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Effect of rice-straw biochar on selective biodegradation of nonylphenols in isomer specificity

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Abstract

In a previous study, we found that rice-straw biochar degraded and removed hydrophobic organic contaminants (HOCs) through coupled adsorption-biodegradation. However, few studies have determined whether biochar affects HOC isomer degradation and isomer-selective biodegradation or whether biochar can alter HOC isomer features, resulting in changes to HOC isomer residues in water environments. In this study, the effects of biochar at two dosages (0.001 and 0.01 g) on the biodegradation of ten isomers of a typical xenoestrogen of nonylphenol (NP) were evaluated. The results revealed that there were no effects of biochar on the adsorption of NP isomers. However, biochar addition affected the biodegradation of a specific isomer without altering the features of the NP isomers. The treatment of NP isomers with *Pseudoxanthomonas* sp. yielded degradation ratios ranging from 60.7 to 100%. At 0.001 g biochar treatment, the degradation of eight NP isomers was enhanced (except for NP₁₉₄ and NP_{193a+b}) due to their bulky structures. The degradation of the ten NP isomers was inhibited when 0.01 g biochar was added. These findings characterized the effects of biochar on NP isomer contaminants and provided basic information for the application of biochar for the remediation of NP isomer contaminants.

Keywords

Nonylphenol (NP); Biochar Isomers; Biodegradation; Remediation; Structure degradation

Introduction

Nonylphenol (NP) is a common industrial antioxidant, an additive in lubricating oils (Eganhouse et al. 2009; Soares et al. 2008) and a raw material in the production of nonylphenol ethoxylates (NPEOs), which are the most widely used non-ionic surfactants.

Since its first reported synthesis in 1940, NP has been in the great demand. Its annual production has reached 48,000 t in China (Anonymous 2015) and 194,000 t in the USA (Anonymous 2004). Approximately 60% of produced NP has been estimated to be released into aquatic environments via wastewater treatment plant (WWTP) effluents, resulting in the frequent detection of NP in the environment (Giger et al. 1984). Worldwide, NP concentrations in aquatic environments range from non-detectable (ND) levels to 4100 ng/L in surface water (Chen and Yeh 2010; Duong et al. 2010; Vethaak et al. 2005) and ND to 32,000 ng/g in sediments (Kawahata et al. 2004; Stewart et al. 2014). NP accumulates in mussels, induces estrogenic effects in aquatic organisms, and causes secondary pollution (Dodder et al. 2014). Therefore, the studies have increasingly focused on the transformation and fate of NP in the aquatic environment (Lu and Gan 2014).

Biochar, a super sorbent of hydrophobic organic contaminants (HOCs), has been considered as a promising material for environmental HOC remediation (Major et al. 2010; Park et al. 2011). The adsorption capacity of biochar for NP has been reported to reach up to $3.3 \pm 0.3 \times 10^4$ mg/kg, suggesting that biochar could be used for immobilizing NP at contaminated sites (Lou et al. 2014). Furthermore, the application of appropriate levels of biochar could enhance NP biodegradation rates and achieve effective coupled sorption-biodegradation remediation (Lou et al. 2015). However, NP is not a single compound but a complex mixture of more than 100 NP isomers (Eganhouse et al. 2009).

Few studies have examined the characteristics of NP isomer degradation and the effects of carbonaceous materials on the degradation process. A few reports have shown that the biodegradation of NP isomers was isomer-specific. For example, Gabriel et al. examined NP biodegradation by *Sphingobium xenophagum*, a bacterial strain isolated from activated sludge, and found that the degradation of 18 NP isomers had degradation rates greater than 80% (Gabriel et al. 2008). Among these isomers, the biodegradation rates of NP₁₂₈ and NP₁₁₉ were as high as 99.7%, whereas those of NP_{193a+b} were only 31.3 and 30.5%, after a 9-day incubation. Lu and Gan evaluated the biodegradability of 19 NP isomers and found isomer-selective biodegradation results in the enrichment of recalcitrant isomers, such as NP_{193a+b}, NP_{110a}, and NP₁₉₄ (Lu and Gan 2014). In contrast, Isobe et al. examined the degradation of ten NP isomers in primary and secondary effluents of WWTPs, showing no isomer selectivity (Isobe et al. 2001). Ikunaga et al. also reported similar results, and no significant variations were observed in NP isomer attenuation by *Sphingomonas cloacae*, another bacterial strain isolated from activated sludge (Ikunaga et al. 2004). Few studies have focused on the influence of carbonaceous materials on the migration and transformation of HOC isomers. Only Gregory et al. evaluated the effect of biochar on the biodegradation of hexachlorocyclohexane (HCH) isomers (i.e., α -HCH and β -HCH) in soil (Gregory et al. 2015). In that study, biochar was found to significantly promote HCH transformation, and α -HCH and β -HCH underwent tenfold and fourfold reductions in concentration, respectively, when compared to the control treatments. Based on these results, biochar could play a special role in affecting the fate of isomer contaminants.

Overall, although appropriate biochar application could accelerate the biodegradation of NP isomers, isomer-selective biodegradation may result in the enrichment of recalcitrant isomers. Therefore, a more comprehensive study on the effects of biochar on the

biodegradation of NP isomers is necessary. The objectives of this study were to (1) evaluate the biodegradation characteristics of ten NP isomers, (2) examine the relationship between structure and biodegradability, and (3) explore the degradation of NP isomers using various biochar dosages. The investigation of the sorption-biodegradation remediation process could provide theoretical support for the engineering applications of biochar-microorganisms in isomer contaminant governance.

Materials and methods

Chemicals and materials

Technical NP (tNP, >99% purity), a mixture of NP isomers with branched side chains (CAS No. 84852-15-3), was purchased from Aladdin (Shanghai, China) and prepared as a concentrated stock solution with acetonitrile. NP₃₆, NP₃₇, NP₁₁₉, NP₁₉₃, and NP₁₉₄ (10 mg/g in hexane; >95% purity) were purchased from ChemCollect GmbH (Remscheid, Germany). NP₆₅, NP₁₁₀, NP₁₁₁, and NP₁₁₂ (10 mg) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, acetone, hexane, and dichloromethane (chromatography grade) were purchased from Sigma-Aldrich.

Biochar was prepared from air-dried rice straw, which was collected from the Hua-jia-chi farm of Zhejiang University in China. Under uncontrolled conditions, the rice straw was burned in a stainless steel plate in an open field. In order to obtain more pure biochar, the samples were treated with 2 M HCl and 1 M/1 M HCl-HF solutions, then washed with distilled water, and oven-dried overnight at 105 °C (Lou et al. 2014).

Strain isolation and identification

A tNP-degrading bacterial strain was isolated from a mixed inoculum from sediments with NP as the sole C source from the Qiantang River in Hangzhou, China, based on previous work (Lou et al. 2015). The cell morphology, physiological characteristics, and 16S rRNA genes of the strain were characterized by the Institute of Microbiology, Chinese Academy of Sciences. The strain was named after *Pseudoxanthomonas* sp. N-1.

Preparation of tNP-degrading inoculum

The strain was enriched in medium (1 L of medium containing 5.0 g of NaCl, 10.0 g of tryptone, and 5.0 g of beef extract; pH was maintained at 7.0–7.2 using 1 M NaOH) and incubated at 30 °C while shaking at 150 rpm. The bacterial culture was centrifuged at 4000g for 10 min after 24 h of growth. After discarding the supernatant, the microorganisms were resuspended in mineral salt medium (1 L of medium containing 4.0 g of K₂HPO₄, 4.0 g of NaH₂PO₄, 2.0 g of (NH₄)₂SO₄, 0.41 g of MgSO₄·7H₂O, 0.01 g of anhydrous CaCl₂, 0.01 g of MnSO₄·H₂O, and 0.01 g of FeSO₄·7H₂O, pH was maintained at 7.0–7.2 using 1 M NaOH), which was repeated four times. The OD₆₀₀ was adjusted to 1.0, and the inoculum was stored in a refrigerator at 4 °C for further experiments.

NP isomer biodegradation experiments

All experiments were conducted in triplicate. The pre-sorption reactors consisted of 50-mL glass centrifuge vials with permeable silica gel stoppers for adequate oxygen exchange.

Each reactor contained 9 mL mineral salt medium with 0-, 0.001-, or 0.01-g biochar and was autoclaved at 121 °C for 30 min twice to sterilize. A stock solution of tNP was added to each vial to a final concentration of 10 mg/L, and the vials were incubated in an orbital shaker at 150 rpm and 30 °C for 24 h for pre-sorption, which was sufficient to reach an apparent equilibrium according to our previous study (Lou et al. 2014). Then, a 1 mL aliquot of prepared tNP-degrading inoculum was added to each reactor, and the reactors were shaken under the same conditions for 0, 1, 2, 4, 8, 12, and 16 days in the dark. At each time point, vials were removed for destructive sampling. To quantify the abiotic loss of NP isomers, sterilized reactors with 0.2 mg/mL sodium azide were operated in parallel. Losses were less than 20% in all sterilized treatments, suggesting that biodegradation was responsible for the removal of NP isomers and the abiotic loss was controllable.

To determine the concentrations of aqueous NP isomers, reactors were centrifuged at 4000 rpm for 10 min to separate the aqueous and solid phases. The supernatants were transferred to another 50-mL glass vial, and the NP isomers were extracted with 15 mL dichloromethane by sonication for 20 min. The extracts at the bottom of the vials were transferred to clean glass vials containing moderate anhydrous sodium sulfate. Subsequently, 10 mL of each extract was transferred to a nitrogen blowpipe and concentrated to 1 mL volumes. The concentrations of the aqueous NP isomers were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The average extraction recovery of the NP isomers ranged from 90 to 110% in these operations.

To determine the concentrations of solid NP isomers, aqueous NP isomers were sampled, and a defined amount of anhydrous sodium sulfate was added to each precipitate. Then, 5 mL methanol was added to extract the NP isomers by 40-min sonication. The extracts were collected in a nitrogen blowpipe. This procedure was repeated four consecutive times to ensure the thorough extractions of the NP isomers. The collected solvents were nitrogen-flushed to dry, dissolved with 1 mL dichloromethane, and analyzed by GC-MS. The average extraction recoveries of the solid NP isomer samples ranged from 95 to 105%.

Sorption experiments

All sorption experiments were conducted in triplicate. Different amounts of biochar (0.001 and 0.01 g) were added to a 50-mL glass centrifuge vial and sealed with a threaded plastic cap. Then, 30 mL of NP solution (10 mg/L), which was prepared from the NP stock solution, and an electrolyte matrix containing 200 mg/L NaCl and 200 mg/mL NaN₃ were added to inhibit biodegradation. The reactors were shaken at 150 rpm on a horizontal shaker at 30 °C for 24 h in the dark. After establishing a sorption equilibrium, the aqueous and solid phases of the NP isomer concentrations were analyzed as described in “NP isomer biodegradation experiments” section.

Analysis of NP isomers

The NP isomers were analyzed using GC-MS (Agilent 7890N-5759C) (Agilent, Santa Clara, CA) with an ion trap mass analyzer. Briefly, samples (1 µL) were introduced into the inlet at 230 °C, and the separation was achieved on a DB-5 capillary column (30 m × 0.32 mm × 0.25 µm; Agilent, Santa Clara, CA). The carrier gas was helium (99.999%) set at a flow rate

of 1.0 mL/min. The column temperature was initialized at 50 °C at 0 min, then ramped at a rate of 20 °C/min up to 180 °C, and further ramped at a rate of 2 °C/min up to 200 °C and held for 2 min. Finally, the temperature was ramped at a rate of 20 °C/min up to 280 °C and held for 2 min. The mass spectrometer was operated in the electron ionization-selective ion monitoring (SIM) mode at 70 eV. Ions with m/z values of 121, 135, 149, 163, 177, and 191 were used for quantifying the different NP isomers. A total of ten NP isomers, including six diastereomers (denoted as a and b), were resolved and identified (Table 1). The most abundant NP isomers were NP₃₅ (12.5%) > NP₁₁₂ (11.6%) > NP₃₆ (10.7%) > NP_{110a} (9.5%).

Data analysis

The biodegradation ratios of the NP isomers were obtained via the loss of peak areas in the total ion chromatograms between the measurement day and the initial day. The NP isomer degradation within 16 days of incubation followed a first-order reaction kinetics model with lag time as shown in Eq. (1):

$$C_t = C_0 e^{-kt(t - t_{\text{lag}})} \quad (1)$$

$$t_{1/2} = \frac{\ln 2}{kt} \quad (2)$$

where C_t and C_0 are the concentrations (mg/L) of the NP isomers at degradation time t and initial time, respectively, t_{lag} (days) is the lag time, k_t (/day) is the degradation rate constant, and $t_{1/2}$ (days) is the half-life period.

Results

Effect of biochar on NP isomer biodegradation

All the ten NP isomers underwent an initial rapid degradation phase, followed by a slow phase as shown in Fig. 1. Without biochar addition, the degradation of NP isomers by *Pseudoxanthomonas* sp. N-1 was found to be generally efficient over a 16-day incubation, and their degradation ratios ranged from 69.7 to 100% with a mean of $88.2 \pm 9.5\%$ (Fig. 1a). The order of the biodegradation ratios of the NP isomers was as follows: NP₃₇ = NP₁₁₉(100%) > NP₃₅ (94.6%) > NP₃₆ (93.4%) > NP₁₁₂ (91.8%) > NP₆₅ (91.3%) > NP_{110b}(91.0%) > NP_{110a} (90.2%) > NP_{111a} (88.5%) > NP_{111b} (86.5%) > NP_{193b} (76.0%) > NP₁₉₄(74.0%) > NP_{193a} (69.7%), which indicated that the biodegradation of the NP isomers by *Pseudoxanthomonas* sp. N-1 varied for different isomers. These results agreed with those of previous studies (Gabriel et al. 2008; Lu et al. 2015; Lu and Gan 2014). Lu et al. found that the removal rates of NP isomers ranged from 90 to 99% depending on the operational conditions and isomer structures in a laboratory-scale continuous flow conventional activated sludge bioreactor (Lu et al. 2015). Gabriel et al. studied the biodegradability of NP isomers by *S. xenophagum* Bayram and observed a degradation ratio order of NP₁₁₉ (99.7%) > NP₃₆(98.8%) > NP₁₁₂ (98.2%) > NP₃₅ (97.6%) > NP₃₇ (96.6%) > NP_{111a} (94.0%) >

NP_{110b}(90.4%) > NP₆₅ (81.0%) > NP_{110a} (77.5%) > NP_{110b} (76.3%) > NP₁₉₄ (61.8%) > NP_{193a}(31.3%) > NP_{193b} (30.5%) (Gabriel et al. 2008). Compared with the order reported in the current study, the results could be conducted that the degradation ratios varied according to the NP isomer and also varied depending on the bacterial species.

With 0.001 and 0.01 g of biochar addition, the biodegradation of NP isomers was significantly affected ($P < 0.01$). Figure 2 displayed the total ion chromatograms of the NP isomers on days 4, 8, and 16 of incubation with 0, 0.001, and 0.01 g of biochar addition, which clearly illustrated the effect of biochar on NP isomer biodegradation. When 0.001-g biochar was added, the profiles of the NP isomers were significantly higher than the profiles of NP isomers without biochar addition on day 4 ($P < 0.01$). However, these profiles were significantly lower than those without biochar on days 8 and 16. This suggested that biodegradation was promoted by 0.001-g biochar, albeit with a time lag. This could be conjectured from Fig. 1b; the biodegradation ratios of most of the NP isomers were higher in the biochar-*Pseudoxanthomonas* sp. N-1 system with 0.001-g biochar. For example, the degradation ratios of NP₃₆ and NP₁₁₂ increased from 93.4 and 91.8 to 100 and 98.5%, respectively, while those of NP₁₉₄ and NP_{193a+b} remained relatively low at 78.3, 70.5, and 76.1%. These lower degradation ratios likely indicated that these isomers would be the main components of residual NP isomers. Furthermore, there were no significant differences in the biodegradation ratios of NP₁₉₄ and NP_{193a+b} compared with those of the control with no addition of biochar ($P > 0.05$).

When 0.01-g biochar was added, the degradation of the NP isomers was significantly inhibited ($P < 0.01$) (Fig. 2). The profiles of the NP isomers were constantly higher than the treatments with 0- and 0.001-g biochar addition; these negative effects of biochar addition are shown in Fig. 1c as well. For example, the biodegradation ratios of NP₁₉₄, NP₃₆, and NP₁₁₂ reduced from 74.0, 93.4, and 91.8 to 60.7, 77.6, and 71.7%, respectively.

Effect of biochar on relative biodegradation ratios of NP isomers

To further elucidate the influence of biochar on the degradation of NP isomers, the biodegradation ratios with biochar were calculated to obtain relative biodegradation ratios of the aqueous, solid, and total phases, as shown in Fig. 3. Generally, the relative biodegradation ratios between the solid and total phases were approximately equivalent and tended to be consistent due to the strong adsorption properties of biochar. The solid phases accounted for 81.0–94.5% of the total NP isomers. Figure 3 displays the changing process of the relative biodegradation ratios of the aqueous, solid, and total phases of the NP isomers observed during the 16 days of incubation as a function of added biochar.

In this study, the degradation of the NP isomers was affected by two factors: (1) the bioavailability of NP isomers, which was evidently restricted due to the strong adsorptive properties of the biochar, and (2) microorganisms, which could be adsorbed by or attached to biochar, form biofilms, and thereby readily promote microorganism growth (Yang and Sheng 2003a, b). The forming of biofilms with the attached abundant microorganisms could be an important mechanism in overcoming the limitations of mass transfer, shortening diffusion distance, and enhancing biodegradation as shown in previous studies (Lou et al. 2015; Mercier et al. 2014). It should be noted that biochar acted in a leading role by

inhibiting or promoting the biodegradation of the NP isomers in separate degradation periods; the degradations required certain durations to enable microorganism growth. In the initial stage (0–6 days), the process was dominated by the inhibitive properties of the biochar. During the mid-stage (6–8 days), the process was characterized by increased biodegradation ratios. By the end stage (8–16 days), the process was stabilized and had lower degradation levels due to the exhaustion of the NP. For example, when 0.001-g biochar was added, before day 6, the relative ratios of the ten NP isomers generally increased but remained less than 1.0. After day 6, these ratios rapidly increased to >1.0 and reached maximum values at day 8. After day 8, the ratios tended to decrease, returning to approximately 1.0 and remained at this low level (Fig. 3). However, with the addition of 0.01 g of biochar, the inhibition of the biodegradation of NP isomers was higher than their promotion due to the reduction in bioavailability by biochar sorption. In this study, when 0.01-g biochar was added, the relative ratios of the ten NP isomers were lower than 1.0 throughout, which was significantly lower than those of the NP isomers under 0.001-g biochar application ($P < 0.01$). The biodegradation process at this higher biochar level also went through the above-described three stages, indicating that stronger sorption and lower biodegradation of the NP isomers occurred. Furthermore, for NP₁₉₄ and NP_{193a+b}, whose degradation ratios were among the lowest, the levels of their relative ratios were much lower (<1.0) than those of other NP isomers, even at 0.001-g biochar dosage treatment, indicating that there were no significant effects of biochar on the degradations of NP₁₉₄ and NP_{193a+b}. However, inhibition effects were likely, especially when treated with 0.01-g biochar.

Effect of biochar on the half-lives of NP isomers

Figure 4 shows the half-lives of NP isomers with various biochar dosages via fitting a first-order kinetic model with lag time. The half-lives of the ten NP isomers ranged from 3.1 (NP₁₁₉) to 6.1 days (NP_{193a}) in the controls without biochar addition. These results agreed with those of previous studies (Lu et al. 2015; Lu and Gan 2014). Past studies have reported that the half-lives of 19 NP isomers ranged from 0.9 to 13.2 days in river sediments under oxic conditions. Of the ten NP isomers examined in this study, the half-life of NP₁₁₉ was the shortest, while those of NP₁₉₄ (5.2 days) and NP_{193a+b} (6.1 and 5.4 days, respectively) were longer than the others, indicating their poor biodegradability. When 0.001-g biochar was added, there were no significant changes in the half-lives of NP₁₉₄ (5.2 days) and NP_{193a+b} (6.2 and 5.1 days, respectively) ($P > 0.05$) compared with those in the control; the half-lives of the other isomers were shortened to 3.0 (NP₁₁₉)–3.6 days (NP_{110b}) ($P < 0.05$), suggesting that the biodegradation of these eight NP isomers was significantly promoted with 0.001-g biochar. Therefore, the addition of 0.001-g biochar likely promoted the biodegradation of NP isomers by accelerating the biodegradation rate (k_t), rather than increasing the biodegradation ratio. Additionally, this figure also shows consistent results described earlier for the degradation process of NP isomers via relative biodegradation ratios: when 0.01-g biochar was added, the half-lives, ranging from 5.1 (NP_{110b}) to 10.0 days (NP₁₉₄), were significantly longer ($P < 0.01$).

Discussion

Mechanism of the effect of biochar on biodegradation of NP isomers

Based on the results described earlier, the biodegradation of the NP isomers by *Pseudoxanthomonas* sp. N-1 was found to be isomer-specific. This isomer-specific degradation was also promoted by suitable doses of biochar. The ten studied NP isomers could be grouped into two categories: those slightly promoted by appropriate biochar as category 1, for instance, NP₃₅, NP₃₆, NP₃₇, NP₆₅, NP_{10a+b}, NP_{111a+b}, NP₁₁₂, and NP₁₁₉, and those with no significant changes due to biochar as category 2, such as NP₁₉₄ and NP_{193a+b}. As described previously (“Effect of biochar on the half-lives of NP isomers” section), the effects of biochar on the degradation of the NP isomers could primarily be caused by adsorption and an abundance of microorganisms. Therefore, the preferential isomer-specific adsorption and biodegradability processes could be important pathways. Thus, we compared the sorption behaviors of these conclusions with the ten NP isomers and explored the relationship between biodegradability and the structures of the NP isomers.

Adsorption of NP isomers by biochar

To demonstrate whether there were isomer-specific adsorptions, the apparent K_d values of the ten NP isomers with the two dosages of biochar were determined based on the separate analyses of the NP isomers in the aqueous and solid phases (Fig. 5) when the adsorption of biochar to the NP isomers reached equilibrium after 24 h (Lou et al. 2014). In general, there were no significant differences among the K_d values for the ten NP isomers ($P > 0.05$). This clearly indicated that there were no significant differences in the sorption of the NP isomers by biochar, i.e., no adsorptive selectivity, as reported by Pinna and Pusino (2013). Pinna and Pusino studied the sorption mechanisms of the *meta* and *para* isomers of the imazamethabenz-methyl (IMBM) herbicide by soil organic matter. They found that hydrogen bonding was the main mechanism influencing IMBM sorption; the sorption mechanisms did not differ between these two isomers (Pinna and Pusino 2013). Buitrón et al. assessed *p*-NP biodegradation and sorption mechanisms in a bioreactor system by quantifying *p*-NP concentrations in liquid and solid phases. Their results showed that 96% of *p*-NP had no isomer-selective sorption properties (Buitron et al. 2015). Based on the non-isomer-specific sorption behavior of the ten NP isomers by biochar, the influence of biochar on the degradation of the NP isomers was likely through the facilitation of microorganism growth.

Effect of biochar on NP isomer-specific biodegradation

Lu and Gan investigated the biodegradability of 19 NP isomers and concluded that the degradation of the NP isomers was isomer-specific. NP isomers with short side chains and/or bulky α -substituents were found to be more recalcitrant to biodegradation (Lu and Gan 2014). Similar results were obtained in this study. The transformations of NP isomers were positively correlated with side chain length and negatively correlated with the complexity of α/β -substituents without biochar amendment. Figure 6 summarizes the relationships between the degradation ratios of the NP isomers and their structural characteristics (i.e., side chain length, α -substituents, and β -substituents) during the 16 days of incubation with or without biochar. These results showed that the isomer-specific

biodegradation behavior of the NP isomers remained unchanged when various dosages of biochar were added. The biodegradation ratios of the NP isomers were used to explore the NP isomer structure-biodegradability relationship. The side chain length was the first factor examined; specifically, NP isomers with longer side chains appeared to be more susceptible to degradation and generally had higher degradation ratios. For example, the average degradation ratios of the NP isomers at different biochar amendment levels with six-carbon side chains were 94.5% (without biochar), 100% (with 0.001-g biochar), and 78.4% (with 0.01-g biochar), higher than those with five-carbon side chains of 91.3% (without biochar), 95.5% (with 0.001-g biochar), and 75.3% (with 0.01-g biochar) and those with four-carbon side chains of 73.2% (without biochar), 75.0% (with 0.001-g biochar), and 63.4% (with 0.01-g biochar), respectively ($P < 0.01$). Therefore, the side chain length of NP isomers could be considered as a potential indicator for estimating their biodegradability.

There were three major types of α -substituents in the NP isomers: α -dimethyl, α -ethyl-methyl, and α -methyl-propyl. These substituents affected the biodegradability of the NP isomers regardless of the level of added biochar (Fig. 6). Generally, the more complex the α -substituent, the less likely the NP isomer could be degraded. The degradation ratios of the NP isomers followed the order of NP₃₆, NP₃₇, NP₁₁₉, NP₃₅ > NP₆₅, NP₁₁₂, NP_{111a+b}, NP_{110a+b} > NP₁₉₄, NP_{193a+b}, indicating that the biodegradability order of these three α -substituents was α -dimethyl > α -ethyl- α -methyl > α -methyl- α -propyl, which suggested that the less bulky substituents were more easily used by microorganisms. The side chain lengths and α -substituents were also considered to affect the biodegradability of NP isomers by another microbial group, *S. xenophagum*. Our results showed that longer side chains and less bulky α -substituents also led to more rapid biodegradation (Gabriel et al. 2005, 2008). The relationship between biodegradability and the β -substituents of NP isomers, which resulted in poor degradation, was similar to that between biodegradability and α -substituent. The biodegradability order of five types of β -substituents was no substituent > β -methyl $\approx$ β -dimethyl > β -ethyl > β -ethyl- β -methyl. This explained the influential role of structure in the biodegradability of isomers.

Based on the spatial structure of ten NP isomers, we can find that the alkyl chain is attached to the benzene ring at the ipso position. Isomers with bulky substituents on the alkyl chain with larger steric hindrance showed higher residues, such as NP₁₉₄ and NP_{193a+b}, which have α -methyl, α -methyl- α -propyl, and β -ethyl (NP_{193a+b} also has β -methyl). These results were in accordance with those in other study (Corvini et al. 2006; Lu et al. 2015). From previous researches, biodegradation pathways of NP isomers could be concluded as three biodegradation pathways: ipso-hydroxylation (Corvini et al. 2006; Gabriel et al. 2008), nitrication at orthoposition of benzene ring (Corvini et al. 2006; Telscher et al. 2005), and alkyl-chain oxidation (Corvini et al. 2006), and the ipso-hydroxylation pathway was supposed to be the dominant pathway due to the strong inhibitive effect of steric hindrance, which drawn to a conclusion that the ipso position may be an important site for NP isomers and could be regarded as the rate-limiting step of biodegradation. Therefore, the steric hindrance especially the ipso steric effect index may be an indicator for evaluating the biodegradability of NP isomers.

The NP isomers were divided into two categories (Table 1) according to their transformation behaviors influenced by biochar. The first type of NP isomers (excluding NP₁₉₄ and NP_{193a+b}) had side chains of five carbons or six carbons, α -substituents of α -dimethyl or α -ethyl- α -methyl, and no β -substituents or methyl-substituted substituents. The biodegradation of this type was promoted when an appropriate biochar was added due to their less bulky structure. The second type of NP isomer consisted of NP₁₉₄ and NP_{193a+b}, whose transformation was recalcitrant due to their complex substituent structure and short side chains of four carbons, even with a suitable biochar. Therefore, it was reasonable to conclude that NP₁₉₄ and NP_{193a+b} may have accumulated after the attenuation of NP isomers in a biochar-microorganism system. This should be taken into consideration when assessing the fate of NP.

Conclusions

The results in this study showed that at a suitable biochar dosage, the degradation of a first type of NP isomer (all isomers except NP₁₉₄ and NP_{193a+b}) was significantly accelerated by biochar, while the degradation of a second type of NP isomer (NP₁₉₄ and NP_{193a+b}) had no significant differences due to a bulky structure. However, the attenuation of all NP isomers was evidently inhibited, when an overdose biochar was added. The NP degradation was isomer-specific regardless of the addition of biochar. The biodegradability was positively correlated with side chain length and negatively correlated with the complexity of the α - and β -substituents. The preferential biodegradation of NP isomers in a biochar-microorganism system can be used to further convert the profile of degraded NP isomers. As a result, isomeric selectivity should be considered to better understand the fate and risks of NP isomers in contaminated environments, especially when biochars are used.

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References

- Anonymous (2004) Demand increase for nonylphenol in China. *Focus Surfactants* 5:3
- Anonymous (2015) Investigation and analysis on the current situation and prospect of nonylphenol Market in China in 2015, pp 1–9
- Buitron G , Torres-Bojorges AX , Cea-Barcia G (2015) Removal of pnonylphenol isomers using nitrifying sludge in a membrane sequencing batch reactor. *Chem Eng J* 281:860–868
- Chen T , Yeh Y (2010) Ecological risk, mass loading, and occurrence of nonylphenol (NP), NP mono-, and diethoxylate in Kaoping River and its tributaries, Taiwan. *Water Air and Soil Pollut* 208:209–220
- Corvini PFX , Schaeffer A , Schlosser D (2006) Microbial degradation of nonylphenol and other alkylphenols—our evolving view. *Appl Microbial Biot* 72:223–243
- Dodder NG , Maruya KA , Ferguson PL , Grace R , Kiosterhaus S , La Guardia MJ , Lauenstein GG , Ramirez J (2014) Occurrence of contaminants of emerging concern in mussels (*Mytilus* spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent. *Mar Pollut Bull* 81:340–34623849955

- Duong CN , Ra JS , Cho J , Kim SD , Choi HK , Park J , Kim KW , Inam E , Kim SD (2010) Estrogenic chemicals and estrogenicity in river waters of South Korea and seven Asian countries. *Chemosphere* 78: 286–29319931116
- Eganhouse RP , Pontolillo J , Gaines RB , Frysinger GS , Gabriel FLP , Kohler HE , Giger W , Barber LB (2009) Isomer-specific determination of 4-nonylphenols using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. *Environ Sci Technol* 43:9306–931319924899
- Gabriel F , Giger W , Guenther K , Kohler H (2005) Differential degradation of nonylphenol isomers by *Sphingomonas xenophaga* bayram. *Appl Environ Microbiol* 71:1123–112915746308
- Gabriel FLP , Routledge EJ , Heidlberger A , Rentsch D , Guenther K , Giger W , Sumpter JP , Kohler HE (2008) Isomer-specific degradation and endocrine disrupting activity of nonylphenols. *Environ Sci Technol* 42:6399–640818800507
- Giger W , Brunner P , Schaffner C (1984) 4-Nonylphenol in sewage sludge-accumulation of toxic metabolites from nonionic surfactants. *Science* 225:623–6256740328
- Gregory SJ , Anderson CWN , Camps-Arbestain M , Biggs PJ , Ganley ARD , O'Sullivan JM , McManus MT (2015) Biochar in cocontaminated soil manipulates arsenic solubility and microbiological community structure. *PloS One* 10(4):1–18
- Ikunaga Y , Miyakawa SI , Hasegawa M , Kasahara Y , Kodama O , Ohta H (2004) Degradation profiles of branched nonylphenol isomers by *Sphingobium amiense* and *Sphingomonas cloacae*. *Soil Sci Plant Nutr* 50:871–875
- Isobe T , Nishiyama H , Nakashima A , Takada H (2001) Distribution and behavior of nonylphenol, octylphenol and nonylphenol monoethoxylate in Tokyo Metropolitan Area: their association with aquatic particles and sedimentary distributions. *Environ Sci Technol* 35:1041–104911347912
- Kawahata H , Ohta H , Inoue M , Suzuki A (2004) Endocrine disrupter nonylphenol and bisphenol A contamination in Okinawa and Ishigaki Islands, Japan—within coral reefs and adjacent river mouths. *Chemosphere* 55:1519–152715099732
- Lou L , Cheng G , Deng J , Sun M , Chen H , Yang Q , Xu X (2014) Mechanism of and relation between the sorption and desorption of nonylphenol on black carbon-inclusive sediment. *Environ Pollut* 190:101–10824735684
- Lou L , Yao L , Cheng G , Wang L , He Y , Hu B (2015) Application of rice straw biochar and microorganisms in nonylphenol remediation: adsorption-biodegradation coupling relationship and mechanism. *PLoS One* 10(9):1–14
- Lu Z , Gan J (2014) Isomer-specific biodegradation of nonylphenol in river sediments and structure-biodegradability relationship. *Environ Sci Technol* 48:1008–101424345275
- Lu Z , Reif R , Gan J (2015) Isomer-specific biodegradation of nonylphenol in an activated sludge bioreactor and structure-biodegradability relationship. *Water Res* 68:282–29025462736
- Major J , Rondon M , Molina D , Riha SJ , Lehmann J (2010) Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil* 333:117–128
- Mercier A , Joulain C , Michel C , Auger P , Coulon S , Amalric L , Morlay C , Battaglia-Brunet F (2014) Evaluation of three activated carbons for combined adsorption and biodegradation of PCBs in aquatic sediment. *Water Res* 59:304–31524813338
- Park JH , Choppala GK , Bolan NS , Chung JW , Chuasavathi T (2011) Biochar reduces the bioavailability and phytotoxicity of heavy metals. *Plant Soil* 348:439–451
- Pinna MV , Pusino A (2013) Influence of the isomerism on the sorption of imazamethabenz-methyl by soil. *Chemosphere* 91:265–26823219405
- Soares A , Guieysse B , Jefferson B , Cartmell E , Lester JN (2008) Nonylphenol in the environment: a critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environ Int* 34:1033–104918282600
- Stewart M , Olsen G , Hickey CW , Ferreira B , Jelic A , Petrovic M , Barcelo D (2014) A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand. *Sci Total Environ* 468:202–21024029692
- Telscher M , Schuller U , Schmidt B , Schaffer A (2005) Occurrence of a nitro metabolite of a defined nonylphenol isomer in soil/sewage sludge mixtures. *Environ Sci Technol* 39:7896–790016295853
- Vethaak AD , Lahr J , Schrap SM , Belfroid AC , Rijs G , Gerritsen A , de Boer J , Bulder AS , Grinwis G , Kuiper RV , Legler J , Murk T , Peijnenburg W , Verhaar H , de Voogt P (2005) An

integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. *Chemosphere* 59:511–52415788174

Yang YN , Sheng GY (2003a) Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. *Environ Sci Technol* 37:3635–363912953876

Yang YN , Sheng GY (2003b) Pesticide adsorptivity of aged particulate matter arising from crop residue burns. *J Agric Food Chem* 51: 5047–505112903968

Fig. 1.

Variations in the biodegradation ratios of ten NP isomers during 16 days of incubation: **a** with no biochar addition, **b** with the addition of 0.001-g biochar, and **c** with the addition of 0.01-g biochar

Fig. 2. Total ion chromatogram of NP isomers after 4, 8, and 16 days of biodegradation with and without the addition of biochar

Fig. 3.

Relative biodegradation ratios of NP isomers in aqueous and solid phase with the addition of 0.001- and 0.01-g biochar

Fig. 4. Changes in degradation half-lives of NP isomers after additions of biochar ($a P < 0.05$; $b P < 0.01$; $c P > 0.05$)

Fig. 5. Apparent partitioning coefficient (K_d) of NP isomers with 0.001- and 0.01-g biochar additions

Fig. 6.

Quantitative relationships between isomer structures and residues after 16 days of biodegradation (NP₁₉₄ and NP_{193a+b} are marked with *red circles* in each treatment)

Table 1Composition and structural features of NP isomers (side chain lengths, α - and β -substituents)

Juelich number	Juelich nomenclature name	Composition (%)	Side chain length	α Substitution	β Substitution
NP ₃₆	4-[1,1,3-trimethylhexyl]phenol	10.7	6	Me, Me	None
NP ₃₇	4-[1,1,4-trimethylhexyl]phenol	6.7	6	Me, Me	None
NP ₃₅	4-[1,1,2-trimethylhexyl]phenol	12.5	6	Me, Me	Me
NP ₆₅	4-[1-ethyl-1-methylhexyl]phenol	4.0	6	Me, Et	Me
NP ₁₁₉	4-[2-ethyl-1,1-dimethylpentyl]phenol	5.9	5	Me, Me	Et
NP ₁₁₂	4-[1-ethyl-1,4-dimethylpentyl]phenol	11.6	5	Me, Et	Me
NP _{111a+b}	4-[1-ethyl-1,3-dimethylpentyl]phenol	8.0 + 7.6	5	Me, Et	Me
NP _{110a+b}	4-[1-ethyl-1,2-dimethylpentyl]phenol	9.5 + 7.6	5	Me, Et	Me, Me
NP ₁₉₄	4-[1,3-dimethyl-1- <i>n</i> -propylbutyl]phenol	4.5	4	Me, Pr	Et
NP _{193a+b}	4-[1,2-dimethyl-1- <i>n</i> -propylbutyl]phenol	4.2 + 4.8	4	Me, Pr	Me, Et
Total		97.6			