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Benzene degradation coupled with chlorate reduction in a soil column study

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Abstract

Perchlorate and chlorate are electron acceptors that during reduction result in the formation of molecular oxygen. The produced oxygen can be used for activation of anaerobic persistent pollutants, like benzene. In this study chlorate was tested as potential electron acceptor to stimulate benzene degradation in anoxic polluted soil column. A chlorate amended benzene polluted soil column was operated over a period of 500 days. Benzene was immediately degraded in the column after start up, and benzene removal recovered completely after omission of chlorate or a too high influent chlorate concentration (22 mM). Mass balance calculations showed that per mole of benzene five mole of chlorate were reduced. At the end of the experiment higher loading rates were applied to measure the maximal benzene degradation rate in this system; a breakthrough of benzene was not observed. The average benzene degradation rate over this period was 31 μmol l^{-1} h^{-1} with a maximal of 78 μmol l^{-1} h^{-1}. The high degradation rate and the necessity of chlorate indicate that oxygen produced during chlorate reduction indeed is used for the activation of benzene. This is the first column study where benzene biodegradation at a high rate coupled with anaerobic chlorate reduction is observed.

Abbreviations: chlorate reducing micro-organisms – CRM; Van Velde Buren – VVB

Introduction

Anaerobic bioremediation is most attractive whenever anaerobic conditions prevail in a polluted soil site. Thus far, anaerobic bioremediation techniques for soils polluted with mobile aromatic hydrocarbons are not often applied. The bottleneck in the application of anaerobic techniques is the supposed poor anaerobic biodegradability of benzene. However, anaerobic biodegradation of benzene under various redox conditions has been described, but only in a few studies the micro-organisms involved are known (Phelps et al. 1998; Rooney-Varga et al. 1999; Ulrich & Edwards 2003). Moreover, only recently the first anaerobic benzene-degrading micro-organism was described. *Dechloromonas* strain RCB is able to degrade benzene coupled with nitrate and perchlorate reduction (Coates et al. 2001). However, the optimal physiological conditions for anaerobic benzene degrading micro-organisms and the precise biodegradation pathway are still unknown. Such knowledge is essential to apply anaerobic bioremediation techniques for soils polluted with aromatic hydrocarbons.

Anaerobic benzene degradation is not often observed because benzene is chemically very stable (Aihara 1992). Benzene degradation requires an initial destabilisation of the molecule. Aerobically, benzene is either converted to phenol or catechol

by means of oxygenases (Yerushalmi et al. 2001). Under anaerobic conditions oxygen is not available. A postulated anaerobic benzene biodegradation pathway is the hydroxylation of benzene to phenol by the incorporation of a hydroxyl group derived from water (Grbic-Galic & Vogel 1987). Other possibilities for the initial step in the anaerobic biodegradation pathway of benzene are fumarate addition, benzene reduction, carboxylation to benzoate or methylation to toluene (Coates et al. 2002).

Chlorate reducing micro-organisms (CRM) reduce chlorate to chlorite, and chlorite is dismutated into oxygen and chloride (Logan 1998; Rikken et al. 1996; Van Ginkel et al. 1996). (Per)chlorate reducing micro-organisms have been studied in the recent years (Logan 1998; Waller et al. 2004; Wolterink et al. 2002; Xu et al. 2003). Due to oxygen formation during chlorate reduction, chlorate is an attractive alternative source of oxygen for the anaerobic biodegradation of persistent aromatic compounds, like benzene. Amendment of CRM and chlorite to ^{14}C-benzene in anoxic soil systems showed production and complete recovery of $^{14}CO_2$ (Coates et al. 1999). One column study showed that bioaugmentation of enriched CRM had a positive effect on anaerobic toluene degradation. Our study evaluates the effect of addition of chlorate on benzene removal in an anoxic polluted soil column. The indigenous CRM in the polluted soil were used to produce oxygen from chlorate, and this oxygen is required for the initial biodegradation of benzene.

Materials and methods

Soil column and medium

Small glass column (60 ml, 2.2 cm diameter, height 15.8 cm) was filled with polluted soil (70 g) from Van Velde Buren (VVB), kindly provided by the sub-department of Environmental Technology from the Wageningen University. This soil was previously characterised (Cuypers et al. 2001). The liquid volume in the column was 22.5 ml. The column was operated in an upflow mode. The artificial chlorate amended (10 mM $NaClO_3$) phosphate buffered (pH 7.0) groundwater consisted of: (in mg l^{-1}) KH_2PO_4 (667), $Na_2HPO_4{\cdot}2H_2O$ (963), $NaSO_4$ (60), $CaCl_2{\cdot}2H_2O$ (110), $MgCl_2{\cdot}6H_2O$ (102), NH_4Cl (27) and $NaClO_3$ (1064) and trace metals ((in μg l^{-1}) $FeCl_2{\cdot}4H_2O$ (20), $MnCl_2{\cdot}4H_2O$ (10), $CoCl_2{\cdot}6H_2O$ (19), $ZnCl_2$ (7), $CuCl_2$ (0.2), $AlCl_3{\cdot}6H_2O$ (1), H_3BO_3 (0.6), Na_2MoO_4 (3.6), EDTA (50), $NiCl_2{\cdot}6H_2O$ (2.4), $NaSeO_3$ (0.15) and $NaWO_4{\cdot}2H_2O$ (0.45)) and vitamins ((in μg l^{-1}) biotin (5), *p*-aminobenzoate (25 sodium salt), pantothenate (5 sodium salt), folic acid (2 dihydrate), lipoic acid (5), pyridoxin (10), nicotinamide (55), thiamine HCl (10), riboflavin (5) and cyanocobalamine (2)). The influent medium was nitrogen flushed. Nitrogen was led through a titanium citrate solution to remove traces of oxygen and maintain anoxic conditions, and pumped *via* a 502S peristaltic pump (Watson Marlow, Falmouth, England) into the soil column. The applied hydraulic retention time (HRT) of the system was 0.8–1.5 day. Benzene was added *via* a 25-ml gastight syringe (Hamilton, Reno, Nev., USA) containing benzene stock solution (3.75–7.5 mM) and a syringe pump (Harvard Apparatus, Southnatick, Mass, USA). All tubing in the column system through which benzene was pumped was from benzene inert material Viton (Rubber B.V, Hilversum, The Netherlands).

Analytical procedures

Benzene influent and effluent concentrations were determined in liquid samples. One millilitre liquid sample was transferred to a 10 ml sample vial (Alltech, Breda, The Netherlands) and sealed with a special and combined magnetic cap with a silicon/teflon liner (Alltech, Breda, The Netherlands). Headspace samples were taken from the vial with a CP9025 headspace sampler (Chrompack, Middelburg, The Netherlands) and were measured on a CP9000 gaschromatograph (Chrompack, Middelburg, The Netherlands) containing a CP porabond Q column (25 m * 0.53 mm; Chrompack, Middelburg, The Netherlands). Carrier gas was nitrogen (7 ml per min) and compounds were detected with a flame ionisation detector (FID). The temperature of the column, injector and detector were 80, 250 and 300 °C, respectively. A temperature gradient program was used to measure benzene (1 min at 80 °C; temperature rise with 20 °C per min for 5 min; 180 °C for 1 min). Solutions with known amounts of benzene were frequently

measured for calibration purposes (benzene detection limit 5 μM).

Benzene in batches were measured *via* headspace analyses using a gaschromatograph (Chrompack 436, Chrompack, Middelburg, The Netherlands) with a capillary column (SIL 5CB, 25 m $\times$ 0.32 mm, DF 1.2, 100 kPa, split flow of 28 ml min^{-1}, Chrompack, Middelburg, The Netherlands) and a FID detector. The column, detector and injector temperatures were 50, 300 and 250 °C, respectively.

Chlorate, chloride and nitrate were determined by suppressor mediated ion chromatography (Dionex, Breda, The Netherlands) and conductivity detection. Eluent consisted of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ at a flow rate of 1 ml min^{-1}. The chromatograph was equipped with an IonPac® AS9-SC column (Dionex, Breda, The Netherlands). Mannitol (10 mM) was added to the samples for stabilisation and sodium fluoride (1 mM) was added as internal standard (both final concentrations in the sample; detection limit for all anions 10 μM).

Batches with column material

At day 500, approximately 1.0 g (wet weight) column material was taken out of the column and transferred into 120 ml batches containing 40 ml of the described column medium under N_2 atmosphere. The bottles were closed with Viton stoppers (Maag Technik, Dübendorf, Switzerland) and aluminium crimp caps. The batches were incu-

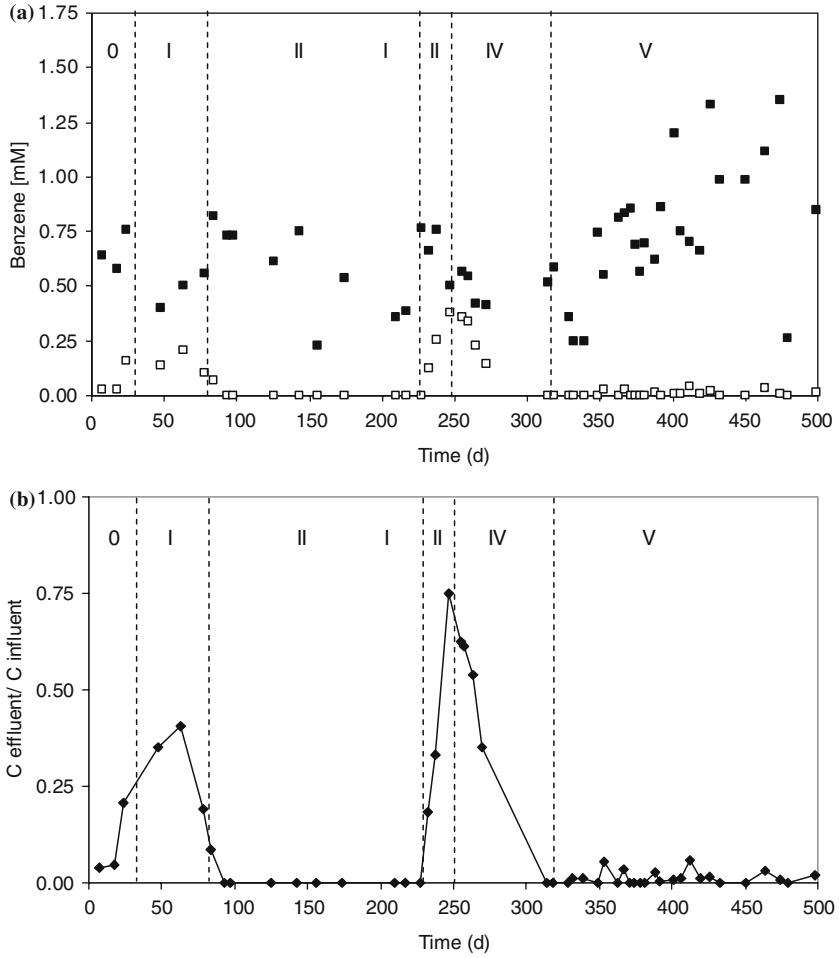

Figure 1. a: Benzene influent (■) and effluent (□) concentrations of the column. b: Relative benzene concentration in the soil column (dashed lines indicate the different period of operation see Table 1).

bated at 20 °C. Heat sterilized controls, and controls without benzene and chlorate were also made. Furthermore, related electron acceptors nitrate (10 mM) and perchlorate (10 mM) were examined to see if they were used as electron acceptor. The experiments were performed in triplicate and controls were conducted in duplicate. Benzene was measured *via* batch headspace measurement and the electron acceptors were analysed by suppressor mediated ion chromatography (Dionex).

Results and discussion

The actual and relative benzene influent and effluent concentration of the column in time are shown in Figure 1. An immediate removal of benzene during the start-up period was observed. The initial benzene removal in the column system could be due to adsorption of benzene to organic matter present in the soil. However, no chlorate reduction or benzene removal was observed in other chlorate amended soil columns with different inocula (results not shown), indicating that benzene adsorption was not likely to occur in these soil columns. In period I, at day 26 benzene was also measured in the effluent, which could indicate a breakthrough of benzene in the system. We discovered that the chlorate influent concentrations were too high (average over the period 22 mM instead of 10 mM) and by restoring the original chlorate concentration complete benzene degradation was observed again in period II. Chlorate showed to have an acute toxic effect on brown alga and was not toxic for bacteria and fungi (Van Wijk et al. 1998). No information is available on the inhibition of chlorate reduction by chlorate itself.

Afterwards, in period III, chlorate was omitted from the influent medium causing an incomplete removal of benzene. This effect clearly showed the necessity of chlorate for the removal of benzene. Benzene degradation coupled with perchlorate reduction was described for an isolated organism (Coates et al. 2001). Furthermore, bioaugmentation with CRM and addition of chlorite had a positive effect on the benzene removal (Coates et al. 1999). However, the application of chlorate reduction by indigenous CRM to stimulate benzene degradation has never been described. In period IV chlorate was re-added and recovery of benzene removal was observed again.

Finally, in period V the maximal degradation rate of this system was determined by decreasing the HRT and increasing the influent benzene concentration. In this period removal of benzene remained high. The average benzene degradation rate over this period was 31 μmol l^{-1} h^{-1} with a maximal benzene degradation rate of 78 μmol l^{-1} h^{-1}. During this period, benzene was completely removed and benzene was not observed in the effluent. This indicates that the maximal degradation rate of the soil column was not yet reached. Table 1 summarises different operational periods that were applied and gives average benzene and chlorate influent concentrations and their removal percentages.

The actual and relative chlorate and chloride influent and effluent concentrations are shown in Figure 2. This figure shows the reduction of chlorate and the expected production of chloride. In period III chlorate was omitted from the influent and this had an effect on the chloride values that were lower and around the expected value of one.

Table 1. Different operational periods with average benzene and chlorate influent concentration (mM) and removal percentage

Period	Days	Benzene concentration	Benzene removal (%)	Chlorate concentration	Chlorate removal (%)	Description of the period
0	0–26	0.66 ± 0.08	90 ± 9	8.0 ± 0.4	38 ± 5	start up
I	27–84	0.57 ± 0.18	68 ± 11	21.8 ± 3.6	15 ± 11	high chlorate
II	85–227	0.59 ± 0.20	99 ± 3	11.5 ± 1.4	28 ± 15	recovery
III	228–247	0.64 ± 0.13	53 ± 29	0		no chlorate
IV	248–314	0.49 ± 0.07	57 ± 26	8.5 ± 0.9	18 ± 13	recovery
V	315–500	0.75 ± 0.30	99 ± 2	9.6 ± 1.2	27 ± 13	high load

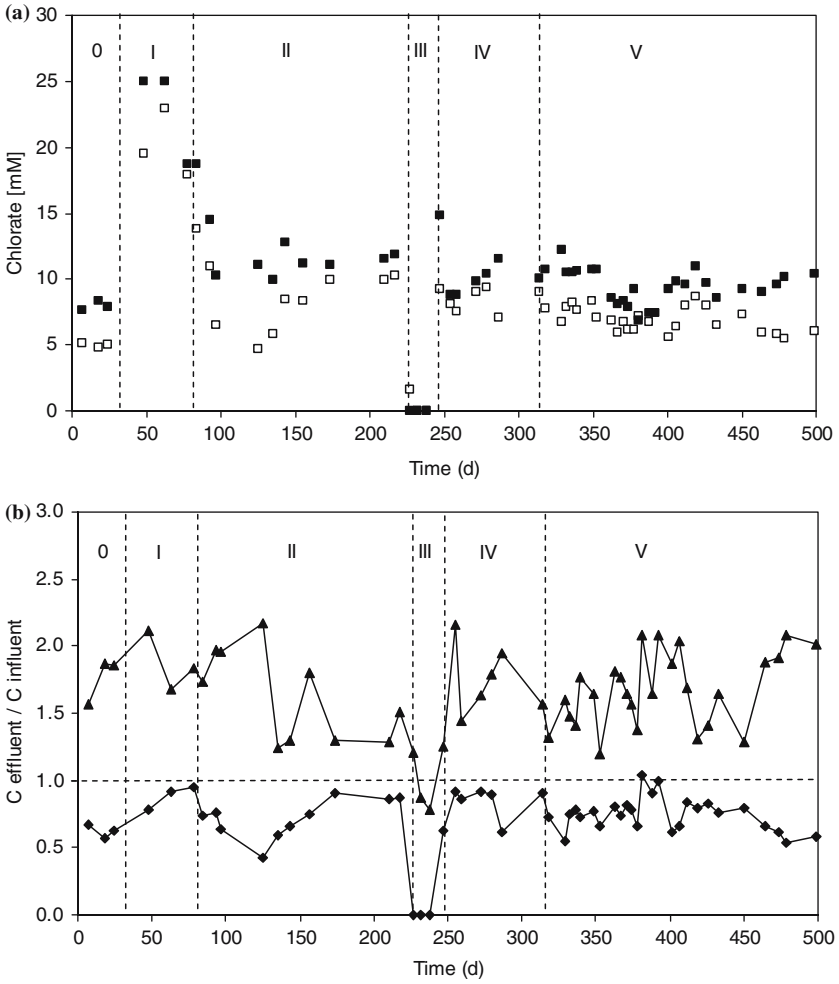

Figure 2. a: Chlorate influent (■) and effluent (□) concentrations of the column. b: Relative chlorate (♦) and chloride (▲) concentration in the soil column (vertical dashed lines indicate the different period of operation see Table 1; horizontal dashed line indicates the value one if no chlorate reduction occurs).

The stoichiometric amount of chlorate required per mole of benzene degraded is five according to the equation (1):

$$C_6H_6 + 5ClO_3^- + 3H_2O \rightarrow 6HCO_3^- + 5Cl^- + 6H^+ \quad (1)$$

The amount of benzene degraded, chlorate reduced and chloride produced were used to calculate the mass balance of the benzene degradation (Figure 3). The average value over all periods was 5.0 with a standard deviation of 3.7. The high standard deviation is due to heterogeneity of the column system leading to fluctuating influent and effluent concentrations of benzene and chlorate. The ratio of the amount of chloride produced and the amount of chlorate reduced is 0.8 with a standard deviation of 0.6 (results not shown).

At the end of the experiment sediment from the column was transferred into batches and benzene degradation was tested. The results of this experiment (Figure 4) showed the removal of benzene in the active batches. If chlorate was omitted benzene degradation did not occur. These results showed again that benzene removal was chlorate dependent as observed in period III for the column experiment. Other related electron acceptors like

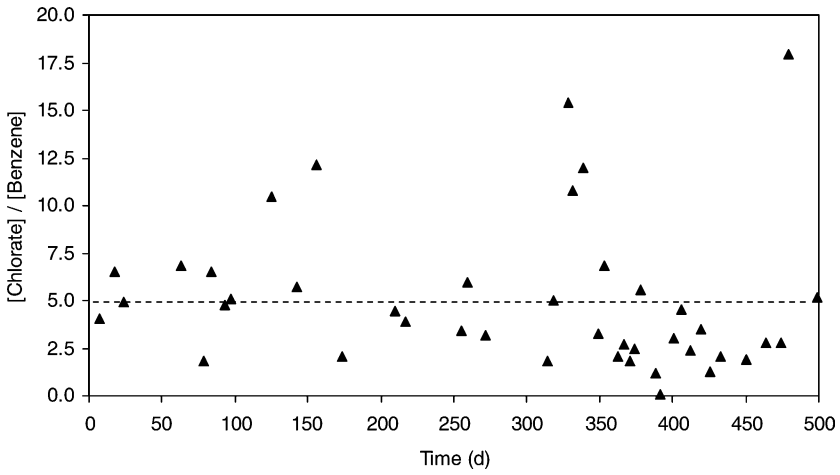

Figure 3. Molar amount of chlorate reduced divided by molar amount of benzene degraded (dashed line indicates the stoichiometric and average value of 5.0).

perchlorate and nitrate were also examined. However, no benzene degradation occurred with these electron acceptors.

The organic matter and other pollutants present in VVB soil (Cuypers et al. 2001) could serve as electron donor for chlorate reduction resulting in the formation of oxygen. However, no chlorate reduction was observed when benzene was not added (results not shown). Therefore, benzene or an intermediate of the benzene degradation served as electron donor for the chlorate reduction, and the produced oxygen can be used for the initial oxidation of benzene. Logan & Wu (2002) speculated that increased toluene degradation under chlorate reducing conditions was due to the leakage of oxygen by the CRM. The transferred oxygen could than be used by toluene oxidising organisms located in close vicinity of the CRM. However, it is also possible that one single organism is responsible for chlorate reduction and benzene degradation just as the isolated *Dechloromonas* strain RCB (Coates et al. 2002). Further research is required to investigate the mechanism of benzene degradation coupled with chlorate reduction.

The application of chlorate as alternative source of oxygen for the removal of anaerobic persistent compounds like benzene, is demonstrated in this

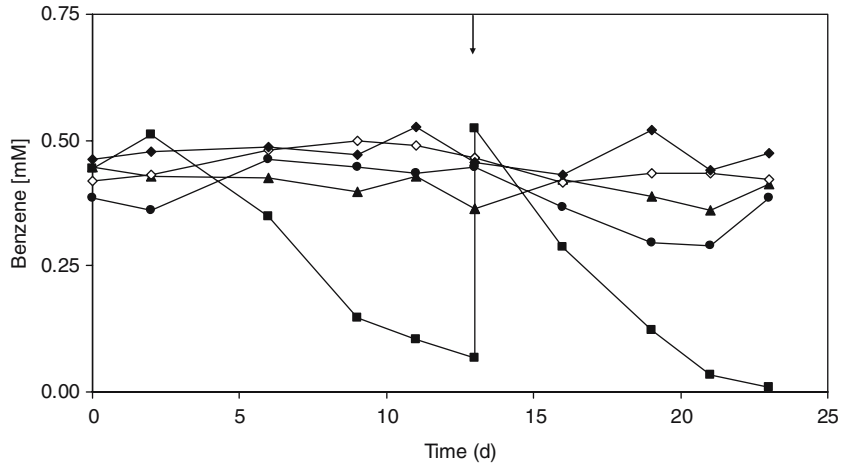

Figure 4. Benzene measurements in batches that were inoculated with the column material at the end of period V (■ active, ▲ chlorate omitted, ● sterile control, ♦ nitrate and ◇ perchlorate as electron acceptor; arrow indicates re-addition of benzene).

study. Addition of chlorate can also be applied for bioremediation of other anaerobic persistent compounds like naphthalene and monochlorobenzene. However, the usage of only chlorate might be insufficient. Dosage of a readily degradable substrate like acetate to enhance chlorate reduction and the production of oxygen may be essential.

For a successful application in the field, the addition of chlorate has to be evaluated from a regulatory point of view and the degradation of other compounds under chlorate reducing conditions has to be studied. This study showed that chlorate alone can be used to stimulate benzene degradation.

Conclusions

Benzene was degraded in an anoxic soil column in which chlorate was added as electron acceptor. Benzene degradation was chlorate dependent in soil column and batch experiments. Mass balance calculations showed stoichiometric benzene removal coupled with chlorate reduction and good recovery of chloride. The average degradation rate in period V of the column was 31 μmol l^{-1} h^{-1}. Batch experiments with column material confirmed the column results and showed that nitrate and perchlorate were not used as electron acceptor.

Acknowledgements

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