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Toxicity of the xenoestrogen nonylphenol and its biodegradation by the alga Cyclotella caspia

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Abstract

Alkylphenols (APs), the breakdown products of alkylphenol polyethoxylates that are widely used as surfactants, have been proven to exert estrogenic effects. With industrial development, higher concentrations of APs are discharged into aquatic environments. Nonylphenol (NP), the most noxious AP, is included in the blacklist of several countries. The toxicity of NP to the alga \textit{Cyclotella caspia} and the biodegradation of NP by \textit{C. caspia} were studied in the laboratory. The median effective concentration at 96 hr (96 hr EC$_{50}$) of NP for \textit{C. caspia} was found to be 0.18 mg/L. Five toxicity and three degradation indices were selected for toxicity and biodegradation experiments, respectively, in five or three concentrations of NP set by the 96 hr EC$_{50}$ of NP. The algal growth rate and chlorophyll $a$ contents decreased as NP concentration increased. The main manifestations of morphological deformity of the cells included volume expansion and the presence of cytoplasmic inclusions (lipid droplets). The abnormality rate of the cells increased with NP concentration and time, and was 100$\%$ at 0.22 and 0.26 mg/L of NP after 192 hr of culture. Superoxide dismutase activity initially increased and then declined at a higher NP toxicity of greater than 0.18 mg/L. After 192 hr of culture, the biodegradation rates of NP by \textit{C. caspia} with initial concentrations of 0.14, 0.18, and 0.22 mg/L were 37.7$\%$, 31.7$\%$, and 6.5$\%$, respectively. The kinetic equation of \textit{C. caspia} biodegradation on NP was correlated with algal growth rate and initial NP concentration.

Key words: alkylphenol; nonylphenol; \textit{Cyclotella caspia}; toxicity; biodegradation

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Introduction

Several organic compounds and metals that interfere with biological endocrine functions are currently identified as environmental hormones. Alkylphenols (AP), lipid-soluble compounds with the chemical structure alkylbenzene ring–OH, are a class of widely distributed organic pollutants in the environment. The toxicity and physicochemical properties of AP change significantly with the connecting position of the alkyl with the benzene ring, the alkyl branching degree, and the alkyl chain length. Alkylphenol polyethoxylate (APEO), a nonionic surfactant in synthetic detergents with a structural formula of alkylbenzene ring–O (CH$_2$CH$_2$O)$_n$H, is a precursor material of AP. The current annual output of APEOs has reached 500,000 tons, 55$\%$ of which is accounted for by industrial products, 30$\%$ for public health products, and 15$\%$ for household and personal care products. In addition, 60$\%$ of the annual APEO output is discharged into different aquatic environments (Mann and Boddy, 2000) and then decomposed gradually into APs.

APs show estrogenic effects on organisms, thereby affecting their reproductive functions. APs are important endocrine disruptors (environmental hormones) recognized as typical exogenous estrogens (Berkner et al., 2004). Moreover, APs have complex mechanisms of genetic toxicity (Liu et al., 2001), reproductive toxicity (Wang and Shen, 1999; Zhang et al., 2003) and behavioral toxicology (Bao, 2001).

Nonylphenol (NP), the most toxic and abundant AP, is listed in the Toxic Substances Control Act of the United States EPA of 1996 (Lee et al., 2001). A total of 67 substances, including NP, are recognized as “chemicals with suspected endocrine-disrupting effects” by the Japanese Ministry of the Environment (Takeda et al., 2000). Some fish exhibiting the “feminized male” phenomenon were discovered in Wuhan East Lake. This phenomenon was attributed to the presence of the pollutants phthalate esters and APs. Therefore, the biological toxicity toward and degradation of APs by organisms have become two research hotspots in recent years.

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The mean NP content in Taihu Lake was found to be 116 μg/L, whereas NP contents in shrimp meat, fish liver, fish fat, fish meat, and shellfish meat were 108, 375, 301, 21, and 1282 times higher than those in water. The concentration of NP in the Jialing River and Yangtze River ranged from 0.02 to 6.85 μg/L (Shao et al., 2002). The threshold concentration of NP for reproductive abnormality is 10 μg/L. This value is recognized by European countries and the United States as the basis for setting environmental standards (Zhou et al., 2003). However, the NP contents in some areas of China are much higher than this value.

Algæ were selected as test organisms for bioassays of chemical risks in several countries. A number of standard test methods for algae were established (Kobraei and White, 1996). The potential of algae to degrade organic pollutants should be given attention. Given their important roles in the degradation of surface water substances (Juhasz and Naidu, 2000; Lu et al., 2001), algae were used as the test organisms in the current study.

Previous studies on APs have used NP as the test compound. In the current study, the toxicity of NP toward algae and the algal degradation of NP were determined under pure culture experimental conditions. The median effective concentration at 96 hr (96 hr EC50) of NP to alga and four toxicity indices were selected, i.e., the algal specific growth rate, algal morphology variation, algal chlorophyll content, and SOD activity. The biodegradation rate and kinetic model for NP biodegradation by algae were also studied.

The results of the present study may serve as a theoretical basis for determining the resistance of algae to AP toxicity and the estrogeneric roles of APs. The degradation and bioremediation abilities of the alga Cyclotella caspia for APs or NP were also studied. Moreover, the degradation kinetics equation was tested in a preliminary fashion to determine the capability of algae to degrade organic pollutants and the underlying purification mechanisms. This study could promote the development of theory on the algal biological degradation of organic matters and establish the application of algae in the biological treatment of organic wastes.

1 Materials and methods

1.1 Determination of the median effective concentration at 96 hr of NP

Algal density was recorded every 48 hr using a counting chamber under a microscope. The growth inhibition rates at different concentrations of NP were calculated according to Eq. (1) (Sepic et al., 2003). The equation relating concentrations with inhibition rates was built using a linear regression method. The 96 hr EC50 of NP was calculated.

\[ I = \frac{\mu_c - \mu_t}{\mu_c} \times 100\% \]  

where, \( I \) (%) represents the growth inhibition rate, \( \mu_t \) (ind/(mL-day)) is the algal growth rate at time \( t \), and \( \mu_c \) (ind/(mL-day)) is the algal growth rate of the control group.

1.2 Toxicity experiments of NP on algae

NP concentration settings: The 96 hr EC50 of NP on C. caspia was the center concentration, and two concentrations were extended anisotropically to each side of this concentration using arithmetic progression forms.

1.2.1 Effects of NP on algal growth rate

The degree of NP toxicity directly reflects changes in algal growth rate. In this study, the algal growth rate was determined by algal cell counting and the corresponding calculation. The algal cell density was expressed as ind/mL.

1.2.2 Effects of NP on algal morphology

Algal cell morphology is the most intuitive index reflecting the responses of cells to external changes. Algal cell morphology changes with the effects of toxins. Changes in algal cell morphology and organelles were observed under a microscope at 400× magnification. The length (μm) and width (μm) of the cells were measured.

1.2.3 Effects of NP on algal Chl-a content

Algae obtain their energy for physiological activities, such as poisonous material degradation, through photosynthesis. The amount of chlorophyll can reflect the photosynthetic ability and physiological status of cells. Chlorophyll content (ρc, mg/L) was obtained using the ethanol extraction method according to Eq. (2):

\[ \rho_c = \frac{[A_{665} - A_{750}] - (A_{665} - A_{750}^\prime}) \times 29.6 \times \frac{V_s}{V_c} \times d }{2} \]

where, \( A_{665} \) and \( A_{750} \) is the absorbance at 665 nm/750 nm, \( A_{665} - A_{750}^\prime} \) is the absorbance at 665 nm/750 nm after acidification, \( V_s \) (mL) is the final volume of the extract solution, \( V_c \) (L) is the centrifugal volume of water, and \( d \) (cm) is the cuvette thickness.

1.2.4 Effects of NP on algal SOD activity

Plants produce a large amount of reactive oxygen species (ROS) when under external stress such as temperature, humidity, moisture, salinity, and nutrients or when contaminated. ROS cause oxidative damage by attacking the proteins, membrane lipids, and other components of cells. Superoxide dismutase (SOD) is a metal enzyme widely distributed in animals, plants, and microorganisms. SOD can catalyze the superoxide anion radical to carry out a disproportionation reaction and clear out the superoxide anions generated in biological oxidation processes (Yu et al., 2005). Superoxide anion free radicals are produced by surfactant stress on plants. Hence, SOD activity was assessed to determine the effect of NPs on algal physiology. SOD activity was determined through the photochemical reduction method using nitro blue tetrazolium (NBT),
which was established by Beauchamp and improved by Bewley (Beauchamp and Fridovich, 1971; Hao et al., 2004). For the calculation of SOD activity (A_{SOD}, U/(mg-min)), activity was determined by the SOD enzyme quantity required to inhibit the NBT reduction rate by 50% in a unit time, as expressed in Eq. (3) (Wang et al., 2000):

$$A_{SOD} = \frac{A_b - A_s}{A_s \times C_p \times T}$$

(3)

where, $A_b$ is the light absorption value in blank tubes for SOD activity determination, $A_s$ is the absorption value of the sample tube, $C_p$ (mg) is the protein content, and $T$ (min) is the reaction time.

1.3 Biodegradation experiments of algae on NP

1.3.1 Biodegradation rate and determination methods

Estimation of the organic matter biodegradation rate is important for predicting its residual concentrations in the environment. The commonly used evaluation indices for biological degradation experiments of surface waters include removal rate (i.e., degradation rate), BOD$_5$/COD ratio, and oxidation rate of organic substances (Gladyshhev et al., 1998). For the determination of degradation rate, the variations in organic matter contents were measured to describe organic matter degradation and evaluate the degradation ability of an organism toward toxic organic compounds (Eq. (4)). The degradation rate ($R, \%$) was used in this experiment as an index to measure the ability of C. caspia to degrade NP.

$$R = \frac{C_1 - C_2}{t_2 - t_1} \times 100\%$$

(4)

where, $C_1, C_2$ (mg/L) represents the concentrations of organic matter at time $t_1, t_2$ respectively.

Three concentrations were selected among the middle values of the five concentrations in the toxicity experiments, i.e., 0.14, 0.18 and 0.22 mg/L. The contribution rates of natural degradation and algal degradation were obtained by using the SPME-GC method to analysis the NP contents in a culture medium free of algae and in algal cells as well as the natural degradation of NP in control samples.

The procedures are as follows: For the pretreatment of samples, a 30-mL of algal liquid was taken from 0.14, 0.18 and 0.22 mg/L culture media every 48 hr till 192 hr and put into glass centrifuge tubes. The samples were then centrifuged at 4000 r/min for 10 min. A 6-mL supernatant in each tube was pipetted out and 10–15 mL of 0.6 mol/L HCl was added to adjust the pH to 3 to analyze NP content for physical degradation. For the control group, 6 mL liquid was subjected to the same procedures. Algae in the bottom of each tube was washed thrice using deionized water, and then centrifuged. Then 10 mL of 6 mol/L NaOH was added into the algal liquid and then mixed fully using a vortex oscillator. Then 0.6 mol/L HCl was added to the algal liquid to adjust the pH to 3, and the sample was centrifuged at 4000 r/min for 10 min. The supernatant was pipetted out to determine the NP content in algal cells and to calculate the biodegradation rate of NP by algae during the 48 to 192 hr period.

For the determination of NP content, 3 mL samples taken from each above 6 mL sample were pre-treated and extracted using the solid phase micro extraction (SPME) method, which included extraction for 60 min and derivatization for 30 min at room temperature. The extracted samples were processed by gas chromatography (GC). High-purity helium was used as the carrier gas. The inlet temperature was maintained at 280°C. The mass spectrometer had an E1 ion source, and the mass scan range of the total ion current was carried out from 50.00 to 550.00 amu. NP peak integration was conducted manually. The integration time was 11.5 to 12.4 min.

1.3.2 Kinetic model for the biodegradation rate of C. caspia to NP

Two equations can be used to describe microbial degradation. One is the power exponent equation, which is mainly used to describe degradation in relation to time. The other is the hyperbolic curve equation or Monod equation, which is mainly used to describe the microbial growth during pollutant biodegradation (Shen, 2003). Aside from their inorganic nutrition, algae can degrade organic pollutants because of their facultative chemoorganotrophic functions. Thus, the degradation mechanisms of algae are different from those of microbes. The kinetic equation of algal degradation of organic pollutants can be expressed as follows:

$$\frac{dC}{dt} = KNr$$

(5)

where, $K$ is the kinetic constant in the second-order reaction, $N$ (ind/mL) is the algal cell density, and $r$ is the algal growth rate.

Equation (6) was obtained after integration of $r = \frac{dV}{dt}$ to give Eq. (5):

$$C = -\frac{1}{2}KN^2 + C_0$$

(6)

Parameters $K$ and $C_0$ were obtained via a linear regression between the square of algal density ($N^2$) and the concentration of organic matter (C, mg/L).

The dynamic equation of C. caspia for the biological degradation ofNP was constructed as follows. The growth curve equations of C. caspia under different NP concentrations were fitted using the Origin software. $K$ and $C_0$ were obtained through a linear regression. The kinetics equation of algal degradation of organic pollutants was estimated using Eq. (6).
1.4 Instruments

The following equipment were used for the experiment: an optical microscope (Olympus CX-31, Japan), a digital camera (Nikon Coolpix 4500, Japan), an algal counting chamber (Institute of Hydrobiology, Chinese Academy of Sciences), a vacuum suction device (SHZ-D(III), YuHua Instrument Co. Ltd., Henan Province, China), a vortex oscillator (100818005, Henry Troemmer, USA), an U-1800 spectrophotometer (W2450, Shimadzu Corporation, Japan), a high-speed refrigerated centrifuge (D-78532, Hettich, Germany), a light incubator (LRH-400-G, Thai-hong medical equipment limited company, Shaoguan, China), a pipetting gun (5 mL: ZX98047 Thermo Scientific, USA; 2 mL: ZX12442 Thermo Scientific, USA; 10-100 μL: DR77261, Dragon, Finland; 100–1000 μL: 12602495, Genex Beta, Finland), and microsyringe (50, 100, and 500 μL, CH-7402 Hamilton Company, USA).

2 Results and discussion

2.1 Determination of the 96 hr EC$_{50}$ of NP

After several experiments, the following four concentrations were selected to test the 96 hr EC$_{50}$ of NP: 0.1, 0.15, 0.2, and 0.25 mg/L. The NP curve was plotted using the NP concentration as the abscissa and the 96 hr growth inhibition rate as the ordinate. Microsoft Excel was used for the linear regression. The regression equation of NP concentration with the growth inhibition rate was obtained as $y = 603.96x - 55.143$ ($R^2 = 0.9475$).

Using the above regression equation, the concentration of NP that inhibited the growth of *C. caspia* by 50% was calculated to be 0.21 mg/L. After the experiments were done in triplicate, the 96 hr EC$_{50}$ of NP to *C. caspia* was found to be $(0.18 \pm 0.02)$ mg/L (mean ± SD). Thus, 0.18 mg/L was used in the succeeding experiments.

Several scholars have determined the varying toxicity levels of APs toward different organisms, among which aquatic organisms were the most studied. The acute toxicity of NP to aquatic animals and plants was summarized by the US EPA. The toxicity of NP to aquatic life ranged from 17 to 3000 mg/L (Desbrow et al., 1996). The species that were greatly affected by different NP concentrations include rainbow trout with 180–270 mg/L, water *Daphnia* LC$_{50}$ (EC$_{50}$) with 84.8–190 mg/L, and *Cladocera* with 20.7–150 mg/L. For algae, the 96 hr EC$_{50}$ of NP on *Scenedesmus obliquus* is 1.0 mg/L (Wu et al., 2003), on *Chlorella vulgaris* is 14.86 mg/L (Jiang et al., 2006), on *Phaeocystis globosa* is 0.42 mg/L (Guan et al., 2011), on *Dunaliella salina* is 1.47 mg/L (Wang et al., 2012), on *Skeletonema costatum* and *Chaetoceros curvisetus* are 0.13 and 0.22 mg/L, respectively (Liu et al., 2012). The 96 hr EC$_{50}$ of NP for *C. caspia* was 0.18 mg/L, which illustrated that *C. caspia* was much more sensitive than most of the organisms, other than *Skeletonema costatum*, which could infer that *C. caspia* could be used as a good indicator for NP toxicity testing.

2.2 NP toxicity to *C. caspia*

Five NP concentration groups in the toxicity experiments were selected for *C. caspia* according to the measured 96 hr EC$_{50}$: L$_1$ group (0.10 mg/L), L$_2$ group (0.14 mg/L), M$_1$ group (0.18 mg/L), H$_1$ group (0.22 mg/L), and H$_2$ group (0.26 mg/L). The algal growth rate, OD, cell morphology, Chl-a content, and SOD enzyme activity were monitored every 48 hr.

2.2.1 Effects of NP on algal growth rate

Figure 1 shows that the L$_1$ and L$_2$ groups stimulated the growth of *C. caspia*. By contrast, the M, H$_1$, and H$_2$ groups inhibited the growth of *C. caspia*. The inhibitory effects of these groups increased as NP concentration increased.

In the L$_1$ and L$_2$ groups, low concentrations of NP stimulated the growth of *C. caspia*. The increase in growth rate and density under low concentrations of NP demonstrated the “poison exciting effect (hormesis)” (Shen and Zhou, 2002). This effect may be attributed to five reasons (Miaich and Richard, 1986). First, low-concentration organic substances can be used as a growth nutrient source, such as for *Anabaena*, which use glucose as its carbon source (Yu et al., 2003). Some organic compounds increase the enzyme activities in algal cells, thereby stimulating algal growth indirectly. The growth of *Chlorella vulgaris* was stimulated by dimethoate in low concentration as the source of phosphorus. Alkaline phosphatase (APA) can also increase algal growth in vivo. APA can hydrolyze phosphate ester into inorganic phosphate used by algae (Tian et al., 1997). Second, the toxicity of pollutants decreases as the concentration of organic matter decreases because of algal degradation. The degradation products of algae can be used as a nutrient source to stimulate...
algal growth. For example, Anabaena can degrade linear alkylbenzene sulfonate, and its degradation products can be used as carbon sources for algal growth (Yan et al., 1998). Organic matter in low concentrations can promote the synthesis of DNA, RNA, and protein within algal cells (Tang and Li, 2000), as well as enhance lipid peroxidation in algal cells within a certain range, thereby stimulating cell reproduction and delaying cellular senescence (Tang and Li, 2000).

In the M, H₁, and H₂ groups, NP in high concentrations inhibited algal growth, which may be attributed to the effect of NP on the cell membranes. Algal biofilms include the plasma, nuclear, thylakoid, mitochondrial, and chloroplast membranes. As an organic matter highly soluble in fat, NP can easily penetrate through the cell membrane, affect multiple targets, and then inhibit cell growth.

### 2.2.2 Effects of NP on algal morphology

The cell morphology of *C. caspia* was observed, including cell size. The cell deformity rate was calculated every 48 hr. Morphogenesis of algal cells under different NP concentrations and different culture time is shown in Fig. 2. The toxic effects on algal morphology were the following: pigment fading, volume enlargement, increased cytoplasmic inclusion content, aggregation in the two ends of the cell, and cell rupture.

In the L₁ and L₂ groups, algal cell deformities were induced, cytoplasmic inclusions accumulated (lipid or oil droplets), and the volume increased slightly (Fig. 2b). In the M group, cytoplasmic inclusions (lipid droplets) aggregated and the cells exhibited distortion and deformation (Fig. 2d). In the M and H₂ groups, the algal cytoplasmic inclusions accumulated in the ends of the cells (Fig. 2n, p), the volume enlarged, and inclusions were highlighted.

![Fig. 2](image_url) Morphology of *C. caspia* under different NP concentrations (40 × 10 magnification).
(Fig. 3).

Figure 3 shows the trends of cell deformity rate under the action of NP. The deformity rate was minimal for the control group. The malformation rates in the L1 and L2 groups were higher than that in the control group. In addition, the deformity rate decreased with time. The deformity rate in the M group reached its maximum in 48 hr, decreased in varying degrees, and then became stable after 144–192 hr of culture. The H1 and H2 groups exhibited strong toxicity on C. caspia, and the algal cell deformity rates remained constant at 100% after 48–192 hr of culture.

Under the toxic effects of NP, algal morphology was changed. Dibutyl phthalate (DBP) can induce algal cell aging, wall thickening, cytoplasmic inclusion aggregation, cell enlargement, and cell division suppression, among other effects (Kuang et al., 2003). Scenedesmus obliquus showed increased cell volume, as well as deformations in individual cell division and in the morphology of colony cells, such as herringbone-, trouser-, chain-, and astral-shaped deformations (Xiong et al., 2002). Antarctic ice algae under ultraviolet radiation exhibited a significant increase in fat granules. Ultrastructural damages under external 1 mg/L Cd2+ stress include inflated chloroplast, disorganized thylakoid, ruptured chloroplast membrane, irregularly shaped mitochondria, and disordered mitochondrial cristae. However, the detailed mechanism remains unclear.

The mechanism of NP toxicity can be explained as follows. The NP in the L1 and L2 groups may directly be used as carbon source by C. caspia. The degradation products may also be used by C. caspia as carbon sources in the biodegradation of NP. The NP in the M, H1, and H2 groups showed greater effects on the growth and physiological characteristics of C. caspia. The first barrier of NP to C. caspia is the cell wall. The cell wall of C. caspia is composed of pectin and massive siliceous matters, with a relatively stronger resistance to external stress than other algae.

NP directly acted on the cell membrane after penetrating the cell wall. The targets of NP attack may include the lipid molecules on the membranes. The lipid molecules were oxidized, and the membrane became thin. Membrane permeability increased, cytoplasmic inclusions escaped, and the cell ruptured (Tang et al., 1998). Large amounts of ROS were produced under NP stress. ROS first attack polyunsaturated fatty acids and lipid peroxidation to affect lipid cell metabolism and increase fat granules (Zhang et al., 2004). The cells showed inclusion (fat granules), aggregation and decreased chloroplasts, which may have caused disruptions in chlorophyll production and lipid metabolism (Xu et al., 2003).

2.2.3 Effects of NP on algal Chl-a content

The Chl-a content showed a similar trend as cell density. That is, Chl-a content decreased with increasing NP concentration (Fig. 4).

The Chl-a contents in the L1, L2, and control groups showed an increasing trend. However, the Chl-a contents in the M, H1, and H2 groups were lower than that in the control group.

Under the “hormesis” function of NP in low concentrations, the Chl-a levels in the L1 and L2 groups were higher than that in the control group after 48–192 hr of culture. In the H1 and H2 groups, the ROS generated by NP also attacked the chloroplast, damaged some substances involved in photosynthesis, and caused information transfer injury in the cascade message transmission during photosynthesis. As a result, the Chl-a contents in the H1 and H2 groups were much lower than that in the control group.

2.2.4 Effects of NP on algal SOD activity

Figure 5 shows that the SOD enzyme activity mainly had two kinds of states. The SOD activities in the H1 and H2
groups were higher than that in the control group in 0–96 hr of culture. By contrast, the SOD activities in the L₁, L₂, and M groups were higher than that in the blank control group after 96 to 144 hr of culture. The determination of protein value after 192 hr of culture showed that the light absorption values of multiple samples were negative; thus, the SOD enzyme activities were not obtained.

NP in low concentrations stimulated algal growth. Thus, the SOD enzyme activities in these groups in the beginning were lower than that in the control group. In the subsequent 48 hr, SOD began to continuously clear the generated oxygen free radicals, and SOD activity continuously increased. After 48 hr, SOD enzyme activity was the same as C. caspia under high concentrations of NP.

Algae exposed to high concentrations of NP showed stress responses. The quantity of free radicals produced in the mitochondria and chloroplasts was increased, and SOD enzyme activity was enhanced immediately. SOD activity decreased substantially after 96–144 hr of culture because the antioxidant defense system in vivo was unable to resist excessive free radicals and collapsed, resulting in algal cell death (Liu et al., 2001b). The protein absorption value in several samples was negative. This result may be due to the following reasons. First, the accumulation of active oxygen caused protein damage (Tang and Li, 2000; Víg and Nemcsók, 1989). Second, C. caspia diminished its capacity of protein production. Algal density decreased with cell death. Therefore, using the same volume of algal liquid, the protein absorption values were negative.

2.3 Biodegradation experiments of algae on NP

2.3.1 Biodegradation rate

Variations in physical degradation rates are shown in Fig. 6a. At 0 hr, NP was not found in the algal cells. The degradation rates were the fastest within 48–96 hr in each group. In addition, the physical degradation rates reduced with time. The physical degradation rate of NP was low. The sum of physical degradation rates in the L₂, M, and H₁ groups were approximately 26.49%, 26.54%, and 25.26% in 192 hr. This phenomenon was similar to the physical degradation of several organic substances.

Variations in biodegradation rates are shown in Fig. 6b. The biodegradation rate increased with time. The rate in the L₂ group decreased from $5.21 \times 10^{-3}$ to $0.71 \times 10^{-3}$ mg/day after 144 hr of culture. In the M group, NP was not degraded by C. caspia after 0–48 hr of culture. However, the degradation rate of NP increased from $0 \times 10^{-3}$ to $3.55 \times 10^{-3}$ mg/day in 48 hr to 192 hr of culture. This trend was similar to the corresponding growth curves of C. caspia. The rate of NP degradation showed a slowly rising trend with time. In the H₁ group, the rates ranged from $0.28 \times 10^{-3}$ to $1.29 \times 10^{-3}$ mg/day.

The biodegradation percentages of C. caspia under 0.14, 0.18, and 0.22 mg/L NP were 37.7%, 31.7%, and 6.5%, respectively, after 0 hr to 192 hr of culture (8 days). C. caspia showed a degree of biological degradation capabili-

![Fig. 5 SOD activity of C. caspia under different NP concentrations.](image)

![Fig. 6 Physical degradation (a) and biodegradation rate (b) of NP.](image)
The biodegradation rate. Severely with high malformation rate, thereby increasing the alkyl group connected to the benzene ring. Mechanism of degradation: The degradation of NP by manganese peroxidase concentration of 0.23 mmol/L. After 12 hr, NP estrogen activity could be removed completely. Therefore, peroxidase probably plays a role in NP biodegradation.

In summary, bacteria and other microorganisms show certain degradation abilities toward APs. However, only a few studies have investigated the ability of algae to degrade APs. At present, algal biological degradation pathways and mechanism research on NP are limited. Thus, the NP metabolites after biodegradation and the underlying mechanisms are not clear.

### 2.3.2 Kinetic model for NP biodegradation by *C. caspia*

The growth curve of *C. caspia* in the L2, M, and H1 groups under the effect of NP were fitted to equations using the Origin software. The kinetic equations of NP degradation by *C. caspia* on NP are shown in Table 1.

The biodegradation kinetic constants of *C. caspia* increased with increasing initial concentration of NP. However, according to the analysis on the different biological removal rates of NP by *C. caspia*, the kinetic constants should be dependent on both algal growth rate and the initial concentration of organic matters.

### 3 Conclusions

Nonylphenol (NP), the most toxic and abundant alkylphenol (AP), is one of the important endocrine disruptors which is discharged into aquatic environments. The 96 hr EC50 of NP for *C. caspia* was 0.18 mg/L, and *C. caspia* showed more sensitivity than Cladocera and Daphnia. Low concentrations of NP (< 0.14 mg/L) stimulated algal growth, whereas high concentrations of NP (> 0.18 mg/L) inhibited algal growth. The inhibition rates ranged from 24.5% to 75.8%. The toxic manifestations of NP on algal cells included cell volume enlargement, cytoplasmic inclusion aggregation, paled chlorophyll, and cell rupture. The contents of Chl-a and SOD activities showed inhibition at higher NP concentrations, which was related to membrane permeability.

*C. caspia* showed low biodegradation ability toward NP compared with its corresponding physical degradation. The biodegradation rates of *C. caspia* for 0.14 and 0.18 mg/L NP were 37.7% and 31.7%, respectively, but almost no effect was exhibited at 0.22 mg/L NP.

<table>
<thead>
<tr>
<th>Group</th>
<th>Biodegradation kinetic equation</th>
<th>N(t)</th>
<th>R²</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>( C = -0.0054N^2 + 0.1123 )</td>
<td>( N(t) = 3.64 - \frac{0.366}{1 + 0.0224t} )</td>
<td>0.9488</td>
<td>0.0108</td>
</tr>
<tr>
<td>M</td>
<td>( C = -0.0093N^2 + 0.1721 )</td>
<td>( N(t) = 1.03 - \frac{0.94}{1 + 0.0224t} )</td>
<td>0.8536</td>
<td>0.1866</td>
</tr>
<tr>
<td>H1</td>
<td>( C = -0.212N^2 + 0.2365 )</td>
<td>( N(t) = 0.275 - \frac{0.175}{1 + 0.0038t} )</td>
<td>0.8458</td>
<td>0.4240</td>
</tr>
</tbody>
</table>

C: concentration of NP; N: density of algal growth; K: kinetic constant.
equation of NP biodegradation was found to be correlated with the algal growth rate and initial NP concentration. It was recognized that algae have significant biodegradation capability for NP in natural waters.

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References


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