6342/NTU201901808

Abstract

Traditional wastewater treatment processes cannot effectively remove pharmaceuticals, resulting in their ubiquitous occurrence in aqueous environments and further undergo various natural attenuation pathways. Both sunlight photolysis and redox reactions resulting from abundantly occurred manganese dioxide minerals are the two vital processes for pharmaceuticals degradation. While pharmaceuticals absorbing solar light, series of photochemical reactions can be induced; a few pharmaceuticals possess self-photosensitization ability—these chemicals are able to be photo-triggered as excited species, which can interact with MnO_2, further affecting the pharmaceuticals degradation and MnO_2 transformation in aqueous systems. This work selects methotrexate, a self-photosensitized pharmaceutical, as the target compound aiming to explore the redox reactions between methotrexate and MnO_2 under sunlight irradiation.

Under simulated sunlight irradiation of the solution containing methotrexate and MnO_2, 29.5 μM of manganese ions were formed after 8 hours reaction ($[methotrexate]_0$ = 20 μM, $[MnO_2]_0$ = 200 μM and pH_0 = 7); higher initial methotrexate concentrations (10–40 μM) lead to an increase in manganese ions formation (0–82.0 μM). On the contrary, for other pharmaceuticals which do not possess self-photosensitized reaction (7-aminocephalosporanic acid and acetaminophen), manganese ions were not detected within 12–14 hours irradiation. This indicated that under sunlight irradiation, the presence

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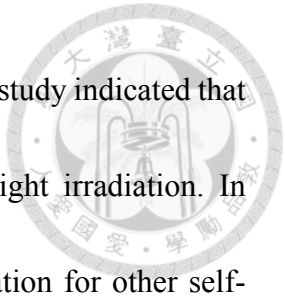
of self-photosensitized compounds accelerates the reduction of MnO_2 into manganese ions. The detailed mechanism and key reactive species participated in the reaction were further elucidated by radicals scavenging and gas-sparging experiments. Isopropanol was used to scavenge hydroxyl radical (•OH) in the solution, and •OH did not affect the rates of methotrexate degradation and manganese ions formation; however, adding sorbic acid to quench triplet excited species significantly inhibited both the methotrexate degradation and manganese ions generation. Additionally, the results of N_2- and O_2-sparging experiments showed that dissolved oxygen inhibited the formation of manganese ions. These results together indicated that triplet excited species generated upon sunlight irradiation of methotrexate is the dominant factor for accelerating the reduction of MnO_2 to manganese ions.

This study also observed that triplet excited species produced from methotrexate photolysis would react with dissolved oxygen to form superoxide anion ($O_2^{\bullet-}$); next, $O_2^{\bullet-}$ will oxidize Mn^{2+} (generated from MnO_2 reduction) into Mn^{3+} ions. Mn^{3+} has oxidizing ability and will further lead to methotrexate degradation. During sunlight photolysis alone, only 60% of methotrexate was degraded within 4 hours, while spiking Mn^{2+} into the solution would substantially enhance the methotrexate degradation (~100% degradation) under irradiation ($[methotrexate]_0$ = 20 μM, $[Mn^{2+}]_0$ = 200 μM and pH_0 = 3); however, after further addition of *p*-benzoquinone to scavenge $O_2^{\bullet-}$, the degradation trend of

doi:10.6342/NTU201901808



methotrexate was similar to its photolysis alone. The results from this study indicated that methotrexate affects the photoredox reaction of MnO_2 under sunlight irradiation. In addition to methotrexate, this work also provides insightful information for other self-photosensitized compounds and their environmental fate in natural water matrices.

Keywords: Self-photosensitized compounds; Manganese dioxides; Simulated sunlight irradiation; Methotrexate; Triplet excited state; Superoxide anion

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Chapter 1 Introduction

1.1 Background

With the advancement of analytic technique and the development of high quality detective instrument, numerous pharmaceuticals have been detected in natural aqueous system (Kim and Carlson 2007, Lindsey et al. 2001, Ryu et al. 2014). Many of these contaminants come from effluents of agriculture (Lee et al. 2019), hospital (Azuma et al. 2019) and wastewater treatment plants (Lin et al. 2009a). These compounds and their metabolism in the environment affect plants and animals, and cause potential risk to human and ecosystem (Ben et al. 2019, Mezzelani et al. 2018). Therefore, over the last two decades, pharmaceuticals in aqueous environment have been a major issue.

Organic contaminants are removed from aqueous system through natural attenuation, including hydrolysis, biodegradation, sorption, photolysis and redox reaction. Manganese oxides are abundant in soil and sediment, and due to the strong reduction potential, they effectively degrade several organic compounds, such as phenols (Stone and Morgan 1984b), anilines (Klausen et al. 1997), fluoroquinolones (Zhang and Huang 2005a) and aromatic N-oxides (Zhang and Huang 2005b). Therefore, the oxidative transformation by manganese oxides is considered to be one of the

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important natural attenuation process. Photolysis, another important natural attenuation, includes direct photolysis and indirect photolysis. In direct photolysis, contaminants absorb photons and break bonds (Zepp and Cline 1977). In indirect photolysis, contaminants are degraded via reactive oxygen species (ROSs), which are generated by photosensitizers. In general, natural photosensitizers contain humic acid (HA), carbonate and nitrate (Zepp et al. 1981). Furthermore, contaminants which also generate ROSs themselves to degrade the parents compounds are so-called self-photosensitized compounds, such as methotrexate (MTX) (Hsu et al. 2019a).

Methotrexate is a major anticancer drug, and is increasingly used to treat cancers and autoimmune diseases (Abolmaali et al. 2013). The physicochemical properties of methotrexate are shown in Table 1, and the concentrations of methotrexate range from <6.25 ng/L in river to 4689 ng/L in hospital effluent (Aherne et al. 1985, Besse et al. 2012, Lin et al. 2014, Yin et al. 2009). In this study, methotrexate is chosen to investigate the mechanism between self-photosensitized compounds and redox reactions of MnO_2 under simulated sunlight irradiation.

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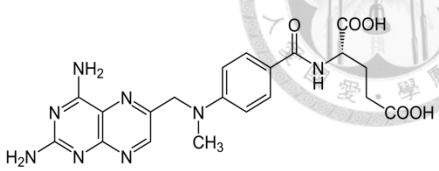
Table 1 Physicochemical properties of methotrexate

	Formula	pK_a	log K_{ow}	Structure
MTX	$C_{20}H_{22}N_8O_5$	2.9, 4.6, 6.6[a]	-1.85[b]	 M.W. 454.44

a (Mioduszewska et al. 2017); b (Hansch et al. 1995)

1.2 Motivation and Hypothesis

Photolysis of pharmaceuticals in different conditions has been discussed in numerous literatures (Boreen et al. 2004, Yamamoto et al. 2009). Redox reaction and sorption effect of manganese dioxide (MnO_2) also has been studied a lot (Liu et al. 2018, Xiao et al. 2013). In contrast, the research of pharmaceuticals photolysis with MnO_2 is not so much, but, in previous studies, MnO_2 undergoes photoreduction with general organic compounds under sunlight irradiation is demonstrated (Bertino and Zepp 1991, Hsu et al. 2019b). Besides, when MnO_2 reacts with natural photosensitizers such as humic acid, the phenomenon of MnO_2 photoreduction becomes more significantly (Spokes and Liss 1995). Self-photosensitized compounds are similar to natural photosensitizers. Both they are transform to triplet excited states, and then generate

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ROSs to degrade contaminants (Hsu et al. 2019a, McNeill and Canonica 2016). Therefore, this study will investigate the interaction between MnO_2 and Self-photosensitized compounds under simulated sunlight irradiation.

As we mentioned in section 1.1, methotrexate is a self-photosensitized compound. Our group previously discovered that the color of experiments solutions containing MnO_2 and methotrexate changed from dark brown to transparent under simulated sunlight irradiation, indicating that MnO_2 particles were reduced to manganese ions. The phenomenon was different from that of most of other compounds. Accordingly, there must be something interesting between MnO_2 and self-photosensitized compounds under simulated sunlight irradiation, and until now there hasn't been any study on this subject. For the first time, this study chooses methotrexate as the target compound to investigate the mechanism between MnO_2 and self-photosensitized compounds. The hypothesis in this study is shown in Fig. 1.

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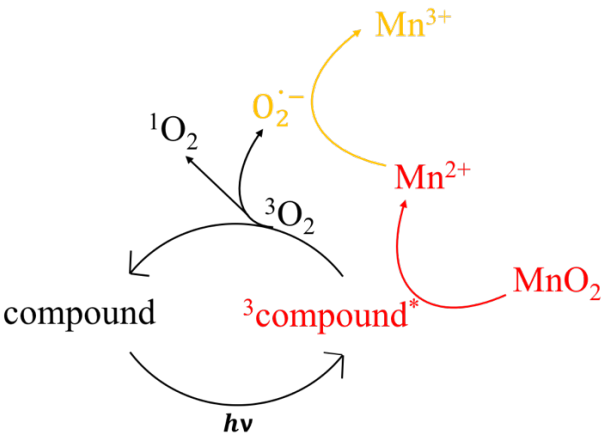

Note: "Compound" is MTX in this work.

"^{3}Compound*" includes all triplet excited species. For example, ^{3}MTX*.

Fig. 1 Hypothesis of reaction scheme on this study

Hypothesis 1: ***MnO_2 is reduced to Mn^{2+} by $^3MTX^*$ under simulated sunlight irradiation.***

Under both light and dark conditions, MnO_2 undergoes redox reactions, and being reduced to Mn^{2+} (eq. (1)). Mn^{2+} is then absorbed to MnO_2, caps vacancy sites, releases an electron, changes the MnO_2 structure to MnOOH or Mn_3O_4 (eq. (2)), and results in decrease of MnO_2 oxidation ability (Hinkle et al. 2016, Lafferty et al. 2010, Wang et al. 2018a, Zhu et al. 2010).

$$MnO_2 + compound \rightarrow Mn^{2+} + byproducts \qquad (1)$$

$$Mn^{2+} + Mn^{4+} \rightarrow 2Mn^{3+} \qquad (2)$$

However, upon irradiation, for compounds that undergo self-sensitization such as

doi:10.6342/NTU201901808

methotrexate, triplet excited state species (^{3}MTX*) will be generated, react with MnO_2, and reduce MnO_2 to Mn^{2+} (eq. (3) & (4)). Because of the reduction of MnO_2, the vacancy on MnO_2 to absorb Mn^{2+} disappears and more soluble manganese ions can be detected.

$$MTX \xrightarrow{h\nu} {}^3MTX^* \qquad (3)$$

$$MnO_2 \xrightarrow{{}^3MTX^*} Mn^{2+} \qquad (4)$$

In order to demonstrate this hypothesis, there are three experiments to be done under simulated solar irradiation:

(1) The effect of different initial concentration of methotrexate reacting with MnO_2.

To test whether different concentrations of reactive species generated upon methotrexate irradiation will have an effect on the appearance of soluble Mn^{2+}.

(2) Methotrexate and MnO_2 with quenchers of hydroxyl radical (•OH) and $^3MTX^*$.

Because isopropanol (IPA) and sorbic acid (SA) are the quenchers for •OH and ^{3}MTX* respectively, this is to verify if these reactive species will have an effect on the formation of soluble Mn^{2+}.

(3) Methotrexate and MnO_2 with N_2- or O_2-sparging.

O_2 will reduce the formation of ^{3}MTX* while N_2 enhances the generation/quantity

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of $^3MTX^*$. Therefore, this is to investigate whether the presence of $^3MTX^*$ will have an effect on the formation of soluble Mn^{2+}.

Hypothesis 2: ***Mn^{2+} is oxidized into Mn^{3+} by superoxide anion ($O_2^{\bullet-}$).***

MnO_2 is first reduced to Mn^{2+} by $^3MTX^*$ (eq. (4)). Additionally, dissolved oxygen ($^3O_2$) will quench $^3MTX^*$, and be transformed into singlet oxygen ($^1O_2$) and $O_2^{\bullet-}$ (eq. (5) & (6)). After that, Mn^{2+} will be oxidized into Mn^{3+} by $O_2^{\bullet-}$ (eq. (7)) (Wang et al. 2018c), then Mn^{3+} will oxidize methotrexate (eq. (8)). Thus, further enhancing methotrexate degradation under simulated sunlight irradiation.

$$^3MTX^* + {}^3O_2 \rightarrow MTX + {}^1O_2 \qquad (5)$$

$$^3MTX^* + {}^3O_2 \rightarrow MTX + O_2^{\bullet-} \qquad (6)$$

$$Mn^{2+} \xrightarrow{O_2^{\bullet-}} Mn^{3+} \qquad (7)$$

$$Mn^{3+} + MTX \rightarrow Mn^{2+} + byproducts \qquad (8)$$

In order to verify this hypothesis, there are three experiments to be done:

(1) Methotrexate and Mn^{2+} under simulated sunlight irradiation.

To test whether Mn^{2+} will react with the reactive species generated upon methotrexate illumination.

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(2) Methotrexate and Mn^{2+} with quencher of $O_2^{\bullet-}$ under simulated sunlight irradiation.

p-Benzoquinone (BQ) is a known quencher for $O_2^{\bullet-}$, and it will be used to investigate whether $O_2^{\bullet-}$ will oxidize Mn^{2+}.

(3) Methotrexate and Mn^{3+} in dark condition.

To investigate whether Mn^{3+} will oxidize methotrexate.

doi:10.6342/NTU201901808

Chapter 2 Literature Review

2.1 Indirect photolysis

2.1.1 Introduction

Photolysis is an important natural attenuation process. After sunlight irradiating, compounds were excited to a higher energy level, either singlet or triplet excited state. Subsequently, it may undergo different pathways which are shown in Fig. 2.1

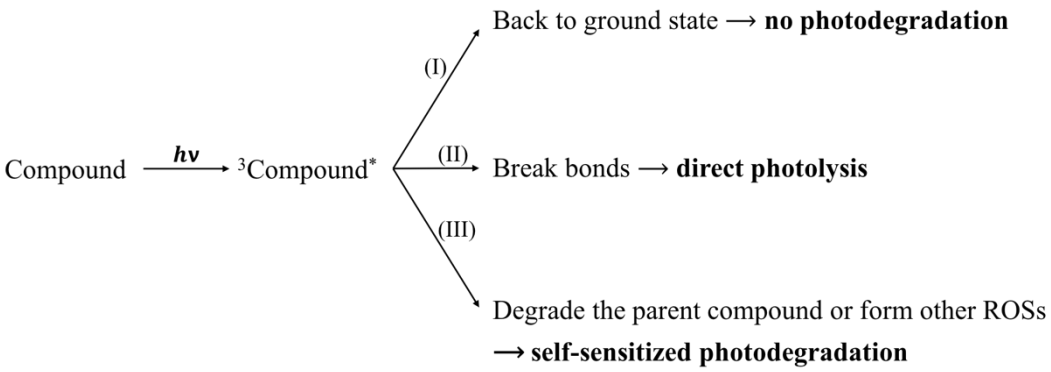

Fig. 2.1 Pathways of triplet excited species

In pathway (I), the excited state species release the energy and back to the ground stat; then, nothing happened to the compound. In pathway (II), compounds absorb enough energy to break bonds and go through direct photolysis. Ultraviolet (UV) direct photolysis is commonly used to degrade pollutants in water (Chen et al. 2016, Luo et al. 2018, Sharpless and Linden 2003). In pathway (III), few of compounds can possibly form ROSs, such as •OH, $O_2^{\bullet -}$ and $^1O_2$, and further react with its parent compound.

doi:10.6342/NTU201901808

For example, methotrexate is a compound that goes through self-photosensitized photodegradation, and generates triplet excited species and $^1O_2$ to degrade the parent compound (Hsu et al. 2019a).

In addition to self-photosensitized photodegradation, there are other indirect photolysis processes happening with natural photosensitizers in environments. Natural photosensitizers can absorb radiation and then generate other reactive species, such as •OH, $O_2^{\bullet-}$ and carbonate radical (CO_3^{2-}) (Andreozzi et al. 2003, Canonica et al. 2005, Wang et al. 2017), to degrade compounds. The most widespread organic photosensitizer is humic acid (Zepp et al. 1981); and inorganic photosensitizers, such as nitrate, free chlorine and hydrogen peroxide (Ribeiro et al. 2019).

doi:10.6342/NTU201901808

2.1.2 Triplet excited states

Upon irradiation, as shown in Fig. 2.2, ground states compounds absorb energy to transform to singlet excited states. Then, because of intersystem conversion, singlet excited states become a lower energy triplet excited states.

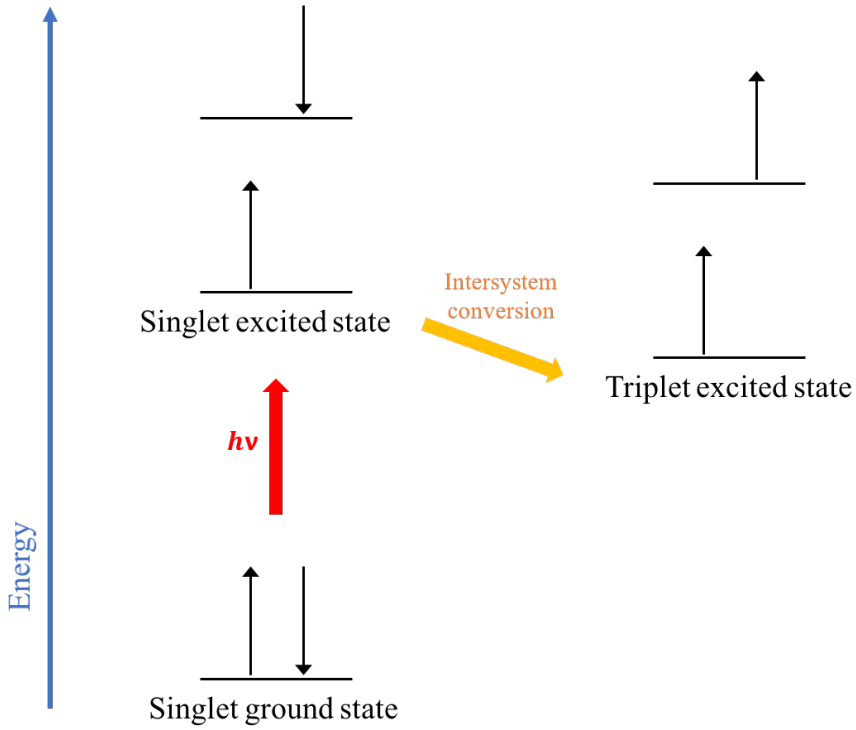

Fig. 2.2 Scheme of compounds states change

When compounds form triplet excited states, they become both better oxidants and reductants than their ground states. Fig. 2.3 shows ground state and triplet excited state electronic configurations of the frontier orbitals.

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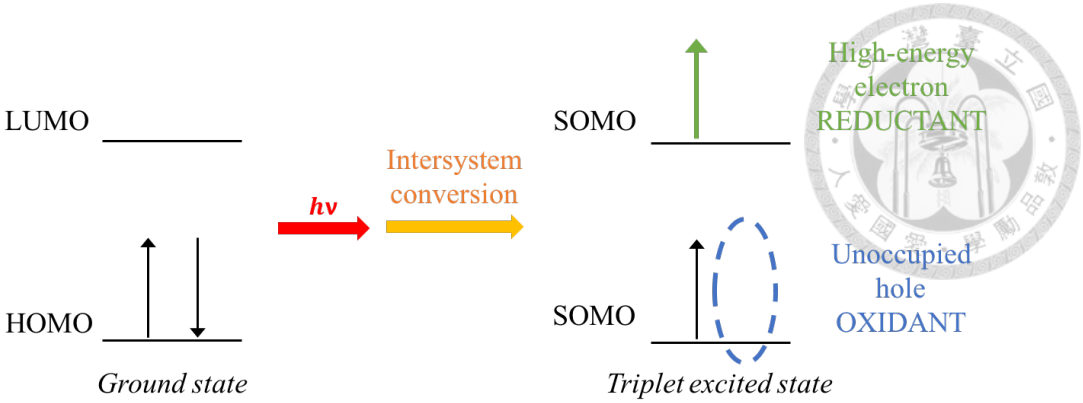

Fig. 2.3 Electronic configurations of ground state and triplet excited state

To be an oxidant, the triplet excited state requires less energy to receive an electron than the ground state. That is because, in the ground state, the incoming electron has to the high-energy lowest unoccupied molecular orbital (LUMO). In contrast, in the triplet excited state, the electron can occupy singly occupied molecular orbital (SOMO) which is lower energy (McNeill and Canonica 2016). For example, triplet excited state of aromatic ketones can oxidize carbonate anion (CO_3^{2-}) into carbonate radical ($CO_3^{\bullet -}$) (Canonica et al. 2005).

With regard to being a reductant, the process is similar to an oxidant. The triplet excited state also requires less energy to release an electron than the ground state because an electron has been already excited to the higher SOMO (McNeill and Canonica 2016). For example, irradiation of dissolved organic matter (DOM) results in an electron transfer to oxygen (Petasne and Zika 1987).

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2.1.3 Natural photosensitizers

DOM is an important component in natural water system, containing humic acid (HA), fulvic acid, hydrophilic acid and other biogenic organic molecules. Humic acid accounts for 50~75% of DOM, and molecular weights are 1000 to 2000 (Thurman 1985).

Humic acid is an important photosensitizer in natural aquatic system (Zepp et al. 1981). Many previous research have reported that during indirect photolysis, humic acid absorbs energy and transforms to triplet excited state; then, the energy is transferred to dissolved oxygen to form $^1O_2$ or $O_2^{\bullet-}$ under UV or solar irradiation (Hoigné et al. 1988, Polewski et al. 2005). Fig 2.4 takes humic acid for example to show the simplified kinetic scheme of photosensitizer excited reaction.

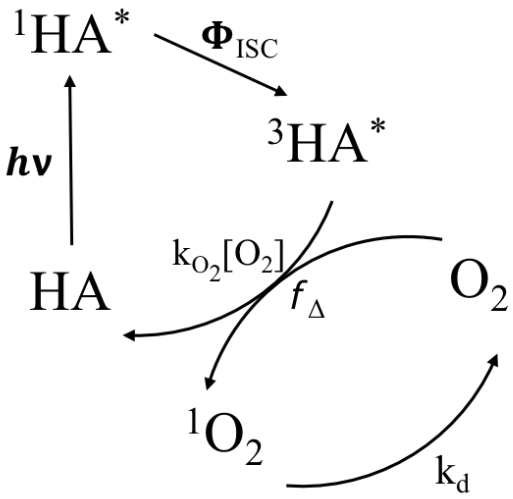

Fig. 2.4 Scheme of excited reaction of photosensitizer (McNeill and Canonica 2016)

doi:10.6342/NTU201901808

Humic acid is excited by the absorption of a photon (***hν***) to form singlet excited state ($^1HA^*$). A part of $^1HA^*$ converts to triplet excited state ($^3HA^*$) because of the intersystem crossing efficiency (Φ_{ISC}) (Sharpless 2012). And then, in natural air-saturated aqueous system, $^3HA^*$ and dissolved oxygen (O_2) react with the rate constant $k_{O_2} = 2\times10^9\ M^{-1}s^{-1}$ (Zepp et al. 1985). The k_{O_2} values are not exactly the same and depend on the various composition of HA. In this reaction, O_2 is regarded as a quencher of $^3HA^*$. Quenching $^3HA^*$ produces $^1O_2$ but the yield of this process (f_Δ) varies from near 0 to near 1, depending on different sensitizers (Wilkinson et al. 1993).

doi:10.6342/NTU201901808

2.1.4 Self-photosensitized compounds

Natural photosensitizers are excited to triplet excited states upon irradiation, and then form ROSs to degrade other compounds. In a few case, compounds that can generate triplet excited states and ROSs to react with the parent compounds are so-called self-photosensitized compounds.

Methotrexate, a chemotherapy agent and immune system suppressant, is a self-photosensitized compound. Under simulated sunlight irradiation, methotrexate generate triplet excited state species and $^1O_2$ to degrade the parent compound (Hsu et al. 2019a). Phenylarsonic compounds (feed additives) and natural dyes (such as indigo dyes and l-benzyl-3,4- dihydroisoquinoline) are excited to triplet excited state and form $^1O_2$ under simulated sunlight irradiation to enhance their own photodegradation rate (Gandra et al. 2006, Martin and Jefford 1981, Xie et al. 2016, Xie et al. 2018). Fluoroquinolone antibiotics, such as ciprofloxacin, danofloxacin, levofloxacin and norfloxacin, also produce $^1O_2$ and •OH upon triplet excited states formation (Ge et al. 2010, Liang et al. 2015, Niu et al. 2016). Tetracycline, another antibiotic, transform to triplet excited state under photoirradiation, then generate $^1O_2$, $O_2^{\bullet-}$ and H_2O_2 ,which participate in the photodegradation of the parent compound (Chen et al. 2008, Werner

doi:10.6342/NTU201901808

et al. 2006). Besides, naproxen is a nonsteroidal anti-inflammatory drug (NSAID), excited to triplet excited state after irradiation, and then form $^1O_2$, $O_2^{\bullet-}$ and •OH (Ma et al. 2014).

Section 2.1.3 mentioned that $^3HA^*$ has high reaction rate constant with O_2 ($k_{O_2} = 2\times10^9\ M^{-1}s^{-1}$). Therefore, dissolved oxygen is seen as a quencher of triplet excited states. In the literature about photosensitizers, conditions of O_2-sparging represent triplet excited species were quenched and ROSs were formed much more than no-sparging. In contrast, N_2-sparging enhances the performance of triplet excited species and reduces the formation of ROSs. However, the effects of N_2- or O_2-sparging are not always the same. The reason is each reactive species has different reactivity to the compounds. Besides, the formation proportion of reactive oxygen species depends on compounds. Table 2 summarize the changes of rate constant in different conditions.

doi:10.6342/NTU201901808

Table 2 Rate constant changes of self-photosensitized compounds in different conditions

Self-photosensitized compounds	Increase initial concentrations	Quench •OH	Quench $^1O_2$	Quench $O_2^{\bullet-}$	Sparge with N_2	Reference
Methotrexate	+	×	×[a]	×[b]	+[a]	(Hsu et al. 2019a)
Phenylarsonic compounds	−	×	−	×	−	(Xie et al. 2016) (Xie et al. 2018)
Naproxen	−	×	−	−	+	(Ma et al. 2014)

+: Higher rate constant than the control group

−: Lower rate constant than the control group; ×: Less effect to the control group

a: High level concentrations of methotrexate

b: No discussion in the study

doi:10.6342/NTU201901808

Photodegradation of methotrexate mainly depends on triplet excited species, especially the pteridine structure in methotrexate and its byproducts. Therefore, having more triplet excited pteridine structure by increasing initial concentrations and extending the lifetime of triplet excited species by N_2-sparging result in the higher reaction rate constant (Hsu et al. 2019a). On the contrary, the photodegradation of phenylarsonic compounds mainly depends on $^1O_2$, so quenching $^1O_2$ and N_2-sparging decrease the reaction rate constant (Xie et al. 2016, Xie et al. 2018). As for naproxen, self-sensitized photooxidation, especially reaction with ROSs, is slower than direct photolysis. Therefore, increasing initial concentrations of naproxen compete for limited quantity of available photons, then decreasing rate constant (Ma et al. 2014).

In summary, self-sensitized compounds are excited to triplet excited states, then dissolved oxygen accept the energy to form ROSs. But effects and formation proportion of ROSs depend on compounds.

doi:10.6342/NTU201901808

2.2 Manganese Dioxide

2.2.1 Characteristic of MnO_2

Manganese oxides are widely distributed in soils and sediments. Although iron(III) oxides are also common in soils and sediments, manganese(III/IV) oxides have higher reduction potential (Li et al. 2003, Murray et al. 1985, Stone 1987).

$$Mn^{IV}O_{2(s)} + 2H^+ + e^- \leftrightarrow \frac{1}{2}Mn^{2+} + H_2O \qquad E^0 = +1.23\ V$$

$$Mn^{III}OOH_{(s)} + 3H^+ + e^- \leftrightarrow Mn^{2+} + 2H_2O \qquad E^0 = +1.50\ V$$

$$Fe^{III}OOH_{(s)} + 3H^+ + e^- \leftrightarrow Fe^{2+} + 2H_2O \qquad E^0 = +0.67\ V$$

Birnessite is one of the most common manganese oxide minerals (Taylor 1968). Concentrations of reducible MnO_2 in different soils were ranged from 1.3 to 960.0 ppm of air-dry soil (Sherman et al. 1942). δ-MnO_2 is a kind of synthetic forms of birnessite-like minerals, and usually used to oxides organic compounds because of the strong sorption capacity and oxidation potential. Table 3 shows researches which applying manganese oxides as reactant to remove pharmaceuticals. Besides, the oxidation capacity of MnO_2 is associated to environmental factors, such as, MnO_2 loading, solution pH , dissolved cations and organic compounds (Stone and Morgan 1984a).

doi:10.6342/NTU201901808

Table 3 Researches applying δ-MnO_2 as reactant to remove pharmaceuticals

Contaminants	**Results**	**Reference**
Acetaminophen (APAP)	>98% APAP was removed at pH 4.0	(Xiao et al. 2013)
Bisphenol A (BPA)	>99% BPA was removed in 6 min at pH 4.5	(Lin et al. 2009b)
Steroid estrogens	The contaminants were almost removed in 220 min at pH 4.0	(Xu et al. 2008)
Cephalosporins	The degradation efficiency depend on the substructure of cephalosporins	(Hsu et al. 2018)
Triclosan	90% Triclosan was removed in 1.6 hrs at pH 5.0	(Zhang and Huang 2003)

doi:10.6342/NTU201901808

2.2.2 Redox reaction between MnO_2 and organics

The redox reaction scheme between organic pollutants (ArXH, X = O or N) and MnO_2 is shown below (Stone 1987, Zhang et al. 2008):

$$> Mn^{IV} + ArXH \overset{K_{eq}}{\leftrightarrow} (> Mn^{IV}, ArXH) \quad (9)$$

$$(> Mn^{IV}, ArXH) \overset{k_1}{\leftrightarrow} (> Mn^{III}, ArX\cdot) + H^+ \quad (10)$$

$$(> Mn^{III}, ArX\cdot) \overset{k_2}{\leftrightarrow} Mn^{III} + ArX\cdot \quad (11)$$

$$> Mn^{III} \overset{fast}{\longrightarrow} > Mn^{II} \quad (12)$$

$$ArX\cdot \overset{fast}{\longrightarrow} products \quad (13)$$

Manganese with different valence is denoted as $>Mn^{IV}$, $>Mn^{III}$ and $>Mn^{II}$. In eq. (9), organic pollutant reacts with MnO_2 surface ($>Mn^{IV}$) to form a precursor complex because of the adsorption reaction. Next, in eq. (10) and (11), electron transfer occurs in the precursor complex, then the complex generates $>Mn^{III}$ and organic radical species ($ArX\cdot$). However, because $>Mn^{III}$ is very unstable in the nature, it may react with other species in the solution, such as, ArXH, $ArX\cdot$, and $>Mn^{III}$. Consequently, in eq. (12) and (13), the unstable $>Mn^{III}$ is transform to more stable $>Mn^{II}$ species, and the organic pollutant is decomposed to byproducts. In addition, the precursor complex formation and electron transfer is regarded as rate-limiting steps (Stone 1987).

doi:10.6342/NTU201901808

After redox reactions, MnO_2 is reduced to Mn^{2+}. When partial MnO_2 still in the solution, Mn^{2+} is then absorbed to MnO_2, caps vacancy sites, releases an electron to form Mn^{3+}, changes the MnO_2 structure to MnOOH or Mn_3O_4 (Hinkle et al. 2016, Lafferty et al. 2010, Wang et al. 2018a, Zhu et al. 2010). Therefore, during the reduction, Mn^{2+} and Mn^{3+} are accumulated to the vacancies of MnO_2. But Mn^{2+}/Mn^{3+} ratio depends on solution pH. Under alkaline conditions, Mn^{3+} accumulates more than Mn^{2+}; in contrast, accumulation is manly Mn^{2+} under acidic conditions. Manganese ions can adsorb to MnO_2 birnessite, and Mn^{3+} adsorb more strongly than Mn^{2+}. The presence of manganese ions on vacancies of MnO_2 can decrease the sorption capacities of metal. Because less MnO_2 vacancies to adsorb with organic pollutant and form a precursor complex, the oxidation ability of MnO_2 strongly decrease (Wang et al. 2018a).

doi:10.6342/NTU201901808

2.2.3 Photochemical reaction between MnO_2 and organics

Redox reaction of MnO_2 in a dark condition has been studied in numerous researches. In contrast, literature about MnO_2 and organic compounds under irradiation is less. In 1991, Dorothy J. Bertlnot and Richard G. Zepp proposed the following scheme of MnO_2 photochemical reaction (Bertino and Zepp 1991).

$$X + MnO_x \leftrightarrow (X--MnO_x) \qquad (14)$$

$$(X--MnO_x) \xrightarrow{hv} (X--MnO_x)^* \qquad (15)$$

$$(X--MnO_x)^* \rightarrow X^+ + Mn(II) \qquad (16)$$

In the scheme, the symbol X--MnO_x represents the surface complex between manganese oxides (MnO_x) and organic compounds (X), such as phenols and DOM. After the formation of surface complex (eq. (14)) and under sunlight, electron transfers from excited DOM to the MnO_x, then the soluble manganese is generated faster than dark conditions (eq. (15) and (16)).

Furthermore, when compounds are photosensitizers such as DOM, different reactive species are formed upon irradiation as we discussed in section 2.1.4.

$$S + O_2 \xrightarrow{hv} S^+ + O_2^{\bullet -} \qquad (17)$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (18)$$

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$$MnO_x + H_2O_2 \rightarrow Mn(II) \qquad (19)$$

One of reactive species generates from photosensitizers irradiation is $O_2^{\bullet-}$ (eq. (17)) (Sunda and Huntsman 1994). $O_2^{\bullet-}$ is a precursor of hydrogen peroxide (H_2O_2) (eq. (18)) (Cooper and Zika 1983). Subsequently, H_2O_2 reduces MnO_x to Mn^{2+} (eq. (19)) (Bertino and Zepp 1991).Therefore, irradiation enhances the formation of dissolved manganese in the solution containing DOM (Sunda et al. 1983, Waite et al. 1988).

$$Mn(II) \xrightarrow{O_2^{\bullet-}} Mn(III) \qquad (20)$$

In system of manganese, it not only goes through reduction reaction but also has oxidation reaction when the solution contains $O_2^{\bullet-}$. Wang et al. demonstrates that Mn^{2+} is oxidized into Mn^{3+} by $O_2^{\bullet-}$ which is generated from humic acid or DOM upon visible light irradiation (eq. (20)); then, Mn^{3+} degrades target compounds efficiently (Wang et al. 2018b, Wang et al. 2018c). Consequently, photosensitizers play an important role in the transform of manganese valence.

doi:10.6342/NTU201901808

Chapter 3 Materials and Methods

3.1 Chemicals and standards

All the target compounds were purchased from Sigma-Aldrich, and of purity over 98%. A stock solution of 1 mM methotrexate was dissolved in 0.05 M NaOH, and naproxen was dissolved in methanol. Other stock solutions were prepared in deionized (DI) water at 2 mM. All the stock solutions were stored at 4°C in brown glass bottles for a maximum of 21 days. The compounds and instruments are listed in Table 4 and Table 5.

Table 4 Information of all chemicals used

Compounds	Suppliers	Purities
Methotrexate (MTX)	Sigma-Aldrich	≥98%
Tetracycline (TC)	Sigma-Aldrich	99.5%
Naproxen (NP)	Sigma-Aldrich	99.5%
7-aminocephalosporanic acid (7-ACA)	Sigma-Aldrich	98%
Acetaminophen (APAP)	Sigma-Aldrich	99%
Isopropanol (IPA)	Mallinckrodt Baker	99.9%
Sorbic acid (SA)	Sigma-Aldrich	≥99%
p-benzoquinone (BQ)	Sigma-Aldrich	≥98%
Potassium permanganate	J.T.Baker	99%
Manganese(II) chloride	Alfa Aesar	97%
Oxalic acid	Sigma-Aldrich	99%
Sodium chloride	Nacalai tesque	≥99.5%
Sodium hydroxide	Sigma-Aldrich	100%

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Compounds	Suppliers	Purities
Hydrochloric acid	J.T.Baker	37.6%
Manganese AA standard	J.T.Baker	1000 ppm
Potassium pyrophosphate	Sigma-Aldrich	97%
Mn(III) acetate dehydrate	Sigma-Aldrich	97%
Methanol	Mallinckrodt Baker	LC grade
Ethanol	Sigma-Aldrich	≥98%
Formic acid	Fluka	≥98%
ammonium acetate	Sigma-Aldrich	7.5 M
Phosphoric acid	Sigma-Aldrich	≥85%
Acetonitrile	J.T.Baker	99.9%

Table 5 Analytical instruments

Instrument	Model type
High performance liquid chromatography (HPLC)	Agilent Technologies, 1200 module
Tandem mass spectrometry (MS/MS)	Applied Biosystems, API 4000 -Electrospray ionization, ESI -Triple quadrupole mass
Diode array detector (DAD)	Agilent Technologies, 1200 module
Atomic absorption spectrometer (AAS)	AAS, Perkin, AA800
UV-vis spectrometer	HACH, DR 6000

doi:10.6342/NTU201901808

3.2 Experimental process

3.2.1 δ-MnO_2 synthesis

δ-MnO_2 synthesis followed the method proposed by Murray (Murray 1974). The stoichiometry of δ-MnO_2 preparation was shown below:

$$2\mathrm{MnO_4^-} + 3Mn^{2+}\ 2H_2O \rightarrow 5MnO_{2(s)} + 4H^+$$

First, 2 L of DI water was sparged with N_2 for about 2 hours. The following solutions were prepared with the sparged water: 80 mL of 0.1 M $KMnO_4$, 160 mL of 0.1 M NaOH, 120 mL 0.1 M of $MnCl_2$. $KMnO_4$ and NaOH were added into the remaining 1.64 L N_2-sparged DI water, then sparged with N_2 for another 0.5 hour. After that, $MnCl_2$ was dropwise added to the N_2-sparged solution throughout about 3 hours while keeping the solution constantly stirred. After the synthesized MnO_2 particles settled down, the supernatant was removed and replaced with DI water several times until the conductivity of solution is less than 2 μS-cm^{-1}. Finally, the volume of MnO_2 suspension was adjusted to about 1 L and stored at 4°C. To quantify the concentration of synthesized MnO_2 stock solution, MnO_2 was reduced to Mn^{2+} by oxalic acid, then determined by atomic absorption spectrometer (AAS) (the concentration of MnO_2 stock solution was 12.15 mM).

doi:10.6342/NTU201901808

3.2.2 Test experiments

Methotrexate, tetracycline, naproxen, 7-aminocephalosporanic acid (7-ACA) and acetaminophen are each of 20 μM was used. Then the pH of experiments was adjusted to 7 before the solution was added 200 μM MnO_2. After that, simulated sunlight irradiation was started immediately.

The experiments were quenched by passing through 0.22 μm PVDF filter membranes. methotrexate and 7-ACA were detected by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS); tetracycline, naproxen and acetaminophen were detected by high performance liquid chromatography-diode array detector (HPLC-DAD); and soluble manganese was detected by AAS. Table 6 to Table 10 show the detailed conditions of analysis.

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Table 6 Analysis method of methotrexate

Mobile phase		A: 0.1% formic acid in DI water B: 0.1% formic acid in methanol	
Source temperature (℃)		500	
Curtain gas		20	
Gas 1/Gas 2		50/50	
Precursor ion (m/z)		455	
Product ion (m/z)		308.2	175.0
MS/MS parameters	DP (volts)	40	40
	CE (volts)	29	52
	CXP (volts)	19	9
Retention time (min)		3.99	
HPLC flowrate (μL/min)		1000	

Time (min)	HPLC gradient of mobile phase	
	Mobile phase A (%)	Mobile phase B (%)
0	100	0
0.5	100	0
2	5	95
4.5	5	95
5	100	0
7	100	0

doi:10.6342/NTU201901808

Table 7 Analysis method of 7-ACA

Mobile phase		A: 5 mM ammonium acetate in DI water B: 5 mM ammonium acetate in methanol		
Source temperature (℃)		500		
Curtain gas		10		
Gas 1/Gas 2		60/50		
Precursor ion (m/z)		271.1		
Product ion (m/z)		211.1	167.1	140.0
MS/MS parameters	CE (volts)	-9	-15	-20
	CXP (volts)	-9	-8	-9
Retention time (min)		4.37		
HPLC flowrate (μL/min)		1000		

Time (min)	HPLC gradient of mobile phase	
	Mobile phase A (%)	Mobile phase B (%)
0	100	0
0.5	100	0
1.5	60	40
3.5	40	60
4	5	95
5	5	95
6	100	0
7	100	0

doi:10.6342/NTU201901808

Table 8 Analysis method of tetracycline

Mobile phase	A: 17 mM phosphoric acid in DI water B: Acetonitrile	
Wavelength (nm)	355	
Retention time (min)	2.8	
HPLC flowrate (mL/min)	0.7	
Time (min)	HPLC gradient of mobile phase	
	Mobile phase A (%)	Mobile phase B (%)
0	90	10
0.5	90	10
1	60	40
3.5	60	40
4	90	10
7	90	10

Table 9 Analysis method of naproxen

Mobile phase	A: DI water B: Acetonitrile	
Wavelength (nm)	230	
Retention time (min)	6.3	
HPLC flowrate (mL/min)	0.6	
Time (min)	HPLC gradient of mobile phase	
	Mobile phase A (%)	Mobile phase B (%)
0	90	10
0.5	90	10
2	60	85
7	60	85
8.5	90	10
11.5	90	10

doi:10.6342/NTU201901808

Table 10 Analysis method of acetaminophen

Mobile phase	A: DI water B: Acetonitrile	
Wavelength (nm)	243	
Retention time (min)	4.2	
HPLC flowrate (mL/min)	0.7	
Time (min)	HPLC gradient of mobile phase	
	Mobile phase A (%)	Mobile phase B (%)
0	70	30
6	70	30

3.2.3 Experiments of MnO_2 photoreduction

All experiment solutions contained 0.01M NaCl, and the concentration of MnO_2 or Mn^{2+} in experiments was 200 μM. To quench reactions for analysis, 3 mL of experiment solutions were rapidly passed through 0.22 μm PVDF filter membranes. The filtrate was used to detect the concentrations of methotrexate with HPLC-MS/MS and the concentrations of soluble manganese ions with AAS.

(1) The effect of different initial concentration of methotrexate reacting with MnO_2.

100 mL of 40, 20, and 10 μM methotrexate were respectively in colorless quartz reactors. After the pH of solutions was adjusted to 7.0±0.1 with HCl and NaOH, the experiment solutions were putted on magnetic stirrers in SUNTEST CPS+.

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Subsequently, 200 μM of MnO_2 was added and simulated sunlight irradiation was started immediately.

(2) Methotrexate and MnO_2 with quenchers of •OH and $^3MTX^*$.

Stock solutions of 1 M isopropanol (IPA) in DI water, and 0.2 M sorbic acid (SA) in ethanol were prepared. Then, 100 mL of 20 μM methotrexate and quencher (20 mM IPA to quench •OH or 10 mM SA to quench $^3MTX^*$) was in colorless quartz reactor and adjusted the pH to 7.0±0.1 with HCl and NaOH. The experiment solutions were putted on magnetic stirrers in SUNTEST CPS+ before 200 μM of MnO_2 was added. Afterwards, simulated sunlight irradiation was started immediately.

(3) Methotrexate and MnO_2 with N_2- or O_2-sparging

The pH of 20 μM methotrexate was adjusted to about 5.7 with HCl and NaOH. Then, 22 mL of the solution was taken into quartz tube and sparged with N_2 or O_2 for 30 minutes. After gas-sparging, the pH of solution increased to 7.0±0.1. Subsequently, the experiment solutions were putted in SUNTEST CPS+ before 200 μM of MnO_2 was added. And then, the experiment solutions immediately started irradiating.

3.2.4 Experiments of Mn^{2+} photooxidation

(1) Methotrexate and Mn^{2+} under simulated sunlight irradiation.

doi:10.6342/NTU201901808

The pH of 50 mL 40 μM methotrexate and 50 mL 400 μM Mn^{2+} were respectively adjusted to 3.0±0.1. Mixing the solutions to make the experiment concentrations of methotrexate and Mn^{2+} were 20 μM and 200 μM respectively. First, to investigate that whether Mn^{2+} reacted with methotrexate, the experiment was started in dark condition with continued stirred. Second, to investigate that whether Mn^{2+} reacted with reactive species which generated because of methotrexate under irradiation, the solution was putted on magnetic stirrers in SUNTEST CPS+ and irradiated for 8 hours.

(2) Methotrexate and Mn^{2+} with quencher of $O_2^{\bullet-}$ under simulated sunlight irradiation.

A stock solution of 100 mM *p*-benzoquinone (BQ), a $O_2^{\bullet-}$ quencher, was prepared in DI water. After the pH of 40 μM methotrexate and 400 μM Mn^{2+} were respectively adjusted to 3.0±0.1, mixing the solutions in colorless quartz reactor to make the experiment concentration of methotrexate was 20 μM and Mn^{2+} was 200 μM. Then, the reactor was putted on a magnetic stirrer in SUNTEST CPS+ and 10 mM BQ was added before simulated sunlight irradiation.

(3) Methotrexate and Mn^{3+} in dark condition.

Mn^{3+} was generated from the reduction of MnO_2 by oxalic acid which could stabilize Mn^{3+} by forming complex (Chen et al. 2013). The pH of 0.5 M oxalic acid

doi:10.6342/NTU201901808

was adjusted to 3.0±0.1, and mixed with the stock solution of MnO_2 to make the total volume of solution was 25 mL for 10 seconds. The time was sufficient to change the color of solution from brown to clear, indicating that MnO_2 was reduced to Mn^{2+} and formed the complex of Mn^{3+}. Then 25 mL of 40 μM methotrexate at pH 3.0 mixed with 25 mL of Mn^{3+} solution in dark condition.

To stop the reaction, the experiment solution was filtered with 0.22 μm PVDF membranes and added high concentration (1 M) of pyrophosphate as the quencher of Mn^{3+}. Mn^{3+}-pyrophosphate formed a stable complex; therefore, Mn^{3+} could be detected with UV-vis spectrometer at 484 nm. In order to quantify concentrations of Mn^{3+}, the standard solution of Mn^{3+} was Mn(III) acetate dehydrate which was prepared with N_2-sparged pyrophosphate (Kostka et al. 1995).

doi:10.6342/NTU201901808

Chapter 4 Results and Discussion

4.1 Formation of soluble manganese ions by $^{3}MTX^{*}$

The preliminary work from our research group discovered that MnO_2 became transparent from originally dark brown color in the presence of methotrexate after irradiating for 4 hours while the solution was still dark brown color without irradiation. This phenomenon is unique and does not occur to many other pharmaceuticals upon irradiation. To test whether only self-photosensitized compounds will cause the same phenomenon, five compounds were chosen to react with MnO_2 under simulated sunlight irradiation.

Methotrexate, tetracycline and naproxen are self-photosensitized compounds (Chen et al. 2008, Hsu et al. 2019a, Ma et al. 2014). Table 11 shows that after simulated sunlight irradiation for 6.5 to 8 hours, manganese ions were detected 29.5, 52.8 and 19.2 μM respectively in the solution of methotrexate, tetracycline and naproxen with MnO_2. However, in the solution of MnO_2 with general compounds (7-ACA and acetaminophen) under simulated sunlight irradiation for 12 to 14 hours, concentrations of manganese ions were under the detection limit of AAS. This result indicated that methotrexate, tetracycline and naproxen these self-photosensitized compounds may

doi:10.6342/NTU201901808

generate ROSs and affect the reduction of MnO_2 under simulated sunlight irradiation. Therefore, the objective of this study is confirming the mechanism between self-photosensitized compounds and MnO_2 under simulated sunlight irradiation. In the following experiments, methotrexate is chosen as the representative of self-photosensitized compounds.

doi:10.6342/NTU201901808

Table 11 Results of compounds (20 μM) and MnO_2 (200 μM) under simulated sunlight irradiation or dark conditions at pH 7

Compounds		**Manganese ions (μM)**		**Self-photosensitized compound (reference)**
		Simulated sunlight irradiation	**Dark conditions**	
Methotrexate (MTX)	chemotherapy agent and immune system suppressant	29.5 (8 hr)	N.D.	Yes (Hsu et al. 2019a)
Tetracycline (TC)	antibiotics	52.2 (8 hr)	N.D.	Yes (Chen et al. 2008)
Naproxen (NP)	nonsteroidal anti-inflammatory drug (NSAID)	19.2 (6.5 hr)	N.D.	Yes (Ma et al. 2014)
7-Aminocephalosporanic acid (7-ACA)	cephalosporin antibiotics	N.D. (14 hr)	N.D.	No
Acetaminophen (APAP)	analgesics	N.D. (12 hr)	N.D.	No

Detection limit of manganese ions: 0.1 ppm = 1.6 μM

N.D.: not detected

doi:10.6342/NTU201901808

4.1.1 Effect of methotrexate initial concentrations

Fig. 4.1(a) shows the photodegradation results of different initial methotrexate concentrations with 200 μM MnO_2 under simulated sunlight irradiation. In the first 1 hour, 9.5, 14.8 and 20.5 μM methotrexate were degraded respectively in the 40, 20 and 10 μM initial concentrations of methotrexate. The degradation efficiency increased with increasing initial methotrexate concentrations possibly due to the increased amount of triplet excited species generated. However, the methotrexate degradation did not fit pseudo first- or second-order kinetic model because the interaction between species in the system were complicated. On the other hand, the concentrations of soluble manganese ions are shown in Fig. 4.1(b). We found that higher initial methotrexate concentrations generated greater amount of soluble manganese ions. After the experiment batches were irradiated for 8 hours, 82.0, 29.5 and 0 μM manganese ions were detected respectively in 40, 20 and 10 μM initial methotrexate concentrations. This result suggested that appearance of manganese ions may be related to triplet excited species or other ROSs which were generated by triplet excited species.

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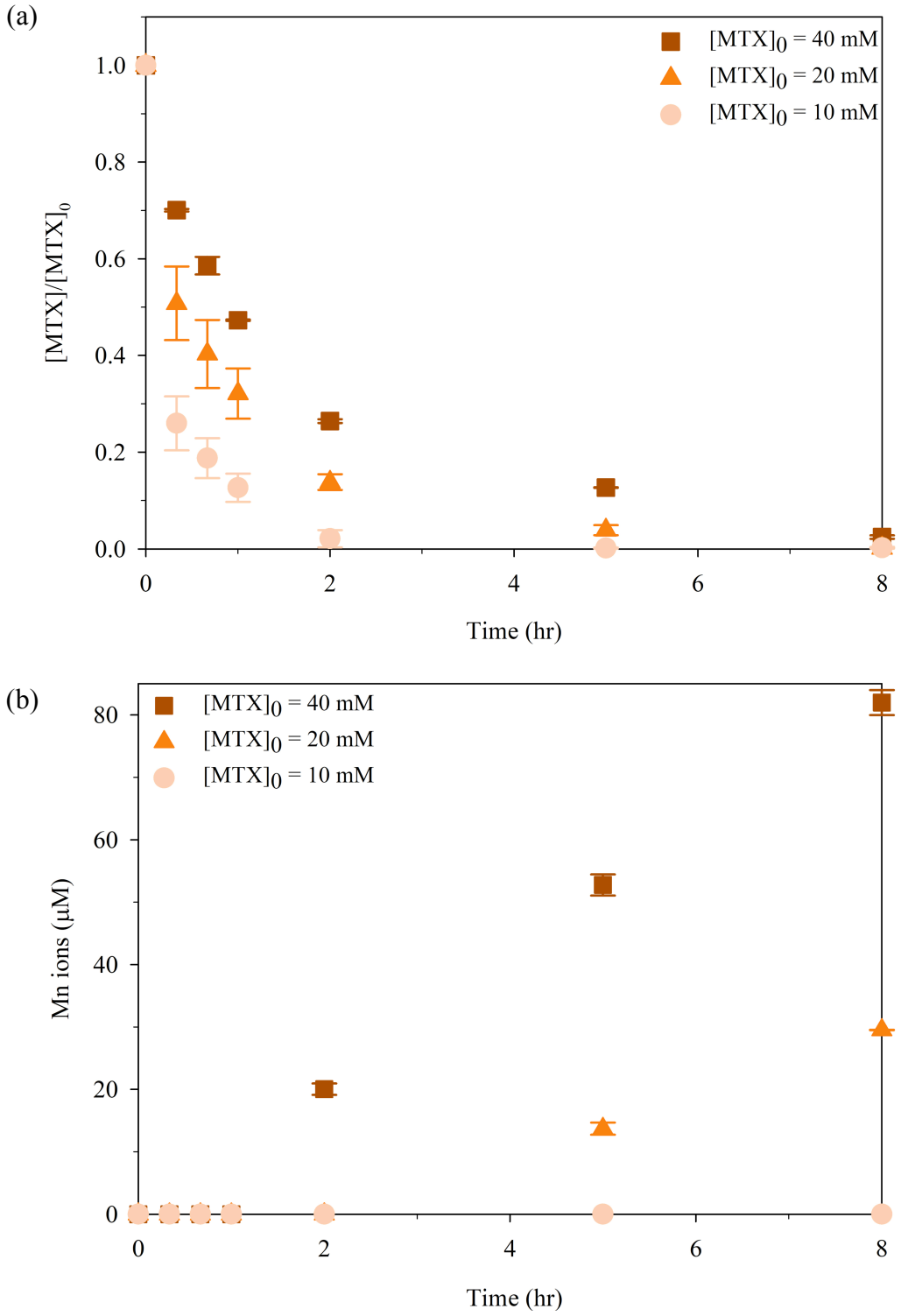

Fig. 4.1 Different initial concentrations of methotrexate with MnO_2 under simulated sunlight irradiation: (a) methotrexate degradation and (b) manganese ions formation ($[MnO_2]_0$ = 200 μM, at pH 7.0)

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4.1.2 Contribution of reactive species

In this system, the reactive species that generated from methotrexate upon irradiation could be •OH, $^1O_2$ and triplet excited species which including the triplet excited state of methotrexate ($^3MTX^*$) and the triplet excited state of the pteridine structure ($^3PT^*$) from the byproducts of methotrexate photolysis (Hsu et al. 2019b).

Isopropanol (IPA) and sorbic acid (SA) were used to quench •OH and triplet excited species respectively. Fig. 4.2(a) shows the methotrexate degradation efficiency (C_t/C_0) and Fig. 4.2(b) shows the concentrations of soluble manganese ions in quenching experiments. The results in Fig. 4.2 indicate that IPA had no obvious effects on degrading methotrexate and producing soluble manganese ions, indicating that •OH provided a low contribution to this system. In contrast, SA, a quencher of triplet excited specie, inhibited photolysis of methotrexate and formation of soluble manganese ions. However, methotrexate was still removed 20% within 20 minutes because of MnO_2 redox reaction. These experiment results indicated that triplet excited species is responsible for the generation of soluble manganese ions.

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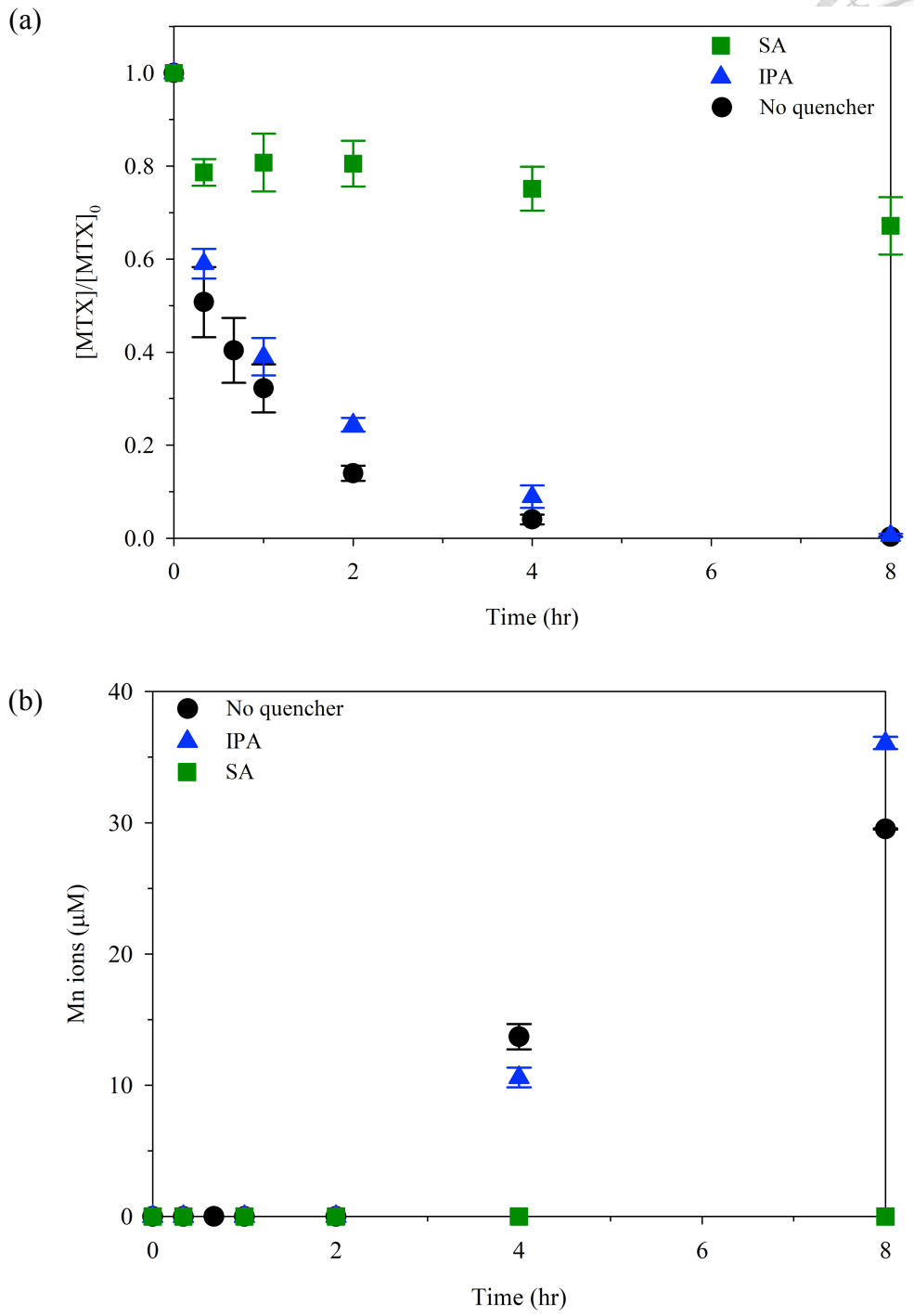

Fig. 4.2 Methotrexate and MnO_2 with quenchers under simulated sunlight irradiation: (a) methotrexate degradation and (b) manganese ions formation ($[MTX]_0$ = 20 μM, $[MnO_2]_0$ = 200 μM, $[IPA]_0$ = 20 mM, $[SA]_0$ = 10 mM, at pH 7.0)

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In methotrexate photolysis, dissolved oxygen absorbs the energy from triplet excited species, quenches triplet excited species and is transformed into $^1O_2$. In order to confirm the effect of triplet excited species or $^1O_2$, MnO_2 and methotrexate reacted with O_2- and N_2-sparging under simulated sunlight irradiation. In oxygen-saturated water, production of $^1O_2$ increased and effect of triplet excited species decreased. As shown in Fig. 4.3(a), in the first 20 minutes, methotrexate degradation efficiency (C_t/C_0) was similar in three different conditions because MnO_2 redox reaction was too fast. But after 2 hours, methotrexate with O_2-sparging was removed 68%, less than the control group (88%) and N_2-sparging conditions (85%). The lower degradation efficiency of methotrexate with O_2-sparging is because $^1O_2$ had a smaller effect on methotrexate degradation (Hsu et al. 2019a). Fig. 4.3(b) shows the concentrations of soluble manganese ions in the gas-sparging experiments. The concentration of soluble manganese ions in the solution with O_2-sparging was 1.6 μM (near the detection limit of AAS). As the result, $^1O_2$ or other reactive oxygen species may not induce formation of manganese ions. On the contrary, experiments with N_2-sparging were detected 2.9 μM of soluble manganese ions earlier than the control group. Therefore, triplet excited species is the reason of inducing MnO_2 reduction under simulated sunlight irradiation.

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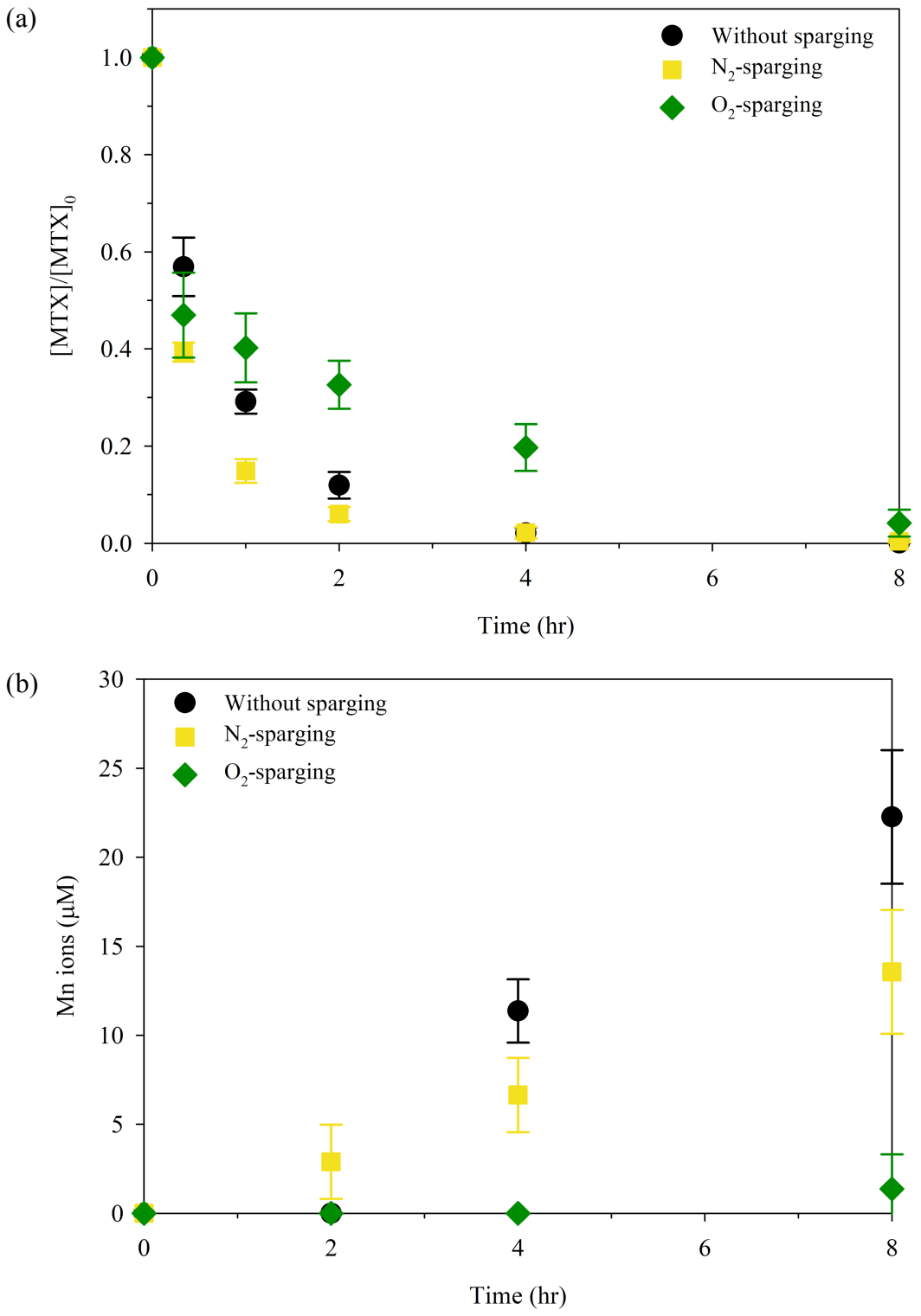

Fig. 4.3 Methotrexate and MnO_2 with N_2- or O_2-sparging: (a) methotrexate degradation and (b) manganese ions formation ($[MTX]_0$ = 20 μM, $[MnO_2]_0$ = 200 μM, at pH 7.0)

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4.2 Oxidation of Mn^{2+} by superoxide anion

From experiments results, we suppose that the system had other reaction to accelerate production of soluble manganese ions. In order to confirm the assumption, methotrexate and Mn^{2+} reacted under simulated sunlight irradiation, and the results of methotrexate degradation efficiency are shown in Fig. 4.4. In 20 minutes, methotrexate which reacted with Mn^{2+} under dark conditions was removed about 10% because of formation of complex between methotrexate and Mn^{2+}, but methotrexate did not degrade after 20 minutes. It indicated that methotrexate and Mn^{2+} did not react under dark condition. In contrast, after 4 hours, 100% of methotrexate was removed in the conditions of reacting with Mn^{2+} under simulated sunlight irradiation, and 60% methotrexate was degraded in only photolysis. The methotrexate degradation rate of reaction with Mn^{2+} under irradiation was faster than that of only photolysis indicating that reactive species generating from methotrexate irradiation may transform Mn^{2+} into other valence and accelerate methotrexate degradation.

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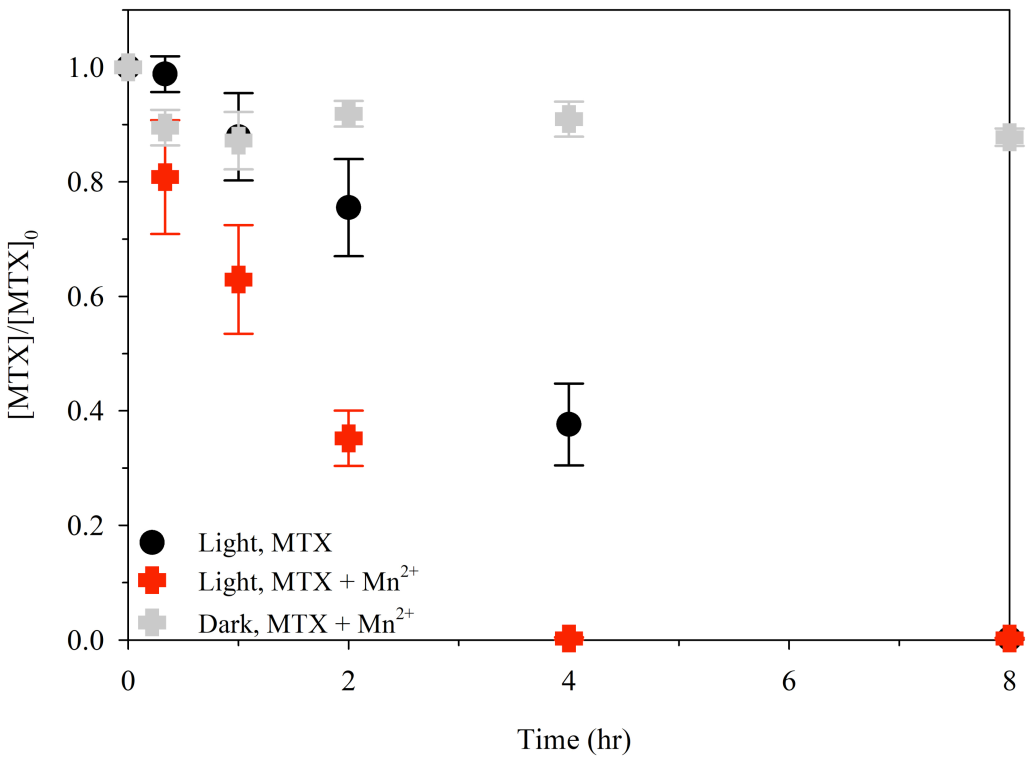

Fig. 4.4 Methotrexate and Mn^{2+} under different conditions ($[MTX]_0$ = 20 μM, $[Mn^{2+}]_0$ = 200 μM, at pH 3.0)

The literature demonstrates that dissolved organic matter as a photosensitizer may be excited to triplet excited state under simulated sunlight irradiation and transform dissolved oxygen into $O_2^{\bullet-}$; and then $O_2^{\bullet-}$ oxidizes Mn^{2+} into Mn^{3+} to oxidize compounds (Wang et al. 2018b, Wang et al. 2018c). Consequently, in order to confirm that my system has similar reactions mentioned above, *p*-benzoquinone (BQ) was used to quench $O_2^{\bullet-}$ in the experiments of methotrexate and Mn^{2+} under simulated sunlight irradiation. As shown in Fig. 4.5, methotrexate degradation efficiency of adding BQ was similar to that of only methotrexate photolysis. The results indicate that methotrexate may generate $O_2^{\bullet-}$ after irradiation, and $O_2^{\bullet-}$ quenching may inhibit the oxidation of Mn^{2+}.

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Therefore, Mn^{2+} oxidation into Mn^{3+} by $O_2^{\bullet-}$ may exist in the system. However, whether Mn^{3+} degrades methotrexate is investigated in the following experiment.

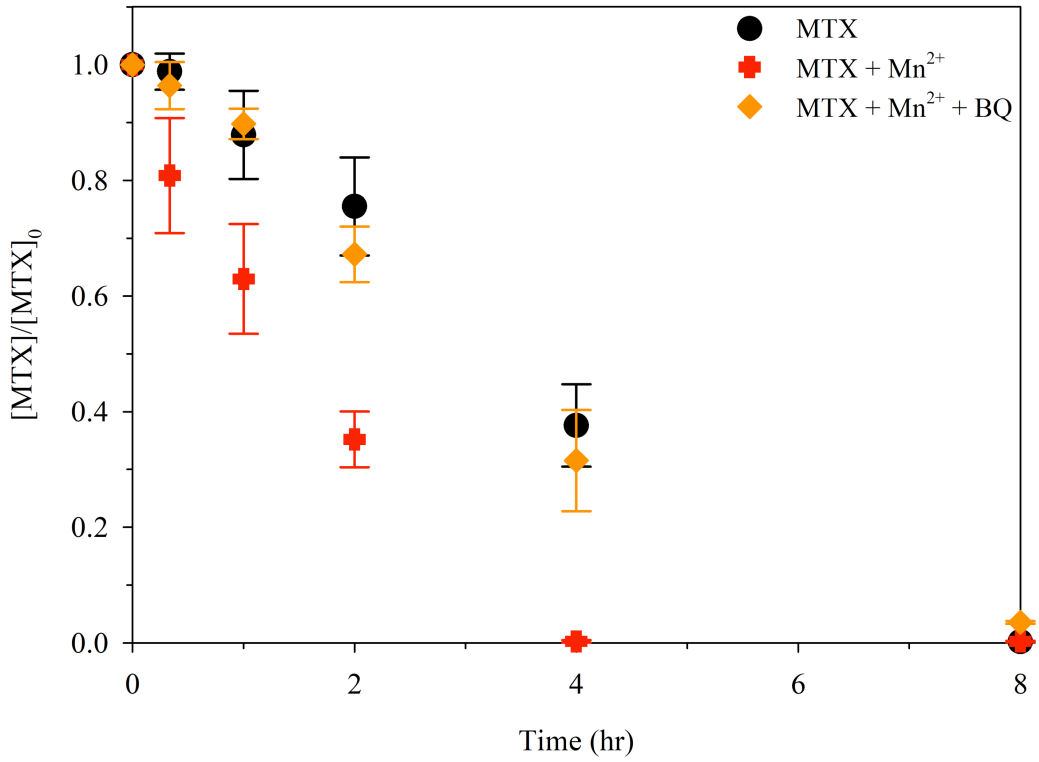

Fig. 4.5 Methotrexate and Mn^{2+} with quencher under simulated sunlight irradiation ($[MTX]_0$ = 20 μM, $[Mn^{2+}]_0$ = 200 μM, $[BQ]_0$ = 10 mM, at pH 3.0)

Mn^{3+} is not stable but has high reduction potential (1.50 V) (Kostka et al. 1995). Numerous studies demonstrate that Mn^{3+} can oxidize compounds effectively (Chen et al. 2013, Wang et al. 2018b, Wang et al. 2018c). In this study, Mn^{3+} was generated from the reduction of MnO_2 by oxalic acid. Fig. 4.6 shows the results of methotrexate degradation efficiency with Mn^{3+} under dark condition. Within 1 hour, methotrexate which reacted

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with Mn^{3+} under dark condition was removed 35%. It indicated that Mn^{3+} may oxidize methotrexate efficiently.

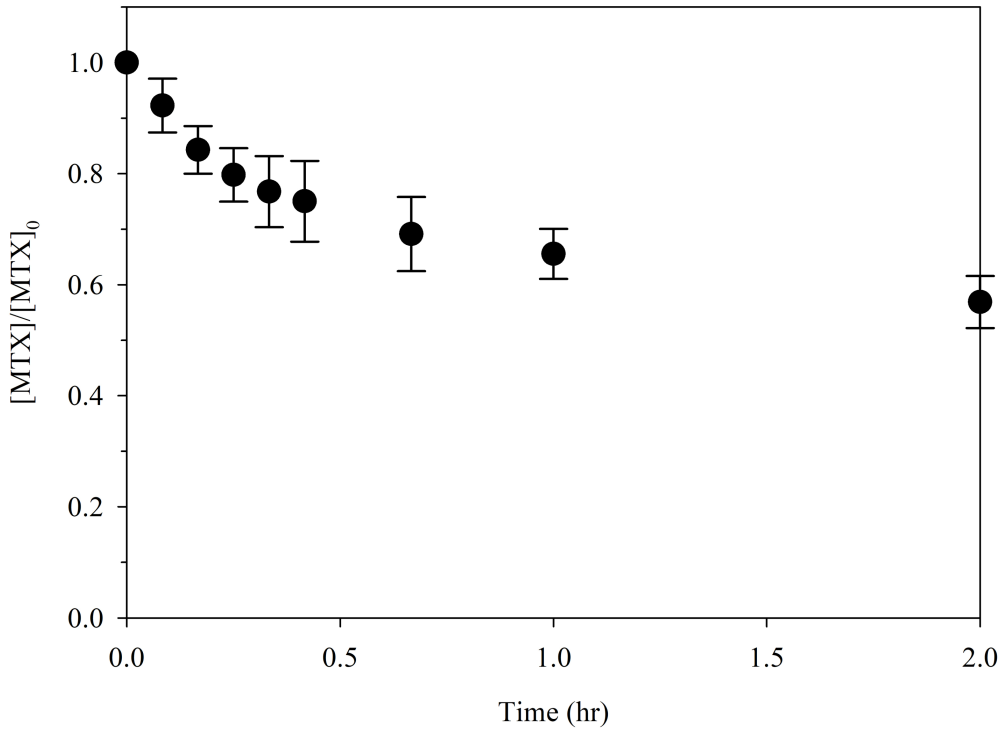

Fig. 4.6 Methotrexate and Mn^{3+} under dark condition
($[MTX]_0$ = 20 μM, $[Mn^{3+}]_0$ = 645 μM, at pH 3.0)

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Chapter 5 Conclusions and recommendation

5.1 Conclusions

1. The self-photosensitized compounds, methotrexate, tetracycline and naproxen, accelerate MnO_2 reduction via sunlight irradiation ($[compound]_0$ = 20 μM, $[MnO_2]_0$ = 200 μM and solution pH = 7).

   - Under simulated sunlight irradiation of methotrexate and MnO_2 at pH 7, manganese ions were produced after 8 hours reaction; higher initial methotrexate concentrations (10, 20 and 40 μM) lead to the increase in manganese ions generation (0, 29.5 and 82.0 μM).
   - For tetracycline and naproxen, 52.2 and 19.2 μM manganese ions were produced after 6.5–8 hours irradiation.
   - For non-photosensitized compounds, 7-ACA and acetaminophen, no any manganese ions were detected after 12–14 hours reaction during the photoirradiation in the presence of MnO_2 ($[7\text{-ACA}]_0$ or $[acetaminophen]_0$ = 20 μM, $[MnO_2]_0$ = 200 μM and solution pH = 7).

2. Triplet excited species were the dominant species to reduce MnO_2 into manganese ions under simulated sunlight irradiation of methotrexate ($[methotrexate]_0$ = 20 μM, $[MnO_2]_0$ = 200 μM and solution pH = 7).

   - Adding IPA (20 mM) to quench •OH did not affect methotrexate degradation

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and manganese ions formation; however, spiking SA (10 mM) to quench triplet excited species obviously inhibited the reaction between methotrexate and MnO_2.

- Compared with no-sparging condition, sparging N_2 into the solution to enhance the performance of triplet excited species resulted in the manganese ions formation earlier. At 1st hour reaction, 2.9 μM of manganese ions formed in N_2-sparging condition while no any manganese ions generated in no-sparging solution.
- By sparging O_2 (for scavenging triplet excited species but also with ROSs generation), the formation of manganese ions has been retarded, indicating the insignificance of ROSs for MnO_2 reduction.

3. Mn^{2+} is oxidized into Mn^{3+} by $O_2^{\bullet-}$ which was generated from sunlight irradiation of methotrexate under aerobic condition; and then Mn^{3+} further degrades methotrexate.

- Under sunlight irradiation alone, only 60% methotrexate was degraded in 4 hours, while addition of Mn^{2+} (200 μM) substantially increased the photodegradation rate to 100%. However, after further spiking BQ (10 mM; to quench $O_2^{\bullet-}$), the methotrexate degradation rate was similar to its photolysis alone.

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- The oxalic acid adding experiment (to generate Mn^{3+} via MnO_2 reduction) suggested that methotrexate can be degraded by Mn^{3+}, while Mn^{2+} did not degrade methotrexate under dark conditions.

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5.2 Environmental implications

Methotrexate, a chemotherapy agent and immune system suppressant, was detected at concentration ranging from <6.25 ng/L in river to 4689 ng/L in hospital effluent (Aherne et al. 1985, Besse et al. 2012, Lin et al. 2014, Yin et al. 2009). Despite the higher methotrexate concentrations (20 μM) studied in this work, other self-photosensitized compounds and natural photosensitizers such as humic acid also exist in natural aqueous system. These photosensitized compounds can transform into triplet excited species and then generate ROSs under sunlight irradiation, further influencing MnO_2 transformation as well as compounds degradation. Considering the fact that sunlight travels into waters about 200 m depth, the photoredox mechanism investigated in this study (Fig. 5) is likely to happen in rivers or top layers of sea which is abundant with manganese oxides.

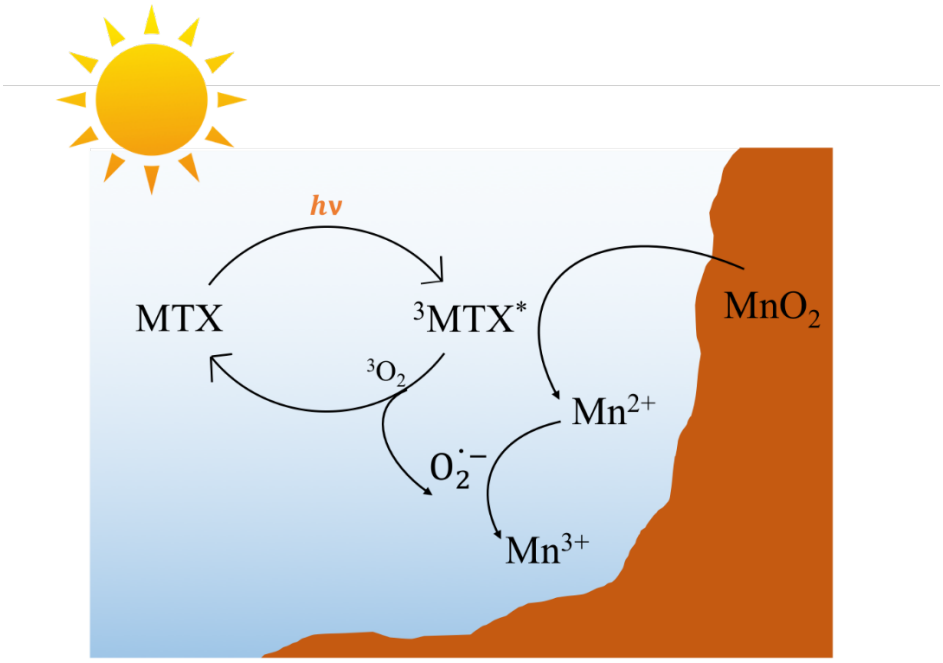

Fig. 5 Redox mechanism between methotrexate and manganese under sunlight

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5.3 Recommendation for future work

The photoredox mechanism between MnO_2 and methotrexate, a self-photosensitized compounds, is demonstrated in this study. For further studies, several recommendations are shown below:

1. In this study, we focused on the observation of methotrexate degradation and soluble manganese ions formation. The change of MnO_2 morphology can be discussed and detected by transmission electron microscopy (TEM) or scanning electron microscopy (SEM).
2. To comprehensively understand the methotrexate transformation under sunlight irradiation of MnO_2, the methotrexate byproducts, total organic carbon (TOC) and change in toxicity are worth exploring to understand the difference compared with those in sunlight irradiation or MnO_2 oxidation alone.
3. In order to confirm the mechanism between methotrexate and MnO_2, the experiments conditions in this study were at high concentration level ($[MnO_2]_0$ = 200 μM and $[methotrexate]_0$ =20 μM). Whether the mechanism under environmentally relevant concentration level ($[MnO_2]_0$ = 1 μM and $[methotrexate]_0$ = 0.1 μM) is similar to this study can be confirmed in the future.
4. In this study, we tested two other self-photosensitized compounds (tetracycline and naproxen). Whether the detailed reaction mechanisms are the same as methotrexate

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should be further confirmed.

5. Iron oxides are another common natural oxidant in environment. Whether the pathway of self-photosensitized compounds and iron oxides under simulated sunlight irradiation is similar to this study can be discuss in the future.

6. Water constituents such as metal cations, metal oxides, and dissolved organic compounds also possibly have influence on the photoredox reaction between methotrexate (or other photosensitized compounds) and MnO_2.

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