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Inhibitory effect of nitrobenzene on oxygen demand in lake sediments

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Abstract

Nitrobenzene is an important raw material and product, which presents a heavy threat to the ecosystem. The potential impacts of nitrobenzene on sediment oxygen demand (SOD) were studied in lake sediment simulating reactors receiving relatively low inputs of nitrobenzene. Oxygen microprofiles were measured in these sediment reactors using microelectrodes. After an initial microprofile measurement as a control, nitrobenzene was added to the overlying water resulting in concentrations of 0, 50, 100, and 150 μg/L. Microprofiles were measured on day 1, 2, 4 and 7 following the addition of nitrobenzene. SODs were determined from the microprofiles using a reaction-diffusion model. Results showed that the SODs increased relative to the initial values measured in the pre-treatment period in reactors exposed to all nitrobenzene concentrations on day 1. However, the values decreased gradually on the following days, which eventually resulted in a 50% loss in SODs after 7 days of exposure to nitrobenzene in all reactors. In addition, the inhibition effect of nitrobenzene on SOD exhibited a weak relationship with its concentration. The microscopic observation and count of algae in the sediment showed that the exposure to nitrobenzene did not change the composition of algae greatly, however, it decreased the number of dominant algae species sharply after 7 days of exposure. These results suggested that nitrobenzene could significantly alter SOD in lakes, which could ultimately affect the pollutant recovery in aquatic-sediment systems.

Key words: nitrobenzene; sediment; inhibitory effect; microelectrode; sediment oxygen demand

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Introduction

Nitroaromatic compounds are important raw materials and products of the chemical industry. They are widely used for the manufacture of pesticides, drugs, explosives, polymers, dyes and rubber chemicals etcetera, and therefore are produced in large amounts (Gatermann et al., 1995). Nitrobenzene, which is a monocyclic nitroaromatic compound and is listed as a priority toxic pollutant by the US EPA, is an important chemical product with annual output of worldwide more than 225,000 tons/yr (Zhao et al., 2003). In the early 1980s, nitrobenzenes were detected in the surface water of Japan, America, and the North Sea (Gatermann et al., 1995). In China, nitrobenzene pollution has been detected in some major rivers (He et al., 2006). As nitrobenzene enters the aquatic system, it could enter and accumulate in sediments and pose dangers to the sediment organisms. The toxicity of nitrobenzene to bacteria, aquatic organisms and terrestrial organisms has been proved by many publications (WHO, 2004).

Sediments are characterized by biogeochemical conditions that vary significantly over short (mm-dm), predominantly vertical, distances with the most intense microbial activity concentrated close to the sediment-water interface (Jourabchi et al., 2008). The carbon and nitrogen cycles conducted by the benthic microorganisms and alga are related closely to the quality of the overlying water. Therefore, it is important and meaningful to evaluate the significance of toxicants on microbial activities. Protein dosage permits an accurate evaluation of biomass variation, and was used as an indicator to evaluate the acute and chronic impacts caused by aromatic hydrocarbons on bacterial communities at beaches by Crapez et al. (2000). Phospholipid analysis permits estimation of total viable biomass by measuring the "phospholipid phosphate" content from phospholipid components of the cellular membranes of microorganisms (White et al., 1979), which is another feasible way to evaluate the impact of toxicants. With further investigation by gas chromatography, high-performance liquid chromatography, etc., researchers have tried to interpret variations in lipid fatty acids patterns to indicate shifts in the relative proportions of different groups of bacteria (Rütters et al., 2002); however, results by different authors are often contradictory (Harvey and Macko, 1997). Besides, with the development of molecular biology, new research results have been brought into the study of biological phases in sediments.

Electrochemical microsensors have been fabricated for measurements of O_2, pH, CO_2, NO_3^-, NH_4^+ and redox potential with a high spatial resolution. These electrodes have been successfully used in sediment measurements (Revsbech and Jørgensen, 1986; Laursen and Carlton,

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1999; Cai et al., 2000; Rabouille et al., 2003; Nakamura et al., 2004). Sediment pore water profiles are commonly used to identify the predominant reactions taking place in aquatic sediments. Amongst the chemical parameters that are measured routinely, the O_2 concentration is particularly instructive (Jourabchi et al., 2008). The sediment ecosystem is a typical bacteria and algae symbiotic system. Algae fix inorganic carbon, assimilate inorganic nutrients and produce oxygen through photosynthesis. Heterotrophic bacteria mineralize organic matter, consume oxygen and transform nutrients. Sediment oxygen demand (SOD) is the total oxygen input from the overlying water due to the microbial activities of bacteria and photosynthesis of algae in sediment, generally represented as oxygen demand per surface area per time, mg/(m^2·day). SOD is a key issue in determining water quality, so it is of importance to identify the processes responsible for SOD and quantify it precisely (Rabouille et al., 2003). Apart from the traditional methods, such as core or benthic chamber incubations (Rabouille et al., 2003), measuring oxygen microprofiles through use of an O_2 microelectrode provides another feasible way to obtain SOD.

Benthic algae are the dominant primary producers in many shallow lakes and streams, providers of habitat and nursery grounds for fish and invertebrates in marine and freshwater systems (Han and Choi, 2005). When pollutants enter the aquatic or sediment environment, the composition or number of benthic algae may change, therefore, it should be possible to assess the ecotoxicological implications of pollutants by using benthic algae as biological indicators, as reported in previous studies (Han and Choi, 2005).

For the reasons mentioned above, this study aimed to investigate the inhibitory effect of nitrobenzene on oxygen demand in lake sediments by *in-situ* microelectrode measurement. By sequentially monitoring the O_2 concentration profiles in sediments exposed to different concentrations of nitrobenzene, the spatial distributions of microbial activities in the inhibitor-treated and untreated sediments were obtained from the measured profiles, which eventually led to the evaluation of the inhibitory effects of the nitrobenzene on the sediments. For comparison, with the help of transmitted light microscopy, the algal composition and number were observed in the upper layer of sediment treated and untreated with nitrobenzene. The decrease in the algae number were quantified to evaluate the impact caused by the nitrobenzene.

1 Material and methods

1.1 Lake sediment

The sediment and overlying water were collected from the Lake of JinChun Yuan at Tsinghua University, China using a grab type sampler. The water qualities in the lake were determined and are listed as follows: pH 7.5–8.0; DO (5.0 ± 0.2) mg/L; COD 30 mg/L; NH_4^+-N 0.63 mg/L; NO_3^--N 0.05 mg/L. Of these, the COD value belongs to the IV type of "Environmental Quality Standards for Surface Water" of China (GB 3838-2002). The nitrogen concentration was relatively small, therefore nitrification and denitrification in sediments were ignored in the following analysis. Within 12 hours after being collected, the sediment sample was sieved through 20 mesh sieve to remove gravel, inorganic particles and large plant stubs and then was transferred to the simulating reactors (Fig. 1). The sediment was stored in the acrylic core of the reactor (5 cm in diameter and 7 cm in depth) and the upper part was filled with overlying water taken from Jinchun Yuan Lake. Connected with a peristaltic pump, the water was recycled from the inlet to the outlet, thereby ensuring the oxygen concentration in the overlying water stayed relatively constant. The recirculation rate was 0.3 mL/sec with a flow rate of 0.00019 m/sec over the sediment. Moreover, cling film was placed on the top of the reactors to decrease evaporation, and the outer surface of each core was covered with black plastic to prevent growth of autotrophs on the inner wall (Laursen and Carlton, 1999). The reactors were maintained with recirculating water for 10 days prior to the experiments.

1.2 Exposure experiment and microelectrode measurement

According to "Environmental Quality Standards for Surface Water" of China (GB 3838-2002), the required nitrobenzene limit for surface water serving as drinking water sources is 17 μg/L. The concentration of nitrobenzene in contaminated surface water was detected at levels as high as 50.97 μg/L in a previous study (Yang et al., 2006). Therefore, we chose the target concentrations of nitrobenzene as 0, 50, 100 and 150 μg/L for the exposure experiment to make it practically significant.

Four simulating reactors were operated in parallel, named Reactor-0, Reactor-1, Reactor-2 and Reactor-3. Reactor-0 was run without nitrobenzene as the control. In the other three reactors, different amounts of nitrobenzene were added to yield the concentrations 50, 100 and 150 μg/L in the overlying water, respectively. Sediment samples were cultured in the simulating reactors for 10 days before exposure to nitrobenzene. Several O_2 profiles in the four reactors were measured at different times

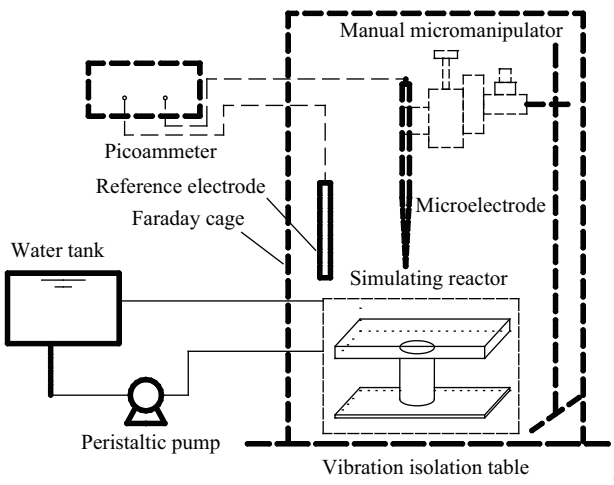

Fig. 1 Simulating reactor and experimental set-up for microelectrode measurements.

and locations in the sediment that were not treated with nitrobenzene; these measurements served as a reference. With exposure to nitrobenzene for 1, 2, 4 and 7 days, O_2 profiles were sequentially and spatially re-measured in the four reactors to evaluate the impact of nitrobenzene on the sediment. During the measurement, the DO concentration in the overlying water was kept relatively stable in the range from 3.5 to 4.5 mg/L. The system for microelectrode measurement is shown in Fig. 1 and was also described in detail in a previous study (Zhou et al., 2008).

1.3 Determination of algal composition and number

After exposure to nitrobenzene for 7 days, 0.01 g sediment samples from the upper 1–2 mm layer of the four reactors were gently collected by scraping, and accurately weighed. The samples were transferred to a flask and water was added to a volume of 25 mL to obtain a concentration of 0.4 g/L. Then 1 mL of the 0.4 g/L sediment solution was extracted and diluted to 100 mL to yield a 0.004 g/L sediment dilution. The diluted suspension was shaken well and 2.5 μL was transferred by pipette to a microscopic cell counting board (DM6000B, Leica Microsystems, Wetzlar, Germany) for observation. The change in algal composition of the sediment caused by nitrobenzene was observed and the dominant algae species was quantified by using standard counting procedures.

1.4 Calculation of sediment oxygen demand and inhibition caused by nitrobenzene

The following assumptions were made in the calculation: (1) oxygen distribution in the sediment reaches a steady state, or $\partial(C,t)/\partial t = 0$; (2) the biochemical reactions in the overlying water are negligible; (3) oxygen transport in the sediment follows Fick's first law of diffusion; (4) the traits of sediment are homogenous in the depth direction.

Based on the assumptions mentioned above, the sediment oxygen respiration rate (SORR, amount of oxygen consumption per unit time per unit volume of sediment) is:

$$\mathrm{SORR} = \Phi \times D_s \times \frac{\mathrm{d}^2 C}{\mathrm{d}z^2} \tag{1}$$

where, C (mg O_2/L) is the oxygen concentration; z (mm) is the depth; Φ is the sediment porosity, equal to 0.8 (Laursen and Carlton, 1999); D_s is the diffusion coefficient of oxygen in pure water, equal to 1.81×10^{-4} m^2/day at 25°C. Historically, oxygen demand in sediment is modelled with a zero-order reaction rate parameter (Hantush, 2007). In this situation, the relationship of oxygen concentration and depth of sediment are:

$$C = az^2 + bz + c \tag{2}$$

where, a, b and c are the fitting coefficients. The second derivative of the parabolic equation is:

$$\frac{\mathrm{d}^2 C}{\mathrm{d}z^2} = 2a \tag{3}$$

Substituting Eq. (3) into Eq. (1) yields:

$$\mathrm{SORR} = \Phi \times D_s \times 2a \tag{4}$$

The program EXCEL was used to determine constant a according to the measured oxygen profiles. If the oxygen penetration depth in the sediment is H (unit of length), the function of sediment oxygen demand is:

$$\mathrm{SOD} = \mathrm{SORR} \times H \tag{5}$$

Inhibition (I) is defined as the ratio of SOD before and after exposure to nitrobenzene.

$$I = \mathrm{SOD}/\mathrm{SOD_U} \tag{6}$$

where, U means the sample without exposure to nitrobenzene.

2 Result and discussion

2.1 SOD and oxygen penetration depth

In the measured oxygen microprofiles of the sediment (Fig. 2), most of the measured oxygen respiration occurred in the top 1.5–2.5 mm of the sediments, in accordance with the reported literature (Laursen and Carlton, 1999; Nakamura et al., 2004). As shown in Fig. 3, the thickness of the oxic layer increased in the next 7 days in the control reactor. However, in all cases of exposure to nitrobenzene, the oxygen penetration depth decreased compared with the initial values measured in the pre-treatment period, and had a tendency of decreasing on day 1 and day 2 and increasing on the following days. Interestingly, in all cases of exposure to nitrobenzene except for the 150 μg/L case, the variation tendency of oxygen penetration depth was just opposite to the SOD tendency as shown in Figs. 3 and 4, which could be explained as oxygen penetrating deeper in sediment under conditions of less oxygen demand in the oxic layer (Fischer et al., 2009). In a word, the decreases in oxygen penetration were significant. However, the thickness of the oxic sediment layer had no relationship to the nitrobenzene concentration.

As shown in Eq. (5), SOD depends on the product of oxygen respiration rate and oxygen penetration depth of the sediment, which is shown in Fig. 4. It should be noted that SOD in the control reactor on day 2 was not depicted due to a bad fitting result. The mean SOD for all reactors was 18.8 ± 1.35 mg/(m^2·hr) in the pre-treatment period. In the control reactor, SOD was maintained relatively stable due to no exposure to nitrobenzene. The addition of nitrobenzene caused a marked increase in SOD after 1 day of exposure for all three levels. However, with increasing exposure time, SOD decreased and showed even lower values on day 4 and 7 than it had been during the pre-treatment period. Overall, the effect of nitrobenzene on SOD was significant. Longer exposure time to nitrobenzene resulted in a larger percentage decrease in SOD. Further discussion on SOD change is presented in Section 2.3.

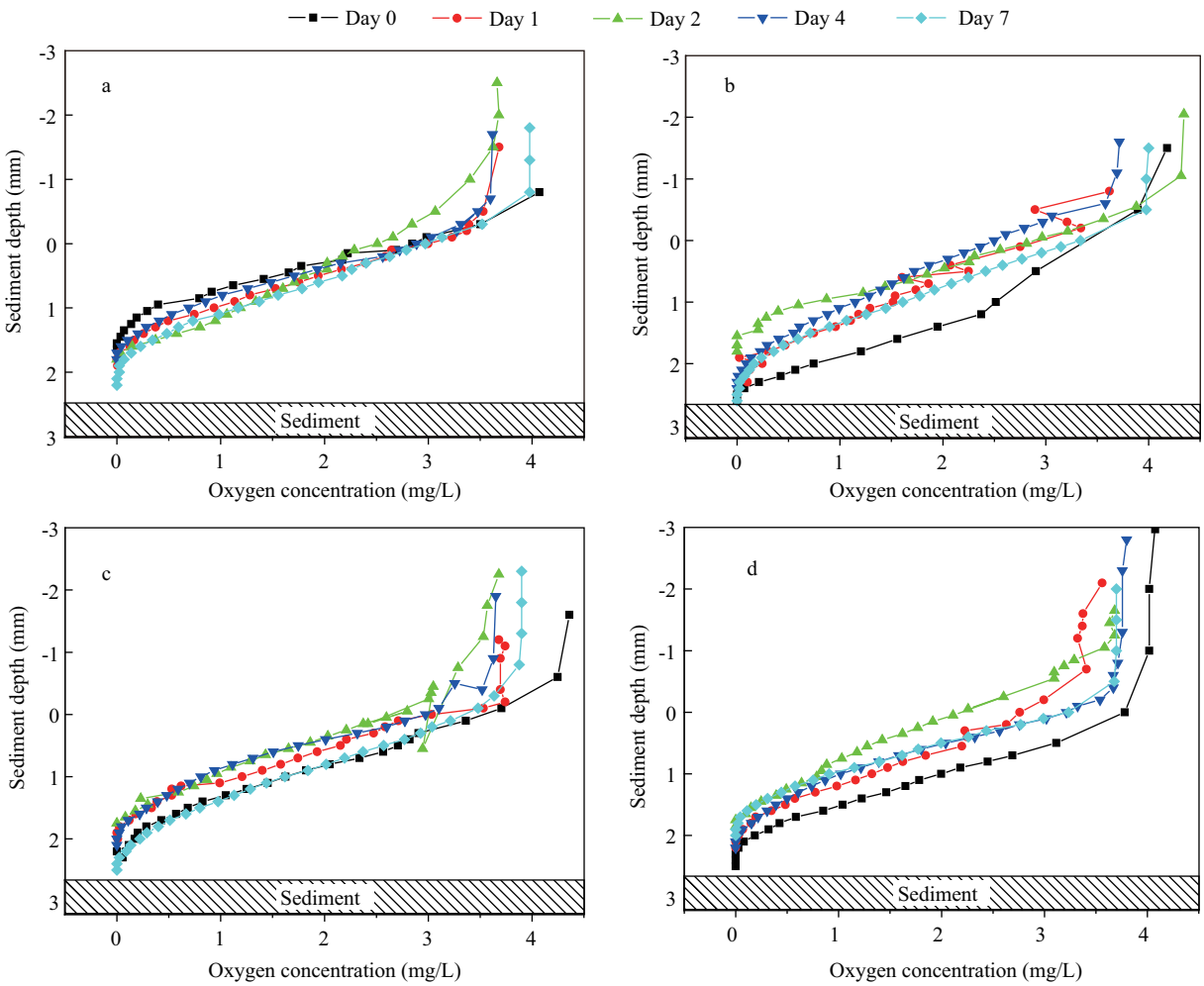

Fig. 2 Oxygen microprofiles measured in four sediment reactors exposed to 0 μg/L (a), 50 μg/L (b), 100 μg/L (c), 150 μg/L (d) nitrobenzene on day 0 (pre-treatment period), day 1, day 2, day 4 and day 7. To show the relationship between oxygen concentration and sediment depth more clearly, depth of 0 on *Y*-axis was used to represent the sediment-water interface, and *Y*-axis were plotted reversely to make the data of sediment depth positive.

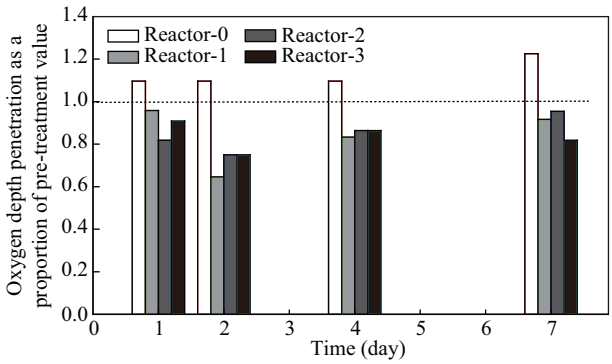

Fig. 3 Effect of nitrobenzene on depth of oxygen penetration into lake sediments. Depth of oxic zone following 1, 2, 4 and 7 days of nitrobenzene exposure is expressed as proportion of the pre-treatment depth.

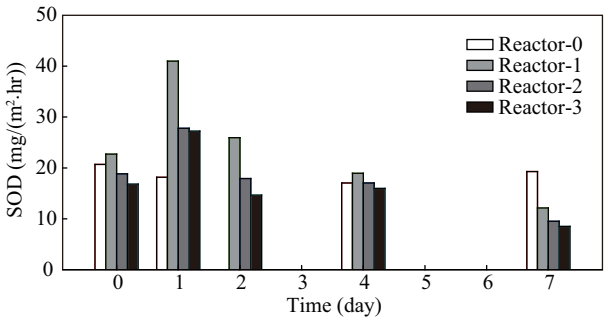

Fig. 4 Sediment oxygen demand obtained from the measured oxygen microprofiles and models described in the text.

2.2 Change in sediment algal composition and number caused by nitrobenzene

Under the microscopic observation, it was found that the sediment algal composition showed little change in the four simulating reactors, indicating that nitrobenzene had little impact on the algal composition in 7 days of exposure. In all four reactors, *Chlorella*, spherical in shape and about 2 to 10 μm in diameter, was the dominant species in the sediment. As determined by the counting procedure, the algal numbers in the four reactors with or without exposure to nitrobenzene for 7 days are shown in Fig. 5. The number of algae decreased sharply with increasing nitrobenzene concentration, which demonstrated that the growth of *Chlorella* was significantly inhibited by nitrobenzene. The result is consistent with previous studies (WHO, 2003).

2.3 Inhibition in terms of SOD caused by nitrobenzene

As shown in Fig. 6, *I* for the control reactor, i.e., SOD as a fraction of pre-treatment value, was close to 1

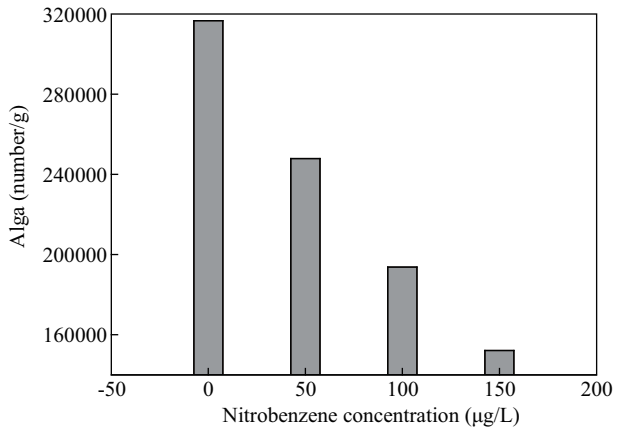

Fig. 5 Algal numbers in four reactors exposed or unexposed to nitrobenzene for 7 days.

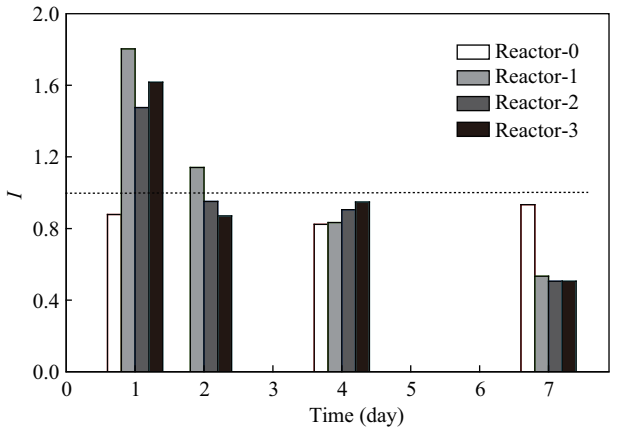

Fig. 6 Inhibition ratio (I) in terms of SOD following 1, 2, 4 and 7 days of nitrobenzene exposure.

due to the lack of exposure to nitrobenzene. The value was somewhat lower than it had been during the pretreatment period, which is in accordance with the result reported by Laursen and Carlton (1999). After 1 day of exposure to nitrobenzene at all three levels tested, the SOD increased sharply compared with that in the pretreatment period. SOD is a complicated indicator, which represents the oxygen consumption by the benthic bacteria (such as respiration and nitrification) deducting the oxygen production due to photosynthesis in the benthic algae. Therefore the SOD change on day 1 could be caused by two possible reasons: (1) the number of benthic algae could decrease after 1 day of exposure to nitrobenzene in similar proportion to the decrease after 7 days of exposure shown in Fig. 5, resulting in a decrease in oxygen production, and therefore correspondingly higher SOD; (2) the respiration activities of bacteria could increase due to use of the nitrobenzene as a substrate instead of an inhibitor, which would be particularly prominent during exposure to the lowest concentration of 50 μg/L nitrobenzene. However, the opposite tendency in SOD change appeared with the increasing of exposure time. The inhibition ratio I was approximately 0.5, which means half of the SOD was lost due to the inhibition of nitrobenzene with 7 days of exposure. Contrary to our expectations, the nitrobenzene concentration had only slight impact on the inhibition ratio, but the ratio was closely related to the exposure time.

The possible reasons explaining the variable inhibition change with the exposure time are as follows. As reported previously, nitrobenzene is very difficult for non-acclimated microorgansims to degrade (WHO, 2003); however, the degradation activity would increase significantly due to the adaptation of the microorganisms in the exposed sediment. According to the results presented by Zhu et al. (2007), the microbial degradation activity of nitrobenzene in sediment treated with 1000 ng/g dry weight increased significantly with the adaptation of the microorganisms to nitrobenzene after 40 hr exposure. Li et al. (2008) also pointed out that as much as 11 mg/L nitrobenzene in a contaminated water sample was removed to a level less than 0.2 μg/L in 8 days. These previous studies indicated that nitrobenzene degradation could potentially occur in our exposure experiment. Many pathways have been proposed for the biodegradation process of nitrobenzene in previous studies. As early as 1993, Nishino and Spain (1993) found that nitrobenzene was reduced to hydroxylaminobenzene via nitrosobenzene by *Pseudomonas pseudoalcaligenes*. In the mixed microflora in the sediment, Li et al. (2008) found that aniline and nitrosobenzene were the two main degradation intermediates. Therefore, we attributed the inhibition change with exposure time to the generation of products due to the long-term biodegradation of nitrobenzene which might change the SOD tendency in the sediment. However, whether the degradation products or intermediates of nitrobenzene are the real reason for the change in inhibition ratio with exposure time needs further investigation. Therefore, more work to reveal the degradation process for nitrobenzene in the sediment will be conducted in the future.

3 Conclusions

The effect of nitrobenzene on bacteria respiration and photosynthesis of microalgae have important implications for the overall metabolism and function of lake ecosystems. During the study, we examined the impact of nitrobenzene on the penetration of oxygen and oxygen demand in sediment through use of a microelectrode. Total oxygen demand increased by 48%–80% in sediments exposed to 50, 100, and 150 μg/L for 1 day of exposure. However, with the increase of exposure time up to 7 days, SOD sharply decreased to half of the initial value without exposure to nitrobenzene; however, the impact had only a slight relationship with the nitrobenzene exposure concentration. Through microcopy, we also found that nitrobenzene did not change the sediment composition of algae species greatly, but did affect the number of dominant algae species, which decreased sharply after 7 days of exposure and was lower when exposed to higher concentrations of nitrobenzene.

Acknowledgments

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