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Environmental Chemistry

Supplementary Material

Enhanced biodegradation of 17*α*-ethinylestradiol by rhamnolipids in sediment/water systems

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The Supplementary material consists of 3 sections (13 pages, S1- S13), including 3 Texts, 2 Tables and 3 Figures.

Contents:

Section 1: Text

Text S1: Procedures and details of extraction and HPLC analysis of EE2

A pipette was used to remove 0.5 mL aliquots of sediments, which were transferred to a 5 mL PTFE centrifuge tube and immediately placed in a vacuum oven (60 °C) to completely dry the sample. The dry weights of samples were measured by analytical balance weights method. Analytical grade methanol (2 mL) was added and the tubes were sealed and shaken. Then, extractions were performed at 25 °C and 225 rpm for 24 h. Following extraction, the tubes were centrifuged for 20 min at 3500 rpm, and the supernatant was taken directly for HPLC analysis.

The HPLC test method for the target substance EE2 is as follows: A Shimadzu (Japan) HPLC system, comprising of an SPD-MZOA photo-diode array (PDA) detector with a Shim-pack VP-ODS liquid chromatography column (particle size 5 μm, 4.6 mm × 250 mm) was utilized. The scanning wavelength was maintained at 205 nm and a mobile phase of acetonitrile (AC)/water solution (50:50 v:v) was applied with a flow rate of 0.7 mL min^{-1}. An injection volume of 10 μL was used and the oven temperature was 35 °C. The EE2 detection limit obtained by this method was 60 μg L^{-1}, and the retention time (RT) of EE2 was 13.1 min.

Text S2: Details of HPLC-PDA gradient scanning of sample

In gradient elution method, the eluent was a mixture of AC and water, with flow rate of 1.0 mL min^{-1}. The gradient elution program was as follows: 15% AC for 1 min; 15–100% AC for 54 min; and 100% AC for 5 min.

Text S3: Details of HPLC-ESI-MS analysis of EE2 metabolic intermediates

In order to improve the ionization efficiency of EE2, the initial concentration of

EE2 in the sediment/river water biodegradation system was increased. The culture supernatant was filtered through a 0.22-μm glass fiber Millipore filter and was used directly for liquid chromatography/mass spectrometry (LC/MS) detection. The sediment was extracted with a mixed solvent of 1:1 (v:v) ethyl acetate/methanol at a constant temperature of 25 °C for 24 h. The upper layer of extract was transferred to a nitrogen purge system and concentrated to dryness. The sample was re-dissolved in a 1:1 (v:v) AC/water solution, which was used for LC/MS detection following Millipore filtration.

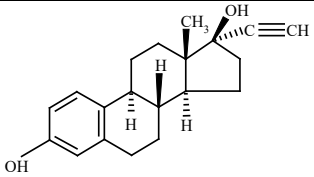
The mobile phase was a mixed solvent of AC and 20 mM ammonium acetate solution. The gradient elution program was as follows: 25% AC, 1 min; 25–50% AC, 23 min; 50–100% AC, 50 min; and 100% AC, 5 min. An Eclipse Plus C18 liquid chromatography column (5 μm, 4.6 mm × 250 mm) was used at a flow rate of 0.8 mL min^{-1}. The injection volume was 10 μL. After the samples were separated via liquid chromatography, they were analyzed using an ion trap mass spectrometer (Esquire HCT, Bruker, Billerica, MA, USA). The MS scanning conditions were as follows: ion source, ESI; capillary voltage, 13 nA; electrospray voltage, 20 psi; nitrogen flow, 10 L min^{-1}; and drying temperature, 350 °C. A similar mass spectrometry detection signal of intermediate peak was obtained both with and without rhamnolipid. The results of the study further proved that the presence of rhamnolipids did not change the metabolic transformation pattern of microorganisms in sediment/river water system to EE2.

Section 2: Tables and Figures

As shown in Fig. S1, the change in EE2 biodegradation rate was significantly affected by variations in rhamnolipid concentrations. The EE2 degradation rate was observed to be marginally higher in low concentration rhamnolipid environments, while an increase in rhamnolipid concentrations over a critical level (below or equal to 2.0 mM under these conditions) resulted in a linear increase in *k*. A maximum concentration dependent rate effect was observed; when rhamnolipid concentrations increased to over 10 mM, EE2 degradation rates began to decline. This was because in experiments containing 10 mM of dissolved rhamnolipid in aqueous solution at room temperature, saturation levels were gradually approached. Exposure to 12 mM rhamnolipid concentrations limited the level of improvement seen in EE2 biodegradation due to glycolipid precipitation from the culture system.

Table S1.

Physicochemical characteristics of 17*α*-Ethynylestradiol

Molecular structure	
Melting point (°C)	182-183
Molecular mass (g/mol)	296
Solubility (mg/L)	3.1 [a]; 4.8 [b]; 2.9 [c]
log K_{ow} [d]	4.15
pK_a [e]	10.21

[a] Yu *et al.,* 2004; [b] Lai *et al.,* 2000; [c] Based on our previous study (Guo and Hu, 2014), the solubility of EE2 in a background solution containing 10 mM NaCl was less than 3 mg L^{-1}. [d] Octanol-water partition coefficient. [e] Deprotonation constant.

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Guo, Y.-P. and Hu, Y.-Y. 2014. Solubilization of moderately hydrophobic 17α-ethinylestradiol by mono- and di-rhamnolipid solutions. Colloids and Surfaces A: Physicochemical and Engineering Aspects **445**: 12-20. doi:10.1016/j.colsurfa.2013.12.076.

Table S2.

Retention time for EE2 and its detected metabolites established by HPLC-PDA chromatography

	M.1	M.2	EE2	M.3
Retention Time (RT, min)	22.1	28.2	29.3	45.4

Fig. S1

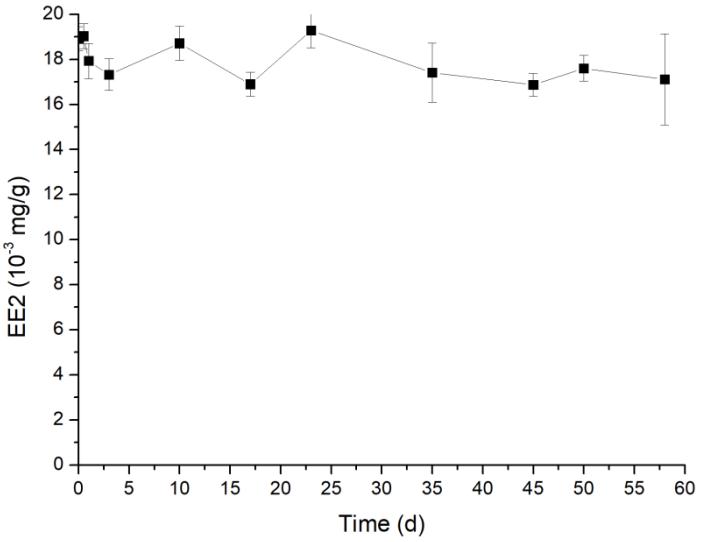

Fig. S1 Concentrations of EE2 in the control experiment

Fig. S2

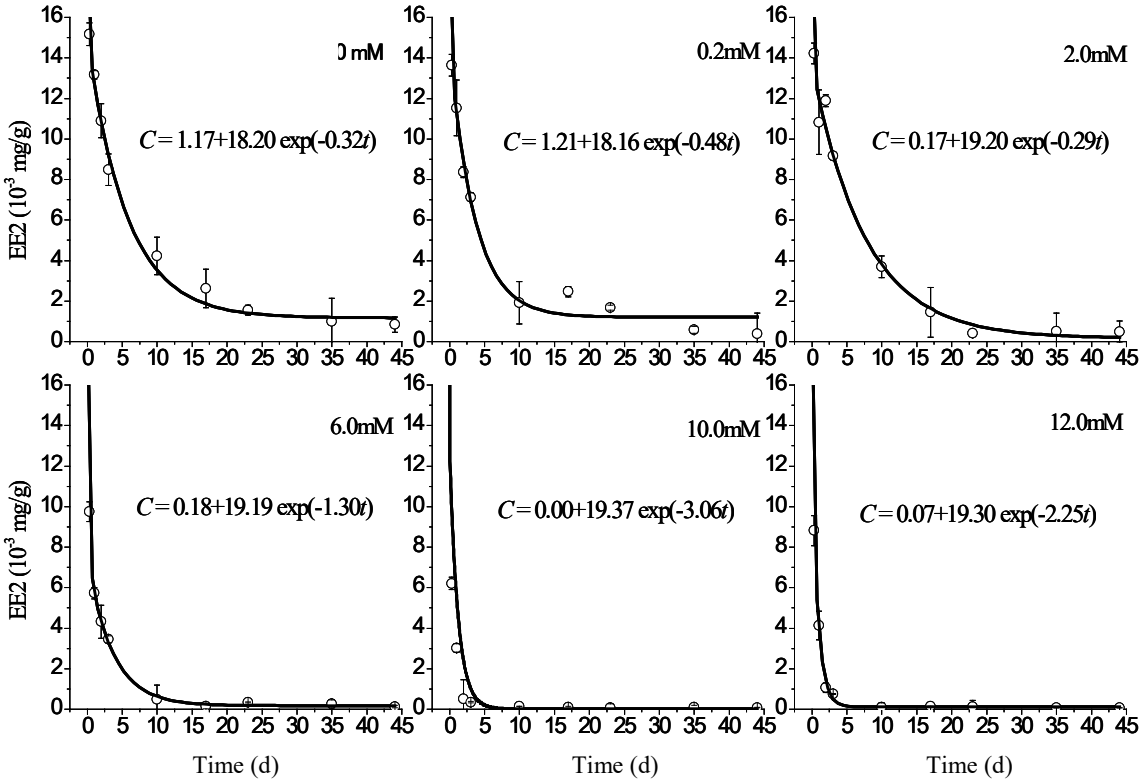

Fig. S2 Fitted kinetic curves of EE2 degradation and the effect of increased concentrations of rhamnolipids within sediment/water systems

Section 3: Effect of glucose on EE2 biodegradation

The mineralization of soil organic matter can be accelerated or retarded by the addition of organic substrates to soil, thus causing positive or negative priming effects, respectively (Hamer and Marschner 2005; Kuzyakov et al. 2000). The activation of microorganisms through easily available substrates is considered as the main reason for the occurrence of positive priming effects in soil. As reviewed by Kuzyakov et al. (2000), one possible mechanism is co-metabolism, i.e., the enhanced degradation of organic matter due to microbial growth and accompanied increased enzyme production.

Hamer and Marschner, (2005) investigated the effects of different substrates on the mineralization of soil organic carbon from different environments of two forest soils and one arable soil. They reported that the priming effects were highly variable in both direction and intensity between substrates and soil samples. The authors consequently hypothesized that the priming effects were not only affected by substrate quality and soil properties, but were also strongly influenced by the composition of the soil microflora, which cannot be easily explained by co-metabolism (Bell *et al.*, 2003).

Recently, Alidina *et al.*, (2014) investigated the role of primary substrate composition and concentration on the attenuation of biodegradable emerging trace organic chemicals. They reported that the composition and concentration of the primary substrate influence the attenuation of moderately degradable chemicals, such as atenolol, gemfibrozil, and diclofenac. The primary substrate represented by the biodegradable portion of bulk organic carbon, occurring in the mg/L range, shapes the microbial community structure in systems where the degradation of trace organic chemicals occurs.

The role of different primary substrates for the attenuation of different trace organic chemicals has been studied previously, such as glucose, acetate, and humic acid (Alidina *et al.,* 2014; Onesios and Bouwer, 2012; Stumpe and Marschner, 2009). Stumpe and Marschner, (2009) reported that addition of glucose induced higher EE2 degradation due to stimulated microbial respiration activity.

Rhamonolipids consist of one or two units of rhamnose linked to one or two fatty acid chains, which have been shown to exhibit good biodegradability and biocompatibility (Abdel-Mawgoud *et al.,* 2010). Mohan *et al.,* (2006) tested the biodegradation kinetics with rhamnolipidic biosurfactants typically used by microorganisms as a single carbon source under aerobic condition. It was found that, during the 50-60 h of cultivation, the oxygen utilization rate of microorganisms reached a maximum within the rhamnolipids degradation system and the degradation achieved the highest rate at the same time.

In fact, to verify whether rhamnolipids could simultaneously act as the primary substrate of indigenous microorganisms during EE2 biodegradation, additional enhancements were added to test the effect of glucose on EE2 biodegradation (Fig. S2). In these tests, 6.9 mM glucose replaced equivalent amounts of mole carbon of 6 mM rhamnolipids. A certain amount of 27.8 mM glucose was converted via equivalent mole carbon amounts of both the rhamnose and carbon chain portions for 6 mM rhamnolipids. Other experimental operations and conditions were similar to those of the shake flask aerobic biodegradation experiments in this study.

Test results showed that EE2 exhibited a slightly improved degradation rate under higher glucose concentration (27.8 mM) compared to the control system without glucose. However, lower glucose concentration (6.9 mM) showed a certain degree of inhibition effect on the EE2 biodegradation. These tests used easily

degradable carbon source (glucose) as the primary substrate. The results indicate that the concentration of bulk organic carbon plays a role in shaping the microbial community and hence affects the degradation of trace organic compounds (Stumpe and Marschner, 2009). Onesios and Bouwer (2012) also found that three of the fourteen PPCPs removals were dependent on primary substrate concentration. Accordingly, it was speculated that rhamnolipids may simultaneously be used as primary substrate by microorganisms during EE2 biodegradation, thus stimulating microbial consortium and influencing EE2 degradation. This constitutes a new problem for the mechanism of rhamnolipids addition and requires further investigations in future.

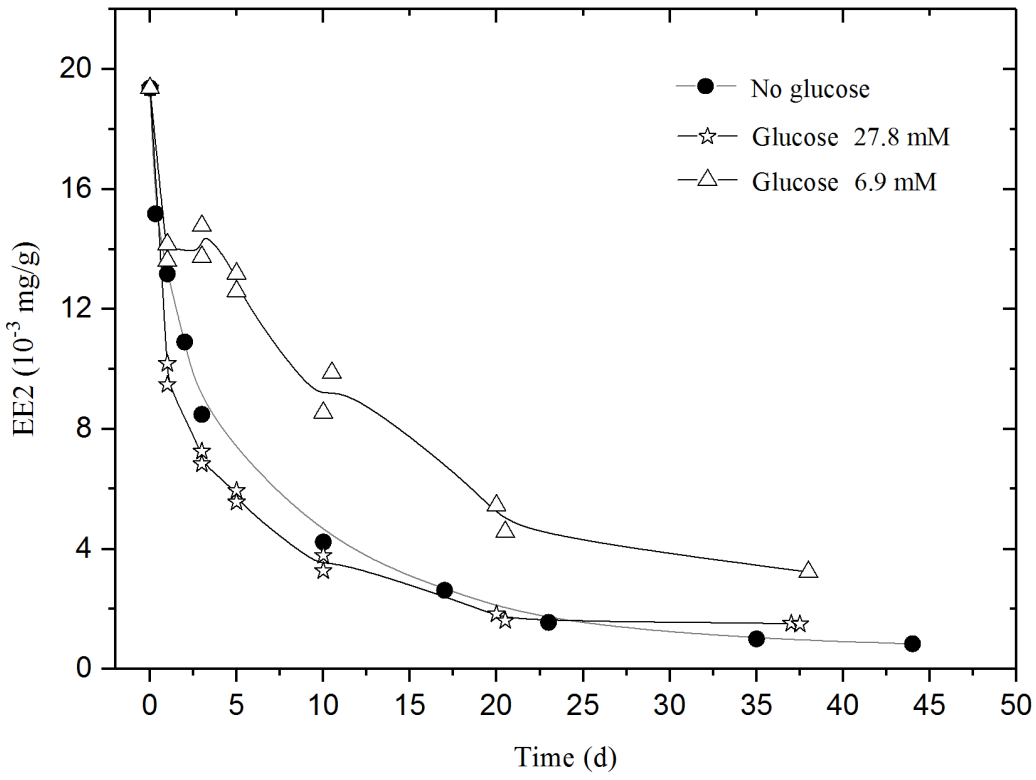

Fig. S3 Effect of glucose on EE2 biodegradation within sediment/water systems

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