**Supplementary Material for**

# Synergistic removal mechanism of tetracycline by ethylenediamine modified magnetic chitosan based Fenton-like catalyst

**Supplementary data**

**Text S1** Materials

Chitosan (85%-90%deacetylated) was purchased from Chemical Reagents Co.,Ltd. of China Pharmaceutical Group. Glutaraldehyde(25%) was from Shanghai Macklin Biochemical Co., Ltd. Epichlorohydrin(AR) and TC(≥99%) were supplied by Aladdin Reagent (Shanghai) Co., Ltd. $FeCl_3 \cdot 6H_2O$ ,$FeSO_4 \cdot 7H_2O$, $CH_3COOH$, NaOH as well as ethylenediamine were of analytical grade and obtained from Beijing Chemical Works.

**Text S2** Characterization methods

Morphology and structure of modified magnetic chitosan beads (EMMCS) were observed by emission scanning electron microscopy (SU-8010, Hitachi, Japan) and transmission electron microscopy (HT7700, Japan Hi-Tech). Functional groups of EMMCS was analyzed by Fourier transflorm infrared(FTIR) spectrophotometry (VERTEX70,Bruker,Germany) in the range of 500-4000$cm^{-1}$.Vibrating sample magnetometer(VSM)( BHV-50HTI, Japan Riken Electronics Co., Ltd.) was taken to measure magntic property of adsorbent; x-ray diffraction(XRD) patterns were recorded using x-ray diffractometer (D8 Advance,Bruker,Germany)with $2\theta$ of 10°-90°.The thermal stability was tested by Thermogravimetric differential thermal-mass spectrometry on-line instrument in the TGA experiment(TG/DTA6300/ Seiko,Japan).

**Text S3** Batch experiment

In the effect of PS concentration experiment, 15mg catalyst was added into 20mL TC solution with initial concentration of 50mg/L shaking well for several hours at different PS concentration (1-40mmol/L).The initial concentration of TC is in the range of 20-200mg/L and the catalyst dosage is 5mg,10mg,15mg,20mg,respectively.In the influence of solution pH,15mg catalysts were added into TC solution with PS concentration of 20mmol/L and the pH was adjusted to 3, 5, 6.5 and 8, respectively. After different time intervals, all the samples were filtered by 0.45 um membranes and determined by Uv-vis spectrophotometer at 358 nm. The removal rate $\eta_t$ (%) was calculated according to equation：

$$\eta_t = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

Where $C_0$ (mg/L) is initial concentration of TC, $C_t$ (mg/L) is the concentration of TC at any time t.

**Text S4** Functional theory calculation analysis

The Gaussian 16 software was used to carry out geometry optimization and single-point energy at the B3LYP/6-311 + G(d, p) level of theory. Electron spin densities based on Natural Bond Orbital (NBO) analysis were used to measure the degree of unpaired spin and charge at different sites in the RhB molecule and radical. The Fukui index was used to explain the regioselectivity for reactive oxidative species (ROS) attacks on the RhB molecule. Particularly, the Fukui function was crucial to DFT, which was frequently used to forecast the reactive sites of electrophilic, nucleophilic, and radical attacks. Fukui function was described as:

$$f(r) = \left[ \frac{\partial \rho(r)}{\partial N} \right]_v \qquad \text{* MERGEFORMAT (1)}$$

where ρ(r) was the electron density at a point r in space, N was the electron number in the present system, the constant term v in the partial derivative was external potential. The amount of electron density distribution around an atom was represented by the atomic population number in the condensed Fukui function (CFF). One method for calculating the Fukui index was as follows:

$$\text{Nucleophilic attack}: \ f_k^+ = q_N^k - q_{N+1}^k \qquad \text{* MERGEFORMAT (2)}$$

$$\text{Electrophilic attack}: f_k^- = q_{N-1}^k - q_N^k \qquad \text{* MERGEFORMAT (3)}$$

$$\text{Radical attack}: f_k^0 = \frac{(q_{N-1}^k - q_{N+1}^k)}{2} \qquad \text{* MERGEFORMAT (4)}$$

where $q_k$ was the atom charge of atom k at corresponding state and the values of Fukui index of the reactive sites were usually larger than other regions.

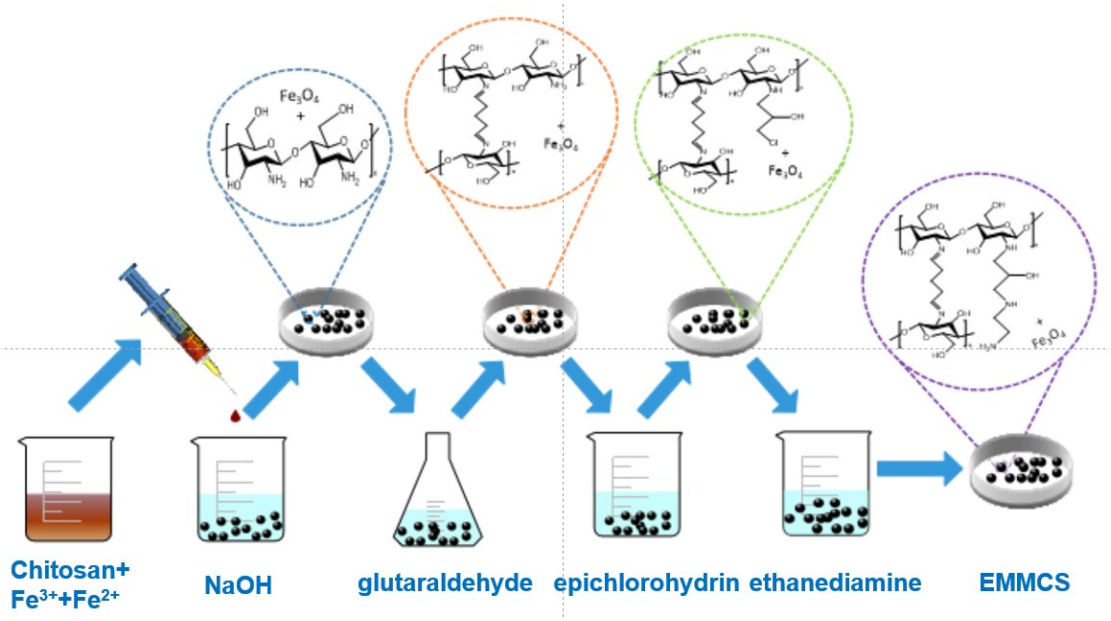
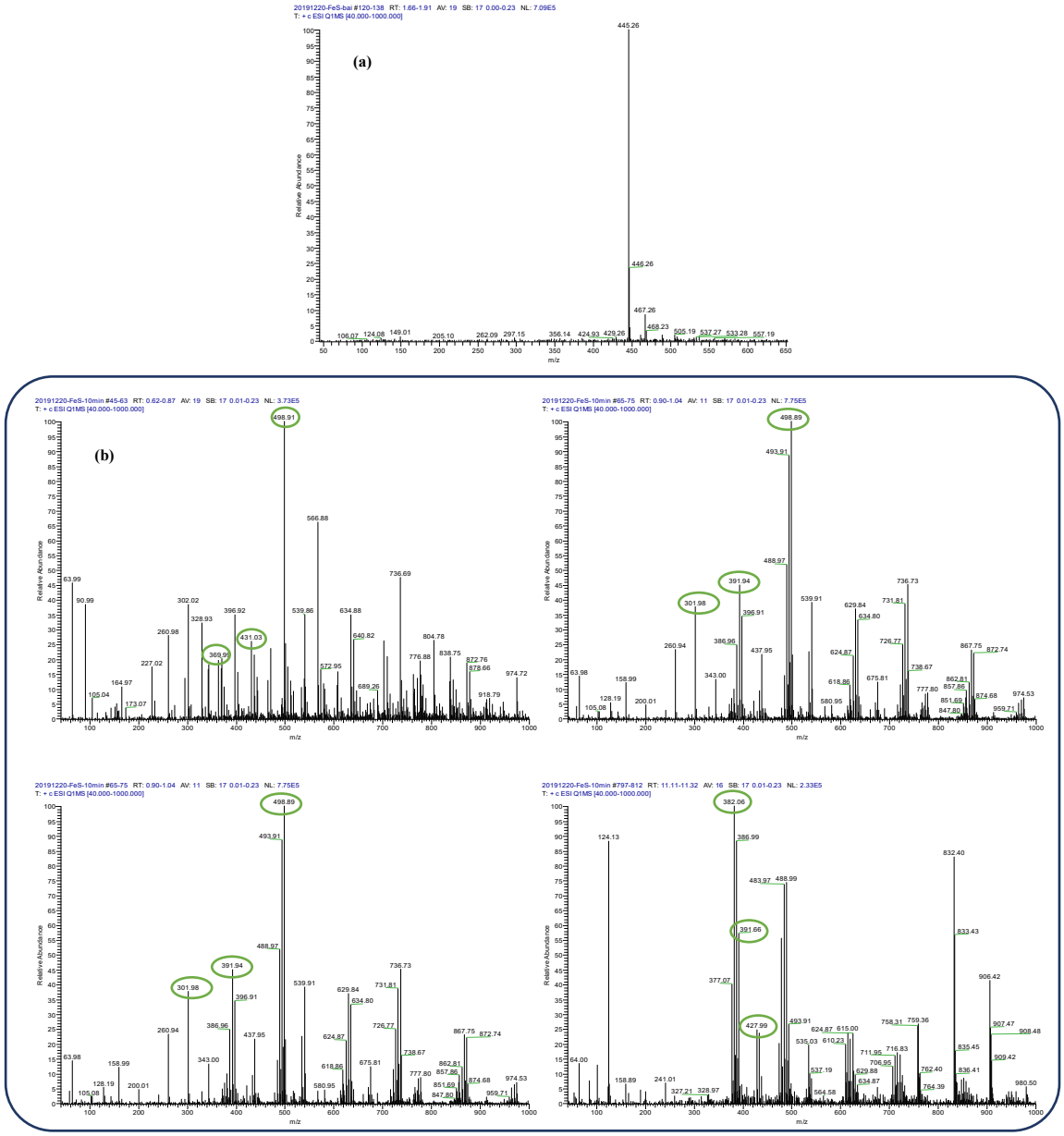



**Fig. S1.** Preparation of modified magnetic chitosan


(a)
(b)

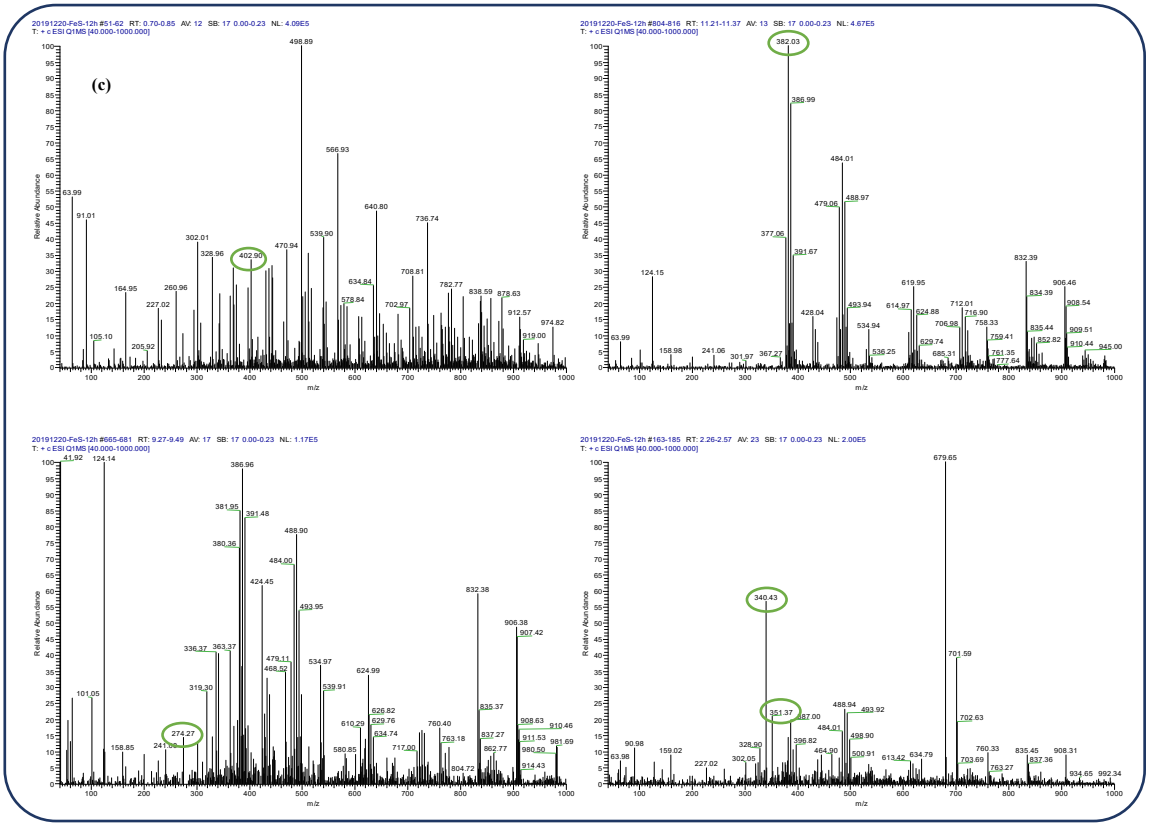



**Fig. S2 (a)** LC-MS spectra of intermediate products **(b)** 10min **(c)** 2h.

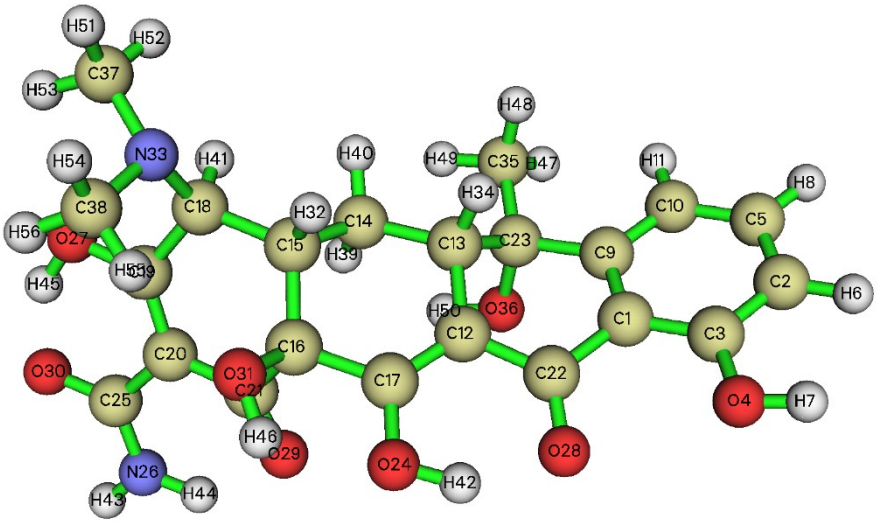



**Fig. S3.** Tetracycline molecular atomic labeling

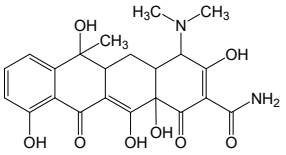
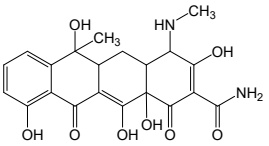
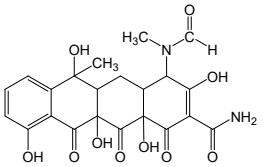
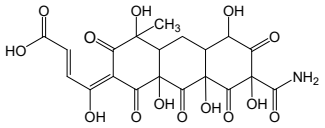
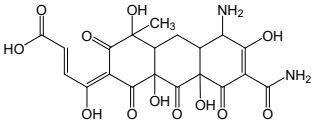
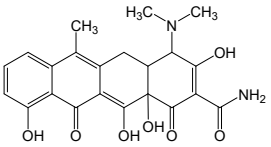
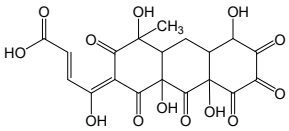
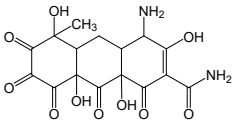
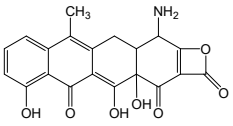
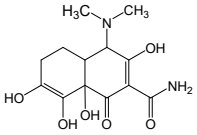
**Table S1.** Hirshfeld charges and calculated $f^-$, f+ and f0 of tetracycline

| Atom | N | N-1 | N+1 | f- | f0 | f+ |
|---|---|---|---|---|---|---|
| 1(C) | -0.0275205 | -0.0085272 | -0.0434532 | 0.0189933 | 0.01746304 | 0.01593277 |
| 2(C) | -0.066003 | -0.0422778 | -0.097928 | 0.02372521 | 0.02782512 | 0.03192503 |
| 3(C) | 0.09262931 | 0.11756706 | 0.06775358 | 0.02493775 | 0.02490674 | 0.02487573 |
| 4(O) | -0.1626767 | -0.1264937 | -0.1812622 | 0.03618305 | 0.02738428 | 0.0185855 |
| 5(C) | -0.0263733 | 0.00179939 | -0.0823459 | 0.02817269 | 0.04207266 | 0.05597263 |
| 6(H) | 0.04055567 | 0.05783642 | 0.0176824 | 0.01728075 | 0.02007701 | 0.02287327 |
| 7(H) | 0.17662513 | 0.19464476 | 0.15851753 | 0.01801963 | 0.01806362 | 0.0181076 |
| 8(H) | 0.04743944 | 0.06578739 | 0.01820498 | 0.01834795 | 0.02379121 | 0.02923446 |
| 9(C) | 0.01139523 | 0.01676207 | -0.0146964 | 0.00536684 | 0.01572924 | 0.02609163 |
| 10(C) | -0.0532653 | -0.0207678 | -0.0764041 | 0.03249758 | 0.02781815 | 0.02313871 |
| 11(H) | 0.03930252 | 0.05430786 | 0.02221618 | 0.01500534 | 0.01604584 | 0.01708634 |
| 12(C) | -0.0622178 | -0.0287956 | -0.0758442 | 0.03342224 | 0.02352431 | 0.01362638 |
| 13(C) | -0.0249827 | -0.0203287 | -0.0272259 | 0.00465402 | 0.0034486 | 0.00224318 |
| 14(C) | -0.047906 | -0.0446139 | -0.05157 | 0.00329214 | 0.00347804 | 0.00366393 |
| 15(C) | -0.0203418 | -0.0154432 | -0.0223289 | 0.00489857 | 0.00344287 | 0.00198716 |
| 16(C) | 0.06856246 | 0.07147048 | 0.06093707 | 0.00290802 | 0.00526671 | 0.00762539 |
| 17(C) | 0.0969892 | 0.11368417 | 0.05961054 | 0.01669497 | 0.02703682 | 0.03737866 |
| 18(C) | 0.02777887 | 0.03852101 | 0.02183735 | 0.01074214 | 0.00834183 | 0.00594152 |
| 19(C) | 0.1297174 | 0.13246881 | 0.08227781 | 0.00275141 | 0.0250955 | 0.04743959 |
| 20(C) | -0.0819613 | -0.0622735 | -0.0974332 | 0.01968779 | 0.01757983 | 0.01547187 |
| 21(C) | 0.12739542 | 0.13443482 | 0.09374104 | 0.0070394 | 0.02034689 | 0.03365438 |
| 22(C) | 0.135434 | 0.14345401 | 0.07124948 | 0.00802001 | 0.03610227 | 0.06418452 |
| 23(C) | 0.08849765 | 0.09223114 | 0.08630925 | 0.00373349 | 0.00296095 | 0.0021884 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24(O) | -0.1609629 | -0.1261567 | -0.1948567 | 0.03480626 | 0.03435004 | 0.03389382 |
| 25(C) | 0.16125675 | 0.1711576 | 0.15083716 | 0.00990085 | 0.01016022 | 0.01041959 |
| 26(N) | -0.1306341 | -0.1135241 | -0.1457334 | 0.01710997 | 0.01610464 | 0.0150993 |
| 27(O) | -0.1448699 | -0.135956 | -0.1840213 | 0.00891394 | 0.02403263 | 0.03915132 |
| 28(O) | -0.223413 | -0.1991877 | -0.2936434 | 0.02422533 | 0.04722783 | 0.07023033 |
| 29(O) | -0.2372861 | -0.2081533 | -0.2804634 | 0.02913277 | 0.03615504 | 0.0431773 |
| 30(O) | -0.2762685 | -0.2484052 | -0.3023396 | 0.02786328 | 0.02696717 | 0.02607106 |
| 31(O) | -0.1983496 | -0.1891109 | -0.2195437 | 0.00923875 | 0.01521642 | 0.02119408 |
| 32(H) | 0.03633268 | 0.04243645 | 0.02665639 | 0.00610377 | 0.00789003 | 0.00967629 |
| 33(N) | -0.0881158 | 0.03163056 | -0.0920413 | 0.11974638 | 0.06183594 | 0.0039255 |
| 34(H) | 0.02818504 | 0.03902232 | 0.01864446 | 0.01083728 | 0.01018893 | 0.00954058 |
| 35(C) | -0.0918929 | -0.0867285 | -0.0982723 | 0.0051644 | 0.00577189 | 0.00637938 |
| 36(O) | -0.2243675 | -0.215422 | -0.231475 | 0.0089455 | 0.00802651 | 0.00710751 |
| 37(C) | -0.0445716 | -0.0171611 | -0.0507571 | 0.02741049 | 0.01679799 | 0.00618548 |
| 38(C) | -0.0535164 | -0.027541 | -0.0577751 | 0.02597545 | 0.01511708 | 0.00425871 |
| 39(H) | 0.02144944 | 0.02826681 | 0.01703188 | 0.00681737 | 0.00561747 | 0.00441756 |
| 40(H) | 0.03302803 | 0.04037227 | 0.01978855 | 0.00734424 | 0.01029186 | 0.01323948 |
| 41(H) | 0.04078224 | 0.05257139 | 0.02637158 | 0.01178915 | 0.01309991 | 0.01441066 |
| 42(H) | 0.13237444 | 0.14337366 | 0.11914821 | 0.01099922 | 0.01211273 | 0.01322623 |
| 43(H) | 0.1357307 | 0.15143954 | 0.11865307 | 0.01570884 | 0.01639324 | 0.01707763 |
| 44(H) | 0.10698437 | 0.1141283 | 0.10051856 | 0.00714393 | 0.00680487 | 0.00646581 |
| 45(H) | 0.12651543 | 0.13514551 | 0.11487357 | 0.00863008 | 0.01013597 | 0.01164186 |
| 46(H) | 0.14987527 | 0.16040112 | 0.13882971 | 0.01052585 | 0.01078571 | 0.01104556 |
| 47(H) | 0.03492406 | 0.04566574 | 0.02396156 | 0.01074168 | 0.01085209 | 0.0109625 |
| 48(H) | 0.03285899 | 0.03850019 | 0.0245935 | 0.0056412 | 0.00695335 | 0.00826549 |
| 49(H) | 0.03009258 | 0.03553897 | 0.02103317 | 0.00544639 | 0.0072529 | 0.00905941 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 50(H) | 0.14684424 | 0.15187144 | 0.13902514 | 0.0050272 | 0.00642315 | 0.0078191 |
| 51(H) | 0.03178184 | 0.05879983 | 0.01933014 | 0.02701799 | 0.01973485 | 0.0124517 |
| 52(H) | 0.02960883 | 0.05202368 | 0.02343843 | 0.02241485 | 0.01429263 | 0.0061704 |
| 53(H) | 0.01769438 | 0.05821366 | 0.00988629 | 0.04051928 | 0.02416369 | 0.00780809 |
| 54(H) | 0.03058741 | 0.05687069 | 0.01743343 | 0.02628328 | 0.01971863 | 0.01315398 |
| 55(H) | 0.02405944 | 0.04001823 | 0.02362126 | 0.01595879 | 0.00819849 | 0.00043818 |
| 56(H) | 0.01420846 | 0.05445043 | 0.00740099 | 0.04024197 | 0.02352472 | 0.00680747 |

**Table S2.** The structural information of the possible intermediates products in EMMCS/PS-TC system obtained from LC-MS

| Reaction time | Compounds | Formula | m/z | Proposed structure |
|---|---|---|---|---|
| 0 | TC | $C_{22}H_{24}N_2O_8$ | 445 |  |
| 10min | B1 | $C_{21}H_{22}N_2O_8$ | 431 |  |
| 10min | B2 | $C_{22}H_{22}N_2O_{10}$ | 475 |  |
| 10min | B4 | $C_{19}H_{19}N_1O_{14}$ | 498 |  |
| 10min | B5 | $C_{19}H_{20}N_2O_{12}$ | 475 |  |
| 10min | B6 | $C_{22}H_{22}N_2O_7$ | 427 |  |
| 10min | B8 | $C_{19}H_{15}O_{13}$ | 453 |  |
| 10min | B9 | $C_{16}H_{16}N_2O_{10}$ | 391 |  |
| 10min | B10 | $C_{20}H_{14}N_1O_7$ | 382 |  |
| 10min | B11 | $C_{13}H_{18}N_2O_6$ | 301 |  |

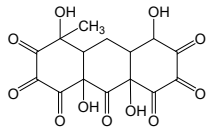
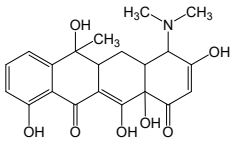
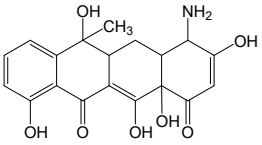
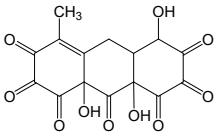
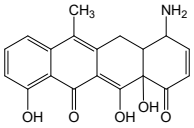
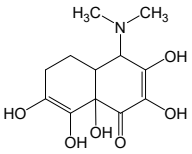
| | | | | |
|---|---|---|---|---|
| 10min | B12 | $C_{15}H_{12}O_{11}$ | 369 |  |
| 12h | B3 | $C_{21}H_{23}N_1O_7$ | 402 |  |
| 12h | B7 | $C_{19}H_{19}N_1O_7$ | 374 |  |
| 12h | B13 | $C_{15}H_{10}O_{10}$ | 351 |  |
| 12h | B14 | $C_{19}H_{16}N_1O_5$ | 340 |  |
| 12h | B15 | $C_{12}H_{19}N_1O_6$ | 274 |  |

**Table S3.** Toxicity of tetracycline and its photodegradation intermediates determined by Toxicity Estimation Software Tool (TEST).

| Products | m/z | Fathead mimmow $LC_{50}$ (96 hr) mg/L | Daphnia minnow $LC_{50}$ (48 hr) mg/L | Oral rat $LD_{50}$ (mg/kg) | Developmental Toxicity | | Mutagenicity | |
|---|---|---|---|---|---|---|---|---|
| | | Predicted value | Predicted value | Predicted value | Developmental Toxicity value | Developmental Toxicity result | Mutagenicity value | Mutagenicity result |
| TC | 445 | 0.2500 | 5.44 | 1524.04 | 0.86 | Developmental toxicant | 0.6 | Mutagenicity Positive |
| $B_1$ | 431 | 0.2500 | 6.99 | 1530.04 | 0.85 | Developmental toxicant | 0.65 | Mutagenicity Positive |
| $B_2$ | 475 | 1.0100 | 60.54 | 1485.97 | 0.85 | Developmental toxicant | 0.7 | Mutagenicity Positive |
| $B_4$ | 498 | N/A | 320.08 | 3099.29 | 0.52 | Developmental toxicant | 0.63 | Mutagenicity Positive |
| $B_5$ | 475 | N/A | 81.03 | 1675.92 | 0.65 | Developmental toxicant | 0.6 | Mutagenicity Positive |
| $B_6$ | 427 | 0.2800 | 1.26 | 1942.14 | 0.92 | Developmental toxicant | N/A | N/A |
| $B_8$ | 453 | 0.0231 | 617.1 | 3222.48 | 0.67 | Developmental toxicant | 0.64 | Mutagenicity Positive |
| $B_9$ | 391 | 0.0191 | 132.13 | 1242.84 | 0.84 | Developmental toxicant | 0.59 | Mutagenicity Positive |
| $B_{10}$ | 382 | 0.2800 | 4.17 | 2916.47 | 0.96 | Developmental toxicant | N/A | N/A |
| $B_{11}$ | 301 | 39.4500 | 24.96 | N/A | 0.8 | Developmental toxicant | N/A | N/A |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $B_{12}$ | 369 | 0.0242 | 1099.42 | 458.55 | 0.72 | Developmental toxicant | 0.33 | Mutagenicity Negative |
| $B_3$ | 402 | 3.3700 | 8.67 | 1320.07 | 0.77 | Developmental toxicant | 0.72 | Mutagenicity Positive |
| $B_7$ | 374 | 1.1600 | 5.82 | 1321.39 | 0.79 | Developmental toxicant | 0.78 | Mutagenicity Positive |
| $B_{13}$ | 351 | 0.0396 | 301.63 | 856.48 | 0.78 | Developmental toxicant | 0.33 | Mutagenicity Negative |
| $B_{14}$ | 340 | 0.1300 | 2.1 | 1160.16 | 0.88 | Developmental toxicant | 0.67 | Mutagenicity Negative |
| $B_{15}$ | 274 | 507.5300 | 53.45 | 1958.34 | 0.63 | Developmental toxicant | 0.37 | Mutagenicity Negative |
| $B_{16}$ | 299 | 2.0000 | 10.17 | 952.02 | 0.88 | Developmental toxicant | 0.66 | Mutagenicity Positive |
| $B_{17}$ | 246 | 3.6800 | 84.14 | 995.5 | 0.85 | Developmental toxicant | 0.44 | Mutagenicity Negative |
| $B_{18}$ | 222 | 29.6900 | 12.49 | 1370.56 | 0.89 | Developmental toxicant | 0.12 | Mutagenicity Negative |
| $B_{19}$ | 173 | 2.4200 | 10.59 | 806.75 | 0.62 | Developmental toxicant | 0.4 | Mutagenicity Negative |
| $B_{20}$ | 227 | 114.4600 | 126.53 | N/A | 0.72 | Developmental toxicant | 0.37 | Mutagenicity Negative |
| $B_{21}$ | 219 | 105.2000 | 157.4 | 942.43 | 0.43 | Developmental NON-toxicant | 0.21 | Mutagenicity Negative |

*The green, blue, pink and red fillings in the table indicate not harmful, harmful, toxic and very toxic respectively. Yellow padding means unpredictable.