

Effects of Ethanol on Benzene Degradation Under Denitrifying Conditions

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Abstract As a popular fuel oxygenate, ethanol frequently co-occurs with petroleum hydrocarbons, including benzene, in groundwater that is contaminated by gasoline. Anaerobic pathways have been identified in benzene biodegradation. Limited reports focus on denitrifying degradation of benzene; however, the role of ethanol in this pathway is unknown. This study investigated the effects of ethanol on benzene degradation under denitrifying condition by using groundwater and sediment samples collected from locations with known history of benzene contamination. Results indicate that benzene can be biodegraded under denitrifying conditions. When concentrations of nitrate were in the range of 480–920 mg/L, there is a critical value in ethanol concentration: Ethanol at concentration less than the critical value enhanced the denitrifying degradation of benzene over a period of time; in contrast, ethanol at concentration higher than the critical value, which was degraded before benzene, demonstrated an inhibitory effect. And the critical value varied with nitrate concentration. It appears that the role of ethanol may be closely associated with its own and nitrate concentrations.

Two mathematical equations were established based on the data and may be used to determine if ethanol presents an enhancing or inhibitory effect on denitrification of benzene. The roles of ethanol in COD/NO₃[−]-N and the subsequent denitrification of benzene were also studied. An optimal COD/NO₃[−]-N ratio of 1.32 was obtained for this testing system, in which the highest rate of benzene degradation can be achieved under denitrifying conditions.

Keywords Benzene · Biodegradation · Denitrification · Ethanol · Groundwater

The phase-out of methyl-tert-butyl ether (MTBE) as a gasoline oxygenate has significantly increased the use of ethanol as an MTBE substitute to mitigate air pollution during combustion (Powers et al. 2001a, b). The recent initiative of renewable fuels further boosts the manufacturing and use of ethanol, which also presents an imminent concern of its release into the environment. The concurrence of ethanol with gasoline releases in the subsurface environment such as groundwater warrants a better understanding of the potential influence of ethanol on the natural attenuation of petroleum hydrocarbons. This is particularly important for hazardous petroleum constituents including benzene, toluene, ethylbenzene, and xylenes (BTEX), which are highly toxic and soluble constituents in gasoline and other petroleum hydrocarbons that frequently occur in the groundwater and expose significant threat to human and environmental health (Corseuil et al. 1992, 1998; Hunt et al. 1997).

Under aerobic conditions, the preferential degradation of ethanol by indigenous microorganisms in the groundwater tend to deplete oxygen, therefore could hinder BTEX

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biodegradation (Corseuil et al. 1998; Hunt et al. 1997). Under anaerobic conditions, the presence of ethanol at 10–110 mg/L was found inhibitory to toluene degradation, but no data was available on the influence of ethanol on benzene degradation (Ruiz-Aguilar et al. 2002). It has been reported that BTEX can be biodegraded in the anaerobic environment, such as under denitrifying conditions (Lovley 1997; Kao and Borden 1997). In a recent study, Ruiz-Aguilar and Alvarez (2005) investigated BTEX degradation in the presence of ethanol under denitrifying conditions, however, the involvement of ethanol in the system was to help deplete oxygen and create an anoxic condition, and benzene was not degraded here. The potential effect of ethanol on Benzene degradation remained unknown.

Easily degradable organic compounds, including proteins, peptides, and ethanol were shown capable of enhancing the aerobic degradation of the recalcitrant organic matters in sediments (Samantha et al. 2000; Lovanh et al. 2002; Lovanh 2004), and it was found that ethanol has the potential to inhibit toluene biodegradation under denitrifying condition (Lovanh 2004). In fact, as one of the most amenable substrates, ethanol within a certain concentration range may enhance microbial activities and growth (Powers et al. 2001a, b), and is subsequently beneficial to biodegradation of contaminants. On the other hand, elevated concentration of ethanol may be toxic to bacteria due to its detrimental effect on cell membranes and other cellular structures (Hunt et al. 1997). So effect of ethanol on benzene degradation may depend on its concentration. But it needs to be proved.

In this study, we investigated the influence of ethanol on benzene degradation under denitrifying conditions. Sediments and groundwater with known history of benzene and nitrate contaminations were collected and used to establish the microcosms. The effect of ethanol at both low and high concentrations (verses nitrate) was studied during benzene degradation under denitrifying conditions. In addition, the relationship of ethanol and $\text{COD/NO}_3^- - \text{N}$ was exploited and a mathematical equation was developed to predict the effect of ethanol on benzene degradation.

Materials and Methods

Unless otherwise indicated, all chemical reagents were of analytical grade and purchased from Xi'an Chemical Regents Company (Xi'an, China). Sediment samples were collected from Weihe riverbed approximately 500 m downstream from Xianyang Bridge in Xi'an (Shaanxi, PR China). The Weihe River (Shaanxi, PR China) has been for decades polluted by petroleum hydrocarbons and nitrate, and the elevated amount of benzene has been detected in the river since 1996. The river water infiltrates through the

sediments and soil and discharges groundwater (Si et al. 2000; Wang and Wang 2004). The riverbed sediments are organic rich, as indicated by the contents of organic carbon and organic nitrogen are 47.72 and 0.41 mg/(dried sediment)g, respectively (Wu et al. 2007), and denitrification was detected in the sediments (Wu et al. 2007). Groundwater was collected from a monitoring well within the same region, nearby Mijiaqiao in Xi'an (Shaanxi, PR China). The possible electron acceptors as nitrate, sulfate and Fe^{3+} are 48.3, 1.34 and 0.35 mg/L in the groundwater with pH 7.53, respectively.

Anaerobic techniques were used in sample collection. Samples were sealed in containers with no headspace and shipped on ice to the laboratory for prompt processing. Both sediments and groundwater samples were processed for baseline physical and chemical characterizations. Sediments were air-dried under room temperature and sieved through a 80-mesh screen before use. Sediments were soaked in hydrogen peroxide (H_2O_2) for 48 h at 40°C to remove organic matters (Xue et al. 1994).

The microcosms were set up by following methods described by Harrison et al. (2003). Each microcosm (1000-mL amber glass bottle) contained sediments (20 g) and groundwater (800 mL). Benzene, nitrate, ethanol and microbicide were amended in corresponding treatments. After vigorous mixing for 2 h under room temperature ($\sim 21^\circ\text{C}$), these bottles were purged with nitrogen gas (N_2) for 30 min to maintain anoxic conditions, and sealed with Teflon-coated stoppers immediately. The microcosms were set up in triplicates and incubated in the dark in an incubator with temperature set at $22 \pm 1^\circ\text{C}$. Samples were taken with syringes and analyzed for concentrations of benzene, NO_3^- and COD.

Microcosms consisted of three groups of treatments. Group 1 microcosms examined benzene degradation under denitrifying conditions. In group 1-I, these microcosms contained benzene (~ 16.4 mg/L) and nitrate (~ 920 mg/L). Controls included the killed that contained 500 mg/L of microicide NaN_3 (Gineste et al. 1998) and background (no nitrate, no NaN_3). In group 1-II, these microcosms had an initial nitrate concentration 45 mg/L and added nitrate 580.31 mg/L on the 36th day. Group 2 microcosms investigated the effects of ethanol on benzene degradation under denitrifying conditions. Two nitrate concentrations were amended into corresponding microcosms. Group 2-I contained ~ 490 mg/L of nitrate and ethanol concentrations ranged from 0 to 513 mg/L. Group 2-II contained 980 mg/L of nitrate and ethanol concentrations ranged from 0 to 893 mg/L. Group 3 microcosms studied the potential influence of carbon/nitrate ratios on benzene degradation under denitrifying condition with ethanol present at 0, 49.59, 98.62 and 171.60 mg/L, respectively. Four sub-batches of microcosms were established in Group 3, the sub-batches

contained ethanol concentrations at 0, 50, 99 and 172 mg/L, respectively. Nitrate concentrations in Group 3 treatments varied from 38 to 2240 mg/L, resulting in COD:NO₃⁻ from 0.4 to 7.0 mg/L.

Benzene and ethanol in solution were analyzed with high-performance liquid chromatography (HPLC, Agilent 1100 Series, Agilent, USA) equipped with an SBC-18 column (150 mm × 4.6 mm (i.d.)) at a flow rate of 1.0 mL/min and column temperature of 30°C. An ultraviolet (UV) detector was used with the wavelength set at 210 nm. The eluent contained methanol/water (40/60 v/v). Benzene and ethanol analysis were conducted immediately after the samples were collected from the microcosms. Detection limit was approximately 0.02 mg/L for benzene, and 0.15 mg/L for ethanol.

Nitrate and nitrite were monitored by using an Ionic Chromatograph (IC, 792Basic IC, Metrohm, Switzerland) equipped with an Supp 5(6.1006.530) column (250 mm × 4.0 mm). Eluent contained NaHCO₃ (1.0 mmol/L)/Na₂CO₃ (3.2 mmol/L)/methanol (3%). Total organic carbon in sediments was quantified by the Tinsley method. The carbon oxidation was made with potassium dichromate under strong acidic conditions (Tinsley 1975; Tan 1996). Chemical oxygen demand (COD) was measured by using a Hach COD analyzer (Loveland, CO, USA) by following method described by Jirka and Carter (1975).

Results and Discussions

Results from Group 1 microcosms are summarized in Fig. 1. It is evident that benzene degradation (from ~16.4 to 8.0 mg/L in 28 days) synchronized well with nitrate decline (from ~920 to 800 mg/L), indicating a potential denitrifying degradation of benzene. This is further supported by the stable benzene and nitrate concentrations in the killed controls that contained NaN₃ (500 mg/L). As also shown in Fig. 1-I, in the background controls that contained no nitrate, benzene concentration stayed unchanged during the test, while system COD decline from ~95 to ~44 mg/L, suggesting active microbial activities but none on benzene degradation when nitrate was absent.

In another microcosm containing nitrate at ~45 mg/L and benzene at ~16.4 mg/L (Fig. 1-II), the depletion of benzene corresponded with nitrate (Fig. 1-II); however, benzene degradation halted when nitrate was completely consumed during the 21 days incubation. This stable benzene concentration remained until nitrate was spiked to 580.31 mg/L on the 36th day, when benzene degradation resumed and proceeded rapidly. Nitrate depletion mirrored the benzene degradation during this process, offering a strong evidence that benzene was degraded under denitrifying conditions.

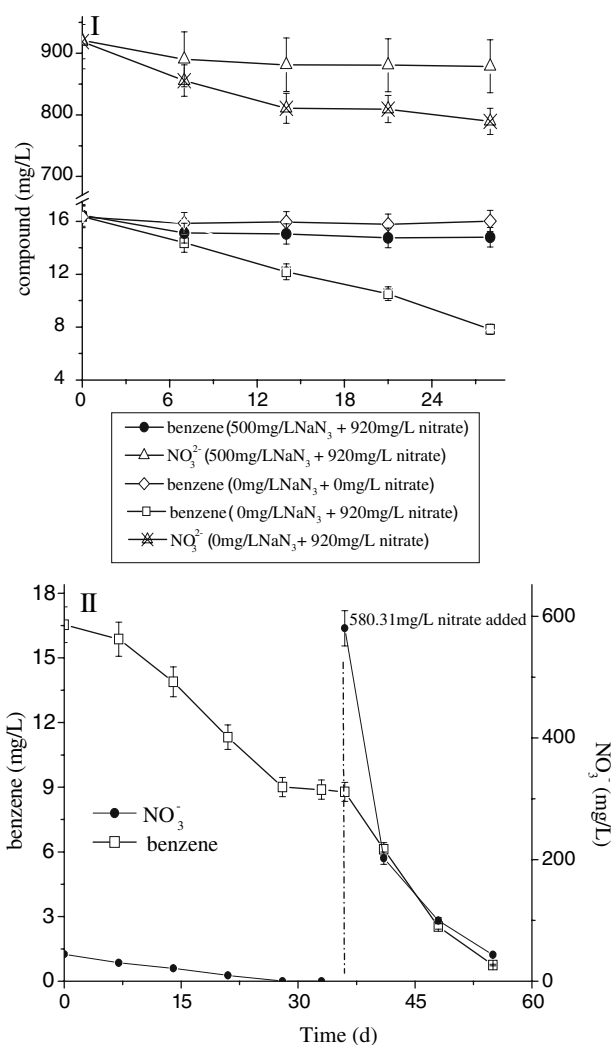
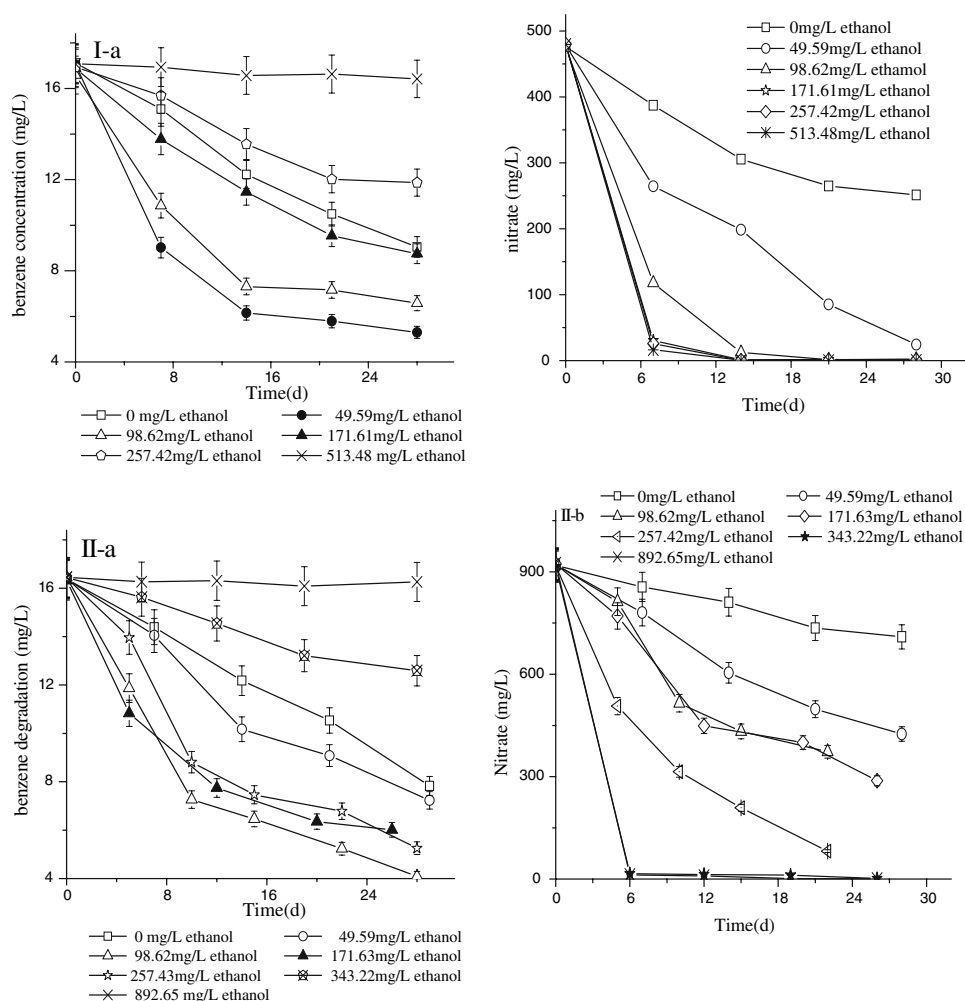


Fig. 1 Benzene profile in presence and absence of nitrate

Group 2 microcosms were established to investigate the role of ethanol in benzene degradation under denitrifying conditions, and their results are showed in Fig. 2.

Results from Group 2-I microcosms are presented in Fig. 2-I with the nitrate concentration 480 mg/L approximately in all treatments. From Fig. 2-I, it is obviously that increasing in ethanol concentration from zero to 171.6 mg/L, decrease in benzene concentration is getting faster with comparing to that in the control test, especially, when ethanol concentration was 49.59 mg/L. These strongly show that ethanol has the potential to enhance benzene degradation. Ethanol's that effect in anaerobic condition was not documented up to now, according to our knowledge. However, when ethanol concentration increases over 171.6–257.4 mg/L, on the contrary, decrease in benzene concentration got more slowly with comparing to that in the control test, even when ethanol concentration reaches 513.48 mg/L, the concentration of benzene refuses to drop

Fig. 2 Effects of ethanol on changes in benzene concentration in the tests with 480 and 920 mg/L nitrate, respectively (nitrate concentrations are 480 and 920 mg/L in the tests I and II, respectively)



during all the test process. Obviously, ethanol also has the potential to inhibit benzene degradation (Fig. 2-I).

So as to the test with about 920 mg/L nitrate (Fig. 2-II). Therefore, the effect of ethanol on benzene degradation is positive or negative that depends on its concentration: ethanol with low concentration has the potential to enhance benzene degradation, and ethanol with high concentration inhibits the degradation. This is following the finding in Lovanh (2004) study on the effect of ethanol on benzene aerobic degradation: ethanol with 1 mg/L enhances, and with 20 mg/L inhibits the degradation. Based on this finding, when ethanol concentration reaches relative high, the degradation should be heavy inhibited so that benzene is a recalcitrant in the conditions. This point is proven in this study when ethanol with 513.48 (Fig. 2-I) and 892.65 mg/L (Fig. 2-II), respectively. Corseuil (1992) and Ruiz-Aguilar et al. (2002) had also found benzene is a recalcitrant under denitrification conditions, which may be mainly due to ethanol with a too high concentration.

Comparing Fig. 2-I and Fig. 2-II, it is found that, (1) there are differences in ethanol concentrations with the

potential to enhance or inhibit benzene degradation strongly, and (2) ethanol with the same concentration has a different effect on benzene degradation, under different concentrations of nitrate. Therefore, the effect of ethanol on benzene degradation has a close relationship with nitrate concentration.

From Fig. 2, It also can be found that, when ethanol has a potential to enhance benzene degradation, the degradation takes place without a lag phase, otherwise, the degradation has a lag phase, and the lag phase is getting longer with ethanol rising in its concentration.

In the term of microbiology, microbe requires suitable concentrations of available organic carbon and nitrate and both in a certain ratio, as known well that COD/NO₃⁻-N ratio should be kept in a certain scale to keep them more actively (Wang and Bartha 1990). Otherwise, the COD/NO₃⁻-N ratio is out the range, microbial activity will be softened. To research the effect of ethanol on the COD/NO₃⁻-N ratio in regard of benzene degradation, different COD/NO₃⁻-N ratios are gotten through adjusting nitrate concentration when ethanol is set at 0, 49.59, 98.62 or

171.6 mg/L, respectively. And their results are showed in Fig. 3, the quasi first-order reaction rate constant of benzene degradation was derived by regression analysis using the relation between the natural logarithm of benzene degradation rate and reaction time. And the optimal COD/NO₃⁻-N ratios and their relative initial concentrations of ethanol and nitrate are showed in Table 1.

In regard with benzene degradation, the optimal COD/NO₃⁻-N ratio varied with ethanol concentration (Table 1). This might imply that there is a difference between the utilities of each kind of available organic carbon so that the requirements for carbon and nitrogen by microbes to keep microbe actively are changed due to the presence of ethanol.

Although the optimal COD/NO₃⁻-N ratio and benzene degradation rate constant as well varied with ethanol concentration (Table 1), there is the biggest one among the benzene degradation rate constants and the relative optimal ratio, which can be called the most suitable COD/NO₃⁻-N ratio. This means that although the presence of ethanol can change the requirements and utilities of carbon and nitrogen by microbes, the most suitable COD/NO₃⁻-N ratio is only one, due to the characters of a certain species of microbes. In this study, the most suitable COD/NO₃⁻-N

ratio is about 1.32. This also calibrates that the theory that microbes including benzene degraders have an endurance of ethanol with a concentration in a certain range (Wang and Bartha 1990; Baker and Herson 1994). When ethanol has a concentration in the certain range, there is a COD/NO₃⁻-N ratio to keep microbes so more actively that benzene was degraded in an ideal rate, and there is one concentration that is the most suitable for microbe activity over the range and there is one biggest rate of benzene degradation. If ethanol concentration outs the range, COD/NO₃⁻-N ratio is much far to the most suitable one, so benzene degradation rate is low and even to zero.

Ethanol is well known as a representative of a carbon and energy source that is likely to stimulate the growth of a variety of microbial populations, including species that can degrade benzene (Hunt et al. 1997). And it is also proven that ethanol can be biodegraded before recalcitrant organic matters including benzene. Especially, Alvarez and his colleagues recently (Corseuil 1992; Corseuil et al. 1998; Hunt et al. 1997) reported that, whenever in aerobic or anaerobic (nitrate or sulfate reduction) conditions, the presence of ethanol has a potential to inhibit the degradations of toluene and ethylbenzene, and even benzene is hardly degraded. Do all these hint that benzene will be

Fig. 3 Benzene concentration varies during the test processes under different concentrations of ethanol and COD/NO₃⁻-N ratios (initial concentrations of ethanol in I–IV are 0, 49.59, 98.62 and 171.60 mg/L, respectively)

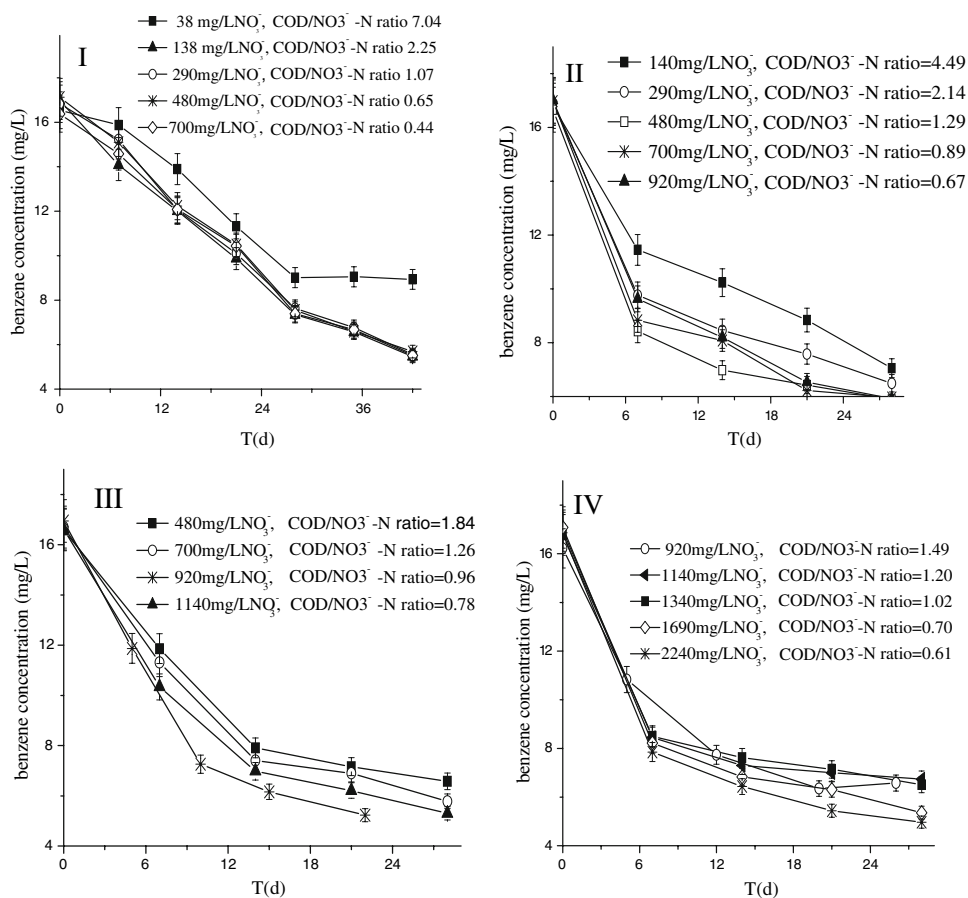
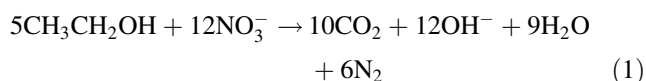


Table 1 The biggest rate constants and their relative COD/NO₃[−]–N ratios in the tests with different concentrations of ethanol

Initial concentration of ethanol (mg/L)	Initial COD value (mg/L)	Better initial concentration of NO ₃ [−] (mg/L)	Better COD/NO ₃ [−] –N ratio	Rate constant (d ^{−1})
0	70	138	2.25	0.29
49.59	173	480	1.61	0.78
98.62	275	920	1.32	0.92
171.6	427	2240	0.84	0.74

degraded after ethanol used up under denitrification conditions, rather than both are degradation in the same time, in other words, impacting of ethanol on benzene degradation is a pulse-like, discontinuous process instead of a continuous process.

Under the studied conditions, nitrite concentration is lower than the detected limiting value (0.2 mg/L). Ethanol was degraded after the following formula (Laurino and Sñeriz 1991):



Therefore, as well nitrate is consumed and the draws in both concentrations are in the ratio 5 mol/L:12 mol/L. Based on this, ethanol concentration and its variation can be determined through the variation in nitrate concentration during this study process, if ethanol is really preferentially used than the other organic matters including the objective compound benzene. In the addition, COD is mainly exerted from ethanol added (1 mg/L ethanol is equal to 2.08 mg/L COD in theory) and the other organic matters in the controlled test, so the concentration of ethanol also can be determined through COD in the study system.

When nitrate is about 920 mg/L, ethanol with 98.62 mg/L concentration has a potential to enhance benzene degradation remarkably (Fig. 3). When the test was conducted over 10 days, decreases in the concentrations of nitrate and benzene approximated to 386 and 9.36 mg/L, respectively. If it is true that ethanol is consumed before benzene, according to the chemical reaction (1), decline in ethanol concentration should be 138 mg/L over 10 days. This means that ethanol concentration should be zero at this time, due its initial concentration (98.62 mg/L). In fact, although ethanol concentration was not monitored, COD value was monitored and was 99.59 mg/L, was bigger than its initial value in the controlled test. That implies ethanol still existed in the system at this moment. Even when the test was conducted over 15 days, the COD value was about 75 mg/L and little higher than its initial value in the controlled test, so ethanol still existed in the system. In the view of nitrate consume, the same phenomenon can be found too. Also based on the chemical reaction Eq. 1, the draws in the concentrations of ethanol and nitrate are in the

ratio 5 mol/L:12 mol/L. So, nitrate with 480 mg/L is needed to use up with 171.6 mg/L ethanol. In fact, in the test with 480 mg/L nitrate (Fig. 2), when the test has been conducted for 14 days, nitrate concentration was still approximately 19 mg/L, and even when the test was lasted for 21 days, was closed to 7 mg/L. These show that ethanol existed for 21 days at least during the whole test process. Therefore, it is a period of time instead of a pulsed transience for impacting of ethanol on benzene degradation, when ethanol has a potential to enhance benzene degradation.

Although ethanol and benzene were degraded in the same time, ethanol is likely to pass through the cellular film and be used by microbes easily (Ruiz-Aguilar et al. 2002), so the speed of COD decline is bigger than benzene degradation in all the test processes. Therefore, when ethanol has an enough high concentration so that inhibit benzene degradation, ethanol was used as a main carbon source and even as the only carbon source by microbes, and benzene degradation occurs after ethanol concentration drawn down enough, so has a lag phase.

It is obvious that, ethanol with low concentration enhanced benzene degradation over a period of time, and with high concentration was degraded before benzene degradation occurred.

Ethanol has a potential to affect benzene degradation, and the characteristics of effect depends on its concentration under the study conditions. From above, benzene degradation relies on the microbes, nitrate (electron acceptors) and available organic carbon. In fact, the possibility and rate of the degradation result in the interactions of these three factors, suitable microbes are musts for benzene degradation, and nitrate, and available organic carbon is essential to the suitable microbe activity. So, if a factor affects one among the three factors, it must affect the degradation. Ethanol with a low concentration stimulates microbe activity, on the contrary, with a high concentration will inhibit microbe activity for its toxicity to microbes. In addition, whenever the ethanol concentration is low or high, ethanol added will vary the concentration of available organic carbon and the ratio of available organic carbon and nitrate in a system. In the view of microbiology, benzene degrader requires a suitable ratio of available organic carbon and nitrate, so the presence of ethanol will

affect benzene degradation and the effect is to enhance or inhibit the degradation depends on its concentration. There is a question which is how to determine the concentration of ethanol is low or high.

According to the chemical reaction (1), the consumptions of nitrate and ethanol should be in the ratio 1:2.81, and declines of both concentrations also follow 1:2.81 under the study conditions. If x and y stand for ethanol and nitrate concentrations, respectively, the following formula are obtained:

$$y = 2.81x \quad (2)$$

Therefore, approximately 480 or 920 mg/L nitrate is used up, and declines in ethanol concentration are 171 and 328 mg/L, respectively. This means that, if nitrate concentration approximate to 480 or 920 mg/L, so ethanol with the concentration bigger than 171 and 328 mg/L, respectively will inhibit benzene degradation, because ethanol is preferentially biodegraded by microbes. If nitrate concentration also is 480 or 920 mg/L, but ethanol with the concentration lower than 171 and 328 mg/L will enhance benzene degradation, respectively. That is proven by this study results (Fig. 2). Therefore, under the study-liked conditions, ethanol concentration is low or high that can be distinguished through Eq. 2.

In terms of environmental microbiology, in order to keep benzene degrader active mostly, there are suitable concentrations of nitrate and available organic carbon and a suitable ratio of the both concentrations (Maier et al. 2000). Due to the effects of ethanol on microbes, nitrate and available organic carbon, when nitrate or ethanol concentration is given, there is a concentration of ethanol or nitrate so that the concentrations of nitrate and available organic carbon and their ratio are closed to the suitable ones, and benzene is degraded quickest. Therefore, in regard of benzene degradation, the initial concentrations of nitrate and ethanol have a relationship. The optimal concentrations of nitrate (y) and ethanol (x) (Table 1) were analyzed by linear regression technique, it was found that the coefficient of correlation is more than 0.97, and the following linear regression equation is obtained:

$$y = 12.26x - 35.42 \quad (3)$$

The degree of confidence (α) was set as 0.01, t -test was done to Eq. 3, and it was found that there is a perfect positive correlation between y and x . Therefore, Eq. 3 can be used to predict the suitable concentration does ethanol has while benzene is degraded in the biggest velocity in the environment with a certain concentration of nitrate.

Base on Eqs. 2 and 3, a diagram can be drawn to show the integrated effect of ethanol and nitrate on benzene degradation under the studied conditions (Fig. 4). The diagram is divided by two lines into three zones. Ethanol

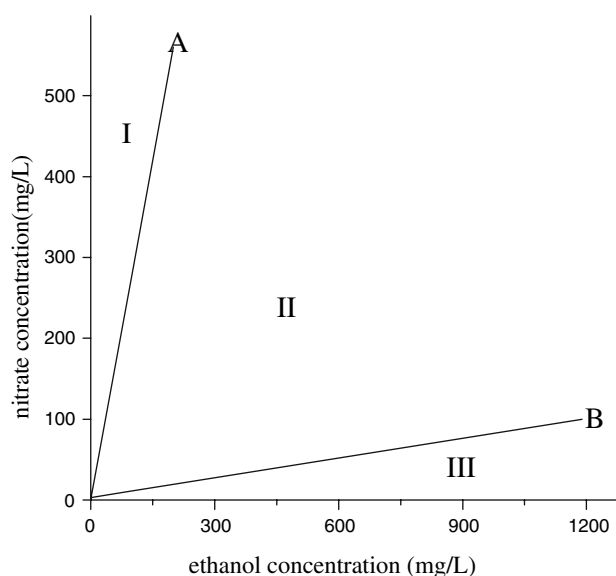


Fig. 4 Zones of integrated effects of ethanol and nitrate on benzene degradation under the studied conditions (zones I and III where ethanol enhances or inhibits benzene degradation, respectively, and zone II where impact of ethanol translates from enhance to inhibit)

with a concentration from the first zone (I) or the third zone (III) has a potential to enhance or forbid benzene degradation respectively, and Ethanol with a concentration from the second zone (II) has a potential from enhancement to inhibition. Therefore, when ethanol with a concentration has a potential to inhibit benzene degradation, increasing the concentration of nitrate can soft the inhibition and even change it to enhancement.

Such as ethanol with 257.4 mg/L, inhibits benzene degradation in the environment with 480 mg/L nitrate, and enhances benzene degradation in the environment with ~920 mg/L nitrate.

Figure 4 is useful to judge the effect of ethanol on benzene degradation in the study-like conditions. Unfortunately, it is not successfully used in other lab and field tests. This shows that the effect of ethanol on benzene degradation has relationships with many other factors, such as composites of available organic carbon and their concentrations (Ruiz-Aguilar and Alvarez 2005). Such studies should be conducted in future. In addition, the critical concentration of ethanol for enhancement and inhibition of ethanol's effect is not found.

Conclusions

Benzene can be degraded linked to nitrate reduction under the study conditions. The presence of ethanol affects the degradation, and the effect depends on its concentration: ethanol with lower concentration has the potential to

enhance benzene degradation, otherwise has the potential to inhibit benzene degradation. In the environment with 440 mg/L nitrate, the concentration of ethanol was lower than 171.60 mg/L, enhanced benzene degradation, and the concentration reached at 257.41 mg/L, inhibited the degradation, and even the concentration closed to 513.48 mg/L, benzene was hardly degraded.

In regard of benzene degradation, although there is an optimal COD/NO₃[−]-N ratio in the environment with a certain concentration of ethanol, one of the ratios is the best. Under the study conditions, the best COD/NO₃[−]-N ratio was about 1.32.

When ethanol has a lower concentration comparing to nitrate concentration in the studied concentration, ethanol is degraded coupled to benzene degradation, and has an impact on benzene degradation over a period of time.

In regarding of benzene degradation linked to nitrate reduction, comparing with the initial concentration of nitrate in the system, ethanol concentration is relative low or high, which can be judged from Eqs. 2 and 3, and what concentration also can be determined when ethanol will enhance benzene degradation mostly or badly.

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