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Enhanced Bioremediation of 4-Chlorophenol by Electrically Neutral Reactive Species Generated from Nonthermal Atmospheric-Pressure Plasma

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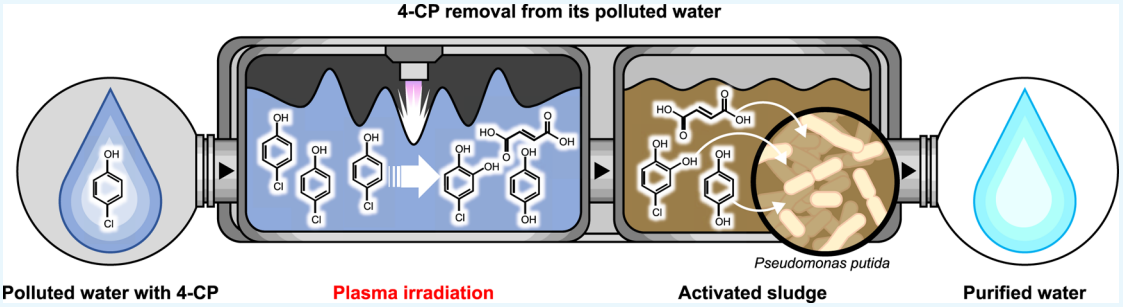

ABSTRACT: 4-Chlorophenol (4-CP) is a chlorinated aromatic compound with broad industrial applications. It is released into the environment as an industrial byproduct and is highly resistant to biodegradation. *Pseudomonas* sp. in the environment and activated sludge are used for 4-CP bioremediation; however, the degradation of 4-CP takes a long time. Consequently, the toxicity of 4-CP is a major barrier to its bioremediation. In this study, we investigated the synergistic effect of electrically neutral reactive species on the bacterial bioremediation of 4-CP. Our results showed that the concentration of 4-CP decreased from 2.0 to 0.137 mM and that it was converted to 4-chlorocatechol (4-CC; 0.257 mM), 4-chlororesorcinol (0.157 mM), hydroquinone (0.155 mM), and trihydroxy chlorobenzene and their respective ring-cleaved products following irradiation of neutral reactive species. These compounds were less toxic than 4-CP, except for 4-CC, which reduced the toxicity of 4-CP to *Pseudomonas putida*. When the neutral reactive species-treated 4-CP fraction was added to *P. putida* cultured in a synthetic sewage medium for 48 h, the 4-CP concentration was reduced to 0.017 mM, whereas nontreated 4-CP (2.0 mM) was hardly degraded by *P. putida*. These results suggest that the biodegradation of 4-CP can be efficiently improved by combining irradiation of neutral reactive species with microbial treatment. The irradiation of neutral reactive species of environmental pollutants may additionally lead to further improvements in bioremediation processes.

INTRODUCTION

Chloroaromatic compounds have numerous industrial applications, including petroleum refining and the production of pesticides, paints, plastics, resins, textiles, iron, solvents, pharmaceuticals, and wood-preserving chemicals.[1−5] The former are released into the environment as byproducts of the latter. Among them, 4-chlorophenol (4-CP) is highly toxic to living organisms owing to its teratogenic, carcinogenic, and mutagenic characteristics as well as its persistence in the aquatic environments.[6−10] Moreover, the environmental and bio-degradation of 4-CP is difficult owing to its low water solubility and vapor pressure characteristics.[11] The US Environmental Protection Agency[12] and the European Union[13] have labeled 4-CP as a "priority pollutant"; that is, its presence in aquatic environments requires constant monitoring and that a concentration of 0.5 mg/L is the upper permissible limit of this compound in public water supply.[14] However, some industrial effluents contain chlorophenols at concentrations of 1.5[15] and 100−200 mg/L.[16] Owing to its broad application and negative health impacts, there is a need to develop an efficient method to remove 4-CP present in the environment and industrial effluents.

Several conventional methods such as adsorption, ion exchange, liquid−liquid extraction, chemical oxidation, and advanced oxidation processes have been used for the removal of chloroaromatic compounds from wastewater.[3,4] However, these expensive methods are not environmentally friendly as they generate hazardous byproducts.[3] Conversely, bioremediation is an effective and environmentally friendly method for the

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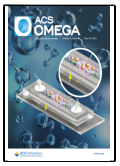

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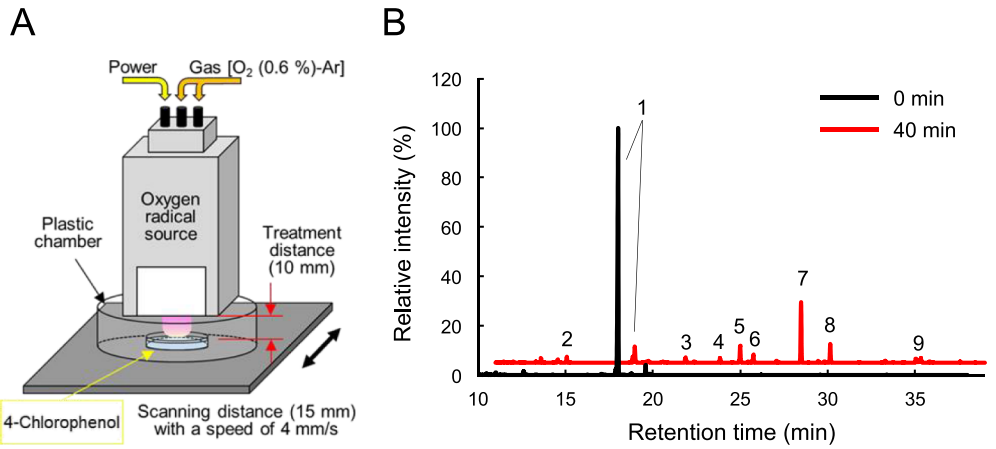

Figure 1. Conversion of 4-CP via neutral reactive species treatment. (a) Schematic diagram of sample preparation for neutral reactive species treatment. Neutral reactive species treatment conditions (*i.e.*, gas mixed with 0.6% O_2 in argon and a treatment distance of 10 mm with a plastic cover) were optimized to obtain the maximal atomic oxygen [O (^3P_j)]. (b) GC−MS chromatogram of 4-CP solution (2.0 mM) treated with the neutral reactive species for 0 and 40 min. Reaction products were trimethylsilylated and analyzed using GC−MS. The identified reaction products are marked with numbers and shown in Table 1.

removal of chloroaromatic compounds from the environment. Moreover, the biodegradation of chloroaromatic compounds has gained increasing attention in recent years owing to the effectiveness of this method, whereby the chloroaromatic compounds are completely mineralized by microorganisms in the environment.[17]

The degradation of CPs and their derivatives using bacteria has been extensively investigated.[17] In previous studies, numerous bacteria such as *Pseudomonas sp*. and *Rhodococcus sp*. that utilize 4-CP as their carbon and energy sources have been isolated from the environment and activated sludge.[18−20] Bacterial degradation of 4-CP occurs *via* either the hydroquinone (HQ)[21] or chlorocatechol (CC) pathway.[22] In the CC pathway *via* meta-cleavage, 4-CC is cleaved to yield a toxic compound, 5-chloro-2-hydoxymuconic semialdehyde (5C2HMS), by catechol-2,3-dioxygenase (EC = 1.13.11.2).[23,24] In several cases, 5C2HMS has been identified as a dead-end product in the degradation pathway of 4-CP.[23,24] Conversely, in the CC pathway *via* ortho-cleavage, 4-CC is eventually transformed to *cis*-dienelactone through the release of chloride ions.[23] However, bacterial bioremediation takes a long time as 4-CP, 5C2HMS, and 4-CC suppress bacterial growth rates.[23] Therefore, the detoxification of 4-CP by bioremediation is not yet an effective method for promoting a sustainable treatment.

In our previous study, we developed a neutral oxygen radical generator based on nonthermal atmospheric-pressure plasma (NTAP) using an oxygen−argon gas mixture.[25−28] The radical generator generates high densities of electrons and atomic oxygen radicals [O(^3P_j)] (10^{16} and 10^{14} cm^{-3}, respectively).[27,28] The use of the radical generator allows on-site generation of radicals, thereby avoiding issues associated with the chemical supply and storage; eliminates the need for a vacuum pump, which enables more concise and practical processes to be conducted at atmospheric pressure in addition to lowering the costs of systems; and enables efficient production of highly reactive atomic oxygen, which speeds up processes. Moreover, chemical conversion using a radical generator is more environmentally friendly than chemical methods as no chemical waste is produced.

To elucidate the usefulness of the electrically neutral reactive species generated from a radical generator in 4-CP conversion, we analyzed the effect of neutral reactive species treatment on 4-CP molecules. Furthermore, we investigated the synergistic effect of neutral reactive species on the bacterial bioremediation of 4-CP. Neutral reactive species treatment results in the dechlorination and ring cleavage of 4-CP, thereby assisting in the detoxification and biodegradation of 4-CP by *Pseudomonas putida*.

RESULTS

Conversion of 4-CP via Neutral Reactive Species Treatment. The effects of neutral reactive species irradiation on 4-CP were examined using gas chromatography−mass spectrometry (GC−MS) (Figures 1B and S1). A solution of 4-CP (2.0 mM) treated by blowing oxygen gas into the solution for 40 min was prepared as a mock control. As there was no decrease in (1.983 ± 0.053) or conversion of 4-CP due to the oxygen gas treatment, we used the nontreated samples as controls in this study. The concentration of 4-CP decreased from 2.0 to 0.137 mM (Peak 1), and it was converted to 4-chlorocatechol (4-CC; Peak 7, 0.275 mM), 4-chlororesorcinol (4-CR; Peak 8, 0.157 mM), HQ (Peak 6, 0.155 mM), and trihydroxy chlorobenzene (Peak 9, not quantified) via neutral reactive species irradiation using the radical generator for 40 min (Figure 1B, Table 1). These results indicate that neutral reactive species irradiation resulted in the hydroxylation and dechlorination of 4-CP. A time course analysis of 4-CP conversion via neutral reactive species treatment using high-performance liquid chromatography (HPLC) showed that the 4-CP concentration in the treated solutions decreased with the increasing treatment time (Figure 2). Additionally, aromatic-ring-cleaved products, including oxalic acid (Peak 2, 0.204 mM), succinic acid (Peak 3, 0.057 mM), fumaric acid (Peak 4, 0.045 mM), and 3-chlorohex-2-ene-1,6-diol (Peak 5, unquantified), were detected using GC−MS. These results indicate that the benzene ring of 4-CP and its derivatives were successfully cleaved via neutral reactive species irradiation (Figures 1B and 2).

The initial concentration of 4-CP was 2.0 mM. The concentrations of 4-CP and the reactants treated with neutral

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Table 1. Detected 4-CP Specific Compounds Derived from Neutral Reactive Species Treatment

no.	identified compound	concentration (mM)
1	4-chlorophenol (4-CP)	0.137 ± 0.016
2	oxalic acid	0.204 ± 0.035
3	succinic acid	0.057 ± 0.004
4	fumaric acid	0.045 ± 0.004
5	3-chlorohex-2-ene-1,6-diol	[a]
6	hydroquinone (HQ)	0.155 ± 0.0013
7	4-chlorocatechol (4-CC)	0.275 ± 0.0019
8	4-chlororesorcinol (4-CR)	0.157 ± 0.010
9	trihydroxychlorobenzene	[a]

[a]These compounds were not quantified because the required reagents were not commercially available.

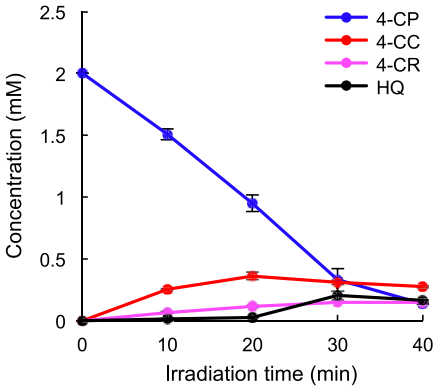

Figure 2. Time-dependent conversion of 4-CP and its derivatives.

reactive species for 40 min were quantified using GC−MS. The reaction products were trimethylsilylated and analyzed using GC−MS. The numbers indicate the GC peaks shown in Figure 1B. The mass spectra obtained for each GC peak are shown in Figure S1.

Quantification of 4-CP and its derivatives was performed using HPLC. Error bars represent mean ± standard error of the mean of three independent experiments.

Effects of Neutral Reactive Species Treatment on Bacterial Growth. To examine the effects of the neutral reactive species treatment of the 4-CP solution on bacterial growth, we cultivated *P. putida* in an OECD synthetic sewage medium containing up to 2.0 mM 4-CP irradiated with or without neutral reactive species (Figure 3). Figure 3 shows the growth curves of cells under various 4-CP concentrations. Compared with that in the control, bacterial growth was inhibited by 68.7, 77.7, and 98.5% in the presence of 0.5, 1.0, and 2.0 mM 4-CP, respectively. Conversely, the growth rates were 129.0, 132.6, and 146.0% in the presence of 4-CP irradiated with neutral reactive species (Figure 4A−D). These findings indicate that the growth of *P. putida* was not inhibited by 4-CP conversion products, suggesting that *P. putida* could assimilate them as energy sources. To comprehensively investigate the toxicity of 4-CP conversion products, we determined the effects of several 4-CP conversion products such as 4-CC, 4-CR, and HQ on bacterial growth (Figure S2). The results showed that the bacterial growth in the presence of a 2.0 mM concentration of the conversion products (4-CR and HQ) was significantly lower than that in the presence of 2.0 mM 4-CP and 4-CC. These results indicate that the 4-CP conversion products, except for 4-CC, generated via neutral

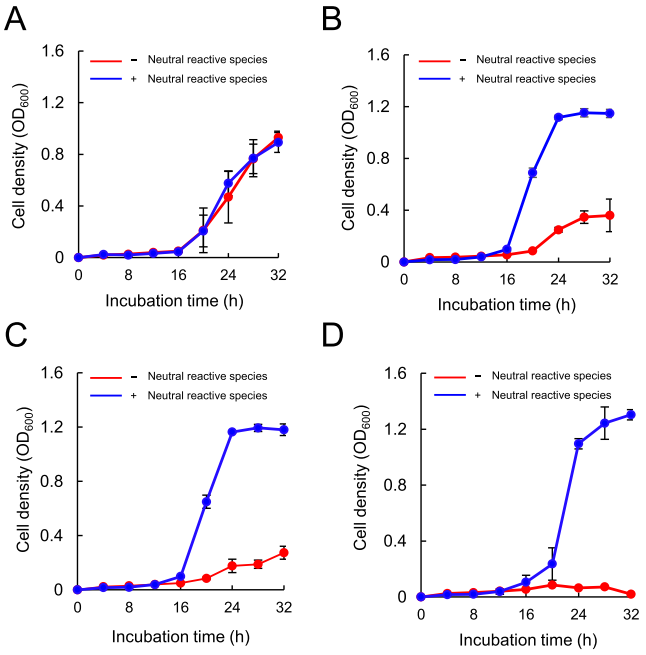

Figure 3. Effect of neutral reactive species treatment of 4-CP on the growth of *P. putida*.

reactive species treatment were less toxic against *P. putida*. Additionally, the concentration of 4-CP (2.0 mM) decreased to 0.137 mM, and it was converted to 4-CC (0.275 mM), 4-CR (0.157 mM), and HQ (0.155 mM) via neutral reactive species treatment. Based on the findings of previous studies, the co-metabolic biodegradation of phenol and 4-CP indicates that low concentrations of phenol can enhance the biodegradation of 4-CP.[29−31] This suggests that the reaction products such as HQ, 4-CC, and/or 4-CR generated from 4-CP may enhance the degradation of 4-CP and its products. Neutral reactive species treatment has considerable potential for the degradation of 4-CP by *P. putida*.

The bacterial cells were grown in a synthetic sewage medium supplemented with 0 (A), 0.5 (B), 1.0 (C), and 2.0 mM (D) 4-CP with or without neutral reactive species treatment. Cell growth was monitored by measuring the optical density at 600 nm. Error bars represent mean ± standard error of the mean of three independent experiments.

Bioremediation of 4-CP by *P. putida*. To investigate the synergistic effects of neutral reactive species treatment on the biodegradation of 4-CP and its derivatives by *P. putida*, the concentrations of these substances were monitored during a 48 h incubation period (Figure 4). The neutral reactive species treatment gradually degraded 4-CP (0.137 mM) and finally decreased the concentration to 0.017 mM after 48 h of incubation with *P. putida* (Figure 4A). The 4-CP derivatives (4-CC and 4-CR) and related cleavage products (oxalic acid, succinic acid, fumaric acid, and 3-chlorohex-2-ene-1,6-diol) were not detected after 48 h of incubation. Conversely, nontreated 4-CP (2.0 mM) was barely degraded by *P. putida*, suggesting that the relatively high concentration of 4-CP inhibited bacterial growth (Figure 4B). These results indicate that the neutral reactive species treatment assists in the assimilation of 4-CP and its derivatives by *P. putida* (Figure S3).

4-CP solutions with (a) or without (b) 40 min irradiation of neutral reactive species were incubated with *P. putida* for 48 h.

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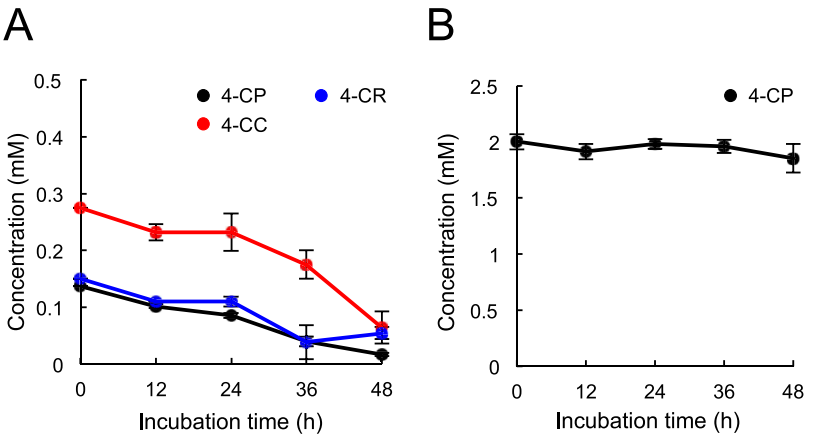

Figure 4. Time-dependent conversion of 4-CP and its derivatives.

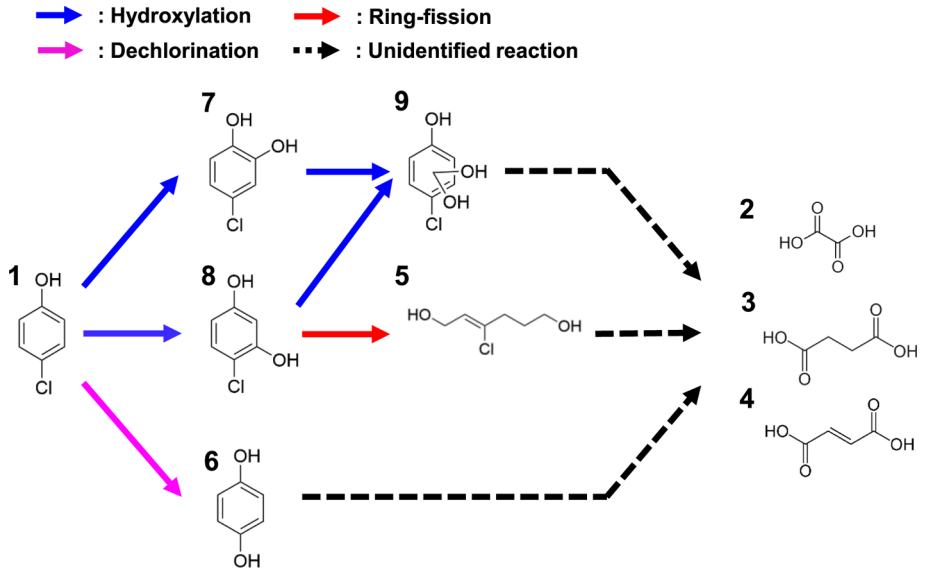

Figure 5. 4-CP oxidation, hydroxylation, dechlorination, and aromatic ring fission via neutral reactive species irradiation.

Quantification was conducted using HPLC. Error bars represent mean ± standard error of the mean of three independent experiments.

DISCUSSION

In this study, irradiation of neutral reactive species resulted in the monooxygenation, dechlorination, and aromatic ring fission of 4-CP. Similarly, in our previous study, we showed that NTAP irradiation results in the hydroxylation, demethylation, decarboxylation, and ring cleavage of the lignin-derived phenolic compound vanillin.[32] Additionally, Shokri et al. (2019) indicated that the 4-CP concentration was decreased by NTAP (O_2 and Ar) irradiation and that one major reaction product (2,3-dihydrofuran) was obtained.[33] Using Fourier transform infrared spectroscopy and ^{1}H nuclear magnetic resonance analyses, Asandulesa et al. (2013) showed that the aromatic ring of benzyl chloride was cleaved and converted to aliphatic groups via NTAP irradiation.[34] Although the exact mechanism of 4-CP conversion and aromatic ring cleavage via neutral reactive species, plasma, or ozone treatment has not been fully elucidated, we speculated that the neutral reactive species irradiation of aqueous samples would likely generate reactive species in both gas and liquid phases and react with 4-CP to promote dechlorination, monooxygenation, and ring cleavage. These suggestions are also presented in Figure 1B and Table 1, showing that the hydroxylation, dechlorination, and aromatic ring fission of 4-CP were initiated via neutral reactive species treatment.

The bacterial degradation of 4-CP occurs *via* the HQ or CC pathway.[17] First, 4-CP is converted to 4-CC or HQ via hydroxylation and dechlorination, and these derivatives ultimately undergo ring cleavage.[21] Intermediates produced in these pathways, such as 4-CP, 5C2HMS, and 4-CC, are toxic compounds that suppress the bacterial growth rate and inhibit bioremediation.[23,24] Therefore, the detoxification of 4-CP via bioremediation is not yet an effective method for the removal of this harmful compound from the environment. In this study, neutral reactive species treatment also involved hydroxylation, dechlorination, and aromatic ring fission reactions. 4-CP was converted to 4-CC, 4-CR, and HQ *via* hydroxylation and dechlorination and finally cleaved to oxalic acid, succinic acid, and fumaric acid (Figure 1B and Table 1). Figure 5 shows the degradation pathways of 4-CP under neutral reactive species treatment. Interestingly, bacterial degradation by intracellular enzymes and modification via

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neutral reactive species treatment detoxified 4-CP in a similar pathway.[17−23]

Among the bacterial and fungal bioremediation strategies, *Pseudomonas* species play important roles in the degradation of environmental pollutants in soil, including 4-CP.[18−20] Thus far, *P. putida* has been utilized for the bioremediation of these chemicals, particularly in activated sludge and bioreactor-facilitated biodegradation.[35] However, bacterial bioremediation takes a long time and does not completely degrade 4-CP as this compound and several of its intermediates suppress bacterial growth.[23,24] Our results indicate that the neutral reactive species treatment assisted the growth of *P. putida* and detoxification and degradation of 4-CP and its derivatives. In other words, *P. putida* assimilated the 4-CP derivatives converted via neutral reactive species treatment as the carbon source. These findings suggest that the neutral reactive species treatment has considerable application potential in bacterial bioremediation strategies in nutrient-poor environments.

Each number indicates the GC peaks shown in Figure 1B and Table 1.

CONCLUSIONS

In this study, we analyzed the effect of neutral reactive species treatment on 4-CP molecules and observed that this treatment reduced the toxicity of 4-CP toward *P. putida* by converting the compound into its derivatives. In addition, plasma-irradiated 4-CP could be used to degrade 4-CP by *P. putida*. These results suggest that the neutral reactive species treatment of 4-CP can significantly contribute to the removal of 4-CP from the environment and industrial wastewater.

METHODS

Chemicals. 4-CP, 4-CC, 4-CR, and HQ used in this study were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Electrically Neutral Reactive Species Treatment of 4-CP. An atmospheric-pressure oxygen radical generator (Tough Plasma FPA10, FUJI CORPORATION, Japan) was used in the study. The radical generator was driven at a high AC voltage of 15 kV (60 Hz, Vpp) using a gas mixture of Ar and O_2. In our previous study, the gas mixture of Ar (4.97 slm) and O_2 (30 sccm) was confirmed as an optimized condition for the production of highly reactive $O(^3P_j)$, with a density of approximately 10^{14} cm^{-3}.[26] Based on this information, the flow rates of Ar and O_2 were set to be 4.97 slm and 30 sccm, respectively, in this study. Additionally, the large amount of Ar used was expected to suppress the three-body collision between O (^3P_j) and O_2, thereby preventing ozone (O_3) production and increasing the supply of atomic oxygen to the samples. Within the radical generator, the gas flow channel is bent at 90°, which prevents the direct exposure of the samples to high-energy photons in the generated plasma. Additionally, an electrically grounded metal plate was placed on the plasma flow channel to terminate charged species in the produced plasma. As shown in Figure 1A, 3 mL of the liquid sample in a Petri dish (38 mm diameter) was placed in the radical generator. The distance between the slit exit (0.5 mm × 16 mm) of the radical generator and the surface of the liquid suspension was fixed at 10 mm. The liquid samples placed on a programmable moving stage were scanned at a speed of 4 mm/s and in a range of 16 mm to improve the uniformity of the radical treatment. A plastic chamber was used to cover the samples to prevent the evaporative loss of 4-CP and its reaction products.

Growth of *P. putida*. *P. putida* KT2440 was obtained from the NITE Biological Resource Center (Tokyo, Japan) and cultured separately in synthetic sewage[36] (16 g/L peptone, 11 g/L meat extract, 3 g/L urea, 0.7 g/L NaCl, 0.4 g/L $CaCl_2$, 0.2 g/L $MgSO_4$, and 2.8 g/L K_2HPO_4) containing a control, 0 mM, and three increasing concentrations of 4-CP—0.5, 1.0, and 2.0 mM, shaken at 300 rpm for up to 48 h at 28 °C. Cell growth in the presence of 4-CP with or without neutral reactive species irradiation was monitored by measuring the optical density at 600 nm.

Analytical Methods. 4-CP solutions treated with or without neutral reactive species were filter-sterilized and analyzed. The residual 4-CP and its metabolic products were analyzed using an HPLC system (JASCO, Tokyo, Japan) equipped with a fluorescence detector (280 nm). The aliquots were separated on a Develosil ODS-HG column (150 mm × 4.6 mm i.d.; Nomura, Seto, Japan) with isocratic elution from water/acetonitrile (20/80) at 1.0 mL/min for 10 min and a linear gradient of 20−100% (v/v) acetonitrile for 15 min. Quantification was carried out using HPLC or GC−MS with calibration curves obtained for 4-CP and its metabolic compound standards.

4-CP solutions (500 μL) were extracted using ethyl acetate (500 μL), dried, trimethylsilylated using 50 μL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (Wako Pure Chemical Industries, Osaka, Japan), and analyzed using GC−MS (GCMS-QP2010; Shimadzu, Kyoto, Japan) on a system equipped with a J&W DB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm thickness; Agilent Technologies, Santa Clara, CA).[37] Glycine (at a final concentration of 50 μM), which is not affected by the neutral reactive species treatment,[38] was added to the 4-CP solutions treated with or without neutral reactive species and used as an internal standard for quantitative analysis using GC−MS.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c01615.

Additional data for the MS analysis of the reaction products generated from 4-CP via neutral reactive species treatment and the growth of *P. putida* with 4-CP conversion products (PDF)

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Author Contributions

#H.K. and K.S. contributed equally to this work. H.K, K.S, S.I, N.I, and MS performed the experiments; H.K, K.S, M.K, and M.S designed the experiments; N.I, M.I, and M.H optimized the oxygen radical generator; and H.K, K.S, and M.S wrote the manuscript. All authors read and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

4-CC, 4-chlorocatechol; 4-CP, 4-chlorophenol; 4-CR, 4-chlororesorcinol; 5C2HMS, 5-chloro-2-hydoxymuconic semialdehyde; CC, chlorocatechol; EU, European union; GC−MS, gas chromatography−mass spectrometry; HPLC, high-performance liquid chromatography; HQ, hydroquinone; NTAP, nonthermal atmospheric-pressure plasma; $O(^3P_j)$, atomic oxygen radicals

■ REFERENCES

(1) Gomez, M.; Matafonova, G.; Gomez, J. L.; Batoev, V.; Christofi, N. Comparison of alternative treatments for 4-chlorophenol removal from aqueous solutions: Use of free and immobilized soybean peroxidase and KrCl excilamp. *J. Hazard. Mater.* **2009**, *169*, 46−51.
(2) Movahedyan, H.; Seid Mohammadi, A.-M.; Assadi, A. Comparison of different advanced oxidation processes degrading *p*-chlorophenol in aqueous solution. *J. Environ. Health Sci. Eng.* **2009**, *6*, 153−160.
(3) Olaniran, A. O.; Igbinosa, E. O. Chlorophenols and other related derivatives of environmental concern: Properties, distribution and microbial degradation processes. *Chemosphere* **2011**, *83*, 1297−1306.
(4) Pera-Titus, M.; García-Molina, V.; Baños, M. A.; Giménez, J.; Esplugas, S. Degradation of chlorophenols by means of advanced oxidation processes: a general review. *Appl. Catal., B* **2004**, *47*, 219−256.
(5) Pi, Y.; Zhang, L.; Wang, J. The formation and influence of hydrogen peroxide during ozonation of para-chlorophenol. *J. Hazard. Mater.* **2007**, *141*, 707−712.
(6) Phornchirasilp, S.; Patel, S. T.; Hanson, J. M.; Newman, H. A. I.; Witiak, D. T.; Feller, D. R. Pharmacologic effects of 4-chlorophenol in rats: comparison to clofibrate. *Proc. Soc. Exp. Biol. Med.* **1989**, *191*, 139−146.
(7) Phornchirasilp, S.; Victor DeSouza, J. J.; Feller, D. R. In vivo and in vitro studies of the hepatotoxic effects of 4-chlorophenol in mice. *Biochem. Pharmacol.* **1989**, *38*, 961−972.
(8) Shehata, M.; Durner, J.; Thiessen, D.; Shirin, M.; Lottner, S.; Van Landuyt, K.; Furche, S.; Hickel, R.; Reichl, F. X. Induction of DNA double-strand breaks by monochlorophenol isomers and ChKM in human gingival fibroblasts. *Arch. Toxicol.* **2012**, *86*, 1423−1429.
(9) Shi, J.; Feng, M.; Zhang, X.; Wei, Z.; Wang, Z. Acute oral toxicity and liver oxidant/antioxidant stress of halogenated benzene, phenol, and diphenyl ether in mice: a comparative and mechanism exploration. *Environ. Sci. Pollut. Res.* **2013**, *20*, 6138−6149.
(10) Yadav, V.; Shitiz, K.; Pandey, R.; Yadav, J. Chlorophenol stress affects aromatic amino acid biosynthesis-a genome-wide study. *Yeast* **2011**, *28*, 81−91.
(11) Nanzai, B.; Okitsu, K.; Takenaka, N.; Bandow, H.; Maeda, Y. Sonochemical degradation of various monocyclic aromatic compounds: relation between hydrophobicities of organic compounds and the decomposition rates. *Ultrason. Sonochem.* **2008**, *15*, 478−483.
(12) Kang, M. G.; Jung, H. S.; Kim, K.-J. Effect of chloride ions on 4-chlorophenol photodegradation in the absence and presence of titanium silicalite-2. *J. Photochem. Photobiol., A* **2000**, *136*, 117−123.
(13) Kristiansen, N. K.; Froeshaug, M.; Aune, K. T.; Becher, G.; Lundanes, E. Identification of halogenated compounds in chlorinated seawater and drinking water produced offshore using *n*-pentane extraction and open-loop stripping technique. *Environ. Sci. Technol.* **1994**, *28*, 1669−1673.
(14) Rodrí, G.; Turnes, M.-I.; Bollaín, M.-H.; Mejuto, M.-C.; Cela, R. Determination of phenolic pollutants in drinking water by capillary electrophoresis in the sample stacking mode. *J. Chromatogr.* **1997**, *778*, 279−288.
(15) Valo, R.; Häggblom, M.; Salkinoja-salonen, M. Bioremediation of chlorophenol containing simulated ground water by immobilized bacteria. *Water Res.* **1990**, *24*, 253−258.
(16) Ettala, M.; Koskela, J.; Kiesilä, A. Removal of chlorophenols in a municipal sewage treatment plant using activated sludge. *Water Res.* **1992**, *26*, 797−804.
(17) Arora, P.-K.; Bae, H. Bacterial degradation of chlorophenols and their derivatives. *Microb. Cell Fact.* **2014**, *13*, 31.
(18) Stolz, A.; Busse, H.-J.; Kämpfer, P. Pseudomonas knackmussii sp. nov. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 572−576.
(19) Knackmuss, H.-J.; Hellwig, M. Utilization and cooxidation of chlorinated phenols by Pseudomonas sp B-13. *Arch. Microbiol.* **1978**, *117*, 1−7.
(20) Fava, F.; Armenante, P. M.; Kafkewitz, D. Aerobic degradation and dechlorination of 2-chlorophenol, 3-chlorophenol and 4-chlorophenol by a Pseudomonas pickettii strain. *Lett. Appl. Microbiol.* **1995**, *21*, 307−312.
(21) Konovalova, E. I.; Solyanikova, I. P.; Golovleva, L. A. Degradation of 4-chlorophenol by the bacterium Rhodococcus opacus 6a. *Microbiology* **2009**, *78*, 805−807.
(22) Bae, H. S.; Lee, J. M.; Lee, S.-T. Biodegradation of 4-chlorophenol *via* a hydroquinone pathway by Arthrobacter ureafaciens CPR706. *FEMS Microbiol. Lett.* **1996**, *145*, 125−129.
(23) Solyanikova, I. P.; Golovleva, L. A. Bacterial degradation of chlorophenols: pathways, biochemica, and genetic aspects. *J. Environ. Sci. Health B* **2004**, *39*, 333−351.
(24) Solyanikova, I.; Golovleva, L. Biochemical features of the degradation of pollutants by Rhodococcus as a basis for contaminated wastewater and soil cleanup. *Microbiology* **2011**, *80*, 591−607.
(25) Inui, H.; Takeda, K.; Kondo, H.; Ishikawa, K.; Sekine, M.; Kano, H.; Yoshida, N.; Hori, M. Measurement of hydrogen radical density and its impact on reduction of copper oxide in atmospheric pressure remote plasma using H_2 and Ar mixture gases. *Appl. Phys. Express* **2010**, *3*, 126101.
(26) Iwasaki, M.; Matsudaira, Y.; Takeda, K.; Ito, M.; Miyamoto, E.; Yara, T.; Uehara, T.; Hori, M. Roles of oxidizing species in a non-equilibrium atmospheric pressure pulsed remote O_2/N_2 plasma glass cleaning process. *J. Appl. Phys.* **2008**, *103*, 023303.
(27) Hashizume, H.; Ohta, T.; Fengdong, J.; Takeda, K.; Ishikawa, K.; Hori, M.; Ito, M. Inactivation effects of neutral reactive-oxygen species on Penicillium digitatum spores using non-equilibrium atmospheric-pressure oxygen radical source. *Appl. Phys. Lett.* **2013**, *103*, 153708.
(28) Ito, M.; Oh, J.-S.; Ohta, T.; Shiratani, M.; Hori, M. Current status and future prospects of agricultural applications using

atmospheric-pressure plasma technologies. *Plasma Processes Polym.* **2018**, *15*, 1700073.
(29) Monsalvo, V. M.; Mohedano, A. F.; Casas, J. A.; Rodríguez, J. J. Cometabolic biodegradation of 4-chlorophenol by sequencing batch reactors at different temperatures. *Bioresour. Technol.* **2009**, *100*, 4572−4578.
(30) Jiang, Y.; Ren, N.; Cai, X.; Wu, D.; Qiao, L.; Lin, S. Biodegradation of phenol and 4-chlorophenol by the mutant strain CTM 2. *Chin. J. Chem. Eng.* **2008**, *16*, 796−800.
(31) Lobo, C. C.; Bertola, N. C.; Contreras, E. M.; Zaritzky, N. E. Monitoring and modeling 4-chlorophenol biodegradation kinetics by phenol-acclimated activated sludge by using open respirometry. *Environ. Sci. Pollut. Res.* **2018**, *25*, 21272−21285.
(32) Ito, S.; Sakai, K.; Gamaleev, V.; Ito, M.; Hori, M.; Kato, M.; Shimizu, M. Oxygen radical based on non-thermal atmospheric pressure plasma alleviates lignin-derived phenolic toxicity in yeast. *Biotechnol. Biofuels* **2020**, *13*, 18.
(33) Hasani, M.; Khani, M.-R.; Karimaei, M.; Yaghmaeian, K.; Shokri, B. Degradation of 4-chlorophenol in aqueous solution by dielectric barrier discharge system: effect of fed gases. *J. Environ. Health Sci. Eng.* **2020**, *17*, 1185−1194.
(34) Asandulesa, M.; Topala, I.; Pohoata, V.; Legrand, Y. M.; Dobromir, M.; Totolin, M.; Dumitrascu, N. Chemically polymerization mechanism of aromatic compounds under atmospheric pressure plasma conditions. *Plasma Processes Polym.* **2013**, *10*, 469−480.
(35) Bhishma, P.-P.; Kumar, A. Biodegradation of 4-chlorophenol in an airlift inner loop bioreactor with mixed consortium: effect of HRT, loading rate and biogenic substrate. *3 Biotech* **2016**, *6*, 117.
(36) *OECD Guidelines for the Testing of Chemicals Proposal for a Revised Guideline 209 Inhibition of Respiration of Activated Sludge*, 2009; pp 1−19.
(37) Sakai, K.; Matsuzaki, F.; Wise, L.; Sakai, Y.; Jindou, S.; Ichinose, H.; Takaya, N.; Kato, M.; Wariishi, H.; Shimizu, M. Biochemical characterization of CYP505D6, a self-sufficient cytochrome P450 from the white-rot fungus Phanerochaete chrysosporium. *Appl. Environ. Microbiol.* **2018**, *84*, No. e01091-18.
(38) Takai, E.; Kitamura, T.; Kuwabara, J.; Ikawa, S.; Yoshizawa, S.; Shiraki, K.; Kawasaki, H.; Arakawa, R.; Kitano, K. Chemical modification of amino acids by atmospheric-pressure cold plasma in aqueous solution. *J. Phys. D: Appl. Phys.* **2014**, *47*, 285403.

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