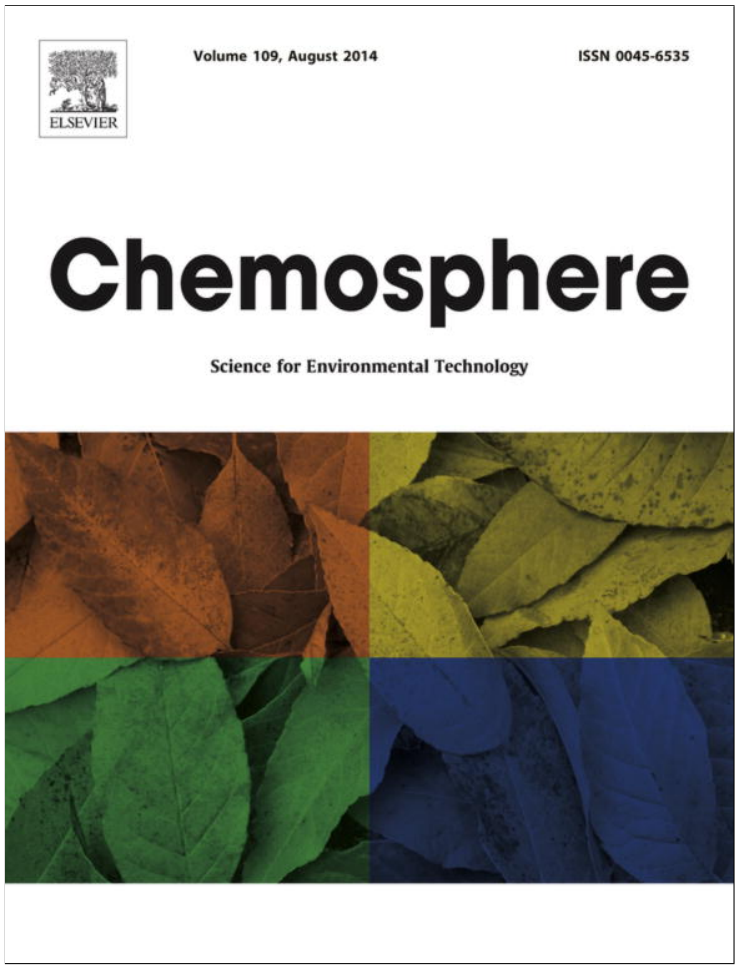

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Technical Note

Hydrogen sulfide alleviates 2,4-dichlorophenol toxicity and promotes its degradation in *Phanerochaete chrysosporium*

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HIGHLIGHTS

- H_2S alleviated 2,4-DCP toxicity and improved its degradation by *P. chrysosporium*.
- H_2S shortened the time required for 2,4-DCP complete degradation.
- H_2S alleviated 2,4-DCP caused oxidative stress and improved cell viability.

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ABSTRACT

In this study, the H_2S donor, sodium hydrosulfide (NaHS) was used to pretreat *Phanerochaete chrysosporium* in order to improve its ability to degrade 2,4-dichlorophenol (2,4-DCP). When pretreated with 100 μM NaHS, *P. chrysosporium* was able to degrade 2,4-DCP completely in 24 h, whereas the degradation efficiency of the untreated control was only 57%. The 2,4-DCP-induced oxidative stress was alleviated by NaHS, and the percentage of surviving cells increased by 32%. H_2S or HS^-, rather than other compounds derived from NaHS, were responsible for promoting 2,4-DCP degradation by *P. chrysosporium*. The results of this study suggest that H_2S treatment is a potential strategy to alleviate environmental stress and improve the efficiency of the biological removal of pollutants from wastewater.

1. Introduction

Chlorophenols are xenobiotic contaminants that are often found in the waste discharges produced during various industrial processes including oil refinery, and the manufacture and processing of petrochemicals, plastics, pesticides, biocides, wood preservers, pulp, and insulation materials (Czaplicka, 2004; Zilouei et al., 2006; Jin et al., 2011). Due to their high toxicity, recalcitrance, bioaccumulation, strong odor emission, persistence in the environment, suspected carcinogenicity, and mutagenicity to living organisms, chlorophenols pose a serious ecological problem as environmental pollutants (ATSDR, 1999; Quan et al., 2004). Among these, 2,4-dichlorophenol (2,4-DCP), which is listed by the US environmental protection agency as a priority environmental pollutant, is considered to promote disturbances in the structure of the cell membrane bilayer (USEPA, 1981; Schüller et al., 2007). Although there have been attempts to regulate the use of 2,4-DCP, large quantities of wastewater containing this pollutant continue to be discharged into water bodies. Biological treatment is a viable alternative for 2,4-DCP removal, since 2,4-DCP can be mineralized by microorganisms under aerobic or anaerobic conditions (Wang et al., 2007). Some microorganisms such as *Aspergillus awamori*, *Bacillus* sp., *Pseudomonas* sp., and *Phanerochaete chrysosporium* have been reported to degrade and transform 2,4-DCP (Stoilova et al., 2006; Matafonova et al., 2006; Ziagova and Liakopoulou-Kyriakides, 2007; Chen et al., 2011). However, this biodegradation process usually takes long time and the removal efficiency is low, especially at later stages, due to the inhibition to microorganisms resulting from 2,4-DCP toxicity (Matafonova et al., 2006; Stoilova et al., 2006).

H_2S has long been considered as a toxic gas and environmental pollutant; however, recently it has gained attention as a novel cell signaling molecule involved in growth, development, and the acquisition of stress tolerance in many organisms, including plants and animals (Li et al., 2011; Lisjak et al., 2013). Recent studies have

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provided evidence that H_2S exerted antioxidant and anti-apoptotic effects, which improved cell viability and survival and protected cells against environmental stress (Zhang et al., 2010; Yu et al., 2011). However, sight has been ignored about introducing H_2S to the wastewater treatment field to explore the potential effects of alleviating the environmental stress and improving the biological treatment efficiency. In this study, sodium hydrosulfide (NaHS), a H_2S donor, was used to explore the effect of H_2S on the degradation of 2,4-DCP by the white-rot fungus *P. chrysosporium*.

2. Materials and methods

2.1. Strain culture

The *P. chrysosporium* strain BKMF-1767 (CCTCC AF96007) used in this study was obtained from the China Center for Type Culture Collection (Wuhan, China). Stock cultures were maintained on malt extract agar slants at 4 °C. Spores were gently scraped from the agar surface and blended in sterile distilled water to obtain a spore suspension. The spore concentration was adjusted to 2.0×10^6 spore mL^{-1} using a turbidimeter (WGZ-200, Shanghai, China). Aqueous suspensions of fungal spores were inoculated into Kirk's liquid culture medium (Kirk et al., 1978) in 500-mL Erlenmeyer flasks. Flasks containing 8×10^6 spores were incubated at 37 °C in an incubator.

2.2. Effect of hydrogen sulfide on 2,4-DCP degradation

After incubation for 3 d, the required amount of 2,4-DCP stock solution was added to the culture medium, to obtain a final concentration of 20 or 50 mg L^{-1}. For NaHS treatment, *P. chrysosporium* were pretreated with 25–200 μM NaHS for 4 h prior to the addition of 2,4-DCP solution. Cultures that were not treated with NaHS or 2,4-DCP were used as controls. A batch of *P. chrysosporium* was autoclaved for 20 min at 121 °C to kill the cells. These dead *P. chrysosporium* were then mixed with 2,4-DCP and cultured under the conditions described earlier to examine the effect of biosorption. Samples were taken every 12 h from 0 to 72 h and analyzed for residual 2,4-DCP concentration. The 2,4-DCP concentration in the aqueous solution was determined using a UV–Vis spectrophotometer (Model UV-2550, Tokyo, Japan) at 306 nm (Chen et al., 2011).

To verify the role of H_2S in the promotion of 2,4-DCP degradation induced by NaHS, a range of sodium salts including those that contained sulfur (100 μM Na_2S, Na_2SO_4, $NaHSO_4$, Na_2SO_3, $NaHSO_3$, and NaAC) were tested under the same experimental conditions.

2.3. Physiological assays

After 72 h of degradation of 2,4-DCP, *P. chrysosporium* pellets were harvested and washed twice with distilled water for performing physiological assays. Cell viability was assessed using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to Luo et al. (2013) with some modifications. Briefly, 0.2 g of *P. chrysosporium* pellets were mixed with 1 mL MTT solution (5 g L^{-1}) and incubated at 50 °C. The reaction was stopped by adding 0.5 mL hydrochloric acid (1 M) to the mixture. The mixture was centrifuged (10000*g*, 5 min) following which the supernatant was discarded and the pellets were agitated in 6 mL propan-2-ol for 2 h. The centrifugation process was repeated and the absorbance of the supernatant was recorded at 534 nm.

The concentration of antioxidant enzymes and lipid peroxidation were measured as described in our previous work (Zeng et al., 2012). Superoxide dismutase (SOD) activity was assessed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride. Catalase (CAT) activity was detected by monitoring the absorbance of hydrogen peroxide at 240 nm. One unit of CAT activity was defined as the decreased absorbance of 0.01 at A_{240} nm min^{-1} under assay conditions.

Lipid peroxidation was evaluated by measuring the concentration of malondialdehyde (MDA), which is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage. The harvested *P. chrysosporium* cells were homogenized in 10% trichloroacetic acid and centrifuged at 2570*g* for 15 min. The supernatant was boiled with thiobarbituric acid for 20 min. The heated supernatant was centrifuged at 2570*g* for 5 min, and the absorbance was measured at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of MDA (155 mM^{-1} cm^{-1}) was used for the calculation of MDA level.

O_2^- production was analyzed according to Lei et al. (2006) with some modifications. The harvested cells were homogenized in 2 mL of 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10277*g* for 10 min at 4 °C. 1 mL of the supernatant was mixed with 0.9 mL of 50 mM potassium phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine hydrochloride. The reaction mixture was incubated at 25 °C for 20 min before adding 1 mL of 17 mM p-aminobenzenesulfonic acid and 1 mL of 7 mM α-naphthylamine. After further incubation (25 °C, 20 min), the absorbance of the mixture was measured at 530 nm by spectrophotometry.

All batch experiments were conducted in triplicates and the data are expressed as mean ± standard deviation. Statistical evaluation of the results was determined by Tukey post hoc test. Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

3.1. Variations of pH

The pH condition influenced the hydrolysis or ionization of HS^- in the solution, resulting in the form of HS^- existed (H_2S, HS^- or S^{2-}). Therefore, the variation of pH in the medium during the degradation process was monitored. As shown in Table 1, the pH did not change much during 2,4-DCP degradation. It fluctuated from pH 4.2 to 5.2. Under this condition, the speciation of sulfide was H_2S or HS^- ($pK_1 = 6.88$).

3.2. Promotion of 2,4-DCP degradation by NaHS

The effect of NaHS on 2,4-DCP degradation was examined using 2,4-DCP at an initial concentration of 20 mg L^{-1} and NaHS at a concentration range of 0–200 μM. Fig. 1 shows that NaHS, a H_2S donor, was able to promote 2,4-DCP degradation by *P. chrysosporium*. This effect of NaHS was more pronounced at a concentration of 50–100 μM, when compared to lower (25 μM) and higher doses (200 μM). Following incubation of 2,4-DCP with inactivated *P. chrysosporium*, it was observed that the concentration of 2,4-DCP remained almost unchanged, suggesting that the removal of 2,4-DCP was mainly through biodegradation and that the biosorption of 2,4-DCP by *P. chrysosporium* mycelium is negligible.

The percentages of 2,4-DCP degradation in 24 h were 57%, 65%, 97%, 100%, and 40% at NaHS pretreatment concentrations of 0, 25, 50, 100 and 200 μM, respectively (Fig. 1a). 2,4-DCP was almost completely degraded when *P. chrysosporium* was pretreated with 50 μM NaHS and no 2,4-DCP was detected when *P. chrysosporium* was pretreated with 100 μM NaHS for a degradation time of 24 h. These results indicate that pretreatment with 50 or 100 μM NaHS not only significantly improved the degradation efficiency,

Table 1
Variations of pH in the degradation medium.

Time (h)	−4[a]	0	6	12	24	36	48	60	72
Control	4.72	4.61	4.54	4.53	4.48	4.47	4.52	4.57	4.55
2,4-DCP	4.70	4.62	4.71	4.76	4.29	4.33	4.56	4.53	4.56
50 μM NaHS	4.71	4.72	4.61	4.54	4.40	4.46	4.55	4.63	4.63
2,4-DCP + 50 μM NaHS	4.75	4.73	4.86	4.99	4.39	4.33	4.40	4.44	4.52
2,4-DCP + 100 μM NaHS	4.79	4.79	4.95	5.12	4.39	4.32	4.37	4.43	4.49

[a] The beginning of NaHS pretreatment.

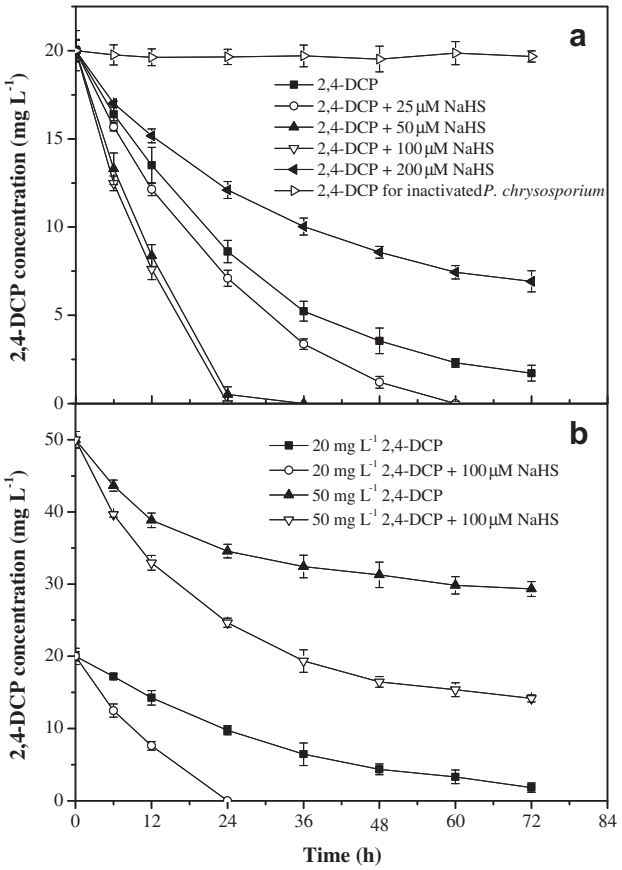

Fig. 1. (a) Effect of NaHS concentration (0–200 μM) on 2,4-DCP degradation in *P. chrysosporium*. (b) Times courses of 2,4-DCP degradation by *P. chrysosporium* with and without pretreatment with 100 μM NaHS.

but also shortened the time required for the complete degradation of 2,4-DCP. At the highest dose (200 μM) tested, the degradation efficiency of 2,4-DCP was even lower than in the untreated control, which indicated that higher concentrations of H_2S released from NaHS may be toxic to *P. chrysosporium*. Similar results were also reported by others (Zhang et al., 2008; Zhang et al., 2010) when the effect of H_2S on wheat seedlings was investigated.

Differences in promotion effects using an initially higher 2,4-DCP concentration (50 mg L^{-1}) are illustrated in Fig. 1b. The degradation efficiency of the control groups (non-pretreatment with NaHS) were 91% and 41% for initial 2,4-DCP concentrations of 20 and 50 mg L^{-1}, respectively. When pretreated with 100 μM NaHS, there was no 2,4-DCP detected in the residual solution, when the initial 2,4-DCP concentration was 20 mg L^{-1}. The degradation efficiency increased to 72% when the initial 2,4-DCP concentration was 50 mg L^{-1}. Although 2,4-DCP was not completely degraded when the initial concentration was 50 mg L^{-1}, the degradation efficiency was relatively higher (72% versus 41%). A higher initial concentration of 2,4-DCP resulted in a decrease in 2,4-DCP degradation efficiency, which could be attributed to 2,4-DCP toxicity toward biological activity (Chen et al., 2011). However, this phenomenon was partly reversed by pretreatment with 100 μM NaHS.

To verify the role of H_2S in the promotion of 2,4-DCP degradation induced by NaHS, a range of sodium salts including those that contained sulfur were used. The results showed that pretreatment with the same concentration of other sodium salts or sulfur-containing components had no significant effect on 2,4-DCP degradation (Fig. 2). From this result, it can be concluded that H_2S or HS^-, rather than other compounds derived from NaHS, were responsible for promoting 2,4-DCP degradation by *P. chrysosporium*.

3.3. Physiological analysis of *P. chrysosporium*

As shown in Fig. 3, *P. chrysosporium* cell viability was reduced in the presence of 2,4-DCP during the degradation process. Death occurred in approximately 35% of the cells after 72 h of incubation with 2,4-DCP at initial concentration of 20 mg L^{-1}. However, death induced by 2,4-DCP was reversed by pretreatment with NaHS (only 15% and 3% of the cells died after pretreatment with 50 and 100 μM NaHS, respectively), which demonstrated that the H_2S donor exerted a strong positive effect against 2,4-DCP toxicity.

In general, oxidative stress has been considered as the first response of cells to unfavorable environments. Therefore, we measured the activity of antioxidant enzymes (SOD and CAT) and lipid peroxidation in 2,4-DCP-stressed *P. chrysosporium*. MDA measurement was used as an indicator of lipid peroxidation, which is linked to the production of O_2^- (Dazy et al., 2009). As shown in

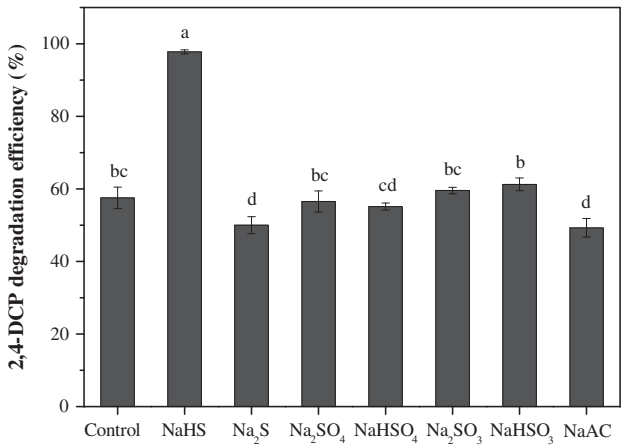

Fig. 2. H_2S or HS^-, but not other compounds derived from NaHS contributed to 2,4-DCP degradation. *P. chrysosporium* was pretreated with a range of sodium salts contained sulfur (100 μM Na_2S, Na_2SO_4, $NaHSO_4$, Na_2SO_3, $NaHSO_3$, and NaAC, respectively) for 4 h. Subsequently, 2,4-DCP was added to a final concentration of 20 mg L^{-1}, and the degradation efficiency at 24 h was investigated. Different letters mean significance of difference between the treatments ($p < 0.05$).

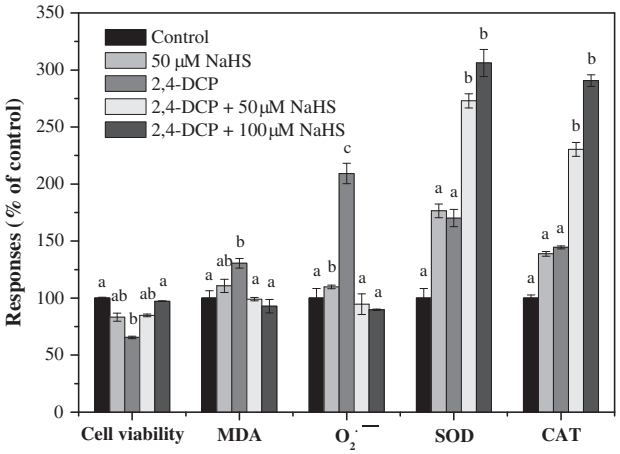

Fig. 3. Physiological properties of *P. chrysosporium* after 72 h incubation with 2,4-DCP. Different letters mean significance of difference between the treatments ($p < 0.05$).

Fig. 3, SOD, CAT activity, MDA level, and $O_2^{\cdot-}$ production were significantly altered in the NaHS-pretreated group when compared to the untreated group (2,4-DCP treated only). The elevated MDA and $O_2^{\cdot-}$ levels suggest that 2,4-DCP stimulated the generation of free radicals in *P. chrysosporium*; however, this elevation was not observed after NaHS pretreatment. NaHS significantly reduced MDA and $O_2^{\cdot-}$ content in 2,4-DCP-treated cells, suggesting that 2,4-DCP-stimulated lipid peroxidation was lowered by NaHS treatment.

On the other hand, the SOD activity of *P. chrysosporium* pretreated with 50 or 100 μM NaHS (2,4-DCP + NaHS) increased significantly compared to other treatments, which was approximately 2.7- and 3.0-fold of that observed in the control, respectively. A similar trend was also observed for CAT activity. The generation of 2,4-DCP-induced reactive oxygen species (ROS), stimulated the production of antioxidant enzymes, which in turn react with ROS to protect the cellular components from oxidative damage. The low MDA and $O_2^{\cdot-}$ levels observed in mycelia pretreated with 50 or 100 μM NaHS, probably due to the significant increase in SOD and CAT activities under these treatments, which indicated that H_2S alleviated the 2,4-DCP caused oxidative stress by increasing activities of ROS scavenging enzymes (SOD and CAT), and played an important role in cell protection. Similar results were also reported when the role of NaHS in alleviating the toxicity of metals and other chemicals that caused oxidative stress was investigated (Zhang et al., 2010; Li et al., 2011).

In the wastewater treatment process, H_2S is produced by the degradation of proteins and other sulfur containing compounds existed in the wastewater, by the activity of sulfate-reducing bacteria (Díaz et al., 2010). Much work has been carrying out to prevent the generation of this gas, which causes a series of problems (Chang et al., 2007; Ramírez et al., 2009). Results from this study provided novel evidence which confirmed that H_2S is beneficial for pollutant removal in wastewater treatment. These findings suggest a new use for H_2S (as an available gas that improves the efficiency of removal of pollutants, rather than a toxic pollutant) in the wastewater treatment process. Further studies are needed to explore the feasibility of H_2S-based technologies to improve wastewater treatment efficiency.

4. Conclusions

In this study, the H_2S donor, NaHS was successfully used to improve the degradation of 2,4-DCP by *P. chrysosporium*. Our results indicated that H_2S or HS^-, rather than other compounds derived from NaHS, were responsible for promoting 2,4-DCP degradation. Our data confirms that H_2S can alleviate 2,4-DCP-induced oxidative stress in *P. chrysosporium* and improve cell viability and survival by increasing 2,4-DCP degradation. Findings in this study suggest that H_2S, a toxic pollutant, has potential applications in wastewater treatment, to improve the biological degradation of pollutants such as 2,4-DCP.

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References

ATSDR, 1999. Agency for Toxic Substances and Disease Registry, Toxicological Profile for Hlorophenols. US Department of Health and Human Services, Public Health Service, GA.

Chang, Y.J., Chang, Y.T., Chen, H.J., 2007. A method for controlling hydrogen sulfide in water by adding solid phase oxygen. Bioresour. Technol. 98, 478–483.

Chen, A., Zeng, G., Chen, G., Fan, J., Zou, Z., Li, H., Hu, X., Long, F., 2011. Simultaneous cadmium removal and 2,4-dichlorophenol degradation from aqueous solutions by *Phanerochaete chrysosporium*. Appl. Microbiol. Biotechnol. 91, 811–821.

Czaplicka, M., 2004. Sources and transformations of chlorophenols in the natural environment. Sci. Total Environ. 322, 21–39.

Dazy, M., Masfaraud, J.F., Férard, J.F., 2009. Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. Chemosphere 75, 297–302.

Díaz, I., Lopes, A.C., Pérez, S.I., Fdz-Polanco, M., 2010. Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion. Bioresour. Technol. 101, 7724–7730.

Jin, X., Zha, J., Xu, Y., Wang, Z., Kumaran, S.S., 2011. Derivation of aquatic predicted no-effect concentration (PNEC) for 2,4-dichlorophenol: comparing native species data with non-native species data. Chemosphere 84, 1506–1511.

Kirk, T.K., Schultz, E., Connors, W.J., Lorenz, L.F., Zeikus, J.G., 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol. 117, 277–285.

Lei, Y., Yin, C., Li, C., 2006. Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. Physiol. Plantarum 127, 182–191.

Li, L., Rose, P., Moore, P.K., 2011. Hydrogen sulfide and cell signaling. Annu. Rev. Pharmacol. Toxicol. 51, 169–187.

Lisjak, M., Teklic, T., Wilson, I.D., Whiteman, M., Hancock, J.T., 2013. Hydrogen sulfide: environmental factor or signaling molecule? Plant Cell Environ. 36, 1607–1616.

Luo, Y.H., Wu, S.B., Wei, Y.H., Chen, Y.C., Tsai, M.H., Ho, C.C., Lin, S.Y., Yang, C.S., Lin, P., 2013. Cadmium-based quantum dot induced autophagy formation for cell survival via oxidative stress. Chem. Res. Toxicol. 26, 662–673.

Matafonova, G., Shirapova, G., Zimmer, C., Giffhorn, F., Batoev, V., Kohring, G.W., 2006. Degradation of 2,4-dichlorophenol by *Bacillus* sp. isolated from an aeration pond in the Baikalsk pulp and paper mill (Russia). Int. Biodeterior. Biodegr. 2006, 209–212.

Quan, X., Shi, H., Liu, H., Wang, J., Qian, Y., 2004. Removal of 2,4-dichlorophenol in a conventional activated sludge system through bioaugmentation. Process Biochem. 39, 1701–1707.

Ramírez, M., Gómez, J.M., Aroca, G., Cantero, D., 2009. Removal of hydrogen sulfide by immobilized *Thiobacillus thioparus* in a biotrickling filter packed with polyurethane foam. Bioresour. Technol. 100, 4989–4995.

Schüller, C., Mamnun, Y.M., Wolfger, H., Rockwell, N., Thorner, J., Kuchler, K., 2007. Membrane-active compounds activate the transcription factors Pdr1 and Pdr3 connecting pleiotropic drug resistance and membrane lipid homeostasis in *Saccharomyces cerevisiae*. Mol. Biol. Cell 18, 4932–4944.

Stoilova, I., Krastanova, A., Stanchev, V., Daniel, D., Gerginova, M., Alexieva, Z., 2006. Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by *Aspergillus awamori* cells. Enzyme Microb. Technol. 39, 1036–1041.

USEPA, 1981. Priority pollutant list. 40 CFR 423, Appendix A. US environmental protection agency, Washington, DC.

Wang, S.G., Liu, X.W., Zhang, H.Y., Gong, W.X., Sun, X.F., Gao, B.Y., 2007. Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor. Chemosphere 69, 769–775.

Yu, Y.P., Li, Z.G., Wang, D.Z., Zhan, X., Shao, J.H., 2011. Hydrogen sulfide as an effective and specific novel therapy for acute carbon monoxide poisoning. Biochem. Biophys. Res. Commun. 404, 6–9.

Zeng, G.M., Chen, A.W., Chen, G.Q., Hu, X.J., Guan, S., Shang, C., Lu, L.H., Zou, Z.J., 2012. Responses of *Phanerochaete chrysosporium* to toxic pollutants: Physiological flux, oxidative stress, and detoxification. Environ. Sci. Technol. 46, 7818–7825.

Zhang, H., Hu, L.Y., Hu, K.D., He, Y.D., Wang, S.H., Luo, J.P., 2008. Hydrogen sulfide promotes wheat seed germination and alleviates oxidative damage against copper stress. J. Integr. Plant Biol. 50, 1518–1529.

Zhang, H., Tan, Z.Q., Hu, L.Y., Wang, S.H., Luo, J.P., Jones, R.L., 2010. Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. J. Integr. Plant Biol. 52, 556–567.

Ziagova, M., Liakopoulou-Kyriakides, M., 2007. Kinetics of 2,4-dichlorophenol and 4-Cl-m-cresol degradation by *Pseudomonas* sp. cultures in the presence of glucose. Chemosphere 68, 921–927.

Zilouei, H., Guieysse, B., Mattiasson, B., 2006. Biological degradation of chlorophenols in packed-bed bioreactors using mixed bacterial consortia. Process Biochem. 41, 1083–1089.