Supporting Information

Reactive Nitrogen Species Mediated Degradation of Estrogenic

Disrupting Chemicals by Biochar/Monochloramine in Buffered Water

and Synthetic Hydrolyzed Urine

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The supplementary information includes 3 texts, 6 tables, and 13 figures.

Text S1. Chemical materials. Sodium hypochlorite solution (NaOCl, 5% purity) was purchased from Aladdin, China. estradiol (E2, 99%), ethynylestradiol (EE2, 97%), potassium iodide (KI, 98%), N,N-Diethyl-p-phenylenediaminesulfate (DPD, 98%), Tertiary butanol ($C_4H_{10}O$, TBA, 99.5%), tetrahydrofuran (($CH_2)_4O$, THF), 5,5-Dimethyl-1-pyrrolidine N-oxide (DMPO, 98%), flavanone ($C_{15}H_{12}O_2$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dimethyl sulfoxide (DMSO), 2,2,6,6-tetramethylpiperidine (TEMP), hydrogen nitrate (HNO_3, 65-68%), hydrochloride acid (HCl, 36-38%), hydrogen peroxide (H_2O_2, 30%), and perchloric acid ($HClO_4$, 70-72%) were purchased from HEOWNS, China. Sodium hydroxide (NaOH, 99%) and ammonium chloride (NH_4Cl, ≥99.8%) were purchased from Kermel, China. Disodium phosphate dodecahydrate (Na_2HPO_4, ≥99%) was purchased from Tianjin Gungfu Technology Development Co., Ltd., China. Deionized water (DI) with resistivity 18.2 MΩ·cm^{-1} was produced by ELGA PURELAB pure water system. The E2, EE2 stock solutions were prepared at 100μM in DI water. The NH_4Cl and FAC stock solutions were prepared at 15mM in DI water. The synthetic hydrolyzed urine was prepared according to the recipe in Table S1.

Text S2. Biochar preparation

Firstly, dry cotton straw, wheat straw and corn straw were ground into powder on a disk-rotating mill and passed through a 40-mesh sieve. Then the 25mL-crucible with straw powder were put into a muffle. The starting temperature was approximately 20℃, then elevated to the desired temperature (elevating 10℃ per minute) and remained for two hours at the desired temperature. After cooling, the biochar was transferred into a beaker and stirred for 1h to reach the dissolving equilibrium. Then, the biochar should be washed for several times until the absorbance of supernatant was less than 0.005 under 254nm in UV/Vis spectrophotometer. Finally, the beaker with biochar solution was put into the drying oven under 80℃ for drying. In this study, Cot350 was used as target biochar.

Text S3. Analytic Methods

HPLC Parameters for E2, EE2, Phenol and Anisole. An eluent of nano-pure water and methanol (30:70, v/v%) was used to separate E2, EE2 and its products at a flow rate of 1.0 mL/min. The peak area of E2 and EE2 was quantified at 280nm. As for phenol and anisole, the ratios of methanol to water were 60:40 and 45:55 respectively and the flow rate was 1.0mL/min. The peak area of phenol and anisole was quantified at 270nm and 220nm, respectively.

EPR Analysis. The parameters for all solid samples were as follows: center field of 3508G, sweep width of 200G, sweep time of 15s, receiver gain of 15dB, attenuation of 26dB, modulation amplitude of 1G, number of scans of 15 and number of points of 1400. The parameters for all liquid samples were center field of 3500G, sweep width of 200G, sweep time of 10s, receiver gain of 30dB, attenuation of 15dB, modulation amplitude of 1G, numbers of scans of 15 and number of points of 1400.

Estrogenic Activity Assessment. The extracted aqueous solution by ethanol required pre-treatment due to the toxic effect of ethanol on yeast before its addition to the yeast solution. Aqueous solution with 1 $g \cdot L^{-1}$ biochar, 0.30 mM NH_4Cl, 0.15 mM FAC, 5 mM PBS, 10 μM E2 (or EE2) was removed 2×0.5 mL solution after 24 h treatment. 1) The first 0.5 mL sample was introduced into 0.5 mL ethanol and 20 μL $Na_2S_2O_3$, and then kept shaking for 20 min under condition of 25 ℃ and 200 rpm. 2) The other 0.5 mL sample was treated as the same as the first sample and filtered through 0.45 μm PET membrane. Then, the solution was blown dry by N_2 after the addition of 1 mL ethyl acetate, and then 1 mL nano-pure water was introduced. The quantification of E2 and EE2 in the two samples were confirmed to be no big distinctive (Fig. S2). The recombined yeast bioassays with human estrogen receptor alpha (αhER) were used to screen the remaining equivalent estrogenic

71 effects of products after treatment. The recombined yeast bioassay was obtained from

72 Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (Beijing,

73 China). The detailed procedures of yeast estrogenic assay was performed according to Ref.

74 2.[1]

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76 **Mechanistic Experiments.** 10 μL TBA was introduced into 10 mL aqueous solution

77 with 10 mg biochar, 0.30 mM NH_4Cl, 0.15 mM FAC, 5 mM PBS and 10 μM E2 or EE2 at

78 the initial reaction, with the aim of quenching the hydroxyl radicals and chlorine atoms.

79 Then, the extraction and analysis experiments were conducted as above. Whether or not

80 the biochar/NH_2Cl system produced a catalytic reaction was confirmed through the cycle

81 experiments. The initial reaction conditions of cycle experiment were 1 g·L^{-1} biochar, 0.30

82 mM NH_4Cl and 0.15 mM FAC in aqueous solution at pH 9 and then periodically 4 mL

83 sample were removed to quantify the monochloramine and FAC through DPD method.[2]

84 Meanwhile, predetermined amount of FAC was introduced to the reaction bottle every 24

85 h, with the aim of keeping the concentrations of NH_2Cl as constant at every 24 h. 0.5 g·L^{-}

86 ^{1} single-walled carbon nanotube solution (SWCNT) was introduced into aqueous solution

87 with 0.15 mM NH_2Cl in a 10-mL bottle and then measured the concentrations of NH_2Cl at

88 different time.

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90 **Analysis of Linear Sweeping Voltammetry.** Five biochar-coated and one SWCNT-

91 coated fluorinated tin oxide glass was used as the working electrode with a Pt counter

92 electrode and an Ag/AgCl (in saturated KCl) reference electrode. A 5 mM phosphate buffer

93 was used as the electrolyte. The current at a working electrode was measured with

94 increasing the potential from 0.2 to1.6 V with a scan rate of 20 mV/s.

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96 **Table S1**

97 Composition of hydrolyzed urine.

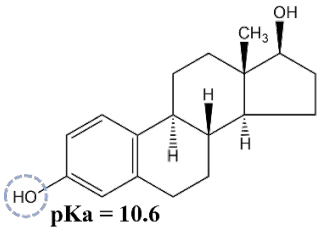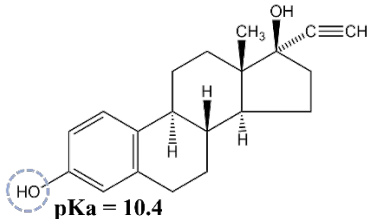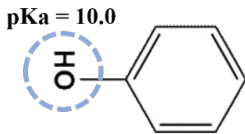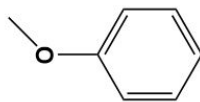| Species	Concentration $(g \cdot L^{-1})$
NaCl	3.50
Na_2SO_4	2.13
KCl	2.98
NH_4OH (conc.)	15.92 (8.5 $mL \cdot L^{-1}$, NH_3, 0.91 $g \cdot L^{-1}$)
$NaH_2PO_4 \cdot 2H_2O$	1.63
NH_4HCO_3	19.76
	pH = 9

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99 **Table S2**

100 Molecular structure and molecular weight of targeting pollutants.

Name	Molecular Structure	Molecular Weight
Estradiol (E2)		272.4
Ethynylestradiol (EE2)		296.4
Phenol		94.11

| Anisole | | 108.14 |

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102   **Table S3**

103   Characteristics of five biochar

	V_t (cm^3·g^{-1})	D_p (nm)	S_{BET} (m^2·g^{-1})
Cot350	0.03514	18.017	39.005
CS350	0.01121	26.5789	8.432
CS700	0.2479	10.3332	479.742
WS350	0.01261	22.8227	11.048
WS700	0.0633	12.4466	101.719

104   S_{BET}: Multi-Brunauer-Emmett-Teller surface area; V_t: total pore volume; D_p: average pore

105   diameter.

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117   **Table S4**

118   SWCNT characterization

Diameter	Element C content	Ash content	Specific surface area
1-3 nm	>85 wt.%	<15 wt.%	400-600 m^2·g^{-1}

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120 **Table S5**

121 Concentrations of PFRs, line width and g-factors of five biochars before NH_2Cl treatment

Samples	g-factor	line width (ΔH_{p-p}, G)	Concentrations of PFRs
Cot350	NaN	NaN	NaN
WS350	2.00275	5.6549	5.75×10^{18}
WS700	NaN	NaN	NaN
CS350	2.00295	5.01043	1.83×10^{18}
CS700	NaN	NaN	NaN

122 Note: NaN means that there was no signals in EPR spectra.

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131 **Table S6**

132 Concentrations of PFRs, line width and g-factors of five biochars after NH_2Cl treatment

Samples	g-factor	line width (ΔH_{p-p}, G)	Concentrations of PFRs
Cot350	2.00252	3.7098	8.25×10^{16}
WS350	2.00281	5.5918	2.70×10^{18}
WS700	NaN	NaN	NaN
CS350	2.00293	5.1186	1.77×10^{18}
CS700	2.0029	3.0208	2.46×10^{16}

133 Note: NaN means there was no signals in EPR spectra.

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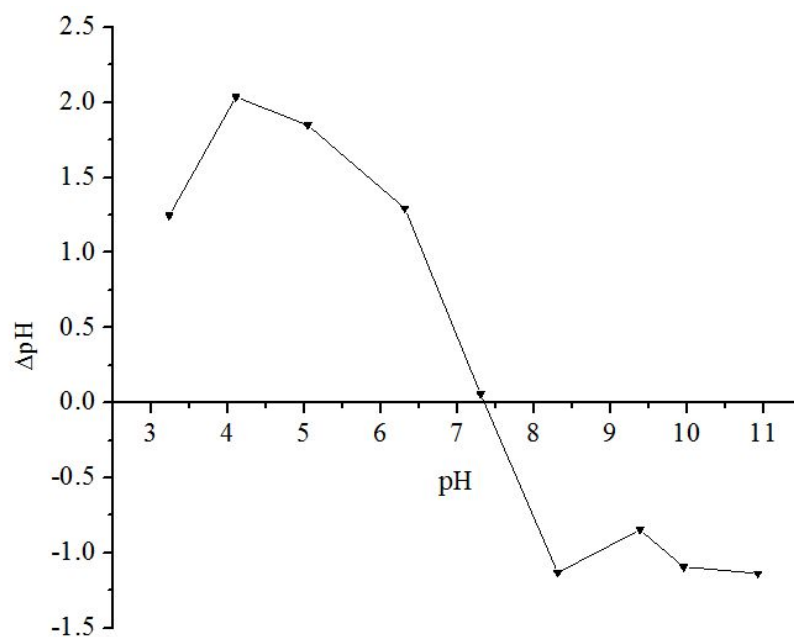
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136 **Fig. S1**. Point of zero charge of Cot350

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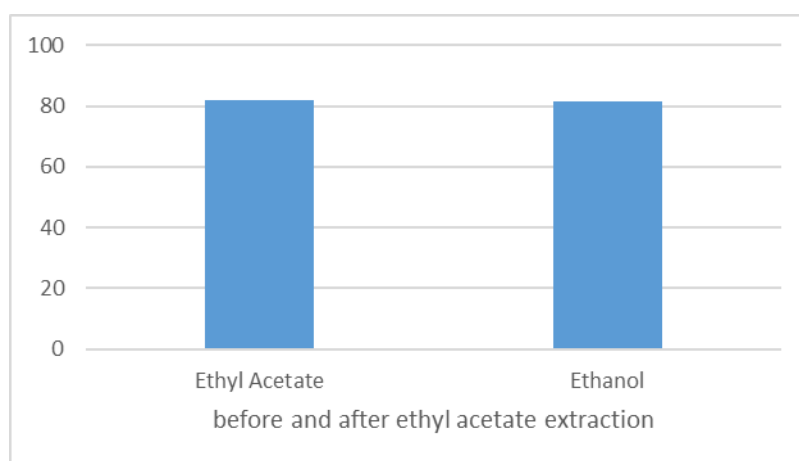
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143 **Fig. S2** Amount of E2 and EE2 measured by HPLC before and after treatment through pre-

144 treatment and ethanol extraction.

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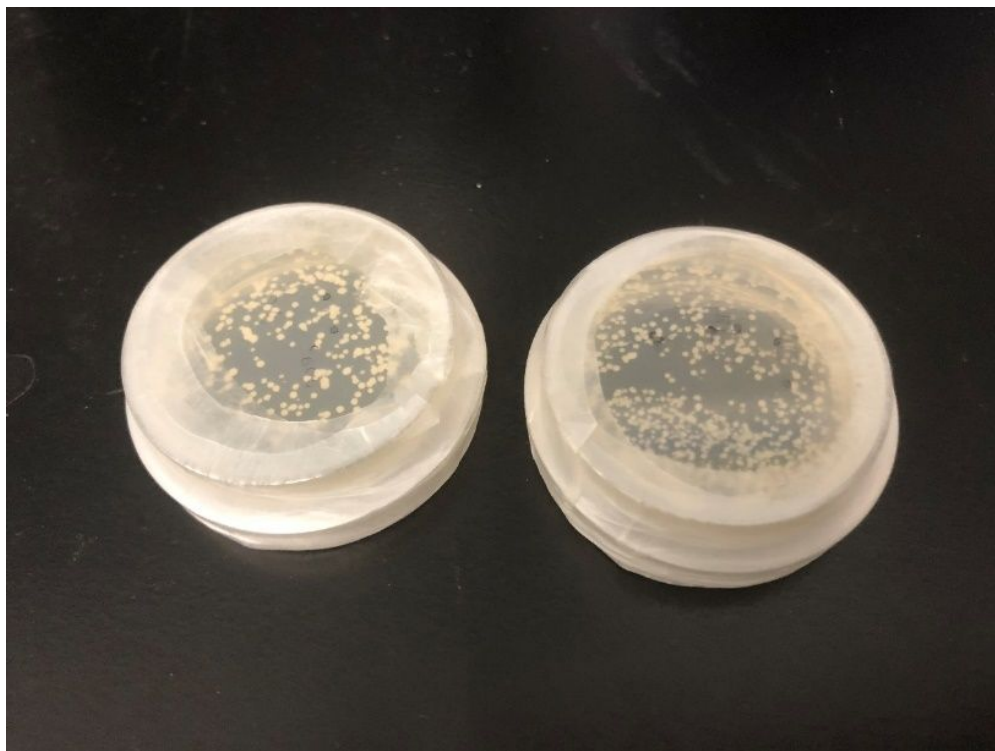
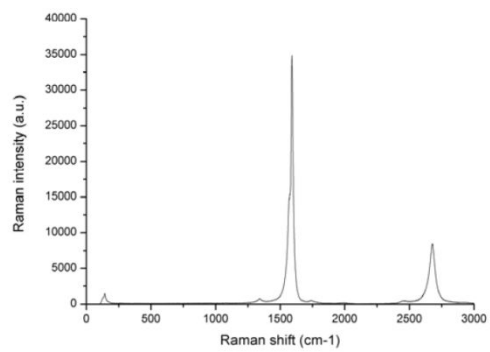146 **Fig. S3** Recombined estrogenic yeast plate.

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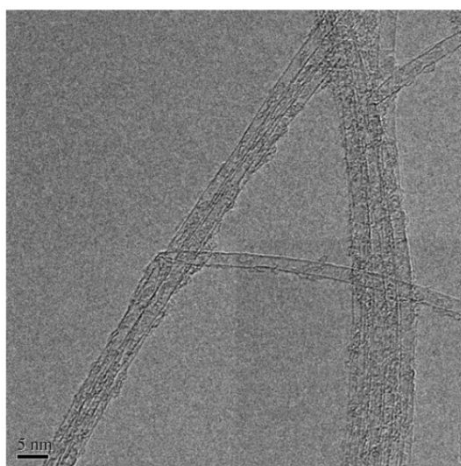
151 **Figure S4.** SWCNT characterization

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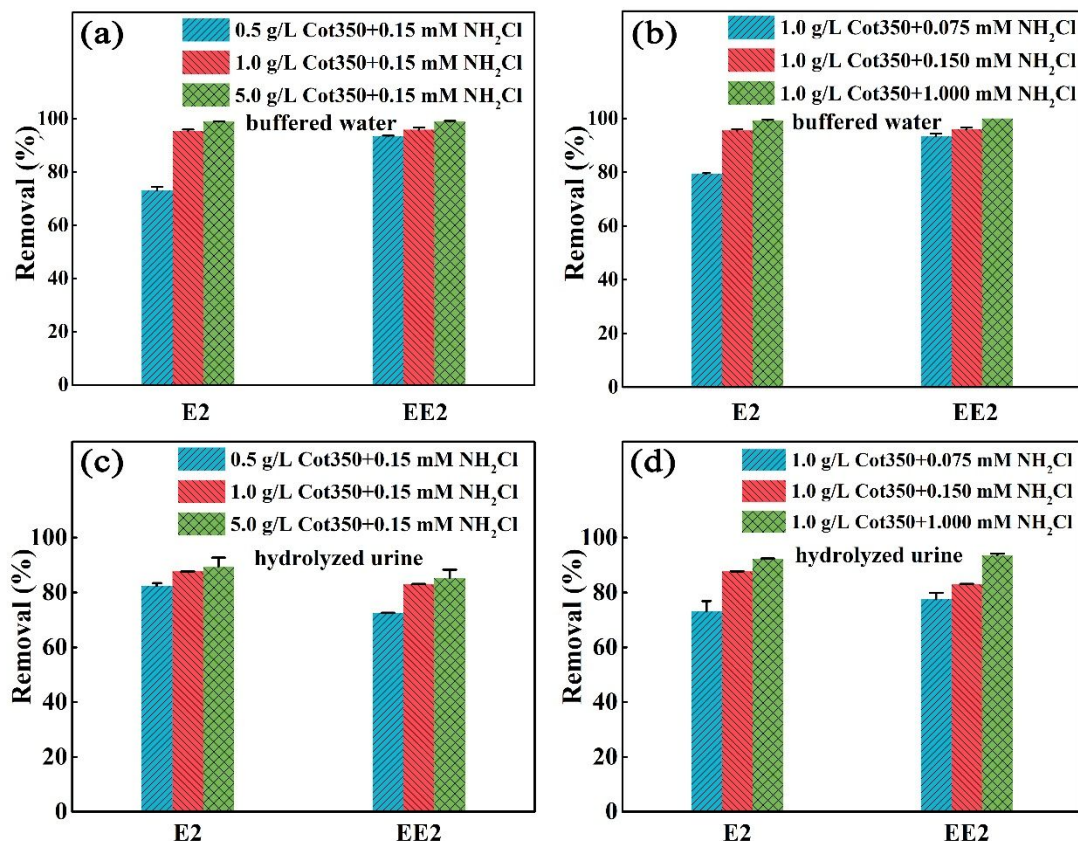
Figure S5. E2 and EE2 degradation within 24h under varying Cot350 and NH$_2$Cl concentrations: (a) in PBS with increasing concentrations of Cot350; (b) in PBS with increasing concentrations of NH$_2$Cl; (c) in synthetic hydrolyzed urine with increasing concentrations of Cot350; (d) in synthetic hydrolyzed urine with increasing concentrations of NH$_2$Cl. Reaction Condition: [Cot350]$_0$ = 0.5, 1.0, 5.0 g·L^{-1}; [NH$_2$Cl]$_0$ = 0.075, 0.150, 1.000 mM; [Phosphate buffer solution, PBS]$_0$ = 5 mM; [E2]$_0$ = [EE2]$_0$ = 10 μM; T = 25 °C; pH = 9.

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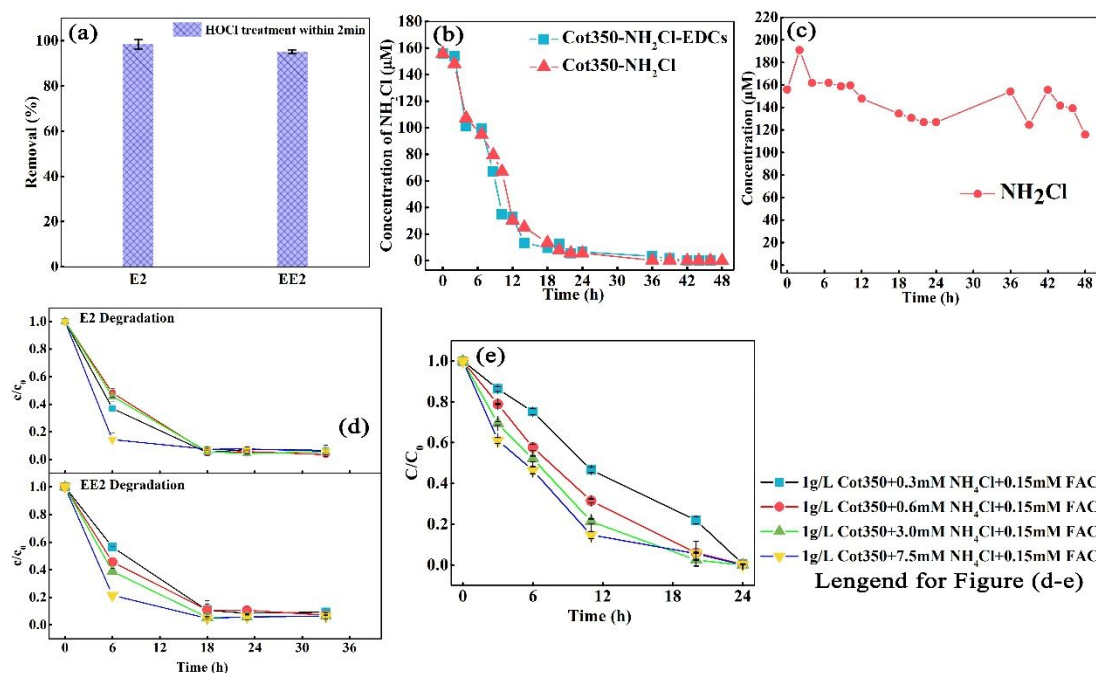
Figure S6. (a) EDCs removal with 0.15 mM hypochlorite within 2min at pH = 9; (b) kinetics of NH_2Cl degradation under Cot350/NH_2Cl and Cot350/NH_2Cl/EDCs within 48h (1 g·L^{-1} Cot350, 0.15 mM NH_2Cl, 10μM EDCs, pH = 9); (c) stability of 0.15 mM NH_2Cl under 200 rpm at room temperature within 48h at pH = 9; (d) kinetics of EDCs degradation with varying concentrations of NH_4^+ at pH = 9; (e) kinetics of NH_2Cl degradation with varying concentrations of NH_4^+ at pH = 9.
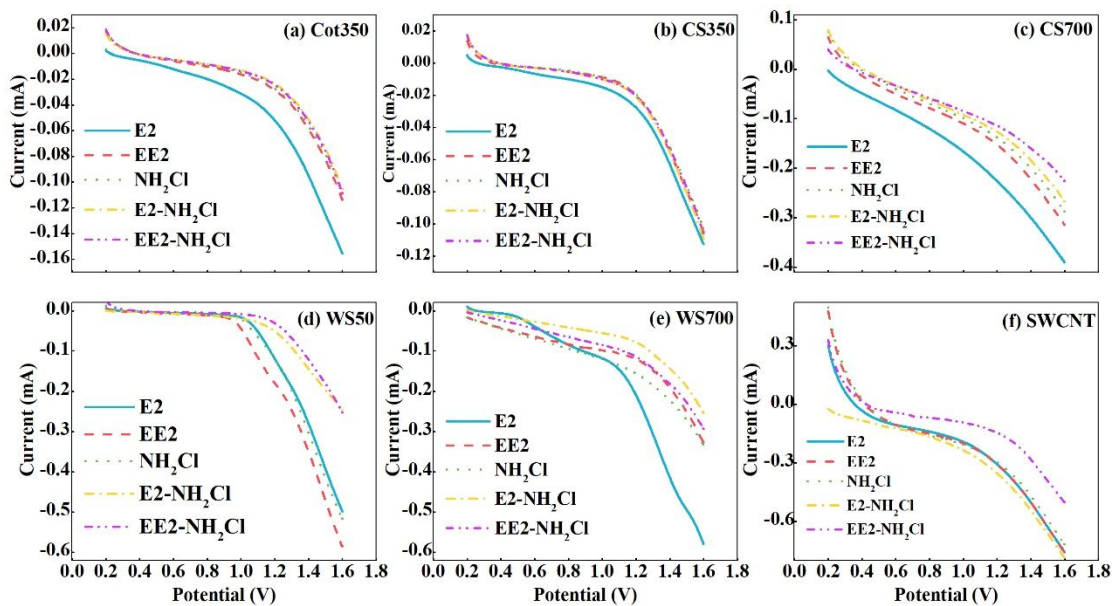
Figure S7. Results of linear sweep voltammetry (LSV) for five biochars (Cot350, CS350, CS700, WS350, WS700) and SWCNT. Reaction condition: $[E2]_0 = [EE2]_0 = 10$ μM; $[NH_2Cl]_0 = 0.15$ mM; $[PBS]_0 = 5$ mM.
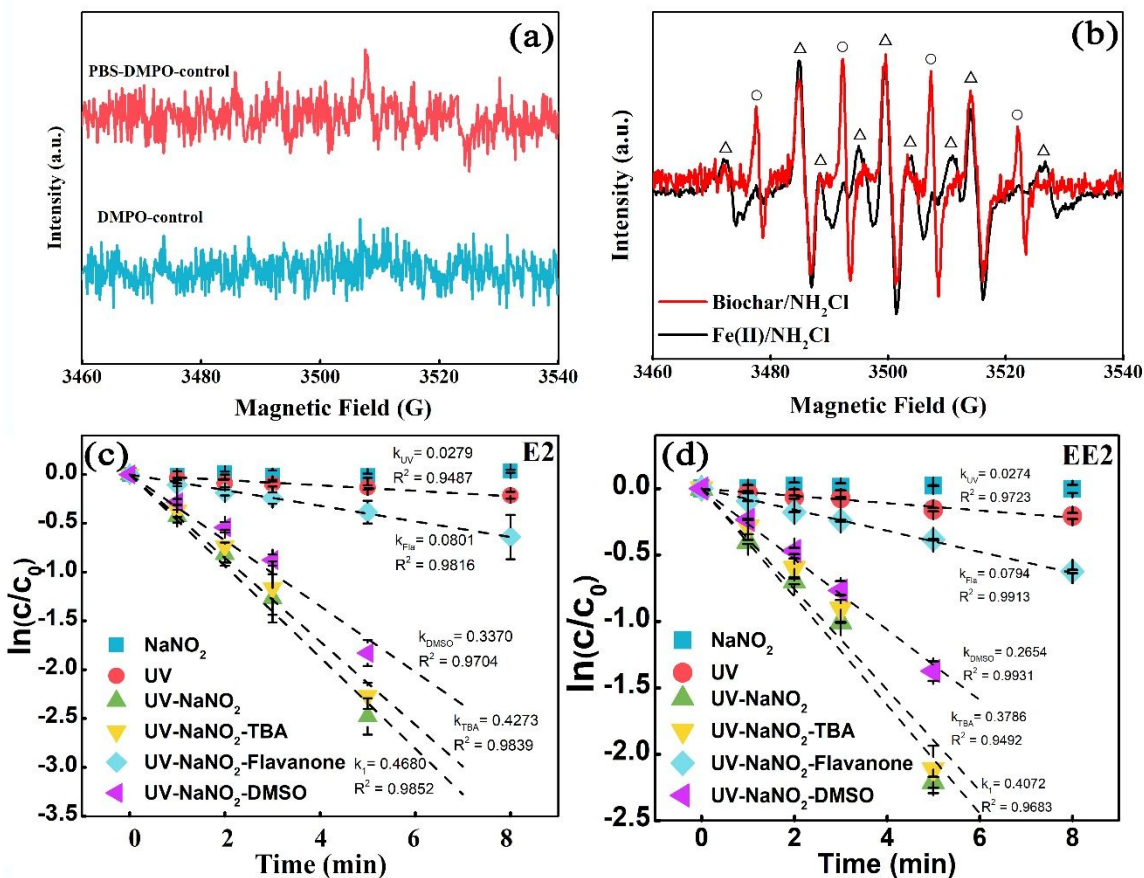
Figure S8. (a) EPR results of DMPO-PBS and DMPO as controls; (b) EPR results of Fe(II)/NH$_2$Cl and Cot350/NH$_2$Cl with addition of DMPO ($\circ$ is •OH, $\triangle$ is •NO); (c-d) UV/NaNO$_2$ for kinetics of E2 (c) and EE2 (d) degradation. Reaction condition: [Cot350] = 1 g·L^{-1}; [DMPO]$_0$ = 150 mM; [PBS]$_0$ = 5 mM; [Fe(II)]$_0$ = 0.179 mM; [NH$_2$Cl]$_0$ = 0.704 mM for Fe(II); [NH$_2$Cl]$_0$ = 0.15 mM for biochar; [NaNO$_2$]$_0$ = 10 mM; [TBA]$_0$ = 1%; [Flavanone]$_0$ = 100 μM; [DMSO]$_0$ = 1%; EPR reaction within 2 min; molar absorption coefficients of NaNO$_2$ ε_{254nm} = 16.75 M^{-1}·cm^{-1}).

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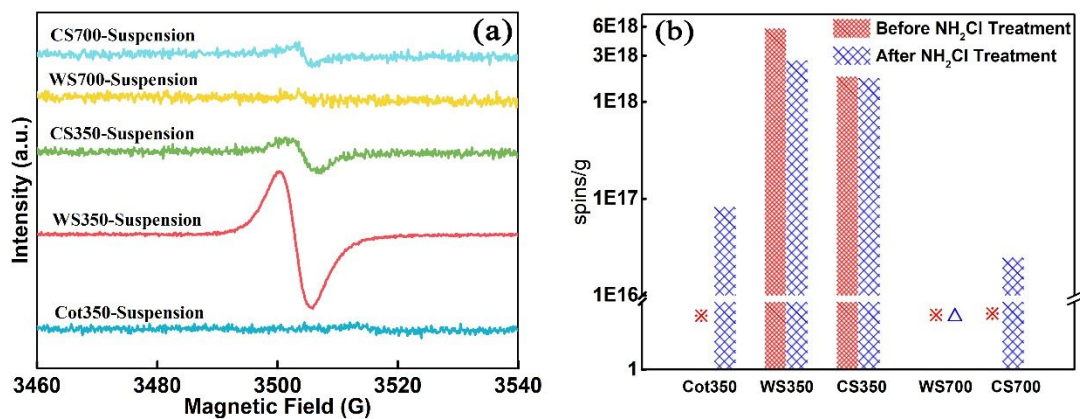
Figure S9. (a) EPR spectra of suspension of five biochars; (b) spin concentrations of five biochars after treatment of 0.15 mM NH_2Cl for 30 min (freeze drying for 24h). ※ is no EPR signal for biochar before NH_2Cl treatment, and Δ is no EPR signal for biochar after NH_2Cl treatment.
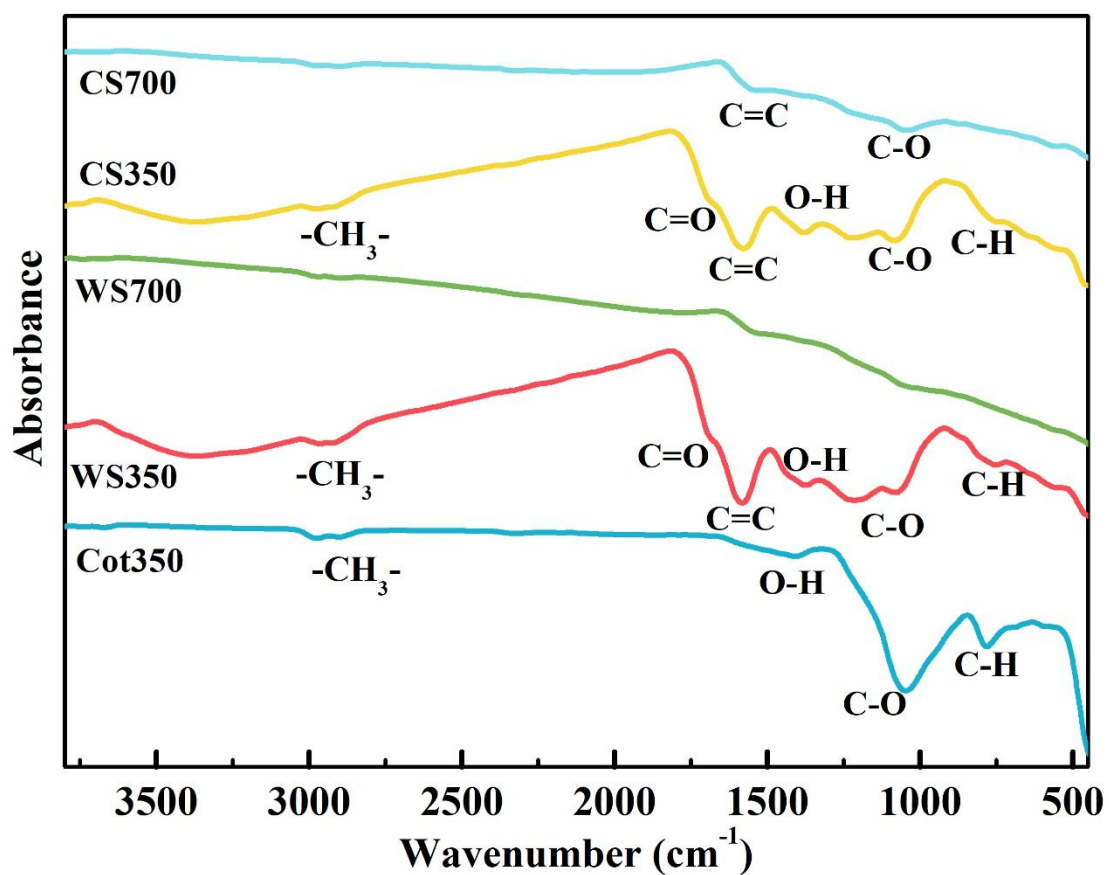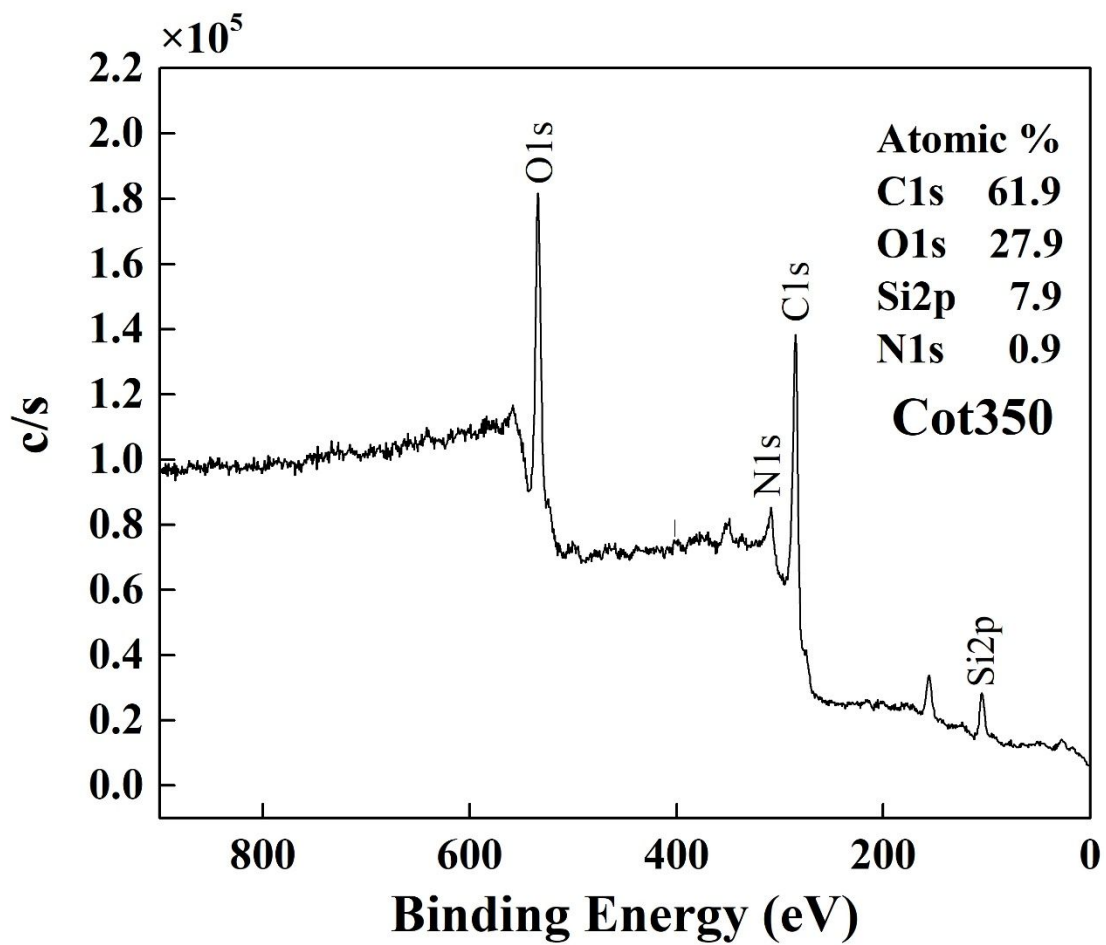
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186 **Figure S10.** Fourier-transform infrared (FTIR) spectra of five biochars.

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188 **Figure S11.** X-ray photoelectron spectroscopy (XPS) results of Cot350.
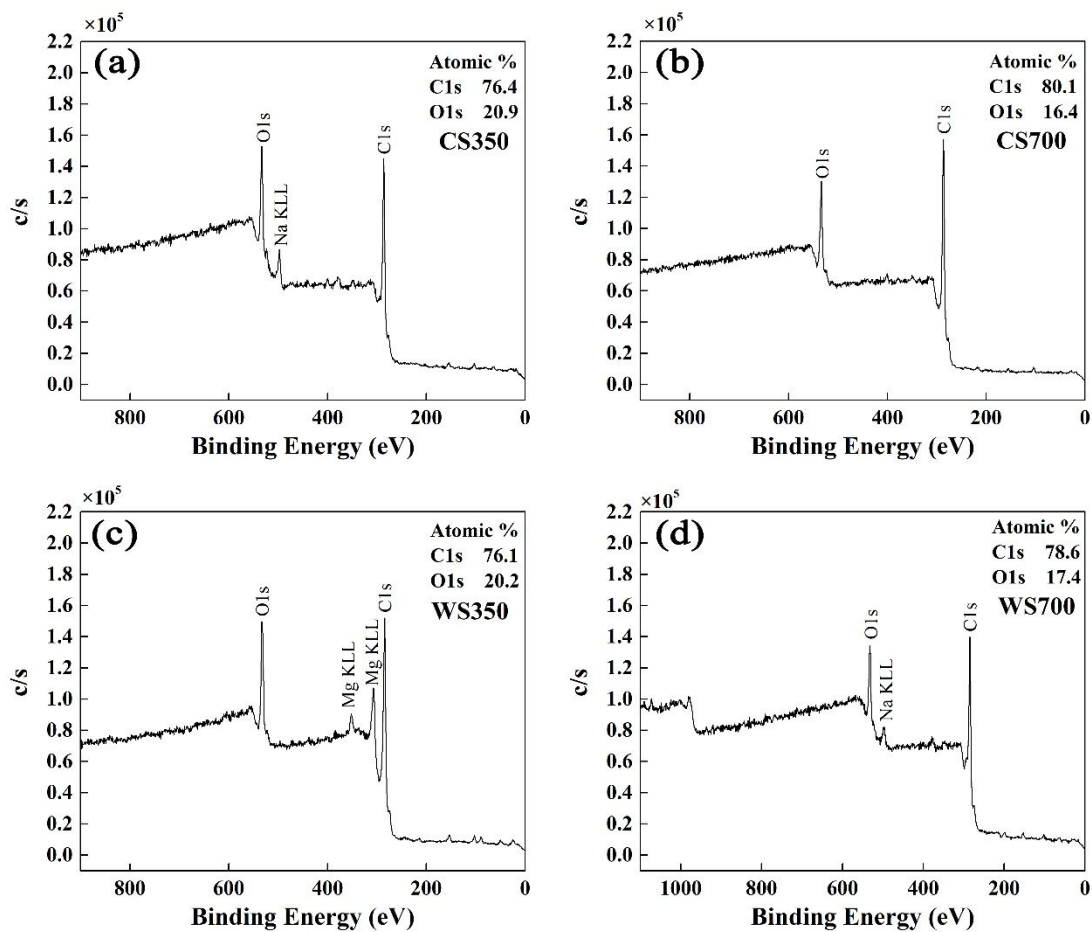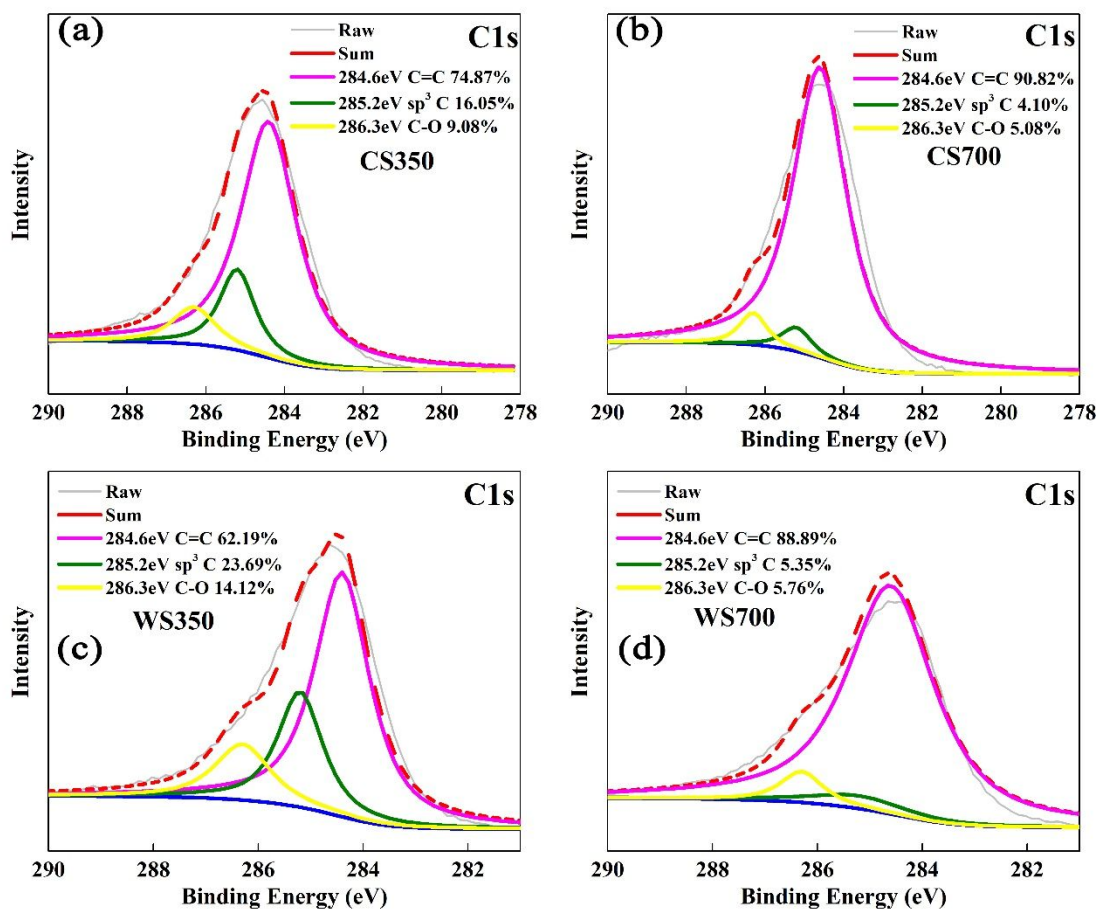
Figure S12. X-ray photoelectron spectroscopy (XPS) results of CS350, CS700, WS350 and WS700.

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Figure S13. X-ray photoelectron spectroscopy (XPS) results of CS350, CS700, WS350
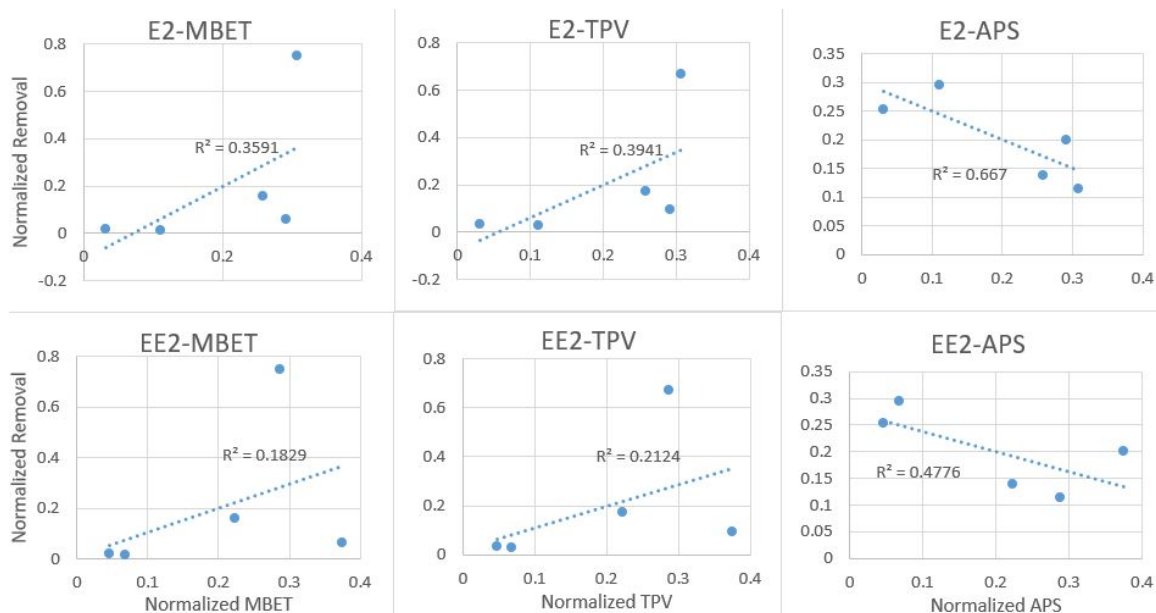
and WS700.

Figure S14. The correlation analysis of the relationship between multi-point BET (MBET), total pore volume (TPV), average pore size (APS) and the degradation of E2 and EE2. Note: the MBET, TPV, APS and removal efficiency were pre-normalized in the convenience of data analysis.

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(1) Ma, M., Rao, K., Wang, Z., Occurrence of estrogenic effects in sewage and industrial wastewaters in Beijing, China. *Environ. Pollut.* **2007,** *147* (2), 331-336.

(2) American Public Health, A., American Water Works, A., Water Pollution Control, F., Water Environment, F., *Standard methods for the examination of water and wastewater.* American Public Health Association.: **1915**; Vol. 2.