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Anaerobic biodegradation of hexazinone in four sediments

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ABSTRACT

Anaerobic biodegradation of hexazinone was investigated in four sediments (L1, L2, Y1 and Y2). Results showed that the L2 sediment had the highest biodegradation potential among four sediments. However, the Y1 and Y2 sediments had no capacity to biodegrade hexazinone. Sediments with rich total organic carbon, long-term contamination history by hexazinone and neutral pH may have a high biodegradation potential because the former two factors can induce the growth of microorganisms responsible for biodegradation and the third factor can offer suitable conditions for biodegradation. The addition of sulfate or nitrate as electron acceptors enhanced hexazinone degradation. As expected, the addition of electron donors (lactate, acetate or pyruvate) substantially inhibited the degradation. In natural environmental conditions, the effect of intermediate A [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H, 3H)dione] on anaerobic hexazinone degradation was negligible because of its low level.

1. Introduction

Hexazinone [3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1H, 3H)dione] is a non-selective herbicide in the triazine family, which is widely used to control a wide variety of broad leaf weeds, grasses and woody plants in forestry field nurseries, sugarcane and pineapple plantations, highway or railway grasses and industrial plant sites [1]. As a contact and residual herbicide, it acts as an inhibitor of photosynthesis. In mainland China, this herbicide is produced by Xinyi Pesticide Factory, Xinyi City, China, and marketed commercially as 5% Sentai G or 25% Sentai SL [2].

Hexazinone is easily moved into the groundwater, and it is often detected in groundwater in the areas used, which raises great concerns about its safety to human health [3]. The previous study on this herbicide mainly focused on residual analysis; intermediates [4]; dissipation [5]; adsorption–desorption [6]; leaching potential and mobility in soils under oxic conditions [7]. The reported field dissipation half-life and organic carbon distribution coefficient (K_{oc}) were 79 days and 34–74, respectively [8]. This herbicide is regarded to be little susceptible to hydrolysis [9] and photolysis [10], and thus the residual activity may be expected to last several months. Metabolites of hexazinone were first identified by Reiser et al. [11] who separated five degradation products in plant seedling. Additionally, Kin and Kimball [12] observed that 3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H, 3H)dione was a predominant metabolite of hexazinone in soils of lowbush blueberry fields in Nova Scotia, Canada. In our previous works, we investigated the behavior of hexazinone under oxic conditions, and isolated two hexazinone-degrading bacterial strains in soil contaminated by this herbicide [2]. Because this herbicide can reach deep horizons in field soils or accumulate in river sediments, where anoxic conditions often prevail. Sediments are usually recognized as both the carrier and potential source of persistent organic pollutants (POPs) in aquatic environments, and removal of POPs from the sediment is therefore of high importance. So far, many researchers have reported anaerobic degradation of organic pollutants in sediments [13,14]. However, no data are available on anaerobic degradation of hexazinone in river or lake sediments. Therefore, this research was carried out: (1) to investigate the fate of hexazinone in four sediments under anoxic conditions; (2) to clarify the relationship between anaerobic degradation of this herbicide and sediment properties; (3) to evaluate the effects of additional electron acceptors, electron donors, and the main intermediate (metabolite A) on anaerobic degradation. The main objective of this study was to obtain initial information on hexazinone degradation behavior, and to supply useful information for the bioremediation and assessment of hexazinone contamination under anoxic conditions.

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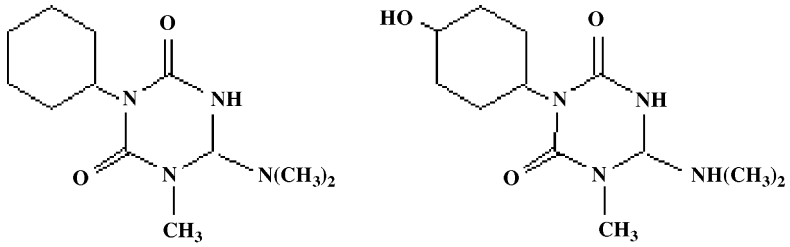

Fig. 1. The chemical structures of hexazinone and its metabolite.

2. Experimental

2.1. Chemicals

Hexazinone (purity 99.7%) was purchased from Shenyang Chemical Engineering Institute, Shenyang, China. Metabolite A (purity 99.0%) [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H, 3H)dione] was obtained gratis from DuPont de Nemours (Experimental station, Wilmington, DE, USA). The stock solutions of hexazinone and its metabolite A, whose structures are shown in Fig. 1, in acetone were prepared freshly every 2 weeks and stored in amber bottles at −20 °C until use. Analytical grade reagents and solvents locally procured were purified and redistilled before use. The water used in this experiment was purified with a Mill-Q-Plus system (Millipore, MA, USA).

2.2. Sampling and sediments

Two sediment samples (designated as L1 and L2) were collected from two sites of Luoma Lake, Xinyi City, China. Contamination of the water and sediment by hexazinone had been detected for years in Luoma Lake (Environmental Protection Bureau of Xinyi City, 2006). The other two samples (named as Y1 and Y2) were sampled from two sites of Ying River, a branch of Huai River that is considered one of the most heavily contaminated streams in Central China. As a large number of industries discharge their treated wastewaters into Huai River, contamination of the water and sediment with various organic pollutants has been noted in this river. The sediment samples (top 10 cm layer) were collected in August 2006. The physico-chemical characteristics of the four sediments and their contamination status are summarized in Table 1. The collected sediments were sieved (2 mm mesh) and stored submerged under water at room temperature until use. Before starting the anaerobic degradation experiment, the sediment sample was equilibrated under submerged conditions at 22 °C for 2 weeks [15].

Table 1
Physico-chemical characteristics of the four sediments investigated in this study

Kind of sediment	L1	L2	Y1	Y2
pH	6.8	6.7	8.8	9.2
Water content (%)	32.1	26.5	35.4	25.1
Total C (%)	3.89	5.64	0.55	0.76
Total N (%)	0.15	0.02	0.03	0.04
Cl^- (mg/kg)	43.05	22.87	13.29	25.48
SO_4^{2-} (mg/kg)	14.86	16.21	4.81	5.12
NO_3^- (mg/kg)	3.51	4.12	5.18	4.23
Soil texture	Sandy loam	Silty loam	Loamy clay	Loamy sand
Hexazinone (ng g^{-1} d.w)	0.19	0.22	ND	ND
Metabolite A (ng g^{-1} d.w.)	ND	0.08	ND	ND

ND indicates the abbreviation of "not detectable".

2.3. Anaerobic hexazinone biodegradation experiment

All experiments were conducted using 125 ml serum bottles, containing 10 g sediment, 30 ml of oxygen-free deionized water, and 5 μg g^{-1} of hexazinone, under a gentle nitrogen flow. The solvent of the stock solution was acetone, which had been proved to be no severe adverse effects on anaerobic microbial activities by our previous study (unpublished data). All of the bottles were flushed with ultra-pure nitrogen gas (99.999%) for 15 min to displace any trace of oxygen from the headspace. The bottles were capped with butyl rubber stoppers, sealed with aluminum crimps and wrapped in aluminum foil to prevent photolysis. The bottles were incubated for 4 months at 30 °C in the dark and without shaking. All experiments were conducted in an anoxic glove box (Forma Scientific, model 1025 S/N, USA). At fixed intervals (0, 10, 20, 40, 80 and 120 days), bottle contents were sampled to determine hexazinone residues.

In order to investigate microbial hexazinone degradation without oxygen, sterilized (ST) sediment samples were prepared in four sediments. 125 ml vials containing 10 g sediment were capped slightly, wrapped in aluminum foil, and autoclaved for 3 h (three separate 1 h treatments) at 121 °C. Then, 50 μg of hexazinone was fortified, followed by adding 30 ml of the sterilized deionized water. The vapor-phase of the vials was replaced by nitrogen gas to produce the anoxic conditions and incubated as described above.

2.4. Effects of electron donors, acceptors and metabolite A on anaerobic hexazinone degradation

The following factors were manipulated to investigate their effects on anaerobic degradation of hexazinone: electron acceptors (sodium nitrate, 20 mM; sodium sulfate, 20 mM); electron donors (sodium acetate, 20 mM; sodium pyruvate, 20 mM; sodium lactate, 20 mM), and metabolite A (0.1, 0.5, or 1 μg g^{-1}). The concentrations of these test chemicals were referred to the report by Lu et al. [16]. The non-sterile (NST) controls were treated without addition of any former test chemicals, and incubated without shaking at 30 °C in darkness. The ST controls were autoclaved at 121 °C for 3 h (three separate 1 h treatments).

2.5. Analytical methodology

Sediment suspension samples (2 ml) were taken at fixed intervals to determine hexazinone residue. Samples were acidified to pH 3 with 1N HCl, and then extracted with 5 ml of methanol with sonication for 10 min. The extracted suspension was centrifuged at 3000 rpm for 10 min, and the supernatant was collected. The remaining sediment sample was reextracted, the combined supernatant was filtered through a polytetrafluoroethylene (PTFE) filter membrane (30 mm diameter, 0.2 μm pore size) to remove any sediment particles, and determined for hexazinone residue by HPLC.

The residue of hexazinone was determined using an Agilent 1100 model HPLC equipped with diodearray detector. The operation was run under the following conditions: cartridge column, Nova-Pak C_{18} (150 mm × 4.6 mm i.d., 5 μm particle size); flow rate, 1 ml min^{-1}; detection wavelength, 247 nm and injection volume, 20 μl. The mobile phase consisted of methanol and water acidified to pH 5 with formic acid, and the gradient chromatograph started with 40/60 methanol–water (v/v) for 7 min then 80/20 for 22 min. An external standard method was used for calibration. Under the above conditions, the retention time of hexazinone was about 16.3 min. At spiking levels from 0.2 to 5 μg g^{-1}, the average recoveries ranged from 74.9 to 91.4%, and the relative standard deviations from 4.2 to 9.1%.

2.6. Statistical analysis

The half-life value ($t_{1/2}$) reported in this study was calculated using the pseudo-first-order model. Microsoft Excel 2003 and Origin 6.0 graphing software were used to fit the data to the model. Analysis of variance (ANOVA) and Duncan's multiple range test were used to determine significant difference at $p < 0.05$ among each treatment using statistical analysis software (SAS Version 8.0).

3. Results and discussion

3.1. Anaerobic hexazinone biodegradation in four sediments

In four anoxic sediments, the remaining percentages of hexazinone at 120 days after treatment (DAT) ranged from 10.8 to 75.7% under NST conditions (Table 2). However under ST conditions, it varied from 71.6 to 76.6%, showing no significant difference among four sediments. In L1 and L2 sediments, the remaining percentages of hexazinone decreased substantially in NST samples (22.8% for L1 and 10.8% for L2) as compared with ST controls (74.8% for L1 and 71.6% for L2), clearly suggesting that microbial action is responsible for the degradation. In contrast, no significant difference was observed between percentages remaining in Y1 and Y2 sediments under ST and NST conditions, indicating that no biodegradation occurred in the two sediments collected from Ying River, a branch of Huai River, China. Briefly, the remaining percentages of hexazinone at 120 DAT under NST conditions were in the following order: Y2 > Y1 > L1 > L2. As a result, L2 sediment showed the highest degradation rate among the four sediments under NST conditions. Throughout the entire incubation period, the loss of hexazinone in ST samples was about 30% of the initial concentration, which probably resulted from the hydrolysis and unextractable adsorption onto the sediment. In the L2 sample, the degradation of hexazione at 120 DAT was less than 30% under ST conditions, whereas the removal was almost 90% under NST conditions.

The degradation of organic chemicals under NST conditions is a result of biological and chemical transformation, while only chemical transformation occurs under ST conditions. Because this experiment was carried out in the dark, photolysis could be negligible.

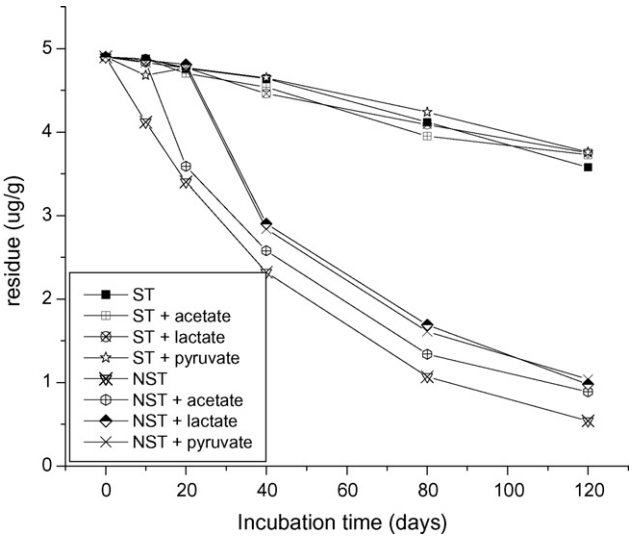

Fig. 2. Effect of electron donors on anaerobic degradation of hexazinone in L2 sediment.

As a result, chemical degradation was mainly involved in hydrolysis in this experiment. Difference between degradation in NST and ST sediments is considered to be attributable to biological transformation. The rate constants and half-lives of anaerobic hexazinone biodegradation are listed in Table 2. The highest biodegradation rate was observed in L2 sediment ($k = 0.01650\ day^{-1}$), and followed by L1 sediment ($k = 0.01066\ day^{-1}$). It is noteworthy that no biodegradation occurred in Y1 and Y2 sediments throughout the entire incubation period, which were both collected from Ying River, a branch of Huai River, China.

Bottoni et al. [7] reported a half-life of 30 days in a soil from Medena province, Italy, and Zhu and Li [9] also reported the comparable field half-life of hexazinone varied from 24 to 74 days. This long half-life of hexazinone in oxic soil may reflect its structural feature, which is recalcitrant to microbial and chemical degradation. As far as our information goes, no comprehensive research data conclusively showed the occurrence of hexazinone biodegradation in river sediments. Data from the present study provide evidence that anaerobic microbial degradation does occur in river sediments. Because L2 sediment showed the highest microbial capacity to degrade hexazinone, it was subjected to further experiments to study the effects of additional electron donors, acceptors and intermediate (metabolite A).

3.2. Properties of sediments in relation to anaerobic hexazinone biodegradation

Hexazinone biodegradation in NST L2 sediment started when the experiment began without an obvious lag period (Fig. 2), as was in NST L1 sediment (not shown in figure), but no biodegradation was found in Y1 and Y2 samples over 120-day incubation period.

Table 2
Percentage of hexazinone remaining after 120 days of incubation, and the degradation kinetic parameters in four sediments

Sample	Percentage remaining (%)		Rate constant (day^{-1})			Half-life (day)		
	ST	NST	ST	BD	NST	ST	BD	NST
L1	74.8 ± 4.6a	22.8 ± 2.4b	0.002415	0.01066	0.01216	287	65	57
L2	71.6 ± 8.9a	10.8 ± 1.6c	0.002783	0.01650	0.01925	249	42	36
Y1	73.2 ± 4.2a	72.9 ± 8.8a	0.002596	–	0.002605	267	–	266
Y2	76.6 ± 7.1a	75.7 ± 3.4a	0.002221	–	0.002224	312	–	309

All values are means ± S.D. of triplicate samples; incubation time is 120 days. Different lower cases (a, b and c) within a column denote the significant difference at $p < 0.05$. BD, ST and NST indicate the abbreviation of biodegradation, sterile and non-sterile, respectively. "–"means no degradation was detected.

Table 3
Biodegradation rate of hexazinone at 120 DAT and the main properties of four sediments

Kind of sediment	L1	L2	Y1	Y2
Biodegradation rate (%)	68.2	82.8	0	0
Total carbon (%)	3.89	5.64	0.55	0.76
pH	6.8	6.7	8.8	9.2
Hexazinone (ng g^{-1} d.w.)	0.19	0.22	ND	ND

At 120 DAT, the biodegradation percentage of hexazinone was 68.2 and 82.8% for the L1 and L2 samples, respectively (Table 3).

The high-to-low order of biodegradation rate for hexazinone was L2 > L1 > Y1 (or Y2). This difference is possibly caused by a difference in the original level of TOC and residual level of hexazinone in the sediments. For example, among the four sediments, the L2 sample had the highest content of TOC and parent compound residue, and thus had the highest biodegradation rate. On the contrary, the Y1 and Y2 samples had very poor TOC and no hexazinone were detected, thus no biodegradation of hexazinone was observed. Hirano et al. [17] investigated the biodegradation of chlordane and hexachlorobenzenes in river sediments, and found that high carbon content and contamination by the target chemicals can enrich microorganisms such as sulfate-reducing bacteria, methanogen and eubacteria which are responsible for degrading organic pollutants. Therefore, high TOC and parent contamination in L2 sediment led to the enhanced biodegradation of hexazinone. In addition, the neutral pH of L2 and L1 sediments may be another important factor in their biodegradation capacity. As reported previously, anaerobic microorganisms can be inhibited at pH values below 6 or above 9 [18]. Chang et al. [19] also reported that the optimal pH for the anaerobic biodegradation of PAH by soil culture was pH 8.0. The fact that no biodegradation occurred in Y1 and Y2 sediments over a 120-day incubation period may be due to their very poor concentration of organic carbon, absence of the parent residue and basic pH values above pH 8.0, which failed to produce suitable conditions for biodegradation.

In L1 and L2 sediments, no obvious lag period for hexazinone biodegradation was observed. The reasonable explanation was that the two sediments were collected from Luoma Lake, where is close to the hexazinone producing factory, and had been contaminated by this herbicide for a long time. The detected residues in L1 and L2 sediments were 0.19 and 0.22 ng g^{-1} d.w., respectively (Table 1). In addition, we also found the major reducing metabolite A at a residual level of 0.08 ng g^{-1} in L2 sediment. During the microbiological acclimation period, little or no biodegradation occurs, which often appears as a lag period in the course of biodegradation [19]. However, the anaerobes in the two sediments finished the acclimation period because of the long contamination time by hexazinone, and acquired the ability to biodegrade this chemical.

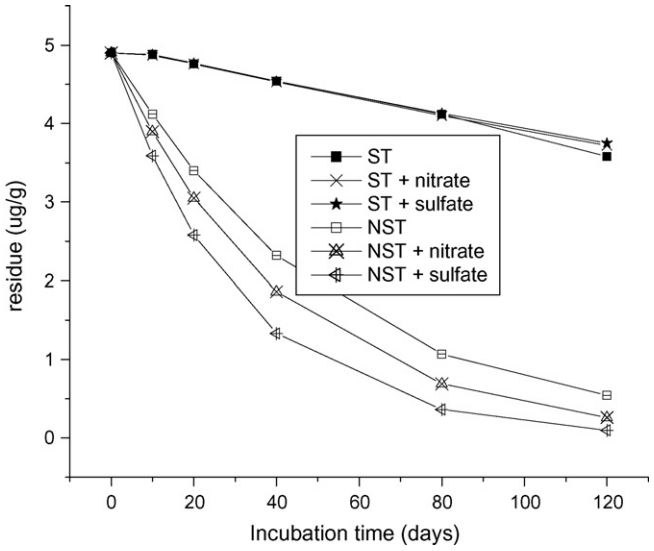

Fig. 3. Effect of electron acceptors on anaerobic degradation of hexazinone in L2 sediment.

These observations suggest that the biodegradation potential to hexazinone in river sediments is strongly related to the sediment properties. Sediments with rich carbon content, contamination by parent chemicals and neutral pH may have a high biodegradation potential for these chemicals because the former two factors can induce the growth of microorganisms responsible for biodegradation and the third factor can produce suitable conditions for biodegradation.

3.3. *Effect of terminal electron acceptor on the anaerobic degradation*

The influence of two electron acceptors (20 mM sulfate and nitrate) on hexazinone degradation was examined using the L2 sample. As listed in Table 4, the addition of electron acceptors had no significant effects on degradation of hexazinone in ST sediments, and the degradation half-lives remained at around 250 days for nitrate-added, sulfate-added treatments and sterilized control. As compared with the NST control, the anaerobic biodegradation of hexazinone was significantly enhanced in the presence of sulfate or nitrate. As shown in Fig. 3, the remaining percentage (10.8%) for the NST control was much higher than those of other treatments (1.9% for sulfate and 5.2% for nitrate). The rate constants for sulfate-added, nitrate-added and control samples were 0.01925, 0.02475, and 0.033 day^{-1} (Table 4), respectively, suggesting that the presence of electron acceptors enhanced biodegradation rate of hexazinone by 20–40% at 120 DAT. Although enhancement of anaerobic biodegradation of other organic contaminants has been reported [20], it is the first report on that of hexazinone caused

Table 4
Effects of addition of different electron acceptors on anaerobic hexazinone degradation

Experimental conditions and parameters		Half-life (days)	Rate constant (day^{-1})	Percentage remaining (%)	R^2
Sterililzed sediment	No acceptor (control)	249	0.002783	71.6a	0.8759
	Nitrate-added	252	0.002750	74.5a	0.9256
	Sulfate-added	251	0.002761	75.1a	0.8149
Non-sterilized sediment	No acceptor (control)	36	0.01925	10.8b	0.9024
	Nitrate-added	28	0.02475	5.2c	0.7954
	Sulfate-added	21	0.03300	1.9c	0.9056

All values are means of triplicate samples; incubation time is 120 days. Different lower cases (a, b and c) within a column denote the significant difference at $p < 0.05$.

Table 5
Effects of addition of different electron donors on anaerobic hexazinone degradation

Experimental conditions and parameters		Half-life (days)	Rate constant (day^{-1})	Percentage Remaining (%)	R^2
Sterile sediment	No donor (control)	249	0.002783	71.6a	0.8759
	Acetate-added	255	0.002718	74.7a	0.8452
	Lactate-added	259	0.002676	75.0a	0.8216
	Pyruvate-added	263	0.002635	75.3a	0.7328
Non-sterile sediment	No donor (control)	36	0.01925	10.8c	0.9024
	Acetate-added	42	0.01650	17.8b	0.9175
	Lactate-added	51	0.01359	19.6b	0.8146
	Pyruvate-added	49	0.01414	20.8b	0.7947

All values are means of triplicate samples; incubation time is 120 days. Different lower cases (a, b and c) within a column denote the significant difference at $p < 0.05$.

by adding terminal electron acceptors. However, it is noticeable that this result is mere an academic observation and that further experiments should be conducted as to its large-scale applicability.

3.4. Effect of additional electron donors on anaerobic degradation

In ST L2 sediment, the degradation half-lives of hexazinone remained at 250–260 days for acetate, lactate or pyruvate-added treatments and control. The addition of electron donors had no significant effect on the degradation under ST conditions, suggesting that electron donors cannot affect abiotic behavior. However, we noted that the anaerobic degradation of hexazinone was substantially inhibited in the presence of electron donors in NST L2 sediment (Fig. 2). The remaining percentage (10.8%) for no addition control was much lower than those of other treatments (17.8% for acetate addition, 19.6% for lactate, and 20.8% for pyruvate addition), as shown in Table 5. The rate constants for acetate-added, lactate-added, pyruvate-added and control samples were 0.01650, 0.01359, 0.01414 and 0.01925 day^{-1}, respectively, in NST L2 sample, indicating that the presence of electron donors decreased biodegradation rate of hexazinone by 17–41%. This result suggests that the electron donors, especially pyruvate, have significant inhibitory effect on anaerobic biodegradation. This inhibition may not be caused by toxic effect on the microbes because these organic compounds are the use carbon sources for heterotrophic anaerobes [16]. The reasonable explanation for this may be that these organic compounds compete with hexazinone for electron acceptors. Similar results have also been reported by many researchers [21]. For example, Lu et al. [16] investigated the anaerobic degradation behavior of nonyphenol polyethoxylates (NEPOs) in sludge, and found that the removal efficiency of NEPOs was greatly decreased by adding organic compounds, especially acetate. Yang et al. [22] also found that the addition of pyruvate as electron donors did not enhance biodegradation, but had an inhibitory effect after 40-day incubation.

3.5. Effect of major degradation intermediate on anaerobic hexazinone degradation

Many researches have proved that metabolite A [3-(4-hydroxycyclohexyl)-6-(dimethyl-amino)-1-methyl-1,3,5-triazine-2,4(1H, 3H)dione], is the predominant intermediate of hexazinone in soils [11,12]. Metabolite A results from hydroxylation at the 4-positon of cyclohexyl group of hexazinone. In our previous study, we also found that metabolite A was one of the two predominant metabolites [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1H, 3H, 5H)trione] and [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H, 3H)dione] in sandy loam soil [2]. Moreover, in this investigation, we also detected this metabolite at a very low level (0.08 ng g^{-1} d.w.) in L2 sediment.

Table 6
Effects of different intermediate concentrations on hexazinone degradation

Concentration of metabolite A ($\mu g\,g^{-1}$)	Half-life (days)	Rate constant (day^{-1})	R^2
0 (no addition)	36.0	0.01925	0.9024
0.1	33.6	0.02063	0.8321
0.5	49.2	0.01409	0.7859
1	66.7	0.01039	0.7916

This metabolite was fortified to the L2 sediment to investigate the effect of typical intermediate on anaerobic hexazinone degradation (Table 6). As shown in Fig. 4, the degradation percentage for the control (no addition) at the end of incubation had no significant difference from that of the treatment at spiking level of 0.1 $\mu g\,g^{-1}$. However, it was sharply decreased from 89.6 to 71.3% when the concentration of metabolite A ranged from 0.1 to 1.0 $\mu g\,g^{-1}$. This result shows that metabolite A could inhibit anaerobic biodegradation of its parent compound only when it was more than 0.1 $\mu g\,g^{-1}$. The reasonable explanation for this may be that high concentration of metabolite A has toxic effect on the anaerobic microorganisms, which subsequently leads to the decrease of the biodegradation efficiency. However, because the concentration of metabolite A in natural environments usually maintains at a very low level (at ng g^{-1} or pg g^{-1} level) that is much lower than 0.1 $\mu g\,g^{-1}$ (the first set concentration in this experiment), it is rational to say that this inhibitory effect of metabolite A on anaerobic hexazinone biodegradation is negligible in most natural environments.

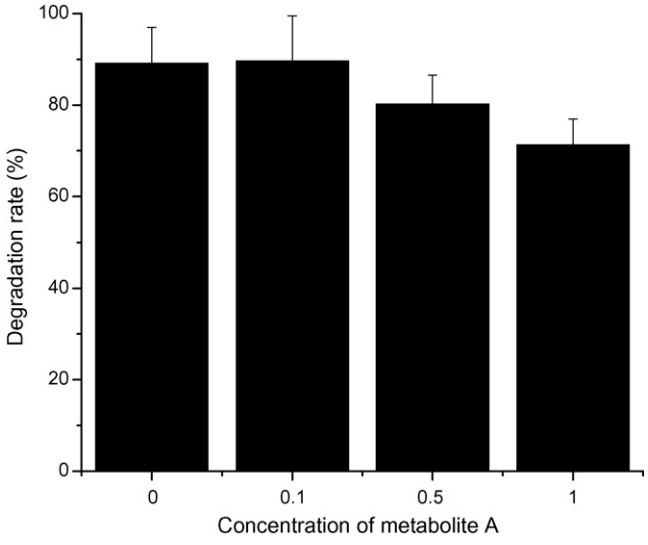

Fig. 4. Effect of metabolite A on the degradation of hexazinone.

4. Conclusions

In this study, we provided evidence that two lake sediments are capable of biodegrading hexazinone under anoxic conditions without any external acceptors. The anaerobic degradation includes chemical and biological transformations, but microbial degradation is a predominant process for hexazionone removal from sediments without any external additional acceptors. Hexazinone biodegradation potential in the sediments varied with sediment properties such as TOC, pH value and contamination by the parent chemical. The L2 sediment sample had the highest hexazinone biodegradation rate, which can be explained by its high content of TOC, neutral pH value, and long-term contamination by hexazinone. The addition of sulfate or nitrate as electron acceptors enhanced anaerobic hexazinone degradation. On the contrary, the addition of electron donors (lactate, acetate or pyruvate) substantially inhibited anaerobic degradation. In natural environmental conditions, the effect of intermediate metabolite A on anaerobic hexazinone biodegradation can be neglected because of its low level in natural environments. To our knowledge, this is the first report on the anaerobic biodegradation of hexazinone in river or lake sediments. These findings have significant environmental implications in terms of the bioremediation and assessment of hexazinone contamination in anoxic environments.

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